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RECENT RESEARCH OUTCOMES IN SCIENCE

Editor: **Shanij K.**

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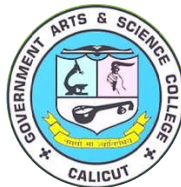
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Shanij K.



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Foreward

It is my previlage and pleasure to present before you the 2nd issue of the 11th volume of Government Arts & Science College Research Journal. This issue is a collection of research articles and review papers from different fields of science that heighlights the theme of the issue “Recent Research Outcomes in Science.” The authors have put sincere effort to identify the gaps in their respective fields and put forward conepts and findings that substantially advances our understanding in Science. I expect this issue to spark an intellectual discussion among the academic and scientific communities and acknowledge and appreciate all those hand and hearts that worked to publish this issue of the Journal.

Dr. Edakkotte Shaji
Principal
Govt. Arts & Science College Calicut

PREFACE

Indeed, it is a delightful moment to write preface to the new issue (Volume - 11, Issue - 2) of the Government Arts and Science College Research Journal. This issue contains research articles and review papers from different disciplines of science. In a time of great change across various fields of science and technology, scientific publications have got their own relevance in substantially advancing scientific understanding in respective fields.

Government Arts and Science College Research Journal provides exciting opportunity and a suitable platform to researchers for publishing their research findings. Sharing of scientific knowledge increases circulation and use of data within the scientific community by encouraging better transparency, enabling reproducibility of research, and informing the larger scientific community. Articles contained in this issue present original research works in different scientific fields that contributes to the wider understanding in respective areas. Review papers, on the other hand, provide comprehensive and contemporary review of a particular theme.

This issue of the Journal is opened with a research article in Statistics entitled 'Non-parametric polynomial density estimation for circular data like wind direction'. In this paper, the authors have proposed a non-parametric estimator for the probability density function of circular data using a sequence of real polynomials.

In the second paper entitled 'Genotoxicity and bacteriological analysis of drinking water sources at an Endosulfan affected region of Kasargod', the genotoxic effect and bacteriological analysis of drinking water sources from one of the Endosulfan affected regions of Kasargod District in Kerala were evaluated. They found out that bacteriological

monitoring approach can detect contamination related issues before drinking water consumption in the exposed regions.

The next article deals with the assessment of the Butterfly diversity of Vimala college campus. The authors have in detail assessed the butterfly diversity of Thrissur Vimala College campus and determined the species diversity and richness of different families. The study revealed 18 species of butterflies belonging to the 5 families with the Family Nymphalidae being represented the most.

The fourth article is a Physics paper that discusses about Z-scan measurements and optical limiting studies of P-nitroaniline. The non-linear optical studies of Z-Scan technique with nanosecond laser pulses at 532 nm was utilized to evaluate the non-linear optical effect embedded within the PNA compound. The obtained nonlinear absorption coefficient and the lower optical limiting threshold pronounce the prominent utility of PNA material in the opto-electronics domain and for the fabrication of optical limiting devices.

The next paper is a communication regarding a new record of cockroach hunting wasp from southern India. The article describes that the species *Ampulex ruficornis* (Cameron) is recorded for first time from southern India and that the key to Indian species of Ampulex Jurine by Anagha & Girish Kumar (2019) is partly modified.

Next paper comes with a discussion on current perspective towards construction and demolition waste management. Authors express the concept that understanding of Construction and Demolition Waste (CDW) is very essential in the thriving economy and its management is one of the current global priority areas. It is a detailed review of the current practices and challenges that influences the construction and demolition waste management in India and around the world. In addition, the study also reviews the recent research in managing CDW.

In the next paper authors have provided new data on the distributions of two crabronid wasp species of the genus

Palarus Latreille, 1802 (Hymenoptera: Apoidea: Crabronidae), viz., *P. comberi* Turner, 1911 and *P. interruptus* (Fabricius, 1787) collected from different localities of India. Intraspecific variations are also discussed in the paper.

Eighth article in this issue is a review about cancer chemoprevention by natural products, which focused on the cancer chemoprevention by natural products in culture as well as animal models. Authors hold that plant extracts and natural compounds purified from plants have been used by humans for many centuries for the treatment and alleviation of a variety of inflammation-related diseases, including cancer. Natural compounds with inhibitory effects on tumorigenesis have been identified from our diet or sources of the diet.

In the ninth article, the authors discuss the results of a comparative study of the super-cooling process and dielectric relaxation of three active pharmaceutical ingredients (APIs) Bezafibrate, Acemetacin and Colchicine. The study was done by broadband dielectric spectroscopy technique in a temperature range of 450K to 123K and in a frequency range of 10mHz to 10MHz to characterize the temperature and frequency dependence of complex dielectric permittivity to estimate the glass forming ability and physico-chemical stability of their amorphous phase. This work, titled 'Dielectric studies of the glass formation in significant active pharmaceutical ingredients: Acemetacin, Bezafibrate and Colchicine' gives a comparison between the dielectric relaxations in an alkaloid drug (Colchicine) derived from plants and synthetic drugs Bezafibrate (fibrate class of drug) and Acemetacin (NSAID class of drug).

The next study reports Butterfly diversity and their feeding preferences in Akode village in Malappuram district in Kerala. The study revealed a total of 45 species of butterflies belonging to five families (Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperidae). Authors have statistically analysed the diversity of butterflies with respect to changing temperature and humidity. Feeding preferences of butterflies on various host plants have also been assessed.

The last article is a Scientific rebut to social defamation done on the constituents of other state clay pots. Authors express the view that the quantitative and qualitative method of analysis could give an exact knowledge on the constituents of the clay pot samples under study and falsify the claims made on the constituents of other state pots. The study is a warning to the common man from making unscientific conclusions and spreading them in the public.

As a concluding remark, I take this opportunity to thank all the contributors and supporters of the journal. On behalf of the Editorial Board, I acknowledge the support given by the Principal, Dr. Edakkotte Shaji. We also acknowledge the support from all the staff members for their direct and indirect involvement in the publication of the Journal.

Shanij K
(Editor)

NON-PARAMETRIC POLYNOMIAL DENSITY ESTIMATION FOR CIRCULAR DATA LIKE WIND DIRECTION

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Abstract: *We proposed a non-parametric estimator for the probability density function of circular data using a sequence of real polynomials. A likelihood cross-validation procedure was used to obtain the smoothing parameter of the estimator. Using the new circular non-parametric polynomial estimator, we fitted the distribution of wind directions using actual daily wind direction data for two years (2000 and 2017), obtained from the Department of Agricultural Meteorology of Kerala Agricultural University, Kerala, India. A comparison of the two charts found no significant changes in the distribution of wind direction between 2000 and 2017.*

Keywords: *actual daily wind direction data, fitting circular distributions, wind direction, non-parametric estimator, likelihood cross-validation procedure.*

Introduction

Circular statistics play an important role in statistical analyses, as numerous data are not linear. Some data are presented as points on the circumference of a circle. For example, if we calculate the arithmetic mean of two angles, say 5° and 355° , in the usual way, we obtain $(5+355)/2 = 180^\circ$ which is illogical as the actual average is 0° , since 360° is the

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same as 0° in the angular measurements (i.e., if the values are $355^\circ, 356^\circ, 357^\circ, 358^\circ, 359^\circ, 0^\circ, 1^\circ, 2^\circ, 3^\circ, 4^\circ, 5^\circ$, the middle value is 0° not 180°).

Various statistical measures for circular data analysis have been explained by Mardia (1976), Batschelet (1981), Fisher (1993), Mardia and Jupp (2000), and Jammalamadaka and Sengupta (2001). Kamisan et al. (2010) assessed the fit of four circular distribution types for the wind direction of the south-westerly monsoon in Malaysia and found that the von Mises distribution was the best fit for the data.

Other distribution types include the wrapped exponential and Laplace distributions (Jammalamadaka and Kozubowski, 2001, 2003, 2004), wrapped skew normal distribution (Pewsey, 2000, 2006), wrapped t distribution (Kato and Shimizu, 2005), probability density function (pdf) for circular log normal, circular logistic, circular Weibull, and circular extreme value distributions (Dattathreya Rao et al., 2007) wrapped normal–Laplace distribution (Reed and Pewsey, 2009), and wrapped Linnik, wrapped geometric stable, wrapped geometric, and wrapped Laplace distributions for integers (Sophy, 2012).

Rosenblatt (1956) introduced a non-parametric density estimation. Prakasa Rao (1983) recounts the early developments in non-parametric functional estimation, as do Silverman (1986), Marron (1987), and Sain and Scott (1996, 2002), and Kim and Scott (2011). Silverman (1981) reported that non-parametric density estimates are useful at all stages of the statistical treatment of data. Hamza (2009) introduced the concept of polynomial density estimation for fitting non-parametric distributions to linear data.

Here, we introduce a new non-parametric polynomial density function for circular data, with the optimal degree of the polynomial k estimated using a cross-validation technique. This new tool is applied to daily wind direction data collected in 2000 and 2017 in the Thrissur district of Kerala by Kerala Agricultural University.

Estimation

Non-parametric polynomial density estimation is used to estimate the distribution of a circular variable. The appropriate degree of the polynomial (k) is fixed using an objective criterion based on the data sample.

We used a technique similar to the kernel method, a concept suggested by Rudin (1976) to prove the Stone–Weierstrass theorem. The Weierstrass approximation theorem is important in mathematical analysis, as it states that every continuous function defined on a closed interval can be uniformly approximated as a polynomial.

Kernel method of estimation

Let X_1, X_2, \dots, X_n be independently and identically distributed (i.i.d.) observations drawn from an unknown distribution function $F(\cdot)$ and probability density function $f(\cdot)$. Here the density function was estimated directly from the data with the help of a series of real polynomials with support $[a, b]$. Without loss of generality, it can be assumed that $a=0$ and $b=1$.

Clearly, the empirical distribution function or sampling distribution function is an unbiased and consistent estimate of F . However, it is a step function and not differentiable. Instead of estimating f directly, we estimate f as:

$$\hat{f}(x) = \int_{-\infty}^{\infty} \frac{1}{h} K\left(\frac{x-t}{h}\right) dF(t)$$

where K is the desired kernel function.

Replacing $F(t)$ with $F_n(t)$ yields:

$$\hat{f}(x) = \int_{-\infty}^{\infty} \frac{1}{h} K\left(\frac{x-t}{h}\right) dF_n(t)$$

where $F_n(\cdot)$ is the sequence of functions estimated from the data.

Then the estimator will be:

$$\hat{f}(x) = \frac{1}{nh} \sum_{i=1}^n K\left(\frac{x - X_i}{h}\right)$$

for every x in \mathbb{R} , where h is band width and K is kernel function. Two fundamental questions are:

- (1) Choice of kernel function (K)
- (2) Choice of band width (h)

In general, K can be any continuous probability density function, and h tends to zero as n tends to infinity. K selection is not that important, but h selection is critical, with two techniques used in practice:

- (1) Least squares cross-validation
- (2) Likelihood cross-validation

Non-parametric polynomial estimator

We propose to replace K with a polynomial of the form:

$$Q_d(x) = c_d(1-x^2)^d, \quad d = 1, 2, 3, \dots \text{ and } -1 < x < 1$$

where c_d is chosen such that:

$$\int_{-1}^1 Q_d(x) dx = 1$$

Next, we define the non-parametric estimator $f_n(x)$ of f as:

$$\hat{f}(x) = \frac{1}{n} \sum_{i=1}^n Q_d(x_i - x)$$

$$\hat{f}_n(x) = \frac{1}{n} \sum_{i=1}^n c_d \{1 - (x_i - x)^2\}^d \dots\dots\dots (1)$$

The degree of the polynomial d is the smoothing parameter of the polynomial estimator and c_d 's are calculated as:

$$c_d = \frac{1}{2} \frac{(2d+1)!}{2^{2d} d!^2}$$

The optimal degree of the polynomial is chosen in a similar way to the optimal bandwidth in the kernel estimator, and based on the quantity $CV(d)$ given by:

$$CV(d) = \frac{1}{n} \sum_{i=1}^n \ln[\hat{f}_{-i}(x_i)]$$

$$\hat{f}_{-i}(x_i) = \frac{1}{n-1} \sum_{\substack{i=1 \\ j \neq i}}^n Q_d(x_j - x_i)$$

where

Hamza (2009) studied the properties of such estimators, which are also extended for right-censored data.

The proposed estimator belongs to the general class of estimators.

Circular case

Let X_1, X_2, \dots, X_n be a continuous sequence of circular variables with probability density function (pdf) f , which we are trying to estimate and support $(0, 2\pi)$. Here, we introduce a non-parametric estimator of f for the circular variable using a sequence of real polynomials. In place of K used in kernel estimators, consider a polynomial of the form:

$$\text{Let } T_k(x) = U_k(1 - \sin^2 x)^k, \quad k = 1, 2, 3, \dots$$

Clearly, $T_k(x) \geq 0$, for every k .

$$\text{Also } T_k(x + 2n\pi) = T_k(x), \text{ for } n = 1, 2, 3, \dots$$

As in the above case of linear data with $Q_d(x) = c_d(1-x^2)^d$, $d = 1, 2, 3, \dots$ and $-1 < x < 1$, Here, $T_k(x)$ replaces $Q_d(x)$, U_k replaces C_d , and $\sin x$ replaces x .

Now choose U_k such that: $\int_0^{2\pi} T_k(x) dx = 1$, satisfying the conditions for a circular distribution.

Next, define the non-parametric estimator $\hat{f}_n(x)$ of f by:

$$\begin{aligned} \hat{f}_n(x) &= \frac{1}{n} \sum_{i=1}^n T_k(\sin(x_i) - \sin(x)) \\ &= \frac{1}{n} \sum_{i=1}^n U_k \{1 - (\sin(x_i) - \sin(x))^2\}^k \end{aligned}$$

The degree of the polynomial k is the smoothing parameter of the polynomial estimator. The constant, U_k can be calculated using the formula:

$$U_k = \frac{1}{2\pi} \frac{2^{2k} k!^2}{(2k)!}$$

Similar to optimal bandwidth selection in the kernel estimator, the optimal degree of the polynomial is selected, based on the quantity $CV(k)$, given by:

$$CV(k) = \frac{1}{n} \sum_{i=1}^n \ln[\hat{f}_{-i}(x_i)]$$

where, $\hat{f}_{-i}(x_i) = \frac{1}{n-1} \sum_{\substack{i=1 \\ j \neq i}}^n T_k(x_j - x_i)$.

The estimation process is similar to the kernel bandwidth parameter of a kernel density estimator (see Silverman, 1986). In kernel estimation, the bandwidth decreases with increasing sample size, whereas in polynomial estimation, the degree of the polynomial increases as the sample size increases. Therefore, polynomial estimation is more robust than kernel density estimation, being more precise as the sample size increases. Moreover, bandwidth is highly sensitive, but the polynomial degree did not affect the estimation of smaller errors in k values. Table 1 presents various k and corresponding U_k values.

Table 1. Various k and corresponding U_k values

k	U_k	k	U_k	k	U_k	k	U_k
1	0.31831	21	1.300439	41	1.811805	61	2.207752
2	0.424414	22	1.330682	42	1.833634	62	2.225701
3	0.509296	23	1.360252	43	1.855206	63	2.243507
4	0.582053	24	1.389194	44	1.87653	64	2.261172
5	0.646725	25	1.417545	45	1.897615	65	2.278701
6	0.705519	26	1.44534	46	1.918468	66	2.296095
7	0.759789	27	1.47261	47	1.939096	67	2.313359
8	0.810442	28	1.499385	48	1.959508	68	2.330495
9	0.858115	29	1.52569	49	1.979709	69	2.347506
10	0.903279	30	1.551549	50	1.999706	70	2.364395

11	0.946292	31	1.576984	51	2.019505	71	2.381163
12	0.987435	32	1.602016	52	2.039112	72	2.397815
13	1.026933	33	1.626662	53	2.058532	73	2.414352
14	1.064967	34	1.650941	54	2.077771	74	2.430776
15	1.10169	35	1.674868	55	2.096833	75	2.44709
16	1.137229	36	1.698457	56	2.115723	76	2.463296
17	1.17169	37	1.721724	57	2.134446	77	2.479396
18	1.205167	38	1.74468	58	2.153007	78	2.495392
19	1.237739	39	1.767338	59	2.171409	79	2.511286
20	1.269476	40	1.78971	60	2.189656	80	2.52708

In polynomial estimation, likelihood cross-validation is used to maximize the score function $CV(k)$, where $CV(k) = \frac{1}{n} \sum_{i=1}^n \log(\hat{f}_{-i}(x_i))$. Since U_k calculations can be difficult due to factorials in the expression, we determined a recurrence

formula where $U_k = \frac{4k^2 U_{k-1}}{2k(2k-1)}$. The choice of k is based on

maximizing the score function $CV(k)$, calculated using the maximum likelihood estimation $\hat{f}_{-i}(x_i)$ by leaving the i^{th} variable in the frequency data. The k , $CV(k)$ curves were plotted to select the maximum $CV(k)$ point. The value of k corresponding to the unique maximum is the optimum parameter for the distribution. Using the k and U_k values, the probability density function can be estimated using Microsoft Excel.

Application of circular distribution for wind direction of Thrissur in Kerala

The Thrissur district in Kerala is characterized by dry weather in summer with intermittent summer showers (March–May), two rainy seasons (southwest monsoon from June–September and post-monsoon from October–November), and pleasant

winter months (December–February). The district has periodic winds from November fortnight to February fortnight, known as *vrischika kaattu*. The name comes from the Malayalam month *Vruschika*, when the wind starts to blow every year. This wind does not pass through other districts in Kerala. The wind is characterized by high speed and no moisture as it passes through the Palakkad Gap of the Western Ghats, where it subsequently gains momentum.

An anemometer was used to measure wind speed and a wind vane to measure wind direction in 16 directions: N (360°), NNE (22.5°), NE (45°), ENE (67.5°), E (90°), ESE (112.5°), SE (135°), SSE (157.5°), S (180°), SSW (202.5°), SW (225°), WSW (247.5°), W (270°), WNW (292.5°), NW(315°), and NNW (337.5°). Calm is noted by ‘C’ and variable by ‘V.’ The wind direction variables were coded from 0 to 16 for ease of analysis. Daily wind directions in the morning and evening, recorded at Kerala Agricultural University, Vellanikkara in Thrissur district of Kerala from January 1 to December 31 in 2000 and 2017, were used to test whether the distribution of wind direction in Thrissur changed over 17 years.

The new tool for determining the probability density function using a non-parametric polynomial estimator was tested using the following formula:

$$f_n(x) = \frac{1}{n} \sum_{k=1}^n U_k [1 - (\sin(X_i) - \sin(x))^2]^k$$
, for $x \in [0, 2\pi]$, X_i is the i^{th} observation. The U_k values were calculated for $k=1, 2, 3, \dots$ using:

$$U_k = \frac{1}{2\pi} \frac{2^{2k} (k!)^2}{(2k)!}$$

where k determines the degree of the polynomial and is the smoothing parameter. To determine the distribution of daily

wind direction in 2000 using the non-parametric estimation, $CV(k)$ was calculated for $k = 1, 2, 3, \dots$

The k , $CV(k)$ curve for 2000 (Fig. 1) shows an optimum k of 62 and corresponding U_k of 2.225701.

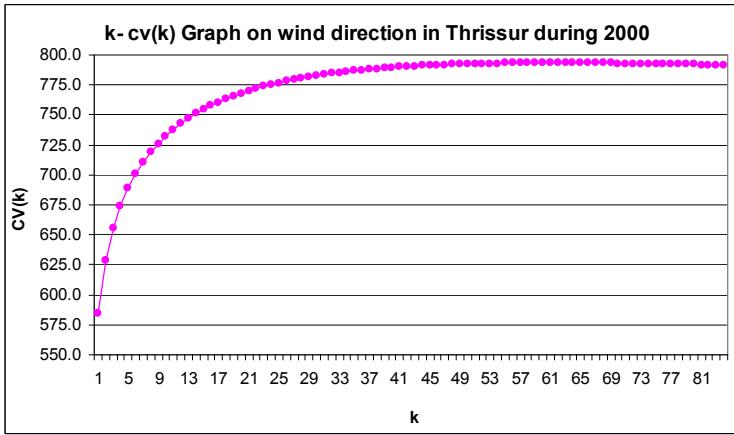


Fig. 1. k , $CV(k)$ curve for daily wind direction data in 2000

Using these values, the distribution of the polynomial estimation is given as:

$$f_n(x) = \frac{1}{365} \sum_{\substack{i=1 \\ j \neq i}}^n 2.225701 \left[1 - \left(\frac{\sin(x_j) - \sin(x_i)}{2\pi} \right)^2 \right]^{62}$$

The k , $CV(k)$ curve for 2017 (Fig. 2) shows an optimum k of 66 and corresponding U_k of 2.296095. Using these values, the distribution of polynomial estimation is given as:

$$f_n(x) = \frac{1}{365} \sum_{\substack{i=1 \\ j \neq i}}^n 2.296095 \left[1 - \left(\frac{\sin(x_j) - \sin(x_i)}{2\pi} \right)^2 \right]^{66}$$

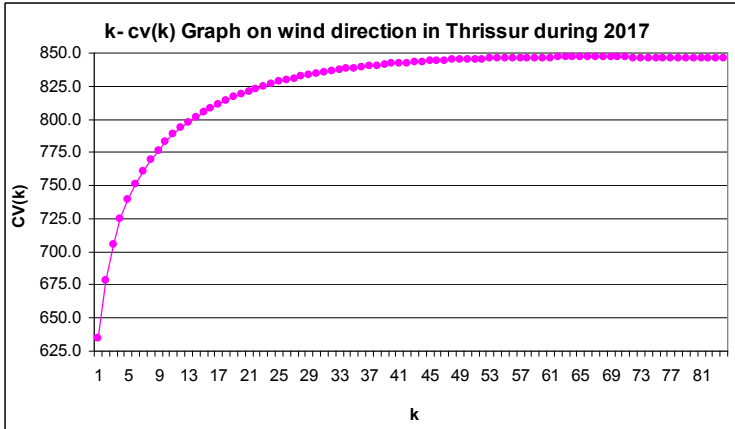


Fig. 2. k , $CV(k)$ curve for daily wind direction data in 2017

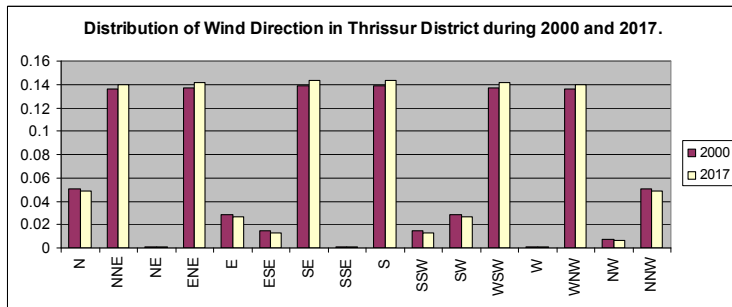


Fig. 3. Distribution of wind direction in Thrissur in 2000 and 2017 using a non-parametric circular polynomial estimator

The Kolmogorov–Smirnov test statistic for the wind direction data in 2000 and 2017 did not significantly differ between years.

