



Energetics Study on Integrated Nutrient Management of Summer Rice (*Oryza sativa* L.) under South Odisha Conditions

M. Sai Ram*, Tanmoy Shankar, Sagar Maitra, Sarath Kumar Duvvada and Dinkar J. Gaikwad

M.S.Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha, India

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*Address for Correspondence

M. Sai Ram

M.S.Swaminathan School of Agriculture,
Centurion University of Technology and Management,
Odisha, India.

Email : sairammasina52@gmail.com



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ABSTRACT

A field trial was carried out at Bagusala Farm of Centurion University of Technology and Management, Odisha during summer season of 2019-20 in sandy clay loam soil study the energetics of integrated nutrient management (INM) of summer rice. The experiment was laid out in Randomized Block Design and replicated thrice. There were eight treatments, namely, 100% RDN (recommended dose of nitrogen), 75 % RDN+25% N through vermin compost, 75% RDN+25% N through FYM (farmyard manure), 50% RDN+50%N through vermin compost, 50% RDN + 50%N through FYM, 50% RDN+25%N through vermin compost + 25% N through FYM, 25% RDN+ 25% N through vermin compost + 50%N through FYM, control (no fertilizer). The rice variety RNR 15048 was used. The treatments with 75% RDN along with 25% vermin compost recorded better results in terms of energy indices like energy output, energy productivity and net energy of (167.16 GJ ha⁻¹), (569.69Kg GJ⁻¹) and (145.29 GJ ha⁻¹). The practice of use of 75% RDN along with 25% vermin compost has recorded highest energy output (167.16 (GJ ha⁻¹), energy productivity (569.69Kg GJ⁻¹).

Keywords: Summer rice, INM, energy input, gross output energy, net energy, energy use efficiency, energy productivity, energy intensity in economic terms.

INTRODUCTION

Rice (*Oryza sativa* L.) as a staple food crop plays an important role in food security of India. Globally, the largest area under rice cultivation is in India (43.8 M ha) and the country produces 112.9 million t of rice with an average productivity of 2.78 t ha⁻¹ (Agriculture Statistics at a Glance, 2018). As rice is the major nutrient draining crop there will be depletion of soil fertility after harvest. The high yielding cultivars require more nutrients and generally these

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are supplied through inorganic fertilizers which results in decline of soil fertility (Imade *et al.*, 2017). The requirement of energy in the form inputs is huge for rice cultivation. Energy being most valuable input in production of agriculture plays a significant role in development process of mankind (Khambalkar *et al.*, 2010). Energy use efficiency is a vital problem of the modern agriculture and there is scope for improvement of energy management in farm production. Efficient energy management helps in making energy policies in agriculture and it saves environment restricting ill effect due to excessive and improper energy use (Singh *et al.*, 2016). Extensive field investigations revealed that locally available organic materials like FYM, and vermin compost can substitute substantial part of nitrogen (N) of total crop requirement. INM can enhance crop productivity with optimum N application and improve N use efficiency because of low rate of mineralization of N and slow release of nutrients in organic manures. Moreover, organic manures have some residual effect for succeeding crops. Among the various inputs, N fertilizers consume the bulk of the energy (non-renewable sources) which needs for substitution through organic manures (Ghosh *et al.*, 2016). Optimization of energy use in agriculture with increase in yield at existing level of energy input on a long run is possible by using greater proportion of renewable energy targeting production sustainability (Singh *et al.*, 2016). Therefore, the study was taken into consideration to estimate the energetic of various INM treatments in summer rice.

MATERIALS AND METHODS

A field trial was carried out at Bagusala Farm of Centurion University of Technology and Management (CUTM), Odisha during summer season of 2019-20 in sandy clay loam soil study the energetics of integrated nutrient management (INM) of summer rice. The field layout was made by adopting completely randomized block design and replicated thrice. The net plot size was 5m x4m. The treatments were T1: 100% RDN (recommended dose of nitrogen), T2: 75% RDN+25% N through vermin compost, T3: 75% RDN+25% N through FYM (farmyard manure), T4 : 50% RDN+50% N through vermin compost, T5: 50% RDN+50 % N through FYM, T6: 50% RDN+25%N throughvermicompost+25% N through FYM, T7: 25% RDN+25%N through vermin compost + 50% N through FYM and T8: Control. The recommended dose of nutrients was 80:40:40 kg/ha of N:P₂O₅:K₂O, respectively. The rice variety used during the season was RNR 15048. The date of nursery bed preparation and sowing seeds in nursery was 23 December 2018. The transplanting was done after 28 days. The total duration of the crop was 120 days. The crop was harvested on 24 April 2019. The climate of the experimental area is sub-humid and semi-arid tropic having a little extreme of weather condition. The weather condition like temperature, rainfall, relative humidity are observed and recorded from the Meteorological Observatory, CUTM, Odisha. During the crop period the mean maximum temperature was ranged between 28.2 to 38.9° C with an average of 34.1°C during the crop period, whereas, the weekly mean minimum temperature during this crop period ranged from 12.0 to 27.4°C with an average of 21.6°C. Mean monthly relative humidity was 81.9%. However, the monthly maximum relative humidity ranged from 77.3 to 92.8 % which was congenial for crop growth. The energy input was worked out by adding of energy equivalents for all inputs used in system represented in Table 1. The gross output energy was calculated by multiplying the produce with grain and straw energy. The energy indices were determined with the help of the following expressions.

$$\text{Energy efficiency (\%)} = \frac{\text{Gross energy output (GJ ha}^{-1}\text{)}}{\text{Total energy input (GJ ha}^{-1}\text{)}}$$

$$\text{Energy productivity (Kg GJ}^{-1}\text{)} = \frac{\text{Grain + Straw yield (Kg ha}^{-1}\text{)}}{\text{Total energy input (GJ ha}^{-1}\text{)}}$$

$$\text{Energy intensity in Economic terms (MJ Rs.}^{-1}\text{)} = \frac{\text{Gross energy output (MJ ha}^{-1}\text{)}}{\text{Cost of cultivation (Rs. ha}^{-1}\text{)}}$$

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RESULTS AND DISCUSSION

Energetics

Energetics namely energy input (GJ ha^{-1}), gross energy output (GJ ha^{-1}), net energy (GJ ha^{-1}), energy use efficiency (%), energy productivity (Kg GJ^{-1}) and energy intensity in economic terms (MJ Rs.^{-1}) of rice which were influenced by different integrated nutrient management levels calculated and represented in Table 3.

Energy Input

Maximum energy input was observed when the crop was supplied with 100% RDN (22.63GJ ha^{-1}) which was followed by 75% RDN+25% N through Vermicompost (21.88GJ ha^{-1}) and 75% RDN + 25% N through FYM (21.86GJ ha^{-1}) while the lower energy input was recorded in the control treatment (16.57GJ ha^{-1}) and that was due to low application of the resources in the treatment. Application of more inorganics in the 100% RDN treatment has showed more energy input compared to other treatments (Halder and Rahman, 2013; Thirupathi *et al.*, 2018)

Gross energy output and net energy

Both gross energy output and the net energy showed similar trend among the different nutrient management treatments. However the higher gross energy output and net energy were recorded with 75% RDN+25% N through vermin compost (167.16GJ ha^{-1} , 145.29GJ ha^{-1}) respectively, which were at par with all the treatments except 25% RDN+25% N through vermin compost + 50% N through FYM and control. The higher yields in 75% RDN+25% N through vermin compost with noticeable share of input energy resulted in obtaining more gross energy output and net energy. These results are conformity with Harika *et al.*, 2020 and Ray *et al.*, 2020.

Energy use efficiency and energy productivity

Energy use efficiency (EUE) and energy productivity (EP) exhibited parallel tendency among the treatments. Maximum EUE and EP were obtained when the crop was supplied with 75% RDN+25% N through vermin compost (7.64% and 547.99Kg GJ^{-1} respectively) which did not show significant differences with other treatments except control; while the minimum EUE and EP were noticed in control treatment (7.27% and 542.81Kg GJ^{-1} respectively). The treatments with replacement of 25%, 50% and 75% replacement of inorganic fertilizers with organic manures found to be effective in obtaining maximum EUE and EP as also observed earlier by Kumar *et al.* (2015).

Energy intensity in economic terms

The energy intensity in economic terms was significantly found highest in 100% RDN (5.09MJ Rs.^{-1}) the treatment with higher inorganic inputs reported the maximum energy intensity in economic terms and the lowest was noticed when the crop was supplied with 25% RDN+25% N through vermin compost + 50% N through FYM (5.09MJ Rs.^{-1}). The higher energy intensity in economic terms is due to enhancement of gross energy output with substantial sum of cost of cultivation. The results are in collaboration with Kumar *et al.* (2019).

CONCLUSION

When the summer rice was supplied with 100% RDN through in organics recorded maximum energy input and energy intensity in economic terms. The highest gross energy output, Net energy, Energy use efficiency and energy productivity were noticed when the crop was provided with integrated nutrients with 75% RDN+25% N through vermin compost.





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Table 1: Energy equivalents for various input and output energy forms

Component	Unit	Energy equivalent (MJ/h)	Source
Cultivator	Hour	3.135	Nassiri and Singh (2009)
Rotavator	Hour	10.283	Nassiri and Singh (2009)
Tractor	Hour	64.80	Devasenapathy et al. 2009
Sprayer	Hour	0.502	Nassiri and Singh (2009)
Adult male	Man per hour	1.96	Soniet al. (2013)
Adult female	Female per hour	1.60	Soni et al. (2013)
Diesel	L	56.30	Nassiri and Singh (2009)
N	Kg	60.60	Kuswardhaniet al. (2013)
P ₂ O ₅	Kg	11.10	Chaudhary et al. (2009)
K ₂ O	Kg	6.70	Chaudhary et al. (2009)
FYM	Kg	0.30	Kizilaslan (2009)
Vermicompost	Kg	0.61	Kizilaslan (2009)
Insecticides	Kg	199.0	Braret al. (2015)
Irrigation	m ³	1.02	Tutiet al. (2012)
Seed	Kg	14.70	Tutiet al. (2012)
Grain	Kg	14.70	Tutiet al. (2012)
Straw	Kg	12.50	Tutiet al. (2012)
Thresher	Hour	7.524	Nassiri and Singh (2009)

Table 2 Energy inputs for different operations in rice cultivation

Name of the Operation	Energy inputs
Nursery Preparation	
Ploughing with cultivator	1 hour tractor + cultivator + 3 L diesel
Seed cost	40 kg/ha
Sowing and nursery bed preparation	2 man-days
Fertilizer	10 kg N and 5 kg P ₂ O ₅
Irrigation	5 ha-cm + 2 man-days
Main field preparation	
Ploughing	4 hrs. Tractor + 4 hrs. cultivator + 12 L diesel
Puddling	4 hrs. Tractor + 4 hrs. rotavator + 12 L diesel
Layout	6 man-days
Uprooting of seedling	15 women days
Transplanting	25 women days
Irrigation	7200 m ³ (18 number of irrigations with 4 ha-cm depth per irrigation)
Labour for irrigation	10 man-days
P ₂ O ₅	60 kg





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K ₂ O	60 kg
Fertilizer application labour	4 man-days
2 hand Weeding	30 women days
Insecticides spraying	2 times
Labour for spraying	4 man-days
Harvesting	20 women days
Threshing with thresher	2 hours
Drying and bagging	1 man-day

Table 3 Energetics of rice cultivation as influenced by integrated nutrient management.

Treatments	Energy input (GJ ha ⁻¹)	Gross energy output (GJ ha ⁻¹)	Net energy (GJ ha ⁻¹)	Energy use efficiency (%)	Energy productivity (Kg GJ ⁻¹)	Energy intensity in economic terms (MJ Rs. ⁻¹)
T ₁ :100% RDN	22.63	166.19	143.56	7.34	547.99	5.09
T ₂ :75% RDN+25% N through vermicompost	21.88	167.16	145.29	7.64	569.69	3.91
T ₃ : 75% RDN + 25% N through FYM	21.86	165.24	143.37	7.56	563.30	4.11
T ₄ : 50% RDN + 50% N through vermicompost	21.12	160.58	139.46	7.60	567.10	2.93
T ₅ : 50% RDN+50 % N through FYM	21.10	158.93	137.83	7.53	562.13	3.25
T ₆ : 50% RDN + 25%N through vermicompost + 25% N through FYM	21.11	157.40	136.29	7.46	556.28	2.93
T ₇ : 25% RDN+25% N through vermicompost + 50% N through FYM	19.58	142.44	122.85	7.27	542.81	2.32
T ₈ : Control	16.57	88.19	71.62	5.32	397.35	2.91
SEm±	-	4.90	4.90	0.23	17.93	0.11
CD (P=0.05)	-	14.9	14.9	0.7	54.4	0.3





A Review on Productivity of Maize Intercropping Systems

Pilli Manasa, M. Sai Ram, Sagar Maitra*, Tanmoy Shankar and Dinkar J. Gaikwad

Department of Agronomy and Agroforestry, M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Sagar Maitra

Department of Agronomy and Agroforestry,
M. S. Swaminathan School of Agriculture,
Centurion University of Technology and Management,
Odisha, India.

Email: sagar.maitra@cutm.ac.in



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ABSTRACT

The greatest challenge of the twenty first century in many developing countries is to maximize the production of basic necessities namely food, fodder, fuel and fiber to fulfill the needs of the increasing human and animal population. The availability of land for agriculture is shrinking day by day and it is converting for non-agricultural purposes. Under this situation, one of the important strategies to increase agricultural output is development of high intensity cropping systems by adopting intercropping. Intercropping systems found to have more advantages than sole cropping as related to income and soil health and other environmental factors. Further, cereal-legume combination of intercropping mostly shows advantages in terms of more productivity, utilization of available resources and soil health improvement. Maize is widely spaced crop and chosen as cereal component to accommodate legumes in a better manner in the space between maize rows. The article focuses on enhancement of productivity of crops and advantages of maize-legume intercropping system.

Keywords: Intercropping, maize, legume, productivity, resource use, competitive functions

INTRODUCTION

In agro ecosystems, intercropping allows better resource use efficiency hence reducing the needs for external inputs and moving towards agricultural sustainability (Beets, 1994; Maitra *et al.*, 2001a; Dariush *et al.*, 2006). It is a practice of growing two or more crops in the same piece of land at the same time (Sanchez, 1976) and intercropping system plays an important role in subsistence food production in developing countries (Tsubo *et al.*, 2001; Maitra *et al.*, 2020). It has been well established that intercropping offers so many potential advantages such as: improved utilization of growth the intercropped species (Banik *et al.*, 2006); direct nitrogen transfer from legumes to cereals in intercropping enhanced productivity due to nitrogen fixation (Mainingi *et al.*, 2001; Maitra *et al.*, 2001b); used as a method of

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controlling weeds, insect pests, diseases (Maitra and Ray, 2019) and control of soil erosion (Jabbaret *et al.*, 2009; Matusso *et al.*, 2012). The sustainable productivity of crops is the need of hour in the present Indian farming. Hence, every possibility for crop intensification with sustainable nutrition should be examined. Under this situation, one of the important strategies to increase agricultural output is development of high intensity sequential cropping and intercropping systems. Intercropping, an age old agricultural practice of cultivating two or more crops in the same space at the same time is generally adopted for more production by utilizing available growth resources. Maize has been recognized as a common component in most intercropping system and it seems to lead as the cereal constituent of intercrop and is regularly combined with dissimilar legumes (Manasa *et al.*, 2018; 2019). The main reason to adopt intercropping is to increase in productivity per unit area per unit time (Willey, 1979; Willey *et al.*, 1983). Moreover, by adoption of intercropping risks in crop cultivation can be minimized and natural resources are fully utilized. This practice is an attractive strategy to smallholder farmers for increasing productivity, utilization of land and labour though intensification of land uses. Furthermore, intercropping cereals with legumes have huge capacity to replenish soil mineral nitrogen through its ability to biologically fix atmospheric nitrogen (Mandal *et al.*, 1991; Giller, 2001, Maitra *et al.*, 2019; Sarath Kumar and Maitra, 2020). Several factors influence the intercropping such as maturity of crop, selection of compatible crops, planting density, time of planting as well as socio economic status of farmers and crops cultivated in the region. In intercropping, land is effectively utilized and Land Equivalent Ratio (LER) is used to measure the productivity of land. Cereal-legume intercropping is commonly practiced worldwide. In the article different aspects of maize-based intercropping system including advantages have been presented in the following paragraphs.

Consideration in maize-legume intercropping system

For the success of intercropping system several aspects need to be taken into consideration before and during the cultivation process (Seran and Brintha, 2010). For example, the potential of cereal-legume intercropping system to provide nitrogen depends in density of crop light interception, crop species and nutrient requirements (Francis, 1989). Despite that, the choice of crops, water management and fertilizer utilization are also important aspects of intercropping system (Maitra *et al.*, 2019). The productivity of an intercropping system is mainly determined by the efficiency of the component crops in utilization of resources. This depends not only on the individual main and component crops of the system, but also time and space dimensions (Willey 1979). It is a common knowledge and observation that when a tall main crop like maize intercropped with a short stature legume component like greengram, blackgram, cowpea, groundnut and so on are the beneficial effects accrue both from the point of better utilization of aerial atmosphere and from the stand point of improved utilization of underground resources. Therefore, the overall productivity of maize based cropping system depends partly on the efficiency of maize crop itself and partly on how well maize utilized the resources and what benefits the legumes got and vice-versa. So the overall productivity of the intercropping of legumes relies on the main crop as well as compatibility with other component. In recent years, it is often recognized that intercropping system can produce higher yield than sole cropping, but there can be problems in assessing the degree of yield advantages. Yet, such an assessment is essential to determine whether a given intercropping combination is indeed better than sole cropping and whether, within that combination one cropping system is better than other. There are number of ways to ensure that intercropping advantages are accurately assessed and most common method is by calculating land equivalent ratio (LER) and area time equivalent ratio (ATER) and base-crop equivalent yield. Spatial and temporal identification of cropping is the need of the day to keep the pace between food grain production and burgeoning population in a densely populated country like India. Though intercropping is an age-old practice, it has attracted worldwide attention owing to the yield advantages (Willey, 1979), if the crops selected are compatible and grown with scientific technology. Intercropping involves the scientific management of compatible crops and genotypes so as to minimize the complex interspecific, inter-varietal and interplant interactions. To achieve this, the competition between component crops may be minimized by selecting crops of different rooting pattern, growth habit and maturity groups having complementary effect, and by adjusting plant geometry, planting time, population levels and other agronomic





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practices in such a way which enables to use the production resources efficiently and enhance productivity per unit area and time.

Maize yield with legume intercropping system

Studies found that intercrops did not affect the maize grain yield significantly reflecting the lack of competition between main and associated crops. Growing greengram, cowpea, groundnut and black gram slightly increased the grain yield of maize. Maximum yield (1589 kg ha⁻¹) of maize was recorded when it was grown in association with blackgram. The sole crop of maize gave significantly higher grain yield than intercropped maize (Sarkar and Shit, 1990). The highest grain yield of maize 4273 kg ha⁻¹ was recorded in sole maize crop but the yield of maize was not reduced significantly when it was intercropped with greengram or with groundnut (Singh and Chand, 1980). However, yield of maize was significantly reduced by intercropping with cowpea showing the competition effect of cowpea with maize, since cowpea had put up quick vegetative growth at early growth stage of maize (Thakur and Bora, 1987). The intercropping of legumes like greengram (3.92 t ha⁻¹), blackgram (3.31 t ha⁻¹) and groundnut (3.65 t ha⁻¹) towards maize yield was comparatively higher than compared with cowpea and soybean (Rajeshkumar *et al.*, 2018). Also the intercropping with soybean and sunflower found antagonistic effect on grain yield of maize (Singh and Awasthi, 1982). The combination of maize cropping with legume fodder like sunhemp had a depressive effect on grain yield of maize due to the vigorous growth of the sunhemp during the growth stages (Thakur and Sharma, 1988; Singh *et al.*, 1988). The contribution of legumes to grain yield clearly showed that the N secretions from legume nodules has its beneficial effect on maize at later stages (Gangwar and Kalra, 1981; Singh and Sharma, 1987). The obvious reason for large yield advantage in intercropping system is that the component crops differed in their use of natural resources and utilized them more efficiently resulting in higher yields per unit area than that produced by their sole crops. The maize grain yield was maximum in maize with groundnut (2:4) intercropping treatment. In another experiment Rashid Saleem *et al.* (2015) reported higher maize yield was noted in sole maize as compared to intercropping of maize + mung bean (Mandal *et al.*, 2014).

Land equivalent ratio (LER)

The LER referred as the proportion/amount of land area that is needed for sole cropping to produce the same yield as the intercropping (Mead and Willey, 1980). LER is mostly preferred and used index for comparisons of intercrop with sole crop (Agegnehu, 2006; Esmaili *et al.*, 2011). LER is an accurate method of assessing the competitive relationship between the intercropped crops, and the overall productivity of intercropping system. The Maximum LER of 1.89 and 1.61 was obtained in maize + blackgram and maize + groundnut intercropping system respectively, which indicates the yield advantage over sole crop of maize (Narasa Reddy *et al.*, 1986; Ambapurkar and Raikhelkar 1988). Also the intercropping of maize with soya bean was found maximum LER when taken in double strips of 90 cm spacing (Ullah *et al.*, 2007). Addo-Quaye *et al.* (2011) found that LER was greater than unity, inclined that maize-soybean intercropping was more productive than monoculture. The incorporation of green gram was also improved the LER. Maize + black gram intercropping recorded the highest LER of 1.40 in 1:1 row proportion and 1.43 in 1:2 row ratio which resulted in 40% and 43% more land use efficiency than pure stand of maize (Tsubo *et al.*, 2001).

Area time equivalent ratio (ATER)

The advantage of intercropping estimated through land equivalent ratio method can mislead sometimes because the conceptual basis in which the monoculture versus intercrop comparisons were made is incomplete. In LER time factor is not taken into consideration. Area Time Equivalent Ratio (ATER) will correct this conceptual inadequacy in LER and enable to assess the land use efficiency along with time use efficiency in crop mixtures in inter cropping system (Hiebsch, 1987). Barik (1997) reported that higher ATER values in maize + green gram, intercropping followed by the maize + groundnut and the values were more than unity which indicated yield advantage in intercropping. Singh *et al.* (2008) recorded an ATER of 1.05 by intercropping greengram in 1:1 row ratio with maize on silty clay loam soils of Jammu and Kashmir. Solanki *et al.* (2011) reported that maize + blackgram and maize + greengram recorded ATER more than one, which indicated better land utilization efficiency than their sole crops. Kheroar and



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Patra (2013) reported maize + black gram intercropping recorded the highest ATER values (1.349 and 1.374 in 1:1 and 1:2 row proportions, respectively) followed by maize + soybean (1.284 and 1.304 in 1:1 and 1:2 row proportions, respectively) indicating higher per day yield from both the systems. Yogesh *et al.* (2014) reported that intercropping system of maize + soybean in 2:6 paired row system (50:75) resulted in significantly higher ATER (1.32) indicating higher per day productivity and spatial complementarity. Jan *et al.* (2016) recorded that maize + black cowpea expressed highest ATER value (1.51) at 2:2 row proportions than other treatments. Khan *et al.* (2018) found that ATER values were greater than unity in all maize based intercropping systems. Maize (100%) + garden pea (66%) in between two lines of maize intercropping system (showed higher ATER value (1.28) than other intercrop combinations which clearly indicating higher yield per day and greater complementarity.

Maize equivalent yield (MEY)

It is noticed that the LER overestimates and the ATER underestimates the land-use efficiency (Maitra *et al.*, 2000). Crop equivalent yield is another expression for evaluating the efficiency of intercropping system (Yogesh *et al.*, 2014). Actually, in maize-legume intercropping system, total yields are converted in the form of base crop (maize) equivalent yield by considering the intercrop yield and market price of maize based crop and associated intercrops. In maize-legume intercropping system it is termed as maize equivalent yield (MEY) and expressed in kg-ha. If the base crop equivalent yield is obtained higher in intercropping combinations than base crop yield, then intercropping is considered advantageous. The greater yields in intercropping is recorded when the component crops show complementary effects amongst themselves and use natural resources efficiently than raised as sole crops (Willey, 1979). The crops with inherent capability can only utilize natural resources efficiently and complementarity plays important role in resource utilization (Maitra *et al.*, 2019). Further, higher yield of both the crops in maize-cowpea intercropping combination was noted than pure stands (Kimou *et al.*, 2017). In soils with low nitrogen content, maize legume intercropping performed well (Vesterager *et al.*, 2008). Yield advantage in intercropping is expressed by crops because of greater use of growth resources like light, water, and nutrients and this efficient use is Crop equivalent yield is an important index in assessing the performance of different crops under a given circumstance. Based on the price structure, economic yield of component crops is converted into base crop yield *i.e.*, Maize equivalent yield. Maize equivalent yield showed marked differences due to inclusion of a short legume like greengram. De *et al.* (1978) observed that maize grown in association with greengram enhanced maize equivalent yield by 93 per cent over monoculture.

Maize equivalent yield was higher by 1.2 t ha⁻¹ when maize was intercropped either with groundnut or soybean over sole maize (Prusty *et al.*, 1987). Thakur and Bora (1987) indicated that maize + blackgram(1:2) resulted in highest maize equivalent yield and it differed significantly from pure stands of maize and blackgram. Intercropping maize and groundnut gave significantly higher maize equivalent yield compared to other cropping systems and sole crop of maize (Mandal *et al.*, 2014). Maize equivalent yield was highest in maize + groundnut (5643 kg ha⁻¹) intercropping system followed by maize + pigeon pea (5487 kg ha⁻¹), maize + blackgram (4948 kg ha⁻¹) and maize + greengram (4860 kg ha⁻¹) (Barik, 1997). Shivay *et al.* (2001) stated that maize equivalent yield was significantly increased due to intercropping. Whereas the highest maize grain equivalent yield was recorded with maize + soybean. Kheroar and Patra (2013) stated that maize with groundnut recorded higher maize grain equivalent yield in both 1:1 and 1:2 proportions of sowing (4,831.45 kg ha⁻¹ and 4,582.36 kg ha⁻¹, respectively), however, the lowest equivalent yield (3,451.94 kg ha⁻¹) was found to be recorded with maize and black gram combination at 1:2 row ratio. They further stated that in maize + legume intercropping, maize yield + extra yield of legumes increased the maize equivalent yield. A study on the effect of intercropping on maize-legume intercropping system on growth yield and competitive ability indicated that planting geometry adopted for paired row hybrid maize (under both of sole and intercropping) was 30 cm/ 80 cm × 25 cm produced greater maize equivalent yield (MEY) than pure stand of maize (Manasa *et al.*, 2018; 2019). On the other hand, greengram and black gram being fast growing shallow rooted crop, utilized the resources from top layer (0-30 cm) of the soil and serving as cover crop conserved soil moisture reduced soil temperature and added organic matter to the soil.





CONCLUSION

The above study from the review, clearly mentioned that maize based intercropping is advantageous in terms of crop productivity and greater use of resources. Further, land is a limiting resource and considering the future need for food it is essential for intensification of crop productivity and maize based intercropping system can assure more food grains production and higher resource utilization targeting sustainable production.

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Scope for Adoption of Intercropping System in Organic Agriculture

Sagar Maitra^{1*} and Harun I. Gitari²

¹Department of Agronomy and Agroforestry, Centurion University of Technology and Management, Odisha, India.

²Department of Agricultural Science and Technology, School of Agriculture and Enterprise Development, Kenyatta University, Nairobi, Kenya.

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*Address for Correspondence

Sagar Maitra

Department of Agronomy and Agroforestry,
Centurion University of Technology and Management,
Odisha, India.

Email : sagar.maitra@cutm.ac.in



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ABSTRACT

Intercropping, also known as multi-cropping or poly culture, is an age-old agricultural practice that involves the growing of diversified crop species in close proximity such that they coexist temporally for a portion of their life cycle. Growing of different plant species together enhances biological diversity and thus ensures greater ecological service, which is important in creation of suitable environment for successful organic agriculture. Further, legume crops in association in intercropping system benefit non-legumes by sharing nutrients, enabling soil biological activities and higher use of available resources. The adoption of Green Revolution Technologies (GRTs) resulted in degradation of natural resources with yield decline or stagnation of major crops. During recent time, organic agriculture has emerged as an economically viable option of farming because of growing demand of the organic products worldwide. In addition to that, organic agriculture has enough potential to assure agricultural sustainability. Under these circumstances, intercropping can play a vital role in organic agriculture for sustainable farm output and the article has focused on beneficial aspects of intercropping system in befitting organic agriculture.

Keywords: Green, culture, environment, farm, agriculture, soil.

INTRODUCTION

Agriculture plays a vital role to human society for its existence as it provides food, natural fibre, fuel and some raw materials for industry along with ecosystem services. Agriculture is the most important source of livelihood that shows prominence in economic progress of a developing nation like India. Indian economy is one of the fastest growing economies among the developing world. In India, agriculture supports employment of 54 per cent of the population and still contributes to satisfactory gross domestic product. The agricultural sector in India has made

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massive pace in the past seven decades. But modern industrialized agriculture is a major source of environmental degradation, contributing to emission of greenhouse gasses and thus causing climate change, reducing freshwater resources, unbecoming soil fertility and contaminating the environment through some man-made practices. These are misuse of natural resources, non-judicious use of chemical inputs for plant nutrition and plant protection and others. Agricultural production system is directly related to the natural resources, which are degrading. Presently, the country produces sufficient food grain, but the modern agriculture flops in attaining the aims of sustainability. The yield plateauing has been observed in the most productive areas of the country where natural resources are over-exploited; pollution due to faulty agricultural practices are prominent and eventually deterioration of natural resources. Sustainability of agricultural production, food and nutritional security necessitates not only that access to sufficient nutritious food to all, but also that the agricultural production system should be lacking any negative ecological effect. In other words, it may be stated that the sustainable agriculture is such a production system that should fulfill the needs of the present without impairing the natural resource and assuring the ability of future generations to meet their needs. That expectation is missing and it is very much clear that modern agriculture will not be able to fulfill the requirements of sustainability.

Cropping Systems in Modern Agriculture

Worldwide cultivable land has been decreased due to increase of global population and industrialization, while global demand for food is increasing. Modern industrial agriculture based on use of huge quantity of chemicals has adverse effect on human and animal health, agro-ecosystem and quality of agricultural products. Furthermore, monoculture has resulted in increased yields, but at the cost of environmental wellbeing. In contrast, sustainable agriculture aims to simulate nature with holistic approach as the pattern for creating agricultural systems by nurturing diversity, integrating plants and animals into a diverse landscape for gratification of the human requirements for today and future. Agricultural production can only be sustained on a long term basis if the land, water and forests on which it is based are not degraded further (Zaman *et al.*, 2017). Creation of multiplicity and its effective execution for sustainable agriculture are very important and intercropping suggests simulating nature's principle of diversity on crop lands. Thus, intercropping can be evaluated as pro-ecological approach of raising of crops, supporting above and below ground bio-diversity and compatible crop husbandry with the aims of sustainable agriculture.

A system consists of different components and these are diligently related with a proper interaction among themselves. Emerson (2007) mentioned that cropping system refers to production of crops in sequences or mixture and the management involved in a specific field for a time period. Generally, farmers adopt various cropping systems to maximize farm output, profitability and production sustainability (Hauggaard-Nieson, 2001). The system approach always aims at better utilization of resources and agronomic manipulations and thus assures sustainable productivity as well as enhancement of cropping intensity. The intensive cropping systems focus on maximization of farm output from unit area. The modern concepts of agronomy clearly narrate that the efficiency of a cropping system hangs on the single crop in mono-cropping and / or different crops cultivated in the sequential or intercropping system. Moreover, time and space dimensions are also considered to measure the efficiency of the system more accurately (Willey and Reddy, 1981; Willey *et al.*, 1983). However, modern agriculture resulted in mechanization, monoculture, cultivation of improved varieties and hybrids of crops, use of chemical fertilizers and nutrients led to a simplification of the interacting components in agricultural systems and ultimately caused genetic erosion, greater susceptibility to abiotic stress and thus vulnerability in cropping system. Restoration of biodiversity through growing of different crops and adoption of farming systems that result in the efficient exploitation of available resources is important for sustaining farm output (Jackson *et al.*, 2007; Scherr and McNeely, 2008). The modern agriculture has assured enhancement in production and productivity, but simultaneously brought uncertainty in farming with a threat to agricultural sustainability (Tilman *et al.*, 2002; Lichtfouse *et al.*, 2009). No sooner had India embraced the Green Revolution in 1960s, than it realized its adverse effects a decade later. The Green Revolution was characterized by mainly research development and initiatives for transfer of technology and no doubt these enhanced agricultural productions worldwide, particularly in the developing countries (Maitra *et*



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al., 2018). The production enhancement noted was mainly due to arrangement of assured irrigation, use of huge quantity of chemical fertilizers and cultivation of ideal types and high yielding varieties of crops. During that period, monocropping became the economically effective path to move. Soon fertilizer shortages and increasing prices developed, which created another problem including lowering of profit. As synthetic fertilizer is a fossil fuel based product, agriculture was gradually becoming dependent on crude oil extraction. Also, environmental complications associated with non-judicious use of fertilizers and other agro-chemicals were becoming prominent. These resulted in surface and groundwater pollution, degradation of soil and ultimately created an imbalance on crop-ecology (Joseph *et al.*, 2018). Monoculture is characterized by devoid of diversity and increases pest, disease and weed problem. The plant protection became difficult because of insufficient population of pest predators (Horrigan *et al.*, 2002), which further encouraged use of more plant protection chemicals. As these and other problems associated with the monoculture system became more apparent, interest in intercropping grew as possibly, part of the solution to achieving and maintaining sustainability (Manasa *et al.*, 2018). Intercropping is one of the many systems that hold great potential in solving future food and economic problems in developing countries. The main reason for the acceptance of intercropping for smallholders is that it has greater stability than monocropping. Due to different aberrant environmental conditions there may be a chance of crop failure is common in fragile ecologies of the developing countries and intercropping ensures stability as it ensures partial restoration of species diversity and improvement of soil fertility (Li *et al.*, 2001; Gitari *et al.*, 2018; Gitari *et al.*, 2020; Manasa *et al.*, 2020), which are not common in monocropping.

Intercropping**A Felt-need**

For achieving sustainable agricultural productivity, the researchers of the developing countries always focus on low-input and energy-efficient agricultural systems (Maitra *et al.*, 2019) and intercropping is considered as a choice. Besides, diversity in agricultural system can be further enriched temporally by crop diversification or spatially by adoption of intercropping systems (Altieri, 1999). Maintenance of on-farm diversity is very much common in many countries and mostly in developing world, where traditional farming systems are observed with cultivation of different crops together in the form of mixed and intercropping (Maitra *et al.*, 2019; Maitra 2020). In an intercropping system, two or more crops may be sown or harvested at the same or different time, but they are grown together for a major portion of their growth periods and the main crop gets more importance for economic gain (Maitra *et al.*, 2001) and additional crop provides support to enhancement of productivity and income. The sequential cropping is no doubt important for increasing the intensity of cropping, but intercropping system benefits a lot by enhancing farm output, monetary advantage and efficient utilization of available resources.

Organic Agriculture: A Way Forward

To accomplish and address social, ecological and economic concerns together, organic agriculture can play a dynamic role. Organic agriculture has a long tradition and heritage in India that progressed over the millennium. Organic agricultural is a complete production system, which improves agro-ecosystem health, inclusive of biodiversity, biological cycles, and soil biological activity. It emphasizes the use of mostly on-farm inputs considering the regional conditions with locally adapted closed farming system. As proposed by International Federation on organic agriculture movement (IFOAM), there are four principles of organic farming (Figure 1), which may be considered as pillars (IFOAM-Organics International, 2018). India has numerous kinds of available organic form of nutrients in different locations of the country, which can be used for supplying nutrients to plants. India initiated the National Program for Organic Production (NPOP) by Agricultural and Processed Food Products Export Development Authority (APEDA) in 2001. The organic production standards prepared by NPOP have been created under guidelines of international organic production standards such as CODEX Alimentarius [United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO)] and IFOAM. The standards followed by NPOP for organic agriculture production and accreditation system have been accepted by European commission, US and Switzerland as it follows the world class norms. In traditional agriculture in India, the



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agriculture production system was fully dependent on organic inputs obtained from fresh or decomposed plant and animal products. The traditional farming system is regarded as production of food and other agricultural products by small and marginal farmers producing for their families and local consumption. During post green revolution era, Indian agriculture system has been changed with introduction of chemical inputs. No doubt, there was enhancement in grain production, but it was achieved at a cost of environmental issues and agriculture in India ultimately has lost its sustainability. Presently, for achieving sustainability, once again agriculturists are opting for organic agriculture. Production of additional food from the same land area without degrading the ecology requires sustainable intensification (Royal Society of London, 2009). By adoption of some agronomic management ill effects on environment can be reduced targeting agricultural sustainability and organic agriculture has enough potential. Organic agriculture is a unique production management system which mostly disregards the usage of artificial inputs (like chemical fertilizers, pesticides, synthetic hormones, food additives and so on) and it rely upon crop rotations and crop diversification, use of crop residues and animal manures, organic wastes, mineral grade rock additives and biological system of nutrient mobilization and non-chemical inputs for plant protection.

However, organic agriculture is not only replacing use of inputs but it is more a farming system approach for creation of a healthy agro-ecosystem targeting the achievement of the four principles of organic farming as advocated by IFOAM. In broad sense we can say organic agriculture is actually a farming system targeted with minimal harm to agro-ecosystems, animals or humans (FAO and WHO, 2007). During recent times, awareness of health and environmental issues in agriculture has been increased which necessitated production of organic food. Organic agriculture is a cost effective and sustainable choice of farming, particularly by the smallholders of rain fed and dryland areas and supports to boost their food and income security. It helps to produce and supply adequate safe and nutritious food to the producers and consumers. Environmental advantages, health benefits and strengthening of farmers' economy are other key factors influencing farmers to shift to organic agriculture.

Organic Agriculture and Intercropping

The success of organic agriculture is lying on some cultural practices of which system approach, crop diversification, inclusion of legumes in cropping system, restoration of soil fertility and reduction in degradation of natural resources and stability in production are important. These basic requirements can be achieved by adoption of intercropping systems. On-farm bio diversity can lead to healthy agro-ecosystems accomplished of maintaining soil fertility and sustaining productivity (Scherr and McNeely, 2008) and intercropping system can create diversity. Crop diversification happens in intercropping system, because two or more crops are cultivated together in the same field. In addition, cultivation of at least two crops of preferably dissimilar type under intercropping creates above ground diversity by attracting pollinator and predators (Maitra and Ray, 2019). The intercropping systems encourages the population of bee and other insect pollinators (Nicholls and Altieri, 2013), which ultimately enhances yield.

Legumes are known to host predators, which can manage pest population dynamics and thus pest management becomes easier (Maitra and Ray, 2019). In organic agriculture, weeds are managed by cultural, mechanical and biological methods. Intercropping system assures less population and growth of weeds because crops cover more ground area suppressing weed growth. Not only the above ground diversity, but also beneficial soil micro-organisms are enriched in cereal-legume intercropping system. Moreover, application of organic manure in soil facilitates suitable environment for increasing the numbers of beneficial micro-organisms. Intercropping cereals with legumes assures presence of soil micro-organisms, namely, *Alphaproteobacteria*, *Betaproteobacteria* and *Cyanobacteria* (Qiao *et al.*, 2012; Li and Wu, 2018). *Rhizobium* colonizes in legumes roots and it has been observed that in *Rhizobium* rich soils, other beneficial microorganisms harbor in rhizosphere and thus soil is enriched biologically favouring crop growth and sustainable yield. Besides, some other free-living soil bacteria like *Pseudomonas denitrificans*, *P. rathonis*, *Bacillus laevolacticus*, *B. amyloliquefaciens* and *Arthrobacter simplex* are observed to enhance plant growth in intercropping. *Rhizobium* species is also beneficial in reduction of harmful pathogens like *Fusarium*spp. and *Phytophthora* spp. (Maitra and Ray, 2019). Thus diversity creates a healthy ecosystem services and sustainability in crop production. In crop-livestock farming system, for nutrient management in organic agriculture



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wastes of livestock and crops as well as manures are used. But in livestock-free system, legumes contribute a lot in organic production. Legumes can fix nitrogen biologically from atmosphere and play a vital role in the N-economy of the system (Amosse *et al.*, 2013). In an experiment on soybean-maize intercropping system, ¹⁵N labeling showed that *Glomus mosseae* and *Rhizobium* SH212 inoculation increased the N transfer from soybean to maize (Senaratne *et al.*, 1995). The fixed N is transferred to non-legume component through root excretion, root and nodule senescence, leaching of N from leaves and leaf fall (Fujita *et al.*, 1992; Ledgard and Giller, 1995). The amount of N fixed biologically and transferred to other non-legume component depends on some factors like legume species, morphology, duration and management practices adopted (Nasar *et al.*, 2020). The short duration green gram (*Vigna radiata* L.) fixes N than long duration red gram (*Cajanas cajan* L.). Moreover, a portion of biologically fixed N is utilized by the succeeding crops, depending upon the quantity of N fixed and utilized (Sarath Kumar and Maitra, 2020). Phosphorus, Fe and Zn nutrition of cereals and/or legumes can be improved by adoption of intercropping through the increased availability of these nutrients in the rhizosphere (Xue *et al.*, 2016). Relay intercropping system of legumes with cereals is beneficial for enrichment of the soil-plant system in N through symbiotic fixation (Amosse *et al.*, 2013).

In Intercropping system, more biomass yield is obtained and crops utilize greater quantity of CO₂ and thus atmospheric pollution is reduced to some extent. Further, renewable solar energy is more utilized by crop mixture for biomass production. Combination of shallow and deep rooted crops are often chosen in intercropping system leading to efficient utilization of soil nutrients by crops from different layers. Maize-legume relay strip intercropping system can minimize soil moisture evaporation than pure stands and under limited water conditions maize-soybean combination is beneficial for maximizing water use efficiency and more yields (Rahman *et al.*, 2017). Combination of shallow and deep rooted crop is advantageous in intercropping not only for utilizing better use of nutrients, but efficient use of soil moisture. Experimental results indicated that finger millet may be benefited by deep rooted pigeon pea by bio-irrigation when biofertilizer inoculation was done with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizo bacteria (PGPR) under dry land conditions (Saharan *et al.*, 2018).

Further, more ground cover in mixed cropping ensures less run-off of water, soil erosion and less nutrient loss from the soil (Dass and Sudhishir, 2010; Gitari *et al.*, 2019). The positive effects of intercropping system are also noted in resource conservation and soil health management as it checks run-off of water, soil erosion and less nutrient loss from the soil (Nyawade *et al.*, 2019). The intercropping combination of finger millet + black gram recorded the lowest runoff (10.2%) and losses of soil and nitrogen, phosphorus and potassium through erosion over sole when sown in contour because black gram covered enough ground area in intercropping with finger millet. The soil fertility status was also improved in finger millet + pulses intercropping, which was due to contribution of leaf fall and biological nitrogen fixation by legumes. Comparatively low soil and nutrient losses from intercropping systems and legume effect contributed to increased nutrient status under finger millet and legumes intercropping (Dass and Sudhishir, 2010). Further, legumes can be a good resource of energy and these can be digested in the bio-gas chamber from which renewable energy can be generated and slurry can be used as nutrient resource. Chemical N fertilizer production system is involved with fossil fuel consumption and emission of greenhouse gases. However, legume-cereal intercrop mixture can assure energy saving and emission saving process with no adverse impact on environment. The enrichment of biodiversity and soil health improvement is possible in intercropping and these enable greater ecosystem services. Thus intercropping system preferably legume as a component may be considered in organic agriculture for multiple benefits targeting production sustainability.

CONCLUSION

Considering the growing demand of organic agriculture, farmers of the developing countries are showing interest in chemical-free agriculture targeting domestic as well as export market. Input management in organic agriculture is also costly because of bulky nature of organic nutrients with low analytical value. Organic agriculture basically relies on system approach and there is enough potential of intercropping system in terms of nutrient management, greater



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resource use, management of pest population dynamics and higher ecosystem services. The present article concludes that intercropping system should be an integral part of organic agriculture assuring sustainability.

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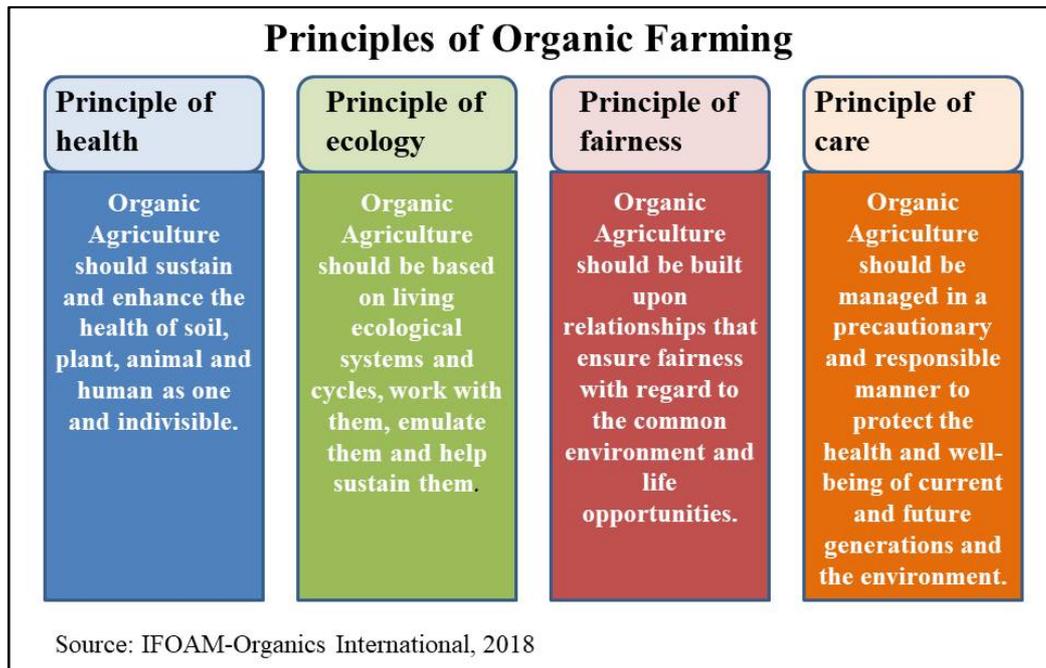


Fig. 1. Principles of Organic Farming





Verification of Asynchronous FIFO Protocol

Sandipan Pine^{1*} and Suman Kumar²

¹Dept of ECE, Centurion University of Technology and Management, Odisha, India.

²Dept of Mech, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Sandipan Pine

Dept of ECE,

Centurion University of Technology and Management,

Odisha, India.

Email: sandipan@cutm.ac.in



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ABSTRACT

The application of FIFO is to pass information from a asynchronous domain to another domain. A lot of design is available but still design synthesis and design analysis is a challenge. Here a technique is adopted to achieve those things using Gray code pointers. This design acts properly almost all the time and chances of error is 1%. Decoding and debugging of errors are also easier here.

Keywords: FIFO. Asynchronous clock domain, Gray code pointers

INTRODUCTION

The application of FIFO is to pass information from a asynchronous domain to another domain. Here both the domains is asynchronous. In this type of communication so they have different read speed and write speed. So passing data is safe here. FIFO pointer generation is a challenge here and another challenge is to find a full and empty condition. Read pointer indicates present location and the write one to the next location in the memory. During reset both will be zero. FIFO pointers need to be synchronized for using in other domain so generation of this pointers are very much important. Full and empty status then will be compared with these pointers. When both the operation i.e read and write pointers are same then the condition is said as empty. This is possible if the last read instruction has been read. So we can say status is Full if last location is written. To differentiate these two extra bit is given to the FIFO pointer. So leaving MSBs if other bits are same we will understand it is Full and when all are same it is empty. Fig 1 shows FIFO pointers. Fig 2 shows FIFO full and empty status.





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Formulation of the Work

Introduction to System Verilog

It is introduced in 2001. It is an improved version of Verilog. A lot of parameter such as productivity, reusability is improved in new version. Hardware description is more firm in this new system due to language enhancement. Test bench generation is much more supportive here.

Introduction to UVM

To support this system Verilog one need a class library which is supplied by UVM. It consists of self-defined base classes. These base classes can be extended to develop verification environment.

Hierarchy of UVM classes is shown in fig 3.

UVM Component Description

It is important to understand the function of each and every object along with the components. It is important to understand the terms and different functions of the file being created. This chapter provides the information about how the UVM frame is created and what every folder and file does. Fig 4 shows the test bench architecture.

Transaction	: DUT processes it directly. This is mainly data.
Agent top	: Agent tops are 2 in test bench. Both has read and write agent. Sequence generation will be outside test bench and in run phase they will be connected.
Agent	: Agent consists of sequence, driver, monitor through with driving the packets of data items from one component to another.
Virtual Sequence	: Here generation of data takes place and it is transferred to the driver.
Virtual Sequencer	: It is known for packet of data items driving from driver.
Environment	: Environment is platform through which driving the packet of data items from one component to another component.
Sequence	: Same as Virtual Sequence.
Sequencer	: Medium from sequence and driver.
Monitor	: All activity will be observed.
Driver	: Driver drives the data at any point.

Logic and Test Cases

This para consists of the logics which are developed so as to drive the data and check the result. There are different waveforms which are generated to observe if the given test case gives the correct result or not. Fig 5 and 6 describes the read and write waveform through Xilinx respectively.

Test case 1	: Here we are verifying reset signal. Result is shown in fig-7
Test case 2	: Here we are verifying the wr_enable. Result is shown in fig-8
Test case 3	: Here we are verifying the rd_enable. Result is shown in fig-9
Test case 4	: Here we are writing and reading single bit. Result is shown in fig-10
Test case 5	: Here we are verifying fifo_full condition. Result is shown in fig-11. Here wr_data is randomized with constraints rst=1'b0 and wr_enable=1'b1
Test case 6	: Here we are verifying fifo_empty. Result is shown in fig-12 Here write_data is randomizing 5 times with constraints rst=1'b0, Wr_enable=1'b1 and reading the data for 8 times with constraints rst=1'b0 and rd_enable=1'b1





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- Test case 7** : Here we are writing and reading multi bit (serial). Result is shown in fig-13. Here wr_data is randomized for 10 to 12 times serially with constraints rst=1'b0, wr_enable=1'b1 and reading data serially with constraints rst=1'b0 and rd_enable =1'b1.
- Test case 8** : Here we are writing and reading multi bit (parallel). Result is shown in fig-14. Here wr_data is randomized for 3 to 4 times serially with constraints rst=1'b0, wr_enable=1'b1 and reading data serially with constraints rst=1'b0 and rd_enable =1'b1.with some delay and repeat the same method for 2 to 3 times.

Analysis

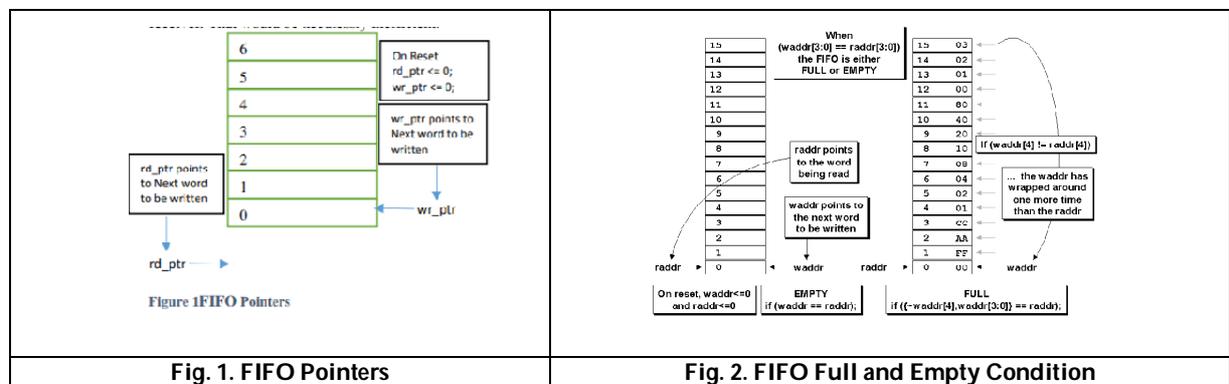
UVM frame for the project is prepared. "Top" is created first. Inside the "Top" we have created "Environment", "Read Agent", "Write Agent" and "Test". Along with these folders "RTL" and "Make File" was also created. There were several other files also created inside the above mentioned folders. UVM frame is finally done followed by work on developing the logic according to the assigned protocol, i.e. FIFO. Logic was also developed for different sequences while a logic was developed by the team to compare the data which we get and the data which is expected. Our objective was to check if the designed concept of the protocol was working without error and efficiently or not. We tried to write a data of 8 bit through the FIFO with respect to wright clock by making wr_enable is „1" and read the data with respect to read clock by making rd_enable is „1". The design successfully writes and read the data. The objective is successfully completed.

CONCLUSION

According to the protocol it is the simplest form of communication between microcontroller and PC. Here data is written and read between two agents. To verify the protocol, we needed to learn a new language along with a new methodology, i.e. System Verilog and UVM respectively.

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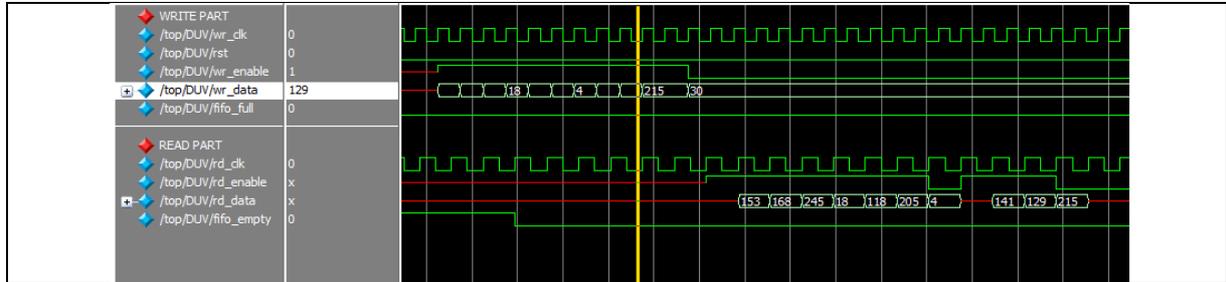


Fig. 9. Test Case 3

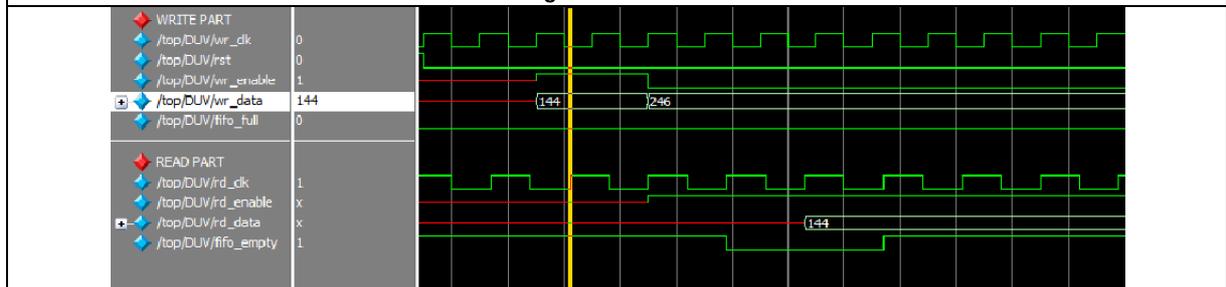


Fig. 10. Test Case 4

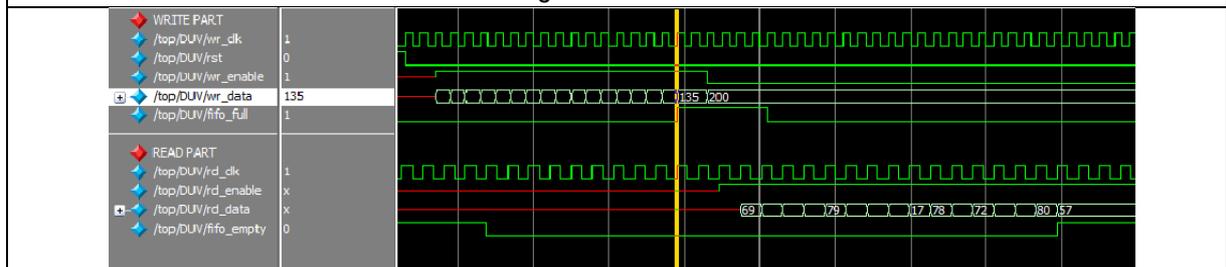


Fig-11 Test Case 5

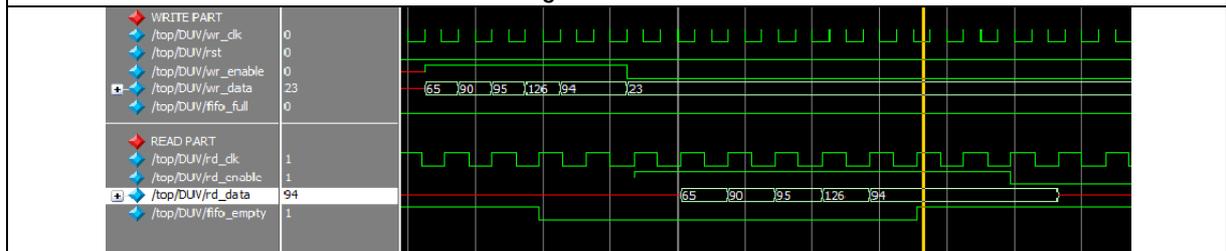


Fig. 12. Test Case 6

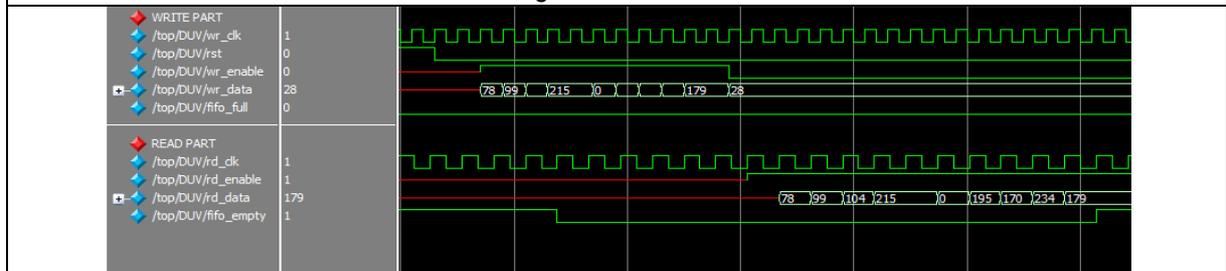


Fig. 13. Test Case 7





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Review of the Progress of Research on Fluid Dynamics

Jaya Prakash Mishra¹ and Tumbanath Samantara^{2*}

¹Research Scholar, Department of Mathematics, Centurion University of Technology and Management, Odisha, India.

²Department of Mathematics, Centurion University of Technology and Management, Odisha, India.

Received: 01 Sep 2020

Revised: 03 Oct 2020

Accepted: 05 Nov 2020

*Address for Correspondence

Tumbanath Samantara

Department of Mathematics,
Centurion University of Technology and Management,
Odisha, India.

Email: tnsamantara@gmail.com



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ABSTRACT

In this literature review paper of advances of fluid dynamics, the research works were done by different researchers during the period from 2013 up to 2020 is discussed. During review process, the different functionalities used in governing equations of flow problems and their effects were studied. Also, different applications of fluid dynamics are acknowledged.

Keywords: Viscous, Bouncy Force, Two phase Flow, Couette flow

INTRODUCTION

We can say that the research on fluid dynamics began with the concept of buoyancy force of Archimedes in around 250 B.C. Then it progressed very slowly up to ninth century. During tenth and eleventh century some advancement occurred in Islamic countries on experimental work with applications of mathematical formula. The real progress of research work in fluid mechanics occurred during seventeenth and eighteenth century due to involvement of Pascal, Newton and Bernoulli. In nineteenth century, many books on fluid dynamics came out to market and a large number of researchers involved. The advancement of numerical method has added a wing in the progress of research work during twentieth century. The research from clear fluid to multiphase, from Newtonian with electrification and magnetism effect of flow field and heat transfer are the challenges of twenty-first century.

Overview on Fluid Dynamics

Okedayo G.T and Abah S.O [29] have considered a numerical study of MHD plane stagnation point fluid flow of temperature dependent viscosity fluid. The flow problem is represented by system of partial differential equation and then it has been transferred into ordinary differential equation. After transforming it has been solved by using "Runge-kutta method followed by shooting technique". The consequence of relevant thermo-physical parameter had



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been studied. A numerical examination of Nusselt number and friction coefficient had been done in this paper. The temperature profile decreases with respect to increasing of magnetic parameter and Prandtl number but the velocity profile decrease when magnetic parameter increase. Maitreejana *et al.*, [22] have conducted a study on an unsteady couette flow of viscous fluid which was incompressible. The fluid passed through a porous medium in a rotating system. The main flow problem has been solved by Laplace transfer procedure. It has been found that when the rotating parameter increases, the magnitude of primary decreases and secondary velocity increases. For any value of rotation parameter and Reynolds number, the asymptotic behavior of the solution has been studied. Mohammed J Uddin *et al.*, [26] have analyzed a numerical conclusion of steady 2-D laminar boundary layer flow of a Newtonian nanofluid which was electrically conducting. The given nanofluid was on a solid vertical plate. The governing flow problem equations are solved by Runge-kutta Fehlberg fourth-fifth order method. As a conclusion they found that when the "Newtonian heating parameter" increases, the "rate of heat and mass transfer" increases and the "Velocity", "Temperature distribution" increases also. Tasawarhayat *et al.*, [40] have analyzed on the 3-D flow of couple stress fluid over a strained surface. The analyzation has been concluded in presence of "Mass transfer and Chemical reaction". Nonlinear flow analysis has been calculated by homotopic approach. They got the conclusion that the "velocity field and boundary layer thickness" were decreasing function of "Time dependent parameter" (A) and "Couple stress parameter" (K). D. Srinivasacharya *et al.*, [4] have conducted a study about the hall and non-slip effect of a electrically conducting couple stress fluid which is in between a parallel disk. The leading flow equations were non-linear "Partial Differential Equation" and concerted to "Ordinary Differential Equation". Then it solved by homotropy analysis method. From the study they concluded that when the "Velocity" decreases, the "Magnetic parameter", "Tangential velocity", "Hall and ion-slip parameter" decreases. In other hand when the "Prandalt number" increases, the "Temperature" increases and "Velocity" decreases. Dhiman Bose and Uma Basu [5] have studied on the incompressible viscous flow for a "Generalised Maxwell fluid". The fluid which was in between two coaxial parallel circular disks. The motion of fluid has been created by rotation of circular disk with different angular velocity. They have used the integral transformation method for solving the governing flow equations. At last they got the analytic solution of velocity fields in series form in terms of "Mittage-Leffler function" for the fluid flow of "generalized Maxwell fluid".

Krishnendu Bhattacharya [21] have made an investigation on transfer of heat in unsteady boundary layer stagnation-point fluid flow. The fluid flow was over a stretching sheet. The governing flow problem equations were converted into "Self-similar ordinary differential equation" by using "Similarity transformation method". Then the converted equations have been solved by "Shooting method" technique. At last this is obtained a dual solution of the heat transfer for the fluid flow. It also found that for both the solution when the Prandtl's number increases, thermal boundary layer thickness decrease whereas rate of heat transfer decrease. G. K. Mahato *et al* [13] studied "Dissipative Effects on MHD Stagnation Point Flow of a Heat Absorbing Nano-Fluid past a Stretchable Surface with Melting". Their concluding remarks are as follows: "Magnetic field and velocity ratio parameter are the reason for enhancement in skin friction while the other parameters are the cause for the decrease in skin friction". "Rate of heat transfer at the surface is getting enhanced by magnetic field, Brownian diffusion, thermophoretic diffusion, heat absorption, viscous dissipation, velocity ratio parameter, and Lewis number while it is getting reduced by melting parameter, and thermal diffusion". Senan Thabet and Thabit H. Thabit [38] have analyzed computational fluid flow simulation around a car model. At last they found aerodynamic coefficients from the computational fluid dynamics simulation and compared them with the available experimental data. Amos S. Idowu *et al.*, [1] have studied on the effect of thermal radiation on inclined magnetic field of MHD fluid flow. The fluid flow is also free convection heat transfer which past through a moving vertical porous plate. The leading equation have been solved by "Implicit finite difference method of Crank- Nicolsan type". The effects of different parameters on "Temperature" and "Velocity field" had been discussed and presented by graphical and tabular form. As results they have analyzed for many values of radiation parameter, thermal grash of number etc. They concluded that the "Velocity" increase with the "Prandalt number" and by increase of "Eekert number", the "Velocity" and "Temperature" decreases. Ebrahim Ahmadloo *et al.*, [9] have discussed about the "Computational fluid dynamics" study of laminar isothermal single-phase flow of water. The flow of water by a hallow helical pipe at various "Reynolds number". It is found that by



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accelerating the force of fluid, create a high-pressure region on the outside of the pipe. When "Turbulence" increases, the "Friction factor" become decreases. Gowdara M Pavithra *et al.*, [12] have deliberated on the numerical study of the construction of an "unsteady boundary layer flow" and "transfer of heat" of a dusty fluid. The fluid was over an exponentially stretching sheet subject to suction. The effects of "thermal radiation", "viscous dissipation" etc. has been studied in the energy equation. The leading equations were solved by "Runge-Kutta-Fehlberg 45 technique". It is concluded that the temperature of fluid phase is greater than dust phase. It was found that when the "unsteady parameter" increases then the "momentum" and "thermal boundary layer thickness" decreases. The increasing of "Prandtl number" decreases the "thermal boundary layer".

K Vajravelu *et al.*, [18] have studied on various thermo-physical property of an unsteady MHD flow. They also have analyzed on the heat transfer of an Ostwald-de Warle fluid which is on a stretching sheet. The thermo physical property like velocity and thermal conductivity of fluid were studied to different varies for temperature. The leading non-linear partial differential flow equations had been changed to coupled, non-linear ordinary differential equation with variable coefficient by using "Similarity transformation method" and had been solved by "Finite difference method with keller-box method" for different values of pertinent parameter. They have found that for reducing the "Velocity boundary layer thickness" and enhancing the "thermal boundary layer thickness", the effect of variable "velocity" and "magnetic parameter" had been used. A mathematical analyzation of "MHD fluid flow" and "Heat transfer" from a "Electrically conducting fluid" to a constant free stream have made by Kalidas das [19]. By using "Similarity transformation technique" the governing partial differential equation had been converted to self-similar ordinary differential equation. Then the flow equations had been solved by using "MATLAB BVP" numerically. The numerical outcomes for temperature and dimensionless velocity has been studied. They have concluded that for decreases of the "Boundary layer thickness", "the thermal radiation", "melting parameter" etc. increases. Moreover, the rate of "heat transfer" decreases for increases of "melting strength" and "magnetic field".

Mohammad Mehdi Rashidi et. al. [25] have analyzed on the homotopy analysis technique to evaluate free convective "Heat and mass transfer" in "2-D MHD fluid flow". The fluid was also steady which is on a stretching vertical structure. "Thermal radiation" and "non-uniform magnetic field" had been considered for this study. The 2-D boundary layer leading partial differential equations had been elaborated by considering "Boussinesq equations" and the ordinary differential equations of momentum, energy and concentration had been elaborated by similarity solution. Then the equations had been solved in presence of "buoyancy forces" analytically. From this study they have concluded that the density was dependent on temperature and concentration. The effect of different parameter like magnetic field, suction, Prandtl number, Grash of number, Biot number etc. has been discussed. Ganewara Reddy, Machireddy and Sandeep Naramgari [11] have conducted a study about heat and mass transfer in carreau fluid flow over a permeable stretching sheet. The study had been continued with convective slip condition with presence of cross diffusion, suction effect, injection effect, and magnetic field. The study gave importance to in metallurgical process with gamma flow, blood flow etc. From this study they have been observed that the heat and mass transfer rate boosted by the power law index. The "Thermal boundary layer thickness" increases with the increase of "strength of applied magnetic field". Also the heat and mass transfer rate of "Carreau fluid" is high in blowing situation.

S Nadeem *et al.*, [36] have conducted a study on the unsteady peristaltic flow of a incompressible "carreau fluid" which was inside an eccentric cylinder. The leading flow equations are investigated under certain condition of "long web length" and "low Reynolds number". The outcoming highly nonlinear equation have been solved by the use of "Series solution method". They found that in peristaltic pumping, the pressure rise curve rise with radius of the cylinder (δ) but fall with Weissenberg No. (We) and material coefficient (n). Aniruddha Mitra et al., [2] have studied on the effect of "Magnetic field" and "Compressibility" on the growth rate of "Rayleigh- Taylor instability". They have also studied about magnetic and hydrodynamic pressure on RT instability. "Dynamics of bubble and spike" had been investigated analytically and numerically. They found that the growth rate decreases nonlinearly by interface parallel magnetic field. Khaled K Jaber [20] did a paper which deals on the effects of viscous dissipation on



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"MHD fluid flow". He has also studied about joule heating on this fluid flow. The equation of momentum and heat equations leads the governing equations which had been solved by "shooting technique". The result which they have found that for increases of the magnetic parameter, the temperature and local Nusselt number increases also and the velocity and skin friction decreases. Both Nusselt number and temperature profile increases with increasing of Eckert number. Mohammed Nasir Uddin *et al.*, [27] have deliberated on unsteady mixed convective boundary layer flow of viscous fluid over a isothermal horizontal plate. The governing partial differential equation had been solved by shooting method after transforming to ordinary differential equation. In the conclusion they found "fluid velocity" and "temperature" decreases but there is no effect on the increasing of the concentration of "Prandtl number". Olubode Kolade Koriko *et al.*, [30] have investigated on a "steady 2-D MHD free convective flow" of micropolar fluid along a vertical porous surface. The governing partial differential flow equations had been transferred into system of non-linear ordinary differential equation and then solved by "Homotopy analysis method". In the result the micro-rotation profile increases by increasing the micropolar parameter. Again when magnetic parameter increases, the microrotation profile and magnetic field in the boundary layer decrease.

P.V Satyanarayan *et al.*, [33] have studied on the effect of heat and mass transfer on MHD oscillating fluid flow with chemical reaction and heat sources. The governing equations have been solved by regular perturbation method. They found that the fluid velocity is maximum magnitude along the centerline and minimum at the walls. E Rahimi *et Al.*, [8] have proposed a study of 2-D viscous fluid flow between slowly expanding and contracting wall with weak permeability. The governing flow problem equations were described by "dimensionless parameters wall dilation rate" (α) and "permeation Reynolds number" (Re). The equations were solved by "Homotopy Analysis Method" (HAM) and "Homotopy Perturbation Method" (HPM). It is observed that for every level of injection, increasing higher axial velocity near the center and lower axial velocity near the wall. Hari R Kataria *et al.*, [14] have studied about boundary layer flow of "Incompressible micropolar fluid" flow under "Uniform magnetic field" theoretically. The governing "energy equation", "angular momentum" and "unsteady boundary layer momentum" had been solved by numerically. They discussed about the effect of "magnetic parameter" (M), "Material parameter" (b) and "Prandtl number" (Pr). In the result they have got that the "temperature" and "velocity" of fluid decreases with increases of the "Prandtl number". The amplitude of "velocity" and "boundary layer thickness" decreases when "magnetic parameter" increases. MizueMunekata *et al.*, [24] have proposed a study on the effect of orbital motion on the "Velocity field" of boundary layer flow on a rotating disk. The motion on the "velocity field" were analyzed experimentally. The velocity field at a fixed orbiter had been measured. The displacement depends on the rotating direction. The shape of 3-D boundary layer flow formed elliptically.

B.J Gireesh et. Al. [3] have investigated on the 2-D mixed convection flow of non-Newtonian fluid in presence of dust particle. Thermal radiation, non-uniform source/sink and viscus dissipation have been implemented for the heat equation. Rosseland approximation had been used for the simulation of thermal radiation effect. The governing heat transport equation were converted into ordinary differential equation and were solved by shooting method. In the result they have found that both "velocity" and "temperature profile" are conquered with thermal radiation. The presence of dust particles gave a great impact on the flow of fluid. E.O Fatunmbi et. Al. [6] have presented a study on 2-D heat and mass transfer of an electrically conducted micropolar fluid flow with velocity and thermal slip condition which was past on a stretching sheet. The governing partial differential flow equation were transferred to non-linear ordinary differential equation and then solved by "shooting method with 4th order Runge-kutta scheme". At last they have found that the "material parameter", "velocity slip" and the "thermal sleep" increasing. The microrotation distribution in the boundary layer flow reduces. T.N Samantara Et. Al.[41] have conducted a study on a problem of laminar mixed 2-D plane wall jet of incompressible carrier fluid. The finite volume fraction, heat due to conduction, viscus dissipation have been taken for the study of heat transfer and skin friction. The governing flow equation have solved by "pertubations on Schlichting's model". In the result many parameters like as "Prandtl number", "Eckrt number", "Nusselt number" had been studied by them. Also it was found that the "Nusselt number" were always increases with increase of above parameters. P Durga Prasad et. Al. [31] have studied on the effect of radiation absorption and chemical reaction on MHD free convective heat and mass transfer flow of a



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nanofluid. The diffusion theorem parameter absorptive enhance the velocity and temperature and skin friction also. The analytical solution of boundary layer flow equations were concluded as oscillatory type and then solved by perturbation technique. They have found on result that the decreasing of species concentration gives the increasing of suction parameter and chemical reaction. There is an effect of magnetic field on skin friction coefficient.

Marcin Pietrzak et. Al. [23] have studied about the calculation of gas void fraction for two phase fluid flow by conventional channels. Both gas-liquid and vapor-liquid two phase flow was investigated in this paper. The approaches were developed for flow conventional channel could be effectively applied for the calculation of gas void fraction in 2-phase flow through a small pipe. RahelehFarokhpour et. Al. [34] have conducted this study which had focused on the dimensional study and scaling rules for multiphase flow and hydrodynamic effects in two-phase flow. They observed about the discrepancies in the pressure gradient of high gas velocity. At last it had been concluded that the matching of "liquid-gas velocity", Froude number and liquid-gas velocity ratio were sufficient for giving a good scaling behavior of the experiment. A modification of the "Adomian Decomposition Method" coupled to the Lesnic's approach to solve solution for initial boundary value problem with mixed boundary value condition has been studied by Nawal Abdullah Alzaid et. Al.[28] by this paper. The methods were used by different forms of heat and wave equation for showing the effectiveness of the modified method. P.K Tripathy & S.K Mishra [32] numerically studied the effect of suspended particulate matter (SPM) on a thermal boundary layer flow over a flat plate. They included the particulate momentum equation normal direction. They solved the non-dimensional governing equations by the help of finite difference technique with non-uniform grid and pointed out that, surface particulate velocity never becomes zero and particulate surface density goes on increasing and never becomes infinity, towards the downstream station. The magnitude of shear stress is increased up to $x = 1.8$ and then decreased in the presence of particles. The heat transfer occurs from plate to fluid, indicating cooling effect on the plate.

E Omokhuale and M.L Jabaka [7] have examined the fluid which was electrically conducted and in the presence of uniform transverse magnetic field. The flow problems were governed as system of partial differential equation and converted into non-dimensional form by the help of non-dimensional variables. Then "Explicit finite difference method" had used to assumed for approximating the velocity, temperature and concentration. At a result when the "suction parameter" increases, then the "velocity", "temperature" and "concentration" of fluid decreases. Jun Liu [17] have given a numerical analyzation of 2-D thermo-magnetic convection of thermal magnetic fluid in this paper. A spectral difference scheme has been employed for solving the governing equations. In the conclusion he found that the electric current wire is fixed in center of annular pipe, two symmetrical circulating flows are produced. T.N. Samantara, S.K.Mishra & T.C.Panda [42] have considered a problem of dusty fluid passing through plane wall jet. They have considered that the fluid is not electrified by outer source rather the dust particles are electrified due to collision among themselves as well as with the wall of the jet. The governing system of nonlinear partial differential equations are solved by using finite difference method of non-uniform grid. It is observed from this paper that the electrification of particles reduces the "velocity" and "temperature gradient", leading to reduction of "skin friction" and "heat transfer". In this paper Rowsanara Akhter, Mohammad Mokaddes [35] have examined that the effect of thermal radiation on the fluid of low Prandtl number. They have discussed about fluid which is also incompressible and electrically conducting. The governing equations as non-linear partial differential equation have solved numerically by finite different method. It was found that by the effect of thermal radiation parameter, fluid velocity, temperature and skin friction were increase.

Farhad A Abbassi et. Al. [10] have studied the laminar forced convection of CuO nanofluid. A "Eulerian two fluid model" is considered for the nanofluid. The governing mass and energy equation has been solved by "finite volume method". From the investigation it had observed that the "Nusselt number" increases when the "expansion ratio" has been decreases as well as increasing in "Reynolds number". The two-phase models are used efficiently instead of single phase model. Hangwei song et. Al. [15] have developed a shale gas reservoir is based on horizontal well production. They analyzed about the "two-phase gas-water flow" in horizontal and highly inclined system. As a





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result, they have got the theoretical distribution models of slug flow were stable. This model is also used to clarify the construction of gas and water two-phase output profile. John kung's and Joseph Nderitu [16] have studied about the estimators of finite population mean by generalized ratio cum product estimator. This estimator is used for two-phase sampling of multi-auxiliary variable. It shows that, this estimator is the most efficient estimator among the all which have used auxiliary variables. Samba bouso and Musandji Fuamba [37] have focused on a determination method for unsteady friction by the "Godunov approach". This approach is in "finite volume method" for employing two-phase flow equation. The main aim of this study to modify the single equivalent 2-phase flow equation. The result shows that the applied approach allows dynamic friction to be taken in account of "finite-volume technique".

CONCLUSION

Reviewing above mentioned papers, it is determined that the research works on the fluid dynamics is a multidirectional task. After reviewing many papers of fluid dynamics, it is observed that the effects of various parameters like "Viscosity", "thermal conductivity parameter", "Prandtl number", "Reynolds number", "Magnetic parameter" etc. on velocity, temperature and concentration profile are studied. It is clear that fluid dynamics has a huge application at every corner of the world.

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Portable Water Testing Instrument for Multipurpose Applications

Sandipan Pine^{1*} and Suman Kumar²

¹Dept. of ECE, Centurion University of Technology and Management, Odisha, India.

²Dept. of Mech, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Sandipan Pine

Dept. of ECE,

Centurion University of Technology and Management,

Odisha, India

Email : sandipan@cutm.ac.in



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ABSTRACT

We have a lot of natural resources in the world. Out of them water is one of the precious. We need water in every part of our life. So testing of this water is very much essential just to make sure that the purpose will be solved. Some water is very good for aquaculture or fish farming but not good for drinking. Different water quality is required for different purpose. Here comes the necessity of a precise, low cost and multipurpose tester. The following design served the purpose. This instrument can measure the temperature, pH and dissolved oxygen in a very quick and precise manner. The design is made very light weight to make it portable and the cost of the product is very less. This is one touch solution for many users who need to measure those three parameters.

Keywords: Water quality. Water Testing, pH meter, Testing Instrument

INTRODUCTION

Water is one of the precious natural resources in the world. We use it for a lot of different purposes. Water are used for Hydropower, Aquaculture, Fish Farming, Irrigation purpose. They are used for domestic purpose or industrial and commercial use. So testing of water is very much essential for each of the applications. During water testing we generally measure the water temperature, pH value, odor, test and colour of the water, total dissolved solid in the water, dissolved CO₂ and O₂, turbidity, hardness, alkalinity, presence of metal and metalloids and so on. This water tester design served the purpose by testing pH, temperature and dissolved oxygen in any water sample in a very easy and precise manner. The result gets available within 1-2 minute. The device is very much light wait and hence portable and cost of the product is very less. So this is a one touch multiple solution device with huge application in testing field. The design involves various sensors like pH sensor, temperature sensor and DO sensor. PCB is designed as per the requirement and all data is synchronized and calibrated to get the correct value with the help of software tools. There are not very much work in application specific testing equipment. pH measurement [1],





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temperature and dissolved oxygen measurement is done separately. Water quality analysis covers all these processes [2] [3] but the targeted measuring instrument is on demand.

Design Flow

The design involves a hybrid approach with the use of hardware and software. The flow is described as shown in fig-1

Design Process

Temperature, pH and DO sensors are procured and required PCB is designed and fabricated with the help of Eagle software as shown in fig 2. ATMEGA 328 Arduino board is used. The circuit diagram is shown in fig-3. Power supply unit is designed with the help of a step down DC to DC transformer, 4.7 Volt Lead Acid Battery Unit as shown in fig-4 Battery level indicator circuit is shown in fig-5 Metal parts including the box is designed with the help of Catia and is shown in fig 6. The final product is shown in Fig-7.

RESULT DISCUSSION

Sample water is taken from 4 different sources of the Centurion University, Paralakhemundi Campus and the result is tabulated as follows.

SI No	Test	Tap water CRC 1	WAPCOS Pond 1	Stp1	Stp4
1	Temperature[°C]	30	29	29	30
2	pH	6.9	7.4	7.6	8.3
3	Dissolved Oxygen[mg/l]	20.1	24.2	10.8	19.1

CONCLUSION

These three data are very much useful for fish farming and this device can give accurate result within couple of minute. The device is very handy too. As the device is designed by looking at precise application it's a low cost solution for the fishery department. Further design can be upgraded with more sensors depending on the requirement and budget.

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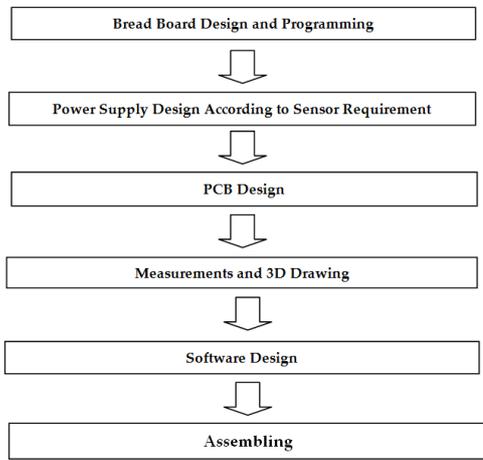


Fig. 1. Design flow

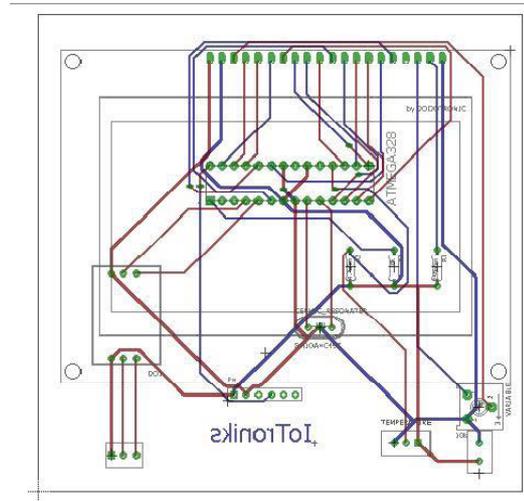


Fig. 2. PCB Design

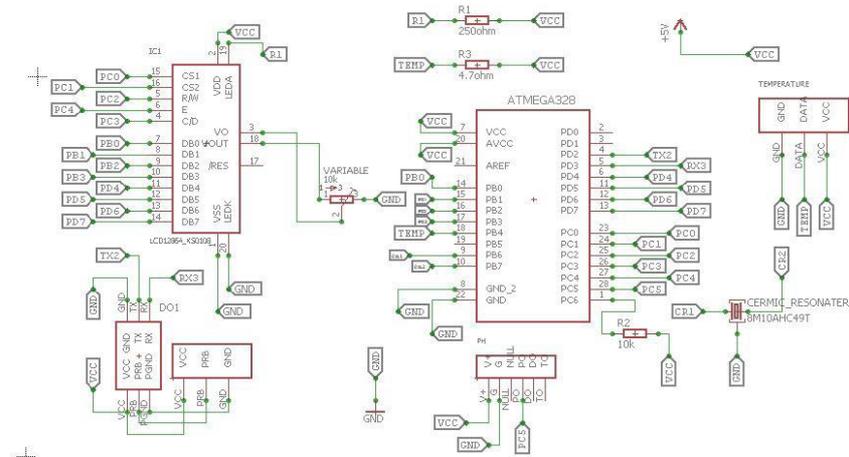


Fig. 3. Circuit Diagram



Fig. 4. Power Supply Unit

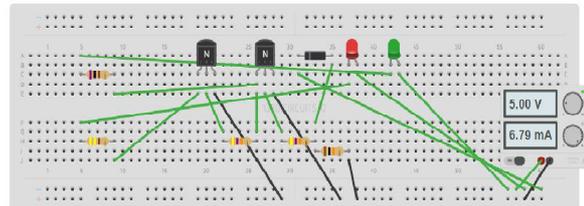


Fig. 5. Battery Level Indicator





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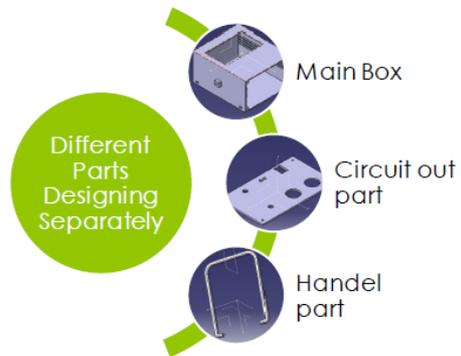


Fig. 6. Metal parts design using Catia



Fig. 7. Final Product





Lucifer – the Intelligent Bike System

Sayyed Viqar Ahmed*

Department of Computer Engineering, Marathwada Institute of Technology Polytechnic College, Aurangabad (MS), India.

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*Address for Correspondence

Sayyed Viqar Ahmed

Department of Computer Engineering,
Marathwada Institute of Technology Polytechnic College,
Aurangabad (MS), India.
Email: sdyusufchem@gmail.com



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ABSTRACT

The purpose of the Lucifer system is to provide automation and security to the rider of the two vehicles. The automation is one greatest thing created ever by human. The Digital world has always given innovative product to the human being. There have been numerous ways and algorithm design to achieve this system. The system is designed in order to provide security and automation to the owner of the two vehicles. Basically the system comprise of both the thing i.e. Hardware and software. The hardware part consist of Arduino nano, Hc-05, ultrasonic sensor, relay module, Isd1820 (voice recorder and voice playing module). The software part consist of Arduino IDE and PCB Express.

Keywords: Hc-05, Voice Re coder, Security, Automation.

INTRODUCTION

There are been various electronic gadgets for the two vehicles for the purpose of safety yet their have been accident because of carelessness and irresponsible of the people [1]. The primary focus of the system is to provide Intelligent Security and automation to the vehicle [2]. Basically the Arduino nano (micro – Controller) is the brain of the system because will help us to achieve our desired System. Basically the system will be embed in our bike to the system with help of ultrasonic sensor [3], relay module & hc-05 (Bluetooth module).

Providing the Security to two vehicles

Security to the two vehicle will provided by using two ultrasonic sensors and a buzzer [4]. These two sensors will attached / Stick to the right side as well as left of the vehicle, the ultrasonic sensor will measure the distance between the two corresponding vehicle, if the corresponding vehicle will come close and arduino will activate the buzzer this will indicate we are getting out of the lane then we will keep our two vehicles in our lane.





Sayed Viqar Ahmed

Providing the automation to two vehicle

Arduino to the two vehicle will be possible with help of ISD1820, relay module , hc – 05 (Bluetooth module) The rider of the vehicle with help of application which connected to our bike system our Bluetooth and it will send command to the relay module to switch on or to switch off the ignition system [7]. The Isd8020 will play the recorded voice when going to start our ignition system with the Bluetooth application

Working of the Lucifer system

Basic Layout

Figure 3 how all the component will be connected to arduino in order to make the Lucifer system. As you can see that our Bluetooth module is connected to the Smart phone as well as to the arduino (micro – controller), our smart phone will be command to the arduino via Bluetooth communication to turn on the ignition System using relay module and then we will get acknowledge through ISD1820 module. Two ultrasonic sensor will calculate the distance between two corresponding [5]. Vehicles, if the vehicles will closer to each other then we will come to know through buzzer. A buzzer will be activated when two corresponding vehicles comes too close other through arduino.

Circuit layout

As we seen fig 4 here, hc – 05 is connected to the arduino via transmitter and Trans –receiver pin of the arduino. The pins of the relay is connected to the Digital pin 3 of the arduino nano, Relay is used to switch the signal of ignition system of the two vehicles ,The Data pins of the ultrasonic to the digital pin 4,5 of the arduino nano , the data pins of the second ultrasonic is connected to the digital pin 6,7 of the arduino nano. The positive pin of the buzzer is connected to the digital pin 8 of the arduino nano. The data pins of the voice recorder is connect to the 9 & 10 pins of the arduino nano and the data out pin are connected to the speaker.

Working Model

Figure 5 show the working model of the purposed system. How the system is going to organize on a breadboard.

CONCLUSION

Thus, we help of the above system we can try to minimize the road accident across the global and this can help many bike rider to ride their vehicle Safely .

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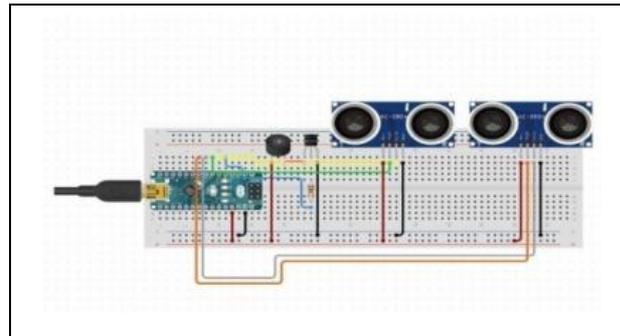


Figure 1 show the how the ultrasonic sensor will be connected to the arduino nano

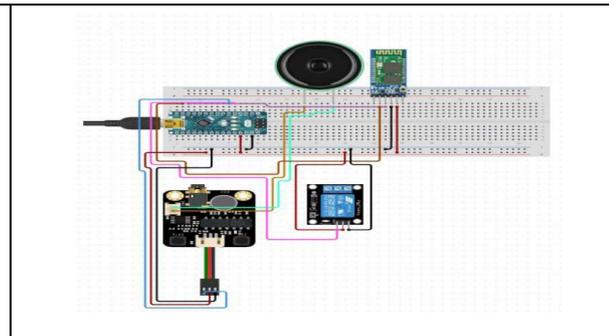


Figure 2 show how hc-05, relay module and voice recorder is connected to the arduino system

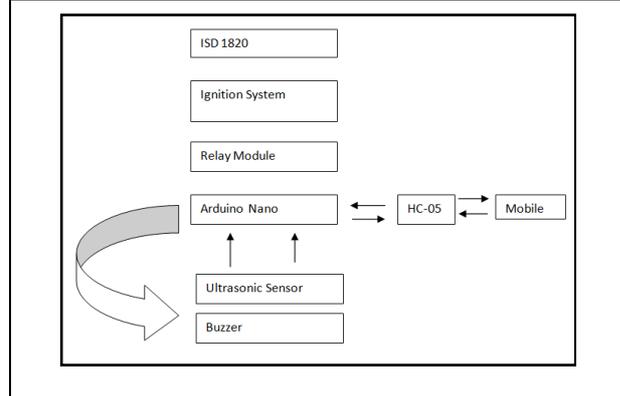


Figure 3 Basic Layout

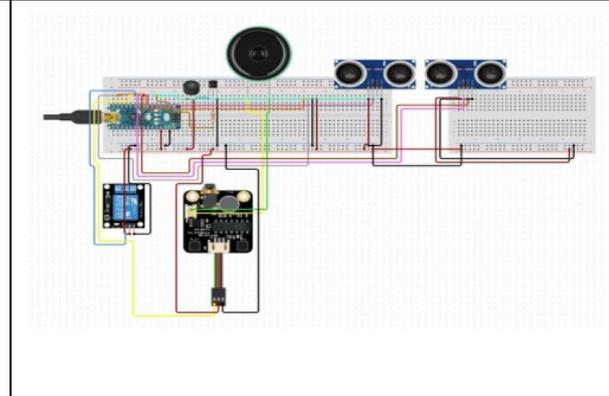


Figure 4 Circuit layout

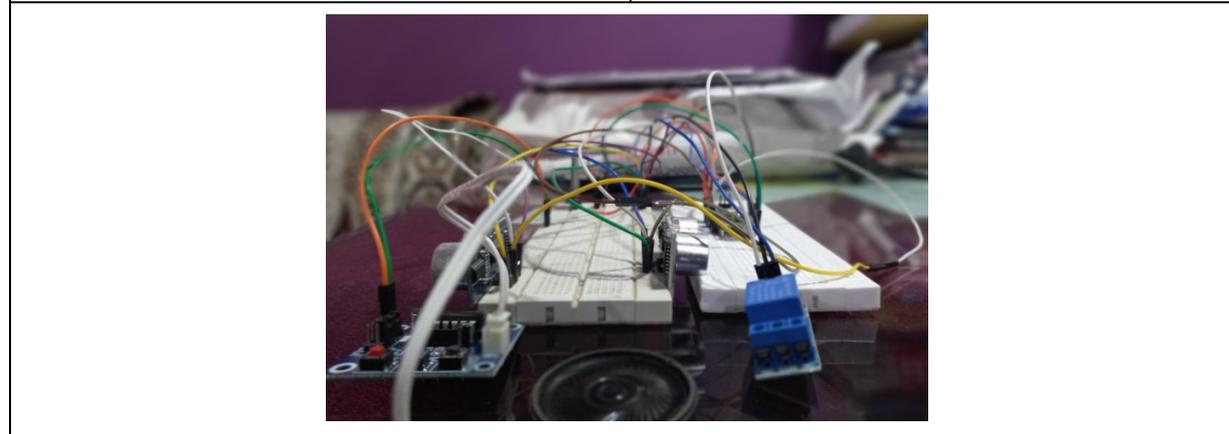


Figure 5 show the working model of the purposed system. How the system Is going to organize on a breadboard





Verification of Universal Asynchronous Receiver / Transmitter Protocol

Sandipan Pine¹ and Suman Kumar²

¹Dept. of ECE, Centurion University of Technology and Management, Odisha, India.

²Dept. of Mech, Centurion University of Technology and Management, Odisha, India.

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Revised: 14 Oct 2020

Accepted: 18 Nov 2020

*Address for Correspondence

Sandipan Pine

Dept. of ECE,

Centurion University of Technology and Management,

Odisha, India.

Email : sandipan@cutm.ac.in



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ABSTRACT

We have a lot of natural resources in the world. Out of them water is one of the precious. We need water in every part of our life. So testing of this water is very much essential just to make sure that the purpose will be solved. Some water is very good for aquaculture or fish farming but not good for drinking. Different water quality is required for different purpose. Here comes the necessity of a precise, low cost and multipurpose tester. The following design served the purpose. This instrument can measure the temperature, pH and dissolved oxygen in a very quick and precise manner. The design is made very light weight to make it portable and the cost of the product is very less. This is one touch solution for many users who need to measure those three parameters.

Keywords: Water quality, Water Testing, pH meter, Testing Instrument.

INTRODUCTION

UART is a physical circuit which can be placed in an IC or Microcontroller. It's full form is Universal Asynchronous Receiver/Transmitter. It is a physical circuit which can be placed in a IC or Microcontroller. UART mainly deals with transmitting serial data and receiving it. Only two wires are required for transmitting and receiving operation, this makes UART more beautiful. Direct communication happens between two UARTs. The transmitter converts parallel data to serial and the receiver converts it to parallel again after receiving. Fig 1 shows UART communication process. Data transmission takes place in asynchronous manner. There is no clock signal available; there is separate start and stop bit available. Starting and ending of packet is defined by these bits.





TEST CASES AND ITS RESULTS

Sequence Logic Developing Method

After creating constructor, we declared an object (req) of uart_wr_txn or uart_rd_txn. Then our sequence items started to randomize with some conditions and rand signals already declared in uart_wr_txn or uart_rd_txn. After randomizing the rand signals the sequence items stop randomizing. This process was continued with different test cases. We are generating a total of eight test cases, four for write sequence and four for read sequence.

Write Monitor Logic Developing Method

At first declared the uart_wr_txn and its handle (data_rcv) and then we created it's handle which is known as data_rcv. As we know sequence generated the data items and sent to driver and driver sent to dut by using interface. Here monitor collected the data signals from virtual interface. It will wait until the load data and busy will be 1 and then monitor will collect the dout from interface, this process will repeated 11 times. At final, monitor send the data to transaction where the data will be print and it will send to scoreboard.

Read Monitor Logic Developing method

Declared the uart_wr_txn and its handle (data_rcv) and then we created it's handle. It will wait until the tx will be 0 and busy will be 1, after that the data frame will create, din data shifted by using shift register and monitor collected the din data from interface. This process will repeated 11 times. At final monitor send the data to transaction where the data will be print and it will send to scoreboard.

Write Driver Logic Developing Method

Declared the uart_wr_txn and its handle txn. As we know the driver sends the data from sequence to dut by using interface, here we declared the reset, tx_enable, din, parity_e_o which send these signals from transaction to virtual interface.

Read Driver Logic Developing Method

Declared the uart_rd_txn and its handle txn, here we developed or set the parity_e_o. As we know, 0 indicates odd parity set and 1 indicates even parity set. For set the parity_e_o we will do the xor operation between txn.dev_data and txn.parity_e_o. After set the parity_e_o, we used one shift register for shifting the 11 bit data one by one. Here we declared the dev_data, reset, rx, rx_enable, parity_e_o which send these signals from transaction to interface.

Test case 1: The Reset signal is verified here. Result is shown in fig-2.

Test case 2: The tx signal is verified here. Result is shown in fig-3

Test case 3: Transmission operation is shown here. Result is shown in fig-4

Test case 4: Same process is done for 5 times here. Result is shown in fig-5

Test case 5: Reset signal is verified here. Result is shown in fig-6

Test case 6: Rx signal is verified here. Result is shown in fig-7

Test case 7: Receiving operation is performed. Result is shown in fig-8

Test case 8: Same process done for 5 times. Result is shown in fig-9

Scoreboard Logic Developing Method

So run phase is executed in parallel for all the components. This phase is preceding by a simulation start phase. In check phase we checked and compared the write and read data, if it is matched then data signals are successful and it will print the uvm_info. If it is not matched then it will show the uvm error Fig 10 shows the transmitter verified output. Here in scoreboard we have verified the start and stop bits of transmitter at tx is successfully transmitted or not.





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Receiver

Here in scoreboard we verified the receiver part of UART that the data sent successfully or not and also verified the parity error that the parity given is successful or not. Fig 11 shows receiver verified output.

CONCLUSION

UART protocol is studied. Here data is transmitted and received between two UARTs within short range. To verify the protocol, we needed to learn a new language along with a new methodology, i.e. System Verilog and UVM respectively. We prepared the UVM frame for the project. We first created the "Top". Inside the "Top" we have created "Environment", "Read Agent", "Write Agent" and "Test". Along with these folders "RTL" and "Make File" was also created. There were several other files also created inside the above mentioned folders. After successfully creating the UVM frame we worked on developing the logic according to the assigned protocol, i.e. UART. Our objective was to check if the designed concept of the protocol was working without error and efficiently or not. We tried to send a data of 8 bit through the UART along with a start bit, a parity bit and a stop bit. The start bit, parity bit and stop bit were used for different indications like if the receiver should be ready to accept the data. The design successfully transmits and receives the data being sent. The objective is successfully completed.

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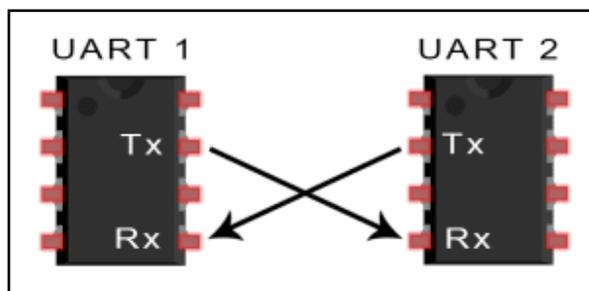
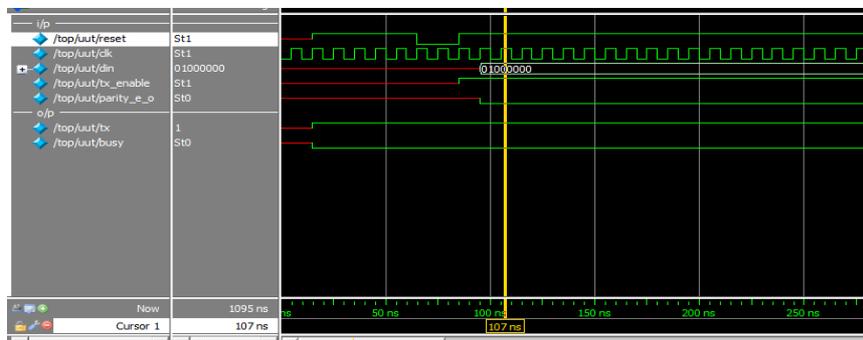


Fig.1: UART Communication





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Fig.2. Test case 1: The Reset signal is verified here.

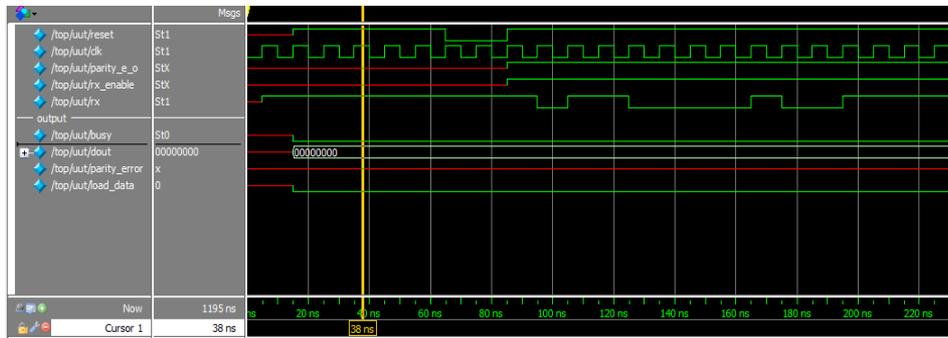


Fig.3. Test Case 2: The tx signal is verified here.

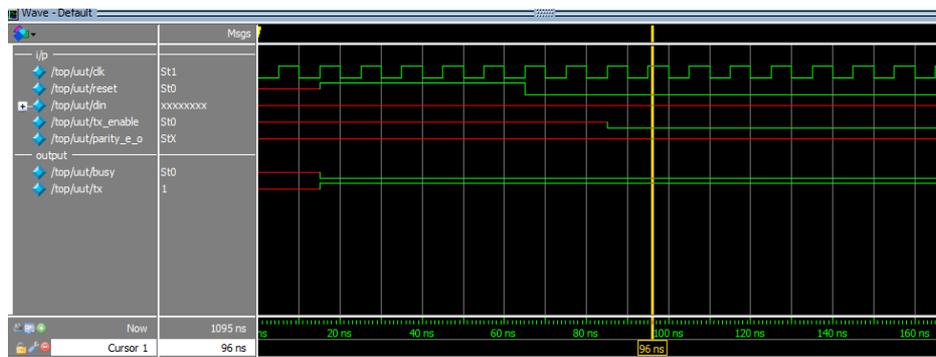


Fig. 4. write sequence: Test Case 3: Transmission operation is shown here.

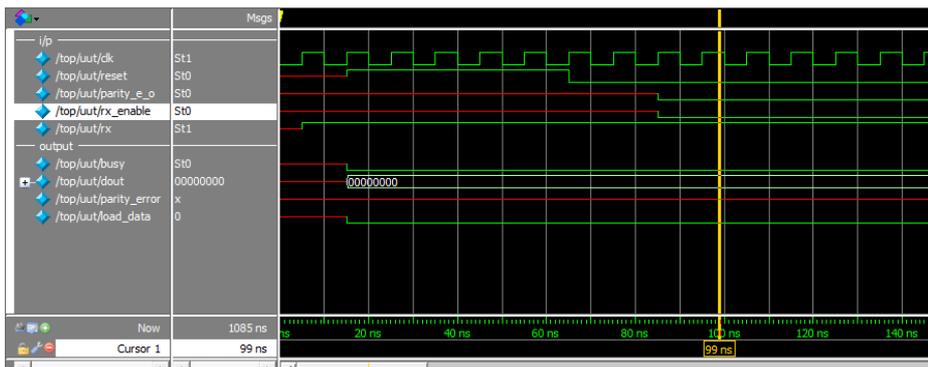


Fig.5. Read sequence: Test Case 4: Same process is done for 5 times here.





