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Organised by
Prof. Ramkrishna More Arts,
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CONTENTS

Regional Disparities In The Socio-Economic Development Ofthe Konkan Region, Maharashtra

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ABSTRACT

Regional disparities prevail within different districts of Maharashtra. Previous work has highlighted the disparities within the districts. The present work is an attempt at bringing out the regional disparities within the tehsils from the Konkan region (excluding Mumbai). Forty-seven tehsils of the Konkan region excluding Mumbai district have been included in the study. The status of development has been worked out on the basis of a number of developmental indicators. The data for the year 2014 with 27 indicators pertaining to agriculture, infrastructure and demography have been employed to obtain the level of development. The developmental level was estimated with the help of composite index based on the best possible combination of all the developmental indicators. The tehsils falling in different levels of development, such as high, middle (moderate) and low have been identified in the study region. It was observed that the correlation between infrastructure and agriculture was better than the one between infrastructure and demographic sector. This indicated that the agricultural development is more sensitive to the infrastructural conditions. For considering the future development of the region, model tehsils have been identified for enhancing the level of overall socio-economic development. Low developed tehsils require improvement of various dimensions in most of the indicators for enhancing the level of overall development.

I. INTRODUCTION

Regional disparities prevail at the international as well as national level and often bring ina socio-economic divide between the regions. Development is associated with growth alongsocial justice where it is intended that the final stage of development should lead to the provisionof increased opportunities to all people for raising their living standard. In order to say that aregion is developed, it is necessary to compare its level of development with some other region.

In this sense disparities are brought forward. The position, a scale that a region or a state orcountry or any other unit has attained in terms of development, is referred to as levels ofdevelopment. Development is a multidimensional process which includes economic, social,political and ecological dimensions of development. The main thrust of this study is on spatialperspective of development.

In India, a notable increase in the net production in agricultural and manufactured goodshas been observedespecially after the green revolution and industrial boom. Despite thesepositive developmental indicators, the states have failed to exhibit any kind of indicator thatdisplayed such activities that would have reduced significantly the level of regional disparities interms of development. During the last two decades, there has been a considerable rise in thestudies on inter-state disparity across the Indian states using sophisticated analytical tools andbetter data giving

emphasis on the level of development and also bringing out the disparitiesamongst the regions. Singh and Srinivasan (2002) carried out a study for the period 1990-91 to 1998-99 and found that the evidence does not permit one to reach very definite conclusion onconvergence or divergence across the major states. The level of socio-economic developmentwas estimated for different states for the year 1971-72 and 1981-82 by Narain et al. (1999) inwhich it was observed that there were wide disparities in the level of development amongdifferent states. The studies regarding evaluation of status of development at district level haveso far been completed for the state of Orissa (Narain et al. 1992, 1993, 2005), Andhra Pradesh(Narain et al. 1994), Kerala (Narain et al. 1994, 2005), Uttar Pradesh (Narain et al. 1995, 2001),Maharashtra (Narain et al. 1996), Karnataka (Narain et al. 1997,2003), Tamilnadu (Narain et al.2000), States of southern region (Narain et al. 1999), Madhya Pradesh (Narain et al. 2003),Assam (Rai and Bhatia 2004), Hilly States (Narain et al. 2004), Jammu and Kashmir (Narain etal. 2005). It was found that entire parts of the low developed districts are not backward but thereare some parts which are also better developed.

In Maharashtra a number of scholars have worked and published their studies on the regionaldisparity within the districts. Brahme et al, (1975) discussed the relative levels of development ofGreater Mumbai, Pune region, Marathwada and Vidharba while working on a publication onregional planning for Marathwada. Levels of development and developmental disparities amongstthe various districts of Maharashtra were brought out as early as in 1980's through a publicationby Shah in 1980. Prabhu and Sarker (1992) identified the levels of development for the districts ofMaharashtra where they employed the data for the years 1985-86. According to this study out of29 districts of Maharashtra, 11 districts emerged as highly developed districts and 15 districtsattained the status of low developed districts. It was interesting to note that these low developeddistricts mostly belonged to the Vidharba and Marathwada regions. Ahuja and Nikam (2015) haveanalysed the inter-district inequality based on the per capita income in Maharashtra for the period 2001-2013. It was observed by Suryanarayana (2009) that half of the Maharashtra state's incomecomes from the four major districts Mumbai, Thane, Pune and Nashik, whereas remaining 31 districts account for the remaining share of states income. Kurulkar (2009) in his study has highlighted the problem of regional disparities in Maharashtra and stated that during the period 1984 and 1994 the regional disparities instead of reducing have actually increased. He also broadly mentioned that from the various studies carried out at the district level, the districts which conspicuously stands out as low developed regions (both at the national and state level) are mostly from Vidharba and Marathwada. Moreover, there are also a few districts which show low level of development like Dhule, Nandurbar, Ratnagiri and Sindhudurg.

To sum up it may be said that so far the regional disparities work is widely carried out from the state to district level. In the present work, an attempt is made to analyse and understand the level of disparity in the development of a region with respect to its smaller administrative unit that is, tehsil. Thus, the main aim of the study is to assess the overall level of development of the Konkan region from Maharashtra State. The specific objectives set are: i) To observe the regional disparities in Agricultural, Infrastructural and Demographical development at tehsil level and ii) To ascertain the developmental distances between the tehsils and obtain the model tehsil for each tehsil.

STUDY AREA
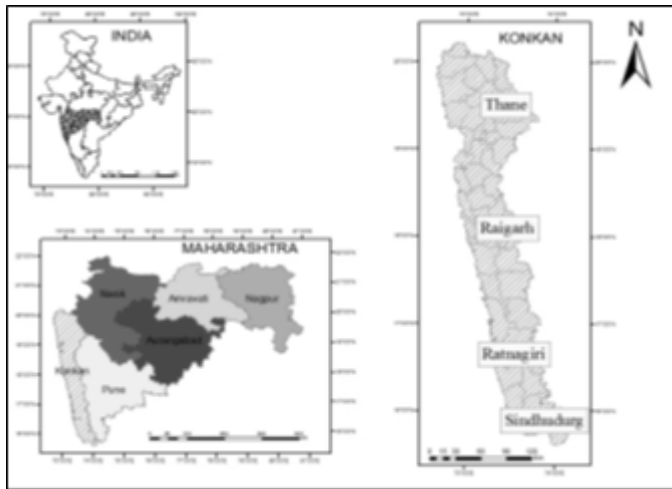
Figure 1. Location of the study area

Konkan region includes five main districts viz: Mumbai (along with its suburbs), Thane, Raigarh, Ratnagiri and Sindhudurg. Figure 1 depicts the location of the study area. For the present study Mumbai is excluded from the Konkan region. The study area falls entirely under the coastal regime and has the spurs of the Western Ghat drained mostly by the steep gradient short distance rivers running westwards and draining into the Arabian Sea. The total number of tehsils within the administrative borders of the four districts is 47. Out of these 15 are from Thane district, 15 from Raigarh, 09 from Ratnagiri and 08 from Sindhudurg.

RATIONALE FOR CHOOSING KONKAN REGION:

Maharashtra is considered as one of the most developed states of India. Maharashtra ranks second in the country according to the population rank with a population of 11.24 crore as per the 2011 census. On the economic front, Maharashtra with its GSDP at Rs. 16, 47,506 crore ranked first amongst the states of India. For administrative purpose Maharashtra is divided into six administrative divisions viz: Konkan, Pune, Nashik, Aurangabad, Amravati and Nagpur. Table 1 depicts the GDP for these six divisions along with the population.

Table 1. Division wise population and GDP in Maharashtra (2011)

Administrative divisions		Population (2011) in '000	GDP(at current price 2014-15) in crore
Pune		23449	3,38,052
Konkan		28601	6,03,481
Konkan	Mumbai	12442	3,34,423
	Rest of Konkan	16159	2,69,059
Nashik		18579	1,84,427
Aurangabad		18731	1,53,885
Amravati		11257	93,796
Nagpur		11755	1,36,493
Maharashtra		11.24 crore	16,47,506

Source: Census of India, District economics and statistical bureau.

Out of the six divisions, Konkan has the highest GDP and also the highest populationindicating highest level of development within the state. However, a closer look at the furtherbifurcation of the Konkan division clearly brings out the disparity between Mumbai and rest of theKonkan. As is observed from the table 1, Mumbai along with its suburbs contributes more than50% of GDP to the Konkan division. Thus in order to understand the developmental disparities inthe Konkan region, one has to exclude Mumbai so that the regional disparities in the region can betruly derived.

II. METHODS AND MATERIAL

DATA AND METHOD:

This study is based on the secondary data which was obtained from various sources. ThePopulation related indicators were taken from the Census of India

handbook (2011). Agriculturaland Infrastructural indicators were obtained from the District Economic and Statistical Bureau,socio-economic abstracts and periodicals publication (2014).

INDICATORS OF LEVEL OF DEVELOPMENT

A total of **27** development indicators have been used in the present study to analyse thelevel of socio-economic and demographic development of different tehsils in the Konkan region.

These indicators are listed in table 2.

Table 2. Indicators used to measure level of overall development

Agricultural indicators	Infrastructural indicators	Demographic indicators
Area under agricultural production in hectares	Number of post offices and PCO	Percentage of total literates
Production of rice and ragi	Number of primary schools	Percentage of urban literates
Fish production	Road length per 100 sq. km/area	Percentage of rural literates
Milk production	Number of secondary schools	Percentage of SC and ST literates
Number of veterinary hospitals	Number of colleges	Infant mortality rate
Percentage of using chemical fertilizers	Number of higher secondary school	Birth rate
Number of total agricultural pumps	Number of PHC (Public Health Centre) & sub centres	Mortality rate

From the detail literature survey carried out the following method was thought to be apt for the present study and thus the same was adopted.

COMPOSITE INDEX OF DEVELOPMENT

The following statistical procedure for estimation of composite index of development is adopted in the study.

i. Standardization of the original data matrix: Each data matrix (X_{ij}) was converted to a standardized data matrix $[Z_{ij}]$. This was thought essential as $[X_{ij}]$ come from different population distribution and they might be recorded in different units of measurement and thus may not be quite suitable for the simple addition to obtain the composite index standardized scores.

$$[Z_{ij}] = \frac{X_{ij} - \bar{X}_j}{S_j} \quad \text{......................eq.1}$$

Here $[X_{ij}]$ is the data matrix with i = 1, 2....n (number of area unit) and j = 1, 2.....k (number of indicators), $\bar{X}_j$ relates to the mean of the jth indicator, S_j is the standard deviation of the j^{th}indicator, and $Z_{ij}$is the matrix of standardized indicators.

ii. Once the standardized matrix was obtained$[Z_{ij}]$ the next step involved identification of the best value of each indicator (Z_{oj}).The best value is either the maximum value or minimum value of the indicator depending upon the direction of the impact of indicator on the level of development.The pattern of development P_{ij} is further calculated using the equation 2.

$$P_{ij} = (Z_{ij} - Z_{oj})^2 \quad \text{.................eq, 2}$$

Pattern of development C_i is given as

$$C_i = \left[\sum_{j=1}^{k} P_{ij}/(c.v.)_j \right]^{\frac{1}{2}} \quad \text{..............eq. 3}$$

Here $(c.v)_j$ is the coefficient of variation of the jth indicator in X_{ij}

iii. Finally, the Composite index D_i is computed using the equation 4.

$$D_i = C_i /C \quad \text{for i = 1, 2,.....n ....eq. 4}$$

Where, $C = \bar{C} + 3S_{Di}$,

$\bar{C}$ = mean of C_i and S_{Di} = Standard deviatrion of C_i

Smaller value of D_i will indicate high level of development and higher value of D_i will indicate low level of development.

ESTIMATION OF DEVELOPMENTAL DISTANCES BETWEEN PAIRS OF TEHSILS

The distance between tehsils is given by d_{ip},

Where, $d_{ip} = \left[\sum_{j=1}^{k} (z_{ij} - z_{pj})^2 \right]^{\frac{1}{2}} \ldots \ldots \ldots \ldots eq. 5$

$$i = 1, 2 \ldots n; \ p = 1, 2 \ldots n$$

Here, $d_{ii=0}$ and $d_{ip=d_{pi}}$, The minimum distance for each row is considered. The critical distance (C.D) is further computed using equation 6.

$$CD = \bar{d} + 2sd \ldots \ldots \ldots eq. 6$$

Where,

$\bar{d} = mean \ of \ d_i$ and $sd = standard \ deviation \ of \ d_i$

IDENTIFICATION OF MODEL TEHSILS

The identification of the model tehsil for each tehsil is obtained by setting a simple criterion. This criterion relates to the comparison of composite index and critical distance of the tehsil under consideration with other tehsil. For the tehsil under consideration that tehsil will be considered as a model tehsil whose composite index is less than the tehsil under consideration and the developmental distance from the tehsil under consideration is greater than or equal to Critical Distance (C.D.) of the other tehsil. Thus, the model tehsil will be a better developed tehsil as compared to the other tehsil. The best value of each developmental indicator of the model tehsil will be the potential target for other tehsil.

In order to achieve a more meaningful categorization, suitable fractile classification from the assumed distribution of the mean of composite indices is employed as given in table 3. For relative comparison, it is assumed that the tehsils having the composite index $\leq$ (Mean – SD) are levelled as high developed, those tehsils with the composite index ranging between (Mean $\pm$ SD) are middle level developed and composite index > (Mean + SD) are low level developed tehsils.

Table 3. Values of Composite Index

VALUE OF COMPOSITE INDEX	LEVEL OF DEVELOPMENT
$\leq$ Mean-S.D.	High
Mean to (Mean + S.D.)	Moderate
Mean + S.D. $\geq$	Low

III. RESULTS AND DISCUSSION

THE LEVEL OF DEVELOPMENT

The composite indices of development (C.I.) have been worked out for different tehsils separately for agriculture, infrastructural service and demographic development. The tehsils have been ranked on the basis of development indices. Table 4 shows the composite indices (C.I.) of development along with the ranks of different tehsils. In this table, a simple ranking of the tehsils on the basis of level of development has been presented.

It may be observed from table 4 that the value of composite indices of agriculture sector ranges from 0.58 to 0.95. Out of 47 tehsils of Konkan region, the tehsil of Alibag is ranked first in agriculture having high agricultural production (rice), fish production, high use of chemical fertilizers and electric pumps and Ulhasnagar is ranked last due to absence of agriculture. The values of composite indices of infrastructural services varies from 0.49 to 0.89.Thane is ranked first in infrastructural facilities with high amenities like educational facilities, transportation facilities and banking. Whereas, Tala ranks last.The values of composite indices of demography vary from 0.43 to 1.56. Panvel is ranked first in demographic sector with high urbanization and high literacy rate and Talsari is ranked last because this tehsil is totally rural.

<div align="center">**Table4.** Composite Index of Development</div>

Sr. No.	Tehsil	Agriculture		Infrastructure		Demography	
		Di	Rank	Di	Rank	Di	Rank
1	Mandangarh	0.84	18	0.83	20	0.99	29
2	Dapoli	0.73	8	0.71	12	0.83	22
3	Khed	0.75	10	0.64	6	0.92	26
4	Chiplun	0.72	7	0.61	3	0.83	22
5	Guhagar	0.77	12	0.76	16	0.90	24
6	Ratnagiri	0.68	4	0.60	2	0.67	16
7	Sangmeshwar	0.71	6	0.67	8	0.94	27
8	Lanja	0.77	12	0.77	17	0.99	29
9	Rajapur	0.72	7	0.69	10	0.95	28
10	Devgad	0.78	13	0.76	16	0.64	14
11	Vaibhavwadi	0.85	19	0.85	22	0.91	25
12	Kankavali	0.74	9	0.74	14	0.60	10
13	Malvan	0.74	9	0.74	14	0.57	09
14	Vengurle	0.82	16	0.81	19	0.69	18
15	Kudal	0.69	5	0.71	12	0.63	13
16	Sawantwadi	0.76	11	0.74	14	0.57	09
17	Doddamarg	0.86	20	0.85	22	0.82	21
18	Uaran	0.88	21	0.84	21	0.47	03
19	Panvel	0.68	4	0.63	05	0.43	01
20	Karjat	0.73	8	0.70	11	0.52	06
21	Khalapur	0.83	17	0.73	13	0.62	12
22	Pen	0.73	8	0.76	16	0.68	17
23	Alibag	0.58	1	0.71	12	0.47	03
24	Murud	0.81	15	0.85	22	0.51	05
25	Roha	0.71	6	0.75	15	0.66	15
26	Sudhagad	0.81	15	0.84	21	1.01	30
27	Mangaon	0.68	4	0.73	13	0.71	19
28	Tala	0.88	21	0.89	24	0.82	21
29	Shrivardhan	0.81	15	0.85	22	0.46	02
30	Mhasala	0.88	21	0.86	23	0.63	13
31	Mahad	0.65	3	0.71	12	0.61	11
32	Poladpur	0.83	17	0.86	23	0.75	20
33	Talsari	0.81	15	0.81	19	1.56	35
34	Dhanu	0.64	2	0.68	09	1.06	31
35	Vikramgad	0.80	14	0.79	18	0.87	23
36	Jwahar	0.74	9	0.76	16	1.11	32
37	Mokhada	0.82	16	0.81	19	1.37	34

38	Vada	0.76	11	0.76	16	0.71	19
39	Palghar	0.69	5	0.65	07	0.55	08
40	Vasai	0.73	8	0.61	03	0.51	05
41	Thane	0.92	22	0.49	01	0.46	02
42	Bhawandi	0.74	9	0.67	08	0.75	20
43	Shahpur	0.72	7	0.67	08	0.69	18
44	Kalyan	0.88	21	0.62	04	0.49	04
45	Ulhasnagar	0.95	23	0.84	21	1.22	33
46	Ambarnath	0.80	14	0.74	14	0.54	07
47	Murbad	0.69	5	0.75	15	0.83	22

Levels of Agricultural Development (2014)
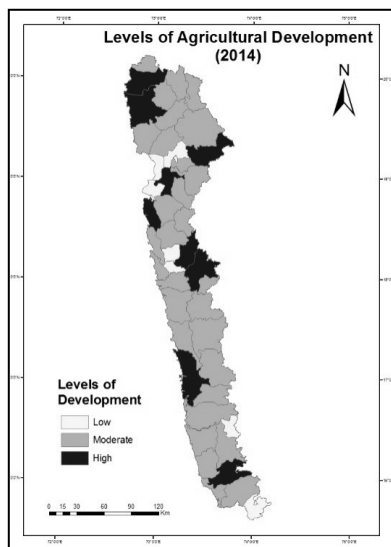
AREA UNDER DIFFERENT STAGES OF DEVELOPMENT:

It would be quite interesting and useful to find out the relative share of area affected, under different levels of development, in the region. The area covered by the tehsils falling under different levels of development is presented in table 5 and Figure 2, 3 and 4.

Levels of Demographic Development (2014)

Levels of Infrastructural Development (2014)
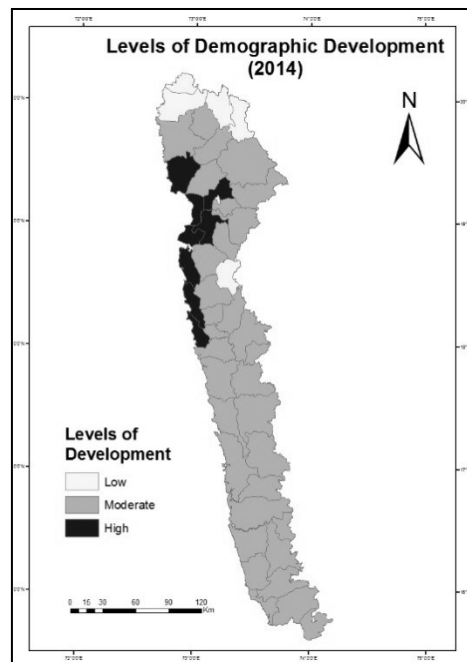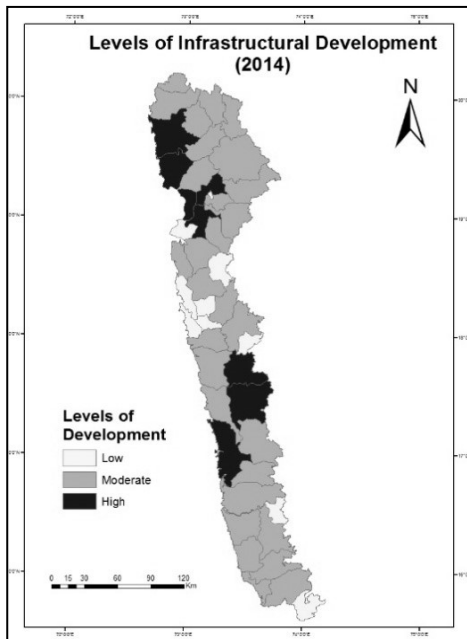
Table 5. Level of development

Sector of economy	Level of development	No. Of Tehsils	Area %
Agriculture	High ($\leq$0.69)	09	24.86
	Moderate (0.69-0.85)	30	68.63
	Low ($\geq$0.85)	08	6.51
Infrastructural facilities	High ($\leq$0.66)	08	19.76
	Moderate (0.66-0.83)	28	68.44
	Low ($\geq$0.83)	11	11.80
Demographic	High ($\leq$0.51)	06	7.47
	Moderate (0.51-1.00)	35	83.78
	Low ($\geq$1.00)	06	8.75

Source : Compiled by authors

The analysis reveals that about 18.70% area is highly developed in all the sectors. In agricultural sector, about 24.86% area is highly developed and 68.63% area is moderately developed and low level developed tehsils cover about 6.51% area.In infrastructural services, about 19.76% area is highly developed, 68.44% area is moderately developed and 11.80% area fall in the tehsils which are low developed. In demographic sector, about 7.47% area is highly developed,83.78% area is moderately developed and only 8.75% area fall in the level of low developed tehsils. It is observed that lowdeveloped tehsils are not as thickly populated as the tehsils belonging to the category of high development.

Table 6 depicts the model tehsils for agricultural development. The analysis reveals that 34 tehsils have model tehsils and there are no model tehsils for 13 tehsils. The composite indicesare equal or the critical distances are equal or high for these thirteen tehsils which leaves them with such a situation. However, these tehsils are the model tehsils of the other tehsils. Shahpur with a composite index of 0.72 is the model tehsil for Vasai, Pen, Karjat and

Dapoli whereas, Alibag with the lowest composite index (0.58) assumes the status of model tehsil for all the tehsils in the study area. Dhanu is the model tehsil for Mahad whereas Guhagar and Lanja are the model tehsils for Vikramgad. Ratnagiri is the model tehsil for Mhasala and Vaibhavwadi. Sawantwadi is the model tehsil for Lanja. Alibag is found highly developed having the least composite index as well as critical distance in agricultural sector.

Table 6. Model tehsils in agricultural development

Sr.No	Tehsils	Model tehsils
1	Mandangarh	Khalapur,Poladpur,Chiplun
2	Dapoli	Shahpur
3	Khed	Bhiwandi,Pen,Vasai,Dapoli,Karjat
4	Guhagar	Sawantwadi,Vada,Khed
5	Lanja	Sawantwadi,Vada,Khed
6	Devgad	Guhagar,Lanja
7	Vaibhavwadi	Khalapur,Poladpur,Murbad,Kudal,Palghar,Mangaon,Panvel,Ratnagiri
8	Kankavali	Bhiwandi,Pen,Vasai,Dapoli,Karjat
9	Malvan	Bhiwandi,Pen,Vasai,Dapoli,Karjat
10	Vengurle	Talsari,Murud,Kshrivardhan,Sudhagad,Ambarnath,Vikramgad
11	Sawantwadi	Khed,Bhiwandi,Jwahar,Malwan,Kankavali
12	Doddamarg	Vaibhavwadi,Mandangarh,Rajapur,Sangmeshwar,Roha
13	Uaran	Doddamarg,Vaibhavwadi
14	Karjat	Shahpur
15	Khalapur	Mokhada,Murud,Vengurle,Talsari,Kshrivardhan,Sudhagad, Ambarnath,Vikramgad
16	Pen	Shahpur
17	Murud	Ambarnath,Vikramgad

18	Sudhagad	Ambarnath,Vikramgad
19	Tala	Doddamarg,Vaibhavwadi
20	Shrivardhan	Ambarnath,Vikramgad
21	Mhasala	Doddamarg,Vaibhavwadi, Murbad,Kudal,Palghar,Mangaon,Panvel, Ratnagiri
22	Poladpur	Mokhada,Vengurle,Talsari, Kshrivardhan,Sudhagad,Ambarnath, Vikramgad
23	Talasari	Ambarnath,Vikramgad
24	Vikramgad	Guhagar,Lanja
25	Jwahar	Bhiwandi,Pen,Vasai,Dapoli ,Karjat
26	Mokhada	Talsari,Murud,Shrivardhan, Sudhagad,Ambarnath,Vikr amgad
27	Vada	Khed,Bhiwandi,Jwahar,Malwan,Kankavali
28	Vasai	Shahpur
29	Thane	Uaran,Kalyan,Tala,Mhasala ,Mahad,Dhanu
30	Bhiwandi	Pen,Vasai,Dapoli,Karjat
31	Mahad	Dhanu
32	Kalyan	Doddamarg,Vaibhavwadi
33	Ulhasnagar	Thane
34	Ambarnath	Devgad,Guhagar,Lanja,

The model tehsils for infrastructural development are represented in table 7. It is observed that 30 tehsils have model tehsils but remaining 17 tehsils fail to have any model tehsils because some of them have the same composite index values and their critical distances are increasing. But these tehsils are the model tehsils of the other tehsils. Kudal, Alibag, Dapoli and Karjat are the model tehsils for various tehsils having composite index 0.71. Thane is the model tehsil for all tehsils which is the highly developed tehsil in the study region having composite index 0.49.