The distributions are shown in Fig. 3, with 0–22.5° coded as N (North), 22.5–45° coded as NNE, etc. as detailed above. The smoothing parameters were $k = 62$ and $k = 66$ in 2000 and 2017, respectively. Fig. 3 gives the combined plot of

distributions in 2000 and 2017. As the plots were similar, it can be concluded that no significant variations occurred in the distribution of wind direction over time.

Conclusion

A new non-parametric polynomial estimator for fitting the distribution of circular data was introduced and applied to actual daily wind directions in the Thrissur district of Kerala, rather than a simulation study of the newly developed distribution. No studies on wind direction in the Thrissur district have been reported, despite its unique *vrischika kaattu* wind. Data were fitted using the newly developed non-parametric circular distribution technique after estimating the degree k of the polynomial (smoothing parameter). The daily wind direction data had a smoothing parameter of 62 in 2000 and 66 in 2017. The data fit revealed no significant differences between average wind directions in the district between 2000 and 2017.

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GENOTOXICITY AND BACTERIOLOGICAL ANALYSIS OF DRINKING WATER SOURCES AT AN ENDOSULFAN AFFECTED REGION OF KASARGOD

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Abstract: *Water quality management requires knowledge about all aspects that can have an impact on water quality. From toxicological point of view the presence of organic micro-pollutant and pathogenic microorganisms in raw water, consequently drinking water are major aspects. Chemical analysis and bacteriological analysis of drinking water sources can reveal the quality and potability of drinking water sources. In this study, the genotoxic effect and bacteriological analysis of drinking water sources from one of the endosulfan affected regions of Kasargod District in Kerala were evaluated. Various reports were published about the pollution caused by the chemical pesticide endosulfan in rural areas of Kasargod district in the year 1980s. More than 50,000 people of this area have been exposed to endosulfan, and over 3000 were affected by various genetic disorders like mental retardation, cerebral palsy, cancer and other diseases like central nervous system disorders, reproductive system disorders etc. Various agency confirmed the presence of endosulfan in drinking water and soil in the exposed area. In this context, here we try to find out the presence of toxic chemicals in drinking water through genotoxicity studies and the bacteriological monitoring approach appears to be able to detect contamination related issues before drinking water consumption in the exposed regions.*

Key words:- *Bacteriological analysis, Endosulfan, Fecal Contamination, Genotoxicity, MPN, Potability*

Introduction

Water is the basic necessity for the functioning of all life forms that exist on the earth. So, safe drinking water is necessary for human health. But the availability of safe drinking water is

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becoming scarce due to water pollution (Malathy B R et al ,2017). This form of water pollution occurs when pollutants are directly or indirectly discharged into the water bodies without adequate treatment to remove harmful compounds. The spraying of endsosulfan in the cashew plantations of Kasaragod district caused a disaster on the biodiversity of the area and also linked to serious health disorders in the citizens residing in these areas (Harikumar P. S. 2014, S.C Bhatia.2008). In 2001, tests carried out by the Centre for Science and Environment in Padre village in Kasaragod confirmed the deadly effects of the pesticide(centre for science & Environment.2001).

Being a universal solvent, water is also a major source of various infections. According to the World Health Organization, 80% of the diseases are water borne. 3.1% deaths occur due to the unhygienic and poor quality of water (Christon J Hurst& Ronald L Crawford. 2007). Among different kinds of environmental pollution, water is the most severe in its implications for the health and wellbeing of people. The specific contamination leading to pollution in water bodies include a wide spectrum of chemicals, pathogens etc. Thedischarge of domestic and industrial effluent wastes, leakage from water tanks, marine dumping, radioactive wastes etc. are the major causes of water pollution. Pathogenic bacteria in water are the major sources of some of the most devastating epidemic diseases in the world such as cholera, typhoid fever etc. Bacteria can cause bacillary dysentery, shigellosis and paratyphoid fever. Fecal contamination of drinking water is another point of concern. The presence of coliform group of bacteria which includes *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* speciesare relatively easy to detect in water, are useful for determining the quality of potable water (Ananthanarayanand Paniker. 2013).

The presence of various chemical agents in water can be determined by checking the genotoxic effects on chromosomes (Theroux, Eldridge, & Mallmann.1943). Genotoxicity is the property of chemical agents to damage the genetic information within a cell by causing mutations, which may lead to cancer or other disabilities. One of the simplest methods to determine genotoxicity is by *Allium cepa* root tip experiment, in which the chromosomal aberrations in the onion root tip cells are identified. This involves the assessment of damage at the cellular and sub-cellular levels. Cells are the primary sites of interaction between chemicals and biological systems. The importance of toxicity assay at this level is based on the assumption that cellular changes are finally reflected in the metabolic and morphological disorders of the organism (Jeffrey C. Pommerville.2014).

Hence, in this study we aim to determine the water quality with respect to bacteriological and genotoxic analysis of drinking water collected from five different water sources in Padre village, one of the most affected endosulfan area in Kasaragod district where the cashew plantations were aerially sprayed with the chemical pesticide endosulfan. After the profound application of endosulfan, serious disorders such as congenital anomalies, mental retardation, cancer, psychiatric cases, epilepsy etc. had started appearing among the human population in those areas (Usha S.2005). The contamination caused by chemicals is identified by genotoxicity studies whereas the bacteriological analysis by MPN method detects the presence of coliforms in drinking water so that we can take necessary steps to improve the quality of drinking water and enhance the potability of water in those area.

Materials and methods

Collection of water samples

The water samples were collected from Padre village in Kasaragod. Sterile bottles were used to collect the water samples. The water samples were properly labeled with full detail of the source, time, place and date of collection. A total of 5 drinking water samples were collected for the analyses. Among these, 3 were well water, 1 was borewell and 1 was pipe water.

Media used

MacConkey broth. Eosin Methylene Blue Agar (EMB), Nutrient Agar

Bacteriological analysis

The bacteriological quality of water was tested by Most Probable Number method (MPN). Bacteriological analysis was done by Multiple Tube Fermentation test, to find the total or presumptive coliform count. Results of multiple tube fermentation tests for coliforms have been reported as Most Probable Number (MPN) index. It is actually a qualitative test rather than quantitative, indicating only the presence of coliforms, not their numerical presentation (Tasnia Ahmed.2013). The MPN test was carried out in three continuous stages: presumptive test, confirmed test and completed test through which the presence of indicator organisms is detected and confirmed (Deepesh Kumar.2013, J. Senthil Kumar.2013).

Double and single strength MacConkey broth with Durham's tube were used for the test. Measured amount of water samples were added using graduated pipettes. Three separate series of test tubes, each containing 3 test tubes i.e., a

total of nine test tubes were set up in a test tube rack. Each tube was labelled with the water source and the volume of sample inoculated. 10 ml double strength macconkey broth was poured into the first set of three test tubes, 9 ml of single strength to the second set and 9.9 ml to the third set. Durham's tube was inserted in an inverted position into all the test tubes. After sterilization, 10 ml water sample was inoculated into the first set of test tubes, 1 ml into the second and 0.1 ml into the third set. The tubes were incubated at 37°C for 24 hours. The presumptive coliform count per 100 ml of water was determined from the tubes showing acid and gas production (E.C.S. Chan 1993, Gerard J. Tortora. 1989). A loopful of culture was taken from each positive test tube and streaked on eosin methylene blue (EMB) agar plates. These plates were then incubated at 37°C for 24 hours. Colonies obtained on the plates were identified by gram staining.

Genotoxic analysis

Onion root tips grown in five different water samples were cut and fixed with carnoy's solution.

Root tip was then placed on a glass slide and gently warmed with 1 N HCl. Acid was removed by washing with water, stained with acetocarmine, and warmed for 1-2 minutes. Cover slip was then placed and squashed with thumb pressure. Excess stain was removed with blotting paper and observed under high power objectives microscope (N. Murugalatha, 2012, Subhash Chandra Parija. 2007).

Result and discussion

Among the five different drinking water samples collected, faecal coliforms were isolated from different water samples. The MPN index (Fig.1) has showed that all the water samples were contaminated with faecalcoliforms and are found to be non-potable. (Table 1)

Table 1. MPN index of different water samples

SI. No	Samples	10 ml	1ml	0.1ml	MPN Index
1	Bore well	3	2	1	150
2	Well	3	3	3	>1100
3	Pipe water	3	3	3	>1100
4	Well	3	3	3	>1100
5	Well	3	1	0	43

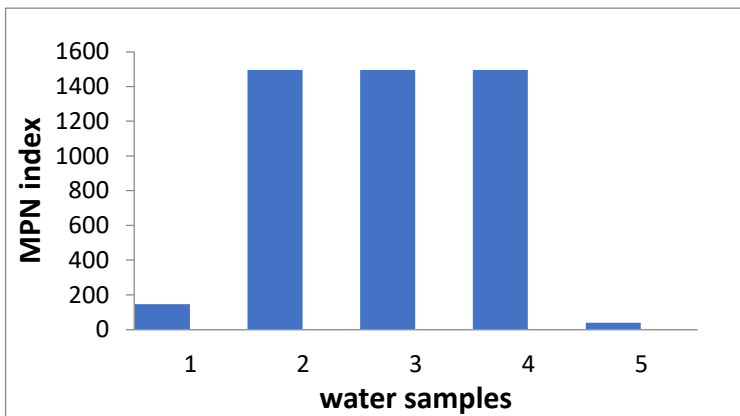


Fig. 1. A graph showing different water samples and their MPN index

Escherichia coli and *Enterobacter* spp were isolated from the EMB agar plates (Table 2) and then gram staining and motility were performed. Biochemical tests were performed for the further confirmation of bacteria. (Table 3)

Genotoxic effect was tested for all the above drinking water samples and no chromosomal aberrations were observed in the mitotic phase of onion root cells (Fig.2). Thus, the watersamples do not cause any genotoxic effect.

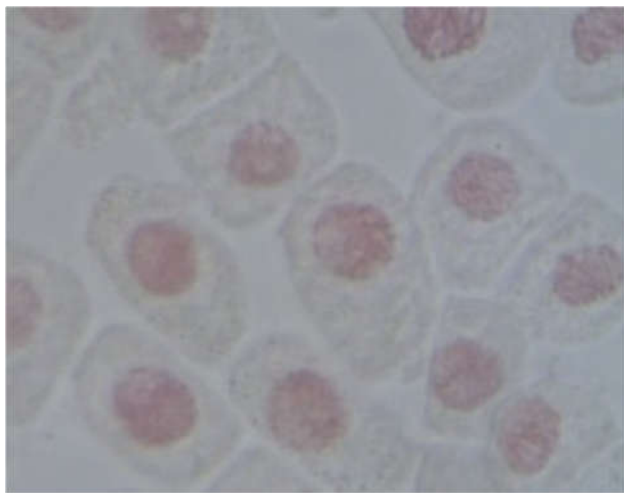


Fig.2. Onion root tip experiment showing no genotoxicity in water samples

Table 2. Characterisation of *E. coli* and *Enterobacter* spp through Gram staining and Motility test

Sl. No	Organisms	Gram staining	Motility test
1	<i>E. coli</i>	Gram negative straight rods.	Motile.
2	<i>Enterobacter</i> sp.	Gram negative rods.	Motile.

Table 3. Biochemical characterization of microorganisms. G-Glucose, M-Maltose, S-Sucrose, M-Mannitol

Sl. No	Organism	Indole	MR	VP	Citrate	Catalase	Oxidase	Urease	Sugar fermentation test			
									G	M	S	M
1	<i>E. coli</i>	+	+	-	-	+	-	-	+	+	-	+
2	<i>Enterobacter</i> sp.	-	-	+	+	+	-	-	+	+	+	+

Conclusion

Bacteriological analysis was done by estimating the total faecal coliforms present in the drinking water samples, which are indicative of the presence of pathogenic bacteria. Results of the bacteriological analysis performed through multiple tube fermentation test (MPN) suggest that all the drinking water samples contain high count of faecal coliforms and are thus recognized as non-potable. Necessary measures were taken for the proper sanitation of drinking water sources. Chlorination of drinking water sources were recommended and performed.

The presence of chemical pollutants in water could cause chromosomal aberrations in the cells, were analysed through *Allium cepa* root tip squash preparation experiment in which onion root tips were grown in the collected water samples and the mitotic phase chromosomes were viewed under high power objective microscope. It was found that all the cells contain normal sets of chromosomes. Thus, it proves the absence of genotoxic pollutants in all the analysed water samples.

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BUTTERFLY DIVERSITY ANALYSIS AT VIMALA COLLEGE CAMPUS

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Abstract: *The aim of our study was to document the butterfly fauna of Vimala college campus and determine the species diversity and richness of different families of butterflies. The study was conducted in two different sites of the campus, namely the Floral Garden and the Botanical Garden. Scan method was used to identify the butterflies. Eighteen species of butterflies belonging to five families were identified and recorded. Species diversity was found to be higher in the Floral Garden compared to the Botanical Garden. This was due to the abundance of host plants in the garden. The family Nymphalidae had the highest Shannon diversity index of 2.217, followed by Papilionidae 1.079, Pieridae 0.694 and Lycaenidae 0.673, while the family Hesperidae showed no diversity with a 0 value. After analyzing the data from all families, we got the overall Shannon index as 1.258 and Simpson index as 0.355. This can be considered as a preliminary study towards butterfly conservation and research.*

Keywords: *Bio indicators, conservation, Lepidoptera, species richness, species diversity, Shannon index, Simpson's index.*

Introduction

Butterflies are the flying beauties in their natural environment. They compete with flowers for attention and admiration. They are referred to as 'flagships' for invertebrate conservation (New et al. 1995). They belong to the order Lepidoptera and sub order Rhopalocera. They are closely related to moths, from which they evolved.

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Butterflies are indicators of a healthy environment and healthy ecosystems. They are one of the important food chain components of birds, reptiles, spiders and predatory insects. They provide a wide range of environmental benefits, including pollination and natural pest control. Butterflies have been widely used by ecologists as model organisms to study the impact of habitat loss, fragmentation and climate change. They are considered as good candidate materials for the study of genetics, insect-plant interactions and co-evolution. Therefore, their diversity becomes an index for detecting the status of a habitat and landscape (Amita and Braj, 2012).

Threats to butterflies largely arise from the human exploitation of forestry, agriculture and other resources. Attitudes towards butterfly collection should be fostered and emphasis should be given to photography, butterfly watching, scientific recording and other non-destructive activities. The Wildlife Protection Act, 1972 (modified in 1989), has enough provisions for conservation of natural resources including butterflies.

Materials and methods

Sampling site

The study was conducted in Vimala college campus, which is located 5kms away from Thrissur town. The total area of the campus is about 29.26 acres. The vegetation mainly comprises of deciduous species, ornamental shrubs, herbs and grasses. During the study, two sampling sites were selected from the campus (Figure 1: Map of Sampling Sites).

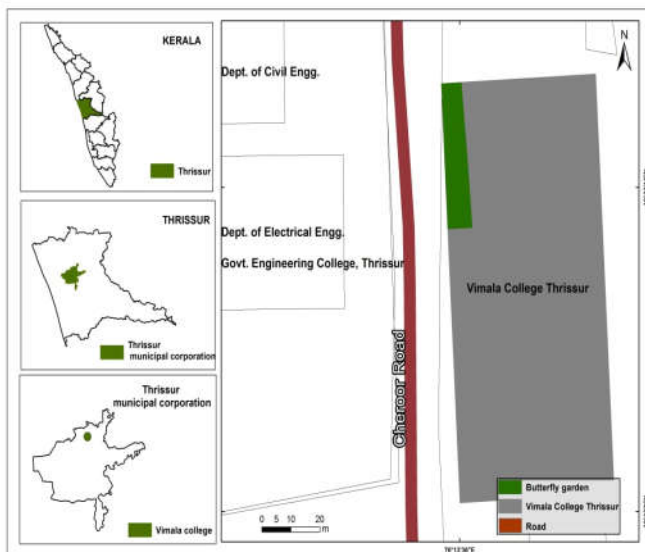


Figure: 1- Map of Sampling Sites.

Site 1: Floral Garden – located on the left side of the campus. Here several flowering plants are grown and displayed. Some butterfly plants like marigolds, poppies, Allamanda, Nerium, Ixora, etc are seen here.

Site 2: Botanical Garden – located in the front right corner of the campus. More than 500 plant species with special arrangements for indoor plants, orchids, water plants, greenhouses and shade houses can be seen here.

Sampling period and time

The butterfly fauna at the campus was surveyed from June to December in the year 2016. Each study site was visited daily between 11:00 AM to 12:00 PM during the college interval hours.

Sampling techniques

The butterflies were observed and recorded by scan method. The photographs of all the butterflies were taken. The

butterfly species were identified with the help of an Entomologist at Kerala Forest Research Institute (KFRI), Peechi.

Data analysis

We calculated the Shannon-Weiner diversity index [$(-\sum p_i \ln p_i)$ where ‘ p_i ’ is the proportion of individuals of i -th species in a whole community.](Shannon and Weaver, 1949), Simpson index [$\{\sum n(n-1) \div N(N-1)\}$ where ‘ n ’ is the number of individuals of each species, ‘ N ’ is the total number of individuals of all species.] (Simpson, 1949) and species richness of the butterfly species identified at the college. The Shannon-Weiner diversity index gives information about the community composition of the species; the higher the number, the higher the species diversity. It accounts for both the abundance and evenness of the species present. Simpson’s index is a weighed arithmetic mean of proportional abundance. It measures the probability that any two individuals randomly selected from a sample will belong to the same species. It is a dominance index as it gives more weightage to the common or the dominant species, whereas the Shannon-Weiner index gives more weightage to the rare species. Simpson’s index ranges from 0 (infinite diversity) and 1 (no diversity), so the larger the value of D , the lower the diversity. Species richness denotes the total number of species observed in an area. It increases with the sample size (Bandana et al.,2021).

Results

During the study, 41 individuals of 18 species of butterflies belonging to the 5 families (Nymphalidae, Papilionidae, Pieridae, Lycaenidae and Hesperidae) were recorded (Table:1, Figure:2). The Family Nymphalidae showed the maximum species richness (23 individuals of 10 species);

followed by Papilionidae (7 individuals of 3 species), Pieridae (4 individuals of 2 species) Lycaenidae (5 individuals of 2 species) and Hesperidae (2 individuals of 1 species). The family Nymphalidae had the highest Shannon diversity index of 2.217 while the family Hesperidae showed no diversity with a 0 value. The overall values of Shannon diversity index, Simpson's index and species richness of the butterfly fauna of Vimala college campus (pooling all families) were 1.258, 0.355, and 18, respectively (Table:4).

Table 1: Butterflies spotted at Vimala college campus

Sl. No.	SCIENTIFIC NAME	COMMON NAME	FAMILY
1.	<i>Hypolimnas bolina</i> (male)	Great egg fly	Nymphalidae
2.	<i>Hypolimnas bolina</i> (female)	Great egg fly	Nymphalidae
3.	<i>Junonia lemonias</i>	Lemon pansy	Nymphalidae
4.	<i>Junonia iphita</i>	Chocolate pansy	Nymphalidae
5.	<i>Tirumala limniace</i>	Blue tiger	Nymphalidae
6.	<i>Phalanta phalantha</i>	Common leopard	Nymphalidae
7.	<i>Ariadne ariadne</i>	Angled caster	Nymphalidae
8.	<i>Neptis jumbah</i>	Chestnut streaked sailor	Nymphalidae
9.	<i>Cupha erymanthis</i>	Southern rustic	Nymphalidae
10.	<i>Ypthima huebneri</i>	Common four rings	Nymphalidae
11.	<i>Papilio demoleus</i>	Lime butterfly	Papilionidae
12.	<i>Papilio polymnestor</i>	Blue mormon	Papilionidae
13.	<i>Graphium agamemnon</i>	Tailed jay	Papilionidae
14.	<i>Catopsilia pomona</i>	Common emigrant	Pieridae
15.	<i>Delias eucharis</i>	Common jezebel vilasini	Pieridae
16.	<i>Chilades pandava</i>	Plains cupid	Lycaenidae
17.	<i>Talicauda nyseus</i>	Red pierrot	Lycaenidae
18.	<i>Borbo cinnara</i>	Rice swift	Hesperidae

Table 2: Butterflies and their host plants

Sl. No.	BUTTERFLY SPECIES	HOST PLANTS
1.	<i>Hypolimnas bolina</i> (male)	<i>Justicia simplex</i>
2.	<i>Hypolimnas bolina</i> (female)	<i>Justicia simplex</i>
3.	<i>Junonia lemonias</i>	<i>Alternanthera sessilis</i>
4.	<i>Junonia iphita</i>	<i>Allamanda cathartica</i>
5.	<i>Tirumala limniace</i>	<i>Allamanda cathartica</i>
6.	<i>Phalanta phalantha</i>	<i>Ixora coccinea</i>
7.	<i>Ariadne ariadne</i>	<i>Tragia involucrata</i>
8.	<i>Neptis jumbah</i>	<i>Hibiscus rosa-sinensis</i>
9.	<i>Cupha erymanthis</i>	<i>Hibiscus rosa-sinensis</i>
10.	<i>Ypthima huebneri</i>	<i>Crossandra infundibuliformis</i>
11.	<i>Papilio demoleus</i>	<i>Ervatamia coronaria, orchid</i>
12.	<i>Papilio polymnestor</i>	<i>Hibiscus rosa-sinensis</i>
13.	<i>Graphium agamemnon</i>	<i>Calliandra cynometroides</i>
14.	<i>Catopsilia pomona</i>	<i>Impatiens balsamina</i>
15.	<i>Delias eucharis</i>	<i>Ixoracoccinia, Hibiscus rosa-sinensis.</i>
16.	<i>Chilades pandava</i>	<i>Crotalaria retusa</i>
17.	<i>Talicauda nyseus</i>	<i>Strobilanthes dyeriana</i>
18.	<i>Borbo cinnara</i>	<i>Lantana camara</i>

Discussion

A total of 18 species of butterflies belonging to 5 families were recorded during our study (Table: 1, Figure: 2). Butterfly diversity was found to be high in the Floral Garden compared to the Botanical Garden of the college campus (Table: 3). The abundance of butterfly may be due to the presence of sufficient number of host plants in the Floral Garden. *Justicia simplex*, *Lantana camara*, *Crotalaria retusa*, *Ixora coccinea*, *Hibiscus rosa-sinensis*, etc are some of the host plants seen in the Floral Garden (Table: 2). In the present study, the family Nymphalidae, (its host plants include *Justicia simplex*, *Alternanthera sessilis*, *Allamanda cathartica*, *Ixora coccinea*, *Tragia involucrata*, *Hibiscus rosa-sinensis*, *Crossandra infundibuliformis*), had the highest species diversity (23 individuals of 10 species), followed by Papilionidae (7 individuals of 3 species). Similar trend was

shown even in the previous study, in the year 2015. A possible explanation for this is the fact that nymphalids are polyphagous in nature, which makes it easier for them to utilize a variety of habitats (Janz, 2005). There is only a slight variation in the total number of species identified, from 22 in 2015 to 18 in 2016 (Figure: 3). This may be due to the climate changes and the variation in the study time. Butterfly conservation is possible through the protection and cultivation of its host plant species, in their respective ecosystems (Mukherjee et al.,2019).