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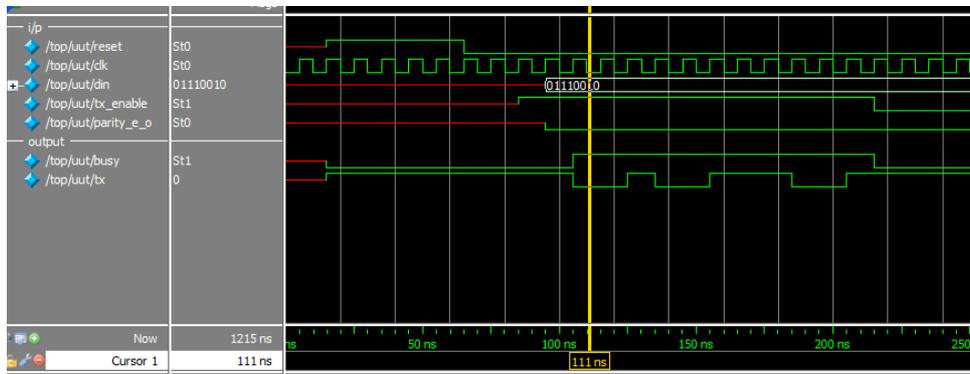


Fig. 6. write sequence: Test Case 5: Reset signal is verified here.

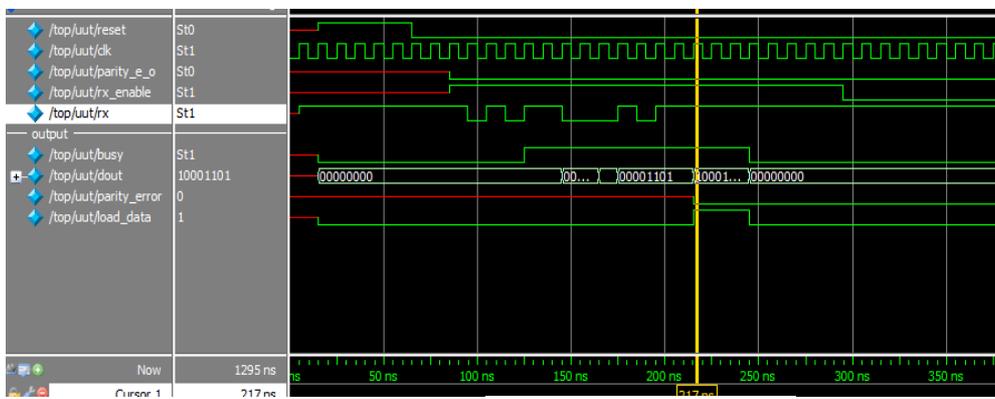


Fig. 7. Read sequence: Test Case 6: Rx signal is verified here.

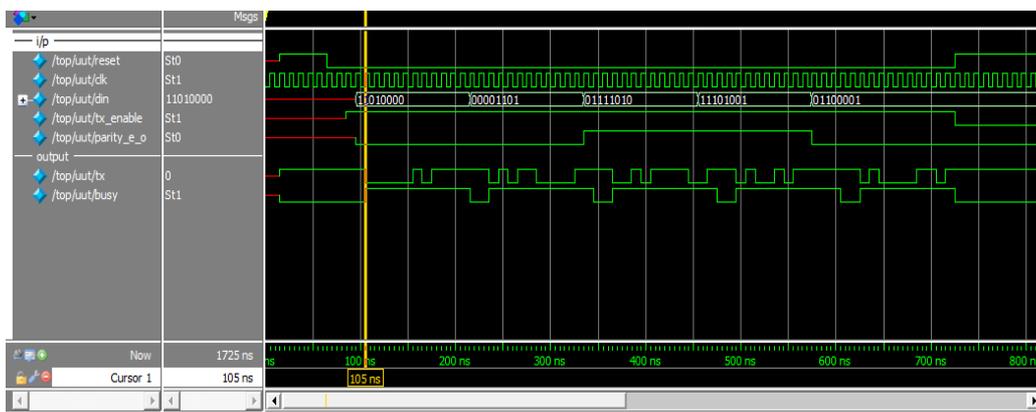


Fig. 8.write sequence: Test Case 7: Receiving operation is performed.





Ramification of Drying Techniques on the Nutrient Quality of *Coriandrum sativum* Linn. Leaves Grown in Ajhai Region, U.P (India)

Sonia Singh^{1*} and Himanshu Sharma²

¹Institute of Pharmaceutical Research, GLA University, 17km Stone, NH-2, Mathura-Delhi Road Mathura, Chaumuhan, Uttar Pradesh, India.

²Department of Computer Engineering & Applications, GLA University, 17km Stone, NH-2, Mathura-Delhi Road Mathura, Chaumuhan, Uttar Pradesh, India.

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*Address for Correspondence

Sonia Singh

Institute of Pharmaceutical Research,
GLA University, 17km Stone, NH-2,
Mathura-Delhi Road Mathura,
Chaumuhan, Uttar Pradesh, India
Email : sonia.singh@gla.ac.in



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ABSTRACT

Coriander (*Coriandrum sativum* Linn.), is an herbal aromatic, annual plant, belongs to the Umbelliferae family, and used as a culinary herb; as a source of aroma compound with multiple biologically, active essential oil components. The aim of the present study was to determine the influence of different drying methods on herbage moisture content and nutrient quality including, protein content, carbohydrate content, and iron content. Leaves of the plant were dried with different drying techniques including, sun drying, oven drying, microwave drying, hot-air-drying and ambient-air-drying. The loss on drying (moisture content) of raw and crude samples of the plant was determined with the help of hot air oven at 105°C for one day. The different leaf extracts (fresh or dried) were determined for the content of protein, carbohydrate and iron, respectively. The purpose was to evaluate the various phenomena to be observed during drying the leaves of coriander. It has been found that the microwave-dried and hot-air oven dried methods were produced the pre-eminent results for preserving most of the nutrients as compared to the fresh herb. As compared to these above methods, ambient-air-dried, hot-air-dried, and sun-dried techniques brought considerable reduction in nutritional values of plant leaf. The optimum and ideal method for drying purpose would be microwave-drying, which require minimum shorter time period (#5 minutes), and leads to provide the most satisfactory retention content of protein and carbohydrates against oven dried which required 18 minutes respectively with 47.97% of iron content.

Keywords: Coriander, Carbohydrate, Drying methods, Food, Iron, Moisture content, Protein.



**Sonia Singh and Himanshu Sharma**

INTRODUCTION

Dhanika consists of dried ripe fruits of *Coriandrum sativum* Linn. (Family: Umbelliferae), has a slender, glabrous, annual herb, cultivated all over region of India, providing characteristic aroma when it has been rubbed in hands [1]. The name 'cilantro' has been randomly as well as frequently employed in American English which referred to as green herb or dried leaves [2]. The whole plant parts are for edible purpose; mainly the fresh leaves and even the dried seeds are commonly utilized in cooking purpose. According to Indian system of traditional medicine, dhaniya is used in the digestive, respiratory and urinary systems disorders. It also has the potential of proving diaphoretic, diuretic, carminative and stimulant activity [3-5].

Vernacular names

Sanskrit : Dhanika, Dhṛṇya, Vitunnaka, Kustumburu

English : Coriander fruit

Hindi : Dhaniya

Drying is important for preserving product quality [6]. Solar and ambient drying are the most commonly employed techniques. Solar and oven drying techniques require heat to eradicate water from food contents through evaporation. Such process ultimately affects most of the nutrient values of edible items either by increasing or decreasing the concentration of some nutrients [7]. Therefore, this study has been conducted to observe the effects of drying techniques on nutrient components of food elements, and also to identify the most suitable methods which not only retain the potency of the nutrients but also enhances their shelf life. Therefore, the better nutritional properties of food supplements are found to be very important for the consumer consumption [8-10]. Among various methods employed for the preservation and conservation of food supplements, drying is an undoubtedly most primitive method. Drying is a technique in which water is removed from food by vaporization or even through sublimation method. These process can have reduced the water availability required for degradation reactions of chemical, enzymatic or sometimes microbial spoilage too. Various factors can influence the drying rate such as transfer of vapor pressures between food and drying air, velocity of air, temperature, and diffusion of moisture among product, thickness rate and surface exposure required for drying [11]. Some of the merit applications of drying techniques are as; (1) food preservation (2) enhancement of shelf life of food via reduced water activity (3) avoid the use of refrigerator application for storage, found very expensive (4) reduction in the storage space requirements and sometimes for transport (5) diversity in the flavor or aroma of the food products, thereby offering the consumer's choice for buying their food products [12,13]. Not only the drying techniques can influence the nutrient ratio, but also it affects the essential oil composition of most of the herbal drugs. For example, it has been reported in the literature that the essential oil concentration of *Calendula officinalis* may get affected, in which monoterpenoid all components would be the most predominant constituent. The hot air drying caused destruction of some of the active constituents [10, 14]. The brief description of chemical constituents present in the Coriander are given in the Table.1 and Figure.1 and Figure. 2. The objective of the present study was to determine the effect of drying method on the nutritional values present in the *Coriandrum sativum* Linn.

MATERIALS AND METHODS

Collection of sample

Fresh leaves of cultivated *Coriandrum sativum* along with petiole were obtained from gardens in the Herbal garden of GLA University, Mathura (India). The leaves were thoroughly cleaned as to remove dirt and other adhered particles/matter and even the damaged or decolorized one. The stalks/ petiole was removed from them. The samples





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of leaves were grouped into 6 batches. One group was kept for 24 hour as fresh sample, immediately after harvesting.

Application of Different drying techniques

The other remaining five groups of Herbal leaves were utilized for different drying experiments (the pictorial representation of drying methods are shown in Figure.3). The drying experiments employed only after the trials had been performed as to obtain moisture content of <25% along with shortest time period of drying, respectively. All the reported data were in triplicate.

Microwave-drying method

About 20 g of fresh leaves of herb were placed onto a glass plate of a microwave oven and dried for 4 minutes.

Hot-air-drying method

Approximately 20 g of fresh leaves were kept on a perforated steel container, and then air-dried with a hand dryer.

Hot air oven-drying method

Around 20 g of raw leaves were evenly placed on baking sheets of a hot air oven at 100 °C for 20 minutes.

Sun-drying method

Around 20 g of fresh leaves were placed on a plate, which has to be covered with a thin sheet of paper. The sample was then kept in the direct sunlight for 8-10 hours at an approx. temperature of 33 °C with a relative humidity of 62%. The covered sheet was turned occasionally in order to allow uniform penetration of the sun rays on the sample.

Ambient air-drying method

20 g of fresh leaf sample was air-dried under dark condition with well-ventilated room for around 7 days at an average temperature of 28 °C with an average relative humidity of 65% [7, 15].

Determination of Moisture content in the samples

The loss on drying of all samples was determined with the help of hot air oven, which has to be kept at 105°C for one day. The moisture content was calculated on the basis of triplicate readings, using the formula described in AOAC 1975 [15,16].

Determination of Protein content as nutritional supplement

According to Mc Clements' method, the UV lamp must be warmed for 15 minutes prior to experiment. The UV wavelength was adjusted to about 280nm, and thereafter it was calibrated using distilled water as blank sample. Around 5g of the leaf samples of each group were crushed thoroughly, and assorted with 30mL of distilled water. The obtained extracts were then diluted with water and collected in the test tubes. All the test samples and the standard, namely tryptophan/ tyrosine (amino acids used for protein determination) were directly measured at an absorbance of 280 nm. All the values were obtained in triplicate form.

Determination of Carbohydrate content

4 g of samples from each group were minced with definite volume of distilled water and were analyzed directly by refractometer. The extracted material was kept at 20°C before analysis. The concentration of carbohydrate content present in the samples was measured at 589 nm. The obtained data were collected in three replicas [17].



**Sonia Singh and Himanshu Sharma****Determination of Iron content**

About 4 g of leaf samples were coarse powdered and mixed with required amount of distilled water. All the samples were then mixed with 50 ml of sodium citrate solution so as to adjust the pH up to 6. Thereafter, about 2 ml of hydroquinone solution (freshly prepared solution) was added to the above extract solution, along with the addition of 5% of ortho-phenanthroline solution. The solution was allowed to stand for about 10 minutes to develop some color formation. The samples were determined for iron concentration at an absorbance of 510 nm using UV spectrophotometry in triplicate form [18].

RESULTS AND DISCUSSION

The coriander leaves used in this experiments have a biological source of dried leaves of *Coriandrum sativum* (Family: Umbelliferae), commonly called as "*Cilantro*". The plant is indigenous in India. Its fragrant leaves smell like lemon thyme. As per literature, the observation of drying effects on the quality and quantity of volatile oil components are found available. But at the same time the impact on the nutritional values are not clearly defined in herbal condiments and spices. The impact on moisture content Figure.4 depicts the moisture contents of raw and dried basil leaves. The results are expressed in percentage wet weight basis. The moisture content of the fresh coriander leaves was $99.26\% \pm 0.54$.

Percentage of moisture content

It has been observed that the leaves dried from hot air dried method had the lowest content of moisture ($14.3\% \pm 0.31$), whereas the direct sun dried leaf samples had the highest moisture content i.e. $19.69\% \pm 0.38$. The leaf samples dried in the ambient temperature at room were relatively containing less percentage of moisture content ($14.87\% \pm 0.09$). The reason for this condition might be due to prolonged drying period. Researchers stated that during prolonged hours of drying, the heat was conducted from the leaf surface into the interior regions of it, which ultimately enhanced the evaporation rate of water from the leaf surface, compared with the diffusion rate into the surface [13]. Therefore, from the above discussion, it must be concluded that the temperature and time were not much effective, and even not optimized to maintain the same percentage of moisture content in all the dried samples. Moreover, the samples obtained had moisture content less than 22%.

Effect on protein content present in the samples

The impact of different drying techniques on protein content in the given drug is shown in Figure.5. The highest protein content found to be present in the microwave dried leaf sample was $4.08 \text{ mg/g} \pm 0.035$, as compared to other methods. This data represents that approximately 89.47% in weight of the protein retained in the microwave dried leaves in comparison to fresh samples. In case of samples obtained from hot air oven were $3.15 \text{ mg/g} \pm 0.13$ i.e. around 69.68% in weight left over in the sample, whereas in ambient temperature, the content of protein would be $0.573 \text{ mg/g} \pm 0.015$ (about 12.56 %) present in the dried leaf sample which is considered to be the smallest percentage. The reason behind this theory is the denaturation of protein present in the cells of the plant leaf may occur due to heating, followed by weak strengthening of 3D conformation of protein tissues. So the prolonged heating time period caused damage of protein content in the plant [19].

Effect on carbohydrate content

Figure.6 shows the impact of different drying techniques in Coriander leaves. During heating, generally starch was converted into dextrin with more loss of water, followed by caramelisation of sugar took place quickly. Most of the low molecular weight carbohydrate compounds lost due to overheating [20]. The observation from the data revealed that all the drying techniques contained appreciable amount of carbohydrates in the leaf samples. But the carbohydrate content found in the hot air dried sample is significantly different as present in the fresh leaves.



**Sonia Singh and Himanshu Sharma****Effect on iron content**

The consequence effect of drying method on the iron content in the given samples is analyzed in Figure.7. The samples obtained from hot air oven contained more iron content as the other dried methods. Approximately 47.97 % weight of iron was possessed in the oven-dried leaf samples, when compared against microwave dried leaf samples (21.95%). When some of the vegetables are cooked, they enhanced the bioavailability of iron content in that. It has been found that the iron content present in the microwave drying samples would be low among all other method, though the microwave drying samples retained the highest percentage of carbohydrates as well as proteins in all other obtained samples. About 12.76% of least iron content found in the ambient air dried leaf samples. Moreover, there was substantial deduction of iron by the application of different drying methods, as compared to that present in the raw samples of coriander leaves. All these methods reflected that they do not favor the optimum retention value of iron content in the plant leaves. The reason behind these effects might be as the heating or excessive heating resulted in oxidative deterioration of iron content by showing increased or decreased level in the leaves. Generally, the microwave dried technique was considered to be the most favorable method in drying of coriander leaves, but the iron levels were found to be present in lower amount than that of the oven dried sample. The nutrient values such as protein, carbohydrate were present in higher amount in microwave dried leaves of coriander with relatively minimum loss in respect with fresh leaves. As the transfer of microwave energy causes rapid evaporation rate of water from the leave surface within a shorter time period, which ultimately causes oxidation reduction, and tend to preserve the nutrient properties in the sample [21,22]. Herbs, condiments and spices have been exported and imported in their dried form for preservation. Through the literature survey, it has been analyzed that the different drying techniques has a significant impact on quality as well as on the quantity of the ethereal oil (essential oils) containing herbal drugs. The concentration of volatile oil components during drying methods depend on various factors including, drying techniques, growth of plant, harvesting time, cultivation condition, and early collection [23, 24].

CONCLUSION

The advanced drying technologies are not only preserving the nutrition value in respect of agricultural production of food crops; but also make their availability throughout the year. These techniques would enhance the food quality with minimum post harvesting reduction. During the completion of the experiment, it has been analyzed that microwave dried method were more efficient in preserving the nutrient quality. Among all the methods, solar drying was found to be cost effective with maximum microbial reduction and lowest moisture content. Therefore, it can be one of the best preserving drying technique with enhanced shelf life property of nutrients.

Conflict of interest

The authors have no conflict of interest.

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Table 1: Chemical composition of *Coriandrum sativum* Linn.

SL.no	Chemical constituents with percentage	References
1	Benzofuran,2,3-dihydro (15.4%), hexadecanoic acid, methyl ester (10.32%) 2,4a-epoxy-3,4,5,6,7,8,-hexahydro-2,5,5,8a-tetramethyl-2h-1-benzofuran (9.35%), 2-methoxy-4-vinylphenol (8.8%)2,3,5,6-tetraflur-2-oanisole (8.62%) 2,6-dimethyl-3-aminobenzoquinone (6.81%) dodecanoic acid (5%)	[23]
2	Linalool (45%), monoterpene esters ; mono- and sesquiterpene hydrocarbons, and ketones (viz., camphor) in reduced amounts, Monoterpene alcohols (78%): monoterpene hydrocarbons (5%), monoterpene esters (2%), monoterpene ketones (1%)	[24]
3	Alcohol: geraniol (1.2%–4.6%), terpinen-4-ol (3%), α -terpineol (0.5%); Hydrocarbons: γ -terpinene (1–8%), r-cymene (3.5%), limonene (0.5%–4.0%), a-pinene (0.2%–8.5%), camphene (1.4%), myrcene (0.2%–2.0%); Esters: geranyl acetate (0.1%–4.7%), linalyl acetate (0%–2.7%)	[25]

Table.2: Values observed in different Drying techniques using Coriander leaves

Nutrient content	Fresh sample	Micro-wave dried	Hot air dried	Hot air oven dried	Sun dried	Ambient air dried
Moisture content(%)	99.27±0.54	18.76±0.36***	14.3±0.30***	19.58±0.26	19.69±0.04***	14.87±0.09***
Protein content(mg/g)	4.56±0.04	4.08±0.04	2.67±0.17	3.15±0.05***	2.93±0.03	0.573±0.01**
Carbohydrate content (mg/g)	24.41±0.26	22.12±0.07**	19.86±0.03***	21.08±0.08***	19.96±0.58***	19.01±0.61
Iron content(mg/g)	1.23±0.001	0.27±0.02***	0.133±0.06***	0.59±0.01	0.21±0.01***	0.157±0.01**

Note: ‘***’ represents p < 0.005; ‘****’ represents p < 0.0001. All values are taken in triplicate form in Mean ± SEM. Different drying methods have been compared with raw leaves of Coriander, using One-way ANOVA statistics

Table 3: Percentage % retention values of different Drying techniques of Coriander leaves

Nutrient content	Percentage (%) retention					
	Fresh sample	Micro-wave dried	Hot air dried	Hot air oven dried	Sun dried	Ambient air dried
Moisture content	100	18.89	14.41	19.72	19.83	14.97
Protein content	100	89.47	58.55	69.68	64.25	12.56
Carbohydrate content	100	90.61	81.36	86.35	81.77	77.88
Iron content	100	21.95	0.81	47.97	17.07	12.76

All values are taken in triplicate form in Mean ± SEM

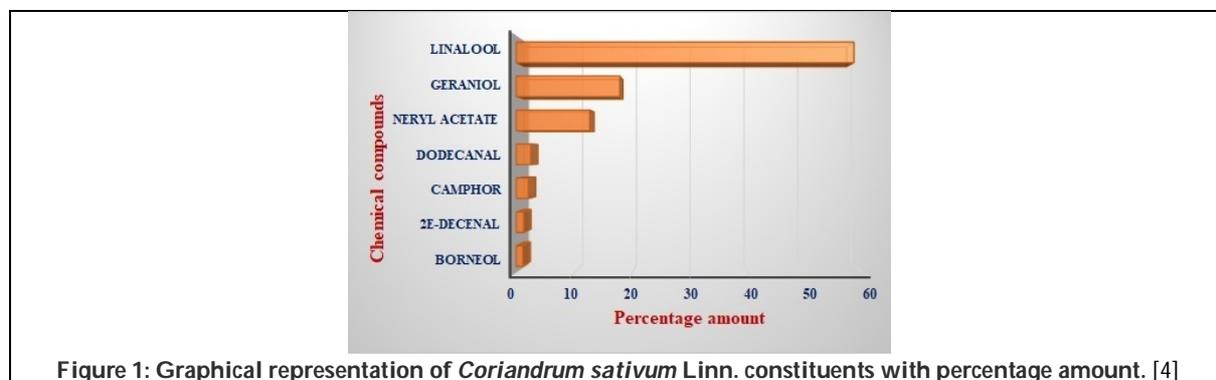


Figure 1: Graphical representation of *Coriandrum sativum* Linn. constituents with percentage amount. [4]





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α-Pinene	γ-Terpinene	α-Cedrene	β-Pinene
α-Farnesene	p-Cymene	Limonene	Linalool
Camphor	Geraniol	Anethol	Citronellal
E-Verbenol	Sabinene	E-Ocimene	m-Cymene
α-Thujene	Cyclooctanol	β-Myrcene	2-Oxabicyclo [2.2.2] Octan-6-ol,1,2,3-trimethyl





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Z-Ocimene	α-Campholenal	Sothujol	Umbellulone
Borneol	4-Terpineol	Terpinyl acetate	Decanal

Figure 2: Structure of Chemical components present in *C.sativum* Linn.

Figure 3: Pictorial representation of Drying methods	Figure 4: Moisture content (%) of <i>Coriandrum sativum</i> Linn. leaf samples. Results are taken in triplicates with Mean± SEM. All values are significant with p < 0.0001
Figure 5: Protein content of <i>Coriandrum sativum</i> Linn. leaf samples. Results are observed in triplicate number Mean ± SEM. The alphabet 'a' represents a significant difference (p < 0.0001), compared with respect to fresh leaves.	Figure 6: Carbohydrate contents of <i>Coriandrum sativum</i> Linn. leaf samples. Results are evaluated in three replicas with Mean ± SEM. The alphabets 'a' and 'b' indicate significant difference (p < 0.05), (p < 0.0001), respectively as compared with crude leaves of Coriander.





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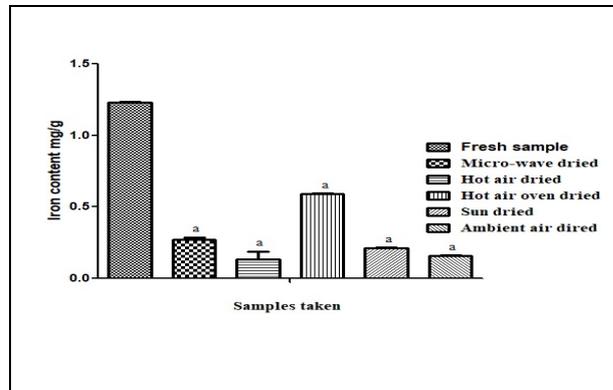


Figure 7: Iron contents of *Coriandrum sativum* Linn. leaf samples. Results are evaluated in three replicas with Mean ± SEM. The alphabet 'a' indicate significant difference ($p < 0.0001$), respectively as compared with crude leaves of Coriander

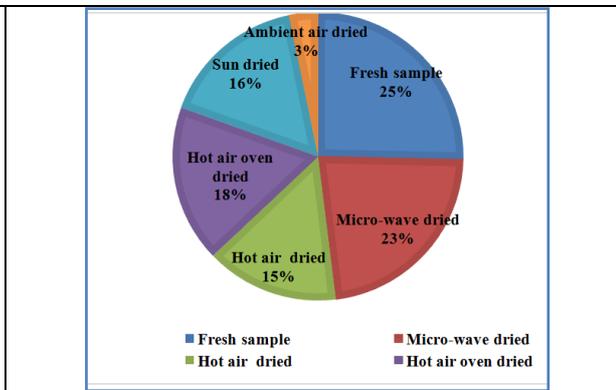


Figure 8: Pie graph of % retention of Protein content

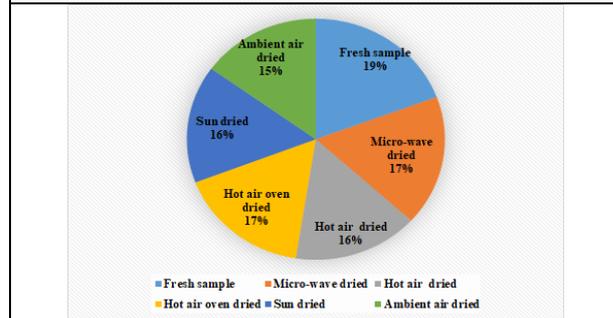


Figure 9: Pie graph of % retention of Carbohydrate content.

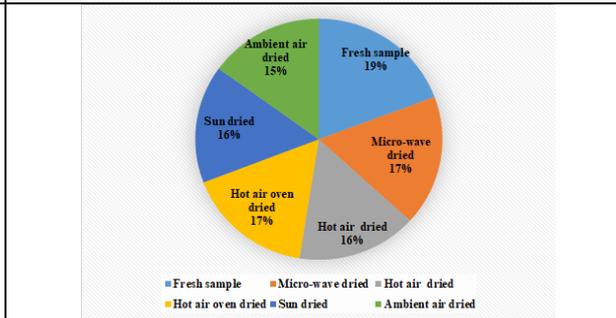


Figure 10: Pie graph of % retention of Iron content





ICT Tools to Design Online Teaching and Learning Materials

Ajit Kumar Pradhan* and Prajna Pani

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Ajit Kumar Pradhan

Centurion University of Technology and Management,
Odisha, India.

Email: ajit.pradhan@cutm.ac.in



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ABSTRACT

The teaching and learning process has undergone a tremendous change amidst COVID-19 pandemic. This has impacted lives across all spheres and education is no exception. At present, many educational institutes have made a shift from face-to-face mode to online teaching and learning. Thus, there is a need to understand some ICT tools including various software and online tools which will be helpful for teachers to prepare and deliver their lessons online effectively. The paper discusses the measures taken by UNESCO and Higher Education during COVID-19. It also makes an attempt to discuss a few tools for teachers which they can use while taking online sessions. The tools include OBS Studio, OpenShot, Screencastify, draw.io, and render forest. This paper discusses each teaching tool and provides how it can be used from a teacher's perspectives. The implications of this paper are highly relevant to teachers who have been assigned to design and deliver online classes during the pandemic.

Keywords: ICT, Teaching, Learning, Software for Pedagogy, Higher Education

INTRODUCTION

Online teaching has become a new reality due to shutdown of many cities across the globe due to Corona virus disease 2019 (COVID-19). This is also true in the context of India and specifically in higher education. Many educational institutions have revised their curriculum by incorporating online courses. Thus, teachers need to enhance their online teaching skills in order to make themselves relevant to the changing demands. The present paper makes an attempt to discuss a few online tools which teachers can use in their online teaching. The discussion is based on language teachers' perspective; however, content subject-teachers can also use the tools to make their online teaching more effective and interesting. In this paper we have made an attempt to find out alternative solutions of some of the issues in higher education pedagogy by using ICT (Information and communication technology) effectively.



**Ajit Kumar Pradhan and Prajna Pani****Impact of COVID-19 on Education**

COVID-19 pandemic has impacted all spheres of lives including education. Educational institutions, from pre-school to university level, have been forced to shut down face-to-face mode of pedagogy. Various educational policies worldwide have emerged due to the pandemic and many countries have recommended for complete closure of educational institutions. As per the report by UNESCO 900, million learners have been directly and indirectly affected. There have been many side effects of the closure of education institutions. Although the primary aim of the closure to stop the spread of the virus in education institutions but the impact was also largely on the socio and economic sectors [3]. UNESCO has provided a few measures to continue with the teaching and learning process both for the students and teachers. Although, there is a closure of educational institutions, the process of education can be transferred to the digital mode using ICT. Table 1 highlights different learning management systems (LMS) and tools for digital learning. However, UNESO has come up with various suggestions to continue with online learning for students and teachers. The Table-1 highlights some of the alternative sources of learning during COVID-19 pandemic closure of educational institutions [2]. Apart from the above sources as highlighted by UNESCO, there are a few tools which will be handy for teachers to design and deliver online classes, which have been discussed in this paper.

Higher Education in India

Although India is the second largest higher education system in the world in terms of enrolment of students, the quality of education is under severe criticism. It is referred as the “island of excellence in a sea of mediocrity” [1]. There is a need to bridge the gap of quality by providing quality education keeping learners’ needs into consideration. Learners should find a direct link between academic studies and industry needs [5]. Due to the complete shutdown of many educational institutions, as a result of the outbreak of COVID-19 pandemic, there is a need to find out alternative ways to pedagogy. Many educational institutions have already started preparing and delivering online programmes. University Grants Commission (UGC) had already launched SWAYAM portal for online courses [6]. Keeping the needs of designing and delivering online courses, the present paper makes an attempt to discuss a few tools which teachers can use in designing their courses.

Tools to Design Online Teaching and Learning Materials**Drawing tools: Mind Master and draw.io**

Teachers can take the help of Mind master and draw.io (see Figure 6) tools to design diagrams. These tools are free open source which can be installed in a computer. Mind master helps in creating mind maps. Mind maps can be used in a lesson as a brainstorming activity which can help developing learners’ schemata and motivate the learners to understand the topic better. Teachers can generate mind maps as per the needs of their lessons. Similarly, draw.io can be installed to generate various types of diagrams. An example of a simple process is shown in Figure 2.

OBS studio

Open Broadcast software is a free online tool which is meant for various purposes as discussed below

- Face recording
- Image recording
- Image and face recording
- Screen recording
- Screen and face recording
- Image slide show
- Image slide show and face recording

A teacher can use the open source tool for recording the class and sharing with his/her students. As there are various features of these tools including image slide show, face and screen recording, it would be handy for a teacher to use this tool for discussion of any topic (Figure 3).





Screencastify

Screencastify or screen video recorder is another tool where there is a facility to record one's audio with screen capture (Figure 4). One can record by screen casting and thus this would be helpful to teachers who wish to develop teaching materials for their students' online learning. It is a very user-friendly tool which can be attached with google chrome. Teachers can develop video lessons by sharing PPTs, lecture notes and any other relevant materials and give voice over.

OpenShot

It is an open-source video editing tool which can be installed in a computer. The video created through open broadcast system and screencastify can be edited through this freely available tool. It has a user-friendly interface where one can easily import videos and images and edit through this platform (Figure 5).

Advantages of using Online Tools

There are many advantages of using the online tools for developing lessons. This will not only be handy during the COVID-19 pandemic shut down of educational institutions, where students can learn through the sources, but also would be a great resource for students in a face-to-face mode, where teachers can play the videos in the classroom and discuss on them. The video lessons are extremely useful for slow-learners, who can view the materials as many times as they require to understand albeit teacher support can also be offered. In this process, learners can become autonomous and take the ownership of their own learning [4].

CONCLUSION

The paper emphasises the use of tools to design and develop online lessons effectively. A teacher can start by designing materials based on his/her course. The tools like Mind Master and draw.io can be used to design charts. After designing materials, one can video record by using tools like Open Broadcast Software and Screencastify. In this process, one can use PPT, videos, or text materials to screen cast. Further, voice over can be given while video casting. After video-audio recording, one can edit the video by using OpenShot tool. Here one can insert any image in the video. Further, the video can be cut into specific lengths as required. After video editing, one can post the video in the required sites, which can be on YouTube or coursera. Thus, the ICT tools are very useful for the teachers to design online teaching and learning materials (Figure 6).

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Table 1. Platforms for online learning for students and teachers (Adapted from UNESCO)

SL No.	Support to Education	Platforms
1.	Digital learning management systems	Edmodo, Google Classroom, Moodle
2.	MOOCs Platforms	Alison , Coursera, EdX, Future Learn, Udemy
3.	Live-video Communication	Hangouts Meet , Teams , Skype , WeChat Work, WhatsApp, Zoom
4.	Tools for Teachers to Create Lessons	MindMaster, draw.io, OBS Studio, Screencastify, OpenShot

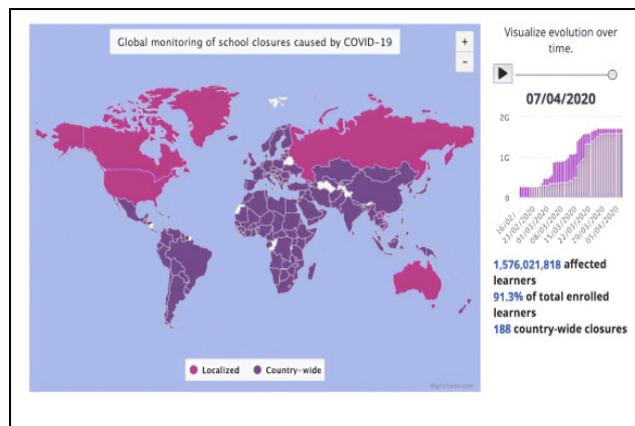


Figure 1. Impact of COVID-19 on closures of educational institutions across globe [2].

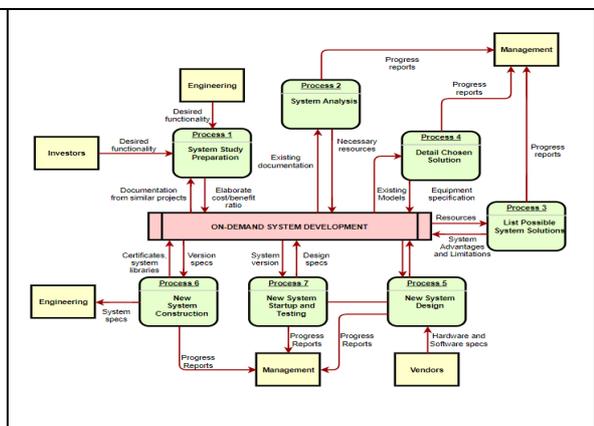


Figure 2. A diagram using drawio tool (Source: <https://app.diagrams.net/>)

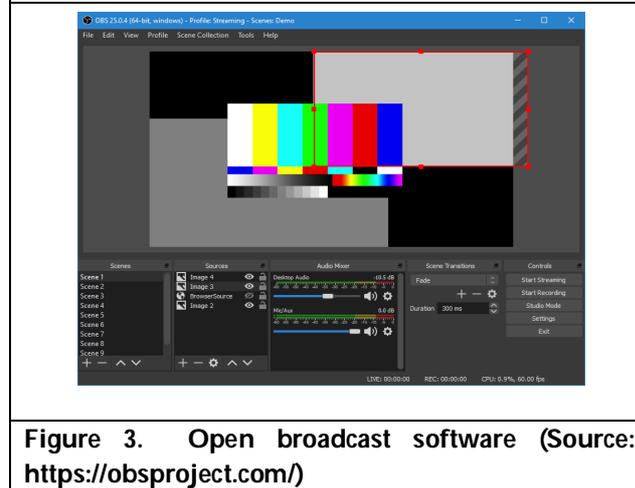


Figure 3. Open broadcast software (Source: <https://obsproject.com/>)

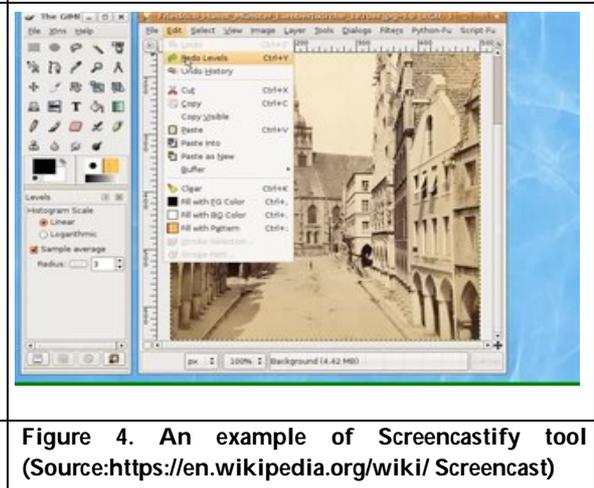


Figure 4. An example of Screencastify tool (Source:<https://en.wikipedia.org/wiki/Screencast>)





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Figure 5. OpenShot tool for video editing
(Source: <https://www.openshot.org/>)

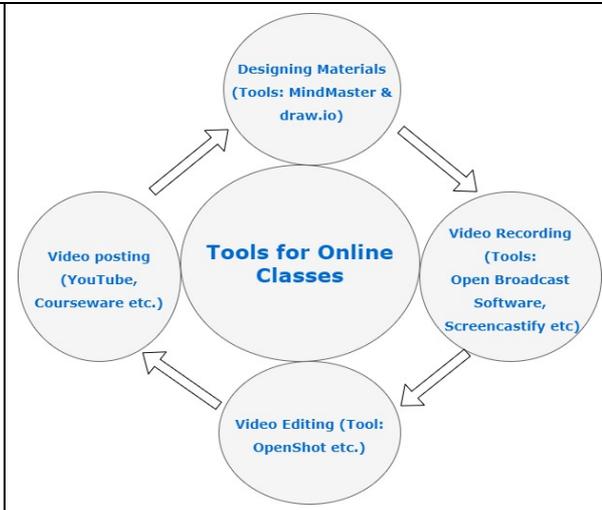


Figure 6. Integrating tools in teaching using draw.io





Electronic Control of a BLDC Motor for an Electric Vehicle

Sandipan Pine^{1*} and B B Choudhury²

¹Research Scholar, Department of ECE, Utkal University, Odisha, India.

²Department of Mechanical Engineering, IGIT Sarang, Sarang, Odisha, India

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*Address for Correspondence

Sandipan Pine

Research Scholar, Department of ECE,

Utkal University, Odisha, India

Email : sandipan@cutm.ac.in



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ABSTRACT

This paper emphasizes on analysis and evaluation of the execution of a permanent magnet brushless DC motor (PMBLDC) drive, controlled by PI controllers. The Controllers are for the PMBLDC motor drive simulated using Dymola Dassault tool and MATLAB tool. Further, the PI controller has been implemented on an experimental BLDC motor set up.

Keywords: Brushless DC Motor [BLDC], Electronic Control Unit [ECU], PI Controller

INTRODUCTION

Brushless dc (BLDC) motors are always given preferences as small horsepower control motors due to their silent operation, reliability, high efficiency, compact form, and low maintenance. However, some difficulties are there in these motor for variable speed operation over last decades continuing technology development in microprocessor, power semiconductors, adjustable speed drivers control schemes and permanent-magnet brushless electric motor production have been combined to enable reliable, cost effective solution for a broad range of adjustable speed applications. BLDC motors are backbone of electric vehicles and the control strategy is very much important. Main control is being done with the help of an electronic control unit [ECU] which can parallelly control a lot of application of the electric vehicle. Present work is a part of larger ECU design of an electric vehicle

Related Work

Current work [1]-[2] has reported that the motor drives with permanent magnet which include the synchronous motor and the brushless dc motor (BDCM) can be a very close rival of the induction motors in servo applications. These PMSM and standard synchronous machines are almost same in nature omitting the fact that PMSM does not have damper windings and the required excitation is not provided by a field winding rather by a permanent magnet. So the required d, q model of the permanent magnet synchronous motor can be found out from the well-known [3] model of the synchronous machine by removing other equations of the field current dynamics and damper





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windings. Transient model of a high performance vector controlled servo drive [4] can be illustrated using the d, q model of the PMSM. BDCM speed servo drive can be checked by using abc phase variable model [5]. Application characteristics of both machines have been presented in [6].

Motor Principle

A BLDC motor can be best described as a permanent synchronous machine accompanied by rotor position feedback. These brushless motors are normally controlled by the help of a three phase power semiconductor bridge. This motor needs a hall sensor to sense the position of the rotor to start and to provide actual commutation sequence to turn on the power module of the inverter circuit. Depending on the position of the rotor, power module are commutated sequentially every 60 degrees. In BLDC these brushes are removed and instead of them electronic commutation is used. Therefore the problem of sparking and wearing out of arrangement is permanently abolished. So BLDC design is strong as compared to dc motor. The basic block diagram brushless dc motor as shown Fig 1. It consist of four major blocks named as permanent magnet-synchronous machine(PMSM), power converter, control algorithm and sensors. The role power converter is to transform power from the source to the synchronous machine, which in turn converts electrical energy to mechanical energy. The main important feature of these BLDC motors is the rotor position sensor. Depending on the rotor position and any type of command signal the gate signal is determined by the control algorithm. Command signal may be in the form of voltage, torque or speed. Gate signal is applied at the power electronic inverter. There are two main classes of BLDC motor namely voltage source based and current source based, which is mainly determined by the control algorithm structure. Both the classes used with permanent magnet synchronous machine with either sinusoidal or non-sinusoidal back emf waveforms .Machine with trapezoidal back emf (Fig.2) may be controlled so as to achieve nearly constan

BLDC Drive System

BLDC is an electronic motor and it needs a 3 phase inverter as shown in fig 3 at the front end. In auto control mode this inverter behaves as an electronic commutator which receives the logical switching pulse from the hall sensors. This drive is also known as electronic commuted motor. Mainly the inverter can run in the following two modes.

- $\frac{2\pi}{3}$ angle switch on mode
- Voltage and current control PWM mode

PI control of the motor

Basic PI control drive is shown in fig 4. It consists of position sensor, speed controller, current controller, reference current generator, and current controlled voltage source inverter. The motor speed is sensed and compared with the reference one and the error signal is fed and processed at PI controller.

$$e(t) = w_{ref} - w_m(t) \dots \dots \dots (1)$$

$w_m(t)$ is compared with the refence speed and the resulting error is estimated at the n^{th} sampling as

$$T_{ref}(t) = T_{ref}(t - 1) + K_p[e(t) - e(t - 1)] + K_I e(t) \dots \dots \dots (2)$$

Where K_p and K_I are the gains of the PI controller. This controller output is treated a the reference torque. Depending on permissible maximum limit of the winding current, a limit is set at the speed controller. As per feedback of the position sensor and controller the reference current generator block generates the three phase reference currents $i_{a,ref}$, $i_{b,ref}$, $i_{c,ref}$. The reference currents have the shape of quasi-square wave in phase with respective back EMF develops constant unidirectional torque as. The PWM current controller regulates the winding currents i_a , i_b , i_c with the small band around. Motor and reference current are compared and switching instructions are created to drive the inverter.





RESULT ANALYSIS

Total control structure is simulated in MATLAB as well as Dymola. Dymola is used to get a better coordination in simulation of a electromechanical structure which is electric vehicle.

MATLAB simulation

The PI control of fig 4 is implemented in MATLAB, which is shown in fig-5, for a reference speed of 1500 rpm and the speed sensor output is shown in fig-6. It is clear from fig 6 that by using close lop PI controller speed is coming to a steady state at 0.05s. At 0.1 s a torque is applied.

Dymola Simulation

Fig 7 shows the PI controller in Dymola. For this design half bridge output, hall sensor output and the inverter current output is shown in fig 8, 9 and 10. Reference rpm is set by setting the voltage source. Fig 11 shows the speed of the motor at a refrence voltage of 24 V. It is clear from fig 11 that the system is stabilizing in 0.3 second.

CONCLUSION

Hence it can be concluded that PI controller is suitable for controlling speed of a BLDC motor because it is stabilizing very fast when the reference is changed. Therefore this design can be implemented on the FPGA board to design the final controller which is a part of the Electronic control unit [ECU]. All the data can be visible on the dashboard for better understanding of the driver. As FPGA is very good for parallel processing all other application can be run parallely and hence it is very good for designing ECU.

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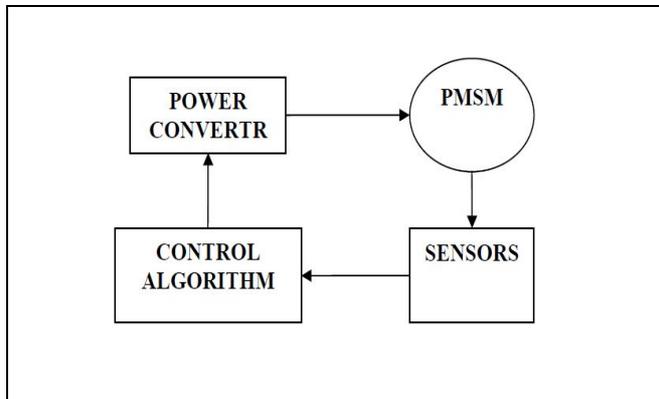


Fig. 1. Basic block diagram of BLDC motor

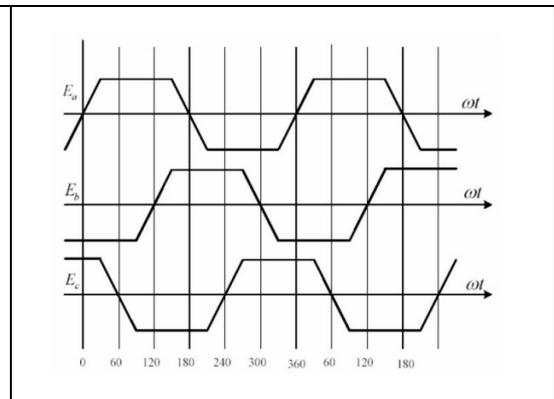


Fig. 2. Trapezoidal Back emf of a 3 phase BLDC motor

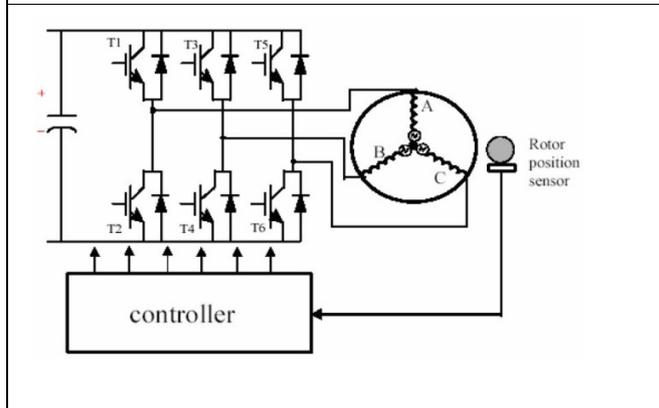


Fig. 3. BLDC drive system

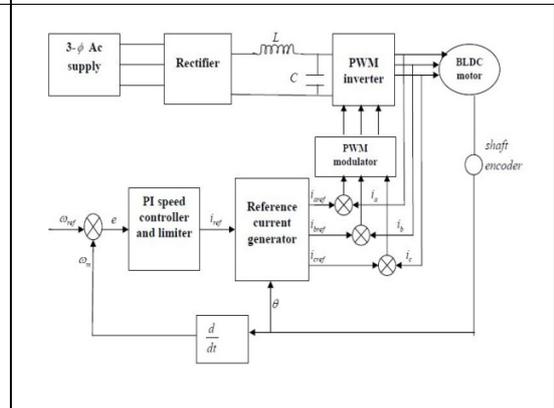


Fig. 4. PI Control of the motor

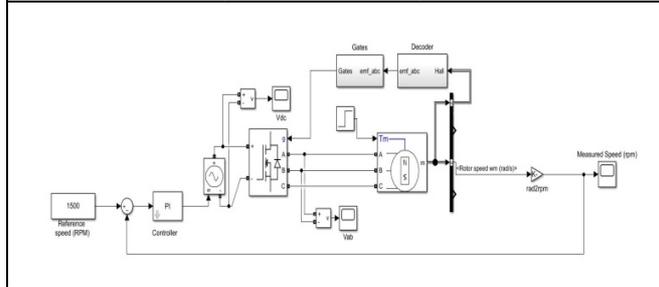


Fig. 5. Design of the PI controller in MATLAB

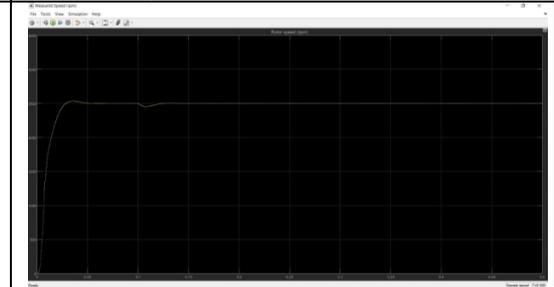


Fig. 6. Speed sensor output

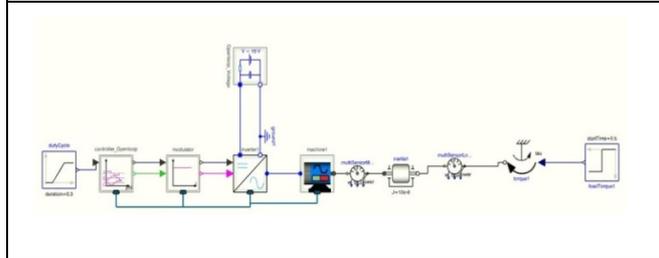


Fig. 7. PI controller in Dymola

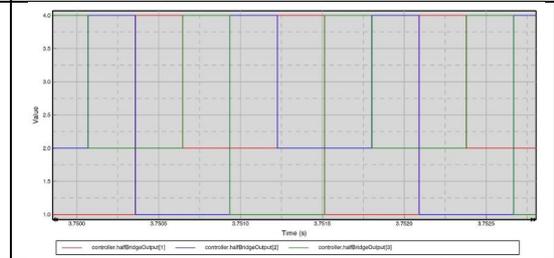


Fig. 8. Half Bridge Output





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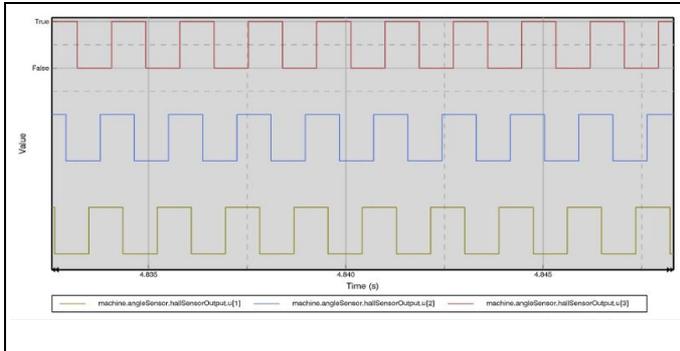


Fig. 9. Hall Sensor output

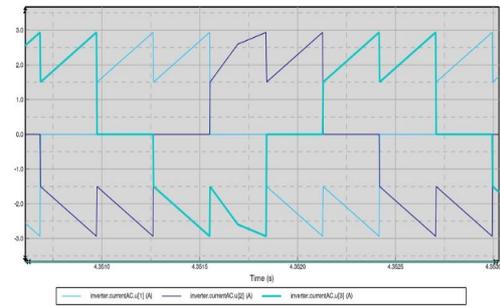


Fig. 10. Inverter current

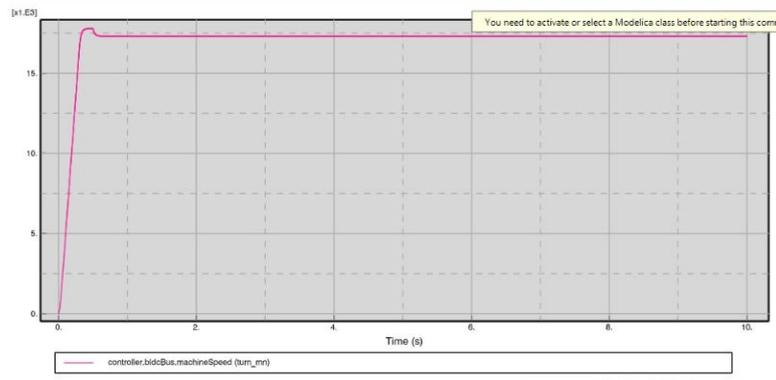


Fig. 11. RPM of the motor at 24 V reference voltage





Intrapersonal Factor of Emotional Intelligence and Communication Skills

Amir Prasad Behera* and Prajna Pani

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Amir Prasad Behera

Centurion University of Technology and Management,
Odisha, India.

Email: amir.prasad@cutm.ac.in



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ABSTRACT

The aim of this paper is to study the correlations between intrapersonal factor of emotional intelligence and communication skills. Self-awareness and self-expression, the EI competencies and skills are the deciding factors in the selection process and performance of an individual in the competitive market. Intrapersonal factor of EI tunes one to recognize one's emotions, feelings, regulates moods and helps one to handle stress; to empathize and to hope. The study is conducted on randomly selected two hundred engineering students and staff of a state private university in Odisha, India. The intrapersonal competency involves being in tune with self-regard, emotional self-awareness, assertiveness, independence and self-actualization. The paper uses descriptive method which aims at quantitatively testing the hypothesis that a correlation exists between intrapersonal factor of EI and communication skills. The quantitative part of the research uses five-point Likert scale questionnaire on intrapersonal and communication skills. The qualitative part of the research is based on literature review. The findings of the study show that there is a significant correlation between intrapersonal factor of EI and communication skills.

Keywords: Emotional Intelligence, Intrapersonal, Communication, Correlation

INTRODUCTION

Intrapersonal is one of the important factors of Baron's EQi model. Individuals who are strong in intrapersonal intelligence are good at being aware of their own emotional feelings, thoughts, emotions and motivations. The practice of intrapersonal factor of EI develops not only a sense of self but also helps in identifying the art of communication. Intrapersonal is defined in the American Heritage Dictionary of the English Language as existing or occurring within the individual self or mind. Graeme and Dimpleby (2006) define intrapersonal communication as communication within the self, and of the self to the self [1]. The value one sees in oneself determines how one





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communicates with others. It is communication that takes place within the individual when he or she is communicating with others, or simply, when he or she is alone and thinking to himself or herself. Good communication with others is a prerequisite for effective intrapersonal communication [2]. Developing effective intrapersonal communication takes discipline and a willingness to slow down your day enough to hear your own thoughts. With time, an effective intrapersonal communication strategy can help one better organize the daily tasks and remain calm in difficult situations. It is considered as the foundation for all communication and a key source for understanding ourselves and our environment [3].

Intrapersonal Factors of Emotional Intelligence

Self-Regard

Self-regard could be defined as the capacity to assess, understand and accept oneself without any bias or prejudice. It is a process of self-evaluation that leads one to discover who he/she really is. It animates one to recognize one's own strengths and vulnerabilities and thus energizes to rediscover one's own potentialities. It empowers one to grow in inner strength that is self-reliance and be assertive while being considerate of others.

Emotional Self-awareness

Emotional awareness is the ability to recognize how and what causes one's own emotions. It not only helps to understand both positive and negative emotion that one goes through and the way one gets affected by it but also those of others. High levels of emotional awareness means one can learn from one's feelings quickly. One can make a sense out of it, and if necessary bring about positive changes in oneself which eventually would lead one to be proactive. It enables one to manage productively one's own emotions.

Assertiveness

Assertive is a core communication skill. It is the ability to be able to express oneself efficiently and productively. Assertiveness also refers to emotional self-expression. It is composed of three key elements: (i) the ability to express one's feelings on an emotional level; (ii) the ability to express one's beliefs and opinions on a cognitive level; and (iii) the ability to stand up for one's point of view, while also respecting the rights and beliefs of others. Assertive people are able to believe in their ability to understand and deal with a situation, their message is clear and easy to understand and they are able to communicate information in calm and controlled manner. Additionally, these people are often guided by their principles, are bold and capable of affirming themselves.

Independence

Independence refers to the capacity of supporting oneself and not being completely dependent on others for their emotions. They are the decision makers of their own lives. They make choices and live by it. They are least bothered or not affected by what others think and say about them. They are driven by choices that revitalize their individuality. They are the ones who are able to bring the most out of themselves and be a source inspiration to others. They are able to bring changes that is lasting and rejuvenating.

Self-actualization

It is the capacity to realize one's own inner abilities to the full. They dream big and are not afraid to make the dream come true. They understand their own uniqueness and so are driven to actualize this uniqueness into a reality. They make choices that are fundamental to their and the humanity's growth.

Objective of the Study

The main objective of the paper is to make a correlational study of intrapersonal factor of EI and communication skills.





METHODOLOGY

This research utilised random purposeful sampling method. The participants consisted of faculty (n=60), staff (n=40) and students (100) from a state private university in Odisha. The sample size represented 134 (67%) males and 66 (33%) females. Their ages ranged from 20 to 58. Out of these, 58% of the participants were between the ages of 20 and 24 years old, 24% were between 25 and 29 years, and the remaining 18% were between 30 to 58 years. Questionnaires were a source of primary data collection for this study. Quantitative data was collected via self-assessment questionnaires comprising a number of scales that tested the constructs of interest. The researcher used the self-report method as it is appropriate for measuring the respondent's subjective attitudes and beliefs about their interpersonal and communication skills. After the study was completed, all data were statistically analysed and interpreted using SPSS 20 Version. In the experimental design, the scores were compared within and between groups of participants to see whether there is any correlation between intrapersonal factor of EI and communication skills. Descriptive statistics was used to find average, below average and above average intrapersonal and communication scores of the participants.

The EI intrapersonal sub-scales and what they assess in the present study is defined in Table 1. The intrapersonal items were in the form of short statements and employed a 5-point response scale. The scale captured EI Intrapersonal items - self-regard, assertiveness, emotional self-awareness, independence and self-actualisation e.g., 'I am self-directed and self-controlled in how think and act'. Participants were required to indicate the degree to which each statement is true of the way they typically think, feel or act on a five-point scale (1=Very Seldom or Not true of me, 5=Very often true of me or True of me). Communication inventory was used to measure the communication skills of all participants of the study. It is a self-report measure that contains 20 items adapted from HTC Consulting. The score of the questionnaire has not been emphasized deliberately as the point is not how high the respondents scored. It is about raising one's awareness on how well one communicates. Responses are captured on a five-point Likert scale. The scale captured communication factors such as Intrapersonal Communication e.g., 'I don't think that my opinion is the most important in the room', Verbal Communication (e.g., 'I am confident when I talk to people and speak clearly without mumbling', Non-verbal Communication e.g., 'I am good at making eye contact with people when I am talking to them', Interpersonal Communication e.g., 'I am open-minded and am willing to change my viewpoint based on the valid opinion of others' [4].

DISCUSSION AND DESCRIPTION

The Pearson's contingency coefficient method was used to estimate the extent of the statistical relationship between two variables (EI intrapersonal and communication skill). Communication variables were independently correlated with EI intrapersonal variables. This procedure in the present study served as a summary reporting tool and was used to analyse survey data. The hypothesis of the study reveals that there exists a positive correlation between the intrapersonal factor of EI and communication skills. The summary of the finding with regard to this hypothesis is given in the following section: Research findings reveal the average scores of communications for faculty (74.8) and staff (73.4). The EI intrapersonal and communication profiles of the participants indicated that they mostly fell into the areas of 'effective functioning' and 'enhanced skills. The findings in Figure 1 seem to be encouraging.

Hypothesis

Null Hypothesis (H₀): There exists no significant correlation between the intrapersonal factor of EI and communication skills.

Alternative Hypothesis (H₁): There exists correlation between the intrapersonal factor of EI and communication skills.





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RESULT FROM TESTING HYPOTHESIS

- Intrapersonal sub-factors of EI (independence, assertiveness, self-actualization) correlate with 9 communication indicators (calm on the point, cool, valuable, clear and concise, encourage, open-minded, reading, shout, intimidated) at 1% significance level.
- Intrapersonal sub-factors of EI (independence, self-actualization) correlate with 11 communication indicators (calm on the point, cool, eye contact, comfortable, message, summarizing, tailor, clear and concise, concentrate, confidence and listen) at 5% significance level.
- Intrapersonal sub-factors of EI (independence, assertiveness) correlate with 11 communication indicators (cool, eye contact, message, shout, intimidated, summarizing, tailor, concentrate, confident, listen and comfortable) at 10% significance level.

Thus, the hypothesis states a significant correlation between the intrapersonal factor of EI and communication skills.

Intrapersonal Factor Correlates with Communication

Thus, the hypothesis states a positive correlation between the intrapersonal factor of emotional intelligence and communication skills. This paper taps intrapersonal capacity and functioning. The sub-factors of the intrapersonal factor in the present study include self-regard, emotional self-awareness, assertiveness, independence and self-actualization. The intrapersonal score of students is found in the domain of effective functioning with 75.1, while the interpersonal scores of staff and students are observed in the domain of enhanced skills with 81.7 and 86.7, respectively.

Sub-factor: Self-regard

The self-regard scale score of all the three sample categories is observed within the area of enhanced skills. Faculty participants are found with the highest score, i.e., 83. The total score of staff is 71 and students are 72.6. The participants seem to have satisfactory degree of self-regard and self-respect.

Sub-factor: Emotional Self-awareness

The results 84 for faculty, 82 for staff and 75.6 for students indicate highly effective emotional self awareness. The emotional self-awareness of faculty is higher compared to students and staff in the area of enhanced skills. The participants are in touch with their feelings and emotions, and usually know what they are feeling and why. These individuals are also comfortable expressing feelings to others. It also indicates an individual who knows how his/her feelings and emotions impact on his/her own opinions, attitudes, and judgments. The participants are able to facilitate interactions by appropriately monitoring their own emotions during exchanges with others.

Sub-factor: Assertiveness

The assertive scores of faculty are 60.6, staff 59.6 and students 58.8 which indicate that the participants fall in the area for enrichment. The participants in this area often lack the ability to express thoughts, feelings, and emotions. These individuals are not confident to participate in conversations / discussions and render opinions openly and in a constructive manner. The individuals often fail to get across their viewpoint to others.

Sub-factor: Independence

The independence scores are 76.6 for faculty, 75 for staff and 73.8 for students. The score of faculty fall within the area of enhanced skills whereas the scores of staff and students are found within the areas of effective functioning. The responses indicate that participants are independent in thinking and also have a strong preference to act independently. These types of people may ask others for advice, but rarely depend upon others to make important decisions on their behalf. These people prefer to be in charge rather than being under the supervision of someone else.





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CONCLUSION

Research findings state that there is a significant and positive correlation between independent variable intrapersonal factor of emotional intelligence (i.e. self-regard, self-actualization, independence, emotional self-awareness and assertiveness) and communication skills. Thus, we can conclude that the educational organizations should pay much attention to both intrapersonal factors of EI and communication skills as they can enhance self-regard, emotional self-awareness, assertiveness, independence and self-actualisation for personal growth and professional success. The paper leaves scope for further research to replicate the findings of the present study in wider samples in educational organizations of the world.

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Table 1: EI intrapersonal sub-scales

EQ-I SCALES	The EI Competencies and Skills Assessed by Each Scale
Self-regard	The ability to respect and accept oneself
Emotional Self-awareness	To be aware of and understand one's emotions
Assertiveness	The ability to express thoughts feelings and emotions
Independence	The ability to act independently
Self-actualization	To strive to achieve personal goals To feel content with one's accomplishment

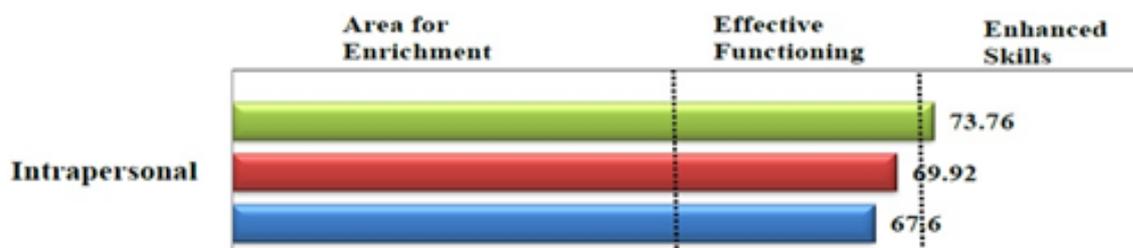


Figure 1: Intrapersonal Scores





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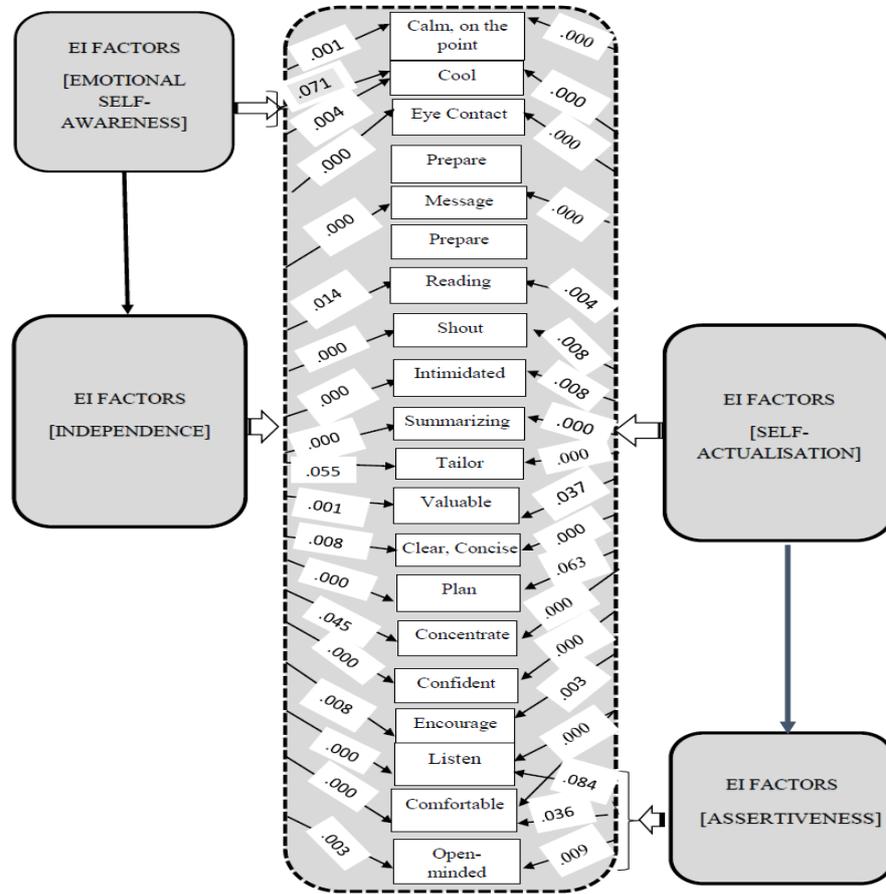


Figure 2: EI Intrapersonal factors correlate with communication





Conservation of Earth's Treasure through Sacred Forests of Uttarakhand, "A Traditional Ecological Heritage"

Brij M.Upreti* and Lalit M Tewari

Department of Botany, D.S.B. Campus Nainital, Kumaun University, Uttarakhand, India.

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*Address for Correspondence

Brij M.Upreti

Department of Botany,
D.S.B. Campus Nainital,
Kumaun University,
Uttarakhand, India.
Email: brijmupreti@gmail.com



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ABSTRACT

The present work is a documentation of sacred forests for its phytodiversity with IUCN status of plants and soil profiling. Present work documented 321 angiospermic plants out of which 37 were trees, 73 were shrubs, 200 herbs and 11 were climbers and total 34 species were identified as IUCN threat category out of which 01 was CR, 07 were ED, 09 were Vu and 17 species were categorised under NT status. Therefore there is an urgent need to protect these sites to conserve regional plant diversity, not only for ecosystem health but also for the benefit of the indigenous tribes who heavily depend on local plants diversity for their day to day requirements.

Keywords: Indian Himalaya, Kumaun Himalaya, Belief system, plant diversity, soil profiling.

INTRODUCTION

Forests represented the feminine principle in prakrti. In the Hindu pantheon, forests have been worshiped as Goddess Aranyani, the Goddess of the Forests and Animals that dwell within them. Forests are the primary source of life and fertility. The forest as a community has been viewed as a model for societal and civilization evolution. The forest thus nurtured an ecological civilization in the most fundamental sense of harmony with nature. The forest as the highest expression of the earth's fertility and productivity is symbolised in yet another form as the Earth Mother having different names at different parts as in Bengal she is associated with Avasthaor or Banbibbi, the lady of the forest, in Bangla Desh, "Bamani", in Assam "Rupeswari" and in Southern India as Vana Devas means forest spirits. Environment provides delight to people leading their life flawlessly. Rivers bliss us with sacred water and provide us health and vegetation. Sun bliss us with peaceful life. Our cows provide us milk. The plant ecology has a great importance to keep the environment in balance. Due to rapid litter decomposition rate, nutrient release into the





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soil of these forests is very high. The soil itself has little nutrients to support the large biomass of the sacred grove. Soil is defined as, an independent body in nature with a unique morphology from the surface down to the parent material as expressed by the sample profiles[1], which has been derived from the Latin word "Solum". The study of soil is known as the 'Pedology' (pedos means earth) or 'Edaphology' (edaphos means soil). The sacred forest of central Himalaya has enormous variations in the climate, topography, and soil conditions, which form a very complex ecosystem [2]. Since, the vegetation zones in this region clearly reflect edaphic and climatic variations and at the same time the knowledge of physical and chemical properties of soils and climatic conditions of different forest types of temperate region of central Himalaya is meagre. However, the present study was undertaken to understand soil properties in relation to traditionally protected and non protected forests structures Uttarakhand.

METHODOLOGY

Extensive field visits were conducted for documentation of plants and ethno botanical information from six sacred forests of Pithoragarh district of Uttarakhand, India (fig 1). The temperature ranged between 3.2°C to 28.7°C during the study period, while rainfall ranged between 7 to 390mm. The knowledge based system methodology for acquisition of local ecological knowledge suggested by Walker [3] and Sinclair & Walker [4] was adopted. The knowledge was collected through repeated, focused interviews, with information bring sought from the location of sacred natural sites related features, local perception about sacredness of the sites and management. Analytical procedures for soil physiochemical characterization was done by various methods viz., soil texture, soil moisture, water holding capacity, soil bulk density, soil porosity, soil organic carbon, available nitrogen, available phosphorus and available potassium. Soil Samples collected from four different depths viz., (i) upper (0–10 cm), (ii) middle (11–30 cm) and (iii) lower middle (31–60 cm) (iv) Lower (61-90 cm) for assessing the physical and chemical properties of the soil in all the selected forest. The pH of soil was determined directly with the help of control dynamics digital pH meter (model Ap 175E/C). Walkley and Black's rapid titration method as modified by [5] was adopted for organic carbon estimation. The factor of 1.724 was used to convert the soil organic matter (%) into organic carbon (%). Available phosphorus was determined in the soil by Olsen [6] method. Potassium was extracted by neutral normal ammonium acetate method and was determined by the flame photometer (Evans Electro Selenium Ltd; Holsted Essex, England). Total nitrogen was measured by using the standard Kjeldhal procedure. All the soil chemical tests were conducted at the soil testing laboratory, Bhawali, Uttarakhand.

Soil moisture: Soil moisture was determined by the following for formula [7].

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight of the soil} - \text{Dry weight of the soil}}{\text{Dry weight of the soil}} \times 100$$

Soil bulk density: Soil bulk density was calculated by following formula [8].

$$\text{Bulk density} = \frac{\text{Mass (g)}}{\text{Volume (cm}^3\text{)}}$$

Soil porosity: Soil porosity was calculated by following formula [9].

$$\text{Porosity (\%)} = \frac{\text{Bulk density}}{\text{Particle density (2.65)}} \times 100$$



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Soil water holding capacity: WHC determined by the following formula [10].

$$\text{WHC (\%)} = \frac{W_2 - W_3 - W_4}{W_3 - W_1} \times 100$$

Where,

W₁ = weight of sieve + filter paper

W₂ = weight of sieve + filter paper + wet soil

W₃ = weight of sieve + filter paper + oven dried soil

W₄ = water absorbed by filter paper

RESULTS AND DISCUSSION

The sacred forests are known by different nomenclature in various parts of the country. As per recent survey for present work it came to know that about 10,874 sacred areas (SA) were studied by around 51 researchers during 1981-2019 (34 years) in 26 states of India [11][12]. Maximum SAs were recorded from Maharashtra (2333) followed by Karnataka (1476), Tamil Nadu (1275), and in Uttarakhand total 138 SAs were studied for their plant wealth, soil profiling, ethnobotanical study, traditional practices and conservation purposes by various researchers [13], [14],[15], [16], [17],[18],[19], [20].

Characterization of Soil Profile

The percentage of sand was maximum in sacred Ratwali forest at 61-90cm depth. The Silt 34.71%, maximum in sacred Betal forest at 61-90 cm depth. The sacred Thal kedar had maximum clay viz. 53.16% at 0-10cm depth. Sacred Thal kedar forest had maximum moisture content (37.16%) at 0-10cm depth. Maximum Water holding capacity recorded in sacred Thal kedar forest (68.74%) at 0-10 cm depth. Maximum bulk density recorded in sacred Ratwali forest (1.34 g/cm³) at 61-90cm depth. Sacred Thal kedar forest had maximum porosity (54.85) at 0-10cm depth. The pH ranged from 5.4 to 7.4 recorded maximum in sacred Ratwali forest at 0-10cm depth. The organic matter percentage ranged 1.8 to 3.86, maximum in sacred Kalika forest at 0-10 cm depth. The sacred Kalika forest had maximum carbon percentage (2.24%) at 0-10cm depth. Sacred Pasupatinath forest had maximum available nitrogen (326kg/h) at 0-10cm depth. Maximum potassium recorded in sacred Kalika forest (0.025) at 0-10 cm depth. Maximum phosphorus recorded in Sacred Ratwali forest (0.014) at 0-10cm depth. Soil physicochemical characterization of sacred forests showed high values at upper layers in comparison to lower layers.

Floristic documentation

Extensive exploration of selected sacred areas of Pithoragarh district (Table 2), shows total 321 plant species under 237 genera and 89 families which can be categorised on the bases of habitat viz. Trees (37), Shrubs (73), Herbs (200) and climbers (11). Dominated tree, shrub and herb species were also evaluated in table 3. Maximum tree species were recorded from SF Pasupatinath, Maximum shrub at SF Thal kedar and maximum herbs were recorded from SF Betal devta (Fig 2).

Threat categorization

Various plants under IUCN category were also documented from sacred forests during present study. Total 34 plant species under 26 families were recorded from sacred forests, which were listed in IUCN category. Out of total 34 species trees were 8, shrubs 13, herbs 12 and climber 1 while out of 34 species 01 was CR, 07 were ED, 09 were Vu and 17 species categorised under NT status. Out of 26 families Berberidaceae and Orchidaceae have highest number of plant species i.e. 03 while Asteraceae, Meliaceae, Rosaceae and Violaceae having 02 species in each. As per extensive field visits it is concluded that habitat destruction is a major cause for plant threat followed by over exploitation.

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CONCLUSION

Present study of sacred forests comprises a diversified flora with a good environmental and edephic factors. The micro climatic conditions of sacred forests which are playing an important role as socio religious institutions as well as reservoir of biodiversity. Uttarakhand Himalaya is serving as repository of many sacred areas having immense biodiversity with many rare and endangered plant species growing in peculiar climatic conditions. Studies showed the role of these sacred sites in reducing soil erosion, preventing landslides and in conferring ecosystem stability. Therefore there is an urgent need to protect these natural heritage sites to conserve regional plant diversity, not only for ecosystem health but also for the benefit of the indigenous tribes who heavily depend on local plants diversity for their day to day requirements.

ACKNOWLEDGEMENT

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Table 1: State-Wise Sacred Areas in India (Total 10,874 Sacred Forests Since 1981 – 2019)

State	No of SG	State	No of SG
Andhra Pradesh	677	Madhya Pradesh	169
Arunachal Pradesh	159	Maharashtra	2333
Assam	29	Manipur	531
Bihar	43	Meghalaya	105
Chhattisgarh	63	Odisha	188
Goa	93	Puducherry	108
Gujarat	42	Rajasthan	570
Jammu and Kashmir	92	Sikkim	16
Jharkhand	29	Tamil Nadu	1275
Karnataka	1476	Telangana	57
Kerala	1096	Uttarakhand	138
Haryana	248	Uttar Pradesh	32
Himachal Pradesh	329	West Bengal	986

Table 2: Brief information of selected sacred forests of Pithoragarh, Uttarakhand, India

Sacred Forest	Altitude (m)	Forest Area (h)	Communities
SF Kalika	1695	145	Rawal, Pant, Joshi, Pathak, Mehta, Bhandari, Karki and Negi
SF Chamunda	1795	159	Pant, Joshi, Upreti, Bhandari, Tamta and Pathak
SF Betal Devta	1504	133	Sirola, Upadhyay, Joshi, Bhandari and Arya
SF Thal kedar	2602	195	Bhatt, Joshi, Ram, Oli, and Negi
SF Psupatinath	1906	153	Joshi and Bisht
SF Ratwali	1807	136	Joshi, Pandey and Mehta

(SF= Sacred Forest)

Table 3: Dominated species in sacred forests of Pithoragarh, Uttarakhand, India

Sacred Forest	Dominated Tree	Dominated Shrub	Dominated Herb
SF Kalika	<i>Cedrus deodara</i>	<i>Sarcococca saligna</i>	<i>Achyranthus aspera</i>
SF Chamunda	<i>Cedrus deodara</i>	<i>Viburnum cotinifolium</i>	<i>Dicliptera bupleuroides /Goldfussia dalhousiana</i>
SF Betal Devta	<i>Quercus leucotrichophora</i>	<i>Desmodium elegans</i>	<i>Achyranthus bidentata</i>





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SF Thal kedar	<i>Quercus leucotrichophora</i>	<i>Daphne papyracea</i>	<i>Achyranthus bidentata /Bidens pilosa</i>
SF Psupatinath	<i>Rhododendron arboreum</i>	<i>Daphne papyracea</i>	<i>Achyranthus bidentata / Goldfussia dalhousiana</i>
SF Ratwali	<i>Quercus leucotrichophora</i>	<i>Daphne papyracea</i>	<i>Achyranthus bidentata/Viola canesens</i>

(SF= Sacred Forest)

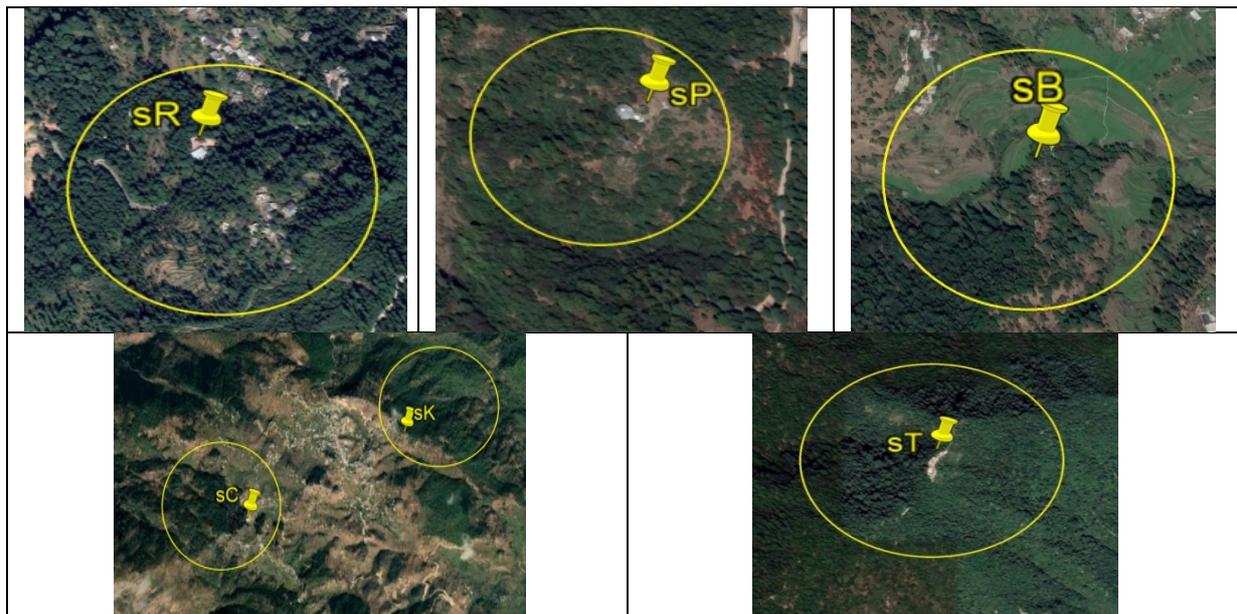


Fig 1: Google earth images of sacred forests, Pithoragarh (SR= Sacred ratwali, SP= Sacred Pasupatinath, SB= Sacred Betal, SC= Sacred Chamunda, SK= Sacred Kalika and ST= Sacred Thal kedar)

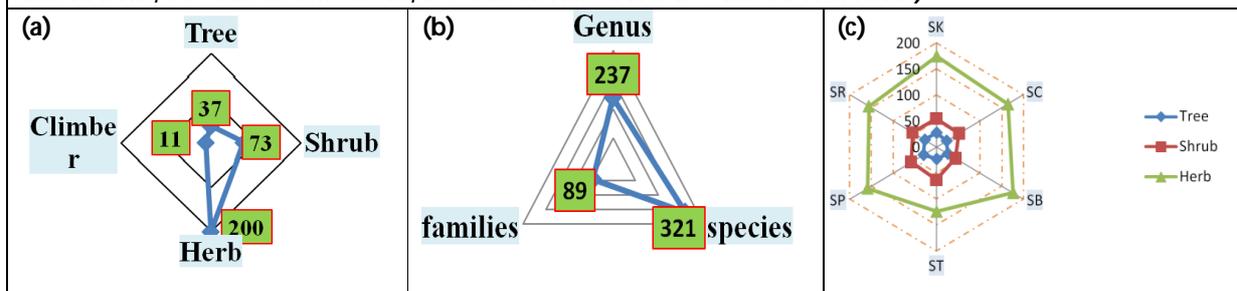


Fig 2: Phytograph of Individual at different habitat level (a), numbers of families, genus, and species (b) and site wise habitat distribution (c)





Battery Performance Optimization of an Electric Vehicle in Modelica

Sandipan Pine^{1*} and B B Choudhury²

¹Research Scholar, Department of ECE, Utkal University, Odisha, India.

²Department of Mechanical Engineering, IGIT Sarang, Sarang, India

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Accepted: 18 Nov 2020

*Address for Correspondence

Sandipan Pine

Research Scholar,
Department of ECE, Utkal University,
Odisha, India.
Email: sandipan@cutm.ac.in



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ABSTRACT

This paper focuses on battery performance optimization of an electric vehicle in Modelica. It's a part of Battery Management System [BMS] which is going to be used in an Engine Control Unit [ECU] design of an Electric Vehicle. Battery is the major concern for any electric vehicle, hence the performance of batteries are simulated for different conditions. Real time data are taken for 1 electric 3 wheelers of 750kg total load and 1.6 m² frontal cross section area. For each condition different battery strength and average speed is taken and the simulation is continued for 7 hour time to understand the discharge of the battery in each case and hence the performance.

Key words: Battery Management System, Electric Vehicle, Engine Control Unit

INTRODUCTION

Modelling of road vehicles is not a task of single discipline rather it needs a multidisciplinary approach to deal with [1]. This task includes modeling with electrical machines, translational mechanics and rotational mechanics, modeling of hydraulic components, electrical converters and many more. Specifically electric vehicle modelling requires direct involvement of mechanical and electrical peoples with their domain knowledge and control strategy. Due to this requirement simulation and modeling of vehicles, specifically electric vehicles, are an area of engineering where Modelica excels. As Modelica is having its internal property to simulate multi domain model, it is the best choice to simulate this kind of designs where multiple domain knowledge are involved. Due to highly reliable simulation results Modelica has more advantages and prospects in modeling multi-domain systems. One such example is the language Modelica [2], which has been used to model and simulate complex multi-domain physical systems in recent years. Its model library covers electrical, mechanical, thermal, control and many other characteristics, providing a basis for unified description of physical systems [3]. Moreover, Modelica supports object-oriented non-causal modeling, which has standardization, openness and extensibility. Modelica is more accurate and



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efficient for complex physical modeling [4]. The battery is essential for the power, safety and economy of an EV. A good design of the battery management system improves battery life and ensures cruising range and vehicle safety [5]. The battery model is used to describe the dynamics of battery operation. The model is indispensable to estimate the battery state of charge (SOC) and simulate the battery management system of an EV. It is difficult to model and simulate the battery management process [6]. This paper will provide models with a detail calibrated level that will help to gain a solid fundamental to build more compete and realistic versions.

Motivation

Electric vehicles are mostly concerned with battery performance. Due to lack of charging points it's not that much popular in rural India. As source of fuels are limited we have no choice left rather shifting to electric vehicle. It gives pollution free environment also; hence it will reduce the greenhouse effect. So by any means this battery problem needs to be resolved. In this scenario Battery Management System has become one of the focused research area nowadays. In this paper battery performance is measured for different average speed and battery power to get an idea of performance. This is the first step in battery management system.

METHODOLOGY**Model Design**

Basic model of the electric vehicle is shown in fig 1 with following components.

Driver

Driver model is a compulsory component in vehicle simulation. Here a very basic driver model is used and the model is created on purpose because it is not available in modelica standard library. It is designed to follow a kinematic drive, whose name is displayed in its icon. It is a block, i.e a Modelica model without any physical connector, which generates signals that should be interpreted to be torque signal: accelerator (above), brake (below), combined (midrange, connected to power train in the figure 1).

Battery

This is the main important thing of this work. Battery pack is simulated for 3 different battery strength, i.e. 100AH, 130 AH and 150 AH with a cell pack of 4 as shown in fig 2.

Electric drive

In this vehicle simplified model, the torque-inertia pair constitutes the electric drive, which receives as input a torque signal, positive when accelerating and negative when braking, and actuates it, considering the dynamics induced by the inertia. The electric drive absorbs or delivers power. This simple model does not show power conversion into electricity. Drive parameters are shown in fig 3.

Gear

Commonly EV s have only a fixed ratio reduction gear. That is the role of gear in the above picture.

Wheel

This is an ideal rolling wheel. Although in real-life cases wheels do not roll ideally. They have some slip, which is below 5% on good tyre-to-tarmac contact, and accelerations below 5-7 m/s². In this paper ideal rolling is considered; the accelerations should be indeed, well below the 5 m/s² threshold. Wheel radius is fixed at 0.5715m, which is shown in fig 4 as per the EV specification.



**Sandipan Pine and Choudhury****Mass**

A very important part, though very simple, is the modelica mechanics rotational components. Mass model called mass. It is the vehicular total mass, as shown in figure5 it is 750kg. At its flange-a it is subject to propulsive force due to power train torque, at its flange-b to the resistance force to movement of the vehicle drag force.

VelSens

This is velocity sensor. It senses the velocity of the vehicle. In modelica environment this sensor creates a communication link between the physical and cyber parts of the simulation model. Looking at things in another way, it plays the role of a vehicle dashboard. The driver has in mind the kinematic cycle he wants to follow, and to do this it acts a closed-loop controller. Therefore actual speed is monitored through the dashboard (which displays the speed measured by VelSens) compares it with the desired kinematic cycle, and acts on its commands (brake, accelerator) so that to reduce or nullify the error.

DragF

This is drag force module. It indicates the resistance of the vehicle for movement. Since this force requires some rather sophisticated code to avoid unexpected effects, this is not a MSL model. It is instead a model extended from modelica mechanics translational interfaces partial friction. Fig 6 shows different drag force parameters

Simulation Result

Simulation is performed in two profiles as bellow. Profile is set through NEDC file of the driver.

Profile 1

Vehicle will ramp up to a speed of 18 kmph in 18 seconds then stay there for 1 hour. Again it will ramp up its speed to 24 kmph in another 6 seconds and stay there for an hour. Then the speed will ramp down to 18 kmph in 6 seconds and again stay there for an hour. Next the speed will ramp up to 32 in 14 seconds and stay there for 1 hour. Then it will ramp up to 40 kmph in 8 seconds and stay there for one hour. Next it will come down 18 kmph in 22 seconds and maintain it for 1 hr. So the average speed in the whole thing is 25 kmph with a max speed of 40 kmph. The whole process is performed under 3 battery specification 100 AH [360000 coulomb], 130 AH [468000 coulomb] and 150 AH [540000 coulomb]. In all the cases battery drainage versus time is shown in fig 7, 8 and 9 respectively.

Profile 2

Vehicle will ramp up to a speed of 15 kmph in 15 seconds then stay there for 3 hour. Again it will ramp up its speed to 20 kmph in another 5 seconds and stay there for an hour. Next the speed will ramp up to 25 in 5 seconds and stay there for 1 hour. Then it will ramp up to 30 kmph in 5 seconds and stay there for one hour. Next it will come down 20 kmph in 10seconds and maintain it for next time. So the average speed in the whole thing is 20 kmph with a max speed of 30 kmph. The whole process is performed under 3 battery specification 100 AH[360000 coulomb], 130 AH [468000 coulomb] and 150 AH [540000 coulomb]. In all the cases battery drainage versus time is shown in fig 10, 11 and 12 respectively

RESULT ANALYSIS

NEDC is prepared to run the simulation for 7 hour [25200s]. The result of the simulation concludes following things. For profile 1 when the average speed is 25 kmph and max speed is 40 kmph, the vehicle runs for 100 km in a single charge of the battery when battery strength is 100 AH. It increases to 126 km for 130 AH and 147.3 km for a 150 AH battery strength. For the second profile when the average speed is 20 kmph and max speed is 30 kmph, the vehicle runs for 104.4 km in a single charge of the battery when battery strength is 100 AH. It increases to 150.6 km for 130 AH and 160.8 km for a 150 AH battery strength. Complete Details is given in following table 1.





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CONCLUSION

Battery performance analysis is very much important. Here the analysis is performed with model of LIPO battery. It is found that if 130 AH battery is used with average speed of 25 kmph speed and max 40 kmph speed, the performance is satisfactory. As the cost of battery also a concern when battery is upgraded, 130 AH is the best performer in all these aspects.

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Table 1.Detail data of speed and battery drainage

Average Speed	Max Speed	Battery Strength	Vehicle movement in a single charge
25 kmph	40 kmph	100 AH	100 km
25 kmph	40 kmph	130 AH	126 km
25 kmph	40 kmph	150 AH	147.3 km
20 kmph	30 kmph	100 AH	104.4 km
20 kmph	30 kmph	130 AH	150.6 km
20 kmph	30 kmph	150 AH	160.8 km

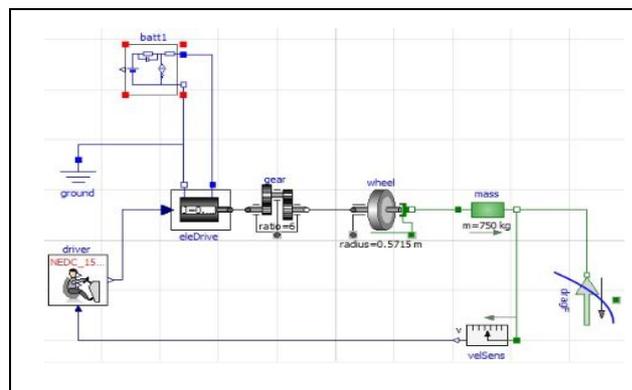


Fig. 1. Basic Model of the Electric Vehicle

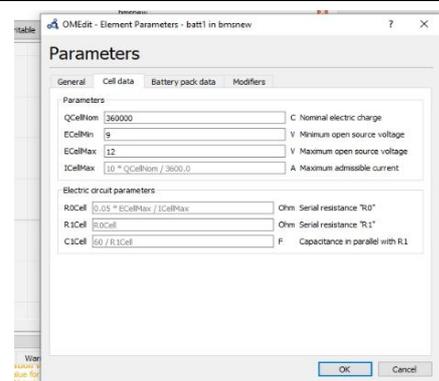


Fig. 2. Battery Cell data





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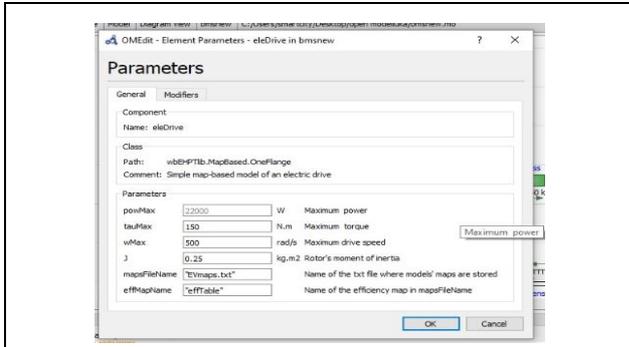


Fig.3.Electric drive parameters

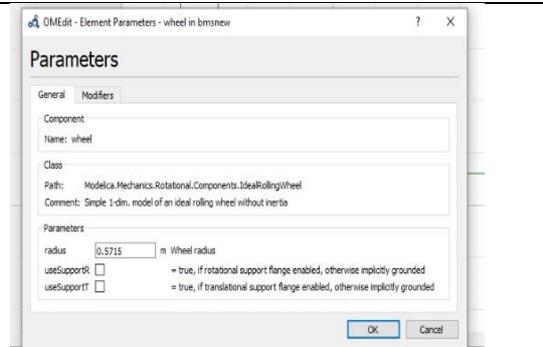


Fig.4.Wheel specification

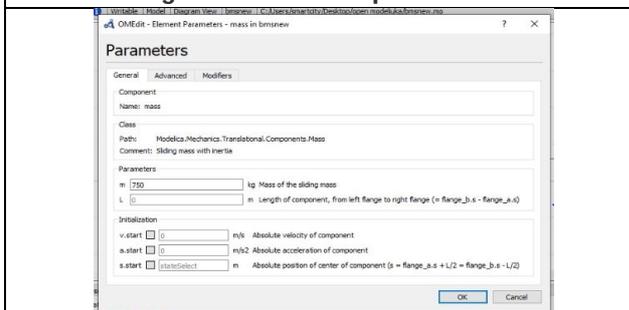


Fig.5.Mass Parameter

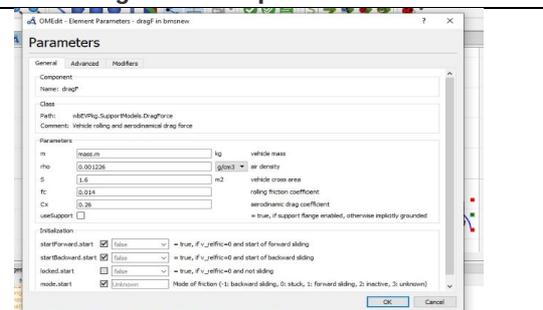


Fig.6.Drag Force parameter

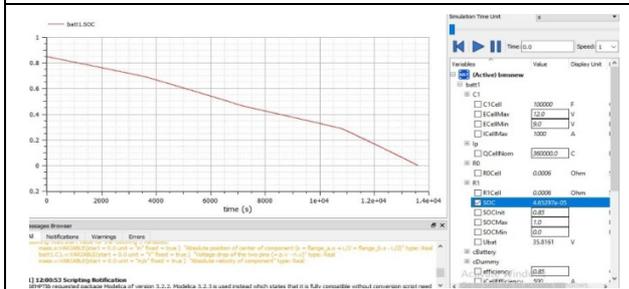


Fig.7.Discharge vs Time with 100 AH battery and 25 kmpl average speed

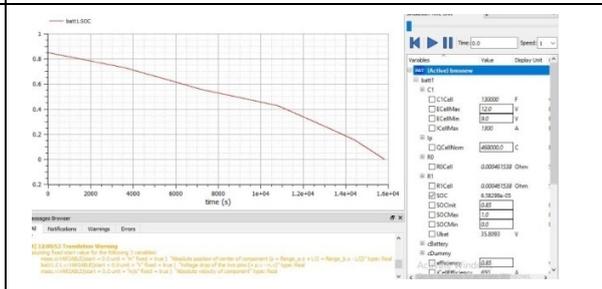


Fig. 8.Discharge vs Time with 130 AH battery and 25 kmpl average speed

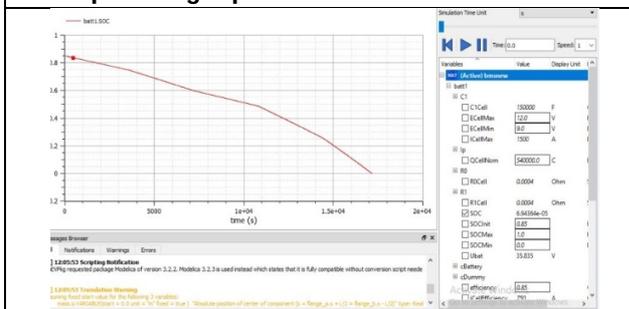


Fig. 9.Discharge vs Time with 150 AH battery and 25 kmpl average speed

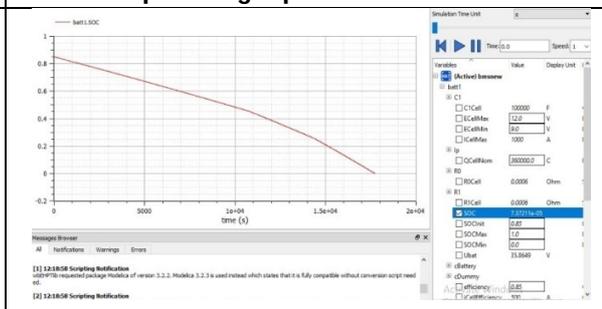


Fig. 10.Discharge vs Time with 100 AH battery and 20 kmpl average speed





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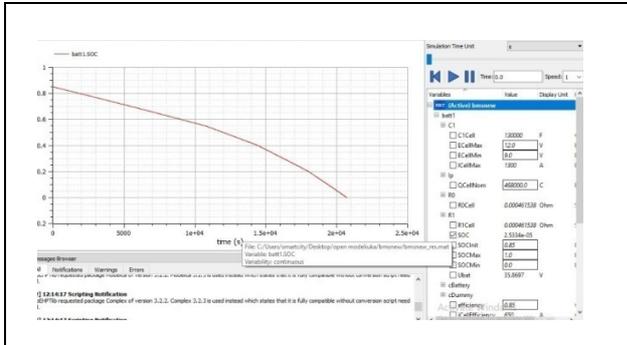


Fig. 11. Discharge vs Time with 130 AH battery and 20 kmpl average speed

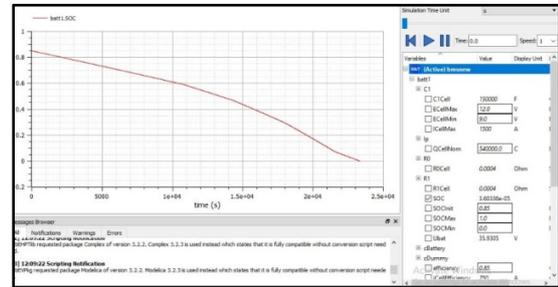


Fig. 12. Discharge vs Time with 150 AH battery and 20 kmpl average speed





Combinatorial Biometrics for Effective Authentication using Statistical Modelling Approach

Sanoop Kumar Parayil^{1*} and Srinivas Yarramalle²

¹Research Scholar, Department of Computer Sciences and Engineering, Centurion University of Technology and Management, Parlakhemundi, Odisha, India.

²Professor, Department of Computer Sciences & Engineering, GITAM Institute of Technology, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India

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*Address for Correspondence

Sanoop Kumar Parayil

Research Scholar, Department of Computer Sciences and Engineering,
Centurion University of Technology and Management,
Parlakhemundi, Odisha, India
Email:p.sanoopkumar@gmail.com



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ABSTRACT

Today most of the organizations are moving towards a secured environment to safeguard their data against vulnerabilities and in this process different applications are considered. As the developments in the area of cloud computing are emerging, in order to safeguard the data and to minimize the cost effectiveness, organizations are moving towards cloud computing domains. However, to ensure the confidentiality of the data and to curtail the vital information, in this article, we address a novel approach by integrating the traits of fingerprints and facial templates to safeguard the data from getting hacked by the unknown agencies or to have complete security about the data during transmission. A methodology is developed using Generalized Gaussian Mixture Model and the derived results are evaluated for accuracy using Precision, Recall and Accuracy, the results showcase an accuracy of around 94%.

Keywords: biometric, security, facial template, authentication, cloud

INTRODUCTION

In the present global scenario, many new technologies and new innovations have been emerged / emerging and these inventions led towards certain advantages to the mankind particularly in the fields of medical science, communication and space technologies. At the same time, these developments have led towards certain disadvantages like providing the scope for stealing the information either by third party hackers or from the unknown identical source residing within the organization. To safeguard this information, many new technologies were developed and utilized with the primary objective of securing the vital information [1–4]. Apart from the

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security concerns, many of the organizations are now emerging themselves into the cloud environment, with the aim of cost effectiveness and also to get benefited with the different services that are rendered by cloud; Infrastructure as a service, Software as a service and Platform as a service. With these migrations, security concerns have been increased, and concealing the information within the organization has become a challenging task.

Guilt agents, from within the organizations, has become a threat to the organizations, whose prime objective is to leak the crucial information during the data transmission [5–8]. Therefore, it has become a crucial task for the organization to safeguard their information not only from the hackers and attackers but also from the guilt agents. With this prime focus, methodologies were developed using MAC binding, IP binding to address the issue of identifying the guilt agents [9, 10]. However, while addressing the security issues in the cloud environment, it is very difficult to note where the data is being stored and who is going to access the data? What is the level of security that is imposed to safeguard the data and the authenticity of the next user who is going to utilize the same cloud facilities after a period of time during the case of public cloud? To address these issues, the present article, drives into this area by proposing the concepts of biometrics. In this regard, every legal user of the company who is going to access the data should leave his templates of face and fingerprints. These two templates are considered as the traits for validating the oneness of the individuals. These templates of each and every individual are acquired and are stored in the database. Each of these templates is normalized and is fixed to a uniform size of 100x100. Each of the faces is considered and only the frontal faces are taken into account. The traits of the fingerprint together with the faces are considered as the parameters for modelling. In order to present the proposed methodology, in this article, we have considered a statistical distribution namely, Generalized Gaussian Mixture Model (GGMM) [11–15]. Each of the parameters of both the traits is considered and is modelled through GGMM and the relevant Probability Density Functions (PDF) are extracted. These values are considered to be vital and they indirectly point to the employee accessing the data.

The rest of the article is articulated as follows. The finer details about each of these biometric traits are presented in detail in the corresponding Section 2 of the article. The Section 3 of the article deals with the Generalized Gaussian mixture model together with its significance of choice. The experimentation is carried out in Section 4 of article. The results derived are presented in Section 5. The final Section 6 of the article. The data is collected from the staff members of Gayatri Vidya Parishad College of Engineering (A) from a set of 500 staff members.

A Brief Insight about Fingerprint Recognition

In this section of the article, we present few interesting points about fingerprints to have a precise knowledge about the domains of interest. The fingerprint consists of two main features called micro features and macro features. Macro features are identified by the naked eye. Among the macro features, ridges and cores are mostly visible by naked eye. Apart from cores, many other features like valley, whorl, delta-delta points, shift delta points are also some of the import features which help in detailed identification of an individual. These features are called as micro features. The other micro features include arches and loops. During the fingerprint identification, the templates that are considered, called as minutiae are compared with that of the fingerprints of the other person regarding the identification.

Fingerprint misrepresentation is a crucial phase in fingerprint authentication system. Misalignment is caused by change in the requested fingerprint image, mainly during the authentication phase. However, during this misalignment, it often affects the authentication phase, accuracy of the match when comparing the query fingerprint with the model in the database during authentication [3]. Another challenge with respect to fingerprint is distortion, which is caused due to poor quality input. This may be due to conditions caused by skin ruptures, cuts or bruises.



**Sanoop Kumar Parayil and Srinivas Yarramalle****Facial Recognition**

Generally, in any facial recognition system, the distance between the eyes containing region of eyebrows, eyes, nose and mouth are considered to be the core information parts. As a result, the acquisition of these properties may be affected due to several variables such as glasses, facial hair, facial impressions, etc., this will lead towards improper acquisition. To overcome this during the facial recognition, each face is considered as a normalized face. Here only the frontal faces are considered. Each face after acquisition is normalized and pre-processed based on the histogram. After pre-processing, each of the faces is normalized to a fixed template size of 100x100.