Table 7. Model tehsils in infrastructural development

Sr. No.	Tehsils	Model tehsils (Infrastructural development)
1	Mandangarh	Talsari,Khed, Panvel
2	Guhagar	Devgad,Vada,Jwahar,Pen
3	Lanja	Guhagar,Rajapur,Dhanu,Shahpur,Sangmeshwar,Bhwandi
4	Devgad	Murbad,Roha,Kankavali
5	Vaibhavwadi	Shrivardhan,Murud,Ulhasnagr,Uaran
6	Kankavali	Malvan,Ambarnath,Savantwadi,Mangaon
7	Malvan	Khalapur,Mahad,Kudal,Dapoli,Alibag,Karjat
8	Vengurle	Vikramgarh,Palghar
9	Sawantwadi	Khalapur,Mahad,Kudal,Dapoli,Alibag,Karjat
10	Doddamarg	Shrivardhan,Murud,Ulhasnagr,Uaran
11	Uaran	Mandangarh,Kalyan,Vasai,Chiplun,Ratnagiri
12	Khalapur	Mahad,Kudal,Dapoli,Alibag,Karjat
13	Pen	Murbad,Roha,Kankavali
14	Murud	Ulhasnagar,Kalyan,Vasai,Chiplun,Ratnagiri
15	Roha	Malvan,Ambarnath,Savantwadi,Mangaon
16	Sudhagad	Mandangarh,Kalyan,Vasai,Chiplun,Ratnagiri
17	Mangaon	Khalapur,Mahad,Kudal,Dapoli,Alibag,Karjat
18	Tala	Mhasala,Poladpur,Vaibhavwadi,Doddamarg
19	Shrivardhan	Ulhasnagar,Kalyan,Vasai,Chiplun,Ratnagiri
20	Mhasala	Shrivardhan,Murud,Ulhasnagr,Uaran

		n,Thane
21	Mahad	Kudal,Dapoli,Alibag,Karjat
22	Poladpur	Shrivardhan,Murud,Ulhasnagr,Uaran,Thane
23	Talasari	Vikramgarh,Lanja
24	Vikramgad	Lanja,Guhagar
25	Jwahar	Murbad,Roha,Kankavali
26	Mokhada	Vikramgarh,Lanja
27	Vada	Murbad,Roha,Kankavali
28	Ulhasnagar	Mandangarh,Kalyan,Vasai,Chiplun,Ratnagiri
29	Ambarnath	Khalapur,Mahad,Kudal,Dapoli,Alibag,Karjat
30	Murbad	Malvan,Ambarnath,Savantwadi,Mangaon

		di
12	Kankavali	Malvan,Savantwadi,Palghar
13	Malvan	Palghar,Ambarnath
14	Vengurle	Pen,Ratnagiri
15	Kudal	Khalapur
16	Savantwadi	Palghar,Ambarnath
17	Doddamarg	Bhiwandi, Poladpur
18	Uaran	Srivardhan
19	Karjat	Murud,Vasai,Kalyan
20	Pen	Ratnagiri,Roha,Devgad
21	Alibag	Srivardhan
22	Murud	Kalyan,Uaran,Alibag
23	Roha	Devgad,Mhasala
24	Sudhagad	Lanja,Mandangarh
25	Mangaon	Vengurle,Shahpur,Pen
26	Tala	Bhiwandi, Poladpur
27	Mhasala	Khalapur
28	Mahad	Kankavali,Malvan
29	Talasari	Mokhada,Ulhasnagar,Jwahar
30	Dhanu	Sudhagad,Lanja,Mandangarh
31	Vikramgad	Dapoli,Chiplun,Murbad
32	Jwahar	Dhanu,Sudhagad
33	Mokhada	Ulhasnagar,Jwahar
34	Vada	Vengurle,Shahpur,Pen
35	Palghar	Ambarnath
36	Vasai	Kalyan,Uaran,Alibag,Thane,Panvel
37	Murbad	Doddamarg,Tala
38	Shahpur	Pen,Ratnagiri
39	Kalyan	Uaran,Thane,Alibag,Shrivardhan
40	Ulhasnagar	Jwahar,Dhanu,Sudhagad,Shrivardhan,Thane,Panvel

Table 8 represents the model tehsils for demographical development. Thane and Panvel are the model tehsils for all other tehsils with the least composite index i.e. 0.43 and 0.46 respectively. Ambarnath, Bhiwandi, Shrivardhan, Khalapur and Poladpur have no model tehsils because composite index values of various tehsils are decreasing but the critical distances are increasing gradually.

Table 8. Model tehsils in demographic development

Sr.No.	Tehsils	Model tehsils(Demographic development)
1	Mandangarh	Rajapur,Sangmeshwar
2	Dapoli	Doddamarg,Tala
3	Khed	Vaibhavwadi,Guhagar
4	Chiplun	Doddamarg,Tala
5	Guhagar	Vikramgad,Dapoli,Chiplun
6	Ratnagiri	Roha,Devgad
7	Sangmeshwar	Khed
8	Lanja	Rajapur,Sangmeshwar
9	Rajapur	Khed,Sangmeshwar
10	Devgad	Mhasala,Kudal
11	Vaibhavwa	Guhagar,Vikramgad

INTERRELATIONSHIPS AMONG DIFFERENT SECTORS

Table 9 depicts the pair wise correlation analysis for the agricultural, infrastructural and demographic development indices. The correlation coefficient

between the development in infrastructural and demographic sectors is found to be significant at 0.05 probability level whereas, the correlation coefficient between the developments in agriculture and infrastructure service facilities significant at 0.01 probability level. On deeper examinations of indicators included under infrastructural facilities, it was found that most of the indicators are highly influenced by the level of education. The agricultural development is found to be significantly affected by the level of education. The growth and progress of agriculture development fully utilise the infrastructural facilities. The level of education and other related infrastructural facilities are found to have a very high significant co-relation coefficient with the demographic development in the region.

Table 9. Pair wise Correlation Coefficient

	Agriculture	Infrastructure	Demography
Agriculture	1		
Infrastructure	0.45**	1.00	
Demography	0.15	0.31*	1

**Significant at 0.01 level

*Significant at 0.05 level

IV. CONCLUSIONS

i. With respect to overall Demographic development, the tehsils likePanvel, Thane, Shrivardhan, Alibag, Uaran, Kalyan, Vasai and Murudare better developed as compared tothe remaining tehsils of the region. On the other hand,Mandangarh, Sudhagad, Uaran, Ulhasnagar, Murud, Shrivardhan, Doddamarg, Vaibhavwadi, Poladpur, Mhasala and Tala are low developed tehsils of the region. Rest of the tehsils have a moderate level of development but they also show an inclination towardsupgrading the developmental level. In demographical development, Thane and Panvel are the model tehsils for all other tehsils with

the least composite index i.e. 0.43 and 0.46 respectively.

ii. The nine tehsils namely Alibag, Dhanu, Mahad, Ratnagiri, Panvel, Mangaon, Palghar, Kudal and Murbad have high developmental level in agricultural sector. Low level of development in the agricultural sector is mostly observed in Vaibhavwadi, Doddamarg, Mhasala, Tala, Kalyan, Uaran, Thane and Ulhasnagar. These are mostly the tehsils which are having more of urban influence. Moderate level of the development in the rest of the tehsils is noted. There is a probability of these tehsils to move towards the higher values of composite index in the present-day scenario of urban expansion. In agricultural sector, Alibag with the lowest composite index (0.58) assumes the status of model tehsil for all the tehsils in the study area.

iii. High level of Infrastructural Development is found in Thane, Ratnagiri, Chiplun, Vasai, Kalyan, Panvel, Khed and Palghar.Whereas, Mandangarh,Sudhagad, Uaran, Ulhasnagar, Murud, Shrivardhan, Doddamarg, Vaibhavwadi, Poladpur, Mhasala and Talaare low developed tehsils of the region. With least variations in the developmental levels of the tehsils the rest of the tehsilshave moderate level of infrastructural development. In infrastructural development, Thane is the model tehsil for all tehsils which is the highly developed tehsil in the study region having composite index 0.49.

iv. Overall development is positively associated with both agricultural development and infrastructural facilities. The impact of infrastructural facilities on overall development is higher than the agricultural development.

v. Wide disparities in the levels of development has been observed in different tehsils with respect to the developmental levels of agriculture, infrastructure and demographic.

vi. Better Development tehsils are found to be thickly populated as compared to low developed tehsils.

vii. Agricultural development along with the better avenues for education, medical facilities and transport systems will enhance the level of overall development

viii. In order to reduce the disparities in development among different tehsils, potential targets of various important developmental indicators are estimated for low developed tehsils. These tehsils required improvements of various dimensions in different indicators for enhancing the level of development.

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Qualitative study of Zooplankton fauna of Satara Bhosale and Satara Tukum Lakes of Pombhurna Tehsil in different Seasons

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ABSTRACT

The present research paper deals with the zooplankton fauna composition in two different fresh water lakes Satara Bhosale and Satara Tukum located in Pombhurna tehsil of Chandrapur district in Maharashtra state, during the period Feb 2016 to Jan 2017 in three different seasons. The studies were focussed on qualitative aspect of zooplankton during 3 different seasons viz. summer, winter and monsoon at different sampling locations. In satara Bhosale lake 34 different species were recorded while in Satara tukum lake waters there are 38 different species thriving. The recorded groups of zooplankton belonged to Protozoa, Rotifera, Cladocera, Copepoda and Ostracoda. The beautiful biodiversity of the animal fauna is represented through these diverse zoopanktonic forms. Through these qualitative studies a beautiful picture of biodiverse zooplanktonic forms of nature emerge on which the world of fishes sustain.

Keywords: Zooplankton, Satara Bhosale, Satara Tukum, Lakes, Qualitative study, Seasons.

I. INTRODUCTION

Water is an essential component for all living organisms. The freshwater ecosystems of the world include pond, lake, river and dams which conserve the nature and other living organisms. Aquatic ecosystems are known to support a wide range of living organisms. Among these zooplankton are the free floating and microscopic animals found in aquatic ecosystem. The zooplankton are important link for fishes as they are used as source of food for life. Zooplankton play an important role in water purification and serve as bio-indicators of water quality (Gannon and Stemberger, 1978; Gajbhiye and Desai , 1981). Abundence of zooplankton depends on the availability of bacterio-plankton and phytoplankton as food.

The zooplankton are broadly classified in various groups as Protozoa, Cladocera, Copepoda, Rotifera and Ostracoda. Many Researchers have studied various aspects of the zooplanktons of water bodies both in India and abroad. Zooplankton are playing important role in biomonitoring of water pollution.

The Zooplankton community fluctuates according to Physico-chemical parameters of the environment and the abundance and composition of zooplankton depends upon the characteristic of water bodies. During last 15 years Indian studies on zooplankton are done by Sehgal *et* al., (2013), Sharma (2007), Thilak (2009), Sharma and Thilak (2000) , Thirupathaiah *et* al (2012), Pawar and Pejawar (2014), Mahajan and Harney (2016), Sarwade and Kamble (2014), Suresh *et* al (2009), Jadhav *et* al (2012), Kadam and Tiwari (2012), Jeelani *et* al (2005), Kamble *et al* (2013), Dede

and Deshmukh (2015), Sitre and Thakare (2013), Joshi (2011), Kumar (2001).

As no previous studies were done by any of the researchers on these two fresh water bodies the present research was undertaken in order to

II. MATERIAL AND METHODS

Study Area

The lakes of Satara Bhosale and Satara Tukum are freshwater perennial lakes located in village Satara Bhosale and Satara Tukum in pombhurna tehsil of Chandrapur district in Maharashtra state (Fig. 1 and 2). The catchment area of the satara bhosale lake is 34 acres while that of Satara tukum is 39 acres. The water of both the lakes is perennial and is utilized for irrigation, washing purpose as well as for pisciculture activities. A large number of major and minor carps are present in waters of both the lakes.

The sampling of zooplankton in satara bhosale and satara tukum lakes was carried out for a year span. Water samples were collected in morning hours between 8 am to 10.00 am every month. The data was recorded seasonally as summer, winter and Monsoon. The zooplankton samples were collected by filtering 50 to 60 litres of water through plankton net made up of bolting silk cloth no.22 and collected samples were fixed in 4% formalin. The qualitative analysis of the organisms is carried out using microscopic study. Samples were examined under the microscope in 10x and 3.2X magnification for identification of zooplankton. The Zooplankton are identified with the help of standard literature up to generic level by using standard keys of Edmondson(1963), Pennak (1978) , Dhanapathi (2000) and APHA (2005).

Figure 1. (a) Satara Tukum Lake

Figure 1. (b) Satara Bhosale Lake

III. RESULT AND DISCUSSION

The present research work reports the zooplankton diversity composition from the lake of village satara Bhosale and Satara Tukum of Pombhurna tehsil of chandrapur district. In total of 34 different species of zooplankton were found in Satara bhosale lake and 38 speceis in satara Tukum lake. The species belongs to rotifer, copepod, protozoa, cladocera and ostracoda groups in both the lakes. According to diversity, Rotifers indicated maximum diversity during the

study period followed by protozoa, cladocera, copepod, ostracoda. Occurrence of indicator species like *Filinia longiseta* and *Brachionus forficula* points out that the lake ecosystems are getting organically enriching due to man made activities.

In Satara Bhosale lake of Pombhurna tehsil of chandrapur district 10 different species of protozoa are observed, 10 differernt species of rotifera are obsereved, 9 different species of cladocera are

observed, 4 different species of copepod and 1 species of ostracoda are observed and recorded (Table 1).

In Satara Tukum lake of Pombhurna tehsil of chandrapur district 12 different species of protozoa are observed, 11differernt species of rotifer are obsereved, 9 different species of cladocera are observed, 4 different species of copepod and 2 species of ostracoda are observed and recorded in one year span

(Table 2) . The total recorded forms are show in Table No.3.In Satara Bhosale only one type of Ostracod was observed *Heterocypris sp.* While there are 2 different forms present in Satara Tukum lake The zooplankton communities respond to a wide range of changing environmental conditions like nutrient input, acidification, sediments and have an immense significance in fisheries sector (Jhingran, 1991). The rotifers have long been identified a indicators of water quality (Arora, 1962). Due to short life cycles rotifers respond quickly to changing environmental conditions and their species composition and standing crop indicates the quality of water in which they are thriving (Chandrasekhar and Kodarkar, 1995; Dhanpathi 1974 b).

In any aquatic ecosystem limnological characteristics can affect both fauna and flora. Biodiversity contribute both directly and indirectly to human needs like food. In last decade people interfere with ecosystem and over exploitation of natural resources resulting in that biodiversity decrease. Biodiversity of zooplankton in lake of satara Bhosale and Satara Tukum. Clearly show that both lake are rich in biodiversity of zoo plankton and need conservation for future generation.

IV. CONCLUSION

In the present research study a total 34 zooplankton were recorded in Satara Bhosale lake and 38 zooplankton were recorded in Satara Tukum lake classified by protozoa, rotifer, cladocera, copepod and ostracoda. Maximum species found in Satara Tukum lake showing pollution.

V. AKNOWLEDGEMENT

Table. 1. Species composition of Zooplankton recorded in lake of Satara Bhosale lake during different seasons.

Sr. No.	Group	Species	Season		
			S	M	W
1	Protozoa	Amoeba radiosa	+	-	-
2		Arcella discoides	+	-	+
3		Arcella vulgaris	+	-	+
4		Chilodonella sp.	+	-	+
5		Difflugia lobostoma	+	-	+
6		Difflugia pyriformis	+	-	+
7		Actinophyris sol.	-	+	+

			S	M	W
8		Centyropyxis sp.	+	-	+
9		Paramecium sp.	+	+	+
10		Vorticella sp.	+	-	-
11	Rotifera	Brachionus calyciflorus	+	-	-
12		Brachionus falcatus	+	-	-
13		Brachionus caudatus	+	+	-
14		Brachionus forficula	+	-	-
15		Filinia longiseta	+	+	-
16		Keratella tropica	+	-	+
17		lecane bulla	+	+	+
18		Polyarthra vulgaris	+	-	+
19		Trichocerea rattus	+	+	+
20		Rotaria neptunia	+	-	+
21	Cladocera	Alonella nana	+	-	+
22		Bosmina longirostris	+	-	+
23		Chydorus sphaericus	+	+	+
24		Ceriodaphnia sp.	+	-	+
25		Macrothrix rosea	+	-	+
26		Moina dubia	+	+	+
27		Alonopsis sp.	+	+	+
28		Diaphanosoma sarsi	+	+	+
29		Simocephalus exspinesus	+	-	+
30	Copepods	Copepod nauplius	+	+	+
31		Cyclops sp.	+	-	+
32		Diaptomus	+	+	+
33		Mesocyclops leucarti	+	-	+
34	Ostra coda	Heterocypris sp.	-	+	-

S= Summer, M= Monsoon, W= Winter

Table. 2: Species composition of Zooplankton recorded in lake of Satara Tukum lake during different seasons.

Sr. No.	Group	Species	Season		
			S	M	W
1	Protozoa	Arcella discoides	+	+	+
2		Arcella vulgaris	+	+	+
3		Centropyxis aculeate	+	-	+
4		Chrysamoeba sp.	+	+	+
5		Difflugia corona	+	-	+
6		Difflugia pyriformis	+	+	+
7		Paramecium bursaria	+	-	+
8		Paramecium caudatum	+	12+	+
9		Pelomyxa palustris	+	-	+
10		Spathidium spathula	+	-	+
11		Urocentrum turbo	+	-	+
12		Vorticella campanula	+	-	+
13	Rotifera	Brachionus falcatus	+	+	-
14		Brachionus forficula	+	-	-
15		Brachionus quadridentatus	+	-	-
16		Filinia longiseta	+	+	+
17		Horaella brehmi	+	-	-
18		Keratella sp.	+	-	-
19		Monostyla bulla	+	-	-
20		Lecane sp.	+	+	+
21		Lecane bulla	+	+	+
22		Platyias quadricornis	+	+	+
23		Rotaria neptunia	+	+	+
24	Cladocera	Alonella nana	+	-	+
25		Bosmina longirostris	+	-	+
26		Chydorus sphaericus	+	+	+
27		Ceriodaphnia	+	+	+
28		Macrothrix rosea	+	-	+
29		Alonella sp.	+	-	+
30		Moina dubia	+	-	+
31		Sida crystalline	+	+	+
32		Simocephalus sp.	+	-	+
33	Copepoda	Copepod nauplius	+	+	+
34		Cyclops sp.	+	-	+
35		Diaptomus forbesi	+	+	+
36		Mesocyclops leucarti	+	-	+
37	Ostra coda	Cypris sp.	+	+	-
38		Stenocypris sp.	+	+	-

S= summer, M= Monsoon, W= Winter

Table 3. Total Recorded Forms in Both the Lakes in a Year.

Sr. No	Group	S.B.	S.T.
1	Protozoa	10	12
2	Rotifera	10	11
3	Cladocera	9	9
4	Copepoda	4	4
5	Ostracoda	1	2
	Total Forms Recorded	34	38

S.B-Satara Bhosale Lake , S.T. = Satara Tukum Lake
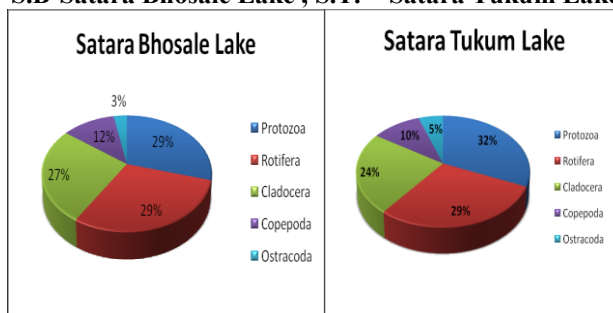
Figure 3. Showing zooplankton species in both lake.

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Geo-strategic importance of Hambantota Port in the Indian Ocean: A Geo-political Analysis

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ABSTRACT

The paper examines the impact of Hambantota port of Sri-Lanka on the geo-political dynamics in the Indian Ocean. Emerging powers like India and China want to control this port to expand their influence in the Indian Ocean. China is among the very few nations in the world which has used geo-politics for its geo-strategic and commercial advantage. It has established a dynamic relationship with many Afro-Asian countries to achieve its aim to become the subsequent superpower. These diplomatic steps precipitating from trade and geo-political ambitions, many a times claimed as a diplomatic and trade inevitability by China, allows it to bargain for the significant infrastructure projects in other countries to sustain its economic growth, and to spread its sphere of influence by investment agreements with the Afro-Asian countries. This aggressive policy of China devoided the competing and neighbouring nations strategic depth in the Indian ocean and African continent. China's current relationship with the world has significantly changed from what it was in the early 1960s, when its boundaries were bordered by its adverse countries, the friends with either the former Soviet Union or United States of America. It was after the India-China 1962 war and the subsequent Indo-Soviet strategic agreement, China chalked out a strategy to contain its potential adversaries through geo-political containments. India on the other hand too, as an emerging nation is trying to reclaim its historic influence in the Indian Ocean. Also, it is converting its brown water navy into a blue water one to protect its economic and security interests.

Keywords: Port, Geopolitics, Indian Ocean

I. INTRODUCTION

Sri Lanka has historical ties with India, the country even being referred as Lankapura in India's mythological texts. The two countries gained freedom almost at the same time from the colonial rule. The later Sri Lankan Civil War and the 13th Amendment brought the two countries closer but also created suspicion between them.

The Hambantota Sea Port of Sri Lanka is very important according to military and civil importance for Sri Lanka, and also the development of this sea port may have geo-economic impact on India and the Indian Ocean. Sea port of Hambantota is planned to develop as a service and industrial port. Hambantota is one of the lowest per capita income regions in Sri Lanka. Thus, the construction of the port will be an important catalyst for a major economic development in Sri Lanka and further it will reduce the prevailing unemployment percentage in the Hambantota region. This port is in the Hambantota District, Southern Regions of Sri Lanka, where the former president of Sri Lanka M. Rajapaksha was elected.

In view of the deeper berth and location advantages at Hambantota, it may be possible to attract most of the port related industries here. Since the maximum draft at Colombo is about 10 minutes for general cargo vessels, manufactures may invest in Hambantota to get the advantage on economies of scale.

Sea-ports are backbone of trade; and play a key role in economic development between India and Sri Lanka, but China got a big chance through developing Hambantota as a sea port.

Background of study:

Since ancient times ports have played an important role in the Indian Ocean, connecting the Indian Sub-continent to the other continents through sea lanes. Many kingdoms of the Sub-continent with coast-lands had well equipped ports which developed as thriving trade centers. Later, the process of colonization of the sub-continent was possible mainly through ports.

Since the Second World War, a new geopolitical order is shaping up in the Indian Ocean Region. Through the decolonization process that began on the shore of the Indian Ocean as early as 1947-48 with the independence of India, Pakistan, Sri Lanka and other countries, the people of the region have regained political control over their respective territories. In the 1960s, the old colonial order was definitively replaced by a new order which we suggest to call an 'Indian Oceanic Order'.

The Indian Oceanic Region, at the end of the Cold War founded expression in a much greater autonomy for States in regards to their International relations, allowing to develop ties with all neighbours as well as with other States. So countries like Sri-Lanka could develop ports like Colombo and Hambantota (now called Magmapura Rajapaksa Port) with the help of other countries in lieu of strengthening bilateral relations. The necessity of other countries can turn into opportunity for these countries in increasing their geopolitical importance.

Sea Ports are the back bone of world trade, and play a key role in the inward and outward movements of goods. Countries who build good sea ports with excellent infrastructure become competitive manufacturing hubs. So it will be important to see how the present and future world geopolitics will be influenced by this geostrategic region, and the role the sea ports will play in it. So far there has been no specific study done on this area where geostrategic importance of ports have been highlighted.

The Indian Ocean has been of great strategic importance for India. The "land locked" nature of the Indian Ocean has given India a commanding position. From the eastern coast of Africa and the shores of the Persian Gulf to the Strait of Malacca, no other country rivals India's dominant location in the Indian Ocean. The strategic importance of this Ocean is further enhanced by the fact that it is accessible from the west and the east through narrow straits only. The Red Sea and the Persian Gulf are the narrow outlets in the west while in the east; there are the Strait of Malacca and the Timor and Arafura Sea.

The Indian Ocean has limited outlets. Before the opening of the Suez Canal in 1869, the only contact of littoral states of the Indian Ocean with the western Countries was via Cape Hope by circumnavigating the whole continent of Africa. On the eastern side hence are two outlets, one through islands of Indonesia and second is the South Australia. Indian Ocean can be choked any time by controlling these outlets. Since the Indian Ocean and the countries surrounding it are very rich in natural resources, such a possibility has considerably enhanced the geopolitical strategy of this Ocean.

