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PART A: SCIENCE AND TECHNOLOGY**





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THE RESARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

BIOMASS FUEL SMOKE AND COPD IN RURAL AREA OF SANGLI DISTRICT.

P.M. Patil

Head, Department of Zoology

Dr. Patangrao Kadam Mahavidyalaya, Sangli

Email – patilpm16@gmail.com

Abstract

On a global scale the household use of solid fuels is the most important source of indoor pollution and the exposure to the byproducts of combustion of biomass fuel particularly wood smoke has been related to chronic obstructive pulmonary diseases. In India 95% households use wood as the primary cooking fuel. The smoke released due to incomplete combustion of unprocessed solid biomass fuel contains high volume and number of health damaging air borne pollutants such as PM, CO, NO₂, SO₂ formaldehyde and other organic compound. Prolong exposure to such air borne pollutants have adverse effect on lung function which causes COPD in which lung functions is reduced. In this study 50 women exposed to biomass fuel were selected from village Malwadi. Information regarding age, height, weight, type of fuel, number of hrs exposed to smoke, no. of yrs., types of kitchen were collected. Spirometry was performed in these women. Spirometric parameter forced expiratory volume per second and forced vital capacity were recorded. In this study we found out of 50 women 18 (36%) were suffered from COPD.

Key words – COPD, Biomass Fuel, FEV1 (Forced Expiratory Volume per one second), FVC (Forced Vital Capacity)

1. Introduction:

On a global scale the household use of solid fuels is the most important source of indoor pollution and the exposure to the byproducts of combustion of biomass fuel particularly wood smoke has been related to chronic obstructive pulmonary diseases. Half of the world population and 75% population of developing countries still depends upon biomass fuel as a primary source of domestic energy for cooking and heating. (Reddy, et al 1990; Desai, et al 2004, Smith, et al.; 2004). Biomass fuel such as wood, plant residues and cow dung especially used for cooking and heating purpose (Nigel Bruce, et al. 1988) biomass accounts for more than 80% of domestic energy in India (Holdren et al; 2000). In India 90% household's use wood or animal dung as the primary cooking fuel (IIPS). The most important factor in the life of average Indian housewife is the domestic cooking. The typical Indian household life revolves around the cooking area and Indian women spent much of the time there. For daily cooking Indian housewife spent on an average more than 6 hours in the kitchen for cooking food (morning and evening). During her lifetime she is exposed to biomass fuel for 30 to 40 yrs. The type of house, location of kitchen and type of fuel used play a significant role on women health.

The smoke released due to incomplete combustion of unprocessed solid biomass fuel contains high volume and number of health damaging air borne pollutants such as (PM) respirable particulate matter PM₁₀, CO, NO₂, SO₂, formaldehyde and other organic compounds (Bruce et al., 2000) Prolong exposure to such air born pollutants have adverse effect on the respiratory system of women which causes COPD. COPD is the inflammation and swelling of the lining of the airways that leads to narrowing and obstruction of the airways. In rural areas, most common cause of COPD is air pollution.

2. Material and Methods:

Total 100 women were participated in this study. The biomass fuel users group was represented by 50 women from Malwadi. Another group of LPG users was represented by 50 women from same villages of Sangli district. All women were 25 years of age or older. Biomass fuel users were from low socio-economic status. All women were interviewed and information was collected about age, height, weight type of house, type of Kitchen, no. of years exposure to biomass smoke, no. of hrs/day exposed to smoke. Spirometry was performed in 100 women, 50 biomass fuel users (subjects) and 50 LPG users (control). Force expiratory volume/1 second (FEV₁%) and ratio of force expiratory volume/1second / force vital capacity were recorded. Women having FEV₁% <70% were considered as COPD.

Statistical analysis:

Z significance test was used. 'Z' value for control and subject was calculated, using formula based on null hypothesis to show significant difference between control and subject.

$$Cal |z| = \left[\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \right]$$

\bar{x}_1 Mean of subject

\bar{x}_2 Mean of Control

σ_1 Standard deviation of subject

σ_2 Standard deviation of subject

n_1 = Number of observations of subjects

n_2 = Number of observations of control

H0: There is no significant difference between control and subject women FEV1.

V/s

H1: There is significant difference between control and subject women FEV1.

Cal |Z| = > table Z = 1.96 at 5% level of significance.

∴ Reject H0

∴ There is significant difference between control and subject.

3. Result and Discussion

Observation on Spirometry of women from Malwadi village

Table No.1 represents observations on Age, years of exposure and spirometry (in percentage) of control and Subject women in the village **Malwadi**. The mean values of Age and years of exposure of control women are 41.34 and 20.26. While mean values of Age and year of exposure of subject women are 42.84 and 21.24. The mean values of FEV₁%, FVC%, FEV₁/FVC%, of control women are 102.03, 94.69 and 87.01 respectively, the mean values of FEV₁%, FVC%, FEV₁/FVC% of Subject women are 73.57, 73.30 and 82.50 respectively. These values are shown at the base of each column in the Table No. 1

The calculated Z value of Age and years of exposure and calculated Z value of FEV₁%, FVC%, FEV₁/FVC% based on null hypothesis are at the last of each column in the Table No.1. The calculated Z value of Age and years of exposure are 1.70 and 0.94. The calculated Z values of Age and years of exposure are less than 1.96 hence there is no significant difference in age and year of exposure of control and subject women. While calculated Z values of FEV₁%, FVC% and FEV₁/FVC% are 6.38, 5.39 and 2.35 respectively. The calculated Z values of FEV₁%, FVC%, FEV₁/FVC% are greater than 1.96 hence there is significant difference in FEV₁%, FVC%, FEV₁/FVC% of control and Subject women. The result is significant at 5% level of significance.

In this study we found that out of 50 women using chulla from rural area Malwadi 18 women were having $FEV1\% < 70$. In Malwadi 18 women were suffered from obstructive type of disorder. Our results are similar with the results of Dennis et al. (1996), Orozco et al. (2006), Caballero et al. (2008). In obstructive type of spirometry pattern there is narrowing of small airways due to chronic inflammation. According to Dennis et al. (1996), Orozco et al. (2006), Caballero et al. (2008) the reduction in $FEV1\%$ & $FEV1/FVC\%$ may be due to chronic inhalation of toxic substances emitted during biomass combustion leading to inflammatory changes in (bronchi and bronchioles).

Observation**Table No. – 1**

**Data of Spirometry (FEV₁%, FVC%, FEV₁/FVC%) of Rural Women from Malwadi
Exposed to Biomass smoke**

Sr. No.	CONTROL					SUBJECT				
	Age	Years	FEV ₁ %	FVC%	FEV ₁ / FVC%	Age	Years	FEV ₁ %	FVC%	FEV ₁ / FVC%
1	31	12	121.33	114.67	86.26	37	18	56.69	62.42	80.10
2	39	21	87.33	58.51	79.39	42	20	57.27	60.82	79.75
3	37	19	98.03	91.30	88.69	38	19	72.88	80.66	75.44
4	41	14	98.03	91.30	88.69	44	22	54.46	67.47	69.05
5	39	22	101.27	96.37	86.02	40	20	42.74	39.34	93.46
6	41	23	101.27	96.37	86.02	38	13	81.44	78.88	89.96
7	44	13	98.03	91.30	88.69	36	17	72.88	80.66	75.44
8	41	21	121.33	114.67	86.26	36	20	67.02	63.68	88.73
9	39	23	98.03	91.30	88.69	38	21	73.94	75.78	82.25
10	40	20	92.79	91.43	86.16	40	20	77.82	72.47	92.79
11	38	13	121.33	114.67	86.26	41	18	98.03	91.30	88.69
12	48	12	121.33	114.67	86.26	42	19	57.27	60.82	79.75
13	41	24	87.33	89.67	79.39	41	14	109.82	98.48	92.27
14	38	21	121.33	114.67	86.26	49	26	42.74	39.34	93.46
15	36	14	98.03	91.30	88.69	38	18	82.46	85.77	82.10
16	39	20	95.07	91.43	86.16	39	21	45.62	58.04	67.93
17	42	23	82.56	61.07	83.04	46	28	45.62	58.04	67.93
18	41	21	87.33	58.10	79.39	47	27	54.55	53.92	87.27
19	38	12	121.33	114.67	86.26	36	17	57.27	60.82	79.75
20	40	23	98.03	91.30	88.69	48	14	21.56	25.74	69.23
21	41	21	121.33	114.67	86.26	42	17	72.88	80.66	75.44
22	40	13	98.03	91.30	88.69	45	25	114.36	102.24	94.30
23	41	23	121.33	114.67	86.26	48	27	42.74	39.34	93.46
24	42	24	101.27	96.37	86.02	49	30	87.58	86.26	88.47
25	43	13	101.27	96.37	86.02	43	22	109.82	98.48	92.27
26	38	20	76.00	77.73	77.19	43	13	166.27	142.11	85.19
27	42	24	98.03	91.30	88.69	44	20	73.79	80.49	76.77
28	48	30	101.27	96.37	86.02	53	31	87.33	89.67	79.39
29	42	14	121.33	114.67	86.26	36	17	51.82	71.25	83.04
30	39	22	98.03	91.30	88.69	40	19	40.96	53.36	64.71
31	43	25	87.58	86.26	88.47	36	17	92.79	91.43	86.16

Sr. No.	CONTROL					SUBJECT				
	Age	Years	FEV ₁ %	FVC%	FEV ₁ /FVC%	Age	Years	FEV ₁ %	FVC%	FEV ₁ /FVC%
32	44	12	101.27	96.37	86.02	38	18	121.33	114.67	86.26
33	43	26	82.56	61.07	83.04	36	18	50.70	53.92	87.27
34	41	21	98.03	91.30	88.69	48	25	99.12	89.06	95.76
35	50	30	101.27	96.37	86.02	49	29	61.43	77.69	66.15
36	42	24	98.03	91.30	88.69	48	28	105.18	102.62	86.38
37	39	13	121.33	114.67	109.00	41	20	101.27	96.37	86.02
38	50	31	98.03	91.30	88.69	44	13	109.82	65.32	92.27
39	41	24	82.56	61.07	70.30	51	30	54.83	53.14	88.20
40	52	33	101.27	96.37	76.19	48	28	61.43	67.01	66.15
41	44	14	92.79	91.43	86.16	48	29	114.36	102.24	94.30
42	37	20	82.56	77.73	128.65	36	19	61.43	77.69	66.15
43	44	25	101.27	96.37	86.02	48	23	101.27	96.37	86.02
44	42	24	98.03	91.30	88.69	38	19	81.61	66.80	83.04
45	45	12	121.33	114.67	86.26	42	20	92.79	91.43	86.16
46	40	22	121.33	114.67	123.22	55	14	23.96	26.32	76.67
47	40	23	82.56	77.73	67.62	46	26	71.93	68.87	92.66
48	39	21	121.33	114.67	86.26	49	28	77.82	72.47	92.79
49	41	14	121.33	114.67	86.26	40	22	54.46	67.47	69.05
50	41	24	98.03	91.30	70.95	42	23	21.56	25.74	69.23
Mean	41.34	20.26	102.03	94.69	87.01	42.84	21.24	73.57	73.30	82.50
Var.	13.42	30.07	181.91	256.14	98.34	25.29	24.78	813.10	531.06	85.24
Sqrt	0.88	1.05	4.46	3.97	1.92					
Z	1.70	0.94	6.38	5.39	2.35					

4. Conclusion :

- Prolonged exposure to biomass fuel smoke in poorly ventilated kitchen causes reduced lung functions and women suffered from Chronic Obstructive Pulmonary Diseases (COPD).
- In subject group the type of COPD observed was obstructive.
- As age and years of exposure increases COPD increases.

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THE RESARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

STUDY OF HEMOGLOBIN IN WOMEN'S BLOOD USING STATISTICAL TOOLS

Dr. Prakash R. Chavan

Head & Assistant Professor, Department of Statistics,
Smt. Kasturba Walchand College, Sangli -416 416,
Affiliated to Shivaji University, Kolhapur, INDIA.

E-mail: prchavan83@gmail.com

Abstract

Good victuals habits always give us healthy welfare. But in this Scurried world people ignore their health. Due to change in lifestyle and Food style we give invitation to many diseases. Iron is essential during times of rapid growth and development, pregnant women and young children may need even more iron-rich Foods in their diet. Body needs iron to make a protein called as "Hemoglobin" which is responsible for carrying oxygen to our body Tissues and it helps to our tissues, muscles to function effectively. In this paper we study Hemoglobin level in women's blood and Impact of diet in Hemoglobin. The relation between Rural and Urban women's Hemoglobin. Check the awareness of hemoglobin level by using statistical tools.

Keywords:: Hemoglobin, statistical tools, lifestyle, Portion, Diet. Introduction

1 Introduction

We've all experienced the sensation of having to stop to catch our breath until our lungs can absorb enough oxygen to transport through the bloodstream to our waiting muscles. Imagine what life would be like if we had to rely only on our lungs and the water in our blood to transport oxygen through our bodies. Fortunately, we have some chemical assistance in the form of the protein hemoglobin [1]. O₂ is a non polar molecule, and therefore does not dissolve well in the aqueous environment of the blood. The evolution of large, multicellular animals depended on

a mechanism that could enhance oxygen delivery to the tissues. Hemoglobin increases O₂ solubility in blood by about a hundredfold. This means that without hemoglobin, in order to provide sufficient oxygen to the tissues, blood would have to make a complete circuit through the body in less than a second, instead of the minute that it actually takes. That would take a mighty powerful heart! In this paper, we will take a detailed look at hemoglobin and its chemical cousin, myoglobin, to see how they work together to deliver O₂ to our waiting muscles [2]. Due to abnormal or low Hemoglobin, the cells in our body will not get enough oxygen. Low Hemoglobin i.e. iron deficiency can cause us to feel tired and extremely low iron levels may cause damage to organs [3].

According to the American Society Of Hematology (ASH), iron deficiency is main cause of anemia. There are many problems caused by low Hemoglobin. Such as rapid or irregular heartbeat, pregnancy complications, delayed growth in infants and children etc. Abnormal or low Hemoglobin is not a big disease. By taking sufficient food we can correct this condition. But if it is untreated, it can lead to other health problems as mentioned above [4].

2. Methodology of Data Collection

For the data collection we chose the method random sampling, by which 100 random women were asked several questions from the questioner. The women considered were from both the urban and rural areas whose different perspectives were recorded.

The questions asked are mainly in the form of rhetorical the answers. Also for the information about the hemoglobin we visited several doctors in Sangli and Miraj city.

3. Statistical tools used for data analysis

Mean:

Mean is a sum of observations divided by number of observations.

$$\text{Mean} = \frac{\sum(xi)}{n}$$

Variance:

The mean of squares of deviations taken from mean is called Variance.

$$\text{Variance} = \frac{\sum xi^2}{n} - \bar{X}^2$$

Standard Deviation: The positive square root of mean of squares of the deviation

Taken from mean is called as standard deviation.

Percentile: The observations P₁, P₂... P₉₉ which divide the total number Of observations into 100 equal parts are called percentile.

Correlation: Chi-square test for independence of attributes: For $m \times n$ contingency table

Let us consider two attributes A and B. Attribute A is divided into m classes as $A_1, A_2, A_3, \dots, A_m$ and attribute B is divided into n classes as $B_1, B_2, B_3, \dots, B_n$.

The variances of cell frequencies can be expressed in the following table known as $m \times n$ manifold contingency table.

(A_i) is a no. of persons possessing attribute A_i .

(B_j) is a no. of persons possessing attribute B_j .

$(A_i B_j)$ is a no of persons possessing attributes A_i and B_j .

Also $\sum (A_i) = \sum (B_j) = N$ Let $(A_i B_j) = O_{ij}$ $m \times n$ contingency table

A/B	B_1	B_2	B_j	B_n	Total
A_1	O_{11}	O_{12}	O_{1j}	O_{1n}	(A_1)
A_2	O_{21}	O_{22}	O_{2j}	O_{2n}	(A_2)
\vdots	\vdots	\vdots		\vdots		\vdots	\vdots
\vdots	\vdots	\vdots		\vdots		\vdots	\vdots
A_i	O_{i1}	O_{i2}	O_{ij}	O_{in}	(A_i)
\vdots	\vdots	\vdots		\vdots		\vdots	\vdots
\vdots	\vdots	\vdots		\vdots		\vdots	\vdots
A_m	O_{m1}	O_{m2}	O_{mj}	O_{mn}	(A_m)
Total	(B_1)	(B_2)	(B_j)	(B_n)	N

The problem is to test that the two attributes A and B are independent or not.

Under null hypothesis,

H_0 : Attributes A and B are independent v/s H_1 : Attributes A and B are dependent.

The theoretical frequency E_{ij} is calculated as follows,

$E_{ij} = \frac{(A_i)(B_j)}{N}$ the table of expected frequencies can be expressed as follows,

E_{11}	E_{12}	E_{1n}
\vdots	\vdots		\vdots
\vdots	\vdots		\vdots
E_{m1}	E_{m2}	E_{mn}

Under the null hypothesis test statistic is,

$$\chi^2 = \sum \sum \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \sim \chi^2 (m-1)(n-1) \text{d.f.}$$

Accept H_0 if calculated $\chi^2 < \text{tabulated } \chi^2$ otherwise reject H_0 .

4. Statistical Analysis

1. Mean

$$\text{a) Rural: Mean} = \frac{\sum f_{imi}}{N} = \frac{1022}{100} = 10.22$$

The Mean HB of rural Woman's is 10.22gm

$$\text{b) Urban: Mean} = \frac{\sum f_{imi}}{N} = \frac{1090}{100} = 10.9$$

The mean HB Of Urban Woman's is 10.9gm

$$\text{c) Combine: Mean} = \frac{\sum f_{imi}}{N} = \frac{2112}{200} = 10.56$$

2. Correlation:

$$\text{1) HB range and vegetarian: } \text{Corr}(X, Y) = \frac{\text{Cov}(X, Y)}{(S.DofX).(S.DofY)} = 0.9907$$

$$\text{2) HB range and non-vegetarian: } \text{Corr}(X, Y) = \frac{\text{Cov}(X, Y)}{(S.DofX).(S.DofY)} = 0.9865$$

3. Percentiles:

$$\text{a) Rural: } P_{50} = 10.23 \quad P_{75} = 11.33 \quad P_{90} = 12.45 \quad P_{97} = 13.33$$

$$\text{b) Urban: } P_{50} = 10.8 \quad P_{75} = 11.82 \quad P_{90} = 12.64 \quad P_{97}$$

$$\text{c) Combine: } P_{50} = 9.5833 \quad P_{99} = 10.51$$

4. Chi-square – test: For finding whether the women's diet and Hemoglobin are dependent or not Observation Table:

Class	O _i	E _i	$\frac{(O_i - E_i)^2}{E_i}$
$(e_1 m_1)$	97	99	0.0404
$(e_1 m_2)$	67	65	0.0615
$(e_2 m_1)$	24	22	0.1818
$(e_2 m_2)$	12	14	0.2857
Total			0.5994

Here calculated χ^2 is 0.5994 and tabulated χ^2 with 1 degrees of freedom at 5% level of significance is 3.841. Therefore Calculated $\chi^2 < \text{Tabulated } \chi^2$

Therefore, we accept H_0 at 5% level of significance

3. Conclusion

1. By comparing both correlations, we can say that, there is positive correlation between Hemoglobin of vegetarian and non-vegetarian women, i.e. they are in same direction.
2. The average value of Haemoglobin in Sangli district is 10.56g
3. The average value of Haemoglobin in Sangli district is 10.56g
4. 50% women have less than 10.23 gm. Haemoglobin.
5. 97% women have less than 13.33 gm. Haemoglobin.
6. Haemoglobin and diet of women are dependent.

Acknowledgement: This Statistical Analysis is based on Primary data collected from Sangli District

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PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

ENUMERATION OF MEDICINAL PLANTS OF SANGLI DISTRICT. MAHARASHTRA.

VIKAS AWALE, HARSHAL WANGIKAR, MANGESH GAVIT

*Department of Botany**Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya, Sangli, 416 416**Email: vikasawale@gmail.com***Abstract**

Sangli district is one of southern district of Maharashtra State. It is situated between the latitudes of $16^{\circ} 43'$ and $17^{\circ} 38'$ N and the longitudes of $73^{\circ} 41'$ and $75^{\circ} 41'$ E. The district is bordered by Satara district on the north – western side. On the north – eastern side it is bordered by Solapur district. On the southern side it is bordered by Belgaum and Bijapur district of Karnataka State. It meets Kolhapur district in south – western side and Ratnagiri district lies on the west of Sangli district. Total area of district is 8501.05 sq. km. and lying mainly in the basin of river Krishna and tributaries Warana, Yerala, Agrni and Man. District has ten talukas, of which Shiralataluka and to some extent Atpaditaluka are hilly, while the greater part of district lies in plains. The average rain fall of the district is 692.40 mm per annum. Potland region of the Shiralataluka and its adjoining is a part of Chandoli Wild Life Sanctuary recently declared as Chandoli National Park and Sahyadri Tiger Project. Another Wild Life Sanctuary in the Sangli district is Sagreshwar Wild Life Sanctuary. Westward part of the district is situated in the Western Ghats ranges shows tropical evergreen, tropical semi-evergreen and tropical moist deciduous type of vegetation, while eastern part and major region of district shows tropical dry deciduous and open thorny scrub vegetation. Various types of vegetation of district harbours variety of medicinal plants. Survey of plant wealth of Sangli district resulted in enumeration of over 300 plant species of some therapeutic value. List of the medicinal plants with their botanical and vernacular names, part of plant used, medicinal values along with their status of occurrence are discussed in the present paper.

Keywords: (Ethno-botany, Plant parts uses, tradition)**1. Introduction**

Plants have been used in all the traditional Indian medicine from time immemorial, particularly in folk medicine and house hold remedies. The world health organization estimated that more than 80% of the world population relies on traditional medicine practices for primary health care needs. Over 75% of the world population is depending upon local health practioners and

tradition medicines for their primary needs (Kattaamani et al. , 2000) India represented by rich traditional practices The present research paper focused on traditional uses of medicinal plant parts and natural diversity . India has rich traditional knowledge of medicine include Ayurveda, Siddha, and Unani use over 7500 plant species have reported. Herbal Medicine are assumed to be a great importance in the primary health care of individual (Sheldon et al., 1997) . In India During last two decades ethnobotanical studies with good scientific base have been appeared.

Table No. 1 LIST OF PLANT AND THEIR USES

Sr. No	Name of the Plants	Family	Local Name	Uses
1.	<i>Annona squamosa</i> Linn	Annonaceae	Sitaphal	Leaves are for destroying worms bred in sores. Seeds are emicid, insecticide.
2.	<i>Argemone mexicana</i> Linn	Papaveraceae	Pivala-dhotara	Seeds are used for laxative and emetic.
3	<i>Cadaba indica</i> Lam.	Cappardiaceae	Kali takali	Leaves are used as anthelmintic , for round worms.
4.	<i>Capparis divericata</i> Lam.	Cappardiaceae	Wghati	Analgesic, aphrodisiac, diuretic
5.	<i>Capparis zeylanica</i> L.	Cappardiaceae	GovindPhal	Sedative and diuretic.
6.	<i>Cleome gynandra</i> L.	Cleomaceae	Pandri-tilwal	Carminative, antispasmodic, anthelmintic.
7.	<i>Helicteris isora</i> Linn	Sterculiaceae	Murud sheng	Demulcent, mild astringent.
8.	<i>Balanaties roxburghii</i> Planch	Simarubaceae	Hinanbet	Seeds used as cough and colic, Bark and leaves used as purgative , anthelmintic.
9.	<i>Clitoria ternatea</i> Linn	Fabaceae	Gokarn	Root are used as laxative diuretic.
10.	<i>Dulburgia sissoo</i> Roxb.	Fabaceae	Sisvi	Dried bark and fresh leaves used as local astringent, haemostatic.
11.	<i>Pongamia pinnata</i> Linn	Fabaceae	Karanj	Oil used in Skin Diseases.
12.	<i>Abrus precatorious</i> Linn	Fabaceae	Gunj	Dental caries, dandruff, Cardiac problems, anti fertility.
13.	<i>Cassia tora</i> Linn	Fabaceae	Takala	Leaves used in Skin diseases, Rheumatism, Leprosy.
14.	<i>Cassia auriculata</i> Linn	Fabaceae	Tarvad	Leaves and Flowers used diabetes, skin disorders, eye disorders.
15.	<i>Acacia nilotica</i> (L.) Wild <i>A. arabica</i> Del.	Fabaceae	Babul	Leaves extract used in diarrhea, blood purification.
16.	<i>Sesbania grandiflora</i> (L.) Poiret	Fabaceae	Shewari	Leaves and Flower extract used in fever, night blindness, Cough, ulcer and muscle relaxant.
17.	<i>Caesalpinia bonducella</i> (L.) Fleming.	Fabaceae	Gajaga	Leaves and seeds used in pain killer, indigestion, piles worms, cough, diabetes, skin disorder.
18.	<i>Butea monosperma</i> (Lam.) Taub.	Fabaceae	Palas	All part of the plant used in fever, diarrhea, haemorrhage, worms,

				scorpion bites and rejuvenative.
19.	<i>Tephrosia purpurea</i> (L.) Pers.	Fabaceae	Unhali	Roots, Seeds and Ash used in diabetes, dysentery, fever, teeth disorders, wound healing cough and snake poisoning, skin disorders.
20.	<i>Erythrina indica</i> L.	Fabaceae	Pangara	Leaves and bark used in bleeding, piles, eye disorders, sleeping problem.
21.	<i>Solanum nigrum</i> Linn	Solanaceae	Kanguni	Whole plant used in Piles, carminative,
22.	<i>Woodfordia fruticosa</i> Salisb	Lythraceae	Dhayati	Bark used in anthelmintic,in thirst , uterine sedative, dysentery, leprosy, erysipelas.
23.	<i>Caesalpinia sappan</i> Linn	Fabaceae	Patang	Wood used as astringent and in dysentery.
24.	<i>Hamiltonia suaveolens</i> Roxb.	Rubiaceae	Gidesa	An infusion of the root is given in courbature.
25.	<i>Calendula officinalis</i> Linn	Asteraceae	Makhmal	Astringent, styptic
26.	<i>Carissa carandas</i>	Apocynaceae	Karvand	Antiscorbutic
27.	<i>Calatropis procera</i> Br.	Apocynaceae	Rui	Latex used as to remove corn, leprosy. Rheumatism, root bark used in alternative, antispasmodic, diuretic, emetic.
28.	<i>Hemidesmus indicus</i> R. Br.	Periplocaceae	Anantvel	Demulcent, Diaphoretic. Diuretic, valuable alternative.
29	<i>Gymnema sylvestre</i> R. Br.	Apocynaceae	Bedkichapal a	Antibiabetic. Diuretic, Astrigent.
30.	<i>Martynia diandra</i> Glox.	Pedaliaceae	Vinchu	Local sedative .