Related Work

In real world, in order to identify a person and grant access to him to his data, devices or systems, we make use of different conventional strategies. These strategies accommodate the use of passwords, personal identification number, smart access cards, access tokens, etc. The use of these conventional strategies may also take a wrong turn if they fall in the wrong hands and make use of a person's identity in an iniquitous act. Such delusive acts can be avoided by the use of human organic properties rather than conventional strategies, in order to identify an individual.

Biometry is a term used to apply statistical analysis to biological data. The word biometric is evolved from a Greek language where bio refers to life and metrics refer to measure. Today, biometrics has evolved as a field of study for establishing individual identities with accurate technologies [6, 16]. The biometric authentication techniques are generally categorized into two classes namely physiological biometrics and behavioural biometrics [17]. In physiological biometric, the authentication is performed based on the characteristics of a person's body such as fingerprint, face, iris, hand geometry, palm print, DNA, etc. The kind of biometrics are based on the physical parameters of the body which cannot be altered without the individual being traumatic. In behavioural biometric technique, the authentication is performed based on how a person uses his body to perform different works. This can identify users based on their characteristics of their unique behaviour like the way they speak so as to consider the pitch and amplitude in their voice as parameters, the way in which they type keys on a keyboard which is normally referred to as 'Keystroke Dynamics', the way in which they perform their signature, etc., are the various forms of non-static biometric systems. There are three important behavioural biometrics which are used for authentication of an individual are voice, signature recognition and keystroke dynamics.

The Biometric System

Verification of an individual is a vital security concern. In the field of pattern recognition, a biometric system makes use of a person's physiological and behavioural features to identify an individual. A biometric system which uses either one physiological or behavioural feature to identify an individual is known as unimodal biometric system. In this system, the verification of the biometric feature is done by comparing the captured biometric trait with the features available in the database used for comparison. If the features match, the system returns 'true', granting access to the individual or else the access is rejected and returns 'false' [18].

There are four major elements in a biometric system. They are acquisition, feature extraction, matching component and decision module. The acquisition module interprets the biometric data from the individual by using different hardware components like camera to capture face and iris, microphone to capture voice, a fingerprint scanner to capture fingerprint, etc. In the feature extraction module, the biometric information captured through various sources are pre-processed to eliminate noise. After pre-processing, the biometric features are extracted using feature extraction procedure which is used to uniquely identify an individual. In the matching component module, these feature vectors are evaluated against the features stored in the database and gives out a score based on the resemblance of feature vector with the features available in the database. The score would be high if the resemblance is high or else, the score would be low. In the decision module, with the similarity score achieved from the previous step claims whether the identity is accepted/ rejected. In order to evaluate the similarity score, a threshold is chosen



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which is dependent on the kind of application [18]. Several researchers have studied and experimented on various individual biometrics traits such as face, fingerprint, iris, voice, etc., for identification and authentication of an individual. Each biometric trait studied by different researchers has applied different algorithms on each trait in order to perform feature extraction and identify its availability in the database. As we consider the face and fingerprint only for the current study, we present a short list of efforts from different researchers to perform facial recognition and fingerprint recognition using different algorithms.

Facial Recognition: R. Senthil Kumar *et al.*, [19]. proposed a feature extraction algorithm which is performed using Haralick feature extraction method. The image classification is done using a method called modified ANFIS classifier. This work is proposed to reduce the time taken for facial recognition and improve the recognition rate. With the use of these methods, the results are observed to be in between 90%-100% accurate for facial recognition and the recognition time is faster than the existing approaches. Atmakuri *et al.*, [20] proposed an authentication framework based on face biometrics. In-order to authenticate a user, the features are extracted from the user's image using geometric approach and matched with the templates stored in the database. This facial recognition gives faster response but has major problem to detect faces when they have variation in the expression of input image with the images in the database [21]. K. Raju *et al.*, [22] proposed a fusion based face recognition technique which uses global features and point based features for performing facial recognition. Global features include the use of Discrete Wavelet Transform (DWT) and Principal Component Analysis (PCA). Point based features include Harris-corner method and Speeded Up Robust Features. This hybrid approach is termed to give around 99% accuracy. Amrutha Bhat *et al.*, [23] proposed that the computers can recognize humans like humans do and used a method to compare the live captured image data with the content available in the database. The comparison is done using Laplacian of Gaussians (LoG) method, Local Binary Pattern (LBP) method and Principal Component Analysis (PCA). Edge detection is done using LoG methods. The LBP and PCA methods are used for feature extraction. Timo Ahonen *et al.*, [24] proposed a paper which performs efficient facial image representation based on texture features in Local Binary Pattern (LBP). The LBP features are extracted by dividing the face into multiple parts and concentrated to a feature vector which is used as a face descriptor. Azad Abdullah Amine *et al.*, [25] conducted an experiment on Cambridge ORL face database for extracting the facial features using DWT and performing feature reduction using LBP algorithm. In this experiment, firstly, the DWT is applied on LBP features and secondly the DWT is applied twice, once on the original image and the second on the LBP features. These two outcomes are compared and shows an improvement in the recognition rate of the image.

D. Sharadamani *et al.*, [26] in the research field of urban development proposed a unique face recognition system in real-time by combining the Local Gradient XOR Patterns (LGXORP) with the Local Orientation Gradient XOR Patterns (LOGXORP). In the proposed method, the feature vector can be constructed by fusing the LOGXORP and LGXORP. Hamayun A. Khan *et al.*, [27] used Yale and AT&T (ORL) benchmark face database to perform facial recognition. The feature extraction is done using Histogram of Oriented Gradients (HOG) and DWT algorithm. The PCA method is used for classification. The recognition rate observed for Yale and ORL databases by using this method are 97.72% and 99.78% respectively. Saloni Dwivedi *et al.*, [28] proposed a face recognition algorithm for real time. The Voila Jones method was used to perform the face detection. The facial recognition is done by using Euclidean face algorithm and Eigen face algorithm. The highest recognition rate when applied to several datasets of live images of four subjects are found to be 90%.

Fingerprint Recognition: Rupali S. Patil [29] proposed a method to extract the texture features of a fingerprint by using discrete cosine transform (DCT). The fractional coefficients of the DCT for multiple feature vector size is used for measuring the accuracy of the system. The experimentation uses 500 fingerprint samples of 100 persons. An accuracy of 91.75% is achieved with feature vectors of size 4x4 when compared with other sizes. Bikash Debnath *et al.*, [30] proposed a reversible circuit which performs fingerprint authentication. The Feynman Gate circuit which is useful for reversible computing is used along with quantum-dot cellular automation (QCA) for the proposed algorithm. A low power nanocircuit is achieved using QCA for the fingerprint authenticator. The proposed circuit is



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justified because of the match in the value of theoretical and simulation experiments. Prashant Bhaskarrao Patil *et al.*, [31] proposed a system to secure the fingerprints when they are used for the purpose of authentication in different authentication systems. In this system, a new virtual thumbprint is generated using distinct thumbprints. The minutiae features from both the fingerprints are extracted to generate a mixed minutiae template. This template is matched with the templates already in the database. The authenticator is successful if the match is found. Ihab AL Rassan *et al.*, [32] proposed a user authentication mechanism using the fingerprint recognition for mobile cloud computing. The fingerprint images of users are captured using mobile camera to access the cloud. The algorithm follows a sequence of steps to perform the match of original image such as converting to grey-scale, edge enhancement, filtering, binarization, thinning, map direction and minutiae extraction. This result is matched with the database for user acceptance in cloud. Sabari *et al.*, [33] proposed a methodology to secure the cloud by eliminating data security concerns using bio-hash function to secure the biometric template. This technique can also be used with other techniques to provide multifactor authentication technique.

All these above systems are also known as unimodal systems as they use a single trait to perform the authentication. But the use of unimodal systems does not ensure performance due to the limitations of unimodal biometric systems [34]. These limitations include variable environment conditions, spoof attacks, nonuniversality, etc., and a single biometric trait is not competent enough for authentication of an individual in all environments. The following list of requirements need to be satisfied to justify if a biometric trait is good one [35]. The requirements include universality, distinctiveness, persistence, collectability, performance, acceptability and circumvention. Universality aims at the possession of the biometric trait in every individual. Distinctiveness sights the ability of the trait to differentiate two people. Persistence points that the trait should be consistent for matching over time. Collectability ensures that the biometric trait should be easily obtainable. Performance focuses the ability of the biometric trait to work in limited resources and operating environments with good speed and high accuracy. Acceptability ensures the use of the biometric trait is accepted by wide range of people. Circumvention conveys that the spoofing of the trait is a difficult task to perform. Hence, we can conclude that there is no single biometric trait satisfies all the requirements in all operational environments [35]. Hence, the use of unimodal biometric do not guarantee performance as required by many real-world applications.

In order to deal with the problems faced by the use of single biometric identifier systems, the idea is to use more than one biometric trait for recognising an individual. This system uses multimodal biometric systems. This system can provide more resistance against spoof as it is difficult to manipulate multiple biometric traits of the same person at the same time. The disadvantages of multimodal biometric system it that they are expensive and requires more resources to store and compute both the biometric traits when compared with the unimodal biometric systems [35]. There are many researchers who also gave a detailed study and made use of the multimodal biometric so as to perform authentication of an individual. The following contents focuses on few of the methods used by different researchers in applying multimodal biometric system. The first two methodologies use two biometric traits and the third one uses three biometric traits for their approach.

Multimodal Biometrics: Manjunathswamy B.E. *et al.*, [36] proposed a multimodal biometric system which uses fingerprint and ECG biometric traits. Initially, using Biopac MP35, the ECG signals are captured from various subjects. The similarity scores of amplitude, angle and interval are extracted from ECG features. The fingerprint minutia features are estimated from the spikes of thinned binary pictures. Sara Zokae *et al.*, [37] proposed multimodal biometrics which uses palm print and ECG biometrics. In order to extract the features of ECG, Mel-Frequency Cepstral Coefficients is considered and PCA to extract the features of palm print. A recognition rate of 94.7% is achieved from the experiments done. Afshan Ashraf *et al.*, [38] described a scheme using biometric fusion of finger vein, fingerprint and retina recognition for a multimodal biometric verification. These three biometric traits are pre-processed and a score level fusion technique is performed. Higher security in this approach can be achieved by performing feature extraction and cryptography. Feature extraction is done using SIFT and minutia algorithms and are classified using Deep Neural Networks.





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Generalized Gaussian Mixture Model

In this article, in order to safeguard the information of the valid user, the templates of face and fingerprints are considered and are fused using the fusing techniques. This data is given to the generalized gaussian mixture model for effective generation of a unique parameter based on the probability distribution function given by:

$$\prod_{j=1}^D \frac{1}{A(\beta, \sigma) \Gamma\left(1 + \frac{1}{\beta}\right)} \exp\left\{-\frac{|x_{ij} - \mu_{ij}|^\beta}{A(\beta, \sigma)}\right\} \tag{1}$$

Where,

$$A(\beta) = \left[\frac{\Gamma\left(\frac{3}{\beta}\right)}{\Gamma\left(\frac{1}{\beta}\right)} \right]^{\frac{\beta}{2}} \tag{2}$$

$$K(\beta) = \frac{\left[\frac{\Gamma\left(\frac{3}{\beta}\right)}{\Gamma\left(\frac{1}{\beta}\right)} \right]^{\frac{1}{2}}}{\Gamma\left(\frac{1}{\beta}\right)} \tag{3}$$

The main advantage of choosing the Generalized Gaussian mixture model is that when $\beta = 1$, it turns to μ Laplace distributions or double exponential distributions. When $\beta = 2$, it behaves as a Gaussian distribution. K denotes the kurtosis, and μ denotes the mean. The kurtosis decides the shape and parameters basing on the shape, the kurtosis can be either a leptokurtic, platykurtic or mesokurtic and shape of the model is decided. Here, in the above equation, β represents the shape.

Metrics for Evaluation

During the evaluation stage, the tested image of the face and fingerprints are compared to each projected exercise images, the training images that are similar to a test images are then used to identify the training images. The evaluation models such as (i) the False Rejection Rate (FRR) and (ii) the False Acceptance Rate (FAR) are considered for evaluation. The formulas for the calculation of the above metrics are given by

$$FRR = \frac{G}{N} \tag{4}$$

where, G is the number of fingerprint traits rejected and N is the total number of genuine tested.

$$FAR = \frac{I}{N} \tag{5}$$

where, I is the number of fingerprints accepted and N is the total number of genuine tested. Evaluation is also further carried out using Accuracy, Precision and Recall. The Accuracy is defined as:

$$Accuracy = \frac{(Number\ of\ Correct\ Predictions)}{(Total\ Number\ of\ Predictions)} \tag{6}$$



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Precision is the fraction of retrieved images that are relevant.

$$\text{Precision} = P(\text{Relevant} | \text{Retrieved}) \quad (7)$$

Recall is the fraction for relevant images that are retrieved.

$$\text{Recall} = R(\text{Retrieved} | \text{Relevant}) \quad (8)$$

The categorization of relevant and non-relevant images is given in Table 1.

EXPERIMENTATION

Each of the input data of the face and the fingerprint that are fused is given to the above model. That is to Eq. (1) and the corresponding PDF is therefore obtained for a particular authenticated user. To test the authenticity, the process is repeated and if the match exists between the PDF of database and the obtained PDF, the data can be transferred or retrieved. In order to experiment the data, each of the fingerprint data and face data against the employees are considered and are stored in the database. The following Table 2 presents the fingerprints together with the facial data.

RESULTS

Each of the fingerprints and facial recognition of the employees are fused and the data is given to the model based on Generalized Gaussian Mixture Model for generation of unique probability density functions. The corresponding details are presented in Table 3. The results derived are evaluated using the Eqs. (4)–(8) and are presented in the following Table 4.

CONCLUSION

In this article, a method is proposed for authenticating the individual based on the biometric traits. The generated dataset is a real-time dataset which is obtained from Gayatri Vidya Parishad College of Engineering (A). This dataset contains 500 staff members along with their facial and fingerprint traits. In this article, a training dataset is generated with 500 samples and for the experimental purpose we have considered a sample set of 23 employees. Each of the input traits are pre-processed and normalized. These combined features of the biometrics are fused and the corresponding probability density function values are generated. Each of the generated values is stored against the database and during the retrieval process these values are compared to identify the most similar values. The retrieved data against these samples are presented in Table 4. From this table, it is evident that the input data matches with the training data and the output derived strengthens the argument.

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Table 1- Categorization of relevant and non-relevant entities

	Relevant	Non-Relevant
Relevant	TP(True-Positive)	FP(False-Positive)
Non-Relevant	FN(False-Negative)	TN(True-Negative)

Table 2- Showcasing the facial representation and the fingerprint against the employee ID

Employee ID	FACE	Biometric	Employee ID	FACE	Biometric	Employee ID	FACE	Biometric
EMP001			EMP009			EMP017		
EMP002			EMP010			EMP018		
EMP003			EMP011			EMP019		
EMP004			EMP012			EMP020		
EMP005			EMP013			EMP021		
EMP006			EMP014			EMP022		
EMP007			EMP015			EMP023		
EMP008			EMP016					





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Table 3- Showcasing the details of PDF values against each of the employees

Employee ID	Face	Fingerprint	Average Value of Fused Image	Employee ID	Face	Fingerprint	Average Value of Fused Image	Employee ID	Face	Fingerprint	Average Value of Fused Image
EMP001			6.32E-5	EMP009			1.15E-4	EMP017			9.13E-5
EMP002			1.06E-4	EMP010			9.35E-5	EMP018			8.42E-5
EMP003			9.02E-5	EMP011			9.6E-5	EMP019			1.14E-4
EMP004			7.98E-5	EMP012			1.01E-4	EMP020			1.42E-4
EMP005			7.8E-5	EMP013			5.53E-5	EMP021			8.89E-5
EMP006			1.48E-4	EMP014			7.24E-5	EMP022			1.07E-4
EMP007			8.35E-5	EMP015			6.93E-5	EMP023			6.55E-5
EMP008			9.78E-5	EMP016			1.02E-4				

TABLE 4- The derived results are presented here

Precision	Recall	Accuracy	FAR	FRR
0.79	0.92	94%	10%	1%





Record of Eco-types of Giant Honeybee, *Apis dorsata* Fabricius (Hymenoptera: Apidae) from Different Geo-locations of India

Bidisha Rajak and Sekarappa Basavarajappa*

Apidology Laboratory, DOS in Zoology, University of Mysore, Manasagangotri, Mysore, India.

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*Address for Correspondence

Sekarappa Basavarajappa

Apidology Laboratory, DOS in Zoology,

University of Mysore,

Manasagangotri, Mysore, India.

Email: ornithoraj11@gmail.com



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ABSTRACT

Native PAGE studies was conducted to characterize *Apis dorsata* worker honey bee isoenzyme pattern variation between samples collected from geographically distinct locations of south India. The alpha and beta esterase, acid and alkaline phosphatase banding pattern showed variation between urban, malnad, semi-arid and arid locations of south India. The Jacquard similarity coefficient for Alpha esterase showed maximum similarity 33.3% between the *A. dorsata* populations inhabited at semi-arid and malnad locations, beta esterase showed maximum similarity 33.3% between the *A. dorsata* population inhabited at malnad and arid locations, acid phosphatase showed maximum similarity 50% with the *A. dorsata* population inhabited between urban locations. The alkaline phosphatase showed maximum similarity 40% with the *A. dorsata* population inhabited between semi-arid and arid locations. Further, there was a considerable variation existed in isozymes for alpha esterase, beta esterase, acid phosphatase and alkaline phosphatase banding pattern that indicated the difference existed among the *A. dorsata* population living at different geo-locations of south India. All these results suggested that there is an intra specific variation existed in *A. dorsata* population surviving at different geo-locations and led to the development of *A. dorsata* eco-race or eco-types in the years to come.

Keywords: *Apis dorsata*, isoenzyme, worker honeybee, south India, Jacquard similarity coefficient.

INTRODUCTION

Researchers are paying more attention on *A. dorsata* population around the world since many years and trying to identify new species or sub-species or eco-races or eco-types of *A. dorsata* if any existed under diversified agro-climatic regions. As, *A. dorsata* is one of the most beneficial insects, produce economically important hive products, which become raw materials for > 200 different by-products [1]. Moreover, *A. dorsata* is potential pollinators; help

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propagate innumerable plant species amidst diversified natural ecosystems by increasing yield in various agricultural and horticultural crops [1]. To help elevate the beekeeping industrial activities and pollination potential, research on pollinators like *A. dorsata* is being carried out and more emphasis is given to explore the enzyme activity that hold a important place to understand eco-races. The enzymes are proteins, few acts as catalytic RNA molecules, commonly as ribozymes [2]. Isozymes are a group of different proteins with similar enzymatic activity and have slight variation in their amino acid composition due to their difference in DNA nucleotide sequence [2]. The only difference among isozymes is the substitution of one to several amino acids. The isozymes are electrophoretically separable, if only there is the larger difference in shape, size or net charge which represents enzymes from different genes which catalyze the same reaction. Iso-enzyme analysis therefore, provides a very conservative estimate for the extent of genetic variability within a population [3]. The iso-enzyme analysis was done by native Polyacrylamide Gel Electrophoresis (non-denaturing) (Native PAGE) that separate proteins; depend on the native charge and size of the proteins moves across the gels. The advantages of native PAGE lies in the separation of protein on native PAGE retain their biological activity, hence allowing the delicate protein to be separated and recovered in a biologically active form. The native PAGE provides useful information such as protein charge or subunit composition. It is generally used when one needs to analyze native, non-denatured proteins such as enzyme activity, receptor binding, antibody binding etc. In such type of analysis, it is necessary to use a non-denaturing system when studying a particular enzyme [4, 5, 6]. Detectable isozymes can arise from three different genetic and biochemical conditions, namely, multiple alleles at a single locus, single or multiple alleles at multiple loci and secondary isozymes usually arise from post translation processing. [7, 8] have reported the variation in morphometric traits of *A. dorsata* found at different geo-locations of southern Karnataka.

However, very few investigations were made on *A. dorsata* speciation and published reports on the extent of species variation in *A. dorsata* are very much demanded. In this regard, several researchers have studied the honeybee proteins and isozymes for diversity studies. [9, 10] have researched on the honeybee proteins for honeybee species diversity. [11,12] have used isozymes to reveal honeybee species diversity. However, the taxonomy and phylogeny of *Apis* species is based on behavioral, morphological but not considered the data derived from molecular techniques. However, gel electrophoresis of certain enzymes used in molecular techniques is most widely applied in insect systematic [13]. In this regard, several researchers have carried out isozyme studies in different honeybee species to reveal allozymes variation and differentiation among four *Apis* species in Sri Lanka [14]. Although, application of molecular genetic data for the study of honeybee population variation and phylogeny began in the late 1980's and early 1990's, only a few published reports are available to predict correlation between the honeybee species with isozyme studies. [15, 16] have conducted the morphometric studies at molecular level with the help of allozymes. Proteins and enzyme polymorphism in honeybees proved to be useful in developmental studies, population genetics and in taxonomic classification [17]. Further, iso-enzymatic work would help understanding the variation if existed among the natural population of *A. dorsata*. The iso-enzyme techniques are important methods to provide a precise tool to study the relationship among insect species. It help understand the phylogenetic relationship and the amount of differentiation existed among the species are in question. Keeping this in mind present study was undertaken by using esterase, acid and alkaline phosphatase isozymes to record the isozymes pattern variation in worker honeybee *A. dorsata* population found at different geo-locations, which experience different ecological conditions to revel eco-types if any at randomly selected few places in south India.

MATERIAL AND METHODS

Apis dorsata worker honeybees were caught alive from different regions of southern Karnataka, which is located amidst south India with the help of honey hunters and were brought to the Laboratory in ice box for analysis. Immediately after the collection, the thorax region of worker bee was homogenized using micro pestle in 30% sucrose solution. The homogenate was centrifuged at 4°C for 20 min at 10,000 rpm and the resultant supernatant was stored at -80°C till further analysis was conducted. The reagents and buffer solutions, Polyacrylamide Gel



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Preparation, buffers and stains preparation for isozymes for Alpha Esterase, Beta esterase, Acid Phosphatase, Alkaline Phosphatase was carried out as per [18]. In the native PAGE gel the lane 'A' represents the urban area – 1 (i.e., *A. dorsata* worker bee population from Manasagangotri Campus in Mysore, southern Karnataka), lane 'B' represents urban area – 2 (i.e., *A. dorsata* worker bee population from Devaraj Market in Mysore, southern Karnataka), lane 'C' represents the semi-arid region (i.e., *A. dorsata* worker bee population from T. Narsipura, southern Karnataka), lane 'D' represents the malnad region (i.e., *A. dorsata* worker bee population from Periyapatanna, southern Karnataka) and lane 'E' represents the arid region (i.e., *A. dorsata* worker bee population from Chamarajanagara District, southern Karnataka). Gel documentation and analysis: The gels were photographed using Alpha InfoTech gel Documentation Image System (USA). The qualitative analysis of isozyme profiles was carried out by calculating the retention factor (Rf) values of the bands based on their movements on the gel against the dye followed by estimation of similarity co-efficients as per [19].

$$Rf = \frac{\text{Distance travelled by the band}}{\text{Total length of the dye.}}$$

Statistical analysis

The data was subjected to multivariate analysis. The cluster analysis was conducted by using un-weighted pair group method for arithmetic averages (UPGMA) as per [20]. The UPGMA was calculated based on their pair wise similarities in the variables (Rf value) and resulting clusters were expressed in terms of Dendrogram.

RESULTS

The polymorphic profile expressed by alpha-esterase, beta-esterase, acid phosphatase and alkaline phosphatase isozymes in the body of worker honeybee, *A. dorsata* collected from different geo-locations of south India are depicted in Tables 1 to 8 and Figures 1 to 4. The polymorphic isoenzyme banding pattern expressed through PAGE for different isozymes are given in Plates 1 to 4. The banding pattern intensity for alpha esterase activity revealed the bands of varying intensities and based on their mobility they were named as Slow (S) and Fast (F) bands. The alpha esterase profile revealed two banding patterns for urban area – 1 (lane A), urban area - 2 (lane B), semi-arid location (lane C), malnad location (lane D) and arid location (lane E) and indicated bands with varying intensities. The fast band has a maximum frequency of 0.382, 0.398, 0.400, 0.414 and 0.441 respectively (Table 1). The slow band has minimum frequency of 0.334, 0.327, 0.328, 0.328 and 0.330 respectively (Plate 1). However, among the *A. dorsata* worker bees collected from semi-arid and malnad locations showed similar banding pattern with 0.328 Rf value (Table 1). Moreover, the Jacquard coefficient similarity showed maximum similarity of 33.3% between semi-arid and malnad locations while other regions revealed no Jacquard coefficient similarity (Table 2). Moreover the dendrogram construction from similarity matrix also showed that semi-arid and malnad are in same clades as they have a similarity percentage 33 however, all other places are lying in separate clades as there is no similarity in the banding patterns given in Figure 1.

Further, the beta-esterase isozyme in the body of worker honeybee, *A.dorsata* revealed two banding patterns for urban area -1 and 2, and semi-arid, malnad and arid geo-locations. The banding pattern intensity for beta esterase activity varying intensities and based on their mobility they were named as slow 'S' and fast 'F' bands (Plate 2). The fast band has maximum frequency of 0.462, 0.385, 0.353, 0.384 and 0.384 respectively, while the slow band has minimum frequency of 0.230, 0.215, 0.200, 0.153 and 0.188 respectively (Table 3.) The *A. dorsata* worker bee population found at malnad and arid geo-locations showed similar Rf values (0.384 each). In addition, the beta esterase activity showed maximum similarity 33.3% each between these two locations in southern Karnataka (Table 3), while other regions revealed no Jacquard coefficient similarity (Table 4). The dendrogram construction from similarity matrix is given in Figure 2. Furthermore, the acid phosphatase profile revealed three banding pattern for urban area – 1 (lane A), urban area – 2 (lane B), semi-arid location (lane C), malnad location (lane D) and arid location



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(lane E) (Plate 3). The banding pattern intensity of acid phosphates revealed varying intensities. Moreover, based on their mobility they were named slow as 'S' intermediate 'IM' and fast 'F'. The fast band for urban area -1 (lane A), urban area - 2 (lane B), semi-arid (lane C), malnad (lane D) and arid location (lane E) showed a varying intensities with maximum frequency of 0.446, 0.430, 0.430, 0.415 and 0.492 respectively (Table 5). Similarly, the intermediate band for urban area -1 (lane A), urban area - 2 (lane B), semi-arid (lane C), malnad (lane D) and arid geo-locations (lane E) showed a varying intensities with maximum frequency of 0.230, 0.230, 0.184, 0.261 and 0.276 respectively (Table 5). However, the slow banding pattern for urban area -1 (lane A), urban area - 2 (lane B), semi-arid (lane C), malnad (lane D) and arid geo-locations (lane E) showed similar Rf values 0.153 (Table 5). Moreover, the Jacquard coefficient similarity showed maximum similarity (50% each) between urban areas 1 & 2 and same is showed in the dendrogram which construction from similarity matrix (Fig. 3), while for other locations there was no Jacquard coefficient similarity recorded (Table 6).

The alkaline phosphatase profile revealed three bands pattern for urban area - 1 (lane A), urban area - 2 (lane B), semi-arid (lane C), malnad (lane D) and arid location (lane E). The banding pattern intensity of alkaline phosphates clearly revealed unique bands of varying intensities and based on their mobility, they were named as slow 'S' intermediate 'IM' and fast 'F'. The band with maximum frequency was 0.466, 0.426, 0.453, 0.440 and 0.413 respectively for *A. dorsata* worker bee sample from urban location - 1 (lane A), urban location 2 (lane B), semi-arid (lane C), malnad (lane D) and arid location (lane E) (Table 7). The intermediate (IM1) band has indicated Rf values 0.266, 0.333, 0.253, 0.240 and 0.266 respectively for *A. dorsata* worker honeybee sample urban location - 1 (lane A), urban location 2 (lane B), semi-arid (lane C), malnad (lane D) and arid geo-locations (lane E) (Table 7). Similarly, for slow band, the minimum frequency was 0.133 among the worker bee collected from different geo-locations (Table 7). For intermediate (IM1) band the Rf value was 0.266 and the banding pattern in *A. dorsata* worker honeybee population found in urban 1 and arid location was almost similar (Table 7). Moreover, the Jacquard coefficient similarity showed maximum similarity 20% between semi-arid and arid geo-locations, showed in the dendrogram construction from similarity matrix (Fig. 4 and Table 8).

DISCUSSION

Among the biochemical markers, isozymes became popular tools for the assessment of genetic diversity, taxonomic relationship and construction of genetic maps [21, 22]. Isozymes acts as a co-dominance marker help distinguish the heterozygous and homozygous conditions in insect population. Several researchers have studied the isozymes to identify and distinguish sub-species or eco-races in *A. mellifera* and *A. cerana* population found at different parts of the world. The banding pattern variation for alpha esterase, beta esterase, acid phosphatase and alkaline phosphatase in the worker bee *A. dorsata* population found at different geo-locations in southern Karnataka revealed surprising results, which could indicate the existence of variation in *A. dorsata* population. Similar types of observation were made in different *Apis* species by [23, 24, 25]. [23] have differentiated *A. dorsata* and *A. florea* population based on isozymes banding patterns. Both *A. dorsata* and *A. florea* population had four distinct bands at pH 5.6 but, there was a difference in the light band.[24] have reported the presence of six bands while [25] have showed the presence of two major bands for esterase in *A. dorsata*. However, during the present investigation, two bands pattern was recorded for alpha and beta esterase enzymes and three bands pattern for acid and alkaline phosphatase enzymes which is on par with the observation of [25]. Esterase enzymes are a group of hydrolytic enzymes, occurring in multiple forms with blood substrate specificity. They perform different physiological functions including defense against foreign substances in the blood [26]. Esterase occurs in numerous forms at distinct genetic loci. The co-dominance inheritance and high degree of variability in banding pattern of esterase enzyme has made reliable marker for studying genetic variability within and between insect populations [27]. Thus, esterase enzymes act extensively on various kinds of substrates and show high polymorphism and variation [28]. During the present investigation, alpha and beta esterase enzymes indicated polymorphism that showed differential expression of these isozymes for urban area – 1 and 2, arid, semi-arid and malnad locations. For alpha esterase, the



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isozyme profile revealed that the urban locations – 1 and 2, semi-arid, arid and malnad locations have indicated two bands pattern while, acid and alkaline phosphatase showed three bands pattern for different geo-locations amidst south India. The beta esterase activity showed different retention factor (Rf) values both for slow and fast bands and indicated considerable variation existed between the *A. dorsata* worker bee population found at different geo-locations in south India. Further, Rf value for 'A' band was almost same (0.384 each) for the *A. dorsata* worker honeybee population found at malnad and arid geo-locations. However for other locations, the statistical analysis revealed significant pattern difference between the worker bee *A. dorsata* populations. The beta esterase activity indicated maximum similarity 33.3% each between malnad and arid locations, while it was zero for other geo-locations and implied that there was a variation in isozyme bands pattern. Further, alpha esterase also revealed two bands pattern with varying intensities both fast and slow bands for worker bee, *A. dorsata* population found at different geo-locations. However, *A. dorsata* worker bee population found at semi-arid and malnad locations showed similar Rf values (0.328 each) for slow bands pattern compared to other locations. Moreover, statistical analysis revealed significant difference in bands pattern between the worker bee *A. dorsata* populations found at different geo-locations in south India. Further, the alpha esterase activity was maximum and Jacquard similarity coefficient was 33.3% between semi-arid and malnad locations. The bands pattern intensity for acid phosphatase showed three bands pattern with varying intensities. Acid phosphatase revealed slow, intermediate and fast bands patterns with varying intensities that varied considerably between the worker bee *A. dorsata* populations found at different geo-locations in south India. Further, there was a considerable variation recorded between slow, intermediate and fast bands patterns for worker bee population found at different geographical locations in south India. Further, beta esterase showed maximum similarity 50% between the *A. dorsata* worker bee populations found at urban areas 1 and 2. All these finding revealed that there is a difference in banding pattern for alpha esterase, beta esterase, acid and alkaline phosphatase. Further, for alkaline phosphatase isozymes for *A. dorsata* worker bee population in semi-arid and arid geo-locations is linked with 20% similarity. Similar types of observations were made by [23, 24 25, 26,27].

However, such types of reports observed during the present investigation on *A. dorsata* are first of its kind in this part of India. Further, the variation in isozyme profile might have influenced by various ecological, edaphic and floristic factors amidst diversified agro-climatic habitats. Because, different geo-locations viz., urban, malnad, semi-arid and arid locations in southern Karnataka amidst south India are experiencing varied weather conditions accompanied by drastic seasonal changes along with diversified flora [29]. This type of observations were widely been noted by [30, 31, 32, 33]. Organisms are also typically exposed to multiple types of environmental variation simultaneously (the 'fundamental niche' of Hutchinson, 1957), such as variations in temperature, nutrient availability, predation and crowding and many others traits exhibit plasticity in response to different types of environmental stimuli [34, 35, 36]. Perhaps, varied environmental conditions might have instinct *A. dorsata* population to show variation in enzymes pattern. Thus, the changes in biochemical characters including isozyme patterns led to cellular differentiation, morphological development and functional specialization of organisms. Enzymes with the similar catalytic activity are made up of numerous subunits. Isozymes are the products of genes through transcription and translation process. Being gene products, isozymes show band intensity that is proportional to the encoding gene [37] and led to a process of formation of new modifications in insect population. Moreover, the insect body size is also closely related to climatic change [38, 39, 40]. Such type of variations might have brought intra-specific variability in *A. dorsata* population living at different geo-locations in south India. [7,8] have recorded the considerable variation in the morphological traits of *A. dorsata* found at different geo-locations of southern Karnataka amidst south India. Perhaps, these factors might have brought change in isozyme activities and led to polymorphism in the worker honeybee, *A. dorsata* population living at different geo-locations in south India. The distance between the isozyme pattern similarities was very less which clearly indicated that there is a variation in the isozyme profile among the worker bee *A.dorsata* population. Cluster tree analysis was carried out by using UPGMA based on genetic distance. For acid phosphatase isozymes, urban locations 1 and 2 are lying in the same cluster and from cluster 2 i.e., urban locations 2 are related to *A. dorsata* worker bee population found at semi-arid population, whereas for *A. dorsata* worker bee population found at malnad and arid locations lying in different cluster. Similarly, for beta esterase, the *A. dorsata* worker bee population found at malnad and semi-arid geo-



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locations indicated 33.3% similarity and were lying in similar clade while all other geo-locations are lying in separate cluster. Similarly, for alpha esterase, the *A. dorsata* worker bee population found at malnad and arid locations indicated 33.3% similarity and were lying in similar clade, while *A. dorsata* population found at other geo-locations are lying in separate cluster. Thus, there is a variation in *A. dorsata* population found at different geo-locations and it may lead to new eco-type development in the years to come. On this line, few more in depth investigations are required and results of such investigations are published elsewhere.

CONCLUSION

The existence of isozymes bands pattern variation between the *A. dorsata* worker bee populations inhabited at different geo-locations of southern Karnataka amidst south India indicated the intra-specific differentiation. Assessment of polymorphisms through isozymes analysis provided a powerful and less expensive tool for evaluating the variability among honeybee population. The changes in biochemical characters including isozyme patterns led to cellular differentiation, morphological development and functional specialization of honeybees. Enzymes with the similar catalytic activity are made up of numerous subunits. Isozymes are the products of genes through transcription and translation process. Being gene products, isozymes show band intensity that is proportional to the encoding gene. The isozymes bands pattern revealed considerable variation among *A. dorsata* worker bee population inhabited at different geo-locations. Alpha esterase showed maximum similarity 33.3% between the *A. dorsata* population inhabited at semi-arid and malnad locations, while there was no similarity existed in the *A. dorsata* population inhabited at other locations. Beta esterase showed maximum similarity 33.3% between the *A. dorsata* population inhabited at malnad and arid locations, while there was no similarity existed in the *A. dorsata* population inhabited at other locations. The acid phosphatase showed maximum similarity 50% with the *A. dorsata* population inhabited between urban location 1 and 2. The alkaline phosphatase showed maximum similarity 40% with the *A. dorsata* population inhabited between semi-arid and arid locations. All these findings revealed that there is a considerable variation in isozymes for alpha esterase, beta esterase, acid phosphatase and alkaline phosphatase bands pattern that indicated the variation existed among the *A. dorsata* population living at different geo-locations of southern Karnataka amidst south India. Along with morphological traits variation even isozyme study revealed pattern variation and there may be intra specific variation in *A. dorsata* population that could lead to development of new eco-type or eco-race in the years to come. However, it is too early to say about sub species, or eco-race or ecotype in *A. dorsata* population in this part of the state. It requires further in depth studies to designate sub-species or eco-type of *A. dorsata* in this part of the State.

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Table 1. Electrophoretic variation of alpha esterase isozymes banding pattern in *A. dorsata* worker bee found at different geo-locations of south India

Geo-location	Banding pattern with Rf Value									No. of Bands recorded
	0.327	0.328	0.330	0.334	0.382	0.398	0.400	0.411	0.414	
Urban location -1 (Manasagangotri Campus, Mysore)	-	-	-	+	-	-	-	+	-	2
Urban location - 2 (Devaraj Market)	+	-	-	-	+	-	-	-	-	2
Semi –arid (T. Narasipura)	-	+	-	-	-	+	-	-	-	2
Malnad (Periyapatnna)	-	+	-	-	-	-	-	-	+	2
Arid (Chamrajnagar)	-	-	+	-	-	-	+	-	-	2

Note: Rf: Retention factor. +: Present; -: Absent





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Table 2. Jacquard’s similarity coefficient percentage from alpha esterase in *A. dorsata* worker bees found at different geo-locations of south India

Geo-location	Location				
	1	2	3	4	5
Urban location -1 (Manasagangotri Campus, Mysore)	100	0	0	0	0
Urban location - 2 (Devaraj Market)		100	0	0	0
Semi-arid (T. Narasipura)			100	33.3	0
Malnad (Periyapatnna)				100	0
Arid (Chamrajnagar)					100

A: Urban area-1; B: Urban area-2; C: Semi-arid region; D: Malnad region and E: Arid region

Table 3. Electrophoretic variation in the beta esterase banding pattern in *A.dorsata* worker bee found at different geo-locations of south India

Geo-location	Banding pattern with Rf Value									No. of Bands recorded
	0.153	0.188	0.200	0.215	0.230	0.353	0.384	0.385	0.462	
Urban location -1 (Manasagangotri Campus, Mysore)	-	-	-	-	+	-	-	-	+	2
Urban location - 2 (Devaraj Market)	-	-	-	+	-	-	-	+	-	2
Semi -arid (T. Narasipura)	-	-	+	-	-	+	-	-	-	2
Malnad (Periyapatnna)	+	-	-	-	-	-	+	-	-	2
Arid (Chamrajnagar)	-	+	-	-	-	-	+	-	-	2

Note: Rf: Retention factor. +: Present; -: Absent

Table 4. Jacquard’s similarity coefficient percentage from beta esterase isozyme in *A. dorsata* worker bee found at different geo-location of south India

Geo-location	Location				
	1	2	3	4	5
Urban location -1 (Manasagangotri Campus, Mysore)	100	0	0	0	0
Urban location - 2 (Devaraj Market)		100	0	0	0
Semi -arid (T. Narasipura)			100	0	0
Malnad (Periyapatnna)				100	33.3
Arid (Chamrajnagar)					100

A: Urban area-1; B: Urban area-2; C: Semi-arid region; D: Malnad region and E: Arid region





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Table 5. Electrophoretic variation in the acid phosphatase banding pattern in *A. dorsata* worker bee found at different geo-locations of south India

Geo-location	Banding pattern with Rf Value									No. of Bands recorded
	0.153	0.184	0.230	0.261	0.276	0.415	0.430	0.446	0.492	
Urban location -1 (Manasagangotri Campus, Mysore)	+	-	+	-	-	-	-	+	-	3
Urban location -2 (Devaraj Market)	+	-	+	-	-	-	+	-	-	3
Semi –arid (T. Narasipura)	+	+	-	-	-	-	+	-	-	3
Malnad (Periyapatnna)	+	-	-	+	-	+	-	-	-	3
Arid (Chamrajnagar)	+	-	-	-	+	-	-	-	+	3

Note: Rf: Retention factor. +: Present; -: Absent

Table 6. Jacquard’s similarity coefficient percentage from acid phosphatase in *A.dorsata* workerbee found at different geo-location of south India

Geo-location	Location				
	1	2	3	4	5
Urban location -1 (Manasagangotri Campus, Mysore)	100	50	20	20	20
Urban location – 2 (Devaraj Market)		100	0	0	0
Semi –arid (T. Narasipura)			100	0	0
Malnad (Periyapatnna)				100	0
Arid (Chamrajnagar)					100

A: Urban area-1; B: Urban area-2; C: Semi-arid region; D: Malnad region and E: Arid region

Table 7. Electrophoretic variation in the alkaline phosphatase banding pattern in *A. dorsata* worker bee from different geo-location of south India

Geo-location	Banding pattern with Rf Value										No. of Bands recorded
	0.133	0.240	0.253	0.266	0.333	0.413	0.426	0.440	0.453	0.466	
Urban location -1 (Manasagangotri Campus, Mysore)	+	-	-	+	-	-	-	-	-	+	3
Urban location -2 (Devaraj Market)	+	-	-	-	+	-	+	-	-	-	3
Semi –arid (T. Narasipura)	+	-	+	-	-	-	-	-	+	-	3
Malnad (Periyapatnna)	+	+	-	-	-	-	-	+	-	-	3
Arid (Chamrajnagar)	+	-	-	+	-	+	-	-	+	-	3

Note: Rf: Retention factor. +: Present; -: Absent

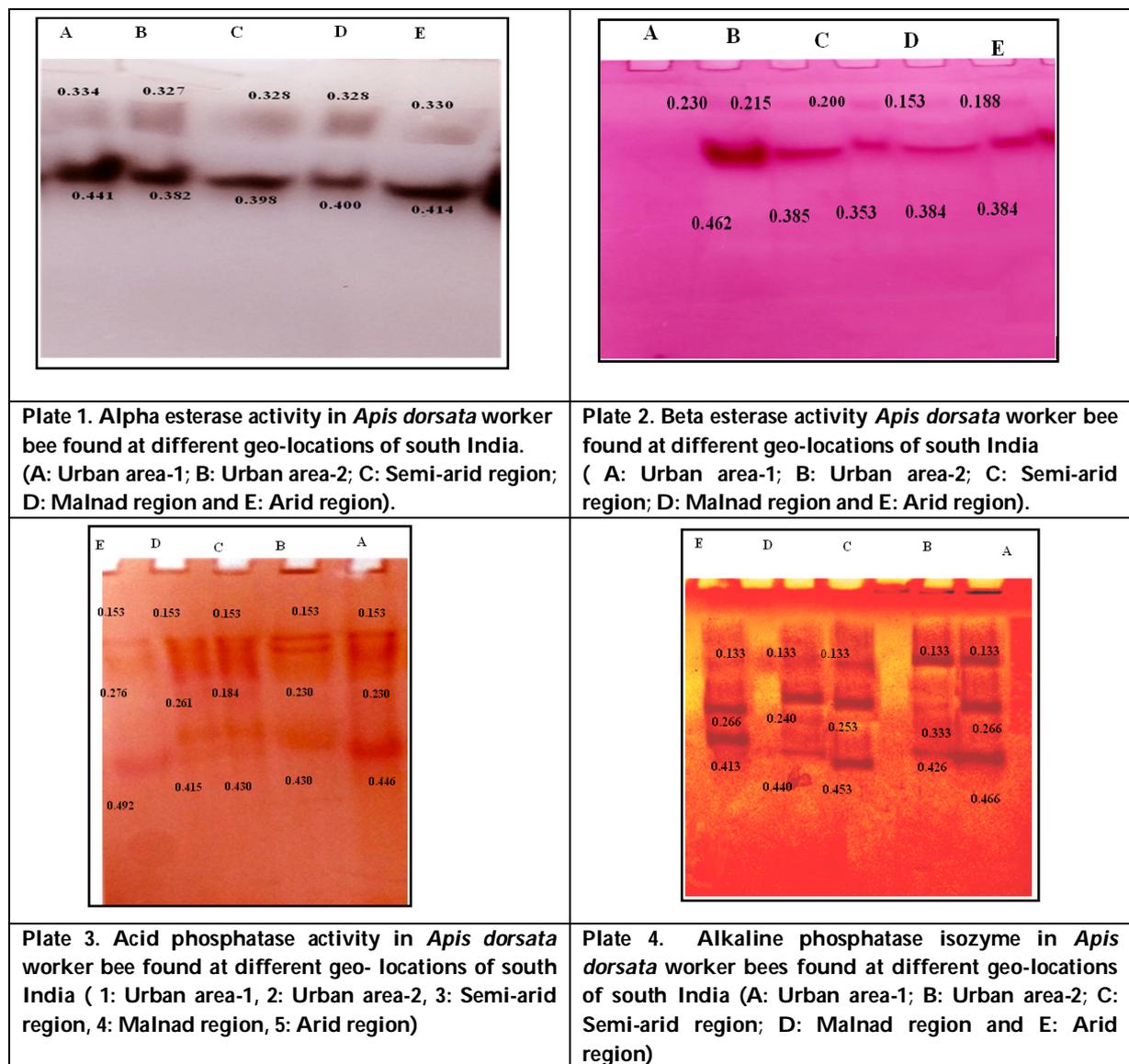




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Table 8. Jacquard’s similarity coefficient percentage from alkaline phosphatase in *A. dorsata* worker bees at different geo-locations of south India

Location	Location				
	1	2	3	4	5
Urban location -1 (Manasagangotri Campus, Mysore)	100	20	20	20	40
Urban location – 2 (Devaraj Market)		100	20	20	16.7
Semi –arid (T. Narasipura)			100	20	40
Malnad (Periyapatna)				100	16.7
Arid (Chamrajnagar)					100





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<p>Figure 1. Dendrogram constructed from similarity matrix presented in Table 2 using SPSS (Method=Ward, Plot=Dendrogram) 1: Urban area-1; 2: Urban area-2; 3: Semi-arid region; 4: Malnad region and 5: Arid region</p>	<p>Figure 2. Dendrogram constructed from similarity matrix presented in Table 4 using SPSS (Method=Ward, Plot=Dendrogram) 1: Urban area-1; 2: Urban area-2; 3: Semi-arid region; 4: Malnad region and 5: Arid region</p>
<p>Figure 3. Dendrogram constructed from similarity matrix presented in Table 6 using SPSS (Method=Ward, Plot=Dendrogram) 1: Urban area-1; 2: Urban area-2; 3: Semi-arid region; 4: Malnad region and 5: Arid region</p>	<p>Figure 4. Dendrogram constructed from similarity matrix presented in Table 8 using SPSS (Method=Ward, Plot=Dendrogram) 1: Urban area-1; 2: Urban area-2; 3: Semi-arid region; 4: Malnad region and 5: Arid region</p>





***Parthenium hysterophorus* a Threat or Beneficial Weed and Management: A Review**

Indranil Singh¹ and Shuchi Kaushik^{2*}

¹Independent Researcher

²State Forensic Sciences Laboratory, Madhya Pradesh, India.

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***Address for Correspondence**

Shuchi Kaushik

State Forensic Sciences Laboratory,

Madhya Pradesh, India.

Email: shuchi.kaushik2@gmail.com



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ABSTRACT

Parthenium hysterophorus known for its proliferative capacity has run wild through many countries over the globe. It has significant impact in America, India, Australia and Africa. It has potential to cause a huge economical loss to country in the form of crop damage, influencing health of humans as well as of livestock. Today it is considered as one of the seven most noxious weed in the world. It is an annual herb that has been native to northern Mexico as well as U.S.A. It has been known for producing thousands of rosettes responsible for toxicity and its rapid spreading over a region. There has been huge loss in crop yield due to either direct competition or due to allelopathy. There are various methods to tackle its uncontrolled growth such as burning, spraying herbicides, biological control in the form of introducing various insects like *Zygogramma bicolorata*, *Epiblema strenuana* etc. In last few decades lot of beneficial prospects have been developed with the involvement of *Parthenium hysterophorus*. It has been used in traditional medicine to treat conditions like inflammation, pain, fever, and diseases like malaria, dysentery etc. It can be used to produce biogas, enzymes and could also take upon heavy metal pollution and acts as bioremediation agent. It can also be used in dye removal and also be used in removal of various menace causing aquatic weeds. It has been proved to be useful in order to maintain moisture of field and could potentially be used as organic manure. It is proved to be better than NPK synthetic fertilizers, provided optimum conditions have been met. The aim of this review article is to explore various dimensions of *Parthenium* existence and how its uncontrolled growth could be tackled.

Keywords: Allelopathy, biological control, traditional medicines, bioremediation, organic manure



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INTRODUCTION

Parthenium hysterophorus also known as carrot grass, white top, congress grass or feverfew belongs to family Asteraceae. At some places it is also known as Santa-Maria or Peterson's curse, 'altamisa' and "Scourge of India". It can survive throughout the year and these herbaceous weeds are obnoxious in nature. There are hardly any efficient benefits of these ephemeral herbs which have been entangled till now. They are prolific weeds known for their vivacious development along with their high fertility rate particularly in hotter atmospheres. These are endemic to America while being local to north-east Mexico. Its expansion has been related to its capability of forming numerous small capitula each with potential to form at least around 5 seeds at maturity. It produces approximately 10,000 to 14,000 seeds in one lifespan which could be dispersed through various mechanisms. Human has been struggling with its rapid spread which has the potential of completely changing the habitat [1]. *Parthenium hysterophorus* can tolerate the extreme of conditions like those in drought scenario or soil condition varying from sandy to wet soils and can complete three generations in a year of time provided it has the favorable growth conditions [2]. It can germinate in wide range of temperature ranging from 10°C to 25°C [3] and requires around 500mm of rainfall in summer season [4]. Last few decades have seen *Parthenium hysterophorus* been spreading throughout the world from India, Africa, and pacific island and especially to Australia [5]. While in India, its emergence has been restricted to about 2000m above the sea level, but cases have been identified where it has been seen to move upward due to human interference and changing climate [6-7]. It has been reported that *Parthenium* possess non-Kranz anatomy with C₃ at the top of leaves and C₄ at the bottom of leaves which follows the Kranz leaf anatomy [8]. Today it is been counted as one of the annihilating and hazardous weeds. It could be found in wide ranges of scenario such as railway tracks, abandoned land, roads, gardens, around buildings etc. which have been favorable for the growth of this weed owing to fact that there is lack of interspecies competition in these areas [9].

Introduction of *Parthenium hysterophorus* in Pune, India was totally a matter of accident, as it came along with PL 480 wheat as a contaminant which was imported from USA in year 1950s. It was carried under Public law 480, which was passed in order to help the country dealing with malnutrition along with insufficient wheat production. Since then, it has spread throughout the country attracting a lot of attention as its growth leads to havoc and destruction of normal flora. It has been found to reduce around 40% of yield in a planted land while itself been able to survive in nutrient deficient conditions [2,10,11]. Further harmful effects on crops growth and yield where *Parthenium hysterophorus* has been growing simultaneously with the crop has been studied by various authors [5,12-13, 96]. It has been estimated that roughly 2 million hectares of land has been under the hazardous influence of *Parthenium hysterophorus*[14]. Another possible mechanism through which *Parthenium hysterophorus* infects and causes shift in natural habitat is either its invasive capacity or its allelopathic effects on plant. It achieves allelopathy through group of allelochemicals. It is the result of parthenin, a sesquiterpene lactone or because of phenolics [15-17]. It acts by undergoing mechanisms like leaching, decaying of fallen parts either by biotic or abiotic means or by going through root exudation [18]. *Parthenium hysterophorus* has many negative impacts on human health when exposed for longer duration. In a study it has been found that it could result into Asthma, respiratory tract infections like bronchitis, along with hay fever, skin lesion and dermatitis [19]. It has potential to greatly affect milk production and meat of animal [20]. The antimicrobial activity of plant is owned to the amount and quantity of its Phyto-constituents. The anti-microbial study of leaf of congress grass has shown that its methanol and acetone extract has potential to act against microorganisms [21].

Growth and Reproduction of *Parthenium hysterophorus*

Parthenium is known for its colonization in wide range of conditions from heavy clays to sandy soil and however it has been known for its better growth in clay soil. It is a highly competitive plant known for its colonizing strength. It can colonize and adapt to wide number of situations from overgrazed pasture to cultivated land, roadside,



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stockyards etc. It could use varieties of means in its spread from one place to another like animals, water, humans, movement of farm products etc. *Parthenium* growth, reproduction and photosynthesis abilities have been highly suppressed under low temperature in winters than in summer. This is due to constrained emergence of seed along with seed to flower ratio and restricted vegetative growth. It was found that below 7°C, rate of photosynthesis highly reduces. Further consideration of CO₂ has resulted into measurable increase in the rate of photosynthesis as well as efficiency in water utilization [6].

Parthenium and its Allelopathic Potential

Allelopathy is considered to be a reason behind reduction in crop along with pasture yield, change in habitat, human health problems and being toxic to nature, livestock etc. These allelochemicals are also the reason behind its ability to dominate over other species and establishment in a new habitat. It has been proven fact that aerial parts of *Parthenium* and weed roots do possess certain chemicals that when extracted with either water or alcohol could lead to reduction in growth and influences its germination. Belgeri and Adkins, 2015 in their work found that response to *Parthenium* seedling was more species specific. It has greater impact over reduction in growth of root as compared to that of shoot. They also found that introduced grasses like Rhodes grass, buffel grass and siratro were least affected with 0%, 8%, and 9% inhibition respectively, as compared to the native grasses like curly windmill grass and cotton panic grass that showed around 54% to 59% of inhibition [22]. Further elucidation has been done to identify its chemical constituents and properties. Sesquiterpene lactone is considered as toxin and has been found to be present in almost all parts of *Parthenium hysterophorus* including pollen grains and trichome. Deeper investigation has resulted into discovery of glycoside parthenin which is bitter and a major sesquiterpene lactone [23]. Along with this, there are other chemicals like sitosterol, caffeic acid, 3,7-dimethylether, quercelagetin, ferulic acid, vanillic acid, p-courmaric, P-hydroxy benzoin and vanillic acid, 6-hydroxyl kaempferol 3-0-arabinoglucoside and some other unidentified alcohols, which are the reasons behind the proliferating nature of *Parthenium* [24]. *Parthenium hysterophorus* isolated from varying geographical positions has revealed different major constituents like dihydroisoparthenin, hysterothrin, hymein, tetraeurin and coronopilin [25]. Another group of workers have isolated a novel compound i.e. allergen named hydroxyproline-rich glycoprotein [27]. Das et al. (2007) in their work mentioned four acetylated pseudoguanolides [26] Venkataiah et al. (2003) in their work reported the discovery of seco-pseudoguanolide named charminarone, a novel sesquiterpenoid [28].

Hazardous Impact of Parthenium Weed

Parthenium a noxious weed is proved to be a significant danger not only for agriculture but has been a causal reason of various diseases affecting human and livestock. It is considered to be invasive alien species in Asia, Africa and Australia as it has been introduced accidentally and lately these plants have developed and established their self-reproducing states [29-30].

Parthenium and Agriculture Productivity

Over the period of time *Parthenium* has emerged as the worrisome issue for farmers. In depth examination of *Parthenium* and its parts in various organic solvents has led us to conclusion that it has potential of severely affecting various parameters of growth. Parameters involving but not limited to it are rate of germination, amount of shoot and root growth, amount of yield and amount of seedling production [31]. *Parthenium hysterophorus* leaf extract in aqueous medium has been found to potentially affecting three major cereal crops grown in and around India i.e. *Oryza sativa*, *Zea mays* and *Triticum aestivum*. In the same work, three other crucifer vegetables along with two from Asteraceae family were also found to be highly affected by *Parthenium hysterophorus* [32]. Khan et al. (2012) has further studied impact of *Parthenium hysterophorus* on *Triticum aestivum* and reported that it is most susceptible to *Parthenium* noxious effect. Another work on *Zea mays* has suggested that inhibition is done by *Parthenium hysterophorus* by inhibiting either seed germination or seedling vigor [34-35]. Fruit development as in case of plants like brinjal, tomato and chili etc. has been impacted by allelopathy of *Parthenium* [36]. 40% loss in production of rice



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and almost around 90% of loss in case of pasture has been reported along with devastating effect on plants like mustard, linseed, and chickpea [37]. In Ethiopia, it has been reported to cause around 40% to 97% of losses of sorghum grain when *Parthenium* is left uncontrolled there [3]. Accumulated *Parthenium* pollen on floral parts of maize plant has potential to cause reduction in grain filling by 50%. Another work suggested that in case *Parthenium* is being burnt as a method of eradication could lead to ash deposition which is further found to be responsible for affecting rate of germination along with reduction in plumule and radical length in case of *Phaseolus mungo* [38]. It too has adverse effect over nodulation in legume plants because its presence has found to have negative impact on biological nitrogen fixation causing bacteria [39]. The weed too has potential to act as alternate host for the insects which are pest for various other plants. Few prominent examples are insect mealy bug, scarab beetle, etc. [3]. Numerous crop viruses along with bacterial wilt pathogen have been recovered from various parts of the plant throughout the world. It is believed to cause infection either in pod formation stage or in the pre-flowering time [40]. In a study, it has been estimated that cost could be double by the time crop take its final form and has been further localized into certain restricted area owing to fact that it has been contaminated with *Parthenium* [41]. Both burnt and unburnt extracts of *Parthenium* have been kept on test for check of its potential in regard to the noxious effect over crop. It has been found that burnt has more potential effect over crop than the unburnt *Parthenium* although both have been found to be toxic against the crop. The reason why burnt has been more toxic is due to its highly alkaline nature and presence of phenolic compounds [42].

Parthenium and Biodiversity

Parthenium is harmful towards the biodiversity of any region, which is being accounted by its allelopathic and invasive attributes that renders *Parthenium* its potential to run wild in ecosystem and to create havoc. It leaves very little or no vegetation in infested territory [30]. Its mere presence in a region could lead to influencing scenario over the diversity and further to their displacement along with imbalance between various forces of nature [43]. *Parthenium* along with another weed known as rubber vine, are seen today as greatest threats to biodiversity as is the case in bioregion of Einasleigh uplands [44]. The most adversely affected regions where significant to complete change in habitat has been seen are, grasslands of Australia, flood prone regions, banks of river and the open woodland [45]. Further investigation in varied regions of world has been done to investigate its effect but has yielded the same conclusion [46-48]. In another work, it has been argued that its secondary metabolite possession has been responsible for the status of invasive alien species [30].

Parthenium Influence over Human and Livestock

Over the period of time, it is appeared that *Parthenium* continuous exposure to people could potentially lead to disease like condition. People living in close proximity to *Parthenium* may also show allergic immune response. Almost all parts of the plant, from root to pollen material, have the potential to induce or to make person prone to *Parthenium* related diseases [50-51]. Conversely, a man who has hypersensitivity may likewise have an unfavorably susceptible response to *Parthenium* despite the fact that they may not be so sensitive to the plant itself. There are many diseases related to *Parthenium* which includes hay fever, allergic bronchitis, contact dermatitis, Phyto photodermatitis etc. [23,51]. Intake of *Parthenium* pollen grains through breathing can cause unfavorable rhinitis that can convert into more severe respiratory disorders such as bronchitis or asthma. *Parthenium* escalated virulence factor is the result of cyclopentene that could lead to intensified result in the form of alteration in chromosome, inhibition of functionally important enzymes or uncoupled phosphorylation. Wiesner et al. (2007) in his work reported that *Parthenium* has the potential to cause skin irritations, cracks in skin, stomach aches, and hand ball pustules in addition to above mentioned diseases. An observation-cum-evaluation in Queensland demonstrated that around 10% of people working in swarmed *Parthenium* zones had showed noticeable hypersensitive to *Parthenium* [1, 52]. About 10 to 20% of people found to be vulnerable to hypersensitivity from *Parthenium* when exposed or remained under its influence for 1-10 years [53]. Studies with *Parthenium hysterophorus* on *Salmonella* and bone marrow of mouse for its mutagenic potential were carried out but the results proved out to be negative for genotoxic potential [54]. In another work, it has been reported that there has been shift of airborne dermatitis towards a pattern that could be mixed to chronic in



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nature [55]. Further Siramarao et al. (1993) found the positive co-relation between the prick test conducted on skin and the presence of *mAb-2* [56]. Gunaseelan (1987) was among the first few researchers to report that *Parthenium* cause toxicity to plants too. Animals that have been feeding on *Parthenium hysterophorus* as well as *Parthenium* pollen infected crop, were found to be positive for Alopecia, diarrhea, reduction in skin pigmentation and dermatitis [49]. Cattle like sheep, buffalo etc. has been found to be with inhibited liver dehydrogenase enzyme along with some degenerative changes in two of the major organs mainly kidney and liver [57]. Further work on *Parthenium* led to the conclusion that consumption of *Parthenium* could even result in deterioration of meat along with milk production [45]. Yadav et al. (2010) has studied correlation between *Parthenium* and WBC count and found a significant reduction in the count in organisms post treated with parthenium. *Parthenium* is found to be a potential cause behind various other diseases like dermatitis, lesion on skin, mouth ulcer and hyper salivation like condition, irritation in eyes etc. In few conditions, it can be very lethal for animal with potential to harm and rupture tissues and organs [58].

Management and Elimination of *P. hysterophorus*

Parthenium hysterophorus has been known for its harmful effects on environment, agriculture, livestock and human. All the very reason it has become very important to control its proliferation or to evolve certain mechanism that could result into its eradication. Today there has been several methods in play to get hold of it like manual and mechanical removal, use of biological or chemical procedure, and preventing seed from spreading [59-60]. However, it has become a matter of concern owing to the fact that it could lead to epidemic proliferation and has a strong reproductive potential [61]. Burning is not something to be suggested as a control measure in case of *Parthenium hysterophorus*. The current observation has revealed that burning *Parthenium* could lead to strong infestation by decreasing pasture competition and favoring *Parthenium* growth. *Parthenium* prevention may be much cheaper as compared to eradication once it has developed to full fledge condition. Hence, many countries prefer to regulate as well as supervise before importing any field yield from *Parthenium* infected region [33]. Spraying weedicides earlier even before they start having their seed could be another method to deal with these noxious weeds, further it should be kept under observation for next two years in case of reemergence of weed. While in the non-crop areas and further areas along railway track or in abandoned land, spraying of 15-20% of salt solution could be effective to get rid of it. And in case of cropped land, one can also spray 1% glyphosate solution in *Parthenium* infested field [62]. Recently Shabbir et al. (2020) showed biological agent when combined with competitive plant like *C.ternatea* and *Asterbla squarrosa* could result into reduction of dry biomass from shoots and also reduction in seed banks with simultaneous decrease into the seedling recruitment in case of upcoming generation [63]. The drawback behind the use of herbicide is pollution and imbalance in ecosystem. Today, biological control has emerged as less expensive, safe for ecosystem and viable in context to environment. Biological control is found to reduce weed population along with enhancement in fodder plant production [64]. Few of notable biological control has been carried out by *Listronotus setosipennis* a stem-boring weevil, *Zygogramma bicolorata* beetle that feed over leaf and *Epiblema strenuana* that is stem-galling moth [95]. These have been successfully employed in order to deal with *Parthenium* havoc [65]. *Psidium guajava* and *Artemisia absinthium* has also shown to significantly impact *Parthenium* germination, development of seedling, and photosynthetic pigment content [66]. Despite of so much of benefits, biological control too has some drawbacks like it consumes very minute amount of foliage and hence insignificant in fast and effective action. It is further worsened by the fact that these weeds do possess higher regenerative potential [67].

Economical Importance of *P. hysterophorus***Health related benefits**

P. hysterophorus has the potential to be used as a medicine. From quiet a long time it has been used as a medicine to treat various diseases like neurological disorders, inflammation, fever, malaria, gynecological ailments, herpes, diarrhea, emmenagogue, urinary tract infections etc. [68]. In another work it has been reported to act as pharmacologically active substance with capability to treat muscular rheumatism [23]. It has been found effective



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against hepatic amoebiasis as well as reported to have possession of anti-cancer properties [23,28,69] were among the first few to report positive result in terms of tumor size reduction and also in cell life span when analyzed *in-vivo* as well as *in-vitro* [69]. Flower when leached out in methanol showed anti-tumor activities while parthenin showed some cytotoxic attribute against T-cell, and cancer cell line [26]. In yet another work it has been found useful to treat hypoglycemia condition with *Parthenium* in diabetic induced rats [70].

Crop production enhancement

Allelopathy could be exploited to be used as a potential substitution to chemical products like fungicides, insecticides, and growth regulators. It could enhance crop yield because of its attributes like disease resistance and at the same time eco-friendly in nature, which could prove very useful in future. It could most probably replace the chemical that is being used as pesticides because of its ovicidal and anti-fleedant properties [71]. In another work, Kishore et al. (2010) reported that *Parthenium* could probably enhance moisture level more efficiently than other attributes of soil like NPK level. It has been reported that it could be used as organic manure too [72,73]. It has been field tested as the potential prospective of green manure against maize, with utmost benefit achieved during administration of 3% parthenin derived green manure treatment. Further elaborating his work, he reported that parthenin could lead to around 42-253% increase in production as compared to only 96% increase when NPK has been administered. Apurva et al. (2010) reported the formation of multi composting to enhance the production without incurring any harmful effects and it has proved to be more beneficial than just parthenin composite [74].

Hussain et al. (2017) in his work tried to form vermicompost of parthenin and to further use it as organic fertilizer on *Abelmoschus esculentus*. As a result, he found that vermicomposting has helped to increase growth rate at same rate of germination along with increase in fruit yield and nutrition enhancement. It has further proved to be free of toxicity and raised the prospect of using it as a boon in future [75].

Bioremediation through *Parthenium*

Heavy metal pollution has emerged as one of devastating cause for degradation of environment. Anthropogenic activities like industries of storage batteries, refineries, casting of different metals like zinc etc. and electroplating adds a huge chunk of heavy metal into environment. At high concentration these can turn out to be carcinogenic and develop ability to induce tumor in normal organisms. This demands for development of a cost-effective way to deal with heavy metal pollution. Bioremediation and Biocontrol are one of the sought approaches to deal with bioremediation and comes into many shapes [77, 78]. A study carried out reported that *P. hysterophorus* can sequester nickel from solution [76]. In the same research it has been argued that addition of Sulphuric acid to treat *Parthenium* biomass could turn out to be an effective, cheap and easy to procure heavy metal remover from aqueous solution. Another research focused toward absorption of Cd (II) from wastewater. Cd (II) has been extensively used in metallurgical, manufacturing of plastic and in the electroplating industry. It is reported to cause various lethal diseases at lower as well as higher accumulation, such as increase in blood pressure, deformity in brain, disorder of urinary tract and the destruction of RBCs etc. Atomic Absorption Spectroscopy (AAS) has verified result further showing the absorption at wide range of concentration with pH ranging from 3-4 with efficiency equivalent to 99.7%. In yet another work, *Parthenium* activated carbon is proved to be over edge as compare to that of activated carbon in availability as well as cost and regeneration [42]. *Parthenium* assisted with ZnO-NP has been shown recently as a potent phytoremediation agent for Pb with 98.2% of accumulation; 86% in case of Ni; and zinc accumulation was 91.4% when they treat fly ash soil containing respective metals [79].

Additional benefits of *Parthenium*

Parthenium hysterophorus has been studied for its ability to produce enzyme like xylanase [14]. Dried leaf powder when sprayed over weeds like water lettuce, *Salvinia* etc. out compete aquatic organisms leading to their death. It has been found that these weeds could get infected with wilting and desiccation in the parts above water when sprayed with *Parthenium* [80,81]. Biomass of weeds has been observed to be highly reduced when *Parthenium* has



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been applied in varying concentration before and after emergence [81,82]. *Parthenium* has been found to be very effective against removal of various kinds of dyes, dissolved heavy metals, and other compounds like phenolics [83]. Nanotechnology has emerged as a wonderful option to tackle various issues in day to day life. It has been highly beneficial for the industry involving imaging, mechanical, electronic, drug delivery, and molecular diagnosis [84,85]. Nanoparticles (NPs) can be broadly classified into organic and inorganic type. NPs can be synthesized from various processes like sol-gel method, thermolysis, hydrolysis etc. [86]. But chemical and physical methods have their own issues leading to the biological synthesis of NPs from Bacteria, algae, fungi and plants extract [87,88]. Recently there has been increase in interest of researcher toward synthesis of NPs from *Parthenium hysterophorus* like TiO₂, AgNPs, and Zinc oxide nanoparticles [89-91]. These nanoparticles were further shown to be useful as antifungal agents, antibacterial, eco-friendly, and in controlling vector born disease [89-91]. Further *Parthenium* has shown magnificent result in the production of efficient biogas when added along with manure [49]. Nargotra et al., (2019) has reported bioethanol production from the *Parthenium hysterophorus* on pretreatment with surfactant that has been assisted with ionic liquid. *Parthenium* has also been studied to produce biogas with the help of anaerobic digestion [92-94].

CONCLUSION

Parthenium is the most common weed found to inhabit almost all over the world. Despite of its harmful effects on the flora and fauna of its habitat, the whole plant or its parts may be used for extracting some useful components from it, which may further be utilized as agricultural, industrial, and pharmaceutical products.

Conflict of Interests

There are no conflicts of interest.

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Histological Studies of Ecotoxicity of Fishes: A Review

Manamohan Polei and Siba Prasad Parida*

Department of Zoology, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Siba Prasad Parida

Department of Zoology, School of Applied Sciences,
Centurion University of Technology and Management,
Odisha, India.

Email: sibaprasad.parida@cutm.ac.in



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ABSTRACT

Since long, the pollution of aquatic ecosystem has become a serious environmental problem throughout the globe. Metals and metalloids with no biological function (As, Cd, Hg, Ni, Pb), pesticides and other persistent organic pollutants such as PAHs, PCBs, dioxins, tributyl tins etc are some of the most common anthropogenic pollutants and when they enter the water bodies, most of these are accumulated in by biota and biomagnifications takes place in the food chain. Being non-biodegradable pollutants, these are difficult to break down to less harmful substances. To know the negative impacts of different pollutants on living organisms particularly fish, biomarkers are applied at different levels (cells, tissues, organism and population) for understanding. Apply of biomarkers at tissue level such as histological alterations in different fish organs give valuable information about the xenobiotic impact. Hence, they are recommended as useful biomarkers in eco-toxicological research, risk assessment and monitoring programmes. Here, we aimed to review the use of histological alterations in the organs of fish such as kidney, liver and gills for ecotoxicological studies on the basis of collected scientific data since the late 1960's in the present paper.

Keywords: Aquatic pollution, fish, biomarkers, histological alterations.

INTRODUCTION

According to Lopez-Bera (1995) "Human activities induce stress on ecological systems by importing pollutants, by modifying habitats, introducing exotic species or removing the native ones and by changing climate". These activities affect the structure and function of natural ecosystem, the survival or the performance of individual organisms and the diversity of life at several levels of organization including the genetic composition, the number of species, the landscapes and the variety of ecosystems. Sekabira et al. (2010); Lushchak (2011); Mohamaddi et al.

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(2011); Ondarza et al. (2010,2011); Periera et al. (2013); Jorundsdottir et al.(2014) has reported that "In recent decades the level of foreign compounds in aquatic ecosystem such as heavy metals, pesticides and other persistent organic pollutants has increased alarmingly as a result of domestic, industrial and agricultural effluents". According to Dutta and Dalal (2008),"the contamination of aquatic systems is treating the living organisms and thus, it has attracted the great attention of many researches around the globe". The Water Framework Directive(WFD) of the European Union Specified the monitoring programmes required to assess the achievement of good chemical and ecological status for all water bodies by 2015 (Sanchez and Porcher,2009).

The Purpose of using Fish in Eco-Toxicological Research

To evaluate the ecological status of rivers, the fish represent one of the key elements according to the Water Framework Directive (Scardi et al., 2008; Hermoso et al., 2010). Fishes are found in different sizes and ages and occupy different trophic levels and in comparison with invertebrates are also more sensitive to many toxicants ,hence they have been found to be good indicators of water contamination in aquatic ecosystems (Powers,1989; Barack and Mason,1990 a,b; Wanderlaar Bonga and Lock, 1992;Wester et al.,1994; Burger et al. 2002). According to Mishra and Shukla (2003)" Fish respond to environmental toxic changes with adapting of their metabolic functions". Many species of fish have been found to be susceptible to environmental pollutants as they are present virtually in all environments.(Van der Oost et al., 2003). According to de La Torre et al. (2005)". Monitoring Sentinel fish species is widely used to assess the degree of accumulation of toxicants and their effect on the health status". As per the findings of Song et al. (2012), fish have well-developed endocrine, osmoregulatory, immune and nervous systems compared to invertebrates, hence they are preferred in toxicological research. It is suggested that " fish may absorb toxicants directly from the surrounding water and sediments(waterborne exposure), or ingest them through contaminated food in the food chain(dietary exposure), enable the assessment of contaminant transfer through the trophic web"(Cid et al., Fisk et al.,2001; Moiseenko and Kudryavtseva, 2001, Rashed,2001; Mondon et al.,2001; Mansour and Sidky, 2002; Usero et al.,2004; Mendil and Uluozlu, 2007; Ozturk et al., 2010; Sounderajan et al.,2010). For the toxicological, ecological and pathological investigations in the teleost fish, the tissues like the gills, kidneys, liver and muscles are the most frequently utilized (Sauer and Watable,1989;Velcheva, 2006; Heir et al.,2009) because these tissues are metabolically active and tend to accumulate toxicants at higher levels (Andres et al.,2000; Karadede and Unlu, 2000; Marcovecchio, 2004). Terra et al. (2008) consider that" toxicants enter the fish organism mainly through the gills and consequently, with the blood they reach the parenchymal organs where they remain for a longer time". The concentrations of toxicants in water where the fish live is reflected by the concentration of toxicants particularly in the gills, where as the concentrations in other organs such as kidney and liver represent storage of toxicants as studied by Kroglund et al.,2008.

Ecotoxicological Research: Fish and Biomarkers

According to Van der Oost et al. (2003);Giari et al. (2008) and Maria et al. (2009) " correlation must be established between external levels of exposure, internal levels of tissue contamination and early adverse effects and determining the extent and severity of such contamination only by the result of water chemical analysis is insufficient and often over estimates the proportion and duration of exposure to the toxic agent". As per the findings of NRC,1987; McCarthy and Shugart,1990; Hitonn et al.,1992; Peakall,1992; Hinton,1994; Bucheli and Fent,1995; Van Gestel and Van Brummelen,1996; Shugart and Theoderakis,1998; Cajarville et al.,2000; Adams et al.,2001; Triebes Korn et al.,2002; Pandey et al.,2003; Ronisz et al.,2004; Weeb et al,2005; Martin-Diaz et al.,2006; Schmitt et al.,2007; Martin-Diaz et al.,2008; Alvarez-Munoz et al.,2009 ; Weeb and Gangon,2009; Domingues et al.,2010; Souza et al.,2013; Munoz et al.,2015, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring and risk assessment programmes in the past 25 years. Fossi and Marsili, 1997; Fossi,1998 have defined the biomarkers as "responses to any exposure evidenced in histological, physiological, biochemical, genetic and behavioural modification". According to Van der Oost et al. (2003) it is defined that "biomarkers are the biological indicators from an exposure to a stressor responding in various ways". Picado et al. (2008) has added that "biomarkers, which act as early warning signals of the presence of potentially toxic xenobiotics, are useful tools for assessing either exposure to, or the effects of these compounds providing information about the toxicant



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bioavailability". Pinto et al. (2009) and Viana et al.(2013) suggest that "the biomonitoring process should include analyses at different levels of biological organisation, from sub-cellular and cellular analysis of tissues and organs, to the population and community levels". Therefore in order to control population effect on the animals that inhabit the water bodies, biomarkers are essential to complement environmental monitoring (Au,2004; Camargo and Martinez,2006; Cazenave et al.,2009).It has been argued by Picado et al. (2008) that "a full understanding of ecotoxicological processes must consider an integrated multi-level approach ,in which molecular impact is related with higher-order biological consequences at the individual, population and community levels". Corresponding to three major parameters, biomarkers have been classified into three separate categories that necessary to conduct ecological risk assessment by NRC (1987); Schlenk (1999) and WHO(1993).To perform such an accurate ecological risk assessment, ecological effects, as well as exposure and susceptibility to contaminants must be well-characterized following identification or formulation of a problem. According to Schlenk. (1999), well-defined biological indicators can be used in certain cases to help ,make inexpensive predictions regarding the exposure (bioavailability) ,effects (mechanism of action) and uncertainty of response (susceptibility) elicited by various anthropogenic substances in each of the processes.

Histological Changes as Biomarkers

Yevich and Yevich,(1994); The et al.(1999); Peebua et al.(2006);Costa et al.(2010)suggested that "histopathology involves the microscopic examination of cell and tissues of an organism and the semi-quantitative determination of histological abnormalities". Greenfield et al.(2008) and Poleksic et al.(2010)explain that "the histological alteration in selected target organs are sensitive biomarkers for xenobiotic effects and they occur earlier and provide a better evaluation of the effects of aquatic pollution than any single biochemical parameters". Therefore to provide additional information to physicochemical analysis in aquatic toxicology and to monitor acute and chronic situations, analysis of histological changes in different fish tissues has been widely used for decades(Wester and Canton,1986;Wester and Canton,1991;Johanson et al.,1993;Perry and Laurent,1993;Schwaiger et al.,1997;Stentiford et al.2003; Schwaiger et al.,2004; Lang et al.,2006; Nero et al.,2006; Monteiro et al.,2008 Van Dyk and Pieterse,2008; Olarinmoye et al.,2009; Van Dyk et al.,2009;Liu et al.,2010;Sousa et al.,2003 a, b).In addition to this, for ecological quality of aquatic ecosystems, several biomonitoring programmes have used the histological alterations observed in different fish organs as biomarkers (Land et al.,2006; Blazer et al.,2007;Pinto et al.,2009). Braunbeck et al. (1990) and Schwaiger et al. (1997) State that "the histological changes can be used as indicators for the effects of various anthropogenic pollutants and they are a reflection of overall health of the entire population in the studied ecosystem". For the diagnosis of direct or indirect toxic effects that affect the animal tissues, the histology is a sensitive tool (Braunbeck and Volk,1993; Bernet et al.,1999; Poleksic et al.,1999; Ferreira et al.,2005;Marchand et al.,2009; Fanta et al.,2003;Van Der Oost et al.,2003;Zimmerli et al.,2007; Van Dyk et al.,2009; Grund et al.,2010; Rajeshkumar and Munuswamy,2011; Paithane et al.,2012). Hence, histological changes as biomarker is considered as an excellent method for the assessment of the environmental quality (Costa et al.,2009).In addition to this, for the assessment of both short and long-term toxic effects, the histopathology is often the easiest method for field assessment(Hinton and Lauren,1990).On the other hand, Wester and Canton(1991) and Yevich and Yevich (1994) state that "the histological methods are relatively labour -intensive and require some experience, but after all they have the considerable advantage that pathological alterations in different tissues (e.g. gills, liver) can be observed individually, thus creating a direct link with physiological links such as growth, reproduction, respiration and nutrition". For the biomonitoring of aquatic pollution in marine ecosystem ,the feasibility of using histopathological parameters in fish as biomarker has been reviewed thoroughly by Au (2004).The study of Rabitto et al. (2005) and Oliveira Ribeiro et al. (2006) reveals that "the exposure of fish to chemical contaminants can induce a number of lesions and injuries to different organs, but the gills and liver represent important target organs suitable for histological examinations in searching for damages to cells and tissues".



**Manamohan Polei and Siba Prasad Parida****Histological Changes in the Gills of Fish**

The gills of fish are multifunctional organs responsible for the exchange of gases, transport of ions, excretion of wastes and regulation of acid-base (Dang et al.,2001;Gernhofer et al.,2001;Evans et al.,2005; Oliveira Ribeiro et al.,2005; de La Torre et al.,2005;Vigliano et al.,2006; Nigro et al.,2006; Salamat and Zarie 2012, Singh,2014).According to Playle (1998), the gills accounts for over 50% of the surface area of the body of a fish, hence it is one of the major target organs for waterborne toxicants. The gills are the first organs which come in contact with the environmental pollutants as they are the main route of the toxicant penetration into fish organism and are sensitive subjects for identifying the effects of water toxicants on the fish organism (Carpene and Vasak,1989; Perry and Laurent,1993; Pourang,1995; Tkacheva et al.,2004; and Rosseland et al.2007).Since the incorporation of toxicants occurs mainly through gills(a respiratory organ),the metabolism of fish acting principally through it can be seriously damaged (Bervoets and Blust,2003; Sloman,2007; Terra et al.,2008). Heier et al.(2009) states that “as the gills of fish can accumulate bioavailable pollutants, their measurement on gills can reflect the speciation of pollutants and in particular metals in water, which make them a useful tool for assessing the bioavailability of metals in water”. All this helps to understand why the gills are more frequently utilised in bioaccumulation studies in teleost fish and pathological damage produced defining the toxicity of the environment, making fish highly suitable for evaluating the health of aquatic system (Mallatt, 1985; Oliveira Ribeiro et al.,2000; Olsviket et al.,2001; Moiseenko et al,2005; Ogundiran et al.,2009).Due to acid rain, heavy metals, and any change in the composition of the environment, the physical and chemical changes of aquatic medium such as acidification, temperature of the water supply takes place and the gills of the fish are very sensitive to these changes which is a very good indicator of waterborne toxicants (Saber,2011).According to Playle et al. (1993) and Teien et al.(2006 a, b) “the surface of gills of fish serves as metal-binding ligands and metal bioaccumulation in particular can occur due to positive charged metal species in the water to negatively charged sites on the gills”.

The morphology of normal gills of most teleost fish species is described in details by Laurent and Perry (1997) and Willson and Laurent (2002). They explained that in most of the teleost fish the gills are typically composed of four pairs of gill arches and they are supported by a skeleton of bone. The gill itself is made up of a double rows of filaments from which the lamellae arise perpendicularly. The filaments arise from the gill arches and supported by cartilage(primary lamellae) from which the secondary lamellae exit. Pavement cells and non differentiated cells compose a squamous epithelium and the primary lamellae are lined by this squamous epithelium. Bellow that epithelium lamellar blood sinuses are present which are separated by pillar cells. The filament is lined by a thick stratified epithelium constituted by several cellular types such as pavement cells, chloride and mucous cells between lamellae. A simple epithelium constitute the secondary lamellae where the exchange of gas takes place. When it comes to biomarkers, the histological alterations of gills are considered as non-specific biomarkers which means that many inorganic and organic contaminants can cause changes in tissue. However, According to Arellano et al. (1999),they are recognised as fast and valid method to determine the damage caused in fish by the exposure of pollutants. Therefore, Au, 2004; Fernades et al.,2007; Flores Lopes and Thomaz,2011; Adeogun,2012; Pereira et al.2013 suggested that the histological changes of gills of fish as useful biomarkers of the contamination of environment. After acute or chronic exposure with sub lethal concentration of toxicants, both in the field and laboratory conditions, there are so many reports found on various histological changes in the gills of fish from contaminated water (Doughtie and Rao,1983, Lauren and McDonald,1985; Alazemi et al.,1996; Majon et al.,2002; Rao et al., 2003,2006; Camargo and Martinez, 2007; Matos et al.,2007; Liu et al.2010; Velcheva et al.,2010,a,b; Muthukumarave et al.,2013; Sousa et al.,2013 a, b),but it is often difficult to decide whether morphological alterations are adaptive or destructive (Tkacheva et al.,2004).In addition, according to Camargo and Martinez (2007), Ayandiran et al. (2009) and Vigarío and Saboia-Morais (2014)” the toxicants could induce the changes in the histology of the gills such as necrosis and degeneration of epithelium” in one hand. But in the other hand, fish are able to develop numerous defence mechanisms, which could prevent the negative effects of the toxicants. Different morphological changes including proliferation of epithelium, fusion and edema expresses this mechanisms.



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Mallat,(1985) and Thophon et al., (2003) have observed that "the presence of edema along with the detachment of the lamellar epithelium is the first sign of pathology in fish and one of the more frequent lesions observed in gill epithelium of fish exposed to different xenobiotics". Many studies have revealed that the interstitial edema is one of the more frequent lesions observed in the epithelium of fish those exposed pollutants such as heavy metals (Karlsson-Norggren et al.,1986;Reid and McDonald;1988;Whang and Tsai,1993;Sala et al.,1995;Bury et al.,1998;Karan et al.,1998;Cengiz and Unlu ,2002;Pane et al.,2004;Cengiz,2006;Nero et al.,2006;Velmurugan et al.,2009). This change in histology can also be due to the exposition to other varieties of pollutants such as endosulphan (Nowak, 1992); Paraquat (Banaee,2013) and drugs (schwaiger et al,2004). Furthermore, Karan et al.(1998);Arellano et al.(1999);Cengiz and Unlu (2002);De Boeck et al (2001);Pane et al.(2004); Schwaiger et al. (2004) and Olurin et al(2006) claim that lifting of lamellar epithelium is another histological change observed, probably induced by the incidence of severe edema. It is supposed by Lauren and McDonald (1985); Haaparanta et al. (1997);Arellano et al.(19999); Allexopoulos et al. (2003) and Van Heerden et al. (2004) that the lifting of lamellar epithelium due to edema could serve as a defence mechanism, because the separation of epithelial lamellae increase the distance across, which the pollutants of water must diffuse to reach the blood stream.

Arellano (1999); De Boeck et al. (2001),Van Heerden et al.(2004); Figueiredo-Fernades et al.(2007); Velmurugan et al.(2007);Mohamed(2009) and Georgieva et al.(2004) reported in different species of fish that, the trace metals induces the hyperplasia in the epithelium of gills. These results were also found in case of fish exposed to other pollutants such as pesticides (Neskovic et al.,1993;Van den Heuvel et al.,2000;Rosety-Rodriguez et al.,2002;Oropeca et al.,2005).De Boeck et al.(2007) reported that "the proliferative changes can increase the water blood distance and reduce the absorption of xenobiotics, but in turn, decrease the respiratory surface area, which reduces the effectiveness of gas exchange ion uptake". According to Fegueiredo-Fernandes et al.(2007),"the proliferation of cell with the thickening of epithelium of gill filament is a histological change, which may lead to fusion of lamellae". After exposure to heavy metals ,the proliferation of cells with thickening of epithelium of gill filament is described by several authors(Arellano,1999; De Boeck et al.,2001;Van Heerden et al.,2004),but these results also were found in fish exposed to other pollutants(Randi et al.,1996;Van den Heuvel et al.,2000;Rosety-Rodriguez et al.,2002).Evans et al.,(2005) says that the fish may be able to survive from the effects of pollution by adapting to apparently pathological symptoms. On the other hand, it is stated by Georgieva et al.(2004) that "the increase in the number of cells would cause an intense secretion of mucous and thickening of mucous layer". Hence the additional significant thickening of the filamentous epithelium and the emergence of fusion could have impact on the osmoregulation and respiration process in the body of fish. Lamellar fusion was found in different fish such as Nile tilapia, *Oreochromis niloticus* exposed to treated sewage water for 96 hours (Fontainhas-Fernandes et al.,2008),in spotted snake head, *Channa punctatus* exposed to sub lethal herbicide concentrations for 96 hours (Butchiram et al.,2009) and in common carp, *Cyprinus carpio* exposed to 0.029 and 0.041mg/L deltamethrin for short-term 96 hours(Cengiz et al.,2006).

Peuranen et al.,(1994) state that "the observation of rupture of the branchial epithelium are considered as direct, dose-dependent deleterious effects of the pollutant, while hyperplasia, lamellar fusion and mucous hyper secretion could be signs of branchial defence responses". The rupture of epithelium could lead to changes in haemoglobin and haematocrit and a negative ion balance and could cause severe disturbances in the gill respiration. Peuranen et al.,(1994) described that this epithelial rupture explain the ion loss from plasma. Such changes has been observed in catfish, *Heteropneustes fossilis* exposed to the 4mg/L malathion for 24,48,72 and 96 hours by Dutta et al.,(1996).After exposure to sub lethal concentration of manocrotophos of the mrigal carp, *Cirrhinus mrigala*, epithelial hyperplasia, curling and fusion of the secondary lamellae were observed (Velmurugan et al.,2007) and this is also noticed in the case of *Gambusia affinis*, the mosquito fish after 30 days of exposure to 0.25-0.50 micro gram per Litre of deltamethrin (Cengiz and Unlu,2006).

Thophon et al.,(2003) and Garcia-Santos et al.(2006) refer that the exposure of fish to heavy metals such as Cadmium, the vasodilation can induce changes in the normal structure of pillar cells with consequent loss of their support function and is probably responsible for the emergence of lamellar aneurysm. Figueiredo-Fernandes et al.(2006) have



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observed similar type of results in Nile tilapia, *Oreochromis niloticus* after 0.5, 1.0 and 2.5 mg/L of copper exposure for 21 days. Mallat(1985) suggests that these lesions are rarely associated to the exposition of metals. Georgia et al.(2014) reported that exposure of sublethal concentrations of pesticides to common carp leads to severe vasodilation. Martinez et al.(2004) and Cengiz, (2006) reported that "aneurism are the most severe alteration in the fish venous system and they are a result from extended vasodilation with the collapse of pillar cells and the breakdown of vascular integrity". Garcia-Santosh et al.(2006) and Stentiford et al.,(2003) observed that "an increased frequency of this serious histological alteration of gills in fish from contaminated areas have affirmed that it can be associated with the presence of toxicants such as metals in the water". It is proved by Monteiro et al.(2005) that aneurisms might be used as a reliable and sensitive biomarker of acute exposure to copper. After the exposure to the pesticides and heavy metals, the necrosis and degenerative changes in the epithelium of gills of fish were reported by Santhakumar et al.(2001), Butchiram et al.(2009) and Hasan et al.(2004). The response of gills of fish to the organic or inorganic pollutants seems not to be affected by the biology of the fish (sex and age) or seasonal factors stated by Au(2004). However, we can add that some species of fish are more resilient to contaminated water (carp and perch) and other are more sensitive to pollutants (trout and salmon). It is important to site here that even all of the above histological changes can be found in case of the fish exposed to toxicants, some of them induce more severe tissue changes than others. According to the study of Oliveira Ribeiro et al.(2006), Although, the tissue preparation for the gill histopathological study is time consuming, in general, for the study of environmental contamination, the histopathology of gills appears to be promising biomarker.

Histological Changes in the Liver of Fish

If, the biological barriers are crossed by toxicants and entered into the blood stream, they will be accumulated and will be reached in the internal organs of fish. To evaluate environmental quality, numerous studies have quantified contaminants in different fish organs, seeking causal relationships with fish health, and based on these, the liver is likely to be the best choice, followed by the kidney and gills (Begun et al., 2004; Pokorska et al., 2012; Majnoni et al., 2013). According to Hinton and Lauren (1990), Van der Oost et al. (2003) and Salmat and Zarie (2012), the liver is the most essential for both metabolism and the excretion of toxic substances as it is a detoxifying organ in the body of fish. According to Mohamed (2009), due to its large blood supply, the liver is a target organ, which causes noticeable toxicant exposure. Moreover, the liver is reported to be the primary organ for bioaccumulation hence, it has been studied extensively in regard to toxic effect of xenobiotics (Hinton and Lauren, 1990; Yilmaz et al., 2007; Van Dyk et al., 2007; Simonato et al., 2008; Madureira et al., 2012; Nunes et al., 2015).

According to Vicenti et al. (2005) and Figueiredo-Fernandes et al. (2007) generally, a teleost fish shows a normal structure with a typical parenchymatous appearance without having any pathological abnormalities. The parenchyma is composed of hepatocytes, epithelial cells with a homogenous cytoplasm and a large central nucleus. The hepatocytes are located among blood capillaries called sinusoids forming a cord-like structure known as hepatic cell cords. The erythrocytes present in the lumen of sinusoids. The venous blood enters the liver via hepatic portal veins caudally and branches into sinusoids. The sinusoids are lined with reticulo endothelial cells and they are surrounded by hepatocytes. According to Hinton and Lauren (1990) and Fernandes et al. (2008), as a monitoring tool, the histopathology of the liver of fish can provide an assessment of the effects of the environmental stressors on fish population. Since the liver plays an important role in many vital functions (Moon et al., 1985; Triebkorn et al., 2002; Figueiredo-Fernandes et al., 2006; Long et al., 2006), it was also proposed to be one of the most reliable indicators for health impairment of aquatic animals by anthropogenic activities (Hinton and Lauren, 1990; Hinton et al., 2001; Stentiford et al., 2003). In coastal waters, several studies carried out and have shown correlation between environmental contaminants, bad quality of water and occurrence of toxicopathic liver lesions in fish (Vethaak et al., 1992; Vethaak et al., 1996; Stentiford et al., 2003; Fiest et al., 2004; Carvallho Neta et al., 2004). Hence, according to Long (2002) and Fiest et al. (2004) "the histopathological alterations of liver and other diseases of fish have been used as indicators of effects of pollution and have been implemented in monitoring programmes." Stentiford et al., (2003) states that numerous categories of pathology of liver are present as reliable biomarkers of toxic damages. It is also found that, the presence of inflammatory lesions, preneoplastic and neoplastic lesions and hepatocellular fibrillar



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inclusions is higher in fish captured in polluted environments than in fish from reference sites. However, the sort of observed histological changes depends on the time of exposition of individual to the pollutants, as well as concentration and type of pollutants (Pinto et al., 2009). Using the quantitative and semi-quantitative electron microscopy, the methods have been described to study the ultra structure of liver of fish (Triebkorn et al., 1997).

Many different toxicants can produce changes in the liver hence, like the histological changes of gills, the hepatic changes are also considered as non-specific biomarkers. According to Van Dyk et al. (2012) and Georgieva et al. (2014) the exposure to different organic pollutants and heavy metals may cause histological changes in the liver and histological investigation of exposed specimen may therefore, produce meaningful result. Figueiredo-Fernandes et al. (2007) suggest that abnormalities develop in the hepatic structure and hepatocytes due to high metal deposition in the liver of fish and that can lead to subsequent death of cells. According to Vethaak et al. (1992); Simpson et al. (2000); Stentiford et al. (2003) a sign of blood quality of water has been marked by the appearance of hepatic lesion and increment in wild fish hence it has been the subject of many studies. According to Pinto et al. (2009), "the sort of histological alterations observed depends on individual exposition time to pollutants, as well as on the type of pollutants and its concentration." According to Strussmann and Takashima (1990), the number of hepatocytes, content of glycogen, area of hepatocytes, content of lipids, core region of hepatocytes in the cytoplasm are some of the morphometric parameters and these are commonly used as an indicator for the metabolic activity of hepatocytes. Biochemical disturbances leads to degenerative changes such as hydropic, vascular, granular and fatty degenerations and this leads to changes in the synthesis of protein impairment of ion regulation, activity of enzymes and the depletion of the resources of energy (Monteiro et al., 2005; Rajeshkumar and Munuswamy, 2011).

The disruption of cellular energy transfer process required for ionic regulation indirectly or directly by denaturation of volume-regulating ATPases leads to swelling of cells (Hinton and Lauren, 1990). Formation of vacuoles in the hepatocytes are reported after the study of alterations in the liver hepatocytes associated with stress by Metelev et al. (2017). Mishra and Mohanty (2008) and Vinodhini and Narayanan (2009) described that "vacuolation of hepatocytes is a common response associated with the exposure to many different toxicants." Such alterations in histology could represent lesions at biochemical levels increasing energy depletion and inhibition of synthesis of proteins. In addition, Velmurugan et al. (2007) studied that after exposure of mrigalacarp, *Cirrhinus mrigala* to the pesticide lambda-cyhalothrin for 10 days, the vacuolation of hepatocytes are found. It was also studied by Fanta et al. (2003) and they found that, the vacuolation of hepatocytes are found in cat fish, *Corydoras paleatus*, after exposure to the pesticide methyl parathion. From the chemically contaminated habitats, Moore et al. (1997) studied the hydropic vacuolation in water flounder, *Pleuronectes americanus*. According to the studies of these authors, the glossy visible foci of vacuolation correspond to the most advanced stage of this lesion and is associated with neoplasias. Stehr et al. (1998) observed and described hydropic vacuolation in the liver of three species of bottom fishes, *Genyonemus lineatus*, *Lepidopsetta bilineata* and *Platichthys stellatus* from the west coast of U.S.A, single large hydropic-appearing vacuole that almost completely filled each affected cells with a little cytoplasm remaining, have been observed by these authors. The vacuoles appeared to be empty, and their edges were smooth, as if they were membrane bounded. Within the vacuoles, the nucleus was located eccentrically. They have also observed that "this lesion can appear like single cell and a small group of two to three cells affected, diffusely distributed throughout the liver in mild cases." On the other hand, they identified a focal form of this lesion with a smaller frequency, where nearly all of the cells within a specific area or "focus" were affected. In Baltic flounder, *Platichthys flesus*, hydropic vacuolation was found by Lang et al. (2006) and this was used as indicator for biological effects of contaminants.

From a metal-contaminated lake, Yancheva et al. (2005) found morphologically altered hepatocytes and adipose cells were resembled with flattened nucleus with peripheral location. Marty et al. (2003) detected some hepatic micro lesions such as hepatic megalocytosis and pigmented macrophage aggregation, fibrosis and lipid accumulation in the demersal rock fish species from prince William Sound, AK, USA, after the Exxon Valdez oil spill in 1989 in his studies. In that study, it was confirmed that in fish the cytoplasm of hepatocytes contained variable amount of round, clear well-demarcated vacuoles with abundant lipid (lipid accumulation) and some cells contained multiple



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cytoplasmic vacuoles. Pinto et al.(2009) suggested several biochemical lesions such as energy depletion, disaggregation of microtubules, inhibition of protein synthesis and shifts in substance utilisation could be represented by histological changes. This lesion can be appeared under two forms such as Foci of variable size or dispense by whole hepatic parenchyma. Cristane de Melo et al.(2008) found that" the cytoplasm of catfish, *Rhamdia quelen* exposed to 0.01 ml/L Folidol 600 , a higher presence of lipid droplets in the first 48h than in the control fish." Arellano et al.(1999), suggested that, change in the fat metabolism of the hepatocytes is indicated by the deposition of lipid. Biagiantirisbourg and Bastide (1995) described that depending on the time of sublethal exposure to the herbicide atrazine, different sizes of lipid droplets are found in the fish thin lip mullet, *Lizaramada*. Several studies demonstrated that due to contaminants, the changes takes place in size, shape and in the number of nucleus of hepatocytes takes place. Paris-Palacios et al. (2000) reported in zebra fish, *Brachydanio rerio* that there is an alteration in the size of the nucleus due to sub-lethal concentration of copper sulphate. Braunbeck et al. (1990 a,b) states that" alteration in the size and shape of nucleus have often been regarding as signs of increased metabolic activity, but may be also of pathological origin." The increase in the volume of nuclei and nucleoli and which lead to necrosis of liver cells are the result of disturbances in the osmotic regulation of cellular membranes (Ahmad et al.,2002).Ayoola(2008) found that the infiltration of leukocytes, presence of vacuoles and increased hepatocyte size with picnotic nuclei in the liver of fish which were exposed to glyphosate.

Coz-Rakovac et al.(2005) and Coz-Rakovac et al. (2008) have described changes like cytoplasmic vacuolation and irregular shaped hepatocytes in the population of sea bass and mullets aggregated around fish farms respectively. The cirrhosis and lysis of hepatocytes and ultimately death of common carp *Cyprinus carpio* due to the accumulation of metals in the liver (Varanka et al.,2001). Due to *in vitro* and *in vivo* sub-lethal exposure to xenobiotics,(4-chloroaniline) of rainbow trout, *Oncorhynchus mykiss*, the proliferation of lysosomes in the hepatocytes takes place(Braunbeck1994).Nero et al.(2006 a, b) observed the pathological changes in the liver in the individuals of yellow perch, *Perca flavescens* and gold fish, *Carassius auratus* held in waters containing levels of oil sands process-affected water following 3-week exposure.In relation to significant changes in the histology of liver, it was dominated by degenerative alterations for both species and, to a lesser extent, cytoplasmic (goldfish) and inflammatory (yellow perch) alterations. In both the species of fishes, nuclear pleomorphism and necrotic foci dominated the degenerative lesions. Under sub-lethal paraquat exposure, Figueiredo-Fernandes et al. (2006) and Ada et al. (2012) found an increased eosinophil cells and macrophages in the liver of Nile tilapia, *Oreochromis niloticus*. After 7.5 and 10mg/L Round up exposure for 6,26 and 96 h in the case of rainbow trout, *Oncorhynchus mykiss* and Martinez(2008) found degenerative changes in the hepatic cytoplasm and nuclei, as well as hypermia in the liver. Ramirez-Duarte et al. (2008) determined LC50 for glyphosate for amazonic Pacu, *Piaractus brachypomus* and found the hyaline droplets and lipid vacuolation like degenerative changes in the cytoplasm of liver of this fish. After the chronic exposure with dioxin and carbaryl in case of Nile tilapia, *Oreochromis niloticus*, Walter et al.(2000) and Matos et al.(2007)determined lipid accumulation in liver respectively. Gultekin et al. (2000) and Tech et al.(2005) were also reported similar results. After the exposure of fungicides for 96h for fish, Gaafar et al.(2010) found single hyaline droplets combined with vacuole degeneration and necrosis in the liver. El-Serafy et al. (2009) exposed the Blue tilapia, *Oreochromis aureus* to sub-lethal (20%,40%, and 80% of LC50) phenol for 7days and determined the reduction in the carbohydrates content in the liver. Pathan et al. (2009 a,b), De Boeck et al.2010) and wiseman and Vijayan (2011) had reported the similar results after exposure to other organic contaminants in case of fish. On the other hand, after the Chlorpyrifos treatment in case of common carp, *Cyprinus carpio*, an increased glycogen level in the liver was found by Ramesh and Saravanan (2008). According to Simenova (1999); Rabbito et al. (2005); Mela et al. (2007) and Carrola et al. (2009),under the influence of metals necrotic areas develop in the liver leading to a serious alterations in its structure. Roberts (1989) explains that 'necrosis can be characterised by nuclear and cytoplasmic alterations, followed by loss of the cellular contours.'"The release of chemical signals takes place in the cells where necrosis occurs and that chemical signals induce proliferation for the substitution of the dead cells, hence the structural and functional condition of the tissue is restored.



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Manahan (1991) states that " the occurrence of necrosis is also a consequence of enzymatic inhibition, damage in the cellular membrane integrity and disturbance in the synthesis of proteins and carbohydrate metabolism." The process of necrosis area in the fish liver, is also related with the concentration of xenobiotics during the detoxifying process. Ayoola (2008) and Olufayo and Alade (2012) consider that " necrosis of some areas in liver tissue were probably resulted from the excessive work required by the fish to get rid of the toxicant during the process of detoxification by the liver." Cengiz and Unlu (2006) determined hepatic necrosis in mosquito fish, *Gambusia affinis* due to sub-lethal effects of deltamethrin. Dutta et al.(1993); Rodrigues and Fanta (1998) and Uguz et al. (2003) have observed necrosis in the liver of fossil cat *Heteropneustes fossilis*, zebra fish, *Danio rerio* and rainbow trout, *Onchorynchus mykiss* exposed to different pollutants respectively. Under the exposure of chloropyrifos and kinalfos to *Chana punctatus* and *Cyprinus carpio*, the infiltration of leucocytes were found in liver (Devi and Mishra, 2003). After acute and chronic exposure with 0.05 mg/L, 0.1mg/L, 0.25 mg/L chlorfos, such types of histopathological changes, as well as, vasodilation in small blood vessels and necrosis in the liver of *Cyprinus carpio* takes place. Yancheva et al.(2005) found "venous hypermia in perch from a metal contaminated lake, which was assumed to be linked to disturbances in the hepatic blood circulation." Most probably due to common vein congestion, it was presented in the major and small blood vessels as well as hepatic sinusoids. It is considered that the hepatic hyperaemia can lead to hepatic necrosis and atrophy (Van Dyk et al.2007). McHugh et al. (2011) found that " the liver alteration in fish exposed to pesticides were mostly associated with circulatory disturbances, related to pathological conditions of blood and tissue fluid flow and regressive changes." They included dilation of blood sinusoids, as well as cytoplasmic granular degeneration and vacuolation of the hepatocytes. Overall, we can conclude here that there are many different mild or severe liver changes. Similarly to the histological alterations in the gills, which can be included by both, inorganic and organic toxicants. It also seems that most of them are destructive rather than adaptive. This can be linked with the inability of the detoxification organ to get rid of the contaminants, thus also indicating biochemical disturbances in the body of fish.