In spite of above mentioned geopolitical limitations, the Indian Ocean has never been a barrier between the countries. On the other hand it has served as a

linkage between the countries lying on its coasts and even further beyond. It has bridges the gap between east and west. It is encircled by 46 countries , 27 littoral including Australia, 7 islands countries and 12 landlocked countries as recognized by the United Nations.

India and Sri Lanka are separated from each other by a narrow and shallow sea called Palk Strait. Dhanush Kodi on the Tamil Nadu Coast in India is only 32 k.m. away from Talaimannar in Jaffna Peninsula in Sri Lanka. These two points are joined by a group of islands forming Adam's Bridge. The northern and north eastern parts of the islands have large number of Tamils who migrated from Tami Nadu to that country.

The maritime boundary between India and Sri Lanka passes through Palk Strait, touching Dhanushkodi. This boundary has remained peaceful barring a few minor clashes between the fishermen of the two countries over the fishing rights. Some bitterness was created over the ownership of kachchitevu Island (area 1.92 s. k. m. from the Tamil Nadu Coast). The problem was resolved with the demarcation of India and Sri Lanka boundary line. This Island was given to Sri Lanka as a result of agreement 1974. The maritime boundary between India and Sri Lank become lively in 1980's with insurgents demanding a separate homeland for Sri Lankan Tamils within the Island.

INFRASTRUCTURE ASPECTS OF PORT OF HAMBANTOTA

Table 1. Main port parameters (Phase II)

1	Design Vessel	100,000 DWT
2	Approach Channel width	21 OM
3	Eastern Break Water	12 M
4	Western Break Water	958 m
5	Turning Circle	600 m
6	Quay Length (Genre Cargo)	600 m
7	Service Quarry	105 m

8	Oil Quarry	610 m/ -17m depth

The Phase-I officially commenced on 15th January 2008 and project is successfully completed. Total construction cost of the project is US $ 501 million and jointly funded by the Ex-Im bank of the peoples Republic of China and Sri Lanka Ports Authority.

Table 2

1	Design Vessel	100,000 DWT
2	Main Container Berth	838.5 M/ -17 M
3	Feeder Container terminal	460 m/ -12 m
4	Multipurpose Berth	838.5 M/ -17
5	Transition Berth	208 M
6	New Oil Terminal	3oo M
7	Harbor Basin	600 M / -17
8	Yard Area (to be completed)	65 Ha.

The phase II was officially commenced on 15th November 2012 and project is completed.

Banking Facility and Tank Farm project

The Banking facility and tank farm at Hambantota has been constructed approximately 1.2 km away from the water front and is connected to the Oil terminal through a pipe line. This facility consist more than 14 tanks with a total storage capacity of 80,000 m^3 fuel and individual, storage capacity of 51,000 m^3 for banker fuel; 23,000 m^3 for aviation fuel and 6000 m^3 for LPG. Apart storing the tank farm comprises of oil blending facility, fuel testing facility, firefighting equipments, truck loading facility etc. Both loading terminals are fitted with flow meters for each product to ensure accuracy of measurement. The project was completed and bunking operations commenced on June 2014.

Present Status:

In order to expand bunker business it has been decided to lease out the facility to an International

bunking service provider. In this regard, calling for expressions of intervals will be carried out in the near future.

The port of Hambantota have approximately 800 hectare (8 km^2) of ample lands. An Industrial zone, is the one of the biggest port related Industrial zone in the south Asian region. The port of Hambantota is basically planned to operate as a Green port as well.

Sri Lanka on 9th December 2017 formally handed over the strategic southern port to China on a 99-year lease.

II. METHODS AND MATERIAL [Page Layout]

The following methodology will be used to analyse Geo-strategic importance of Hambantota port in the Indian Ocean.

Analyzing the long term strategic importance of Hambantota port, the data related to export and import, the connected routes, the frequency of transportation, total input and output of the port, cargo handled in a year, number of vessels visits in a year, service rendered as a port of call etc. will be collected.

The study will also analyze the socio-political impact of the port.

OBJECTS OF STUDY

To study India's stake in the Indian Ocean and the importance of Hambantota port according to Indian perspective.

1. General Study of Importance of Ports in World Geopolitics:

World Geopolitics has become very intense in recent years and Geo-strategic ports have become immensely important to control maritime trade, exploitation of ocean resources and for naval dominance. This is also true in the Indian Ocean where emerging powers like India and China are in race for a stronger foot hold.

The Indian Ocean has great strategic importance for India. The "Land Locked" nature of the Indian Ocean has given India a commanding position from the Eastern Coast of Africa and the shores of the Persian Gulf to the Strait of Malacca. No other country rivals India's dominant location in the Indian Ocean. But in real sense the big world powers are expanding their strategic weapons in the Indian Ocean for their world interest and it is a challenge for India. How finely it is tackled by India, is an important study factor of this thesis. So the study of development of Hambantota in the Indian Ocean is a part of this view.

2. Study of Hambantota Sea-port and to predict its geo-political ramification:

When we see the geomorphological characters of Southern India and Sri Lanka, the Hambantota Sea-port of Sri Lanka is very important according to military and civil importance for Sri Lanka. Also, the development of this Sea-port may have geo-political impact on India and the Indian Ocean. Sea-port of Hambantota is planned to develop as a service and industrial port. Hambantota is one of the lower per capita income regions in Sri Lanka. Thus, the construction of a port in Hambantota will be an important catalyst for a major economic development in Sri Lanka and also it will reduce the prevailing unemployment percentage in the Hambantota region.

In view of the deeper berth and location advantages at Hambantota, it may be possible to attract most of the port related industries. Since the maximum draft at Colombo is about 10 mile for general cargo vessels, manufacture may invest economy of scale.

So the relation of Kochi Sea-port of India and one of developing a sea-port in Tamil Nadu near Kanyakumari in India and the Colombo and Hambantota sea-port are very important backbone of trade, and play a key role in economic development between India and Sri Lanka. But China got a big opportunity to develop Hambantota as a sea-port. The involvement of Chinese companies in the

development of Hambantota port have been claimed by some analysts that it is part of China's "String of Pearls strategy". Other analysts have argued that, it would not be in Sri Lanka interests to allow the China's navy's access to the port and in any event the exposed nature of the port would make it of dubious value to China in time of conflict.

ATTITUDE OF THIS RESOURCE STUDY

Here attitude means a complete mental state involving benefits, values and dispositions to act in a certain ways.

In this resource study the conceptual basis of geopolitics in its embryonic form can be traced back to a handful of pioneering works of Alfred Thayer Mahan, Friedrich Ratzel, Halford Mackinder, James Fairgrieve and Rudolf Kjllen. Subsequently, Nicholas Spykman and Alexander P. de Seversky also contributed their thoughts to both Mahan and Mackinder and advanced what seemed to be contrary views on the relative importance of sea power and power for global dominance. In his perspective on classical mercantilism and the clash of nations competing for maritime trade, Mahan advocated sea power as the path to national greatness. He was hopeful of the recurrence of the phenomenon in the twentieth century.

When we see the physical geographical area of Southern India and Sri Lanka it can be seen that Hambantota Sea Port of Sri Lanka is very important according to military and civil importance of Sri Lanka. Sea Port of Hambantota is planned to develop as a service and industrial port.

On the other hand, construction of the port in a dense vegetative region have raised environmental concerns. There was blasting of rocks on the continental shelf to deepen the originally shallow shelf for passage of large vessels. This has caused huge damage to the ecosystem. Many environmental NGO's therefore have opposed and demonstrated against the project. Since the construction of ports, local fishermen are prohibited

to catch fish here. It has created problems for their traditional livelihood.

International aspect and Geo-strategic importance:

Thayer Mahan had stated about a century ago that whoever controlled the Indian Ocean would dominate Asia that this Ocean was the key to seven seas in the 20th century. For that is why the all world power like U.S.A., Russia, China and others are strengthening their sea powers. In the recent visit of U.S. President Barak Obama to India, on the event of Republic day on 26th day of January 2015 as a chief guest, both leaders, Indian Prime Minister Modi and Obama declared the strategy of the countries having access to the Indian Ocean. Country that dominates the sea has a decisive advantage over its rivals because:

- ✓ It can control the maritime trade which is assuming added importance.
- ✓ It can facilitate easy transportation of its troops to fight in any littoral area and thus dominate it.
- ✓ It can use its Navy for blockade and for short and long bombardment and for landing troops and material at a given point and time. If pressed the enemy can even be retreated to the high seas.

So here we can see that, because of the competition with U.S.A. and India, China is planning to increase its influence in the Indian Ocean as a sea power. If India desires to become a strong sea power in the Indian Ocean, it should have control of strategic ports. World Geopolitics have become very intense in recent years and geostrategic ports have become immensely important to control maritime trade; exploitation of ocean resources and for naval dominance.

Sea Ports are backbone of world trade and play a key role inward and outward movement of goods. Countries which build good sea ports with excellent infrastructure become industrial and trade hubs. The passage of sea lanes of oil has attracted the attention of U.S.A. and its allied NATO nations in the Indian

Ocean. India too wants to secure her interest by preparing to play a more pro-active role.

One of the important player, but still considered small country in the Indian Ocean is the Island of Sri Lanka whose strategic location in the Indian Ocean with important ports makes it a potential ally in the Indian Ocean. It's very close proximity to India has increased its importance from India perspectives.

III. CONCLUSION

So my opinion is that, time has come for India to develop a parallel port in the Indian peninsula to balance the new Geopolitical paradigm in the Indian Ocean.

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Synthesis of Plant Growth Promoter (Natural Liquid Fertilizers) from Medicinal Plant By Fermentation Method

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ABSTRACT

A chemical based plant growth promoter normally affect particular organ or function of plant, whereas a fermented product show effect on the total plant system helping it to achieve better results as compared to a chemical plant growth promoter and reduce the side effects on the soil or the overall plant growth system. Plant growth promoters could be harmful for human health if not applied in adequate doses and convenient time on plants. In present investigation leaves of various medicinal plant e.g. Ricinus communis (Caster), Annona squamosa (Custard apple), Tinospora cordifolia (Gulvel), Azadirachta indica (Neem Tree), Nicotiana tobacum (Tobacco) taken in equal proportion and fermented for one month in cow urine (Gomutra). Filtered it and filtrate was taken as end product. Different concentrations (5, 10, 30 and 50 µl/ml) of fermented product were applied to Mung (Vigna radiata) seed germination. Growth of seeding was compared with control. Result showing that lower concentration increase seed germination and higher concentration decrease seed germination than control (Without treatment). We can conclude that this product can be use as growth promoter at low concentration at germination level.

Key words: Growth promoter, Vigna radiata, seed germination

I. INTRODUCTION

In recent years, use of continuous chemicals and intensive cultivation has been reduced soil organic materials and micronutrients. It is approved that continuance of these conditions lead to loss of biological diversity, agro-ecosystem disorder and destruction of soil structures. Plant active principles are chemical compounds present in the entire plant or in specific parts of the plant that confers them therapeutic activity or beneficial effects (Martins *et al.*, 2000) . Hence use of this plant property to produce herbal growth promoter is eco-friendly and cost effective. In recent years, Thai farmers mostly used fermented plant extracts (FPEs) as natural liquid fertilizers because it can easily produced from agricultural products or

agricultural waste. These FPEs can promote plant growth and act as bio-control agents depending on the type of plants being used (Kantachote and Charernjiratrakul, 2008). Since FPEs are useful to reduce problems associated with the use of chemical fertilizers and pesticides, they are now being widely applied in organic agriculture, natural farming and IP farming.

However, there is very little scientific information to support the use of FPEs. FPEs are product of lactic acid fermentation and most of the available scientific information is concerned with the production and use of them as beverages (Kantachote and Charernjiratrakul, 2008; Prachyakij et al. 2008).

Habitats and some physiological properties of Lactic acid bacteria (LAB) and yeasts are confirmed that normally found in fermented plant products (Oboh, 2006; Okada et al. 2006; Olstorpe et al. 2008). We therefore thought of the possibility that an FPE from leaves of medicinal plant might be useful as a potential liquid fertilizer and could assist farmers because it would be easy and cheap to prepare and make use of what is at present a common but non utilized resource. Hence, the aims of this study were to investigate effect of fermented pant extract on seed germination

AIMS AND OBJECTIVES

To observe effect of fermented plant extract as Growth promoter/ liquid fertilizer

II. MATERIAL AND METHODES

• Synthesis of plant Growth Promoter (Natural Liquid Fertilizers):

Leaves of various medicinal plant e.g. *Ricinus communis* (Caster), *Annona squamosa* (Custard apple), *Tinospora cordifolia* (Gulvel), *Azadirachta indica* (Neem Tree), *Nicotiana tobacum* (Tobacco) taken in equal proportion (250gm) and fermented for one month in cow urine (Gomutra). Filtered it and filtrate was taken as end product.

• Effect of plant Growth Promoter (Natural Liquid Fertilizers)on seed germination:

Phytotoxicity can assess by a seed germination assay, it is one of the most common techniques (Kapanen and Itavaara, 2001). The graded concentrations (5,10,30 and 50 μl/ml) of fermented plant extracts

(FPEs) were added aseptically to sterilized petriplates lined with Whatman no. 1 filter paper. Surface sterilized seeds of Mung were germinated (20 seed per plate) in each concentration of PEFs. Similar experiment without PEF was conducted as control. Distilled water was used as a control set for the testing of seed germination (Hoekstra et al. 2002; Fuentes et al. 2004). After 10 days of treatment, seedlings were harvested and shoot and roots of seedling were separated. Seedling growth in terms of root length, shoot length, fresh weight and dry weight were recorded and results were compared to see effect of PEFs on seed germination and early seedling growth.

III. RESULTS AND DISCUSSION

Results pertaining to seed germination and early seedling growth clearly indicate that PEFs at lower concentration promoted seed germination and seedling growth, but at higher concentration reduced seed germination and seedling growth. Lower concentrations of PEFS (5,10 μl/ml) showed significant enhancement in shoot and root lengths, however higher concentrations (50 μl/ml) of PEFs showed decreased root length, shoot length and total seedlings height. There was no major difference in root shoot ratio in all treatments however an decreasing trend was seen from lower to higher concentrations. Similar trend as in seedling height was seen in case of fresh and dry weight of the seedlings after PEFs treatments (Table. 1).

Table 1. Effect of Fermented product on seedling growth in Mung

Concentration(μl/ml)	Shoot Length (cm)	Root length (cm)	Total seedlings Height (cm)	Root-Shoot Ratio	Fresh Wt. (mg)	Dry Wt. (mg)
00	3.05	1.76	4.81	0.58	529.12	73.38
05	4.86	2.48	7.36	0.51	676.28	90.44
10	6.45	2.88	9.33	0.45	768.92	112.38
30	5.85	2.40	8.25	0.41	698.08	101.12
50	230	0.93	2.66	0.40	356.26	41.42

Fruits and vegetables are a rich source of B (Bellaloui and Brown, 1998). Fermentation process has provided a relatively high amount of plant nutrients particularly B, therefore after an appropriate dilution the PFEs may be a suitable liquid fertilizer. It has long been recognized that GAs play an important role in the stimulation of seed germination (Chen et al. 2001), thereby the FPEs may also induce seed germination. Other plant nutrients particularly P, Mg, Mn and B may also be present at appropriate concentrations to stimulate seed growth (Bellaloui and Brown, 1998). The numbers of lactic acid bacteria (LAB) and yeasts that were present during a wild forest noni (Morinda coreia Ham) fermentation, the changes in its physicochemical properties and levels of plant nutrients were investigated (Duangporn K e*t al.*2009) .Higher concentration decreases growth there may be increased concentration of citric acid. Results indicate that plant nutrients present in the FPE were at an appropriate level for potential use as a liquid fertilizer, particularly for the micronutrients such as B, Mn and Zn.

IV. CONCLUSION

From this project, we can be concluded that fermented plant extract can increase seed germination at lower concentration can use as herbal growth promoter or liquid fertilizer and at higher concentration decrease growth of seedlings can used as herbicide. Based on these results and because of high chemical herbicide and growth regulator costs for farmers, herbal growth promoter and herbicide can be replaced by them. In addition to, they had not any environmental pollution and risks for human being

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Microbial degradation of Dimethoate and Parathion

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ABSTRACT

Organophosphorus pesticide is a group of pesticides which inhibit the enzyme cholinesterase and hence cause neurological disorders in insects, humans and pest. This group includes Dimethoate, Parathion pesticides which are commonly used in agriculture. Present study includes degradation of these pesticides using soil isolates, so that an adverse effect of these pesticides on humans will be decreased. In this study, primary screening and secondary screening was carried out to isolate the organisms from the soil which can degrade these pesticides. In primary screening, soil samples were collected from the fields where these pesticides are commonly used. Ninety six isolates were obtained on Minimal medium which contains pesticide as a sole source of carbon. These isolates include both bacteria as well as fungi. In secondary screening, different pesticide concentrations were tested, four isolates could efficiently degrade maximum upto 19gm% of pesticide. Biochemical tests and online gene based tools BLAST and FASTA were used for identification of bacterial isolate. The biodegradation of Dimethoate was found out to be plasmid mediated.

Keywords: Organophosphorus pesticide, Primary screening, Secondary screening, Plasmid mediated

I. INTRODUCTION

Pesticide is a substance or mixture of substances intended for preventing destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal foodstuff, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.[3]

Pesticides can be classified by target organism, chemical structure, and physical state. Pesticides can also be classed as inorganic, synthetic, or biological (biopesticides), although the distinction can sometimes blur. Biopesticides include microbial pesticides and biochemical pesticides. Plant-derived pesticides, or "botanicals", have been developing quickly. These include the rotenoids, nicotinoids, and a fourth group that includes strychnine and pyrethroid scilliroside.

Pesticides can be classified based upon their biological mechanism function or application method. Most pesticides work by poisoning pests. A systemic pesticide moves inside a plant following absorption by the plant. With insecticides and most fungicides, this movement is usually upward (through the xylem) and outward. Increased efficiency may be a result.

Systemic insecticides, which poison pollen and nectar in the flower, may kill bees and other needed pollinators.

Subclasses of pesticides include herbicides, insecticides, fungicides, rodenticides, pediculicides and biocides.

Most organophosphorus pesticides are used in agriculture and they are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid. [5] Organophosphorus pesticides are highly toxic and easily absorbed through the skin. Poisoning may also occur through the mouth. There are many effects when inhaled. The first effects are usually respiratory and may include bloody or runny nose, coughing, chest disorder, difficult or short breath. These may include vomiting, Diarrhea, abdominal cramps, headache, eye pain &blurred vision. Severe poisoning will affect the central nervous system lack of co-ordination & eventually paralysis of the body extremities & respiratory muscles.

Despite their high toxicity, Dimethoateand Parathion pesticides are still extensively used all over the world for its broad spectrum of action. Dimethoate and Parathion inhibit the enzyme choline esterase which is required for normal functioning neurotransmitter and hence cause severe neurological disorder in humans. [2]

Due to its toxicity, it is important to remove Dimethoate and Parathion from environment. A variety of physical & chemical methods are available to treat soil, contaminated with hazardous material. But many of these physico- chemical treatments do not actually destroy hazardous compounds so the role of microorganisms in bioremediation is important because of their ability to degrade hazardous compound into harmless ones. For microbial degradation,the target pesticide will be able to serve as the sole carbon source & energy for micro-organism.

II. MATERIALS AND METHODS

A. **Sample Collection**: Soil sample were collected from the fields of five different locations in Maharashtra namely, Theur, Lonikalbhor, Shirval, Urulikanchan. Dimethoate and Parathion pesticides are commonly used in the fields of these areas.

B. **Pesticide used**: Dimethoate (Anu Products Pvt. Ltd.) and Parathion.

C. **Primary screening**: Soil samples collected are diluted in sterile distilled water upto10^{-9}.Minimal medium with 1gm% of respective pesticide was used for spread plate technique. Plates were incubated at 37^0C for 48-72 hours.

D. **Secondary screening**: Individual isolate obtained at primary screening was inoculated on minimal medium with different pesticide concentration(1gm% to 30gm%). Plates were incubated at 37^0C for 48-72 hours.

E. **Identification of bacterial isolates:** Based on colony characters and biochemical tests bacterial isolates were identified [6]. Gene sequence of isolated bacteria was obtained from Geneome Bio Pvt. Ltd., Pune. BLAST and FASTA, these online tools were used for complete identification.

F. **Isolation of plasmid:** Plasmid isolation using miniprep system was performed on the isolate showing maximum pesticide degradation [7].

III. RESULT AND DISCUSSION

At primary screening 96 isolates were isolated which could grow on the Minimal medium with 1gm% of pesticide. These isolates include both fungal and bacterial isolates.

At secondary screening four isolates were isolated which could degrade 19gm % of pesticide. This is the maximum concentration of the pesticide which can be degraded. Above this concentration no growth was observed.
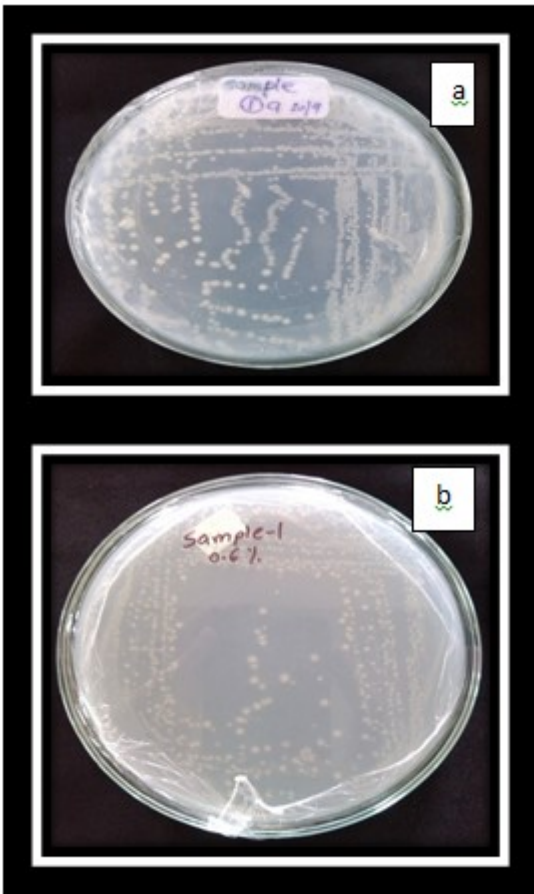
Figure 1. At secondary screening, different concentrations of pesticides were tested. 1.a shows the growth of bacterial isolate on minimal media containing Dimethoate. 1.b. shows the growth of bacterial isolate on minimal media containing Parathion.

According to the results (Table 1), and referring to Bergey's Manual of Determinative Bacteriology 8th Edition, the isolated organismmay belong to *Pseudomonas spp.*

Table1. Biochemical tests with the results

Test performed	Results
Nitrate reduction	Positive
Indole test	Negative
Glucose fermentation	Positive
Catalase test	Positive
Oxidase test	Negative

According to BLAST & FASTA organisms may be *Pseudomonas spp.*A1113 Figure 3, shows the FASTA results of the bacterial isolate .
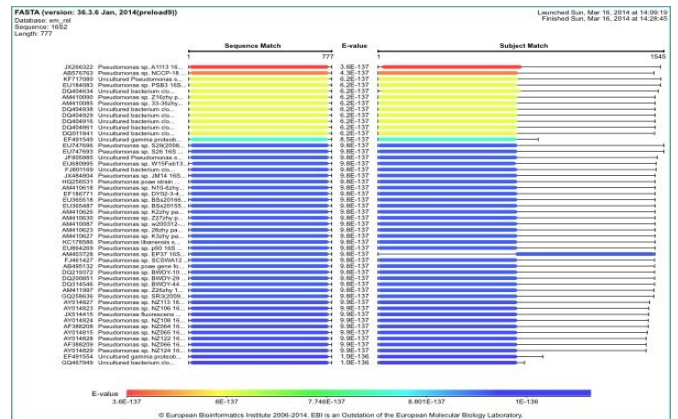
Figure 2. FASTA result shows that the E-value is minimum for *Pseudomonas spp.*A1113.Hence, the gene sequence obtained matches maximum with *Pseudomonas spp.*A1113.

Dimethoate and Parathion both are organophosphorus pesticide which has approximate half-life as long as 206 days at 25[0]C if not degraded[2]. On the basis of the results presented here, we propose that microbes can degrade organophosphorus pesticides efficiently. Isolates obtained can degrade these pesticides more rapidly. *Pseudomonas spp.*A1113 use these pesticides as sole source of carbon and hence grow on the minimal medium and hence can be used for decontamination of pesticide polluted areas.
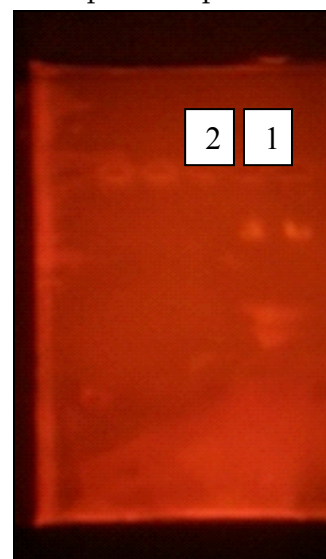
Figure 3. Plasmid bands observed under U.V transilluminator after gel electrophoresis. Lane 1 from right

contains PUC18 (Control) and lane 2 shows the plasmid band isolated from *Pseudomonas spp.*A1113
The plasmid was isolated from *Pseudomonas spp.*A1113. The Dimethoate degrading property of organisms was plasmid mediated which can be confirmed by plasmid curing.