CONCLUSION:

In this present paper emphasis was laid only on less known medicinal uses of plant with different mode of application. Proper scientific evaluation of this plant might lead to the discovery of some interesting and some fruitful information. The use of plant resources as remedies as ancient as man himself.

Total 30 number of species of plants from 13 families were recorded which are used medicinally by the Vaidu people of the Sangli District. The name of the plant with family, local uses and medicinal uses of the plant which were enumerated (Table 1.)

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

SOLVATOCHROMIC FLUORESCENCE BEHAVIOR OF 2-AMINO-3-CYANO-5-BROMOSPIRO(5H-INDENO [1,2-b] PYRAN-4,3'-INDOLINE)-2',5,-DIONE IN VARIOUS SOLVENTS AND ITS INTERACTION WITH BOVINE SERUM ALBUMIN

Shilpa Y. Salunkhe^{1,2}, Dhanaji G. Kanase², Govind B. Kolekar^{1*}.

1 Name of Institute (Department of Chemistry, Shivaji University, Kolhapur, M. S., India, 416008.)

2 Name of Institute (Department of Chemistry, Dr. P. K. Mahavidyalaya, Sangli, M. S., India, 416101.)

Email: gbkolekar@yahoo.co.in

Abstract:

A 2-Amino-3-cyano-5-bromospiro (5H-indeno [1, 2-b] pyran-4, 3'-indoline)-2',5,-dione (ACBSIPID) was synthesized by Knoevenagel reaction. The solvatochromic fluorescence behavior of ACBSIPID has been investigated in various polar and non-polar solvents which featuring electron withdrawing $-\text{CN}$, $-\text{C}=\text{O}$ groups, electron donating $-\text{NH}_2$, $-\text{NH}$, $-\text{Br}$ groups and conjugated pi electron system. The fluorescence of the compound exhibits bathochromic shift and correlated with the solvent polarity. The interaction of bovine serum albumin (BSA) with ACBSIPID was also investigated. The fluorescence intensity of BSA quenches with increasing concentration of ACBSIPID and quenching is in accordance with Stern-Volmer equation.

Key Words: Knoevenagel reaction, Fluorescence quenching, Solvatochromic effect.

1. Introduction:

Isatin is an indole derivative broadly presents endogenously in both human and other mammalian tissues and fluids probably as a result of the tryptophan metabolic pathway. The versatile molecular architecture makes isatin as a model platform for structural modification and derivatisation. Many isatin substituted compounds exhibit an extensive range of biological activities such as anticancer, antidepressant, anticonvulsant, antifungal, anti-HIV and anti-inflammatory activity [1]. The isatin derivatives also have anti-mycobacterial, antiviral and analgesic activities [2]. Likewise, synthesis and pharmacological application of various spirooxindol-furan derivatives have been reported [3]. Consequently, we have synthesized a novel isatin derivative [2-Amino-3-cyano-5-bromospiro (5H-indeno [1, 2-b] pyran-4,3'-indoline)-2',5,-dione] (ACBSIPID) via Knoevenagel reaction [4]. It is an anticancer compound effective against breast cancer cell lines MCF7, MDA-MB435. Therefore, we have chosen synthesized drug for the study of solvatochromic fluorescence behavior in various solvents and its interactions with BSA.

Fluorescence radiation always gets at longer wavelength than that of the Excitation. Emission is typically less than the absorption. Fluorescence naturally occurs at longer wavelengths or lower energies. This phenomenon was first detected by Sir G. G. Stokes in 1852. It is the characteristic of fluorescence emission. One mutual cause of the Stoke's shift is the fast decay to the lowest vibrational level of first excited energy level [5]. The separation between excitation and emission band maxima is known as Stoke's shift. When the emission band lies within 30 to 50 nm of excitation wavelength, measurement problems can get up due to difficulty in separating the Rayleigh scatter of the excitation light from the emission band. The connections of solute molecules with solvent generally introduce higher spectral red shifts of fluorescence. These shifts are sometimes solvent specific and are also called Stokes shift [6]. It is common fact that the position of absorption bands of a compound in solutions alters as a result of interactions of a solute with solvent molecules. These relations can be classified into non-specific, i.e. caused by polarity effects, and specific such as hydrogen bonding or electron donor-acceptor interactions. The influence of a solvent on spectral behavior of a compound is called solvatochromism [7,8]. The fluorescence of synthesized compound ACBSIPID reveals considerable red shifts and it correlated with solvent polarity. The study of the solvent sensitive emission shows, ACBSIPID is a good drug and therefore to be used as a fluorescent probe.

Yet, there are examples of current studies of the solvent impact on electronic absorption spectra of several organic compounds which have been designated in terms of their interactions with the solvent [9,10]. Newly some marine alkaloids such as dihydropyrimidine-5-carboxylate

have been synthesized and used as fluorescent probes. They show remarkable biological activities like potent HIV-gp-120-CD 4 inhibitors as well as anti-HIV agents [11]. One of the important fluorescent probes BODIPY, boron dipyrromethane was used to recognize the properties such as chemical and photostability, relative high absorption coefficients and fluorescence quantum yields [12]. The effect of the pyrimidine additives on the dye sensitized solar cell performance was also investigated [13]. The influence of solvents on the spectral properties of molecules normally mentioned as solvatochromism has been examined for many years [14].

Figure 1 displayed the scheme of synthesis and molecular structure of ACBSIPID. It is an anticancer compound active against breast cancer cell lines MCF7, MDA-MB435. It is synthesized in our laboratory by three-component condensation of 5-bromoisatin, malononitrile and 1, 3 indandione in DMF at room temperature [4].

In this paper, we were reported on the analysis of fluorescence spectral properties of ACBSIPID in different solvents. From these measurements Stoke's shift ($\Delta\nu$) was determined. Solvent polarity and local environment have deep effects on the emission spectral properties of fluorophore molecules. The effect of solvent polarity is one cause of the Stoke's shift, which is one of the observations in fluorescence.

2 Experimental:

2.1 Materials and Methods:

Bovine Serum Albumin was purchased from Himedia Chemical Company. All chemicals and solvents used for synthesis of ACBSIPID and spectroscopic data analysis were of analytical reagent grade and used lacking of further purification. ACBSIPID was synthesized by using the method reported in literature [4] and it was purified by re crystallization from ethanol. The fluorescence quenching spectra were recorded on PC based Spectrofluorophotometer (JASCO Japan FP-8300). Excitation and emission slit width was fixed to 10 nm. The stock solutions of concentration (0.5×10^{-3} M) were prepared by dissolving the pure solid compound in different solvents. The fluorescence emission spectra were monitored at different excitation wavelengths between 200 to 550 nm.

3 Results and Discussion:

3.1 Study of solvatochromic effect:

For study the solvatochromic effect, excitation and emission fluorescence spectra of ACBSIPID were measured in different solvents such as acetone, methanol, ethanol, acetonitrile, ethyl acetate etc. at room temperature and predicted spectroscopic data such as excitation and emission maxima, Stoke's shifts are listed in Table 1. The fluorescence quenching occurs in

region 380-550 nm with maximum emission at 424 nm, and fluorescence spectra are depicted in Figure 2. The emission spectrum of compound exhibited a red shift (from 415 to 424 nm).

The interaction between solvent and fluorophore affect the energy difference between the ground and excited state. To a first estimation this energy difference is a property of refractive index (η) and dielectric constant (ϵ) of the solvent which is usually explained by the Lippert-Mataga equation [15, 16].

$$\Delta\nu = \nu^{\text{abs}} - \nu^{\text{em}} = [(\mu_s - \mu_g)^2 / hca^2] \Delta f + \text{Const.} \quad (1)$$

$$\Delta f = (\epsilon - 1/2\epsilon + 1) - [(\eta^2 - 1)/(2\eta^2 + 1)] \quad (2)$$

Where $\Delta\nu$ = Stoke's shift in cm^{-1} , $h = 6.6262 \times 10^{-34}$ J is Plank's constant, $c = 2.99 \times 10^8$ ms^{-1} is velocity of light and 'a' is Onsager cavity radius, ϵ and η are dielectric constant and refractive index of the solvent, respectively. The obtained results were presented in Table 1 indicates that Stoke's shift increases with increasing polarity. This shows good correlation coefficient in excited state and also the dielectric solute-solvent interactions are responsible for the observed solvatochromic shift for the present molecule.

3.2 Study of Fluorescence quenching:

Serum albumin is one of the most rich carrier proteins which play an important role in transport and disposition of endogenous and exogenous compounds present in blood. Protein drug interaction plays an important role in pharmacokinetics and pharmacodynamics of the drugs. In a study techniques regarding the communications of drugs and protein, fluorescence methods are great supports in the learning of relations between drugs and plasma proteins in general and serum albumin in specific because of their high sensitivity, rapidity, and ease of application [17]. In this study, the concentration of BSA solutions was stable at $1.0 \times 10^{-5} \text{ mol L}^{-1}$ and the concentrations of ACCSIPID different from 0 to $1.2 \times 10^{-5} \text{ mol L}^{-1}$. A fluorescence spectrum of BSA with increasing addition of ACBSIPID was recorded upon excitation at 280 nm at room temperature and is depicted in Figure 3. This clearly indicates that, there is gradual decrease in fluorescence intensities of BSA with increasing concentration of ACBSIPID with blue shift from 336 to 331 nm. It suggests that the fluorescence chromophore of serum albumin is placed in a more hydrophobic environment subsequent the addition of ACBSIPID. The fluorescence quenching effect was due to the formation of non-fluorescent complex between drug and protein [18]. Moreover, the kinetics of quenching of BSA was studied by using Stern-Volmer equation [19] by plotting F_0/F versus concentration of quencher (Figure 4).

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (3)$$

Where F_0 and F are the fluorescence intensities of donor in presence and in absence of acceptor, $[Q]$ is the concentration of quencher, K_{SV} is the Stern-Volmer quenching constant, K_q is the

bimolecular quenching rate constant, and τ_0 is the lifetime of the fluorophore in the absence of quencher [20]. The formation of complex was again confirmed from the values of quenching rate constants, K_q which is calculated using the equation,

$$K_q = K_{SV} / \tau_0 \quad (4)$$

Where K_{SV} is obtained from slope, i.e. 3.1257 M^{-1} and K_q was found to be $3.1257 \times 10^6 \text{ M}^{-1} \text{ S}^{-1}$.

4 Conclusions:

The compound ACBSIPID was synthesized and characterized by NMR, IR spectral data. The photophysical properties of ACBSIPID were examined by fluorescence spectra. The Stokes shift increases with rise in solvent polarity. Fluorescence intensity of BSA was reduced with increasing concentration of ACBSIPID due to formation of drug-protein complex and quenching is in accordance with Stern-Volmer relation. The Stern-Volmer constant (K_{SV}) is 3.1257 M^{-1} , quenching rate constant K_q is $3.1257 \times 10^6 \text{ M}^{-1} \text{ S}^{-1}$.

5 Tables:

Table 1: Spectroscopic data of ACBSIPID in various solvents.

Solvent	Solvent Polarity Δf	λ_{ex} (nm)	λ_{em} (nm)	ϵ ($\text{M}^{-1} \text{ cm}^{-1}$)	Stoke's shift $\Delta \nu$ (cm^{-1})
Methanol	0.3087	285	425	32.7	11503
Acetone	0.2847	330	420	20.7	6494
Ethanol	0.2974	286	417	24.3	10985
Ethyl acetate	0.1995	368	416	6.02	3135
Acetonitrile	0.0211	370	415	37.5	2931

6 Figures:

Figure 1. Synthesis and Molecular Structure of ACBSIPID.

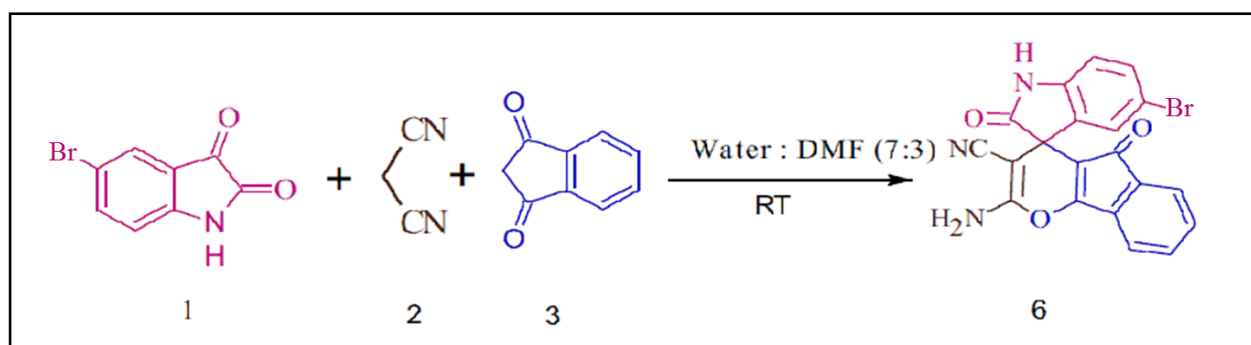


Figure 2. Fluorescence spectra of ACBSIPID in various solvents at $C = 1 \times 10^{-4}$ M.

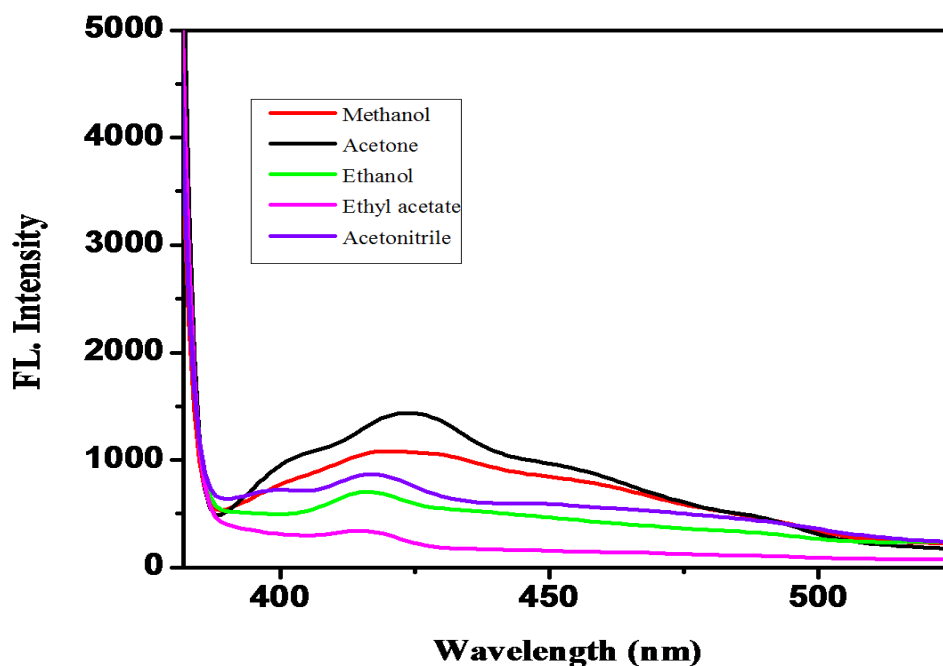


Figure 3. The fluorescence quenching spectra of BSA in the presence of ACBSIPID, $C_{BSA} = 1.0 \times 10^{-6}$ mol L⁻¹; $C_{ACBSIPID}/(10^{-6}$ mol L⁻¹) a-g: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ($T = 301$ K, $pH = 7.4$, $\lambda_{ex} = 280$ nm).

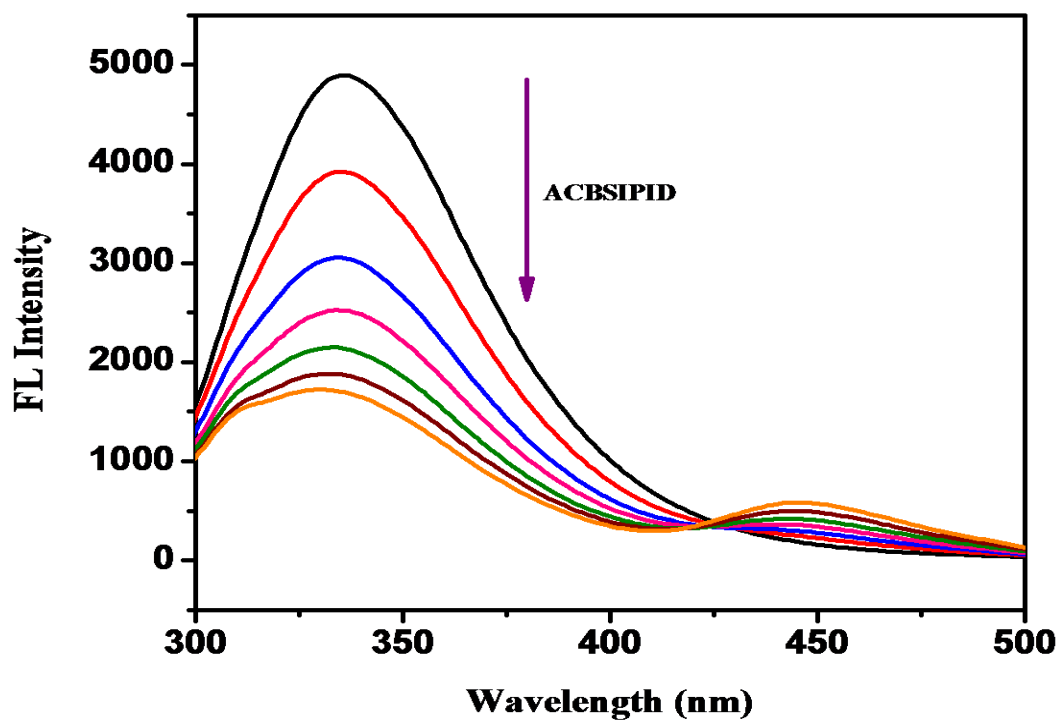
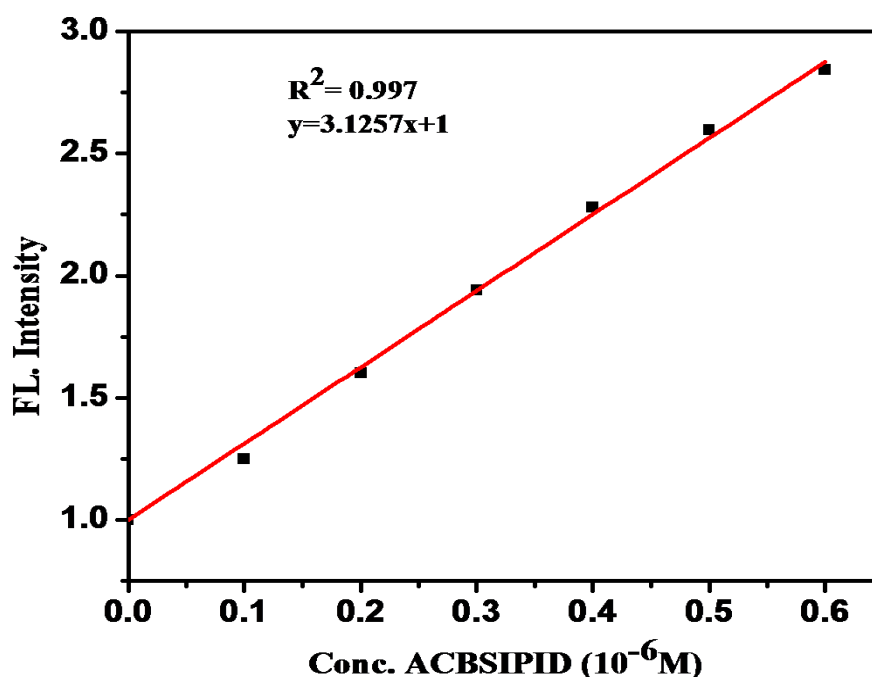


Figure 4. Stern-Volmer Plot for BSA and ACBSIPID.**7 References:**

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PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

THE LATITUDINAL TREND VARIATION IN TCO OVER INDIA

R. P. Pawar¹, O. M. Patil¹, S. T. Mane², S.B. Wategaonkar³, D. P. Nade², D. G. Kanase^{*2}¹*Center for Space and Atmospheric Science, Department of Physics, Sanjay Ghodawat University Kolhapur*²*Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya (Shivaji University, Kolhapur), Sangli*³*Sanjay Ghodawat Polytechnique, Atigre**Corresponding author email: dgkanase@gmail.com (D.G. Kanase)

Abstract

To study the latitudinal variation in total column ozone (TCO) during the period of 22 years from 1986 to 2008 over eight multiple stations of the India. We have used, the linear regression analysis technique to identify the trend variations in the TCO time series. The period includes three solar cycles (i) 22nd solar cycle (September 1986 to July 1996), (ii) 23rd solar cycle (August 1996 to November 2008). Our results agree with solar cycles and TCO trend shows the increasing mode from the 23rd solar cycle. This may result of the Montreal Protocol. The latitudinal variations in TCO trend is may due to the Brewer-Dobson circulation. This work will help to understand the dynamics of the stratospheric ozone layer over the India.

Keyword: stratospheric ozone, solar cycle, trend analysis.

1. Introduction

Life on earth is sustained due to stratospheric ozone layer because it observe that the high energetic radiation, ultraviolet (UV) radiations, are coming from the sun. Basically ozone is a greenhouse gas and minor constituent in the lower atmosphere of the earth. However, small variations in ozone can impact on the local climate and weather and globally as well. The atmospheric ozone is classified into two types, stratospheric ozone (good ozone) and tropospheric ozone (bad ozone). Good ozone absorbs UV radiations while bad ozone acts as a pollutant and it is harmful to the biosphere as well. The research on the stratospheric ozone has been accelerated after the detection of so called ozone holes (Farman et al., 1985) over the Antarctic region in 1985. The halogen compounds such as CFC are responsible for the

destruction of ozone over the polar region (Crutzen, 1970; Stolarski and Cicerone, 1974; Molina and Rowland, 1974). The TCO represents all the ozone molecules in the atmospheric column having 1 m^{-2} cross-section. The unit of TCO is Dobson unit.

Many researchers have reported that the anthropogenic emissions have a minor effect on the concentration of stratospheric ozone over the tropical region (e.g. Chakrabarty et al., 1998; Nade et al., 2019; Nade et al., 2020). The tropical region is the region where the density of population and biodiversity is high. However, the decrease in ozone is not been observed or severe over the tropical region. But a small decrease in ozone can modify incoming UV radiation, which is harmful to the biosphere. Thus, investigation of the long-term variations in ozone over the tropical region is more important than mid latitude and polar region. The stratospheric ozone naturally varied due to the effect of solar activities, QBO, ENSO etc. The study of atmospheric dynamics and weather condition will help to make policies in developing country like India.

Many researchers have carried out the long term trend variations in the TCO. First time, Chakrabarty et al. (1998) have studied the TCO, measured by the ground based Dobson spectrophotometer at six different stations in the period of 23 to 45 years from 1957 to 1996. Herein, they have observed decreasing trend in TCO. Tondon and Attri (2011) used TCO data collected from different stations over India from 1979 to 2008 and studied the trend analysis. They observed decrease in ozone concentration over the stations at northern part of India. Recently, Potdar et al., 2018 have calculated the long term trend in the TCO over Indian region during recent solar cycle. The first section is about the introduction, the second section is about the data and methodology and final section presents the results and discussions.

Table 1: The list of the station considered with their latitude, longitude and altitude.

Name	Latitude	Longitude	Altitude (meters)
Trivandrum	8.48	76.95	60
Kodaikanal	10.23	77.47	2343
Bangalore	12.98	77.58	609
Hyderabad	17.38	78.47	358
Nagpur	21.1	79.05	308
Benares	25	83	228
New Delhi	28.67	77.22	220
Srinagar	34.08	74.83	1586

Data

We have collected the merged ozone data (MOD) of the TCO at different pressure levels measured by Solar Backscatter Ultraviolet (SBUV) instrument from NASA website (<https://acd-ext.gsfc.nasa.gov/anonftp/toms/sbu/MERGED/>). This data is continuously updated from 1979. Bhartia et al., 2013 given the algorithm of MOD-TCO data sets. There are 15 stations where the over pass MOD-TCO data is available, but we have considered only those stations having same

longitude with different latitudes. This will helps us to understand the latitudinal trend variation in TCO. The lists of the stations are shown in Table 1.

2. Methodology

We have used the standard method of linear regression analysis to calculate the trend in the TCO time series for multiple stations of the India. The linear model as,

$$TCO = trend \times (m + k) \quad (1)$$

where, TCO monthly time series obtained by taking averages of daily TCO in month. Trend represented the linear trend in the time series. 'm' represents month number and 'k' is the intercept. Finally, least square method is used to find trend and k.