Histological Changes in the Kidney of Fish

The main organ involved in the maintenance of homeostasis of body fluid is the kidney in vertebrates. Through evolution, the morphology and function of the kidney have been modified to fulfil different physiological requirement and the widest range of kidney types is found in fishes (Hentschel and Elger, 1989). Hinton et al. (1992); Evan, (1993); Ojeda et al. (2003) suggests that the kidney along with the gills and intestine are responsible for the excretion and the maintenance of the homeostasis of the body fluids in the teleost. WHO,(1992);Hinton et al.1992; and Eisler, (1998), the kidney produces urine and acts as an excretory route for the metabolites of a variety of xenobiotics to which the fish may be exposed. The metabolic waste products such as ammonia and creatinine containing nitrogen are also excreted by the kidney. Cengiz, (2006) suggest that a vital function related to the balance of water and electrolytes and the maintenance of a suitable internal environment is performed by kidneys of fish as in higher vertebrates. Mobjerg et al. (2004) describe that " the kidney of all vertebrates is made up of nephrons and nephrons are functional and structural unit of kidney and take part in adaptation process. "The kidney of teleosts consists of two parts called anterior kidney known as head kidney and posterior kidney known as body or trunk kidney. According to Takashima and Hibiya (1995) the anterior kidney is pronephric so far as the embryology is concerned. Donaldson (1981) and Wendelaar Bonga, (1997) states that the anterior kidney of fish is very important for stress response as it is integrated into the endocrine system of the fish and is mediated by the hypothalamus-sympathetic nervous system-chromaffin tissue(HSC)axis. In addition, the endocrine elements-the chromaffin cells and internal tissue are present in the head of kidney and these are located around the blood vessels. The posterior kidney contains the nephrons with variable quantities hemopoetic and lymphoid tissue in the interstitium. The nephrons composed of renal corpuscles that are made up of the glomeruli, Bowman's capsule, renal tubules and collecting ducts. Federova (1998) and Mobjerg et al.(2004) states that there are three types of kidneys found in the vertebrates and they are the pronephros , mesonephros and metanephros. Hickman and Trump, (1969) suggests that incase of teleosts, the morphology of kidney of each species often reflects the osmotic demands placed on the organism by the environment. Thus, the fresh water fishes are faced with the need to produce copious amounts of dilute urine and therefore, their kidneys have well developed renal corpuscles in both proximal and distal segments



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in the tubules. In contrast, according to Yousin et al (1989), "marine fishes have to conserve water and produce small amounts of concentrated urine and consequently their kidneys are either aglomerular (with no renal corpuscles) or with poorly developed renal corpuscles." Hinton and Lauren,(1990),Ortiz et al. (2003), Kurtovic et al.(2008) suggest that in the case of fish the renal lesions might be expected to be good indicators of environmental pollution as the kidney receives largest portion of post branchial blood. Therefore, Hinton et al.(1992); Olivera Ribeiro et al.(1996); chwager (2001) and Pacheco and Santos (2002) consider that " the effects of pollutants on fish kidneys have been studied in some species and the severity of damage seen depends on the sensitivity of the species to the substances released into the environment similarly to the gills and liver."

According to Takashima and Hibiya (1995) "the most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscles, such as dilation of capillaries in the glomerulus and reduction of Bowman's space." The et al. (1997),Thophon et al. (2003) reported that the histological alterations in the kidney of fish were found at the level of tubular epithelium and glomerulus following the exposure of fish to the toxic agents such as pesticides. Yeldrim et al. (2006) reported that the formation of hyaline droplets results from tubular reabsorption of plasma protein lost to the urine by glomerular damage. The degeneration of hyaline in kidney was studied and observed by Boran et al.(2010) in rainbow trout *Oncorhynchus mykiss*, after 0.01 mg/L to 2.00mg/L maneb and 0.20mg/L to 3.90 mg/L carbaryl exposure for 24,48,72 and 96h. After the exposure to lindane, Ortiz et al.(2003) observed the increased space within the Bowman's capsule and shrinkage of the glomerulus. Tilak et al.(2005) reported the decrease in the size of convoluted tubules and formation of vacuoles after 10 days of exposure to butachlor. Thophon et al.(2003) described that frequent exposure to metals causes alteration in the glomerulus and the tubules after exposure to the sub-lethal cadmium in perch, *Lates calcarifer*. Due to exposure of trout, *Salmo trutta* and tilapia, *Oreochromis mossambicus* to mercuric chloride, the swollen cells of bowman's capsule and melano macrophages in the kidney were found by Handy and Penrice (1993). Veiga et al. (2002) found similar type of changes in the fishes exposed to organic contaminants and it was also found by Schwaiger et al.(1997);Pacheco and Santos in mixed environmental contaminants. Srivastava et al.(1990) exposed the fossil cat, *Heteropneustes fossilis* to chlorpyrifos for 96h and reported that necrosis of kidney tubules, dilation of lumina of tubule, shrinkage of glomerular tuft, vacuolation of blood cells in the glomerular tuft takes place in this case. Banerjee and Bhattacharya (1994) reported that after the treatment of elsan for 90 days in case of *Channa punctatus* leads to karyolysis and precipitation of cytoplasm which resulted in a significant decrease in the dimension of Bowman's capsule and glomerulus and loss of the regular shape of the tubule.

Das and Mukherjee (2000) exposed carp *Labeo rohita* to 1/10 and 1/15 sub-lethal doses of hexachlorocyclohexane for a test period of 45 days and reported the tubular dilation, necrotic changes characterised by karyolysis at the nuclei of the affected cells in the carp. After exposure of *Muguli* species, *Cyprinus carpio* and *Barbus* sp. to lindane (γ-hexachloro-cyclohexane-γ-HCH-) Ortiz et al. (2003) found tubular necrosis, vacuolisation and desquamation of tubular epithelial cells of kidney. Lauren et al (1989) fed the antibiotic fumagilin to the rainbow trout, *Salmo gairdneri* and found that there is an increase in of hemosiderin or melanin-like intertubular deposits, tubular degeneration and eosinophilic, proteinacious inter tubular cast and hyaline droplets in the kidney. Hyaline droplet formation results from tubular reabsorption of plasma protein lost to the urine by glomerular damage. Intratubular casts are markers of damage to the tubule cell themselves. The thickening and sloughing of the glomerular epithelium in the kidney was found in the channel cat fish, *Ictalurus punctatus* after an intramuscular injection of gentamycin sulphate (Rolf et al.,1986). Due to the exposure of carp to sub-lethal terbutary, Vesilek et al. (2010) found the cellular alterations such as destruction of the tubules in the caudal kidney. After the chronic exposure to atrazine in case of rainbow trout, Fischer-Scherl et al. (1991) observed there is a change in the renal corpuscles and tubules in the kidney. From DDT-affected area, Mc Hugh et al.(2011)observed vasculature of renal tubules, dilation of glomerular capillaries and generation of hyaline droplets in the kidney of tigerfish, *Hydrocynus vittatus*. Camargo and Martinez (2007) found " glomerular expansion and absence of the Bowman's space and tubule cells with hypertrophied nucleus, tubule



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starting the regeneration process. Occlusion of the tubular lumen and cloudy swelling degeneration in a neotropical fish caged in an urban stream."

Histological alterations in the posterior kidney of white fish fed with high dose diets observed by Ptashynski and Klaverkamp (2002) in their study, they revealed that the kidney may be a target organ for nickel toxicity. In case of control fish, the trunk kidney involved in excretory functions was formed by a large number of nephrons each having a renal corpuscles. The corpuscle was the proximal part of the tubular, which consists of two parts, a glomerulus and a capsule. The histopathological abnormalities in kidney of silver carp, *Hypophthalmichthys molitrix* were the dependant. Hyperplasia in tubules and hypertrophic nuclei and haemolysis of erythrocytes were observed in the kidney of fish treated with nickel for 10 days. In case of the fish exposed to nickel for 20 days, the cytoplasmolysis, vacuolisation and karyolysis were seen in the tubules of the kidney. In case of 30 days of treatment, at the end of 30 days of treatment, syncytical condition and pyknotic nuclei with aggregation of nuclei and ruptured cells were seen due to the damage of plasma membrane. The structure of glomerulus was disrupted and the convoluted tubules and uriniferous tubules were enlarged. Athikesavan et al. (2006) exposed the silver carp *Hypophthalmichthys molitrix* to a sublethal concentration of Nickel for 30 days and observed the hyperplasia, haemolysis, hypertrophy, karyolysis, vascularization, ruptured cells and pyknotic nuclei in the kidney in this fish. According to Weber et al. (2003) "the occurrence of dilated tubules appears to be a consequence of dead and dying epithelial cells while a thickening of Bowman's capsule can arise as a result of fibrosis." Palet al. (2011) observed the fish exposed to dietary heavy oil and found the dilation of the capillaries of glomerulus, haemorrhage in the Bowman's space in the renal corpuscles of exposure groups and necrosis of the epithelium of the tubule in the kidney. Similar structural changes were also observed by schwaiger et al. (1997) in brown trout, *Salmo trutta*, *F. favio* and stone loach, *Barbatula barbatula* and in *Astyanax altiparanal* by Silva and Martinez, (2007), collected from polluted urban stream containing high amount of PAHs and other chemicals. Banaee et al. (2013) exposed the rainbow trout, *Oncorhynchus mykiss* to 0.1 and 0.2mg/L of diazinon for a period of 28 days and observed glomerular lesions, enlargement of space inside Bowman's capsule, shrinking of glomerulus, decreasing of the tubular lumen, necrosis and degeneration of tubules in the tissue of kidney in this fish. Gabriel et al. (2007) studied African catfish, *Clarias gariepinus* with a dose dependent relationship to fuel oil and reported the tubular necrosis in this fish. It was observed from the investigated sites that the histological alterations in the posterior kidney of *Astyanax altiparanae* were in complete contrast to those from the reference site in respect of type, severity and number of lesions observed. The changes of tissues in the reference fish were light where as the lesions found in fish from the studied stream were more severe and in some cases irreparable, such as necrosis of epithelial cells of the tubule which reflects the poor quality of water of this urban stream. In general, morphological and histological structure of the kidney of fish is well-studied. However, on the basis of the observed literature we can say that in the histopathological studies, the kidneys are not as widely as the gills and liver but yet the results are very useful in terms of contamination of water and its effect on fish.

CONCLUSION

As a whole, we can conclude here that the changes in histopathology in target organs of fish such as the gills, liver and kidneys have been successfully applied in ecotoxicological research and risk assessment programmes in the last few decades. Even though, these biomarkers are non-specific, time-consuming (but relatively cheaper) and require knowledgeable and well trained experts, they provide accurate data on the effects of different contaminants which on the other hand, can signal for alterations at lower biological organizations. We therefore, suggest that histopathology should be included in the monitoring programmes on contaminated aquatic ecosystems along with other biomarkers and analysis of water and sediments.





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Application of Nanobiosensors in Detection of Mycotoxins

Ravikiran Regeti^{1,2}, Raghu Gogada² and Praveen Boddana^{1*}

¹Department of Plant Pathology, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati, Odisha, India.

²Department of Biochemistry and Plant Physiology, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati, Odisha, India.

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*Address for Correspondence

Praveen Boddana

Department of Plant Pathology,
M.S. Swaminathan School of Agriculture,
Centurion University of Technology and Management,
Paralakhemundi, Gajapati, Odisha, India.



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ABSTRACT

Mycotoxin analysis in food and feed is essential to ensure the food quality and safety. Major mycotoxins include aflatoxins, deoxynivalenol, ochratoxin, zearalenone, patulin and fumonisin. Most of the mycotoxins proved as strong carcinogenic agents such as AFs B1, patulin etc..., others are under suspicion to have carcinogenic effects. Mycotoxin-contaminated food and feed pose a serious threat of mycotoxicoses. The conventional methods are constantly improving, advanced research trends are looking for innovative solutions. Nanotechnology approaches are most promising, effective, and low-cost way to minimize effects of mycotoxins. Nanomaterials implementation in the fabrication of nanobiosensors and their use for the detection of the mycotoxins in food and feed is the key of interest in this review

Keywords: Nanobiosensors, Nanomaterials, AFs B1, patulin, mycotoxicoses.

INTRODUCTION

The word mycotoxin originated from the greek word mukos meaning “fungus” and latin word toxicum referring “poison” is secondary metabolite produced by fungus, that colonizes on crops either during harvesting or mycotoxins affects agricultural products and most importantly cereals and cereal-based foods. Most commonly found molds produce and excrete mycotoxins. Around 300 and 400 different types of mycotoxins are known today, the acute toxicity of these mycotoxins results in serious human and animal health problems. Mycotoxins cause deleterious health effects and their levels have been strictly regulated especially in food and feed.



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Food quality and safety significantly through the use of advanced micro and nanosensors and tracking systems. The development approaches of micro or nanosensor devices for analysis of toxins is increasing due to their extremely attractive characteristics and applications. The novel electron transport properties make them highly sensitive at low level detection. Nanotechnology research is interface between biology, chemistry, material science, physics and engineering where ultra precision engineering can be combined with nanostructured materials and molecular manipulation to produce novel device. Lab-on-a-chip devices are examples of micro/nanotechnology systems approaches which can be used for the analysis of food contaminants such as mycotoxins for on- site analysis. These devices can be cost effective and highly beneficial for the food industry in ensuring high safety and quality of the food and also for risk assessment and management. The use of nanomaterials such as semiconductors and conducting polymer nanowires, and nanoparticles (silica nanoparticles, carbon nanotubes, nanoparticles quantum dots, dendrimers, gold nanoshells, noble metals nanoparticles super paramagnetic, polymeric nanoparticles) for biosensor applications are expanding rapidly.

BIOSENSORS

Biosensor is defined as a bioanalytical device incorporating a molecular recognition element associated or integrated with a physicochemical transducer (Tothill & Turner, 2003). Simpler and faster analytical procedures based on biosensors has emerged in scientific literature as a very promising alternative. Biosensors are combination of biological component with a physico-chemical detector or transducer may vary from optical, piezoelectric element, electrochemical etc. the transducer transforms the signal received from interaction between analyte and biological these signals displayed by signal processor into user-friendly manner digital output signals. The specific mycotoxin recognized of interest, the optimal recognition materials (sensing receptor) need to be integrated on the surface of the sensor. Especially the important of small molecular weight toxins with diverse structural similarities as the sensitivity and specificity of the sensing molecules will play an important role in the range of the different types of sensing receptors are used as sensing layers in biosensors reviewed recently.

Types of biosensors

There are different types of biosensors such as optical biosensors, electrochemical biosensor, enzyme-based biosensors, immunochemical biosensor in which monoclonal and polyclonal antibodies are used (Baeumner, 2003. Gaag et al. 2003). SPR biosensor was developed by the use of SPR (surface plasma resonance), which has been proved as the most reliable and promising biosensor for detection of AFs. The principle of surface plasma resonance biosensor is based on the binding of biological molecules on a thin metal film will acts as a sensor. The activity of the sensor depends on the binding and dissociation of interacting molecules at the metal surface responsible for the mass concentration changes. These change are measured by the detector element. The free electron of sensor metal plate absorbs the light of specific wavelength at a specific angle of incidence, than the intensity of the reflected light decreases. This phenomenon is dependence on the angle of incidence change because of changes in the mass concentration on the surface of the sensor. Surface plasmon resonance (SPR) biosensor developed by (Gaag et al. 2003). The principle of surface plasmon resonance (SPR) developed for the analysis of four types of mycotoxins. Specific antibodies are immobilised on the sensor surface to which four serially connected flow cells are attached, than the analysis of the four different types of mycotoxins at a time is possible. Electrode based biosensors developed by Tudorache & Bala, (2007), whole cell-based biosensors developed by Baeumner, (2003), whole organism-based biosensors, amperometric biosensors, acoustic biosensor, potentiometric biosensors, optical biosensors and colorimetric biosensors developed by Rana et al., (2010).

Nanobiosensors

Nanobiosensors are basically the sensors which are made up of nanomaterials, the nanotechnology advancement is the development of nanobiosensors from existing biosensors. Traditionally used transducers in biosensors replaced by nanoscale devices. These nanobiosensors ultrasensitive due to involvement of nano material in it. These are the

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materials with dimensions between 1 and 100 nanometers. The size of these materials makes them special as they have most of their constituent atoms located at or near their surface and have all vital physico-chemical properties highly different from the same materials at the bulk scale. Sensing mechanism of the biosensor technology can play very efficient role. Many nanomaterials have been developed on the basis of their electronic and mechanical properties for their use in improved biological signaling and transduction mechanisms. Different kinds of nanomaterials such as super paramagnetic nanoparticles, CNT, metallic nanoparticles including gold, dendrimers, quantum dots, silica and other noble metal nanoparticles, etc. are used in development of nanobiosensors (Otlés & Yalcin, 2010). Different types of nanomaterials can be used for different applications of nanobiosensors. Many factors which govern the use of a particular kind of nanomaterials for biosensing applications. These factors are the ingredients of their chemical and physical properties along with their energy sensitive and selective responses. Nanomaterial enhances activity of nanobiosensors at the level of two elements of biosensors, at bioreceptor level and at transducer level. Because of the high surface area to volume of nanomaterial, can act better at bioreceptor level by providing more surface area for biomolecules attachment and pose an ability to detect target biomolecules even at low concentration. The use of CNT in biosensors demonstrated by Katz & Willner (2004). The hybrid semiconductor nanoparticles and biomolecules will form nanowires and used as nanobiosensor. This phenomena of nanoparticles integration into biomolecules helpful for the fabrication of bio-electronic devices and lead to research for bio-nanomaterials (Tothill, 2009). The potential use of nanotechnology in food and feed is to monitor the conditions of packaged food and feed during storage and transport with the help of nano sized sensor incorporated into them (Chaudhary & Castle 2011).

TYPES OF NANOMATERIALS

Nanoparticles

Metallic nanoparticles are major area of interest as they possess numerous possible applications in the development of nanobiosensor. The electronic, chemical & physical properties of nanoparticles make them different from the bulk metal properties. Nanoparticles used in the immunosensor assay developed by Paniel et al. (2010) for detection of AFsM1 in milk. Super paramagnetic nanoparticles are used to provide the surface for binding the conjugate of AFsM1-horseradish peroxidase (AFM1-HRP) and antibody anti-AFM1. The complex has an ability to attach the magnet because of super paramagnetic nanoparticles. Will helps to separate AFs present in milk and gives their concentration on amperometer. Application of different nanomaterials in nanobiosens studied by Zhang et al. (2009). Gold nanoparticles have immense value in development of variable nanobiosensors for quantitative detection of polyionic drugs like protamine and heparin. Liu et al. (2008) used gold nanoparticles for the synthesis of gold immune chromatographic strips for detection OTA from coffee.

For the preparation of nanobiosensors Fernandez-baldo et al. (2011) used magnetic nanoparticles, which are used for the detection of OTA. Nanoparticles help to enhance the immobilisation of biologically active elements available in microfluidic system for the fast and accurate detection of OTA in apples. ZEA mycotoxins detection, based on gold nanoparticles immunochromatographic (ICG) assay by using monoclonal antibodies. For on-site screening test of ZEA, ICG strips are very accurate, sensitive and rapid. Detection limit is 30 mg/kg in corn. These biosensors developed by Shim et al., (2009). Magnetic nanoparticles for immobilisation on graphene oxide and formed magnetic nanocomposites. These are used as bioreceptor for adsorption of AFsM1 biomolecules. Nanomaterials when applied at transducer level, it may increase the electron transfer by behaving as an active quencher of electron and improves the signal amplification. It, enhances transducers activity (Gan et al., 2013).

Gold Nanoparticles

Physicochemical properties of gold nanoparticles, such as fluorescence quenching, electrochemical activity, surface-enhanced raman scattering (SERS), and localized surface plasmon resonance (LSPR). Gold NPS shows intense surface plasmon absorption bands, it facilitates easy adoption of these particles in the colorimetric aptasensors. And



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they exhibit size and distance dependent optical properties which is of great interest in colorimetric aptasensor design (Amina Rhouati et al., 2017). Gold nanoparticles provide suitable environment for the biomolecule immobilization and facilitates the electron transfer between immobilized sensing probes and electrode surfaces, which resulted in intensive use of gold nanoparticles for development of electrochemical aptasensors with enhanced analytical performance (Xie, d.et al., 2014; Taghdisi, S.M. et al., 2016). The gold nanoparticles is also exploited for forster resonance energy transfer (FRET)-based aptasensors. Gold nanoparticle based fluorescence aptasensors have been employed to detect mycotoxins such as ochratoxin (Liu,Y et al.,2016), and aflatoxins (Sabet, F.S et al., 2017) based on different mechanisms. Mainly the nanoparticle have been employed to mediate the quenching of fluorescent dyes, increasing the sensitivity and efficiency of the assays. The two main approaches that have been utilized: (a) molecular beacons (b) hybrid structures with DNA.

Carbon Nanotubes

In 1991Iijimadiscoverednanotubes, a japanese scientist followed by the development of laboratory synthesis of fullerenes. To synthesis of the CNT, pyrolysis of hydrocarbons on metal surfaces is done. The four-step mechanism of the CNT synthesis research showed by (Rao et al., 2001).Fullerenes, single-or-multi-walled carbon nanotubes, and graphene are called carbon-based nanomaterials (Cha, c et al., 2013).Researchers applied in various apt a sensing designs with electrochemical, fluorimetric, colorimetric, outcomes for detection of different analytes. Graphene employed mostly in electrochemical aptasensor designs, nanotubes are mostly assayed by monitoring the fluorescence response. Extraordinary electronic transport properties of individual graphene sheets have been demonstrated in several areas since the exfoliation and characterization of graphene have shown in 2004(Novoselov, K.S et al.,2004).It has found widespread application in electrochemical analysis owing to their unique conductivity (Zhang,Y et al., 2005).Carbon nanotubes can act as quenchers for different fluorophores by electron transfer process, which gives low background with high signal-to-noise ratio (Zhu, Z et al., 2010).The fluorescence quenching is led between nanotubes and fluorophore labeled aptamer because of the π - π stacking interaction between the carbon nanotubes and DNA bases of the aptamer once they are close to each other(Wang,S-E&SI, S 2013).Signal will be recovered by addition of the target molecule, or complementary strand of the ssDNA.

Guo et al. (2011) developed fluorescent aptasensor, which is selective and sensitive for OTA, SWCNT act as a quencher of fluorescence made by carboxy-fluorescein attached aptamer specific to unfolded free molecules of toxins. MWCNT used in amperometric nanobiosensor developed by Zachetti et al. (2013) for the detection of CIT toxins contamination in the rice samples. They used MWCNT containing redox mediators for the carbon paste electrode formation, which is again coated by dialysis membrane were used for cit detection. The carbon-carbon chemically covalent bonds and crystalline structure gives specific properties such as elasticity, strength, and great conductivity. The novel adsorbents of mycotoxins are graphene oxide,graphene, nanodiamonds, fiber, fullerenes, and nanotubes have great potential. The nanocarbon structures are amphoteric and surface of the these structure could be protonated or deprotonated in which results in the binding capacity of nonpolar or polar compounds. The nanotubes peculiarly carry the structure-dependent mechanical and electronic characteristics, which make them to develop potential for various field applications. The multiple use of CNT in the development of superior quality of electrochemical nanobiosensor for the mycotoxins detection in food studied by Subramanian & Jerzy (2008). Gan et al., (2013) applied cadmium telluride-CNT (CDTE-CNT) for the detection of AFM1 in milk. The CDTE-CNT are used to form signals tag in sensor development by attaching the AFs M1 antibodies on their surface. Mycotoxin STE can be detected by electrochemical biosensors, which are designed with the use of CNT (Yao et al., 2006).

Nanorods

Gold nanorod (GNR) used by (Xu et al. 2012) for the fabrication of optical biosensors and these GNR are applied at bioreceptor level. The sensor is developed to detect the AFs B1 with a high sensitivity up to 0.04 ppb in the samples, this nanobiosensor with low detection value is the greatest achievement in the field of nanobiosensor development.(Xu et al. 2013)report showed the label-free construction of optical biosensor requiring minimum time with a single-step assay for AFs B1 by GNR competitive dispersion. GNR are used as a platform for sensing purpose

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because of its high ionic strength and stability and it does not require any stabilising agents. (Wang et al., 2010) have synthesised electrochemiluminescent aptamer biosensor by some modification on gold electrode (modified by gold nanoparticles) using a label n-(aminobutyl)-n-ethylisoluminol for the detection of OTA.

Quantum dots (QDs)

QDs are bright NPS which have been applied frequently as fluorescent probes (Wegner, K.D.&Hildebrandt, N 2015) also known as zero dimensional particles. The special photophysical properties such as wide absorption spectra along with narrow photoluminescence spectrum. QDs present 10–20 times more brilliant fluorescence and their photo durability is 100 times better than organic dyes(Chan, W.C.& Nie, S.1998), which make them one popular choice in designing fluorescence based aptasensors. QDs are often used as FRET donors for organic dye acceptors (Petryayeva, E et al., 2013) and their emission can be size-tuned (Martin-Palma, R.J. 2009). Different-colored QDs by a single light source, and they will still produce specific, narrow and symmetric emissions of different colors, which can be point of interest for multiplexing and array development (Hayat, A. et al., 2013).

Antifungal nanomaterials cause mold inhibition and mycotoxin production

By mediating antifungal nanoparticles can prevent mycotoxin, its easy to produce in large scale. Orientation of antifungal strategy is in two directions. 1. antifungal compound is encapsulated into a polymeric nanocage. 2. Inhibition effect will reach by nanoparticles alone. This method mainly depends on metal nanoparticle which is stable, act immediately, and offer the possibility of green synthesis. The green synthesis is the main advantage to form nanobiocomposites by using micro-organism, plant, and animal sources which show less toxicity and improved their main features. The main disadvantage of this method is the instability in air, although nanopolymers allow cargo release under the appropriate conditions like wise higher temperature, ph change, presence of enzymes.

Ideal properties of nanomaterials

Nanoparticles are noble metal nanoparticles, nanowires, nanoshells, quantum dots, polymeric nanoparticles, nanotubes, etc. (Katz & Willner, 2004; Malhotra et al., 2014). Detection of one or more multitoxins at a time is possible (Gaag et al., 2003). Time required and minimum assay time, for mycotoxins assay is low, about 4–8s for constructed nanobiosensors (Xia & Ning, 2011). Sensitivity is higher for mycotoxins even at low concentration, 0.5nm concentration of tyrosinase enzyme for tyrosinase biosensor (Yotova et al., 2013). Its cost-effective (Xu et al., 2012). The unique feature is portability, nanobiosensor are portable and they can be use for on-site mycotoxins detection in fields or in storage or at retailers shop (Moon et al., 2013).

Detection of mycotoxins

Mycotoxins detection in the food industries, there are listed many nanobiosensors for contamination in food or any gases released due to microbial growth within the packaged food. Zhang et al., (2014) developed fluoro immunoassay by using quantum dots as fluorescent label, for AFsB1 detection by conjugation of monoclonal antibodies and CDTE quantum dots. This nanobiosensor is developed to detect contaminants and their secretion in peanuts. To detect OTA, procedure and synthesis of nanobiosensors reported by wang et al. (2010). For the detection OTA from contaminated wheat grains gold electrode is altered by GNP and the n-(aminobutyl)-n-ethylisoluminol is specifically labeled. Paniel et al., (2010) developed electrochemical biosensor by using magnetic nanoparticles coated with antibodies specific for AFsM1 from food. Horse radish peroxidase (HRP) was used as tag for the competitive immunoassay and it gets linked to AFs M1 to form a conjugate, which is used as a tracer. This sensor worked on the basis of competition for antibodies between tracer and antigen (AFs M1). The equilibrated mixture is deposited on carbon electrode to which mediator MPMS (5-methylphenazinium methyl sulphate) will be added. Enzymatic reactions will be takes place at the electrode are detected by amperometer. The detection limit of such sensor is 0.01 ppb having good reproducibility. The conventional optical biosensors are modified by the use of nanomaterial like GNR and proved better in the sensation purpose for AFs B1 at very low level of mycotoxins existence 0.04 ppb in food commodity (Xu et al., 2012).



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Yao et al. (2006) developed a novel nanobiosensor for the detection of STE using CNT, sterigmatocystin (STE) is the second most important mycotoxin. The modified CNT by the binding of AF-detoxifzyme, AF-detoxifzyme deposits on the electrode of MWCNT and it gives the notable signals, which helps to detect mycotoxin. To detection of STE, the enzyme AF oxidase gets immobilised on chitosan-SWCNT, which was attached to poly-ophenylenediamine electrode modified by gold. This electrochemical nanobiosensor have the ability to detect the mycotoxin at very low concentration up to 3 ng/ml (Chen et al., 2009). Optical biosensor by using GNR dispersion on a competitive basis for detection of AFs B1 was constructed by (Xu et al. 2013). GNR dispersion also increases and absorption intensity of UV-Visible spectra varies followed by the concentration of AFs B1 increases in solution. The changes in the spectra and hydrodynamic size of GNR are recognised as indicator of sensors. This strip assays with nanoparticles used for conjugation to detect OTA. The strips were designed on the basis of competitive assay between OTA in food and OTA-BSA conjugated on the surface of gold nanoparticles with the immobilised OTA antibodies. Moon et al., (2013) fabricated immunosensor based on micro fluidic competitive assay, in which platform for immobilisation of biomolecules was used magnetic nanoparticles (MNPS) for OTA quantification (Fernandez-baldo et al. 2011). GNP immunochromatographic strips were constructed for the detection of OTA in coffee samples (Liu et al. 2008).

For OTA detection electrochemical biosensor is developed by the use of MNPS in combination with square wave voltammetry (Fernandezbaldo et al., 2010). Modified aptasensor based on SPR principle for OTA by the use of GNR, is the selective in mechanism and can be reused for seven times by giving the heat treatment at 70c in methanol each time (Park et al. 2014). Nanobiosensor by the conjugation of gold electrode coated with modifier developed by(Evtugyn et al. 2013). The modifier is developed by electro-polymerised neutral red and suspension of dendrimeric polymer botlorn-h30 with GNP, this apparatus runs on the basis of OTA-specific aptamer form a bonding between sulphur and gold from gold nanoparticles, and thiolated aptamer, respectively. Their interaction leads to the conformational changes and makes aptamer to detect OTA the range of 0.02 nm concentration. The applicability of different nanomaterial for OTA detection reviewed by Hayat et al. (2013). They reviewed on magnetic nanoparticles, quantum golddots and carbon-based nanomaterials for the fabrication of aptasensor specific for OTA. Yuan et al. (2014) developed manganese dioxide nanosheets for OTA biosensing purpose by the use of ssDNA probe. Silver nanocluster with combination fluorescent DNA scaffolds for the detection of OTA at 2pg/ml concentration developed by Chen et al. (2014). SPR nanobiosensor fabricated for fumonisin detection by using polyclonal antibodies adsorbed on the gold film substrate coupled with glass prism in the kretschmann configuration is developed (Mullet et al. 1998). Immunochromatographic biosensors are favourite due to the use of monoclonal antibodies in its design, which makes them function specific. Immunochromatographic nanobiosensor (strips) developed by Shim et al. (2009)by using monoclonal antibodies functionalised with GNP (ICG). These ICG strips are found to be useful tool for onsite and rapid screening of ZEA in corn samples. Because of the small size and minimum assay time, these ICG strips are applicable for onsite detection of mycotoxins.

Maragos (2012) developed a biosensor based on the bilayer interferometry principle, in which colloidal gold is used for the amplification of signals received from transducers for the DON in wheat. For the to detection of mycotoxin in rice samples Zachetti et al.,(2013) developed biosensor. Contamination of rice by citrinin was detected with amperometric nanobiosensor by using MWCNT and some other reagents. This nanobiosensor showing reproducibility, repeatability and good correlations in spectrophotometric and electrochemical methods. Simple optical method to detect mycotoxin PAT in apple juice based on MIPS capped MN-doped ZnS quantum dots (MIP-QDS). The nanosensor fabricated by using 6- HNA as the dummy template, 3-aminopropyltriethoxysilane (APTES) as the functional monomer and tetraethoxysilane (TEOS) as the cross-linker through the surface imprinting process. This optical sensor selectively recognizing and binding the target PAT from different analogues 2-HNA, CA and 5-HMF and various mycotoxins like AFs B1, OTA and DON. The impact of media pH, dosage of sensors and incubation time on phosphorescence quenching of the nanosensor was investigated. A phosphorescent method was developed in aqueous solution for determination of PAT. The proposed method was feasible to detect PAT in real apple juice with consistent accuracy compared to HPLC (Wengang Zhang et al., 2017).





Advantages and limitations of nanobiosensors for detection mycotoxin

Nanosensors can improve food, feed and environment sectors including food processing, agriculture, air and water quality monitoring, and packaging and transport. Increased mycotoxins contamination, required monitoring and its control. Due to their sensitivity and tunability multi array sensors based on micro and nano systems are the best tools for diagnostics and risk assessment for the researchers (Tothill, 2011). On-site detection of mycotoxins by nanobiosensors are able to provide rapid, robust and cost-effective quantitative methods due to development of ultrasensitive devices in nanobiosensors. Highly sensitive have ability to detect even ultra-low concentration of mycotoxins in food and feed. Its required comparative less manpower, etc. (Kerman et al., 2008; Otles & Yalcin, 2012; Tothill, 2009).

Nanobiosensors has some limitations also, to develop successful commercial products that should compete with traditional methods of mycotoxin analysis. Mainly the complex molecular structures of toxins present in wide range of concentrations need to be detected. Similarly, there is need to increase and maintain the stability of some nanomaterials like quantum dots, which reduce the aggregation in use conditions and also should reduce their cost. In spite of all the characteristics and advantages of nanobiosensors, there are no reports on the controversy for the use of nanobiosensors in detecting mycotoxins presence in food. But, need to focus on its controversial part such as stability and nano-wastes from fabricators. However, these problems can be easily resolved by ensuring the control and limited use of nanobiosensor and also focus needs to be given on its safe disposal after use.

CONCLUSION

Nanobiosensors technology has been proved to be a very significant blessing in the development of biosensors. In this review, summarized most of the findings of mycotoxins detection by nanobiosensors. Many researchers find the potential and the exceptional properties of nanomaterials. The combination knowledge of disciplines from nanotechnology, toxicology, and agricultural practices is on the rise of nanobiosensors development. Nanobiosensors development is the sophisticated for mycotoxins detection in food and feed. Further research should carry on to investigate the detailed toxicity and efficiency testing as is also required in other nanotechnology disciplines. It will bring a promising resource towards the public health concern for mycotoxin-free food and feed.

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Free Learning Day, Celebrating Democracy in the Academic Learning Setup

Suchismita Nayak* and Amiya Singh

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Suchismita Nayak

Centurion University of Technology and Management,
Odisha, India.

Email: suchismita.nayak@cutm.ac.in



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ABSTRACT

Learning is an ongoing process. Learning helps in obtaining the ability to understand, grow knowledge and develop skill of an individual student. The transformation which occurs by learning remains forever with an individual. When democracy is added in the learning process it drives the democratic merit in the education and bring positiveness in the learning process. This article completely focus on free learning, where the pupils are given a chance to learn as per their interest area is concerned and to gain relevant knowledge. The article here exhibits and highlight that free learning is a democratic learning activity which results in forecasting the efficiency of the learners and brings vision to imagination or creativity of this learning affair with positive outcomes.

Keywords: knowledge, democracy, leaning, students, 21st century.

INTRODUCTION

Education is an enlightening experience and learning is the acquisition of knowledge or skills through study, experience, or being taught. Before, the conventional education method was in great demand in schools, colleges and even in universities. Conventional or customary education principle idea is to pass on the next generation, the values and etiquette and community based practices. These teachings and learning are very much beneficial to all individuals and now in this 21st century it is being followed by all educational sectors from schools to universities but the dilemma in the traditional method of learning is that the students rarely get freedom to speak out and share their views and ideas, they also become less focused [1]. Lack of limberness in the traditional or customary education fails to cater the need of 21st century students. A democracy in education system is very essential. The students learn at a faster rate if a bit democracy is given and that empower them to showcase their talents by participating in activities based on their subject matter, exploring their own field of interest as well by sharing their ideas [3] [4]. We all know that we are living in the modern world and similarly our education system is also becoming modern day by day



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which entail us to move accordingly. It is the demand of the modern century to provide democracy in education system which can bring flexibility in learning process and now most of the educational organisations are following this technique which is considered to be the most effective one in enhancing the skills of the students. In this context, Centurion University of technology and management, a multi sector university provides "Choice Based Credit System" (CBCS) system for the students, who have full flexibility to choose the subject of their choice of the courses offered. This system somehow dispense the democracy to the students to select the subject papers they are interested for which help the students perform better as they are not forced or pressurized to take the subject they don't want.

Setting the context: Centurion University as an accouchement to innovative learning approach

Centurion University of Technology and Management, enacted by the State Legislature of Odisha is a young multi sector university that extend freedom to the entire team as well as the students to use their creative mind set, input the design in a pragmatic way and bring on prolific upshots. The belief, trust or the confidence this young university has on its unit or team, works as latent qualities or abilities that supports for the development of new pedagogies with alternative method of teaching and learning that lead to future success or usefulness. Here this article talks about the free learning day, a democracy in learning to motivate, inspire and develop self-determination among the pupil who adore and enjoy learning. Free learning day can be considered as a new learning methodology that proves if students are given opportunity to do something worthwhile, which not only develop consciousness but also ignites the spark for skill development further resolving the unemployment factor, a matter or situation regarded as unwelcome or harmful and needing to be dealt with and get the better out of it. Centurion University demonstrate that acquiring or allowing new teaching and learning approach works as an incantation for the upgradation of the students keeping in mind the future perspectives essential in this 21st century.

Project Conceptualization

Multitudinous specification are there of free learning in different educational organisations all over the globe. The evolution of free learning day took place back in the month of January 2020. It was declared that all the team members of school of vocational education and training has to participate in an organizational meet, which was organized by Gram Tarang, a social out rich of Centurion University. This organizational meet happens every year, but this time it was a like challenge for the school of vocational team members to participate in the annual meet. The problem was, the meet was scheduled on a working day and that day was not declared as a holiday and the students were supposed to come and attend the classes as usual. Here the question arise, how the students will be kept engaged in a productive way, as all the faculties including the office staff of school of vocational education were to attend the annual meet conducted by Gram Tarang. So finally it was decided by the head of the school to convert this structured academic day in to a free learning day a, a day of democracy in learning. The idea was that the students were to completely let free in that particular day to search and select their field of interest and to attend any class or workshop they feel like, as for example photography, welding workshop, ceramic workshop, electrical workshop, wood engineering workshop, fabrication and automotive workshop, textile apparel center and even horticulture and dairy farm within the 40acres area of the Centurion university. It sounds quit interesting and amazing that in this free learning day, there was no structured syllabus and no academic timetable to be followed by the students. At the same time it was also necessary to keep a track of this free learning day to be an outcome oriented. Whether the students will utilize it in a positive way or not, the outcome was to be presented by the students the very next day. The outcomes to be recorded and presented by the students were as follows:

- (i) Their attendance proof
- (ii) The record of the whole day activity from 9:30 a.m. – 5:10 p.m.
- (iii) To prepare a presentation on; Why they opted that specific area, what was their learning outcome after attaining the class, workshop of their own choice including horticulture and dairy farm and how their approach towards their interest zone can be a part of their skill development and enhance their career in near future.



**Suchismita Nayak and Amiya Singh****Free learning as a democratic learning**

Education has a broader meaning. It can be considered as an act of learning interesting and new things. Yes it is true going to schools, colleges and universities with a prescribed syllabus helps an individual to acquire knowledge to be certified. It has been observed that there are subjects in which the students lack their interest but as designed and being a part of the syllabus or curriculum they have to go through those subjects whether they find it interesting or not, to be certified for further higher education and for jobs, whereas there are certain areas in which the students have interest but unable to explore and work on it as because it is not in their syllabus. World is changing, accordingly everything is changing swiftly including the education system. The syllabus structure, the teaching and learning methodology are changing keeping in mind the needs and demands of the present generation. In some point of times it is also necessary to give freedom to the students to come out of the academic structured curriculum boundaries and to explore something new and interesting from their point of view and which creates a positive vibe within them and can also inspire and motivate them to be adventurous, creative and to do something worth while [3][4]. Yes free learning can be considered as a new learning methodology where students are completely free to participate or attend a class or workshop of his or her choice. It is a democratic learning platform, where students are given opportunities to explore the areas of interest they have within them. This free learning approach which was initiated by the school of vocational education, a unit in Centurion University has lots of positiveness in it like: Interest Level Inspiration, Stress Release, and Social Skill Development.

Interest Level Inspiration

As we all know that each and every individual has his or her own interest to learn something of their choice and to use it in a productive way. But if we take into account students hardly get such types of opportunities to focus and work on their interest areas because of bulky academic curriculum and of long scheduled classes with lots of classwork and homework. But if they get a chance or scope, they can prove their efficiency, the skill they possess and also can succeed in designing their own career creating employability opportunities for themselves and also creating jobs for others [7]. Free learning is such a platform which can inspire the pupil to focus on their personal interest of learning. Motivation by faculties and educational organization can inspire them to work on their interest areas. The students took this opportunity in a very positive way [3]. They were very much happy and got inspired by the decision of this free learning day, because they got a scope to explore their interest and to learn something which was of their choice and not in their syllabus. This free learning day inspired them to know and learn what they want and the best part of it was they were free to do so. The encouragement from trainers in the workshop increased their interest level to know more about that particular subject matter even if it was not in their syllabus.

Stress Release

Free learning is really a stress release booster for all students, where they are free to learn or explore in those areas which is not in their course but of their choice and interest. The students feel free and relaxed as they get an opportunity to take a break out of their regular academic schedule. Doing something new always release the pressure and makes an individual happy which works as a rejuvenating mechanism for the body and mind [8]. This rejuvenation increases positiveness in an individual's thought process and build in the creativeness and keeping the mind free from regular day routine. Engaged in an enjoyable activity which we have interest and liking make us tireless or anxious while working on it. Relaxation is a disposition of mind where we feel calm and can get by our stress or anxiety. Here free learning which was decided to keep the students engaged in the field of their choice also worked as patent medicine for the students to release their stress.

Social Skill Development

The students need to be a social butterfly. It is very much important for every individual to improve their social skills which are very necessary for life itself. Free learning helps students in developing social skills and when honed well leads to contentment [2], release stress and also helps to magnify or uplift self-esteem as well as interpersonal relationship [9]. Development of social skill in the students help them to strengthen their communication skill with



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their peer and as well as with their trainers and lecturers which help them to identify individuals and develop better relationship leading to explore in the interested field. Without communication skill it becomes difficult to exercise on any task given or to do something of our own interest. Yes it's true that social skill makes an individual student to be efficient enough to advance in career prospective.

Responsibility Development

It is important for every individual to be responsible. Specially the students must have an obligation to do something worthy [19]. Free learning invigorate the students to showcase on what they are learning and how they are learning it. This unconventional way of learning induce the pupils to accept responsibility for decisions they make about learning something new and productive. Being responsible and accountable is considered to be most distinctive for a student to perform a task and to excel in it. This free learning platform enables the student to be answerable and responsible for what they learn and to what degree the learning will be related to the skill development as well as taking employability into account for their own development as well as taking into account the betterment of the society [20].

Free learning, a root to student- oriented learning**Query Based Learning Method**

Questioning, especially one expressing doubt or requesting information leads an individual to the depth in a particular subject matter. It acts as a pillar for the students in their learning process. The thirst of gaining knowledge in an area of interest, enhance and encourage the students to ask questions to themselves and try to equate the issues accordingly. This type of learning technique works as a skill developing mechanism, for the students and they participate in it actively [10]. It will be not wrong, if we say this process of learning can be aired with the free learning method. Query based learning approach is considered to be one of the positive sign towards the development of the skill of a student in the field of his/her interest and at the same time boosts the lecturers and trainers to have more and more conceptual as well as practice based knowledge to cater the needs of the students[16]. Facilitation is also the key aspect of this query based learning method. As students have interest in different discipline and accordingly their research on that particular subject matter take them to another level ahead [17]. By attaining the maturity level to study, research and excel in that specific domain, they also contemplate how their previous findings and skills can be associated to other area of inquisitiveness. It has rightly been said by great people, "Interest on subject matter frequently evolves in inquiries leading to steam into a new zone of knowledge".

Personalized Learning Method

Free learning method acts as a root to develop personalized learning. This type of learning give rise to curiosity which later leads to the development of skills. Personalized learning is completely based on student's choice and their willingness to learn something new and which sounds interesting to them and which is beyond their structured syllabus. In personalized learning, the plans made to accomplish or reach the desired level is generated by students. Personalized learning motivate the students to have possession on the subject matter in which they have interest and how to reach the target level [11][13]. Students get more self-assessed about their strength and incapability's and try to fill-up the gaps, learn what they want to. The highlighted part of personal or personalized learning, which is a gift of free learning, is that it enables the flow of logicity and consistency with clarity in the student and increase the focus to work on the decisions taken by them on the specific subject matter or technology and grow their skill. Personalized learning is the evidence of progress in the interest area by promoting learning achievement. It enhances the communication skill of an individual by seeking advice, listening and incorporating others in their learning as well as in the skill development process. It also strengthens collaborative working which proves to be more beneficial at the workplace in future. All these entities developed in a student grants success at the end.



**Suchismita Nayak and Amiya Singh****Kinesthetic Learning**

It is a learning process where an individual learn by physically participating in it. Free learning creates the chance to develop kinesthetic learning which can also be named as hands-on learning. This learning method helps the students to participate in doing or creating something productive [12]. This learning process proves to be very much effective because students learn by engaging themselves in any field which is more activity oriented not just only sitting in class room and listening lectures [18]. Here the students get an opportunity to introduce themselves to the selected area and discover new things and acquire new and interesting knowledge out of it which will always last with them till the lifelong. This method of learning is possible if we believe in our students and give them a chance of this free learning or we can say democracy in learning system.

Free Learning and Letter of Gratitude

At first it was simply a thought to engage the students to do something productive on a particular day, where no subject faculties will be present to take regular classes but at the same time, the students were free to attend any class or workshop within the campus to broaden his or her knowledge about their own university as well as about different interesting courses and workshops within the campus. The thought turned out to be interesting, productive with positive outcome. This one day activity, inspired the students a lot and they took this opportunity to convey their gratitude to the head of the school, for giving them such a wonderful day of free learning which turned out to be rewarding.

Good evening sir.

Today is the best experiment day for me

And that is just because of you.

As you have told.

"You are FREE to learn ANYTHING"...

So today I spent my day just like a very special gift which I am opening slowly slowly :) so ,

1. Today I visited one of the Gram Tarang buildings and there I met

Mr. Parthasarathi Mohanty (Sr. General Manager - production), and queried about the role of Gram Tarang and the services and skills it provides as well as about the benefits. Very nicely he explained me and some of my fellow batch mates about # Gram Tarang Employability and Training services Pvt.Ltd, and also about CDG (Coffee Day Global).

2. After that I visited a Gram Tarang workshop where e-rickshaw is made and there I met Mr. Jakesh Ranjan Sahoo. And he also explained very nicely about how e-rickshaws as well as the parts are made.

I hope we will get more free learning day in every week from your side so that we can go through all these things in details because one day is very less time for me.

And at last thanks to give free learning day.

Thanks you sir.

Avinash Kumar

Diploma civil branch.

Centurion University

Odisha

Respected sir

I request you to give me one free day every week because last time I learned many things about campus. But this time I would like to do something creative like I don't know about the MSC course, so I would like to go there and sit with them for one class and learn something about what the course is all about and in the second half I will





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share my experience and try to do something creative.

Thank you for giving me this beautiful day which is very special for me. Thank you once again sir.

Thank you.

Nandini kumari

181207140029.

Respected Sir,

I am Md Altamas (mechanical Engineering) 1st year, writing with a request to you. Ones we got an opportunity to experience free learning day. That day's experience helped me a lot. Finding out new things in my university campus. I request you to give that opportunity again, at least ones in a week. I want to experience some new things in my college. I want to study about the different kind of machines there in the campus. I want to do some extra study of my interest rather than my course please give us this opportunity at least ones a week.

Yours sincerely

Md Altamas

These are the evidences that proves, a belief can act as a great change in developing something new and interesting among the students. And this happened with the diploma students of Centurion University, who tried to utilize the free learning day in a very constructive and effective way.

CONCLUSION

Learning is the process which not only develops knowledge but also brings behavioural changes by experiments and experiences. Free learning acts as a mother which gives birth to distinct learning methods that helps a student to excel in their desired areas. Free learning can be considered as democratic learning because of its characteristics, where the students are let free to study, explore new things and increasing the enthusiasm to learn and do something productive and output oriented for their own development as well as to perform exceptionally well for the betterment of the society.

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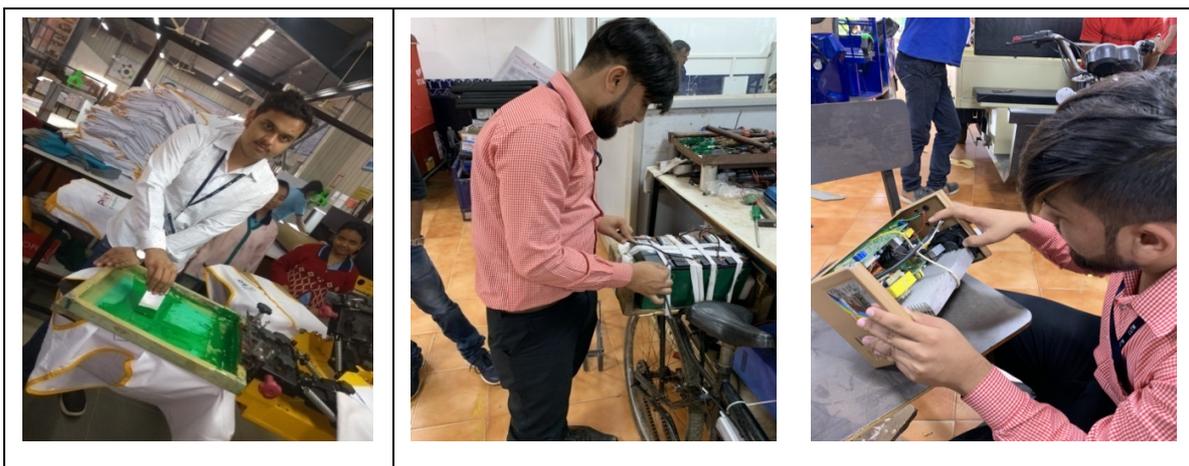


Fig.1. The picture indicates that a diploma student from Civil Engineering branch has interest towards textile

Fig.2. The above pictures indicate that a student of Mechanical Engineering branch showing his personal interest in the configuration parts of E-Rickshaw in E-Rickshaw workshop.





RESEARCH ARTICLE

Occurrence and Distribution of Micro Plastic Litters in the Beaches of Palk Bay and Gulf of Mannar Coast, India.

Hari Vignesh Murugesan*, Ramakritinan C.M, Anand Muthusamy and Santhanakrishnan Malairaj

Department of Marine and Coastal Studies, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Pudumadam, Tamil Nadu, India

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*Address for Correspondence

Hari Vignesh Murugesan

Department of Marine and Coastal Studies, School of Energy,
Environment and Natural Resources, Madurai Kamaraj University,
Pudumadam, Tamil Nadu, India

Email : coolhari019@gmail.com.



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ABSTRACT

Plastics has numerous and huge extraordinary properties; it is easy to access, lightweight, durable and low production cost. All the plastic wastes end up into the marine environment due to improper disposal and management. Plastic has long persistence, mainly due to waves, UV from the sun and by biological degradation, plastics lead to tiny particles said to be the microplastics (size <5 mm). In India, Palk bay and Gulf of Mannar consists of highly populated coral reef islands and the plastic impacts adjacent to the coral reef ecosystem are very scarce. The size wise distribution of microplastics was not dependent on tide, wind, currents and other factors. The present study concludes that the variations in distribution of microplastic litter depends on the macro litters distribution along the beaches. The primary source for the plastic pollution in the Palk bay and Gulf of Mannar beaches were from local fishers, pilgrims, tourists and other local inhabitants. Further the FTIR analysis is performed to find out the functional group of various types of plastics distributed in the Palk Bay and Gulf of Mannar.

Keywords: Microplastics, Beach sediments, FTIR, PalkBay and Gulf of Mannar

INTRODUCTION

The production of plastics in the world wide was reached to 1.5 million tonnes per year in 1950. Currently it is estimated by 250 million tonnes per year and increasing by 10% each year (Plastic Europe, 2008). Stable increase in plastic and other litter usage leads to serious litter pollution along the coastal areas that increase global interest on study of sources, distribution and risk assessment. In addition, anthropogenic, non-degradable materials are well-known for fragmentation in the coastal and marine environments which leads to formation of micro litter particles. Due to their buoyant and persistent properties, these micro litters have a potential to become widely dispersed in the





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coastal marine environment through the hydrodynamic processes and ocean currents. These were not visible to naked eye and it is said to be the micro plastics and the size 5.0mm and below (GESAMP, 2015). These were ingested in a variety of organisms including protists (Christaki et al., 1998) copepods (e.g., Cole et al., 2013), annelids (e.g., Besseling et al., 2013, Wright et al., 2013), echinoderms (Della Torre et al., 2014; Kaposi et al., 2014; Nobre et al., 2015), cnidaria (Hall et al., 2015), amphipods (Thompson et al., 2004, Ugolini et al., 2013), decapods (e.g., Watts et al., 2014), isopods (Hämer et al., 2014), molluscs (e.g., Avio et al., 2015a), fish (e.g., Pedà et al., 2016), and birds (Tanaka et al., 2013). and indirectly consumed by the Human beings. India is among the major plastic consumers in the world and generates approximately 5 to 6 million tons of plastic waste annually. Tamil Nadu is one of the highest dense populated state, of the Indian subcontinent, with Bay of Bengal to the east and the Indian Ocean to the south. In Tamil Nadu, Ramanathapuram is the second largest town by population. Here, the marine environment plays a vital role in the people’s revenue. Ramanathapuram district consist of Palk Bay and the Gulf of Mannar with 21 small islands. Very few studies were carried out the sources and distribution of marine micro plastics in the Palk Bay and Gulf of Mannar Coasts. Therefore, it was aimed to study the occurrence and distribution of marine micro plastic litters in the selected beaches of Palk Bay and Gulf of Mannar coasts, India.

Study Area

Table 1. Sampling sites along palk Bay and the Gulf of Mannar with GPS coordinates

Name of the Landing centers	Latitude	Longitude
Pudumadam	9°16'26.41"N	79°00'04.85"E
Mandapam (South)	9°16'37.41"N	79°09'15.63"E
Mandapam (North)	9°16'36.68"N	79°09'03.78"E
Dhargavalasai	9°19'32.93"N	79°01'19.08"E



Figure 1. Google Map showing Palk Bay and the Gulf of Mannar region with sampling sites

Methodological approaches

In the present study, the quantification of micro plastic litter was done based on the standard protocols developed by Besley et al., (2016) for beach sand. The beach sands from both high tide as well as low ride marks in the selected beaches such as Pudumadam and Mandapam Boast Jetty in the Gulf of Mannar and Dhargavalasai and Mandapam in the Palk Bay coasts were collected. For sample collection, the quadrat at a size of 50 sq. cm was made using a wooden frame. It was placed on the high and low tide marks of selected locations and removed top layer of the beach sand upto 2-5 cm depth within this quadrat frame. The collected samples were individually mixed with 3 litres of concentrated saline and allowed to settle for 60 minutes. The samples were filtered using 20, 120 and 500 micron mesh sieves. The filtrate was allowed to dry and weighed using upto 0.01 mg accuracy and observed under





Labomed Trinocular microscope. Then the FTIR analysis is performed to find out the functional group of various types of plastics distributed.

RESULTS

The total micro plastic litters collected in high tide mark at Pudumadam and Mandapam beaches in the Gulf of Mannar was 226.9 and 246.2 mg kg⁻¹ respectively and the samples collected in low tide mark were 276.0 and 322.0 mg kg⁻¹ respectively. In the meanwhile, the micro plastic collected in the high tide mark at Dhargavalasai and Mandapam in the Palk Bay Coast were 309.6 and 248.2 mg kg⁻¹ respectively and in the low tide mark, the quantities were 353.1 at Dhargavalasai beach and 311.5 mg kg⁻¹ at Mandapam beach (Figure-1). The size wise distribution of micro plastics was not dependent with tide. In Pudumadam beach, Gulf of Mannar, the low tide mark showed highest quantities i.e., 163.4mmg kg⁻¹ of 20 µm size. Though the quantities of micro plastics segregated in both low and high tide marks at Mandapam beach showed that the highest amount was noticed in 20 micro size in low tide mark and 120 micron size at high tide mark. In Palk Bay coast of Dhargavalasai beach, the 20 micro sizes was maximum in both low and high tide marks while, in the Palk Bay region of Mandapam coast, 500 micro size plastic litter was highest at high tide mark and 200 micro size was highest in low tide mark (Figure 2). From the observed peak values the peak value ranges from 350 to 4500, and the mostly obtained peak ranges refers the functional groups were strong and medium amide and amine, Strong alkanes, strong aldehydes, Ketones, Broad hydrogen bond and Carboxylic acids, Carboxylic esters, Alkyne OH bends, Poly sulphides and Aryl sulphides, From these functional groups the final plastic compounds found were Polyethylene, Polypropylene, Polystyrene, Nylon, polyvinylchloride, Polyacrylic, Poly acrylonitrile, Polyurethane, Rubber foam and Cellulose acetate (Figure 4 to 11) (Johnson, 2015).

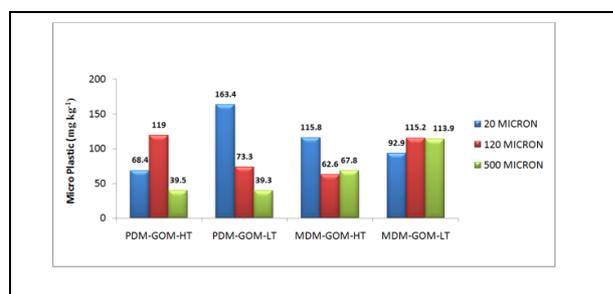


Figure 2: Size wise distribution of micro plastic litters at selected location in the Gulf of Mannar Coast

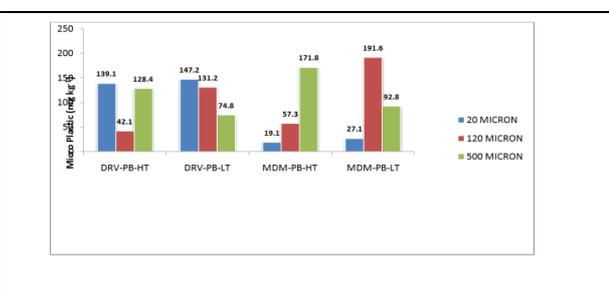


Figure 3: Size wise distribution of micro plastic litters at selected locations in the Palk Bay. (PDM: Pudumadam ; MDM: Mandapam; DRV: Dhargavalasai ; GOM: Gulf of Mannar; PB: Palk Bay; LT: Low Tide; HT: High Tide).

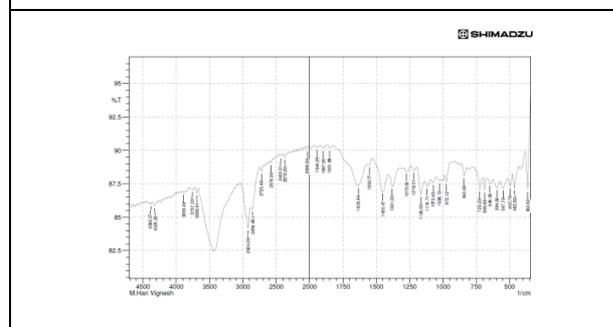


Figure 4: FT-IR graph shows that functional group peaks of the microplastics in Pudumadam coast low tide mark

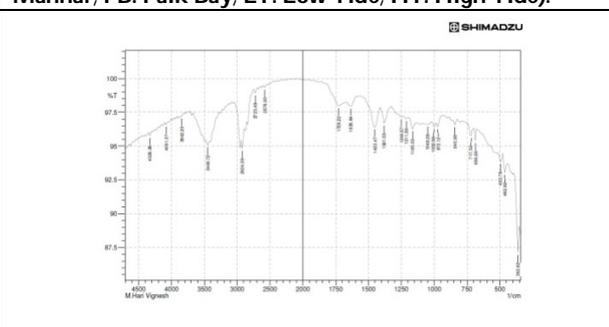


Figure 5: FT-IR graph shows that functional group peaks of the microplastics in Pudumadam coast high tide mark





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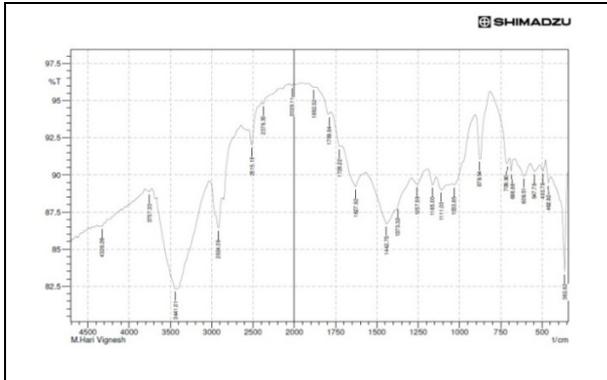


Figure 6: FT-IR graph shows that functional group peaks of the microplastics in Dargavalasai coast high tide mark.

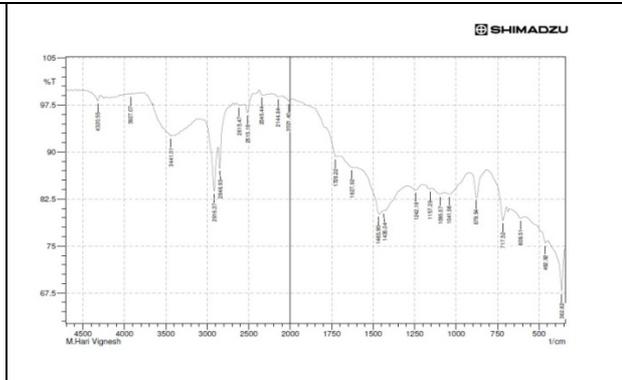


Figure 7: FT-IR graph shows that functional group peaks of the microplastics in Dargavalasai coast low tide mark.

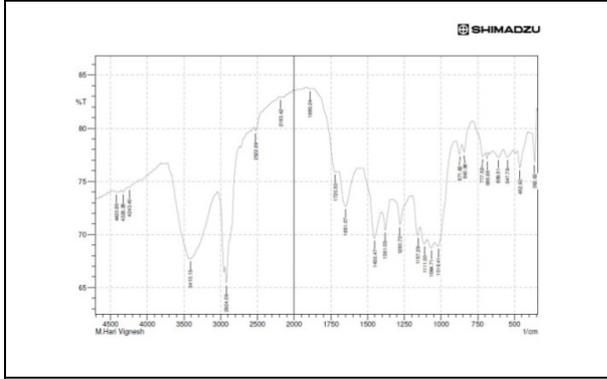


Figure 8: FT-IR graph shows that functional group peaks of the microplastics in Mandapam (South) coast high tide mark.

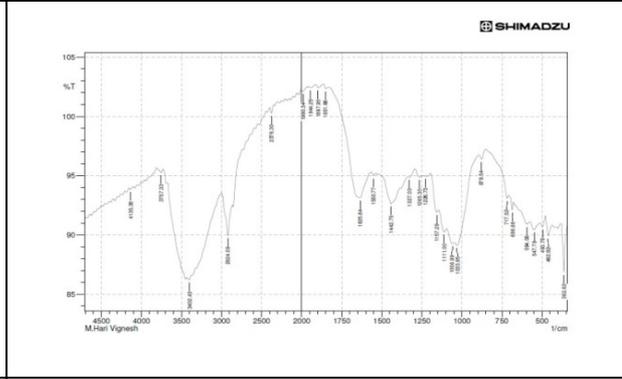


Figure 9: FT-IR graph shows that functional group peaks of the microplastics in Mandapam (South) coast low tide mark.

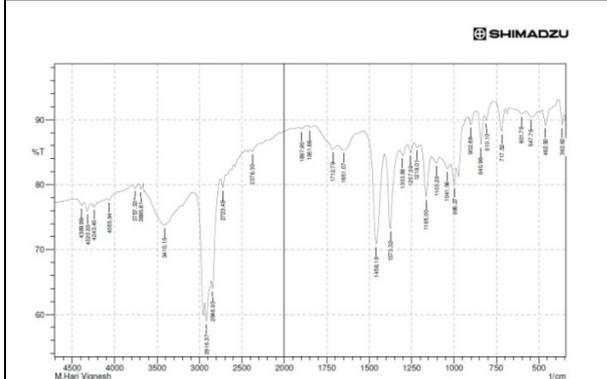


Figure 10: FT-IR graph shows that functional group peaks of the microplastics in Mandapam (North) coast high tide mark.

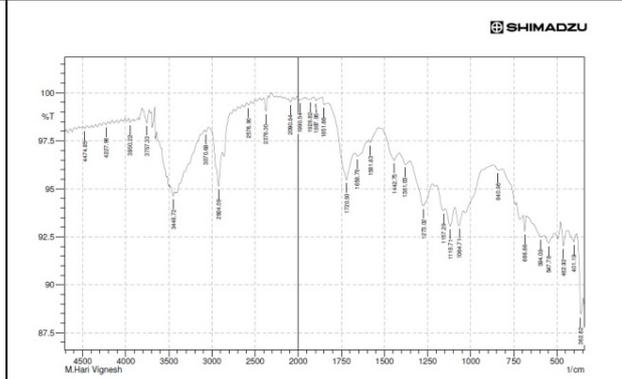


Figure 11: FT-IR graph shows that functional group peaks of the microplastics in Mandapam (North) coast low tide mark.





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CONCLUSION

There was no direct relationship between tide and different size of micro plastics in the beach sands collected at all four study sites while the low tide mark noticed higher quantities of total micro plastics in all four sampled locations and the beach of Dhargavalasai noticed maximum quantity i.e., 353.1 mg kg⁻¹. This concludes that the higher accumulation of micro plastic litters in the low tide mark increases the chance of ingestion of micro litter particles by benthic marine organisms. Of the three different sized micro plastic litter collected, both high tide and low tide marks of Mandapam beach, Palk Bay coast reported highest quantity of 120 and 500 micro sized particles i.e., 171.8 and 196.6 mg kg⁻¹ respectively. Microplastics occurrence in the Gulf of Mannar and Palk Bay shows the abundance of Plastic usage across the coastal areas, The Government should take respective actions such as plastic banning or the alternative use of plastics will create a plastic free coastal zone.

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A Retrospective Study of Drug Related Problems in Patients Undergoing Elective Total Joint Arthroplasty of the Hip or Knee

Sriram K^{1*}, Soundarrajan K², Sreedevi Sajiv³, Thoufeeque K Rahim³ and Yalamati Uday Sri Sai³

¹Assistant Professor, Department of Pharmacy Practice, Vinayaka Missions College of Pharmacy, Salem, Tamil Nadu, India.

²Assistant Professor, Department of Orthopedics, Vinayaka Missions Kirupananda Variyar Medical College and Hospital, Salem, Tamil Nadu, India.

³PharmD Intern, Vinayaka Missions College of Pharmacy, Salem, Tamil Nadu, India.

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*Address for Correspondence

Sriram K

Assistant Professor,
Department of Pharmacy Practice,
Vinayaka Missions College of Pharmacy,
Salem, Tamil Nadu, India.
Email: shriramkasi@gmail.com



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ABSTRACT

The surgical replacement of joints with an artificial material is known as arthroplasty. The main aim of this study is to evaluate the drug-related problems in patients undergoing elective total joint arthroplasty of the hip or knee. This retrospective study was carried out for 6 months from November 2018-April 2019 in which 100 patients were selected for the study by considering the inclusion and exclusion criteria. A total of 100 prescriptions were analyzed in arthroplasty undergone people. Out of the total number of patients analyzed male (53%) patients mostly underwent arthroplasty procedure than females (47%). Patients of age group of 61-70 were mostly found to have undergone arthroplasty (Male – 31.9% and Female – 30%). Diabetes Mellitus 48% are the most seen co-morbid condition in our study along with Hypertension 20%. Total Hip Replacement surgery was the most performed arthroplasty in our study (71%) and only 29% of the patients underwent Total Knee Replacement surgery. Our study shows that the prevalence of Drug-Related Problems (DRPs) was mostly found in postoperative cases of 90.6% whereas that of home medications was only 0.5% and the potential indication was 8.7%. The DRPs were classified according to type and category and the number of DRPs were found to be as follows: inappropriate dose or strength in postoperative medication 24.5%, inappropriate frequency post-operative medication 75%, the omission of medication in postoperative medication 57.5%, contraindication in postoperative medication 1.1%, incorrect drug in-home medication 0.5% and other 8.7 % were found in potential indication.

Keywords: Arthroplasty, Prehabilitation, Postoperative Medication, Drug-Related Problems.





INTRODUCTION

Arthroplasty is defined as the essential surgical replacement of a joint with artificially produced material which is fixated in the bone (joint replacement, endoprosthetic surgery, alloarthroplasty). Total replacement refers to the replacement of all the joint surfaces concerned while partial replacement involves the replacement of only one or some of the surfaces but not the entire joint. Hip and knee joints are the most frequently replaced, but endoprosthetic implants are also used to replace other joint functions, such as shoulder or elbow joints (1)(2). The most common reason for joint replacements is joint surface destruction from wear of the cartilage lining due to degenerative diseases such as osteoarthritis, fractures and other changes in bone and connective tissue structures. Under certain circumstances, these can lead to permanent loss of function, permanent pain and impaired mobility of the affected joint, as well as a decrease in quality of life (1). If these symptoms cannot be treated otherwise, replacement with an artificial joint becomes necessary in order to avoid secondary complications and to restore the patient's ability to participate adequately in everyday life. The causes and consequently also the risk of requiring joint replacements are largely dependent on age. On average, patients are aged between 60 and 70 years when they receive an artificial hip or knee joint replacement for the first time(1)(3). Patients undergoing any of the orthopaedic surgery are often recommended for certain preparations before their surgery which includes going for prehab and preadaptation clinics. Prehabilitation or prehab is a form of strength training, aims to prevent injuries before the actual occurrence. Since rotator cuff and elbow injuries are common among athletes in a wide range of sports, training the muscles surrounding these vulnerable joints can prevent injuries sustained from repeated wear and exertion. Prehab can be applied to people awaiting orthopaedic surgery. The intention is that the healthier they are when they have surgery the quicker they will recover from surgery. Strengthening exercises and massage therapies are tailored to the patient so that even those with end-stage arthritis can safely do some form of exercise and improve their outcome. It improves quadriceps strength before knee surgery and hence the support of the knee joint post-surgery. The prehab and preadaptation clinics include scheduled diagnostic tests to be performed and the patient assessment regarding the surgery, patient counseling to be given and their needs to be fulfilled on discharge. These preparations aim at preventing postoperative complications and also reducing the number of days the patient is admitted to the hospital. The pharmacist has a greater role in providing these preparations. These studies show that their work and involvement plays importance in reducing the incompatibilities in the medication history. Adequate care should be given to lessen several chronic medical conditions that occur before the patient undergo surgery. The main focus is on increasing or improving the quality of patient's distorted functional capacity and to enable the patient to combat with the surgery. Through relevant assessment and management escalation of patient medication therapy can be achieved. These show that clinical activities of pharmacists improve patient care(4).

METHODS

The patients who undergone arthroplasty were identified and their medical records containing prescriptions were examined retrospectively. The study was conducted by selecting patients who were admitted from November 2018 to April 2019. All the patients included in the study had undergone assessment at a pre-hab before surgery. The case records were collected from Vinayaka Missions Kirupananda Variyar Medical College & Hospitals, Salem, Tamil Nadu. All the relevant and necessary data is collected from patient case sheets. The data collected include demographic details, past medical and medication history, co-morbidity, number of drugs per prescription, presence or absence of drug-related problems. A sample of 100 cases was randomly selected from the list of patients who underwent arthroplasty. Those patients whose medical records were unavailable were excluded. The patients who were given more than one medication before surgery were included in the study. And the patients with at least one drug-related problem is taken as the primary outcome. Then the number of these problems arising from patient age, the category, type, and severity of the problem. Then later drug-related problems were examined with simultaneous preparations of charts. They were categorized according to home medication, postoperative medication, or potential indication (5).





RESULTS

Prevalence of Drug-Related problems

Among the 100 patients selected for this study, 53% were women and 47% were men and their mean age was 60 years. At least one drug-related problem was reported by 99% of the study population and thus a total of 172 problems were detected during this study for an overall mean of 1.73 drug-related problems per patient. Only 1% of the patient had no drug-related problems, whereas 35% had a single drug-related problem. 54% had 2 problems, and 10 had 3 drug-related problems.

Drug-related problems with chronic medical conditions

In the study population, 25% of the patients were with chronic medical conditions in which 48% patients were diabetic and 20% patients had hypertension and 8% patients had a past medical history of Chronic obstructive pulmonary disease, and 4% patient had coronary artery disease and 8% patients had lumbar spondylitis and 8% patients were with hypothyroidism and 4% patient had cough with expectoration (6).

Surgery

Among 100 surgeries, 71 surgeries were of total hip replacement and 29 surgeries were of hip replacement. The results were shown in table 1.

Types of Drug-Related Problems

Out of 23 patients, only one home medication-related problem was identified which was found to be an incorrect drug 4.3%. The most common type of drug-related problem related to post-operative medication was the omission of medication 99%, followed by inappropriate dose or strength 48%, followed by inappropriate frequency 13%, followed by contraindication 2%. A total of 15 problems were related to potential indications for drug therapy, this category included patients with a diagnosis of congestive heart failure, diabetes mellitus, hypertension, chronic obstructive pulmonary disease.

The severity of Drug-Related Problems

Of the 172 drug-related problems identified in this study, 57.5% were potentially harmful, 25.5% were deemed to require monitoring, and 16.8% were not considered harmful, according to the index categorization system of the National Coordinating Council for Medication Error Reporting and Prevention. Of the 172 problems related to home medications, 0.5 % was not considered harmful, the problem was an incorrect drug given to the patient which is an ayurvedic medicine. Of the 156 postoperative drug-related problems, 64.7% were deemed potentially harmful and 35.2% were deemed to require monitoring. The potentially harmful problems related to postoperative medications included an order for tramadol "as needed" with no maximum dose or frequency. And the other problem was the order of postoperative thromboprophylaxis was missing for 99 patients out of 100. Of the 15 potential indications for drug therapy identified in this study, 0% were deemed potentially harmful and 100% were deemed to require monitoring (6)(7). The results were shown in table 2.

CONCLUSION

Arthroplasty is an orthopedic surgical treatment wherein the articular floor of a musculoskeletal joint is replaced, made over, or realigned through or a few different methods. It is an optionally available method this is performed to relieve ache and repair characteristic of the joint after harm through arthritis or a few different form of trauma. Two kinds of arthroplasty surgical operations exist (7). Joint resection entails disposing of a part of the bone from stiffened joint, developing an opening b/w the bone and the socket, to enhance the variety of motion. Interpositional reconstruction is a surgical operation to reshape the joint and upload a prosthetic disk b/w the 2 bone forming the





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joint (8). The prosthetic may be fabricated from plastic and metallic or from frame tissue which includes fascia and skin. Elbow, shoulder, ankle, and finger joints are much more likely to be handled with joint resection or interpositional reconstruction. This is normally reserved for human beings over the age of 60. The observe becomes carried out through choosing the affected person who had been admitted b/w November 2018 – April 2019. Among the hundred sufferers decided on for this observation 53% had been women and 47% had been men (imply age become 60 years). Among a hundred surgical procedures, seventy-one surgical procedures are of a general hip substitute and 29 of hip substitute. Of the 172 drug-associated troubles recognized on this observe ninety-nine had been doubtlessly harmful (57.5%), (25.5%) had been deemed to require tracking and (16.8%) had been now no longer taken into consideration harmful. In this observation, sufferers who underwent general joint arthroplasty skilled many drug-associated troubles. Pharmacists might also additionally have possibilities to optimize affected person care through identifying, resolving, and stopping drug-associated troubles on this affected person population(9)(10).

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Table 1: Baseline Characteristics 100 of Patients Admitted for Elective Total Joint Arthroplasty

Characteristic	No. (%) of Patients
Age in years (mean)	60
Sex	
Male	47
Female	53
Chronic medical conditions	
Diabetes mellitus	12
Hypertension	5





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Hypothyroidism+Hypertension	2
Coronary artery disease	1
Chronic obstructive pulmonary disease	2
Lumbar spondylitis	2
Cough with expectoration	1
Type of Surgery	
Hip	71
Knee	29

Table 2: Prevalence of Drug-Related Problems (DRPs) By Category

Category of DRPs	No. (%) of Patients with 1 or More DRPs (n = 100)	Percentage of DRPs
Home Medication	1	0.5%
Postoperative	156	90.6%
Potential indication	15	8.7%

Table 3: Examples of Drug-Related Problems by Category and Type

DRP Category and Type Home medication	Example
Therapeutic duplication	Prescriptions for both losartan and candesartan
Incorrect formulation	Prescription for nifedipine regular release when XL (extended-release) formulation was intended
Inappropriate dose or strength	Prescription for metoprolol 5 mg when dosing at the home was 50 mg
Inappropriate route	Prescription for brimonidine eyedrops to be administered to both eyes when home the prescription was for the left eye only
Inappropriate frequency	Prescription for metoprolol regular release to be given once daily instead of twice daily
Omission of medication	The patient was taking ramipril at home but had no prescription for this drug while in hospital
Incorrect drug	The patient was taking formoterol at home but had a prescription for salmeterol in hospital
Contraindication	Prescriptions for ramipril and spironolactone with high potassium obtained during the pre-admission clinic, with no serum electrolytes ordered on hospital admission
Other	Allergy assessment required (morphine allergy recorded in the chart as "nausea and vomiting")
Postoperative medication	
Drug-allergy interaction	Prescription for morphine for a patient with documented allergy to codeine
Therapeutic duplication	Prescription for both warfarin 5 mg daily and warfarin 7.5 mg daily
Inappropriate dose or strength	Lorazepam 5 to 10 mg prescribed instead of the intended dose of 0.5 to 1 mg
Inappropriate frequency	Prescription for lorazepam "as needed", with no dosing frequency specified





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Omission of medication	Order for preoperative antibiotic missing Order for postoperative thromboprophylaxis missing
Contraindication	Celecoxib prescribed for a patient with a recent history of a bleeding ulcer
Potential indication for drug therapy	Patient with myocardial infarction but no Prescription for β -blocker Patient with a diagnosis of diabetes mellitus but no prescription for angiotensin-converting enzyme inhibitor Patient with a diagnosis of coronary artery disease but no prescription for a statin

Table 4: Number of Drug-Related Problems by Type and Category

Type Of Drug-Related Problem	No. Of DRPs For Home Medication(n=1)	No. Of DRPs For Postoperative Medications (n=156)	No. Of DRPs For Potential Indications (n=15)
Drug-allergy interaction	0	0	0
Therapeutic duplication	0	0	0
Non-formulary drugs	0	0	0
Incorrect formulation	0	0	0
Inappropriate dose or strength	0	42(24.5%)	0
Inappropriate route of administration	0	0	0
Inappropriate frequency	0	13(7.5%)	0
Omission of medication	0	99(57.5%)	0
Contraindication	0	2(1.1%)	0
Incorrect drug	1 (0.5%)	0	0
Other	0	0	15(8.7%)





Review on Ethno Medicinal, Phytochemical and Pharmacological Constituents of *Mimusops elengi*

Bikash Samantaray*

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Bikash Samantaray

Centurion University of Technology and Management,
Odisha, India



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ABSTRACT

This review is an attempt to focus on different Ethno medicinal, Pharmacological, Phytochemical and chemical Constituents reports on *Mimusops elengi* which is a potent medicinal plant distinctive of Sapotaceae family. It is mostly known as Bakul, an ornamental plant, well known for its fragrant flowers mostly cultivated in gardens in India. Simply all the different parts of the plant like plant, root, leaf, seed, fruit, bark are used in various ways to cure different human ailments. Preclinical researches have shown that some part of its phytochemicals have Anti-inflammatory, Analgesic, Hypotensive, Antibiotic, Antimicrobial, Antihyperlipidemic, Cytotoxic, Antioxidant, Antipyretic, Gingival bleeding, Congestive enhancing, Gastric ulcer activity.

Keywords: *Mimusops elengi*, Pharmacological, Phytochemical, Antiulcer

INTRODUCTION

The most part of the whole world, specially undeveloped and developing countries relies upon specially on the traditional system of medicine. As herbal medicines are higher safety with no side effect and are easily available with low cost, the use of various plants and their products in medicines is getting popularised. Plants have been a significant source of medicine for millennia as they are considered as chemical stores which contain various chemical compounds such as glycosides, saponins, lactones, alkaloids, oils and resins, which act on human body in different ways. certain phytochemicals synthesized in different parts of medicinal plants which are useful for the maintenance of human health as well as other animals.[1] As per The application of traditional medicine are established on thousands of years of analysis, observations and belief, which help in the development of modern medicine Promising outcomes from researches on different medicinal plants have forced scientists to study the pharmacological properties of the phytochemicals, as herbal medicines are used to cure broad variety health problems like cough and cold to cancer treatment [2,3].



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Mimusops elengi, which is often known as Bakul belongs to Sapotaceae family mostly found in different parts of Indian subcontinent like India, Bangladesh, Pakistan. *Mimusops elengi* is regarded as a holy plant among Hindus and has acquire prime place in religious texts .its dried twigs were used in religious rituals, its fruits were also used as staple diet by sages in the ancient Indian civilization. Due to its tremendous medicinal properties, it has produced supreme contribution to the field of science from ancient times to the modern researches. [4,5,6]. The aim of the current review is to systemize the detail information about different properties of *Mimusops elengi* so that researchers will get more clarity on different properties of it for their further research.

Scientific classification:

Family	: Sapotaceae
Kingdom	: Plantae
Order	: Ericales
Genus	: <i>Mimusops</i>
Species	: <i>M. elengi</i> L
Botanical name	: <i>Mimusops elengi</i>

Vernacular names of *Mimusops elengi*

Hindi	: mulsari, sinhakaraka
English	: bullet wood, spanish cherry
Odia	: baula, kirakauli
Bengali	: Bakal, Bakul, Bohl, Bukal
Sanskrit	: bakula, kesara, madhugandha, Udumbara
Tamil	: vagulam, magadam, muhunain
Malayalam	: Bakulam, Elengi, Ilanni, Iranni, Makuram
Telugu	: bogada-manu, bogada
Assamese	: Gokui
Singhali	: muhulla, muhunaminn-mal,
Marathi	: ovalli
Punjabi	: Maulsari, Maulsiri
Guajarati	: Babhuli, Bolsari, Varsoli, Vovoli
Burmese	: Kaya
Nepalese	: Bakulapuspa
French	: karanicum
Unani	: Moolsari
German	: Affengesict
Malaysian	: Enengi

Botanical Description

Mimusops elengi Linn is a small to large ornamental evergreen tree with a growth height of 12-14cm with dark green colours leaves and leathery with heavy margins. White colour flowers have aroma in nature. Trunk is short erect, base rounded. It has 2.5 cm long elliptical shape berry with it and changes to yellow colour with sweet test. In the rainy season fruition occurs containing one seed with ripe berry and has solitary brown colour seeds. The barks are gray in colour with rough surfaces due to presence of the longitudinal fissures and cracks.[12,13]

Phytochemical analysis

Mimusops elengi has been explored phytochemically by various researchers and found number of chemical constituents [14].



**Bikash Samantaray****Leaves and Roots**

Leaves of this plant is dark green colour with sharp margins and ethanolic extract of leaves gives beta-carotene, glucose, quercitol hentriacontane etc. A new phytochemicals was isolated from the roots are steroidal saponin, 5-alpha-stigmast-9(11) en-3-o-beta-d-glucopyranosyl and (1-5) -o- beta D-xylofuranoside [15,16].

Stem bark

Bark of *M.elengi* consists of tannin, saponin and colouring matter. Starch and ash of it forming inorganic salt and saponin in hydrolysis produce beta-amyrin and bassis acid [17]. Many more phytochemicals extracted from the bark like betulinicacid, urosolicacid, taraxerol and fatty acid esters of alpha spinasterol. Steam distillation of bark sample produces volatile organic compounds of 0.17%. Major constituents in its are hexadecenoic acid, disobutylphthalate, alphacadinol octadecadienoic acid [18].

Fruits and Seeds

Seeds of bakula showed the presence of 21% fatty oil and 2% of saponin. Both fruit and seed consist of different types of phytochemicals which have lots of medicinal uses. Ursolicacid, quercitol, beta-d glycosides of beta sitosterol. Two novel triterpenoid saponins mimusopin and mimusops in were extracted from the seeds of *Mimusops elengi* [20].

Pharmacological Activities

Mimusops elengi shows different pharmacological and biological activities such as antibacterial, anthelmintic, antiviral, antifungal, antihyperlipidemic, antioxidant, antihyperglycemic, cognitive enhancing, diuretic effects, anticariogenic, cytotoxic activities, free radical scavenging, etc. due to presence of various of active phytochemicals.

Antimicrobial Activity

Streptococcus mutans bacteria which causes dental caries was isolated from dental caries patient were tested against methanol, ethyl acetate, ethanol and hexane extracts of *Mimusops elengi* and all the extracts showed antibacterial activity against *Streptococcus mutans* [21]. For antibacterial activity through well diffusion method, methanol, petroleum ether, water and acetone extracts of bark were investigated against five different dental infection microorganisms like *Streptococcus salivarius*, *Streptococcus mutans*, *Streptococcus sanguis*, *Staphylococcus aureus*, *Lactobacillus acidophilus* and *Candida albicans*. Aqueous and methanolic extracts showed greater activity in comparison to acetone petroleum and ether extracts due to more phytoconstituents were percolated in it [22]. salivary micro flo collected from children age between 6 to 12 year were screened against acetone extract of bark by "paper disc diffusion" method, acetone extract exhibited antimicrobial activity [23]. Against some pathogenic bacteria like Kanamycin, Ethyl acetate, Methanol, Petroleum ether solvent extracts of leaves, bark and fruits were tested for their antibacterial activity. In comparison to leaves and barks, fruit extracts were found little potent against most of the screened organisms and were inactive against the fungus *Trichoderma viride*. Against *Trichoderma viride* and *Bacillus subtilis*, Leaves extracts displayed good activity but it displayed inactive against *Helminthosporium sativum* [24].

Anti-bacterial Activity

For antibacterial activity of the leaves of *Mimusops elengi*, different solvent extracts of leaves were prepared and tasted against six different bacterial strains of both gram positive strains like *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae* and gram negative strains such as *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa*. All the tested extracts displayed noteworthy antibacterial activity. Methanol extract displayed high inhibitory zone between different solvent extracts of *Mimusops elengi* leaves followed by petroleum, ether ethanol and chloroform [25]. From the seeds of *Mimusops elengi*, two antibacterial compounds such as 3,3',4',5,7-pentahydroxyflavone and 2,3-dihydro-3,3',4',5,7-pentahydroxyflavone exhibited strong inhibitory activity against Gram-negative and Gram-positive bacteria [26] By using spectrophotometric method, Ethyl acetate, ethanol, dichloromethane, methanol, methanol-chloroform, chloroform, acetone, ethanol-water, acetone-water and methanol-



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water extracts of fruit, seed and bark were tested for antibacterial activity against gram negative and gram positive strains. Against all the bacterium, stem bark extracts exhibited antibacterial activity while seed and fruit extracts were found inactive [27].

Antifungal Activity

To evaluate antifungal activity, different extracts such as, methanol, ethyl acetate, petroleum ether, from fruits, leaves and bark of *Mimusops elengi* were tested against some pathogenic fungi. In comparisons to leaves and bark extracts of *Mimusops elengi* fruit extracts showed little potent against most of the experimented organisms. Similarly leaf extract exhibited good quality against the fungus *Trichoderma viride* whereas fruit extract were inactive against it [28].

Antiulcer Activity

For the comparison of antiulcer activity between petroleum ether extracts and alcoholic extract of *Mimusops elengi* bark was tested on rats and it was concluded that alcoholic extract of *Mimusops elengi* bark has tremendous antiulcer activity than petroleum ether extracts [29].

Wound Healing Activity

For wound healing activity, methanolic extract from *Mimusops elengi* bark was investigated in three types of wound models on mice such as dead space, the incision and the excision model in the form of ointment. In comparisons to standard drug Betadine ointment, the extract ointments exhibited significant response in terms of wound closure time, wound contracting ability, dry granuloma weight and tensile strength [30].

CONCLUSION

As modern technology tremendously improved in production of synthetic drugs, most of the world population still wants medicines from plant origin so the pharmacologists are focusing more to develop new drugs from plant origin. From various experiments it has been found that it is an important medicinal herb having various phytoconstituents which are responsible for antioxidant, antimicrobial, anticancer, antihyperglycemic, and protective effects on different key organs such as heart, kidney, liver and nerves. Most of the part of this plant like fruit, leaf, bark, flowers and seed are used to cure many diseases .due to which it has attracted many researches to develop new formulations which will be beneficial to whole human society.

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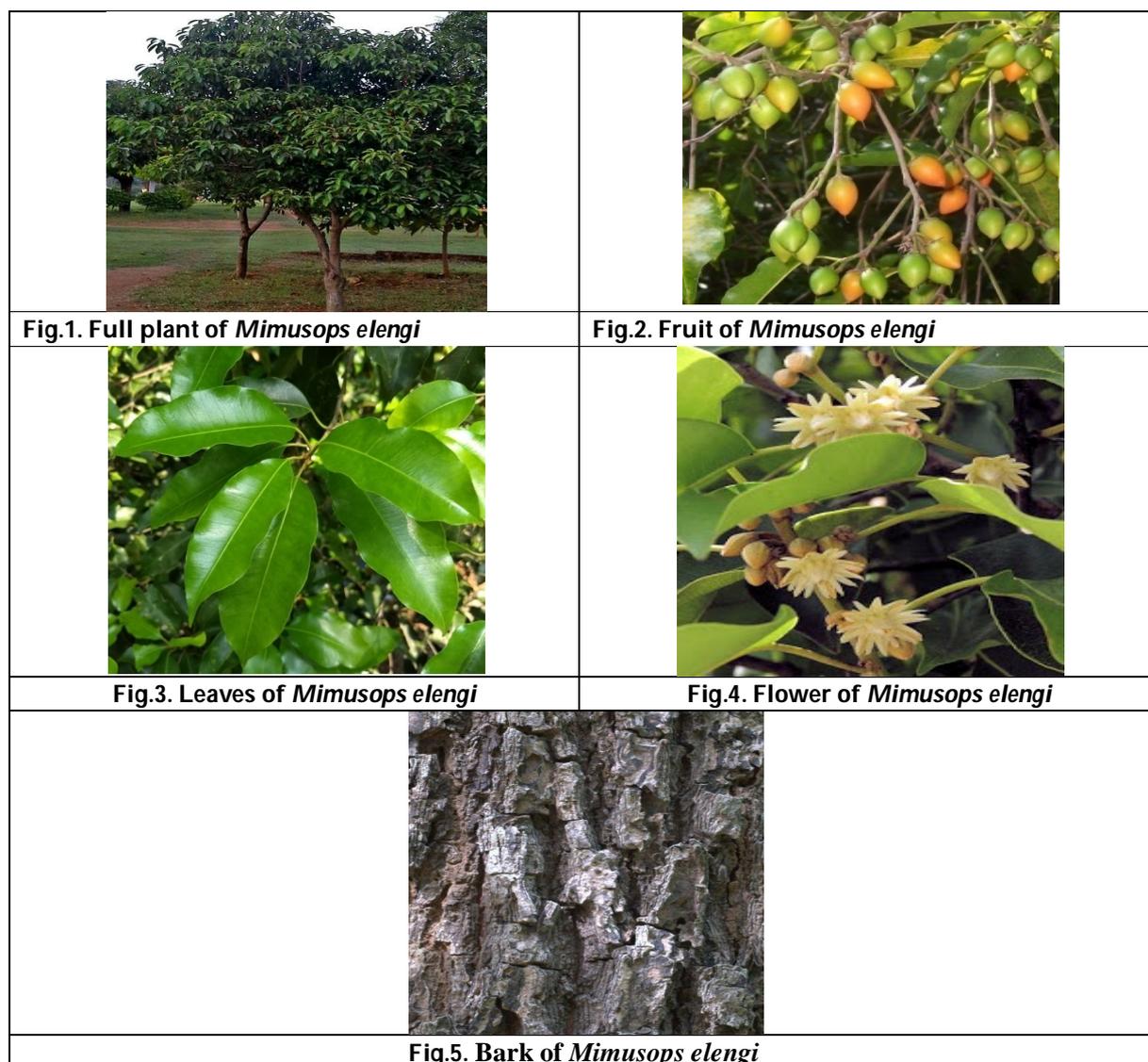




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Table 1. Ethno medicinal uses

SI. No.	Plant part used	Ethnomedicinal uses	References
1	Root	Cardiotonic Diuretic, astringent, and stomachic	7
2	Leaves	to treat bleeding from the gums and gingivitis	8,9
3	Flowers	an expectorant and in liver complaints and asthma	8
4	Fruits	to treat bleeding from the gums and gingivitis	7,9
5	Seeds	obstinate constipation	7,10
6	Bark	wounds and ulcers	9,11





Potential Impact of COVID-19 Pandemic on Co-Morbidities

Bharat Mishra¹, Anu Sebastian^{2*}, Chippy Jose³, Ceenu Jose³, Juvanitta Sabu³ and Anusha Shaji²

¹Professor and Head, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

²Assistant Professor, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

³Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

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*Address for Correspondence

Anu Sebastian

Assistant Professor,

Department of Pharmacology,

Nirmala College of Pharmacy,

Muvattupuzha, Ernakulam, Kerala, India.

Email: anusbtn@gmail.com



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ABSTRACT

Corona Virus disease (COVID-19) is a global pandemic mainly effect on the respiratory system of the human. It is caused by severe acute respiratory syndrome coronavirus-2 which invades cells through the angiotensin converting enzyme 2. There are various ways in which this pandemic impact existing public health issues. Here we disclose the interaction between COVID-19 and other diseases. Various studies show that patients with other diseases like respiratory syndrome diseases, cardiovascular diseases, digestive system diseases, liver diseases etc. are highly susceptible to COVID-19. The main reason for this is the reduced immunity in such patients. The patients with respiratory system disease such as COPD, asthma etc. is highly vulnerable for COVID-19 as their respiratory system is already impaired. Like that the mortality rate and severity of disease is high in COVID patients with other diseases. In COVID patients with chronic liver diseases, liver tissue may get injured, and the virus damages the digestive system directly or indirectly through an inflammatory response. In most of the cases, COVID patients with other disease are at high risk and they should provide with special care.

Keywords: COVID-19, SARS-COV-2, ACE-2 receptor, Respiratory diseases, Cardiovascular diseases, Gastrointestinal diseases, Hepatic diseases, Neurological diseases.



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INTRODUCTION

Disease outbreak have ravaged humanity from prehistory to modern times. On the 31st December of 2019, a cluster of cases like pneumonia was reported in the city of Wuhan, Hubei province in China to the World Health Organization. Coronavirus disease- 2019 is an infectious disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) which belongs to the same family of severe acute respiratory syndrome (SARS) and middle east respiratory syndrome. Typical symptoms of COVID-19 include fever, sore throat, cough, fatigue, sputum production, headache and shortness of breath. However, recent reports and studies show that COVID-19 also have other non- respiratory manifestations. Diagnosis is often complicated by patients who are asymptomatic. Those with pre-existing disease conditions, such as diabetes, hypertension, pulmonary, cardiac and kidney diseases are considered to be at a higher risk of developing severe diseases.

Recent studies and researches have shown that angiotensin converting enzyme 2 is the functional receptor of SARS-COV-2 cases can worsen and lead to an acute respiratory distress syndrome (ARDS) or pneumonia. Treatment for this pandemic is symptomatic and oxygen therapy represents as the main intervention for the patients with severe infection. As millions of patients are fighting with COVID-19 pandemic in this situation, investigations into the incidence and clinical management of COVID-19 in patients with other diseases, including are ongoing with intensity. Thus, in the health care setting, the possible impact of COVID-19 in patients with other diseases should be considered.

Impact of Corona Virus Disease on Respiratory System

COVID-19 mainly infect on the respiratory tract in humans. ARDS and pneumonia leads to death in severe cases. The primary symptoms of COVID like breathlessness, dry cough, loss of smell is also related to respiratory system. So, it is important to lock into the impact of COVID-19 on respiratory system diseases like COPD, Asthma, TB, etc. Across the world around hundred millions of people were affected by lung diseases. Millions of deaths are also due to chronic respiratory diseases (CRD) like asthma, COPD. The risk of COVID 19 on patients with CRD is based on various features like their age, socioeconomic status etc. Lower immunity level, impaired respiratory functions and infective treatments may increase their susceptibility to infection [1].

COPD & COVID 19

COPD on chronic obstructive pulmonary disease is a group of progressive lung diseases. The conditions common in COPD is emphysema and chronic bronchitis. Emphysema slowly destroys air sacs in lungs and bronchitis causes inflammation and affects respiratory system. The COPD patients are susceptible to severe COVID 19. The reason for COPD patient's causes worse outcome of COVID 19 that cellular machinery for entering of SARS-COV-2 is become light in the patients [2].The entry is facilitated by the fusion of virus with ACE2 receptor. In COPD patients and in smokers the ACE2 expression has elevated compared to the controlled subjects [5]. It should also note that ACE2 expression alone has not been shown to present increased vulnerability or increased acuteness of disease [2].

Management of COPD

The main challenge faced during this pandemic regarding the management of COPD that whether the usual methods of pharmaceutical for COPD can still apply. The common medications used by the COPD patients are inhaled, (IES) and systemic (SCS). Corticosteroids, short and long acting beta2 agonists and short and long acting muscarinic antagonists. Recent studies show the possibility that exposure to inhaled corticosteroids could reduces the viral entry. But due to the absence of data that explains about its benefits and harm IES and other long acting corticosteroids should not withdraw nor should rapidly increase their use as a precaution for COPD patients during pandemic [2]. The historical data for systemic corticosteroids in viral pandemic are not much favorable. Some studies related to SARS showed the inconveniences including delayed viral clearance and increased rate of psychosis. So far, the most favorable data on corticosteroids and COVID 19 are from a randomized controlled trial of dexamethasone (RECOVERY) performed in U.K, which indicated a one third reduction in mortality [4]. As a result of this recovery



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trial, dexamethasone becomes a standard treatment of COVID 19 patients as well as those with COPD. The impact of pandemic reduced the clinical visits of COPD patients to avoid exposure to the virus. This led to the delayed care and increased risk [2].

Asthma

Asthma is an inflammatory disease of the airway in which airways get narrowed and swell and may produce extra mucus. People with asthma are being at a lower risk for severe COVID 19 than people with COPD. This difference is due to the difference in the ACE2 expression. The messenger RNA expression level of ACE2 was significantly reduced in asthmatics. But there is no significant decrease was seen for ACE2 expression in COPD patients [3].

Management of asthma

The main challenge in the management of asthma during this corona virus pandemic is concerning with the use of oral corticosteroids (OCS). There is no relevant data's regarding treatment with inhaled corticosteroids susceptibility to SARS-COV-2[8]. There are many asthma patients who were treated with biologics. The biologics target type2 inflammatory pathways and are successful at decreasing escalation, maintaining control over asthma symptoms, and lowering systemic steroid use. The global initiative for asthma (GINA) suggests that patients with asthma should continue to uses their ICS during COVID 19 epidemic. To reduce the need of OCS in severe asthma patients' biological therapies should be used [8]. According to American Academy of Allergy, Asthma and Immunology [AAAAI] there is no evidence that indicates immune response to COVID 19 will impaired in asthma patients treated with anti-IL5, anti-IL4/anti-IL3 medications [8].

COVID 19 and tuberculosis

Tuberculosis [TB] is a disease caused by a bacterium known as *Mycobacterium tuberculosis*. This bacterium generally attacks lungs but it can affect any part of body such as kidney, spine and brain. The bacteria are spread by respiratory droplets like cough, saliva, or by kissing or by shared drinks. TB disease management needed frequent clinical assessment, testing, contact tracing, treatment regimes for effective eradication. Lockdown, self-isolation, quarantine during this pandemic has reduced the diagnosis of new TB patients. Though mode of spread differs slightly, both TB and COVID 19 transmitted by close contact between people [6]. And both of them have some similarities in clinical features. Simultaneous infection of SARS-COV- 2 and *Mycobacterium tuberculosis* can occur during the current COVID 19 pandemic and as such damage caused by precedent TB infection can predispose the patient to develop COVID 19 [7]. Irrespective of expected temporal association between COVID 19 and TB, both of them may have synergistic effect in social and economic impact worldwide [6].

Impact of COVID 19 on Cardiovascular Diseases

The relationship between COVID 19 and cardiovascular system has been an important topic. Patients with pre-existing hypertension or diabetes mellitus appear to be high risk of having severe form of diseases [9]. Many potential mechanisms are explained for the link between COVID 19 and CVD, they include virus induced inflammation, high risk of thrombosis, ischemia due to increased oxygen demand, microvascular ischemic injury and the accelerated immunologic response mediated injury, acute coronary diseases and heart failure are a higher risk of mortality. The exact mechanisms of cardiac involvement in COVID 19 is under studies [11]. ACE2 mediated direct myocardial involvement is one of the potential mechanisms. Cytokine storm, hypoxia induced excessive intracellular leading to cardiac myocytic apoptosis are the other mechanisms [10].

Potential Mechanisms of CVDS Induced by COVID 19

Imbalance in RAA system

The fusion of virus and host cell is mediated by human Angiotensin Converting Enzyme. The angiotensin 1 is converted to angiotensin 2 by ACE and angiotensin 2 leads to vasoconstriction, elevated blood pressure. ACE 2 is



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present in human alveolar epithelial cells and which is a gateway of virus entry and also mediate lung injury and lung infection caused by virus invasion. Various studies show that plasma angiotensin 2 level has increased in COVID 19 patients [10].

Inflammatory storm

After fusion with host cell the viruses get replicated which leads to lung inflammation, edema, degeneration etc. They cause the release of pro inflammatory cytokines. The accumulation of cytokinin in injury site results in inflammatory storms, stimulating body immune response, increased damage of myocardial cells etc [10].

Hypoxemia

The COVID 19 causes lung injury which leads to hypoxemia, the partial presence of circulating oxygen and oxygen saturation decreases continuously, which leads to accumulation of oxygen free radicals, lactic acid and other metabolites. Due to this condition the demand for energy and blood is intensified and prone to cardiac dysfunction and heart failure [10].

Stress response

The activation of human immune and inflammatory response is the first and strongest reaction of virus invasion. The interaction among tissues, organs and pathogens are a complex stress process. Emotional responses like anxiety, fear, also increases stress in patients. As a result, catecholamines are released in large quantities which lead to elevated levels of blood pressure [10].

Myocardial injury

If a patient were confirmed or suspected as COVID 19 having palpitation, chest tightness, shortness of breath after exercises, a sudden acute myocardial infarction, or heart failure occur immediately [10]. The diagnosis of myocardial injury in moderate to severe COVID 19 patients is by increased troponin level. The cytokinin storm causes raised vascular permeability and myocardial oedema with transient damage to myocardium [11]. When COVID patient shows increased respiratory rate, low BP, ECG with prolonged QRS interval it might involve heart and there is a possibility for myocarditis. Caring of COVID 19 related myocardial injury is by using present guidelines for the treatment of viral myocarditis, including the use of standard heart failure therapies and supportive measures [11]. Supportive measures include mechanical ventilation, extracorporeal membrane pulmonary oxygenation [ECMO], temporary pacemaker implementation etc [10].

Hypertension

Various studies show that hypertension is closely related to severity of COVID 19 infection. COVID 19 infects human by the fusion of spike protein on its surface with ACE2 on the alveolar membrane. This will activate the immune system and release cytokinin and inflammatory factor [10]. The ACE inhibitors or ARBs are not associated with the susceptibility of COVID 19 infection. So, the treatment with both of the can be continued in patients with hypertension. In patients having both hypertension and COVID their treatment using ACEI or ARB depend on their condition. In mild cases the treatment continues and in severe cases the treatment plan is decided after careful monitoring. It is recommended to delay the use of ACEI in patients with COVID 19 to avoid dry cough [10].