IV. CONCLUSION

In present study, biological degradation of these pesticides has been carried out using soil isolates which include both fungal and bacterial isolates. Bacterial isolate identified as *Pseudomonas spp.*A1113 can biologically degrade these pesticides maximum up to 19gm%. The Dimethoate degrading property of organisms was plasmid mediated which can be confirmed by plasmid curing, using different curing agents. Hence, *Pseudomonas spp.*A1113 can be used to degrade this pesticide, which would serve the ecofriendly and cheap way of degradation.

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Protein, Ascorbic Acid and Antioxidative Enzymes Alterations In The Digestive Gland of Lamellidenscorrianus Due to Heavy Metals from Different Reservoirs of Nashik District. (M.S.)

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ABSTRACT

The heavy metals Zn, Cu, Pb and Cd concentrations were determined in surface water and the freshwater bivalve lamellidens corrianus were collected from Girna, Ozarkhed, Chankapur and Gangapur reservoirs of Nasik district during summer, monsoon and winter seasons. The biochemical components proteins, ascorbic acid and oxidative stress indicator parameters like activity of antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), the levels of antioxidant scavenger molecules, reduced glutathione(GSH) and lipid peroxidation (LPO) were estimated from digestive glands of the freshwater bivalve lamellidenscorrianus. The results demonstrate that the level of proteins, ascorbic acid, LPO and activity of GST were lowest and activity of antioxidant enzyme CAT, GPx and SOD were highest at Gangapur reservoir and lowest at Girna reservoir. The results also indicates that the level of LPO and activity of GST were lowest and CAT, GPxand SOD activity were highest in monsoon, while level of LPO and activity of GST were highest and CAT, GPx and SOD activity were lowest in summer season at four reservoirs in digestive glands oflamellidenscorrianus. The mean values of heavy metals Zn, Cu, Pb and Cd concentrations in surface water were highest at Girna reservoir and lowest at Gangapur reservoir. Therefore, it was concluded that Girna reservoir was more polluted than other studied reservoirs.

Keywords: Lamellidenscorrianus,heavy metals, proteins, ascorbic acid, antioxidant enzymes.

I. INTRODUCTION

Consumption of aquatic food highly contaminated with heavy metals may form a significant pathway to metal contamination in the human being and creating public health problems wherever man is involved in the food chain (Otitoloju and Don-Pedro, 2004; Lodhi et. al., 2006; Yigit and Altindag, 2006; Sarabject and Dinesh, 2007; Medeiros et al., 2012). The toxicant bioaccumulation became a topic of public and scientific concern early in the 1950s (Barron, 2003). Heavy metal pollution poses a great potential threat to the environment and human health. A wide range of metal pollution or stresses are also responsible for the secretion or suppression of the proteins (Iwata et al., 1998 and Kohler et al., 2001) in the body of organism. Ascorbic acid is well known to inhibit oxidative damage against metal toxicity (Houston and Johnson, 2000; Rao et al., 2001; Nandi et al., 2005). Ascorbic acid helps to maintain the oxidation-reduction potential of the cell at the stabilized level. Antioxidant property of ascorbic acid helps to prevent free radical formation from toxic water-soluble molecules which may cause cellular injuries and diseases. The study of antioxidant enzymes in conjunction with trace metal

body burden contribute to a more comprehensive picture of environmental pollution and biological responses in bivalves representing useful reference value for future heavy metal pollution assessment. Several studies reported that accumulated heavy metal stress causes biochemical alterations in organism (Verlecar et al., 2008; Zhanget al., 2010; Rajkumar and Milton, 2011).

II. METHODS AND MATERIAL

Four reservoirs of Nasik district were selected for the study. The digestive glands of five animals of lamellidens corrianus, species was collected seasonally during November 2010 to October 2011 from four water reservoirs of Nashik district.Protein content of the tissues was estimated by method of Lowry's (Lowry et al., 1951). Estimation of ascorbic acid was carried out by the method of Roe (1967). Lipid peroxidation (LPO) was determined by the method of Oshkawa et al., (1979). Glutathione-S-transferees (GST) activity was assessed by the method of Habig et al., (1974). The amount of reduced glutathione (GSH) in the samples was estimated by the method of Boyne and Ellman (1972). Superoxide dismutase (SOD) activity was estimated by the method of Beauchamp and Fridovich (1973). Catalase (CAT) is measured using hydrogen peroxide as a substrate by (Aebi, 1984). Glutathione peroxidase (GPx) was assayed according to the procedure of Rotruck et al., (1973) with some modifications. Results are expressed as mean ± standard deviation (S.D.). The ANOVA test was used in order to access whether biochemical constituents are varied significantly between the reservoirs, seasons and bivalve species. The probabilities less than 0.05 (p<0.05) were considered statistically significant. All statistical calculations were performed with SPSS 21.0 version.

III. RESULTS AND DISCUSSION

To monitor the heavy metal pollution of Girna, Ozarkhed, Chankapur and Ganapur reservoirs of Nasik district, biochemical components, Proteins, Ascorbic acid and the activity of antioxidant enzymes (SOD, CAT, GPx and GST) and level of GSH and LPOwere measured in digestive glands of the bivalve species, Lamellidens corrianus, collected during three seasons. The obtained results are presented in table nos. 1 to 4.The knowledge of these biomarkers will provide information on metal pollution in the reservoirs. In the present investigation the digestive glands were selected for the study because in bivalve the digestive glands are the main site of metal accumulation as it contains higher level of metallothenin (Pipe et al., 1999; Canesi et al., 2008; Waykar and Shinde, 2011; Deshmukh, 2013). In the present investigation the biochemical constituents like protein, ascorbic acid contents were determined from soft body tissues like mantle, gills, digestive glands and whole soft body tissues of bivalve species, Lamellidenscorrianusinhabiting the four reservoirs of Nasik district during three seasons.

In the presence of reactive oxygen species (ROS), proteins can be damaged by oxidative attack, results in site-specific amino acid modifications, fragmentation of the peptide chain, and aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis (Grune, 2000). The observed low level of protein contents in different tissues indicate that, environmental stress reduces the rate of protein synthesis or increase the proteolysis to cope with the high energy demands under toxicants stress (Vincent et al., 1995; Waykar and Lomte, 2001a). Pottinger et al., (2002) reported that at high pollution stress, protein synthesis can be suppressed representing disturbance of regular metabolic processes.

The results showed low level of ascorbic acid contents in different soft body tissues of bivalves collected from Girna reservoir than other three studied reservoirs, might be due to bivalve species inhabiting at Girna reservoir were exposed to higher level of pollutant, the contaminants may exert stress on bivalves.

Number of researchers reported that due to toxicant stress ascorbic acid content was decreased. Nawale (2008) reported a decrease in ascorbic acid content in freshwater bivalve, Lamellidenscorrianus after chronic exposure to lead nitrate and sodium arsenate.

In the present seasonal study, the lowest protein, ascorbic acid contents were observed in different soft body tissues of bivalve sampled during summer season, might be due to bivalves were exposed to higher level of pollutant in summer than winter and monsoon seasons.Digestive glands often show higher level of antioxidant enzymes (Irato et.al., 2003). In the present investigation obtained results showed the highest level of lipid peroxidation and glutathione-S-transferase activity and lowest activity of superoxide dismutase, catalase and glutathione peroxidase and low level of reduced glutathione (GSH) in the digestive glands of freshwater bivalve Lamellidens corrianuscollected from Girna reservoir than other three studied reservoirs. On the other hand results showed the lowest level of lipid peroxidation and glutathione-S-transferase activity and highest activity of superoxide dismutase, catalase and glutathione peroxidase and level of reduced glutathione (GSH) in the digestive glands of bivalve species collected from Gangapur reservoir than other three studied reservoirs. Rajkumar and Milton (2011) reported increase of lipid peroxidation in P. viridis along with increase in concentration of cadmium, copper, lead and zinc in short-term chronic toxicity test.

In the present investigation the highest activity of glutathione-S-transferase (GST) was observed in digestive glands of three bivalve species collected from Girna reservoir than other three reservoirs might be due to bivalve species were exposed to higher level of pollutants than other three reservoirs. Higher GST activity at Girna reservoir in the digestive glands of the freshwater bivalve might be related to the capacity of the digestive glands to metabolize xenobiotics, eliminate waste products (Gamble et al., 1995) and it also suggests the protective action against reactive oxygen radicals. Increase of GST enzyme activity indicating activation of detoxification mechanism in the digestive glands could be a good indicator of pollutant exposure. Increase of GST activity can therefore be due to increased detoxification of hydroperoxides. Bouraoui et al., (2009) reported a parallel increase in GST activities as well as in LPO levels in H. diversicolor exposed to a mixture of BaP and Cu (1 μM) for a short-period.

It was observed that bivalve species collected from Girna reservoir showed low level of GSH in digestive glands than other three studied reservoirs, this might be related to the bioaccumulated level of heavy metals in bivalve species. The results indicate that bivalve species inhabiting in environments with higher level of metals. Dafre et al., (2004) observed decreased GSH level in the mussel Perna perna, after exposure to lead. Nedjoud et al., (2009) also reported a decrease in GSH in H. aspera after exposure to high concentrations of metalicdust.The antioxidant defense enzyme system comprises several enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx). Many of these antioxidants interact in a concerted manner to eliminate reactive oxygen species and prevent damage to cellular components. These enzymes activities can be altered by reactive oxygen species (ROS) and therefore they may represent indicators of oxidative stress (Pavlovic et al., 2004; Valavanidis et al., 2006). Altered antioxidant enzyme activities are frequently used as indicators of oxidative stress (Cargnelutti et al., 2006; Banni et al., 2008; Bocchetti et al., 2008 Zhou et al., 2008). In the present investigation it was observed that, bivalve specis collected from Girna reservoir showed the lowest activity of SOD, CAT, and GPx than bivalves collected from other three studied reservoirs, might be in response to bioaccumulated levels of metal in bivalves. Numerous researchers showed that the toxicants induces the LPO formation, increases the activity of GST, decrease the GSH level and alter the antioxidant enzyme (SOD, CAT and GPx) activities in

mollusk (Vasseur and Leguille, 2003; Box, et.al.2007; Osman et.al.2007; Deshmukh, 2013)

IV. CONCLUSION

In the present study obtained results showed the low level of proteins, ascorbic acid, highest level of lipid peroxidation and glutathione-S-transferase activity and lowest activity of antioxidants enzymes superoxide dismutase, catalase and glutathione peroxidase, low level of reduced glutathionein digestive glands of freshwater bivalve species, Lamellidens corrianus collected from Girna reservoir than other three studied reservoirs. Thus results clearly indicated that Girna reservoir was more polluted by heavy metals than other three studied reservoirs. The results demonstrate that bivalves living at Girna reservoir were more under environmental stress than bivalves living at other three studied reservoirs. In the present study results also showed the lowest levels of proteins, ascorbic acid, GSH and lowest activity of SOD, CAT and GPxand highest level of LPO and highest activity of GSTin the digestive glands of bivalve species in summer season than monsoon and winter seasons. This indicates that in summer bivalve species wereunder more environmental stress than in winter and monsoon seasons.

Table 1. Seasonal variations in heavy metal concentrations from surface water samples from different reservoirs of Nasik district.

Parameters	Seasons	Zn	Cu	Pb	Cd
Girna	Summer	437.21±5.81	134.27±1.56	110.72±1.95	23.92±0.95
	Monsoon	299.50±3.09	97.98±3.09	92.61±1.07	2.42±0.63
	Winter	329.07±4.73	113.42±1.42	105.73±1.64	16.72±0.79
Ozarkhed	Summer	408.39±5.46	112.51±2.17	104.42±2.42	15.57±1.24
	Monsoon	258.39±4.12	85.32±1.53	81.20±1.82	08.62±0.83
	Winter	293.65±4.59	98.62±1.90	93.62±2.13	11.40±0.92
Chankapur	Summer	381.32±5.81	108.83±1.94	98.81±1.94	15.12±1.14
	Monsoon	235.16±4.26	80.93±1.08	74.38±1.62	08.16±0.72
	Winter	276.64±4.56	95.84±1.17	85.42±1.87	09.83±0.87
Gangapur	Summer	359.15±5.72	98.26±2.14	95.37±2.42	12.51±0.82
	Monsoon	225.09±5.27	74.42±1.45	62.53±1.86	06.37±0.65
	Winter	254.70±4.75	85.11±1.63	79.23±1.92	08.48±0.74

± indicate standard deviation

Table 2. Profile of Protein contents in different soft body tissues of freshwater bivalve Lamellidenscorrianusfrom different reservoirs of Nashik district (Values are in mg/100mg dry tissue weight).

Reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Girna	39.28 ±0.78	50.90 ±1.38	47.51± 1.28	50.43± 1.63	60.11± 1.43	58.05± 1.63	48.52± 1.32	59.57± 2.43	56.10 ±2.34	46.19± 1.93	57.13 ±2.32	54.13± 1.85
Ozarkhed	41.73 ±1.98	52.64± 1.85	49.34± 1.59	51.42± 1.97	62.78± 2.14	60.46± 1.92	49.29± 1.82	61.72± 1.87	58.24± 1.93	48.00± 1.63	59.63 ±2.03	55.82± 1.74
Chankapur	42.08 ±2.04	53.72± 1.87	49.92± 1.68	52.60± 1.24	64.18± 2.05	60.90± 2.42	50.46± 1.93	63.04± 1.73	58.92± 2.04	48.83± 1.58	60.07 ±2.28	56.19± 1.83
Gangapur	43.51 ±0.71	53.98± 1.68	51.38± 1.76	53.81± 1.39	64.89± 1.92	61.00± 2.08	51.72± 1.84	63.91± 1.86	60.13± 2.28	49.47± 1.47	62.01 ±1.96	58.05± 1.77

± indicate standard deviation

Table 3. Profile of Ascorbic acid contents in different soft body tissues of freshwater bivalve Lamellidenscorrianus from different reservoirs of Nasik district (Values are in mg/100mg dry tissue weight).

Reservoir	Mantle			Gills			Digestive glands			Whole soft body tissue		
	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win	Sum	Mon	Win
Girna	0.627 ±0.009	0.934 ±0.018	0.835 ±0.018	0.767 ±0.018	1.108 ±0.026	1.002 ±0.021	0.876 ±0.018	1.307 ±0.029	1.093 ±.0.022	0.714 ±0.010	1.103± 0.025	0.926 ±0.018
Ozarkhed	0.662 ±0.016	0.957±0.013	0.869 ±0.012	0.795 ±0.023	1.143 ±0.018	1.048 ±0.017	0.897 ±0.019	1.364 ±0.026	1.154 ±0.014	0.741 ±0.009	1.135± 0.021	0.987 ±0.014
Chankapur	0.681 ±0.014	0.974±0.019	0.893 ±0.016	0.819 ±0.017	1.154±0.016	1.103±0.019	0.943 ±0.014	1.394 ±0.022	1.165 ±0.018	0.785 ±0.014	1.146± 0.018	1.007 ±0.010
Gangapur	0.694 ±0.012	0.985±0.012	0.903±0.010	0.848 ±0.015	1.187±0.012	1.109±0.028	0.968 ±0.016	1.397 ±0.019	1.193 ±0.023	0.797 ±0.016	1.164± 0.015	1.034 ±0.016

± indicate standard deviation

Table 4. Profile of lipid peroxidation level, reduced glutathione level and activity of antioxidant enzymes in the digestive glands of freshwater bivalve, Lamellidenscorrianus from different reservoirs of Nasik district.

Reservoir	Sampling seasons	Lipid Peroxidation (LPO)(nmol MDAformed / mg protein)	Glutathione-S-transferase (GST)(nmol CDNB conjugate formed / min / mg protein	Reduced glutathione (GSH) (μM / gm wet tissue)	Superoxide dismutase (SOD) (U / mg of protein)	Catalase (CAT) (U/mg of protein)	Glutathione peroxidase (GPx) (μg of GSH utilized / min/ mg of protein)
Girna	Summer	211.08±3.24	231.57±4.08	6.05±0.53	114.82±2.12	91.32±1.72	35.54±1.88
	Monsoon	142.84±3.05	150.18±2.92	8.79±0.61	156.42±2.82	129.46±2.46	49.75±1.63
	Winter	185.20±2.92	193.48±3.34	7.81±0.59	123.06±2.63	115.39±2.09	45.83±1.55
Ozarkhed	Summer	197.32±2.04	217.73±4.18	7.93±0.62	126.91±2.46	96.38±1.72	36.39±1.21
	Monsoon	128.25±1.93	132.92±2.38	8.64±0.58	172.04±2.58	137.81±2.08	51.04±1.55
	Winter	172.14±1.90	172.38±2.13	8.07±0.71	149.28±2.61	121.08±1.94	48.57±1.36
Chankapur reservoir	Summer	191.48±1.72	208.35±4.35	11.23±0.87	137.47±2.76	101.35±2.34	38.76±1.29
	Monsoon	123.16±2.07	127.11±3.81	12.56±1.14	189.28±3.04	148.17±1.93	54.69±1.57
	Winter	163.40±2.18	165.54±3.72	11.98±1.12	148.61±3.17	123.51±2.05	49.63±1.42
Gangapur reservoir	Summer	179.33±3.05	203.93±4.03	13.27±1.21	145.39±2.90	105.47±2.00	42.32±1.35
	Monsoon	109.52±2.85	112.42±3.35	14.78±1.29	195.72±2.47	154.82±1.83	59.17±1.69
	Winter	158.32±3.04	159.02±3.72	14.09±1.08	157.67±2.18	128.62±1.62	51.08±1.47

± indicates the standard deviation

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Development and Validation of Solvent Extraction Method for Spectrophotometric Determination Cu(II) by Using SALENH₂ Ligand

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ABSTRACT

In present investigation we demonstrated first time the green chemistry route of synthesis of N,N'-bis (salicylaldehyde) ethylenediimine ligand (SALENH2). The ligand has four coordinate sites and form stable complex with Cu(II) ion at pH 8 to 11. The complex is yellow coloured and insoluble in water but highly soluble in CHCl₃. The complex was found to stable in CHCl₃ for long time and is violet coloured. The absorbance spectra of complex show broad peak in visible region with maximum absorbance at 558 nm. The calibration curve method was developed for selective analysis of Cu(II) and was validated in context with AAS method. The sample analysis showed that developed method by is highly efficient and selective for the analysis of Cu(II) in presence of other metal ions.

Keywords: Solvent extraction, Cu- SALEN, analytical method development, spectrophotometry

I. INTRODUCTION

Solvent extraction is the method of quantitative separation of substances usually from aqueous phase to organic phase (extracting solvent) which is immiscible with water. The extracting solvent is so selected that compound is highly soluble in it and can be easily separable from aqueous phase [1]. Solvent extraction methods are applicable for selective separation as we as quantitative analysis of substances. The technique is well demonstrated for selective separation of metal ions from aqueous phase in to organic phase and for quantitative analysis by spectrophotometry.[2] In the technique, the importance is choice of suitable ligand which form stable complex with metal. Furthermore complex should be highly soluble in organic phase. Transition metal complexes are usually coloured hence after extraction they can be easily quantitatively analysed by spectrophotometry. For selective determination of metal ion of interest from mixture of metal ions, suitable complexing agent must be selected which show distinct colour with analyte metal ion from other metal ions in the sample. Such method spectrophotometric method possesses high importance as sensitivity of these methods is high and low cost of equipment.[3] Thus, herein we demonstrate the synthesis of salicylaldehyde ethylenediamine Schiff's base (SALENH₂) ligand by green chemistry route and its application for the solvent extraction and quantitative analysis of Cu(II) metal ion from micronutrient sample.

II. METHODS AND MATERIAL

a. Synthesis of Ligand:

Ligand was synthesized by green chemistry route under ambient condition. To 5 ml pure salisylaldehyde liquid 2 ml ethylenediamine was added drop wise with constant stirring. This resulted into formation dark yellow coloured crystalline

product. The product is the ligand of interest i.e. SALENH₂. Ligand is purified by crystallization using methanol as a solvent. TLC was recorded in pure methyl acetate. Melting point was recorded by usual method.

b. Analytical Method Development for Cu(II) analysis by using SalenH₂ ligand:

In first step of analytical method development standard Cu(II) 0.01M concentration was used. In first step effect of pH was studied on percent extraction.[5] Into 2 mL Cu(II) solution 2 mL ligand solution and 10 ml water was added and pH was adjusted to 5. Similarly six more solutions were prepared of pH was adjusted to 6, 7, 8, 9, 10 and 11. Each of this solution was extracted with 5+2 ml chloroform (two times) and absorbance spectrum was recorded in visible region. From observed absorbance optimum pH for extraction was decided.

c. Quantitative analysis:

Standard stock solution (0.01M) of Cu(II) was prepared from A.R. Grade CuSO₄·5H₂O was prepared in water and used in further studies for the preparation working standards of Cu(II). Linearity range and detection limit was obtained by reported method. Afterword, the known solution of Cu(II) was analysed by calibration curve method so as to validate the method. Finally copper alloy sample was analysed by validated spectrophotometric method and results were confirmed by reported atomic absorption spectroscopic (AAS) method.

III. RESULTS AND DISCUSSION

a. Synthesis and purity of Ligand:

Synthesised by ligand was purified by crystallisation method using in 1:1 water methnol mixture. M.P was recorded which is found to be 123ºC which matches with reported value (125º C). Further purity was checked by TLC (Fig.1). The synthesised product shows Rf value is 0.53 which is different from starting material (solvent system) methyl acetate using silica gel as stationary phase and N-hexane + Methyl acetate as mobile phase.
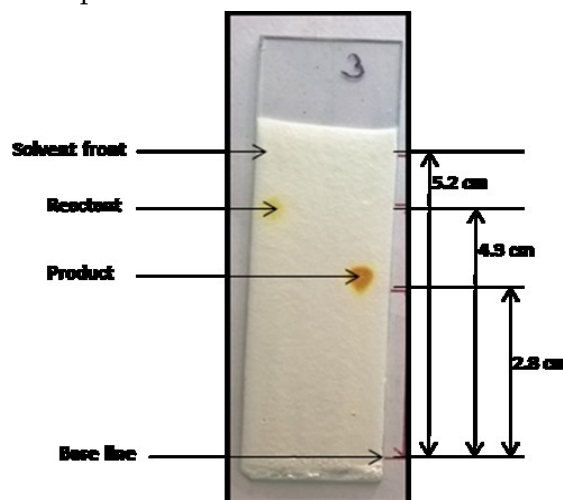
Figure 1. TLC of ligand and starting material

The purity of product was quantitatively checked by non aqueous titration method. 100 mg of synthesised ligand was dissolved in 10 ml Glacial acetic acid and titraed against 0.1M HClO₄ using crystal violet as an indicater. The observed purity of ligand was found to be 98.72% for which structure is represented in fig.2.[5]
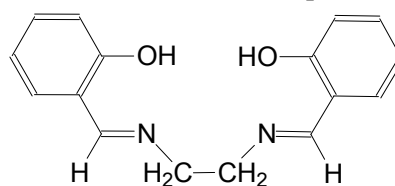
Figure 2. Structure of ligand

b. Effect of pH on Extraction of Cu(II):

In 1st step we have studied the effective pH on complexation of ligand with Cu(II).[6] The complaxation was performed at the different pH (5, 6, 7, 8, 9, 10,11). The complex was extracted into chloroform (CHCl₃) and absorbance was recorded 560 nm. 560 nm is a wavelength at which Cu-SALENH₂ ligand show maximum absorbance (Fig.3). The result of effect of pH on extraction of complex represented in Fig-4.
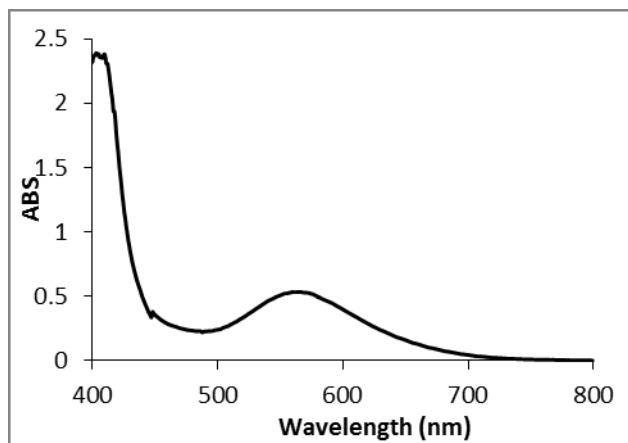
Figure 3. Absorbance spectra of Cu-SALEN Complex
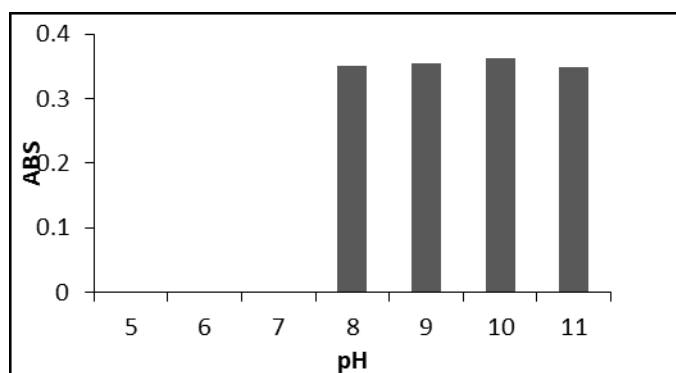
Figure 4. Effect of pH on extraction of Cu-SALEN complex

The results clearly indicate that alkaline pH is good for extraction of this complex into $CHCl_3$ and pH 10 is best for the quantitative extraction of the complex.

c. Linearity Rang:

Beers law ($A = \varepsilon bC$) is obeyed over the limited range of concentration of analyte. In most of the cases good relation in concentration and absorbance is observed over the range of absorbance from 0.1 to 0.9.[7] Thus we have selected the concentration range of Cu (II) which gives the absorbance near to this range. For Cu (II) concentration it was found to be 0.0002 to 0.001 M. Over this concentration of Cu (II) absorbance was found in the range of 0.139 to 0.650 (fig. 5). The linearity in absorbance is indicated by R^2 value which is 0.998 i.e. well above the 99.8% confidence level as required for quantitative analysis, theoretically.
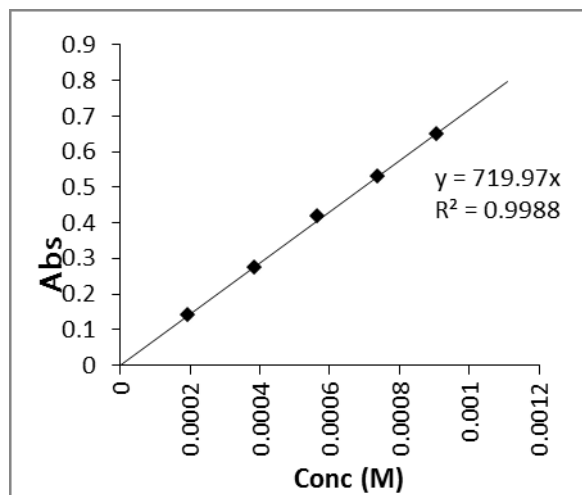
Figure 5. Determination of Linearity range

d. Method validation by calibration curve method:

For method validation we have used calibration curve method.[8] Calibration curve was set by using 0.2 ml to 0.1 ml Cu(II) and extracted with 5+2 ml $CHCl_3$. Sample of known concentration was used. From stock sample 0.3 and 0.5 mL solution i.e. 0.000291M and 0.000476 M Cu(II) was used for extraction. By using calibration curve method conc. of Cu(II) in sample were calculated. The observed amount was found to be 0.000287 and 0.000466 M. These values lie in the range of 95 to 105% of expected value i.e. in the 98% confidence limit. This indicate that the developed method of Cu(II) analysis by using $SALENH_2$ ligand can be used for the quantitative analysis of Cu(II).[9]

e. Analysis of sample and its confirmation by AAS:

We have analysed the sample of plant micronutrient supplement for the Cu(II) content by developed method. 1 g Micronutrient powder sample was dissolved in 250 ml water and 0.5ml H_2SO_4 solution was prepared. From this solution 5 ml was used for analysis. Calibration curve method was used. Calibration curve was set by using 0.2 ml to 1.0 ml of Cu(II) of 0.01M. Absorbance of extracted solution was recorded at 560 nm. The observed content in micronutrient sample was 3.63±0.08% by developed method. The same sample was analysed by known AAS method. By AAS percentage of Cu(II) in sample was found to be 3.64±0.06%. The result of analysis of

developed method is very close to AAS method which is well known and precise method of analysis of trace metal in presence of other metals.