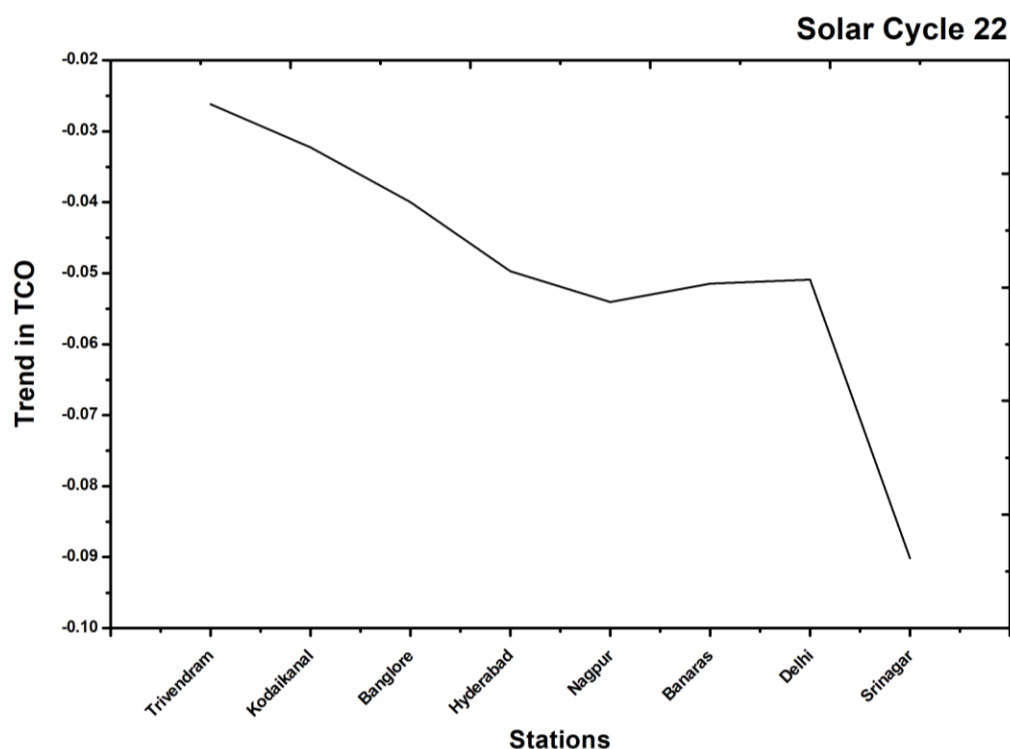


Figure 1: The latitudinal variation in trend of TCO in 23rd solar cycle at multiple stations over India.

3. Result and Discussions

The average of the daily TCO of corresponding month is considered to calculate the monthly mean TCO time series. This TCO dataset is free from daily and weekly fluctuations. We have considered the data from 22nd solar cycle to 23rd solar cycle data set.

Trend analysis

The trend analysis method is used to detect the increasing or decreasing nature of the data sets. Herein, we have applied the linear regression analysis to the monthly mean TCO time series from 22nd solar cycle and 23rd solar cycle.

The trend in TCO from 22nd Solar cycle:

The time spans of the 22nd solar cycle were 10 years from September 1986 to July 1996. Figure 1 illustrates the latitudinal variation in the trend of TCO over all stations for the selected period. We found that the highest decreasing trend at Sri-Nagar station and the lowest decreasing trend at Trivandrum. It indicates a decreasing trend from low latitude to high latitude. Particularly, at Srinagar station, the highest negative trend is may due to specific chemistry at this station. The formation of ozone is higher as in lower latitude than high latitude, but due to the Brewer Dobson circulation, ozone is found at high latitude. Our results are well agreed with Butchart 2014. In this circulations the ozone from troposphere of tropical region is driven into the stratosphere and it is driven further to higher latitude stratosphere (Brewer 1949). The anthropogenic emissions contain ozone depleting substances (ODS) which are may responsible for such decreasing trend. Our results are not only limited to local variations in ozone (decreasing trend) but also globally. This explains by Tondon and Attri., (2011) and they have suggested the trend variations of the TCO for the same period.

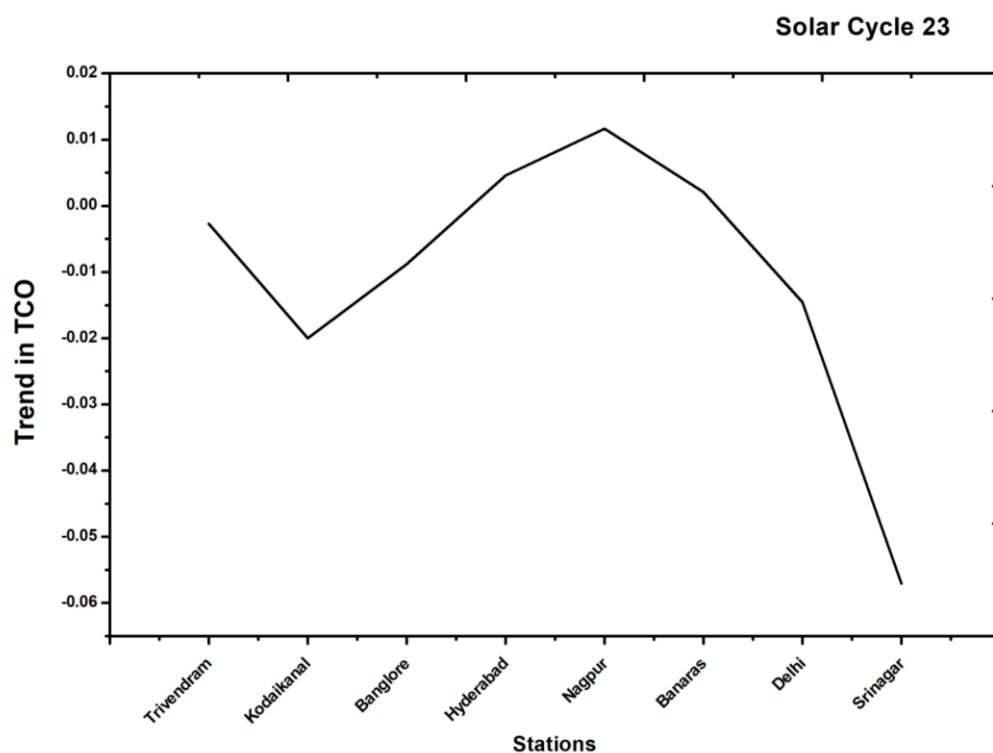


Figure 2: The latitudinal variation in trend in 24th solar cycle at different stations over Indian region

The trend in TCO during 23rd Solar cycle:

The 23rd solar cycle was time spans of 12 years from August 1996 to November 2008. Figure 2, shows the linear trend in TCO time series for all the stations during the 23rd solar cycle. The trend in this period is lower as compared to the 22nd solar cycle. The decrease in trend is only be explained by the increase in ozone after the Montreal protocol which was put forth in 1997. This treaty bans the use of ODE's which is responsible for the increase in the ozone in this period. We can say that the ozone is recovered in this period. Weatherhead et al., (2006) found the

recovery of the ozone over global scale for the same period. WMO 2014 report also found the increase in the TCO in this particular period.

Summary and conclusions

The variation in the linear trend with respect to solar cycle over the Indian region at multiple stations have been carried using the standard statistical methods. We have considered 22nd solar cycle and 23rd solar cycle for the trend analysis of TCO over India and we found that decreasing trend in the TCO during the 22nd solar cycle at all stations. It may be due to the global decrease in ozone during the same period. In the next, 23rd solar cycle, we found that the linear trend in the decreasing trend is lowered. The international treaty Montreal protocol may be responsible for the same. The latitudinal trend variation of TCO shows the effects of the Brewer-Dobson circulation in both the solar cycles. This study will help to understand the ozone dynamics over the Indian region.

Acknowledgement

The authors are thankful to National Aeronautics and Space Administration (NASA) <https://acd-ext.gsfc.nasa.gov/anonftp/toms/sbu/MERGED/> for providing MOD data free for public domain and also thankful to National Oceanic and Atmospheric Administration (NOAA).

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

TOTAL HAEMOCYTE COUNT CHANGES DURING THE DEVELOPING STAGES OF *ANTHERAEA PROYLEI* J.

V. S. Kumbhar

Dr. Patangrao Kadam Mahavidyalaya, Sangli, M.S., India, 416101

Email: varshakumbhar2@rediffmail.com

Abstract

The present study was carried out on the total haemocyte count in the developing stages of *A. proylei* J. The THC steadily increases during the developmental stages, attains its peak in the 5th instar and steeply declines in the pupa. The total haemocyte count in female larva is higher than male.

Key words: Haemocytes, total haemocyte count, differential haemocyte count,

Antheraea proylei.

Introduction

Antheraea proylei is a temperate species of tasar exploited for commercial silk production in Sub-Himalayan Oak-tract extend from Jammu and Kashmir in the west to Manipur in the East embracing Himachal Pradesh, Uttar Pradesh (Uttarakhand), Meghalaya, Mizoram and Nagaland. Mainly five species of Oak are the food plants of this species in their native region Viz. *Q. incana*, *Q. himalayana*, *Q. dealbata*, *Q. serrata*, *Q. semiserrata* (Jolly *et al.*, 1979).

The chemical composition of haemolymph is highly variable among the species and at different developmental stages of the same species (Florkin and Jeuniaux, 1974). Haemolymph, the only extracellular fluid in insects has diverse functions such as immunity, transport and storage of the products required for cellular metabolism. (Pawara and Ramakrishna, 1977; Mullins, 1985)

During metamorphosis there is loss of water and changes in the distribution of water and ions between body compartments. Dramatic changes in body mass, water content, and haemolymph volume and osmolality have been described during the pupal adult transformation of Lepidoptera (Nicolson, 1976; Jungries *et al.*, 1982).

The circulating body fluid, haemolymph contains various types of haemocytes. The haemocytes firstly classified in to four types (Cuenot, 1896). Metalnikov (1908) described their occurrence as floating cells in the haemolymph of the larvae of wax moth *Galleria melonella* and classified them as lymphocytes, spherule cells, leucocytes, and oenocytoids, probably basing on some analogy with mammalian blood cells. Hollande (1909) studied the free floating cells in the haemolymph of certain coleopterans and the larvae of some Lepidoptera and described their cytoplasm filled with granules which he regarded as enzyme containing bodies. Muttokowshi (1924) distinguished the free haemocytes of certain insects as chromophite leukocytes and amoebocytes, both are variable in size but the latter type larger as well as faintly staining. Paillot (1933) for the first time gave a more complete review on the study of blood corpuscles in insects including the larvae of Lepidoptera and Hymenoptera. He grouped them into micro-nucleocytes, macro-nucleocytes, micro-nucleocytes with spherules and oenocytoids.

After this classification, Jones (1962) revised this classification and proposed a dynamic scheme of classification, mentioning nine types of haemocytes. Finally Price and Ratcliff (1974) reconsidered this classification and classified the haemocytes in to six types viz., prohaemocytes, granulocytes, spherulocytes, plasmatocytes, oenocytes and coagulocytes.

The lepidopteran insect blood cells are grouped in to six main classes by the workers Jones, (1962); Gupta, (1979); Ratcliffe, and Rowley, (1979); Arnold, (1982); Rowley and Ratcliffe, (1981) that are prohemocytes, plasmatocytes, granulocytes, spherule cells, adipohemocytes and oenocytoids. Like wise in another insect and same types of haemocytes are observed (Gotz and Boman, 1985; Gupta, 1979 and Gupta, 1994). During the

physiological activities like moulting, growth and development the total and differential hemocytes are often studied (Bahadur, 1993).

Mangalika *et al.*, (2010) reported that the haemocytes are very vital component of the insect immune system and are biochemically very sensitive having multiple functions such as nodule formation, phagocytosis and encapsulation as defense mechanism, synthesis and transport of nutrients and hormones for proper growth and wound healing by way of connective tissue formation. In the silkworm, there are five types of haemocytes prohaemocytes, granulocyte, spherulocyte, plasmatocyte, and oenocytoid (Nittono, 1960; Akai and Sato, 1979; Wago, 1991; Yamashita and Iwabuchi, 2001).

In the present investigation total haemocyte counts were studied in first instar to fifth instar larvae and male and female pupa.

1. Material and Methods

All larval stages and pupa of *Antheraea proylei* J. used in the present investigation. They were maintained under laboratory conditions. During this study they were fed on the leaves of *Terminalia catappa*. Before taking haemolymph, the caterpillars were immersed in hot water (51°C to 52°C) for 2.3 minutes to give a clear view of each haemolymph type (heat fixed) and the haemolymph was obtained by amputation of their legs (Cunet, 1896; Gupta, 1979). The total haemocyte counts were taken by Naubaur's haemocytometer (Pilat, 1935; Jones, 1962).

Results

The total haemocytes count is larger in females than males. The total haemocyte count is $10216/\text{mm}^3$ in female and $9165/\text{mm}^3$ in male. As depicted in table 1 the THC starts at low value i.e. $670 \pm 10/\text{mm}^3$ in the 1st instar larva, increases gradually up to 5th instar larva i.e. $10216 \pm 101/\text{mm}^3$ and declines steeply in pupa stage. In pupal stage the THC count is higher in female pupa $268 \pm 70/\text{mm}^3$ than male pupa $198 \pm 80/\text{mm}^3$. In adults failed to yield an adequate amount of haemolymph.

Table 1 Total haemocyte count changes during the postembryonic development of *A. proylei* J.

Sr. No	Developing stages	THC/mm ³
1	I instar	670 ± 10
2	II instar	1050±50
3	III instar	1672±92
4	IV instar	5066±120
5	V instar Female	10216±315
6	V Instar Male	9165 ±312
7	Male pupa	198±80
8	Female pupa	268±70

2. Discussion

The THC count in *A. proylei* was 10216/mm³ in female and 9165/mm³ in male. In *Prodenia aeridania*, Rosenberger and Jones (1960) showed that THC did not vary significantly in sixth instar larvae as they approached pupation. Laigo and Paschke, (1966) reported in *Trichoplusiani* larvae, counts varied from about 14,000 mm³ to 25,000/mm³ with no apparent trend. In *A. proylei* total haemocyte number increases from 1st instar (670±10 THC/mm³) to 5th (10216±315 THC/mm³) instar larvae and declines in pupal stage in male (198 ± 80 THC /mm³) female (268 ± 70 THC /mm³) like in *Euroa declarata* cell numbers increases from 6,000/mm³ to 20,000/mm³ in second to sixth instars (Arnold and Hinks, 1970).

In THC count in holometabolus increases during the larval stages attains its peak by at end of 5th instar and declines in the pupa, because of elevated rate of mitosis that characterizes all other tissues during this period of active growth and increases number of haemocytes. Haemocytes are involved in intermediary metabolism such as protein synthesis, transport of nutrients, phenol metabolism, growth stimulation (Wigglesworth, 1959; Crossley, 1979) some authors demonstrated that ecdysone enhances the rate of mitosis in hemocytes (Hoffman, 1970; Hinks and Arnold, 1977; Prasada Rao *et al.*, 1984). Since the ecdysone titre is high towards the latter part of each instar (Hinks and Arnold, 1977). The steep declines in haemocyte count in the pupa of present insect (from 10,216/ mm³ in 5th instar to 198/ mm³ in male pupa and

268 mm³ in female pupa) is in agreement with most of the other reports. Andrade *et al.*, (2003) reported decreasing tendency in the number of hemocytes in *A. gemmastalis* during larval period which was also observed in the present study.

The pattern of total haemocyte count during post embryonic development is very similar to early reported literature on holometabola i.e. of increases during the larval stages, attains its peak of end of 5th instar and declines in the pupa. The reason is increase in rate of mitosis that characterizes all other tissues during the period of active growth and it may also increases the number of haemocytes. Due to active growth during the larval stages, intermediary metabolism process should be higher and therefore needs the services of a large number of haemocytes. Some authors demonstrated that ecdysone enhances the rate of mitosis in haemocytes (Hoffman, 1970; Hinks and Arnold, 1977; Prasada Rao *et al.*, 1984).

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES OF SOIL FROM REGION OF CHANDANAPURI, SANGAMNER TEHSIL, (M.S.) INDIA.

*Shantaram Bhoje¹, Pankaj Bhoje², Nalesh Bhiram³, Pradnya Bhalerao⁴

1. Department of Zoology, Shri Pundlik Maharaj Mahavidyalaya, Nandura Rly., Dist.

Buldana(M.S.), India. Email id: shantarambhoje8@gmail.com

2. Kr. Vasant Rao Narayanrao Naik Shikshan Prasarak Sanstha's, Arts, Commerce and Science College Nashik.

3. Department of Zoology, Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya Sangli. E.mail id: naleshbhiram22@gmail.com

4. Department of Zoology, S. N. Arts, D. J. Malpani Commerce and B. N. S. Science College, Sangamner, Dist. Ahmednagar (M.S.), India.

Abstract:

Soil is one of the most important components of Agriculture. Soil is mixture of organic matter and minerals that can support, plant life. Now a day's large number of fertilizers are used in the Agriculture instead of natural manure. Which increases crop productivity while decreases the soil quality so, attempt has been made to analyze the Various Physico-chemical properties of soil such as pH, Alkalinity, EC, Chloride, Calcium Carbonates, Carbonates, Calcium, Magnesium and Organic matter which helps to farmer to maintain the nutrient requirement for the plant and crop.

Keywords: Physico-chemical; pH; Alkalinity; EC; Chloride; Chandanapuri; Calcium Carbonates; Magnesium; Organic matter.

Introduction

Agriculture is the art of cultivating the soil for the purpose of producing more abundantly crop that is necessary for the substance of man & his domesticated animals. (Soil Sci.book by

Dr. J .A. Daji et.al). The soil is the most important to fulfillment of all the basic need of human being. Soil is an important component of our farming. Agriculture is the most important sector of Indian economy. Indian agriculture sector accounts for 18% of India gross domestics products and provide employment to 50% of the countries workforce ([http: www.omicsonline.org](http://www.omicsonline.org)).

The soil is a natural body of mineral and organic material differentiated into horizons, which differ among themselves as well as from underlying materials in their morphology, physical make-up, chemical composition and biological characteristics (Solanki and Chavda, 2012). Soil is a mixture of Organic matter and minerals that can support plant life. In India now a day's large numbers of fertilization are used instead of manures. Due to this crop productivity is increasing speedily but the quality of soil support decreases. So, it becomes essential to analyze the soil parameters. It is a real time to carry out the physic-chemicals analysis of soil because as with the increasing use of chemical fertilizer to the soil, it is difficult to control the adverse effect of the chemical's fertilizers to the soil, plant, animal's % humans being. (Rg. j. Agriculture & forestry sci. by Borkar A.D).

The soil is polluted directly as a result of activity, due to the quality of soil is bad and it becomes harmful to plant life, soil microbe and another organism many agricultural fields are polluted. Pollution is one of the problems in India, out of this soil pollution is one. The physico-chemical parameters of the soil indicate the quality of soil.

1. Materials and Methods

Study Area

Chandanapuri is a village located in Sangamner Tehsil, Ahmednagar District, lies between latitudes 19°29'46,08" N and longitudes 74°11'35,41"E with an altitude of 613m above the mean sea level.



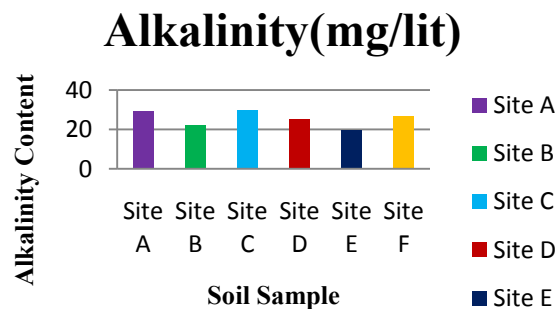
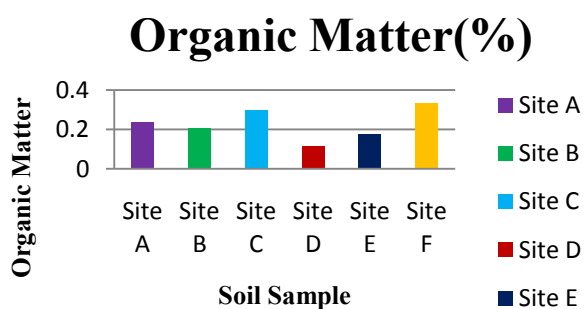
Image- Study area of village Chandanapuri (Source from Google Earth

Methods:

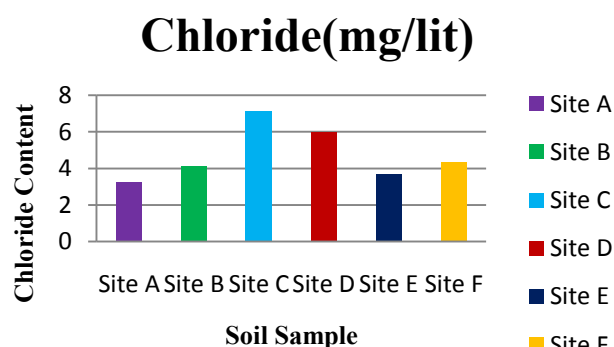
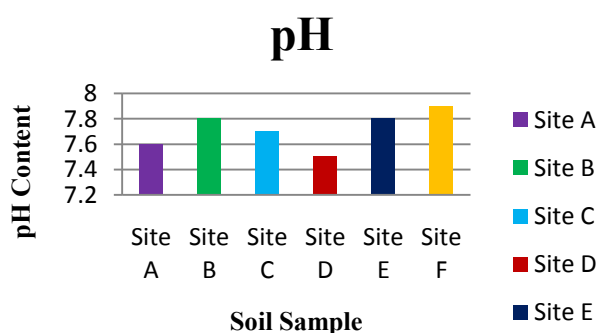
The soil samples were collected from six different sites in Chandanapuri. Soil sample collected in the depth of 0 – 20 cm from the surface of soil from different places. The chemicals and reagents used for the analysis were of A. R. Grade. Standard instrumental and non-instrumental

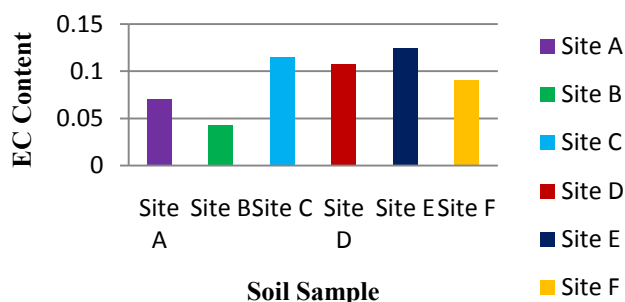
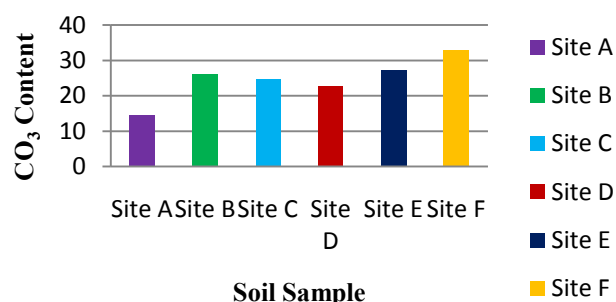
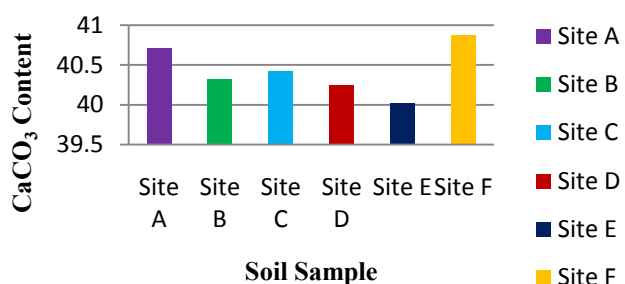
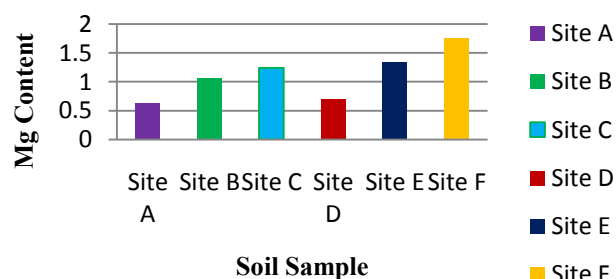
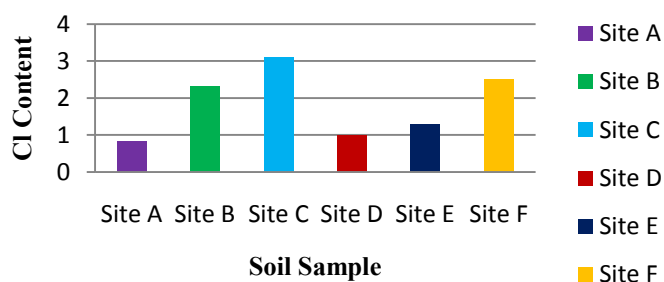
methods were used for estimation of parameters. Experiment was performed to evaluate the soil quality parameters such as Colour, pH, Alkalinity, EC, Chloride, Calcium Carbonates, Carbonates, Calcium, Magnesium and Organic matter. The procedure is followed according to the book as Handbook of soil analysis of J.C. Tarafdar & B. K. Yadav.

Parameter Sample	Color	pH	Alkalinity (mg/lit)	EC	Chloride (mg/lit)	CaCO ₃ (%)	CO ₃ (mg/lit)	Ca (mg/lit)	Mg (mg/lit)	Organic matter (%)
Site A	Black	7.6	29.25	0.071	3.26	40.72	14.56	0.82	0.63	0.2382
Site B	Black	7.8	22.05	0.043	4.16	40.32	26.04	2.3	1.07	0.2058
Site C	Brown	7.7	30.00	0.115	7.15	40.42	24.64	3.1	1.24	0.2968
Site D	Black	7.5	25.00	0.108	5.98	40.25	22.69	1.0	0.71	0.1153
Site E	Black	7.8	19.75	0.125	3.68	40.02	27.16	1.3	1.34	0.1741
Site F	Black	7.9	26.75	0.091	4.32	40.88	33.04	2.5	1.75	0.3358



The given table shows variation in physicochemical parameters according to different soil samples.