Heart failure

Congestive heart failure occurs when the heart muscle does not pump enough blood that is required by the body. Certain conditions like narrowed arteries or high BP may weakens the heart to fill and pump efficiently. Compared to general population the mortality rate of patients with CVD including heart failure is very high. Patients with chronic cardiac conditions including CHF are predisposed to respiratory complications [11]. So, the susceptibility of such patients to COVID 19 is very high. Patients with CHF may follow up via telemedicine of patients with CHF the guidelines directed medical therapies can be continued. Among the experimental approaches' hydroxychloroquine is

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used to treat COVID 19 but it could prolong GT intervals and causes arrhythmias. Mechanical support is given to selected patients with refractory shock [11].

Impact of COVID 19 on GIT

Digestive symptoms in patients with COVID 19

Some studies found that digestive symptoms are a common complaint in patients with COVID 19. Many patients initially present with diarrhea, anorexia and vomiting along with respiratory symptoms. Patients with digestive symptoms were likely to suffer the liver injury and a lower monocyte count [20]. There are many reasons why this COVID 19 pandemic seems to cause the digestive symptoms. SARS-COV 2 can invade the human body by binding to the human angiotensin converting enzyme 2 receptor [20]. So, SARS- COV-2 damages the digestive system directly or indirectly through an inflammatory response [21]. According to the studies the high expression of ACE2 is the intestinal tract makes the colon and small bowel susceptible to the infection [21].

Upper gastrointestinal bleeding in COVID 19 patients

Upper gastrointestinal bleeding (UGIB) has a potential impact in patients having COVID 19. Some study reports found that patients who had overt signs of acute UGIB (i.e., hematemesis tarry stool or coffee ground vomitus) have a positive diagnosis of COVID 19 infection. The UGIB is routinely managed mainly by the endoscopists after clinical stabilization within 24hr [22]. If patients are more critical, i.e. if there is high flow or non-invasive positive pressure support, it is important to stratify them. In case of poor response of medical management, upper GI endoscopy is to be considered. In UGIB patients, thrombotic and other systemic complications remain the main as a challenge [22].

Peptic & gastric ulcers related with COVID 19

The pathogenesis of peptic ulcer caused by SARS-COV-2 is explained by different mechanisms, including stress resulting from the direct gastric epithelial damage, acute illness etc. If thromboprophylaxis remains there is a relatively higher risk of GI bleeding. So, the clinician should be alert and should check routinely for its occurrence by hemodynamic stability [23]. Gastrointestinal symptoms such as diarrhea, vomiting and abdominal pain have been reported in patients having gastric ulcers related to COVID 19. The electron microscopy in biopsy and autopsy samples showed active virus replication in the small and large intestines and evidence from SARS studies indicates that the corona virus has tropism in the GI tract, so that SARS COV RNA can be easily detected in stool samples from the SARS patients. The fecal-oral transmissions should be prevented because this could be an additional route for the spread of SARS-COV-2. Some patient's studies say that, patients are really suffering from perforated gastric ulcers secondary to infection by SARS-COV-2 [24].

Laryngopharyngeal reflux disease in COVID 19 patients

Some studies focused on the GI comorbidity specifically on the laryngopharyngeal reflux disease [LPRD] to investigate the impact of gastrointestinal [GI] disorder on COVID 19 patients. The most common symptoms are fever, cough, nausea, vomiting, diarrhea etc [25]. Virus mainly affect the GI motility and function, leading to repeated reflux of GI content into larynx and it will further facilitates the attack of the virus on the respiratory system. The reflux symptom index (RSI) is used for the assessment of the presence and severity of commonly reported symptoms in patients having LPRD. An RSI score higher than 13 are considered to be abnormal and indicative of this disease. LPRD, a common digestive disorder pre existence cannot be determined due to the following reasons; if the past medical history was documented via self-report or due to the lack of awareness etc. The RSI may help to assess the risk of LPRD on patients [25].

Inflammatory bowel disease in COVID 19

Patients with IBD have an increased risk for COVID 19. IBD patients those under immunosuppressive treatment might have higher viral load, prolonged viral shedding and impaired antibody response. Patients with active IBD, older age and presence of comorbidities were found to have a higher risk of COVID 19 pneumonia and death. The



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patients with IBD and COVID 19 had a higher rate of clinically or endoscopically active diseases and higher baseline C - reactive protein values and fecal calprotectin when compared with IBD patients without COVID 19 [26].

Active Inflammatory Bowel Diseases

Those patients with active IBD should avoid systemic steroids as much as possible because doses above 20 mg per day have an immunosuppressive effect that may increase the risk of SARS-COV-2 infection [27].

Impact of COVID 19 on Kidney

Previous studies found that the incidence of acute kidney injury (AKI) and related kidney problems are up to 15% in COVID 19 patients. Even though the exact mechanisms of SARS-COV-2 associated with renal damage is not fully known, studies shown that the cellular components such as ACE2, cathepsin L(CTSL) are highly expressed in kidneys. Kidney dysfunction is characterized by the elevated levels of creatinine, uric acid, blood urea nitrogen (BUN). The presence of fragments of COV in the patient blood & urine and the high ACE2 expression in the kidney implicate COVID 19 in the renal system [29]. Kidney damage may occur through the effects of COV- infected ACE 2. The patients those undergone continuous kidney replacement therapy (CKRT) also have a higher risk for the occurrence of COVID 19 [30]. The anticoagulant treatment could improve the kidney functions in both patients and underscores the possible benefit of a timely diagnosis and kidney infraction in COVID 19 patients with acute kidney injury [28].

COVID 19 & Urological Problems

Previous studies showed that 5.74% of the COVID 19 patients had positive viral RNA in urine samples. Acute kidney injury (AKI) can be a manifestation in patients with COVID 19. COVID 19 had a strong interaction with ACE2 receptor. So, the virus must be able to spread to the kidneys via blood stream and also expressed in testis and bladder [31]. Cervix is the main part of female genital tract system which could be affected by the virus. However, women represent a huge spectrum of gynecological pathology; cervix is much seriously affected by the virus. There are main two clinical implications on potential observation of SARS-COV-2 presence in cervix. The first implication concerns the presence of virus. The second implication concerns its impact on the HIV infection. SARS -COV-2 positive patients should be followed up regularly to optimize the early detections of lesions [32]. If the patient has additional symptoms such as fever along with urinary symptoms, we can't assume that fever is attributed to an underlying urological cause. Procalcitonin might serve as marker to preliminarily differentiate between COVID-19& urological infections. Procalcitonin levels may rise in bacterial, parasitic & fungal infections, but not during the viral infections [31].

Impact of COVID 19 on Hepatic System

Due to the impact of COVID 19, the liver and pre-existing liver diseases may influence the clinical course of COVID 19 in various ways. Depends on the nature of liver diseases, the risk factor for the course of COVID 19 also change [13]. On that account, we sum-up, the proof for the different liver diseases involved in the peril for COVID 19 infection. And also impart some recommendations for the management of patients with liver disease and SARS-COV2.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a malignancy of the liver that occurs predominantly in patients with underlying chronic liver disease and cirrhosis. HCC patients are at high risk severity in SARS-COV-2 infection and more likely to be admitted in the intensive care unit. During this pandemic situation of COVID 19, the HCC patients are now becoming life threatening, due to delayed observations and diagnosis. Because the risk of COVID 19 patients with HCC is remain vague. The delayed treatment were mostly trans-arterial procedures and also thermal ablations [33] and the consequence will be bard for HCC patients.



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First of all, test the patient for SARS-COV-2 infection, if it is positive, hold up the procedures for 7-14 days until the potent test become negative.

Early stage of HCC: Liver transplant, loco regional ablation [17]

Intermediate Stage of HCC: Trans-arterial procedures [17]

Advanced stage of HCC: Systemic therapies, palliative radiotherapy [17].

Blood test should be carried out in 1-3 months.

Cirrhosis

Cirrhosis also known as liver cirrhosis or hepatic cirrhosis is a condition in which the liver does not function properly due to long term damage. This damage is characterized by the replacement of normal tissue by scar tissue. Cirrhotic patients are always vulnerable to raise bacterial infection, primarily due to the displacement of bacteria from gut to the extra intestinal lymph nodes [16] after the immune system shows dysfunctionality. Liver is the primary organ for protein, liposaccharide and some cytokines like TNF- α , IL-6 etc. but all these letup for patients having cirrhosis [15, 16]. The mortality rate of COVID 19 patients with cirrhosis is high, because of organ failure and other complications like gastrointestinal bleeding, obstructive jaundice so on. And also, they are liable to evolve major respiratory problems like arterial hypoxemia, Porto pulmonary hypertension etc [16]. The morbidity and mortality rate are inflated for advanced cirrhosis in children, often caused by genetically [13]. Even so, there is a high risk of patients with SARS-COV-2 infection and cirrhosis compared with COVID 19 alone.

Management

Patient should take vaccination for *Streptococcus pneumonia* and influenza [13]. Also, they should take care of themselves and adopt guidelines offered by the physician.

Liver Transplantation

The immune suppressed liver transplant recipient bitter to face a harsh situation on this pandemic circumstance of COVID-19. On this elevated pandemic condition, if the transplant recipient is a geriatric, besides comorbidities [13] have to confront a high risk. By usage of calcineurin inhibitors like cyclosporin can also cause kidney failure [13]. The risk factor should also be faced by LT candidates. The LT candidates should be alert or aware about the infection during the major surgery [14]. The risk factors like malignancy, metabolic disorders etc. However, the immune suppressed patients can also produce atypical symptoms. The hospital acquired infection can also generate in the course of diagnosis and therapeutic procedures on transplantation [14].

Management

By the administration of drugs for SARS-COV2 infection, the drug dosage level of calcineurin inhibitors should also be checked. Avoid the frequent visit to the hospital for diagnosis.

Autoimmune Hepatitis

Autoimmune Hepatitis is a disease in which body's own immune system attacks the liver and causes it to become inflamed. The COVID-19 disease may give rise to the abnormalities in liver enzymes [18]. The utilization of high dose of steroids is a risk factor for the AIH patients may even cause death. Abruptly, AIH patients can cause jaundice and requires an immediate supervision [12]. During this pandemic condition, the immune suppressive therapy is cut back for AIH patients having COVID-19 [18].



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Use web-based consultation with general practitioner. Avoid intrusive diagnostic approaches like liver biopsy, endoscopy, if it is possible [18].

Impact of COVID19 on Neurological Patients

The prospective targets for COVID-19 infection are brain and skeletal muscles due to the angiotensin converting enzyme 2 [19]. The ACE receptors are highly sensitive to SARS-COV2 infection during the cases of intra cerebral hemorrhage, ischemic stroke etc. The mechanical ventilation is needed for the patients having deficiency in axonal involvement and respiratory failures. During the COVID-19 pandemic, the stroke is a milestone for mortality and morbidity [19].

Management

The scheduled patient must attend in the hospitals or neurological centers alone. The operative decision always taken in accordance with medical practitioner.

CONCLUSION

The pandemic COVID-19 became vulnerable to the whole world. But it mostly encountered by the patients with other severe diseases like cancer, respiratory problems, GIT disease, neurological problems etc. The availability and the facilities for the health care information in various disease become deficit during this situation. In conclusion, prevention is better than treatment. So, we should face this strenuous challenge and provide tele medicine to monitor patients and provide SARS-COV2 surveillance.

ABBREVIATIONS

ACE	Angiotensin converting enzyme
COPD	Chronic obstructive pulmonary diseases,
IES	Inspiratory efforts sensation,
ARB	Angiotensin receptor blockers,
CTSL	Cathepsin L1

CONFLICT OF INTEREST

There is no conflict of interest found among authors.

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Phytochemical Screening, TLC Profiling and FTIR Analysis of Flower, Leaf and Seed of *Cascabela thevetia* L.

Pravin Solanki¹, Anjali Shukla², Siddhi Panchal¹, Dilip Vasava¹ and Nainesh Modi^{2*}

¹Department of Chemistry, Gujarat University, Navarangpura, Ahmedabad, Gujarat, India.

²Department of Botany Bioinformatics, Climate Change Impacts Management, Gujarat University, Navarangpura, Ahmedabad, Gujarat, India.

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*Address for Correspondence

Nainesh Modi

Department of Botany Bioinformatics,
Climate Change Impacts Management,
Gujarat University, Navarangpura,
Ahmedabad, Gujarat, India.
Email: nrmodi@gujaratuniversity.ac.in



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ABSTRACT

The plant *Cascabela thevetia* L. of Apocynaceae family is comparatively understudied in terms of functional group analysis. Genus *Cascabela* is widely recognized by its phytomedicinal and ethnomedicinal attributes. The study is aimed to evaluate the qualitative analysis, Fourier transform infrared (FT-IR), thin layer chromatography (TLC), as a justification for its use in traditional medicine. Phytochemical evaluation confirmed the presence of phenols, tannins, steroids, sugar, protein and saponins which was later confirmed by TLC (Thin layer Chromatography). Methanol extract of *C. thevetia* showed the presence of steroid (Rf values L, F, S: 0.88, 0.88, 0.55), phenols (Rf values L, F, S: 0.76, 0.70, 0.76) and tannins (Rf values L, F, S: 0.96, 0.73, 0.92). Moreover, FT-IR spectroscopy revealed the presence of Amine, Alkene, Aromatic ring, Carbonyl, Aldehyde, Alcohol, Phenol, Alkyl, Ethers and Alkyl halide. The findings of this analysis suggest that the hydro-methanolic extract from *S. urens* root exhibit antioxidant activity quantified by its ability to scavenge DPPH; antimicrobial activity displayed appreciable microbial sensitivity. These properties are associated with the presence of high phenolic content, different secondary metabolites, and their functional groups. The results are suggestive that *S. urens* root is rich in bioactive compounds, which serve as a novel natural source for potential therapeutic applications.

Keywords: *Cascabela thevetia* L, methanolic extract, Phytochemical screening, TLC, FT-IR





INTRODUCTION

Mankind has always been under threat of diseases and ailments. To cure such diseases, nature has gifted various plants to humans. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature. In the past, almost all the medicines used were from the plants, the plant being man's only cure for ages even today. In 1985, the World Health Organisation (WHO) estimated that about 80% of the world's populations still rely mainly on traditional remedies such as on herbs for their primary health care needs [1]. In recent times, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine [2,3,4]. During the last two decades, there has been an increasing interest in the study of medicinal plants and their traditional uses in different parts of India. Medicinal plants like *Cascabela thevetia* (L.), *Centella asiatica* (L.) Urban, *Solanum trilobatum* (L.), *Ocimum sanctum* (L.), *Eclipta alba* (L.) Hassk. and *Justicia adhatoda* (L.) Nees. have been used to cure various ailments for several thousand years. Medicinal plants and herbs have been proven to be an abundant source of biologically active compounds, many of which have been the basis for new pharmaceuticals. Metabolism is essential to all living things and in plants there are two forms namely primary and secondary metabolism, thus plants have two forms of metabolites termed primary and secondary metabolites [5,6]. Therapeutically, the secondary metabolites are either used singly in combination or as starting material for other medicines [7,8]. Secondary metabolites are produced by plants mainly as products of primary metabolism and as part of the defence mechanisms of plants. Phytochemicals such as alkaloids, Tannins and flavonoids are examples of secondary metabolites produced by plants, from which the plants are thought to get their healing properties [9].

The presence of phytochemical compounds in the plants indicates its medicinal potential. Phytochemical analysis gives an idea about the different class of phytoconstituents present in the crude drug which are responsible for the different therapeutic activity. The presence of tannins shows plant possess anti-parasitic, antiviral and antibacterial activities. Steroids act as signaling molecules and are important against cardiotoxic activity. Phenols are used as antiseptic and active ingredient in some oral analgesics such as carmax and chloraseptic spray. Intimation of the chemical segments of plants is desirable because such information may be of great worth in revealing new sources of economic compound as tannins, oils, gums, precursor for the synthesis new chemical substance which can be used in drug [10]. From the above preliminary phytochemical studies (Qualitative chemical test) the strong target of *C.thevetia* as a whole plant in methanol extract alone, were taken for the further study of thin layer chromatography. Methanolic extracts gives an impressive result that directly showing the presence of diverse phytochemicals. FT-IR has played an important role in pharmaceutical analysis in recent years [11]. Moreover, spectroscopy has come into the picture as one of the major tools for biomedical reorganization and has made noteworthy progress in the field of clinical evaluation. Discovery of the actual value of a ethnomedicinal plant as well as discovery of a therapeutic agent solely depends upon the knowledge about the phytochemical composition of the plant. *Cascabela thevetia* L. belongs to family Apocynaceae (Figure 1) is a small evergreen shrub or small tree. Commonly it is known as Mexican oleander, Still tree, Yellow oleander or Current tree [12]. *C. thevetia* is native to Mexico, central America and south America [13]. *C. thevetia*, a non-edible oil-seed bearing tree species that grows naturally in north-east India. The plant has innumerable medicinal properties like antidiabetic, antimalarial, antimicrobial, antioxidant etc. as reported in ethnomedicinal and research articles [14, 15]. The plant contains a higher magnitude of cardiac glycosides especially in the fruit which makes it lethal. But from the literature survey, it is found that the bark of the plant has several important traditional uses for treating different diseases and disorders including diabetes and malaria [14,16]. *C.thevetia* is a potential feedstock for biofuel production using both chemical and thermo chemical conversion processes [17]. A considerable number of literatures have been reported on the phytochemistry of leaves, flowers and seeds of *C. thevetia*. No reports are available on the functional group analysis of the *C. thevetia*. The study was therefore targeted at determining suitable solvent for phytochemical screening and TLC profiling of *C. thevetia* plant. The aim of the current study was to accomplish the phytochemical screening, TLC profiling and functional group analysis as a justification for its use in traditional medicine.





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MATERIAL AND METHOD

Plant Collection

The samples of *C. thevetia* were collected from Navarangpura, Gujarat, India, (23.03°N and 72.53°E) authenticated and submitted (Voucher Specimen- GUCT01, GUCT02) to Plant systematics division, Department of Botany Bioinformatics and Climate Change Impact Management, Gujarat University Ahmedabad, Gujarat, India, during the month of January 2020. After collection samples were cleaned properly, and washed thrice, the fresh leaves, seeds and flowers were air-dried, pulverized by using electrical grinder, passed through a 20-mesh sieve pestle and stored at 4°C for further use.

Chemicals and Reagents

Anisaldehyde, Vanillin, Methanol, N-butanol were purchased from Sigma Aldrich Co. Toluene, FeCl₃, Acetic acid (glacial), Sulphuric acid, Chloroform and Acetone used in the present study was bought from (ACS chemicals). The rest of the chemical used in the study was achieved it locally and were of analytical grade.

Preparation of Plant Extract

The powdered sample (leaf, flower and seed) were pulverised and subjected to successive extraction with organic solvent methanol (10 g /100ml) using Soxhlet extraction method. A sample of powdered plant material (< 220 micro meter) was placed into an extraction thimble (80×22 mm, DWK life sciences, India) and extracted at 65°C with 100 ml methanol for 16 hrs with 3-4 cycles/h. The collected extracts were filtered with Whatman No. 1 filter paper and the solvent was then evaporated by rotary evaporator (IKA- RV 10 Digital, India) at reduced pressure for 30 min at a controlled temperature of 40°C and stored for further analysis at 4°C. The resulted extracts were used for preliminary phytochemical screening, TLC profiling and FT-IR analysis.

Bioassay

Phytochemical analysis

The powdered sample of *C. thevetia* leaves, flowers and seeds were subjected to qualitative analysis for identification of tannins (Gelatin test), glucose (Molisch's test), alkaloids (Dragendorff's reagents), Coumarins (FeCl₃ test), Saponins (Foam test), flavonoids (alkaline reagent) and steroids (Liebermann – Burchard test) according to the standard methods described by Harbone and Siddiqui [18-19].

Thin Layer Chromatography

Thin Layer Chromatography (TLC) is an important analytical tool in the separation, identification and estimation of different classes of natural products. The different solvent systems of different polarities were prepared and TLC studies were carried out. Crude extracts were applied on pre-coated TLC plates (Silica gel 60, UV 254 nm pre-coated 7×3 cm, 200 µm layer thickness, aluminium- backed mean particle size of 10-12 µm particle size distribution of 5-10 µm, Merck) by using capillary tubes and developed in a TLC chamber using suitable mobile phase for Tannin (CHCl₃:MeOH: water = 6.5: 3.5: 1.0), Steroid (n-butanol: MeOH: Water = 16.0: 4.0: 0.2) and Phenol (Toluene: Acetone = 9.0: 1.0). The developed TLC plates were air dried and observed under UV light at 254 nm. They were later sprayed with solution containing vanillin/ H₂SO₄ (1g of vanillin in 100 ml of H₂SO₄), anisaldehyde sulphuric acid and FeCl₃ spray for tannins, steroid and phenols respectively and heated at 120° C until maximum colour formation occurred. The movement of the analytes was expressed by its retention factor (R_f) values were calculated for different samples as shown in equation [18,20].

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR is operably the most influential tools for recognize the types of chemical bonds (functional group) available in compounds. The extracts were examined under FT-IR spectrophotometer (Bruker alpha eco ATR, USA). The peak





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values of the FT-IR were recorded using Opus software. Each plant sample was loaded in FT-IR and all the spectra were recorded at room temperature ($25 \pm 2^\circ\text{C}$) within the range of 4000 to 400 cm^{-1} in transmittance mode [21-22].

RESULTS

Phytochemical screening

The preliminary phytochemical screening results of methanol extract were given in table (1). Phytochemical screening extracts of *C. thevetia* confirmed the presence of phenols, tannins, steroids, sugar, protein and saponins. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Glycosides are absent in all methanolic extract of seed, flower and leaf.

Thin Layer Chromatography (TLC)

The TLC results of the methanol extract along with images of the developed plates are given below in table (2) and figure (2). MeOH extract showed the presence of Steroids, Tannins and Phenols. Methanol extract showed the presence of steroids confirmed by pinkish brown colour of the spot after anisaldehyde sulphuric acid spray. A phenolic compound was confirmed after its blue and red fluorescence when viewed under UV transilluminator. Tannin were present, it was ensured by the blue spot after FeCl_3 spray.

Fourier Transform Infrared Spectroscopy (FT- IR)

C. thevetia flower exhibited the varied characteristic bands ranging from 4000 cm^{-1} to 500 cm^{-1} (Table 3(a)) and the FT-IR spectrum is shown in figure (4). Band at 3319.60 cm^{-1} appeared as broad peak which could indicate the O-H stretching vibration (strong) presence of alcohol and phenol groups. Band at $2923.19\text{--}2854.48\text{ cm}^{-1}$ for Aldehyde (fermi doublet, medium). Band of strong intensity were recognized at 1740.52 cm^{-1} indicate the presence of carbonyl group (C=O) like aldehyde, ketones, acid halides, amides, lectum, amino acids, esters, carboxylic acid and anhydrides. The band obtained at 1379.54 cm^{-1} indicates the presence of amine, Band at 1626.09 cm^{-1} assigning bands of C=C, C=N, N-H indicating possible groups like amines, amides, Unsaturated aliphatic and heterocycles, at 1061.03 cm^{-1} which could indicate the C-O stretching vibration presence of ethers and esters. The results of *C. thevetia* seeds functional group analysis confirmed the presence of phenols, alkyl group, alkene, C-H aromatic ring, amines and ethers which reveals major peaks at 3316.37 , 2924.38 , 1639.71 , 1512.91 , 1372.49 and 1281.43 cm^{-1} respectively (Figure. 3(b) and Table : 3(b)). The methanolic extract of *C. thevetia* leaves FT-IR analysis results proved the presence of phenol, alkyl group, alkene, alcohol, amine, ether and alkyl halide compound which shows major peaks at 3284.89 , 2925.59 , 1610.99 , 1413.71 , 1360.30 , 1244.53 and 549.90 cm^{-1} respectively (Fig.3(c) Table 3(c)).

DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extract. There are records that show the benefits of these compounds detected from *C. thevetia* for example: Steroids are well known to be crucial for their cardiogenic, insecticidal and antimicrobial properties. Steroids have at least two functions, as precursor in the formation of other steroids e.g. Cholesterol and Sitosterol are precursors in the formation of saponins. They are also used in nutrition, herbal medicine, cosmetics and they are routinely used in medicine because of their profound biological activities [23-24]. Tannin is reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin is able to inhibit HIV replication selectivity and is also used as diuretic [25-26]. Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars [27]. The results suggest that there were not much differences in phytocompound profiles among the methanolic extract of seed, flower and leaf TLC method are best choice for the identification of secondary metabolites present in the plant. Plants and herbs contain higher number of secondary



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metabolites as they are amenable for various biological activities in human beings and animals. A significant number of studies have been accomplished to obtain purified plant chemical in the plants. Methanol extract of *C. thevetia* showed the presence of steroid (R_f values L, F, S, = 0.88, 0.88, 0.55), phenols (0.76, 0.70, 0.76) and tannins (0.96, 0.73, 0.92). The R_f values of each phytoconstituents available in methanol extracts in different mobile phase is indicate in figure (3). Phenolic compound and tannins are the main group of compounds acting as primary antioxidant or free radical scavengers [28]. Methanolic extract of *C. thevetia* were found to be similar to the previous investigations reported in this plant [29]. The present results are in good agreement with the earlier finding. TLC was performed with an aim of separating the individual components present in the extracts and to find out the suitable solvent systems which will be helpful in isolation of the components by column Chromatography. TLC is usually done for a better identification of the bioactive compounds. Different R_f values of the compounds provides an idea about their polarity that may also help in selecting a particular solvent system for further isolation of any compound from the plant extracts using chromatographic & spectroscopic techniques [30-31]. Most of the functional groups were found common for methanolic extract of seed, flower and leaf (fig. 5).

The FT-IR spectrum in figure (4) confirmed the presence of functional groups (aromatic ring, alkyl substituted ether, alcohol, alkane, carbonyl, amine). The absorption at 3319.60 cm^{-1} is due to the stretching of hydroxyl groups that are the present in the extract (Table 3(a)). The band at 2924.30 cm^{-1} is due to the symmetric stretching of saturated (sp^3) carbon (table 3(b)). The band at 1512.90 cm^{-1} is due to C=C stretching associated with the aromatic skeletal mode of the extracts. The vibrational absorption band at 1360.39 cm^{-1} was assigned to rocking of methyl group. A notable band at 1244.23 and 1059.89 cm^{-1} can be assigned to C-O stretching. A band at 549.90 cm^{-1} represent the aromatic-H out of plane bending. The FT- IR spectrum was utilized to find the functional group of the active components present in extract in reference to the peak's values in the region of IR radiation. Once the extract was passed into the FT-IR, the functional groups of the components were separated in reference to its peak's ratio. The Functional group analysis (table 3) revealed the presence of steroids, Phenols and tannins due to O-H stretching. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition. Functional group OH was found to be present uniformly in all the samples [32]. Further advanced spectroscopic studies are required for the structural elucidation and identification of active principles present in the methanolic extract of *C. thevetia* plant.

CONCLUSION AND FUTURE SCOPE

The principal objective of the study is to identify the presence of different secondary metabolites and their functional groups. The presence of these bioactive metabolites implies that the methanolic leaf, flower and seed extract of *C. thevetia* has quite a few pharmaceutical potentials that would have quite several medicinal applications. This primary information will simplify in leading further studies on the discovery of bioactive ingredients, resolve their efficacy by in vivo studies, and demonstrate their safety and effectiveness in clinical trials. The study suggests that crude extracts may possesses promising antimicrobial and antioxidant activity; thus, organic antioxidant and antimicrobial medicines can be generated for possible therapeutic applications. This is the fundamental collective report on phytochemical screening, TLC and FTIR on the Leaf, flower and seed of *Cascabela thevetia* L.

ABBREVIATION

C. thevetia: *Cascabela thevetia* L F S : Leaf Flower and Seed ; FT-IR: Fourier Transform Infra-Red; TLC : Thin Layer Chromatography

CONSENT FOR PUBLICATION

Not applicable





AVAILABILITY OF DATA AND MATERIAL

Not applicable

COMPETING INTERESTS

All the authors approve that they have no conflicting interests, which may seem to have affected the research documented in this paper.

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AUTHORS CONTRIBUTION

PS and AS performed the experiments and provided major contribution in writing the manuscript. SP, DV and NM was associated in supervising, advising positioning and structuring the manuscript. All authors read and approved the final manuscript.

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STUDY INVOLVING PLANTS

As per the local and national guidelines and legislation and the required or appropriate permissions and/or licenses for the study.

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Table 1: Phytochemical screening of Methanolic Extract of *Cascabela thevetia* L.

Tests for secondary metabolites	Seed	Flower	Leaf
PHENOLS			
Ferric chloride	A	A	A
Lead acetate	P	P	P
Potassium dichromate	P	P	P
Alkaline reagent	A	A	A
TANNINS			
Ferric chloride	A	A	A
Lead acetate	A	A	P
Potassium dichromate	P	P	P
STEROIDS			
Lieberman -sterol test	P	P	P
Lieberman -burchard test	A	A	P
GLYCOSIDES			
Kelle- kiliani test	A	A	A
SUGARS			
Molies's test	P	P	P
Benedict's test	A	A	A
Iodine test	A	A	A
Fehling test	A	A	A
PROTEINS			
Millan's test	A	P	A
Ninhydrien test	P	A	A
SAPONINS			
Frothing test	P	P	P

Table 2: TLC Profile of Methanolic Extract of *Cascabela thevetia* L.

Phytochemical	Solvent system	Spraying reagent	Remarks
Tannins	CHCl ₃ :MeOH: Water (6.5: 3.5: 1.0)	(0.5 W/V) vanillin spray	Present
Steroids	n- Butanol:MeOH: Water (16.0: 4.0: 0.2)	Anisaldehyde sulphuric acid	Present
Phenols	Toluene: Acetone (9.0: 1.0)	FeCl ₃ Spray	Present



Pravin Solanki *et al.*,Table 3: FT-IR Analysis of Methanolic Extract of *Cascabela thevetia*L. (A) Seed, (B) Flower and (C) Leaf

(A) FT-IR Analysis of Methanolic Extract of <i>Cascabela thevetia</i> L Seed					
Sr.no	Peak values (Cm ⁻¹)	Frequency range cm ⁻¹	Intensity	Functional group	Functional groups name
1	3319.60	3500-3200	Strong, broad	O-H Stretching vibrations	Alcohol, Phenol
2	2923.29-2854.48	2900-2700	Medium	CHO (Fermi doublet)	Aldehyde
3	1740.82	1850-1650	Strong	C=O stretching vibrations	Carbonyl
4	1626.09	1650-1550	Medium	C=C, -C=N, Unsaturated heterocycles	Alkene, Aromatic ring
5	1379.54	1400-1300	Medium	C-N Stretching vibration	Amine

(B) FT-IR Analysis of Methanolic Extract of <i>Cascabela thevetia</i> L Flower					
Sr.no	Peak values (Cm ⁻¹)	Frequency range cm ⁻¹	Intensity	Functional groups	Functional groups name
D 1	3316.31	3500-3200	Strong, broad	O-H stretching vibrations	Alcohol and phenol
2	2924.40	3100-2800	Medium	C-H stretching vibrations	Alkyl group
3	1629.71	1650-1550	Medium	C=C, C=N,	Alkene, Aromatic ring
4	1512.91	1450-1600	Medium	C-H aromatic ring	Aromatic ring
5	1372.49	1400-1300	Medium	C-N stretching vibration	Amine
6	1281.43	1300-1000	Strong	C-O-C and C-OH ethers,	Ethers, alcohol

(C) FT-IR Analysis of Methanolic Extract of <i>Cascabela thevetia</i> L Leaf					
Sr.no.	Peak values (Cm ⁻¹)	Frequency range cm ⁻¹	Intensity	Functional groups	Functional groups Name
1	3284.90	3500-3200	Strong, broad	O-H stretching vibrations	Alcohol and phenol
2	2926.59	3100-2800	Medium	C-H stretching vibrations	Alkyl group
3	1610.99	1650-1550	Medium	C=C, C=N, Unsaturated heterocycle	Alkene, Aromatic ring
4	1413.71	1420- 1330	Medium	O-H bending alcohol	Alcohol
5	1360.39	1400-1300	Medium	C-N stretching vibration	Amine
6	1244.23	1300-1000	Strong	C-O-C and C-OH	Ether, Alcohol
7	549.90	500-800	Strong	C-Cl stretching vibration	Alkyl halide





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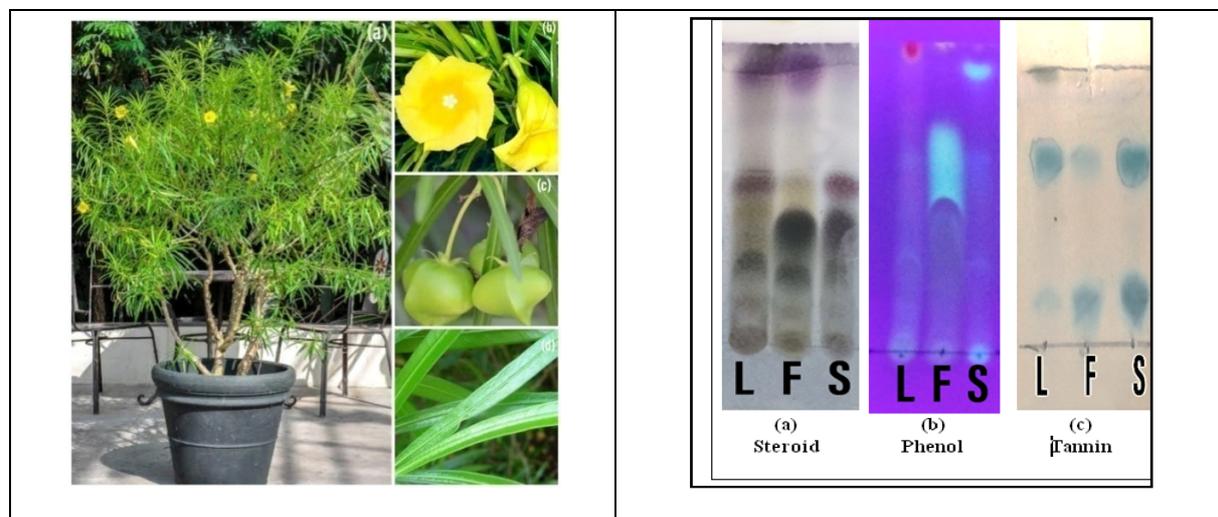


Figure 1: *Cascabela thevetia* L. (A) Whole Plant, (B) Flower, (C) Seed And (D) Leaf

Figure 2: TLC Profile Image Of Methanolic Extract Of *Cascabela thevetia* L. (L= Leaf, F=Flower, S=Seed)

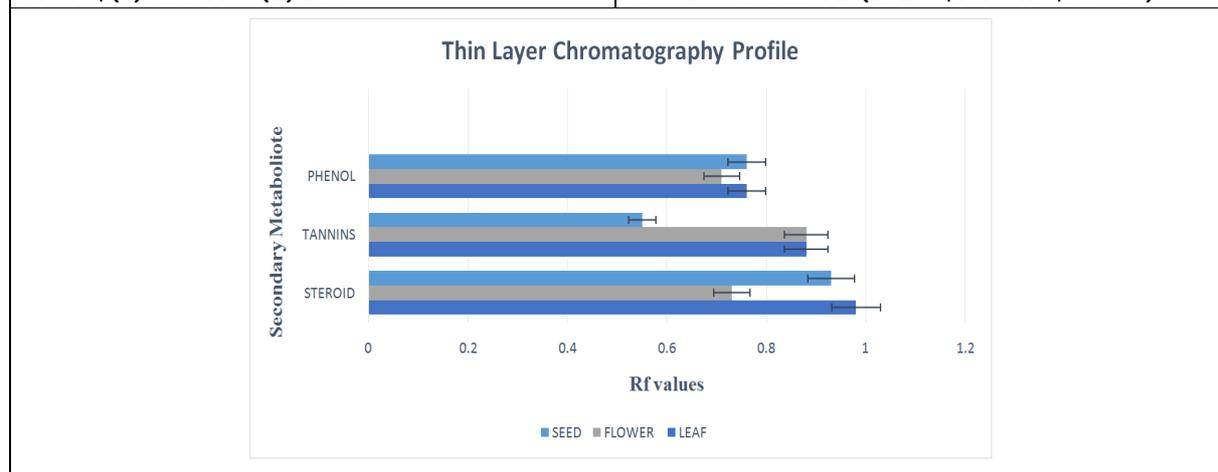
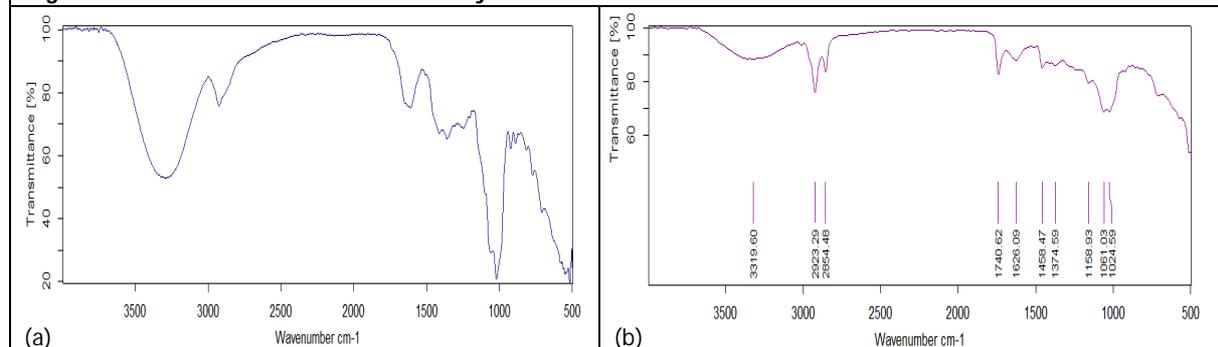


Figure 3: Rf values of for various secondary metabolite in methanolic extract of *Cascabela thevetia* L.





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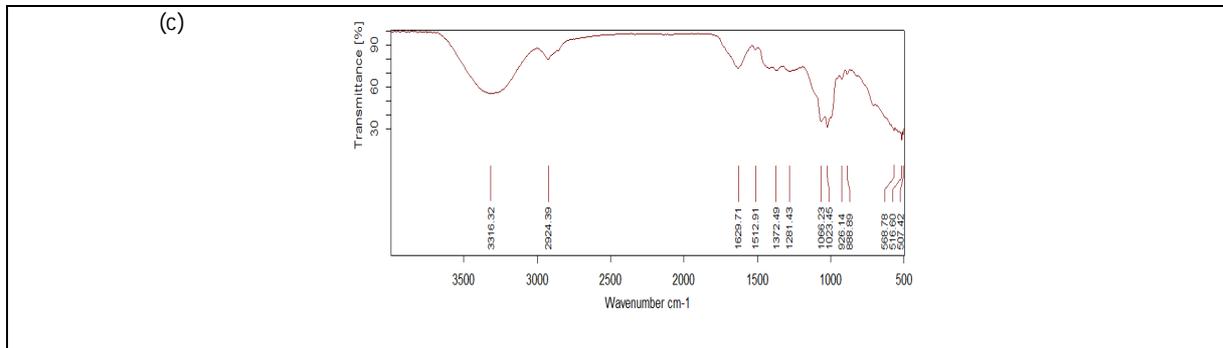


Figure 4:FT-IR spectrum of methanolic extract of *Cascabela thevetia* L.(a)Flower (b) seed and (c) leaf

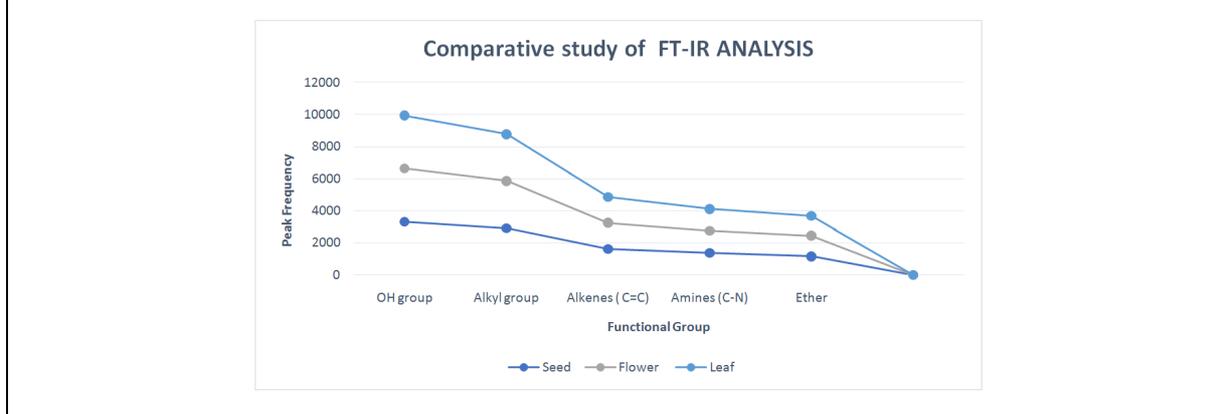


Figure 5:FT-IR Analysis of methanolic extract of *Cascabela thevetia* L.





Effects of Social Determinants on Economic Progress and Expansion Female Education and Health

Vipin Jain¹ and Rohin Garg^{2*}

¹Director/Principal, Professor, Teerthanker Mahaveer Institute of Management and Technology, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India.

²Associate Professor, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), Rajkot, Gujarat, India.

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*Address for Correspondence

Rohin Garg

Associate Professor,

Department of Anatomy,

All India Institute of Medical Sciences (AIIMS),

Rajkot, Gujarat, India.

Email Id: rohingarg99@gmail.com



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ABSTRACT

The socioeconomic factors present different social and private benefits and investment cost for female education, which creates variable private and social rates of return on female education in our country. Thus, leading to different amounts and resources to be invested in female education. Some still argue that the female education has direct and significant correlation with economic growth in developing countries like ours, which is based on the fact that intrinsic ability for both men and women are same and equal. To understand the effect of social determinants on women health and education; a cross-disciplinary study was conducted at Public Health Department, TMMC & RC and Teerthanker Mahaveer Institute of Management & Technology, Moradabad, UP, India. The study was conducted for six months in an area of 15 kilo meters around our collage, including 1,852 study samples. The age group selected was 18-62 years. Considering and analyzing level of education, it was observed that only 963 women were educated and were able to produce an evidence of the same, amounting mere 52% of the total. Out of the educated women 702 had attained primary education i.e. 73%. Secondary education was acquired by 183 women i.e. 19% and only 77 women were found to be able to get tertiary education amounting 8% of the educated pool. Only 77 of the educated women i.e. 8% continued education after the age of twenty, rest dropped out of school before that. The study described investments in women's health and education, and its impact on economic growth of the society. The habits of good health among women is important for child development and also for the production of future human capital. Non availability of primary health care and primary education still stand out as a major concern.

Keywords: Socioeconomic, Cross-Disciplinary, Economic Progress, Female Education, Health.





INTRODUCTION

An effort has been made to study and analyze both female education and health aspect in a systemic manner. So, according to The World Bank's 1993 World Development Report which concluded that health issues in women are not intrinsically, but also instrumentally essential for a society's development [1,2]. In the year 1994, the International Conference on Population and Development (ICPD) with about 20,000 delegates from 179 countries, came forward to discuss global development challenges. Now nearly 20 years have passed since the ICPD, and recent research has shown how investments in health and adequate health policies affect economic growth and development through different sources [3,4]. It includes labor, productivity, savings, education, and age format [5,6]. The research are yet to deliver is an exclusive investment policy describing where and how to provide resources in order to regulate fruitful health and economic gains. Now considering role of female education in economic growth we need to understand the status of women education first. Female enrolled are more in the developed areas and in urban areas than in the developing regions or let's say rural areas. The socioeconomic factors present different social and private benefits and investment cost for female education, which creates variable private and social rates of return on female education in our country. Thus, leading to different amounts and resources to be invested in female education. Some still argue that the female education has direct and significant correlation with economic growth in developing countries like ours, which is based on the fact that intrinsic ability for both men and women are same and equal. This results that if more women are educated, the total level of intrinsic ability of society is higher both economically and intellectually. Therefore, female education has more marginal returns in the developing economies. Majority of diseases affect both sexes differently, but due to the social and biological factors, women and men are exposed to differing types of risks to health. This can be under stood with an example as, the primary risk factor for lung disease in men is considered smoking, but in case of women it is household air pollution [7]. Also, women live longer than men, with the gender gap in life expectancy increasing from 4.8 to 5.7 years on an average [8]. In young adult women it was observed that morbidity was high and they have a heavier burden of years of life lived with certain disability [9]. Women were seen to face constant obstacles in achieving sound care and preventing diseases; namely gender discrimination, lack of education, and domestic violence, etc., [10]. Not only in our country but also globally, have great health discrepancies in existence. The data analyses from the Global Burden of Disease Study in 2013 also shows that the risk of maternal mortality is many times higher among women in developing countries than in developed countries [11]. A number of barriers are observed in female education especially in the developing countries, which has led to low female enrolment in educational institutions. Poverty being one of the major reasons that fewer women attend schools. It has been a trend in developing countries that, women from poorer families give up education before men do. The idea of female education has higher opportunity cost due to greater responsibility in the household and social norms was observed throughout. In some parts of the developing countries, the concept of early marriages, might unable women to pursue higher education.

MATERIALS AND METHODS

To understand the effect of social determinants on women health and education; a cross-disciplinary study was conducted at Public Health Department, TMMC & RC and Teerthanker Mahaveer Institute of Management & Technology, Moradabad, UP, India. The study was conducted for six months in an area of 15 kilo meters around our collage, including 1,852 study samples. The age group selected was 18-62 years. The area included semi-rural and township areas, with both residential and commercial units. Those without any permanent address were excluded from the study. The analysis is based upon three main headings; (a) women education (b) women health and (c) economics. Our aim was to focus on women's health and education in particular, which did not include effects on men's health. First set of questions included the level of educational qualification, number of years enrolled in education was there any financial return due to the education attained, what was their annual income if any and obstacles they faced during their education. The second set of question included the age at they were married, pregnancy status, past pregnancy, time gap between pregnancy, any acquired diseases, work related diseases and an





average expenditure on health per annum. Subject's identity was kept anonymous and confidentiality was kept as promised.

RESULTS

Table 1 shows the general characteristics of females enrolled in the study. Considering and analyzing level of education, it was observed that only 963 women were educated and were able to produce an evidence of the same, amounting mere 52% of the total. Out of the educated women 702 had attained primary education i.e. 73%. Secondary education was acquired by 183 women i.e. 19% and only 77 women were found to be able to get tertiary education amounting 8% of the educated pool. Only 77 of the educated women i.e. 8% continued education after the age of twenty, rest dropped out of school before that. (Graph1). Out of total 1,852 women only 593(32%) had any sort of income, rest 1259 (68%) women were housewives or were unemployed. Out of 593 (32%) of earning women only 148 (25%) women were earning due to their educational qualification. Out of the 593 women only 249 women (42%) had an annual income above two lacs. (Graph 1,2) While listing the barriers faced during education, surprisingly there were hardly any incidence where subjects were not interested in education. Where 39% reported financial conditions to be the reason behind dropping out of education, 48% reported social stigma to be the reason. Hardly 2% of the subjects showed no interest in education at all. Whereas all 98% of the subject wanted to pursue a higher level of qualification. Now considering the health aspect in the study samples out of 1,852 women 722 were married before the age of 20 years of age. (39%). Fertile women documented to a total number of 1,629(88%) out of which 521 females were currently pregnant (32%) and about 1,124 women had a gap of at least two years between two pregnancies (69%). Of the total 1852 study samples 667 women were found to be suffering from an acquired disease mainly work related (36%). About 1,019 (55%) did spend more than fifty thousand rupees per annum on their health with a valid record (Graph 3).

DISCUSSION

The study so conducted has shown interesting facts pertaining to women health and education, which involved a life span approach and has selected study samples from a holistic view, including women's reproductive, sexual, physical and mental health, educational status, levels of education and productivity of the education too. While most of the literature defines women's health as limited to reproductive health only, we made an effort to analyze how women's overall health and educational status relates to economic development of the society. Further an attempt was made to understand, how women's health and education and its economic importance beyond reproduction and fertility. Our study acknowledges the relevance of women's mental health also, and its progressive effects on economic outcomes. The study is non bias and was kept away from social stigmas. But, still there may remain reporting biases in the real text on women's health and economic development [12]. The cross-disciplinary nature of our analysis is one of its strengths, but a strict divide and lack of cross-referencing was observed between the health/education and the economic outcomes. A cross-disciplinary method is highly recommended so as to promote more efficient use of existing knowledge. A fewer educated woman reduces human capital thus leading to poor economic growth in the society. So, a poor quality of human capital decreases productivity and economic growth. Also, health related issues and lack or poor quality of education are the factors affecting human capital adversely. It should be understood that an equal schooling system and standards has positive correlation to investment rate indirectly through a higher human capital. A direct relation between female education and fertility rate is observed, by reducing population growth, lowering burden and dependency. Female education also reduces fertility rate by increasing literacy and eventually decreasing mortality rate. As female education improves quality and the economic standards. A number of barriers are observed in female education in developing economies. Thus, leading a low female enrolment rates in jobs and service sector. Poverty being one of the many reasons that fewer women enroll and attend school or colleges. Many women from poorer families had given up education before men do. The female





education shows an opportunity cost due to greater responsibility in the household and social norms like early marriage. In early marriages as a social norm might deprive women to pursue higher education.

CONCLUSION

As the conclusions of this study are based on study groups in low- and middle-income groups of the society thus, increasing credibility of the analysis. The study described investments in women's health and education, and its impact on economic growth of the society. The habits of good health among women is important for child development and also for the production of future human capital. Non availability of primary health care and primary education still stand out as a major concern. Promotion of higher education among women has a significant effect on economic growth and development, but still availability of primary and secondary education plays the foundation for economic growth in Indian women so as to make them independent to make choices for themselves.

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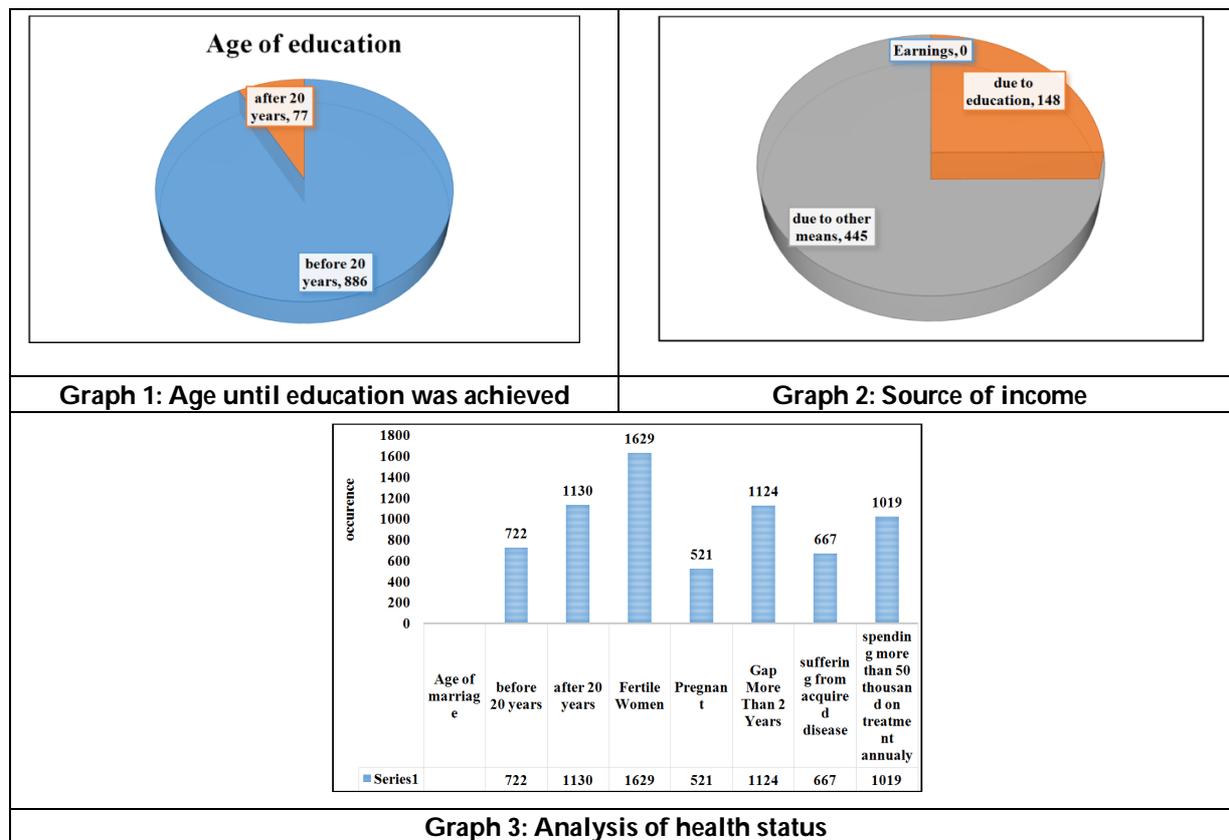




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Table 1: General characteristics of females

Education	n	Health	n
Educated women	963	Age of marriage	
Primary	703	Before 20 Years	722
Secondary	183	After 20 Years	1130
Tertiary	77	Fertile Women	1629
Age of education		Pregnant	521
Before 20 Years	886	Gap More Than 2 Years	1124
After 20 Years	77	Suffering from acquired disease	667
Barriers		Spending more than 50 thousand on treatment annually	1019
Financial	376		
Social	462		
Income			
Above 2 Laks	249		
Below 2 Laks	344		
Earnings			
Due to Education	148		
Due to Other Means	445		





Emotion Recognition Model Based on Dynamic Time Warping and Generalized Gamma Distribution

Sreenu Ponnada^{1*}, Srinivas Yarramalle², Nagesh Vadaparathi³ and Surya Prasad Potnuru⁴

¹Department of CSE, Vishnu Institute of Technology, Bhimavaram, Andhra Pradesh, India.

²Department of CSE, GIT, GITAM, Visakhapatnam, Andhra Pradesh, India.

³Department of Information Technology, MVGR College of Engineering, Andhra Pradesh, India.

⁴Department of ECE, MVGR College of Engineering, Andhra Pradesh, India.

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*Address for Correspondence

Sreenu Ponnada

Department of CSE,
Vishnu Institute of Technology,
Bhimavaram, Andhra Pradesh, India.



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ABSTRACT

In this article, the detection of voice emotions using Generalized Gamma Distribution (GGMM) is introduced. Emotion recognition helps out in identifying the innate expressions of people from the language database. This article describes Dynamic Time Warping (DTW) technology, which uses speaker-independent emotion detection using 39 MFCC functions. A tone of approximately 900 isolated word samples from five different words emotions are collected and recorded with a sampling frequency of 15 to 200 kHz. 39 MFCC functions are used to generate the training and test models. In this proposed work, the Melting frequency Cepstral coefficients (MFCC) coefficients are taken from the speech database, a prototype version of each word in the vocabulary is recorded by using DTW and the incoming emotions with each word are calculated. The GGMM is used to classify emotions. The experiments are carried out using MFCC, delta coefficients (Δ MFCC) as well as the delta-delta coefficients ($\Delta\Delta$ MFCC).

Keywords: Emotion Recognition, MFCC, Dynamic Time Warping, Generalized Gamma Distribution, Gaussian Mixture Model

INTRODUCTION

Language is one of the most capable forms of automatically understanding a human's thoughts, in addition to individual facial expressions. Emotions play an important role in surveillance systems; lie detection, clinical assistance, and video games are also referred to as complementary emotion detection situations [1]. It must be borne in mind that a technical approach can only be focused on realistic decisions about the form, magnitude and number

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of emotions when considering classification in practice. By adapting and restricting this number and type of recognizable feelings to the requirements defined in the application, robust classification can be assured. There is no criterion for the classification of emotions in technical recognition, however. It is also preferred to differentiate between a given set of discrete feelings [2] [3] [4]. Much of today's approaches to understanding speech feelings are focused on global statistics of phrases [5] [6] [7] [8]. Interpersonal communication is a type of interaction that includes the exchange of ideas and mutual emotions. The information is conveyed by using the gestures and voices during human-to-human interaction. The language is a special form of sound and is a basic way to transmit information between different people. The words are not just parts of a language. The acoustic properties of speech during an interaction also have important emotional properties. There are emotions in every part of the language and transfer of the linguistic emotions is done from one communicator to another. Due to the exchange of emotions during an ongoing conversation, the emotional state of a participant can easily be triggered by the speaker's emotional state, and leads to a change in the style of speech [9] [10]. In order to explain emotions dependent on speech Production, much research has been conducted. A speech recognition system's performance is also characterized by accuracy and speed with advances in both hardware and software DSP (digital signal processing). Despite all these improvements, the output of the machine cannot be compared with its human counterparts, particularly the recognition of emotions independently of the speaker. In this article, emotions are classified using MFCC properties and DTW. The recognition accuracy of MFCC function is thought to mimic the perception of the human ear. The remainder of the contribution is as follows. Part 2 of the article focuses on relevant work in this research area and the feature extraction based on MFCC coefficients is described in Part 3. Part 4 describes Dynamic Warping and Part 5 presents a Generalized Gamma Distribution. Parts 6 and 7 summarize the final results of Par 5. The last Part 8 summarizes the article.

RELATED WORKS

A new spectrum modeling feature (MSFs) was proposed by Wu et al. [9] that recognizes the human sense of speech. A convenient feature derived from the long term spectrum evoked by long term voice using the modeling filter bank and control bank for attenuation of speech. The acoustic frequency and timely frequency shift components were obtained by this approach to provide critical data from typical short-term spectral properties. The radial basic function (RBF) SVM has been adopted for classification purposes. The Berlin dataset is used to evaluate the MSF. In an experimental result, MSFs show potential performance compared to MFCC and visual sequence prediction coefficients (PLPC), and in this article the classification accuracy attributed to is approximately 91.6%. In recent years, speech statistics have been used to describe emotions. Cao et al. [10]. proposed an SVM classification to synthesize emotion recognition information to solve the binary classification problem. This sorting method guides the SVM algorithm for specific emotions, treats the data from each messenger as a specific query, and then combines all the custodians' predictions to implement multiple class prophecies. An SVM rating has, above all, two advantages for training and testing grades in a speaker - it is independent of taking certain data from a speaker. Second, he thinks that the intuition that every speaker can express in a mixture of emotions recognizes the strong emotion. Sequencing methods have a great advantage in terms of accuracy over Traditional SVM in two public active emotional speech datasets, Berlin and LDC. In both active and spontaneous data, this includes neutral intense emotional practices; achieved SVM based on higher sequencing accuracy in recognizing emotional applications of normal SVM techniques, in this article 44.4% of accuracy is achieved by the authors. Through the 3-level speech emotion recognition process, Chen et al [11]. Suggested improving speech recognition in an individual speaker. This method coarsely sorts the various emotions and then uses the Fisher level to pick the required attribute. Multilevel classification based on SVM takes as an input parameter the Fisher level output. Furthermore, in the scale and classification of 4 comparative studies, the principal component analysis (PCA) and the artificial neural network (ANN) are used. Fisher + SVM, PCA + SVM, Fisher with ANN, and PCA to the four comparative studies. Fisher is higher than PCA, and SVM is more prevalent than ANN for ranking because of a sense of appreciation in an autonomous speaker. The acceptance rates for the three levels are 86.5%, 68.5% and 50.2%, respectively.



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A new method for classifying the emotions of signals was introduced by New et al.[12]. To distinguish speech signals and the individual classifier, this method uses the short-term log frequency (LFPC) and HMM power coefficients separately. This technique separates the emotion into 6 groups and then the new system was trained and evaluated using private data. By comparing the LFPC with the ordered MFCC and Cepstral coefficients (LPCC) calculation, the suggested method output is assessed. The outcome indicates 78 % and 96 %, respectively, of the average and best achieved classification accuracy. Wrong et al.[13] provided trees with a large number of attributes to identify emotions without relation to any language or grammar with a random tree set (ERF Trees), and an unanswered problem remains. A small amount of data is added to this system with a large number of attributes. In addition, ERFT outperforms common dimensionality reduction techniques such as PCA and recently developed multivariate scaling (MDS) and ISO Map. This approach has improved the degree of understanding of emotion. Optimal accuracy achieves the maximum accuracy of 82.54 % with a female data set with 16 features, whereas the lowest rate is just 16 % for 84 features with the natural data set. Narayanan [14] proposed understanding a basic sense of terrain from the call centre application using speech signals. This research focuses on the identification of negative and non-negative emotions such as anger and happiness; emotions, including phonetics, vocabulary, and communication, are identified using different types of knowledge. In addition, to obtain data on emotional intelligence at language level, theoretical information content of emotional emotion was presented. In order to deal with various types of properties, both the k-NN and linear separation classification are used. The experimental results indicate that a mixture of acoustic and linguistic data is used to produce the best results. The findings indicate that the accuracy of the classification improved by 40.7 percent for males and 36.4 percent for females by integrating three information sources instead of a single source. Compare the relevant market development area with precision from 1.4% to 6.75% for men and 0.75% to 3.96% for men and 0.75% to 3.96% for women.

In order to understand the frequency of supposed emotional events, a novel method suggested by Arias et al.[15] uses an impartial model. The aim of obtaining the natural variability of F0 contours is a new approach assisted by functional data analysis (FDA). PCA is known to be used as a speech perception recognition feature for a particular F0 contour. The empirical result shows that in binary classification, the precision of the proposed method exceeds 75.8 %. With a lot of F0 numbers, this means 6.2 % higher than a studied benchmark method. The approach is assessed using the SEMAINE dataset. In order to recognize the feeling of speaking, the results express this, using a form-based approach in reality practice can be successful. A versatile model proposed by Grimm et al. [16] uses emotional primers for emotional expression recognition. Three measurements were taken from 3 different emotional primate values called phantom, activation, and leadership. Estimated values of these factors are in the range [-1, +1]. A text and image-based approach has been introduced in assessing core emotions and get the best moderator. Both fuzzy and predictive logic based on a rule are used to capture acoustic properties such as energy, core, and spectral specifications. The validations are validated by testing the EMA and VAM datasets where the feeling and sensation of speech without energy is applied. A hierarchical computational framework for explaining emotions was proposed by Lee et al [17]. This is accompanied by a set of binary sequences, which map a speech signal to one of the corresponding emotion groups. To minimize the key error, it is best to resolve the classification function at a different level in a tree. By using AIBO, the classification system is evaluated and USC IEMOCAP databases are used for evaluation. Compared to the SVM baseline, the final outcome increases the overall accuracy of progress archives by 72.44 percent-89.58 percent. The outcome verified that the classification method described was effective in classifying emotional speech in several databases.

In order to classify emotions and classify classes, Albornoz et al [18] studied a new spectrum property. Based on phonological characteristics and the new hierarchy classification, emotions were gathered in this analysis. Based on unique configurations and input properties, various classifications such as HMM, GMM, and MLP were tested in order to build a new rating system for classifying emotions. The proposed approach is revised twice, first of all using a set of main performance characteristics and selected Secondly, the main classification output is similar to the explorer by class of aggregate features. The experimental findings in the Berlin dataset show that with cross validation, the hierarchical approach outperforms the best traditional navigator. For instance, the output of the

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HMM standard mode reached 68.57 % and the hierarchy model reached 71.75 %. Yang and Lutter [19] have implemented a new collection of harmonic features for speech-sensory recognition. It begins from the predicted signals of expression, followed by the pitch histogram's spherical autocorrelation. It tests the frequency of two distinct lengths, resulting in a harmonic or discordant appearance. The Bayes player plays an important role in the classification stage with the emergence of a Gaussian class.. The results of experiments based on Berlin University database using fit features reflect in the recognition performance improvement. Wu et al. [20] suggested a fusion-based technique for speech recognition using several groups and acoustic-prosodic characteristics (AP) and semantic pamphlets (SLs). In this fusion mode, the AP features are first extracted, and then there are 3 basic classification forms, namely GMM, SVM, MLP and Meta decision tree (MDT). The relationship between the rules of emotional association (EAR) and emotional states in emotional recognition is given by the presented Maximum Entropy Model (Max End). For the interpretation of the emotional recognition outcome, the combined outcome is taken from SL-based and AS-based in the last state. The experimental results on a private dataset provide performance based on 80% of MDT files using SL 80.92 identity files and 83.55% using AP and SL files.

MFCC BASED FEATURE EXTRACTION

An significant part of emotion recognition is the extraction of characteristics, on the basis of which the remainder of the classification and recognition relies on the acoustic pattern of speech signals. The MFCC features function better for emotion recognition as LPC, LPCC with different experiments [22], [23], [24] [26]. No standard number of MFCC voice recognition coefficients exists in any literature. The MFCC is a compact way of representing spectral sound information. 20 Mel Triangle Filters are built with a 50 percent overlap. To obtain a coefficient, the spectrum is applied from each philter so that the first 13 coefficients are considered as characteristics and then modified to the Mel scale by making use of the conversion relationship given below.

$$f(\text{Mel}) = 2595 * \log_{10} (1 + 1/100) \dots \dots \dots (1)$$

13 MFCC coefficients are considered due to the fact that it provides better recognition accuracy than others coefficients.

DYNAMIC TIME WARPING (DTW)

The most convenient way for individuals to connect is through the voice portrayed as a sound. The sound properties [27] [28] [29] [30] [31] [32] [34] are all distinct. A special algorithm, DTW, which measures the similarity of a model having different time zones, is required to recognize sound compatibility. With smaller distances produced, the two models of sound are more similar. If the two sound models were similar, the two voices would be identical. The initial data from the method of speech recognition is translated into frequency waves. The volume, pronunciation time, and noise around the recording impact the distance created by speaking out. The optimal distortion path between two speech signals is determined by the DTW algorithm and is used for the output distortion values and distance. The direction of the implies the greater the similarity between the two patterns that are produced. There could be different times for two words from the same user in the same word.

Database collection

The five emotions, namely sad, happy, angry, surprised and neutral, are collected from the students of GITAM University. 200 isolated words with a variable time constraint between 15 and 200 kHz sampling frequency are collected from each person and a total of 900 samples are collected for the experiment. Of 900 samples, training uses 600 and testing takes 300. 39 MFCC functions are used to create the training models.





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Generalized Gamma Mixing Model

A continuous likelihood distribution using 3 parameters is the generalized gamma distribution. They are $a > 0$, $d > 0$, and $p > 0$, which is a two-parameter gamma distribution generation. Since many distributions are often used in survival analysis (exponential, Weibull, and gamma distributions) for parametric models, they are generated in special cases. In this article, to categorize the emotions produced from EEG signals, a generalized gamma distribution is projected. In general, the underlying feelings expressed by each of the signals can be interpreted when signals from the brain are collected, based on the strength levels. The description of signal variations is carried out as follows. Tail distributions are more fitting and the most suitable distribution is classification of the signals with the smallest variations. Generalized Gamma Distribution displays the random variable X with the generalized gamma, in short generalized gamma (a, β, γ). A generalized gamma random variable, "x" with scale parameters, " α " and the shape parameters " β " and " γ " is given as:

has a probability density function $f(x) = \frac{\gamma x^{\gamma\beta-1} e^{-(x/\alpha)^\gamma}}{\alpha^\gamma \Gamma(\beta)}$ $x > 0$(2)

Experimental Evaluation

A database is used to demonstrate the proposed method with 300 speakers having different dialects, with five basic emotions being sad, happy, angry, bored and neutral.

The proposed algorithm model is given as

Phase -1: Extraction of MFCC coefficients.

Phase -2: Clustering data having different sample sizes as input for DWT.

Phase -3: Extraction of emotional characteristics from DWT and get the PDF using the general gamma distribution

Phase 4: Follow steps 2 and 3 for testing purposes.

RESULTS

The emotional speech signal to be evaluated is trained and defined in part 4 of the paper, and the properties acquired are compared based on MFCC coefficients with the current emotions. The characteristics of the test emotion are contrasted with true truncated values using the Gaussian Mixture Model by using the emotions that exist in the database, and the results of the proposed model are tabulated in the form of an uncertainty matrix and are displayed in Tables 1 and 2. The developed model is also compared using a standard data set in Berlin and the obtained results are shown in Tables 3 and 4. From the above Fig. 2 & Fig. 3, which demonstrates the accuracy of the emotions related to both male and female subjects? The experimental values clearly depict that the accuracy is higher for the proposed model.

CONCLUSION

In this work, a novel method of recognizing emotions using the generalized gamma distribution is developed. Rating of the emotions is done by GITAM students using different dialects. Recording of these emotions is done from five different emotions in 20 ms. The speech database is based on an active sequence of a short emotional phrase consisting of 5 distinct emotions from 200 speakers (students) from different parts of India. Features are extracted and the feelings of test speakers are measured and categorized. In Table 1 and Table 2, the results obtained are shown in the exchange matrix for both sexes. From the table, it clearly indicates that for some emotions, the recognition rate is 90%, and for other emotions, the recognition rate is almost 85%. The developed method is also tested for different sample size. This output is compared with the output of existing GMM based model. From Table 1 and Table 2 it is clear that the proposed method exceeds the performance of existing model. The overall emotion rate is over 80% which shows that the developed model works well in identifying emotions.





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Table 1: Confusion Matrix for identifying various Emotions of Male Subjects

Stimulation	Recognition Emotion (%) w.r.t proposed model					Recognition Emotion (%) using GMM				
	Angry	Happy	Sad	Neutral	Boredom	Angry	Happy	Sad	Neutral	Boredom
Angry	92	9	1	0	0	88	0	8	4	0
Happy	6	86	0	8	0	10	72	0	18	0
Sad	0	0	93	7	0	0	8	80	12	0
Neutral	0	7	6	88	0	10	0	10	65	15
Boredom	0	8	8	4	80	0	16	0	8	76

Table 2. Confusion Matrix for identifying various emotions of Female Subjects

Stimulation	Recognition Emotion (%) w.r.t proposed model					Recognition Emotion (%) using GMM				
	Angry	Happy	Sad	Neutral	Boredom	Angry	Happy	Sad	Neutral	Boredom
Angry	88	6	4	2	0	82	0	0	4	4
Happy	4	89	0	7	0	11	69	0	10	10
Sad	2	2	90	6	0	0	9	83	7	0
Neutral	0	9	2	89	0	10	0	12	70	18
Boredom	0	6	6	8	82	0	10	8	10	72

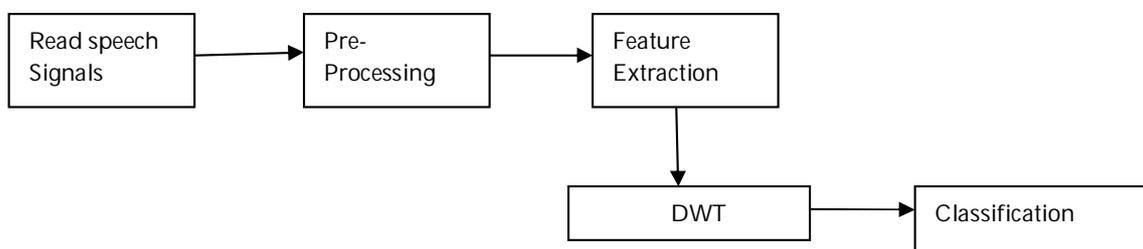


Figure. 1 Block Diagram for Emotion Recognition





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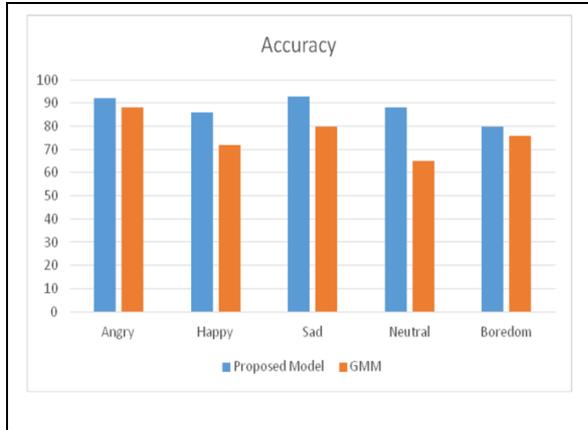


Figure 2: Comparison of emotions related to Male Subjects

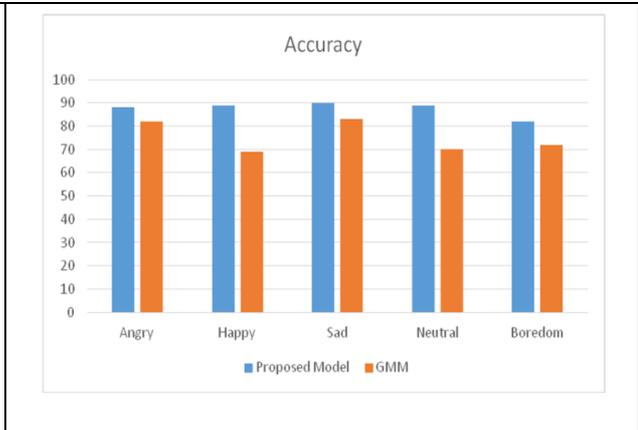


Figure 3: Comparison of Emotions related to Female Subjects





Antibacterial Activity of Unripe Avocado Fruit Peel (*Persea americana*) and its Constituents against Multidrug-Resistant Urinary Tract Infection Pathogens

Louis M. R. Lima Mirabel¹, V. Pushpa Rani^{1*}, Padma Krishnan², Kedike Balakrishna³ and Antony Stalin⁴

¹PG and Research Department of Advanced Zoology and Biotechnology, Loyola Institute of Frontier Energy (LIFE), Loyola College, University of Madras, Chepauk, Chennai, Tamil Nadu, India.

²Department of Microbiology, Dr.ALM PG IBMS, University of Madras, Taramani, Chennai, Tamil Nadu, India.

³Entomology Research Institute, Loyola College Campus, Chennai, Tamil Nadu, India.

⁴State Key Laboratory of Subtropical Silviculture, Department of Traditional Chinese Medicine, Zhejiang A&F University, Hangzhou 311300, China.

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*Address for Correspondence

Louis M. R. Lima Mirabel

PG and Research Department of Advanced Zoology and Biotechnology,
Loyola Institute of Frontier Energy (LIFE),
Loyola College, University of Madras,
Chepauk, Chennai, Tamil Nadu, India.

E.mail : admirabel15@gmail.com / push.rani76@gmail.com



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ABSTRACT

The chloroform and methanol extracts of the leaves and unripe fruit peel of *Persea americana* were tested for antibacterial activity against MDR bacteria viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Staphylococcus aureus*. The well diffusion method was used. The chloroform and methanol extracts demonstrated a preciable activity against the tested organisms. The antibacterial activity of all the extracts was tested at the concentration of 4.0 mg/ml. The extract of the leaves showed activity against the tested organisms comparable with the standard azithromycin(30µg/ml). The chloroform extract of the unripe fruit peel showed lesser activity. The methanol extract of the unripe fruit peel showed the highest activity with *K.pneumoniae* as the most sensitive organism. Bioactivity guided fractionation of the methanol extract of the fruit peel resulted in the isolation and identification of the active constituent as the flavonoid glycoside hyperin (Quercetin -3-O-β-D-Galactopyranoside). *K.pneumoniae* was the most sensitive organism with MIC value of 16 µg /ml. Docking analysis of hyperin with murF ligase enzyme of *K.pneumoniae* showed appreciable binding.

Keywords: *Persea americana*, Antibacterial activity, MDR strains, Hyperin.





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INTRODUCTION

Plants contain many biologically active structurally diverse compounds that are useful as drugs, lead structures or raw materials and are used primarily for curing mild or chronic diseases[1-2]. Herbal medicines are in high demand around the world for primary healthcare because of their broad biological and medicinal activities, higher safety margins, and lesser costs [3]. Plants are rich in secondary metabolites as steroids, terpenoids, alkaloids, tannins, flavonoids etc. which possess antimicrobial properties. Unfortunately, the increasingly widespread use and misuse of antibiotics have led to the rapid appearance of antibiotic-resistant strains today. Many infections are caused by microorganisms that fail to respond to conventional treatments. Urinary tract infection (UTI) is one such disease caused by multidrug-resistant bacteria and defined as the presence of a threshold number of pathogenic bacteria (10^5 CFU/mL) in the urine. Invasive/progressive infections of the tract with an increased bacterial population cause cystitis, urethritis and pyelonephritis. The Center for Disease Control and Prevention (CDC) estimates that in the US, more than 2 million people are infected every year with antibiotic-resistant microbes, of which 23,000 die due to these infections. The multi-drug resistant microbial strains are continuously increasing due to the abundant use of broad-spectrum antibiotics, intravenous catheters, immunosuppressive agents and organ transplantation [4]. Therefore, there is a need for isolation and identification of new bioactive chemicals from new sources including plants that can be used alone or in combination with other agents to control infectious diseases.

Persea americana Mill.(Lauraceae) is known as Avocado, is an evergreen tree grown and also cultivated in the tropical and subtropical regions of the world. It is edible. In traditional medicine, avocado is preferred for many ailments like cardiac, skin problems, hypertension, diabetes, bronchitis, and diarrhea [5,6]. Many pharmacological attributes are reported as antifungal, antibacterial, antiviral, antitubercular activity, anti-inflammatory, antihypertensive, hypocholesterolemic, vasorelaxant, analgesic, hypotensive activity, an anticonvulsant effect, cytotoxicity, wound healing, antiulcer, antihepatotoxic, antioxidant, acetyl Co-A carboxylase inhibition, nitric oxide and superoxide generation inhibition, skin lysyl oxidase inhibition and insecticidal activity [5-7].

Research work on *Persea americana* fruit pulp, leaf, seed and peel extracts and compounds having antimycobacterial effect has already been reported [7,8,]. Antibacterial activity of avocado seeds of Margarida' variety was studied against isolates of human and fish origin of *Streptococcus agalactiae*. Similarly, avocado seeds worked effectively against clinical bacterial isolates [9-11]. In the food industry, to assist in the prevention of food contamination during handling, especially against the bacteria *Staphylococcus aureus*, the avocado extract from a variety of Quintal peels have been used. The ethanol extracts of epicarp, seed, and cultivar combinations of avocado displayed activity against several bacterial species including *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium*, and *Enterobacter aerogenes* and fungi viz., *Aspergillus flavus*, *Penicillium spp.*, and *Zygosaccharomyces bailii* [12]. Work has also been done on the effect of the leaf and bark extracts of *Persea americana* on some pathogenic bacteria. Antimicrobial peptide (Defensin PaDef) from *Persea americana* pulp showed activity against *Escherichia coli* and *Staphylococcus aureus* [13].

In this communication, we report the antibacterial activity of unripe avocado fruit peel and leaves against a few antibiotic-resistant urinary tract infection (UTI) pathogens viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Staphylococcus aureus*. The methanol extract of the unripe fruit peel showed higher activity and the active constituent was identified as the flavonoid glycoside hyperin [Fig.1]. Docking analysis of hyperin with the target enzyme murF ligase of *Klebsiella pneumoniae* is also reported.





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MATERIALS AND METHODS

Botanical material

The leaves and fruits of Avocado were collected from Kodaikanal, Dindigul district, Tamil Nadu. The identity of the materials was authenticated by Dr. Jeyajothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu.

Extraction

The collected leaves and unripe fruit peel were shade dried and coarsely powdered. 250 grams of each was extracted with chloroform and methanol in the cold (48 hr.). The extracts were filtered through Whatman no:1 filter paper and concentrated in a rotary evaporator. The extracts were finally dried in vacuum. The extracts were stored at 4°C in airtight containers until further use.

Test Microorganisms

Antibacterial activity of the plant extracts were tested against multidrug-resistant Gram-positive isolates of *Staphylococcus aureus* and Gram-negative bacterial isolates viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*. They were obtained from the Department of Diagnostics, The King Institute of Preventive Medicine & Research, Guindy, Chennai, Tamil Nadu. Reference strains *E. coli* MTCC443 and *S. aureus* (MTCC 740) were used for the study. Azithromycin (30µg/disc) and 10% DMSO were used as positive and negative controls. The bacterial strains were re-identified and characterized before use. The microorganisms were repeatedly subcultured on sterile nutrient agar media to obtain pure isolates. A loop full of test organism was inoculated on glycerol broth and incubated for 24 h at 37° ± 1°C and maintained at -20°C.

Antibiotic Susceptibility Testing of the study isolates

Antimicrobial susceptibility testing of the study isolates were performed by Kirby Bauer disc diffusion method according to the recommendations of the Clinical Laboratory Standards Institute by using the following antibiotic discs (14) viz., norfloxacin (10 µg), ampicillin (10 µg), gentamicin (10 µg), cefotaxime (30µg), ceftazidime (30 µg), cotrimoxazole (25 µg), nitrofurantoin (300 µg), imipenem (10 µg), amikacin (30 µg), ceftazidime / clavulanic acid (30/10 µg) and antibiotic resistance was determined by the diameter of inhibition zones around the antibiotic discs.

Antibacterial Susceptibility Testing of the Extracts with study isolates

The inocula were prepared by inoculating the test organisms in nutrient broth, and they were incubated for 24 hours at 37°C. The cultures were diluted to 0.5 McFarland turbidity standard after the incubation. 0.2 milliliter of the cultures was further diluted in normal saline and were inoculated onto solidified nutrient agar using a glass rod by spreading technique. The ability of the various extracts to inhibit the growth of the clinical test organisms was determined using the agar well technique. The inoculated nutrient agar plates were allowed to dry. After this, wells were bored on the surface of inoculated agar plates using 4mm cork borer. 0.2 ml of the different concentrations of each extract were transferred into the well using Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were incubated at 37°C for 24 hours. The experiment was performed in triplicate and the resulting zones of inhibition were recorded as mean ± standard error.

Fractionation and isolation of the active constituent hyperin (Quercetin -3-O-β-D-Galactopyranoside)

The most active methanol extract of the unripe fruit peel (25 gm.) was subjected to column chromatography over silica gel (Acme 100-200 mesh). The column was eluted with solvents of increasing polarity in the order hexane, chloroform, ethyl acetate, methanol and their mixtures. The fractions were monitored by TLC (Merck 60 F₂₅₄ silica gel coated Aluminium plates, layer thickness 0.2mm) and similar fractions were combined. Five major fractions were finally obtained. Fraction four eluted with ethyl acetate -methanol 4:1 gave the active compound hyperin. It showed





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single spot on TLC over silica gel (Developing system, BuOH – 27% HOAc 1:1, $R_f=0.7$). The compound was finally purified by preparative RP- HPLC (Shimadzu LC-10 AT VP HPLC system, hyper SIL R BDS C-18 column, 250 x 4.6 mm, 5 μ m size). Isocratic elution with acetonitrile /water (8 : 2 v/v), was carried out for 20 mins, rate of flow 1.0 ml/min, detector wavelength 220 nm. Hyperin was eluted at $R_T= 6.307$.

Antibacterial activity

Agar diffusion assay of the extracts

Antibacterial activity of the crude extracts was screened against five different drug-resistant bacterial strains by the agar well diffusion method. The culture plates were aseptically prepared with nutrient agar media. 100 μ l of overnight grown bacterial culture (10^5 cells ml⁻¹) was inoculated on to the culture plate using a sterile L-shaped glass rod to get a confluent distribution of bacteria. Agar well (6mm dia) was made with the help of sterile cork borer and 25 μ l, 50 μ l, 75 μ l and 100 μ l of test solution were added in four different concentrations (1mg, 2mg, 3mg, and 4mg). For comparative evaluation, standard antibacterial agent azithromycin (30 μ g/well) was used as a positive reference control and 10% DMSO with sterile distilled water was used as a negative control. Then, the culture plates were incubated for 24 h at 37°C during which the activity was noted by the presence of the inhibition zone around the well. The zone of inhibitions was calculated as in mm (diameter). Bacterial growth inhibition was determined as the diameter of zones of inhibition around the well. These were examined following the standard antibiotic susceptibility testing as per the National Committee for Clinical and Laboratory Standards Institute in document M07-A9 (CLSI document M100-S24, 2015) [14].

Minimum inhibitory concentration (MIC) of hyperin

The minimum inhibitory concentration of compound hyperin was tested using the microdilution method. The test sample was first dissolved in 10% dimethyl sulfoxide (DMSO). The solution obtained was added to Mueller Hinton Broth (MHB), and serially diluted two-fold (in a 96-well microplate) to attain dosage ranges. Bacterial inoculum (1.5×10^6 CFU/mL) prepared in MHB was then added. The turbidity of the microbial suspension was adjusted with a densitometer to 0.5 McFarland turbidity standard that is equivalent to 1.5×10^8 CFU/mL and was introduced to the microplate. The plates were covered with a sterile plate sealer, agitated to mix the contents of the wells using a shaker and incubated at 37°C for 24 hours. Wells containing MHB and 100 μ l of inoculum was used as a negative control. Azithromycin (30 μ g/ml) was the reference antibiotic. The MICs of samples were detected after the addition of (40 μ L) of 0.2 mg/mL p-iodonitrotetrazolium violet and incubated at 37°C for 30 min [15]. Viable bacteria reduced the yellow color dye. MIC was calculated from the lowest sample concentration that exhibited complete inhibition of bacterial growth.

Molecular docking studies

Preparation of the protein and ligand

The structure of ligand quercetin-3-O-galactoside was drawn by ChemDraw Ultra v12, and its bond length and structural properties were checked. The PRODRG server [16] was used to optimize, and energy minimizes the ligand molecule. Due to the unavailability of the 3D structure of murF Ligase from *Klebsiella pneumoniae* (UniProtKB (ID: A6T4M9) EC: 3.1.1.7.), the 3D structure was modeled by Swiss Model based on its potential template protein structure (crystal structure of *Escherichia coli* udp murnac-tripeptide d-alanyl-d-alanine-adding enzyme (murF) at 2.3-angstrom resolution) (PDB ID: 1GG4). To check the structural quality of the developed models, it was evaluated by PROCHECK analysis using the Ramachandran plot on the SAVES server (<https://servicesn.mbi.ucla.edu/SAVES/>). PyMol visualized the finalized model. The CASTp server analyzed the active sites of modelled murF Ligase.

Protein-Ligand docking

The modeled target protein murF Ligase was docked with the ligand quercetin-3-O-galactoside using Auto Dock tools [17] (ADT version 1.5.4 and 4.2 programs) and followed the procedures of Balamurugan *et al.*, 2012 [18]. The grid box points were assigned 100 x 100 x 100 with the default grid spacing of 0.375 Å. The Lamarckian Genetic





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Algorithm with the population range of 150 individuals with a modified cut off rate 0.02 was used to identify the significant docking pose from the 10 generations. Based on the least binding energy values, the finest docking orientation was retrieved. PyMOL viewer and LigPlot program developed the related hydrogen bonds and hydrophobic interactions.

RESULTS AND DISCUSSION

Antibacterial activity of the extracts

The antibacterial activity of the leaf extracts against the tested MDR bacteria are given in table 1 at the concentrations of 1,2,3,4 mg/ml. Both the chloroform and methanol extracts demonstrated appreciable activity against the tested organisms comparable with the standard azithromycin (30µg/ml). The antibacterial activity of the chloroform and methanol extracts of the unripe fruit peel are given in table 2. The chloroform extract showed lesser activity. The methanol extract showed the highest zone of inhibition of 18mm for *K.pneumoniae* at 4mg/ml while azithromycin (30µg/ml) was inactive.

Characterisation of hyperin

Hyperin was obtained as pale yellow solid from methanol (mp 254°, Yield 500 mg). It gave positive ferric reaction for phenol and answered Shinoda test for flavonoid by giving reddish pink colour with magnesium and hydrochloric acid. It was analysed for C₂₁H₂₀O₁₂ by ESI-MS. UV: Δ_{max} MeOH 255,296(SH),363nm. IR :ν_{max}KBr 3433,3033(hydroxyl), 3065 (aromatic), 2961, 1640 (flavones C=O), 1623,1562 (aromatic), 1494,1408, 1307, 1159, 1073(sugar C-O), N27, 913, 849, 778, 746. ESI-MS (-ve mode) m/z 463[M-H]⁻ (+ve mode) m/z 465[M+H]⁺. The physical and spectroscopic data of hyperin are comparable with those reported on literature (19,20 &21). The identity of the compound was confirmed by direct comparison using HPLC with an authentic sample of hyperin (Sigma-Aldrich).

Antibacterial activity of hyperin

The antibacterial activity of the active constituent hyperin was also determined microdilution method. The activity was studied against the same MDR bacteria. The MIC values are given in table 3. Azithromycin (30µg/ml) was used as the standard. *Klebsiella pneumoniae* showed highest sensitivity of 16µg/ml while *Staphylococcus aureus* and *Escherichia coli* showed MIC value of 32 µg/ml. *Proteus vulgaris* and *Salmonella typhi* were less sensitive with MIC greater than 32 µg/ml.

There are many reports on the antibacterial activity of avocado fruit as given in the introduction, however, there are only two reports on MDR bacteria. Lucky and Jonathan, 2017 [22], studied the aqueous and ethanol extract of the leaves. The ethanol extract showed higher activity however, contrary to our report, both aqueous and ethanol extract showed no activity against *k.pneumoniae* at studied concentrations. Tchanaet *al.*, 2014, [23], studied the antibacterial activity of selected medicinal plants of Cameroon against selected Gram negative MDR bacteria. *Persea americana* seed methanol extract showed MBC of 250 µg/ml against *E.coli*.

Docking analysis of Hyperin

The target protein murF Ligase from *Klebsiella pneumonia* was homology modeled and its structure was validated by Ramachandran plot. The isolated compound hyperin (Quercetin-3-O-β-D-galactoside) was docked with the modelled murF Ligase using Auto Dock tools. The compound showed strong binding with the active sites of murF Ligase and packed well in the active pocket given in figure 2 & 3. In the C ring, the hydrogen atom of 5-OH shows binding with ASP 331, while the oxygen atom of 7-OH in A ring shows binding with SER 339. The oxygen atom of 3''OH of the galactose unit shows binding with VAL313. The oxygen atom of 4''OH of the sugar unit shows binding with HIS 281 and ASN 282, while the hydrogen atom shows binding with PRO 278. The Binding Energy, Inhibition Constant, Van der Waal hydrogen bond desolvation energy, and Ligand efficiency are given in Table 4.





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CONCLUSION

In this communication, the antibacterial activity against MDR bacteria of the successive chloroform and methanol extract of leaves and peel of avocado unripe fruit is given. Five MDR bacteria have been studied. The methanol extract of the peel of unripe fruit showed highest activity. The active constituent flavonoid glycoside hyperin showed appreciable activity. The highest activity of MIC, 16 µg/ml was observed against *K.pneumoniae*. This study shows that *Persea americana* fruit may be used as a good antibacterial agent. It can be noted that *P.americana* fruit products are already in use.

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Table 1: Antibacterial activity of unripe *Persea americana* leaf extracts against multidrug-resistant bacteria using agar well diffusion method

Tested organisms	Tested extracts mg/ml								Azithromycin 30 μ g/ml
	PALC				PALM				
	1mg	2mg	3mg	4mg	1mg	2mg	3mg	4mg	
<i>Staphylococcus aureus</i>	7	9	10	13	-	10	12	13	13
<i>Escherichia coli</i>	7	8	10	12	-	-	9	13	14
<i>Klebsiella pneumoniae</i>	8	9	11	13	-	8	10	12	15
<i>Proteus vulgaris</i>	10	11	12	13	-	-	-	9	12
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	12

PALC- *Persea americana* Leaf chloroform extract; PALM- *Persea americana* Leaf Methanol extract; Azithromycin- Standard antibacterial agent.

Table 2: Antibacterial activity of unripe *Persea americana* fruit peel extracts against multidrug-resistant bacteria using agar well diffusion method

Tested organisms	Tested extracts mg/ml								Azithromycin 30 μ g/ml
	PAFC				PAFM				
	1mg	2mg	3mg	4mg	1mg	2mg	3mg	4mg	
<i>Staphylococcus aureus</i>	-	-	10	12	9	12	12	14	-
<i>Escherichia coli</i>	-	-	-	10	9	11	13	16	15
<i>Klebsiella pneumoniae</i>	-	9	10	12	11	13	15	18	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	8	10	-
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	-	-

PAFC- *Persea americana* fruit chloroform extract; PAFM- *Persea americana* fruit Methanol extract; Azithromycin- Standard antibacterial agent.





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Table 3: Minimum inhibitory concentration of hyperin

Tested organisms	Tested compound µg/ml	Azithromycin 30µg/ml
<i>Staphylococcus aureus</i>	32	32
<i>Escherichia coli</i>	32	32
<i>Klebsiella pneumoniae</i>	16	>32
<i>Proteus vulgaris</i>	>32	32
<i>Salmonella typhi</i>	>32	>32

Table 4: Docking analysis of hyperin

Ligand	Protein PDB ID	Binding amino acid Residues	Binding Energy (kcal/mol)	Inhibition Constant	VDW_HB desolv_energy (kcal/mol)	Ligand efficiency
Quercetin-3-O-β-D-galactoside	murF Ligase (Model)	PRO`278/O, HIS`281/HE2, ASN`282/2HD2, VAL`313/HN, ASP`331/OD1, SER`339/HG	-4.8	304.68 (uM)	-8.2	0.15

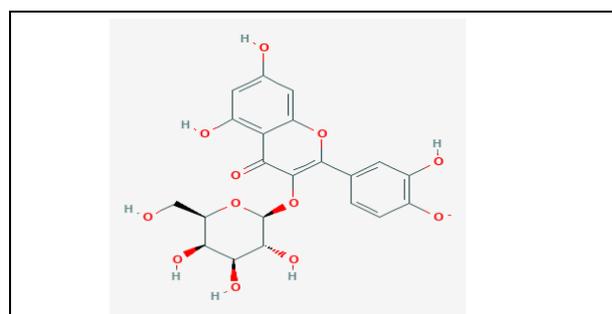


Figure 1. Structure of Hyperin

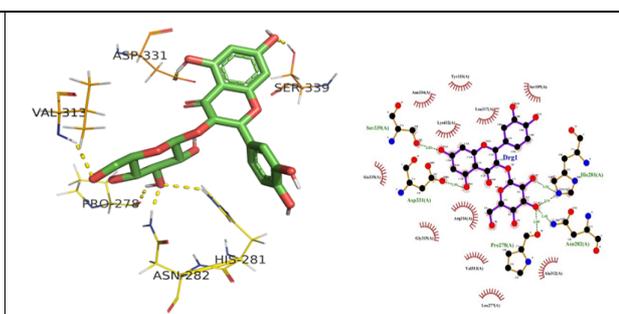


Figure 2&3. Docking analysis of hyperin

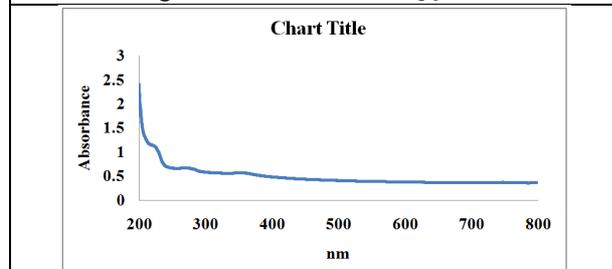


Figure 4. UV spectrum of hyperin

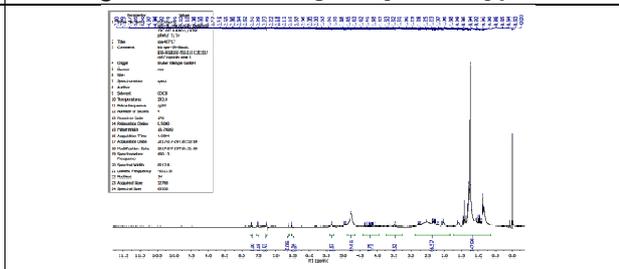


Figure 5. IR spectrum of hyperin

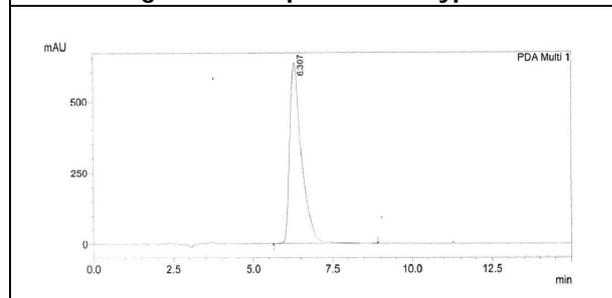


Figure 6. HPLC of hyperin

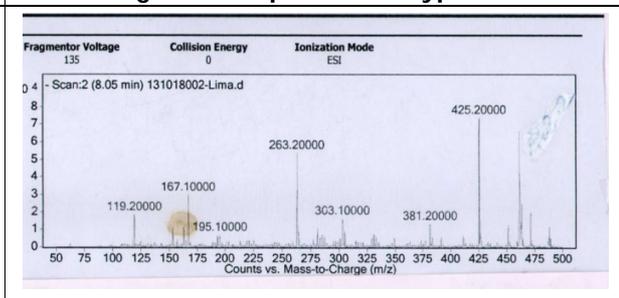


Figure 7a. ESI-MS -ve mode of hyperin





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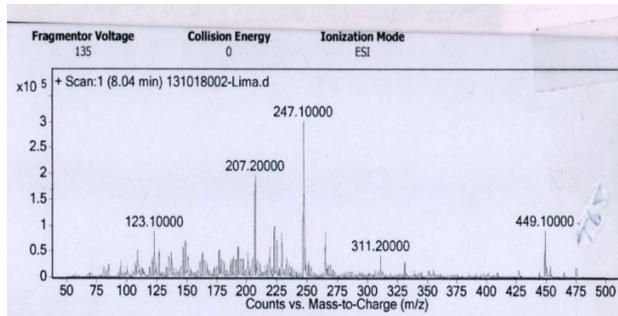


Figure 7b. ESI-MS +ve mode of hyperin





Key Role of *Syzigium cumuni* in Mitigation of Blood Protein Biochemical Parameter in Hyperglycemic Male Mice

Kumari Rekha*

Department of Zoology, TMBU, Bhagalpur, India.

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*Address for Correspondence

Kumari Rekha

Department of Zoology, TMBU,
Bhagalpur, India.

Email: jankumarirekha@gmail.com/pintumgr@gmail.com



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ABSTRACT

The objective of this study was to assess restorative impact of *Syzigium cumuni* to mitigate blood protein levels in hyperglycemic male mice. Hyperglycemic condition was induced in male mice by injecting Alloxan Monohydrate which led to deficiency in insulin secretion in the mice. Fixed dose of *Syzigium cumuni* seed powder were given along with food to hyperglycemic mice and blood protein level were observed at a regular intervals for three weeks. *Syzigium cumuni* seed powder restores the histoarchitecture of pancreatic β cells and stimulates the secretion of pancreatic insulin in hyperglycemic male mice. Blood proteins level were found to return to their normal levels. It is well established that both proteins and amino acid ingestion stimulate insulin secretion. This effect may be responsible for a reduction in blood sugar level [3]. The present study thus concludes that oral administration of the powder of *Syzigium cumuni* seed powder have significantly reduced plasma lipid levels associated with diabetes mellitus. Thus it can be affirmed that *Syzigium cumuni* seed powder prevent as well as reverse the plasma lipid profile, thus emphasizing the protective role against diabetes induced hyperlipidemia. Further studies on the active components of *Syzigium cumuni* seed powder and mechanism(s) of its protective effect against diabetic hyperlipidemia are needed.

Keywords: Hyperglycemia; Histoarchitecture; *Syzigium cumuni*, Diabetes, Insulin

INTRODUCTION

The present study explores the restorative impact of *Syzigium cumuni* to mitigate blood protein levels in hyperglycemic mice a distinctive allusion with mice. The diabetes mellitus disease (DMD) commonly referred as diabetes is a significant public health problem. Predicting the disease at the early stage can save the valuable human resource. Voluminous datasets are available in various medical data repositories in the form of clinical patient records and pathological test reports which can be used for real-world applications to disclose the hidden

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knowledge. Diabetes mellitus (DM) also known as simply diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. Jamun also called jambul, java plum in English and botanically accepted as *Syzygium cumuni* (Linn) Skeels, is native to India and indigenous part of Indian folk remedies. In Ayurveda, its fruits, seeds, bark and leaves are used as medicine for treating bleeding disorders and other diseases. Nowadays, its seeds famous as anti-diabetic medicine. Jamun (botanically known as *Syzygium cumuni* (Linn) Skeels is known for its seasonal perishable jamun berry, which is also named as java Plum (in english) and Jambul. It belongs to the Myrtles family Myrtaceae. Jamun tree is evergreen plant grows up to 30m in height and has a girth of around 3.6 m. The jamun tree grows in tropical and sub-tropical parts of the India and found in Indian Subcontinent. Jamun tree is widely distributed forest tree found in India, Sri Lanka, Malaysia, Bangladesh and Australia. Jamun fruit is oval in shape. In immature stage, Jamun fruit is green in color. Its color changes to crimson black with ripening. The seed in the fruit bears about 25% of its weight and fruit pulp and peel have 75 % of its total weight. Ripe jamun fruits are odourless and juicy.

Botanical Classification

Kingdom	-	Plantae
Sub-Kingdom	-	Viridiplantae
Infra Kingdom	-	Streptophyta (Land Plants)
Super Division	-	Embryophyta
Division	-	Tracheophyta (Tracheophytes or Vascular Plants)
Sub Division	-	Spermatophyta (Spermatophytes or Seed Plants)
Class	-	Magnoliopsida
Super order	-	Rosanae
Order	-	Myrtales
Family	-	Myrtaceae
Genus	-	<i>Syzygium</i>
Species	-	<i>Syzygium cumuni</i>

Pharmacological studies have expanded the biological activities of *Syzygium cumuni*, which include antihyperglycemic, anti inflammatory, antibacterial, cardio protective, anti diabetic and antioxidant [1]. Alloxan is one of the usual substances utilized for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of pancreas. Alloxan causes a massive decrease in insulin released by the destruction of beta cells of the islets of Langerhans, thereby inducing diabetic insulin deficiency leads to various metabolic variations in the animals. Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of β -cells of pancreatic islets.

MATERIALS AND METHODS

Fresh *Syzygium cumuni* seeds collected from PG Dept of Botany, TMBU Bhagalpur were air dried and reduced to coarse powder with the help of mortar and pestle and kept in airtight container until the time of use stored at room temperature and relative humidity of 43%. Swiss albino mice *Mus musculus* weighing about 30 ± 5 gram were maintained at the Animal House of University Department of Zoology, T.M. Bhagalpur University, Bihar, India, under standard environmental conditions and fed with standard diet. Food and water were given ad libitum. Rice



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husk was used as bedding material and changed daily. The mice of 12 weeks of age were acclimatized in the laboratory condition for one week before the experiment [16,13]. The drug Alloxan-monohydrate was purchased from Loba Chemicals, Mumbai. All other chemicals used in the entire experiments were of analytical grade. The experimental animals were kept on fast before induction of diabetes. Diabetes was induced intraperitoneally by administering alloxan- monohydrate [9]. Total dose of Alloxan monohydrate (450mg/kg/bw) was administered in three injections at intervals of 48 h (150mg/kg/bw) each time.

The experimental mice were divided into three groups of 10 animals each.

Group-I (Control)

Group-II (Diabetic control)

Group-III (Diabetic fed with *Syngizium cumuni* seed powder).

The total experimental protocol was maintained for 21 days (3 weeks) after induction of diabetes [16]. Experiments were performed on the frequency of 1, 7, 14 and 21 days for all the test animals.

Method of Blood Sample Collection

At the end of experimental protocol, blood samples were obtained from animal by puncture from the tail of the mice; using disposable needles (25 G) fitted with plastic syringe and was carefully transferred to collecting tubes containing 4% of sodium citrate. The blood collection tubes were kept cool on ice and transported to the laboratory where blood biochemical analysis was performed [5].

Blood biochemical analysis**Plasma protein**

Plasma protein was estimated by Biuret method [8].

PROCEDURE

- 2ml of blood sample was taken in a centrifuge tube rinsed with an anticoagulant solution (3% sodium citrate) and centrifuged at 3000 rpm for 15 minutes.
- The separated plasma was taken in another test tube.
- For blank, 3 ml of sulphate-sulphite, 0.2 ml distilled water were added from this solution
- Then 2 ml above solution was taken in another test tube.
- 5 ml of Biuret reagent was added to this.
- Protein reacts with Biuret forming a co-ordinate complex of blue-purple colour with a specific absorbance at 540 nm.
- By comparing the absorbance value of the standard solution with the absorbance value of sample solution, protein was estimated.
- The concentration of the plasma protein was estimated with the help of standard curve prepared with bovine albumin.