Table 3: Distribution of butterflies in the college campus

BUTTERFLY SPECIES	FLORAL GARDEN	BOTANICAL GARDEN
<i>Hypolimnas bolina</i> (male)	+	+
<i>Hypolimnas bolina</i> (female)	+	+
<i>Junonia lemonias</i>	+	
<i>Junonia iphita</i>	+	
<i>Tirumala limniace</i>	+	
<i>Phalanta phalantha</i>	+	
<i>Ariadne ariadne</i>	+	
<i>Neptis jumbah</i>	+	
<i>Cupha erymanthis</i>		+
<i>Ypthima huebneri</i>	+	
<i>Papilio demoleus</i>	+	+
<i>Papilio polymnestor</i>	+	
<i>Graphium agamemnon</i>	+	
<i>Catopsilia pomona</i>	+	+
<i>Delias eucharis</i>	+	+
<i>Chilades pandava</i>		+
<i>Talicauda nyseus</i>	+	
<i>Borbo cinnara</i>	+	

Table 4: Diversity indices for each butterfly family identified from Vimala college campus as well as the overall values when data from all families were pooled together.

Family	Shannon Index	Simpson Index	Species Richness
Nymphalidae	2.217	0.075	10.000
Papilionidae	1.079	0.238	3.000
Pieridae	0.694	0.333	2.000
Lycaenidae	0.673	0.400	2.000
Hesperiidae	0.000	1.000	1.000
All families	1.258	0.355	18.000

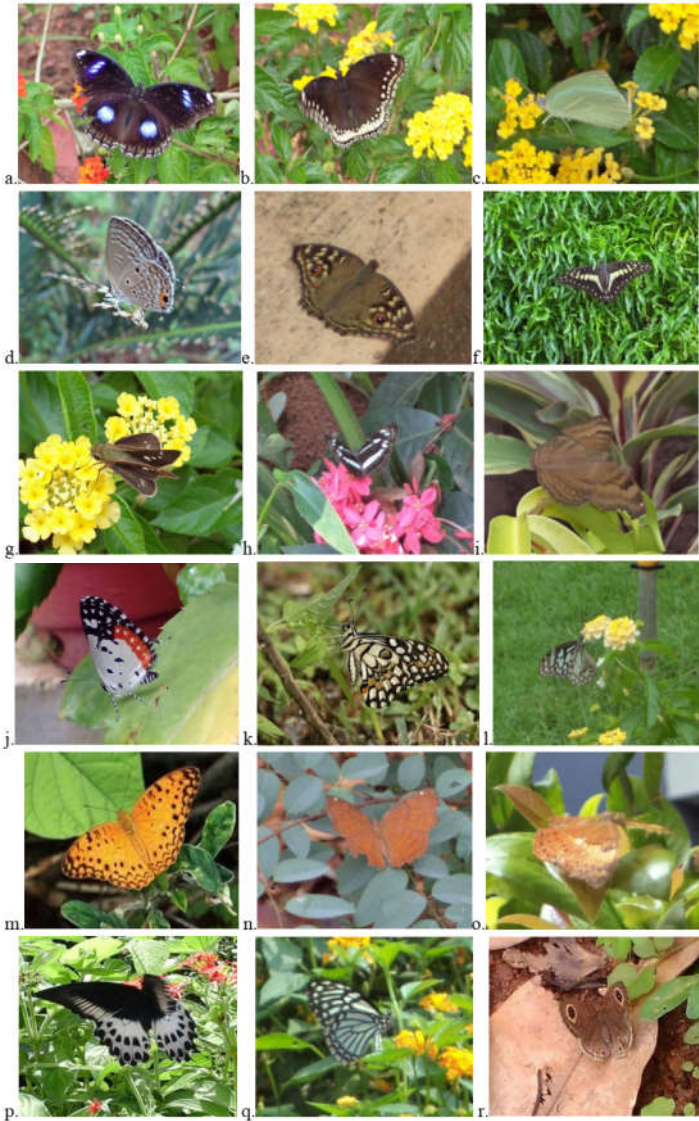


Figure: 2- Butterflies identified from Vimala college campus, a- *Hypolimnas bolina*(male), b- *Hypolimnas*

bolina(female), c- *Catopsilia pomona*, d- *Chilades pandava*, e- *Junonia lemonias*, f- *Graphium agamemnon*, g- *Borbo cinnara*, h- *Neptis jumbah*, i- *Junonia iphita*, j- *Talicauda nyseus*, k- *Papilio demoleus*, l- *Tirumala limniace*, m- *Phalanta phalantha*, n- *Ariadne ariadne*, o- *Cupha erymanthis*, p- *Papilio polymnestor*, q- *Delias eucharis*, r- *Ypthima huebneri*.

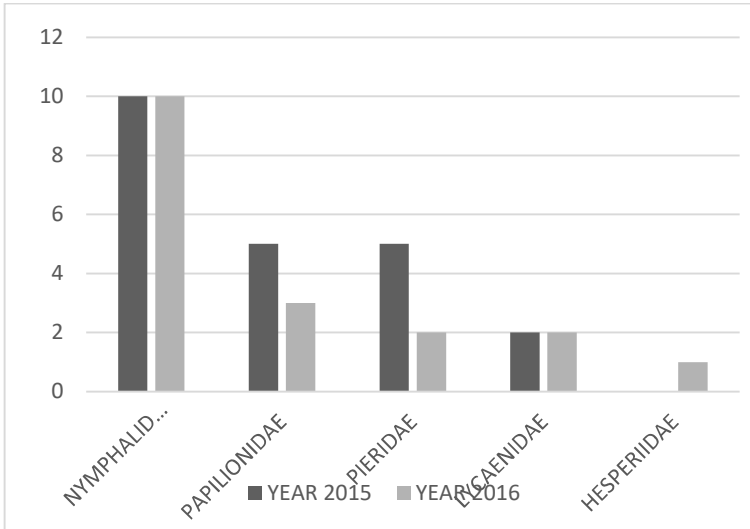


Figure: 3- Taxonomical analysis in the years 2015 and 2016.

Conclusion

Butterflies are the dominant components of the food web of life. They are diverse and ecologically important bioindicators. In Vimala college campus, 18 species of butterflies from 5 families were reported. They reflect a healthy patch of ecosystem in the heart of the city. If landscaping and maintenance of campus garden is carefully planned, the diversity of butterflies may increase in the college campus, providing a rich ground for butterfly conservation as well as for research.

Creating habitats for butterflies would help in conserving not only butterflies but also other insects, birds and mammals. Present study reveals that the study area provides favourable ecological conditions and habitat for butterflies. The highest number of species was recorded from Site- 1 (Floral Garden). It might be due to the favourable climatic conditions and also due to the presence of sufficient host plants for the development and the growth of butterflies.

From the conservation point of view, butterflies play an important role as ecological indicators and as vectors of cross pollination in plant propagation. They also act as a food source to predators like birds, spiders, lizards and other animals. Conserving butterflies will improve our whole environment and enrich the lives of people, in the present and in the future.

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Z-SCAN MEASUREMENTS AND OPTICAL LIMITING STUDIES OF P-NITROANILINE

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Abstract: Good quality single crystals of P- nitroaniline (PNA), an organic nonlinear optical compound, were grown by exploiting the slow evaporation solution growth technique. The non-linear optical studies of Z-Scan technique with nanosecond laser pulses at 532 nm was utilized to evaluate the non-linear optical effect embedded within the PNA compound. The obtained nonlinear absorption coefficient (β) ($5.5 \times 10^{-11} \text{m/W}$) and the lower optical limiting threshold (1.08J/cm^2) pronounce the prominent utility of PNA material in the opto-electronics domain and for the fabrication of optical limiting devices.

Key Words: Nonlinear optical material, optical limiting, optical limiting threshold, saturable absorption, two photon absorption, Z-scan

1. Introduction

In recent years, nonlinear optical materials have achieved significant importance due to their potential applications in emerging technological fields (Arjunan et al. 2010; Zhang et al. 2010). The fast advancement in optoelectronics field necessitates the search for efficient non-linear optical materials for its utilization in optical telecommunication, frequency shifting, electro-optic shutters, laser-fusion mechanism and for medical diagnostics (Tatsuno et al. 1992). Nonlinear optical crystals generally create short

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wavelength of radiation from near infrared region and longer wavelength of visible range which finds wide usability in solid state tunable lasers (Hu et al. 2005). Organic nonlinear optical materials have attracted a great deal of interest as they possess large optical susceptibilities, wide transparency range, ultrafast response time, high optical damage threshold and maneuverability for device applications. An organic system acquires these unique characteristics because of the occurrence of charge delocalization creating π -electron conjugated molecules which results in high optical nonlinearity to enhance the molecular hyperpolarizability (Nalwa and Seizo Miyata, 1997). A series of heterocyclic aromatic nitro derivatives have been reported for its non-linear optical activity (Prabhu et al. 2015; Vedyappan et al. 2019). Nitroanilines belong to the class of so-called push pull structures due to the intramolecular charge transfer interaction between the NH_2 electron donor moiety and NO_2 electron acceptor moiety through the phenyl core. P-nitroaniline is pronounced as the simplest nitroaniline derivative possessing both donor and acceptor groups connected through a π -conjugated aromatic ring network (Khalil et al. 1973). The photochemical features and biological applications of the p-nitroaniline compound has been the subject of investigation for the past few decades (Sinha and Yates, 1991). Besides, through the present study we adopt a potentially effective strategy to assemble the non-linear optical properties of a prominent third order nonlinear optical crystal, P-nitroaniline (PNA), which remains unexplored till now. Moreover, third order NLO materials have attained wide exposure because they are likely to play a significant role as optical limiters in the photonics regime, for the protection of optical components from the intensive laser radiation. Hence, through the present investigation extensive study has been carried out to verify the optical limiting efficiency of the synthesized compound of PNA.

2. Material Synthesis

The compound under investigation namely P-nitroaniline (PNA) is an analytical reagent purchased from Sigma Aldrich chemicals. It was purified by repeated crystallization from ethanol solution employing slow evaporation. The solution was filtered through a Whatmann filter paper of grade 1 to remove the suspended impurities. After successive crystallization, some good quality pale yellow coloured needle like seed crystals were obtained within 10 days. This was used for performing further characterizations.

3. Results and Discussion

3.1. Z-Scan Measurement

The Z-Scan is a highly sensitive method to determine the third order nonlinear optical activity of materials employing the principle of spatial beam distortion (Sheik-bahae et al. 1989). The technique measures the transmittance of a narrowly focussing Gaussian laser beam passing through the sample, as a function of the position of the sample with respect to the focal axis (Naseema et al. 2012). The open aperture Z-Scan experiment was carried out by a Q-switched pulsed wave Nd:YAG laser of 532 nm wavelength possessing a pulse width of 9 ns and the pulse rate of 10 Hz. The open aperture plot of the PNA sample is shown in Fig.1. From the figure, it can be noticed that the normalized transmittance of the Gaussian beam is found to be decreasing when approaching the focus ($z=0$) indicating a valley profile. This linear transmittance of the sample clearly exhibits the occurrence of Reverse Saturable Absorption (RSA) in the sample, where the absorption cross section increases with the input intensity moulding the sample less transmissive (Naseema et al. 2010). The decrease of normalized transmittance illustrates the strength of optical nonlinearity within the sample attributed to Two Photon Absorption (TPA). To confirm the veracity of the proposed

mechanism, the effect of Saturable Absorption (SA) has to be taken into account along with the TPA cross section. In order to find the nonlinear absorption coefficient of the sample, the experimentally obtained Z-Scan data was fitted with a theoretical model of pulse propagation equation given by

$$\frac{dI}{dz} = -\left[\frac{\alpha_0}{\left(1 + \frac{I}{I_s}\right)} + \beta_{eff} I\right]I \tag{1}$$

Where z symbolizes the propagation distance of the sample.

Hence the intensity dependent nonlinear absorption coefficient(α) can be written by incorporating β for RSA and I_s for SA mechanism as

$$\alpha(I) = \frac{\alpha_0}{\left(1 + \frac{I}{I_s}\right)} + \beta_{eff} I \tag{2}$$

Here α_0 denotes the unsaturated linear absorption coefficient, β refers to the two photon absorption coefficient, I , represents the excitation intensity and I_0 reflects the saturation intensity.

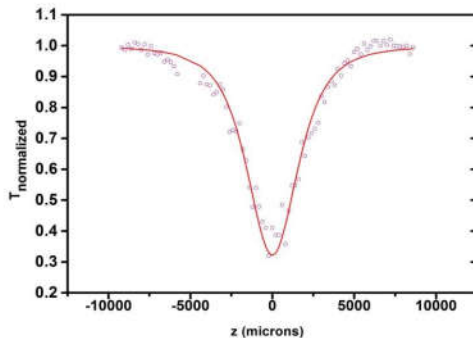


Fig.1. Open aperture Z-Scan curve of PNA crystal

By the accurate fitting of the pulse transmission equation on the experimentally obtained Z-Scan open aperture curve, the values of β and I_s was obtained to be $5.5 \cdot 10^{-11} \text{m/W}$ and $5 \cdot 10^{12} \text{W/m}^2$. The enhanced value of β is mainly attributed to the organic nature of the chosen material. The compound, P-nitroaniline is pronounced as the simplest nitroaniline derivative possessing both donor and acceptor moieties with NH_2 and NO_2 groups connected through a π -conjugated aromatic ring network. The delocalization of electrons via the efficient charge transfer interaction between the donor and acceptor groups thereby enhances the molecular polarizability and thus the optical nonlinearity in the material.

The slight deviation of the experimental curve from the theoretical model may be attributed to the slow relaxation of the excited levels of the sample, occupied by the irradiation of intense laser radiation when compared to the absorption strength. When $I < I_s$, the situation is equivalent to TPA coefficient and hence the effect of ' β ' can itself provide the best fit. The obtained value of non-linear absorption coefficient (β) was compared with some organic derivatives as given in Table.1.

Table.1. Comparison of β values with some organic compound derivatives

Some organic compound derivatives	Nonlinear absorption coefficient (β) ($\cdot 10^{-11} \text{ m/W}$)
Toluidine tartrate	2.6 [13]
2-methyl-5-nitroaniline	1.4 [14]
4-methylanilinium 3,5-dinitrobenzoate	0.58 [15]
2-(4-methylphenoxy)-N'-{(1E)-(4-methylphenyl)methylene}acetohydrazide	0.44 [16]
P-nitroaniline	5.5 [present work]

3.1.1. Optical Limiting Property

Optical limiters are materials that can strongly attenuate intense optical beams and thereby protect human eyes, optical elements from laser induced damage (Naseema et al. 2011). The optical limiting curve of the PNA sample was extracted from the open aperture Z-Scan data as shown in Fig.2. The plot exhibits a reduction of the normalized transmittance expressed as a function of input fluence. It is seen that the output transmittance remains in a saturated state up to an input energy of $1.08\text{J}/\text{cm}^2$. But with the further increase of energy, the transmittance starts decreasing from a limiting amplitude which is commonly termed as the Optical Limiting Threshold (O_{LT}). The low value of O_{LT} demonstrates that PNA compounds possess excellent optical limiting properties. Hence recommends this organic material of PNA to be used for the fabrication of optical limiting devices by finely tuning the transmittance value employing TPA theory. Optical limiters found extensive applications in passive mode-locking, pulse compression and optical device protection from high energy laser beams. The input laser fluence corresponding to the different z-position is extracted from the open aperture data using the relation

$$F(z) = 4(\ln 2)^{1/2} \frac{E_{in}}{\pi^{3/2}(z^2)} \quad (3)$$

Where E_{in} stands for the input laser energy.

From the plot, it is established that the transmitted intensity of the Gaussian laser beam acquires a saturated value at $1.08\text{J}/\text{cm}^2$ and starts decreasing gradually with the further increase of input energy. The saturated value is defined as the optical limiting amplitude of the PNA material. The pi-electron

delocalization and charge transfer contributes to the optical non-linearity in the material. Lower optical threshold value of 1.08 J/cm^2 signifies the title compound to be utilized as a promising optical limiter in extensive fields of human eye protection, optical sensor protection, pulse compression and passive mode-locking operations (John et al. 2020).

The optical limiting amplitude value of PNA was compared with the values of organic compounds for establishing the capability of title compound for opto-electronic applications. The comparison study is gathered under Table.2.

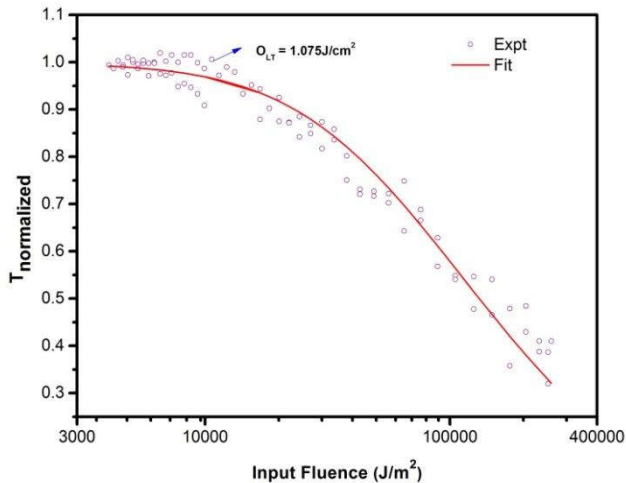


Fig.2. Optical limiting curve of PNA crystal

Table.2. Comparison of O_{LT} values with some organic compound derivatives

Some organic compound derivatives	Optical limiting threshold value, O_{LT} (J/cm ²)
(2E)-1-(Anthracen-9-yl)-3-(4-methoxyphenyl)prop-2-ene-1-one	14.70 [19]
4-dimethylaminopyridinium 3,5-dinitrobenzoate	10.64 [20]
4-methylanilinium 3,5-dinitrobenzoate	7.08 [15]
1,5-Diaminoanthraquinone	5.62 [21]
P-nitroaniline	1.08 [present work]

4. Conclusion

Good quality single crystals of P-nitroaniline (PNA), an organic nonlinear optical compound, was grown using the slow evaporation solution growth technique. Third order nonlinear optical property of PNA compound was evaluated using Z-Scan technique with nanosecond laser pulses at 532nm. The molecules in which donor and acceptor groups connected at the terminal positions of a pi bridge to create highly polarized molecules could exhibit large molecular nonlinearity. The enhanced values of non-linear absorption coefficient, β ($5.5 \times 10^{-11} \text{m/W}$) and the lower optical limiting threshold (1.08J/cm^2) are mainly attributed to the delocalization of electrons via the efficient charge transfer interaction between the donor and acceptor groups in the material. The obtained results pronounce the prominent utility of PNA material in the opto-electronics domain and for the fabrication of optical limiting devices.

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**NEW RECORD OF *AMPULEX RUFICORNIS*
(CAMERON, 1889) (HYMENOPTERA: AMPULICIDAE)
FROM SOUTHERN INDIA**

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Abstract: *The species *Ampulex ruficornis* (Cameron) is recorded for first time from Southern India. Key to Indian species of *Ampulex* Jurine by Anagha & Girish Kumar (2019) is partly modified here.*

Key words: *Ampulex, key, record.*

Introduction

The genus *Ampulex* Jurine is the largest genus within the family Ampulicidae and the members are commonly known as cockroach wasps. It is a widespread tropical genus distributed in Australia, Ethiopian, Nearctic, Neotropical, Oriental and Palearctic Regions. One hundred and thirty three species are recorded in this genus worldwide, of which twenty six species are known to occur in India (Anagha & Girish Kumar, 2019; Pulawski, 2021). Five species of *Ampulex* namely *A. ceylonica* Krombein, 1979, *A. compressa* (Fabricius, 1781), *A. dissector* (Thunberg, 1822), *A. major* Kohl, 1893 and *A. sadiyae* Anagha & Girish Kumar, 2019 are recorded from Kerala so far (Anagha & Girish Kumar, 2019).

Ampulex ruficornis (Cameron, 1889) is slender ant-like cockroach hunting wasp of the family Ampulicidae with rufous and black colouration. The species is characterised by the apically nodose first abdominal segment and abruptly angulate mandible of female (Krombein, 1979). The species has been

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reported from Northern India (West Bengal), Laos, Sri Lanka, and Thailand (Cameron, 1889; Krombein, 1979; Ohl *et al.*, 2014; Dollfuss, 2017; Pulawski, 2021). In the present paper, *A. ruficornis* is newly recorded to Southern Indian fauna and a modified part of key to Indian species of the genus *Ampulex* (Anagha & Girish Kumar, 2019) is provided.

Materials and Methods

The species *A. ruficornis* (Cameron) was collected from ZSI Campus, Kozhikode, Kerala and Kollur, Mookambika Wildlife sanctuary, Karnataka. The specimens mounted on entomological pins were studied under LEICA M205 stereoscopic binocular microscope and images were captured using attached LEICA DFC 500 camera. Images at varying depth were stacked using Leica Automontage Software V3.80 and the final illustrations were processed for contrast and brightness using Adobe® Photoshop® CS5 (Version 6.1) software. Key to Indian species of *Ampulex* Jurine by Anagha & Girish Kumar (2019) is partly modified in the couplets 23 and 24 here. The collected specimens were added to the ‘National Zoological Collections’ of ZSIK.

Museum abbreviations: **OXUM:** Hope Department of Zoology, Oxford, England; **ZSIK:** National Zoological Collections, Western Ghat Regional Centre, Zoological Survey of India, Kozhikode, India.

Results

***Ampulex ruficornis* (Cameron, 1889) (Figs 1–6)**

Rhinopsis ruficornis Cameron, 1889: 115, ♀. Holotype: ♀, India: Barrackpore (OXUM), type identity established by Krombein, 1979: 11.

Ampulex constanceae: Bingham, 1897: 255 (in revision of aculeates of British India), corrected to *Ampulex ruficornis* by Krombein, 1979: 11.