EC(10^{-3})**CO₃(mg/lit)****CaCO₃(%)****Mg(mg/lit)****Ca(mg/lit)****Discussion**

Physical and chemical properties of soil samples were studied. pH 7.5 to 7.9, the above 7.5 value of pH shows basic nature. The electric conductivity study of soil shows variation in EC values between 0.043 to 0.125. The alkalinity of soil samples varies from 19.75 to 30mg/ml. Chloride of soil samples varies from 3.26 to 7.15. Calcium carbonate varies from 40.02 to 40.88%. The total carbon varies from 14.56 to 33.04mg/ml. Calcium varies from 0.82 to 3.1g/ml. Magnesium varies from 0.63 to 1.75mg/ml. Percentage of organic matter shows varies from 0.1153 to 0.338.

This study gives the information about the nature of soil, present nutrients in soil, according to this information farmers arrange the number of which fertilizers and nutrients needed to soil for increasing the percentage yield of crop and it also helps to choose the right crop according to type of soil.

3. Conclusion

This conclusion can be drowned in the fact that these physicochemical parameters of soil samples showed dissimilar values at different places. This can be due to irregular distribution of different parameters present in soil. Such type of monitoring soil sample is beneficial to know the concentration of various parameters present in soil samples.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

CHARACTERIZATION OF EXOCHELIN AN EXTRACELLULAR IRON CHELATOR SIDEROPHORE OF *PSEUDOMONAS STUTZERI* OF SGM 1 STRAIN

S. D. Adole*, S.M. Chavhan*

Vidyabharati College Seloo, Dist- Wardha 442104 ,Maharashtra- India*

K.V.N.Naik's Arts, Commerce and Science ,Nashik 422002,Maharashtra- India*

*E-mail: surajadole18@gmail.com**shubhamchavhan808@gmail.com*

Abstract

The present research deals with extraction, characterization and identification of extracellular iron chelator molecules siderophore of an indigenous salt tolerant bacterium *Pseudomonas stutzeri*SGM-1. This is the first report known so far for any genus to produce Exochelinsiderophore other than *Mycobacterium*. In iron limited conditions the strain does not only survived but also yielded the iron chelator molecules the Siderophore of hydroxamate as well as a mixed type. The plant growth promoting features of siderophore produced as well as the strain are of great interest to use this Organic Chelator and its producing strain as a bioinoculants for effective use of available micronutrients in agricultural soils to nourish the plants to yield high quality food from such soils to feed the growing need of food by world's population.

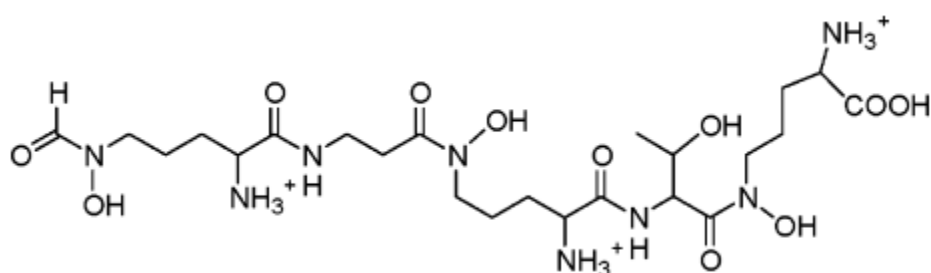
Keywords: Iron, Chelator, Siderophore, *Pseudomonas stutzeri*, bioinoculant

1. Introduction

Iron uptake into mycobacteria is mediated by the exochelins are low molecular weight siderophores of, as yet, chemically undefined structures. Exochelin MS (Structure) is an extracellular siderophore and is reported so far to be produced by *Mycobacterium* species only e.g. the nonpathogenic *Mycobacterium smegmatis*. Considering the chemistry of Exochelin MS is a formylated pentapeptide (N-(δ -N-formyl, δ N-hydroxy-R-ornithyl)- β -alaninyl- δ N-hydroxy-

Rornithinyl-R-allo-threoninyl- δ N-hydroxy-S-ornithine) with three hydroxamic acid groups for Fe^{3+} chelation. In general, siderophores are low molecular weight compounds that can chelate ferric iron from many insoluble compounds in the environment. Ranging in size from 500 – 1500 Daltons, they are synthesized by many microbes when growing under low iron conditions. The siderophores are synthesized by the bacteria under iron deficient conditions. Siderophore helps in chelating iron forming a complex. This complex is taken in inside the cell selectively to obtain the iron collected by the released siderophore. For this active transport is required because the iron needs to be transported against the iron concentration gradient into the cell. (Clement et al, 2004). No other bacterial genus and including all known *Pseudomonas stutzeri* strain is till date reported to produce the siderophore Exochelin, this the Exochelin production by *Pseudomonas stutzeri* SGM-1 is the first report as per knowledge.

Exochelin:



Exochelin producing bacteria reported so far:

Genus & Species	Mnemonics
<i>Mycobacterium neoaurum</i>	Exo-MN
<i>Mycobacterium smegmatis</i>	Exo-MS
<i>Mycobacterium leprae</i>	Exo-ML
<i>Mycobacterium bovis</i>	Exo-MB
<i>Mycobacterium tuberculosis</i>	Exo-MT

2. Materials and Methods

Screening of isolate to grow under iron depletion:

Succinate medium-A: For testing the siderophore producing ability of the organism Succinate Media was used. The media ensures that there is no iron in it medium to test of siderophore production by micro-organisms efficiently. The media (100 mL) was prepared as follows.

Table: Solution D: - This solution was prepared 50ml with components listed

Components	g/50 ml
Yeast extract	1
Tryptone	2
Water	50ml

1. Solution D1:- 4 g of dextrose was dissolved in 20mL water.
 2. Solution C: - 0.15 g of calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was dissolved in 1 mL of water.
 3. Solution B:-0.5 g of Magnesium sulfate hepta-hydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) was added to 10 ml of water.
 4. Solution A: 0.20 g of dipotassium hydrogen phosphate and 0.50 g of ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ was added mixed in 17.4 mL water.
- The above said solutions were autoclaved separately at 121°C for 20 mins, 15 lbs
Now these above solutions were mixed as shown below to obtain the succinate medium.

2% inoculum was added to the medium. The flask was kept in a shaking incubator at 37 °C at 121 rpm for 24 hrs.

The cell culture was collected in centrifuge tube and then centrifuged at 8000 rpm at 40 °C in REMI centrifuge to pellet down the cells. The supernatant was then checked for the presence of siderophore production by using the CAS Reagent described below

- a. Chrome Azurol Sulfonate (CAS) dye assay:- The blue dye contains iron in its structure as seen in figure. The micro-organisms when grown in iron free medium could produce siderophore which was secreted into the surrounding that the supernatant collected above. To the 1 ml supernatant, 0.5 ml of CAS was added and mixed properly and kept for 2 minutes.
- b. The absorption spectra of the supernatant alone and CAS treated supernatant were obtained.
- c. Thin layer chromatography: To identify the type of siderophore synthesized thin layer chromatography was done. The solvent system used was n-butanol: acetic acid: water (12:3:5). The silica gel adsorbs the polar molecules while the non-polar ones get mobilized with the non-polar solvent system. Thus separation of polar and non-polar molecules can be achieved by this technique. Concentrated spots were spotted 1 cm above on a 2×7 cm aluminum supported TLC silica gel plates. After the spotting was done the plates were allowed to dry and then they were kept in an enclosed chamber containing the solvent system. Care was taken that spots were not in direct contact with the solvent system. It was allowed to run until the solvent front came within 1.5 cm of the top of the plate. The plate was removed and dried. The plates were then developed using 0.1 N FeCl_3 prepared in 0.1 N HCl the color of the spot indicates the type of siderophore obtained. If the spot appears wine colored it shows presence of hydroxamate type and if the spot appears to be dark gray then catecholates are indicated.

Siderophore production, isolation and extraction:

Siderophore production was carried out in M9 medium by *Pseudomonas stutzeri*. After completion of incubation period, cell mass was separated by centrifugation at 8,000 rpm at 4°C for 15 min.

Solvent extraction:

The supernatant of M-9 was extracted with ethyl acetate three times. Then ethyl acetate was evaporated in a rotary evaporator. Yellow color solid compound was obtained at bottom of RB indicating the presence of Siderophores.

Still the separated flow through separating funnel was showing positive CAS activity (presence of siderophore). The flow through fraction collected was passed through activated XAD-4 column to let the Siderophores selectively bind to resin. The bound siderophore was then eluted with HPLC grade methanol. This methanol was again evaporated with ROTA vapor and re-suspended in 2ml of methanol.

TLC of siderophores:

The supernatant of M-9 extracted with ethyl acetate in siderophore was spotted on silica gel TLC plates (5X10 cm silica gel 60F 254, Merck, Germany). To achieve best resolution of the extracted siderophores, the chloroform and methanol (1:1), n-Butanol: Acetic Acid: Water (BAW, 3:1:1) system was used.

Purification of Siderophores:

Ion exchange column chromatography:

The supernatant of M-9 extracted with ethyl acetate was acidified to pH 6 with 1N HCl and passed through Amberlite XAD 4 resin (Sigma, Aldrich, USA) column (2.5 × 30 cm). It was washed with five to ten bed volumes of water to remove all unbound components of the medium. It was followed by elution with methanol: Water (70:30) solution. Presence of siderophore was confirmed by CAS reaction and TLC.

Chemical Characterization of Purified Siderophore:

Spectral Scan Analysis

Samples are prepared in MQ water for spectral analysis.

High performance liquid chromatography (HPLC) analysis of purified siderophores:

Analytical HPLC of purified siderophores was carried out on Chromleon (c) Dionex version 6.60 SP8 build 1544 (Switzerland) using REFTEK, Pinnacle II C18 reverse phase column (250 × 4.6 mm, 5 μM integrated pre column) as stationary phase with detector (UV-Visible, PDA 100) and methanol: water (8:2 v/v) as mobile phase at flow rate of 1 ml min⁻¹ at 25°C and at 220 nm. Siderophore separation was carried out by Preparatory HPLC (LC-8A, Sa20A Preparative, Shimadzu) using same mobile phase.

Infra-Red (IR) spectroscopy analysis of purified siderophores:

Purified siderophore sample was dried and subjected to FTIR-8400, Shimadzu; Japan, IR spectrophotometer for the determination of functional groups. Spectra were recorded in 4000-cm⁻¹ - 600-cm⁻¹ range.

Mass Spectroscopy

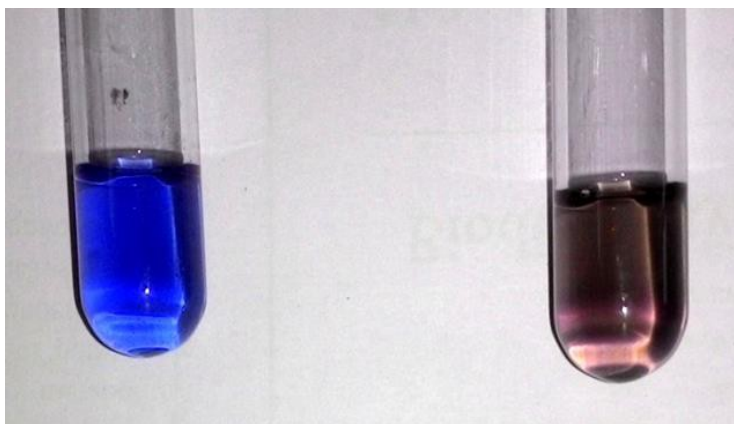
The Exo-MS preparation was also subjected to high resolution liquid chromatography/mass spectrometry (HR-LC/MS) using Agilent Technologies Q-TOF Mass Spectrometer system at Central Instrumentation Facility (CIF), Savitribai Phule Pune University, Pune, India. The solvent system was 95 mL of 0.1% of Trifluoroacetic acid (TFA) in water with 5 mL of 0.1% TFA in 90% Acetonitrile (ACN) for 22 min. Then, 5 mL of 0.1% of Trifluoroacetic acid (TFA) in water with 95 mL of 0.1% TFA in 90% Acetonitrile (ACN) for 9 min. This was followed by the first solvent mixture for 5 min. The flow-rate was 0.4 mL/min for 35 min with maximum pressure of 1200 bar.

3. Result and Discussion

CAS (Chrome Azurol Sulphonate) assay:

Qualitative estimation:

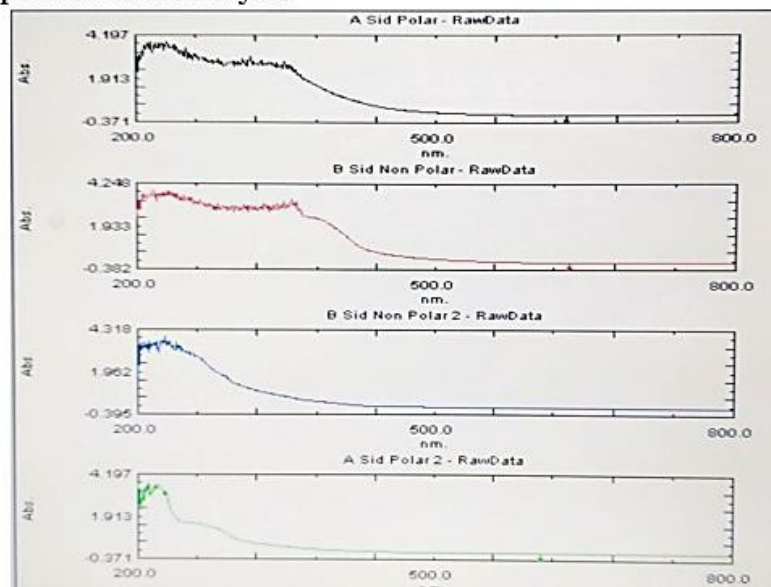
The isolate grew exponentially in M-9 minimal medium and after completion of incubation period the cell free supernatant was obtained by centrifugation at high speed and at low temperature. The 1 ml of supernatant obtained was treated with 1 ml of Chrome Azurol-S (CAS) reagent for the detection of successful Siderophore production. Supernatant upon addition of CAS reagent turned pinkish orange indicating presence of Siderophore in it.



This synthesized siderophores were analyzed by UV-Vis Spectrophotometry (Jasco V630, USA) by scanning from 800nm to 200nm. The supernatant containing Siderophore and reacted with CAS showed absorption elevation in visible region due to complex formation with CAS.

The supernatant was analyzed by spotting TLC on silica supported by aluminum and with n-Butanol: Acetic Acid: Water (B:A:W) as a solvent system. After completion of solvent run the TLC plate was exposed to Short and Long UV and was resulted in shining two spots under both the UV lights. The two spots were visualized under UV indicating the mixture of two UV active compounds in the supernatant.

○ Spectral Scan Analysis



Exochelin molecule is reported earlier with same molecular formula but different structural formula. Basically this exochelin molecule is composed of following moieties in its structure

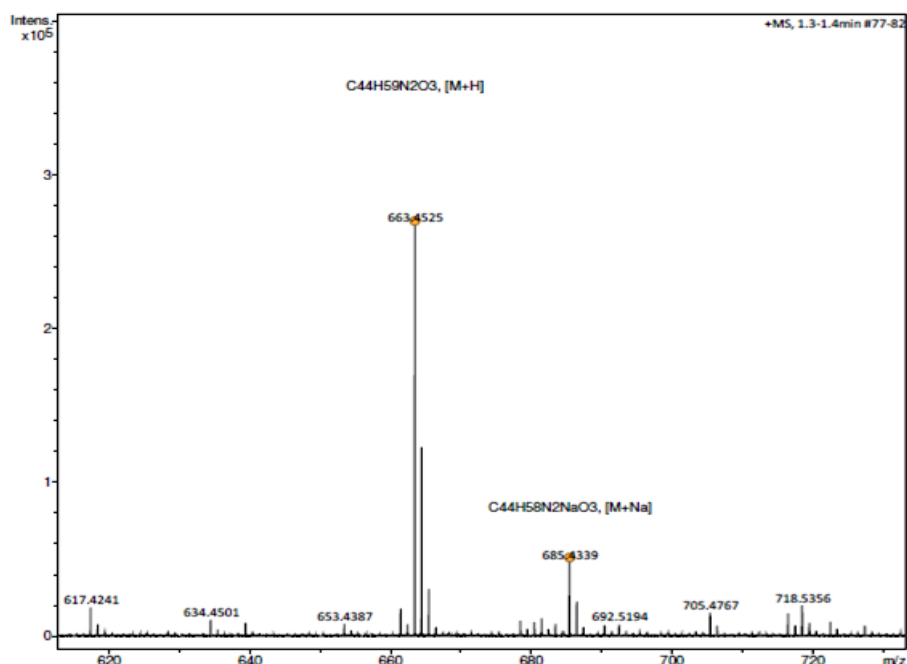
3-Hydroxamate

4-Carboxylate and

3-Primary Amines.

Exochelin molecule is a peptide containing Threonine, β -Ornithine and β -Alanine monomer units.

Following are some Patents of Exochelin molecule based on its Structural variations and one recently reported HR-MS data resembling with the HR-MS data of the exochelin molecule in the present study.



The Exochelin synthesis by *Pseudomonas stutzeri* will be carried out on a large scale, it will be purified and will be used for further analysis. ^1H , ^{13}C , ^{15}N NMR, NMR-TOCSY, NMR-HQ-COSY will be used for structural elucidation of the exochelin produced by *Pseudomonas stutzeri*. Role of produced exochelin to chelate plant essential micro-nutrients other than Fe will be studied.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

ALBINO MICE GENOTOXICITY OF ENDOSULFAN IN FEMALE SWISS ALBINO MICE

Sharma A*, John.P.J**, and Bhatnagar P*.

Department of Zoology, IIS (Deemed to be

University) Jaipur, Rajasthan

*** Centre for Advanced Studies in Zoology, University of Rajasthan, Jaipur-302004, India.*

**Email: dranju1110@gmail.com*

ABSTRACT

Endosulfan an organochlorine pesticide is widely recommended for the control of various crop pests viz. cotton, rice, pulses, plantation crops, fruit crops and vegetables. The residues of Endosulfan and its metabolites reportedly find their way into various organisms including human beings through the contaminated air, water and food which in turn produce various toxic effects. Therefore the aim of the study was to investigate the genotoxic effects of Endosulfan on female Swiss albino mice. Two groups of female mice were administered 5.6mg/kg. bw/day (high dose) and 2.8mg/kg. bw/day (low dose) of Endosulfan by oral intubation for 5 days. Control group was administered plain tap water. Animals from all the three groups were sacrificed. For *in vivo* genotoxic study the femur bones of the treated animals were removed and bone marrow cells were examined. One hundred well spread metaphases per animal were examined to determine the frequency of various chromosomal aberrations like chromosome break, chromatid break, dicentric rings, centric rings, exchanges, fragments, translocations, polyploidy, pulverization, and total number of aberrant cells. The present study reveals that genotoxicity of Endosulfan was elicited by various chromosomal aberrations which were found to be more in the higher dose group.

Key words-Endosulfan, Genotoxicity, Chromosomal aberrations, female Swiss

1. Introduction

Pesticides are economically important chemicals. Their use in agriculture has increased crop yields leading to a decrease in food costs. At the same time, concern for the possible threat to human health has been increasing. Pesticides, in addition to their intended effects, are sometimes found to affect non-target organisms, including humans [Chantelli-Forti, et. al., 1993; Chaudhuri, et. al. 1999]. The exposure to

these toxic chemicals may alter the genetic material of the non target organisms specially females, which may affect them and their descendants. Genotoxicity of pesticides for non-target organisms and their influence on ecosystems are of worldwide concern [Pimentel, et. al., 1998]. Because of the potential environmental impact connected with the introduction and heavy use of pesticides, it is necessary that the genotoxic potential of these agents should be studied.

Previous studies have demonstrated that some pesticides have mutagenic and clastogenic activities (aberrant metaphases) in several biological test systems [Siroki, et. al., 2001; Celik, 2003]. However, additional well-conducted in vitro and in vivo genotoxic studies are necessary to assess the possible health risks associated with the extensive use of pesticides.

Endosulfan is a widely used insecticide belonging to the cyclodien group of organochlorine pesticides. It was considered a safer alternative to other organochlorine pesticides in many countries since the 1970's. But in the last two decades several countries have recognized the hazards of wide application of this pesticide and have banned or restricted its use. India is one of the nations, which has imposed no ban or restriction on Endosulfan. In India, it is widely used against a variety of agricultural pests without much of protective measures as mask, gloves etc. During 1999–2000, about 81,000 metric tons of Endosulfan was manufactured in India. A ban on Endosulfan exists in Kerala imposed through a Court Order, which came as a result of a public pressure following the poisoning of many villagers due to aerial spraying of the chemical [Devakumar, 2002].

Endosulfan is highly toxic, regardless of route of its exposure, causing in-coordination, imbalance, difficulty in breathing, gagging, vomiting, diarrhea, agitation, convulsions, loss of consciousness, and central nervous system disorders [Dutta, et. al., 2003]. Chronic exposure results in liver enlargement, seizures, reduced growth and survival, changes in kidney structure and blood parameters. Endosulfan is known to damage the endocrine system, nervous system, circulatory, reproductive, respiratory and excretory systems and developing foetus [Renber, 1981; Naqvi and Vaishnavi, 1993; Paul, et. al., 1995; Sinha, et. al., 1995]. Endosulfan has been evaluated for genotoxicity in a variety of in vivo and in vitro assays and the results of these assays have been both positive and negative [Usha Rani, et. al., 1980; Sobti, et. al., 1983; L'Vova, 1984; Dzwonkowska and Hubner, 1986; Usha Rani and Reddy, 1986; Dubois, et. al., 1996]. These contradictions indicate that more investigations must be carried out to fulfill this area of study and to give clear idea about the effects of this pesticide since, the complete genotoxic impact of the compound on health of agriculture workers and consumers is still largely unknown. Studies have also showed that female species are more sensitive to the lethal effects of Endosulfan than male species [Paul, et. al., 1995]. Therefore an attempt was made to analyze the possible effects of Endosulfan on female mouse bone marrow cells. This study is expected to furnish further data on how Endosulfan acts on somatic cell line.

2. Materials and Methods

2.1 Test organism

Swiss albino mice aged 30-40 days were obtained from a closed random bred colony of Indian Veterinary Research Institute, Bareilly, U.P.. They were housed in a well ventilated hygienic vivarium at a temperature of 29 ± 2 °C (relative humidity 33-40%) with natural light and dark cycles. The animals were kept in polypropylene cages with wood shavings spread on the floor. Standard mice feed was obtained from Hindustan lever Ltd, Delhi, India and drinking water was provided ad libitum.

2.2 Test chemicals

The technical grade Endosulfan (99% pure; mixture of α -Endosulfan and β -Endosulfan) for the present study was generously gifted by Excel India Pvt. Ltd, Ahmedabad, Gujarat, India.

2.3 Test procedure

The acclimatized females were divided into three groups of four animals each, two experimental and one control group. Two groups of female mice were administered 5.6 mg/kg. bw/day (high dose) and 2.8 mg/kg. bw/day (low dose) of Endosulfan by oral intubation for 5 days. The doses were decided on the basis of recommended dose of commercial formulation of Endosulfan for the field spray. Control group was administered plain tap water.

Bone marrow chromosome aberration assay—Animals were sacrificed by cervical dislocation, and the genotoxic effect was evaluated by the bone marrow chromosome aberration assay (Adler, 1984). About 2 h before sacrifice, colchicine (0.25%) was injected intraperitoneally to produce mitotic arrest. Both femora were dissected out and cleaned for any adhering muscle. The epiphysis was cut off and bone marrow from each bone was flushed out with a syringe into a pre-numbered 15 ml centrifuge tube in sufficient quantity of 0.9% NaCl solution. This suspension was centrifuged for 10 min at 1000 rpm and then supernatant was gently poured off without disturbing the pellet of bone marrow cells. The pellet was dispersed by gentle shaking and then 0.075 M KCl (prewarmed at 37 °C) was added drop wise with agitation to approximate 5 ml. After that, cells were incubated in this solution at 37 °C for 15 min. The bone marrow cell suspension was then centrifuged at 1000 rpm for 10 min and the supernatant was discarded off. The cells were resuspended by slowly adding 5 ml freshly prepared cold fixative (1:3, Glacial acetic acid: Methanol) with agitation. The cells were allowed to fix at room temperature for 20 min in the fixative. The solution was again centrifuged (10 min at 1000 rpm) and the fixative was replaced with fresh fixative. This process was repeated for 4-5 times after allowing the cells in fixative for 10-15 min to assure proper fixation. Finally, the cells were centrifuged, the supernatant was eliminated and a concentrated cell suspension was prepared in 0.5 to 1 ml fixative.

Slides were prepared by the air-drying method. Slides were stained with Giemsa stain (4% in phosphate buffer) for 15 min and rinsed with double distilled water. Ten slides were prepared for each animal. One hundred well spread metaphases per animal were examined to determine the frequency of various chromosomal aberrations like chromosome break, chromatid break, dicentric rings, centric rings, exchanges, fragments, translocations, polyploidy, pulverization, and total number of aberrant cells.

2.4 Statistical analysis-

The data obtained was statistically analyzed using student's t test.

3. Result and Discussion

The results of the present investigation are summarized in the Table -1 and figure-1, 2

A dose related increase in the frequency of chromosomal aberrations occurred. Chromosomal aberrations in controls comprised acentric fragments, chromatid breaks and chromosomal breaks only, whereas in both the treated groups, increase in dicentrics, centric rings, fragments, chromosomal breaks, chromatid

breaks and translocations were recorded. The low dose (2.8mg/kg. bw/day) caused a significant increase in the number of fragments. Increase in Acentric fragments, chromatid breaks and chromosome breaks were significant after exposure to the higher dose (5.6mg/kg. bw/day). The percentage of aberrant cells was 5% in control group while it was 11.5% and 29% for the Low Dose and High Dose group respectively.