Calculation – $A = \epsilon bc$ (The Beer-Lambert Law)

Where A is absorbance (no units, since $A = \log_{10} P_0/P$); ϵ is the molar absorptivity with units of $L \text{ mol}^{-1} \text{ cm}^{-1}$; b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained, expressed in centimeters; c is the concentration of the compound in solution, expressed in mol L^{-1} [8].





RESULT

The plasma protein of control Group-I mice reduced from 6.73 ± 0.17 gm/dl to 5.7 ± 0.07 gm/dl at 1st week to 3rd week. A significant decrease was observed in plasma protein value on first week (5.47 ± 0.06 gm/dl), second week (4.68 ± 0.15 gm/dl) and 3rd week (3.63 ± 0.09 gm/dl) in Group-II when compared to the values of control mice. It was found that at 3rd week the level of plasma protein recovered from 3.63 ± 0.09 gm/dl to 5.28 ± 0.09 gm/dl in Group-III when compared with diabetic mice of Group-II. ANOVA when applied between treatment group and with residual groups were found to be insignificant at day 1 but significant at second week, 2nd week and highly significant at 3rd week at 95 % and 99 % of confidence ($p < 0.05$, $p < 0.01$).

DISCUSSIONS

In the present investigation alloxan monohydrate induced *Mus musculus* for different time duration (1st week, 2nd week and 3rd week) showed decreased protein values (Table:1). The cause of protein depletion may be due to elevation in the activity of proteases and subsequent elevation in the free amino acid content and activity of amino transferase protein depletion may lead to cannibalism by diabetic mice [6, 14]. It is well established that both proteins and amino acid ingestion stimulate insulin secretion. This effect may be responsible for a reduction in blood sugar level [3].

CONCLUSIONS AND FUTURE PROSPECTS

In the present study *Syzygium cumuni* seed powder exhibits antidiabetic effects improving insulin action in the body, can be widely utilized by the traditional healers for the treatment of various types of diseases especially diabetes and related other complications. The optimum dosage for both conditions need to be established via clinical studies involving human subjects. Before utilization there is a need to ascertain its safety on prolonged consumption on vital organs of the mice, determine the acute, sub chronic toxicity level as well as LD 50. These data are more important for highlighting the commercial and worldwide use of *Syzygium cumuni* seed powder as a diabetic prevention and curative natural product.

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Conflict of Interests

I have no conflict of interest.

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Table.1. Analysis of Blood Biochemical Changes in Mice due to Diabetes and their Amelioration by *Syzygium cumuni* Seed Powder

PLASMA PROTEIN GRAM /100 ml ± SE	Group-I	6.73±0.17	6.56±0.13	6.43±0.14	5.7±0.07
	Group-II	6.54±0.13	5.47±0.06	4.68±0.15	3.63±0.09
	Group-III	6.64±0.15	6.11±0.14	6.16±0.13	5.28±0.09





Fuzzy Solutions for Delay Differential Equations

D.N.Chalishajar¹ and R.Ramesh^{2*}

¹Department of Applied Mathematics, Virginia Military Institute (VMI), Lexington, VA 24450, USA.

²Associate Professor, Department of Science and Humanities, Sri Krishna College of Engineering and Technology,Coimbatore,Tamil Nadu, India.

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*Address for Correspondence

R.Ramesh

Associate Professor,

Department of Science and Humanities,

Sri Krishna College of Engineering and Technology,

Coimbatore,Tamil Nadu, India.

Email: rameshrajappa1982@gmail.com



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ABSTRACT

The theory of fuzzy differential equations has become an important area of investigation in recent years, stimulated by their numerous applications for instance, problems from biology, ecology and so on. This manuscript is mainly concerned with the existence of second order fuzzy neutral functional impulsive integro-differential equations. The idea of delay differential equations has applied in this work since it has many applications in population dynamics, epidemiology, and immunology. The results are obtained by using suitable fixed point theorem.

Keywords: Neutral functional differential equation, fuzzy solution, contraction mapping, delay, fixed point.

INTRODUCTION

In this work, we investigate the existence and uniqueness for the following second order fuzzy neutral functional impulsive integro-differential equations with delay using Banach fixed point theorem.

$$\frac{d}{dt} \left[x'(t) - g \left(t, x_t, \int_0^t a(t, s, x_s) ds \right) \right] = Ax(t) + f \left(t, x_t, \int_0^t b(t, s, x_s) ds \right) \dots \dots \dots (1)$$

$$x(t) = \varphi \in \mathbb{E}^n \dots \dots \dots (2)$$

$$\Delta x|_{t=t_k} = I_k(x(t_k^-)), \dots \dots \dots (3)$$





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$$\Delta x'|_{t=t_k} = J_k(x(t_k^-)) \dots\dots\dots(4)$$

where $t \in J = [0, T], t \neq t_k, A: J \rightarrow \mathbb{E}^n$ is fuzzy coefficient, \mathbb{E}^n is the set of all upper convex semicontinuously fuzzy numbers on $\mathbb{R}, g: J \times \mathbb{E}^n \times \mathbb{E}^n \rightarrow \mathbb{E}^n, f: J \times \mathbb{E}^n \times \mathbb{E}^n \rightarrow \mathbb{E}^n$ and $a, b: J \times J \times \mathbb{E}^n \rightarrow \mathbb{E}^n$ are fuzzy nonlinear regular functions and $I_k \in C(\mathbb{E}^n, \mathbb{E}^n)$ are bounded functions, $\Delta x(t_k) = x(t_k^+) - x(t_k^-)$, where $x(t_k^+)$ and $x(t_k^-)$ signifies the right limit and left limit of $x(t)$ at $t = t_k$ respectively. In recent years, fuzzy differential equations have gained a great deal of attention. They are very useful to explain the complex performance of uncertain systems. Chalishajar et al [1] investigated the existence of fuzzy solutions for nonlocal impulsive neutral functional differential equations. Mondal [2] investigated differential equation for interval valued fuzzy numbers. Qiu et al. [3] analyzed the fuzzy differential equations in the quotient space of fuzzy numbers. Ramesh and Vengataasalam [4] established existence and uniqueness theorem for fuzzy impulsive differential equations.

In biology, physics and engineering one can find the major role of delay differential equations. They are more significant than equations without delays because of their ability to define natural phenomena. For instance, both man-made and natural, in varied areas such as medicine, biology, economics and engineering involve time delays. There exists an extensive literature dealing with functional differential equations and their applications. In many areas of applied mathematics neutral functional differential equations arise and for this motive these equations have much attention in the last decades. These equations have major impact in the field of engineering and biological processes. Kavitha et al. [5] examined existence for a second order differential inclusions in Banach spaces with infinite delay. For more details, refer [6].

The concept of impulsive differential equations is better than differential equations without impulse effects. These equations have applications in biological systems, mechanical systems, population dynamics, financial systems and optimal control, for more details refer [7]. Benchohra et al. [8] discussed the fuzzy solutions for impulsive differential equations. Chalishajar and Ramesh [9] studied the controllability of fuzzy dynamical system. However, fuzzy solutions for second order has not yet been studied in detail when compared to that of ordinary second order differential equations. So, in this work, we have studied fuzzy concept with delay. The structure of this work is organized as follows: Preliminaries is reserved for basic definitions and preliminary facts which will serve as a motivation to get existence results. Then, we establish second order fuzzy solutions for neutral functional impulsive integro differential equations with delay. Finally, conclusion is provided. Our approach here depends on Banach fixed point theorem.

Preliminaries

In this section, preliminary facts and definitions of fuzzy differential and integro differential equations, which will be used throughout this work, are presented. The set of all nonempty compact, convex subsets of \mathbb{R}^n is denoted as $CC(\mathbb{R}^n)$ and define $\mathbb{E}^n = \{u: \mathbb{R}^n \rightarrow [0,1]$ such that u is compact, normal, fuzzy convex and upper semi-continuous. The following basic preliminaries are found in [10].

Definition 1: "The distance between two non-empty bounded subsets A and B of \mathbb{R}^n is defined by the Hausdorff metric

$$\Omega_d(A, B) = \max \left\{ \sup_{a \in A} \inf_{b \in B} \|a - b\|, \sup_{b \in B} \inf_{a \in A} \|a - b\| \right\}$$

where $\| \cdot \|$ denotes the Euclidean norm in \mathbb{R}^n . Thus $(CC(\mathbb{R}^n), \Omega_d)$ is a separable metric space and complete."

Definition 2:"We express the complete metric Ω_∞ on \mathbb{E}^n by

$$\Omega_\infty(u, v) = \sup_{0 < \alpha \leq 1} \Omega_d([u]^\alpha, [v]^\alpha) = \sup_{0 < \alpha \leq 1} \{ |u_1^\alpha - v_1^\alpha|, |u_r^\alpha - v_r^\alpha| \}$$





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for any $u, v \in \mathbb{E}^n$, satisfying $\Omega_d(u + w, v + w) = \Omega_d(u, v)$. Hence, $(\mathbb{E}^n, \Omega_\infty)$ is a complete metric space."

Definition 3: "The supremum metric Ω_H on $C(J, \mathbb{E}^n)$ is defined by

$$\Omega_H(u, v) = \sup_{0 \leq t \leq T} \Omega_\infty(u(t), v(t)).$$

Hence $(C(J, \mathbb{E}^n), \Omega_H)$ is a complete metric space."

EXISTENCE RESULTS

In order to define the fuzzy solution for the second order problem (1) – (4), consider the space $\Theta = \{x: J = [0, T] \rightarrow \mathbb{E}^n\}$ and define $\Theta' = \Theta \cap C(J, \mathbb{E}^n)$.

Definition 4: A function x is called a mild solution for the problem (1)-(4) if the following is verified.

$$\begin{aligned} x(t) &= P(t)\varphi(0) + Q(t)[x_1 - g(0, \varphi, 0)] \\ &+ \int_0^t P(t-s)g\left(s, x_s, \int_0^s a(s, \tau, x_\tau) d\tau\right) ds \\ &+ \int_0^t Q(t-s)f\left(s, x_s, \int_0^s b(s, \tau, x_\tau) d\tau\right) ds \\ &+ \sum_{0 < t_k < t} P(t - t_k)I_k(x(t_k^-)) \\ &+ \sum_{0 < t_k < t} Q(t - t_k)J_k(x(t_k^-)) \dots\dots\dots(5) \end{aligned}$$

Theorem 1 : Assume that

(H1) $Q(t)$ and $P(t)$ are fuzzy numbers, where $[Q(t)]^\alpha = [Q_t^\alpha(t), Q_r^\alpha(t)]$, $Q(0) = I$, $Q_j^\alpha(t) (j = l, r)$ is continuous with $|Q_j(t)| \leq K, K > 0$. This holds for $P(t)$ with $|P_j(t)| \leq N, N > 0$ for all $t \in J = [0, T]$.

(H2) There exists a constant $d_1 > 0$ such that $\Omega_d([x_1]^\alpha, [y_1]^\alpha) \leq d_1 \Omega_d[x(t)^\alpha, y(t)^\alpha]$

(H3) For the function $g: J \times \mathbb{E}^n \times \mathbb{E}^n \rightarrow \mathbb{E}^n$, \exists constants $d_2 > 0, d_3 > 0$ such that

$$\begin{aligned} &\Omega_d([g(s, \chi_1(s), \kappa_1(s))]^\alpha, [g(s, \chi_2(s), \kappa_2(s))]^\alpha) \\ &\leq d_2 \Omega_d([\chi_1(\theta)]^\alpha, [\chi_2(\theta)]^\alpha) + d_3 \Omega_d([\kappa_1(\theta)]^\alpha, [\kappa_2(\theta)]^\alpha), \end{aligned}$$

where $\chi_j(s), \kappa_j(s) \in \mathbb{E}^n, (j = 1, 2)$

(H4) For the function $f: J \times \mathbb{E}^n \times \mathbb{E}^n \rightarrow \mathbb{E}^n$, \exists constants $d_4 > 0, d_5 > 0$ such that

$$\begin{aligned} &\Omega_d([f(s, \chi_1(s), \kappa_1(s))]^\alpha, [f(s, \chi_2(s), \kappa_2(s))]^\alpha) \\ &\leq d_4 \Omega_d([\chi_1(\theta)]^\alpha, [\chi_2(\theta)]^\alpha) + d_5 \Omega_d([\kappa_1(\theta)]^\alpha, [\kappa_2(\theta)]^\alpha), \end{aligned}$$





where $\chi_j(s), \kappa_j(s) \in \mathbb{E}^n, (j = 1,2)$

(H5) For the nonlinear continuous functions $a: J \times J \times \mathbb{E}^n \rightarrow \mathbb{E}^n$ and $b: J \times J \times \mathbb{E}^n \rightarrow \mathbb{E}^n$, $\zeta_j(s) \in \mathbb{E}^n (j = 1,2)$, \exists constants $d_6 > 0$ and $d_7 > 0$ such that

$$\Omega_d([a(t, s, \zeta_1(s))]^\alpha, [a(t, s, \zeta_2(s))]^\alpha) \leq d_6 \Omega_d([\zeta_1(\theta)]^\alpha, [\zeta_2(\theta)]^\alpha),$$

$$\Omega_d([b(t, s, \zeta_1(s))]^\alpha, [b(t, s, \zeta_2(s))]^\alpha) \leq d_7 \Omega_d([\zeta_1(\theta)]^\alpha, [\zeta_2(\theta)]^\alpha),$$

(H6) There exists constants $d_8 > 0$ and $d_9 > 0$ such that

$$\Omega_d([I_k(x(t_k^-))]^\alpha, [I_k(y(t_k^-))]^\alpha) \leq d_8 \Omega_d([x(t)]^\alpha, [y(t)]^\alpha),$$

$$\Omega_d([J_k(x(t_k^-))]^\alpha, [J_k(y(t_k^-))]^\alpha) \leq d_9 \Omega_d([x(t)]^\alpha, [y(t)]^\alpha),$$

where $x(t), y(t) \in \Theta'$.

(H7) If $KN(\frac{1}{N}(d_1 + d_9) + \frac{d_8}{K} + \frac{1}{K}(d_2 + d_3 d_6 \frac{T}{2})T + \frac{1}{N}(d_4 + d_5 d_7 \frac{T}{2})T) < 1$, then (1)-(4) has a fuzzy solution which is unique.

Proof: Define $\Psi x(t) \in \Theta'$ by

$$\begin{aligned} \Psi x(t) &= P(t)\varphi(0) + Q(t)[x_1 - g(0, \varphi, 0)] \\ &+ \int_0^t P(t-s)g\left(s, x_s, \int_0^s a(s, \tau, x_\tau) d\tau\right) ds \\ &+ \int_0^t Q(t-s)f\left(s, x_s, \int_0^s b(s, \tau, x_\tau) d\tau\right) ds \\ &\quad + \sum_{0 < t_k < t} P(t-t_k)I_k(x(t_k^-)) \\ &+ \sum_{0 < t_k < t} Q(t-t_k)J_k(x(t_k^-)) \end{aligned}$$

Then, for $x, y \in \Theta'$, we have

$$\begin{aligned} &\Omega_d([\Psi x(t)]^\alpha, [\Psi y(t)]^\alpha) \\ &\leq \Omega_d([P(t)\varphi(0) + Q(t)[x_1 - g(0, \varphi, 0)] \\ &+ \int_0^t P(t-s)g\left(s, x_s, \int_0^s a(s, \tau, x_\tau) d\tau\right) ds \\ &\quad + \int_0^t Q(t-s)f\left(s, x_s, \int_0^s b(s, \tau, x_\tau) d\tau\right) ds \\ &\quad + \sum_{0 < t_k < t} P(t-t_k)I_k(x(t_k^-)) \end{aligned}$$





$$\begin{aligned}
 & + \sum_{0 < t_k < t} Q(t - t_k) J_k(x(t_k^-))^\alpha, \\
 & [P(t)\varphi(0) + Q(t)[y_1 - g(0, \varphi, 0)] \\
 & + \int_0^t P(t - s) g\left(s, y_s, \int_0^s a(s, \tau, y_\tau) d\tau\right) ds \\
 & + \int_0^t Q(t - s) f\left(s, y_s, \int_0^s b(s, \tau, y_\tau) d\tau\right) ds \\
 & + \sum_{0 < t_k < t} P(t - t_k) I_k(y(t_k^-)) \\
 & + \sum_{0 < t_k < t} Q(t - t_k) J_k(y(t_k^-))^\alpha \\
 & \leq K d_1 \Omega_d([x(t + \eta)]^\alpha, [y(t + \eta)]^\alpha) \\
 & + N \int_0^t (d_2 \Omega_d([x(s + \eta)]^\alpha, [y(s + \eta)]^\alpha) \\
 & + d_3 d_6 \int_0^s \Omega_d([x(\tau + \eta)]^\alpha, [y(\tau + \eta)]^\alpha) d\tau) ds \\
 & + K \int_0^t (d_4 \Omega_d([x(s + \eta)]^\alpha, [y(s + \eta)]^\alpha) \\
 & + d_5 d_7 \int_0^s \Omega_d([x(\tau + \eta)]^\alpha, [y(\tau + \eta)]^\alpha) d\tau) ds \\
 & + N d_8 \Omega_d([x(t)]^\alpha, [y(t)]^\alpha) \\
 & + K d_9 \Omega_d([x(t)]^\alpha, [y(t)]^\alpha)
 \end{aligned}$$

Therefore,

$$\begin{aligned}
 \Omega_\infty(\Psi(x), \Psi(y)(t)) & = \sup_{0 < \alpha \leq 1} \Omega_d([\Psi x(t)]^\alpha, [\Psi y(t)]^\alpha) \\
 & \leq K d_1 \sup_{0 < \alpha \leq 1} \Omega_d([x(t + \eta)]^\alpha, [y(t + \eta)]^\alpha) \\
 & + N \int_0^t (d_2 \sup_{0 < \alpha \leq 1} \Omega_d([x(s + \eta)]^\alpha, [y(s + \eta)]^\alpha) \\
 & + d_3 d_6 \int_0^s \sup_{0 < \alpha \leq 1} \Omega_d([x(\tau + \eta)]^\alpha, [y(\tau + \eta)]^\alpha) d\tau) ds \\
 & + K \int_0^t (d_4 \sup_{0 < \alpha \leq 1} \Omega_d([x(s + \eta)]^\alpha, [y(s + \eta)]^\alpha) \\
 & + d_5 d_7 \int_0^s \sup_{0 < \alpha \leq 1} \Omega_d([x(\tau + \eta)]^\alpha, [y(\tau + \eta)]^\alpha) d\tau) ds
 \end{aligned}$$





$$+Nd_8\Omega_d \sup_{0<\alpha\leq 1} ([x(t)]^\alpha, [y(t)]^\alpha)$$

$$+Kd_9\Omega_d \sup_{0<\alpha\leq 1} ([x(t)]^\alpha, [y(t)]^\alpha)$$

Since, $\Omega_H(\Psi x, \Psi y) = \sup_{0\leq t\leq T} \Omega_\infty(\Psi x(t), \Psi y(t))$.

Then,

$$\Omega_H(\Psi x, \Psi y) \leq Kd_1 \sup_{0\leq t\leq T} \Omega_\infty([x(t+\eta)]^\alpha, [y(t+\eta)]^\alpha)$$

$$+N \int_0^t (d_2 \sup_{0\leq s\leq T} \Omega_\infty([x(s+\eta)]^\alpha, [y(s+\eta)]^\alpha)$$

$$+d_3d_6 \int_0^s \sup_{0\leq \tau\leq T} \Omega_\infty([x(\tau+\eta)]^\alpha, [y(\tau+\eta)]^\alpha) d\tau ds$$

$$+K \int_0^t (d_4 \sup_{0\leq s\leq T} \Omega_\infty([x(s+\eta)]^\alpha, [y(s+\eta)]^\alpha)$$

$$+d_5d_7 \int_0^s \sup_{0\leq \tau\leq T} \Omega_\infty([x(\tau+\eta)]^\alpha, [y(\tau+\eta)]^\alpha) d\tau ds$$

$$+Nd_8 \sup_{0\leq t\leq T} \Omega_\infty([x(t)]^\alpha, [y(t)]^\alpha)$$

$$+Kd_9 \sup_{0\leq t\leq T} \Omega_\infty([x(t)]^\alpha, [y(t)]^\alpha)$$

$$\leq KN\left(\frac{1}{N}(d_1 + d_9) + \frac{d_8}{K} + \frac{1}{K}(d_2 + d_3d_6\frac{T}{2})T + \frac{1}{N}(d_4 + d_5d_7\frac{T}{2})T\right)\Omega_H(x, y)$$

By the hypothesis (H7), Ψ is a contraction mapping. So by Banach fixed point theorem, it is determined that (5) has a unique fixed point.

CONCLUSION

In this work, we have discussed the second order neutral functional impulsive integro differential system with delay. We can extend our study using various fixed-point techniques to study the nature of the dynamical system. Also, we can extend this work to nonlocal case, since non local conditions has varied applications in pollution control, pharmacokinetics and in various physical and biological phenomena.

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Menopause Significance of Nutraceuticals

Richa Sharma^{1*} and Tarvinder Jeet Kaur²

¹Research Scholar, Department of Home Science, Kurukshetra University, Kurukshetra (Haryana), India.

²Professor, Department of Home Science, Kurukshetra University, Kurukshetra (Haryana), India

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*Address for Correspondence

Richa Sharma

Research Scholar,
Department of Home Science,
Kurukshetra University,
Kurukshetra (Haryana), India
Email : richa87sharma75@yahoo.com



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ABSTRACT

Aging population, changing lifestyle, increasing health consciousness and rapid advances in modern medicine are the significant elements for the upsurge of the nutraceutical market worldwide. Since thousands of years medical benefits of food have been explored and nutraceuticals have been found to have many nutritional and therapeutic benefits. Nutraceutical may be defined as any food substance containing health promoting components that help in disease prevention beyond basic nutritional functions. A natural aging in women life resulting from cessation of ovarian function leading to the end of the reproductive years is known as menopause. During menopausal transition, estrogen deficiency leads to numerous physiological and psychological symptoms among women. They are prone to a number of long term degenerative diseases such as obesity, osteoporosis, cardiovascular disease and cancer that decrease the overall quality of life. In the current scenario, nutraceuticals have gained immense popularity and preferred in combating menopausal symptoms and associated health complications. In whole, nutraceuticals have a bright future in the food industry sector and can play a key role in promoting good health and quality of life of postmenopausal women.

Key words: Menopause, nutraceuticals, herbs, menopausal symptoms, health benefits, hot flashes

INTRODUCTION

Menopause is a normal physiological phenomenon which signifies the end of the reproductive years in middle aged women. It is defined as “the permanent cessation of menses for 12 months or more due to the loss of follicular activity of the ovaries”[1]. During menopausal phase, women may experience several biological and psychosocial changes which can have a deleterious effect on their quality of life [2]. Vasomotor symptoms, bone loss, sleep





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disturbances, urinary tract infections, sexual discomfort, increased cardiovascular risk, low libido and loss of skin elasticity are the common manifestations of menopause. As there is withdrawal of estrogen during menopausal phase it leads to high blood pressure, impaired glucose tolerance, vascular inflammation, hyperlipidemia and coronary endothelial dysfunction and shifting from a gynoid to an android fat distribution [3]. Owing to increased longevity, better economic conditions and fast paced lifestyle changes postmenopausal health has emerged as an important public health concern in India. Treatments like hormonal replacement therapy (HRT), allopathic and homeopathic medications are available for managing the complaints and health risks associated with menopause but they are having adverse effects and are expensive as well. Therefore, majority of the women have been reported to prefer non prescribed and self- medicated therapies such as herbal medicines, vitamins and minerals, acupuncture and meditation[4]. Studies regarding postmenopausal health issues and their remedial measures are lacking in India. Thus the present study was undertaken to explore the role of nutraceuticals for relief of menopausal ailments among postmenopausal women.

METHODOLOGY

Literature Collection - The relevant studies were searched by screening the Pubmed, Medline, Science Direct and Google Scholar database. References of included studies and related articles were also reviewed to find other relevant research papers.

RESULTS

Nutraceutical

A nutraceutical can be defined as “a non- toxic food or food component which has scientifically proven potential health benefits such as prevention and treatment of disease”[5]. They contain the desired amount of carbohydrates, protein, lipids, vitamins, minerals and other necessary nutrients [6]. Nutraceuticals can include isolated nutrients, herbal products, dietary supplements and diets to genetically engineered foods and processed products such as cereals, soups and beverages [7].

Classification of nutraceutical

On the basis of chemical composition nutraceuticals are classified under the following heads:

- Nutrients – “Any substance or ingredient with well known nutritional benefits” eg. minerals, vitamins, amino acids and fatty acids.
- Herbals – Plant products available in the form of extracts, pills or concentrates eg. aloe vera gel, evening primrose oil.
- Dietary supplements – A product consisting of one or more ingredients (amino acid, mineral, vitamin, herb) that is able to supplement the diet and marketed in the form of capsules, tablets, softgels, gelcaps, liquids and powders eg. green tea, fish oil[8].
- Medical foods – Specific therapeutic foods that are formulated for the therapeutic management of a specific disease or condition. An example of health bars with added medications, lactoferrin for immune enhancement, transgenic plants for oral vaccination against infectious diseases[9].
- Functional foods - A food product having potential health benefits in addition to the basic traditional nutrition eg. oat bran for heart disease.

Role of various herbs in menopausal symptoms

Black cohosh (*Actaea racemosa* or *Cimicifuga racemosa*)

Black cohosh is a herb indigenous of North America. The roots and underground stems of the plant are used for



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making herbal preparations and available in different forms like liquid extracts, dried extracts, powdered form and pills. It has been reported for managing a variety of menopausal symptoms such as vaginal dryness, nervousness, irritability, insomnia and heart palpitations along with vasomotor symptoms (hot flashes, night sweats) [10]. Recently the researchers have found it as a safe herbal medicine for consumption during menopause [11].

Red clover (*Trifolium pratense*)

The active isoflavones present in red clover are biochanin A and formononetin. The beneficial role of isoflavones present in red clover for providing relief in hot flashes during menopause period has been successfully established [12]. In addition, it has positive effects in skeletal and cardiovascular health of menopausal women [13]. The aerial parts of the red clover being rich in isoflavones are used for the production of food supplementations for postmenopausal women having menopausal complaints and complications[14].

Evening primrose oil (*Oenothera*)

Evening primrose oil is abundant in gamma- linolenic acid which is involved in the production of hormone-like substances called prostaglandins. Maharaj et al [15] reported that 70.4 percent of the postmenopausal women aged 50 to 65 years used herbal remedies for menopausal symptoms and complications with evening primrose oil the most commonly used. Another study conducted by Farzaneh et al [16] to assess the efficacy of evening primrose oil (EPO) in treating hot flashes found that the frequency and severity of hot flashes were reduced in the subjects who consumed evening primrose oil.

Dong quai (*Angelica sinensis*)

Also known as female ginseng, dong quai is one of most frequently recommended Chinese herb. Traditional systems of Chinese medicine have been using dong quai for thousands of years for the treatment of reproductive problems especially symptoms associated with menopause. It is mostly prescribed in combination with other herbs as a valuable female tonic worldwide [17]. This natural agent contains phytoestrogen as well as vitamin A, E and B12 and is helpful in alleviating the symptoms of hot flashes and vaginal dryness. It also helps in combating with the stress and mood swings prevalent during the menopausal phase[18].

Motherwort (*Leonurus cardiaca*)

A medicinal herb in the mint family with antioxidant and anti inflammatory activity and considered primarily as a cardiogenic for the treatment of heart problems, especially palpitations since ancient times. The German Commission E has acceded it as a remedy for certain cardiac disorders like nervousness, tension, anxiety along with thyroid hyperfunction [19]. It is considered as a "superior antispasmodic and nervine" and recommended for women suffering from postmenopausal problems. It is usually available in many menopausal formula in combination with black cohosh.

Ginseng (*Panax*)

It is a multipurpose herb having medicinal properties and is used as a part of complementary and alternative medicine in China for centuries. Numerous studies have revealed that ginseng has a potential therapeutic effect in treating menopausal depression, sexual dysfunction and poor quality of life [20,21]. The German Commission E has highlighted the use of ginseng as a herbal tonic for alleviating mental fatigue and improvement in work capacity and concentration [22].

Maca root (*Lepidium meyenii*)

It is effective in combating hot flashes and vaginal dryness, increases the libido, decreases the risk of osteoporosis and memory loss. It also comprises of some beneficial components such as magnesium,calcium, zinc and iron along with various vitamins[23]. Nicole et al. [24] concluded that maca root reduced psychological symptoms such as anxiety and depression in postmenopausal women.





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Licorice (*Glycyrrhiza glabra*)

Licorice can be consumed in many forms such as candies, capsules, herbal tea and liquid extract. Nahidi et al., [25] concluded that licorice helps in reducing the frequency and severity of hot flashes in postmenopausal women. Abdollahi F et al.[26] and Menati L et al., [27] have reported positive effects of licorice on the quality of life (QOL) of postmenopausal women.

Valerian (*Valeriana officinalis*)

Use of valerian has been proved to be effective in decreasing the frequency of hot flashes and thus could be considered as a potential treatment of hot flashes among the postmenopausal women [28]. A randomized placebo controlled clinical study reported that there was a significant improvement in quality of sleep in postmenopausal women suffering from insomnia [29].

Chaste tree berry (*Vitex Agnus castus*)

It has been traditionally used for postmenopausal women due to its therapeutic properties. It has hormone stabilizing effects which help in relieving vasomotor symptoms experienced during menopausal phase [30]. Abbaspoor et al., [31] studied efficacy of consuming chaste tree berry among postmenopausal women and observed a statistically significant improvement in the symptoms of depression, anxiety, insomnia, night sweats, headache and hot flashes among women consuming chaste tree berry.

Soy (*Glycine max*)

Soy and soy products have generated a lot of interest because of abundant presence of phytoestrogens such as genistein, daidzein, biochanin A and formononetin. Kyoko et al., [32] found in his study that soy isoflavone supplements reduced the intensity of hot flashes by 26 percent. Ahsan and Mallick [33] also reported a significant improvement in psychological symptoms among postmenopausal women on soy isoflavone supplementation. Symptoms such as menopausal depression, anxiety and irritability were mild to moderate in nature and improved by 25, 21 and 19 per cent, respectively. Maximum improvement was observed in severe symptom of fatigue (31%). Phytoestrogens present in soy prevent women from bone loss and helps to maintain a healthy heart[34]. Soy protein has been reported to positively influence bone and calcium balance in menopausal subjects, especially women not on HRT [35].

Flaxseed (*Linum usitatissimum*)

Flaxseed has been widely used by food industries as an important functional food ingredient. It is a rich source of dietary fibre, protein, phytoestrogen and α -linolenic acid (ALA). Ghazanfarpour et al., [36] suggested that flaxseed and *H. perforatum* has a beneficial effect on the vasomotor symptoms experienced by postmenopausal women. Another previous study by Dew and Williamson [37] also demonstrated beneficial effect of flaxseed on hot flashes.

Ashwaganda (*Withania somnifera*)

Several in vitro and in vivo studies have proven the ability of ashwaganda to exhibit anti-inflammatory, anti-oxidative, anti-anxiety and anti-stress properties[38]. It has a vital role in fighting mood swings which is a common symptom among postmenopausal women [39]. A recent study reported that ashwagandha is useful for improving energy levels as well as reducing stress related disorders [40].

Shatavari (*Asparagus racemosus*)

Shatavari has phytoestrogenic properties and acts as a hormone modulator by naturally rebalancing estrogen levels. A study demonstrated the role of shatavari in reducing vasomotor symptoms night sweats and hot flashes in postmenopausal women [41]. It can be effectively used as an alternative to synthetic HRT for the management of menopausal problems [42].





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Therapeutic benefits of nutraceuticals in postmenopausal health complications

Menopausal period is characterized by many symptoms such as nervousness, anxiety, night sweats, hot flashes, vaginal atrophy and reduced libido [43]. There is also a considerable decrease in the levels of antioxidant defense mechanism which leads to an increased incidence of obesity, hypertension, dyslipidemia, cardiovascular diseases, diabetes and cancer among postmenopausal women. In the present scenario, nutraceuticals are given much importance in comparison to HRT because of their multidimensional therapeutic benefits in alleviating menopausal symptoms [44]. Research carried out in last few decades have also documented the efficiency of nutraceuticals in the management and prevention of many chronic health complications [45].

Obesity

During menopause, reduced estrogen levels lowers the metabolic rate which leads to loss of subcutaneous fat and an increase in abdominal fat. Nutraceuticals such as momordica charantia (MC), conjugated linoleic acid (CLA), polyunsaturated fatty acid and capsaicin are potentially effective in weight reduction [46].

Momordica Charantia (MC)

It is a popular fruit in Asia, South America, India and East Africa and is also used as traditional medicine in diabetes and obesity [47,48].

Chitosan

Chitosan is a type of dietary fibre known for controlling body weight by reducing fat absorption. A clinical study conducted to test the efficacy of chitosan among obese subjects found significant decreased BMI among the chitosan fed group [49].

Conjugated linoleic acid (CLA)

It is the most common omega-6 fatty acid found in flaxseed, fish, oils and poultry eggs. A study on the ability of CLA to promote weight loss found that consumption of conjugated linoleic acid decreased adiposity among overweight men[50]. Similarly, a study conducted on overweight men and women having metabolic syndrome demonstrated that 3 g mixed CLA per day reduced body weight without having any adverse effects on glucose or liver metabolism[51].

Polyunsaturated fatty acid

Polyunsaturated fatty acids (PUFAs) namely, docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) are considered to possess anti-inflammatory properties [52]. They help in balancing components of the triad of inflammation, fatty acid metabolism and adiposity [53].

Buckwheat seed

Buckwheat seed proteins function like dietary fibre and play a beneficial role in obesity and constipation [54]. Green tea extract and 5-hydroxytryptophan have been reported to be helpful in weight loss, by increasing the energy expenditure and decreasing appetite, respectively [55].

Capsaicin

Red chili pepper contains a biologically active ingredient called capsaicin. Numerous clinical studies have demonstrated therapeutic benefits of capsaicin in obesity treatment and insulin resistance[56,57]. Animal studies have suggested that capsaicin plays a role in improving the uptake of glucose in muscle cells by insulin stimulation [58].

Osteoporosis

Osteoporosis is a common type of bone disorder. In women, postmenopausal status is a risk factor for osteoporosis due to lack of beneficial effects of estrogen.

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Calcium and vitamin D

Calcium and vitamin D has a vital role in bone remodeling [59]. Supplements of vitamin D and calcium are recommended for proper bone metabolism. Functional food such as vitamin D-fortified milk can be used for combating osteoporosis [60]. A study reported improvement in bone mineral density (BMD) score in more than three fourth of the patients consuming vitamin D supplements [61].

Isoflavones

Isoflavones prevent bone loss and thus help in the preservation of the bones. Various studies have demonstrated the efficacy of soy isoflavone in improving postmenopausal bone health [62,63]. A study confirmed the association of high isoflavone diet with (BMD) bone mineral density in both spine and hip region in postmenopausal women [64]. Basse et al., [65] also reported in his study that isoflavones prevent skeletal morbidity in postmenopausal women.

Hypertension

Elevated blood pressure is a modifiable risk factor of coronary heart disease. Nearly one half (45%) of heart attacks and more than one-half of the strokes are caused due to hypertension [66]. Due to fall in estrogen levels after menopause, the rate of hypertension is increased among postmenopausal women [67].

Polyphenols

Park et al [68] reported that on grape seed extract supplementation for six weeks among prehypertensive subjects, systolic blood pressure (SBP) and diastolic blood pressure (DBP) significantly reduced by 5.6 and 4.7%, respectively in prehypertensive subjects. Grape and wine contain polyphenols which have been found to reduce elevated blood pressure [69]. Ried K et al., [70] concluded in his study that polyphenols in cocoa products was linked to a decrease in blood pressure. Numerous clinical studies have also noticed the efficacy of consumption of cocoa consumption in reducing the mean arterial pressure and diastolic blood pressure [71]. Rostami et al [72] also reported the beneficial role of flavones in cocoa in reducing blood pressure among diabetic and hypertensive patients [72].

Vitamin C

A negative correlation between plasma vitamin C level and blood pressure was observed among healthy young adults which further resulted in reduced risk of associated vascular events. So vitamin C has been considered as an important factor in regulating blood pressure among healthy young adults [73].

Potassium

Vegetables, fruits and low fat milk and milk products are rich in potassium. Several clinical investigations have concluded that intake of potassium lowers blood pressure (BP) significantly among both hypertensive as well as nonhypertensive patients [74].

Dyslipidemia

Dyslipidemia is a medical condition characterized by elevated levels of blood lipids. It is a major causative factor for cardiovascular diseases. As the ovaries stop producing required amounts of estrogen during menopausal phase, hence postmenopausal women become more prone to dyslipidemia and cardiovascular diseases [75,76].

Fish oil

Fish oil contains omega-3 fatty acids which exhibit cardioprotective effects on the cardiovascular system of the individuals [77].





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Soy milk

Wofford et al.[78] reported that soy milk reduced plasma blood lipid levels viz. triacylglycerols, LDL- cholesterol and total cholesterol significantly. There was an average of 2 per cent reduction in LDL and total cholesterol as compared to milk protein carbohydrate.

Cardiovascular disease (CVD)

Epidemiological studies have suggested that the occurrence of cardiovascular disease events begin a decade earlier in men as compared to women [79,80]. With the attainment of menopause, due to low estrogen levels there is an adverse effect on plasma lipoprotein metabolism which leads to cardiovascular diseases among postmenopausal women [81,82]. A study reported that arterial stiffness also known as marker of vascular aging was more prevalent in post-menopausal women as compared to premenopausal women [83].

Nutraceuticals useful for the management of cardiovascular disease (CVD) are :

Psyllium fibre

It is a soluble dietary fibre source which possesses hypoglycemic and hypocholesterolemic properties [84,85]. It has been shown to be beneficial for the decrease of LDL levels in humans[86]. Ganji and Kuo[87] investigated the effect of psyllium fiber supplementation for six weeks on the serum lipid profile of hypercholesterolemic premenopausal and postmenopausal women. The results revealed that there was a significant reduction in the total cholesterol concentration in post-menopausal women but not in premenopausal women. Efficacy of 5.2 g of psyllium fiber intake has been reported to be effective in significant improvement in glucose and lipid values among type 2 diabetic patients [87].

Isoflavones

Soy isoflavones being low in saturated and high in polyunsaturated fat reduces the chances of developing coronary heart disease by improving lipoprotein levels [88]. A study conducted on 350 healthy postmenopausal women aged 45 to 92 years by Hodis et al [89] also confirmed the role of soy isoflavones in hindering the development of subclinical atherosclerosis.

Vitamin B

Several observational studies have highlighted the potential role of vitamin B in preventing cardiovascular diseases [90]. Folate, vitamin B6 and vitamin B12 are vital for lowering plasma homocysteine levels which would also lower the heart disease risk [91].

Omega-3 fatty acids

Alpha-linolenic acid (ALA) present in flaxseed exhibits positive effect in reducing blood lipids and thus combating cardiovascular diseases [92]. Hu et al[93] found a significant inverse relationship between dietary omega-3 fatty acids and coronary heart disease risk in women. Rodriguez et al. [94] also concluded that α -linolenic acid (ALA) intake from flaxseed helps in lowering the risk of cardiovascular disease.

Polyphenols

Flavonoids represent the most abundant polyphenolic constituents in plant foods. Extracts from green tea significantly decreased triglycerides, total cholesterol and low density lipoprotein in postmenopausal subjects without major side effects [95].

Diabetes

Middle aged women are more prone to type 2 diabetes as compared to younger women [96]. Due to the high prevalence of insulin resistance and excess adiposity, midlife women have a significantly greater risk of developing diabetes [97]. Some of the major nutraceuticals known to be used in diabetes are -





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Dietary fibre

Soluble dietary fiber is effective in lowering postprandial glucose levels and increasing insulin sensitivity in diabetic as well as healthy subjects [98]. Abutair et al [99] concluded that supplementation of soluble fibre helps in improving glucose metabolism in patients with type 2 diabetes. Several prospective studies suggested that the risk of having diabetes can be reduced by 21 per cent by increasing two servings of dietary whole grains per day [100]. Kapoor et al [101] reported a significant decrease in the blood glucose level among the menopausal diabetic women after the flaxseed supplementation. The decrease in the blood glucose levels could be attributed to the delay in blood glucose absorption due to the fibre present in the flaxseed.

Alpha-lipoic acid

In type 2 diabetic subjects, α -lipoic acid has been reported to improve insulin sensitivity by approximately 18–20 per cent [102]. Ziegler et al [103] analyzed the positive impact of α -lipoic acid in improving acute symptoms of diabetic neuropathy.

Aloevera extract

Aloe vera extract consists of polysaccharides, anthraquinones and lectins, which are known to possess hypoglycaemic activity beneficial for diabetes [104].

Calcium and vitamin D

Calcium and vitamin D supplementation may have a significant contribution in the prevention of diabetes by suppressing the release of parathyroid hormone (PTH) and preserving insulin sensitivity [105].

Polyphenols: Red wine grape pomace flour and grape seed polyphenols intake led to a significant reduction in blood glucose levels [106]. Guilford et al. [107] also associated the regular consumption of red wine with approximately one-third reduction in the risk of type 2 diabetes.

Cancer

Many epidemiological and experimental studies have explored the role of estrogen exposure in the etiology of cancer. A woman who experiences menopause after age 55 has longer exposure to estrogen which may be on increased risk of breast, uterine and ovarian cancers [108]. The duration of estrogen exposure is found to be closely related to the risk of breast cancer risk. For each 1 year delay in the occurrence of menopause, there is a 3 per cent increase in the risk of developing breast cancer [109].

Flavonoids

Flavonoids are a group of phenolic compounds found naturally in fruits, vegetables, fruits, red wine and tea. Flavonoids having anticarcinogenic and cardioprotective properties reduce the risk of estrogen induced cancers by blocking the enzymes producing estrogen [110]. As consumption of fruits and vegetables help in the prevention of human cancers especially breast cancer [111,112], flavonoids have been recognized as a prospective cancer-preventive component of vegetables and fruits [113,114].

Omega 3 fatty acids

Omega-3 intake has been found to lower the serum steroid sex hormone levels in moderation which are contributing in breast cancer development among the obese postmenopausal women [115]. The data analyzed from 16 prospective cohort studies investigating the possible association of omega-3 intake and breast cancer indicated a reduction in breast cancer risk in individuals with higher intakes as compared to those with lower intake of dietary PUFA [116]. A clinical study on obese Mexican women reported correlation of higher intake of omega-3 fatty acid intake with decreased risk for breast cancer [117].



**Richa Sharma and Tarvinder Jeet Kaur****Lycopene**

Lycopene is a carotenoid mainly present in tomatoes, papaya, guava and water melon [118]. Evidence suggests that lycopene present in vegetables and fruits exhibit cancer-protective effect by decreasing oxidative stress and damage to DNA [119]. A meta analysis conducted to examine the correlation between dietary lycopene consumption and ovarian cancer risk revealed that intake of dietary lycopene reduced the risk of developing ovarian cancer in postmenopausal women by 3.7 per cent, suggesting an inverse relationship between dietary lycopene and risk of ovarian cancer [120].

Curcumin

Turmeric contains a well known antioxidant called curcumin which has medicinal properties. It helps in managing metabolic disorders and inflammatory diseases due to its anti inflammatory, antioxidative and anticarcinogenic properties[121]. A research study reported that curcumin competitively curbed endogenous estrogen and therefore, was responsible for the repression of growth of breast cancer cells [122]. Curcumin was found to exhibit anti-proliferative, pro-apoptotic and anti-migratory activities of endometrial tumor cells and thus was considered to be as a potential anti cancerous agent against endometrial cancer in women [123].

CONCLUSION

Since last few years due to increasing concern of consumers regarding their health and sickness, nutraceuticals have been developed to manage various nutrition related diseases. The nutraceutical industry has seen maximum growth in last decade and is still showing impressive growth rates. Postmenopausal women face a lot of menopausal symptoms and complications are at a greater risk of degenerative diseases such as cardiovascular disease, osteoporosis and cancer. As nutraceuticals can provide a suitable alternative for postmenopausal women who are unwilling to go through hormonal replacement therapy so they are becoming more inclined to nutraceuticals. Nutraceuticals have numerous special health benefits which have been scientifically proved so their recommended intakes will help menopausal women to live a optimal life free of medical complications.

Conflicts of interest

There are no conflicts of interest.

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Table 1. Various herbs useful in menopausal symptoms

HERBS	ORIGIN	ACTIVE COMPONENT	BENEFITS IN MENOPAUSAL SYMPTOMS
Black cohosh	North America	Remifemin	Hot flashes, night sweats, vaginal dryness, heart palpitations, sleep disturbances and irritability
Red clover	Europe, Western Asia	Isoflavones (biochanin A, formononetin)	Hot flashes, vaginal dryness, decreases the risk of osteoporosis and cardiovascular disease
Evening primrose oil	North America	Gamma-linolenic acid	Hot flashes, night sweats
Dong quai	North western China	Ligustilide	Hot flashes, vaginal dryness, stress and mood swings
Motherwort	Asia and South eastern Europe	Leonurine	Menopausal anxiety and stress
Ginseng	China and Korea	Ginsenosides	Depression, stress, decreased libido, fatigue
Maca root	South America and Spain	Macamides	Hot flashes, vaginal dryness, libido, menopausal anxiety, depression, memory loss
Licorice	Europe and Asia	Glycyrrhizin	Hot flashes
Valerian	Europe	Gamma-aminobutyric acid (GABA)	Hot flashes, sleep disturbances
Chaste tree berry	Southern Europe and Mediterranean region	Flavonoids	Hot flashes, night sweats, anxiety, depression, insomnia
Soy	Southeast Asia	Isoflavones (formononetin, biochanin A, daidzenin and genistein)	Hot flashes, fatigue, menopausal depression, irritability and anxiety
Flaxseed	Western Asia, Middle east, India	Lignans	Hot flashes
Ashwagandha	India	Alkaloids, saponins, steroidal lactones	Mood swings, menopausal anxiety, stress, fatigue
Shatavari	India	Saponins, isoflavones	Hot flashes, night sweats

Table 2. Therapeutic benefits of nutraceuticals in various postmenopausal health complications

NUTRACEUTICAL	SOURCE	THERAPEUTIC BENEFIT
Dietary fibre	Fruits, oats, lentil, nuts, seeds and beans	Lowers blood glucose levels, reduces risk of cardiovascular disease, maintains a healthy weight
Polyphenols	Onion, apples, grapes, dark chocolate, green tea, broccoli	Lowers blood sugar levels, boosts heart health, reduces risk of cancer
Alpha lipoic acid	Spinach, broccoli, rice bran, yam, potato, yeast, tomato	Lower blood glucose levels, reduce inflammation




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Vitamin C	Indian gooseberry (amla), orange, guava, lemon, kiwi, tomato	Regulates blood pressure, reduces risk of cardiovascular disease
Calcium	Milk and milk products, green leafy vegetables, soy and soy products, fortified cereals	Improves bone mineral density
Vitamin D	Sunlight, cod liver oil, fatty fish such as tuna, salmon, vitamin D fortified foods eg. orange juice, milk products, breakfast cereals	Helps in calcium absorption, maintains bone health
Isoflavones	Soyabean and its products, lentils, beans, peas	Protects against cardiovascular disease, osteoporosis, cancer
Lignans	Seeds (flax, pumpkin, sunflower, poppy, sesame), whole grains and bran (wheat, oat, rye)	Reduces the risk of hormone associated cancers (breast, uterine and ovarian cancer) as well as cardiovascular disease
Saponins	Peas, soybeans, spinach, tomatoes, potatoes, alfalfa and clover	Protection from cancer
Omega -3 fatty acids	Fish (salmon, mackerel), flax seeds, chia seeds, walnut	Reduce blood pressure and blood lipids, improve blood vessel function





Non – Functional Pancreatic Neuroendocrine Tumor: Apperception of Molecular Mechanism and Genetic Intervention

Anitta Thomas¹, Bharat Mishra^{2*}, Elza Baby¹, Annamol Joshy¹, Sijin Biju¹ and Aleesha R³

¹Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

²Professor and Head, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

³Assistant Professor, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

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*Address for Correspondence

Bharat Mishra

Professor and Head,
Department of Pharmacology,
Nirmala College of Pharmacy,
Muvattupuzha, Ernakulam, Kerala, India.
Email: bharatekansh@gmail.com



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ABSTRACT

Non-Functional Pancreatic neuroendocrine tumors (NF-PNETs) are rare heterogeneous group of neoplasms that are slow progressing with non-specific symptom presentation, but hostile with high metastatic potential and may become incurable once they progress to inexpugnable metastatic disease. Thus they are identified only in the advanced stages of disease, when it starts producing symptoms related to physical size of tumour and metastasis which may decrease the overall survival rate. These are generally sporadic, and the pathogenesis of sporadic PNETs occurs by the loss of chromosome 1, 3p, 6q, 11q, 17q, 22q and gain of chromosomes 4q or 9q, usually characterized by germline or somatic mutations involving MEN-1, TSC1/2, ATRX and DAXX genes. Non familial NF PanNETs can occur in association with Multiple Familial Endocrine tumor syndromes such as Multiple Endocrine Neoplasia Type-1 (MEN-1), von Hippel-Lindau (VHL), Tuberous Sclerosis Complex (TSC), Neurofibromatosis (NF) etc. The neuroendocrine phenotype is proven by the immune-histochemical detection of the Neuro-endocrine markers synaptophysin and / or chromogranin A (CHGA). Epigenetic modifications and differential micro RNA expression are involved in the dysregulated signaling pathway of PNETs. Aggressive surgical intervention, including formal pancreatic resection and /or resection of metastases, radiofrequency ablation (symptomatic relief) is associated with improved survival. Understanding the genetic susceptibility and molecular mechanism are essential to provide genetic counseling and best preventive care. This overview provides a comprehensive aspect of molecular and genetic intervention of Non-Functional Pancreatic neuroendocrine tumors in order to understand the molecular mechanisms leading to mutation of tumour suppressor gene and to enhance the overall survival rate of patients.

Keywords: Non functional Pancreatic Neuroendocrine Tumor, Sporadic, MEN1, VHL, Chromogranin A.



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Non-Functional Pancreatic Neuro-endocrine tumors embodies the largest group (about 60-90%) of PNETs that emanate from pancreas. They are generally symptomless, which may or may not secrete non-specific markers such as Chromogranin A (CHGA), Neuron-specific enolase (NSE), Pancreatic Polypeptide (PP), Human Chorionic Gonadotropin (HCG), Alpha Fetoprotein (AFP) etc., [1] which are used as biomarkers in disease detection. On the other hand functional PNETs are symptomatic with excessive hormonal secretion. These include Insulinomas, glucagonomas, gastrinomas, GRFoma, Somatostatinomas, VIPomas and ACTHoma (rare) [2]. As NF-PNETs show quiescent advancement for a long time without producing warning signs of excess hormones, they result in morbidity and mortality by intruding normal tissues and by metastasizing at higher rate [3, 4]. They can be identified only in the advanced stages of disease, when it starts producing symptoms related to physical size of tumour and metastasis including distant metastasis [5, 6]. Incidence of venous tumor thrombus is also reported with NF-PNETs [7]. Besides, the size of lesions at diagnosis has considerably decreased, rendering additional difficulty in detection of such tumors [8]. NF-PNETs are prominent over functional PNETs. Most of NF-PNETs are sporadic, meanwhile approximately 10% of PNETs are associated with inherited genetic syndrome [9] such as Multiple Endocrine Neoplasia type 1 (MEN 1), von Hippel Lindau disease (VHL), Neurofibromatosis type 1 (NF1), Tuberous sclerosis (TSC), etc. With each of these genetic disorders showing autosomal dominant inheritance [10] and all function as tumor suppressor genes [11]. The inherited germ line loss of various tumor suppressor genes is the major etiology [3]. This review sheds light on understanding the genetic susceptibility and molecular mechanism of non-functional pancreatic neuro-endocrine tumors, which is essential to provide genetic counseling and preventive care to patients as well as for increasing the overall survival rates.

METHODOLOGY

This selective literary research was accelerated by Pub Med, Google Scholar, Science direct, Hindawi, and Springer. Each article was carefully studied and a database regarding Non functional pancreatic neuro-endocrine tumors, its molecular mechanism and genetic interventions was explored. This database also highlights the epidemiology, prognosis and staging, diagnosis, clinical presentation of the same. Search results including pancreatic neuro-endocrine tumors, genetic alterations in various genetic syndromes associated with this tumor was discovered. The citations of the selected papers were used for the accretion of additional relevant publications.

Epidemiology

Gastro entero pancreatic neuro-endocrine tumours exhibits preponderance among all other neuro-endocrine tumours [12]. From Surveillance, Epidemiology and End Results (SEER) database of different countries it is clear that the incidence of NF-PNETs are increasing with the more frequent use and augmenting acuity of cross-sectional imaging [12, 13, 3] and autopsy studies [18]. Fitzgerald et al on reviewing State-wide Tumour Registry in the time period of 16 years from 1986-2002, reveals a greater than two fold increase in the incidence of NF-PNETs [15]. In Ontario, Canada a six fold increase was discerned around 1994 to 2009 (from 0.1 to 0.6 per 100000 persons) [14]. From the recent studies conducted it is clear that the incidence of non functional PNETs (90.8%) are far more compared to functional PNETs [16]. Considering the genetic interventions MEN Type 1 contributes 20-80% of PNETs with majority of NF-PNETs, Von Hippel Lindau disease about 10-17% with 98% NF-PNETs [5]. Incidence of PNETs increases significantly after the age of 40 with peak incidence around age 65. The median age at diagnosis in a study conducted was 63 years [17, 13]. According to SEER database of the year 1973-2000 peak incidence of NF-PNETs was found to be 6.9/100000 between the age group 70-79 in males (9.4) and females (5.1). PNETs diagnosed in the age of 60-70's would be at advanced stage and exhibit poor 5 year survival [12,17]. Occurrence of NF-PNETs show slight male predominance (55.2%) compared to females (44.8%). In the study conducted 88.3% of patients had solitary primary tumour while 11.7% had two or more tumours. Considering race, Whites (84.3%) had shown predominance than Blacks (9.4%) [16, 13]. Overall survival of patients with functional PNETs is higher compared to NF-PNETs [16].



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Staging and Prognosis

The stage of a cancer explains how much cancer is in the body and it helps in determining how serious the cancer is and how best to treat it [19]. The present classification and staging systems are proposed by the World Health Organization (WHO), the European Neuroendocrine Tumor Society (ENETS), and the American Joint Committee on Cancer (AJCC). The WHO system is based on the tumor proliferation rate. In 2010 the PNETs were classified into 3 main groups by this system: Grade 1 tumor (< 2 mitosis/10 HPF and Ki-67 index \leq 2%), grade 2 (2-20 mitosis/10 HPF and Ki-67 index 3%-20%) [Grade 1 and Grade 2 are called NETs i.e. NET G1, NET G2] and grade 3 (> 20 mitosis/10 HPF and Ki-67 index of > 20%) [Neuro - endocrine carcinoma G3]. [2, 20] Ki-67 is a nuclear protein that is expressed only during active phases of cell cycles. NET-G1 and NET-G2 tumors are considered to be well-differentiated neoplasms. ENETS and AJCC staging is based on the TNM i.e. tumor-nodes-metastasis classification. The AJCC proposed a specific TNM staging system for PNETs in 2010, it comprise local disease (stage I), locally advanced/resectable tumors (stage II), locally advanced/unresectable tumors (stage III), and distant metastatic tumors (stage IV). The AJCC TNM system is based on 3 key informations which are The size and extent of the main tumor (T), The spread to nearby lymph nodes (N), The spread (metastasis) to distant sites (M). Table 1 shows the two TNM based staging system by AJCC and ENETS. [2,19,20].

Both the ENETS and the AJCC system provide important prognostic information for PNETs. According to a study including more than 1000 patients, the American Joint Committee on Cancer, the WHO 2010, and the European Neuroendocrine Tumor Society classification systems all resulted to be independent prognostic factors for survival [8]. Some other factors can also be important in determining a person's prognosis which are not formally a part of the TNM system, it includes 1) Tumor grade, it describes how faster the cancer is likely to grow and spread. In PNETs important part of grading is measuring how many of the cells are in the process of dividing into new cell and it is determined by mitotic count and Ki-67 index 2) Tumor functionality, the prognosis for pancreatic NETs can be affected by whether the tumor is functioning or non-functioning [19]. Some recently recognized independent prognostic factors include - (i) The existence of calcification at preoperative imaging found to be related to tumor grade and metastatic lymph node numbers. (ii) Distant metastases and its progression time are survival predictors, independent from the Ki-67 index. (iii) Both lymph node involvement and lymph node ratio are related to the tumor recurrence after surgery. (iv) The absence of symptoms in NF-PanNETs found to be related with a better prognosis, independent from the tumor stage. (v) Recently peritumoral vascular invasion is known as an independent prognostic factor (vi) With different cut-offs (55–75 years), older age is related with a higher mortality rate [8].

The 5-year overall survival (OS) and the median for patients affected by NF-PanNETs are 43% and 38 months respectively. Spread of tumor is another important prognostic factor, with median OS falling from 124, 70, and 23 months for patients with localized disease, regional tumor involvement, and metastatic disease, respectively [8]. Clinical feature prognostic factors like gender, age, and function were analyzed in a study. The data from it shows that, in case of gender there was no difference in prognosis between male and female patients and in case of age there was no significant difference between young patients and old patients [21].

Clinical Presentation

NF-PNETs remain asymptomatic until it reaches advanced stage. Symptomatology of tumour is related to the mass effect of the primary tumour or metastasis [24]. Major symptoms of PNETs include weight loss, jaundice, and abdominal pain. Other minor symptoms include nausea, anorexia, intra-abdominal bleeding or a palpable mass. The majority of metastasis is found in liver, other locations like spleen, brain, adrenal, peritoneum, bone have also been reported [16]. Most of the liver metastasis occurring in NF-PNETS and symptomatic patients may be multifocal or bilobar. NF-PNETs either do not produce hormone (such as calcitonin, neurotensin, ghrelin, chromogranin A or pancreatic polypeptide) or produce it in low rate to not cause any of the symptoms. Approximately 10% of the PNETs will be associated with an inherited genetic syndrome. E.g. Multiple endocrine Neoplasia type 1 (MEN1) is an inherited autosomal dominant disease with hyperparathyroidism (100%), pituitary tumors (<50%) and PNETs up to



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75%. PNETs are found almost exclusively in duodenal somatostatinomas and rarely in neurofibromatosis type 1 (0-10%). Tuberous sclerosis (TS) are associated with PNETs (rare condition) and it may be either functional or non functional. NF-PNETs are difficult to be distinguished from functional tumors. Some of the imaging techniques such as ultrasound, computed tomography, endoscopy etc; have been useful to detect PNETs greater than 2cm, but they are ineffective visualizing PNETs less than 5mm. Positive staining with chromogranin A and synaptophysin confirms the presence of tumour and these are elevated in 60-100% of NF-PNETs. Another tool for detection of PNETs is the somatostatin receptor scintigraphy, which is based on the presence of somatostatin receptors. [23]. The clinical presentation of patients depends upon the advancement of disease as well as on the treatment offered. [25]

Pathogenesis

Tracing the origin of PNETs we are ultimately left to the conclusion that it is generally sporadic. It can also happen in accordance with the multiple familial endocrine tumor syndromes such as multiple endocrine Neoplasia syndrome type 1 (MEN1), von Hippel Lindau disease (VHL), neurofibromatosis type 1 (NF1), and tuberous sclerosis (TSC). Several studies have exposed the molecular heterogeneity of the disease functioning at distinct levels. They also look into the involvement of germ line mutations, gene fusions and clinically relevant molecular subgroups. The sporadic NF PNETs are due to the inherited germ line loss of the respective tumor suppressor gene. Chromosomal alternations cause PNETs where usually there is a loss of chromosomes 1, 3p, 6q, 11q, 17p or 22q or by the gaining of a chromosome 4 or 9q however random chromosomal changes are also possible [26].

Genetic Variations in PNETs

Although, the exact pathogenesis mechanism that results in PNET tumor genesis is unknown. These tumors generally display DAXX/ATRX and MEN 1 mutations or protein impaired expression. The loss of this ATRX/DAXX in PanNETs leads to ALT phenomenon, chromosomal instability and higher tumor stage. When compared to carcinoma tumorigenesis, NENS has got PTEN and TSC1/2 that perform as active driver mutants with a frequency of inactivating lesions [26]. (Table 2).

Chromosomal and Genetic Alterations

(i) In about certain cases it is reflected that chromosomal / epigenetic alterations can lead to neuroendocrine transformation hence we cannot simply depend on multinational events alone to study all cases of NEN. We can recognize four PanNENs subtypes through CNV analysis and whole –genome sequencing. They are thus defined on the basis of their chromosomal variations: loss of chromosome 11q where MEN1 reside. (ii) A recurrent pattern of whole chromosomal loss (RPCL) in association with higher mitotic index, ALT and ATRX/DAXX inactivation (iii) Patterns of chromosome gaining, complementary to losses of the RPCL group and associated with higher risk of metastasis. (IV) germ line mutations in base-excision repair (MUTYH) DNA Methylation alteration along with which Hyper-Methylation of RASSF1A, HIC-1, CDKN2A, VHL, and MGMT genes are also associated with PanNETs can also be associated with PanNETs [27] (Table 3).

Genetic Syndromes

Men Type 1

MEN type 1; a rare autosomal dominant disorder caused by the germline mutation in MEN 1 gene but also occurs sporadically. The molecular genetic studies revealed the presence of de novo mutations of MEN 1 gene in the patients affected. It is found that gene carriers most frequently develop tumors in the parathyroid glands (95%), the anterior pituitary (20–40%), and in the endocrine cells of the pancreas/duodenum (40–80%). The most fatal of the MEN1 lesions are the pancreatic. MEN 1 genes (consist of 10 exons) located on chromosome 11q13 codes for a scaffold nuclear protein called Menin (610 amino acids) involved in a large no of biologic functions like chromatin modification, transcription, cell division, protein degradation, DNA repair. More than 300 germline mutations results in loss of heterozygosity of Menin. Menin is involved in binding and inactivation of many nuclear transcription factors like JunD, SMAD3, mSin3a, trithorax family histone methyl transferrase complex, up regulation of cell cycle

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inhibitors expression (p27^{KIP1} and P18^{Ink4c}) [28, 32]. Somatic mutations in MEN 1 are frequently detected in NF sporadic PNETs and are commonly associated with LoH of the remaining WT allele. NF PNETs has inactivating MEN 1 mutations and allelic loss of chromosome 11q13 (MEN 1 locus). An alteration in the protein expression is also found which is due to the fact that the majority of them (mainly truncating mutations) occur within regions of the gene associated with subcellular protein localization. Repeated inactivating mutations and chromosomal rearrangements in chromatin-remodeling genes (SETD2, ARID2, KMT2C (MLL3) and SMARCA4) were recently documented in PanNETs with SETD2 and ARID 1A mutations present in 18 and 13% of advanced WD- PanNETs respectively. Mutations in DAXX which is a histone H3.3 chaperone recruited by ATRX, occur in 25% of panNETs. Local DNA damage increases by its depletion, results in the loss of structural integrity at telomeres and development of endocrine tumors. Mutations in genes involved in HR DNA repair have also been identified [30].

Von Hippel – Lindau disease (VHL)

A unique autosomal dominant disease characterized by either single retinal or cerebellar hemangioblastoma (HB) or renal cell carcinoma; pheochromocytoma etc. pNETs can be detected in 12-17% of patients with VHL and can be malignant in 17% of patients showing metastatic diseases. VHL gene is an oncosuppressor gene located on the short arm of chromosome 3p25–26 which codes for two proteins (pVHL) of 213 and 610 amino acids respectively. These proteins form an ubiquitin complex with cullin 2, Rbx1 and VCB (Elongin B) that degrades the alpha subunit of hypoxia inducible factor (HIF) [28, 29]. Even though the literal mechanism of VHL is unknown the dysregulated vessel neogenesis is expected to be one of the input mechanisms. According to Knudson's two-hit hypothesis of tumorigenesis, tumor formation initiated when the two proteins are inactivated. Either a deletion or mutation in wild type allele develops tumors by the somatic inactivation of VHL gene (sporadic cause) and germline mutations by inheriting the genetic trait. In normal conditions, HIF coordinates the cells response to hypoxia that is when normal oxygen levels are present VCB-CUL 2 complex binds to the alpha subunit of HIF1 and HIF 2, hence mediates the degradation of absent VHL protein function or protein abnormal. These perform the oncosuppression function by either directly through VHL mediated effects or through the degradation of HIF and indirectly through HIF mediated effects. Whenever there is a functional loss of VHL protein transcriptional regulation processes initiates and led to HIF mediated angiogenesis. HIF enhances glucose uptake, resulting in the uncontrolled production of mitogenic factors such as VEGF (Vascular Endothelial Growth Factor) PDGF (Platelet Derived Growth Factor), TGF (Tumor growth Factor).

Possible mechanisms that initiates tumorigenesis

Either increase in VEGF or PDGF or both results in HIF mediated angiogenesis. An increased level of VEGF increases the vascular permeability of the tumour vessels and generally ensuing in peritumoral edema and cysts. HIF mediated carcinogenesis through the overproduction of TGF that stimulates the over expression of epidermal growth factors (receptors for TGF) that creates an autocrine loop. Other possible mechanisms like disruption of normal cell cycle, increased angiogenesis and abnormalities in the extracellular matrix are also possible in VHL [31, 5].

Von Recklinghausen's Disease or Neurofibromatosis Type 1 (NF1)

An autosomal dominant caused by the germ line mutation of NF 1 gene (oncosuppressor gene of 50 exons) located on chromosome 17q11.2 coding for neurofibromin. This product called neurofibromin is a GTPase has a pivotal role in mitogenic Ras pathway especially the mTOR signaling pathway [28] mTOR has an important role in the cellular growth and inputs like nutrients, growth factors, nutrients and hypoxia induced stress, hence suppression of this signaling pathway needed for oncosuppression. The structural loss of this protein thus prevents this inhibition and initiates tumorigenesis [28].



**Anitta Thomas et al.****Tuberous sclerosis complex 1/2 (TSC1/2)**

Tuberous sclerosis complex is a high penetrance autosomal dominant syndrome, allied to mutations in TSC and TSC 2 genes. Chances of getting NF PNETs with TSC are very rare with a frequency of 1.8% to 9%. These are mainly associated with germ line mutations, and sporadic mutations. Pan NET are persistent with somatic mutations in TSC 2. LoH or large chromosome 16 deletions consequently inactivate the remaining, allele. TSC genes codes for two proteins called hamartin and Tuberin that dimerise to form a regulatory protein upstream of phosphatidylinositol 3 – kinase (P13K) and mTOR signaling pathway responsible for the cellular growth. The dysregulation of the mTOR pathway initiates tumorigenesis [30].

Diagnostic Methods

NF-PNETs are normally large in size and have a heterogeneous structure. Irregular calcification, necrosis, and cystic changes are possibly observed. The different methods for the diagnosis of this disease includes routine sectional imaging techniques such as Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), Endoscopic Ultrasonography (EUS), and Somatostatin Receptor Scintigraphy (SRS) [5].

Imaging Techniques**Computed Tomography (CT) and Magnetic Resonance Imaging (MRI)**

The best imaging is obtained by the contrast agent administration, since PNETs are having a hyper vascular structure. Anatomical CT or MRI of abdomen and pelvis are important for the evolution of pancreatic, liver, lymph node and peritoneal metastases [5,3]. Contrast-enhanced CT scans are more accurate in detecting PNETs greater than 2 cm and have a sensitivity range of 63%–82% and specificity range of 83%–100%. In tumors smaller than 2cm sensitivity is decreased. However, symptomatic non-functional and functional tumors with hormone syndrome are generally greater than 2–3 cm at presentation [8]. The noncontrasted phase, contrasted arterial phase, and portal venous phase are found in a typical CT scan [5].

Currently, PNETs are well visualized in MRI with the advent of new technology in MRI sequences. The advantages of MRI may include non exposure to ionized radiation and excellent soft tissue contrast resonance. The diagnostic PNETs studies by MRI report 80% sensitivity and 100% specificity. In MRI the PNETs are visualized as hyper vascular lesions of low T1 signal intensity or moderate T2 signal. MRI is reported to be more effective in detecting liver metastasis compared to SRS and CT [5,36].

Endoscopic Ultrasonography (EUS)

It provides a high-resolution image of the pancreas with high-frequency transducer. Especially for the diagnosis of pancreatic lesions smaller than 1cm EUS can be used. The reported sensitivity of EUS is found to be 79-100%. EUS has an important role in early detection and follow-up of multifocal PNETs that are common among patients with MEN-1 and VHL syndromes. The EUS-guided thin needle biopsy provides the preoperative histopathological examination and it has an vital role in the discrimination of guided NF- PNETs from pancreatic adeno carcinomas and other pancreatic lesions [5, 36].

Somatostatin Receptor Scintigraphy (SRS)—Octreoscan

High levels of somatostatin receptors are expressed in PNETs including non-functional tumor. Since most of the PNETs include somatostatin receptors on their cell membranes, it is possible to visualize the PNET cells via radioactive-labeled somatostatin analogues. SRS is known as octreoscan because Indium 111 labeled DTPA octreotide is a normally used agent in it. The major use of SRS is in diagnosing NF-PNETs, gastrinomas and glucagonomas. One of the advantage of SRS is that it can scan not only the abdominal region but also the whole body rapidly with regards to the presence of a metastasis [5, 36].





Biochemical marker scanning

A most widely used tumor marker for NF-PNETs includes chromogranin A (CgA), neuron-specific enolase (NSE), and pancreatic polypeptide. In NF-PNETs Chromogranin A and pancreatic polypeptide were recommended as circulating tumor marker, but the percentage of patients with increased pancreatic polypeptide levels is lower than that of patients with increased chromogranin A levels. In earlier laboratory workup in NF -PanNETs comprehends chromogranin A, with a sensitivity of 72–100% and a specificity of 50–80%, and neuron-specific enolase (NSE) with sensitivity of 30–40% and a specificity of up to 100%. Other markers such as pancreatic polypeptide (pp), pancreastatin, and human chorionic Gonadotropin α and β may be used in the diagnosis of GEP-NET. [5, 8, 36]

Treatments of NF PNETs

Treatment should primarily be personalized by considering the prognostic features as well as life expectancy of the patient. Treatment of PNETs relies on many factors such as the severity of symptoms, grade of tumor, presence of a hereditary syndrome and whether the tumor is well or poorly differentiated. The principal curative treatment is the surgical resection [35] and palliative reductive surgery [33]. Surgical resection is advised in both sporadic and MEN 1 related non functional PNETs. A study conducted by Jan Franko et al on 2158 patients suggests surgical therapy for improving overall survival in patients with localized, metastatic NF-PNET [34]. Liver transplantation can be adopted in several rare liver metastasis patients. Other interventional loco regional therapies such as Selective Hepatic Arterial Embolization and Radiofrequency Ablation and medical therapies can be used in progressive loco regional disease and liver metastasis.

CONCLUSION

Pancreatic neuroendocrine tumors are uncommon neoplasms but its prevalence is increasing due to advancement in imaging techniques. Since, non-functional pancreatic NETs are indolent and slow advancing, without symptoms and diagnosis is common mainly in the advanced stages of disease and overall survival rates are less compared to functional PNETs. Even though in majority of cases it is sporadic, nearly 10% are associated with genetic syndromes like MEN1, VHL, etc. If the genetic and molecular interventions of NF-PNETs are studied elaborately, then we can increase the overall survival rate of patients with the use of targeted therapies. This also helps in early diagnosis in patients.

Abbreviations

NF-PNETs	Non-Functional Pancreatic neuroendocrine tumors
PNETs	Pancreatic neuroendocrine tumors
MEN-1	Multiple Endocrine Neoplasia Type-1
VHL	Von Hippel-Lindau
TSC	Tuberous Sclerosis Complex
VEGF	Vascular Endothelial Growth Factor
TGF	Tumor growth Factor
PDGF	Platelet Derived Growth Factor
NF	Neurofibromatosis
CHGA/Cg A	chromogranin A
NSE	Neuron-specific enolase
PP	Pancreatic Polypeptide
HCG	Human Chorionic Gonadotropin
AFP	Alpha Fetoprotein
SEER	Surveillance, Epidemiology and End Results
WHO	World Health Organization





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ENETS	European Neuroendocrine Tumor Society
AJCC	American Joint Committee on Cancer
OS	overall survival
TS	Tuberous sclerosis
RPCL	recurrent pattern of whole chromosomal loss
HB	hemangioblastoma
HIF	Hypoxia Inducible Factor
NET	neuroendocrine tumors
WT	Wild type
GTP	Guanosine triphosphate
mTOR	Mammalian Target of Rapamycin
LoH	Loss of Heterozygosity
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
EUS	Endoscopic Ultrasonography
SRS	Somatostatin Receptor Scintigraphy

Conflict of interest

The authors have no conflict of interest to declare.

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Table 1- ENETS and AJCC TNM grading system for PNETs [2]

	ENETS	AJCC
T1	Tumor limited to pancreas, <2 cm	Tumor limited to pancreas, <2 cm
T2	Tumour limited to pancreas, 2–4 cm	Tumor limited to pancreas, >2 cm
T3	Tumour limited to the pancreas, >4 cm, or Tumor invading duodenum or common bile duct	Tumor extension beyond pancreas, but not involving celiac axis or SMA
T4	Tumor invades adjacent structures	Tumor involves celiac axis or SMA
N0	No regional lymph node metastasis.	No regional lymph node metastasis
N1	Regional lymph node metastasis	Regional lymph node metastasis
M0	No distant metastasis	No distant metastasis
M1	Distant metastasis	Distant metastasis
	Stage I-T1N0M0 Stage IIA-T2N0M0 Stage IIB -T3N0M0 Stage IIIA -T4N0M0 Stage IIIB -any T, N1M0 Stage IV -any T, any N, M1	Stage IA -T1N0M0 Stage IB -T2N0M0 Stage IIA -T3N0M0 Stage IIB -T1-3N1M0 Stage III -T4N0-1M0 Stage IV -any T, any N, M1

Table -2 Percentage of mutant genes in sporadic PNETs from an exom study in 68 patients [26]

PERCENT	Mutant gene
44%	MEN 1
43%	DAXX /ATRX
14%	Mtor

Table -3 Description about Genetic Syndromes associated with NF PNETs [28]

Syndromes	Chromosome/ gene	Protein	Gene function, main molecular consequences	Patients with pnets
MEN TYPE1	11q13, MEN 1	Menin	Oncosuppressor deregulation of JunD, Smad3, p27KIP1, p18Ink4	20-60%
von Hippel Lindau Disease(VHL)	3p25-26, VHL	VHL protein	Oncosuppressor overexpression of HIF and VEGF	5-17%
Von Recklinghausen's disease	17q11.2, NF1	Neurofibromin	Oncosuppressor deregulation of Ras pathway mTOR	Rare
Tuberous sclerosis complex	9q34, TSC1 16p13.3TSC 2	Tuberin	Oncosuppressor deregulation of mTOR pathway	Very rare





Fuzzy EOQ Inventory Model for Price-Dependent-Demand of Deteriorating Items

S.K. Indrajitsingha¹, A.K.Sahoo^{2*}, P.N. Samanta³, U.K.Misra⁴ and L.K. Raju⁴

¹Department of Mathematics, Saraswati Degree Vidya Mandir, Neelakantha Nagar, Berhampur, Odisha, India.

²Department of Mathematics, Berhampur University, Berhampur, Bhanja Bihar, Odisha, India.

³Department of Mathematics, Berhampur University, Berhampur, Bhanja Bihar, Odisha, India.

⁴NIST, Palur Hills, Berhampur, Odisha, India.

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*Address for Correspondence

A.K.Sahoo

Department of Mathematics,
Berhampur University, Berhampur,
Bhanja Bihar, Odisha, India.

Email: ajit2ksahoo@gmail.com / <https://orcid.org/0000-0003-3053-7557>



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ABSTRACT

The objective of this study is to develop an EOQ inventory model for non-instantaneous deteriorating products, in fuzzy approach. Due to lockdown and shutdown, during this pandemic COVID 19, customers have faced financial crises which lead to the fluctuation of market demand followed by other system parameters of like vegetables and bakery items. Since classical EOQ model has many unrealistic parameters, some of the inventory management systems are fit for such situations but most of the business firms are dealing with parameters which are highly uncertain in nature. In the present study, we assume demand, deterioration, holding cost, backlog rate are constant in Crisp model, on the other hand in fuzzy model they are considered as triangular fuzzy numbers. Graded Mean Integration Representation Method (GMIR), Signed Distance Method (SDM) and Centroid Method (CM) are used to defuzzify the total average cost function. The result obtained in these methods is compared with the help of numerical example and sensitivity analysis is also carried out to explore the effect of change of different system parameters and validation of the proposed model.

Keywords: Demand, Deterioration, partially-backlogging, Graded Mean Integration Representation Method, Signed Distance Method, Centroid Method.

AMS Classification No. 90B05





INTRODUCTION

The concept of inventory management was developed by Whiteman Harris in 1963 by deriving square root formula for ordering inventory. This is attracted by many entrepreneurs towards inventory control. Thereafter researchers in different fields have realized the fact and gave special attention towards development of inventory modeling by assuming different parameters related to it. Deterioration plays a crucial role in inventory modeling. Deterioration is defined as decay, damage, spoilage, pilferage, evaporation of products over time during their storage period in which it loses its value in such a way that it is not possible to use as its original purpose. Ghare [1] is the first researchers to propose the concept of deteriorating products in inventory model. Many researchers have given considerable attention to develop inventory models with deteriorating items. Apart from this, order quantity of products is another important parameter which affects the inventory directly. Therefore optimizing the ordering quantity gives more profit for an organization. There are various papers published in this aspect. Padmanabham and Vrat [2] proposed an inventory model of deteriorating items for initial stock dependent consumption rate of exponentially decaying products without shortages in which optimum ordering quantity is obtained with total system cost. Mishra and Mishra [3] developed an EOQ model of deteriorating items with stock dependent demand rate. Chang et al. [4] proposed an inventory model of non-instantaneous deteriorating items with shelf space dependent demand in the retail outlets and considering zero ending inventories when shortages are not desirable. Goyal et al. [5] studied the model when the ordering policy is a function of price and stock level with partially backlogging condition. Singh et al. [6] demonstrated an EOQ inventory model of deteriorating items where deterioration is reduced by applying preservation technology. Since high stock level in a shelf of a supermarket attracts more customers to buy the product, they considered stock dependent demand with trade credit policy. Tiwari et al. [7] developed a fuzzy inventory model of non-instantaneous deteriorating items with selling price dependent demand where green technology applied to reduce the carbon emission without affecting the total profit. Cardenas-Barron et al. [8] derived an EOQ inventory model with non-linear holding cost, non-linear stock dependent demand with trade credit policy and partially backlogging rate.

In real life inventory modelling, we face numerous vague values of the parameters like demand, deterioration, holding cost, shortages etc. Hence, on modelling the inventories, the organization needs to reach a fine balance between high and low inventory level to optimizing the total average cost. There are many uncertainty and imprecision occurring in daily life to find out the very accurate result. To avoid these kinds of uncertainty, researchers classically modeled by using probability theory. However in certain situations, uncertainties are due to fuzziness, and such cases are dilated in the fuzzy set theory. The concept of fuzzy set theory was developed by Zadeh [9] in 1965. Thereafter, Kaufmann and Gupta [10] extends the concept of fuzzy set theory by developing arithmetic operations. Zimmerman [11] proposed the application of fuzzy set theory in modelling. Consequently a large number of research papers published on inventory modelling in fuzzy approach. Park [12] proposed an EOQ inventory model in fuzzy approach. Yao and Lee [13] developed an inventory model using fuzzy set theory. Chang [14], Yao et al. [15], Wu and Yao [16], Nezhad et al. [17], Jaggi et al. [18], Indrajitsingha et al. [19,20,21].

In 2019, Ghasemkhani et al. [22] developed an integrated production inventory model in fuzzy environment considering multi product, multi-period with heterogeneous fleets and time windows in a distribution network. Panja and Mandal [23] developed a green supply chain inventory model in which type-2 fuzzy credit period is constructed and demand as function of green degree and retailers selling price. Recently, Srinivasan and Vijayan [24] presented an EOQ inventory model with worsening materials in fuzzy sense by assuming triangular fuzzy numbers. Generally almost all products deteriorate in course of time. However some items maintain their quality for a certain period of time and after that deterioration occur as usual. Such types of items are called non-instantaneous perishable like cake, bread, vegetables etc. In this paper, we studied an optimal order quantity decaying non-instantaneous inventory model with price sensitive demand in fuzzy approach. The shortage is allowed with partially backlogging. Since the parameters like demand, deterioration and holding cost fluctuate in market; we





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assume them as triangular fuzzy numbers. The fuzzy total average cost is defuzzified by three methods such as graded mean integration representation method, signed distance method and Centroid method and the results so obtained are compared. The rest of the paper is organized as follows: In section 2, we provide the fundamental notation and assumptions. In section 3, a mathematical model is formed with a valid solution procedure. Numerical examples are given in crisp as well as in fuzzy environment in section 4 for validation of the imposed model. In section 5, sensitivity analysis and managerial insights of the optimal solution with respect to major parameters are carried out by using Mathematica 11.1 software. Lastly in section 6, the article ends with some concluding remark and given suggestion for future research.

Notation and Assumptions

The following notations and assumptions have been used throughout the paper

p	Unit selling price	s	Unit shortage cost
a	Demand coefficient	σ	Unit lost sale cost
b	Constant, $b \geq 1$	\tilde{a}	Fuzzy demand coefficient
T	Length of replenishment cycle	\tilde{b}	Fuzzy demand constant
v	The time at which inventory level becomes zero.	$\tilde{\theta}$	Fuzzy deterioration coefficient
θ	Deterioration coefficient, $0 < \theta \leq 1$.	\tilde{k}	Fuzzy backlogging rate
t_1	The time after which deterioration starts.	\tilde{h}	Fuzzy holding cost
h	Unit holding cost	$I_1(t)$	Initial inventory at any time t during the interval $[0, t_1]$
q_1	Initial inventory level at the beginning of each cycle.	$I_2(t)$	Inventory at any time t during the interval $[t_1, v]$
q_2	Backordered quantity during shortage period.	$I_3(t)$	Inventory at any time t during the interval $[v, T]$
Q	Total ordering quantity	\widetilde{TC}	Fuzzy total average cost
A	Ordering cost	\widetilde{TC}_g	Fuzzy total average cost in Graded Mean Integration Representation Method
c	Unit purchasing cost	\widetilde{TC}_s	Fuzzy total average cost in Signed Distance Method
k	Backlogging rate	\widetilde{TC}_c	Fuzzy total average cost in Centroid Method
		$I_1(t)$	Initial inventory at any time t during the interval $[0, t_1]$

Assumptions

- Demand depends upon selling price of the market.
- Deterioration is non-instantaneous in nature.
- Market demand exists in an infinite time horizon T .
- Replenishment or repair is not allowed.
- Shortages are allowed and are partially backlogged.
- Since demand, holding cost, deterioration and backlogged rate are uncertain in nature, they are considered as triangular fuzzy number.

Mathematical model : formulation and Solution procedure

Here, we have formulated a mathematical framework of inventory problem for non-instantaneous deteriorating products having market price dependent demand rate. The replenishment cycle is T which is fixed. Initial inventory level q_1 together with back-order inventory q_2 at the beginning of each cycle in which time $t = 0$. Since the products are non-instantaneous deteriorating in nature, the inventory decreases due to demand only during the interval $[0, t_1]$. Then deterioration starts after time $t = t_1$ and inventory level depletes due to combined effect of market demand and deterioration. At time $t = v$, the inventory level becomes zero and shortage starts in the interval





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$[v, T]$ which is considered as partially backlogged. The behavior of the system (Inventory-time graph) is shown in the fig-1 below:

Therefore the governing differential equation of the system can be derived as follows:

Crisp Model

$$\frac{dI_1(t)}{dt} = -\frac{a}{p^b}, \quad 0 \leq t \leq t_1 \dots\dots\dots(3.1)$$

$$\frac{dI_2(t)}{dt} = -\theta t I_2(t) - \frac{a}{p^b}, \quad t_1 \leq t \leq v \dots\dots\dots(3.2)$$

$$\frac{dI_3(t)}{dt} = -\frac{a}{p^b}, \quad v \leq t \leq T \dots\dots\dots(3.3)$$

with boundary conditions

$$I_3(v) = 0, I_2(v) = 0, I_1(t_1) = I_2(t_1) \dots\dots\dots(3.4)$$

The solutions of the above differential equations (3.1), (3.2) and (3.3) with the help of boundary condition (3.4) are

$$I_1(t) = \frac{a}{p^b} (t_1 - t) + \frac{a}{p^b} \left\{ (v - t_1) + \frac{\theta}{6} (v^3 - t_1^3) \right\} e^{-\frac{\theta t_1^2}{2}}, \quad 0 \leq t \leq t_1 \dots\dots\dots(3.5)$$

$$I_2(t) = \frac{a}{p^b} \left\{ (v - t) + \frac{\theta}{6} (v^3 - t^3) \right\} e^{-\frac{\theta t^2}{2}}, \quad t_1 \leq t \leq v \dots\dots\dots(3.6)$$

$$I_3(t) = \frac{a}{p^b} (v - t), \quad v \leq t \leq T \dots\dots\dots(3.7)$$

Using the initial condition $I_1(0) = q_1$, we have

$$q_1 = \frac{a}{p^b} t_1 + \frac{a}{p^b} \left\{ (v - t_1) + \frac{\theta}{6} (v^3 - t_1^3) \right\} e^{-\frac{\theta t_1^2}{2}} \dots\dots\dots(3.8)$$

Total average cost per unit time can be formulated as

$$TC = \frac{1}{T} \left[\begin{matrix} \text{Purchasing cost} + \text{Holding cost} + \text{Shortage cost} \\ \text{+ Lost sale cost} + \text{Ordering cost} \end{matrix} \right] \dots\dots\dots(3.9)$$

Purchasing cost (PC)

$$PC = \left[\frac{a}{p^b} t_1 + \frac{a}{p^b} \left\{ (v - t_1) + \frac{\theta}{6} (v^3 - t_1^3) \right\} e^{-\frac{\theta t_1^2}{2}} + \frac{\theta a}{p^b} (T - v) \right] c \dots\dots\dots(3.10)$$

Inventory holding cost (HC)

$$HC = h \left[\begin{matrix} \frac{a}{2p^b} t_1^2 + \frac{a}{p^b} \left\{ \left(vt_1 - \frac{t_1^2}{2} \right) + \frac{\theta}{6} (v^3 - t_1^3) t_1 - \frac{\theta}{2} (vt_1^2 - t_1^3) t_1 \right\} \\ + \frac{a}{p^b} \left\{ \frac{v^2}{2} + \frac{\theta v^4}{8} - \frac{\theta v^3}{24} - \left(vt_1 - \frac{t_1^2}{2} \right) - \frac{\theta}{6} (v^3 t_1 - \frac{t_1^4}{4}) + \frac{\theta}{6} \left(\frac{vt_1^3}{3} - \frac{t_1^4}{4} \right) \right\} \end{matrix} \right] \dots\dots\dots(3.11)$$

Shortage cost (SC)

$$SC = s \int_v^T \frac{a}{p^b} dt = \frac{sa}{p^b} (T - v) \dots\dots\dots(3.12)$$

Lost sale cost (LC)

$$LC = (1 - k) \frac{\sigma a}{p^b} (T - v) \dots\dots\dots(3.13)$$

Ordering cost (OC)

As per notation, we have





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$$OC = A \dots\dots\dots(3.14)$$

Hence the total average cost of the system per unit time is given by

$$TC(v) = \frac{1}{T} \left[\begin{array}{l} \left\{ \frac{a}{p^b} t_1 + \frac{a}{p^b} \left\{ (v - t_1) + \frac{\theta}{6} (v^3 - t_1^3) \right\} e^{-\frac{\theta t_1^2}{2}} + \frac{\theta a}{p^b} (T - v) \right\} c \\ \left\{ \frac{a}{2p^b} t_1^2 + \frac{a}{p^b} \left\{ \left(vt_1 - \frac{t_1^2}{2} \right) + \frac{\theta}{6} (v^3 - t_1^3) t_1 - \frac{\theta}{2} (vt_1^2 - t_1^3) t_1 \right\} \right. \\ \left. + \frac{a}{p^b} \left\{ \frac{v^2}{2} + \frac{\theta v^4}{8} - \frac{\theta v^3}{24} - \left(vt_1 - \frac{t_1^2}{2} \right) - \frac{\theta}{6} (v^3 t_1 - \frac{t_1^4}{4}) + \frac{\theta}{6} \left(\frac{vt_1^3}{3} - \frac{t_1^4}{4} \right) \right\} \right\} h \\ \left. + \frac{sa}{p^b} (T - v) + (1 - k) \frac{\sigma a}{p^b} (T - v) + A \right] \dots\dots\dots(3.15)$$

To minimize the total average cost $TC(v)$ per unit time, the value of v that minimizes $TC(v)$ can be obtained by solving the equation

$$\frac{dTC(v)}{dv} = 0 \dots\dots\dots(3.16)$$

satisfying

$$\frac{d^2TC(v)}{dv^2} > 0 \dots\dots\dots(3.17)$$

Fuzzy Model

Due to uncertainty in the market, it is not easy to define all the system parameters precisely. We assume some of these parameters $\tilde{a}, \tilde{b}, \tilde{\theta}, \tilde{k}$ and \tilde{h} may change within some limits.

Let $\tilde{a} = (a_1, a_2, a_3)$, $\tilde{b} = (b_1, b_2, b_3)$, $\tilde{\theta} = (\theta_1, \theta_2, \theta_3)$, $\tilde{k} = (k_1, k_2, k_3)$ and $\tilde{h} = (h_1, h_2, h_3)$ are considered as triangular fuzzy numbers. Then total average cost per unit time in fuzzy environment is given by

$$\tilde{TC}(v) = \frac{1}{T} \left[\begin{array}{l} \left\{ \frac{\tilde{a}}{p^{\tilde{b}}} t_1 + \frac{\tilde{a}}{p^{\tilde{b}}} \left\{ (v - t_1) + \frac{\tilde{\theta}}{6} (v^3 - t_1^3) \right\} e^{-\frac{\tilde{\theta} t_1^2}{2}} + \frac{\tilde{\theta} \tilde{a}}{p^{\tilde{b}}} (T - v) \right\} c \\ \left\{ \frac{\tilde{a}}{2p^{\tilde{b}}} t_1^2 + \frac{\tilde{a}}{p^{\tilde{b}}} \left\{ \left(vt_1 - \frac{t_1^2}{2} \right) + \frac{\tilde{\theta}}{6} (v^3 - t_1^3) t_1 - \frac{\tilde{\theta}}{2} (vt_1^2 - t_1^3) t_1 \right\} \right. \\ \left. + \frac{\tilde{a}}{p^{\tilde{b}}} \left\{ \frac{v^2}{2} + \frac{\tilde{\theta} v^4}{8} - \frac{\tilde{\theta} v^3}{24} - \left(vt_1 - \frac{t_1^2}{2} \right) - \frac{\tilde{\theta}}{6} (v^3 t_1 - \frac{t_1^4}{4}) + \frac{\tilde{\theta}}{6} \left(\frac{vt_1^3}{3} - \frac{t_1^4}{4} \right) \right\} \right\} \tilde{h} \\ \left. + \frac{s\tilde{a}}{p^{\tilde{b}}} (T - v) + (1 - \tilde{k}) \frac{\sigma \tilde{a}}{p^{\tilde{b}}} (T - v) + A \right] \dots\dots\dots(3.18)$$

Now, we defuzzify the fuzzy total average cost $\tilde{TC}(v)$ by using Graded Mean Integration Representation Method (GMIR), Signed Distance Method (SDM) and Centroid Method (CM).

(i). By using Graded Mean Integration Representation Method (GMIR), the total average cost is given by

$$\tilde{TC}_g(v) = \frac{1}{6} [\tilde{TC}_{g1}(v), \tilde{TC}_{g2}(v), \tilde{TC}_{g3}(v)]$$

$$\text{Hence, } \tilde{TC}_g(v) = \frac{1}{6} [\tilde{TC}_{g1}(v) + 4\tilde{TC}_{g2}(v) + \tilde{TC}_{g3}(v)] \dots\dots\dots(3.19)$$

To minimize the total cost function $\tilde{TC}_g(v)$ per unit time, the value of v that minimizes $\tilde{TC}_g(v)$ can be obtained by solving the equation

$$\frac{d\tilde{TC}_g(v)}{dv} = 0 \dots\dots\dots(3.20)$$

$$\text{Satisfying } \frac{d^2\tilde{TC}_g(v)}{dv^2} > 0 \dots\dots\dots(3.21)$$





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(ii). By using Signed Distance Method (SDM), the total average cost is given by

$$\widetilde{TC}_s(v) = \frac{1}{4} [\widetilde{TC}_{s1}(v) + 2\widetilde{TC}_{s2}(v) + \widetilde{TC}_{s3}(v)] \dots\dots\dots(3.22)$$

To minimize the total cost function $\widetilde{TC}_s(v)$ per unit time, the value of v can be obtained by solving the equation

$$\frac{d\widetilde{TC}_s(v)}{dv} = 0 \dots\dots\dots(3.23)$$

Satisfying $\frac{d^2\widetilde{TC}_s(v)}{dv^2} > 0 \dots\dots\dots(3.24)$

(iii). By using Centroid Method (CM), the total average cost is given by

$$\widetilde{TC}_c(v) = \frac{1}{3} [\widetilde{TC}_{c1}(v) + \widetilde{TC}_{c2}(v) + \widetilde{TC}_{c3}(v)] \dots\dots\dots(3.25)$$

To minimize the total cost function $\widetilde{TC}_c(v)$ per unit time, the value of v that minimizes $\widetilde{TC}_c(v)$ can be obtained by solving the equation

$$\frac{d\widetilde{TC}_c(v)}{dv} = 0 \dots\dots\dots(3.26)$$

Satisfying $\frac{d^2\widetilde{TC}_c(v)}{dv^2} > 0 \dots\dots\dots(3.27)$

Solution procedure

The objective is to minimize the total average cost function per unit time. The necessary condition of existence of the solution is $\frac{dTC(v)}{dv} = 0$ provided that $\frac{d^2TC(v)}{dv^2} > 0$. By applying the following systematic algorithm, we can get the optimal solution of the given problem.

ALGORITHM

- Step-1 : Start
- Step-2 : Assign the values of the parameters $a, b, p, t_1, \theta, \sigma, h, A, c, T, k$ and s .
- Step-3 : Find $\frac{dTC(v)}{dv}$.
- Step-4 : Solve the equation $\frac{dTC(v)}{dv} = 0$ and find the value of v .
- Step-5 : Evaluate $TC(v)$.
- Step-6 : Find $\frac{d^2TC(v)}{dv^2}$.
- Step-7 : If the value of $\frac{d^2TC(v)}{dv^2} > 0$, then we get an optimal solution and go to Step-9.
- Step-8 : Otherwise go to Step-2.
- Step-9 : Stop

Numerical Example

In this section we considered a numerical example for validation of the proposed model.





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Crisp model

Let us consider the following parametric values with their appropriate units

$$\begin{array}{llllll}
 a = 1500 \text{ units,} & b = 2.4, & P = 4 \text{ \$/unit,} & t_1 = 3 \text{ days,} & \theta = 0.01, \\
 \sigma = 4 \text{ \$/unit,} & h = 0.2 \text{ \$/unit,} & A = 200 \text{ \$/order,} & c = 3 \text{ \$/unit,} & T = 20 \text{ days,} \\
 k = 0.7, & s = 7. & & & &
 \end{array}$$

The values of different parameters considered here are realistic, though these are not taken from any case study. Corresponding to these input values, the optimum value of $v = 13.2501$ days, $Q = 1140.84$ and the optimum value of $TC(v) = \$ 399.522$.

Fuzzy model

$\tilde{a} = (1400, 1500, 1600)$, $\tilde{b} = (2.3, 2.4, 2.5)$, $\theta = (0.008, 0.01, 0.012)$, $k = (0.5, 0.7, 0.9)$ and $\tilde{h} = (0.1, 0.2, 0.3)$ be consider as triangular fuzzy numbers and $P = 4$ \\$/unit, $t_1 = 3$ days, $\sigma = 4$ \\$/unit, $A = 200$ \\$/order, $c = 3$ \\$/unit, $T = 20$ days, $s = 7$. Then the fuzzy total average cost can be determined by

1. Graded Mean Integration Representation (GMIR) Method is $\widetilde{TC}_g(v) = \$ 393.498$ with $v = 13.596$ days and economic order quantity $Q = 1180.1$
2. Signed Distance Method (SDM) is $\widetilde{TC}_s(v) = \$ 390.486$ with $v = 13.7284$ days and economic order quantity $Q = 1199.73$
3. Centroid Method (CM) is $\widetilde{TC}_c(v) = \$ 393.498$ with $v = 13.596$ days and economic order quantity $Q = 1180.1$

Sensitivity analysis

Due to uncertainties present in any decision-making situation, sensitivity analysis will give us a significant assistance in decision making process. Sensitivity analysis and managerial insights are performed here to analyze the effects of change of different system parameters on the optimal solution. We use Mathematica 11.1 software for calculation of the total average cost function. Let us consider the following parametric values with their appropriate units.

$$\begin{array}{llllll}
 a = 1500 \text{ units,} & b = 2.4, & P = 4 \text{ \$/unit,} & t_1 = 3 \text{ days,} & \theta = 0.01, \\
 \sigma = 4 \text{ \$/unit,} & h = 0.2 \text{ \$/unit,} & A = 200 \text{ \$/order,} & c = 3 \text{ \$/unit,} & T = 15 \text{ days,} \\
 k = 0.7, & s = 7. & & & &
 \end{array}$$

Corresponding to these input values, the optimum value of $v = 13.2501$ days, $Q = 952.386$ and the optimum value of $TC(v) = \$ 347.827$.

Managerial insights

From the example we see that in fuzzy model, total average cost is minimum than that of crisp model. It is also found that Centroid method provides best result as compared with Graded mean integration representation method and Signed distance method. From the above table it is found that, by increasing the demand parameter b per unit and other parameters unaltered, fuzzy total average costs $\widetilde{TC}_g(v)$, $\widetilde{TC}_s(v)$ and $\widetilde{TC}_c(v)$ decrease together with economic order quantity Q , without affecting v . Increase in the demand parameter ' a ' per unit and without changing other parameters, it can be concluded that the fuzzy total average costs derived by three different methods, $\widetilde{TC}_g(v)$, $\widetilde{TC}_s(v)$ and $\widetilde{TC}_c(v)$ remain same with order quantity Q and v . It is observed that when the value of deterioration coefficient θ per unit increases and keeping all other parameters constant, fuzzy total average costs $\widetilde{TC}_g(v)$, $\widetilde{TC}_s(v)$ and $\widetilde{TC}_c(v)$ per unit time also increase with the decrease of order quantity Q and v . As the value of the holding cost h per unit increases, it is observed that the fuzzy total average costs $\widetilde{TC}_g(v)$, $\widetilde{TC}_s(v)$ and $\widetilde{TC}_c(v)$ increase with





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decrease of economic order quantity Q and v . Increase of the rate of backlogging value k per unit and at the same time keeping all other related parameters unchanged; we found that there is very slow decrease in fuzzy total average costs $\widetilde{TC}_g(v)$, $\widetilde{TC}_s(v)$ and $\widetilde{TC}_c(v)$, per unit time, economic order quantity Q and v .

CONCLUSION

In real markets, it is very common that many products like vegetables, bakery deteriorate during their storage period or at their warehouses in some time interval. Hence in the In this present study we assume deterioration as triangular fuzzy number due to uncertainty. The public demand always fluctuate that means it may go up and down in the market depending upon the selling price of the products. So we also consider demand parameter as triangular fuzzy number. Apart from these two parameters, we assume holding cost and backlogging rate as triangular fuzzy numbers. For defuzzification, we used Graded Mean Integration Representation Method, Signed Distance Method and Centroid Method. We develop the model both in crisp and fuzzy approach and is concluded that fuzzy total average cost gives more approximate value as compared to crisp model. Moreover, in defuzzification process, it is observed that Centroid method provides minimum total average cost per unit time. The graphical representations are additionally provided here to visualization of the effectiveness of the related parameters. Moreover, this model is highly essential at the time of lockdown and shutdown of the daily markets during the pandemic COVID 19 in the countries like India, Pakistan, Bangladesh, Srilanka, Nepal, Bhutan, Maldives, Mymar, Mauritius where just in time delivery is not possible. In future aspect, one can extend this paper by adding preservation technology in deterioration rate and by considering trade credit policy.

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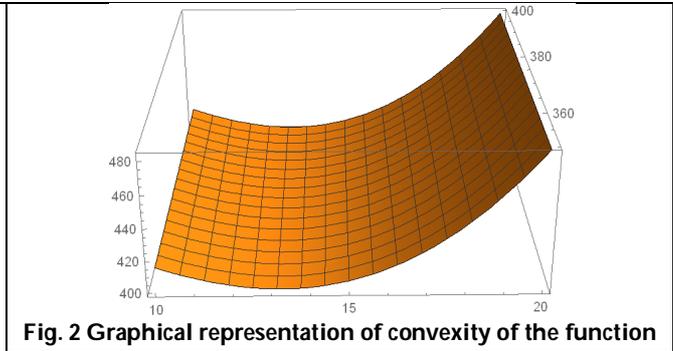
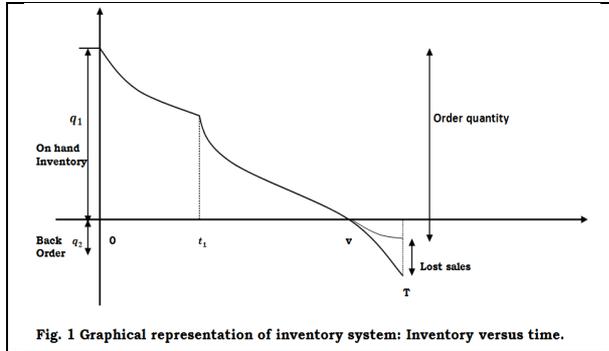
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Table 1: Effects of parameters on the optimal total average cost

		GMIR			SDM			CM		
		v	$\widetilde{TC}_g(v)$	Q	v	$\widetilde{TC}_s(v)$	Q	v	$\widetilde{TC}_c(v)$	Q
\tilde{a}	(1300,1400,1500)	13.2501	325.528	888.894	13.2501	325.528	888.894	13.2501	325.528	888.894
	(1400,1500,1600)	13.2501	347.827	952.386	13.2501	347.827	952.386	13.2501	347.827	952.386
	(1500,1600,1700)	13.2501	370.127	370.127	13.2501	370.127	370.127	13.2501	370.127	370.127
\tilde{b}	(2,2,2,3,2,4)	13.2501	398.799	1097.51	13.2501	399.415	1099.27	13.2501	400.031	1101.02
	(2,3,2,4,2,5)	13.2501	348.9	955.441	13.2501	349.137	956.968	13.2501	349.973	958.495
	(2,4,2,5,2,6)	13.2501	305.461	831.76	13.2501	305.928	833.09	13.2501	306.395	834.42
$\tilde{\theta}$	(0.01,0.02,0.03)	10.8683	382.556	899.163	10.9791	380.965	901.018	11.0899	379.373	902.873
	(0.02,0.03,0.04)	9.45367	403.355	862.44	9.4941	402.756	863.271	9.53454	402.158	864.101
	(0.03,0.04,0.05)	8.62467	415.573	837.013	8.64494	415.269	837.485	8.66522	414.965	837.956
\tilde{h}	(0,2,0,22,0,24)	12.8274	356.589	918.418	12.8302	352.532	928.751	12.833	356.476	929.083
	(0,22,0,24,0,26)	12.4243	364.799	906.139	12.423	364.749	906.154	12.4216	364.7	906.168
	(0,24,0,26,0,28)	12.0312	372.413	885.753	12.0418	372.369	886.309	12.0524	372.325	886.864
\tilde{k}	(0,3,0,4,0,5)	13.5192	349.561	944.748	13.5191	349.558	944.826	13.519	349.556	944.904
	(0,4,0,5,0,6)	13.4302	349.014	946.381	13.4301	349.011	946.462	13.4301	349.088	946.542
	(0,5,0,6,0,7)	13.3405	348.434	948.978	13.3404	348.431	949.06	13.3403	349.428	949.141







Psycho-Social Factors Influencing Mental Illness in Person with Intellectual Disability

S. Karthikeyan ^{1*}, C. N. Ram Gopal ² and K. Sagayaraj ²

¹Clinical Psychologist, Lecturer and Head in Charge, Department of Clinical Psychology, National Institute for Empowerment of Persons with Multiple Disabilities (Divyangjan), East Coast Road, Muttukadu, Kovalam Post, Chennai, Tamil Nadu, India.