Material examined. India: Kerala, Kozhikode district, ZSI Campus, Jaferkhan colony, 1 ♀, 25.iii.2021, Coll. J.B. Terine, ZSIK Regd. No. ZSI/WGRC/IR/INV/ 18309. Karnataka, Udupi district, Kollur, near Mookambika Check dam, 1 ♂, 5.ix.2021, Coll. V.D. Hegde & Party, ZSIK Regd. No. ZSI/WGRC/IR/INV/ 19352.

Diagnosis. Female. Head not elongate behind eyes; frons finely granulate; clypeus with a median carina that project at apex in to sharp tooth, two tooth on either side of lateral margins of clypeus; mandible abruptly angulate at basal third of outer margin (Fig. 2); pronotum with a median furrow; mesoscutum and mesopleuron with close fine punctures and scattered small punctures; posterolateral angle of propodeal dorsum with small blunt tooth (Fig. 3); first abdominal segment nodose at apex (Fig. 6); forewing with infumation in second submarginal cell, at apex of first submarginal cell, and at apex of submedian cell (Fig. 5).

Male. Sculpturing as in female; mandible normal with rather evenly rounded outer margin; clypeus without tooth on either side of clypeal margin (Fig. 8).

Variability. Colour of male is found to be more rufous than that of female except for antennae; forewing weakly infumated compared to female.

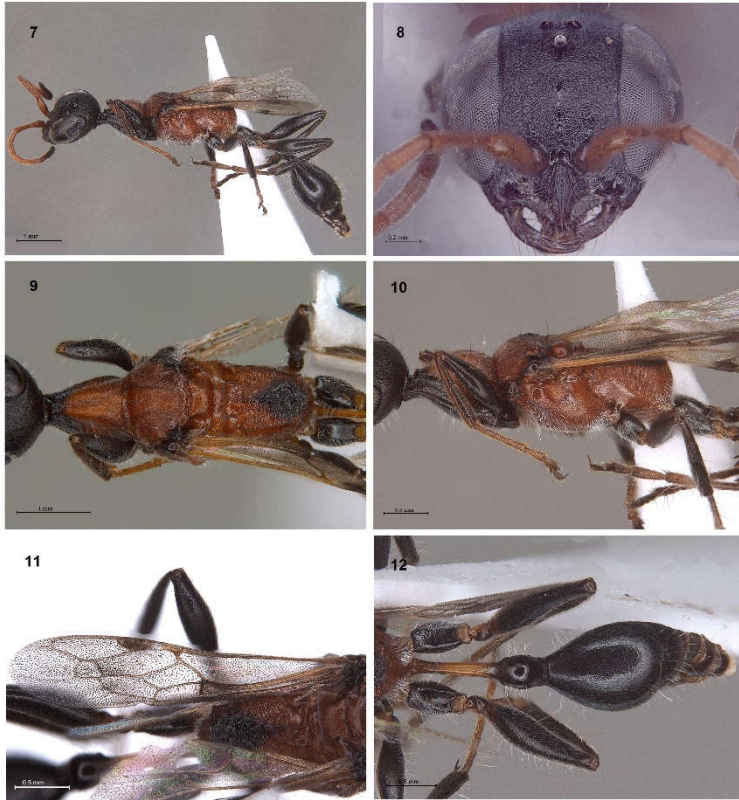
Distribution. India: Kerala (new state record), Karnataka (new state record), West Bengal. *Elsewhere*: Laos; Sri Lanka; Thailand (Cameron, 1889; Krombein, 1979; Ohl *et al.*, 2014; Dollfuss, 2017; Pulawski, 2021).

**Modified part of key to Indian species of *Ampulex* Jurine
(from Anagha & Girish Kumar, 2019)**

22. Postero-lateral angle of propodeal dorsum with long curved tooth. ***ceylonica* Krombein**
— Postero-lateral angle of propodeal dorsum with blunt tooth. 23
23. Mandible abruptly angulate at basal third of outer margin (Fig. 2); petiole rufous ***ruficornis* (Cameron)**
— Mandible normal with evenly rounded outer margin; petiole white. ***sadiyae* Anagha and Girish Kumar**



Figures 1-6. *Ampulex ruficornis* (Cameron) ♀. 1. Habitus, lateral view; 2. Head, frontal view; 3. Mesosoma, dorsal view; 4. Mesosoma, lateral view; 5. Fore wing; 6. Metasoma, dorsal view.



Figures 7-12. *Ampulex ruficornis* (Cameron) ♂. 7. Habitus, lateral view; 8. Head, frontal view; 9. Mesosoma, dorsal view; 10. Mesosoma, lateral view; 11. Fore wing; 12. Metasoma, dorsal view.

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PERSPECTIVE TOWARDS CONSTRUCTION AND DEMOLITION WASTE MANAGEMENT: INSIGHT INTO CURRENT PRACTICES, CHALLENGES AND APPLICATIONS

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Abstract: *Understanding of Construction and Demolition Waste (CDW) is very essential in the thriving economy and its management is nowadays a global priority. In this scenario, awareness of CDW management is need of the hour. Before implementing rules, first, there is a need to understand the CDW generation. Present study investigates the detailed review of the current practices and challenges that influences the construction and demolition waste management in India and around world. In addition, study reviews the recent research in managing CDW. Some of the potentially significant field of application such as reusability of waste soil from railway construction sites as soil amendments, highway embankment application, conversion of lignocellulose based CDW to bioethanol, traffic orientation sensor production etc.*

Key words: *CDW; Waste recycling; Demolition; Disaster waste*

1. Introduction

In the last few decades, there is a trend of immense urbanisation which uplifted unceasing construction globally. According to Klepa et al., 2019 issues related to the environment as well as the dearth of natural resources lead to exploring advanced concepts and solutions for environmental sustainability. The regenerative capacity of earth is totally

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disturbed by the improper management of natural resources and leads to environmental degradation (Ram et al., 2020). A sustainable resource management and waste utilisation is inevitable for fuelling development. Million tonnes of Construction and Demolition Waste (CDW) are generated every year, contributed by faster-growing cities, leading to detrimental effects on the environment. There are a number of ways that cause CDW to cause an impact on environment such as accumulation of solid waste, contribution of greenhouse gas emission etc (Klepa et al., 2019; Esa et al., 2017).

Construction and Demolition Waste (CDW) is defined as material which is surplus and damaged while construction and demolition process (Wang et al., 2020, Chen et al., 2020, Yuan and Shen 2011). According to Contreras et al., 2016 financial crisis for authorities as well as public administration will occur if CDW will dump in a public urban area near the road and other places, leading to economic impact. Several researchers proposed number of approaches to diminish CDW. Martose categorised CDW by its huge volume and mass. CDW can be broadly classified into natural as well as man-made sources (Menegaki et al., 2018). It can be further classified into the following sources (Figure 1). Umar et al., 2020 highlighted that when materials are being recycled, leading to low energy demand rather than new stock, which leads to decrement of transportation needs.

This study is organised into four different sections: an outline of debris discarded in Indian construction industry and other parts of the world is focused on the first section, the second section investigates the technological advancement to minimize the generation of CDW, followed by the third section, demolition waste after a disaster event and, the challenges in managing CDW and finally conclusion is drawn.

2. Outline of debris discarded in Indian construction industry and other parts of the world

Different countries have different methods of CDW disposal. Whether the nation is developing or developed one, the insight and understanding of controlling these wastes vary from one to another. Akhtar and Sarmah 2018 highlighted that the use of aggregates in concrete that are recycled are not permitted by Indian Standards. In India, the estimation of CDW is less focussed. In order to manage CDW, Indian Government introduced CDW management regulations and developed a hierarchy related to the management rule i.e., reduce>reuse>recycle>landfill (MoEFCC 2016, CPCB 2017). According to Jain et al., 2020, in India, about 100-300 million tonnes of CDW generated in a single year.

Apart from India, CDW management is a major challenge facing globally. Wu et al., 2019 reported that CDW amount that generated worldly every single year is greater than 10 billion tonnes, out of which US produce 700 million tonnes than 800 million tonnes produced by European Union (Chen et al., 2020). Wang et al., 2020 and Rodríguezetal., 2015 highlighted that CDW recycling is a challenge in Spain.

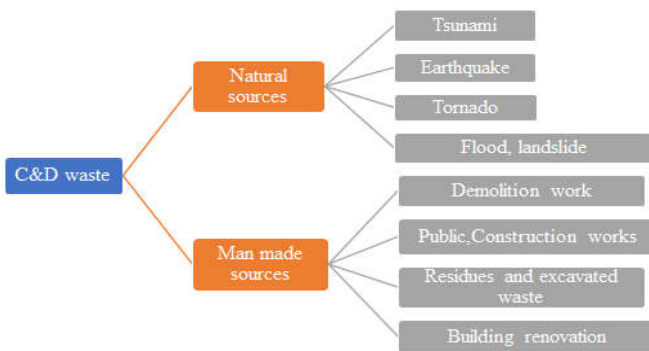


Fig. 1 Classification of CDW according to origin (Menegaki et al., 2018)

Economic development is one of the major reasons behind the huge generation of CDW (Wang et al., 2020, Li et al., 2016). Huang et al., 2017 reported that in China, the annual output of CDW is above 1.5 billion tonnes, causing severe problems to the environment. In China, these CDW dumped in landfills and the recycling rate of CDW in China is only about 5% (Huang et al., 2018). Huang et al., 2018 conducted CDW management through the Reuse, Reduce, Recycle principle. The above-mentioned study revealed that the very first barrier of CDW in China includes inappropriate urban planning, lack of guidance and underdeveloped market. According to a report by Zhang et al., 2019 about two billion tonnes of CDW is produced yearly in China, which contributes to 30-40% waste from municipalities and this value is higher than CDW developed by other countries. CDW recycling is less than 5% in China, with a reason that only a few domestic engineering projects which utilize recycled CDW are there.

About 97% of solid CDW, a balance of 3% are treated with incineration or recycling. When compared to developed countries, CDW recycling rate in developing and underdeveloped countries is less. This may be due to lack of available data (Umar et al., 2021).

According to the critical comment of Akhtar and Sarmah 2018, India and China have less focus on data collection, monitoring on waste recycling and reuse especially in the case of CDW. They also commented that CDW recycling helps to improve the economy rather than energy conservation. Common CDW management scenario is landfilling, incineration and recycling (Ram et al., 2020; Ortiz et al., 2010).

2.1 CDW recycling

CDW like concrete, brick as well as rock waste could be used as alternative aggregate by recycling while glass, metal as well as plastic can be removed through a process called sorting. When glass and plastic are not removed, it reduces the strength of aggregate which are recycled (Zhang et al., 2019).

3. Technological advancement to minimize CDW

In recent decades, there is much focus on the environmental impact of CDW minimization. The engineering applications of CDW must be well-explored to reduce the detrimental effect on the environment. Table 1 shows some of the studies related to CDW applications.

Table 1 Applications of CDW management

Literature study	Study region	Type of waste focussed	Applications
Jafari et al., 2011	Sweden	Lignocellulosic C&D waste	Bioethanol
Azambuja et al., 2018	Brazil	MDF, MDP, plywood or timber	Particleboard production
Yilmaz et al., 2018	Turkey	Concrete blocks, brick and plaster	Backfill material for mining industry
Zhang et al., 2019	China	Building related C&D waste	Highway embankment
Jelic et al., 2017	Belgrade	Concrete, facade, bricks and asphalt	Radionuclide sorption
Klepa et al., 2018	Brazil	Construction waste	Thermoluminescence sensor in highway traffic control
Contreras et al., 2016	Brazil	C&D waste	Low cost bricks for masonry walls
Han et al., 2018	South Korea	Waste soil generated from renovation of old rural railway	Reusability
Arulrajah et al., 2020	Australia	Concrete aggregates, crushed bricks, recovered plastic	Railway capping material
Saberian et al., 2020	Australia	Demolition waste mixed with	Pavement base/ subbase application

		crushed glass, crumb rubber	
Reis et al., 2019	Brazil	Inorganic sludge from the inert part of C&D waste	<ul style="list-style-type: none"> ▪ Phosphate removal from aqueous effluents ▪ Potential fertiliser

3.1 Robotic system for automatic CDW collection and sorting: a new technological solution

Wang et al., 2020 proposed a robotic system of advanced level for collection and sorting of CDW automatically. The objective was to plan a prototype which can patrol accurately in many different surroundings and CDW with higher altitudes. Major steps in CDW are categorization and shaped construction waste collection. For grabbing as well as for moving, the author proposed the robot prototype. For collecting shaped debris like cable and pipe, Wang et al., 2020 developed extraction classification technology. Apart from all these, Wang et al., 2020 introduced Simultaneous Localization and Mapping (SLAM) technology to assure accuracy and rate of success of robot prototypes.

3.2 Recycling as a management strategy

CDW can either be hazardous or non-hazardous. Non-hazardous which includes woody material. In 2018, Azambuja et al conducted a study using the CDW wood residues that are recycled for the manufacturing of particleboard. In the study, they split the particles in accordance with the material source as medium-density fibreboard, timber, plywood in equal parts. Rather than that the physical, as well as mechanical properties, were evaluated and the result shows that there is a strong bond between the particles of any CDW woody material. Studies from this literature showed that all residues that are tested offer some potentiality for usage in medium-density particleboard,

the residue addition upgraded the non-destruction testing prediction.

3.3 Utilization as cemented paste backfill material

As reported by Yilmaz et al., 2018 in the mining industry as a result of the ore processing operation, an increment of solid waste as well as mill tailings are generated per year. Cemented paste backfilling considered a good technique for mill tailings management and hence reducing environmental impacts and thereby increasing economic benefits (Yilmaz et al., 2018, Ercikdi et al., 2017). According to Yilmaz et al., 2018 the mining industry could be a recent field of CDW use in order to fill the voids formed during underground mining.

3.4 Reusability of waste soil from the demolition of a rural railway

Projects related to the railway produce enormous volume of CDW. These include ballast, sleepers, sub-base soil, which can contribute to environmental problems (Han et al., 2018). Also, rural railways have a lower chance of pollution as compared to urban railways and hence fewer studies are seen. With this aspect Han et al., 2018 evaluated railway pollutants which are common like heavy metals, polycyclic aromatic hydrocarbons, petroleum hydrocarbons and chemical properties of soil formed from the reconstruction of old railway in Wonju, South Korea. Peas (*Pisum Sativum*) were cultivated on lab-scale to assess waste soil reusability as a soil amendment. Their result suggests that these waste soil formed are cleaner and applications which are properly applied to these soil waste can improve the condition of soil and yield of the crop.

3.5 CDW in Highway Embankment

The feasibility of using CDW which are recycled is studied by Zhang et al., 2019 for the highway embankment application. CDW are used in asphalt and cement concrete, granular as well as sub-base in pavement engineering (Zhang et al., 2019). Their study showed that embankment application found to utilize recycled material rather than potential application. But the limitation of this study is that, it investigated only one source of CDW with one blended gradation. Hence further studies should focus onto material source and blended gradation. In highway embankment the cost-effectiveness as well as environmental impact must be evaluated for the use of recycled CDW.

3.6 In Highway Traffic Control

Klepa et al., 2018 introduced traffic-oriented sensor by reusing CDW and explained environmental and economic viability. The reviewed literature gives the chance of reusing CDW to obtain traffic sensor and helps to mitigate the environmental impact. Cost analysis showed that the produced traffic sensor by Klepa et al., 2018 is three times cheaper than commercially available one. As stated by Klepa et al., 2018 the incorporation of waste in the production process is environment friendly and efficient way to re-route them from landfilling. The application of CDW as traffic sensor is remarkable, however it is noticeable that when compared to enormous generation of CDW, only a lesser percentage of CDW contributes for utilizing as traffic sensors.

3.7 Low-cost brick with excellent physical properties

Contreras et al., 2016 used CDW as substitute of aggregate to produce brick. Contreras et al., 2016 mentioned

about possibility of using debris from CDW, lime and cement as raw material in new material production, less-cost brick for wall.

3.8 Lignocellulosic CDW to Bioethanol

While constructing 180m² home at USA, around 1.7 tonnes lignocellulosic waste are produced (Jafari et al., 2011). CDW including plywood, chipboard, oriented strand board and wallpaper are converted into bioethanol through Separate Hydrolysis and Fermentation. Their findings indicated that likewise other lignocellulosic biomass, pre-treatment and hydrolysis of CDW done using baker's yeast for producing ethanol with 70.5 to 84.2% theoretical yield. From the study of Jafari et al., 2011 it is clear that their result indicated that from one Kg of CDW, 160 ml bioethanol can be best produced.

4. Demolition waste after a disaster

Disasters also have impact on environment and can contribute to tremendous amount of waste. According to Domingo and Lee, 2017 these types of debris generated are unavoidable. When a disaster hits, many rescue activities and building rehabilitation are some of short- and long-term programs.

4.1 Management strategies for Disaster waste

CDW need to be addressed efficiently. Sustainable management of waste helps in minimizing waste generation and can helps in encouraging reuse of waste. But CDW management after a disaster is more difficult than normal CDW management (Domingo and Lee, 2017). As stated by Faleschini et al., 2017 one of the parameters that influenced CDW management is financial issue. According to Domingo and Lee, 2017 CDW generation is unavoidable issue. Man-

made disaster is mainly by human impacts while natural disaster can be tsunami, earthquake etc. According to Faleschini et al., 2017 three phases are their when deals with disaster management, i.e., pre-disaster, event, post-disaster. So, aim of all these in CDW management is to reduce risk.

4.2 Suggestions to improve Disaster Waste Management

According to Domingo and Lee, 2017 by improving facilities in waste processing, upbringing legislations, by co-ordinating training programs are suggestions to improve disaster waste management.

5. Challenges in Managing CDW

While reviewing literatures many studies have shown that every country has hindrance that need to overcome. Still many challenges are there to handle. According to Reis et al., 2019 the biggest challenge is to improve the right management among a large amount of solid waste formed. According to Wang et al., 2020, less co-ordination and poor awareness are some culprits that leads to inefficient CDW management. Agamuthu2008, highlighted that major challenge is lack of proper legislature that governs CDW management. Second challenge is characterising CDW. One of the research gaps that found while reviewing literatures is that there is no information regarding the role of micro-organisms in treating CDW. Hence more research work need to be focussed on these areas.

6. Conclusion

Rapid industrialisation and urbanisation, generates huge amount of construction and demolition waste (CDW) into the environment. Now a days, management of these waste is a major challenge faced by both develop and developing countries. Present study investigated the current management

practices adopted world-wide. The study finds out that, developing countries must monitor the current system and improve as explained in the above sessions. In addition, study reviews the recent research in managing CDW. Some of the potentially significant field of application such as reusability of waste soil from railway construction sites as soil amendments, highway embankment application, conversion of lignocellulose based CDW to bioethanol, traffic orientation sensor production etc. It is also clear from this study that some challenges need to overcome. Continuous monitoring and tremendous effort must be taken to manage CDW.

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RANGE EXTENSIONS OF TWO SPECIES OF *PALARUS* LATREILLE, 1802 (HYMENOPTERA: CRABRONIDAE) WITHIN INDIA

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Abstract: *New data on the distributions of two crabronid wasp species of the genus Palarus Latreille, 1802 (Hymenoptera: Apoidea: Crabronidae), viz., P. comberi Turner, 1911 and P. interruptus (Fabricius, 1787) collected from different localities of India. Intraspecific variations are discussed.*

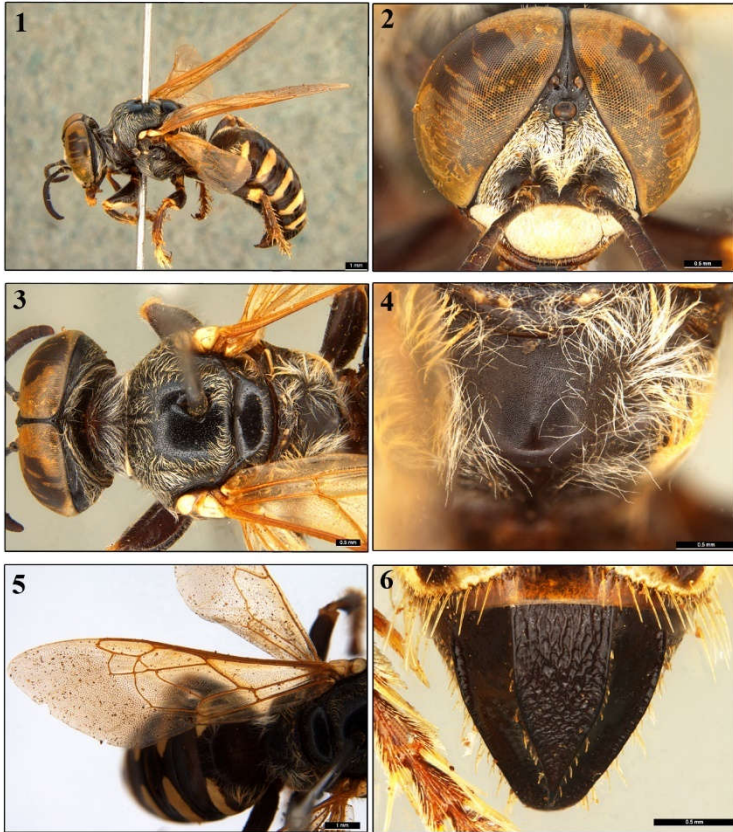
Keywords: *Bee/wasp killers, Crabroninae, India, New record, Palarini*

Introduction

The genus *Palarus* Latreille, 1802 is composed of thirty two species and additional three subspecies, are distributed mainly in the Africa, Southern Asia and Southern Europe (Bohart & Menke, 1976; Pulawski, 2021). Hitherto, six species are reported from India (Pulawski, 2021). *Palarus* are medium to large sized, stout bodied wasp with short and stout thorax. Generally, their gaster is with yellow band or spots, and legs are yellowish or reddish (Bohart & Menke, 1976). They prey on aculeates of the families Apidae, Ichneumonidae, Mutillidae, Pompilidae, Scoliidae, Sphecidae, Tiphidae and Vespidae, and dipterans of the families of Bombyliidae and Stratiomyidae (Dufour, 1841; Fabre, 1856; Girard, 1879; Nicolas, 1894; Brauns, 1911; Ferton, 1912; Ahrens, 1925; Sato, 1926; Cherian and Mahadevan, 1937; Honoré, 1941; De Beaumont, 1949; Scobiola, 1951; Móczár, 1952; Grandi, 1954

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& 1961; Tsuneki, 1969; Simon Thomas, 1972; Botha, 1975; Clauss, 1985; Gayubo *et al.*, 1992; Blagoveshchenskaya, 1994; Kazenas, 1994, 2001; Shkuratov, 2001). *P. interruptus* (Fabricius) is reported as the pest of Indian honey bee (*Apis cerana* Fabricius) (Cherian and Mahadevan, 1937).



FIGURES 1–6. *Palarus comberi* Turner, female. 1. Habitus, lateral view; 2. Head, frontal view; 3. Head and mesosoma, dorsal view; 4. Propodeum, dorsal view; 5. Forewing; 6. Last metasomal tergum.