Bone marrow cells of both groups also showed a high incidence of polyploidy and pulverization, and this frequency increased as the dose increased. Genotoxic potential of pesticides is a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. To study their genotoxicity various pesticides have been tested by a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage. The chromosome aberration assay is used extensively in population monitoring, since it is recognized as a reliable biomarker for documentation of genotoxic effects due to exposure (Sierra-Tores, et. al., 1998) therefore in the present study genotoxicity was assessed by chromosome aberration assay.

A dose related increase in the frequency of chromosomal aberrations occurred. Chromosomal aberrations in controls comprised acentric fragments, chromatid breaks and chromosomal break only, whereas in both the treated groups, increase in dicentrics, centric rings, fragments, chromosomal breaks, chromatid breaks and translocations were recorded. The low dose (2.86mg/kg. bw/day) caused a significant increase in the number of fragments. Increase in Acentric fragments, chromatid breaks and chromosome breaks were significant after exposure to the higher dose (5.6mg/kg. bw/day) group. In the results of this study fragments and breaks were the main type of aberrations and they increased significantly with increase in dose. This is in concomitance with the observation that the majority of chemical mutagens / clastogens are capable of inducing fragments and breaks (Hassan, 1996).

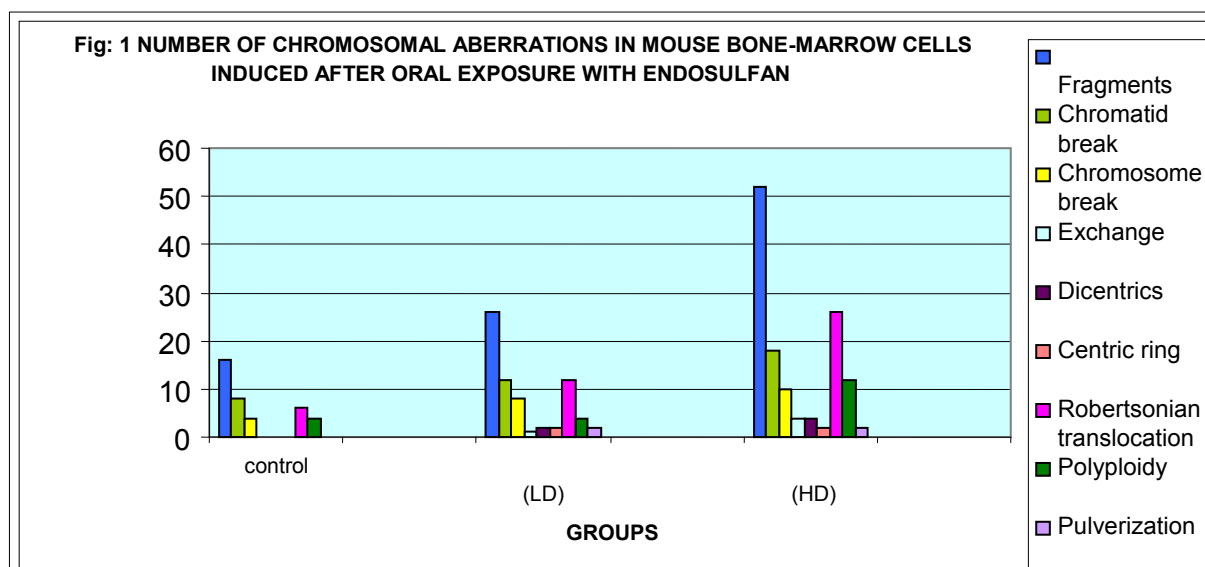
Table-1 Number of chromosomal aberrations in mouse bone-marrow cells induced after oral exposure with Endosulfan.

Treatment / Dose Endosulfan	Total no. of examined metaphases	No of metaphases with different types of chromosomal aberrations							No of abnormal metaphases (%)	No of aberration / cell	No of metaphases with	
		Fragments	Chromatid break	Chromosome break	Exchange	Dicentric	Centric ring	Robertsonian translocation			Polyploidy	Pulverization
Control	400	16	8	4	0	0	0	6	5 ± 0.70	0.11 ± 0.026	4	0
2.8mg/kg bw/day (LD)	400	26*	12	8	1	2	2	12	11.5 ± 1.65	0.14 ± 0.016	4	2
5.6mg/kg bw/day (HD)	400	52**	18**	10*	4	4	2	26	29 ± 2.48	0.29 ± 0.02	12	2

*Significant difference (P<0.05)

** Highly significant difference (P<0.01)

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The results of this study indicate that oral gavage treatment of Swiss albino mice with Endosulfan induced a dose dependent increase in the number of chromosomal aberrations in their bone marrow cells. This is in agreement with recent study which found increased CA frequency in human lymphocytes after in vitro exposure to commercial formulation of Endosulfan, Endocoel (Gadhia, et. al., 2005). Similar formulation of Endosulfan is also known to induce significant sperm abnormality and to reduce sperm count in male Swiss albino mice when administered intraperitoneally (Khan and Sinha, 1996).

The percentage of aberrant cells was 5% in control groups while it was 11.5% and 29% for the Low Dose and High Dose groups of Endosulfan respectively; this confirms the clastogenic potential (aberrant metaphases) of Endosulfan. Bone marrow cells of both groups also showed a high incidence of polyploidy and pulverization, and their frequency increased as the dose increased.

Robertsonian Translocations were also observed in the present study. These are centric fusions between acrocentric chromosomes and one of the commonest forms of chromosomal rearrangement in man. They played an important role in the evolution of both plants and animals (Durante, et. al., 1994). In the present study the number of Translocations increase as the dose increase and were found to be maximum i.e 26 in the high dose group.

These results correspond to some earlier studies in which Endosulfan was found to cause chromosomal aberrations in hamster and mouse, sex-linked recessive mutations in *Drosophila*, and dominant lethal mutations in mice (Naqvi and Vaishnavi, 1993). Studies in human cells both in vitro and in vivo also showed that Endosulfan caused the occurrence of sister chromatid exchanges indicating chromosomal damage (Sobti, et. al., 1983). A team of researchers in Japan found further evidence of Endosulfan genotoxicity using sister chromatid exchanges, micronuclei, and DNA strand breaks as detected by single cell gel electrophoresis as biomarkers (Yuquan Lu, et. al., 2000).

Cytogenetic analysis of bone marrow cells and spermatogenic cells of male albino rats did not reveal any significant effect on chromosomes using Chromosomal aberration biomarker after oral dose 55 mg/kg/bw of Endosulfan. No increase in chromosomal aberrations or sister chromatid exchanges was seen in greenhouse workers in Italy who were exposed to complex mixtures of pesticides that included Endosulfan (Scarpato, et. al., 1996; 1997).

Chromosomal aberrations are usually considered to be derived from unrepaired or misrepaired DNA lesions induced by exogenous or endogenous exposure to DNA-damaging agents and have been proved to be a biomarker for cancer risk by several large and long term prospective studies (Hagmar, et. al., 1994; 1998) so it may be stated that at least some pesticides, which induce CA, may be carcinogens. The data of the present study also confirms the increased rate of chromosomal aberrations; which can be considered an evidence of clastogenic potential (aberrant metaphases) of Endosulfan.

Acknowledgements:

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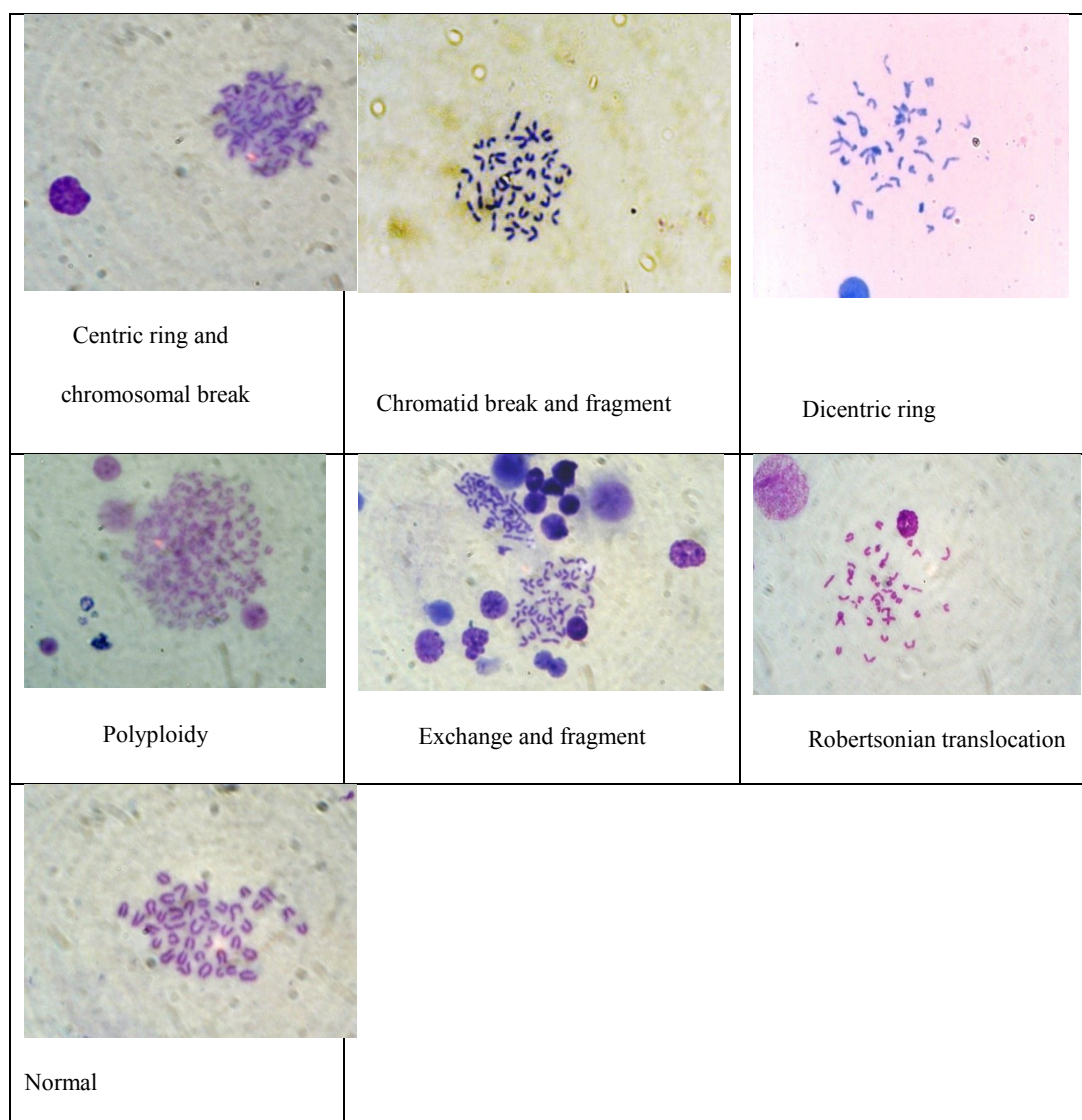


Fig: 2 Photo micrograph of various chromosomal aberrations in mouse bone-marrow cells induced after oral exposure with Endosulfan.

THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

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DEVELOPMENT AND VALIDATION OF A HPLC-UV METHOD FOR DETERMINATION OF FORMALDEHYDE IN LEVOFLOXACIN HEMIHYDRATE DRUG SUBSTANCE

Santosh Patil¹, Poonam Koppula¹ and K. S. Kumar^{2*}¹*Ipca Laboratories Ltd., Vadodara - 391445*²*School of Sciences, GSFC University, Vadodara, Gujarat, 391750***Corresponding author: School of Sciences, GSFC University, Vadodara, Gujarat, 391750**E - mail address: ksk.india@gmail.com, ORCID: 0000-0002-8334-2350*

Abstract

Trace level determination and quantification of potentially genotoxic impurities (PGIs) in drug substances is a challenging task. Formaldehyde is a known PGI and analysis of it is very taxing due to its volatility, low molecular weight, high polarity and absence of any chromophore. The present study demonstrates development and validation of an HPLC-UV method for quantification of formaldehyde in Levofloxacin hemihydrate. The LOD and LOQ achieved are 0.01 and 0.03 % w/w respectively. The calibration curve was found to be linear over a range of 0.03% to 0.15%. The validated method is precise, sensitive, accurate and has been successfully utilized to ascertain formaldehyde content in scale up batches of bulk drug.

Key words: Genotoxic impurity, HPLC-UV, derivatization, active pharmaceutical ingredient

1. INTRODUCTION

Levofloxacin is one of the most promising fluoroquinolone antimicrobials¹ that have excellent activity against several bacterial strains like gram positive aerobic pathogens responsible for atypical pneumonia². Levofloxacin is chemically known as [9-fluoro-2,3-dihydro-3-methyl-10-

(4-methyl-1-piperazinyl-7-oxo-7Hpyrido[1,2,3-de]-1,4-benzoxazine)-6-carboxylic acid] (**Figure 1**) and demonstrates antibacterial activity via antagonism of the interaction between bacterial DNA gyrase and cell DNA. Levofloxacin has high tissue distribution making it effective in treatment of bronchoalveolar (BAL) and bone diseases³.

Figure 1. Chemical Structure of Levofloxacin Hemihydrate

Formaldehyde is utilized as an important entity in the synthesis of many pharmaceuticals and specialized chemicals⁴. It is highly reactive, volatile, toxic and exposure to it is a significant concern for human body. It has been classified as 'known human carcinogen' by the US National Toxicology Program since 2011⁵⁻⁶.

Analytical monitoring of potentially genotoxic impurities in drug substances is nowadays greatly emphasized by regulatory bodies. The International Conference on Harmonization (ICH) and The European Medicines Agency has dedicated guidelines for identification and quantification of impurities in new drug substances⁷⁻⁸. In 2014, these bodies also has formulated M7 guidelines for identification, categorization, qualification and control of PGIs in pharmaceuticals⁹⁻¹⁰.

Due to increased review by regulatory agencies with respect to the potential hazards, there has been an increase in number of analytical methods for determination of PGIs in drug molecules. Based on the maximum daily dose of Levofloxacin Hemihydrate, formaldehyde needs to be controlled at a limit of 0.1%. In the present study, considering the overview facts, a sensitive HPLC - UV method was developed and validated by utilizing derivatization technique.

2. MATERIALS AND METHODS

Materials

Formaldehyde was purchased from S D Fine Chem Ltd, Mumbai. The solvents acetonitrile, methanol and ortho phosphoric acid were purchased from Merck, India. 2,4-Dinitrophenyl Hydrazine AR was purchased from Molychem, Mumbai, India. Water used during this study was from Milli Q system.

Instrumentation

The analysis was carried out using Waters Alliance HPLC system (e2695 separating module) (Waters Co., Milford, MA, USA) with a Ultraviolet - Visible detector (Waters 2489) with an auto sampler and column heater. Data were collected and processed using EmpowerTM software (Version 3) from Waters.

Chromatographic Conditions

The method for formaldehyde was developed, validated and applied to study the estimation of formaldehyde in Levofloxacin Hemihydrate. The mobile phase was filtered through 0.45 μ filter (Millipore) and degassed using sonicator. Formaldehyde was analyzed using Inertsil ODS 3V (250mm \times 4.6mm, 5.0 μ m) column set at 25°C with mixture Solution-A, Solution-B and orthophosphoric acid (60:40:0.1 v/v) as the mobile phase in isocratic mode. Solution -A is water and Solution-B is pre-mixed solution of acetonitrile and methanol in 1:1 ratio. A flow rate of 1.0 mL/min with an injection volume of 10 μ L and an absorption wavelength of 355 nm were used. The run time was 30 minutes and the retention time was 18.3 minutes for formaldehyde.

Preparation of derivatizing solution, standards stock, working standard and sample solutions

1 mg/mL derivatizing solution of 2,4-Dinitrophenyl Hydrazine (DNPH) was prepared by using acetonitrile. Diluent was prepared by using mixture of derivatizing solution of 2,4-Dinitrophenyl Hydrazine (DNPH) and water in ratio 5:95 v/v. Formaldehyde standard stock solution of 0.0675 mg/mL concentration was prepared in water. Working standard solution was prepared by diluting 1.0 mL of Formaldehyde standard stock solution to 100 mL volumetric flask containing 10 mL of 1% solution of ortho phosphoric acid, made to the volume by using diluent. The solution was allowed to stand for 4 hours at room temperature before injection.

Sample solution was prepared by dissolving approximately 25 mg of Levofloxacin Hemihydrate in 10 mL of 1% solution of ortho phosphoric acid and made up to the volume of 100 mL by using diluent as derivatizing solution. The solution was allowed to stand for 4 hours at room temperature before injection. Blank solution was prepared by transferring 10 mL of 1% solution of ortho phosphoric acid into 100 mL volumetric flask and made up with volume by using diluent.

3. RESULTS

Method development and optimization

Based on maximum daily dose of selected drug substance, formaldehyde is required to be controlled to a limit of 0.1 % since the drug substance is administered orally. Formaldehyde is volatile in nature and does not have any chromophore and therefore chromatographic method development was initiated by using gas chromatograph (GC) with FID detector. During development, it was found that GC method is quite sensitive to detect the formaldehyde peak after derivatization, however the analysis becomes difficult in Levofloxacin Hemihydrate drug substance due to its instant crystallization in organic solvents. Eventually, High Performance Liquid Chromatography (HPLC) technique was tried for the determination and estimation of formaldehyde content in drug substance. But due to the absence of chromophores in formaldehyde, it could not be detected by using UV detector in HPLC. Hence, a derivatizing agent, namely 2,4-Dinitrophenyl hydrazine, was selected to introduce UV chromophores into a carbonyl group of formaldehyde. Formaldehyde and 2,4-Dinitrophenyl hydrazine form an instant reaction at 1:1 mole ratio and immediately form formaldehyde-2,4-dinitrophenyl hydrazone derivative at room temperature (**Figure 2**), which is UV active and shows UV maximum at a wavelength of 355 nm. Based on peak response obtained for formaldehyde-2,4-Dinitrophenyl hydrazone complex, content of formaldehyde was estimated. The retention time for the Formaldehyde derivative was observed at 18.4 minute. The objective of this work was to develop an analytical method, which could not only separate formaldehyde complex peak from drug substances and its related components but also could quantify at least less than 30% to the specification limit of formaldehyde which is 0.1%. Several development trials were carried out by using water and acetonitrile as mobile phase and using ODS column but poor peak shape was observed. In some cases there were interference from sample matrix observed at the peak of Formaldehyde. Desired resolution and sensitivity was achieved by using 5 μ m particle size, Inertsil “ODS-3V” HPLC column of 250 mm length, 4.6 mm internal diameter having 17% of carbon load. Elution mode was selected as isocratic with Solution-A and Solution-B, 1:1 (v/v) as mobile phase. Impurity was monitored by using UV detector as shown in **Figure 3** for chromatograms of standard solution, Sample as such and sample spiked with formaldehyde. Three batches of Levofloxacin Hemihydrate were analyzed for Formaldehyde content and it was not detected in all the three batches.

Figure 2. Reaction scheme of Formaldehyde derivative formation

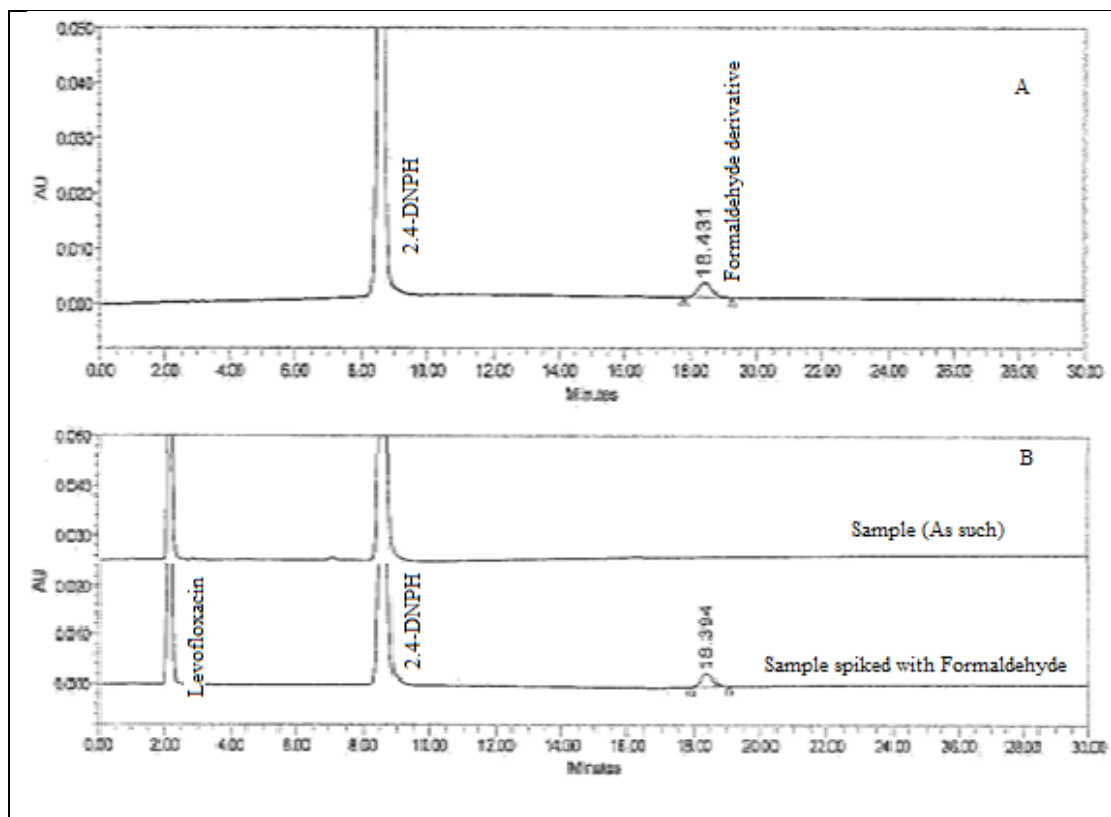


Figure 3. Representative chromatogram (A) formaldehyde-2,4-Dinitrophenyl hydrazone (derivative), (B) Sample chromatogram overlay As such and spiked with Formaldehyde.

Method validation

The method was validated according to validation of analytical procedures provided in the ICH guidelines¹¹⁻¹² and the results are presented in **Table 1**.

Specificity

Specificity was used to test the ability of the method to eliminate the effects of all interfering substances on Formaldehyde derivative peak, specifically by comparing the chromatograms to the blank samples. The validated method showed that there is no interference observed at the peak of interest.

Linearity and range

A linear relationship was observed between the area response for Formaldehyde derivative and corresponding concentrations. The calibration curves exhibit a linearity over a range of 0.03% to 0.15% with respect to sample concentration for formaldehyde with regression coefficient value greater than 0.9800. The method provided a good correlation between the area response and component concentration.

Sensitivity

The LOD was evaluated by determining the minimum levels of concentration of Formaldehyde derivative that could be detected using this analytical method. The LOQ was studied by estimating the minimum concentration that could be quantified with acceptable accuracy and precision. The precision study was also carried out at LOQ level by injecting six preparations of all three impurities. LOD, LOQ and Percentage RSD for the area response of each impurity at LOQ level for Formaldehyde derivative are shown in **Table 1**.

Precision

The precision for the said Formaldehyde derivative was evaluated by injecting six individual Levofloxacin Hemihydrate samples spiked with 0.1% of Formaldehyde. Percentage relative standard deviation for actual recovered amount was calculated and were within 15% confirming the good precision of the developed analytical method.

Accuracy

Accuracy was studied by analyzing three replicates at four different concentration levels: at LOQ, 80%, 100% and 150% (Table 1). The observed accuracy values were within a range of 87.5% to 97.1% for Formaldehyde derivative. This indicates high accuracy of new method developed.

Table 1: Method validation summary

Parameter	Formaldehyde derivative
System Suitability, RT min	18.4
Linearity	0.9880

Correlation coefficient (r)	
Detection limit, LOD (%)	0.01
Quantitation limit, LOQ (%)	0.03
Precision %RSD. LOQ, (n = 6)	4.93
Method Precision %RSD. (n = 6)	0.5
Accuracy at LOQ level (n = 3), % Avg Recovery	90.3
Accuracy at 80% level (n = 3), % Avg Recovery	89.5
Accuracy at 100% level (n = 3), % Avg Recovery	97.1
Accuracy at 150% level (n = 3), % Avg Recovery	87.5
Sample-1	ND
Sample-2	ND
Sample-3	ND

4.CONCLUSION

An isocratic reversed phase - HPLC method was successfully developed for the estimation of Formaldehyde in Levofloxacin Hemihydrate drug substance. The method developed is simple for the purpose of quantification for the current specified limit 0.1%. The method validation results proved that the method is specific, linear, precise and accurate and can be applied for estimation of Formaldehyde in Levofloxacin Hemihydrate for monitoring drug safety. Also, this method can be easily adopted for the determination at trace level i.e., less than 1 ppm level

considering formaldehyde as known genotoxic by using appropriate sample preparation technique based on solubility of individual molecule

Acknowledgement

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PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

SYNTHESIS OF COUMARINE DERIVATIVES CATALYZED BY Ni- SUBSTITUTED HPA

Amit R. Supale¹ and Sandip R. Sabale²*1 Department of Chemistry, Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya, Sangli, M.S., India, 416416**2 Department of Chemistry, Jaysingpur College, Jaysingpur 416101
Email: amitsupale@gmail.com*

Abstract

A methodology for synthesis of coumarin derivatives by using Ni-MPA catalyst is reported. The reaction was carried out in ethanol under reflux condition. The method gave good yields of products in short reaction time compared with previous methods. This methodology offers significant improvements for the synthesis of coumarin derivatives.

Keywords: Heteropoly acid, Pechmann condensation, Coumarin derivatives, One pot reaction.