²Counseling Psychology, Faculty of Allied Health Sciences (FAHS), Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai, Tamil Nadu, India.

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*Address for Correspondence

S. Karthikeyan

Clinical Psychologist, Lecturer and Head in Charge,
Department of Clinical Psychology,
National Institute for Empowerment of Persons with Multiple Disabilities (Divyangjan),
East Coast Road, Muttukadu, Kovalam Post,
Chennai, Tamil Nadu, India.
Email: karthikeyan.clinpsy@gmail.com



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ABSTRACT

The prevalence rate of psychiatric disorders are 4 to 5 times higher for people with intellectual disability than the general population (Rush et al., 2004). Most of the persons affected by intellectual disabilities with mental illness go unnoticed with regard to their etiological condition. This study would expect to create an impact in providing awareness amongst the common public and professionals working in the field of rehabilitation to identify and to make possible efforts in preventing the potential factors attributing to such condition, which in turn will prevent a person with single disability becoming a person with multiple disabilities. By using the purposive sampling method parents of persons with intellectual disabilities were only incorporated for this study. The participants responses were collected by using Kuppaswamy Socio-Economic Status Scale (2014) and Reiss Screen for Maladaptive Behavior (2009) and the data were analysed with SPSS. The results of the study confirm the fact that psychosocial factors are influencing the psychotic features in persons with intellectual disabilities.

Keywords: Intellectual disability, Mental illness, Psychiatric disorders, Psychotic features.





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INTRODUCTION

Mental Health is a vital element of a decent quality of life. It becomes an important goal for all individuals as well as persons with Intellectual Disability (ID) and other disability condition. In mental health, we can see individuals with good mental health to individuals having diagnosed and treated with mental illness. Each individual varies in their position at different points in their life. An individual with good mental health can have a positive relationship with individuals around them, they will have the ability to reason and assume, and they will also feel in control of their emotions. Similarly, Mental illness could be a general term, pertaining to a group of illnesses that disrupt the person's ability to work and perform in traditional daily living activities and interact. Mental illness can come into an individual's life lasting from a couple of weeks or months to years. It is not mental retardation; rather it causes severe disturbances in a person which can be seen in their behavior, mood, thought processes, social and relationships. Intellectual disability or mental retardation is often explained as below average intellectual functioning coincident with deficits in adaptive functioning. Individuals with intellectual disability have considerably a lot of issue than others in learning new things, understanding ideas, finding solution, concentrating and basic cognitive process. Consequently, they need additional support to find out and reach their full potential. Intellectual disability is commonly present from a person's early years.

Irrespective of their level of function, mental illness can affect people with intellectual disability similar to people without a disability. Data shows that there is a higher risk of mental illness for those with intellectual disability. Some sources point out that higher prevalence rates, but the estimated prevalence is at 33% (Quintero & Flick, 2010). It is difficult to assess and diagnose mental illness when the individual has an intellectual disability. Because an individual with intellectual disability cannot express the symptoms or even identify their feelings which makes it harder to assess mental illness. Other reasons are the unusual or infrequent presentation of signs and symptoms; behavioral and physical issues are usually managed by medications which can act as masking of mental illness. It is also difficult to identify the patterns of illness with missing or inconsistent historical information.

We can see people with intellectual disabilities with varies types of mental illness. But the most common types of mental illness are affective disorder, avoidant disorder, anxiety disorder, personality disorder, psychotic disorder. Underlying medical conditions, specific brain dysfunctions, or mental illnesses such as psychosis, depression, paranoia could be the reasons for behavior problems found in persons with intellectual disability. It is also to be noted that psychosocial and biological factors both contribute to mental illness with intellectual disability. It is been estimated that prolonged exposure to negative social conditions and the attitude of the public towards people with mental retardation attributes in developing mental illness. Added to this, the perception of having a retarded condition in a person overshadows the symptoms of mental illness (Reiss, S., et. al 1982). This in-turn affects family members to seek any interventions that is required to treat the illness condition.

Both mental retardation and mental illness are classified under Persons with Disabilities Act (1995) and terms, that existence of the above two conditions existing in the same individual as Multiple Disability as per National Trust Act, 1999. Thereby, the mental illness symptom to the existing condition of intellectual disability turns a person with a single disability to multiple disabilities. In connection with that this study would empirically prove that preventing the potential negative psychosocial condition will prevent a person with single disability becoming a person with multiple disabilities.

METHODOLOGY

Aim

The aim of the study is to identify the predominant psychosocial and behavioural conditions attributing to psychotic features in persons with intellectual disabilities.





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Objectives

- (i) To find the relationship between psychosocial factors that are influencing the psychotic features in persons with intellectual disabilities
- (ii) To find the relationship between behavioural and physical signs of depressive symptoms that are influencing the psychotic features in persons with intellectual disabilities

Hypothesis

1. H₁ There would be a significant relationship between aggressive behaviour and autistic features in persons with intellectual disabilities
2. H₁ There would be a significant relationship between behavioural and physical depression in persons with intellectual disabilities
3. H₁ There would be a significant relationship between dependent personality and avoidant behaviour in persons with intellectual disabilities
4. H₁ There would be a significant relationship between the negative psychological condition (psychosis and paranoia) in persons with intellectual disabilities

Research Design

Exploratory Research Design was adopted for this study

Sampling Technique

Purposive sampling technique was used in this study. The population of the study participants are parents having persons with intellectual disabilities affected with mental illness.

Sample Descriptions

A total of 312 parents having persons with intellectual disabilities and willing to voluntarily participate in the study were incorporated. The samples were collected from the various rehabilitation institutions and special schools for intellectual disabilities in and around Chennai, providing rehabilitation services for the age group of 15 – 30 years in individual and group forms.

Inclusion Criteria

- Parents having their wards with intellectual disabilities age ranging 15 to 30 years, with mild to a profound category of intellectual disability holding National ID Card for mental retardation / intellectual disabilities issued by the State Government of Tamil Nadu.
- Both parents and single parent having persons with intellectual disabilities.

Exclusion Criteria

- Caretakers other than parents of persons with intellectual disabilities.
- Parents with poor clarity of information due to specific reasons like sensory impairments or dementia.

Tools Used

Kuppaswamy Socio-Economic Status Scale (2014): The social demographic details were collected by using this scale. It categorizes socio-economic status by consumer price index and its various forms have been providing statistical standardization over the decade. Test-retest reliability of the scale is found to be $r=0.92$ and it is found to have good validity as well.

Reiss Screen for Maladaptive Behaviour (2009): This tool consists of 38 items. It was developed by Steven Reiss. This schedule aims to measure maladaptive behaviour and more specifically, to aid in the identification of

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individuals who require a further psychiatric evaluation. It has been used in adults with mild-to-profound levels of mental retardation. It divides maladaptive behaviour into eight dimensions: aggressive behaviour, psychosis, paranoia, depression (behavioural signs), depression (physical signs), dependent personality disorder, avoidant disorder and autism. Items are rated on a 3-point Likert scale. Each of the 38 items on the Reiss Screen is scored as follows, No problem indicate 0, Problem indicate 1, Major Problem indicate 2. The scale's Cronbach's alpha internal reliability was 0.84 and validated for the different population as well.

PROCEDURE

The participants were approached individually and in group forms. The need for the study and introductory comment about the study was addressed by the researcher. Anonymity and participant confidentiality were assured.

Data Analysis: The analysis of the collected data was computed by descriptive statistics mean (M), standard deviation (SD); and inferential statistics Pearson correlation coefficient by using SPSS.

RESULTS

From the table 1 result, it can be inferred that the negative psychosocial condition such as aggressive behaviours and autistic features have a significant moderate positive correlation and the P-value was found to be 0.500 at 0.01 significance level. This study is well correlated and supported by the (Fitzpatrick et al., 2016; Luiselli, 2009) where aggression is clearly found to be associated with the negative outcome of autistic features in children with mental illness, hence hypothesis 1 is accepted. A study conducted by (Farmer et al., 2015) indicated that sex difference is not associated with aggression hence we have not incorporated comparison of sex difference in the participants and also it further reveals that younger children with ID shows more aggression than individual with older age. Behavioural disorders are more often found in the persons with intellectual disability regardless of the underlying etiology and such negative behaviours are often chronic and more than one may be presented in every individual (Ageranioti-Bélanger et al., 2012) such as aggressive behaviours which may include (destructive, hostile, impulsive, tantrums) and autistic features (echolalia, object attachment, self-stimulating behaviour, unusual movements, withdrawn) which have been empirically studied and found to be correlated in this study.

Table 2 represents the relationship between behavioural depression and physical depression in persons with ID and it was found to be a moderate positive correlation with a P-value of 0.618 at 0.01 significance level. (Hartley & Maclean, 2009) emphasises that stress is the major cause for depression in persons with ID and this was influenced by social interaction, negative attribution style, avoidant behaviour and less coping strategies. (Goodwin, 2006) explained that depressive symptoms are most commonly associated with a variety of physical or somatic presentation of the individual and it may exhibit in different behavioural forms as well which was correlated in this study and found to be significant hence hypothesis 2 is accepted.

Table 3 referring the relationship between dependent personality and avoidant behaviour in persons with intellectual disabilities and found to be have weak relationship between the variables. The P-value was low and positively correlated with 0.440 at 0.01 significance level. Avoidant behaviour is a relatively common disorder and associated with distress, impairment and disability. A study by (Gjerde et al., 2012) proved that avoidant and dependent characteristics are influenced by heritable factors and no evidence of environmental factors are shared between them. Since the participants of this study vary from a different social and environmental background the weak positive correlation is represented hence hypothesis 3 is partially accepted.

Table 4 corresponds to the relationship between the negative psychological condition (psychosis and paranoia) in persons with intellectual disabilities and the P-value was found to be positively highly correlated with the value of 0.881 at 0.01 significance level. Several studies confirmed that social environmental factors influence the risk of onset psychotic disorders and psychosocial related stress associated with risk and relapse of psychosis (Veling et al., 2016).



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(Coopet et al., 2007) found that psychosis has a high prevalence and higher incident rate in individual with ID than the general population. Psychotic conditions involve mood swings, impulsivity and it is highly correlated with paranoia (Wilkinson et al., 2012) which was empirically confirmed in this study as well hence hypothesis 4 is accepted.

DISCUSSION

Basically, the negative psycho social factors such as stress, depression, anxiety, hostility are created by the social environment and not presented by default in any individual. General population may not be aware of these influences or sources of such negative factors but they find the way to get rid of that in order to have the healthy quality life style. This opportunity is not being given for people with intellectual disabilities due to their condition but it's the responsibility of every mental health professionals to provide necessary help that is required. Just like the physical illness, mental illness is also treatable and curable to some extent. From this study we emphasis the prevention of single disability becoming multiple disabilities in terms of preventing the negative psycho social factors.

CONCLUSION

The study confirmed the fact that psychosocial factors are influencing the psychotic features in persons with intellectual disabilities. It empirically reveals the significant relationship between aggressive behaviour and autistic features, behavioural and physical depression, dependent personality and avoidant behaviour, negative psychological condition (psychosis and paranoia) in persons with intellectual disabilities. Though the fact that genetic influence these negative psychosocial factors, it was mostly influenced by the social-environmental situation. Hence adequate modification is needed in the psychosocial factors to reduce the mental illness in the persons with intellectual disability.

Conflicts of Interest: Nil

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Table 1. Shows the relationship between aggressive behaviours and autistic features in persons with intellectual disabilities (N = 312)

	Mean	SD	P-Value	Sig. (2-tailed)
Aggressive behaviours	2.22	1.664	.500**	.000
Autistic features	2.14	1.717		

** Correlation is significant at the 0.01 level (2-tailed).

Table 2. Shows the relationship between behavioural depression and physical depression in persons with intellectual disabilities (N = 312)

	Mean	SD	P-Value	Sig. (2-tailed)
Behavioural depression	2.46	2.047	.618**	.000
Physical depression	2.46	2.077		

**Correlation is significant at the 0.01 level (2-tailed).

Table 3. Shows the relationship between dependent personality and avoidant behaviour in persons with intellectual disabilities (N = 312)

	Mean	SD	P-Value	Sig. (2-tailed)
Dependent personality	3.05	1.644	.440**	.000
Avoidant behaviour	2.60	1.492		

**Correlation is significant at the 0.01 level (2-tailed).

Table 4. Shows the relationship between the negative psychological condition (psychosis and paranoia) in persons with intellectual disabilities (N = 312)

	Mean	SD	P-Value	Sig. (2-tailed)
Psychosis	2.27	2.386	.881**	.000
Paranoia	2.01	1.999		

**Correlation is significant at the 0.01 level (2-tailed).





Potential Upshots of COVID-19 Pandemic on Comorbidities

Alby Manoj¹, Aleesha R^{2*}, Bharat Mishra³, Aiswarya VS¹, Divya Merine¹ and Nithin Thomas¹

¹Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

²Assistant Professor, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

³Professor and Head, Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

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*Address for Correspondence

Aleesha R

Assistant Professor,

Department of Pharmacology,

Nirmala College of Pharmacy,

Muvattupuzha, Ernakulam, Kerala, India.

Email: aleeshar23@gmail.com



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ABSTRACT

The 21st century has witnessed a global pandemic due to the world wide spread of corona virus. The novel (SARS -COV2) corona virus is highly infection and spreads alternatively from person to person. It introduced in to the human population as a highly pathogenic one and was declared by the WHO as pandemic. Thus, the urgent need for the control of the disease leads to the exploration in therapeutic options. Currently, the FDA has not approved any drugs for COVID 19. However, an array of drugs that are being studied for the treatment of COVID 19 in clinical trials around the globe. Scientists all over the world are in the quest for developing a better and effective therapy for curing COVID 19. A large number of suggestions and recommendations are being exposed into the light of treatment of the disease. The recommendations are based on the evidence from the clinical trial and from the knowledge of treatment of other viral infections. This review mainly highlights the insight into drugs discovery, various strategies that are followed for drugs discovery and mentioning some of the therapeutic approaches- antiviral drugs and other immune based therapies. The main therapeutic options include potential antiviral drugs, blood derived products, Immunoglobulins, Immunomodulators and other adjunctive therapy. This review spotlights the systematic review on the role of various therapeutic options in management of the disease

Keywords: Covid19, Remdesivir, Chloroquine, Ivermectin, Lopinavir, Immunoglobulins, Corticosteroids, Interferon.



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The ghastly infectious disease COVID 19 is caused by a novel corona virus. In 2020 January Wuhan, China was the first country to get conquered by virus and within months it spread worldwide and became a disaster and officially known as Severe Acute Respiratory Syndrome Coronavirus-2(SARS Cov-2) which is global issue of health concern. They are single stranded RNA virus that belongs to Coronaviridae family. Many countries are trying to defeat the pandemic by the use of antivirals, oxygen therapy, mechanical ventilation [1]. By concerning the severity WHO announced that COVID19 can be characterized as a pandemic on 11th March 2020 [2]. WHO ordered the countries to meet needed measures to tackle the situation ,treat ,diagnose and decrease transmission. The symptoms associated more often are respiratory illness. Transmission occurs mainly by means of infected droplets. The incubation period ranges from 2-14 days normally. Since the novel Corona has afflicted the world we should clutch to the precautions by WHO. Normally virus change or mutate after infecting a person. This almost proved true in case of corona virus also. After testing it showed two strains L and S. S is older and L was common in early stages [3]. Management of this infection was heterogeneous across the countries regarding Indications for virological testing and asymptomatic contacts, Indications for low dose CT, Therapeutic options and follow up. Treatments usually done are PCR, LDCT of chest for positive and with Hydroxychloroquine and later Azithromycin was added to Hydroxychloroquine when found to have synergistic action [4]. As per recent reports there are 216 countries or areas with cases and till 18th September 5.21 Million confirmed cases and 84.372 deaths in world [WHO].The tally of new cases in Asian countries has never fallen to zero and instead doubling day by day. FDA has launched an emergency program for corona treatments, Corona virus Treatment Acceleration Program (CTAP). It brings methods to move new treatments to patients and also investigate the presence of any harm [5]. As now no vaccines and accurate treatments have discovered, potential medications used are Remdesivir , Chloroquine, Tocilizumab , Covalent plasma and vaccine immunization [6]. Many clinical and treatment trials are going on and moreover drug development is a multistep process which takes more than five years to check safety and efficacy. Many vaccines have entered phase II and III. Here this paper highlights about drug discovery on COVID19.

Insight into Drug Discovery

Amidst the pandemic the scientists are working really very hard in order to develop an effective treatment for the disease. Therapies including drugs are still under investigation. It's known that there are no specific antiviral drugs that have proven effective for COVID 19. However drugs repurposing are being tested to see if they have the capacity to fight against the virus that causes COVID 19. But difficulties are still there to develop treatment for viral infection since the virus adapt easily and are having plenty of ways to mutate thereby developing resistance to drugs that are being given [7].

Drug Discovery Strategies

In order to achieve an effective treatment certain tactics are needed to be installed. Extensive researches concerning the drug discovery streamlined many drug discovery strategies. They include :Drug repurposing (also called drug repositioning)- the investigation of existing drugs for new therapeutic purposes- is one line of scientific research that have been developed in order to achieve safe and effective COVID 19 treatment. Many existing broad spectrum antiviral agents that were once proven to cure other viral infections are being researched as COVID 19 treatment and among them some are getting into a way to clinical trials [8]. Another approach for drug discovery is chemical library screening- molecular libraries and databases that allow verification of millions of potential agents; These compounds or database are later evaluated by antiviral detection test [9]. One of the crucial method for drug discovery is the targeted therapy, precisely developed to disrupt the crucial steps if viral infection [10]. For example:A notable drug target may be the cellular enzymes that help to attach fatty acids to cysteine cluster in cytoplasmic tail of S. Another important dimension in the quest for drug development is to develop drugs targeting receptor mediated endocytosis and viral budding [11].





Therapeutic Approach to Covid-19

Based on evidences from laboratory, animal and clinical studies the drugs which are advices for the treatment of COVID-19 are required to be both viral multiplication limited and offset tissue damage triggered by an inappropriate immune reaction. Some of the clinically used medications under the evaluation for the treatment of COVID-19 can be categorised into Potential antiviral drugs(Remdesivir, Chloroquine or hydrochloroquine, Ivermectin, Lopinavir-Ritonavir), Blood derived products (Convalescentplasma), Immunoglobulins , Immunomodulators (Corticosteroids, Interferon, Interleukin 6 inhibitors) and Adjunctive therapy (Vitamin C, Vitamin D)[12].

Remdesivir (Gs- 5734)

It is an antiviral drug developed by Gilead Science in 2014 for Ebola treatment [13]. Antivirals that inhibit protease inhibitors and nucleotide/nucleoside analogues that inhibit RNA synthesis have been used for SARS COV- 2. Remdesivir is most potent drug of that class. Remdesivir is metabolised to active nucleoside triphosphate by host. Remdesivir,GS 5734 and active form GS 44152 which is adenosine analogue [14]. The competitive inhibitor of viral DNA dependent RNA polymerase Remdesivir is an investigational nucleoside analogue with molecular formula C₂₇H₃₅N₆O₈P and mass 602.23 Da. Remdesivir inhibits viral replication, reduce viral loads decrease pathological process relieve mild symptoms and improve pulmonary lesions. A number of in vitro and in vivo studies have been carried out with mouse and non- primates. The studies proved that duration of therapy extends from 5-10 days. In-vitro studies show that Remdesivir is effective for SARS COV with EC₅₀ value 0.069 and MERS COV with EC₅₀ value 0.074µM. The involvement of non-structural proteins such as nsp5 nsp8 came up in inhibitory mechanism of active triphosphate molecule of Remdesivir against MERS-COV. Another study conducted in Vero E6 cells proved that Remdesivir is most potential antiviral drug by inhibition of replication of SARS COV- 2 with EC₅₀ of 23.15µM. In case of in vivo studies Remdesivir proved to reduce the concentration of virus in infected mice and also reduced lung lesions by inhibiting replication in pulmonary organs of Rhesus Macaques and also reduced viral load within 7 days without any pulmonary disease. It also inhibits COV-229E and COV-OC43 replication depending upon dose, which cause upper respiratory infection in children and asthma and COPD in adults [15]. The adverse effects are nausea, vomiting, elevated transaminase and and elevated prothrombin time. Gilead Science says that Remdesivir should not be used with Rifampin which is a strong inducer of CyP P450, OATP and also not recommended with chloroquine and 2- hydroxy chloroquine as they decrease antiviral activity [16].

Chloroquine / Hydroxychloroquine

Chloroquine is used to treat malaria since 1947. Hydroxychloroquine, a less toxic derivative of chloroquine with a hydroxyl group at the end of the side chain is also used to treat auto immune disorder [17]. Both was found to have activity against coronavirus. They have immunomodulatory effect and decrease the cytokine production [18]. Both were first found effective on a Chinese COV- 19 patient. Clinical safety profile of HCQ is better than CQ and higher daily doses is possible with less drug- drug interaction. In France, the early treatment with HCQ to which they added azithromycin when found synergistic action. But the risk associated with that is QT prolongation and arrhythmia. A demonstration of a study was presented by a Chinese team which proved that HCQ inhibits SARS COV- 2 in vitro with EC₅₀=0.72% µM and CQ EC₅₀= 5.47% µM which was less potent [19]. Some reports have come up explaining the mechanism of AZM/ CQ/ HCQ. The possible mechanism would be binding of receptor binding domain of spike (s) protein of virus to cell surface ACE2 that aids in viral entry and replication occurs in host cell. It was found that main protease (M^{pro}) act as a suitable site for drugs inhibiting viral replication. CQ act by elevating endosomal p^H and eliminating virus-endosomal fusion [20]. Many in vitro studies conducted in Vero E6 cells contributed to the efficiency of HCQ and believed that this is due to lysosotropic effect of drug. These were the two drugs which gained most public attention and trolled as “biggest game – changers in the history of medicine” and at the other extreme “useless and dangerous” [21]. Studies show reduction in viral load in naso-pharangeal samples within seven days with HCQ and AZM [22]. The distance between therapeutic and toxic dose is limited and its poisoning is life threatening [23]. Advance effects include abdominal pain, anorexia, skin reactions, hypoglycemia



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etc. Cardiac adverse effects include QT prolongation, Torsade de Pointer and ventricular arrhythmia and cause cardiac deaths [24].

Ivermectin

Ivermectin is a FDA approved drug as antiparasitic. It was recommended as drug for COVID19 by the end of March 2020. Several in vitro studies conducted by Australian scientists proved that Ivermectin could reduce viral content viral RNA 500 folds and stop replication within 48 hours and then no more reduction seen [25]. It acts by the mechanism of inhibiting nuclear import of viral and host proteins [26]. IMP α/β 1 is the dependable for RNA virus, and Ivermectin inhibits the import [27]. An experienced team of doctors together on 19th July 2020 conducted vast studies and concluded by the following words " Ivermectin in the dose of 12mg BD alone or in combination with other therapy for 5-7 days may be considered as safe therapeutic option for mild , moderate or severe cases of COVID 19 infection. It is cost efficient especially when other drugs are very costly and not easily available". Clinical trials currently use dose from 200-1200mcg/kg body weight for 3-7 days which found excellent results. Most important is that zero toxicity is seen in Ivermectin [28]. It can also be used in combination with HCQ and Azithromycin because Ivermectin by acting on importin α/β dependent transport of virus, they inhibit RNA replication and also HCQ inhibits the entry of virus into host. Then both are found to have synergistic action and no drug interactions. But yet no studies have been conducted on this [29]. A drawback found is that Ivermectin is not recommended in pregnancy since mice, rat show teratogenic effect [30].

Lopinavir-Ritonavir

Lopinavir-ritonavir is a combination of protease inhibitors that are commonly used in the treatment of Human immuno deficiency virus. While considering the therapy for COVID 19 lopinavir-ritonavir is being used as an off-label treatment and have to be considered at earlier of the disease progression. Even though some side effects may include with the therapy. Gastrointestinal side effects can be observed commonly in patients with lopinavir-ritonavir treatment. In such a case then discontinue the treatment [31]. Lopinavir is having a low oral bioavailability and is extensively metabolised by CYP3A4 isoenzyme. Hence there arises the need for co administration of lopinavir with ritonavir in order to accomplish high drug concentration so as to inhibit viral replication. The molecular target of lopinavir/ritonavir is the main protease (3CLpro) in SARS-CoV-2 infected cells. 3CLpro is a major CoV protease that helps in the cleavage of the large replicase polyproteins during viral replication. However there are no biochemical and molecular studies that confirms the interaction and associating this with clinical efficacy of the protease inhibitor. Some of the reported results of lopinavir-ritonavir obtained from studies in different cell lines, animal models, and patients are not so convincing in their inhibition action in human corona viruses [32]. Apart from symptomatic and supportive care little benefits are attained by improving the clinical outcome of hospitalized patients with mild/moderate COVID-19 by this treatment [33]. However In hospitalized adult patients with severe Covid-19, no visible benefits were observed with lopinavir-ritonavir treatment beyond standard care. Future trials in patients suffering from severe illness may help to confirm or exclude the possibility of a treatment benefit. Anyhow, during the SARS outbreak, treatment with lopinavir - ritonavir, lead to some success in nonrandomized clinical trial. Patients with SARS-COV treated with lopinavir / ritonavir showed a progressive decrease of viral load and reduction of the composite adverse outcomes [34].

Convalescent Plasma

Convalescent Plasma is plasma that is collected from people who have recovered from any disease and the person blood is assumed to have antibodies against that particular disease. It can be defined as a passive antibody therapy, and it is being contemplated as an optimistic and potential therapeutic consideration since the beginning of the COVID-19 outbreak. The donation and preparation of convalescent plasma is based upon some selection criteria referred by various national and international guides. Some of the criteria a person must follow in order to donate the convalescent plasma include: They have to be tested positive for COVID19, and once recovered, they should have no symptoms for 14 days, currently test negative for COVID19, and should have a high plasma antibody level. The



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blood types of both the donor and recipient must be well matched. Once donated, the plasma is screened for other infectious diseases. A donor produces enough plasma to donate to one to three patients. Convalescent plasma seems to retain its importance as an alternative treatment method for patients with COVID-19 infection until a specific effective anti-viral agent or vaccine is found [35]. Convalescent plasma therapy can be considered as a safe and effective therapy for COVID19 patients. The critical efficacy can be achieved only by the early administration of convalescent plasma. However there are some limitations like transfusion associated reactions and other limitations the use of other treatment regimens including antiviral medications along with convalescent plasma transfusion, which was inevitable. A key benefit of the convalescent plasma therapy is that this treatment is provided by people who have been infected previously with corona virus and these people are immediately available. It is also cost effective. If treated at proper time, it may prevent patient getting into serious stage of the disease [36]. A productive convalescent plasma contains high-titer specific antibodies which bind to the antigen and neutralize the viral particles, thereby preventing the access of viral particles to other uninfected cells. Convalescent plasma therapy helps in gradually improving patients' survival, reduce hospitalization period. This considerably reduces the treatment costs. A series of case studies in china reported that the patients who received the CP therapy resulted in increased oxygenation and reduced viral load. There were no severe adverse effects reported from transfusion of convalescent plasma in recipients. Protection may last from weeks to month. Pathogen reduction therapy is available for the effective reduction of infectious pathogens in convalescent plasma. It adds a security for the use of convalescent plasma, particularly with those viruses whose pathogenesis and immune responses are not well known [37].

Immunoglobulins

Immunoglobulins are glycoprotein molecules produced by plasma cells and they hold a critical part of the immune response by precisely recognizing and binding to a specific antigen such as bacteria, virus and further helps in their destruction. Immunoglobins either Polyvalent Immunoglobulin or hyper immune immunoglobulin is considered as the fastest therapeutic option in the outbreak of emergent infection. Currently available IVIG products like Gamunex-C and Flebogamma DIF contain antibodies reacting against SARS-CoV-2 antigens, however further studies are needed to prove its functionality and safety for COVID-19. From the knowledge of some studies it has been observed that patients developing a more severe clinical course of SARS-CoV-2 infection are likely to have higher plasma levels of pro-inflammatory cytokines, and there are possibilities for a 'cytokinestorm' associated with the disease severity. Since IVIGs are found to be effective in the treatment of inflammatory disorders it can be considered as a therapeutic option for hyperinflammation in patients with severe COVID-19. The immunomodulatory effects of IVIG products perhaps be advantageous in COVID-19 disease management, but this has to be clinically confirmed [38]. Continuous infusion with IVIG increase the level of Ig G in serum, that efficiently neutralize pathogens in the respiratory tract of the patient. This helps in the faster recovery of the patient and reduce the disease span. It also helps in improving the body's defence, block the receptors of the target cell and prevent the further damage from pathogen. Also they inhibit the production of inflammatory factors and thus reduce the inflammatory issues encountered by patients [39]. The therapy using immuno-globulins in the treatment of COVID-19 is efficacious, along with potential adverse sequels. Intravenous immunoglobulin (IVIG) is a polyvalent IG prepared from the plasma of thousands of donors and it can be diversely used for treatment of primary and secondary immunodeficiencies, autoimmune disorders inflammatory conditions, neuro-immunologic disorders etc [40]. Recent data from the studies in patients with the use of IVIG was reported with significant clinical benefits with good tolerance and was reported with a reduction in the use of mechanical ventilation. Moreover, this choice helps to avoid use of steroids that established a reduction of viral clearance. Also, the respiratory function of the patient is significantly improved. From a brief report it can be suggested that high-dose IVIg (0.3–0.5g per kg weight per day for five days) was used in 3 patients as an immunomodulator. None of the 3 patients reported any adverse effects and they were clinically improved shortly after the administration. Thus high-dose IVI g could be considered a promising option at the early stage in patients with COVID-19. It is considered as a great choice of immune modulatory therapy for autoimmune or inflammatory disease and for prophylaxis and treatment of severe infections including COVID19 [41].





Corticosteroid

COVID-19 is now a sweeping pandemic worldwide. The disease progresses in various faces in which first phase includes primary antiviral immune response that ultimately leads to activation of inflammatory cytokines and second phase which suggests hyper inflammatory condition. Corticosteroids are considered a good choice as it causes immune suppression and also reduce chances of pneumonia. When steroids were tried in critical patients with SARS COV-1 and influenza a viral pneumonia, it shows that mortality rate has been reduced. Methylprednisolone was given parenteral and have shown quicker action compared to dexamethasone. In addition, it also suggests high rates of survival and reduced ARDS. Since the conflict exists regarding whether corticosteroids can be used as a permanent, safer and good choice due to several adverse outcomes of these drugs, WHO analyses data for and against the use of steroids and stated that routine usage of steroids should be avoided for COVID-19 patients [42]. Methyl- prednisolone was used in SARS COV-1 patients to reduce the levels of IL-8, IP-10 and now 'the survival sepsis campaign' suggests the low dose steroid therapy to effectively manage intense activation of pro inflammatory cytokine and chemokine. In short, use of methylprednisolone and dexamethasone were beneficial in patients who were supplemented with oxygen therapy as indicated by 'Recovery trial' conducted in UK. In people suffering from influenza pneumonia, studies indicated increased mortality and increased length of ICU stay. Majority due to mono suppressant effect of steroids. The studies show that steroids may increase the risk of developing a systemic complication. High dose steroid therapy resulted in temporary psychosis during SARS COV-1. MERS COV patients who were treated with steroid experienced sustained viral replication and even renal replacement therapy. And prolonged use leads to glaucoma, cataract hypotension and weight gain. Although many studies support the use of corticosteroids we need even more evidence studies to explain the benefits of corticosteroids in covid19 [43]. Inhaled corticosteroid for COVID-19 study conducted open FAFELY group suggests that patients with comorbidities like asthma COPD developed worse covid19 clinical outcome with usage of inhaled corticosteroid as harmful. And it was found that there was 50% reduction in ARDS in high risk patients who were using ICS before hospital administration [44].

Interferon

Interferon is considered a choice for treatment in covid-19 due to their antiviral property but they are not yet suggested as a relevant one since studies have been continuing. Interferon's have not shown much bonus in the later phase of infection. It has shown there is no sufficient evidence to prove the same. Also, these drugs have toxic outcome when administered. In this study it was found that administered of inhaled interferon decrease the chance of developing critical condition of the disease. Its effect was also studied after parenteral administration. Deaths were reported but those subjects have not completed the full dose of interferon therapy. Studies were also conducted for combination therapy such as interferon beta -1b along with lopinavir or ribavirin. And it was concluded that interferon beta-1b with or without ribavirin has a good effect then administered within 7 days of hospitalization [45].

Interleukin - 6 Inhibitors

Interleukin 6 is a cytokine that is, associated with inflammation, infection and immune regulation. Cytokine storms syndrome is a manifestation in covid-19 and it is believed that IL-6 has a significant role in this, as per some studies due to the raised plasma levels of IL-6 during this condition. It is not only associated with inflammation and pyrexia, but also with increased chances of ARDS. Thus IL-6 was considered a pro inflammatory molecule. So that inhibition of IL-6 activity leading to reduction of inflammation became a material of study. IL-6 inhibitors (tocilizumab, sirilumab) were under study for the treatment of covid-19. It was tested in patients with low oxygen requirement. Many studies revealed that IL-6 inhibitors have several adverse outcomes. It had increased the risk of infection, bacterial pneumonia, bowel perforation etc. Drugs were also sounds ineffective when given at much later period of disease progression [46]. But many other studies indicated that IL-6 is an anti-inflammatory agent and not a pro inflammatory one. It is at this point that the necessity of much more detailed an elaborate study becomes important and hence these drugs are not yet considered relevant for COVID-19 [47].





Vitamin C

Vitamin C also known as ascorbic acid, is an essential vitamin found in various food sources and sold in the form of dietary supplement. They are necessary for the growth, development and repair of all body tissues. Vitamin C is a nutrient which is involved in many body functions, including collagen formation, absorption of iron, maintenance of cartilage, bones and teeth. It is required for the functioning of several enzymes and is important for the immune system. Vitamin C is having a very important role in responding to respiratory defence mechanisms thereby preventing viral infections. Administration of vitamin C can reduce the severity and duration of pneumonia in elderly patients. They also act as a powerful antioxidant thereby, protecting various bio molecules from oxidative damage and dysfunction. In addition, they are having anti-histamine properties also. Their vital action against respiratory infection makes the interest of supplementing vitamin C in COVID-19. During the outbreak of SARS COV 1 IN 2003, the use of vitamin C was found to be useful for the nonspecific treatment for serious viral respiratory tract infections [48]. Like vitamin D, vitamin C also known to act as a modulatory drug in the innate and adaptive immunity of the body. Vitamin C has beneficial effects in patients with pneumonia especially in children and adults. Also the cytokine storm during COVID 19 infection includes secretion of IL- 6 which is the sudden response triggered by the pro inflammatory cytokines, IL-1 β and TNF $-\alpha$. Vitamin C reduces the levels of these pro inflammatory cytokines and they increase anti-inflammatory cytokines like IL-10. The role of IL-10 is that, they act as negative feedback mechanism with IL- 6 which controls inflammation, crucial in COVID- 19 [49]. Recent trials conducted with patients having sepsis, high dose vitamin C is found to be effective as the cytokine storm observed in COVID- 19 results in increased oxidative stress which can be managed by large dose of anti-oxidants. High dose intravenous vitamin C was also successful in 50 moderates to severe COVID-19 patients in China. Recent NIH expert panel document also mentioned that vitamin C (1.5g/kg body weight) is safe and does not have any major risk factors [50]. Therefore, vitamin C supplementation is a reliable choice of micronutrient for COVID-19 patients having their deficiency. However further investigation is required including additional scientific experiments and well-designed clinical studies.

Vitamin D

Vitamin D is a group of fat soluble secosteroids which are enhancers for intestinal absorption of compounds like calcium, magnesium, phosphate and associated with other biological effects. In humans, Vitamin D is available in the form of vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Humans obtains it through animal food sources in the form of fatty fish such as salmon, mackerel and tuna, and vegetable sources such as assun-exposed yeast, mushrooms and so on. Vitamin D act as a modulatory drug in the innate and adaptive immunity of the body. They fight against the invading pathogens by suppressing cytokine production, thus enhances the innate immune system. They also respond to infections associated with the body in case for decreasing the over-activation of adaptive immune system, thereby involved in the pathogenic load. Recent studies suggest that vitamin D supplementation has been found to be safe and effective in preventing acute respiratory tract infections. The intake of vitamin D is found to increase the gene expression related to anti-oxidation. Hence, they have potential antimicrobial activity and suggested to treat COVID-19 infection. A large population-based nutrition survey was conducted for exploring the correlation between vitamin D levels and occurrence of respiratory tract infections. The result shows that patients with severe vitamin D deficiency is suffering from pneumonia and the admissions to intensive care units and mortality rate were higher .At the same time, patients with sufficient vitamin D levels shown to be positively associated with better outcomes in certain respiratory infection such as mycobacterium tuberculosis and influenza virus infections [51] .Vitamin D in the form of calcitriol exhibits anti-inflammatory effects in acute lung injury and ARDS. They produce these effects through modulating expression of ACE enzymes. Hence SARS-CoV-2 which utilizes Angiotensin converting enzyme as an entry receptor into the lung cells cannot down regulates its expression. Randomized trials have been shown to have protective effects against respiratory tract infections; therefore, people who are at higher risk of vitamin D deficiency should consider taking vitamin D supplements. Administration with daily or weekly modest doses of vitamin D in COVID-19 patients in whom vitamin D levels are deficient may afford some benefit to an extent without causing any adverse effects or risk factors [52].



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CONCLUSION

COVID-19 is a global pandemic caused by SARS-CoV-2 hits most of the countries in the world and affected millions of human populations. The virus became a life threatening one to each and everyone in the earth. Since the vaccine against the disease is yet to be available in the market, the mankind should be aware about the things that may be taken into consideration for improving the immunity and the health measures that to be taken care of. In this critical situation the necessity of administration of drugs which are useful in the disease are very important. The disease may vary from asymptomatic cases, mild symptoms to life-threatening complications such as ARDS, multi organ failure, sepsis, and death. The WHO has also planned a large global trial known as “Solidarity Trial” mainly to generate a robust clinical evidence to combat this pandemic. As there is no specific treatment till date, prevention is the only measure to contain the infection. Even a small negligence in following the preventive measures would be very expensive for the mankind. The famous quote says “United we stand and divided we fall.” Therefore, it is the duty of every citizen of India to abide by the rules and regulations led by our government, let's come together and fight against this pandemic.

Conflict of Interest

The authors have no conflict of interest to declare.

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RESEARCH ARTICLE

To Determine the Efficacy of Percutaneous Trigger Finger Release in Patients with Trigger Finger Visiting a Tertiary Care Center

Faisal Abdul Jabbar¹, Syed Amir Jalil¹, Muhammad Naseem¹, Zeeshan Idrees¹, Abdul Azeem Khan^{2*} and Mohammad Ali Merchant²

¹Department of Orthopedics, Abbasi Shaheed Hospital, Karachi, Pakistan.

²Tabba Heart Institute, Karachi, Pakistan.

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*Address for Correspondence

Abdul Azeem Khan

Tabba Heart Institute,
Karachi, Pakistan.

Email: axeemkhan@hotmail.com



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ABSTRACT

Trigger finger is caused by formation of nodule or thickening of A1 pulley by its fibrocartilage metaplasia resulting in entrapment of the flexor tendon. Conservative treatment of this condition consists of NSAIDs, splint immobilization and steroid injection into the tendon sheath. Failure of the conservative treatment is the indication of an open release. Percutaneous release of trigger finger is advised by several authors. The purpose of this prospective study was to determine the efficacy of percutaneous trigger finger release in patients with trigger finger visiting a tertiary care center. A prospective, observational, case series study carried out in department of Orthopedics Surgery Abbasi Shaheed Hospital, Karachi for duration of six months from 15st April 2016 to 15th October 2016. Patients with diagnosis of trigger finger were enrolled. Detailed history, physical examination and biochemical measurements were recorded. Patients were followed to see for outcome variable i-e efficacy of percutaneous release. Total 315 patients fulfilling the inclusion criteria were included in this study. Some patients with single digit and some with more than single digit involvement, so the total number of involved digits were 475, with no comparable difference in outcome between digits of same individual, of either gender or any selected age). The mean \pm standard deviation age of study population was 42.28 ± 10.961 years. 219 (69.52%) were males and 96 (30.48%) were females. 189 (60%) patients did not have initial treatment while 126 (40%) had initial treatment. On analysis of frequency of outcome variable 265 (84.13%) had efficacy. We recommend percutaneous trigger finger release technique as an effective procedure for releasing trigger.

Keywords: Trigger Finger, Open Release, Percutaneous Release.





INTRODUCTION

Stenosing tenosynovitis (Trigger Finger) is caused by nodule or thickening of flexor tendon, which catches on the proximal edge of first annular pulley (A1) when the finger is actively flexed [1]. Trigger finger is one of the most frequent pathological conditions in hand surgery with incidence rates of 2.2% through the life in adults and up to 10 % in diabetics [2]. Trigger finger is a common finger condition, thought to be caused by inflammation and subsequent narrowing of the A1 pulley. Primary trigger finger occurs up to six times more frequently in women than men and the onset is usually in the middle fifth to sixth decades of life [3,4]. Green developed a classification for trigger finger according to severity of symptoms. In grade I, there is only pain and tenderness at the A1 pulley. In grade II, there is catching of digits. In grade III, there is locking of digits, which is passively correctable. In grade IV, fixed locked digits are present [5]. When conservative treatment (corticosteroid injections and splints) fail, surgical release either with open volar approach or with minimally invasive percutaneous approach is indicated [6]. Even though open release is a simple procedure, follow up series has documented poor results secondary to complications including complex regional pain syndrome, infection, stiffness, nerve injury and dissatisfaction rates as high as 17-26 % [7]. Percutaneous approach has been reported as a safe, effective procedure with clinical efficacy of 98.75 % [6]. It is a quick procedure with significant good results in short term post-operative rehabilitation [8]. It is also possible to use ultrasound-guided percutaneous release to achieve a success rate of 100 % [8]. No major complication has been reported after percutaneous release. The rationale of this study is to determine the efficacy of percutaneous release of trigger finger in terms of improvement in symptoms. Based on literature search, this study would be an initiative as it has been found that minimal research has been done on this topic so far, especially in Pakistan. Favorable results, if found, can encourage its use as a safer, cost-effective and less time consuming procedure.

METHODOLOGY

A descriptive case series study conducted in department of Orthopedics Surgery Abbasi Shaheed Hospital, Karachi for duration of six months from 15th April 2016 to 15th October 2016. Patients in between 20-60 years of age presented with duration of trigger finger with at least two months of history were included in the study while those with co-morbidities or known case of diabetes, Hypertension, Ischemic heart disease, chronic renal disease, Carpal tunnel syndrome being diagnosed on EMG and NCV, Osteoarthritis of hand being diagnosed on clinical or radiographic evidence were excluded from the study. All data was collected and recorded in Performa. Permission for the study was requested from Research evaluation unit, College of Physicians & Surgeons and ethical review committee of the institution (ASH/KMDC), while permission for data collection was taken from the Head of Orthopaedic Surgery, Abbasi Shaheed Hospital, Karachi. Informed and written consent was sought from all participating patients. The respondents were assured of the confidentiality of the information that they provided. OPD follow up was carried at 2nd, 4th and 8th week for assessment of symptoms. Final outcome was assessed at 8th week, when pain and restriction of flexion and extension (catching) was assessed. Efficacy was noted as yes or no, as per operational definition. SPSS version 11.0 was used to analyze the data. It was calculated for quantitative variables, like age, number of digits involved, and duration of trigger finger. Frequency and percentage were calculated for qualitative variables, like gender, grade and efficacy (yes/no). Effect modifiers like, age, gender, duration of trigger finger were controlled through stratification. Chi-square test was applied to see the effect of these on outcome variables and $P < 0.05$ was taken as significant.

RESULTS

Three Hundred and fifteen patients fulfilling the inclusion criteria were included in this study some patients with single digit and some with more than single digit involvement, so the total number of involved digits were 475, with



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no comparable difference in outcome between digits of same individual, of either gender or any selected age). The mean \pm standard deviation age of study population was 42.28 ± 10.96 years. 219(69.52%) were males and 96(30.48%) were females, 189(60%) patients did not have initial treatment while 126(40%) had initial treatment. On analysis of frequency of outcome variable 265 (84.13%) had efficacy (Table 1). Stratification of age, gender, number of digits, initial treatment taken and duration of disease is mentioned in tables 2.

DISCUSSION

Stenosing tenosynovitis, trigger digit, is a condition for which many treatment modalities have been recommended. NSAIDs, splinting and injection of steroid have been advocated by number of authors. These conservative modalities have been successful in 57% to 97% of cases [9-11]. Marks and Gunther in their series of 108 trigger digits found 92% of trigger thumb were cured with single dose of steroid injection and 97% cured after repeated injections [12]. Corticosteroid injection therapy appeared to be more successful in a semi-acute setting and less useful in more advanced cases. The success rate when triggering had been noticed less than a month before diagnosis was higher than in patients who had been symptomatic for 6 months or more (88% vs. 65%) [13]. Other studies demonstrated even lower success rate 55% Newport et al. [14], 45% Rhodes et al., when symptoms had been present for more than 6 months. Failure of these methods has resulted in the need for surgical release of the A1 pulley [15]. Many of the bone associated problems are common among developing countries due to lack of awareness and decreased availability of essential nutrients [16]. Moreover, it has also been found that majority of the health conditions are influenced by gender, working status and age specifically due to health-related quality of life and associated physical disabilities [17,18]. Satisfactory results of 60% to 100 % have been reported after open release with 7% to 28% of complications, such as digital nerve injury, infection, stiffness, weakness, scar tenderness and bowstringing of the flexor tendons [13,19-22]. Percutaneous release of the trigger digit was first described by Lorthior in 1958 using a fine tenotome. He reported good results in all 52 patients with no neurovascular complications. Tanala et al. reported 64.3% excellent, 9.5% good, 8.1% fair and 18.1% poor results following subcutaneous release of 210 trigger digits with a fine scalpel (Bain 1995) [23]. Lyu in 1992 performed a closed tenotomy with a curved knife blade in 16 trigger thumb and had high success rate without damaging the digital nerve [24]. Ha et al. in 2001 used a special blade with a hook end and reported 92% (72 of 79) satisfactory result [25]. Park et al. in 2004 used similar blade with a hook (HAKI knife) for percutaneous release of locked trigger digit with 91% (107 of 118) success result [26]. Jongjirasiri (2007) used 15° full handle knife for percutaneous release of trigger digits with success rate of 92.9% (314 of 334) [27]. Percutaneous division of the A1 pulley using a 21 gauge needle was first reported by Eastwood et al., in 1992 with success rate of 94% in 35 trigger digits [28]. The average distance from A1 pulley to the digital nerves in the thumb is 2.9 mm at the metacarpophalangeal crease [29,30]. This proximity of the digital nerve to the A1 of the thumb has prompted some authors to recommend that this should not be treated by percutaneous release [28-30]. However other authors have safely used percutaneous release technique for trigger thumb [31-35]. Maneerit et al. recommended that in order to prevent digital nerve damage the needle should be held above the tendon in the midline of the thumb and radial approach should be avoided [36]. Secondly, the needle should be inserted a few millimetres distal to the metacarpophalangeal flexion crease. Thirdly, the thumb should be held in full extension during the procedure as this will move the tendon and A1 pulley anterior to the neurovascular bundle. Fourthly, the forearm should be placed in hypersupination to place the palmar surface of thumb in a horizontal plane for good orientation. Several authors [33,37] performed percutaneous release of trigger digits by needle with success rate ranging from 91% to 96%. Cihantimur et al. and Gilberts et al. claimed success rate of 100% with this method of treating trigger digits [32,37]. None of the patients in the above studies had digital nerve injury after percutaneous release of trigger digits, including thumb.

CONCLUSION

We recommend percutaneous trigger finger release technique as an effective procedure for releasing trigger finger.





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Table 1: Analysis of baseline characteristics of study

Variables		Mean
Age		42.28
Number of digits		1.5
Duration of trigger finger (months)		5.7
		%
Gender	Male	69.52
	Female	30.48
Initial treatment	Yes	40
	No	60
Efficacy	Yes	84.13
	No	15.87





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Table 2: Analysis of various factors with efficacy

		Efficacy		p-value
		Yes	No	
Age	<i>Below 40 years</i>	107	19	0.440
	<i>40 years and above</i>	158	31	
Gender	<i>male</i>	188	31	0.138
	<i>female</i>	77	19	
Initial treatment	<i>yes</i>	108	18	0.321
	<i>no</i>	157	32	
Duration of disease	<i>2-6 months</i>	78	16	0.417
	<i>More than 6 months</i>	187	34	

*p-value <0.05 is considered significant





Iron(III) Chelates Derived from Schiff Bases of 4-Benzoyl-3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one: Synthesis and Spectroscopic Investigation

Jatin D. Patel*

Shri Alpesh N. Patel P.G. Institute of Science and Research, Anand, Gujarat, India.

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*Address for Correspondence

Jatin D. Patel

Shri Alpesh N. Patel P.G. Institute of Science and Research,
Anand, Gujarat, India.

Email: drjdpatel@gmail.com



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ABSTRACT

4-benzoyl-3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one (BMMP) and its schiff bases with aniline (A), o-chloroaniline (OCA), m-chloroaniline (MCA), p-chloroaniline (PCA), o-toluidine (OT), m-toluidine (MT), p-toluidine (PT) have been synthesized and well characterized. These schiff bases are used for the synthesis of seven Iron(III)chelates. All the synthesized chelates were well characterized by IR, NMR, elemental analysis, magnetic properties and thermo gravimetric analysis.

Keywords: 4-benzoyl pyrazolones, Iron(III)chelates

INTRODUCTION

The heterocyclic pyrazolone derivatives have found wide applications in various disciplines including catalysis, materials science, bio- and medicinal chemistry. The 1-phenyl-2-pyrazolin-5-one derivatives react with acid chlorides to give substitution at 4th position of the pyrazolone, giving the 4-benzoyl pyrazolones. Large number of schiff bases of 4-benzoyl pyrazolones has been analyzed for their metal complexation study [1-3]. 4-benzoyl pyrazolones have proven as versatile ligands in coordination chemistry as an –ON bidentate ligand. Several reports in the literature on metal complexes of 4-benzoyl pyrazolones for d-block metals are available which display good anticancer, antimalarial, antifungal, antibacterial and catalytic activities [4-6]. To the best of our knowledge, the complexation of schiff bases of 4-benzoyl pyrazolones with Iron metal is not been reported yet. Herein, we report the first synthesis and spectroscopic study of Iron (III) chelates of schiff bases of 4-benzoyl pyrazolones.





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EXPERIMENTAL

Materials

All the chemicals used in the present study were of the best quality. Dioxane was obtained from Merck(india) Ltd., Bombay and used after purification. [7] Absolute alcohol obtained from Baroda Chem. Industry Ltd., Dabhoi, was used after distillation. All the amines were obtained from Sisco-Chem. Pvt. Ltd., Mumbai and used after purification. Calcium hydroxide and acetyl chloride were obtained from Samir Tech. Chem. Pvt. Ltd., Vadodara. Sodium acetate was obtained from Sarabhai M. Chemicals Pvt. Ltd., Baroda. DMF was obtained from Spectrochem Pvt. Ltd., Bombay. All other chemicals used were of reagent grade.

Synthesis of Ligands

The ligands used in the present study were prepared in two steps:

- (i) Synthesis of 4-benzoyl-3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one (BMMP). [8]
- (ii) Synthesis of Schiff bases of BMMP. [9]

Synthesis of 4-benzoyl-3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one (BMMP)

The 3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one (MMP) (0.1 mole, 20.1 g) was placed in a flask equipped with a stirrer, separating funnel and reflux condenser. It was then dissolved in dioxane (80 ml.) by application of heat. To the reaction mixture, calcium hydroxide (0.2 mole, 14.81 g) was added, followed by the dropwise addition of the benzoyl chloride (0.1 mole, 18.01 g). At this stage the mixture became a thick paste and its temperature also increased, as this being an exothermic reaction. The reaction mixture was then refluxed for half an hour. The resulting calcium complex was then decomposed by pouring it into the dilute hydrochloric acid (200 mL, 2M). The resultant coloured crystals were then collected on a Buchner-funnel and recrystallized from an acidified methanol-water mixture (Scheme 1).

Synthesis of Schiff bases of BMMP

An identical procedure has been followed in the preparation of all the Schiff bases used in the present study. They were prepared by refluxing the equimolar quantities of 4-benzoyl-3-methyl-1-(4-methylphenyl)-2-pyrazolin-5-one (BMMP) with aniline (A), o-chloroaniline (OCA), m-chloroaniline (MCA), p-chloroaniline (PCA), o-toluidine (OT), m-toluidine (MT), p-toluidine (PT) in absolute alcohol for 2 hours. The products thus obtained were filtered and recrystallized from ethanol (Scheme 1). The physical properties of ligands are listed in Table 1

Synthesis of the Iron (III) chelates

Ferric nitrate salt was dissolved in a minimum amount of hot ethanol. The hot ligand solution (in DMF) in slight excess over the required metal: ligand ratio, was added dropwise with constant stirring. To the resulting mixture 2 grams of sodium acetate was added and then the mixture was refluxed for 2 hours. The resulting mixture thus obtained was then concentrated to half of its original volume. The product was filtered and washed several times with hot water and finally with hot ethanol. The product was dried at 45 °C (Scheme 2). The yields of the chelates were almost quantitative.

RESULT AND DISCUSSION

All the synthesized Fe(III) Chelates were analyzed by their colour, composition, conductivity and magnetic properties. IR spectra and electronic spectra has been also measured for the synthesized chelates. The conductivity data of the chelates are listed in Tables 3.1. All the chelates have some solubility in DMF, in which the molar conductance has been measured. The molar conductivities of the chelates are found to be lower than the reported range for the 1:1 electrolytes, indicating that they are non-electrolyte in nature.





Magnetic Properties

The room temperature magnetic moments of the chelates are given in Table 2. The magnetic moments of all the Fe (III) chelates are abnormally high having octahedral structure, suggesting their ferromagnetic behaviour. [10-15]

Infrared Spectra

The ligands used in the present study, can take any of the following forms (Fig. 1). [16-21]. The infrared spectra of all the ligands show medium broad band with some structures in the region 3600-3000 cm^{-1} , indicating the presence of strongly hydrogen bonded OH [22-24]. The ligands as well as chelates show absorption in the region 3000-2800 cm^{-1} which may be due to the $\nu_{\text{C-H}}$ [24-26]. The free ν_{OH} is generally found between 3650-3500 cm^{-1} , similar to that of alcoholic ν_{OH} [27-28]. The ν_{OH} of all the ligands show bands in the region 3500-3000 cm^{-1} . This indicates the involvement of a 5-OH group in the intramolecular or intermolecular hydrogen bonding with the π -electrons of the azomethine group and may also correlated with the lone pair of nitrogen [29, 30]. This also suggests that the ligands exist in the enol form (II) in the solid state [31-36]. All the ligands show fairly strong absorption band in the region 1280-1290 cm^{-1} , which may be due to the O-H deformation vibration in hydrogen bonded ring. On coordination this band disappears or its intensity decreases. This may be due to the deprotonation of the 5-OH group of the ligands [37-41]. All the chelates show a characteristic band of intermediate intensity at $\sim 1095 \text{ cm}^{-1}$ which may be assigned to ν_{CO} . [42-44] On coordination this band is shifted towards higher frequency indicating that oxygen of the 5-OH group of the ligands has taken part in the coordination [45-46]. All the ligands show sharp and strong band due to the $\nu_{\text{C=N}}$ of the azomethine group at $\sim 1620 \text{ cm}^{-1}$. The observed low energy shift of this band in the chelates suggests nitrogen coordination [47-48]. All the ligands as well as the chelates show band at $\sim 1595 \text{ cm}^{-1}$ due to $\nu_{\text{C=N(cyclic)}}$. The $\nu_{\text{C=N(cyclic)}}$ is observed at the same energy in all the chelates, indicating the non-participation of the cyclic nitrogen in the coordination [49-50].

Electronic Spectra

The spectra of high-spin octahedral iron (III) complexes have not been adequately characterized [51]. In view of the oxidizing nature of the iron(III) species, charge transfer transition from ligand to metal ion are quite common and these render the interpretation of electronic spectra more difficult. [51-54] However, in the present study, all the chelates show the presence of a very broad band in the region 19000-24000 cm^{-1} (Table 3), which may be a combination of several bands corresponding to the ${}^6\text{A}_1\text{g}$ to ${}^4\text{T}_1\text{g}(\text{G})$, ${}^4\text{A}_1\text{g}(\text{G})$ and ${}^4\text{E}_\text{g}(\text{G})$ transitions. [51,55-57]

Thermo gravimetric analysis (TGA)

The TGA data provide important experimental evidence in determining the number of water molecules present in the chelates. No weight loss at lower temperature suggests the absence of water molecules. This group of the metal chelates is found to be stable up to 200°C without any weight loss. Then they undergo sudden decomposition, which might be due to the loss of the ligands. On the basis of TGA and analytical data of these chelates correspond to the general formula ML_3 group. All the thermograms of the Iron (III) chelates are of this nature. A representative thermogram of Fe (BMMP-OT)₃ is shown in Fig. 2.

${}^1\text{H}$ NMR Spectra

The signals due to methyl protons appeared as singlets in the range δ 1.56 – 2.42 ppm. In some cases, signals of CH_3 groups are overlapped and all of these signals are so closely spaced that it is difficult to assign each signal to a particular methyl group unambiguously. Moreover, such assignment will not contribute to establish the geometry of the ligands, therefore, no attempt has been made for such assignment. In the aromatic region, a few doublets and in few cases, some overlapping doublets/multiplets are observed in the range δ 6.49 - 7.92 ppm. These doublets/multiplets are due to aryl protons of three benzene rings. All the ligands show signal at $\sim \delta$ 12.9 ppm which may be due to the enolic proton (5-OH). The downfield shift of the observed signal may be due to the presence of the strong intramolecular hydrogen bonding. This signal is disappearing on deuterium exchange studies. This indicates and confirms the presence of structure II (Fig. 1) for the ligands.





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CONCLUSION

A novel series of Iron (III) chelates of schiff bases of 4-benzoyl pyrazolones has been synthesized and characterized. The protocol is found to be simple and highly efficient for the preparation of iron (III) chelates using simple synthetic methodology.

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Table 1. Physical Properties of Ligands

Ligands	Melting Point °C	Found (Calculated) Percentage			Yield %
		Carbon	Hydrogen	Nitrogen	
BMMP-A	154	78.89 (78.44)	5.63 (5.76)	11.01 (11.44)	67
BMMP-OCA	144	81.02 (80.10)	5.45 (5.60)	11.51 (11.68)	65
BMMP-MCA	153	79.66 (80.10)	5.31 (5.60)	11.44 (11.68)	64
BMMP-PCA	161	80.03 (80.10)	5.87 (5.60)	11.74 (11.68)	65
BMMP-OT	165	77.99 (78.70)	6.01 (6.07)	11.10 (11.02)	69
BMMP-MT	173	77.93 (78.70)	6.87 (6.07)	11.35 (11.02)	68
BMMP-PT	184	78.61 (78.70)	6.38 (6.07)	11.37 (11.02)	68





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Table 2. Colour, Composition, Conductivity and Magnetic Data of Fe(III) Chelates

Chelate	Colour	Found (calculated) percentage				Λ^a_M	μ_{eff} . B.M.
		Metal	C	H	N		
[Fe(BMMP-A) ₃]	Brown	4.76 (4.80)	74.59 (74.46)	5.51 (5.73)	10.73 (10.85)	3.71	Ferromagnetic
[Fe(BMMP-OCA) ₃]	Yellowish brown	4.48 (4.57)	70.92 (70.85)	5.11 (5.20)	10.29 (10.33)	3.21	Ferromagnetic
[Fe(BMMP-MCA) ₃]	Yellowish brown	4.48 (4.57)	70.65 (70.85)	5.09 (5.20)	10.37 (10.33)	7.17	Ferromagnetic
[Fe(BMMP-PCA) ₃]	Yellowish brown	4.52 (4.57)	70.78 (70.85)	5.11 (5.20)	10.41 (10.33)	3.79	Ferromagnetic
[Fe(BMMP-OT) ₃]	Blackish brown	4.59 (4.64)	75.01 (74.85)	6.18 (6.03)	10.38 (10.47)	7.13	Ferromagnetic
[Fe(BMMP-MT) ₃]	Blackish brown	4.66 (4.64)	74.66 (74.85)	6.09 (6.03)	10.44 (10.47)	4.15	Ferromagnetic
[Fe(BMMP-PT) ₃]	Blackish brown	4.67 (4.64)	74.81 (74.85)	6.51 (6.03)	10.47 (10.47)	3.09	Ferromagnetic

a = Ohm⁻¹.cm².mole⁻¹ in DMFTable 3. Electronic Spectral Data of Fe(III) Chelates (cm⁻¹)

Chelate	Observed transition energies
[Fe(BMMP-A) ₃]	23752
[Fe(BMMP-OCA) ₃]	19230
[Fe(BMMP-MCA) ₃]	23753
[Fe(BMMP-PCA) ₃]	24691
[Fe(BMMP-OT) ₃]	21929
[Fe(BMMP-MT) ₃]	22222
[Fe(BMMP-PT) ₃]	23696

Table 4. ¹H NMR Spectral data of ligands

Ligands	Methyl protons (δ ppm)	Aryl protons (δ ppm)	-OH ⁺ (δ ppm)
BMMP-A	---[1.56, 2.34]--- Singlet 6H	---[7.08 - 7.90]--- Multiplet 14H	12.98 Singlet 1H
BMMP-OCA	---[1.59, 2.35]--- Singlet 6H	---[6.61 - 7.91]--- Multiplet 13H	12.84 Singlet 1H
BMMP-MCA	---[1.57, 2.36]--- Singlet 6H	---[6.70 - 7.89]--- Multiplet 13H	12.99 Singlet 1H
BMMP-PCA	---[1.58, 2.36]--- Singlet 6H	---[6.65 - 7.89]--- Multiplet 13H	12.99 Singlet 1H

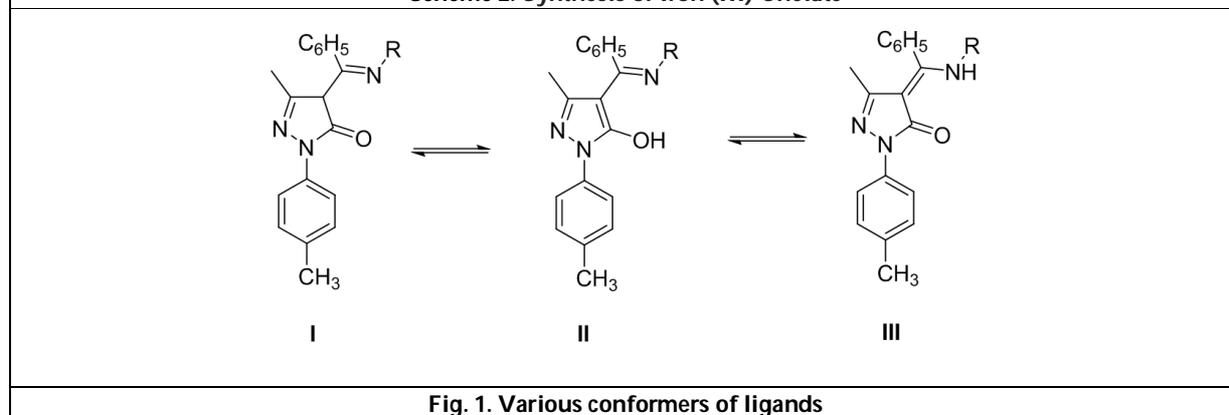
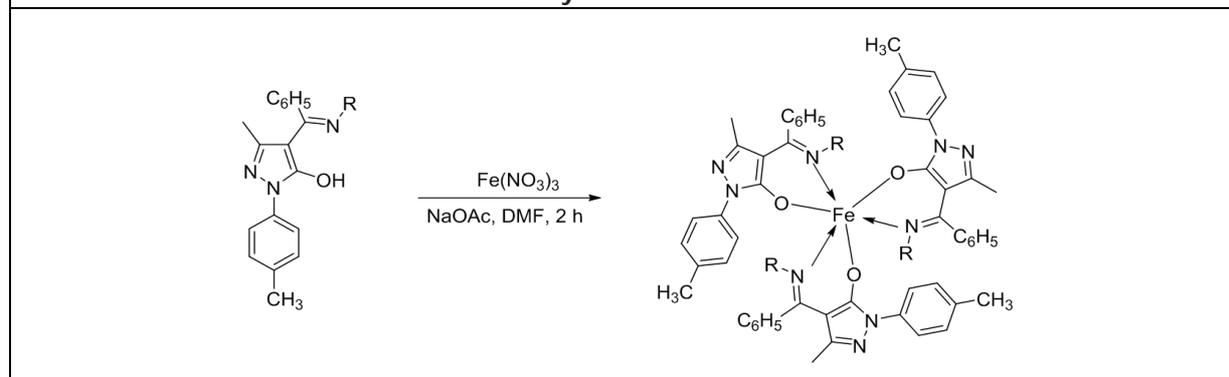
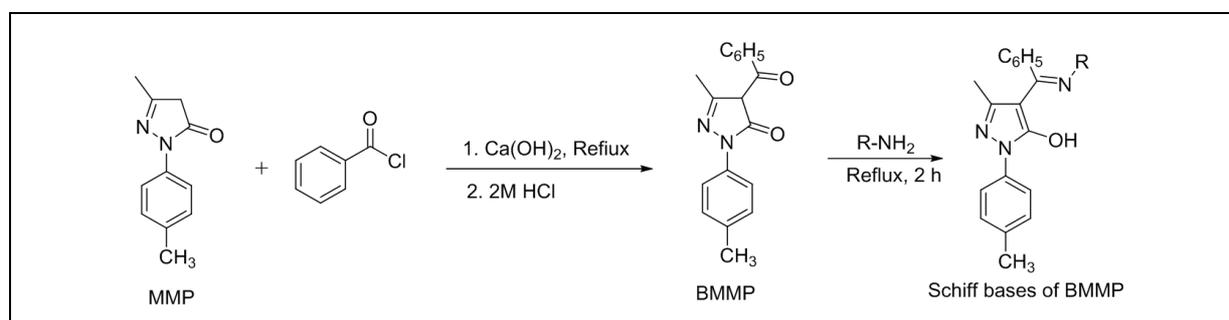




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BMMP-OT	---[1.58, 2.34, 2.42]--- Singlet 9H	---[6.56 - 7.39]--- Multiplet 13H	12.83 Singlet 1H
BMMP-MT	---[1.56, 2.15, 2.34]--- Singlet 9H	---[6.49 - 7.92]--- Multiplet 13H	12.98 Singlet 1H
BMMP-PT	---[1.56, 2.21, 2.34]--- Singlet 9H	---[6.67 - 7.91]--- Multiplet 13H	12.93 Singlet 1H

*Signal disappeared in CDCl₃ + D₂O





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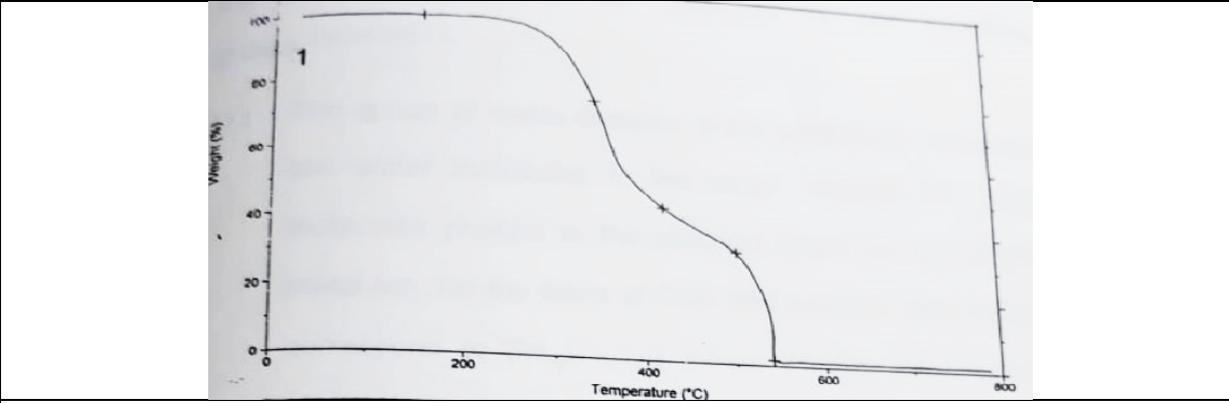


Fig. 2. TGA of Fe(BMMP-OT)₃





Recent Advances and Modern Methods of Medicinal Plant Analysis

Sobhan Babu^{1*} and Kumudhavalli²

¹Research Scholar, Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu, India.

²Department of Pharmaceutical Chemistry, Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu, India.

Received: 03 Sep 2020

Revised: 05 Oct 2020

Accepted: 09 Nov 2020

*Address for Correspondence

Sobhan Babu

Research Scholar,

Vinayaka Mission's College of Pharmacy,
Salem, Tamil Nadu, India.

Email: drjdpatel@gmail.com



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ABSTRACT

The analysis of medicinal plants has had a long history, and especially with regard to assessing a plant's quality. The first techniques were organoleptic using the physical senses of taste, smell, and appearance. Then gradually these led on to more advanced instrumental techniques particularly the development of chromatographic and spectroscopic methods and the hyphenation of these techniques like HPLC, TLC, Mass spectroscopy, DNA Barcoding Techniques, The DNA barcoding, DNA molecular markers, Metabolomics are the stepping stones towards quality standardization. Different branches of science like cell biology, organic chemistry, bioinformatics, statistics and many more will help to establish the efficacy of the herbal medicinal plant.

Keywords: Medicinal plants, analytical techniques, HPTLC, DNA bar coding method

INTRODUCTION

Medicinal plants have been a resource for healing in local communities around the world for thousands of years. The phytochemicals rich in plants have shown to be beneficial for prevention of diseases as well as long-term health. Plants are generally consumed as sources of essential compounds such as saccharides, coumarins, lignans, flavonoids, terpenoids, and steroids¹. The separation of phytochemicals is a process of isolating the constituents of plant extracts or effective parts one by one and purifying them into monomer compounds by physical and chemical methods. Classical isolation methods, including solvent extraction, precipitation, crystallization, fractional distillation, salting-out, and dialysis, are still used commonly at present. On the other hand, modern separation technologies such as column chromatography, high performance liquid chromatography, ultrafiltration, and high performance liquid drop countercurrent chromatography also play an important role in the separation of

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phytochemicals. Chromatographic techniques are characterized by high separation power; plant material generally needs extensive sample treatment before it can be injected into GC for final analysis.² Medicinal plants are widely used, nowadays, for the preparation of various pharmaceutical forms, or as food additives; therefore, the research focuses on validated methods of analysis by single or hyphenated HPLC. According to a report of world health organization (WHO)[1], 70% of the world population uses medicinal plants to cure diseases through their traditional practitioners. India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. In Indian subcontinent, plant oriented drugs have been used extensively from a very long time. According to a survey conducted by WHO, traditional healers treat 65% patients in Sri Lanka, 60% in Indonesia, 75% in Nepal, 85% in Myanmar, 60% in Pakistan, and 90% in Bangladesh. In India, 80% of the population especially in villages is getting health care by traditional practitioners (Hakims) who prescribe herbal preparations [3]. Modern strategies for drug discovery emphasize on availability of some simple and inexpensive biological assays to evaluate medicinal potential of plant species. So in our present study various plants used by traditional healers were analyzed for their medicinal value. Medicinal plants contain active chemical constituents in any of their parts like root, stem, leaves, bark, fruit and seeds. These compounds either act on different systems of animals including man and act through interfering in the metabolism of microbes infecting them. In either way the bioactive compounds from medicinal plants play a determining role in regulating host microbe interaction in favors of the host.

Sample preparation for final analysis

Plant material is a very complex matrix composed of a great variety of organic compounds. Sample preparation, even for chromatographic techniques of high separation power, is a difficult process composed of many operations. The basic operations include (not necessarily all are applied in a given analysis) prewashing, drying plant material or freeze drying, grinding which is aimed at obtaining a homogenous sample and often improving the kinetics of analyte extraction.

Thin Layer Chromatography

This technique is used for the detection, identification, quantification of the herbal drug phyto-constituents. HPTLC is a sophisticated form of TLC. But still TLC is a crude method. This is used for preliminary drug analysis and helps to identify the solvent system which gives the maximum separation. Merits-The main advantage of this process is its simplicity, adaptability, simple sample preparation, and sensitivity of the test. This test can be performed on the crude extracts of the plant drugs to estimate the phytochemicals present in the drug.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC is a more sensitive method of separation of the herbal drugs which uses less amount of sample and solvent but gives maximum, sharp and better separations of compound. This technique can also be used for stability studies and pharmaceutical testing. This technique complies with Good Lab Practices (GLP) and Good Analytical Laboratory Practices (GALP). The newer techniques used concomitantly are Forced Flow Planar Chromatography (FFPC) Rotation Planar Chromatography (RPC), Over-Pressured Layer Chromatography (OPLC), And Electro Planar Chromatography (EPC). Merits-Detection of adulterants in small amount of sample plant drugs can be done. It separates at a high speed and different samples can be analyzed concurrently. It may be a bit expensive but sample required is less, and accuracy of results are greater than TLC. High Pressure Liquid Chromatography(HPLC)- It is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50 μ m) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption. It is the most extensive application in the analysis of Herbal medicines.





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Mass Spectroscopy (MS)

Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecule. Combining chromatography with mass spectrometry provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method.

Differential Scanning Calorimeter (DSC)

DSC is a thermal analysis apparatus measuring how physical properties of a sample change, along with temperature against time. It has many applications including the analysis of proteins, carbohydrates and nucleic acids.

Capillary Electrophoresis (CE)

It is versatile and powerful separation tool with high separation efficiency and selectivity when analyzing mixtures of low-molecular- mass components. However, the fast development in capillary electrophoresis.

DNA Barcoding Techniques

The accurate identification of medicinal plants in relation to their purity and quality as well as safe application has become increasingly important. DNA barcoding is an established technique that uses the sequence diversity in short, standard DNA regions for species level identification. It is primarily used to identify known species by comparing their unique barcode sequences to reference sequences in public databases, as well as to facilitate species discovery. DNA barcoding provides a more rapid, subjective and accurate identification compared with traditional methods.

CONCLUSION

To develop and instrumental technology advances, it is clear that we will be able to delve further and further into the chemical composition of medicinal plants and develop more advanced techniques for the detection and quantification of adulterants and contaminants. However, it should be considered that although these technological advances give us this opportunity, more traditional organoleptic analysis also provides us with essential sensory information regarding medicinal plant quality. The various analytical methods multiple applications. The need of study should be clear in the mind of the researcher to choose the correct analytical method. The recent advances in each technique will provide a finer and detailed information about the medicinal plant, its active principle or its extract. This information will certainly benefit to identify, authenticate and give qualitative as well as quantitative information of the plant.

Future

The next steps in analytical advancement in combination with technological improvements will most likely occur in the realm of artificial intelligence. Analytical approach can only provide a partial perspective on complex multicomponent preparations. So future improvements in this area may not entirely on developing ever more complex analytical techniques, but in implementing best practice throughout all stages of the production and supply of herbal medicines.

Conflict of Interest:

Nil



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Iradina Simulation of Alkali-Ion Having Energy 200 keV on Vanadium Nanoparticles

Satyanarayan Dhal^{1*} and Narayan Gouda¹

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Satyanarayan Dhal

Centurion University of Technology and Management, Odisha, India.

Email: satyanarayan.dhal@cutm.ac.in



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ABSTRACT

We have utilized iradina simulation, a GUI package to realize the impact of 200 keV sodium ion impact on vanadium nanoparticles as the target atoms and the production of vacancies, displaced atoms, and replacement collisions. This simulation data is certainly supportive to elucidate the phenomena that occurred after the impacts with energetic alkali ions like sodium before the experimental works. We have simulated for the transport 10^7 ions through a single cell of vanadium nanoparticle and then added up to draw the conclusions regarding defects produced. Atomic displacements occurred 2000 on a single cell per single ion incidence whereas the vacancies found to be 1500 and the replacement collisions remained 500.

Keywords: iradina, Vanadium Nanoparticles, Monte-Carlo Simulation

INTRODUCTION

Irradiation induced impact on nanomaterials has been considered is a significant research area. Nanomaterials owing to their large surface area have gained always prime importance for various industrialists. Vanadium nanoparticles are highly effective materials for numerous industrial applications. Several works have been already accomplished by various scientists especially in the nuclear research field worldwide. For instance, D. Braski [1] reported the impact of neutron irradiation on vanadium alloys and the hardening in the materials after the irradiation has been studied and compared with stainless steel. Even Helium ion had also been bombarded on vanadium to produce bubble and blister on the surfaces for varying the target temperatures [2]. H. Watanabe studied the impact of alteration of temperature on pristine vanadium and their alloys because of the bombardment of neutrons in high flux isotope reactor [3]. Apart from bulk, vanadium oxide nanotubes also have been irradiated with a laser to visualize the variation in the microstructure where the tubes have been converted into nanoparticles [4]. In other words, experimentally several works have been carried away. But a Monte-Carlo simulation should be accomplished before the experiments. Here, we have reported the monte Carlo simulation on vanadium nanoparticles using 200 keV sodium ions using iradina. In one of our earlier work, we have accomplished this work using IM3D [5].

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Simulation Details

Iradina is grounded on the binary collision approximation that is designated by a 3D rectangular network, from which we can create arbitrary target geometries. Iradina is open-source and licensed [6]. It is having a GUI version. Iradina displays a three-dimensional target structure inside the rectangular network of a large number of cells. It can provide the output data regarding the displaced atoms from the target due to the bombardment made by incident ions. Apart from that it also displays the vacancies database, implanted, and replacement collisions which are very highly essential to understand the bombardment induced modifications in the nanomaterials. Here we have reserved vanadium nanoparticle as the target body and simulated using 200 keV sodium ions [7]. We have considered the diameter of a single cell of vanadium as 80 nm. This simulation provides the number of displaced atoms of vanadium nanoparticles after the impact of sodium ions. These displaced atoms after the collision cascade reach different spaces inside the target material. Some of them create a vacant position in the lattice forming vacancies. Some of These displaced atoms may replace the vacant positions designated as replacement collisions. In other words, the displaced atoms are the summation of vacancies produces and replacement collisions. We have verified this data in this simulation process.

RESULTS AND DISCUSSION

Iradina provides precise simulation than SRIM regarding the implantation of matter. Iradina supports flexible 3D target definitions which is appropriate for irradiation of nanostructures [7]. We have simulated for the transport 10^7 ions through a single cell of vanadium nanoparticle, and consequently, the number of immobile ions after they are being stopped is added up for each cell. This, in turn, can create detailed information about the number of Frenkel-pairs and other defects shaped. This code also aids to display the cross-sectional spreading of ions that are implanted (not shown here) and the corresponding damage present in the nanomaterial. The image in fig.1. shows the average vacancies (shown in light blue color) produced in one single cell of vanadium nanoparticle is about 1.5×10^8 . We have simulated for 100000 ions. Hence, the vacancies produced per single ion is 1500. The arrow key in the image indicates the direction of incidence of the ions. The right side of the fig.1.1. shows the actual image drawn from the simulation package whereas the left side of the image has been drawn using a graphical software namely the gnu plot [8]. The image in fig.2. shows the average displacements (shown in dark green color) produced in 1 single cell of vanadium nanoparticle is about 2×10^8 . Therefore, the displaced vanadium atoms produced per single ion is about 2000. The image in fig.3. revealed the average replacement collisions (white dots) produced in one single cell of vanadium nanoparticle is about 0.5×10^8 . Therefore, the replaced vanadium atoms produced per single ion is about 500. Therefore, our previous assumptions which were based on the theoretical calculation used in SRIM [9-21] is proven correct.

CONCLUSIONS

We have simulated the irradiation of 200 keV sodium ions on a vanadium target using iradina and verified the theoretical assumption regarding vacancies, displaced atoms, and replacement collisions. We have obtained that the target displacements due to the summation of vacancies i.e. 1.5×10^8 and the replacement collisions 0.5×10^8 .

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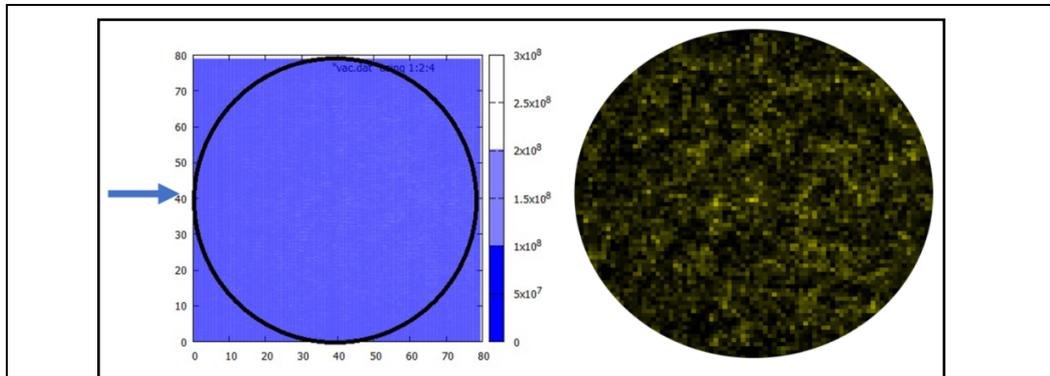


Figure 1. Vacancies distribution of 200 keV sodium ion on Vanadium using IRADINA.

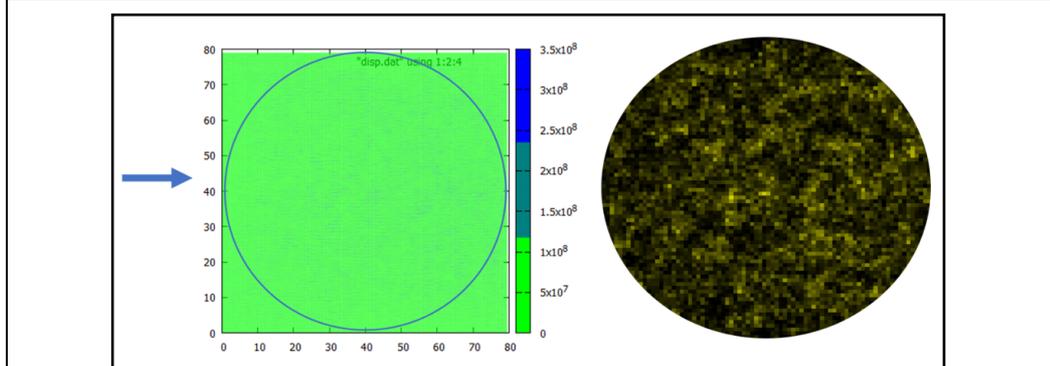


Figure 2. Atomic displacements occurred in the Vanadium because of 200 keV sodium ion incidence.

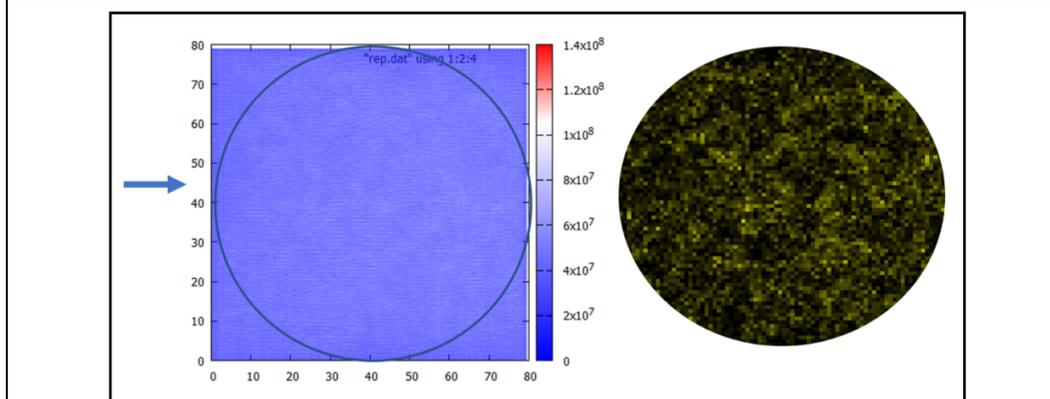


Figure 3. Replacement collisions occurred in the Vanadium because of 200 keV sodium ion incidence.





***In silico* Analysis of Gas Permeability Properties of Polyvinylchloride and Poly Methyl Methacrylate Composite Blend**

Rachayita Pradhan¹, Prasant Behera², Bijaya Kumar Singh³ and Arun Kumar Pradhan^{1*}

¹Department of Chemistry, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

²Department of Chemistry, Govt. Polytechnic, Mayurbhanj, Odisha, India.

³Department of Chemistry, Aska Science College, Aska, Ganjam, Odisha, India.

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***Address for Correspondence**

Arun Kumar Pradhan

Department of Chemistry,
School of Applied Sciences,
Centurion University of Technology and Management,
Odisha, India.

Email: arunkumar.pradhan@cutm.ac.in



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ABSTRACT

A blend may be a mixture of quite one components. The specified property of a mix is its homogeneity. The composition of polyvinylchloride and poly methyl methacrylate to supply desired different gas permeability properties of the blend was explored using Biovia Materials Studio. The composition of the blend was analyzed with reference to permeability properties. The molar volume decreased with increase in polyvinylchloride fraction and density in crises with increase in polyvinylchloride fraction. The permeability properties of the composite were studied supported permeability of oxygen, nitrogen and CO₂. The results showed that the permeability for all the gases decreased with increase in mass fraction of polyvinylchloride. This study will help determine pairs without performing laboratory experiments saving materials, money and time.

Keywords: Blend, Molar volume, Density, Permeability of gases.

INTRODUCTION

Blends or composites are materials containing quite one components. The components don't lose their identity within the mixture. They combine and contribute to the property of the blend thereby improving the standard of the fabric. Development of one material with the specified property involves significant research and time. a mix utilizes the benefits of various materials, mix them to urge the specified property. Thus a mix saves time to develop a replacement material thereby reducing the value of development of products with desired

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properties. Polymer blends are often made from two or more polymers or fibres and polymer, or particles and polymer. Nano material modified polymers paved the thanks to multi functional materials. Polymers including carbon based (graphene, carbon nano-tube) nano-materials [1] have drawn attention. Biodegradable polymers- natural fibre composites [2] are reported to reinforce mechanical properties and water resistance. Researchers are performing on fire retardant/fire proof materials [3]. There are reports of inorganic additives in polypropylene; which will enhance flame retardancy without increasing the load [4]. Researchers have emphasized on synthesis and production of lightweight composite materials having high strength important for enhancing fuel efficiency within the field of transportation [5]. There are applications of composites in structural Engineering thanks to high strength to weight ratio and resistance to corrosion. Thus, optical fiber reinforced polymers, latex polymer cementitious composites [6] were developed for construction of bridges, light rail transit, mining and tunneling, retaining walls and other waterside buildings. All the above mentioned examples relied on laboratory experiments. Usually blends are prepared by trial and error method. Thus it involves wastage of materials, time and money. Thus, researchers have focused on the utilization of in silico approach to develop new blends. Software (Materials Studio [7]) are wont to identify compatible pairs. Acrylic polymers have a good sort of applications in dentistry as denture bases, artificial teeth, denture repair materials, maxillofacial appliances for skeletal defects etc. [8], bone repair [9], dispersing agent, superabsorbent polymer, ion-exchange resin, etc.,[10].

MATERIALS AND METHODS

Software used

Materials studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques and standard algorithms to predict the extent of interaction.

Methodology

The structures of polyvinylchloride were fed to the synthia menu of Materials Studio. it had been then run different weight fractions of the components. Different properties of the composite were displayed during a tabular form. The values were wont to plot graphs to spot the effect of weight fraction of polyvinylchloride on the mechanical properties of the composite.

RESULTS AND DISCUSSION

In this work the utilization of of polyvinylchloride and polymethyl methacrylate as potential components of a composite was analyzed using Biovia Materials Studio. BIOVIA Materials Studio Synthia uses pre-defined correlations (advanced quantitative structure-property relationships). Group additive methods were used for several years to predict the properties of polymers also as small molecules. These methods are extremely fast and straight forward to use. Consequently, they're of greatest utility when a rapid estimate of a property is required without an in depth understanding of the atomistic interactions that produce there too. However, the principal shortcoming of those methods is their dependence upon a database of group contributions. Thus, if a polymer contains a gaggle that the group contribution can't be estimated, then the property of that polymer can't be calculated. To overcome this limitation, the tactic implemented in Synthia uses topological information about polymers within the predictive correlations. The connectivity indices derived from graph theory are employed. Thus, no database of group contributions is required and properties could also be predicted for any polymer composed of any combination of the subsequent nine elements: carbon, hydrogen, nitrogen, oxygen, silicon, sulfur, fluorine, chlorine, bromine.



**Rachayita Pradhan et al.,****Molar volume**

It is the volume occupied by one mole of a substance. Fig. 1 shows that the molar volume of the composite decreases linearly with increase in mass fraction of polyvinylchloride. Increase in density indicates decrease in porosity. A higher porosity will enhance the surface area making it suitable for absorption/adsorption applications. Fig.2 shows that the density of the composite decreases linearly with increase in mass fraction of polyvinylchloride

Permeability of gases**Permeability of gas of oxygen**

Permeability is the rate at which the gas can pass through the polymer membrane after the gas has come to equilibrium. Lower permeability indicates longer time lag for the gas to pass through the membrane. Fig.3 shows that the permeability of oxygen through the composite decreases with increase in mass fraction of polyvinylchloride

Permeability gas of nitrogen

In Fig.4 shows that the permeability of nitrogen through the composite decreases with increase in mass fraction of polyvinylchloride.

Permeability gas of carbondioxide

In Fig.5 shows that the permeability of carbon dioxide through the composite decreases with increase in mass fraction of polyvinylchloride. Thus the results indicated that an increase in of polyvinylchloride fraction reduces the permeability of different gases. The rate of permeability might be influenced by the molecular weight of the gases.

CONCLUSIONS

The possibility of use of polyvinylchloride to make a homogeneous blend was explored using Biovia Materials Studio. The composition of the blend was analyzed with reference to permeability properties. The molar volume decreased with increase in polyvinylchloride fraction. The permeability properties of the composite were studied supported permeability of oxygen, nitrogen and CO₂. The results indicated that the permeability for all the gases decreased with increase in mass fraction of polyvinylchloride usually components for a mix are identified experimentally. This *in silico* study will help determine components of a mix without performing laboratory experiments saving materials, money and time.

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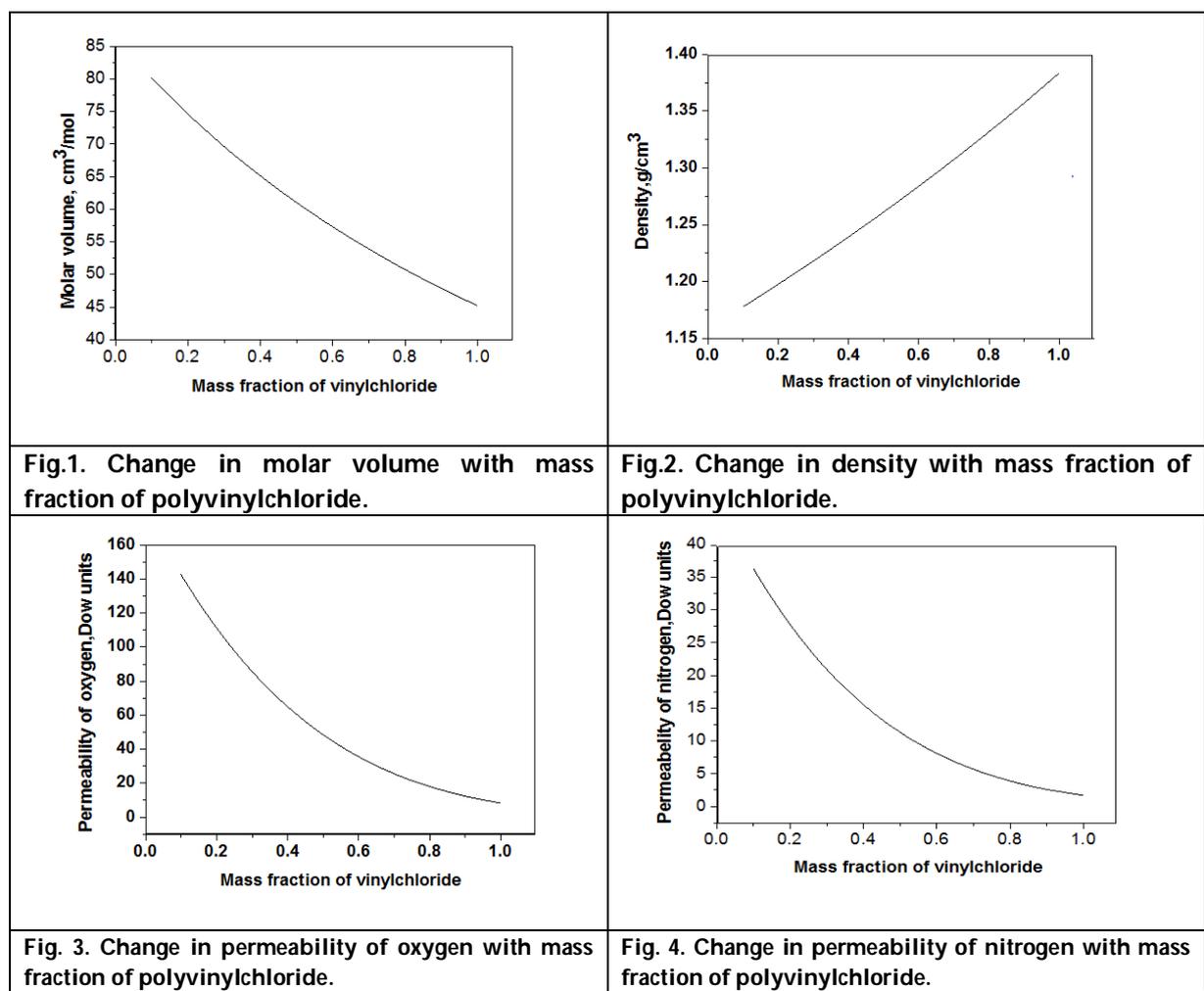
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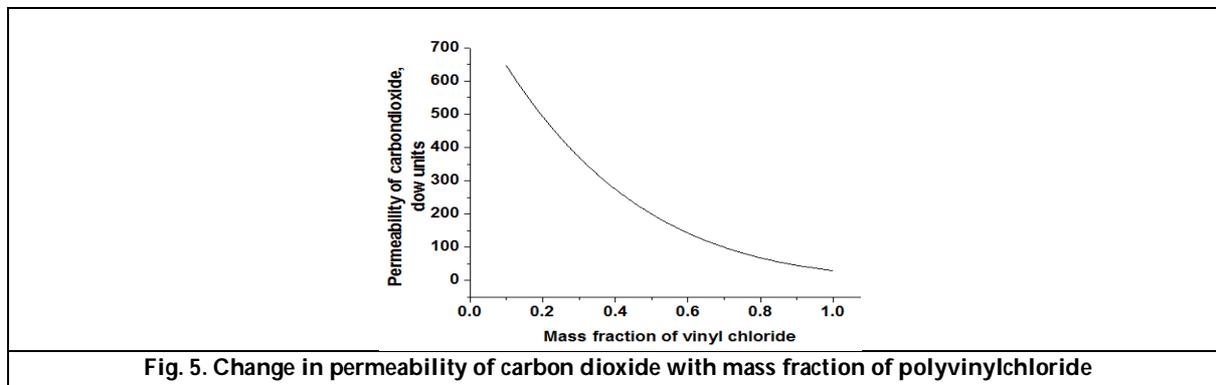
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Phylogenetic Analysis of Five *Garcinia* Species from Assam, India, by ITS1 Sequences Data

Nabajyoti Gogoi^{1*}, Ankur Gogoi¹, Bijoy Neog² and Dibyojyoti Baruah²

¹Centre for Biotechnology and Bioinformatics, Dibrugarh University, Assam, India.

²Plant Molecular Biology Laboratory, Department of Life Sciences, Dibrugarh University, Assam, India.

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*Address for Correspondence

Nabajyoti Gogoi

Centre for Biotechnology and Bioinformatics,
Dibrugarh University,
Assam, India.

Email : nabajyotigogoi@dibru.ac.in



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ABSTRACT

Garcinia is well known for their edible fruit and a number of species from the wild have been selected and cultivated not just for their fruits but also for other uses such as vegetables and traditional medicine. Phylogenetic relationships of five wild species of *Garcinia* were determined using the sequence of the internal transcribed spacer region1 (ITS1) and also standardized the isolation of DNA from this genus. The 4X CTAB, 3% PVP in lyses buffer with higher concentration of NaCl was found to be more efficient for the extraction of pure and high amount of DNA. The multiple sequence alignment and phylogenetic analysis revealed that *G. xanthochymus* and *G. dulcis* are closet species compare to the other species. However, the alternation of DNA base pairs in many position of ITS1 regions also detected among the species. This study is the first-time report of molecular identification of five *Garcinia* species and a landmark towards the future exploration of this genus biodiversity of Assam, India.

Keywords: CTAB, DNA-Barcode, *Garcinia*, ITS1

INTRODUCTION

DNA barcoding is an advanced technique, which provides rapid identification of species without considering the morphological trait. Usually, in Barcoding a relatively small DNA fragment is used as a tag, to identify, define or discover a species. In DNA barcoding minor differences of nucleotides in the particular gene loci of different organisms are targeted for identification and separation. Later, the gene is sequenced to determine the base-pair position and differences and then it is submitted in the barcode database. These nucleotide sequences could be retrieved through the digital libraries like NCBI and similar species could search through the BLAST algorithms and;

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hence, an unknown species can be identified by scientist around the World. An ideal DNA barcode should normally be distributed in short sequence of DNA (400-800 bp) among the population (the genus or species), which able to be simply amplified by PCR and easily to characterized [1]. The internal transcribed spacer (ITS) region belonging to the nuclear genome is a non-functional RNA sequence located between 18S and 25S rRNA coding regions. The ITS1 is present between 18S and 5.8S rRNA and ITS2 is present between 5.8S and 25S rRNA [2]. However, the genus *Garcinia* Linn. belongs to Clusiaceae (Guttiferae) family (Plantae Guttiferae, latex bearing plants) popularly known as mangosteen plant, the largest genus of this family [3]. They are an evergreen glabrous tree or shrubs usually dioecious, some are monoecious and most of them are growing in evergreen and semi-evergreen forest with a relatively wild monsoon [4,5]. The genus, popularly called Thekera (in Assamese), has been utilized as a medicine to cure various ailments, such as diabetes, jaundice, anti-obese drug and liver diseases [6]. Earlier, renowned taxonomist Hooker [7] and Kanjilal [8] had reported few species of Assam, but controversial addressing regarding the synonyms has been reported in the course of time. The present investigation was designed to analyze the phylogenetic relationship and genetic distance among the five *Garcinia* L. of Upper Assam, using DNA-barcode.

MATERIALS AND METHODS

Genetic material collection

Five genotypes of *Garcinia* L. were collected from the Natural habit and Homestead garden. The latitude and longitude of the genotypes were recorded in 60CSx, Garmin system (Table 1).

Chemicals and Reagents

For the DNA isolation and PCR reactions, chemicals were obtained from the SRL, Merck, Hi-media and Sigma Aldrich. The primers were synthesized in the Eurofins Scientific, Pvt Ltd, Bangalore, India. The chemicals and buffer were used in the DNA isolation were prepared in de-ionized Millipore water.

Isolation of DNA: The preserved fresh leaves were used to isolate the DNA. DNA was isolated from the leaves according to Doyle and Doyle [9] methods with slight modification. The protocol was standardized by changing the concentration of CTAB and other chemical composition in extraction buffer.

Quantification of DNA

The yield of DNA per gram of leaf tissue extracted was measured using a UV Spectrophotometer (Eppendorf BioSpectrometer basic) at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. The DNA concentration and purity were also determined by running the samples on 0.8% agarose gel, based on the intensities of the band when compared with the Lambda DNA marker (DNA ladder). The nucleic acid concentration was calculated following the procedure of Sambrook et al. [10]. The obtaining DNA samples were diluted by serial dilution methods with nuclease-free water as a dissolved solution and make the concentration 40-50ng for the PCR amplification.

PCR reaction for ITS-1 region amplification

ITS-1(5.8S, partial sequence) regions of five species were amplified by polymerase chain reaction (PCR). Primers were designed using an online tool Prime3 (<http://bioinfo.ut.ee/primer3-0.4.0/>). The primers used for the ITS1 region amplification were shown in Table 2. The PCR reaction (for ITS) were performed in a total volume of 20µL in 0.2ml PCR tube, containing 10X PCR buffer without MgCl²⁺, 25mmMgCl²⁺, 50ng/µL Template (DNA sample), 10 pmol/µL primer, 500U Taq-polymerase, 10mm dNTP and Nuclease-free water. Volumes of each component were shown in Table 3.



**Nabajyoti Gogoi et al.,****Thermo profiling of PCR**

The PCR thermocycling profiling consists of one initial denaturation cycle 94°C for 5 minutes, followed by 36 cycles of 30 second 94°C, annealing temperature 45 seconds at 46-60°C (depending on primer), 1 minute at 72°C for extension cycle, and final extension cycle was 10 minutes at 72°C, then reaction was held at the 4°C until the electrophoresis analysis.

Molecular identification by sequencing the ITS1 regions

The amplified ITS1 regions of each species were quantified by Agarose gel electrophoresis and the pure products were sent to the Eurofins Scientific, Pvt Ltd, Bangalore, India, for the DNA sequencing. The sequencing was done by Sanger's principles, and the Fasta format was used for further analysis.

Consensus sequence and BLAST analysis

The both forward and reverse sequence of each species were further used for creating the Consensus sequence with the Codon Code Aligner (version: 8.02; Centerville) software. Then the consensus sequences were used for the search of similar sequence by BLAST algorithm in NCBI and submitted to the NCBI-GeneBank Database [11].

Phylogenetic analysis

The similar sequences were found in BLAST analysis downloaded in Fasta format then aligned with ClustalW, the phylogenetic analysis was conducted for each species individually and together using the Maximum likelihood method. MEGA (Version.6, Developer: The Pennsylvania State University) software was used for the experiment.

RESULTS AND DISCUSSION

Genomic DNA isolation and Quantification

It was observed that after applying the different concentration of CTAB, the high amounts of DNA were extracted in the 4X CTAB buffer only. Other modifications like using the high RPM in configuration, increasing the PVB concentration, using more amounts of NaCl, etc. helped to access the high amount of DNA. The isolated DNA was subjected to the Agarose (0.8% w/v) gel electrophoresis to determined the size of the genomic DNA and check the purity. The RNA contaminated DNA was treated with the RNase to remove the RNA. The isolation of pure, intact, and high-quality DNA is very crucial for any molecular studies [12]. However, DNA isolation from plants is usually a challenging task due to the presence of polysaccharide and secondary metabolites like phenol, flavonoids, tannin, saponin, etc. We have already reported that the *Garcinia* spp. contains a high amount of polyphenols [13]. Therefore it was necessary to remove the all polyphenols to access the pure quality of DNA. In other words, DNA isolation methods need to be adjusted to each plant to plant and even to each plant tissue because of the presence of these metabolites in different quantity [14]. The different parameter of chemicals used in the DNA isolation needs to be standardized sometimes too isolated the high-quality DNA, in spite of following the conventional Doyle and Doyle methods. However, the CTAB and PVP concentration are the most important chemicals for the lysis buffer to be standardized to access the high quality of plant DNA [15].