Materials and Methods

This study is based on the specimens collected from various localities of India. The specimens have been studied and photographed using a Leica Stereo microscope model LEICA M 205A with LEICA DFC 500 Camera. The species are identified by using the key of Pulawski and Prentice (2008). All the identified specimens are deposited at Western Ghat Regional Centre, Zoological Survey of India, Kozhikode (ZSIK).

The following abbreviations are used for the Museums in the text: BMNH: The Natural History Museum, formerly British Museum (Natural History), London, Great Britain; NHMW: Naturhistorisches Museum, Wien, Austria; ZMUC: Zoological Museum, University of Copenhagen, Copenhagen, Denmark; ZSIK: Western Ghat Regional Centre, Zoological Survey of India, Kozhikode, India.

Results

Palarus Latreille, 1802

Palarus Latreille, 1802: 336. Type species: *Tiphia flavipes* Fabricius, 1793 [= *Palarus rufipes* Latreille, 1812], junior secondary homonym of *Palarus flavipes* (Fabricius, 1781), by monotypy.

Diagnosis: Clypeus yellow, tripartite; inner orbit converging above; ocelli mostly deformed or flattened; antennal socket not contiguous with fronto clypeal suture; face above antennal socket swollen; galeal crease reduced to short longitudinal remnant; mandible usually notched or angulate on external ventral margin; labrum short, broadly triangular; forewing with three submarginal cells, second submarginal cell triangular or petiolate (Bohart & Menke, 1976; Pulawski & Prentice, 2008).

Distribution: Africa, Southern Asia and Southern Europe (Bohart & Menke, 1976; Pulawski, 2021).

***Palarus comberi* Turner, 1911**

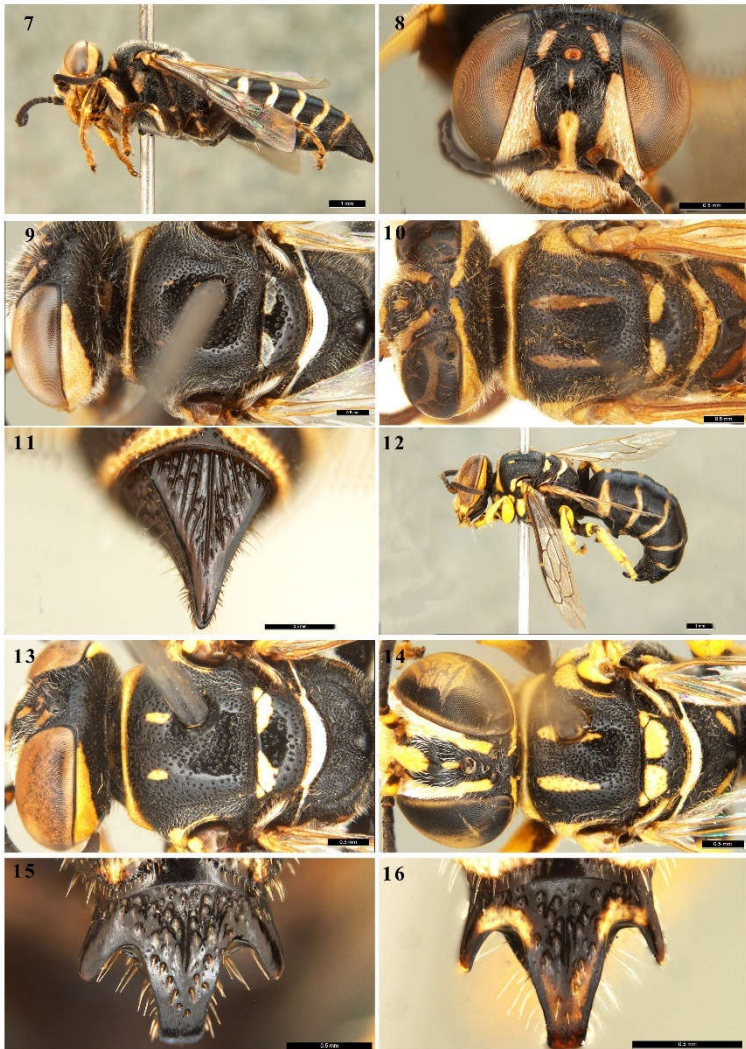
(Figs 1–6)

Palarus comberi Turner, 1911: 483, ♀, ♂. Holotype: ♂, Pakistan: Karachi (BMNH). Paratypes: India: Gujarat: Deesa (BMNH).

Diagnosis: Female. Length: 12–12.3 mm. *Coloration:* mandible basally yellowish white, pre-apically ferruginous and apically black; flagellum, thorax, and propodeum black with yellowish maculae on lateral pronotal collar, pronotal lobe feebly at posterior margin, pre-episternum, scutellar flange inconspicuously, postscutellum and postero-lateral metanotal edge, tegulae, and humeral plate (Figs 1, 3 & 4); coxae ferruginous; femora dark brown to black, fore and mid femora with apicoventral maculae, hind femora maculated at apex; tibiae ferruginous, fore and mid tibiae maculated basally and apically; tarsi ferruginous, fore basitarsus maculated dorsally; gaster blackish brown, all terga except last tergum with yellow interrupted fascia at apex (Fig. 1).

Postocellar area with obtuse crest (Fig. 2); vertex without punctures along edge of crest; mid basitarsus with about thirty spines; hind ocelli oblong and slightly sunken along admedian line; first flagellomere $3.21 \times$ its apical width; forewing and pygidial plate as in figures 5 & 6.

Material examined: India: **Diu**, Nagoa Beach (20°41'52"N, 70°55'37"E), 1 female, 14.vii.2014, Coll. P. Girish Kumar, ZSIK Regd. No. ZSI/WGRC/IR/INV/18169.



FIGURES 7–16. *Palarus interruptus* (Fabricius). 7–11 female. 7. Habitus, lateral view; 8. Head, frontal view; 9. Head and mesosoma, dorsal view (Nedumkayam specimen); 10. Head and mesosoma, dorsal view (Parbatidanga specimen); 11. Last metasomal tergum; 12–16 male: 12. Habitus, lateral view; 13. Head and mesosoma, dorsal view (Nedumkayam specimen); 14. Head and mesosoma, dorsal view (Kannapuram specimen); 15. Last metasomal tergum (Nedumkayam specimen); 16. Last metasomal tergum (Kannapuram specimen).

Variation: microsculptures on propodeal dorsum (Fig. 4) of the studied specimen seems to be slightly different from fig.13a of Pulawski and Prentice (2008).

Distribution: India: Diu (**new record**), Gujarat, Rajasthan. *Elsewhere*: Iran, Pakistan, Saudi Arabia, UAE (Pulawski and Prentice, 2008; Pulawski, 2021).

***Palarus interruptus* (Fabricius, 1787)**

(Figs 7–16)

Bembix interrupta Fabricius, 1787: 295, sex not indicated. Lectotype: ♀, India: no specific locality (ZMUC), designated by Pulawski and Prentice, 2008: 367.

Crabro interruptus Fabricius, 1787: 295, sex not indicated. Lectotype: ♀, India: no specific locality (ZMUC), designated by Pulawski and Prentice, 2008: 367.

Vespa indica Gmelin, 1790: 2769. Substitute name for *Vespa interrupta* (Fabricius, 1787: 286) Gmelin, 1790: 2769, junior secondary homonym of *Vespa interrupta* (Fabricius, 1787: 295) Gmelin, 1790: 2762.

Palarus interruptus: Dahlbom, 1845: 468 (new combination, in key to four species of *Palarus*).

Palarus orientalis Kohl, 1885: 422, ♂. Holotype: ♂, Sri Lanka: no specific locality (NHMW). Synonymized with *Palarus interruptus* by van der Vecht, 1961: 24.

Diagnosis: Female. Length: 14.10–15.33 mm. *Coloration*: head black with whitish or yellowish maculae on clypeus, along mid line of frons below mid ocellus, lateral sides of frons below mid ocellus (extending to hind ocelli), outer surface of eye on vertex (Fig. 10). Thorax and propodeum black, except

following yellowish or whitish: anterior pronotal rim, propleuron, pronotal lobe on posterior margin, precoxal lobe, preepisternum dorsally, anterior part of metapleuron, ventral metapleuron flange, submedial line and antero lateral markings of scutum, one large and one small spots on both sides of base of scutellum, scutallar flange, postscutellum, postscutellar flange, and postero-lateral portion of propodeum (Figs 7, 9–10, 12–14). Leg colouration in female: femur brownish black except fore and mid femora apico-ventrally and hind tibia apically with maculae, all tibiae brownish black except fore and mid tibia with yellow maculae, all tarsi brownish except fore basitarsus with yellow maculae (Fig. 7); leg colouration in male: fore and mid femora with apico-ventral maculae and hind femur maculated at apex, all tibiae and tarsi yellow (Fig. 12).

Genal setae straight and which shorter than basal mandibular width; first flagellomere 1.2–1.4 × its apical width; punctures on terga I & II minute and indistinct mesally, in female, posterior ocellar distance and ocello ocular distance approximately equal, last tergum of male truncated at apex (Figs 15 & 16).

Material examined: India: **Kerala**, Kannur district, Kannapuram (11°57'55"N, 75°19'07"E), 1 male, 8.v.2019, Coll. C. Charesh, ZSIK Regd. No. ZSI/WGRC/IR/INV/18170; Malappuram district, Nedumkayam (11°17'31"N, 76°20'22"E), 1 male & 1 female, 29.ii.2020, Coll. Tessa Rajan, ZSIK Regd. Nos. ZSI/WGRC/IR/INV/18171 & 18172; **West Bengal**, Malda district, Parbatidanga (25°05'34"N, 88°25'51"E), 1 female, 27.vii.1987, Coll. K.P.M. Mukherjee & Party, ZSIK Regd. No. ZSI/WGRC/IR/INV/18173.

Variation: Hind femoral outer surface without any preapical spine in all the studied specimens. Least inter ocular distance of

the studied specimens is $4.13\text{--}4.37 \times$ mid ocellar width instead of $3.60 \times$ in female described by Pulawski and Prentice (2008); yellow maculae is absent behind hind ocelli on vertex in both female and male from Nedumkayam, Kerala (Figs 9 & 13). Maculae on lateral sides of frons, below mid ocellus sometimes extending to hind ocelli after an interruption (Fig. 8). Female specimen from Nedumkayam is devoid of yellow maculae postero-laterally on propodeum (Fig. 9), but small postero-lateral dirty yellow-coloured maculae is present in male specimens from Nedumkayam (Fig. 13). Maculae on metasternum present in Kannapuram male specimen, which is absent in both male and female specimen of Nedumkayam. These variations are insufficient to consider them as different species. Pulawski & Prentice (2008: 367-369 pp) well explained the range of variations in different characters especially the extend of yellow maculations at vertex, metasternum, postero-lateral sides of propodeum and pygidium within *P. interruptus*.

Distribution: India: Gujarat, Kerala, Tamil Nadu, West Bengal (**new record**). *Elsewhere:* Sri Lanka (Pulawski and Prentice, 2008; Pulawski, 2021).

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CANCER CHEMOPREVENTION BY NATURAL PRODUCTS

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Abstract: *Plant extracts and natural compounds purified from plants have been used by humans for many centuries for the treatment and alleviation of a variety of inflammation-related diseases, including cancer. The National Institutes of Health, after extensive research over the last 50 years, has recently stated that a diet rich in fruits and vegetables can reduce the risk of many diseases including cancer. In fact, a number of natural compounds with inhibitory effects on tumorogenesis have been identified from our diet or sources of the diet. This Review will focus on the cancer chemoprevention by natural products in culture as well as animal models.*

Keywords: *Plant extracts, Cancer, Chemoprevention, Natural products*

Introduction

Cancer chemoprevention, a term coined by Sporn for the protective effects of retinoids (Sporn and Newton, 1979), is defined today as the blocking or suppressing of the carcinogenic process by one or several compounds (Wattenberg, 1985). Several natural products and dietary components have been shown to function as cancer

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chemopreventive agents. These natural products may disrupt many signaling pathways, including transduction of cell surface (epidermal growth factor) or nuclear (estrogen) receptors via inhibition of their associated tyrosine kinase activities that regulate mitogenic signaling cascades. (Block *et al.*, 1992). Alternatively, cytoprotective signal transduction pathways may be activated in a concentration and time dependent manner. People, who have diet consisting of fruits and green-yellow vegetables, have lower risk of many kinds of cancer (Block *et al.*, 1992).

Vinblastin and vincristine were first introduced in the late 1960s and have contributed to long-term remissions and cures with childhood leukemia, testicular teratoma, Hodgkin's disease and many other cancers (Xu and Xu, 2020). Guggulsteron obtained from the gum resin of *Commiphoramukulwas* was found to suppress inflammation by inhibiting iNOS expression (Meselhy, 2003). Guggulsteron also suppressed the constitutive NF- κ B activation expressed in most tumour cells (Shishodia and Aggarwal, 2004).

Chemoprevention by naturally occurring Sulfur compounds

Among naturally occurring products, Sulfur containing compounds (OSCs), especially garlic compounds (GCs) and isothiocyanates (ITCs) represent two important and promising chemopreventive families because of their potent chemopreventive effects in various *in vivo* and *in vitro* models.

a) Garlic constituents

Garlic, *Allium sativum*, has been well known for its medicinal properties since time immemorial. Egyptian records dating to about 1550 B.C mention garlic as a remedy for a

variety of diseases (Block, 1985). Experimentally garlic and its associated Sulfur components are reported to suppress the incidence of tumours of rodent models in breast, colon, skin, uterus, oesophagus and lung (Amagase and Milner, 1993). Epidemiological findings also demonstrated an inverse relationship between garlic consumption and the incidence of stomach cancer, colorectal cancer and prostate cancer. Two pathways are involved in the conversion of natural garlic to Sulfur compounds. The first pathway is natural aging bioconversion, which leads to the formation of mainly water soluble Sulfur compounds such as S-allyl cysteine (SAC), and S-allylmercapto cysteine (SMAC). The second pathway is cell decomposition to allicin, which again breaks down rapidly under uncontrollable chemical reactions to produce odorous oil soluble Sulfur compounds namely Diallylsulfide (DAS), Diallyl disulfide (DADS), Diallyltrisulfide (DATS) and Ajoene.

Chemopreventive effects of garlic constituents are based on: i) enhancement of the activity of specific mixed–function oxidases that depress the activation of carcinogens(Wu *et al.*, 2005);ii) induction of phase II enzymes which enhance detoxification and excretion of potential carcinogens and reduction of the formation of DNA adducts (Ameen *et al.*, 2003); iii) increased synthesis of GSH, an endogenous tripeptidethiol that directly protects cells from damage by free radicals and iv) induction of apoptosis (Wu *et al.*, 2005).

Diallylsulfide (DAS), a flavour component derived from fresh garlic, has been shown to protect against chemically induced toxicity and carcinogenesis in animals. Modulation of the metabolism of the carcinogen by DAS is considered as one of the possible mechanisms for protection against cancers.

Several studies showed that DAS could modulate hepatic drug-metabolizing enzymes (Wargovich *et al.*, 1992).

Diallyl disulfide (DADS) is an oil soluble Sulfur compound from garlic that is produced as a result of decomposition of allium. About 60% of garlic oil was reported to be DADS, indicating that it is the most prevalent oil soluble garlic constituent. Studies conducted by Sundaram and Milner(1996)proved that the inhibition of proliferation of human tumour cells by DADS is through alteration in calcium homeostasis.Studies also revealed that oil soluble organosulfur compounds in garlic were more effective in inhibiting *in vitro* growth of tumour cells while water soluble compounds had little or no inhibitory effect. Earlier studies also demonstrated the immunomodulatory, antimetastatic and antiangiogenic activity of DAS and DADS (Kuttan and Kuttan, 1999; Thejass and Kuttan 2007). It has been shown that metabolism of DADS leads to the formation of allylmercaptan, which contributes to the anticarcinogenic effect against N-nitrosodiethylamine-induced fore stomach cancer in mice (Sundaram and Milner, 1996).

b) Isothiocyanates (ITCs)

Isothiocyanates (ITCs) are hydrolysis products of a group of naturally occurring thioglucoside compounds, glucosinolates, found in cruciferous vegetables such as watercress, brussels sprouts, broccoli, cabbage, horseradish, radish and turnip (Conaway *et al.*, 2002). ITCs inhibit cancer formation or reduced cancer growth in various tissues such as rat lung (Hecht *et al.*, 2002); oesophagus; liver and small intestine.

ITCs pertube several steps in carcinogenic process by:

i) blocking DNA damage by both inhibition of carcinogen

activation through inhibition of Phase I enzymes (mainly cytochrome p450) and detoxification of reactive carcinogens through induction of phase II enzymes (glutathione-s-transferase); ii) inhibiting cell growth by cell cycle arrest and iii) removing premalignant and malignant cells through activation of apoptosis (Conaway *et al.*, 2002). Benzyl isothiocyanate, Allyl isothiocyanate (AITC), Phenethylisothiocyanate and Sulforaphane are examples of ITCs that induce GST and block carcinogenesis.

Allyl isothiocyanate (AITC) which exist in nature as the glucosinolate precursor, sinigrin, has been reported to induce GST activity in the liver and small intestine of the rat. Studies conducted earlier in our laboratory proved the immunopotentiating activity of AITC and Phenyl isothiocyanate (PITC) and found that both these compounds enhanced stem cell proliferation. These compounds also inhibited pulmonary lung metastasis. DAS, DADS, AITC and PITC have been reported to reduce cyclophosphamide induced urotoxicity (Manesh and Kuttan, 2005) and leucopenia associated with radiation (Manesh and Kuttan, 2006).

Sulforaphane is present in several cruciferous vegetables, but predominantly in broccoli. Previous studies demonstrated that Sulforaphane induces high levels of mammalian phase II enzymes via an antioxidant response element (ARE)-mediated transcriptional activation (Fahey *et al.*, 1997). In addition, Sulforaphane reduced breast cancer incidence and minimized the size of the tumour in a rat model (Fahey *et al.*, 1997).

Overall it is well established that ITCs and garlic constituents (GCs) affect xenobiotic metabolizing enzymes in such a way that carcinogens are less activated or detoxified and

excreted rapidly or that DNA damage is circumvented (Conaway *et al.*, 2002). Knowledge of mechanism of cancer chemopreventive effect of ITCs and GCs sheds more light not only on the beneficial effects to humans of garlic and cruciferous vegetables, but may also pave the way for the development of GCs and ITCs for dietary supplementation or even cancer therapeutic drugs.

Chemoprevention by Curcumin

Curcumin (diferuloyl methane), has been shown to suppress carcinogenesis of the skin, liver, lung, colon, stomach and breast. It has also been shown to inhibit the proliferation of a wide variety of tumour cells in culture and to promote apoptosis through Bid cleavage, cytochrome-c release, caspase-9 activation and then caspase-3 activation (Antoet *al.*, 2002). Curcumin mediates this wide variety of therapeutic effects by regulating the transcription factors NF- κ B and activator protein, suppressing I κ B α kinase and c-Jun N-terminal kinase and inhibiting expression of COX-2, cyclinD1, adhesion molecules MMPs, iNOS, Bcl-2, Bcl-X_L and TNF (Antoet *al.*, 2002). Some synthetic curcuminoid derivatives have also been shown to inhibit tumour specific angiogenesis.

Chemoprevention by naturally occurring Polyhydroxyphenols

Intake of certain kinds of polyhydroxy phenols such as flavanoids or lignans in the diet has been correlated with low incidence of colon cancer and breast cancer. Tangeritin occurring in tangerine peel was found to inhibit leukemia HL-60 cell growth partially through induction of apoptosis (Hirano *et al.*, 1995). Epigallocatechingallate (EGCG), a polyphenol from green tea, has been shown to stimulate apoptosis of various cancer cell lines such as prostate, lymphoma, colon and

lung (Katiyar, 2006). It also strongly induced DNA fragmentation in PC-9 cells and inhibited TNF- α gene expression.

Conclusion

Cancer prevention by natural products has received considerable attention in the recent years. Chemoprevention, which is referred to as the use of natural products to intervene the multistage carcinogenesis, has emerged as a promising approach to reduce the risk of cancer and its progression. Higher intake of vegetables and fruits protects against cancers of the stomach, oesophagus, lung, oral cavity and pharynx, endometrium, pancreas and colon. The types of vegetables and fruits that most often appear to be protective against cancer are raw vegetables, followed by cooked allium vegetables, carrots, green vegetables, cruciferous vegetables and tomatoes. This reminded us what Hippocrates (460–377 BC), acknowledged as “Father of Medicine,” remarked almost 25 centuries ago: “Let food be thy medicine and medicine be thy food.”

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DIELECTRIC STUDIES OF THE GLASS FORMATION IN SIGNIFICANT ACTIVE PHARMACEUTICAL INGREDIENTS: ACEMETACIN, BEZAFIBRATE AND COLCHICINE

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Abstract: A comparative study of the supercooling process and dielectric relaxation of three active pharmaceutical ingredients (API) Bezaifibrate, Acemetacin and Colchicine was done by broadband dielectric spectroscopy technique in a temperature range of 450K to 123K and in a frequency range of 10mHz to 10MHz to characterize the temperature and frequency dependence of complex dielectric permittivity (ϵ^*) in order to estimate the glass forming ability and physico-chemical stability of their amorphous phase. The presence of α process and secondary process was confirmed in the three APIs selected for some test frequencies. The real and imaginary part of the complex dielectric permittivity is found to be dependent on temperature and frequency in all three APIs. The measure of deviation from the Debye relaxation and the stability of the amorphous phase of the title APIs were estimated from the fit of the dielectric loss curve at an arbitrary temperature in supercooled liquid state. This work gives a comparison between the dielectric relaxations in an alkaloid drug (Colchicine) derived from plants and synthetic drugs (Bezaifibrate (fibrate class of drug) and Acemetacin (NSAID class of drug). The structures of the title APIs were optimized by Density Functional Theory (DFT) method using Gaussian software.

Keywords: Dielectric relaxation, Bezaifibrate, Acemetacin and Colchicine, Density Functional Theory

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Introduction

Poor solubility of drugs has created a huge loss in the pharmaceutical industry as many of the potential drugs including life saving drugs were excluded from production due to this problem. Amorphous pharmaceuticals have become the focus of research recently in order to solve this issue due to its better solubility than crystalline counterpart (Sun et al., 2012). However, the amorphous drug has poor physico-chemical stability due to its metastable state and hence all the advantages of amorphous form may be lost within the shelf life (Craig et al. 1999). Molecular mobility and crystallization are the key factors for the instability of the amorphous phase. Therefore, in order to design storage conditions for the safe storage of amorphous drugs during shelf life, it is essential to understand the nature of molecular relaxations in the supercooled liquid and glassy states and glass forming ability.