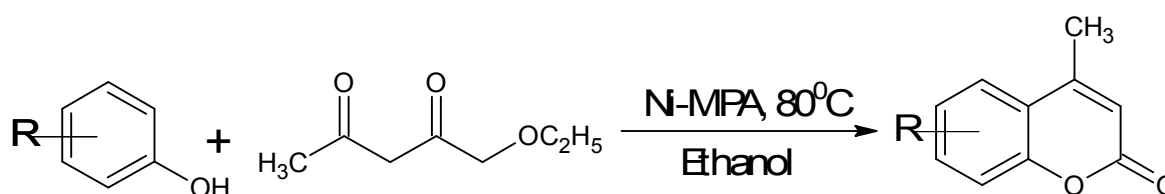
1. Introduction:

Coumarin derivatives have wide range of applications in medical science, biomedical research, and many industrial branches [1]. They are useful because of their biological activities such as antibacterial, antimicrobial, anticoagulant, anti-inflammatory, anticancer, anticonvulsant, antioxidant, antifungal, and anti-HIV [2]. Apart from this they are also used as optical brighteners, photosensitizers, fluorescent and laser dyes, and additives in food, perfumes, cosmetics, and pharmaceuticals [3]. These are important because of their use as intermediates in various drug synthesis [4]. By considering above utilities numerous efforts have been taken to develop novel methods to synthesize coumarin derivatives. Several methods are available for one-pot synthesis of coumarin derivatives, including Pechmann condensation, Knoevenagel condensation, Baylis-Hillman reaction, Michael addition, Kostaneck reaction and Heck-lactonization

reaction [2]. Pechmann reaction is most widely used [5], which involves the condensation between phenols and β -keto esters, in the presence of an acid catalyst. Various homogeneous and heterogeneous catalysts such as H_2SO_4 , TFA, various Lewis acids, cation-exchange resins, zeolites and many other have been successfully utilized for their synthesis [6]. Still majority of methods suffer one or more drawbacks such as long reaction time, poor yield, catalyst recyclability. Hence still there is need to develop suitable catalyst which can overcome above drawbacks.

Heteropoly acids are very important due to their different physicochemical properties. They have been widely used as homogeneous and heterogeneous catalysts [7] in different organic transformation reactions. They have proved to be very good acid and redox catalysts [8]. Heteropoly acids possess catalytic property because of special arrangement of anionic units and the presence of different types of metals. Depending on the arrangement of ions they have different types such as Keggin, Dawson, Waugh, Anderson type and many. The keggin type has been used mostly [9]. There has been lot of reports indicating use of molybdophosphoric acid as catalyst in acid catalysed organic transformations. It has high Bronsted acidity and possess high thermal stability. It has so many advantages over regular solid acid catalysts.

In continuation of our work [10] on utilization of heteropoly acids in catalytic transformation, we have modified the molybdophosphoric acid using Ni as substituent. The synthesised Ni-MPA is tested for its catalytic activity for synthesis of coumarin derivatives. The reaction is carried out at room temperature in ethanol as shown in scheme 1.



Scheme 1: Synthesis of coumarin derivatives

2. Experimental section:

Preparation of Catalyst: 5 gm of Molybdophosphoric acid is mixed with 1.1 gm of nickel nitrate in aqueous medium. The mixture was stirred for 2 hours. Excess water was removed on water bath. Resultant compound is dried in oven at 100°C for about 12 hrs. After this compound was transferred in silica crucible and dried in Furness at a 300° C for about 3 hrs. This catalyst is denoted by Ni-MPA which is used for carrying out organic transformation.

General Procedure for Synthesis of Coumarin Derivatives:

In 25 ml round bottom flask phenol (1 mmol) and ethyl acetoacetate (1 mmol) were taken. 10 mol% of Ni-MPA was added as catalyst. 2.5 ml of ethanol as a solvent was added to it. The

reaction mixture was refluxed in oil bath at 80⁰ C. After regular interval of time the TLC was performed. After completion of the reaction the mixture was extracted with ether. Organic layer was dried on anhydrous magnesium sulphate. Solvent was evaporated to get required product. The product was recrystallized, and M. P. was recorded.

3. Result and discussion:

To study the catalytic activity of Ni-MPA, we have chosen phenol as a model substrate and reaction was carried out by simply mixing it with ethyl acetoacetate in ethanol. The corresponding Coumarin derivative was obtained in high yield (80%). The effect of catalyst concentration was also tested. Various percentage of catalyst loading was used. It has been observed that 10 mol% of catalyst Ni-MPA is enough to carry out the reaction. At higher percentage of catalyst concentration, the reaction does not show improvement in yields of Coumarin derivatives. The results are summarized in **Table 1**.

Table 1 Effect of catalyst concentration

Sr. No.	Catalyst (mol%)	Time (h)	Yield (%) ^b
1	1	8	58
2	2	8	60
3	5	2.5	75
4	10	2	80

^b – isolated yield

The reaction is tested by using substituted phenols. The reaction does yield product for p-nitro phenol. The results are summarized in Table 2.

Table 2 Synthesis of coumarin derivatives:

Entry	Phenol	Time (h)	M. P. °C [8]	Yield (%) ^b
1a	Phenol	2	85	80
1b	Resorcinol	1.5	187	85
1c	p-nitrophenol	10	--	0
1d	a-Naphthol	2.2	155	55

^b - isolated yield.

4. Conclusions:

The reported method is simple and efficient for synthesis of coumarin derivatives. The reaction gave high to moderate yield of products after simple workup procedure. The reaction is also completed in moderate time in comparison with reported methods. Thus, the methodology is economically viable.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

EFFECT OF PLANT EXTRACTS (MURRAYA KOENIGII, EUCALYPTUS, AND RICINUS COMMUNIS) AGAINST THE RED FLOUR BEETLE (TRIBOLIUM CASTANEUM HERBST)

*Nalesh Bahiram¹, *Shantaram Bhoje², Ashutosh Nirbhavane³

1. Department of Zoology. Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya Sangli.

E.mail id: naleshbahiram22@gmail.com

2. Department of Zoology. Shri Pundlik Maharaj Mahavidyalaya, Nandura Rly., Dist. Buldana.

E.mail id: shantarambhoje8@gmail.com

3. School of Architecture and Science Technology, Yashwantrao Chavan Maharashtra Open University, Nashik.

Email id: ashunirbhavane@gmail.com

Abstract

Stored grains, cereals are important resources of the food. The effective conservation of this resource is very important of subsistence of mankind. But the storage grain pest and some other insects are destroying stored food or other valuable organic matter. That damage is mostly influenced by *T. castaneum* insect. In this study we are using some plant extract which is effective against the stored grains pests. First, we identified the plant which produces antibacterial secondary metabolites. The plant species are *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri), and *Ricinus communis* (Castor), for the extraction we use the dry leaves. The plant extract is diluted with ethanol at various concentrations and used in experiment. Some plants are very effectively work against the pest and some show intermediate effect. The all process is without any harmful chemical and very effective. It can be handled easily by any person. No toxic effect on human health and no side effect and economically also low value.

Keywords: Stored grains, Secondary metabolites, Pest, *T. castaneum*.

1. Introduction:

Store grain pest:

The store grain pest is an insect that destroys stored food or other stored valuable organic matter (i.e., Grains & Pulses). The wheat grains are mostly damaged by *S. oryzae*, *R. dominica*, *T. granarium*, *T. castaneum* and *S. cerealella* in storage (Mookherjee et al., 1968). Stored grains, cereals & pulses are important sources of the organic matter, therefore effective conservation of this resource is very important for life. Maize, Rice, and Wheat are most consumed grains, while, chickpea supplement world food demands (Wondatir et al., 2015). There are two types of pest: Primary pest and Secondary Pest.

Primary pest

The Primary pests include the lesser grain borer, Granary weevil and Rice weevil. (Manual on the Prevention of Post-harvest Grain Losses (GTZ). Lesser grain borer (*Rhyzopertha dominica*). The lesser grain borer has a dark colored and cylindrical in shape with the hidden head. This species is known to damage stored, corn and wheat cereal crops with the seeds become hollowed out husks. However, large infestations require more control, including complete fumigation (Pai, A. January 2010).

Secondary Pests

Secondary grain insects feed on broken grain and any powder products left as a result of the broken grain. The pests belong to genus *Tribolium*, beetle species and moth species. ("Pest Web / Lesser Grain Borer").

Model Organisms:

Rust-red flour beetle (*Tribolium castaneum*).

Morphology:

The Rust-red flour beetle is a red-brown beetle; it is cosmopolitan in distribution with an exoskeleton that darkens in color. They can produce up to 1000 eggs and lay them inside the damaged grain with parts of the larvae able to use the damaged grains and cereal as their food source. The beetle measures 1/8 of an inch in length. The adults live for more than three years (Walter, 1990). Setiferous patch present on the male posterior side of the fore femur, while females have no such setiferous patch. Female lays 2-10 eggs each day throughout most of her adult life. Under optimal conditions of temperature (35°C) and relative humidity (75%), egg-laying can increase at a rate of 70-100 times a month (Herrman, 1998). They hatch within 5 to 12 days. Beeman et al. (2012) reported that the duration of egg ranged from 3 days at 30°C and 2 days at 34°C.

Habit & Habitat:

Generally, beetles cannot be found only inside grain products, but also found in cracks and crevices where grain may have spilled. Pests are attracted towards grain with high moisture content and can cause a grey tint to the grain they are infesting. The red flour beetle attacks stored grain products such as flour, cereals, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Weston and Rattlingourd, 2000).

2. Materials and Methods:

The study was conducted in laboratory condition maintaining temperature and humidity of $29 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ respectively, in order to assemble valid and reliable data for achieving a meaningful result and conclusion.

1) Rearing and culture of insect:

Population of *T. castenum* was cultured in a controlled environmental room at 29°C and 75% R.H under continued dark photoperiod. The food media was broken wheat grain (Padin et al., 2013). They were obtained from the laboratory stock culture. The *S. oryzae* lesser grain borer was reared on uninfected whole rice (Ashouri et al., 2010). One hundred *T. castenum* adult of mixed sex were put in 500ml glass jars containing 200g of wheat grains (Daniel et al., 2013). The jars were covered with muslin cloth held in place with rubber band for ventilation. The jars were kept in the laboratory maintaining temperature at $29 \pm 2^\circ\text{C}$ and relative humidity at $70 \pm 5\%$ respectively, for two months to multiply insect number. To ensure continuous supply of adequate adults, the rearing procedure was repeated with different batches of insect. After 48h, the adults and subsequently these adults were used for the experiments.

2) Preparation of powder:

The fresh upper leaves of *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri), and *Ricinus communis* (Castor) were dried in the shade at room temperature for 7 days and finally at 45°C in a hot air oven for 48h before grinding into powder by grinding machine (Padin et al., 2013). The powders were carefully placed inside air tight containers and kept until the experiments (Ashouri, 2010).

3) Extraction by Soxhlet apparatus:

For the extraction, crude extracts of the following dried plants leaves were used: *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri) and *Ricinus communis* (Castor). These leaves were ground in an electric grinder to obtain a powder. Maceration was performed in solvents of different polarity (Ethanol) for 72h with the aim of extracting different plant components. The extraction of each plant sample was done in about 12h. Soxhlet Extraction Apparatus was used to extract each plant leaves compounds by dipping 50g of powder in 250ml ethanol according to the procedure described (Valladares et al., 1997). Each plant leaves were done by placing flasks in the Soxhlet Apparatus. Each plant leaves were poured into a filter tube made from filter paper on one end of the cap. A flask which contained 500ml of ethanol was placed under this glass

tube 3 times for 24h per batch. After 3 days, the solvent was evaporated by heat lamp. The extracts were stored at 4°C prior to application.

3. Results:

A. Mortality effect of dry plant powder on insects:

Table 1.1: *Murraya koenigii*

Sr.No.	Weight of dry plant powder	No. of <i>T. Castenum</i> exposed	No. of <i>T. Castenum</i> dead after some hours			Total percentage of morality
			24 h	48 h	72 h	
1)	Control	10	-	-	-	00%
2)	0.5gm	10	-	0	01	10%
3)	1 gm	10	-	01	02	20%
4)	2.5gm	10	-	01	03	30%
5)	5 gm	10	02	03	04	40%

Table 1.2: *Eucalyptus*

Sr.No.	Weight of dry plant powder	No. T. <i>Castenum</i> exposed	No. T. <i>Castenum</i> dead after some hours			Total percentage of morality
			24 h	48 h	72 h	
1)	Control	10	-	-	-	00%
2)	0.5gm	10	01	02	03	40%
3)	1 gm	10	01	03	04	40%
4)	2.5gm	10	02	03	04	40%
5)	5 gm	10	03	05	06	60%

Table 1.3: *Ricinus communis*

Sr. No.	Weight of dry plant powder	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> dead after some hours			Total percentage of morality
			24 h	48 h	72 h	
1)	Control	10	-	-	-	00%
2)	0.5gm	10	01	02	03	30%
3)	1 gm	10	01	03	05	50%
4)	2.5gm	10	04	05	06	60%
5)	5 gm	10	05	06	07	70%

B. Mortality effect of plant extracts on insects:**Table 1.4: *Murraya koenigii***

Sr. No.	Conc. of plant extracts in percentage	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> dead after some days			Total percentage of morality
			1 day	2 day	3 day	
1)	Control	10	-	-	-	00%
2)	1%	10	01	03	04	40%
3)	2%	10	00	02	05	50%
4)	5%	10	00	03	06	60%
5)	10%	10	01	03	07	70%

Table 1.5: *Eucalyptus*

Sr. No.	Conc. of plant extracts in percentage	No. T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> dead after some days			Total percentage of morality
			1 day	2 day	3 day	
1)	Control	10	-	-	-	00%
2)	1%	10	02	04	06	60%
3)	2%	10	01	02	07	70%
4)	5%	10	02	03	06	60%
5)	10%	10	01	06	09	90%

Table 1.6: *Ricinus communis*

Sr. No.	Conc. of plant extracts in percentage	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> dead after some days			Total percentage of morality
			1 day	2 day	3 day	
1)	Control	10	-	-	01	10%
2)	1%	10	-	01	04	40%
3)	2%	10	01	03	04	40%
4)	5%	10	04	06	06	60%
5)	10%	10	06	08	08	80%

C. Repellency effect of dry plant powder on insects**Table 1.7: *Murraya koenigii***

Sr.No.	Weight of dry plant powder	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after somehours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	-	01	01	10%
2)	0.5gm	10	-	01	02	20%
3)	1 gm	10	01	02	03	30%
4)	2.5gm	10	02	03	03	30%
5)	5 gm	10	03	04	04	40%

Table 1.8: *Eucalyptus*

Sr.No.	Weight of dry plant powder	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after some hours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	01	-	01	10%
2)	0.5gm	10	01	03	04	40%
3)	1 gm	10	01	04	05	50%
4)	2.5gm	10	03	06	06	60%
5)	5 gm	10	05	07	08	70%

Table 1.9: *Ricinus communis*

Sr.No.	Weight of dry plant powder	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after some hours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	-	-	-	00%
2)	0.5gm	10	01	02	03	30%
3)	1 gm	10	07	06	05	50%
4)	2.5gm	10	07	08	05	60%
5)	5 gm	10	06	04	08	80%

D. Repellency effect of plant extract on insects:**Table 1.10: *Murraya koenigii***

Sr.No.	Conc. of plant extract percentage	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after some hours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	01	02	-	00%
2)	1%	10	02	03	04	40%
3)	2%	10	02	04	05	50%
4)	5%	10	04	05	06	60%
5)	10%	10	05	06	07	70%

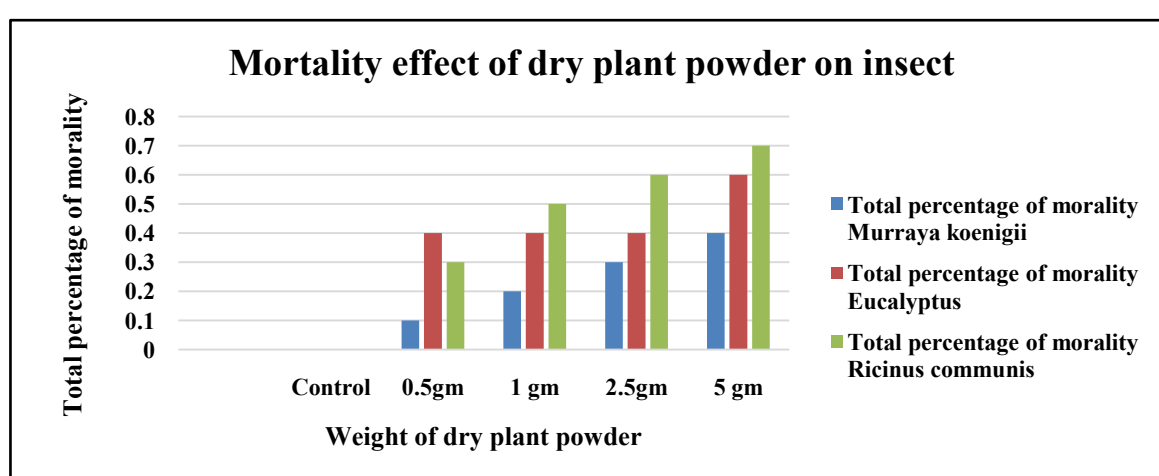
Table 1.11: *Eucalyptus*

Sr.No.	Conc. of plant extract percentage	No. T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after some hours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	-	01	01	10%
2)	1%	10	01	02	03	30%
3)	2%	10	03	02	04	40%
4)	5%	10	03	04	05	50%
5)	10%	10	05	06	07	70%

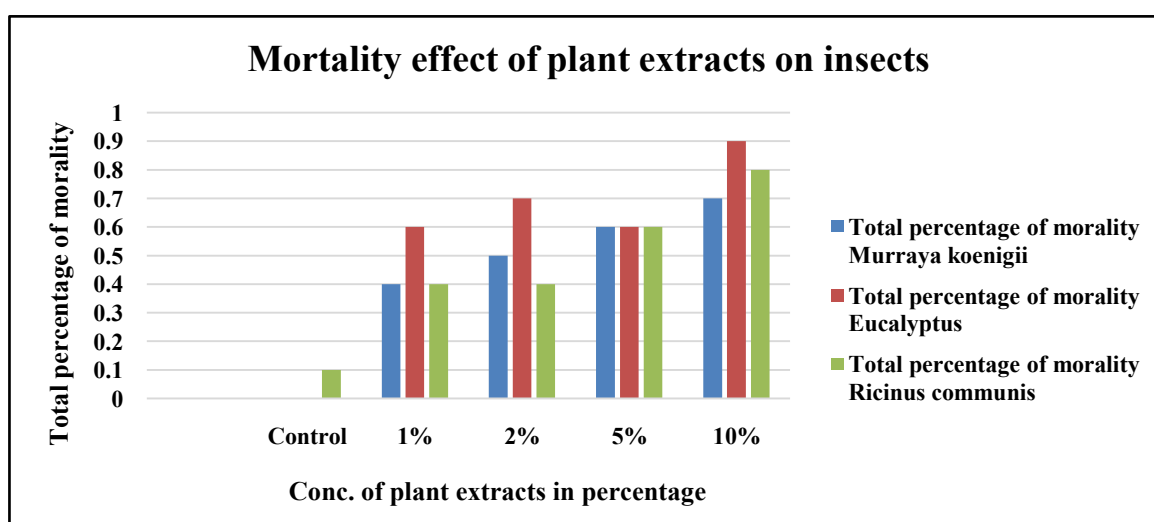
Table 1.12: *Ricinus communis*

Sr.No.	Conc. of plant extract percentage	No. of T. <i>Castenum</i> exposed	No. of T. <i>Castenum</i> repelled after some hours			Total percentage of repellency
			24 h	48 h	72 h	
1)	Control	10	1	-	-	00%
2)	1%	10	03	04	03	30%
3)	2%	10	04	05	05	50%
4)	5%	10	04	07	07	70%
5)	10%	10	06	07	08	80%

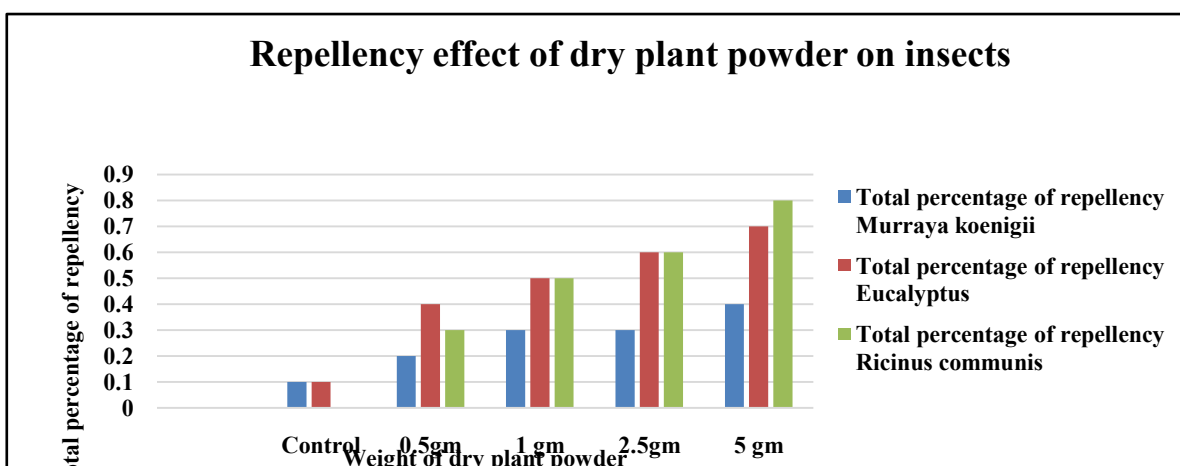
Graph: 1 Mortality effect of dry plant powder on insect:



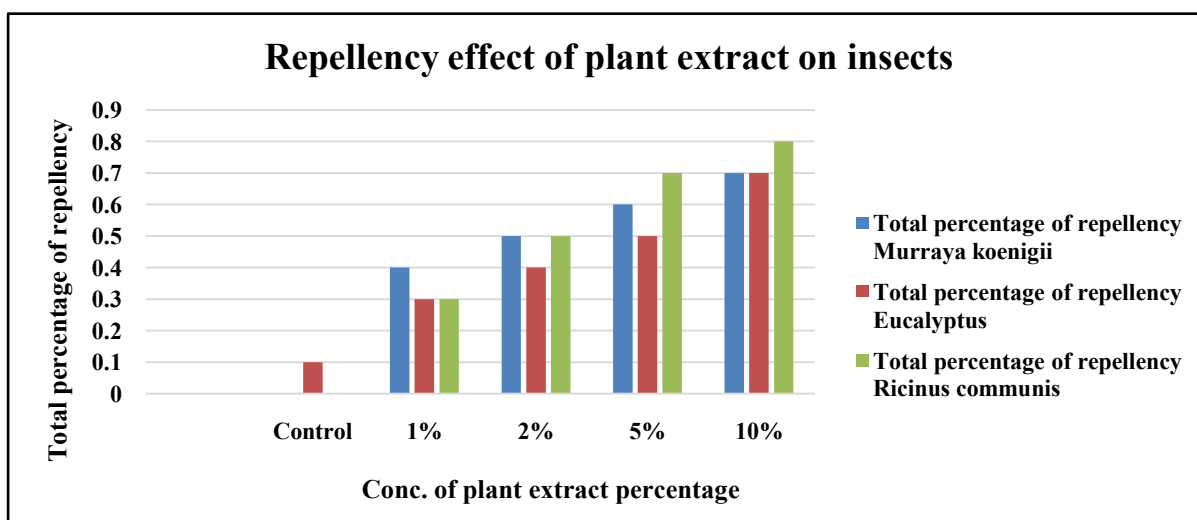
Graph: 2 Mortality effect of plant extracts on insects:



Graph: 3 Repellency effect of dry plant powder on insects:



Graph: 4 Repellency effect of plant extract on insects:



Discussion

Many researchers investigated the compounds in plants that have a variety of properties including insecticidal activity, repellence to pests, insect growth regulation, toxicity to Store grain pest and agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens. Extracts of *M. communis* were highly repellent against beetles as compared to other extracts. (Khan & Marwat 2004) evaluated the leaves, bark and seeds of bakain (*Melia azadarach*) and Ak (*Calotropis procera*) powder against lesser grain borer (*R. dominica*). In Bishkatali plant extracts found both chloroform and ethyl alcohol they show remarkable residual effects on *T. castaneum* by reducing the production of F_1 progeny and/or by increasing the population mortality Moreira et al., (2007).

In the present study the data indicated that: *Murraya koenigii* dry powdered shows significant toxicity against mortality among *Tribolium castenum* the recorded Maximum %mortality was 40% while the minimum toxicity 20% (Table 1). The tested plant extracts showed slight toxic

effect against *T. castaneum*. The most effective mortality was observed in extract of *Ricinus communis*. The maximum mortality was 70% and minimum 30% (Table 1.3) respectively.

Murraya koenigii powder was least effective to cause mortality of *T. castaneum* with Minimum of 40% (Table 1.1). Mortality effect of plant extracts on insect *Murraya koenigii* plant extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded Maximum % mortality was 70% while the minimum toxicity 40% (Table 1.4). *Eucalyptus* plant extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded Maximum % mortality was 90% while the minimum toxicity 60% (Table 1.5).

Ricinus communis extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded Maximum % mortality was 80% while the minimum toxicity 40% (Table 1.6). Repellency effect of dry plant powder on *Murraya koenigii* powder was least effective to cause repellency among *Tribolium castaneum* the recorded Maximum % mortality was 40% while the minimum toxicity 20% (Table 1.7). *Eucalyptus* plant powder was moderate effective to cause repellency among *Tribolium castaneum* the recorded Maximum % mortality was 70% while the minimum toxicity 40% (Table 1.8).

Ricinus communis powder was high effective to cause repellency among *Tribolium castaneum* the recorded Maximum % mortality was 80% while the minimum toxicity 30% (Table 1.9). Repellency effect of plant extract on insects *Murraya koenigii* plant extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded Maximum % mortality was 70% while the minimum toxicity 40% (Table 1.10). Repellency effect of plant extracts on insect *Eucalyptus* plant extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded Maximum % mortality was 70% while the minimum toxicity 30% (Table 1.11). Repellency effect of plant extracts on insect *Ricinus communis* plant extract shows significant toxicity against mortality among *Tribolium castaneum* the recorded maximum % mortality was 80% while the minimum toxicity 30% (Table 1.12).