IV. CONCLUSION

The ligand can be synthesized by very simple and rapid method by mixing molar proportions of salicyladehyde and ethylendiamine which is environment friendly method with high yield of product. Solvent extraction method for selective analysis of Cu(II) was developed and validated by using SALENH$_2$ ligand of which validation was done by comparing results by AAS method. The results of analysis shows that developed method is highly efficient for the analysis of Cu(II) in presence of other metal ions.

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Green Approach for Preparation and Characterization of Silver Nano Particles by using Citrus Limon

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ABSTRACT

In this study, simple approach was applied for synthesis of silver Nanoparticles using Citrus Limon fruit and leaf extract. Reduction of Ag^+ to Ag^0 was confirmed by the color change of solution from colorless to brown. Its formation was also confirmed by using UV–Visible spectroscopy by recording spectra at different intervals. In this work we develop a simple technique to synthesize $AgNO_3$ nanoparticles using Silver nitrate in aqueous solution. Among a variety of Nanoparticles, they have advantages because of their properties. This method is not only rapid and one step but eco-friendly, non-toxic also and an alternative conventional methods. It is also advantages for durable textile finish on cotton and silk fabrics. Remarkable antifungal activity has been observed in the treated fabrics as well as antifungal and antimicrobial activity.

Keywords: Silver Nanoparticles, Plant Extract, UV/Vis-Spectrophotometry.

I. INTRODUCTION

Nowadays, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols. Green synthesis approaches include biological and irradiation method which have advantages over other physical and chemical methods involving chemical agents associated with environmental toxicity. Selection of solvent and eco-friendly non-toxic reducing and stabilizing agents are the most important factors which must be considered in green generating of Nanoparticles.

We have synthesized silver Nanoparticles by using plant extracts of *Citrus Limon* which acts as catalysts. The main aim of this is to study an assay method for Synthesis and characterization of silver Nanoparticles and characterization using UV/Vis-Spectrophotometer and SEM techniques.

II. METHODS AND MATERIAL

Experimental Work
Preparation of Citrus Lemon Extract

The *Citrus Limon* leaves washed with deionised water to remove dust and finely cut into small pieces. The pieces of leaves were boiled in a 100 mL of deionised water for 30 minutes to digest it till 10 mL of water and allowed to cool. The cooled solution was filtered and stored in a refrigerator for 6 hours. It was filtered and used as a *Citrus Limon* extract solution.

Synthesis of Ag Nanoparticles

In the preparation of Ag Nanoparticles, $AgNO_3$ (0.1 N) was first dissolved in 25 ml of conductivity water and mixed with 25 ml of *Citrus Limon* extract solution under vigorous stirring by using magnetic stirrer at room temperature for 30 minutes. A grey precipitate was collected by filtration and washed with conductivity water several times, and finally dried at

100°C for 2hours in an oven. The synthesized Ag Nanoparticles were characterized using UV/Vis-Spectrophotometer and scanning electron microscopy (SEM).

III. RESULTS AND DISCUSSION

UV/Visible Analysis of the reaction

The reduction of silver ions was characterized by measuring the UV–visible spectrum of the reaction medium at 3 h after diluting a small amount of the sample into conductivity. UV–visible spectral analysis was done by using UV/Vis spectrophotometer (SHUMADZU, UV-1800).

Table 1. Determination of λ_{max} for Silver Nitrate solution

Wavelength /nm	Absorbance
250	0.27317
300	**0.77479**
350	0.01928
400	0.01693
450	0.01627
500	0.01547
550	0.01538
600	0.01552
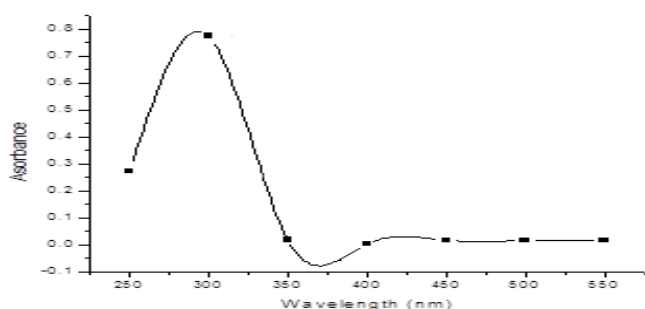
Graph 1. Calibration curve of absorbance vs. wavelength for Silver nitrate solution by UV/VIS Spectrophotometry technique

SEM of Silver Nanoparticles

Scanning electron microscopy (SEM) provides size of the Nanoparticles which confirms the size of silver Nanoparticles. The average size of an individual particle is estimated to be 80 nm.
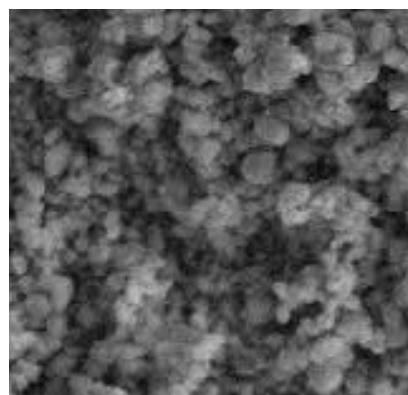
Figure 1

IV. CONCLUSION

We have successfully synthesized silver Nanoparticles. Biological methods are a new approach for synthesizing Nanoparticles using natural obtained plant extracts. These methods are accepted due to low cost and material availability.

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Study of oral microflora and application of oral deposition (local antibiotic) to avoid oral infection in RPD/CD (removable partial denture) patients

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ABSTRACT

Oral cavity is open growth system with an interrupted introduction and removal of microbes and their nutrients of all the types of microbes living in, our mouth in abundant number. It has been estimated that more than 600 different types of bacteria are present in saliva. Due to which periodontal diseases are occur severely. In which Streptococcus mutans, Staphylococcus aureus , Enterococcus feacalis, Streptococcus pyogens, Lactobacilli sp. are common infectors and results into dental carries and common encountered problem in dentistry is loss of teeth and consequential replacement by RPD/CD.However it has been reported that the presence of a prosthesis (RPD/CD) in oral cavity promotes the condition for establishment and accumulation of micro-organism. It increases infection to edentulous space and adjacent healthy teeth. Various antibiotics has proven to be effective in showing antimicrobial activity against gram positive and gram negative bacteria and fungi. So, instead of using oral drug (chlorohexidine) , the application of oral depositors reduces the chances of oral infection related to RPD/CD and minimize the formation of MDR sp. of micro-oraganism including bacteria and fungi. It also protects the liver and nerve system from damage due to excessive use of antibiotic.
Keywords: Oral microflora, RPD/CD, oral deposition

I. INTRODUCTION

Oral cavity is an open growth system with an uninterrupted introduction and removal of microbes and their nutrients .It offers diverse habitat where different spp. of microorganism can prosper. The primary requisite for any group of microbes to flourish in a niche and is their ability to adhere to tooth surfaces and multiply in shielded environment like periodontal packet and RPD crevices. The aggregates of microorganism that reside on the surface have traditionally referred to as plaque because of its yellowish colour of characteristics microorganism found there (1).

The aggregate of microorganism that reside on the surface and in deep layer of skin, in saliva and oral mucosa as well as in conjunctiva and in gastrointestinal tracts. The microflora exsisting in oral cavity is called oral microflora(7). Oral microbiology is the study of microorganism of oral cavity and their interactions between oral microbes or with their host.

The environment present in mouth allows the growth; the presence of nutrients, epithelial debris and secretions makes the mouth a favourable habitat for a variety of microorganism. Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from mouth to stomach where acid sensitive microbes are destroyed. The oral cavity represents a unique environment. Oral microbiology

at early stage: Although minute and primordial, bacteria were incredibly versatile and diversified judging by their number and biomass, they are arguably the most successful living organism on earth. They can tolerate environmental extremes and colonize almost every habitat on Earth, including human oral cavity (8).

At the time of birth oral cavity is composed solely of the soft tissue of lips, cheeks soft palate and tongue. Kept moist by salivary gland secretion. *Streptococcal spp*. will adhere strongly to gum and soft tissue. With the creation of gingival crevice area, increase the habitat for variety of anaerobic species (3). One of the commonly encountered problems in dentistry is loss of teeth and consequential replacement. Along with the restoration of function and aesthetic, removable prosthesis may change the oral ecology either qualitatively or quantitatively, such as increasing the total amount of oral microorganism or increasing a certain part of the oral microflora(8).

Disease due to bacterium can be prevented by maintenance of good oral hygiene and regular dental check-up; prophylactic antibiotic may be needed prior to major dental work and incident infections (3). All medications have side effects, when used appropriately antibiotic are relatively safe with typically few side effects. However some antibiotics are notorious for producing side effects that can be especially intolerable. An antibiotic side effect is known as an unwanted reaction that occurs in addition to the desirable therapeutic action of the antibiotic. Like any drug antibiotic side effect can occur and many interfere with patient's ability to tolerate and finish the course of medication (4).

Usually antibiotic treatment should not be stopped without a health care providers approval; all medication should be finished. Stopping antibiotic early due to side effects may allow the infection to worsen and may lead to antibiotic resistance, making an antibiotic less effective. Even if the infection appears to have clear up before all of the medication is gone, the full course of antibiotic treatment should always be completed.

Antibiotic resistance is one of the biggest threats to global health food security and development today. Antibiotic are medicine use to prevent and treat bacterial infection, antibiotic resistance occurs when bacterial genes changes in response to use of these medicine or antibiotic. Bacteria become antibiotic resistant, these bacteria may infect human and infection the cause by resistant bacteria are harder to treat than sensitive bacteria(Guidelines by WHO). Antibiotic resistance is raising dangerously high level in all parts of world. A new resistant mechanism is spread and emerging globally. A growing list of infections such pneumonia, tuberculosis, blood poisoning, gonorrhoea and food born disease are become harder sometimes impossible to treat as antibiotic became less effective. Therefore to prevent and control and spread antibiotic resistance in different species of microbes, health professional can only dispense or local antibiotic when patient need, according to guideline given by WHO. Hence the scope of present study is cure oral infection arises due to RPD/CD are treated with application of oral depositors (antibiotic) RPD wearer patients

II. MATERIAL AND METHODOLOGY

A sample of 30 patients was included in study, 23 were females and 7 were males visiting prosthodontic clinic at Akola city in month August – September 2017.Complete history and examination was performed for study of common oral microflora and further study of oral depositors. All patients with denture RPD/CD subject to bacteriological examination. The oral rinse technique was used to study the oral flora. Patients were ask to rinse thoroughly and vigorously with 10 ml of sterile distilled water. The water was then cultured using streak plate method on Blood agar plate, culture for 24 hrs and incubated the Corn meal agar for suspected

Candidal growth was identified by conventional recognised method ,incubated at 23-27 degree Celsius for 3-4 days(1).

Composition of Blood Agar

Table 1

Peptone	0.5%
Beef extract	0.3%
Agar-Agar	1.5%
NaCl	0.5%
Sheep blood	5%
Distilled water	1000mL
pH	7.3

Composition of Corn Meal Agar

Table 2

Corn meal infusion form	50grams
Agar-Agar	15grams
pH	6.2
Distilled water	1000mL

RPD/CD in oral cavity promotes the condition establishment and accumulation of microorganism, it increase the infection to edentulous space and adjacent healthy teeth. In this study application of various local depositors were used in combined powder form i.e. Amoxicillin : Clavulanic acid potassium salt(5:1) , Amphicillin Sodium Salt, Erythromycine, Oxacillin sodium salt, Niastasin (Hi Media, Mumbai). These local depositors subject to apply on inner part of RPD/CD where it come in contact with edentulous space(10).

Under the study RPD/CD patients was asked to wear prosthesis with oral depositor application continuously for minimum period of 3 days. Oral rinse water technique was use to study for growth of microorganism. The rinse water was then cultured using streak plate method on blood agar and corn meal agar for bacterial and fungal growth respectively.

III. RESULTS

Sample Characteristics:

Table 1 illustrate the age, sex and distribution of sample among RPD/CD wearer. Age range of patient was 31-85 years. Mean age was 50.70 years and Mode was 40 years. It was found that 76.7% females and 23.3% males were carry prosthesis. Age range majority of patient 41-60 years was 60.1% while 31-40 years and 61-above 80 was nearly 40%.

Table 3. AGE, SEX AND DISTRIBUTION OF RPD/CD SAMPLE

Age Group	Sex		Total %
	Female No. (%)	Male No.(%)	RPD/CD
31-40	4(13.3)	3(10)	7(23.3)
41-50	8(26.7)	2(6.7)	10(33.4)
51-60	8(26.7)	-	8(26.7)
61-70	3(10)	1(3.3)	4(13.3)
71-85	-	1(3.3)	1(3.3)
Total	23(76.7)	7(23.3)	30(100)

Microbiological Findings:

Table 4 illustrate the no of patients having the occurance of *Streptococcus mutans* ,*Streptococcus pyogens, Staphylococcus aureus ,Enterococcus feacalis* and *Candida albicans* before and after application of oral depositors. It was found that there was 80% irradication of infectious microbes from oral cavity in RPD/CD wearer patients. Among 30 patients only 20% patients were found with *Streptococcus mutans , Streptococcus* pyogens with alpha and beta haemolysis on Blood Agar and *Staphylococcus aureus* shows Coagulase positive test along with haemolysis on Blood Agar respectively.

Table 4 Summarised the changes in predominantly cultured species in oral flora prior and after application of oral depositors in RPD/CD patients 20% among 30 does not show any change in

predominantly cultured microorganism after using combination of oral depositors after using minimum 3 days.

Candida albicans 26.66% was the most cultivated microorganism among RPD/CD wearer it shows almost total decrease in its population than pre treated visit .Among 30 patients the percentage of *Streptococcus mutans* (43.33%) ,*Stretpcoccus pyogens* (13.33%), S*taphylococcus aureus* (10%) decreses upto 10%, 3.33%, 6.66% respectively while *Enterococcus feacalis* (6.66%) was not found in culture after application of local depositors.

Table 2 .Predominantly Cultured Oral Flora Distribution By Rpd/Cd Before And After Application Of Oral Depositors

Name of Microorganism	Common flora in RPD/CD pts.		Oral flora after application of depositors	
	No. of patients	%	No. of patients	%
Streptococcus mutans	13	43.33	3	10
Streptococcus pyogens	4	13.33	1	3.33
Staphylococcus aureus	3	10	2	6.66
Enterococcus feacalis	2	6.66	-	-
Candida albicans	8	26.66	2	6.66
Total	30	100	6	20

A wide variety of the predominatly cultured bacterial strain were found among females some strains were mostly cultured from female including Streptococcus mutans and Candida albicans. Table 3 illustrate sex wise distribution of microorganism inRPD/CD patients. Among 30 ,60% females having *Streptococcus mutans(26%)* ,*Streptococcus pyogens(6.66%), Staphylococcus*

aureus(6.66%) ,*Enterococcus feacalis(3.33%)* and *Candida albicans(16.66%)* while 40% males having *Streptococcus mutans(16.66%)* ,*Streptococcus pyogens(6.66%), Staphylococcus aureus(3.33%)* ,*Enterococcus feacalis(1.33%)* and *Candida albicans(10%)*.

Table 5. Predominantly Culturd Microorganisms Distribution In Both Sexes

Name of Microorganism	SEX		Total
	Female	Male	
Streptococcus mutans	8(26)	5(16.66%)	13(43.33%)
Streptococcus pyogens	2(6.66%)	2(6.66%)	4(13.33%)
Staphylococcus aureus	2(6.66%)	1(3.33%)	3(10%)
Enterococcus feacalis	1(3.33%)	1(3.33%)	2(6.66%)
Candida albicans	5(16.7%)	3(10%)	8(26.66%)
Total	18(60%)	12(40%)	30(100%)

IV. DISCUSSION

The result of this study revealed that a fairly considerable change in oral flora does occur following the application of depositors. This is very acknowledge in literature and is of particular concern since oral treatment and essential to maintain oral health. This can be universal rather there is use of local depositors in RPD/CD patients. Host and properties of mouth are internal factors which is responsible for development of microorganism. Antimicrobial susceptibility depends upon particular bacterial strain, PH, nutrient availability, material, type, design of prosthesis and oral hygiene.

This study gives new drug delivery system because local drug delivery has been chosen as a better

treatment protocol over systemic therapy which maximise efficiency and minimise side effects. Dr. Sneha Gada and her co workers performed work on this new drug delivery system using 150 gms of Flucanazole powder and compare modulation efficiency with systemic therapy

Although fungi represents a minor percentage of oral microflora Candida albicans gives rise to dental Candidosis and stomatitis .The fact that this is the most common investigated microorganism is not surprising considering that one of the most common cause for RPD/CD wearer hence by using local depositors Niastasin (powder form) fungal growth can be minimise. Wala M. Amin and his co workers gives a new form of intraoral delivery of antifungal drug for treatment of denture induced oral Candidacies. Following the guidelines of WHO , health and dental experts should recommend local depositors and tropical antibiotic in permissible quantity which checks the formation of new resistant strain of bacteria and fungi.

In future scientist can use various combination of these drug in denture fixative and denture material, Dr. Addy from Dental School ,Cardiff performed work on in vitro studies into the use of denture base and soft liner material as carrier for drug in mouth in his study use of chlorohexidine acetate was incorporated in acrylic gel soft liner to minimise bacterial infections.

RPD/CD changes oral ecology which increases the risk of oral infection. Thus this study is imperative that a factor such as oral depositors maintains healthy oral function and environment. This study assure and maintained healthy oral function in RPD/CD wearer patients and prevent from organ damage from higher antibiotic dose which checks to form new resistant species of microbes.

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Review on Agricultural Potentials of Nanotechnology

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ABSTRACT

Nanotechnology proved as a new hope of ray for almost every aspect of lifestyle of human being. Properties of nanomaterials are diverse in nature and significant for their functions. Advancement of nanotechnology is applicable for food, medicine, computer sectors. The remarkable properties of nanomaterials have enabled them to be used in agricultural sector too. To increase productivity at the same time disease management is important zone to be considered. In a given review paper it is considered regarding the crop management, crop protection pest control and utilization of nanotechnology based resources to achieve maximum production in an agricultural point of view.

Keywords: Agriculture, Crop protection, Nanomaterial's, Pest, Productivity.

I. INTRODUCTION

Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. Nanotechnology can work from the top down (which means reducing the size of the smallest structures to the nanoscale e.g. photonics applications in nanoelectronics and nanoengineering) or the bottom up (which involves manipulating individual atoms and molecules into nanostructures and more closely resembles chemistry or biology). The definition of nanotechnology is based on the prefix "nano" which is from the Greek word meaning "dwarf". In more technical terms, the word "nano" means 10^{-9}, or one billionth of something. For comparison, a virus is roughly 100 nanometres (nm) in size. The size of the double helix of DNA on the nanoscale is about 2 nm wide. The word nanotechnology is generally used when referring to materials with the size of 1 to 100 nanometres, however it is also inherent that these materials should display different properties from bulk (or micrometric and larger) materials as a result of their size. These differences include physical strength, chemical reactivity, electrical conductance, magnetism, and optical effects. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering.

"Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nm, where unique phenomena enable novel applications." To this, it is useful to add two other statements to form a complete definition. First, nanotechnology includes the forming and use of materials, structures, devices, and systems that have unique properties because of their small size. Also, nanotechnology includes the technologies that enable the control of materials at the nanoscale.

Properties of Nanomaterials

a) Small particle size results in to new particle properties, which can also introduce new risks. Nanoparticles have a very large surface area which typically results in greater chemical reactivity,

biological activity and catalytic behaviour compared to larger particles of the same chemical composition (Garnett and Kallinteri 2006; Nelet al. 2006).

b) Nanomaterials also have far greater access to our body (known as bioavailability) than larger particles, resulting in greater uptake into individual cells, tissues and organs. Materials which measure less than 300 nm can be taken up by individual cells (Garnett and Kallinteri 2006), while nanomaterials which measure less than 70 nm can even be taken up by our cells' nuclei, where they can cause major damage (Li et al., 2005).

c) Unfortunately, the greater chemical reactivity and bioavailability of nanomaterials may also result in greater toxicity of nanoparticles compared to the same unit of mass of larger particles of the same chemical composition (Oberdörsteret al. 2005a; Oberdörsteret al., 2005b). Other properties of nanomaterials that influence toxicity include: chemical composition, shape, surface structure, surface charge, catalytic behaviour, extent of particle aggregation (clumping) or disaggregation, and the presence or absence of other groups of chemicals attached to the nanomaterial (Brunner et al. 2006; Magrezet al., 2006; Sayeset al., 2004; Sayeset al., 2006). Some nanomaterials have proved toxic to human tissue and cell cultures in in vitro (test tube) studies, resulting in increased oxidative stress, production of proteins triggering an inflammatory response (Oberdörsteret al., 2005b), DNA mutation, structural damage to cell nuclei and interference with cell activity and growth, structural damage to mitochondria and even cell death (Li et al., 2005). Nanomaterials now in commercial use by the food industry, such as nano titanium dioxide, silver, zinc and zinc oxide have been shown to be toxic to cells and tissues in in vitro experiments and to test animals in in vivo studies. Nanomaterials have such diverse properties and behaviours that it is impossible to provide a generic assessment of their health and environmental risks.

d) The shape, charge and size of different particles can influence their kinetic (absorption, distribution, metabolism and excretion) and toxic properties. For this reason even nanomaterials of the same chemical composition which have different sizes or shapes can have vastly different toxicity (Sayeset al. , 2006).

The population is increasing and subsequent worldwide demand for food has urged for a better protection of agricultural crops from the infestation by different groups of insects. This initiated the intervention of modern techniques for the development of novel strategies of plant protection. Over the past decade, there has been a considerable amount of active research on the possible application of nanotechnology in the current agricultural practices including development of novel plant-protection products. In particular, designing of nanoformulation of different insecticides has emerged at high speed and which can be basically attributed to the fact that the composition of many conventional insecticides are feebly water soluble and require a delivery system for their application in the field.