Colchicine is an alkaloid extracted from the roots of *Colchicum autumnale* L, antimitotic, anti-inflammatory and antineoplastic effects (Brossi et al. 1988) is used for treatment of gout and Mediterranean fever, however antimitotic anti cancerous effect of Colchicine was reported (Bhattacharyya et al. 2008). Colchicine, is also an alkaloid drug popularly used for its. It is extracted from the plants of the genus *Colchicum* (autumn crocus) and is used to treat gouty arthritis for many years (Roberts et al. 1987; Alabed et al. 2014). Mediterranean fever, Pericarditis (Cocco et al. 2010) and Behçet's disease (Cerquaglia et al. 2005). The bioavailability of Colchicine is around 45% (Rochdi et al. 1994). Acemetacin belongs to the class of non-opioid analgesic and is commonly used

in relieving pain. Bezafibrate belongs to the class of fibrates used to treat hyperlipidemia to lower LDL cholesterol and triglyceride in the blood and increase HDL. Bezafibrate reduces the incidence of Myocardial Infarction in patients with metabolic syndrome (Tenenbaum et al. 2005). Supercooling a liquid is the most commonly used method for making glass. However, the molecular processes by which liquids acquire amorphous rigidity upon cooling are not fully understood (DeBenedetti and Stillinger 2001; Lubchenko and Wolynes 2007; Sahra et al. 2019; Ediger et al 1996). The glass formation study of alkaloids and non-alkaloid pharmaceuticals shed light for researchers who are interested in deep understanding of liquid-glass transition phenomenon. The comprehensive chemical structures of pharmaceuticals studied by dielectric spectroscopy has helped researchers in this field to have more understanding to the field of liquid-glass transition (Schammé et al. 2016). This study will not only benefit the pharmaceuticals, but also give insights into the glass transition phenomenon.

Dielectric spectroscopy played a major role in understanding the dynamic response of the glass formers like for example the detection of excess wing (Davidson and Cole 1951) at a higher frequency than the α relaxation peak (a hidden intermolecular relaxation called as Johari-Goldstein relaxation which is considered to be the universal feature of glass forming liquids, as the glass transition phenomenon is still considered as an unresolved

problem (Roggendorf 1992). The temperature dependence of the complex dielectric constant (ϵ^*) of the title APIs while quench cooling was studied for arbitrarily selected frequencies and analyzed the dielectric relaxation exhibited by the samples which are characteristics of glass formers. The frequency dependence of complex dielectric constant (ϵ^*) of the title APIs for an arbitrary selected temperature in super cooled liquid state is analyzed by fitting it to the Havriliak - Negami (HN) equation (Havriliak and Negami 1967) and Kohlraw-William-Watts (KWW) function (Safna Hussan et al. 2018; Safna Hussan et al. 2016; Safna Hussan et al. 2017; Sailaja and Shahin Thayyil 2014). Above glass transition temperature (T_g) ($T > T_g$), glass formers normally exhibit α -relaxation which arises due to the cooperative effect of neighboring molecules and in glassy state ($T < T_g$) secondary relaxation processes (β -, γ -, δ - etc.) arises due to the motions of a single molecule alone or due to subgroups of a molecule (Capaccioli et al 2011). Secondary relaxation can be Johari-Goldstein (JG) relaxation (β -process) or non-JG. JG β relaxation is intermolecular in nature while if the relaxation is due to intra molecular motions it is called as non-JG relaxation. JG β -relaxation is considered as the universal property of glass forming liquids, which is correlated to the primitive relaxation through Ngai's coupling model (Ngai 2003). This study is not only important for pharmaceutical industry but also give new insights to glass formation phenomena of complex glass forming systems.

2. Experimental details

2.1 Dielectric study

Complex dielectric constant of the samples were measured by using a broadband dielectricspectrometer (Novocontrol GmbH, Germany frequencyrange- 10 mHz–10 MHz).The sample (50mg of crystalline powder) is kept between two stainless steel electrodes (30mm diameter) and keeping two narrow Teflon spacers of 50 μ m thickness toget an empty cell capacitance of approximately 100 pF. The temperaturecontrollerworks by dry nitrogen-flow supplied by theNovocontrol Quatro cryosystem having a temperature stability better than ± 0.1 K.For cooling curves (temperature dependance of complex dielectric constanteach of the samples was heated above their respective melting point and then quenchcooled to 123.15K for glass formation. This procedure was repeated for all the three samples. The frequency dependance curve of complex dielectric constant at an arbitrary temperature in the supercooled liquid state were measured isothermally, after stabilizing the temperaturefor about500swere fitted with non-linear curve fitting routineof Levenberg Marquadt algorithm using WINFIT software Version 3.2provided by Novocontrol.

Acemetacin, Colchicine and Bezafibrate (crystalline powder form) were purchased from Sigma-Aldrich (purity > 98%) and were used without any further purification. The IUPAC name of Acemetacin is 2-[2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetyl] oxyaceticacid (Molecular formula: $C_{21}H_{18}ClNO_6$, Molecular mass: 415.826 g/mol, melting temperature: 424.65K). The IUPAC name of colchicine is (1E)-N-[(7S)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetra hydro benzo[a]heptalen-7-yl] ethanimidic acid and empirical formula

is $C_{22}H_{25}NO_6$, (molecular weight is 399.437g/mol, melting point 429K). The IUPAC name of Bezafibrate is 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid, Molar mass 361.819 g/mol molecular formula is $C_{19}H_{20}ClNO_4$ and melting point 459K. The chemical structure of Acemetacin, Bezafibrate and Colchicine are shown in figure 1.a and 1.b and 1.c respectively.

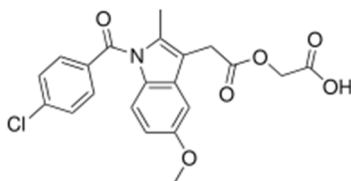


Figure 1.a. Chemical Structure of Acemetacin

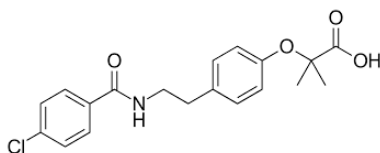


Figure 1.b. Chemical Structure of Bezafibrate

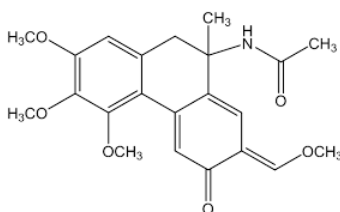


Figure 1.c. Chemical structure of Colchicine

3. Results and Discussion

The optimized geometry of the bezafibrate, acemetacin and colchicine by Density functional theory method in B3LYP level of theory using 6-311g++(d,p) basis set, using the Gaussian 09 package (Frisch 2009) are shown in figures 2,3 and 4 respectively.

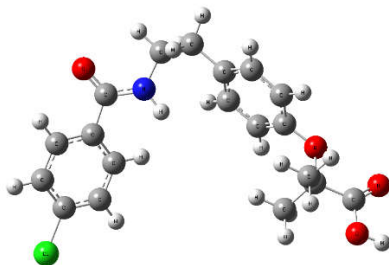


Fig.2 Optimized structure of bezafibrate

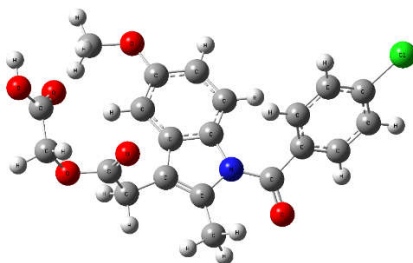


Fig.3 Optimized structure of acemetacin

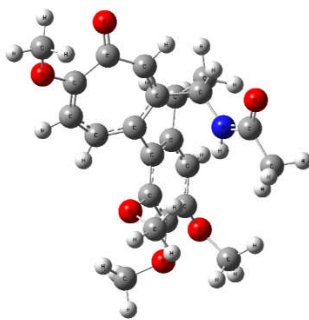


Fig. 4 Optimized structure of Colchicine

From the figures 2, 3 and 4, the molecular structures are found to be complex and hence the glass formation study of these title APIs are worth studying. Due to the presence of side groups in the structures of all the title APIs, secondary relaxation is expected.

3.2 Dielectric study

The temperature dependence of real part (ϵ') of the complex dielectric constant (dielectric permittivity) $\epsilon^*(f)$ at arbitrary selected constant frequencies $f = 10^2, 10^3, 10^4$ and 10^5 Hz in the super cooled liquid states of Colchicine, Bezafibrate and Acemetacin while cooling from their corresponding melt are shown in figures 2 and 3.

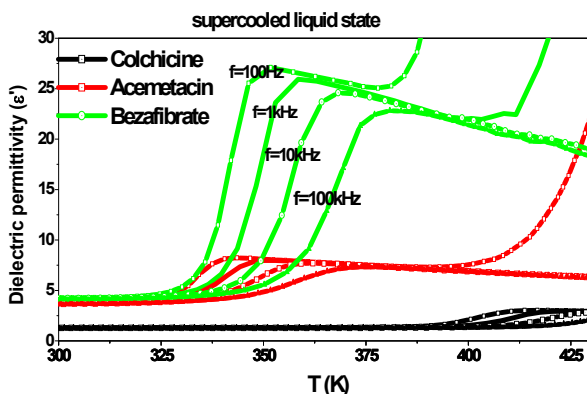


Figure 5 Temperature dependence of dielectric permittivity in the supercooled liquid state of Colchicine, Bezafibrate and Acemetacin at frequencies $f=100\text{Hz}$, 1kHz , 10kHz and 100kHz

Bezafibrate has higher values of dielectric permittivity (ϵ') than acemetacin which in turn is higher than colchicine. In figure 5, ϵ' is found to increase with temperature and becomes

maximum value at a particular temperature for a given frequency and then decreases which is due to structural molecular relaxation (α relaxation) attributed to the global molecular mobility and is an intrinsic characteristic of all glass formers. As frequency is increased the temperature at which the dielectric permittivity becomes maximum (ϵ_m) shifts to higher temperatures, as thermal energy increases the molecular motion becomes faster and hence there is increase in global molecular mobility. For a given frequency structural relaxation starts in lower temperature in acetaminophen than that of bezafibrate whereas in Colchicine, the α process starts only at a temperature 50K higher than that of acetaminophen and bezafibrate. This may be due to the comparatively higher degree of complexity in the structure of colchicine. The increase in dielectric permittivity in bezafibrate due to α process at all selected frequencies is found to be around ten times bigger than that of acetaminophen and even more than that of colchicine which indicate that the global molecular mobility and temperature dependence of molecular relaxation is very much higher in bezafibrate compared to acetaminophen and colchicine.

The temperature dependence of real part (ϵ') of the complex dielectric constant (dielectric permittivity) $\epsilon^*(f)$ at arbitrary selected constant frequencies $f = 10^2, 10^3, 10^4$ and 10^5 Hz in the glassy states of Colchicine, Bezafibrate and Acetaminophen while cooling from their corresponding melt are shown in figures 7 and 8.

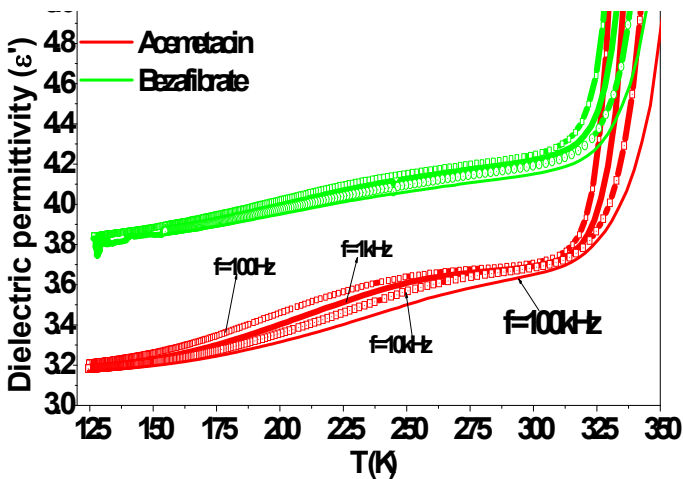


Figure 6. Temperature dependence of dielectric permittivity in the glassy state of Bezaifibrate and Acemetacin at frequencies $f=100\text{Hz}$, 1kHz , 10kHz and 100kHz

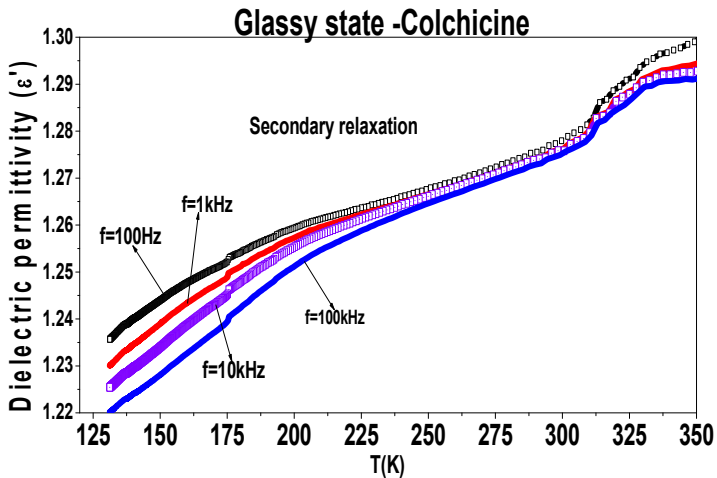


Figure 7. Temperature dependence of dielectric permittivity in the glassy state of Colchicine $f=100\text{Hz}$, 1kHz , 10kHz and 100kHz

From figures 6 and 7 secondary relaxation is observed in all the title APIs which is evident from the comparatively small values of dielectric permittivity and it is clear that α

process is strongly dependent on temperature than secondary relaxation. The secondary relaxation is found to cease at 123K at all the title APIs. The secondary relaxation is comparatively stronger in acetaminolol than that of colchicine and bezafibrate. Secondary relaxation in colchicine has lower value of dielectric strength than that of acetaminolol which in turn is lower than that of bezafibrate

The temperature dependence of imaginary part (ϵ'') of the complex dielectric constant $\epsilon^*(f)$ (dielectric loss) at arbitrary selected constant frequencies $f = 10^2, 10^3, 10^4$ and 10^5 Hz in the super cooled liquid and glassy states of Colchicine, Bezafibrate and Acemetacin while cooling from their corresponding melt are shown in figure9.

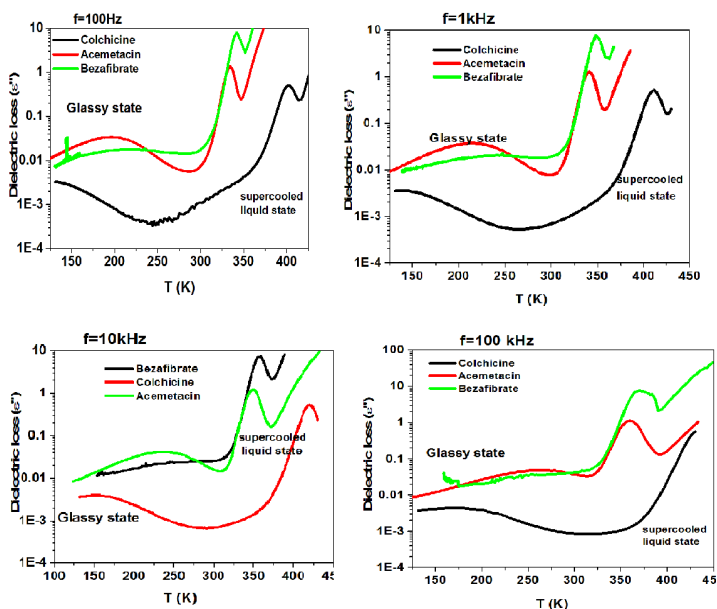


Figure 8 Temperature dependence of dielectric loss in the supercooled liquid and glassy states of Colchicine, Bezafibrate and Acemetacin at arbitrary fixed frequencies (a) $f=100\text{Hz}$ (b) $f=1\text{ kHz}$ (c) $f=10\text{ kHz}$ (d) $f=100\text{ kHz}$

The presence of molecular relaxation in the title APIs in the supercooled liquid and glassy states is confirmed by a change in the real part of dielectric constant with increase in temperature, on the other hand, a peak is observed in the imaginary part of dielectric constant (dielectric loss(ϵ'')) corresponding to structural(α process) and secondary relaxation at a particular temperature depending on the frequency. Figures 8.a to 8.d shows the temperature dependence of dielectric loss for bezafibrate, colchicine and acetaminophen at arbitrary fixed frequencies of $f=100\text{Hz}$, 1kHz , 10 kHz and 100 kHz respectively. From the figure 8, for all frequencies, Bezafibrate have higher value of peak dielectric loss than that of acetaminophen which in turn is higher than that of colchicine similar to the dielectric permittivity. The temperature at which the dielectric loss becomes maximum is also higher for bezafibrate than that of acetaminophen which in turn is higher than that of colchicine. However, the secondary relaxation is more prominent in acetaminophen than that of bezafibrate and colchicine. As frequency increases the peak dielectric loss also shifts to higher temperature in all title APIs similar to dielectric permittivity. There is a correlation between the temperatures at which the dielectric permittivity and dielectric loss changes during quench cooling. The temperature at which the maximum dielectric loss due to secondary relaxation is higher for acetaminophen than bezafibrate which in turn is higher than that of colchicine. The temperature at which dielectric loss peak (α process) of all the title APIs are found to increase with higher frequencies, similar behavior is observed in brucine (Afzal et al 2018). Acetaminophen is found to show secondary relaxation for larger temperature range than that of the other two title APIs in a temperature lower where α relaxation is observed. The increase in dielectric loss at temperatures after the α peak

for all frequencies in figure 8 were observed in colchicine, acetaminophen and bezafibrate due to dc conductivity contributed by the translational motion of ions.

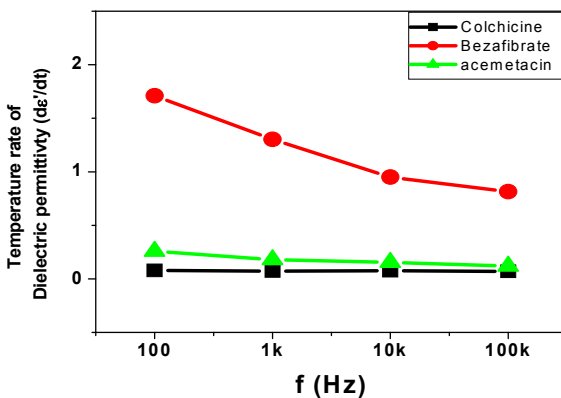


Figure 9. The temperature rate of decrease of dielectric permittivity in supercooled liquid state at selected frequencies for colchicine, bezafibrate and acetaminophen.

Figure 9 shows the rate of decrease of dielectric permittivity with temperature in the supercooled liquid state during quench cooling at four selected frequencies for colchicine, bezafibrate and acetaminophen. This will give an idea of the measure of easiness of the molecular motion in the supercooled liquid state (molecular mobility) to undergo orientation polarization as thermal activation energy changes. It is clear from the figure 9 that in bezafibrate, molecules in supercooled liquid state has higher molecular mobility and is strongly dependent on temperature than that of acetaminophen which in turn is stronger than that of colchicine. Complexity of the structure, intermolecular interactions, hydrogen bonding may be the reason for the difference in the differences in the temperature rate of decrease of dielectric permittivity.

Complex dielectric permittivity, [$\epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega)$] was measured for an arbitrary selected temperature in supercooled liquid state and was fitted by Havriliak - Negami (HN) equation and shown in figure 10.

$$\epsilon^*(\omega) = -i \left(\frac{\sigma_{dc}}{\omega \epsilon_0} \right) + \epsilon_\infty + \frac{\Delta\epsilon}{(1+(i\omega\tau_{HN})^\alpha)^{\beta_{HN}}} \quad (1)$$

Where ϵ_∞ is the high frequency limit of ϵ' , $\Delta\epsilon$ is the dielectric strength, and τ_{HN} is the characteristic relaxation time of the medium, σ_{dc} is the dc conductivity, α and β are fractional shape parameters ($0 < \alpha < 1$ and $0 < \beta < 1$) which describe the symmetric and asymmetric broadening of the dielectric loss curves respectively.

The HN fit parameters are show in table 1

Table 1 HN fit parameters for Colchicine, Bezafibrate and Acemetacin

Sample	T[K]	α	β	τ_{max} [s]	$\Delta\epsilon$
Colchicine	375	0.382	0.751	4.74	1.41
Bezafibrate	313	0.147	0.695	4.88×10^{-02}	1.92
Acemetacin	317	0.117	0.570	5.79×10^{-01}	4.72

The HN fit values of the title APIs shown in table 1 reveals the behavior of a complex glass former obeying HN equation. From the values of the structural relaxation times τ_{max} , the glass transition temperature of the title APIs are below the selected arbitrary temperature as at glass transition temperature the value of $\tau_{max}=100$ s. From the comparison of the values of dielectric strengths in Table 1, we found that acemetacin have maximum dielectric strength than bezafibrate and colchicine which suggest high polarity of acemetacin compared to the other two APIs. The values of the symmetric

and asymmetric stretching parameters α and β reveals that all the title APIs shows non Debye type of relaxation and has medium value of deviation from Debye relaxation. The dielectric loss spectra in supercooled liquid state in most glass formers normally is non-exponential in nature due to the distribution of molecular relaxation times indicating their dynamic heterogeneity and is fitted by Kohlrausch-Williams-Watts function (equation 6)

$$\phi(t) = \exp\left(-\frac{t}{\tau_\alpha}\right)^{\beta_{KWW}} \quad (6)$$

Where $\phi(t)$ is the relaxation function, τ_α is the relaxation time due to α process and β_{KWW} is related to the distribution of structural relaxation times in different temperatures in supercooled liquid state. The Kohlraw-William-Watts (KWW) function (Williams and Watts 1970) describes the degree of non-exponentiality of dielectric loss curve and is used to find the asymmetrical stretching parameter β_{KWW} which measures the asymmetric distribution of relaxation time and its deviation from Debye relaxation ($\beta_{KWW}=1$). The breadth of the structural relaxation peak was described using stretching parameter β_{KWW} ($0 < \beta_{KWW} \leq 1$) which can be determined by fitting the α -peak in the frequency domain by the one-sided Fourier transform of the KWW function. Taking one side Fourier transform of the Kohlrausch–Williams–Watts (KWW) function (Williams and Watts 1970) β_{KWW} is determined for the arbitrary temperature for the title APIs, and found that the experimental data of acemetacin, bezafibrate and colchicine fitted well to the KWW function and are shown in figure 10.