4. Conclusion

It is concluded that all components of each botanical were found to be effective as repellent against *Tribolium castaneum* Herbst. The repellency of bitter taste was better as compared to other repellents. The pest mortality rate was maximum at 10% concentration after 24 and 48 hours of time interval. The effectiveness of *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri) and *Ricinus communis* (Castor) were also found to be significant. The toxicity of *Ricinus communis* (Castor) was lesser than *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri). Moreover, *Murraya koenigii* (Curry leaves), *Eucalyptus* (Nilgiri) showed more mortality and repellent effect than only *Ricinus communis* (Castor). The results show an evaluated plant extracts in this study may be useful in repellent and toxicant formulations against *T. castaneum*.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

ETHNOBOTANICAL ASPECTS OF KOKANA TRIBE IN KALWAN TEHSIL NASHIK DISTRICT, MAHARASHTRA

Gavit, M.P., Awale V. B., Wangikar H. V.

Department of Botany

Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya Sangli, 416 416

Email: mangeshgavit439@gmail.com

Abstract

The present investigation is an attempt to document ethnobotanical uses of plants by Kokana Tribe in Kalwan tehsil Nashik district, Maharashtra. Kokana tribe is one of the primitive tribe is accustomed with medicinal, edible and other economic uses of the plants. They were used many herbal remedies to cure many diseases. In the present study, documentation of traditional knowledge associated with 25 plant species were documented during survey. The information is based on correct botanical identification with local name, family, part used, disease treated and administration of 25 plant species.

Keywords: (Ethnobotany, Kokana, Bhagat, Kalwan)

1. Introduction

The scientific study of the traditional knowledge & customs of a people concerning, Plants & their medicinal, religious & other uses. In this field, People's are exploring the knowledge of indigenous people. In India various indigenous communities are living in different states, Maharashtra is one of the state in which there are Mahadeo-koli, Katkari, Bhill, Kokana and Warli dominant Scheduled Tribes. Kalwan is one of the tehsil of Nashik district situated on latitude 20.47 and longitude 74.02.









Kokana is one of the dominant Scheduled Tribe of Kalwan, as well as they are also living in the hilly parts of Satana, Surgana, and Peth Talukas of Nashik. Dongarya Dev is family God and language is Kokani. Women wears Padki and Sarees (lugada). Men's wear shirt and pant, older people still wearing Sadara and Langot. Farming carried out by conventional methods; Recently, They were started technical farming. They are celebrating all festivals of Hindu religion as well as Waghbaras, in which they Worship to Tiger. In Dongarya Dev, they worship to Nature. They are also interested in hunting, fishing. Due to the poverty and unavailability of medical facilities, people are preferred to Bhagat. He is a traditional healer. He diagnosed and cures diseases. By

different plant based medicines this knowledge is transferred to descendents only. There is no documentation of this knowledge. So, collection of data and its documentation is very important

The said tribe is distributed sporadically in Nashik district with its own characters, features, association with plants. Hence present investigation was undertaken.

2. Methodology

We have collected this information from physician (Bhagat) PundlikChanduGavit. He gives us information about collection time, used parts, and how to use in his language (kokani). We have translated it into English. The authentication of plants is done by using Flora's, Image comparison and subject experts. In observation table data is arranged as Plants Scientific name, Local name, Family, disease treated, used part and administration of that particular plant part or extract.

			
<i>Geodorum densiflorum</i>	<i>Sterculia villosa</i>	Joining of Hand Fracture by using paste of <i>Sterculia villosa</i> roots	Vaidya Pundlik Gavit showing how to collect <i>Geodorum densiflorum</i>
			
Roots of <i>Plumbago zeylanica</i>	Seed of <i>Butea monosperma</i>	Fruit of <i>Careya arborea</i>	Fruit of <i>Terminalia chebula</i>

3. Results and Discussion

During this study it was observed that the Kokana tribe of Kalwan tehsil used many wild plants as a medicine to cure the various ailments. They were well acquainted with the plants of surrounding forests and knew the different medicinal values of plants.

In this study we have collected medicinal information regarding to 25 plants. Out of 25 medicinal plants used by Kokana tribe, all plants were belongs to Angiosperms. Dicots are represented by 21 species and monocots were represented by 04 species. Among these plants, Root and Tuber of 06 species were used to cure ailments, as well as leaf of 04 species, Stem and Stem bark of 04 species, seeds of 04 species, fruits of 04 species, Latex and Sap of 03 species and Gum of 01 species were utilized.(Table No. 1).

Some medicinal plants are also used as food material such as *Ensetesuperbum*, *Agave sissalana* etc.

Observation Table : 1

Sr. No	Botanical Name	Local Name	Family	Disease are Treated	Plant part used	Administration
1	<i>Solanumvirginianum</i> L.	Kanteringani, Bhuringani	Solanaceae.	Tooth -Ache	Seed	Take Pinch of Seeds (~ 2 gm.) and polish with any edible oil and put on hot iron plate and inhale the smoke in mouth.
2	<i>Buteamonosperma</i> (LAM)Taub	Palas	Leguminosae	Kidney stone	Seed	Small piece of seed (~0.3gm) taken with water per day.
3	<i>Pongamiapinnata</i> (L) Panigrahi	Karja,karaj	Leguminosae	wound	Seed	Seeds crushed and prepare powder (~2gm). Apply it on wound.
4	<i>Cardiospermumhalicacabum</i> Mhnt	Dabbadana	Sapindaceae	Dog bite	Seed	Take mature Seeds (~5 gm), and fried in oil. Crushed and prepare powder. It mixed with curd (~100 gm) and gives to infected

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5	<i>Curculigoorchioides</i> Gaertn	Kidmari	Hypoxidaceae		wound of animals	Root	Roots (~5 gm) given with 1 Roti.	Dog twice a day.
6	<i>Geodorumdensiflorum</i> (Lam)Schltr	Haryakand	Orchidaceae		Stomach -ache	Tuber	Small piece (~1 gm) of tuber is eaten, whenever stomachache.	
7	<i>Cissampelospareira</i> L	Tanel	Menispermaceae		Stomach -ache & dysentery	Root	Small piece (~1 cm) of root is eaten.	
8	<i>Plumbagozeylanica</i> L	Chitrak	Plumbaginaceae		Arthritis	Root	Prepare root paste (~5 gm) and apply it in cut made on both upper and lower side of knee	
9	<i>Hemidesmusindicus</i> (L.) R.Br	Kumsadi	Apocynaceae		Common cold	Root	Small part (~2 gm) of root is eaten.	
10	<i>Sterculiavillosa</i> Roxb. Ex Sm.	Udala	Malvaceae		Bone Fracture	Root	100gm Root paste is wrapped around fracture and bind with Bamboo strips.	
11	<i>Terminaliaarjuna</i> (Roxb.)Wight	Arjunsadada	Combretaceae		body pain	Stem Bark	Bark (~1 kg) is boiled in water and this water used for bath.	
12	<i>Moringaoleifera</i> Lam	Shevaga	Moringaceae		Arthritis	Stem Bark	Bark (~1 kg) is wrapped around the leg for two hours.	
13	<i>Sennatoria</i> (L.) Roxb	Tarota.	Leguminosae		Migraine	Stem	Stem cut into bits. Earring prepared from seven bits and wears it in ear of affected side.	
14	<i>Holopteleaintegrifolia</i> Planch.	Papada.	Ulmaceae		Tooth -ache	Stem bark	Bark (~5 gm) boiled in edible oil; cool it and two to three drops apply in ear.	
15	<i>Cannabis sativa</i> L	Ganja.	Cannabaceae		Appetite	Leaf	Half teaspoon dry leaves powder (~2gm) mix in one teaspoon honey and eats.	

		Rui	Apocynaceae	body pain	Leaf	
16	<i>Calotropis gigantea</i> (L) Dryand		Apocynaceae	body pain	Leaf	2-3 Leaves warmed and apply on affected parts.
17	<i>Agave sisalana</i> Perrine	Kekat, Ghaypat	Asparagaceae	Cough	Leaf	Tender leaves warmed and extract Juice (~5 ml). Mixed it in milk (100 ml) or water (100ml) and drink
18	<i>Xanthium strumarium</i> L	Landaga, Zalmani	Compositae	skin disease	Leaf	Take fresh Leaves (10gm) and extract juice. It applied on infected parts till disease cured
19	<i>Terminalia chebula</i> Retz	Hirda, Balhirda	Combretaceae	Tooth -ache	Fruit	Coal powder of fruits (~2gm) is used to tooth wash.
20	<i>Careya arborea</i> Roxb	Kumbhi	Lecythidaceae	Indigestion	Fruit	Dry Pulp powder (~5gm) is given with water (100ml).
21	<i>Helicteres isora</i> L	Murudsheng	Malvaceae	Stomach -ache	Fruit	Dry fruits (~5 pods) kept in water (100ml) to 1-2 hours. Water is drink after filter.
22	<i>Baliospermum montanum</i>	Datara.	Euphorbiaceae.	Skin diseases	Latex	Latex applied on infected body parts.
23	<i>Ensete superbum</i> Roxb	Kavadar, Rankel	Musaceae.	Fever	sap	Take ~5ml sap and mixed in water (100ml) and drink
24	<i>Argemone mexicana</i> L	Pivaladhotar ^a	Papaveraceae	Mouth ulcer	Latex	Take latex (0.5ml) and applied to affected parts.
25	<i>Pterocarpus marsupium</i> Roxb	Bivala	Leguminosae.	Cough	Gum	Gum (1gm) kept in mouth and chewed

Conclusion

Tribal people through their traditional knowledge were well known about the medicinal plants and their uses. They clearly know that when and which part of plant used to treat a particular disease.

They were collect all these plants from nature, in this list some plants are on high risk Status of IUCN, so there scope to develop different propagation techniques and domesticate such medicinal plants for further use.

As well as there is need to chemical analysis of that particular plant and standardization to use against particular disease.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

STUDY OF PISCINE DIVERSITY OF KRISHNA RIVER DISTRICT SHAMLI, UTTAR PRADESH (INDIA)

NeerajMalik¹, Praveen Kumar Singh²

¹Assistant Professor Department of zoology S.M. college, Chandausi, Dist-Sambhal[U.P] PIN CODE 244412

²Assistant Professor 2Department of zoology D.N. College Meerut
E-mail-neeraj.malik1979@gmail.com

ABSTRACT

The studies of fish fauna of different water bodies were made by different worker however the study of ichthyofauna of the Krishna river District Shamli, Uttar Pradesh is scanty. The study area (Stretch) is 35km in District Shamli in three different site of river Krishna.

The study revealed the presence of 12 species of fish belong 5 order in (Cypriniformes, Perciformes, Siluriformes, Synbranchiformes and Osteoglossiformes) These fish belong into 6 families which are Cyprinidae, Channidae, Siluridae, Bagridae, Mastacembelidae, Notopteridae. Cyprinidae family is dominant over other families. The paper describes the detailed species composition their relative contribution and also some important point that may help to better understand the current scenario of piscine diversity in Krishna river District Shamli, Uttar Pradesh (India)

Key word- (Krishna river, Diversity, Cyprinidae)

1. Introduction: -

Uttar Pradesh located in the northern part of India cover on area of 243,268 km with a population of 199,581,000 people (around 200 million, 2011 census). As such, it is the most populous state of India as well as the most populous sub-national entity of the world. In fact, a nation in its own right, Uttar Pradesh would be the fifth most populous country of the world.

Ichthyofaunal diversity refers to variety of fish species: dependent on context and scale, it could refer to alleles or genotype within fish population to species of life forms within a fish community and to species of life forms across aqua regimes [4]

Biodiversity indicates the potential of any aquatic system and also depicts its trophic status.

Fish constitute half of the total number of vertebrates in the world and live in almost all conceivable aquatic habitats. Around the world approximately 22,000 species of fishes have been recorded out of which 11% are found in India.

i.e., about 2,500 species of fishes of which 930 live in fresh water and 1,570 are marine [9,19]

India is one of the mega biodiversity countries in the world and occupies the ninth position in terms of fresh water mega biodiversity [16]

Studies on taxonomy (Ichthyofaunal diversity) have been of immense interest to researchers of all times [1,2,5]

However, there are still a large number of habitats/regions for which the ichthyofaunal diversity is still to be reported. Moreover, such habitats are being exploited for various resources and also, they experience the natural climate change that is bound to impact its faunal diversity and abundance. One such habitat, that lacks any published scientific information on ichthyofaunal diversity is the Krishna river district Shamli, Uttar Pradesh. Therefore, this study was undertaken with the objective to explore the ichthyofauna of this region.

1.1 STUDY AREA: -The geographically coordinate of district Shamli is 29°27'0" N 77°19'0"E Shamli is district in India state of Uttar Pradesh. This district was carved out from Muzaffarnagar district on 28 sep-2011 as Prabhudhnagar and rename Shamli in July 2012 Shamli is the head quarter of distt. Shamli is located along the Delhi-Saharanpur (National Highway-709B) Total Area covered- 1,063 km.

It is bounded by the distt of Saharanpur, Muzaffarnagar and Baghpat. It is separate by Yamuna river to Haryana state. Due to changing course of flow of Yamuna river, the adjoining area of district change frequently.

1.2 ABOUT THE KRISHNA RIVER: - Krishna river is a Tributary of Hindan river. Krishna the mightily Indian river originates from the Saharanpur, remains the source of water for millions of Indians. From its source its entry in Hindan. It travels a distance of around 225 km. Shamli is a city in Northern India on the bank of the Krishna river north of Delhi. It is an agriculture pilgrimage center. Shamli lies along the Krishna river at the boundary between the Indo-Gangetic plain.

River Krishna called also Krishniriver. River Krishna ultimately join river Hindanat Barnava (village) DisttBaghpat.

River Krishna run in disttShamli 70 km. This is a study area(35streach).

2. Materials and Methods: - The collection of fish was made from the site with help of local fisherman by fishing net during the period-March-2019 to February 2020.

The collected fishes were preserved in 4% formalize and brought laboratory for identification. The collected fishes were identified and measurement like total length, snout length, head length was taken. Fish identification was done with help of standard taxonomic reference [3,18,14]

3. Results and Discussion: - The total fish species (12) belonging to 5 order in Krishna river District Shamli (Table1). On basis of percentage composition and species richness, order Cypriniformes was dominant (6 species) followed by Perciformes (2 species), SiluformesSynbranchiformes andOsteoglossiformes(One species each) (Fig-1)
Cypriniformes>Perciformes = Siluriformes>Synbran-
chiformes = Osteoglossiformes.

Fish diversity of 6 familiesnamelyCyprinidae (50%) Channidae (17.52%), Siluridae (8.12%), Bagridae (8.12%) Mastacembelidae.(8.12%) Notopteridae (8.12%)(Table-2 fig-2)

Cyprinidae>Channidae>Siluridae>Bagridae= Mastacembelidae= Notopteridae,

Family Cyprinidae was represented by two species of genusLabeo. one speciescirrhinus and catla and Cyprinus, and also Tor- while Family Channidaeshouldtheir presencechanna, and family Siluridae, show by wallago, and Bagridae show their presence one species mystus, and while family - Mastacembelidae show theirpresence by Mastacembelas species and Notopteridae family show their presence by Notopterus species.

In these reported fishes Cyprinidae family was more dominant manyresearches have reported the strong dominanceCyprinidae family in their investigation in ichthyofaunaldiversity.

Cyprinidae family was dominant with 8 species in Ekruchlake Solapur district [15] In the Nira river also Cyprinidae family dominant and have 10 species. [17]

[6] recorded 16 fish species belonging. Five families,In thekallor river. [7] recorded 13 fish speciesbelongin two order (Physostomous and Perciformes) in Bhima River at Pedgaon in Ahmednagar District (Maharashtra).

[8] reported 23 was spices belonging to 07 order where Cyprinidae family was dominant with 11 species from JalgaonreservoirSolapur district (Maharashtra).

[11] recorded 23 fish species belonging to six order in masooli reservoir, District Parbhani (Maharashtra).

Table - 1 Fish fauna in Krishna river District(Shamli)

S.No.	Taxonomic position and scientific Name	Local Name	Frequency
	01. Order Cypriniformes Family Cyprinidae		
1.	<i>Catlacatla</i> (Ham.)	katla	Common
2.	<i>Cirrhinus mrigala</i> (Ham.)	Naini	Common
3.	<i>Cyprinus carpio</i> (limm)	Common carp	Rare
4.	<i>Labeorohita</i> (Ham.)	Rohu	Common
5.	<i>Labeogonius</i> (Ham.)	Kheri	Rare
6.	<i>Tor tor</i> (Ham.)	Mahaser	Rare
	2. Order - Siluriform Family-Siluridae		
7	<i>Wallago attu</i> (Bloch and schneider)	Lanchi	Rare
	3. O - Siluriformes . F - Bagridae		
8	<i>Mystus seengala</i> (sykes)	Malli, Singhara	Rare
	4. O - Perciformes F - Channidae		
9.	<i>Channa punctatus</i> (ham)	sawli	rare
10.	<i>Channa monrullius</i> (ham)	sawl, Guldar	rare
	4 O-Synbranchiformes F- Mastacembelidae		
11	<i>Mastacembela samatrus</i> (Lacepedes)	Bomcha	Rare
	(5) O- Osteoglossiformes F- Notopteridae		
12	<i>Notopterus</i> (pallas)	Patra	Rare

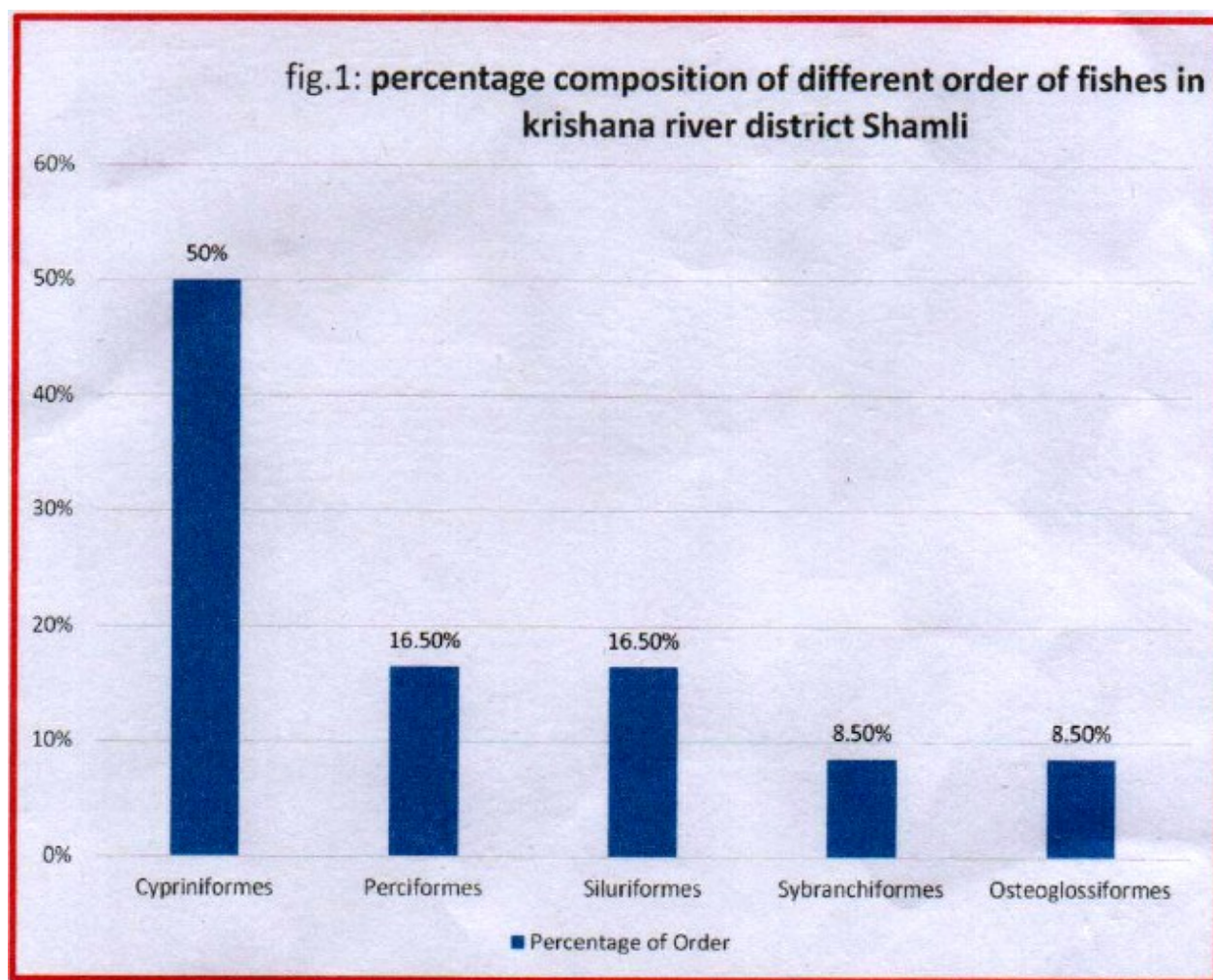
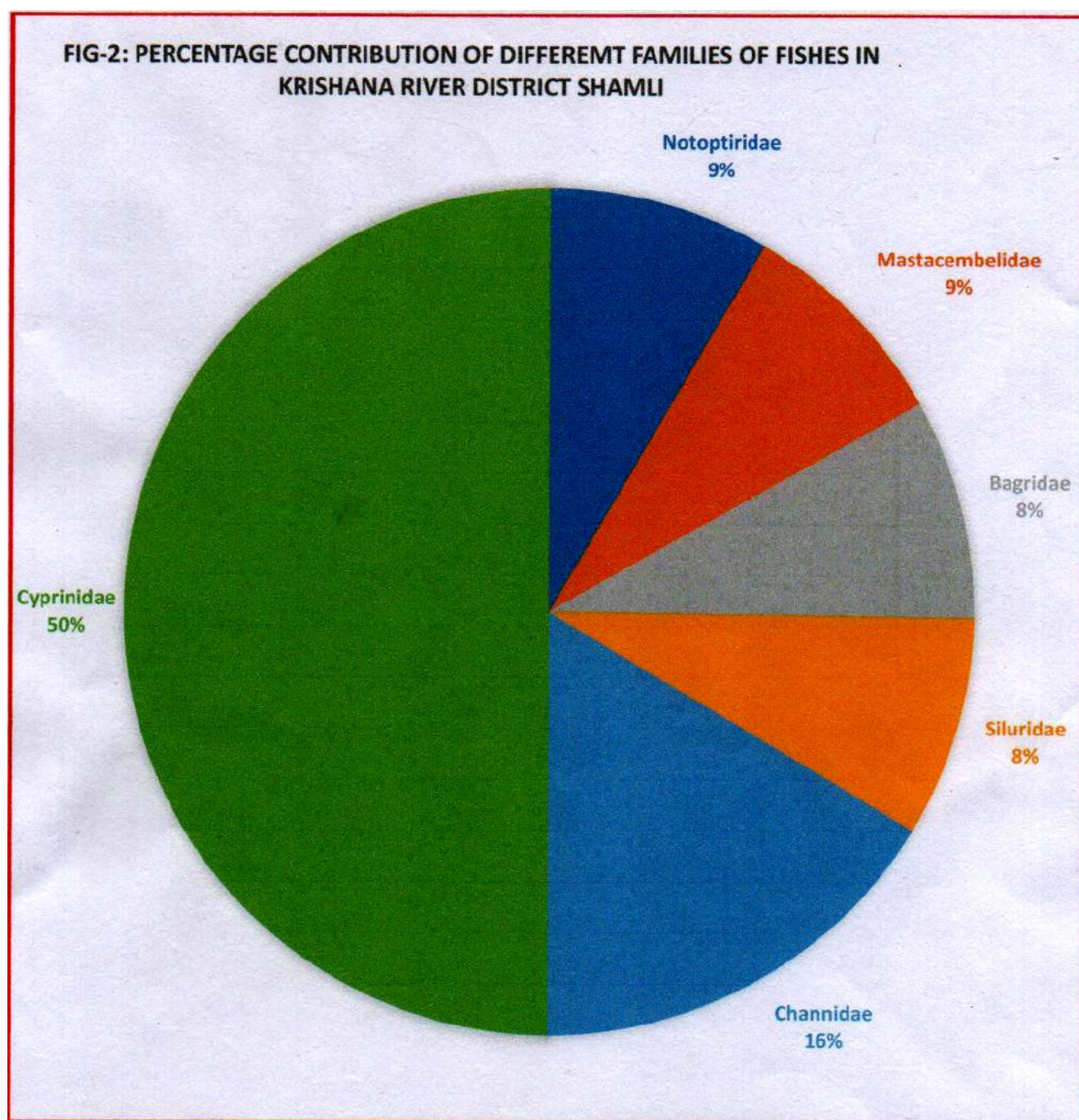


Table 2: - Family wise contribution of the fish Fauna in Krishna River District Shamli.

S.No.	Families	No of Genus	No of species	% Contribution
1	Cyprinidae	05	06	50.00
2	Channidae	01	02	16.40
3	Siluridae	01	01	8.40
4	Bagridae	01	01	8.40
5	Mastacembelidae	01	01	8.40
6	Notopteridae	01	01	8.40
	Total	10	12	100



[10] Cyprinidae family have 15 species in Muzaffarnagar district.

[16] observed 11 species under to genera under Cyprinidae family from Harsul Savangidam in the District-Aurangabad (Maharashtra).

[19] observed that ichthyofauna of Ambadidam belong to 08, order, 11 families, 22 genus, and 27 species where Cyprinidae was dominant in Ambadidam, Taluka, kannad, District Aurangabad (Maharashtra).