Compared to bulk substances, nano-insecticides have added many advantages such as: (a) There is less environmental contamination due to reduction in rate of pesticides application. (b) Efficiency of chemical and natural insecticides is enhanced by controlled release. (c) Renders insecticides more susceptible to photodegradation. (d) Safe handling with reduced toxicity risks to animals. (e) Less toxicity towards non-target organisms compared with bulk. Among other benefits, nanoformulation of many natural insecticides (e.g. Neem oil) has protected them from premature degradation in the environment and thus helped in delivering maximum impacts on the target organisms. Polymer-based nanoformulations have been exploited for the encapsulation of most of the insecticides. Different polysaccharides like chitosan, alginates, starch and polyesters like poly-ε-caprolactone, polyethylene glycol have been considered for the synthesis of nanoinsecticides. The first formulation containing polymer for controlled release of biocides dates from the early 1970's. With the growing awareness for environmental pollution,

application of biodegradable and biocompatible polymers of natural origin is preferred over the synthetic ones. The metabolites produced from the degradation of such polymers are of little concern. On the other hand, the growing general trend of preferring polymeric nanoformulations by researchers can be correlated to the manifestation of higher efficacy in insecticidal property of the encapsulated ingredient compared to commercial formulations. The efficacy tests have been confirmed from many field studies for different target organisms. Slow release, protection against degradation, and low solubility of the encapsulated insecticide are the most important features of polymeric nanoformulation making them first choice for nanoencapsulation. This valuable information has paved way to further development and practical application of polymeric nanoinsecticides with huge potential. Different form of polymer-based or non-polymer-based nanoformulations, such as nanosphere, nanocapsule, nanogels, micelles, nanofibers, nanometals and nanoemulsions has been proposed for encapsulation of insecticides. Among these, nanocapsules are by far the most widely used for controlled release of insecticides. Very recently, a novel concept of hybrid nanoformulation (encapsulation of nanoemulsion or liposome coating) has been suggested for the controlled release of some insecticides. However, the efficacy of the proposed novel approach needs to be tested for a broad spectrum line of insecticides. This is highly anticipated that application of nanoinsecticides for plant protection inevitably results in new benefits to human and environmental health. However, environmental safety issue on application of nanoinsecticides has been recently addressed. In order to ensure efficacy, most of the nanoinsecticides have been designed for slow release and allowing them persistence in the environment. Thus, it is important to investigate the environmental fate processes for both nanocarriers and the nanoformulated insecticides. Existing regulatory protocols for environmental risk assessment are mostly applicable to the bulk insecticides and cannot access the nanoformulated

products because of different properties. For a fair risk assessment of the fate of nanoinsecticides, a new framework has to be developed and practiced in near future (Das et al. 2013).

Plant pathogens and pests are of the major factors limiting crop productivity. In crop sciences, nanotechnology can be used for the production of nanocapsules for delivery of pesticides, fertilizers, and other agrochemicals (Jhaet al., 2011). Nanotechnology for the control of plant diseases is a promising technique in plant pathology either by providing controlled delivery of functional molecules or as diagnostic tool for disease detection, an important step in plant disease treatment (Sharon et al., 2010). Encapsulation of herbicides could provide improvement in their application. For example Sulfonylurea herbicides are applied through the soil to control Orobanche spp., but several applications are needed to achieve effective control (Joel et al., 2007). Several studies were conducted using nanosized particles to control fungal pathogens such as Pythiumultimum, Magnaporthegrisea, Colletotrichumgloeosporioides, Botrytis cinereand, Rhyzoctoniasolani, as well as bacterial disease including Bacillus subtilis, Azotobacterchrococuum, Rhizobium tropici, Pseudomonas syringaeand, Xanthomonascompestrispv. Vesicatoria (Park et al., 2006). In Palestine, nanotechnology might be used for the control of several plant pathogens such as powdery mildews on grapevine and leaf spot on olive trees.

Nanotechnology and agricultural production developments

In the near future, nanostructured catalysts will be available which will increase the efficiency of pesticides and herbicides, allowing lower doses to be used. An agricultural system widely used in the USA, Europe and Japan, which efficiently utilises modern technology for crop management, is called Controlled Environment Agriculture (CEA). CEA is an advanced and intensive form of hydroponically based

agriculture. Plants are grown within a controlled environment so that agricultural practices can be optimized. The computerized system monitors and regulates localised environments such as fields of crops and irrigated water. CEA technology provides an excellent platform for the introduction of nanotechnology to agriculture. Nanotechnological devices for CEA that provide "scouting" capabilities could tremendously improve the grower's ability to determine the best time to harvest the crop, the vitality of crop, and food security issues, such as microbial or chemical contamination.

Nanoparticles and plant disease control

Some of the nano particles that have entered into the arena of controlling plant diseases are nanoforms of carbon, silver, silica and alumina-silicates. At such a situation, nanotechnology has astonished scientific community because at nano-level, material shows different properties. The use of nano size silver particles as antimicrobial agents has become more common as technology advances, making their production more economical. Since silver displays different modes of inhibitory action to microorganisms (Young, 2009), it may be used for controlling various plant pathogens in a relatively safer way compared to commercially used fungicides. Silver is known to affect many biochemical processes in the microorganisms including the changes in routine functions and plasma membrane (Pal et al., 2007). The silver nanoparticles also prevent the expression of ATP production associated proteins (Yamankaet al., 2005). In a nutshell, the precise mechanism of bio-molecules inhibition is yet to be understood. Thus, use of nanoparticles has been considered an alternate and effective approach which is eco-friendly and cost effective for the control of pathogenic microbes (Kumar and Yadav, 2009; Prasad et al., 2011; Swamy and Prasad, 2012; Prasad and Swamy, 2013).

These nanoparticles have a great potential in the management of plant diseases compared to synthetic fungicides (Park et al., 2006). Zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles are effective antibacterial agents (Shah and Towkeer, 2010). The increased ease in dispensability, optical transparency and smooth-ness make ZnO and MgO nanostructures an attractive antibacterial ingredient in many products. Both have also been proposed as an anti-microbial preservative for wood or food products (Aruojaet al., 2009; Sharma et al., 2009). Properly functionalized nanocapsules provide better penetration through cuticle and allow slow and controlled release of active ingredients on reaching the target weed. The use of such nano-biopesticide is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional chemical pesticides (Bariket al., 2008).

Silica nanoparticles a potential new insecticide for pest control

Application of nano-silica to the tomato plants may minimize the problems caused by Spodopteralittoralis. It provides a moderate degree of resistance, but presents the advantage of being feasibly integrated to other management tactics in controlling this pest. Nano-silica sprays affect the feeding preference of the Spodopteralittoralis, thus increasing the resistance of tomato plants. Concomitantly it affects biological parameters of the insect such as longevity and nymph production, thus reducing the reproductive potential of females on tomato plants and therefore reducing the insect population density, damages and yield losses to the crop. In conclusion, nano-silica is effective against Spodopteralittoralisand would therefore be a useful component of an integrated pest management strategy (El-Bendaryand El-Helady, 2013).

Pest Management

Insecticides are used in different ways, based on the physical-chemical characteristics of the each chemical substance, the area that needs to be covered and the target. Typical application of insecticides in crops is made by spraying a solution, emulsion or colloidal suspension containing the active chemical compound,

which is made by a vehicle which may be a hand pump, a tractor or even a plane. This mixture is prepared using a liquid as a carrier, usually water, to ensure a homogenous distribution. Other methods for applying insecticides are through floggers or granule baits embedded with the active compound, among others that are less used. However, due to several degradation processes, such as leaching or destruction by light, temperature, microorganism or even water (hydrolysis), only a small amount of these chemical products reaches the target site. In this case, the applied concentrations of these compounds have been much higher than the required. On the other hand, the concentration that reaches its target might be lower than the minimum effective one.

Nanoparticles as pesticides

Nanoparticles are also effective against insects and pests. Nanoparticles can be used in the preparation of new formulations like pesticides, insecticides and insect repellants (Bariket al., 2008; Gajbhiyeet al., 2009). Torney, (2009) reviewed that nanotechnology has promising applications in nanoparticle gene mediated DNA transfer. It can be used to deliver DNA and other desired chemicals into plant tissues for protection of host plants against insect pests. Porous hollow silica nanoparticles (PHSNs) loaded with validamycin (pesticide) can be used as efficient delivery system of water-soluble pesticide for its controlled release. Such controlled release behaviour of PHSNs makes it a promising carrier in agriculture, especially for pesticide controlled delivery whose immediate as well as prolonged release is needed for plants (Liu et al., 2006b). According to Wang et al. (2007), oil in water (nano-emulsions) was useful for the formulations of pesticides and these could be effective against the various insect pests in agriculture. Similarly, essential oil-loaded solid lipid nanoparticles were also useful for the formulations of nano-pesticides (Liu et al., 2006b). Nanosilica, a silica product, can be effectively used as a nanopesticide.

Bariket al., (2008) reviewed the use of nano-silica as nano-insecticide. The mechanism of control of insect pest using nano-silica is based on the fact that insect pests used a variety of cuticular lipids for protecting their water barrier and thereby prevent death from desiccation. But here, the nanosilicaparticles when applied on plant surface, cause death by physical means of insects by being absorbed into the cuticular lipids.

It has been observed that the control efficacy against adult T. castaneumwas about 80%; presumably due to the slow and persistent release of the active components from the nanoparticles (Yang et al., 2009). The applications of diverse kind of nanoparticles viz. silver nanoparticles, aluminium oxide, zinc oxide and titanium dioxide in the management of rice weevil and grasserie disease in silk worm (B. mori) are caused by Sitophilusoryzaeand baculovirusBmNPV (B. morinuclear polyhedrosis virus, respectively (Goswamiet al., 2010). Teodoroet al. (2010) studied the insecticidal activity of nanostructured alumina against two insect pests viz. S. oryzaeL. and Rhyzoperthadominica(F.), which are major insect pests in stored food supplies throughout the world. Significant mortality was observed after 3 days of continuous exposure to nanostructured alumina-treated wheat. Therefore, compared to commercially available insecticides, inorganic nanostructured alumina may provide a cheap and reliable alternative for control of insect pests, and such studies may expand the frontiers for nanoparticle-based technologies in pest management.

Biological studies were performed on cotton plants infested with aphids to estimate the direct contact efficacy of nanosphere formulations on insects. The systemic effect of nanoformulation was studied from their ability to penetrate through the plant and reach the sap. The nanosphere formulations performed better than the reference to control the infestation at all the doses used due to their enhanced systemicity. The use of porous hollow silica nanoparticles (PHSN),

with a shell thickness of nearly 15nm and a pore diameter of 4–5 nm, for providing shielding protection to pesticides from degradation by UV light was reported (Li et al., 2007). PHSN carriers improved the photostability of the pesticide, avermectin, loaded into the inner core and avermectin showed a typical sustained-release pattern from the carrier. Hence, such carriers have a promising future in the sustained-release pattern applications of various photosensitive components. The effects of slow/controlled-release fertilizers (for regulated, responsive and timely delivery) cemented and coated by nanomaterials; clay-polyester, humus-polyester and plasticstarch on crops were studied with wheat (Zhang et al., 2006).

II. CONCLUSION

Agricultural pest is serious problem which reduce the crop yield in every country. Increasing food demands of growing population need to increase the crop productivity. Unnecessary using of chemical pesticides cause problem to ecosystem and time consuming IPM programme, it is necessary and urgent to use alternatives to control pest. Nanotechnology is best approach against agricultural pests. Various authors studied the nanoparticles like carbon, silver, silicon, oxide of zinc and magnesium are effective against the various microorganisms and insect pests.

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Study of Physico-Chemical Parameters of Bhima River, Indapur Tehsil (Maharashtra: India)

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ABSTRACT

The research article is carried out on the study of water quality with reference to Bhima River. The monthly changes of river water quality were analyzed by different parameters such as pH, temperature, dissolved oxygen (D.O.), carbon-dioxide, hardness, chloride and alkalinity were investigated to assess the suitability of water for various different purposes. The investigated parameters determined showed the fluctuations in the parameters. These fluctuations in parameters can be caused due to various reasons such as industrialization, over population, man- made activities, pollution and many more due to which water is being highly polluted and that adversely affect aquatic as well as terrestrial life. It is important to check the quality of water before drinking because there are many water borne diseases which adversely affect human population which later can cause death. These parameters can easily describe the quality of water as well by graphical representation it is relevant to find fluctuations month to month.

Keywords: Bhima River, Water quality, Physico-chemical parameters.

I. INTRODUCTION

Water is the basic need of all living beings. It is impossible to live without water. Water is the gift by nature to all of us. This natural resource is diminishing day-by-day due to ignorance towards it. There are various sources of water in India in form of lakes, rivers and reservoirs which are used for various purposes such as irrigation, fish production, generation of hydro power plants, industrial water supply, drinking and many more. In last few decades, there has been a tremendous increase in the demand for fresh water due to rapid growth of population and the accelerated pace of industrialization, Shivashranappa and Yalakpalli; (2012). The quality of water is essential for human health as well as to maintain biotic and ecological ecosystem. But now-a-days the water quality has become degraded due to various untreated sewage, industrial, agricultural and domestic waste which has adverse effect on the flora and fauna for the aquatic ecosystem.

All living organisms on the earth need water for their survival and growth. As of now only earth is the planet having about 70 % of water, Patil*et.al*;(2015).In South Asian countries such as Nepal, India and Bangladesh, pollution of river is more severe and critical near urban stretches due to huge amount of pollution load discharged by urban activities, Joseph and Jacob;(2010). Domestic effluents continuously resulting in degradation of this habitat at alarming rate, Zingede *et.al*; (1994). The physico-chemical parameters are essential for determining water quality. The temperature is depending on the solubility of oxygen in water. DO (dissolve oxygen) is important to maintain biological life of water. The

source of DO is atmosphere and photosynthesis of aquatic plants. Low oxygen is responsible for killing aquatic flora and fauna. pH (PotentiaHydrogeni) is the negative logarithm of hydrogen ions which indicates water is acidic or basic. This is measured in scale of 0 to 14. pH value 7 is neutral, less than 7 is acidic and greater than 7 is basic, Dixit *et.al*,(2013) The hardness of water is caused due to detergents and soaps used for washing clothes which makes water harsh. Alkalinity is caused by dissolved bicarbonates. The CO_2 from atmosphere and respiration of soil organism dissolves Mg and Ca in the water which produces hardness and alkalinity of water. Chloride is present in almost all natural water bodies which increase as increasing salinity. The lotic ecosystem, which deals with this research work, is Bhima River in Bhigwan (Tehsil-Indapur). BhimaRiver is Major River in south India. It flows through Maharashtra, Karnataka, Telangana states and then enters the Krishna River. In Bhigwan there is an industry BILT (Ballarpur Industries Limited) is located at village called Paundhwadi. This company provides paper and paperboards mainly coated papers. BILT has been granted clearance from Maharashtra State Pollution Control Board. The waste water in BILT is generated from paper, oil machine. These are being treated in ETP (Effluent Treatment Plant) which is useful in generation of waste water. The Indapur tehsil is at the stage of development and has major sugarcane factories which are provided with treatment facilities. The effluents are treated by sludge process which requires few months for stabilization. The under treated effluents used for irrigation causes ground water as well soil pollution.Some factories discharge it's effluents directly on land which further goes to the water and

pollutes it.The site of collection is under rural area where education is low. The people living around these area are unaware about good hygiene practices as well as there is improper drainage system. Due to this the sewage is dispersed in river water. This leads to the contamination of water and this can be observed by studying different physico-chemical parameters. The main aim and objectives of this research article is to find out the monthly variation of physico-chemical parameters from October 2017 to March 2018.

II. MATERIALS AND METHODS

For determining the physico-chemical parameters, water sample were collected between 8:00-8:30am from the site of the collection. For estimation of dissolve oxygen content, the water sample was fixed immediately on the field and estimated in the laboratory using Wrinkle's method. Temperature and pH were recorded on the field by centigrade thermometer and pen pH meter respectively. The carbon-di-oxide, alkalinity, hardness, dissolved oxygen, chloride where investigated by titration method on the same day in the laboratory, (Golterman *et.al*, 1978 and APHA, 1985).

Table 1. Method of Analyzing parameters.

SR.NO	WATER QUALITY PARAMETERS	METHOD OF ANALYSIS
1	Temperature	Thermometer
2	pH	pH meter
3	Dissolved oxygen	Winkler's method
4	Carbon-di-oxide	Phenolphthalein method
5	Alkalinity	Titration
6	Chloride	Mohr's method
7	Hardness	EDTA method

III. RESULT AND DISCUSSION

The investigation involves Physico-chemicals parameters of Bhima River in Bhigwan (Tal- Indapur) to find out the month variations. Physico-chemicals parameters like pH, temp., DO, CO_2, alkalinity, Cl and hardness were tested in month of October and November 2017.In the study water temperature range was 27^0C (October), 21^0C (November) and 19^oC. pH range was 6.4 (October), 5.0 (November) 5.0(December). Tazzwell (1957) suggested that minimum of 3mg/l dissolved oxygen is necessary for

healthy life of fish and aquatic life. In the study dissolve oxygen range was 0.12 mg/l (October), 1.12mg/l (November) and 0.42mg/l which is not suitable for flora and fauna of the river. CO_2 range was 15 mg/l (October), 9.0 mg/l (November) and 5.0mg/l (December) which depends on respiration of the aquatic animals. The estimated hardness of water range was 310 mg/l (October), 240 mg/l (November) and 222mg/l (December) it depends on Ca, Mg salts from detergents and soaps used for washing cloths by villagers. Alkalinity range was 28 mg/l (October), 102mg/l (November) and 51.6mg/l(December). The chloride range was 226.92mg/l (October), 70.97mg/l (November) 17.9mg/l which depends on pollution by organic matter.

Table 2. Monthwise physicochemical parameters of Bhima River.

SR.NO	PARAMETERS	MONTHS		
		OCTOBER	NOVEMBER	DECEMBER
1	Temperature	27°C	21°C	19°C
2	pH	6.4	5.0	5.0
3	Dissolved oxygen	0.12mg/l	1.12mg/l	0.42mg/l
4	Carbon-di-oxide	15mg/l	9mg/l	5mg
5	Alkalinity	38mg/l	114mg/l	51.6mg/l
6	Chloride	226.92mg/l	70.97mg/l	17.99mg/l
7	Hardness	310mg/l	240mg/l	222mg/l
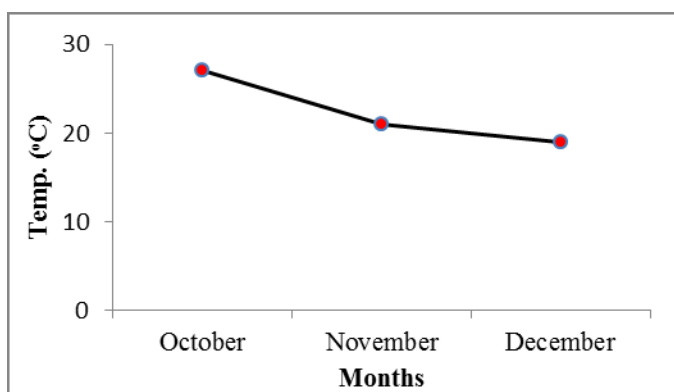
Figure 1. temperature (°C)
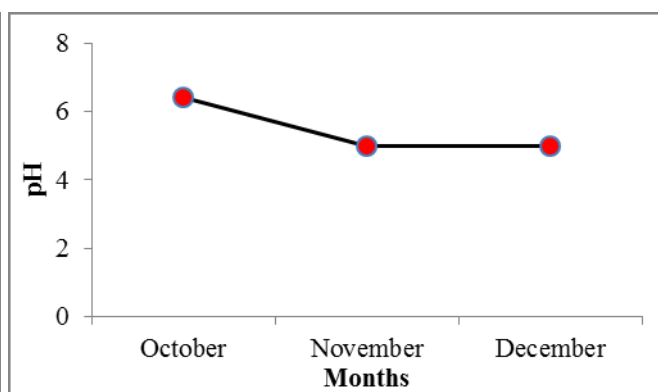
Figure 2. pH
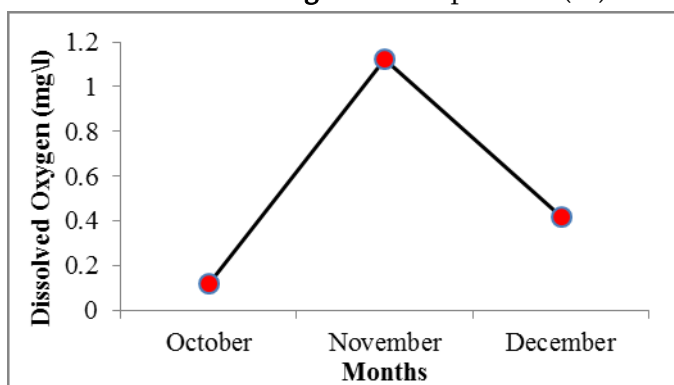
Figure 3. Dissolved Oxygen (mg\l).

Figure 4. CO_2 (mg/l)

Figure 5. Alkalinity (mg/l)
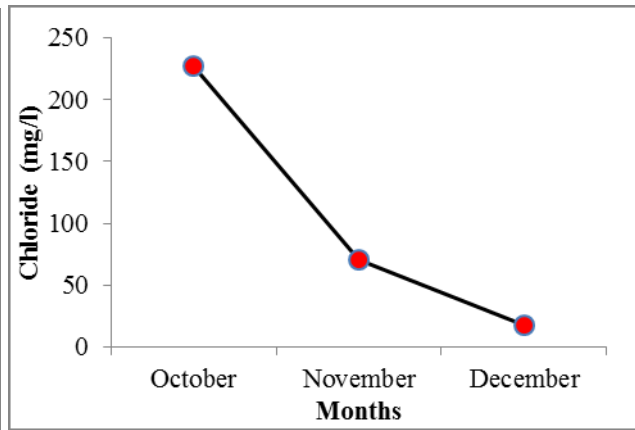
Figure 6. CO_2 (mg/l)
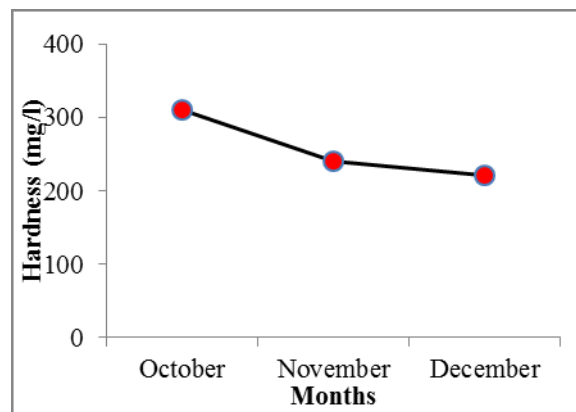
Figure 7. Hardness of water (mg/l).

Figure 1, The above graphical representation is of temperature from month of october to december. The temperature shows the gradual decrease according to the data. The decrease in temperature may be due to winter season which results water to be more cold. **Figure 2,** The above graphical representation is of pH from month of october to december. It is essential to find water is acidic or basic. According to the data from month october to december water is found to be acidic in nature which is not suitable for aquatic life. Figure 3, The above graphical representation is of dissolved oxygen (D.O.) from month of October to December. It is one of the important parameters. It plays a important role in maintaining the aquatic life. According, to the data the D.O. was very low in October it raised upin November and again decreased in December. This fluctuation during months may be depending on the amount of atmosphere oxygen dissolved as well as photosynthesis by aquatic plants. As, the amount of D.O. is very low it is not suitable

for the flora and fauna and may cause death. Figure 4, The above graphical representation is of carbon-di-oxide from month of October to December.