The addition of NaCl at higher concentrations than 0.5M, along with CTAB, is known to remove polysaccharides during DNA extraction [16, 17]. The concentration of NaCl varied with plant species in a range between 0.7M [18] to 6M [19]. We standardized the protocol by increasing the concentration of CTAB, PVB, and NaCl to isolate the high amount of DNA. The present investigation revealed that 4X CTAB with 3% PVP and 2M of NaCl in lysis buffer is the effective concentration to isolate the high amount of DNA (Fig1). Asish et al. [20] reported the similar observation supported the present investigation, they observed the high quality of DNA can be isolated from the *Garcinia* species with the 4X concentration of CTAB in lysis buffer which is more efficient to subjected to the PCR for molecular profiling. It has been also reported that the high amount of β -mercaptoethanol help to removes the high amount



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polyphenols from the plant tissue [21]. Therefore, in the present study high amount of β -mercaptoethanol was used which made the protocol practically viable for extraction of high-quality DNA. A number of earlier workers [22, 23] also recommended the use of PVP with the molecular weight of 10,000 at 2% (w/v) to address the problem of phenolics. PVP with low molecular weight has less tendency of precipitating with the nucleic acids as compared to PVP with the high molecular weight [24]. In the present investigation, we used the higher molecular weight PVP with 3% concentration to remove the polyphenols from all species, and thus we yielded a high amount of polyphenols-free DNA. Pure DNA can only be subjected to the PCR for any kind of molecular analysis. It can be measured by taking OD at A_{260}/A_{280} , ranging from 1.78 to 1.84 and A_{260}/A_{230} less than 2 suggesting pure DNA without proteins and RNA contamination [25]. The purity and amount of DNA were measured in the present investigation by taking the OD at A_{260}/A_{280} , and the amount was calculated in Eppendorf Bio-spectrometer. DNA extracted in the present study showed the OD=1.6-1.8 and amount were recorded 30ng/ μ L-150ng/ μ L. The amount of 40-50ng/ μ L DNA was subjected to PCR for ITS1 amplification.

ITS1 amplification and Sequencing

The DNA with the highest purity of five species viz. *G. morella*, *G. pedunculata*, *G. xanthochymus*, *G. dulcis*, and *G. lanceifolia* were subjected to the PCR amplification of ITS1 region using the above-mentioned primers. The efficiency of the PCR product was very good and the size of the amplicons was predicted almost 250bp as compared to the DNA ladder. The amplicons were then sequenced, annotated and submitted to the Genebank, NCBI. The ITS1 sequences obtained from the Genebank, with the accession numbers (Table 4) were used for further study.

BLAST analysis

The sequences of accession numbers were acquired by Gene Bank were subjected to the BLAST analysis. The n-BLAST were performed in the default algorithm, the higher E-value and similarity percentage (92-99%) sequence were found appeared as the same species in the NCBI database. The similar sequences were downloaded in FASTA format and multiple sequence alignment was performed for all the sequences by ClustalW and created a phylogenetic tree using MEGA software.

Phylogenetic tree analysis

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-458.9456) is shown (Fig 3). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 32 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 163 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The bootstraps of 1000 are performed showing the accuracy of the results. The phylogenetic tree revealed that the molecular identification of these five species belongs to the same branches of species retrieved from the database. In the present investigation, the *Clusia* species (Accession no: MF188242) was taken as an outgrowth to create a rooted phylogeny tree.

Sequence alignment and phylogenetic tree of five species

Multiple alignment and phylogenetic analysis among the five sequences help to understand the nucleotide composition and genetic distance among the species. The present investigation showed that among the five species the ITS1 regions contain different numbers of base pairs. Adenine (A) was recorded highest in *G. pedunculata* and lowest in the *G. dulcis*. Thymine (T) content was highest in *G. dulcis* and lowest in *G. lanceifolia*. Guanine (G) found highest in both *G. xanthochymus* and *G. dulcis* and lowest in the *G. lanceifolia*. Cytosine (C) highest in the *G. xanthochymus* and lowest in the *G. lanceifolia* (Table 5). The multiple sequence alignment showed the distinctive nucleotide in 5, 6, 11, 25, 30, 35, 36, 37, 47, 62- 66, 67, 70-76, 83, 85, 92, 95, 105, 108,109,110, 111,14,18,19, 35 positions,



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these could be used as a DNA barcoding. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-525.4267) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 6 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 160 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

The phylogenetic tree revealed that *G. morella* and *G. lanceifolia* are located at the same branch, while *G. xanthochymus* and *G. dulcis* in another same branch. It can be said that *G. morella* and *G. lanceifolia* are as similar species as *G. xanthochymus* and *G. dulcis*, while *G. pedunculata* are genetically dissimilar with other species. Sequence analysis of internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) between the small subunit (18S) and the large subunit (25S) of nrDNA has been used as a source for analysis of genetic relationships within genera and among closely related genera in many angiosperms [26]. Usually, the entire ITS regions are classified into ITS1 and ITS2, and the size of both regions varied in the plant to plant ranges less than 300 in angiosperm [27]. It has been used frequently to resolve genetic relationships among many plant taxa [28] because it was proven to contain enough genetic information [26]. In the present study, we found the sizes of ITS1 regions of the five species ranges between 254-298bp. The similarity search by BLAST and phylogenetic analysis revealed the identification of these five species with the similar species present in the NCBI database. A similar observation was reported by Yapwattanaphun et al. [28] for efficient DNA barcoding to identify *Garcinia* species. Multiple sequence alignment of the sequence data showed the variation in the ITS1 regions in different positions. A similar observation was made by Liu et al.[29] in *Garcinia* species of China. The Euclidean distance and phylogeny analysis revealed the closeness and distances exhibited among the five species. The present investigation suggests that in the lysis buffer 4X CTAB with 3% of PVB and 2M of NaCl are the optimum concentration for the extraction of the high quality of DNA from the *Garcinia* species. It is observed that the ITS1 region is an efficient barcode to study the phylogenetics of this genus. The sequence data revealed the alternation of the base pairs in the different position of the ITS1 region among the species. The present study found that *G. xanthochymus* and *G. dulcis* are the closet group depending on the ITS1 region sequence.

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Table 1. Collected accessions and their location. GM= *Garcinia morella*, GP= *Garcinia pedunculata*, GX= *Garcinia xanthochymus*, GD= *Garcinia dulcis*, GL= *Garcinia lanceifolia*

SI No	Accession	Location	District	Latitude (N°)	Longitude (E°)
1	GM	Dhakuakhana, Hiloidari	Lakhimpur	27°13'43"	94°25'38"
2	GP	Lakhimipathar, Sadiya	Tinsukia	27°55'22"	95°45'18"
3	GX	Dhakuakhana ghilamara	Lakhimpur	27°18'59"	94°21'32"
4	GD	Jokai forest, Dibrugarh	Dibrugarh	27°23'02"	94°56'06"
5	GL	Bahana, Jorhat	Jorhat	26°48'37"	94°14'21"

Table 2. Primers for ITS1 region amplification. T_m= Melting temperature of Primer, T_A= Annealing temperature in PCR

SI. No.	Primer name	Nucleotide sequence	T _m	T _A
1	ITS1 Forward	AGCACATGGCTCCTCAA	59.4°C	58°C
2	ITS1 Reverse	AGACGTGCCCTCAACCA	57.3°C	

Table 3. Chemical profiling in PCR for ITS1 amplification

Reagent name	Volume (µL)
Template 50ng/µL	2µL
10 p mole primer Forward	0.5µL
10 p mole primer reverse	0.5µL
10X PCR buffer without MgCl ²⁺	2µL
25mm MgCl ²⁺	2.5µL
10mm d NTP	1µL
500 U Taq- polymerase	1µL
Nuclease-free Water	10.5µL
Total Volume	20 µL

Table 4. Accessions numbers of ITS regions acquired from the Genebank, NCBI

Species	Accession number
<i>G. morella</i>	MF990624
<i>G. pedunculata</i>	MF990623
<i>G. xanthochymus</i>	MF990622
<i>G. dulcis</i>	MF990625
<i>G. lanceifolia</i>	MF990621

Table 5. Nucleotide composition of five sequences.

Species	A	T	G	C	Total
<i>G. morella</i>	68	61	66	59	254
<i>G. pedunculata</i>	80	65	68	65	278
<i>G. xanthochymus</i>	77	70	78	73	298
<i>G. dulcis</i>	67	75	78	70	290
<i>G. lanceifolia</i>	71	53	58	56	238
Average	72.6	64.8	69.6	64.6	271





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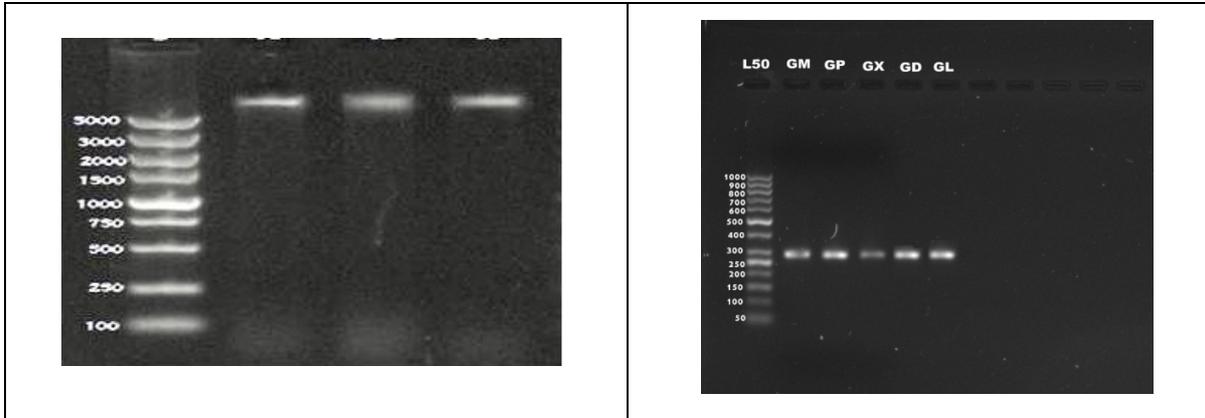


Fig1.Determination of the genomic DNA size using the DNA ladder

Fig.2. PCR amplicons of ITS1 regions, L50= 50 bp DNA ladder, GM= *Garcinia morella*, GP= *Garcinia pedunculata*, GX= *Garcinia xanthochymus*, GD= *Garcinia dulcis*, GL= *Garcinia lanceifolia*

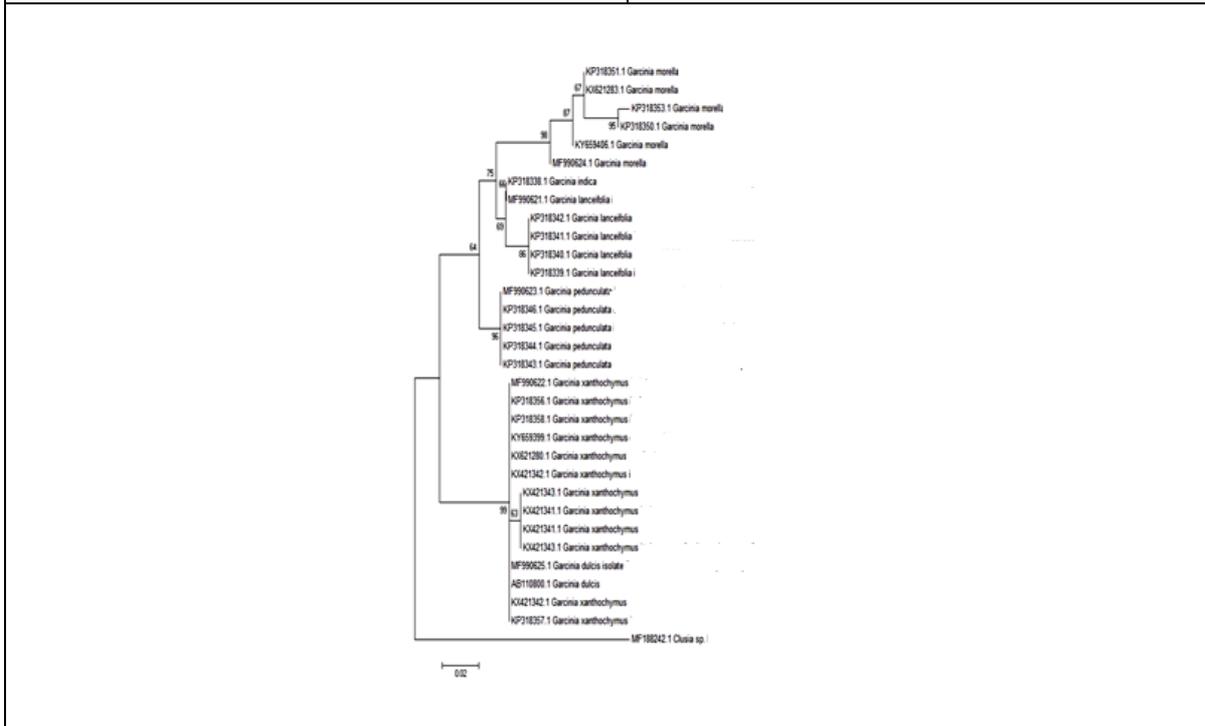


Fig.3. Molecular Phylogenetic analysis by Maximum Likelihood method.





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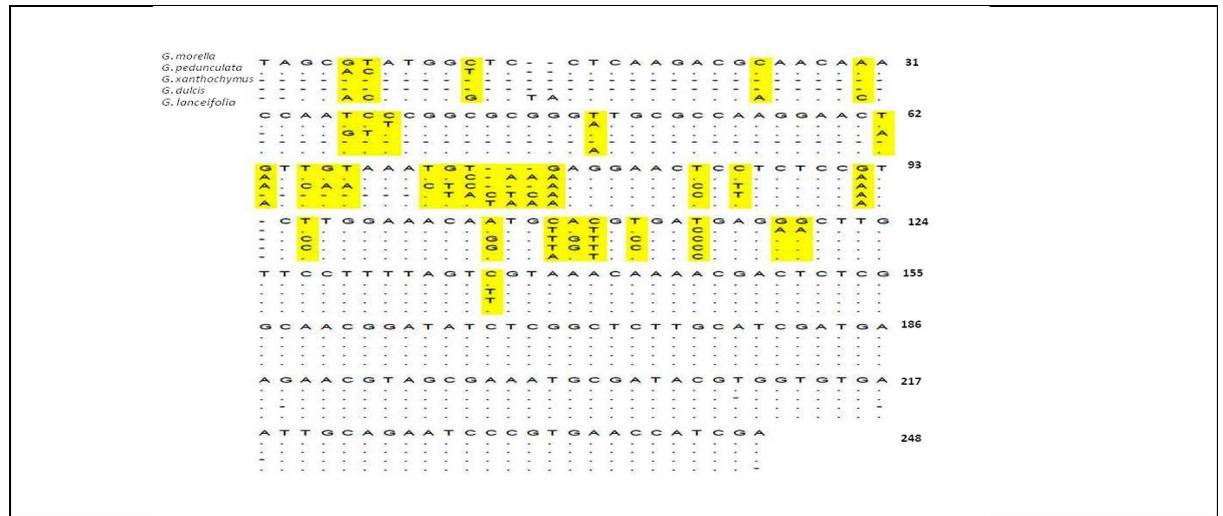


Fig.4. Multiple sequence alignment of ITS1 regions of five *Garcinia* species.

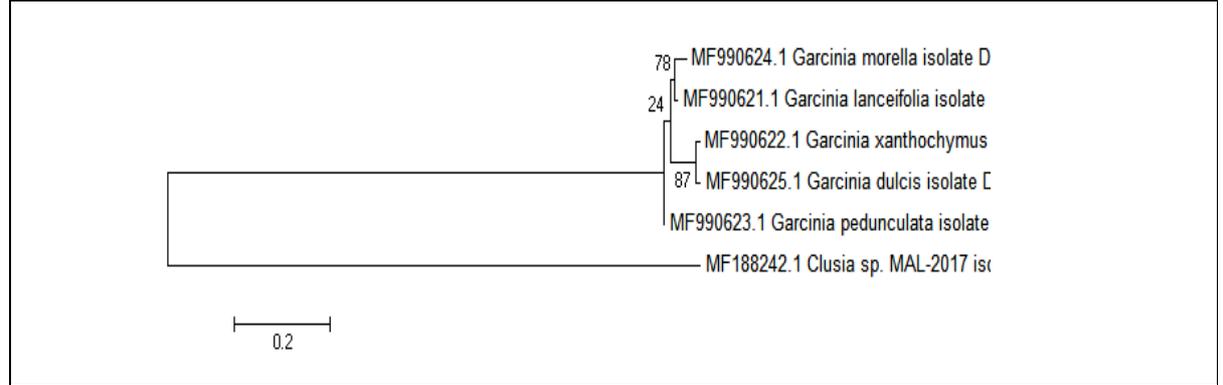


Fig.5. Phylogenetic tree of five *Garcinia* species, considering *Clusia* spp. as an outgrowth.





Collision Dynamic Simulation of Sodium-Ion Having Energy 200 keV on Vanadium Nanoparticles using IM3D

Satyanarayan Dhal^{1*} and Narayan Gouda¹

Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Satyanarayan Dhal

Centurion University of Technology and Management,
Odisha, India.

Email: satyanarayan.dhal@cutm.ac.in



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ABSTRACT

We have employed the IM3D simulation to understand the collision dynamics of 200 keV sodium ion impact on vanadium nanoparticles as the target atoms. This simulation data is really helpful to explain the phenomena happened after the collisions with energetic sodium ions before moving towards the costly experiments. The projected average range of the sodium ions obtained nearly 0.9 nm at which nearly 8600 number of ions have been intruded. Target displacements due to the vacancies i.e. 1.7×10^6 and the replacement collisions 0.8×10^6 remained 1.8×10^6 within an error bar of 33 %. They also induce phonons from the vanadium materials apart from atomic collisions.

Keywords: IM3D, Vanadium Nanoparticles, Monte-Carlo Simulation

INTRODUCTION

Vanadium Nanoparticles [1] are generally 10 - 80 nano-meters (nm). They are considered as highly potent materials for various industrial applications. For instance, Vanadium pentoxide nanoparticles have been synthesized using the spray pyrolysis process [2] which enhanced the electrochemical process that are suitable for lithium batteries. The polymer photovoltaic efficiency can be enhanced using the hybridization with the arrays of zinc oxide nanorods by introducing a vanadium oxide buffer layer [3]. Nanosized V_2O_3 material, owing to its higher catalytic surface with diameter less than 30 nm have a toxicity effect in human endo- and epithelial lung cells [4]. In other words, apart from beneficial effects, it also has some destructive impacts. Hence, it is necessary enough to know the ion or electron irradiation impact on vanadium nanoparticles. To keep in minds, here we have used IM3D [5] simulation to derive the data's related to irradiation induced defects. These datasets are significant for the purpose of going directly to the experiment. IM3D is a three-dimensional Monte-Carlo code that provides the simulation for the transport of ions and the creation and further distribution of defects within various materials. It can utilize randomly complicated three-dimensional targets consisting of different elements of varying geometry. The change in the target material like atomic displacement, radiation damage, sputtering, phonon production etc. can be determined using IM3D. Output

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files generally remain in the extension name of .cfg, or. msh which can be plotted by Atom Eye [6] or Gmsh [7]. Here we have taken vanadium nanoparticle on a silicon substrate and simulated using 200 keV sodium ions.

Simulation Details

This simulation is dynamic in nature and which may provide better results than SRIM. SRIM [8 – 10] provides the results for layered structure where as IM3D provides the simulation data for 3D target structure. We can utilize IM3D, an open source package to use ions up to having atomic number 92 and energy ranging from eV to Giga-electron volt. We can employ different shape distribution of ions. Arbitrary complex targets may be constructed using different algorithms. Also, different input shapes can be used to produce target surfaces. We may even calculate the stopping power of a target compound using Bragg's rule. This simulation presents the number of displacement collisions designates the number of vanadium atoms that were in the cascade with energies greater than their displacement energy. A vacancy is always being created as a hole when a recoil atom transfers from its original location. Replacement collisions does the job of decreasing the number of vacancies. If an ion handover extra than its displacement energy, and the primary atom, after the bombardment, stops, then it simply replaces that atom in the target and there is no vacancy created. In other words, the sum of vacancies and replacement collisions creates the target displacements. We have discarded the silicon substrate from the simulation input parameters.

RESULTS AND DISCUSSION

Hereby, the detailed simulation results are plotted below:

The image in Fig 1 shows the projected average range of the sodium ions having energy 200 keV in a vanadium target is nearly 0.9 nm. At a distance 0.9 nm, almost 8600 number of sodium ions have been penetrated inside the target. Because of collision cascade, the ion energy gradually reduces and when it reaches zero, it eventually stops. Almost all the ions will be stopped by a distance of 2.5 nm. The image in Fig 2 revealed the maximum displacement of recoiled cascade vanadium atoms due to sodium ions having energy 200 keV occurs at a distance of nearly 0.5 nm. The maximum atoms displaced at that position is nearly 1.8×10^6 vanadium atoms. The image in Fig 3 shows the atomic vacancies created in the vanadium target because of 200 keV sodium ions. The maximum number of vacancies of recoiled cascade vanadium atoms due to sodium ions having energy 200 keV occurs at a distance of nearly 0.5 nm. The maximum atoms displaced at that position is nearly 1.7×10^6 vanadium atoms. The target displacements confirm the summation of vacancies i.e. 1.7×10^6 and the replacement collisions 0.8×10^6 (Fig 5) within an error bar of 33 %. This error may be beyond the scope of this simulation package. The image in Fig 1.4 revealed the corresponding maximum interstitials produced in the vanadium target because of 200 keV sodium ions at a distance of nearly 0.75 nm. The maximum interstitial atoms positioned at that position is about 1.6×10^6 vanadium atoms. The image in Fig 1.5 clearly shows the corresponding replacement collisions in the vanadium target because of 200 keV sodium ions at a distance of nearly 0.45 nm. The replacement happened is about 0.8×10^6 vanadium atoms. The image in Fig 1.5 clearly shows the corresponding replacement collisions in the vanadium target because of 200 keV sodium ions at a distance of nearly 0.45 nm. The replacement happened is about 0.8×10^6 vanadium atoms. The blue curve in Figure 6 shows the number of phonons and the red curve shows the emission of electrons occurred in the Vanadium because of 200 keV sodium ion incidence. They are about 6×10^7 number of phonons. If incident energy is less than displacement energy, a replacement collision happens releasing phonons. This type of collision is common in single element targets like vanadium with large recoil cascades. our previous assumptions which were based on the theoretical calculation used in SRIM [11-23] is proven correct.

CONCLUSIONS

We have simulated the collision dynamics of 200 keV sodium ions on a vanadium target. We have found



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that the target displacements due to the summation of vacancies i.e. 1.7×10^6 and the replacement collisions 0.8×10^6 remained intact within an error bar of 33 %. They also induce phonons from the vanadium materials apart from atomic collisions. These simulated data's which we have derived here, will be beneficial prior to the experiments.

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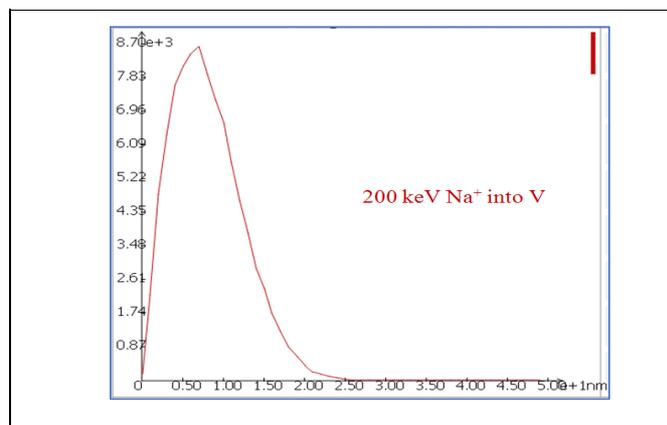


Figure 1. Depth distribution of 200 keV sodium ion on Vanadium using IM3D.

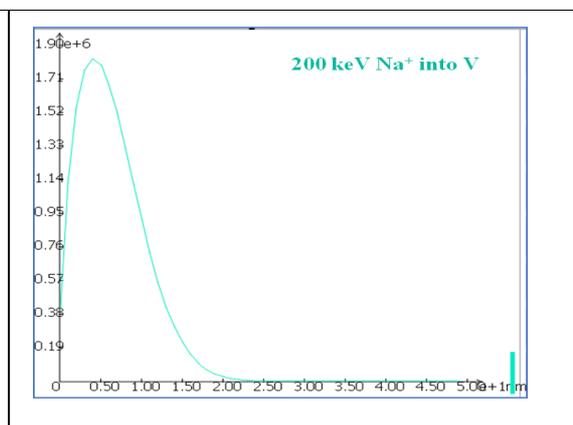


Figure 2. Atomic displacements occurred in the Vanadium because of 200 keV sodium ion incidence.

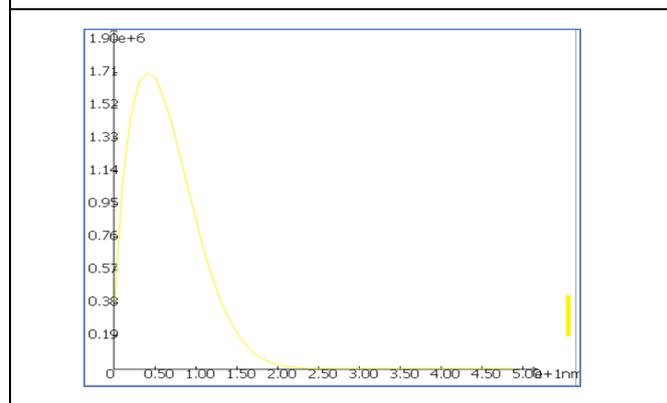


Figure 3. Atomic vacancies occurred in the Vanadium because of 200 keV sodium ion incidence.

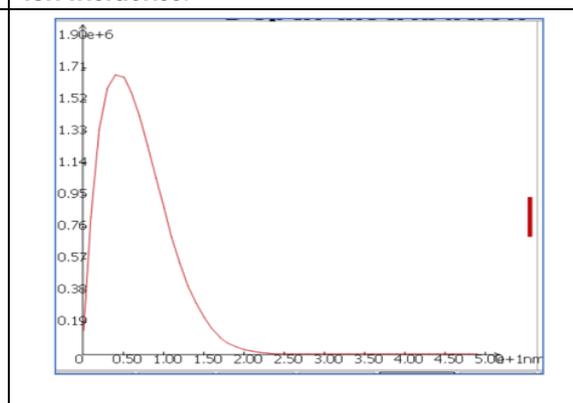


Figure 4. Interstitials occurred in the Vanadium because of 200 keV sodium ion incidence.





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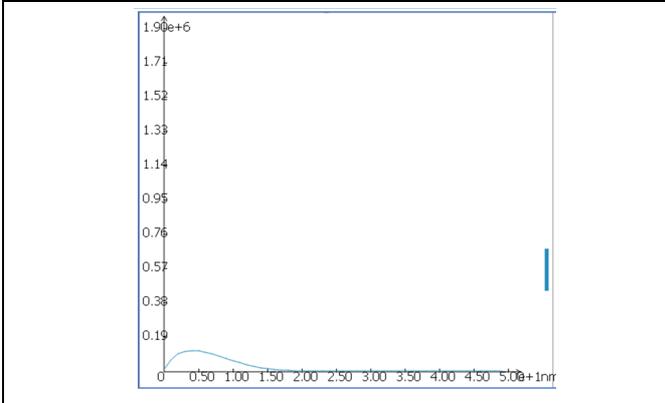


Fig 5 Replacement collisions occurred in the Vanadium because of 200 keV sodium ion incidence.

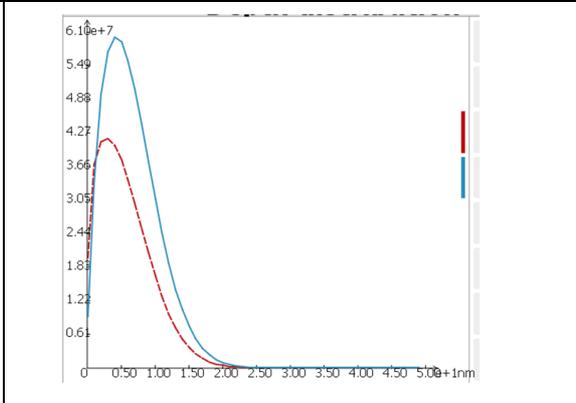


Fig 6 The number of phonons (blue line) occurred in the Vanadium because of 200 keV sodium ion incidence.





***In silico* Analysis of Different Mechanical Properties of Polynylon 12 and Polyethylene-Isophthalate Compatibility in a Blend Properties in Mass Fractio of Polynylon 12**

Prateet banajyotshna sahoo¹, Bijaya kumar Singh² and Arun Kumar Pradhan^{1*}

¹Department of Chemistry, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.

²Department of Chemistry, Aska Science College, Aska, Ganjam, Odisha, India.

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***Address for Correspondence**

Arun Kumar Pradhan

Department of Chemistry, School of Applied Sciences,
Centurion University of Technology and Management,
Odisha, India.

Email: arunkumar.pradhan@cutm.ac.in



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ABSTRACT

Combination of two or more components results in the formation of a blend. The compatibility of Polynylon12 and Polyethylene-isophthalate were studied to form a miscible blend using Bio-via Materials Studio. The mechanical properties of the composite were studied based on bulk modulus, shear modulus, Young' modulus, Poisson ratio. The results indicated that the values of all the properties increased with increase in mass fraction of polynylon 12. This study will not only help to determine pairs without performing laboratory experiments but also less time consuming and low cost process.

Keywords: Blend, bulk modulus, shear modulus, Young' modulus, Poisson ratio.

INTRODUCTION

In general, formation of blends & composite can be made with the combination of more than one component where the blending of the composite is done without losing their identity. Quantity of the material can be improvised by mixing up different components as the blending brings multiple properties. A continuous effort in the field of research brings desired property in a single material. Thus the blending of different materials leads towards the formation of new material with desired properties by making it cost and time effective. Polymer composites can be prepared with the combination of two or more polymers, or fibers & polymers, or particles and polymer. Nano material modified polymers facilitated to multifunctional material. In the present day Scenario, polymers coupled with carbon based (grapheme, carbon nanotube) nano materials [1] have brought greater importance. In biodegradable polymer, natural fiber composites [2] is used to enhance mechanical properties and water resistance.

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Now a days, widespread research is being conducted on fireproof materials [3]. Research says that inorganic additives in Polypropylene can enhances flame retardancy without adding much weight [4]. Another study on fuel efficiency transportation says that the fuel efficiency can be enhanced through synthesis and production of light weight composite materials of higher strength [5]. There are applications of composites in structural engineering too because of high strength to weight ratio and resistance to corrosion. Thus glass fiber reinforced polymers, latex polymer cementitious composites [6] where developed for construction of bridges, light rail transit, mining and tunneling, retaining walls and other water side buildings. All the above mentioned examples are based on laboratory experiments. Basically trial and error method is being used for the preparation of blends as it is a cost effective and less time consuming method. Hence, use of silico approach to develop new blends has been emphasized. Software (Materials studio {7}) has been used to identify compatible pairs.

MATERIALS AND METHODS

Software Used

The Biovia software having Material studio module (Dassault Systemes of France) was utilized to study the machine learning techniques and standard algorithms to assume the interaction level.

Methodology

By using the build menu of Materials Studio, the Polynylon12 and Polyethylene-isophthalate were prepared. The components used in blends calculation menu of Materials studio were utilized for the optimization of the structural components. The Polynylon 12 was used as the base and Polyethylene-isophthalate was used as screen. Various data were generated by the blends analysis menu of Materials studio after the calculation and the compatibility of the components to form a blend was analyzed. The structures Polynylon12 and Polyethylene-isophthalate of were fed to the synthia menu of Materials Studio. The components were run to different fractional weight which were then displayed in a tabular form according to their variable properties. To identify the effective weight fraction of polynylon 12, different values were used to plot the graphs on the basis of mechanical properties of the composite.

RESULTS AND DISCUSSION

In this process, Polynylon12 and Polyethylene-isophthalate were used as potential components of a blend was analyzed using Biovia Materials Studio. Using Biobio Materials Studio, polynylon 12 and Polyethylene-isophthalate potential components of a composite was analyzed under this process. While evaluating a wide range of polymer properties, Biovia Materials Studio Synthia uses pre-defined correlations (advanced quantitative structure-property relationships). To evaluate the properties of polymers as well as small molecules, Group additive methods were used from many years. These methods have one of the greatest utility when a rapid estimation of property is required without a detailing understand of the atomistic interactions which give rise to it and also they are extremely fast and easy to use. The dependence upon a database of group contributions are the principal shortcoming of these methods. Hence, the property of a polymer cannot be calculated unless the polymer containing group for which the group contribution is not estimated. The method used in Synthia, topological information about polymers is used in the predictive correlations to overcome the limitations. Due to the implementation of graph theory that is derived from connectivity indices, no database of group contributions is required. Properties can be predicted for any polymer which is generally composed for any combination of the following nine elements: carbon, hydrogen, nitrogen, oxygene, silicon, sulfur, fluorine, chlorine, bromine.





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Bulk modulus

Bulk modulus is the measure of the decrease in volume with an increase in pressure. Figure 1 shows that the bulk modulus of the composite decreases linearly with increase in mass fraction of polynylon 12 and certation mass fraction that remain constant.

Shear modulus

It is defined as the ratio of shear stress and shear strain. It shows the response of the composite to shear deformation. Figure 2 shows that the shear modulus of the composite decreases linearly with increase in mass fraction of polynylon 12 and certation mass fraction that remain constant.

Young's modulus

It is defined as the ratio of stress and strain. It compares the relative stiffness of the composite. Figure 3 shows that the Young's modulus of the composite decreases linearly with increase in mass fraction of polynylon 12 and certation mass fraction that remain constant.

Poisson ratio

It is the ratio of lateral strain to longitudinal strain. Figure 4 shows that the Poisson ratio of the composite increases linearly with increase in mass fraction of polynylon 12.

CONCLUSIONS

By using Biovia Materials Studio, the probability of using polynylon 12 and polyethylene-isophthalate to form a homogeneous blend was explored. According to the studies of bulk modulus, shear modulus, Young' modulus, decreases linearly with increase in mass fraction of polynylon 12 and certation mass fraction that remain constant but Poisson ratio of the composite increases linearly with increase in mass fraction of polynylon 12. Experimental identification were generally of blend components. The study of in silico helps to determine the components of a blend without performing laboratory experiments saving materials, money and time.

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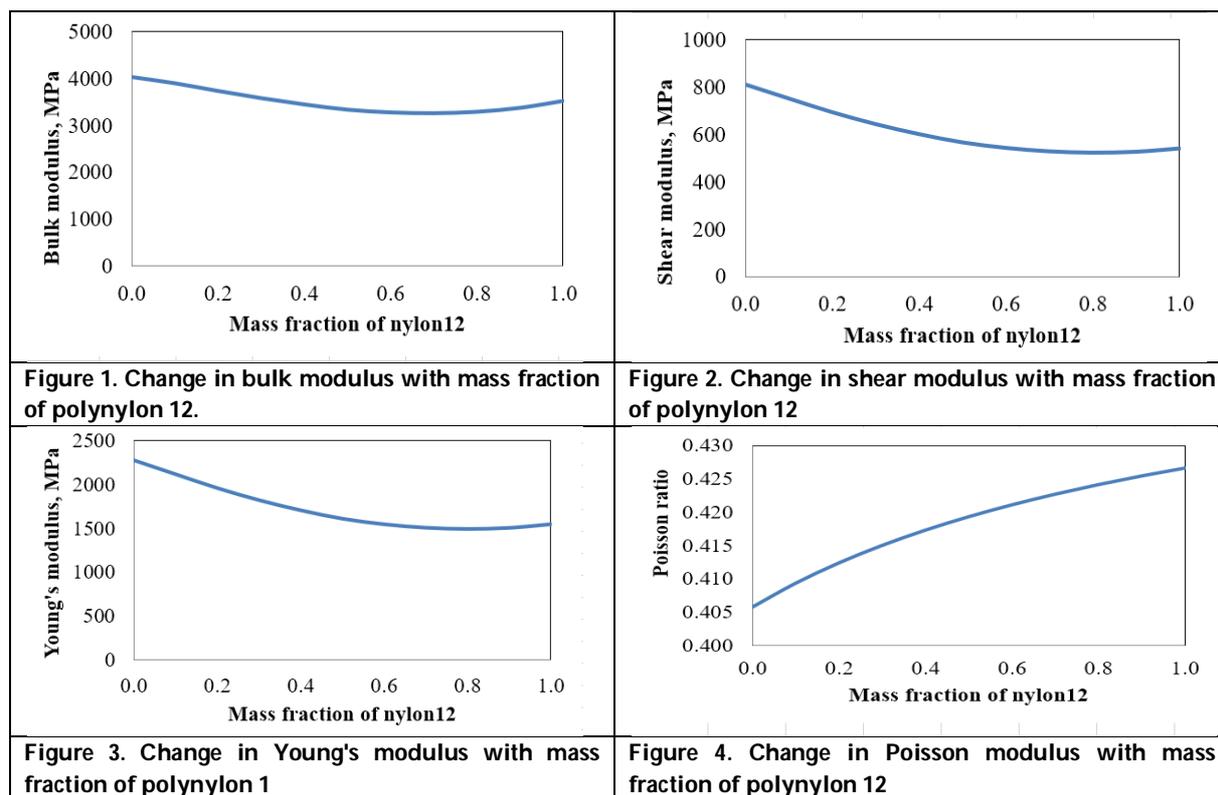
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Review on Battery Management System

Smitanjali Rout* and Abhinna Chandra Biswal

Department of Electrical and Electronics Engineering, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Smitanjali Rout

Department of Electrical and Electronics Engineering,
Centurion University of Technology and Management,
Odisha, India.

Email : smitanjalirout19@gmail.com



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ABSTRACT

A crucial task for battery management systems (BMS) is the role to ensure the protection of the lithium - ion battery and prolong their maining life of the batteries. Few pitfalls occur in traditional BMS, such as poor scalability and inadequate versatility. Now a day's batteries have many applications in power industries, such as electric vehicles (EVs) and hybrid electric vehicles. For efficient operation a good battery management system (BMS) is essential. In this paper there is a brief review on different stages of BMS, which emphasizes on modeling of batteries, its state estimation as well as charging of batteries etc. Second, it explores common types of batteries used in EVs, followed by the introduction of core innovations used in BMS. Various types of batteries are studied, including the electric model, the thermal model, and the coupled electro thermal model. Battery management system and safe operation of cell and module. Battery state calculation for the state of charge, health condition, aging and internal temperature are closely analyzed and battery energy management system and optimization are studied and finally battery charging and discharging technique methods are addressed. Further battery discharging, Depth of discharge (DOD) control, prediction of battery life and SOC has been optimized for advanced technique in BMS.

Keywords: Battery management system, battery modeling, battery state estimation, battery charging

INTRODUCTION

Electric vehicles (EVs) and hybrid electric vehicles (HEVs) present an excellent alternative to the current fossil fuel powered vehicles. It would replace the conventional internal combustion (IC) engine -based vehicles. Day by day it is found a wide range of change in the design, performance and operation of the technology for EV and HEV. Due to advantages such as high energy density, low air emissions and long cycle life, batteries have been commonly applied as the power source for EVs and HEVs [1]. Because of gas emission laws and air pollution automobile manufactures

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were forced to manufacturing is increasing today. Low noise and zero emission increase the preferences for electric vehicles it requires special attention to the operation and maintenance. Some specific factors should keep in mind such as the charging or discharging property, current or voltage supply, temperature etc. For healthy operation of the vehicle all the safety measures must be taken from the battery side. The battery management system (BMS) therefore plays a critical role in maintaining battery protection and efficiency. The BMS consist of battery modeling system, internal state estimation and battery charging system. In BMS the most prominent factor is the designing of the battery. The battery must have high current density, high energy density and high temperature withstand property. Therefore, modeling of battery is important. In other aspect analyze the behavior of the battery, real time control, battery state monitoring, thermal management as well as fault diagnostic would be easy after proper battery management. Some factors in the battery management system needs proper estimation methods to examine such as the state of charge (SOC), state of health (SOH) and internal temperature as these are effects on the operation of the batteries. Again, battery charging strategy system also its own importance in BMS.

The battery charging strategy depends upon the effective and efficient operation of the battery with damage protection system. So, a well-designed charging strategy must be adopted in order to improve the energy conversation efficiency. The charging time must be suitable for the life span of the battery. It is found that fast charging gradually slows the service life of batteries followed by increasing the temperature and hence energy losses [2]. The objective of this paper is to provide a brief review of key technologies in the BMS of EVs, particularly for battery modeling, state estimation and battery charging. Latest methods are discussed first to resolve the question of battery simulation of electrical and thermal characteristics. The models of excited batteries are used to analyses the battery's electrical and thermal behavior. The related battery SOC and internal temperature isolated or combined state calculation methods are also checked. Battery charging approaches are discussed on the basis of battery models and estimated internal states, along with optimization algorithms for improving the performance of these charging approaches. The majority of this article is formulated as follows. Section II addresses several common types of battery used for BMS in EVs and main developments. Then, in Section III, battery electric models are reviewed together with thermal models as well as coupled electro thermal models. In Section IV, the battery management system and safe operation of battery cell and battery module has been discussed. Section V, relies on a detailed analysis of the SOC calculation of the battery, the SOH calculation, the battery aging the internal temperature estimate and the joints state estimate. In section VI, about cell balancing and major 2 types of cell balancing are reviewed. In section VII, about energy management and optimization has been discussed. Then in section VIII, discusses several charging approaches and discharging approaches. In section IX, future trends of smart batteries have been discussed. Finally, this is concluded in section X.

Battery types and key technologies for BMS

As the only power supply system in the EV is battery, several types of batteries are used in it to provide continuous power. Here discussion is on some commonly used batteries and the key technology of BMS.

Types of battery used in EVs

Depending upon the recharging method the batteries are classified as primary battery and secondary battery. The characteristic of the primary battery is that, it cannot be reused after gating discharged and hence there should be availability of the sufficient battery for the operation. But the secondary batteries can be reused [3]. After discharged it can be recharged for the next time use. Hence the secondary batteries are suitable for continuous operation. Therefore, in EVs and HEVs different secondary batteries are used. The secondary batteries are also chosen based on some characteristics like, life cycle, energy loss, power and current density etc. There are some lists of such batteries which are in generally used in EVs. These are lithium-ion (Li-ion), lead acid, nickel-cadmium (NiCd) and nickel- metal hydride (NiMH), etc. Some key characteristics of these popular batteries illustrate in Table. 1. It is found that Li-ion battery has some advantages over other such as, its service life cycle is around 6 to 10 years, it does not contain any toxic material which is less hazardous for environment, and more over it has high safety level. Therefore, it is most reliable for power supply application in EVs.



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Key technologies for BMS

For the application of battery for electric vehicle special attention must be taken in order to maintain proper temperature, adequate charging and discharging so as to maintain the service life of the battery. Because improper charging and improper temperature control may lead in the deterioration of the life of the battery in EV. Along with that as the battery consists number of cells connected in series or parallel combinations to fulfill the high energy requirement of EV, focus should be given in the connection of batteries. Hence, it needs very care full design of the batteries in BMS for the protection and safe operation point of view [4,5]. This paper comprises the analysis of key technologies such as battery modeling, battery state estimation and battery charging. These are helpful in designing an effective BMS for the EVs. The analogy among these key technologies are as shown in Fig. 1.in the EVs the battery parameters like current and voltage can be sensed directly by on board current sensor and voltage sensor. And by the help of thermocouples or any temperature sensor the surface temperature easily detected. Then the battery independent or joint state estimation SOC or internal temperature can be found by suitable battery models and estimation model. After gating these two electrical and thermal characteristics of the battery, the charging strategy can be properly optimized by algorithms in the way of fast charging, suitable temperature rises and highly efficient energy conversion. As a result, an equilibrium condition can be maintained from initial state to final stage among all the parameters of the BMS. During the process if any fault or abnormality will occur then in the system the protection or fault control mode will enable and clear or eliminate the so cause. Therefore, these two key technologies i.e. battery modeling state estimation and the battery control mechanism in BMS are the most relevant focus point in the paper.

Battery Modeling

As the name indicates here, we have to choose or model such a battery which will appears most suitable for the EVs. This is the preliminary stage in the battery management system. Gradually various battery models with different level of accuracy as well as different staged of improvements are on the progress. The various models are the battery electric model, battery thermal model, and battery coupled model. The battery modeling is shown in Fig. 2.

Battery Electric Model

From the figure the battery model is the first stage of modeling of battery. It essentially includes the electrochemical model [6-9], the reduced order model [10-13], the analogous circuit model [14-17] and the data driven model [18-20]. Rahman et al., [6] proposed that the electrochemical battery model should have the capacity to capture the dynamics of spatiotemporal battery connection, the electrode potential for each stage and the kinetic Butler to track the inter calculation reaction of the electrochemical model. Then the electrochemical behavior of the battery can be studied through particle swarm optimization (PSO) method in the electrochemical model. Where critical parameters are optimized Sung and Shin [7] conveyed that in electrochemical model the accuracy a high degree of computational efforts must be there. Then a model implementation scheme had been developed to model electro chemical model. The most predominant factor of electro chemical model is that, here the electrochemical process with in the battery has been accurately studied. In the real time application, it is necessary to analyze the electrochemical composition of the battery. But it is not so easy to get this parameter. This parameter can be estimated by computational process after solving many partial differential equations for this. The higher order electrochemical equations can be reduced to lower order equation by taking suitable assumptions. Han et al., [10], for example, suggested as estimated method for collecting solid phase diffusion and battery electrolyte concentration distribution, then developed a condensed physics based electrochemical model to predict the SOC of Li-ion battery. Zou et al., [11] suggested a reduced order electrochemical model to predict discharge power under different conditions for the LiFePO₄ battery, then based on this reduced -order battery model robust SOC estimation was achieved. This simplified reduced order approach is very much suitable for real time application of the batteries, but in this process some information loss may happen. In equivalent circuit model all the circuit parameters such as voltage sources, resistance and capacitance are trapped together in order to find out the battery electrical behavior. Due to its simplicity in modeling and availability of the small number of model parameters the equivalent circuit model is largely accepted in battery real time application. A standard battery equivalent circuit model structure is shown in fig.3. In an analogous circuit model, the resistor





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capacitor (RC) networks are connected to the electrical behavior of the battery, such as charge transfer or diffusion on process. Based on the number of RC networks, the model order varies. First and second order models have been seen be more common and higher order models are not required in many cases [14]. For the widely used battery lumped parameters equivalent circuit model, Nejad et al., [15] presented a critical analysis regarding power prediction and better performance for SOC, the RC network model should better. In data driven model the input and output signal of the battery and their inter relation between them have been studied. To describe battery electrical behaviors, numerous data driven models such as natural networks [18, 19] and support vector machine (SVM) [20] were adopted. The efficiency of the model powered by battery data is strongly dependent on both test data and training approaches. Test data should cover enough battery operation ranges in order to achieve acceptable model accuracy as well as good generalization ability. After that, the parameters of the methods of training are appropriately gathered and analyzed. In addition, adaptive data driven methods [21, 22] can be used to produce improved performance in the simulation of batteries. As discussed, improper temperature management may lead to deterioration of the life of battery as well as the reliability of its use. Hence special care must be taken in the thermal control of battery in EVs [23]. In thermal model various thermal behavior of the batteries are being studied. This model consists of heat generation model, heat transfer model, reduced-order thermal model and data-driven model. Here the case study of heat generate model, where all the methods have been discussed regarding the heat generation in the batteries. How the heat is generating and why it is generated etc. For that numerous methods are there, such as activation, concentration and ohmic losses. Three popular approaches to assess the heat generation in the batteries are illustrated in Eq.(1), Eq.(2) and Eq.(3), which have been widely applied in real-time applications [24–27].

$$Q_1 = R * I^2 \dots\dots\dots(1)$$

$$Q_2 = I(V - OCV) \dots\dots\dots(2)$$

$$Q_3 = I(V - OCV)IT \frac{dOCV}{dT} \dots\dots\dots(3)$$

Where,

R is the initial resistance of the battery, I and V stand for the battery current and voltage, respectively, the open circuit voltage of the battery Q₁ stands for the generation of battery heat, which is mainly caused by the broad current crossing the internal resistance of the battery, Q₂ stands for the generation of battery heat caused by the over potentials through the RC networks, Q₃ stands for the battery, within or outside the battery heat transfer takes place by three methods. One is conduction, second is convection and third one is radiation [28]. Developed a three-dimensional distributed parameters heat transfer model to investigate the geometrical current and heat distribution inside the Li-ion battery. This is as follows;

$$\frac{\partial_p C_p T_{3c}}{\partial t} = -\nabla(k_{3c} \nabla T_{3c}) + Q \dots\dots\dots(4)$$

it can be also expressed as [29]

$$\frac{\partial_p C_p T_{3c}}{\partial t} = -\frac{\partial}{\partial x} \left(k_x \frac{\partial T_{3c}}{\partial x} \right) - \frac{\partial}{\partial y} \left(k_y \frac{\partial T_{3c}}{\partial y} \right) - \frac{\partial}{\partial z} \left(k_z \frac{\partial T_{3c}}{\partial z} \right) + Q \dots\dots\dots(5)$$

Where,

p stands for the battery density, C_p stands for battery heat capacity, k_{3c} is the coefficient of battery thermal conductivity (along three dimensions: k_x, k_y, k_z), Q means battery heat generation. Assuming a uniform distribution of heat with in the layers of the battery and considering one dimension of heat conduction (e.g., x dimension), then a reduced one-dimension heat conduction model can be developed [30] as follows,

$$\frac{\partial_p C_p T_{1c}}{\partial t} = -\frac{\partial}{\partial x} \left(k_x \frac{\partial T_{1c}}{\partial x} \right) + Q \dots\dots\dots(6)$$

In one-dimension heat transfer model the temperature gradient can be found out in only one direction. The three-dimensional heat transfer model is capable of detecting the hot-spots by subjugating the inside temperature distribution in the battery. This can be done in high heat generation application in EVs. But the computational heat transfer model which are basically used in offline simulation in state of real time application due to its lengthy





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process. From all heat transfer methods. Here, heat has been distributed uniformly in all layers as well as inner and outer surface of the battery. Then a popular two-stage thermal model for battery cell [31–33] can be derived as:

$$C_{q1} \frac{dT_{in}}{dt} = k_1(T_{sh} - T_{in}) + Q \dots\dots\dots(7)$$

$$C_{q2} \frac{dT_{sh}}{dt} = k_1(T_{in} - T_{sh}) + k_2(T_{amb} - T_{sh}) \dots\dots\dots(8)$$

Where,

T_{in} and T_{sh} stands for the internal and surface temperature of the battery, T_{amb} means the ambient temperature around the battery, C_{q1} and C_{q2} stand for the internal and surface thermal power of the battery, k_1 stands for the heat conduction between the surface of the battery and the interior, k_2 stands for the heat conduction between the environment and the surface of the battery. The battery thermal management cannot be completed with the study of only these two stages. Hence battery reduced thermal model has proposed, which holds good in the battery thermal management [34–36]. In Ref. [34], the order of a Li-ion battery model has been reduced by converting the one-dimensional boundary-value problem into a low-order linear model in the frequency domain. In reduced order model the temperature prediction is approximately same as experimental data and the simulation of the three-dimensional elements. Hu et al. [36] proposed a reduced-order state space model of battery pack based on a computation alfluid dynamics (CFD) model using the singular value decomposition method. the proposed model is more advantageous than the CFD model as less computational cost is involved.

Battery coupled electro-thermal model

As the name here the electrical model and the thermal model are analyze together based on their respective parameters. The battery electric behaviors are current, voltage and SOC. The battery thermal behaviors are internal temperature between the levels and the surface temperature of the battery. In this literature both lumped parameter and distributed parameters of different coupled electro-thermal models have been developed [37–39]. In addition, a three dimensional electro thermal model was proposed by Gout a m et al [40] to estimate battery SOC and quantify the generation of heat .The coupled model consists of a 2D potential distribution model and a 3D distribution model of temperature .The most efficient method of heat transfer is the battery SOC and temperature conduction. On the basis of model, propagation under both steady and dynamic currents is determined effectively. A reduced low temperature electro thermal model was suggested and validated by three cathode content batteries. In Ref [41]. This reduced model is sufficiently precise to establish quick heating under temperature condition and an optimal charging strategy. In order to examine the effects of different battery operations such as coolant flow rate and discharge current on the battery temperature. Basu et al [42] proposed a coupled three-dimensional electro thermal model. Based on the battery temperature, touch resistance is checked to play a critical role in battery temperature.

Battery Management System

Multiple battery cells need to be inter connected to build desirable power and energy ratings in order to implement battery technologies for PEV and BESS applications. Energy ratings for PEVs typically vary from 25 kWh to 65kWh, while BESSs vary from 10kWh to 200 MWh.Fig.4 provides an analogous model for N battery cells. Where battery cells are attached in sequence, the circuits based current sources remain the same. If the battery module is attached to the M battery branch (attached series) in parallel. Fig.5 indicates the Thevenin based electrical model for battery module. Protection, stability and cost efficiency should be taken into consideration when several battery cells are linked together, So the Battery Management system (BMS) is essential [104]. BMS main function can be classified approximately as: 1) secure operations (monitor individual cell voltage, current, charge rate and temperature to ensure that they are all within limits), 2) calculation of battery states like state of charge (SOC) and state of health (SOH) and 3) providing cell balancing for multi-cell applications, (Fig.6).

Safe Operations

BMS prevents the battery from discharging and charging below a certain SOC when fully charged [105]. In addition, BMS can ensure that the rates of charge and discharge are all within the protection spectrum. In addition, it is





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important to limit the operating temperature. Batteries of Lithium ion typically work from 0 degree and 45 degree for charging 20 degree and 60 degree for discharging, depending on the rechargeable battery. The temperature of the battery cell also applies to the performance of the battery cell due to internal resistance and loss of capacity [24]. Finally, the charging and discharging speeds of battery cells for BESS and PEV applications need to meet the specification of power grid operators and consumers of PEV. Charging and discharging speeds should also conform with the restrictions on battery chemistry.

Battery State Estimation

Here broadly battery state estimation has been reviewed with special emphasis on battery key states. This includes SOC estimation, SOH estimation, internal temperature estimation and joint state estimation.

SOC Estimation

State of charge is the capacity of an electric battery related to its charge capacity and expressed in the percentages. For fully charged battery the SOC is 100% and for full discharge the SOC is 0%. It is a key concept in calculating the exact SOC of the battery in BSM to get an idea about efficient and effective operation of the battery [43]. For SOC calculations there are two main methods are there. These are direct calculation and model-based calculation. In direct calculation method directly measured the battery current and voltage. Here SOC is calculated in two different methods Ampere- hour (Ah) or coulomb counting method and open circuit voltage (OCV) based method. Among these two methods Ah methods is simplest method to calculate SOC, which is illustrated as follows,

$$SOC(k) = SOC(k_0) + \int_{k_0}^k \eta I(t) dt / C_n \dots \dots \dots (9)$$

Where,

$SOC(k_0)$ is the accepted initial SOC, η stands for battery charging or discharging efficiency, C_n stands for the nominal capacity of the battery, $I(t)$ is the actual value that is positive for charging and negative for discharging current. But it depends upon some factors which is badly affect the accuracy of the calculation. These are current measurement and error accumulation. In addition, it is difficult to reliable calculate the initial SOC in real-time application. Especially when the battery is only charged within a small range. e.g. 10% to 90%. Hence it is little bit challenging in Ah method in calculating initial SOC and current.

It has been suggested that the battery SOC and OCV have anemone nonlinear relationship. Therefore, the use of OCV has become a powerful and common method after enough rest to estimate battery SOC, and in many application it has been used [44, 45]. In OCV method the calculation of the battery SOC value is up to a high accurate but, its resting time put this method think before apply, Here after disconnecting the load current and to regain its equilibrium It takes a long time period (for example, under low temperature condition, the length of LiFePO4 battery is often longer than two hours.) Furthermore, with battery aging and temperature changes the relation between OCV and SOC also changes. Owing to this limitation the OCV method faces restriction in its use in EVs. This issue can be solved if the OCV can be obtained in real time to allow SOC estimation while driving. Hence for the online prediction of the battery SOC the model-based approach has been developed. Here it should be focus on a battery model; which would be suitable for the state estimate. To predict battery SOC. The battery equivalent circuit model [46] and the electrochemical model [47,48] are typically chosen in the standard state space format. In these battery models, one of the state variables is SOC. Furthermore, for calculation of the SOC in online mode various state observers like such as Kalman filter (KF), extended Kalman filter (EKF), adaptive Kalman filter (AKF), unscented Kalman filter (UKF), slide mode observer and H filter are chose [49,53]. In finding the values in this model-based approach the accuracy will be depended upon the training of the battery models, the adopted state observers, and the parameters tuning. Such as the main parameters in the model and the KF observer noise covariance matrix. Furthermore, the efficiency of the SOC battery estimation by these various observers is validated only under restricted test data conditions, and an accurate trust zone is typically difficult to achieve, there is some deviation in the experimental data from different test conditions.





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SOH Estimation

SOH represents the standard operating hour of a battery. The exact operating time cannot be said before. One can predict the approximate time period for a battery which are used in EVs. There is no single definition for the battery SOH. A general description of battery SOH can be given as:

$$SOH(t) = SOH(t_0) + \int_{\tau=t_0}^{\tau} \delta_{func}(I, T, SOC, others) d\tau \dots\dots\dots(10)$$

Where,

SOH(t_0) is the original SOH battery, δ_{func} is an ageing rate feature that relies heavily on many variables such as current temperature, SOC, others reflect other stress factors such as mechanical vibrations and over-potential. Gradually with the passage of time the internal resistance of the battery increases, which results in the deduction of the battery capacity. Thus, the internal resistance or functional power of the SOH battery can be calculated as a kind of prediction regime for improvements in the field of computer science [54]. Several approaches were suggested to estimate SOH battery, which are classified into three categories, namely methods of model free, model based and data mining. Because of the aged power C_{aged} or the increased internal resistance R_{in} , the model free process, the battery SOH can simply be described as

$$SOH = \frac{C_{aged}}{C_n} * 100\% \dots\dots\dots(11)$$

$$SOH = \frac{R_{in}}{R_n} * 100\% \dots\dots\dots(12)$$

Where C_n and R_n account for the new battery's nominal power and internal resistance without being used. Pursuant to the SOH definition in Equations. In order to assess the battery's aged capacity and increased internal resistance, the regular capacity test [55] or pulse current test [56] can be added. But this method is not recommended due its adverse effect in the operation of the EVs as here full discharge is being carried out using controlled current and temperature. The battery electrochemical impedance spectroscopy (EIS) method is recommended due to the availability of more information. It will more effective than the direct measurements of C_{aged} and R_{in} . Therefore, it is accepted by many researchers for health diagnostics of batteries EIS [57-60]. However, specialized instruments are required for the on-board calculation and application of the EIS battery, which will restrict its applicability. In comparison, it often takes a long time to perform a complete EIS exam. On the one side, the battery capacity or internal resistance is used as the time-varying parameters based on the battery equivalent circuit model for the model-based process.[15] and a model of electrochemistry [61]. For finding the SOH the internal resistance as well as the capacity of the battery will be calculated. Before that various observations are chosen. These are particle filters [62, 63], Kalman filters [64–66] and sliding model [67–69], here also battery life cycle model has been analyzed to estimate the battery degradation based on the stress factor. Defining the battery capacity loss C_{loss} as:

$$C_{loss} = \frac{C_n - C_{aged}}{C_n} * 100\% \dots\dots\dots(13)$$

Then C_{loss} can be further expressed as,

$$C_{loss} = \delta_{func}(f) Ah^z \dots\dots\dots(14)$$

Where,

f stands for the stress factors that cause performance depletion, Ah implies the cumulative current through-put, z is a parameter of power law, δ_{func} reflects the impact on the degraded capacity of the battery of stress factors. Degradation of battery capacity is related to battery current, temperature, SOC and methods of charging, etc. Therefore, several studies to establish battery ageing dynamics have been suggested. For example, Wang et al., [70] establish a generalized cycle-life model for LiFePO₄ cell by the help of the power law equation. Here the discharge rate is not considered. Only the consideration is the wide range of current and temperature variation. A similar cycle-life model was validated in Ref. [71] to predict battery capacity loss at a low SOC level at a constant temperature. Omar et al., [72] proposed a model which predict the degrading of the battery as per aging both in case of charging and discharging. In Ref. [73], a cycle-life model based on the profiles suggested by the VDA (German Organization of



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the Automotive Industry) was validated for the Li-ion battery. In order to catch the battery capacity loss over data mimicking real cycling conditions, Suri and Onori [74] suggested a control-oriented cycle-life model. In Ref. [75], a complex cycle-life model was proposed according to a mechanistic and prognostic model to capture the dynamics of power depletion under the conditions of varying load for large-format Li-ion batteries. Gao et al., are in addition, since the SOH battery changes at a much slower pace compared to the SOC battery, broader battery activity ranges and more test data are required to train the battery cycle life model. Gao et al., [76] survey different charging condition to inspect the battery aging. It was found that active material loss is being affected if the charging current is less than 1 C, and the lithium loss if the cut-off voltage is less than 4.2V, then it was proposed an experimental model which enable a relation batted graded battery capacity and charging stresses. To increase the service life of battery the by optimizing the real time operation the life-cycle model plays significant roles.

But the working load is the main research aspect for the researchers. Under real-time applications their accuracies cannot be predicted hundred percentages. Besides, as battery SOH changes at a much slower rate as compared with the battery SOC, wider ranges of battery operation and more test data are required to train the battery cycle- life model. This leads in application restriction in engineering applications. In addition, relative to the battery SOC, as battery SOH shifts at a much slower pace, wider ranges of battery operation and more test data are required to train the battery cycle-life model. Data mining approaches have also been used for battery SOH estimation [77–87], similar to battery SOC estimation. In an alternate power train using SVM, Nuhicet et al., [79] suggested a battery health diagnostic and prognosis approach that relies only on on-board observable details, e.g., battery terminal voltage and current and operating temperature. An SVM approach for estimating the SOH battery was also suggested by Klass et al., [80]. Using load collectives to provide the input and output vectors of the specified SVM training data collection, a new data-processing approach is proposed. A data-driven classification method, i.e. the K-nearest neighbor (KNN) method, was proposed by Hu et al, [81] for battery capacity estimation. Again, only the observable on-board signals are needed, i.e., voltage and current. The characteristic characteristics of the charge curve that are representative of the SOH battery are established, and then the method of KNN is used to 'learn' the relationship between the SOH battery and these characteristics from data. In Ref. [82], a data mining method was proposed to estimate the battery SOH using clustering and neural network technologies based on historical distributions of appropriate data such as battery current, voltage and temperature. The average calculated SOH error can be less than 2.2 percent in realistic settings. A health indicator (HI) extraction and transformation framework to estimate the remaining useful life of the battery (RUL) was proposed by Liu et al. [85]. With the optimized HI, the relevance vector machine (RVM) algorithm will attain the fulfilled RUL calculation. Other data-driven methods have also been implemented for better battery SOH estimation, such as Naive Bayes [84], Bayesian learning [83,86], Bayesian Monte Carlo [87].

Battery Aging

Capacity attenuation and improved internal resistance [107] are two major manifestations of the ageing of lithium-ion batteries. Increasing internal resistance in the operating phase would increase the energy loss of lithium-ion batteries, which will affect the battery's performance.

Internal Temperature Estimation

Battery temperature is another important factor influencing the performance of the battery in many respects, such as lifespan, quality of energy transfer, stability and protection. Using appropriate thermal sensors or thermocouples, the surface temperature is simple to calculate directly. But the battery's internal temperature is an internal state that is hard to directly quantify. The disparity between the battery surface and internal temperatures can be very important (e.g., in high-power systems, often greater than 12 ° C [88]). Overheated internal temperatures will speed up the ageing of the battery and even cause safety concerns such as fires and explosions [89]. Therefore, calculating the surface temperature of the battery is inadequate for battery safety. Proper internal temperature estimating approaches can not only avoid harm to the battery, but also allow BMS to establish appropriate energy saving strategies. The injection of the correct micro-temperature sensors into the battery cell [90,91] is one simple process.



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However, due to the accessory manufacturing specifications and instruction difficulties, these approaches are often of high cost and complexity. A variety of improved battery temperature estimation methods have been suggested, including thermal model-based approaches that typically follow a thermal battery distributed battery model [92] or a thermal battery lumped-parameter model [93,94]. In battery thermal models, the internal temperature is also chosen as one of the state variables, and for on-line internal temperature calculation, different state observers are adopted. These model-based approaches can be easily implemented to estimate battery internal temperature on-line with good estimation results by combining the necessary state observers. Since the battery thermal models focus primarily on heat generation characteristics and thermal boundary conditions information, these model-based methods often have difficult problems such as tuning parameters and collecting valuable thermal information. Considering that the EIS measurements of the battery are also related to the difference of battery temperature [95], the internal temperature of the battery can therefore also be measured using EIS measurements. Srinivasan et al. [96] discovered an inherent relationship between the internal temperature of a cell and an easily detectable electrical parameter, namely the phase change between the sinusoidal current applied and the induced voltage, allowing the internal temperature of the battery to be determined instantaneously.

Analysis of the electrochemical cause of this observed interaction. The value of this approach is that this interaction found is almost entirely independent of the SOC of the battery. In addition, 40-200 Hz is the specified optimum frequency range of the sine excitation signal, allowing a quick calculation, which is another benefit for on-board applications. In order to track the complex temperature activity of the carbon anode in a Li-ion cell during charging and discharging operations, a four-probe measurement technique was subsequently developed [97]. From Zhu et al. [98] also suggested using the calculated EIS data to predict battery internal temperature. The effect of the SOC, SOH and temperature batteries on the EIS measurements is analyzed, and the frequency of the excitation signal is chosen so that the measured EIS is temperature dominant and independent of the SOC and SOH batteries. Rajmaker et al., [99] have suggested methods for battery sensor less temperature calculation using EIS measurements and compared the efficiency of various EIS-based estimates of battery temperature. Methods of estimation [100] under both thermal equilibrium states and complex load conditions. It should be noted, however, that certain methods of battery internal temperature estimation using the measurement of battery impedance at a single frequency can only provide an 'average' battery temperature under inhomogeneous or transient temperature distribution conditions, rather than the temperature distribution area or the peak internal temperature. In addition, to estimate the internal temperature of the battery, data-driven approaches such as neural networks can be adopted. Data-driven approaches that are entirely free of context information can capture the extremely nonlinear performance of battery dynamics. Liu et al., [101] suggested a hybrid data-driven approach to estimate Li-ion battery internal temperature based on the linear neural network model. Good estimation precision is achieved after EKF filtering and this method can be extended to other types of batteries with minor modifications.

Joint State Estimation

The joint state measurement of battery SOC and internal temperature, which plays an important role in certain regulation and equalization applications of batteries, can be accomplished according to coupled electro-thermal models capable of simultaneously representing battery electrical and thermal behaviors. The main step in achieving joint state estimation is to first construct a simple and precise electro-thermal battery model, then it is possible to apply reasonable estimation methods such as slide mode observer, Kalman philter observer accordingly. Bizeray et al., [102], for instance, suggested a thermal-electrochemical model to achieve a combined Li-ion battery calculation. Battery SOC and internal temperature can be determined by an updated EKF after solving the partial differential equations of the coupled model by an orthogonal collocation method. In order to capture battery electrical and thermal activities, Zhang et al., [103] suggested a coupled thermoelectric model. A non-linear look-up table clearly defines the relationship between battery resistance and internal temperature, so it is possible to estimate battery SOC and internal temperature simultaneously.



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Cell Balancing

Considering that several battery cells are attached in series and parallel to a battery module, the mismatch of the capacity and voltage of specific battery cells would cause the battery module to be inefficient. First of all, uneven temperature distribution or ageing characteristics could cause capacity degradation of specific cells for series linked cells. While these cells enter full charge state during the charging cycle, they will continue to charge until the majority of the cells are fully charged. This will cause the degraded cells to overcharge, which will begin to build up to their early failures. The short circuit of one of the cells for parallel cells would cause all the parallel cells to discharge through the failed one, which further damages the module of the battery. Therefore, for BMS, the Cell Balancing feature is required to ensure the reliability of the battery module. Passive Balancing and Active Balancing can be commonly known as Cell Balancing.

Active Balancing

Active Balancing is a way of moving energy from high average energy cells to low average energy cells by means of the flying capacitor Charge Shuttle Charge Distribution Way or the transformer Inductive Shuttle Charge Distribution Method [105].

Passive Balancing

The other approach is to turn unwanted energy into heat passively. The charge frequency of multiple battery cells can be equalized by dissipating the disproportionate energy of the high voltage cells to a bypass resistor. This approach is inexpensive and simple, but it has an efficiency of 0 percent and the time of equalization is very long [105,106].

ENERGY MANAGEMENT AND OPTIMIZATION

We understand that charging process, current rate, number of charging / discharging cycles, SOC, temperature, balance control algorithm are essential performance factors for the battery and battery pack with what is presented in the previous section. In fact, the cycle is always placed on the demanded load during the discharge, so the discharge time is not controllable. As a result, battery performance optimization only takes into account the charging process, while the overall internal resistance varies with temperature. Different charging methods and various equalization techniques of the distributed battery energy storage system will be defined and evaluated from a performance perspective in this section.

Charging Methods of Battery Cell

The battery and battery management system (BMS) are two elements of a battery system in the majority of li-ion battery applications. Battery charging plays an important role in the BMS, where battery performance is highly affected by the charging algorithms.

Constant Trickle Current Charge

To recharge a fully discharged battery or to maintain the charge in a fully charged battery, Trickle charging utilizes a very low current of a magnitude around 0.1C. To prolong its life, a discharged battery is usually trickle-charged for a short period. But with the limit of the usage state, for the entire charging time, the high-performance trickle charging cannot be widely used as the charging algorithm

CC/CV Charging

The constant current- constant voltage (CC / CV) charging algorithm is well developed and widely adopted among all charging algorithms due to its simplicity and simple implementation [108]. A constant current is added to the battery charging for the CC / CV charging algorithm before the battery voltage increases to a predefined safety limit at which CV starts and the charging current is exponentially decreased accordingly. CC / CV can not only support multiple chemistries in this process, but also restrict the current and thus prevent overcharging of the battery, resulting in a high charging efficiency. There are several other versions of the CC / CV charging algorithms besides

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the traditional CC / CV charging algorithm, such as the double-loop power charger (DL-CC / CV) [109] and the boost charger (BC-CC / CV) [110], which are updated by the traditional CC / CV charging algorithm. A charging configuration very similar to the standard CC / CV can be accomplished by the DL-CC / CV, and without detecting the charging current, the same output is accomplished in the hardware implementation at the simplest and lowest cost, thus improving reliability. However, prior to charging, the BC-CC / CV requires complete discharge of the battery, which makes this charging algorithm inefficient. To apply the CC / CV, named as the FL-CC / CV [111] and the GP-CC / CV [112], two more algorithms using the fuzzy-logic control and the grey-predicated control. Two more algorithms, called FL-CC / CV [111] and GP-CC / CV [112], respectively, use the fuzzy-logic control and the grey-predicated control to execute the CC / CV. The charging current of the CV mode of these versions of the CC / CV charging algorithm is greater in the higher current portion and smaller in the lower current portion than the current in the CV mode of the normal CC / CV charging algorithm. Therefore, over the same time of the CV mode, more power can be charged into the battery, leading to a higher charging efficiency. And several other algorithms, such as PLL-CC / CV, IPL-CC / CV, etc.

Optimal Charging

Optimum lithium-ion battery charging is studied in [113], which focuses on optimizing the performance of Li-ion battery charging. Electronic resistance and the effect of polarization resistance are studied in this method. With two nonlinear differential equations, this problem structure results in a two-point boundary value question. To solve this problem, optimization methods are used. And the result shows that for different initial SOC, the optimal performance varies and the start of charging from a higher initial SOC results in better charging performance. Centered on fuzzy logic [114], neural networks [115], grey system theory [116], genetic algorithm [117], and ant-colony system algorithm [118], more complex optimal charging strategies have been proposed, which can also result in better charging performance.

Multistage Current Charging Algorithm (MSCC)

One of the most popular methods for fast charging is the multi-stage constant current charging (MSCC)[119] method. First of all, the batteries are subject to various charging patterns and their respective charging times (CT) and discharge capacities are registered. To find the optimum charge pattern (OCP), various optimization methods are then used. The charging speed is usually higher and the charging performance is better than that of the CC / CV.

Pulse Charge

A swift and powerful charging algorithm for lithium-ion batteries [120] has been claimed to be the pulse charge. Using an electronic network model, the effects of the pulse charge on lithium-ion batteries were analyzed. The findings of the simulation give insight into the effect of the pulses, such as diffusion, displacement, electrochemical reactions and heat generation, on the internal phase. The charging time is seen to be less than that of the CC / CV and the charging performance is higher and the life of the cycle is longer.

Energy Management of Battery Pack

Due to manufacturing method limitations and different charging / discharge conditions, there are often unavoidable variations between batteries in terms of power and internal resistance with repeated use, which eventually results in inconsistent stage-of-charge (SOC) and causes the battery pack power to decrease and over-discharge / over-charge even battery lifetime reduction [121]. The charge equalization controller is therefore necessary to keep the battery stack SOC stable, significantly extend the service life and boost the performance and efficiency of the lithium-ion battery.

PI Controller

A PI controller based on a closed loop is implemented to control the balance current. In dealing with extremely non-linear balancing systems during the balancing process, the PI controller with constant parameters is not adaptive.





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The device is not stable if these parameters are too high, especially when the voltage gap is small. The balancing current is too weak if these parameters are too low, resulting in slow balancing speed and low efficiency [122].

The Fuzzy Logic Controller (FLC)

The FLC [123] is designed for balancing circuit power. It turns out that the FLC will change the balancing currents adaptively. If the voltage gap is large, a large balancing current can be set to speed up the balancing process. It will set small balancing current to minimize energy consumption when the voltage difference is small. This increases the speed of balancing and the effectiveness of balancing. In each voltage case, this FLC has greater adaptability compared to the PI controller and does not need to be tuned. The performance from both speeds is enhanced.

Intelligent Control

We understand from the analysis that the balancing controller is commonly built with different balancing structures. Different optimal methods are used for different optimal objectives to boost the optimization of the parameters of the PI controller, which will lead to better efficiency [124]. Particle swarm optimization (an equalization control algorithm) is one of these optimal methods that has been used to optimize the parameters of the PI controller that produce the converter’s controlled pulse width modulation switching signal.

Li -ION BATTERY CHARGING AND DISCHARGING

CVL & DVL of Battery

That’s why an electric car must control its battery power by means of a battery management system. Electric vehicle rely on battery operated electricity. The reliability of car batteries in electric car is a great prospect. For each battery to operate, there is a healthy voltage range. In this range, to prevent damage to the battery, the battery should be charged and discharged. The Battery, Therefore, if the cell in the serial battery stack exceeds the limit of the charging voltage or the controlling feature of the discharge voltage, or if the discharge phase must be halted without destroying the cell, it has a charge voltage limit (CVL) and discharge voltage limit (DVL) [134,135]. In the configuration of the battery safety system, this should be taken into account in the requirements. CVL and DVL range of a battery shown in fig.7.

Battery Discharging Process

In electric vehicles, the control of the discharge of batteries. It is relevant because it checks the available battery power and calculates the available battery range. During the battery C_n , battery nominal capacity (Ah), I working current(A)The battery balance is carried out using the support in the evaluation when considering the characteristics of the voltage drop. And by comparing the currents, the balancing current is measured. The cost per battery. You can determine the voltage dropping factor as follows [136].

$$r_v = - \frac{du}{dt} \dots\dots\dots(15)$$

Where,

U_n – battery nominal voltage (V), r_v – battery working current (V.h-1), u – battery working voltage (V)

Battery Charging Process

There are several charging approaches used for Li-ion battery charging. Ongoing current charging is the most common solution. Descriptions of the optimal current for charging are provided in the battery data sheet. The battery charge voltage of the battery is much higher than the discharge voltage at the same charge state. This is because when the battery is running, any voltage drops. It is packed with internal resistance. The battery is supplied with a charging voltage far greater than the discharge to tackle this issue. The Li-ion battery is usually charged at constant current, and the constant voltage battery charge is carried out until the maximum charge is completed before the



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constant current shut-off voltage is reached. The battery voltage is increased when the constant current is charged and the charging current is greatly reduced when it is shifted to a constant voltage. The existing minimum number is known as the maximum charge. Fig.9 explain the charging characteristics of Li-ion battery. For Li-ion batteries, there are several different charging methods accessible. One of them is Fast Charging. Not all Li-ion batteries have fast charging capabilities. High-power Li-ion batteries are capable of fast charging, such as the Valance IFR26650PC high-current battery and the A123 high-current ANR26650m1B battery. A battery is charged up to 80 percent of its SOC value at a steady high current rate during fast charging. And, it charges the remaining 20 percent total at constant voltage. A Charging Control Unit is installed in electric vehicles. The technology is known as the device for smart charging and that is shown in the fig.10. Charges are configured by the smart charging unit's users. The available battery charge was determined by the charging control system and previous running cycles were controlled by the vehicle. Then, with the user's preference in mind, the machine creates a determined charge pattern. A management framework is defined in for smart charge charging [137]. A time-controlled charging pattern is described. Charging time is estimated by coordination between the battery control system and the energy management system [137]. The energy monitoring system provides a time estimation by the power management system of how long it is essential to monitor the charging time and power. The user's perspective is not provided here. Based on the battery management system's response, the energy control system measures the charging time. The fig.11 shows the charging pattern mechanism that is controlled by time.

FUTURE TRENDS OF SMART BATTERIES

A promising research field for BMSs is the smart battery. For smart batteries, there are some potential trends:

- Communication via wireless. Wireless systems can achieve low costs due to the drop in connectivity elements such as wiring harnesses and connectors. This also decreases the sophistication of construction and performance. Furthermore, there would be a substantial improvement in versatility and efficiency since there are no specifications for fixed communication wiring [125].
- Direct juggling technique for bypass. Direct bypass circuits require only a few switches to bypass or insert cells, unlike dc / dc converter balancing circuits. The benefits of direct bypass are high efficiency, easy and simple.
- The stability of BMSs can be improved by wireless connectivity. In certain tough situations, such as high vibration environments for EVs, it may handle the possible physical breakdown of connectors, cables and harnesses.
- The direct bypass cycle allows the cells rest time to complete chemical reactions, so it is possible to prolong the life of the battery cells and the entire battery pack. In high-frequency mode, the dc / dc converters are worked, so the bypass time is too short to be an adequate rest period for cells.
- The cost of the system rises due to the large number of controllers compared to traditional BMSs. Wireless communication technology used in smart batteries, however, can lower the cost of wiring, and the simple half-bridge circuit with fewer components can also save the cost. The cost advantages. Control, low switching frequency, fewer parts, and low cost [126] are therefore provided in the fourth method.
- Strategy for SOC and SOH decisions. The SOC is able to represent better cell states compared to the voltage decision strategy and is thus more suitable for direct bypass circuits. Many researchers have contributed to SOC estimation in recent years and obtained those outcomes. In addition, SOH is supposed to be part of the decision strategy and to combine with SOC in order to evaluate cell states and also to realize cell balancing [127].
- Regulation of rest time. The rest time provides the cells with healing time, which can reduce cell tension. Combined with a direct bypass balancing strategy, rest periods can increase the battery capacity and increase the battery life [128].
- Architecture Spread. With the advancement of wireless networking systems, without being limited by communication cables, cells can communicate with each other. The master controller will be eliminated and the battery mechanism will improve in stability and flexibility [131].
- Fast charging pulse frequency technique [129]-[130]. The pulse frequency technology decreases the charging time for low-temperature batteries by around 1°C and thus increases the life of the battery.



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- Reconfigurable Architectures for battery management. Reconfigurable techniques can randomly regulate the number of cells to be connected in series or in parallel, thereby allowing the battery pack voltage or power to be increased or decreased [132]. The power, lifespan, protection and reliability of the battery packs can be improved by the reconfigurable battery system [133].

CONCLUSIONS

Key technologies in the BMS of EVs have been reviewed in this paper, especially in the fields of battery modelling, state estimation and battery charging. Battery modelling together with the estimations of battery internal states and parameters play a vital role in revealing a hologram of battery operating status in the applications of EVs firstly. After capturing these key states, suitable battery charging approach can be designed to protect battery against damages, improve efficiency of energy conversion, and prolong the battery lifetime. However, most of the key technologies in the BMS are achieved and validated in specific test conditions. The modelling, estimation and charging performance in real-world applications that would be different from the test conditions, or in a worse-cases scenarios difficult to guarantee. Therefore, to explore the limitations or to develop a confidence interval of the presented algorithms and approaches are required to tackle this challenging issue.

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AUTHORS PROFILE

Smitanjali Rout is pursuing her Doctoral Fellowship in Electrical and Electronics Engineering Department of Centurion University of Technology and Management, Bhubaneswar Campus. She completed M. Tech. from the same university. Her interest is in AI/ML application to renewable energy resources.



Dr. Abhinna Chandra Biswal completed M. Tech and Ph. D. in Electrical Engineering Department, IIT, Kharagpur. He is currently serving as Professor in Electrical & Electronics Engineering department of Centurion University of Technology and Management, Bhubaneswar, prior to this he was working in ABB Ability Innovation Center. His research interests are Deep Learning, Machine Learning, Electricity market, Power System Analysis.





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Table 1 Popular types of battery in EVs

Battery Type	Life Cycle	Nominal Voltage(V)	Energy Density (Whkg ⁻¹)	Power Density (Wkg ⁻¹)	Charging Efficiency (%)	Self – Discharge rate	Charging Temperature (°C)	Discharging Temperature (°C)
Li-ion	600-3000	3.2-3.7	100-270	250-680	80-90	3-10	0 to 45	-20 to 60
Lead acid	200-300	2.0	30-50	180	50-95	5	-20 to 50	-20 to 50
NiCd	1000	1.2	50-80	150	70-90	20	0 to 45	-20 to 65
NiMH	300-600	1.2	60-120	250-1000	65	30	0 to 45	-20 to 65

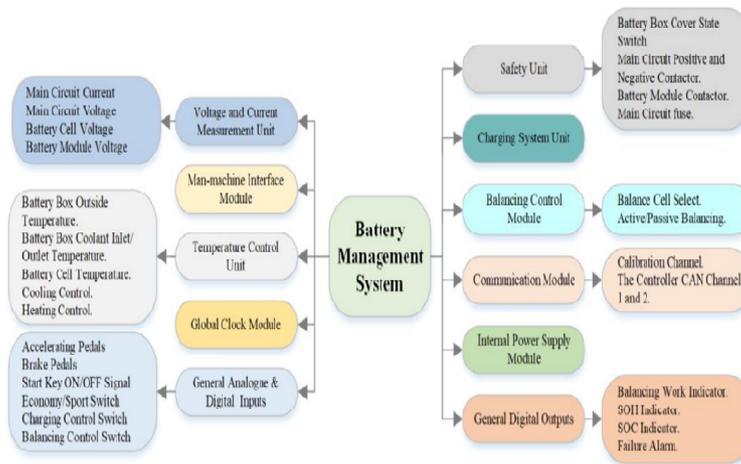


Fig.1: The relation of key technology in the BMS

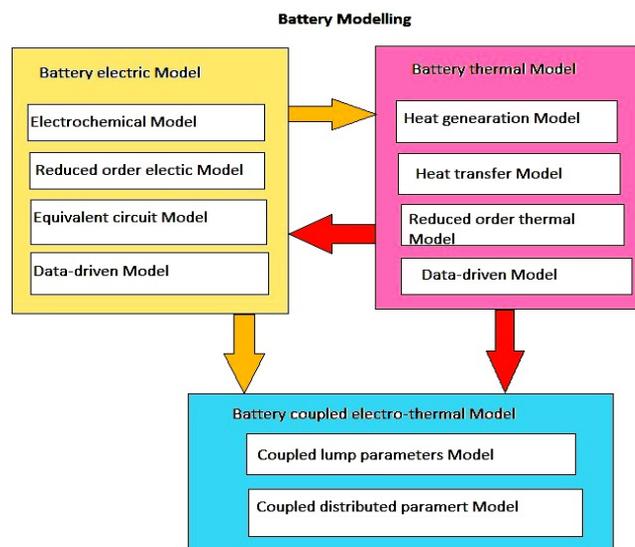


Fig . 2: Three classifications of battery modeling





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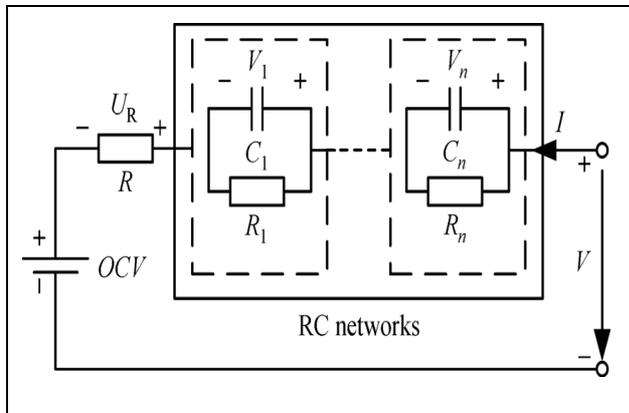


Fig. 3 Typical framework of a battery equivalent circuit model.

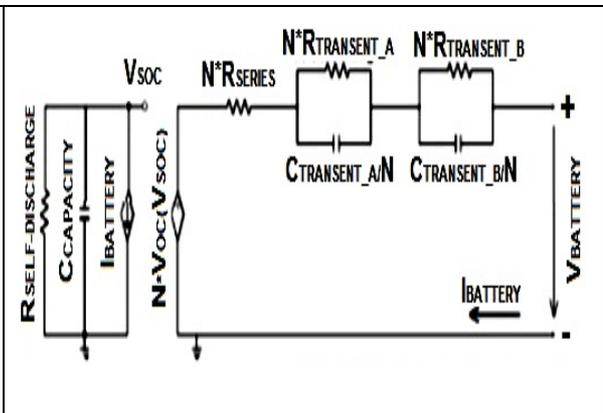


Fig.4: Improved Thevenin-based electrical Battery Model

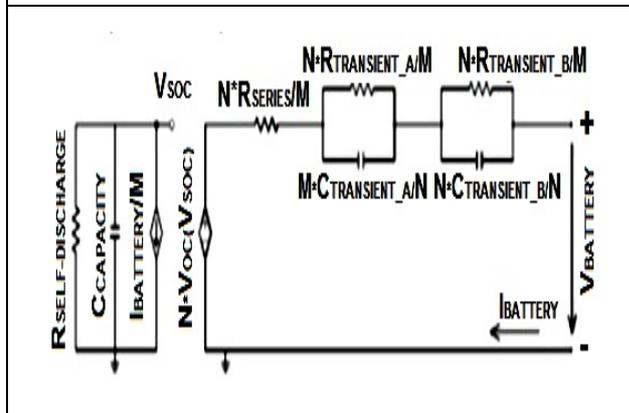


Fig. 5: Improved Thevenin-based electrical battery model for Battery Module

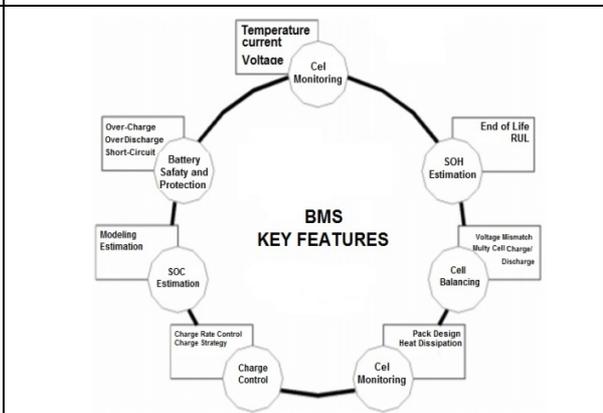


Fig.6: The BMS features [105]

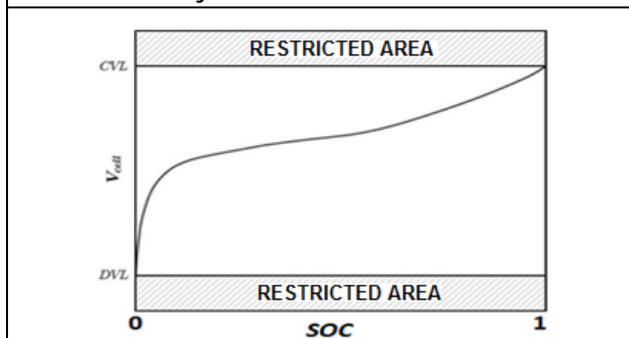


Fig.7: CVL and DVL range of a battery

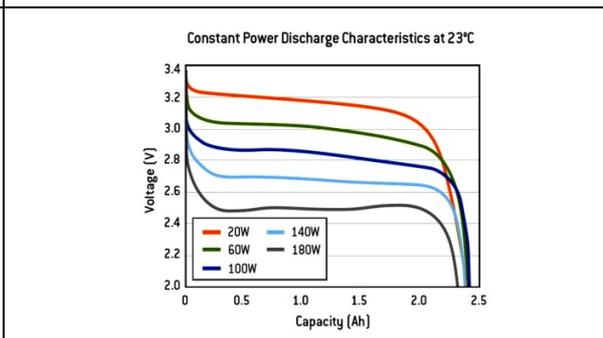


Fig. 8: Battery voltage diagram to different discharging capacity





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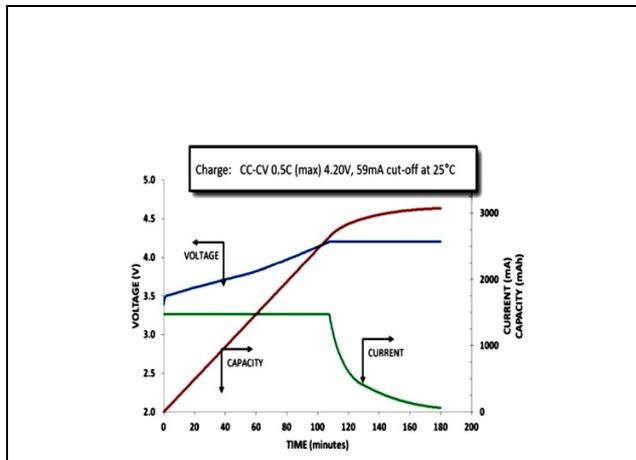


Fig.9: Charging characteristics of Li-ion battery

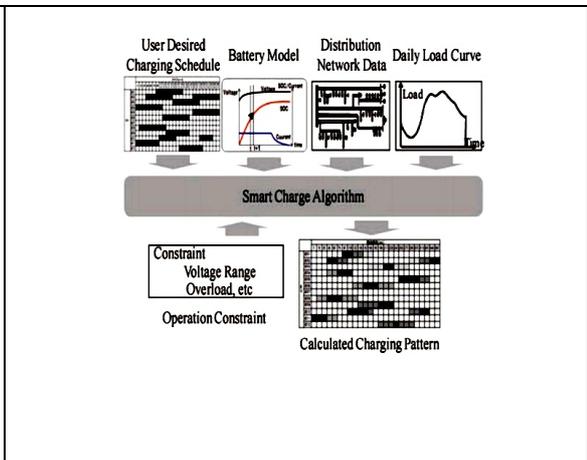


Fig.10: Smart charging process of electric vehicles [137].

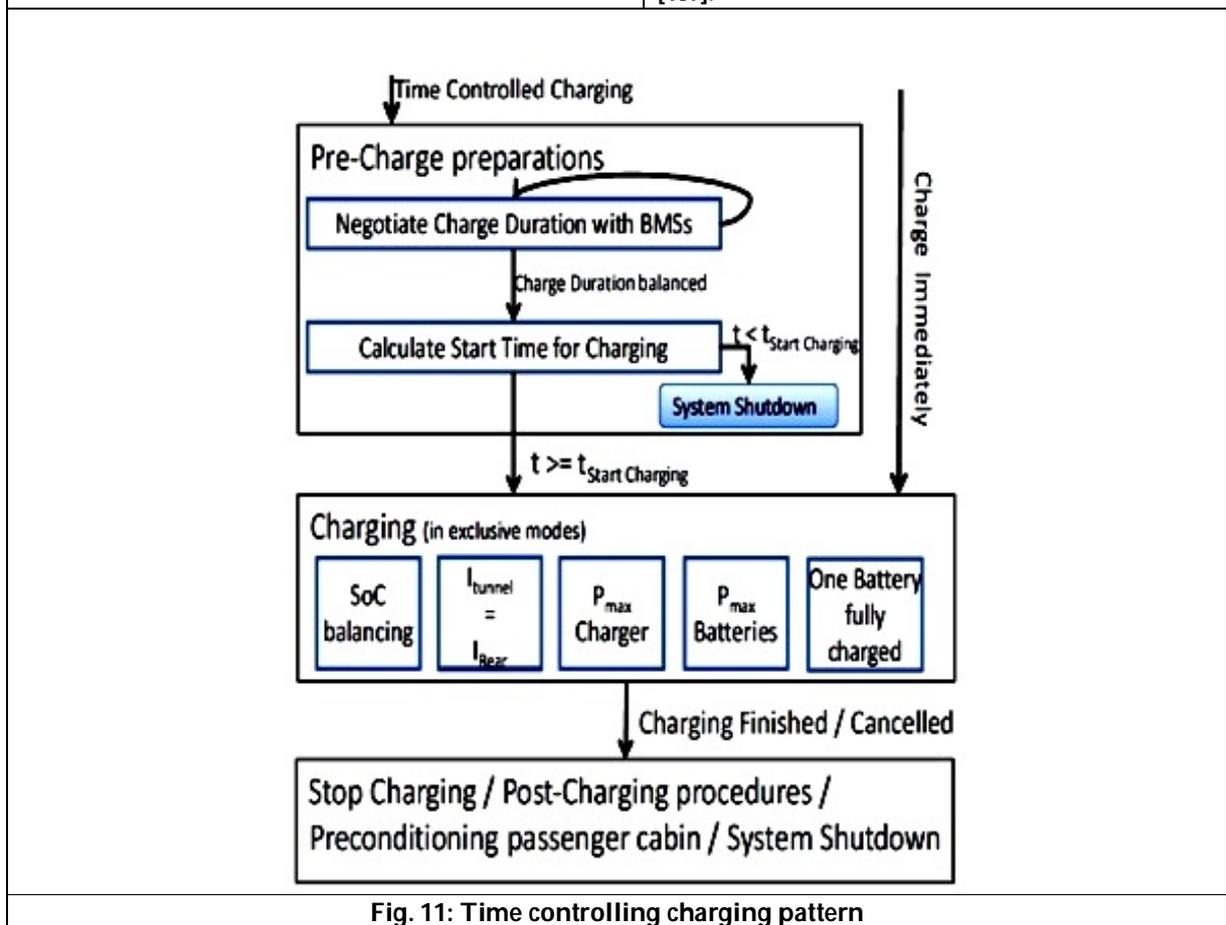


Fig. 11: Time controlling charging pattern





Studies on Fish Diversity in Sanaghagara Waterfall of Keonjhar District

Sukanya singh¹ and Sitaram Swain^{2*}

Department of Zoology, School of Applied Science, Centurion University of Technology and Management, Odisha, India.

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*Address for Correspondence

Sitaram Swain

Department of Zoology, School of Applied Science,
Centurion University of Technology and Management,
Odisha, India.

Email: sitaram.swain@cutm.ac.in



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ABSTRACT

India is highly diverse in nature having a wide range of fish fauna. Odisha exhibit about 13.92% of total freshwater fish fauna of India. Odisha is highly blessed with a wide range of fish fauna. This fauna should not be used only for economic purposes, also necessary to save the aquatic environment. There are 32,000 species of fishes in the world. Fresh water fishes are important component of global biodiversity. Varied freshwater resources of Orissa blessed with diverse fish fauna. *Cypriniformes* is the most dominant order and *Cyprinidae* is more diverse family. This study has conducted to observe the fish diversity near sanaghagara waterfall at Keonjhar district during November 2019 to February 2020. There are about eleven species identified during this study. It is necessary to gain knowledge about the fish diversity. This study may provide an information about assessment of fish diversity and management of fisheries in this local tourist area of Keonjhar district.

Keywords: Fauna, Sanaghagara waterfall, fish diversity, cyprinidae.

INTRODUCTION

India is highly diverse in nature having a wide range of fish fauna. Odisha exhibit about 13.92% of total freshwater fish fauna of India. Odisha is highly blessed with a wide range of fish fauna. This fauna should not be used only for economic purposes, also necessary to save the aquatic environment. There are 32,000 species of fishes in the world [1]. Fresh water fishes are important component of global biodiversity [2]. Fish is considered as gill bearing, cold blooded, aquatic vertebrate that lacks limbs and lives wholly in water and breathes using lungs mostly. Fish diversity indicates the various types of fish fauna present in a particular habitat. The development of fisheries in these fresh water resources needs to be increased through the scientific development. In the present investigation, species diversity, distribution management was studied in sanaghagara waterfall in keonjhar district. The diversity of fish species and distribution are determined by biotic and abiotic factor operating on a range of spatial scales. Fishes are

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most diverse among vertebrates but have not been well studied. Currently, the global fish diversity has been estimated to be about 28,900, which could increase to 32,500 when the World's ichthyofauna has been completely inventoried [3]. The fresh water fish distribution pattern varies globally [4]. Each country has a specific fish fauna, but most of the species belong to five taxa: the *Siluriformes*, the *Cypriniformes*, the *Charciformes*, the *Perciformes* and the *Cyprinodontiformes*. Fish populations in India are about 11.72% of species, 23.96% of genera, 57% of families and 80% of the global fishes. The uniqueness of majority of the freshwater ecosystems in terms of habitat features and fauna make them extremely vulnerable to change [5]. As a consequence, the global freshwater biodiversity is declining more rapidly than the terrestrial biodiversity. Freshwater fishes are highly threatened taxa [6]. Habitat alteration, pollution, overexploitation and introduction of non-native species were identified as the main causal factors for the rapid decline of fish species.

MATERIALS AND METHODOLOGY

The entire study was undertaken in the Sanaghagara in the locality of Keonjar district. The waters course of Sanaghagara is "Machha kandana". The height of this waterfall is about 30.5 metres and it has its significance in tourism of Odisha. It spreads over 488 about 0.8 Km away from Keonjhar town of Odisha. The area spread over 488 Hectare. This study site enriches with biodiversity and mixed with forest vegetation All the fishes are collected from the local fishers of a nearby village of Sanghagara. The study was conducted from the months of September, 2019 to January, 2020. Twelve numbers of samples were collected in that waterfall. Most of the fishes have been collected with the help of fishermen directly from the waterfall area and some fishes are also collected from nearby market. Collected fishes were preserved in 10%formalin in the laboratory for the further analysis. Majority of the fishes were identified on the study site and rest were identified with the help of key [7,8] .

RESULT

These collected species were identified and arranged in phylogenetic hierarchy. These were identified and arranged according to their taxonomic order, family, genus and species. The economic importance and their conservation status were taken into consideration while doing so. From this study, Cyprinidae family has shown the most abundant family contributing 50% of the total species of all families found in Sanaghagara waterfall. During this biodiversity study about eleven fish species has found in the study area. The list of fishes available during survey period that happens on September (2019) to February(2020) in the area. The study recorded 11 species belonging 7 families and 5 orders. *Mola carplet*, *Elongate glassy perchlet*, *Nile tilapia*, *Mystus gulio*, *Puntius sophore* ,*Catla catla* ,*Labeo rohita*, *Ailia coila*, *Pangasius bocourti*, *Bronze featherback* and *labeo calabasu* are collected and identified during the study Edible fish means fish which are safe for the human consumption and ornamental fishes are used as decorative purpose. These animals are well known because they can be seen every day in the home aquarium, ornamental pond, pet store, and public aquarium [9, 10, 11], while at the same time they are unknown because knowledge regarding their health care is limited.

CONCLUSION

The fresh water fishes are the most diverse vertebrate taxa, yet the least studied group because of their complex life history pattern. However, our results showed that the species richness of fishes was strongly influenced by stream size. The larger streams had more number of species than the small stream. However, the freshwater fishes are also the most threatened group after amphibians because of constant pressure on their habitats by human activities. Therefore it would be important to inventory and to understand the relationship between fish and habitat particularly in waterfalls. The present work on fish diversity of Sanaghagara waterfall clearly shows that this particular area is endowed with variable type of fishes. Most of the species are consider as economic and edible



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fishes. The study clearly indicates the abundance of the species mostly belonging to the family cyprinadae and order cypriniformes. Study on their habit and habitat are required for assessing their export potential looking at the demand in the international market. This diversity is not only the wealth of our world but it also has some serious implications on fishery. Thus there is an urgent need for proper investigation and documentation of this fish diversity in order to develop a fresh water fish diversity.

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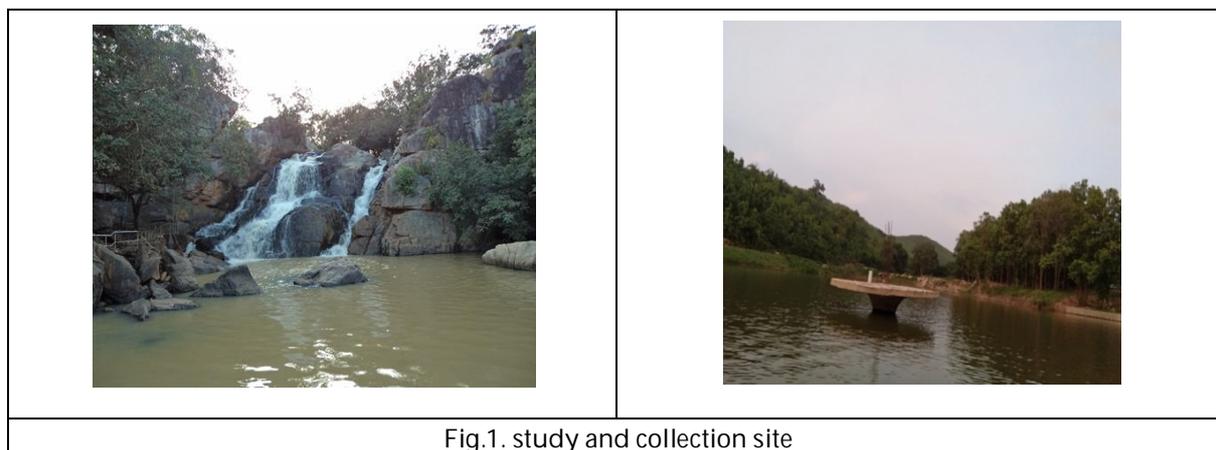


Fig.1. study and collection site





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Table.1 Fish species collected from Sanaghagara waterfall

SI No.	common name	Scientific name	family	Order
1	Elongate glassy perchlet	<i>Chanda nama</i>	Ambassidae	<i>Paciformes</i>
2	Mola (mahurali)	<i>Mola carplet</i>	Cyprinidae	<i>Cypriniformes</i>
3	Pool barb (karandi)	<i>Puntius sophore</i>	Cyprinidae	<i>Cypriniformes</i>
4	kantia	<i>Mystus gulio</i>	Bagridae	<i>Siluriformes</i>
5	Kala bainshi	<i>Labeo calabasu</i>	Cyprinidae	<i>Cypriniformes</i>
6	Rohu (Rohi)	<i>Labeo rohita</i>	Cyprinidae	<i>Cypriniformes</i>
7	Catla (Bhakur)	<i>Catla catla</i>	Cyprinidae	<i>Cypriniformes</i>
8	Basa	<i>Pangasius bocourti</i>	Pangasiidae	<i>Siluriformes</i>
9	Bronze featherback (fali)	<i>Notopterus Notopterus</i>	Notopteridae	<i>Osteoglossiformes</i>
10	Mango fish	<i>Nile tilapia</i>	Cichlidae	<i>Cichliformes</i>
11	Gangetic ailia	<i>Ailia coila</i>	Ailliidae	<i>Siluriformes</i>

Table.2 Showing the fish species are edible or ornamental

SI No.	Common name	Edible	Ornamental
1	Elongate glassy perchlet	+	+
2	Mola carplet	+	+
3	Pool barb	+	–
4	Kantia	+	–
5	Kala bainshi	+	–
6	Rohu	+	–
7	Catla	+	–
8	Basa	+	–
9	Bronze featherback	+	+
10	Mango fish	+	–
11	Gangetic ailia	+	+