[21] ichthyofaunal diversity comprised of 8 families namely Cyprinidae (30%), Siluridae (5%), Bagridae (5%), Heterospneustidae (5%) and Claridae (5%), Channidae (20%), Centropomidae (5%), and Anabantidae (5%). These found in Mawana region of District Meerut.

[22] theichthyofaunal diversity of collected and identified fish species belong to 11 families and 24 genera recorded from the Nandurbar District.

[24] ichthyofaunal diversity in Nakana lake (Distt DHULE (Maharashtra) is 15 species Cyprinidae in dominant with 8 species (53.33%) from over all the reported families.

[25] Ichthyofaunal diversity. 61 fish species belonging to 38 different genera, 19 different families and 9 different order. The order cypriniforms was dominant with 23 species in water resources of western U.P.

[23] In Yamuna river show 63 fish species were present in the river which belonged 8 order, 18 families, 39 genus and 63 species dominate Cyprinidae family which have 40% species.

[10] A total of 36 fish species belonging to 6 order were collected from various water resource of the Bijnor District.

Abundance wise distribution suggests common catlacatla, cirrhinus mrigala, Labeo roheta, other species are in rare categories (Cyprinus carpio, Labeo gonius, tortor, wallago attu, mystus seengala, channa punctatus, channa pamasulis), Mastacembela armatus, Notopterus notopterus, fish are very poor density in Krishna river.

All these fish species not found all over year it found only in July to September because these time, Rannie season and East Yamuna canal are linked in the Krishna river.

The information on the fish diversity and its distribution and habitat is of great importance in study for ecosystem dynamics and planning and implementing sustainable development program and utilization of natural resources.

4. Conclusion: - The aim of the study was identified fish species in the study area. Based on the present study, it may be concluded that in Krishna river fish diversity is very good condition before 15-20 year. Fish diversity in the river but Nowadays is very poor condition because Anthropogeny activities like deforestation, overfishing, sand mining, recreational activities organic and inorganic pollution. It is suggested that more studies should be undertaken to generate the basic biological information on the ichthyofaunal of the region such information may be utilized 1. Develop scientifically sound management strategy by government and non-government organization.

2. Awareness of public and use scientific approach for fishing capture. 3. The use of illegal method to catch fishing should to be banned in this river.

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

STUDY OF PEROXIDASE AND ACID PHOSPHATASE ENZYME ACTIVITY DURING SENESCENCE OF IPOMOEAE CARNEA JACQ.

*Vikas S. Salunkhe¹, Hemant S. Joshi¹, Anuja H. Joshi¹

¹Department of Botany

Bharati Vidyapeeth's

Matoshri Bayabai Shripatrao Kadam Kanya Mahavidyalaya, Kadegaon Email –
drvikassalunkhe@rediffmail.com

Abstract:

The genus *Ipomoea* comprises the largest number of species within the Convolvulaceae. Recently *Ipomoea carnea* Jacq is recognized in two sub species i.e *Ipomoea carnea*, sub sps *carnea* Jacq and *Ipomoea carnea* sub sps *fistulosa* Mart-ex- choicy which are studied for activity of peroxidase and acid phosphatase during senescence. The present communications reports higher values of both enzymes in both the taxa viz., *Ipomoea carnea*, sub sps *carnea* and *Ipomoea carnea* sub sps *fistulosa* (2.12, 2.10; 3.75, 3.62) during senescence. This confirms the role of peroxidase and acid phosphatase in senescence.

Keywords: Peroxidase, acid phosphatase, senescence, *Ipomoea carnea*

1. Introduction

The genus *Ipomoea* comprises the largest number of species within the Convolvulaceae. Throughout the world *Ipomoea* is usually estimated to contain 500 species (Mabberley, 1989; Mc Donald and Mabry, 1992). However Austin

and Huaman (1996) believed that *Ipomoea* is more likely to contain 600-700 species. *Ipomoea carnea* Jacq is one of the major weeds in India.

Ipomoea carnea Jacq from above is characterized by showy and pale rose coloured corolla with long tube. Generally the plant is about 20 feet in height and woody in nature. It is introduced in many gardens of Bombay presidency.

The *Ipomoea carnea* Jacq occurs all over the world but it is a native of South America. It occurs in many states of India. In Maharashtra these species are commonly occurring in all the districts and cultivated as hedge plant and termed as weed. It does not require any special type of climate and soil. It is popular amongs the farmers with local name as “Garvel” or “Besherm”. Recently *Ipomoea carnea* Jacq is recognized in two sub species i.e *Ipomoea carnea*, sub sps *carnea* Jacq and *Ipomoea carnea* sub sps *fistulosa* Mart-ex- choicy. These two species are studied in present investigation.

2. Material and Method

I) PEROXIDASE:-

Peroxidase from fresh plants leaves was determined by the method described by Maebly (1954). The enzyme was extracted by homogenizing the plant material (0.5 g) in 10 ml ice cold water. It was filtered through two layered cheese cloth and the filtrate was centrifuged for 15 minutes at 50000 rpm. at 0°C to 4°C and supernatant was used as an enzyme source. Enzyme assay mixture contained 2 ml of 0.1 m phosphate buffer (pH 7) 1 ml of 20 mm guiacol and 0.5 ml of enzyme. The reaction mixture was started by addition of 0.04 ml of 10 mm H₂O₂. The change in optical density (OD) due to oxidation of guiacol was recorded per minute at 470 nm on spectrophotometer with frequent stirring of the reaction mixture with glass rod. Enzyme activity is expressed a change in OD min⁻¹ g⁻¹ fresh tissue.

II) ACID POSPHATASE: -

The enzyme was isolated from fresh leaves by the method of Mclachlan(1980). The enzyme was prepared by homogenizing 0.5 g of plant leaves in 10 ml of 0.1 m acetate buffer (pH-5) with a mortar and pestle. The extract was filtered through the muslin cloth already mistered with acetate buffer and the filtrate was centrifuged at full speed of 5000 rpm for 10 minutes. The supernatant was stored at 0 to 4 c and used as an enzyme source.

Enzymes assay mixture contained 3 ml of p-nitrophenol phosphate (0.1 mg. ml⁻¹) 2 ml of 0.1 m acetate buffer (pH-5) and 1 ml of enzyme. Enzymatic reaction was initiated by the addition of enzyme and was stopped by the additional of 1.5 ml of 1.68 N NaOH. Yellows colored complex p- nitrophenol produced because of reaction between enzymatic breakdown of p-nitrophenol phosphate and NaOH was estimated spectrophotometrically at 420 nm. The enzyme activity was expressed as change in $\Delta OD \text{ hr}^{-1} \text{ gm}^{-1}$ fresh tissue.



Ipomoea carnea subsp. carnea



Ipomoea carnea subsp. fistula

3. Result and Discussion

ACTIVITIES OF ENZYME DURING SENESCENCE:

1) PEROXIDASE:

The enzyme peroxidases an indicator of respiration rate (Harvortz et al, 1968). There are several reports regarding the peroxidase activity during senescence. In case of detached tobacco leaf segment, Parish (1968) noticed increase in peroxidase activity. The activities of several enzymes either generating or decomposing O_2 or H_2O_2 were investigated during the course of senescence in detached wheat and rice leaves in light and darkness by Kar and Feierabend (1984). The increase was higher in the dark than in light. According to those workers the increased peroxidase activity accompanies the senescence of detached leaves.

Pilet et al (1970) have reported that peroxidase can bring about oxidation of opines and hence its increase in senescent leaves may induce hormonal imbalance. However Parish (1968) suggested that increase in peroxidase activity is one of the most reliable indicators of maturity and senescence. According to Mukharjee and Rao (1993) peroxidase activity increased constantly during leaf maturation and much higher level was during senescence.

According to Rane and Chavan (1993) change in peroxidase activity during senescence of detached leaf segment in darkness of groundnut cultivars TMV-10 and JL-24 should that in both the cultivars there was continuous increase in peroxidase activity as

senescence progressed. Liu Yang et. al. (2019) has reported peroxidase activity in *Ipomoea batatas*. They effectively precipitated peroxidase by ammonium sulphate at 60% saturation or higher.

Patel et. al. (2008) purified heme peroxidase MGP from the latex of *Ipomoea carnea* subsp. *fistulosa* (morning glory) belonging to the Convolvulaceae family using ammonium sulfate precipitation, anion exchange, hydrophobic interaction, and gel filtration chromatography.

Our result of present investigation (Table No. 1) clearly indicates that the peroxidase activity is increased during the senescence of both the species of *Ipomoea carnea* Jacq.

2) ACID PHOSPHATASE:

Acid phosphatase is one of the important enzymes of phosphorous metabolism which is involved in breakdown of several phosphates including sugar phosphates and even ATP (De Levand Sacher 1970). Acid phosphatase has multiple molecular forms perticuraly with respect to possible changes during maturation and senescence of plant tissues (Baker and Tekeo 1973). In certain plant tissues like leaf discs of *Rheo discolor*, acid phosphatase increased considerably (De Leo and Sacher 1970). According to Baker and Tekeo (1973) this significant rise in acid phosphatase prior or during senescence of certain plant tissue is not clear and they reported that it is not general phenomenon of senescence of plant tissue. This enzyme is involved in autolytic process during senescence. An increase in acid phosphatase involve in catabolic process during rice leaf senescence was found by Kar and Mishra (1976). Besford (1979) reported that the acid phosphatase activity in the expanding leaves was greater on a fresh wet basis than in the fully expanded or mature leaves.

Recently Rane (1991) has observed the activity of acid phosphatase on groundnut and found that acid phosphatase activity is elevated during course of induced senescence in the two groundnut cultivars. Rane also found that the increase in acid phosphatase in senescence of groundnut leaf takes place depending upon decrease in proline level during senescence. Thus during leaf senescence process and phosphatase may play a critical role particularly with respect to translocation of phosphorus.

Zink (2011) studied the levels and developmental patterns of the two acid phosphatases in the two strains of *Ipomoea* sp. (morning glory) grown *in vitro* by influencing differently by gibberellic acid (GA3). Durmus et. al. (1999) purified

and characterized acid phosphatase from *Ipomoea batatas* using spectroscopic techniques.

In the present investigation we found increased acid phosphatase activity in the senescent leaves of both the species of *Ipomoea carnea* Jacq.

Table No. 1

Activities of enzyme Peroxidase and Acid phosphatase Green and Senescent leaves

Ipomoea carnea Jacq :-

Plant Material	Leaf Stage	Peroxidase Activity	Acid Phosphatase Activity
<i>Ipomoea carnea sub sp. carnea</i>	Green	1.27	2.43
	Senescent	2.12	3.75
<i>Ipomoea carnea sub sp. fistulosa</i>	Green	1.18	2.28
	Senescent	2.10	3.62

Values are expressed in $\Delta OD \text{ hr}^{-1} \text{ gm}^{-1}$ fresh tissue.

4. Conclusion-

In the present investigation the activity of peroxidase and acid phosphatase enzyme increased during the senescence of both the sub species of *Ipomoea carnea* Jacq. The metabolites decrease and catabolic activities increased during senescence.

5. Acknowledgment-

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THE RESEARCH VIEW: A MULTIDISCIPLINARY JOURNAL

PART A: SCIENCE AND TECHNOLOGY

Recent Trends in Pure and Applied Sciences (RTPAS-2021)

Dr. Patangrao Kadam Mahavidyalaya, Sangli

WATER QUALITY ANALYSIS OF FOUR LAKES IN KADEGAON TEHSIL, MAHARASHTRA, INDIA

***Vikas S. Salunkhe¹, Hemant S. Joshi¹, Anuja H. Joshi¹**

¹Department of Botany

Bharati Vidyapeeth's

Matoshri Bayabai Shripatrao Kadam Kanya Mahavidyalaya, Kadegaon

Email ID- drvikassalunkhe@rediffmail.com

Abstract

It is believed that life started with water on earth in form of unicellular micro-organisms. More than 75 % of area of earth is covered by water and the rest by land mass. Even a healthy human body consists of more 70% of water by mass. Humans use water for domestic as well as industrial purposes. Hence the water quality parameters are at most important. The present communication is about the water quality parameters such as alkalinity, BOD, hardness, chlorine, etc from lakes in and around Kadegaon tehsil. Three samples of four water bodies from Kadegaon tehsil viz., Kerli lake, Hingangaon lake, Shivajinagar lake and Tadsar lake were test for water quality parameters such as pH, electric conductance, temperature, total alkalinity, BOD, total hardness, residual chlorine and total dissolved solids. The results show the water quality is good in the tehsil. But still proper care and maintenance should be taken of these water bodies.

Keywords: Water quality, parameters, alkalinity, chlorine, hardness, BOD

1. Introduction

It is believed that life started with water on earth in form of unicellular micro-organisms. Also oxygen which is necessary for survival of living organisms is formed by photolysis of water. Hence, water plays a very important role in survival and evolution of living organisms on earth. More than 75 % of area of earth is covered by water and the rest by land mass. Even a healthy human body consists of more 70% of water by mass (4).

Humans use water for domestic as well as industrial purposes. Hence, it must be said that human welfare is directly dependent on water. Hence the water quality parameters are at most important. These parameters will help to decide the usage of water. About 30 % of urban and 70 % of rural population in India do not have access to safe drinking water as per the WHO (5). Water quality has become a major global concern due to increasing human developmental activities. The present communication is about the water quality parameters such as alkalinity, BOD, hardness, chlorine, etc from lakes in and around Kadegaon tehsil.

Kadegaon is a tehsil in Sangli district of Maharashtra state, India. Four lakes were selected for the analysis. Soyabean, Jowar, Maize, Pigeon pea, sugarcane and grapes are the important crop plants here. The pesticides, fertilizers and insecticides used for farming ultimately lead to the water pollution in this area. Also few industries present in the study area are responsible for water pollution here. Kadegaon tehsil has the total population of 1, 43,019 according to Census 2011. Out of the total population, children account for 11%. So the water sources in this area need to be evaluated for the health concerns of children population. Also 86.2% of the 69,793 working population are permanently employed either in industries or farming (2). Such a large

population is depended directly or indirectly through agriculture or industries on water bodies. Hence, the authors have investigated the some water bodies in Kadegaon tehsil for their quality.

2. Material and Methods

The water samples were collected from four different lakes in Kadegaon tehsil viz., Kerli, Shivajinagar, Hingangaon and Tadsar. The locations of these water bodies are given in the **Table No. 1**. The collections were made in the month of February 2020. Three samples from each water body were collected in sterile glass bottles with rubber corks. The water samples were immediately brought into the laboratories for further analysis.

Sr. No	Location	Coordinates
1	Nerli Lake	17°17'12.1"N 74°18'55.5"E
2	Shivajinagar Lake	17°18'49.7"N 74°17'50.1"E
3	Hingangaon Lake	17°24'08.8"N 74°20'38.8"E
4	Tadsar Lake	17°15'07.0"N 74°19'49.1"E

Table 1 Locations of sample collectionPhysicochemical analysis of water

1. pH- The pH of the water sample were determined by using digital pH meter.
2. Temperature- The temperature of the water sample was measured by using thermometer.
3. Electric conductivity- For measurement of conductivity, EC meter was used.
4. Total dissolved solids- Total dissolved solids denote mainly the various kinds of mineral present in water. These can be determined as the residue left after evaporation of the filtered sample.
5. Total alkalinity- It is determined by titrating the sample against Sulphuric acid using bromocresol green as indicator.
6. BOD- It was estimated by Wrinkler's method.

7. Total hardness- Hardness was determined by titrating against EDTA using Erichrome Black-T indicator.

8. Residual Chlorine – It was estimated by iodometric titration method.

The present study involves the analysis of water quality in terms of physicochemical parameters (1). Water Quality Index (WQI) was calculated for collecting water samples. Water Quality Index was calculated based on physicochemical parameters and the standards of drinking water quality by the World Health Organization (WHO) is given in **Table No. 2**. The calculated WQI values of the samples are classified according to the five types, which are given in the following **Table No. 2**.

Sr. No.	Water Quality	WQI Value
1	Water Unsuitable for drinking	>300
2	Very poor water	200-300
3	Poor water	100-200
4	Good water	50-100
5	Excellent	<50

Table 2 Classification water quality index based on WQI value

3. Result and Discussion

Three samples of four water bodies from Kadegaon tehsil viz., Kerli lake, Hingangaon lake, Shivajinagar lake and Tadsar lake were test for water quality parameters such as pH, electric conductance, temperature, total alkalinity, BOD, total hardness, residual chlorine and total dissolved solids. The results show the water quality is good in the tehsil (3,6).

Fig. nos. **1, 2, 3, 4, 5, 6, 7 and 8** show the results of the water sample and **Table no. 3** shows the comprehensive results of water samples. From the results, pH, conductivity, temperature, hardness, residual chlorine and total dissolved solids are within the limits of IS and WHO while, total alkalinity and

BOD are above the optimum levels. BOD is a measure of the amount of oxygen required to remove waste organic matter from water in the process of decomposition by aerobic bacteria. The higher values of BOD indicate organic pollution in these water bodies. Also the higher temperatures are responsible for increase on BOD values as at higher temperature the planktonic population increases. Higher alkalinity suggests industrial or agricultural discharges in the water bodies.

Parameters	Kerli Lake	Hingangaon Lake	Shivajinagar Lake	Tadsar Lake	IS and WHO water quality parameters
1. Total alkalinity (mg/lit)	245 ± 0.41	198 ± 0.55	212 ± 0.32	210 ± 0.98	200
2. pH	7.0	7.2	7.3	7.1	6.5-8.5
3. Electric conductivity (mhos.cm ⁻¹)	0.413 x 10 ⁻³	0.458 x 10 ⁻³	0.502 x 10 ⁻³	0.462 x 10 ⁻³	0.75 x 10 ⁻³
4. BOD (mg/lit)	1365 ± 0.85	1402 ± 0.78	1524 ± 0.68	1204 ± 0.89	350
5. Temperature (°C)	29.68 ± 0.35	28.98 ± 0.68	28.10 ± 0.45	30.01 ± 0.12	-
6. Total Hardness (mg/lit)	152 ± 0.98	132 ± 0.47	102 ± 0.67	120 ± 0.49	300
7. Residual chlorine (mg/lit)	0.2 ± 0.11	0.1 ± 0.09	0.1 ± 0.07	0.1 ± 0.07	0.2
8. Total dissolved solids (ppm)	424 ± 0.21	325 ± 0.78	365 ± 0.49	226 ± 0.77	500

Table 3 Results of Water Quality Value

Table No. 4 and **Fig. no. 9** show the Water quality index of the water bodies ranging from 52-89 which indicates good water. But the WQI of the Kerli lake (89.04858195) was on the higher side which is the matter of concern (**Table No. 2**).

Location	WQI Value	Water Quality
Kerli Lake	89.04858195	Good water
Hingangaon Lake	52.88729455	Good water
Shivajinagar Lake	54.48578061	Good water
Tadsar Lake	52.76628362	Good water

Table 4 Water Quality Index

4. Conclusion

The results of four water samples from the four lakes indicate good quality of water according to IS and WHO standards. But still proper care and maintenance should be taken of these water bodies. Care such as chlorination of water, reduction of use of inorganic chemicals in agriculture, no discharge of industrial effluents in the water bodies can prolong and enhance the use and quality of water. It shall help in improving health of the people.

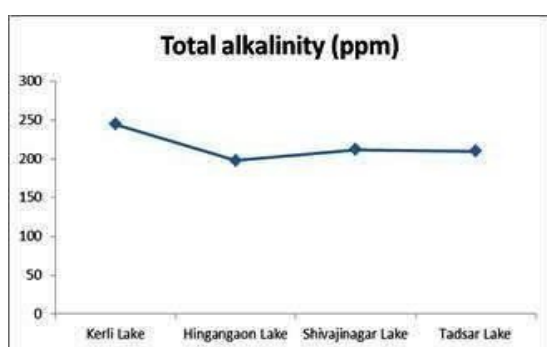


Fig No. 5 Total alkalinity

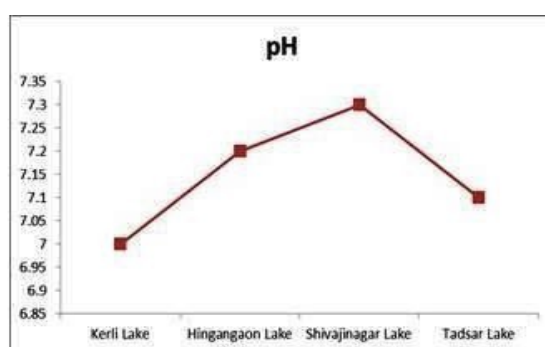


Fig No. 6 pH

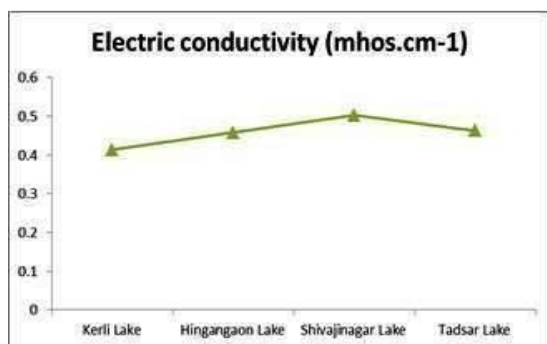


Fig No. 7 Electric conductivity

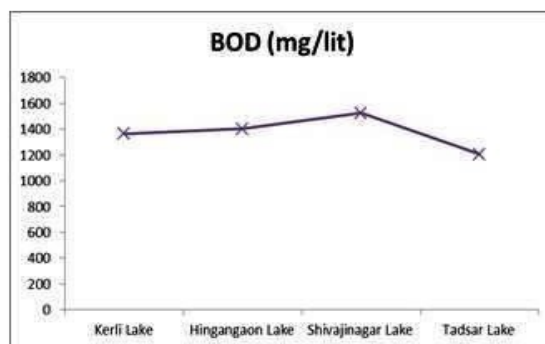


Fig No. 8 BOD

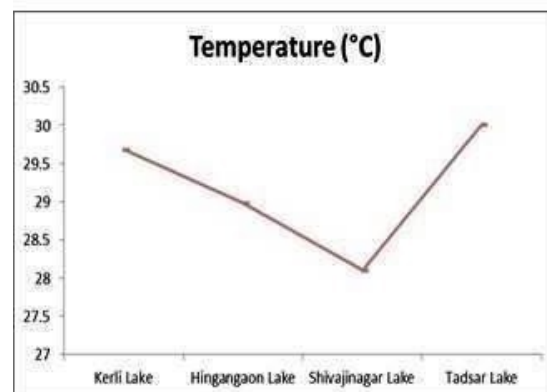


Fig No. 9 Temperature

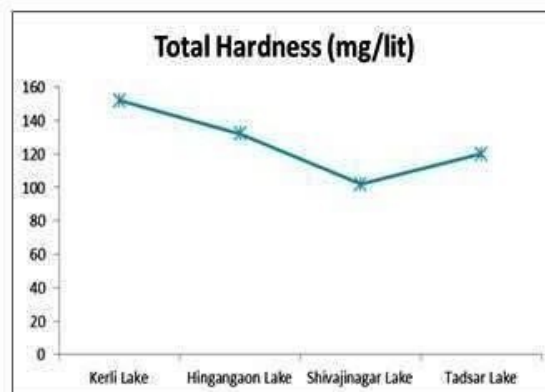


Fig No. 10 Total Hardness

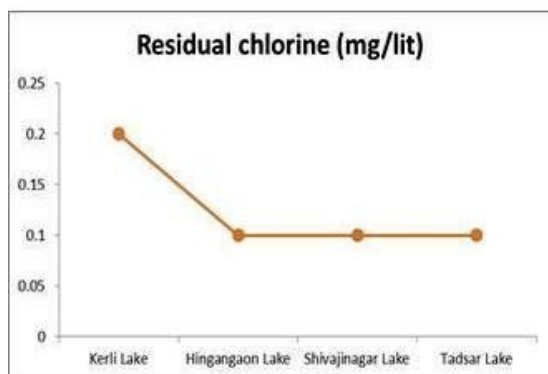


Fig No. 11 Residual chlorine

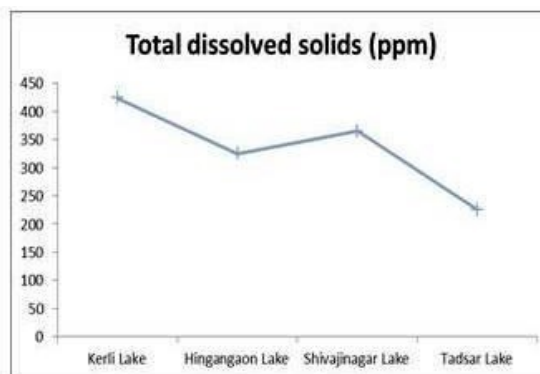


Fig No. 12 Total Dissolved Solids

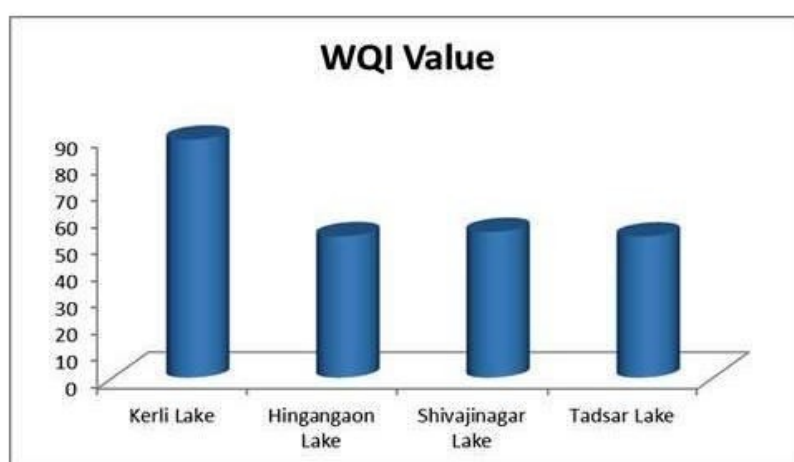


Fig No. 13 Water Quality Index Values

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