According, to the data the CO2 decreased monthly. It depends on the atmosphere as well as respiration of the aquatic organisms.**Figure 5*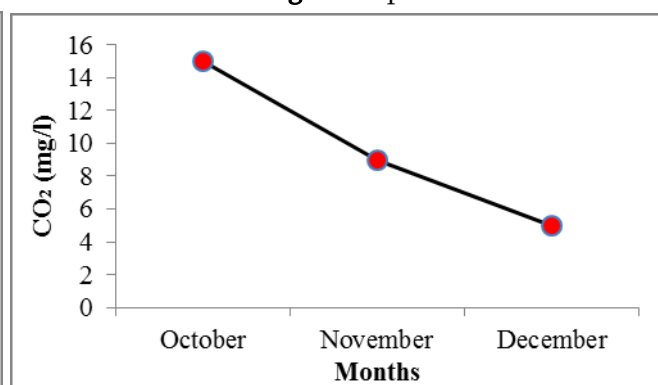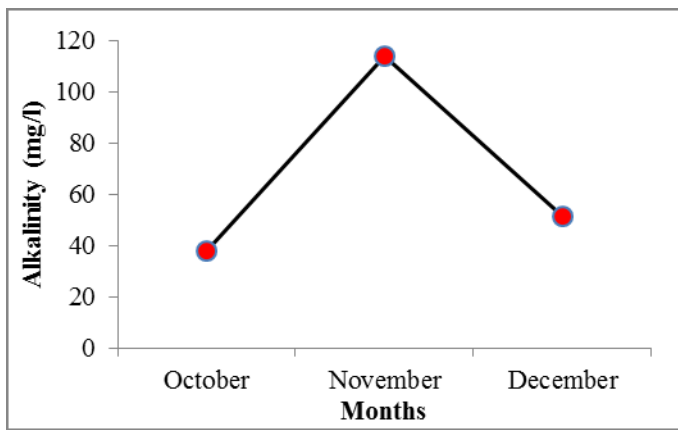* The above graphical representation is of alkalinity from month of October to December. According, to this data the alkalinity shows greater fluctuations, as it is lower in October increases highly in November and again decreases in December. Alkalinity is important in stabilizing pH as well has capacity to neutralize acids. It is caused by dissolving bicarbonates. Figure 5 The above graphical representation is of chloride from month of October to December. According to the data, there is a greater fluctuation in month of October and November and less fluctuation in month of November and December compared to October. It depends on the contamination as well salinity of water. Figure 7, The

above graphical representation is of hardness of water from month of October to December. The hardness of water shows gradual decrease every month. The hardness is caused due to detergents and soap which makes water hard and destroys the capacity of water. Calcium and magnesium are the important ions that are responsible for hardness

IV. CONCLUSION

From the above experimentation result of three months it can be concluded that the water is not suitable for use. The water is highly polluted according to the data analyzed. The water has very low amount of oxygen content where no organism can survive for long period of time. As well, the water is found to be acidic which is again neither suitable for aquatic life not suitable for use of human. The water is contaminated which can't be drink. The local peoples live there use non-sanitary toilets, poor drainage system, the domestic waste are directly thrown into the river water, the clothes, vessels as well as animals are washed into the river by many people's these causes severe contamination of water. This water is directly drunk by the village peoples by whom they may suffer from many water borne diseases It is important to aware as well as educating people for hygiene and sanitary purpose. The strict action should be taken for contaminating the water. The water should always be analyzed before drinking to be safe from diseases. For healthy, living life is not only necessary to have a healthy food, it is also important to drink clean water too.

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Studies on Physico-Chemical Aspects Khandala Water Tank of Osmanabad District Maharashtra

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ABSTRACT

The present paper deals with the studies on physico-chemical aspects of Khandala water tank of Osmanabad district (M.S.) India. The water parameters such as temperature, pH, D. O., Free CO2, hardness of water, total alkalinityand chlorinity were analyzed. The detailsstatus of tank was discussed in text. The present work was carried out during the year 2017 with its different seasons (January to December).

Keywords: Physico-chemical aspects – Khandala watertank,Osmanabad.

I. INTRODUCTION

Water quality is ever-changing entity and no water body has a persistently constant water quality in progression time. Today's world facing a problem of pure drinking water.The quality of water in an ecosystem provides significant information about the available sources for supporting life in that ecosystem. The present investigation was made on Khandala water tank. There is no any back record found on the same hence this task was undertaken. Many workers worked on this aspect they are Breet (1950), Bharadawaj and Sharma L.L. (1999), Choudhary N.K. & others (1979), Goel P.K. and V.R. Chouhan (1991), Chavan R.J. and A.D. Mohekar (1999) etc.

II. MATERIALS AND METHODS

The present investigation was undertaken from January to December (2017). Water samples were collected in the morning hours – from the Khandala water Tank. The temperature and pH was recorded on the spot and remaining parameters were analyzed in the laboratory by using standard literature given by APHA (1985 and 1991) and Trivedy and Goel (1986).

III. RESULT AND DISCUSSION

Table 1. Physico-chemical profile of Khandala watertank.

Sr. No.	Parameters	Range
1	Temperature (water)	18 to 38℃
2	pH	6.4 to 8.0
3	Dissolved Oxygen (mg/lit)	3.4 to 6.9
4	Free CO_2 (mg/lit)	0.5 to 1.9
5	Hardness of water (mg/lit)	75 to 141
6	Total Alkalinity (mg/lit)	61 to 93
7	Chlorinity (mg/lit)	13 to 25

The physico-chemical profile of a Khandala water tank will discussed as below,

Temperature (water): Generally the water quality depends on the atmospheric as well as water temperature of water body. The water temperature ranged between 18 to 38℃. It was maximum in the month of May and minimum in the month of

December. There is rise in water temperature leads to spared up the chemical reactions accelerates in the water body.

pH:the pH is most important abiotic factor that serves as an index of water pollution of the water body. The pH was recorded with the help of pocket digital pH meter. Majority of the water body are slightly alkaline or basic in nature because of the presence of carbonates as well as bicarbonates present in the water.It varies from 6.4 to 8.8. The maximum pH was recorded in the month of March and minimum in the month of October.

Dissolved Oxygen: The dissolved oxygen in the water body is very important parameter for aquatic animals because all the aquatic animals it used for respiration.In the water body the quality of D.O. depend on the photosynthetic activity of aquatic plants. It was determined by Winkler's method. It ranged from 3.4 to 6.9 mg/ lit. The highest values of D.O. observed in the month of May and lowest in the month of November. The cooler water can carry more amount of D.O. than warmer water of the water body.

Free CO_2: The amount of Free CO_2 is due to the respiration of aquatic animals.Free CO_2 was determined by titration method. It varies from 0.3 to 1.9 mg/ lit. The maximum values were recorded in the month of April and absent in the month of January. The Free CO_2 was increases due to the respiration and whereas decreases due to the photosynthesis activity.

Hardness: Generally the hardness of any water body will be due to the amount of calcium as well as magnesium salts present in the water. It indicates the level of carbonates and bicarbonates in the water. It was estimated by EDTA method. It was recorded highest (141 mg/lit.) in the month of April and minimum (75 mg/ lit.) in the month of July. So this water was suitable for the growth of aquatic animals.

Chlorinity: Chloride is an ion that is released in to surface water through the breakdown of salt compounds. Although salts are naturally occurring mineral, elevated levels in surface waters may be attributed to various human activities. It was recorded 13 to 25 mg/lit. The higher value impact on water quality as well as damage to vegetation.

IV. ACKNOWLEDGEMENT

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Limnological and Correlation Studies of Sonala Dam, Sonala, Distt. Washim, (M.S.)

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ABSTRACT

Sonala dam is a perennial reservoir in Maharashtra, where limnological studies were conducted from Februay 2012 to January 2013. The reservoir is mainly used for drinking water supply to nearby villages and for irrigation. The nearby villages also use the water for bathing and washing purposes. The present investigation deals with the estimation of current status of physico-chemical characteristics of Sonala dam. Water samples were analysed from six sampling stations every month. The physico-chemical parameters varied seasonally. Physico-chemical factors like temperature, conductivity, pH, free carbon dioxide, biological oxygen demand and chloride recorded higher values during summer season. Similarly, during monsoon season turbidity, total dissolved solids, phosphate and nitrate; and in winter season dissolved oxygen were in maximum concentration. pH remained alkaline throughout the study period at all the sampling stations. The correlation matrix of physico-chemical factors have been computed and analysed. Correlation coefficient showed positive and negative relationship. Further, the statistical correlations have revealed DO and BOD exhibited an inverse relationship with each other. The observations revealed that the physico-chemical parameters of the water were within the permissible limits and can be safely used for domestic, irrigation and pisciculture.

I. INTRODUCTION

Fresh water is a natural resource of fundamental importance. India has vast fresh water resources in the form of both lentic and lotic ecosystems. The lentic ecosystems include ponds, lakes, tanks and reservoirs [21]. Reservoirs play a vital role for anthropogenic activities like domestic, agriculture and aquaculture. Dams are the most important water resource. Regrettably, dams are being polluted by injudicious disposal of sewage and human activities. The dams are always the victim of the negative impact of development. The nature plays major role to lower the water pollution but the process is very slow [19]. Analysis of water is important to preserve and protect the natural ecosystem. To assess the quality of water, analysis of physico-chemical parameters of water is essential for the best usage like irrigation, drinking, and other purposes. Different aspects of water are monitored to determine the quality of water. To employ scientific method for aquaculture, the study of different water parameters is very important for understanding of the metabolic events in aquatic ecosystem. The parameters influence each other as well as they govern the abundance and distribution of the flora and fauna. Pollution of water bodies first affects its physico-chemical quality and then systematically destroys the community disrupting the decline food web. Physico-chemical properties were influence by seasons and also anthropogenic activities like agriculture, urbanization, domestic sewage etc. in the catchment area resulted in deterioration of water quality [26]. The statistical correlation has been used to develop mathematical relationship for comparison of physico-chemical parameters [5].

There is a lack of baseline data on limnological characteristics of Sonala dam reservoir, Sonala, Distt. Washim. Therefore, the present study was undertaken to monitor the water quality of Sonala reservoir, to determine the seasonal changes in water quality parameters and to find the relationship between different physico-chemical parameters.

II. MATERIALS AND METHODS

Study area

Sonala Dam is a fresh water reservoir constructed in the year 1981. It is located at 77^0, 12', 30" Longitude and latitude of 20^0, 19', 00" in Sonala village of Washim district in Maharashtra (India). The reservoir is constructed on the River Adan a tributary of River Godavari. The Sonala dam is an earthen dam with 19.20 meter maximum height and 446.90 hector submergence with 132.50 square Km. of catchment area. The reservoir stores rain water received from adjoining catchment area. The reservoir is presently used for irrigation and drinking purpose for regional rural areas and is much influenced by anthropogenic activities.

Methods

The study was carried out for 12 months from February 2012 to January 2013 during different seasons at six sampling stations. The selection of six sampling stations was made on the basis of human and other domestic activities. The seasons defined as monsoon (June to September), winter (October to January) and summer (February to May). For physico-chemical analysis of water of Sonala dam, water samples were collected from six sampling stations every month in the forenoon (between 7:00 am to 9:00 am.). Unstable parameters like pH, temperature, Electrical Conductivity (EC) and Dissolved Oxygen (DO) were measured at the sites. To study other physico-chemical parameters, like free CO_2, chlorides, ammonia, nitrate, phosphate and Biochemical Oxygen Demand (BOD), water samples were collected in 5 liters plastic can, brought to the laboratory, analyzed

and calculated as per standard formulas and methods. [3, 12, 18, 30].

Statistical analysis

The correlation between various physico-chemical parameters of water samples were analyzed statistically conducting Pearson correlation analysis.

III. RESULTS AND DISCUSSION

Physico-chemical parameters (Mean ± S.E) of Sonala Dam obtained during the present investigation (February 2012 to January 2013), is presented in Table 1. The statistical analysis of Pearson's correlation coefficient is presented in Table 2.

Water temperature

In present investigation, the temperature of Sonala dam water ranged from 20.42 ^{0}C to 26.2 ^{0}C. Low water temperature was recorded during the winter season and the higher water temperature recorded during the summer season. High water temperature during the summer season was observed due to low water level, high temperature and clear atmosphere. Similar findings were recorded [17, 23]. Low temperature during winter season might be due to high water level, lesser solar radiation and low atmospheric temperature [4]. Water temperature showed significant positive correlation with conductivity (r= 0.667), TDS (r= 0.701) and BOD (r= 0.781) at p<0.01, however showed significant negative correlation with dissolved oxygen (r= -0.923 at p<0.01) and ammonia (r= -0.655 at p< 0.05).

Electrical conductivity

During the study period conductivity ranged from 440 μmhos/cm to 618μmhos/cm. Conductivity of dam water was seen maximum during summer and decreased during winter season [25]. Higher values of conductivity during summer months may be due to the increased concentrations of salts in reduced quantity of water in reservoir. However, during the

winter season and rainy season there was more inflow of water, which diluted the pollutants to some extent and lowered the ionic contents of the water. Pearson's correlation coefficient of conductivity showed significant positive correlation with CO_2 (r= 0.848) , PO_4 (r= 0.837) and BOD (r= 0.930) at $p < 0.01$.

Turbidity

The turbidity value of the Sonala dam was recorded between 18.8 to 26.71 NTU. The maximum turbidity during monsoon season and minimum during winter season was observed [10]. Maximum values of turbidity in dam water during monsoon may be due to increase of suspended particulate matter in water and influence of flow of surface runoff from the catchment areas [1, 27]. Lower turbidity was observed during the winter months due to increased water level of reservoir which diluted the pollutants in water [14]. Turbidity showed high significant positive relationship ($p<0.01$ level) with total dissolved solids (r=0.828) and nitrate (r= 0.739) whereas chloride (r= –0.832) and ammonia(r= –832) showed negative relationship ($p<0.01$ level) and ($p<0.05$ level) respective.

Total Dissolved Solids (TDS)

The data obtained from the present study revealed that the TDS value ranged between 235.71 to 432.6 mg/L. Maximum value was observed during monsoon season and minimum during the winter season [28]. The low values of total dissolved solids during winter months might be due to settling of suspended particles and trapping of dissolved solids by organisms. Total dissolved solids showed significant positive correlation with water temperature (r= 0.701) at 0.05 level and nitrate (r= 0.834) at $p< 0.01$ level, whereas pH(r= –0.680), ammonia (r= –0.842), dissolved oxygen (r= –0.607) and chloride (r= –0.595) showed negative relationship ($p<0.05$ level) [24].

pH

The results from present, investigation indicated that, the average pH value observed ranged 7.23 to 7.60. The pH showed seasonal change and fluctuation in values, which were mainly due to photosynthetic activity of phytoplankton and other higher aquatic plants [17]. Decrease in pH values during winter season is mainly related to the high bicarbonate content, while the uptake of CO_2 by phytoplankton decreased as a result of increase in concentration of bicarbonate [15]. Minimum in winter season could also be due to short day length and decreased photosynthetic activity [25]. pH remained alkaline throughout the study period at all the sampling stations. During the study period, pH showed significant positive correlation with ammonia (r= 0.834) at $p<0.01$, whereas significant negative correlation with nitrate (r= -0.620) at $p<0.05$.

Free Carbon dioxide (CO_2)

In the present investigation, CO_2 ranged from 14.62 to 33.52 mg/L. Maximum value was recorded during the summer season and minimum during winter season. The maximum concentration of CO_2 in the water during summer season may be attributed to microbial degradation of organic matter and respiratory activities of aquatic flora and fauna [20]. During the study period CO_2 showed significant positive correlation with chloride (r= 0.860), phosphate (r=0.962) and biological oxygen demand (r=0.809) at ($p<0.01$ level

Chloride

Chloride is an anion in water. It occurs in fresh water in the form of salt of sodium, potassium and calcium. In the present investigation, the values of chloride ranged from 52.64 to 89.65 mg/L. The maximum value was obtained during the summer season and minimum value was recorded during the rainy season [11]. This may be due to the concentration of sewage and increased intensity of human activities associated with dam water. During the rainy season indicated low concentration of chloride may be due to dilution of pollutants in more quantity of water [23].Statistically, chloride showed significant positive correlation with phosphate(r=0.845) and CO_2(r=0.860) at $p<0.01$ level, whereas significant negative correlation with nitrate (r= -0.764) at $p<0.01$ level.

Dissolved oxygen

Dissolved oxygen, an important abiotic factor regulates the life of animals in water. In the present investigation, dissolved oxygen was in the range of 6.33 to 9.54 mg/L. The minimum values were recorded during the monsoon season and the maximum value was recorded during the winter season. Dissolved oxygen showed high significant positive relationship ($p < 0.05$ level) with ammonia ($r = 0.674$), whereas biological oxygen demand ($r = -0.907$) at $p < 0.01$ level and phosphate ($r = -0.602$) at $p < 0.05$ level showed negative relationship. During winter months higher value of DO was observed due to decreased solar intensity and reduced temperature of water, which reduced the microbial activities and their consumption of DO from water [8]. On the other hand higher solubility of oxygen at low temperature of water is responsible to maintain the more DO values in dam water [21].

Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand is an important parameter which is used as an index of organic pollution in water. Presence of oxidizable organic matter in water results in increased BOD, as more the amount of oxygen is required to degrade it biologically. The biochemical oxygen demand (BOD) reported from Sonala dam ranged from 6.0 to 10.3 mg/L. The maximum demand of oxygen in the water was recorded during summer season and the minimum demand was recorded during the winter season. Higher values during the summer season were due to increased metabolic activities of various aerobic and anaerobic micro-organisms on bottom sediments and organic matter, which increased BOD. It is evident from the minimum value of BOD harvested during the winter season that, decreased in values of BOD, may be caused due to reduction of microbial activities at low temperature. However, the comparatively more water and dilution effect is also responsible to reduce the BOD values during winter days [6, 9]. Statistically, BOD showed significant positive correlation with phosphate ($r = 0.855$), conductivity ($r = 0.930$), CO_2 ($r = 0.809$) at $p < 0.01$ level and temperature ($r = 0.781$) at $p < 0.05$ level.

Ammonia

Ammonia is an end product of decomposition of nitrogenous organic matter; it is also the excretory product of aquatic fauna. During the present study, the value of ammonia ranged from 0.63 to 2.04 mg/L. Ammonia showed high significant positive relationship ($p < 0.01$ level) with pH ($r = 0.717$) and DO ($r = 0.674$) at $p < 0.05$ level. Ammonia showed high significant negative relationship with nitrate ($r = -0.864$) at $p < 0.01$ level and temperature ($r = -0.655$), turbidity ($r = -0.580$) and TDS ($r = -0.842$) at $p < 0.05$ level.

Phosphate

Phosphate is a key nutrient that resulted in an enrichment of natural water owing to its immense importance towards biological productivity. In the present investigation, the phosphate was in the range of 0.13 to 0.25 mg/L. The concentration of phosphate was maximum during monsoon and minimum during the winter season. These domestic activities in the vicinity of the dam resulted in the increase in the percentage of phosphate in water. Agricultural practices in the basin of river and the use of cow dung as manure by farmers, during summer season polluted the water and constituted the major source of phosphate [13]. During the winter season relatively low level of phosphate

Table 1. Average with standard error values of physico-chemical parameters at Sonala Dam (2012-13)

S.No	Parameters	Year 2012 -2013		
		Summer	Monsoon	Winter
1	Temperature (^{0} C)	26.21±1.6	25.83±.84	20.42±0.66
2.	Conductivity (µmhos/cm0	618±7.9	499±17.7	440±10.03
3	Turbidity (NTU)	19.41±1.2	26.71±1.63	18.8±2.26
4	Total dissolved solids (mg/L)	337.62±36.76	432.6±40.29	235.71±35.62
5	pH	7.60±0.04	7.45±0.06	7.23±0.06
6	Carbon dioxide (mg/L)	33.52±1.54	14.62±2.14	18.39±0.20
7	Chloride (mg/L)	89.65±1.8	52.64±5.65	75.50±5.25
8	Dissolved Oxygen (mg/L)	7.13±0.84	6.33±0.46	9.54±0.52
9	Biochemical Oxygen Demand (mg/L)	10.3±0.53	7.43±0.30	6.0±0.44
10	Ammonia (mg/L)	2.04±0.025	1.30±0.046	0.63±0.19
11	Phosphate (mg/L)	0.16±0.015	0.25±.002	0.13±0.007
12	Nitrate (mg/L)	1.19±0.02	4.01±	0.05±0.006

has been reported. This may be attributed to the abundance of phytoplankton in the water. Assimilation of phosphates by phytoplankton population reproduction for their growth and is responsible to decrease the levels of phosphates in the months of winter [7, 8]. Phosphate showed positive correlation with conductivity (r=0.837), CO2 (r= 0.962), chloride (r=0.845) and BOD (r= 0.855) at p<0.01 level, whereas showed significant negative relationship with DO (r= -0.602) at p<0.05 level.

Nitrate

Nitrate is an important nutrient that results in enrichment of natural water. Large quantity of nitrate leads to eutrophication. Nitrate is an indication of nitrogen richness in an aquatic system primarily attributed to animal origin [29]. In the present investigation, nitrate ranged from 0.05 to 4.01mg/L. During the study period maximum values of nitrate were observed during the monsoon season, this may be due to the influx of runoff water from the agricultural fields and more quantity of sewage and wastes brought by water from catchment area increased the level of nitrates in dam water [27]. Nitrate showed high significant positive relationship (p<0.01 level) with TDS (r=0.834) and turbidity (r=0.739), whereas showed significant negative relationship with pH (r= -0.620) at p<0.05 level.

IV. CONCLUSION

Fluctuations in various physico-chemical parameters were monitored during monsoon, winter and summer seasons. The study of correlation coefficient between various physico-chemical factors indicated that water temperature varied with the variation of atmospheric temperature. The present study shows that, the water of Sonala dam exhibits high concentration of turbidity and total dissolved solids, which may be attributed to large flow of surface, run off from catchment areas and increase of suspended particulate matter in water. The pH of water tends towards alkaline nature. High value of ammonia was recorded, may be due to the death and subsequent decomposition of phytoplankton and excretion of ammonia by planktonic organisms. Maximum BOD was recorded in summer season. The values of BOD showed inverse trend with the values of dissolved oxygen. High values of BOD were due to the metabolic activities of various aerobic and anerobic micro-organisms on bottom sediments and organic matter. High concentration of chloride during summer season may be due to sewage and human activities performed. Phosphate and nitrate values were higher during monsoon season and lower during winter season. Higher values during monsoon season may be due to the use of chemical fertilizers in crop fields of catchment areas and surface runoff. The correlation coefficient indicates positive and negative correlation of physico-chemical parameters with each other.

The selected dam, reservoir was found to be affected by anthropogenic activities, yet the overall dam is not considered as more polluted in nature. Not much pollution was observed, though results of some of the parameters, values were reported slightly above the permissible standards of WHO. The reason may be uncontrolled use of dam water for waste disposal and anthropogenic activities. The data harvested from the present study exhibits that the degree of contamination of the lake water is greater in summer months. It may be concluded that, on an average the water reservoir of Sonala dam is not significantly polluted. Comprehensive monitoring and proper management could be enough to make the water less polluted. This study may be helpful in sustainable management of the reservoir.

Table 2. Matrix showing correlation & significance level of physico-chemical & biotic parameters of water from Sonala dam during 2012-2013

	Wt	CON	TUR	TDS	pH	CO_2	Chlo	DO	BOD	NH3	PO₄	NO₃
Wt	1											
CON	0.667*	1										
TUR	0.392	-0.245	1									
TDS	0.701*	0.229	0.828**	1								
pH	-0.506	-0.29	-0.438	-0.680*	1							
CO₂	0.426	0.848**	-0.525	-0.15	0.044	1						
Chlo	0.012	0.556	-0.832**	-0.595*	0.419	0.860**	1					
DO	-0.923**	-0.823**	-0.157	-0.607*	0.536	-0.56	-0.168	1				
BOD	0.781**	0.930**	-0.118	0.355	-0.248	0.809**	0.487	-0.907**	1			
NH3	-0.655*	-0.345	-0.580*	-0.842**	0.717**	0.159	0.535	0.674*	-0.383	1		
PO₄	0.455	0.837**	-0.509	-0.093	0.102	0.962**	0.845**	-0.602*	0.855**	0.114	1	
NO₃	0.392	0.022	0.739**	0.834**	-0.620*	-0.47	-0.764**	-0.357	0.078	-0.864**	-0.38	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

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Synthesis of Silver and Gold Nano-particles Using co-precipitation Method

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ABSTRACT

In continuation of the efforts a chemical method for in situ synthesis of gold and silver nanoparticles using co-precipitation method under ambient conditions has been reported. $NaBH_4$ was used as a reducing reagent to perform the reaction. The obtained nanoparticles have been investigated by Transmission Electron Microscopy. The process is easy to make and size can be controlled using reduction reagent.

Keywords: Gold nanoparticle, Silver nanoparticle, transmission electron microscopy, nanoparticles, co-precipitation synthesis

I. INTRODUCTION

Over the past few decades, nanoparticles of noble metals such as gold and silver displayed noticeably varied physical, chemical and biological properties from their bulk counterparts. Nano-size particles of less than 100 nm in diameter are presently attracting increasing interest for the broad variety of new applications in diverse fields of industry.[1] Nanoparticles are of immense scientific significance as they viaduct the gap between bulk materials and atomic or molecular structures[2]

Metal nanoparticles particularly gold and silver have fascinated significant importance in biotechnology, bioengineering, textile engineering, water treatment, metal-based consumer products and other areas, electronic, magnetic, optoelectronics, and information storage, catalysis, optics, sensing, imaging and biomedical devices [3].

Metal nanoparticles can be prepared by two routes, the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates [4-6]. Number of methods have been developed for the preparation of metal nanoparticles, such as photolytic reduction[7], radiolytic reduction [8],sonochemical method [9], solvent extraction reduction [10], microemulsion technique [11] polyol process [12] and alcohol reduction [13].

Recently, chemist, physicist and material scientists have shown great significance in the development of new methods for the synthesis of nanomaterials. Physical and chemical properties of these materials are highly size dependent therefore, it is important to develop novel techniques for the synthesis of smaller and monodispersed nanomaterials.

In this paper we have reported a one pot synthesis method to prepare pure gold and silver nanoparticle using co-precipitation method with uniform size at ambient condition (air atmosphere and at room temperature) by sodium borohydride as a reducing agent. The samples were characterized using transmission electron microscopy (TEM).

II. MATERIALS AND SAMPLE PREPARATION

In the presented work, we have used silver nitrate ($AgNO_3$), chloroauric acid ($HAuCl_4.H_2O$), triply distilled water (H_2O), 1 % tri-sodium citrate and $NaBH_4$. All used chemicals were of synthesis grade and all the solvents are distilled prior to use.