From the values of β_{KWW} determined from KWW fits for acemetacin, bezafibrate and colchicine, it is found that these

APIs have intermediate degree of non-exponentiality and deviation from Debye relaxation(β_{KWW}) in these arbitrary temperatures in the supercooled liquid state. However the primitive relaxation frequency (f_0) as calculated using Ngai's Coupling model corresponds to the frequency where excess wing is normally observed and a remarkable deviation of the dielectric loss curve in the high frequency flank is observed in figure 10 in the three APIs, which may be due to the presence of hidden intermolecular JG relaxation which is hidden by α relaxation, such behavior is observed in many pharmaceuticals (Sailaja et al. 2013).

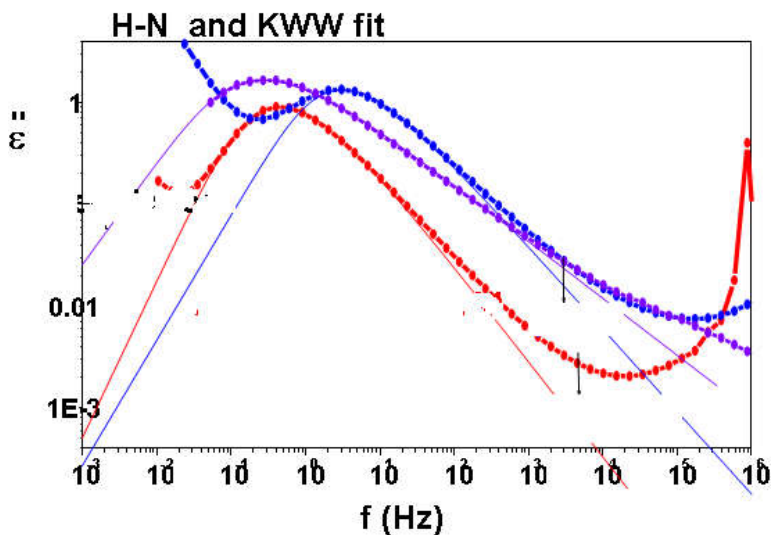


Figure 10. Havriliak-Negami and KWW fit of acemetacin, colchicine and bezafibrate at an arbitrary temperature in supercooled liquid state. Primitive relaxation frequency found by coupling model is also shown.

CONCLUSIONS

Dielectric study of supercooling process and molecular relaxation of three APIs Bezafibrate, Colchicine and Acemetacin were done and characterized the temperature and

frequency dependence of real and imaginary part of dielectric constant in supercooled liquid state. Primary (structural) and secondary relaxation were found to present in three APIs studied. The orientation polarization in Bezafibrate has stronger temperature response than that of colchicine and acetaminophen. Secondary relaxation process is found to be weaker in colchicine and is strongest in acetaminophen. Acetaminophen has more dielectric strength than that of bezafibrate and colchicine which may be due to presence of polar subgroups. The value of β_{KWW} determined from KWW fits for acetaminophen, bezafibrate and colchicine, it is found that these APIs have intermediate degree of non exponentiality and deviation from Debye relaxation (β_{KWW}) in this arbitrary temperatures in the supercooled liquid state. However the primitive relaxation frequency (f_0) as calculated using Ngai's Coupling model corresponds to the frequency where excess wing is normally observed and a remarkable deviation of the dielectric loss curve in the high frequency flank is observed in figure 10 in the three APIs, which may be due to the presence of hidden intermolecular JG relaxation which is hidden by α relaxation.

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A PRELIMINARY STUDY OF BUTTERFLY DIVERSITY AND THEIR FEEDING PREFERENCES IN AKODE, MALAPPURAM, KERALA, INDIA

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Abstract: *A preliminary study was conducted to document butterfly diversity of Akode village of Vazhakkad Grama panchayath, Malappuram district during the period from November 2020 to March 2021. A total of 45 species of butterflies belonging to five families (Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperidae) were identified from the study area. Out of these, members of the family Nymphalidae were the most dominant with highest species (20) and the least number of species belonging to Hesperidae (4). Among the 45 species observed, Common bush brown and Chocolate pansy was the most predominant species. The diversity of butterflies with respect to changing temperature and humidity level has been analyzed statistically by correlation coefficient. The analysis of the influence of environmental variables on butterfly fauna indicates that butterflies are more abundant during high humidity (81%) and average temperature (26°C). At high temperature (31°C) showed a negative correlation and high humidity (81%) showed a positive correlation. The feeding preference of butterflies on various host plants revealed that they primarily feed on nectar and sugars from flowers, tree sap and rotten foods. One of the interesting facts noticed during the study was feeding by certain butterflies like Banded blue Pierrot, Common Pierrot, Chocolate pansy, Grey count etc. on rotten foods/ fruits, animal droppings and carrion.*

Keywords: *Butterfly diversity, Host plant, Akode village, Kerala*

INTRODUCTION

Butterflies are the most well-known, wellstudied well studiedeasily recognizable orders of insects and important component of biodiversity and have gained much global

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attention (New,1991; Ghazoul, 2002). The degree of diversity depends upon the adaptability of a species to a particular micro habitat. There are about 18,000 species of butterflies in the world and they serve as important plant pollinators in the local environment, and help to pollinate hundreds of economically important plant crops (Shields, 1989). They are indicators of a healthy environment and healthy ecosystems in terms of anthropogenic disturbance and habitat quality (Kocher and Williams, 2000). They form an important part of the food chain of birds, reptiles, amphibians, spiders and predatory insects. Butterflies and their caterpillars are depending upon specific host plant for food, thus the diversity of butterflies indirectly reflects overall plant diversity especially that of shrubs and herbs in the given area. Most of them are strictly seasonal and prefer only particular set of habitats (Kunte, 1997). According to Gaonkar (1996), India hosts 1,501 species of butterflies, of which peninsular India hosts 350, and the Western Ghats 331 out of which 316 species have been reported from Kerala. Recent data by Varshney and Smetacek (2015) indicates that there are 1318 species in India. Some of the significant contributions to our understanding of butterfly diversity and abundance, on aspects such as habitat association, effect of disturbance and area clearance, seasonal abundance and migration patterns, on conservation etc were made by many researchers (Kunte *et al.*, 1999.; Krishnakumar *et al.*, 2008., Sreekumar and Balakrishnan, 2001; Kunte, 2000). The present study aimed to examine the butterfly diversity, abundance and the feeding preference of butterflies on various host plants in the Akode Village of Vazhakkad Grama Panchayath, Malappuram district. The study will be helpful by adding a documentation of biodiversity of butterflies in some local areas

of Malappuram district and also to remind the conservation of both butterflies and their preferred habitats.

MATERIALS AND METHODS

The present study was conducted in Akode village, a place belonging to Vazhakkad Grama Panchayath of Malappuram district which is surrounded by local paddy fields and patches of plantain and Areca palm cultivation with a latitude and altitude of 11°14'34" N, 75°55'30"E (Figure 1 & 2). The study area consists of around 0.5 acres of land with a wide variety of grasses, herbs, shrubs and trees along with a ground layer of fallen leaves that provide both nectar and host plants also with associated fauna too. The sampling of butterfly diversity was done during the period from November 2020 to March 2021. The survey was carried out by steadily walking along the survey routes and recording butterflies observed within 10 m width along the routes, using line transect method (Yamamoto, 1975.; Pollard and Yates, 1993). This method has been extensively used to survey and monitor butterfly population and communities. The survey was repeated four times per month twice in a day. The transects were visited between 9:00 am to 1:00 pm in morning and 3:00 pm to 5:00 pm in the evening.



Figure 1-satellite view of study area Figure 2:view of study area

During the survey, the observed butterflies were photographed by using a mobile camera having 405

ppi density. The captured species were identified using the field guides “A Concise Guide To Butterflies & Moths” (Elizabeth Balmer, 2007., “Butterflies of India” (Arun Pratap Singh., 2011). During the study, the collection of butterflies was avoided as part of conservation policy (National forest policy). It was not possible to distinguish between closely related species for many individuals during flight. These counts were excluded from the sample. To note the host plant preference a thorough observation was made during the study period. Two components namely alpha diversity and species evenness were calculated by using appropriate statistical diversity indices. The Shannon diversity index is used for calculating the alpha diversity and the species abundance has been calculated by another diversity index named Pielou’s evenness index and has been calculated by appropriate statistical formulas. The effect of two environmental parameters such as temperature and humidity on butterfly diversity has been statistically analyzed by correlation coefficient, calculated by Karl Pearson correlation coefficient method. It is a mathematical method for measuring linear relationship between two variables.

RESULTS

Butterfly diversity

A total of 45 species belonging to five families of the order Lepidoptera were recorded from a sample of 405 individuals in the study sites over a period of five months (November 2020 to March 2021). The checklist of the butterfly species observed in the study area is presented and tabulated (Table 1). The maximum number of species were recorded in the family Nymphalidae (20 species), followed by Papilionidae (9 species), Lycaenidae, Pieridae (6 species) and Hesperidae

(4 species). Nymphalidae represented the greater number of genera (16) and then Lycaenidae (6), Pieridae (5), Hesperidae and Papilionidae (4) respectively. Therefore, the percentage of occurrence of butterfly diversity in the present study revealed that Nymphalidae were the most commonly recorded family, accounting for 45% (n=20) of total species recorded, followed by Papilionidae with 20% (n=9), Pieridae 14% (n=6), Lycaenidae 11% (n=6) and Hesperidae 9% (n=4) (Figure 3). From the visits and observation during the study period, it is clear that both species and individual number of butterflies were higher in the morning than those in the afternoon. In terms of species diversity, the most abundant species recorded in the present study includes Banded bush brown (23 individuals) followed by Chocolate pansy (22 individuals) and Angled castor (21 individuals). All these three species belong to the family Nymphalidae. The species most abundant in the family Papilionidae is Common mormon whereas Common emigrant and Three spot grass yellow is the most commonly found species in the family Pieridae. Common Pierrot is the most abundant species found from family Lycaenidae. Rice swift is the most abundantly found species from the family Hesperidae. At the generic level, Nymphalidae showed the greater abundance (204) with 16 genera and 20 species whereas the lowest abundance was shown by Hesperidae (10) with 4 genera and 4 species. The number of species and abundance of species in each family was represented in Figure 4. The checklist of butterflies also indicated their status and migration. In the present study, the butterflies whose number of sightings below 5 is considered as the status of 'rare' (R) whereas those which ranges between 5-15 are 'common' (C) and above 15 are regarded as 'very common' (VC). Out of 45 species of butterflies 13 are very

common (VC) and 15 rare (R), whereas 17 are common (C). Accordingly the most frequently sighted species was Banded bush brown (23) and least sighted was Southern bird wing, Jezebel, Indian palm bob and Dark palm dart (1). (Table-1). The present study also documented the migratory status of different butterflies in the study area and out of 45 species, five species of butterflies show migration which include Blue mormon, Crimson rose, Common emigrant, Common crow, Blue tiger (Table 1).

**Table1 : Checklist of butterflies reported from study area:
Butterfly diversity Index**

Sl. No	FAMILY	COMMON NAME	SCIENTIFIC NAME	NO. OF SPECIES	STATUS
1	NYMPHALIDAE	Angled castor	<i>Ariadne ariadne</i>	21	VC
2		Blackvein sergant	<i>Athyma ranga</i>	4	R
3		Blue moon	<i>Hypolimnas bolina</i>	5	C
4		Blue tiger	<i>Tirumala limniace</i>	6	C, M
5		Chocolate pansy	<i>Junonia iphita</i>	22	VC
6		Common castor	<i>Ariadne merione</i>	7	C
7		Common sailor	<i>Neptis hylas</i>	16	VC
8		Common four ring	<i>Ypthima huebneri</i>	8	C
9		Common five ring	<i>Ypthima baldus</i>	7	C
10		Common lascar	<i>Pantoporia hordonia</i>	2	R
11		Commander	<i>Moduza procris</i>	4	R
12		Banded bush brown	<i>Mycalesis mineus</i>	23	VC
13		Evening brown	<i>Melanitis leda</i>	11	C
14		Gladeye bush brown	<i>Mycalesis patina</i>	9	C
15		Glassy tiger	<i>Parantica aglea</i>	7	C
16		Grey count	<i>Tanaecia lepidea</i>	7	C,II
17		Grey pansy	<i>Junonia allites</i>	3	R
18		Common crow	<i>Euploea core</i>	17	VC, M

19		Medus Brown	<i>Orsotriaena medus</i>	16	VC
20		Rustic butterfly	<i>Cupha erymanthis</i>	9	C
21	PAPILIONIDAE	Southern bird wing	<i>Troides minos</i>	1	R
22		Blue mormon	<i>Papilio polymnestor</i>	9	C, M
23		Common mormon	<i>Papilio polytes</i>	18	VC
24		Common rose	<i>Pachliopta aristolochiae</i>	6	C
25		Common mime	<i>Papilio clytia</i>	4	R
26		Crimson rose	<i>Pachliopta hector</i>	6	R, I, M
27		Common banded Peacock	<i>Papilio buddha</i>	3	R
28		Malabar raven	<i>Papilio dravidarum</i>	2	R
29		Tailed jay	<i>Graphium agamemnon</i>	6 C	C
30	PIERIDAE	Common emigrant	<i>Catopsilia pomona</i>	19	V, C, M
31		Common grass yellow	<i>Eurema hecabe</i>	17	VC
32		Common wanderer	<i>Pareronia valeria</i>	11	C
33		Jezebel	<i>Delias eucharis</i>	1	R
34		Psyche	<i>Leptosia nina</i>	16	VC
35		Three spotted grass	<i>Eurema blanda yellow</i>	19	VC
36	LYCAENIDAE	Common imperial	<i>Cheritra freja</i>	3	R
37		Banded blue pierrot	<i>Discolampa ethion</i>	17	VC
38		Common cerulean	<i>Jamides celeno</i>	22	VC
39		Common pierrot	<i>Castalius rosimon</i>	5	C
40		Monkey puzzle	<i>Rathinda amor</i>	6	C
41		Quaker	<i>Neopithecops zalmora</i>	3	R
42	HESPERIIDAE	Dark palm dart	<i>Telicota</i>	1	

			<i>ancilla</i>		R
43		Grass demon	<i>Udaspes folus</i>	2	R
44		Inadian palmbob	<i>Saustus gremius</i>	1	R
45		Rice swift	<i>Borbo cinnara</i>	6	C

Butterfly diversity index Table 4 shows the butterfly species diversity index in the present study area. The diversity indices like Shannon Index ('H') and Pielou's evenness index were calculated as diversity indices. In order to assess the species richness of the butterfly diversity in the study area the Shannon diversity index was calculated and presented (Table 2). It is a measure of diversity that combines species richness and (number of species in a given area) and their relative abundances. Pielou's evenness index measures the diversity along with species richness. While species richness is the total number of different species in a given area, evenness is the count of individuals of each species in an area. A calculated value of Pielou's evenness ranges from 0 (no evenness) to 1 (complete evenness). The diversity indices like Shannon-Weiner (H) and Pielou's (J) were calculated which could analyze both species richness and evenness into a single value. Wiener Diversity index (H). Species diversity was found highest in the family Nymphalidae (2.805.), while as it was lowest in Hesperiiidae (1.080). The overall calculated Shannon index value of the study area was found to be 3.5251926 which means the study area is enriched with butterfly diversity. diverse. The evenness indices (Pielou's evenness index) obtained in the study area was calculated as 0.9 which indicated a high evenness in the study area .(Table - 2).

Table-2 Measures of diversity index calculated for each family:

SS(J)				
1	Nymphalidae	20	2.805571575	0.921514501
2	Papilionidae	9	1.896898103	0.4800762084
3	Pieridae	6	1.638219215	0.3707350775
4	Lycaenidae	6	1.49754846	0.3720292226
5	Hesperiidae	4	1.088899975	0.4729032505
Overall Shannon-Wweiner diversity index				3.5251926
Overall Pielou's evenness index				0.9260586

Effect of Temperature & Humidity on Butterfly Diversity

Environmental factors, especially temperature and humidity has been a great influence on the butterfly diversity during the study period conducted from November 2020 to March 2021 (five months). The highest average temperature observed was in March with 31°C and lowest in January with 26°C. Similarly the average humidity observed is high in November with 81% and low in January with 70%. The average humidity, temperature and number of species recorded in each month are presented and tabulated. From the data presented, it indicates that high humidity (81%) and moderate temperature (28°C) favour a high butterfly population, with a total of 39 species recorded species were recorded during the month of November 2020 from the study field. Comparatively to the above observation a low butterfly population (15)) was noted during the month of March where the recorded average temperature and humidity was 31°C and 66% accordingly. Therefore, from the data obtained during the study period indicated that a medium temperature and high humidity is favourable for high butterfly diversity.

Table – 3. List of Host plants in the Study area:

Sl no	Family	Common Name	Host plant family	Host plant
1	NYMPHALIDAE	Angled castor	Fabaceae	<i>Mimosa pudica</i>
2		Blackvein sergeant	Oleaceae	<i>Ligustrum sinense</i>
3		Blue moon sergeant	Verbenaceae	<i>Lantana camara</i>
4		Blue tiger	Verbenaceae	<i>Lantana camara</i>
5		Chocolate pansy	Fabaceae	<i>Vigna radiata</i>
6		Common castor	Fabaceae	<i>Mimosa pudica</i>
7		Common sailor	Lamiaceae	<i>Ocimum tenuiflorum</i>
8		Common four ring	Verbenaceae	<i>Lantana camera</i>
9		Common five ring	Poaceae	<i>Axonopus compressus</i>
10		Common lascar	Euphorbiaceae	<i>Macaranga peltata</i>
11		Commander	Rubiaceae	<i>Mussaenda laxa</i>
12		Banded bush brown	Lamiaceae	<i>Ocimum tenuiflorum</i>
13		Evening brown	Lamiaceae	<i>Tectona grandis</i>
14		Gladeye bush brown	Poaceae	<i>Axonopus compressus</i>
15		Glassy tiger	Verbenaceae	<i>Lantana camara</i>
16		Grey count	Apocynaceae	<i>Catharanthus roseus</i>
17		Grey pansy	Malvaceae	<i>Hibiscus calyphyllus</i>
18		Indian crow	Apocynaceae	<i>Hemidesmus indicus</i>
19		Medus brown	Sapotaceae	<i>Manilkara zapota</i>
20		Rustic butterfly	Salicaceae	<i>Flacourtiamontana</i>
21		Southern bird wing	Lamiaceae	<i>Clerodendrum paniculatum</i>
22		Blue mormon	Hydrangeaceae	<i>Hydrangea</i>

				<i>macrophylla</i>
23	PAPILIONIDAE	Common mormon	Rutaceae	<i>Murraya koenigii</i>
24		Common rose	Rubiaceae	<i>Ixora species</i>
25		Common mime	Rubiaceae	<i>Ixora species</i>
26		Crimson rose	Rutaceae	<i>Murraya koenigii</i>
27		Common banded peacock	Rubiaceae	<i>Ixora species</i>
28		Malabar raven	Lamiaceae	<i>Clerodendrum paniculatum</i>
29		Tailed jay	Magnoliaceae	<i>Michelia champaca</i>
30	PIERIDAE	Common emigrant	Fabaceae	<i>Cassia species</i>
31		Common grass yellow	Asteraceae	<i>Tagetes erecta</i>
32		Common wanderer	Rubiaceae	<i>Ixora species</i>
33		Jezebel	Lamiaceae	<i>Clerodendrum paniculatum</i>
34		Psyche	Polygonaceae	<i>Persicaria virginiana</i>
35		Three spotted grass yellow	Lythraceae	<i>Cuphea hyssopifolia</i>
36	LYCAENIDAE	Common imperial	Anacardaceae	<i>Mangifera indica</i>
37		Banded blue pierrot	Rahmanaceae	<i>Ziziphus jujuba</i>
38		Common cerulean	Asteraceae	<i>Cosmos sulphureus</i>
39		Common pierrot	Rahmanaceae	<i>Ziziphus jujuba</i>
40		Monkey puzzle	Anacardaceae	<i>Mangifera indica</i>
41		Quaker	Rutaceae	<i>Glycosmis pentaphylla</i>
42		HESPERIIDAE	Darkpalm dart	Lythraceae
43	Grass demon		Apocynaceae	<i>Catharanthus roseus</i>
44	Indian palm bob		Arecaceae	<i>Cocos nucifera</i>
45	Rice swift		Rubiaceae	<i>Ixora sp</i>

Correlation Coefficient of Environmental Variables and Butterfly Diversity

The nature and strength of relationship between two variables is described in terms of correlation coefficient. In the present study, Karl Pearson's correlation coefficient method is used to determine the correlation between temperature and butterfly diversity, as well as the humidity and butterfly diversity. The normal range of calculated value of correlation coefficient is from -1 to +1 and the value close to +1 shows a perfect positive correlation and the value close to -1 shows perfect negative correlation. A value of zero indicates there is no correlation at all. Here, in the present study, the correlation coefficient of temperature and butterfly diversity is -0.750555407, indicating a perfect negative correlation and the calculated value of correlation coefficient of humidity and butterfly diversity is +0.727831786, showing a positive correlation.

Correlation coefficient between temperature and butterfly diversity has been presented as a scatter as scatter plot diagram (Figure 5) and this diagram clearly indicated a strong negative correlation between temperature and butterfly diversity. That is, as the temperature increases the species diversity decreases. In order to correlate humidity and butterfly diversity, a similar scatter plot diagram has been presented (Figure 6). Humidity and butterfly diversity showed a positive correlation from the scatter plot diagram where both humidity and butterfly diversity increases

diversity in the Study area

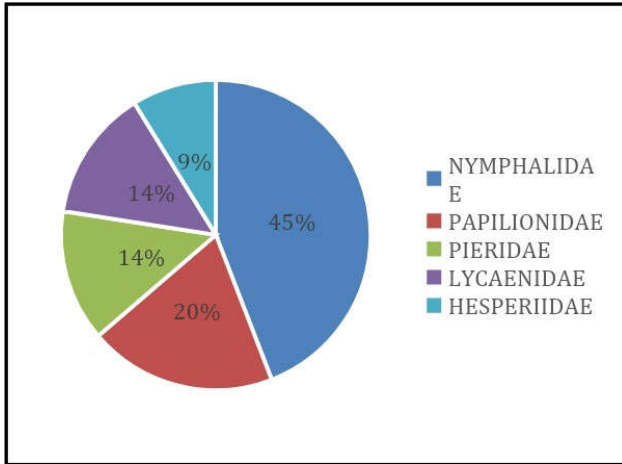


Figure-3. Percentage of Occurrence of Butterfly Family

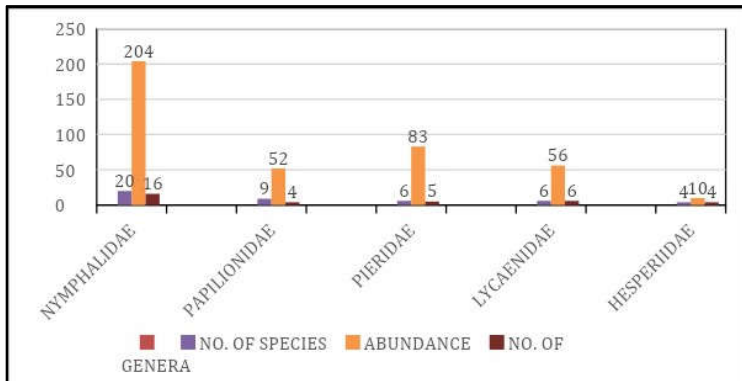


Figure-4 Family wise Number of Genera, Species and Abundance of Butterflies

Feeding and host Preferences of Butterflies

The study also comprises the observations of feeding behaviour of butterflies from the study area. Most of the butterfly families observed from the study area were found to

feed on nectar from a variety of host plants. Among the butterfly families, the members of Nymphalidae preferred some different food items such as rotten fruits and other kitchen waste rather than nectar. This kind of feeding was commonly observed in species like Common sailor, Commander, Grey count, Nigger, Common bush brown, Common evening brown, Chocolate pansy etc. The study also highlighted a general understanding of host plant selection by butterfly fauna in the study area which is tabulated and presented in Table 3 . Most of the butterflies like Common mormon, Crimson rose, Common rose, Common four ring etc., feeds on nectar plants like *Lantana camara*, *Hibiscus*, *Clerodendrum*, *Ixora species* flower and leaves which flower almost continually. Butterflies of Lycaenids and Pierids mostly feed on flowers of the Compositae family like *Cosmos sulphureus* , *Tagetes erecta*, *Cassia* etc. The most commonly selected host plants in which the butterfly feed belongs to the family Rubiaceae (*Ixora*), Vebernaceae (*Lantana camara*) and Lamiaceae (*Clerodendrum paniculatum*).

Discussion

The present study analyzed the diversity of butterfly assemblages in a rural area namely Akode , a small village in Malappuram district which revealed that the family Nymphalidae occupied highest position in terms of species diversity and abundance, followed by Papilionidae, Lycaenidae , Pieridae, and Hesperidae. Similar observations were also found in many previous studies on butterfly diversity done in Kerala, by .Radhakrishnan (2000) who listed 40 species of butterflies ,where maximum number found in Nymphalidae (32 species) followed by papillionidae (13 species). Similarly studies on the butterflies of Silent valley National park by George Mathew and Rahmathulla in 1993 listed about 100 species of butterflies belonging to five families with the highest

number in Nymphalidae family . The predominance of Nymphalidae family was also reported by different researchers (Nair, 2014; Ajayan, 2017; Senthilmurugan, 2005; Harsh, 2014). Members of the family Nymphalidae were always dominant in the varied habitats because most of them are polyphagous in nature, consequently they were able to survive in almost all the habitats. Additionally, many species of this family are strong, active fliers that might help them in searching for resources in large areas (Eswaran and Pramod, 2005).The least abundance of butterflies was noticed in the family Hesperiiidae, and similar observation is obtained in the study of “Bbutterfly diversity in Ghandruk area of mid-mountain, Nepal by (Ditya Rai, 2008). This may be partly due to the sampling bias since Hesperiiids exhibit crepuscular habit , i.e., they are active at early morning and to a lesser extent in the evening. They are also active in the shade (Kkunte, 2000). Thus, from the both these index values, it is clearly indicated the presence of good butterfly diversity and evenness in the study area

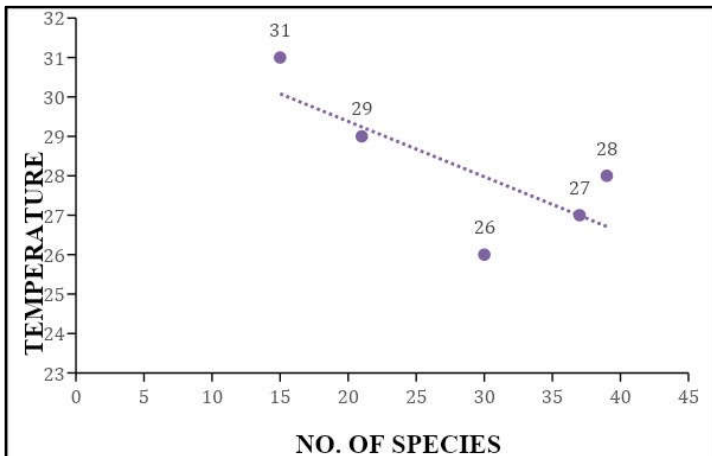


Figure 5. Scatter plot diagram of temperature and butterfly diversity

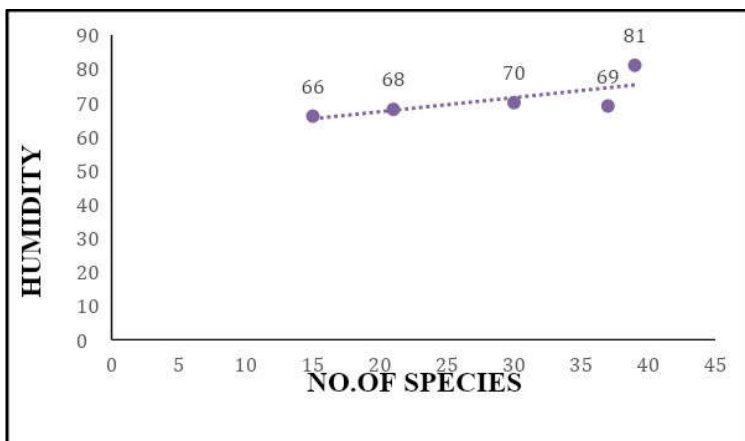


Figure6-Scatter plot diagram of humidity and butterfly diversity

The study revealed that the diversity and species richness of butterflies were significantly high and their population is correlated across the gradient of temperature and relative humidity. In the present study the butterfly diversity was most dominant during the month of November with a temperature of 28°C, i.e. Medium ie Medium temperature is favourable for high butterfly diversity. The temperature was high during the month of March with 31°C and hence the diversity was very less. The lowest temperature was observed in January with January with with 26°C and diversity were comparatively low. In general butterflies favours damp-warm climatic condition than dry hot condition. Freitas *et al.*, (1997) reported the optimum temperature for butterflies to actively search for food is between 23 to 30°C. The studies on the effect of temperature on the butterfly community at the forest area of Manokwari, Papuabarat studied by Panjaitan *et.al.*, 2016) showed the average temperature for butterfly abundance ranges

between 24 to 26°C. In this study, the diversity of butterflies showed a general trend of increase with that of high humidity. Contrary Contradictory to the present observation, the number of butterfly species affected by humidity and canopy cover in secondary forest whereas the butterfly abundance is influenced by wind speed, light intensity, temperature and rainfall on habitat, gardens and settlements. Study of Bhusal and Khanal in 2008 reported that, the abundance of diverse butterfly species was positively affected by approaching warmer days, high humidity and more rainfall which is in conformity with the present observation of butterfly diversity and humidity. This study was limited to analyzing the effect of temperature and humidity as explanatory variables affecting assemblages of butterflies. The effect of temperature and humidity on the butterfly assemblages need to be analyzed further with other environmental factors such as host plant type, host plant range, and habitat fragmentation (Soga and Koike, 2012).

The observation on feeding preferences of certain members of butterfly families, especially Nymphalidae revealed some interesting facts like feeding on rotten fruits and other kitchen wastes, tree sap etc. These observations were supported by Krenn (2008) who observed the feeding behaviour of neotropical butterflies and reported that additionally some nectar feeding nymphalid butterflies there is a rich fauna of non nectar feeding nymphalids which prefer fermenting fruit, tree sap, or other rotting substances. Another interesting mode of feeding noticed during the study was in the members of family Lycaenidae. The Blue banded pierrot, Common imperial and Common cerulean were found to feed on bird droppings. A similar observation was noticed by Rima *et.al.*, (2016) where several types of puddling sources were used by butterflies viz. mud or wet soil, dung, carrion,

wet sands and wet bricks, human sweat, bird-dropping, rotten fruits and flowers. The hHost plant specificity and butterfly fauna has been observed during the study period exhibited that certain species of butterflies revealed some sort of specificity. Members of Papilionidae are highly host specific to plants belonging to Rubiaceae and Rutaceae. Like wise, the Nymphalidae mostly preferred on the host plants of the family Verbanaceae and Fabaceae where as Pieridae and Lycaenidae mostly visited on the host plants of the family Rhamnaceae and finally Hesperidae is specific on the plants palnts of the family Areaceae. Balakarishna and Hemachandra (2016) studied the butterfly diversity and its host specificity of Permude village in Dakshina Kkannada district revealed that the members of Papilionidae was found associated with plants belonging to Rutaceae, Annonaceae, Loraceae and Aristolochiaceae; and Nymphalidae on Apocynaceae , verbenaceae, Asteraceae and Fabaceae. Thus, the present study has revealed that taxonomically different host plants are used by particular butterfly families.

CONCLUSION

Butterflies are the most fascinating group of insects, admired even by those who have the vaguest of science. They are the wonders of nature with diverse and vibrant colour, nature, size and habitats with aesthetic value. The present study reveals a higher butterfly diversity due to a vast variety of host plants in the study area. During the study 45 species were identified belonging to five families. Nymphalidae was the most dominant family and Hesperidae was least dominant. Common bush brown and Chocolate pansy were was the most predominant species. Among the butterflies identified, Blue tiger, Common crow, Common emigrant and Crimson rose are migrant species and species like Crimson rose, Grey count and

Common wanderer are coming under Schedule I & II of Wild life protection Act 1972. An analysis on influence of environmental variables on butterfly diversity indicates that an average temperature and high humidity favour for high butterfly diversity. Regarding the feeding habit preferences, adult butterflies feed mainly on nectar and other sugars, such as those contained in the sap of wounded trees and rotting fruits. Some butterflies live on pollen rather than nectar, but also some feeds on animal droppings and dead animals in order to obtain nutrients such as carbon, nitrogen and amino acids. Some can even absorb human sweat that contains electrolytes they find valuable. Butterflies maintain the ecosystem by acting as pollinator, prey, biological pest control, make genetic variation in plants, and increase environmental beauty, decrease the level of carbon dioxide in air. The findings of the present study highlighted that the study area as a perfect habitat for butterflies. If the improvement and care of gardens are carefully planned, the diversity of butterflies may increase in such less disturbed areas providing a rich ground for butterfly conservation as well as for research. This study will also add to our future attempts in understanding the complex nature of mutualistic contact between butterflies and flowering plants that is important for continuity of ecosystem services as well as are essential for the better conservation and management of the environment

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A SCIENTIFIC REBUT TO SOCIAL DEFAMATION – ANALYSIS OF CLAY POTS

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Abstract: *Science is in fact a saver of human kind. It unveils the mysteries of what is happening around us and contributes to the survival of the existing life, which ultimately points to serve the mankind. The significance of this project also lies on the ground of being a saver of a community. The quantitative and qualitative method of analysis could give an exact knowledge on the constituents of the clay pot samples under study. Our experiment concludes by falsifying the claims made on the constituents of other state pots. This project is a scientific answering to the social defamation and also stands to warn the common from making unscientific conclusions and spreading them in the public.*

Key words: *Pot, clay, Red oxide, SEM-EDAX, Qualitative and Quantitative analysis*

Introduction

Pottery is one of the oldest humankind's handicrafts. Even writing, the very method of communication used to create this article, came after the first pots. And like many things, it's theorized that it was discovered by complete accident.

In ancient times, people would transport water in hand woven baskets. The water, especially that from rivers, would have some clay in it. As the clay dried out, it would take on the shape of the basket. Eventually, people realized that these clay linings could be used as sturdy containers. They gathered clay,

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shaped it, and baked in the sun or hot ashes, sometimes decorating them with primitive tools. Thus, the first clay pots (and by extension, all of pottery) were born.

Nowadays, pottery is made by forming a ceramic (often clay) body into objects of a desired shape and heating them to high temperatures (600–1600 °C) in a bonfire, pit or kiln and induces reactions that lead to permanent changes including increasing the strength and rigidity of the object. Much pottery is purely utilitarian, but much can also be regarded as ceramic art.

Clay minerals are composed essentially of silica, alumina or magnesia or both, and water, but iron substitutes for aluminum and magnesium in varying degrees, and appreciable quantities of potassium, sodium, and calcium are frequently present as well. Clay-based pottery can be divided into three main groups: earthenware, stoneware and porcelain. Earthenware clay is fired at a low temperature to harden but not vitrify so it is still somewhat porous. Terra cotta is the most common earthenware for cooking.

Potters fired finer clays at higher temperatures, this created strong pots that were naturally non-porous these are stoneware. It is used not just for cooking but as decorative pieces as well. However, the durability and non-porous qualities keep it a popular choice in the kitchen to this day.

Porcelain is a special type of pottery made from a mix of kaolin and feldspar, the result was a very beautiful but fragile item. It was so popular that potters across Europe and the rest of Asia began developing new techniques in hopes of replicating Chinese porcelain. Whether or not they were successful in capturing the same beauty, however, is up to personal preference.

The main pottery spots in India are Rajasthan, Assam, Goa , Kerala , Tamil Nadu, Uttar Pradesh , Andhra Pradesh and Bihar. The Kerala culture and traditions have close relation with the favors of nature. Clay pots adding tang to our food items is a common site in all Keralite kitchen. Also the ceramic clay pots have become one of our favorite decor item. In one way or other, people stay in touch with the nature and our traditions through clay pots and this particular industry has much credits in Indian society.

Objective of the study

Science is the only authentic means of approaching, studying and answering all the questions of what, why and how that arises around us while dealing with our daily life. The role of science in an increasingly fragmented and digital society, as well as its significance for politics and civil society, will be redefined. Science has an important social role and at the same time bears great responsibility precisely when the situation is not clear. People trying to make conclusions from the casual observations on the ground of uncontradicted experience leads to unscientific inductions. Such initiations paves way to dangerous social stigmas which are likely to spread among the common people apace.

This project addresses such a social issue that took place in Mukkam, Kozhikode district, Kerala. The studies are to falsify the claims of a common man regarding the composition of clay pots used for cooking that is sold by the Tamil natives. The main claim was that, the pots are not made of clay, while they are marble pieces coated with mud. Since there was no scientific means of approaches adopted for making the conclusions, their claims were unscientific. Unfortunately, the video establishing the claims reached

hundreds through social media platforms and it got much acceptance among the common people in this locality. Subsequently it fell on accusing the Tamil sailors of the clay pots and they absconded.

Conclusions formed of inaccurate information and often deliberately published or shared in media outlets and social media, fake news can be a dangerous way of obtaining information. When sharing news, people often don't stop and think about whether the information could be fake and, in an age of instant internet access and social media platforms, news is shared to millions at the touch of a button. And therein lies the danger.

We understand it as the social responsibility, especially of those from chemistry background to research on such claims and come up with a scientific reasoning for our conclusions. The project objects to check for the truth in the claims, to falsify the claims, thereby to apprise the trend of people in making inference in subjects they are illiterate and most importantly to aware the people to stop and think on whatever heard, read or seen.

Materials and Method

Experimental

Three set of samples were taken for the analysis. Sample A is red oxide, sample B is other state pot and sample C is local pots. Sample B collected from the customers who bought it from Tamil natives. Sample A & B collected from local market.

Qualitative analysis of samples

Sample A was used as such while sample B & C was collected by scraping the surface of the pot which gives fine powder and was used for analysis. Qualitative analysis of each

sample was carried out by systematic procedure employed in chemistry graduation course. Each sample was dissolved in concentrated HCl before carrying out qualitative analysis.

Quantitative Analysis of samples

Quantitative analysis of each sample was carried out by SEM EDX method from Nano Reseach Center, Karunya University Coimbatur.

Results and Discussion

The qualitative analysis of three samples was done to identify elements present in the sample. At first identification of groups was done followed by confirmation of group cations. The result of qualitative analysis depends on concentration of cations present in the stock solution. Interestingly three samples confirmed presence of iron owing to higher concentration of iron. Sample B & C showed presence of group 4 & 5 cations. The amount precipitate was too less to be separated and to carry out further confirmation of cations.

Table 1- Qualitative analysis of sample A, B & C

Sl. No.	Sample	Cation groups identified	Cations confirmed
1	A	Group 2	Not confirmed
		Group 3	Fe ³⁺
2	B	Group 3	Fe ³⁺
		Group 4	Mn ²⁺
		Group 5	Not confirmed
3	C	Group 3	Fe ³⁺
		Group 4	Mn ²⁺
		Group 5	Not confirmed

Quantitative Analysis

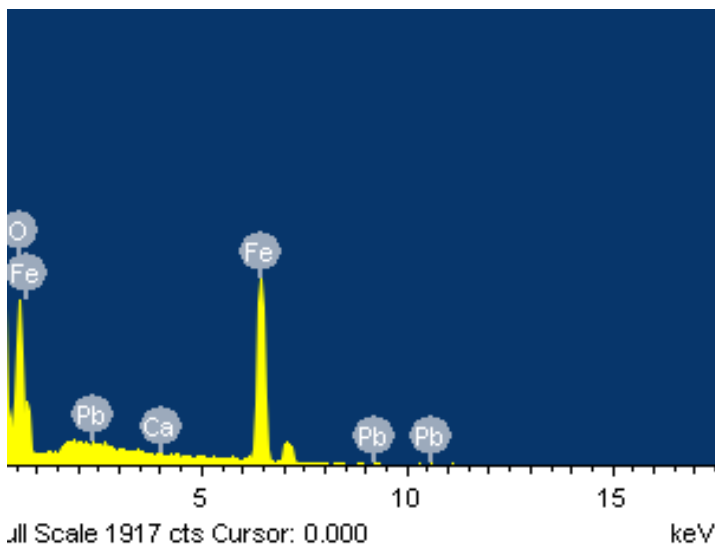


Fig1- SEM EDX spectra of sample A

Table 2 Quantitative estimation of sample A

SI No	Element	W%	Atomic %
1	O	31.87	62.12
2	Ca	0.30	0.23
3	Fe	67.27	37.56
4	Pb	0.56	0.08

Quantitative analysis clearly establishes various elements present in all three samples. Sample A as given in graph1 and table 1 contains iron and oxygen as major elements. Trace amount of lead and calcium are also present in the sample A, may be as impurity.

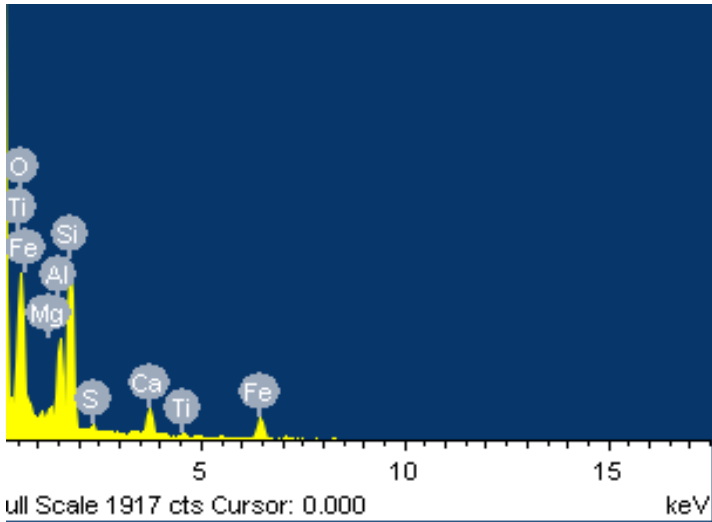


Fig2- SEM EDX spectra of sample B

Table3- Quantitative estimation of sample B

Sl. No.	Element	W%	Atomic %
1	O	55.20	70.86
2	Mg	1.74	1.47
3	Al	9.12	6.94
4	Si	19.95	14.59
5	Ca	4.17	2.14
6	Ti	0.99	0.42
7	Fe	7.65	2.82

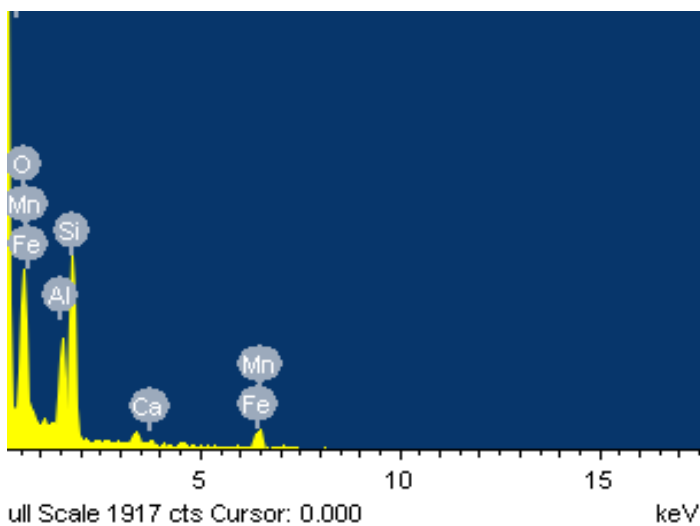


Fig3- SEM EDX spectra of sample C

Table 4- Quantitative estimation of sample C

Sl. No.	Element	W%	Atomic %
1	O	55	70.23
2	Al	11.05	8.37
3	Si	24.51	17.83
4	Ca	0.79	0.4
5	Mn	0.32	0.12
6	Fe	8.32	3.04

Quantitative analysis of sample B & C shows the presence of oxygen, silicon, aluminum and iron as common and major elements in both. Trace amount of magnesium, calcium and titanium are present in sample b where as a little amount calcium, manganese are present sample C. The studies shows that the elements present in other state pot and local pot are almost the same whereas the elements in red oxide is not

exactly those present in the pots. Oxygen and iron are the main elements in red oxide. The similarity in the constituents of the pots indicates that they are made from clay material. The trace difference in the elements of the both can be understood from the difference in the clay of different regions.

Hence from the studies we could falsify the claim that the other state pots are not made of clay but are marble coated with red oxide. The elements of other state pot was identified almost similar to the constituents in our local pots therefore, the claims are verified to be unscientific.

Conclusion

Science is in fact a savior of human kind. It unveils the mysteries of what is happening around us and contributes to the survival of the existing life, which ultimately points to serve the mankind. The significance of this project also lies on the ground of being a savior of a community. The quantitative and qualitative method of analysis could give an exact knowledge on the constituents of the clay pot samples under study. This technique easily drew a conclusion for our cause of study. We found that the constituents of the other state clay pots and the local clay pot from the area of study were almost the same, which concludes that both of them are made of same material, that is clay. At the same time the elements of red oxide which is claimed to be the main component of other state pots was different from it.

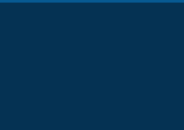
Our experiment concludes by falsifying the claims made on the constituents of other state pots. This project is a scientific answering to the social defamation and also stands to warn the common from making unscientific conclusions and spreading them in the public.

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