Silver nanoparticles were prepared by the reduction of silver nitrate using co-precipitation process. 1 % tri-sodium citrate and $NaBH_4$ are acts like reducing agents for this process.

Gold nanoparticles were also prepared by the reduction reaction using co-precipitation method. Gold chloride (4%) solution and sodium citrate solution were made in deionized H_2O. The addition of sodium citrate solution to the gold chloride initiated series of reduction reaction characterised by changes in the color of the initial gold chloride solution. The silver and gold nanoparticles were concentrated by centrifugation of the reaction mixture at 10,000 rpm for 10 min and then were collected. The obtained nanoparticles were stored at room temperature in dark bottles.

III. RESULTS AND DISCUSSION

Previous studies showed that [14-16] several of reduction reactions are characterized by changes in the color as the reaction progressed. The Ag nanoparticles reaction mentioned in this work also showed the change in color as our reaction progressed – the solution were transparent liquid and it changed

to pale yellow followed by the formation of colloidal solution, as shown in Figure 1.
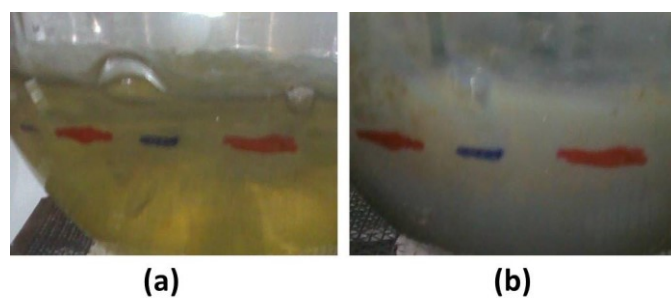
Figure 1. The change in sequence of the color of the reduction reaction during silver nanoparticle formation. (a) After some time during boiling the liquid changed to pale yellow color. (b) At the end of the reaction we have observed the colloidal solution.

A similar reduction reaction (change in color) was observed during gold nanoparticle reaction. However the color changes from pale yellow to blackish purple, as shown in Figure 2. These colloidal solutions were centrifuged for 10000 rpm and the nanoparticles were stored in dark bottles which were used for further characterizations.

Figure 2. The change in sequence of the color of the reduction reaction during gold nanoparticle formation. (a) After some time during boiling the liquid changed to pale yellow color. (b) At the end of reaction the color was changed to blackish purple color.

The changed in color of the both reaction i.e. for silver nanoparticle and gold nanoparticle confirmed that the reactions were successfully carried out and on can expect that the silver and gold nanoparticles were formed.

Transmission Electron Microscopy Study

Transmission is used as a primary tool to determine physical characteristics and the size of the nanoparticles. The samples were scan at various scan sizes from 20 nm – 200 nm as shown in Figure 3.

From the figure it is clear that the nanoparticles of silver and gold are formed with a fairly even size. Hence the distribution is also fairly even size distribution. Many particles fell within in ˜ 20 nm particle size for both silver and gold nanoparticles.

From the figure (especially 20 nm scaled figure – third from left in for silver and gold system) some nanoparticles aggregated and formed a comparative bigger size. It is observed that the normal size nanoparticles were coagulating around the large particle in a circular order. However, the average still these bigger particles are in nano-meter size.
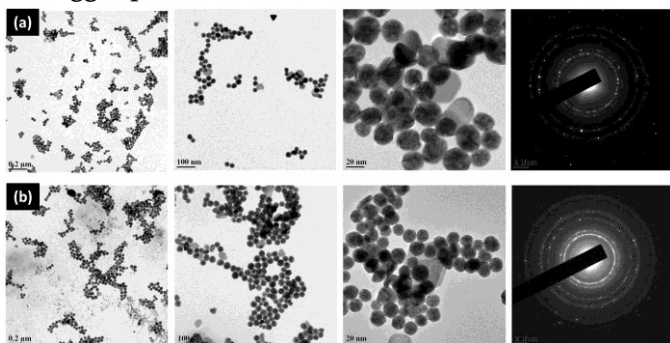
Figure 3. High-resolution TEM images of silver nanoparticle at various scan. (a) 0.2 μm scanned Ag-nanoparticles. (b) 20 nm scanned Ag-nanoparticles to show that the distribution is nearly uniform. (c) 20 nm scanned Ag-nanoparticles to show the formation of aggregated nanoparticle.

Thus from the above observation, it confirmed that silver and gold nanoparticles were successfully synthesized using co-precipitation method and NaBH4 as a reduction agent.

IV. CONCLUSION

The study shows that the silver nanoparticles prepared using co-precipitation method using NaBH4 as a reducing agent. The distribution of particles was uniformly distributed and the nanoparticles size was ˜ 20 nm.

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Phytochemical screening of some pteridophytes from Ratnagiri District of Maharashtra

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ABSTRACT

Pteridophytes are resistant to microbial infection which may be one of the crucial factors for their evolutionary success and the fact that they lasted for more than 350 million years. The present study screened the phytochemical properties of ten pteridophytes. The parameters studied are Moisture, Ash, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Sodium, Zinc, Ferrous, Copper, Manganese, Molybdenum, and Boron. The study revealed the presence of many medicinally active constituents in ten species investigated, suggesting that several pteridophyte species have the potential to synthesis useful metabolites.

Keywords: Pteridophytes, chemical, medicinal importance, metabolites.

I. INTRODUCTION

It has been suggested that the distribution of ferns is mostly determined by factors of climate and habitat due to relatively low limitations in ferns for dispersal and establishment[1]. Appropriate plant nutrition is crucial to plant health and for adequate growth of the plants with maximal productivity. There are ample amount of evidences of herbs used in the treatment of diseases and for revitalizing body systems in almost all ancient civilization. There are many references for use of plant has curative properties in various diseases. Among the nutrients in plants N, P, K, Ca, Mg and S are in large quantities while Na, Zn, Fe, Cu, Mn, Mo, and B are in trace amount. The immense potentiality of Pteridophytes in modern medicine. Some common known taxa used in ayurvedic and homeopathic and siddha system of pathy are believed by tribal, rural and common people of whole world. This is common known facts that the herbal medicines having no side effect, thus, pteridophytic plants used as better herbal preparation, but before its use requires proper testing, screening, and validity of compounds [2]. The determination of macro and micronutrients in plant tissues is an important measure, used to analyze plant nutrient status, resistant to microbial infection, and to evaluate the possible requirements of soil type for their better growth also it gives information about soil type of that locality. Ferns and fern allies have engaged the attention of the botanists and horticulturists because of their beauty and graceful foliage. Besides this, these have been successfully used in the past in Ayurvedic, Unani, Siddha, Homeopathic and other preparations. For their use as horticultural plant or in the medicinal preparations, ferns are being removed from their natural shady habitats in the forests [3], which draw our intension towards the conservation of these much neglected group. The medicinal values of ferns have been known to many for more than 2000 years. The Greek botanist Theophrastus (327-287 B.C) mentioned medicinal values of ferns in his book Historia plantarum. Phytochemical analysis ferns of India are studied by many pteridologists uses [4,5,6,7]. May published a detailed review the uses of ferns and listed 105 medicinal ferns. The microbial resistance potential of

some pteridophytes is also reviously studied. In our study ten medicinally ferns of Ratnagiri district of Maharashtra have been assessed for their phytochemical screening.

II. METHODS AND MATERIAL

The material was collected from Ratnagiri district which is situated between 17.2478 N and 73.3709 East. The average temperature range is about 20°-40° with 3364 mm annual average rainfall. During present investigation ten species of pteridophytes M. minuta L, C parasitica (L.) H.Lev, P. lanceolata, Farwell., A. Philippens. L., A.hohenackeranum (Kunze) T. Moore A.incisum Forssk., P.vittata L., P.calomelanos (L) Link., H.crenatum (Forssk.) Kuhn D. quercifolia (L.) J.Smith. from Ratnagiri District of Maharashtra state were collected for analysis. The specimens were identified by the Pteridophytic flora of South India [8]. Plant species collected and washed with water to remove soil and debris then plants were dried in oven and powdered and powdered material were directly subjected to analysis in Atomic absorption spectrometry (AAS).

III. RESULTS AND DISCUSSION [Page Style]

The phytochemical components of each extract are presented separate in fig, 1 to 15 viz. Moisture, Ash, Nitrogen, Phosphorous, Potassium, Calcium, Magnesium, Sulphur, Sodium, Zinc, Ferrous, Copper, Manganese, Molybdenum and Boron. Element concentration in 10 pteridophytes (whole) where measured by relative methods of AAS using multi element standards as comparators these are listed. In order to compare the main elements concentration on each species for N, P, K, Ca, Mg, S, Na, Zn, Fe, Cu, Mn, Mo, and B are plotted in separate Fig (1-15).

The moisture content in M. minuta have highest percentage (13.7%) and lowest in A. philippens (0.6%) followed by P. lanceolata, P. calomelanos, C. parasitica, D. quercifolia, A.hohenackeranum, P. vittata,

H.crenatum, A. incisum (Figure 1). Highest Ash Produced in M. minuta (33.56%) and lowest in C. parasitica (0.5%) followed by P. lanceolata, A. Philippens A.hohenackeranum, A. incisum, P. vittata, P. calomelanos, H.crenatum,, D. quercifolia (Figure 2). Highest Nitrogen content present in A.Philippens (1.79%) and lowest in D. quercifolia , M. minuta, H.crenatum equally i.e. (0.5%) followed by A.hohenackeranum, P. vittata,P.calomelanos, C. parasitica, A. incisum, P. lanceolata (Figure 3). Highest Phosphorus content present in H.crenatum (0.28%) and lowest in A.hohenackeranum followed by P. lanceolata, A. Philippens, A. incisum, M. minuta, P. vittata, P. calomelanos, D. quercifolia, C. parasitica (Figure 4). Highest Potassium content present in A.hohenackeranum (1.89%) and lowest in A. incisum (0.21%) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippens, P. calomelanos, D. quercifolia (Figure 5). Highest Calcium content present in A. hohenackeranum (2.83%) and lowest in A. incisum (0.31%) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippens, P. calomelanos, D. quercifolia (Figure 6). Highest Magnesium content present in A. hohenackeranum (6.30%) and lowest in A. incisum (0.70%) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippen, P. calomelanos, D. quercifolia (Figure 7). Highest Sulphur content present in A. hohenackeranum (1.81%) and lowest in P. lanceolata (0.48%) is followed by A. incisum, A. Philippens, C. parasitica, D. quercifolia, M. minuta, P. calomelanos, H.crenatum, P. vittata. (Fig 8). Highest Sodium content present in A. hohenackeranum (2.61%) and lowest in A. incisum (0.29%) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippens, P. calomelanos, D. quercifolia (Figure 9). Highest Zinc content present in A. hohenackeranum (120 PPM) and lowest in A. incisum (13.32 PPM) is followed by H.crenatum, M. minuta, P. lanceolata, P. vittata, C. parasitica, A. Philippens, P. calomelanos, D. quercifolia (Figure 10).

A. Highest Ferrous content present in A. hohenackeranum (128.5 PPM) and lowest in A. incisum (14.28 PPM) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippens, P. calomelanos, D. quercifolia (Figure 11). Highest Copper content present in A. hohenackeranum (54.18 PPM) and lowest in A. incisum (6.02 PPM) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, P. vittata, A. Philippens, P. calomelanos, D. quercifolia (Fig12). Highest Manganese content present in A. hohenackeranum (180.2 PPM) and lowest in A. incisum (20.02 PPM) is followed by C. parasitica, H.crenatum, M. minuta, P. lanceolata, , P. vittata, A. Philippens, P. calomelanos, D. quercifolia(Fig13).. Highest Molybdenum content present in A. hohenackeranum (9.72 PPM) and lowest in A. incisum (0.21 PPM) is followed by C. parasitica, P. vittata, M. minuta, A. Philippens, P. calomelanos, D. quercifolia, P. lanceolata, H.crenatum. (Figure 14).Highest Boron content present in A. Philippens (8.22 PPM) and lowest in , P. calomelanos (1.27 PPM) is followed by A.hohenackeranum, H.crenatum, C. parasitica, P. lanceolata, , D. quercifolia, A. incisum, P. vittata, M. minuta. (Figure 15).

Out of 1000 species of pteridophytes occurring in India, 170 species have been informed to be used as food, flavor dye, medicine, bio fertilizers, oil, fiber and biogas production. The medicinal importance of pteridophytes against bacteria, fungi, virus, cancer rheumatism, diabetes, inflammation, consultant, fertility, diuretic pesticides, hepatoprotective and sedative has been reported besides sugar, starch, proteins and amino acids ferns contain a variety of alkaloids , glycosides, flavonoids, terpenoids, sterols, phenols, sesquitorpens etc. as potential components used in various industries [8]. Nitrogen is required to debelope proper green colour in ferns; weather primary macronutrients Nitrogen (N), Phosphorus (P), Potassium (K) are needed for growth of ferns, three secondary macronutrients: Calcium (Ca), Sulphur (S), Magnesium (Mg) are also important for growth of fern

and fern allies. The micronutrients/ trace minerals: which required by pteridophytes are Boron (B), Manganese (Mn), Iron (Fe), Zinc (Zn), Copper (Cu), Molybdenum (Mo).
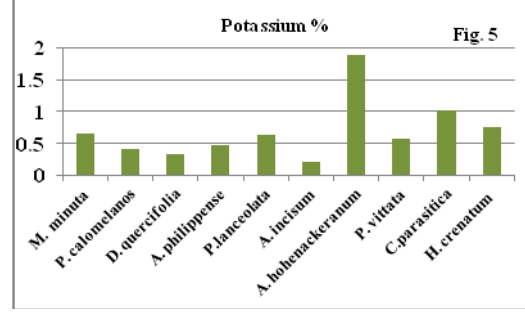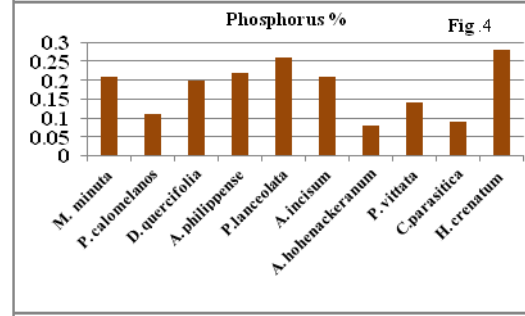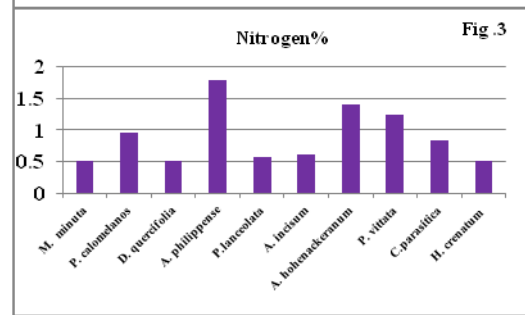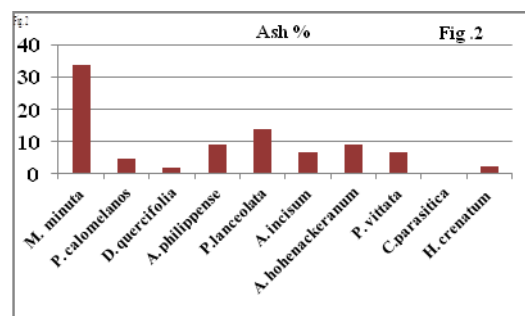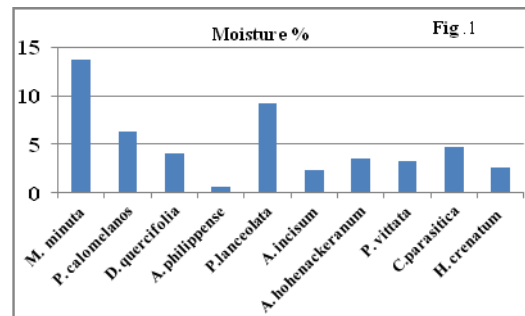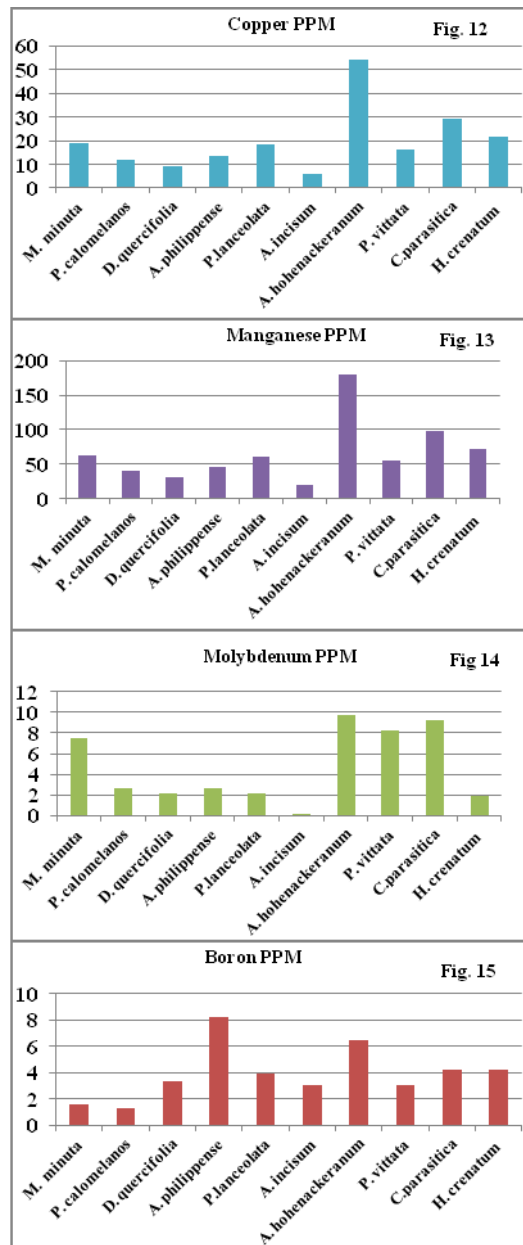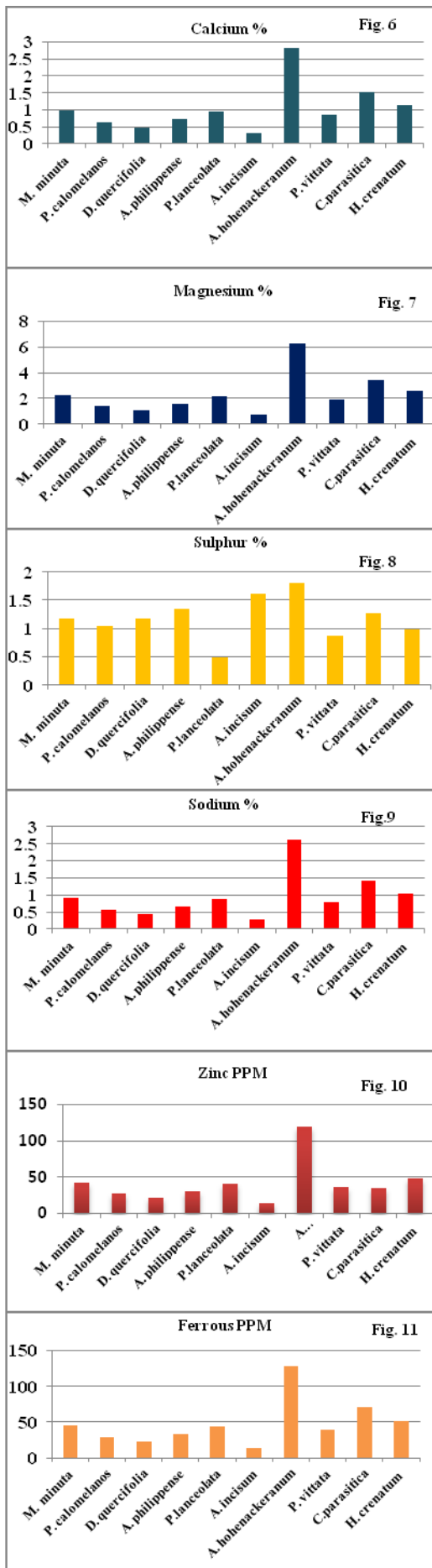
Figyre (1-15). Phytochemical screening of ten pteridophytes from Ratnagiri district of Maharashtra .
1. Moisture (%) 2. Ash (%) 3. Nitrogen (%) 4. Phosphorus (%) 5. Potassium (%) 6. Calcium (%) 7. Magnesium (%) 8. Sulphur (%) 9. Sodium (%) 10 Zinc PPM 11.Ferrous PPM 12. Copper PPM 13. Manganese PPM 14. Molybdenum PPM 15. Boron PPM

IV. ACKNOWLEDGMENT

V. CONCLUSION

Current work would be helpful for developing and updating the database of the studied species and also to undertake specific project based on conservation strategies of the species.

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Fern and fern allies diversity from the Northern Western Ghats of Maharashtra

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ABSTRACT

Ferns and fern allies are seedless vascular plants traditionally referred to as pteridophytes. Pteridophytes are major components of tropical flora, but they show lower extant species diversity than seed plants. Western Ghats, is one of the world's richest areas in terms of its diversity of pteridophyte species, with an occurrence of no less than 320 species. Although this number appears to be an overestimate due to the presence of taxa that are typically synonymized in families with no published revisions of the flora. Thus, the present study was undertaken to analyse present status of pteridophytes with respect to their distribution, habitat and conservation status of the world's most fascinating and important area for pteridology.

Keywords: Fern and fern allies, Western Ghats, distribution, habitat, conservation

I. INTRODUCTION

Pteridophytes form a noticeable part of vegetation all over the world. They are a group of plants having importance in phylogeny and evolutionary biology, because these plants explain the evolution of vascular system and clearly replicate the processes of that have gone into the emergence of seed habit in plants. About 10,000 species belonging to 305 genera of pteridophytes occur in the wild flora of the World of which around ca. 191 genera and 1080 species are distributed in different biogeographical regions of India mainly diversified in Himalayas, Western Ghats and Eastern Ghats [1,2]. Pteridophytes have played a significant role in creating the early land flora as they come forward in the process of the evolution of land plants [3]. A comprehensive study of ferns and fern allies of Northern Western Ghats hills of Maharashtra has not been conducted till date, except for some reports by [4,5,6]. In the recent years, however the pteridological studies have pulled out in various parts of the country, many questions related to the pteridophytic diversity of the Western Ghats remain unexplained and hence, more studies are required for developing in situ and ex situ conservation strategies for this wonderful and important group of plants. Hence the present study was undertaken.

II. METHODS AND MATERIAL

An extensive floristic survey was carried out between July, 2006- September, 2016 to different parts of the Northern Western Ghats of Maharashtra, in the rainy seasons. Specimens of pteridophytes were collected and identified with the help of different floras. Soil samples were also collected from 10-20 cm depth and analysed for different characteristics with standard methods in the laboratory. Temperature and humidity of the study region was measured with the help of a thermo-hygrometer (M288CTH) and light intensity was measured with a digital light meter (TES-1332A) in the field.

III. RESULTS AND DISCUSSION

The Western Ghats transverse the states of Tamil Nadu, Kerala, Karnataka, Goa, Maharashtra and South Gujrat. The Western Ghats presides over the ecology and biogeography of Peninsular India [7]. The Western Ghats harbour about 320 species of ferns and fern allies with more species diversity in the southern part. The major families of pteridophytes found in the Western Ghats are Aspleniaceae, Polypodiaceae, Thelypteridaceae, Selaginellaceae, Pteridaceae, etc. Whereas on the generic level, maximum diversity is observed in the genus Asplenium, Selaginella, Pteris, Athyrium, Diplazium, etc. The Western Ghats also harbors endemic species like Polystichum manickamii, Cyathea nilgiriensis, Bolbitis semicordata, Selaginella radicata, etc. The habitat of the pteridophytes consists of microclimatic conditions with special preference for moist and shady places and a minor disturbance in their microclimate conditions can lead to loss of large number of species (Dudani et al., 2012). In the present investigation major fern species occurred are Osmunda huegeliana Presl., Bolbitis appendiculata (Willd.) Iwats., Bolbitis subcrenatoides Fres.-Jenk., Bolbitis preslina (Fee) Ching., Lygodium flexuosum (L.) J.Sm., Lygodium microphyllum (Cav.) R. Brown, Pityrogramma calomelanos(L.) Link, Pteris biaurita Linn., Pteris pellucida Pr. Cheilanthes tenuifolia (Brum.) Sw., Cheilanthes farinosa (Forssk.) Kaulf., Adiantum capillus- veneris Linn., Adiantum philippense L., Pteridium aquilinum (L.) Kuhu., Lindsaea heterophylla (Bedd.) Bak., Athyrium hohenackerianum (Kze.) Moore Athyrium falcatum Bedd., Tectaria coadunata (Wall.ex.Hook.et Grev) C. Chr., Nephrolepis auriculata (L.) Trimen., Asplenium laciniatum D. Don, Thelypteris interrupta(Willd.) K. Iwatsuki, Blechnum orientale Linn., Pyrrosia adnescens (Sw.) Ching., Microsorium membranaceum (D.Don.) Ching., Pteris ensiformis Burm. f. There is much diversity of ferns in this area may be due to its weather conditions and moist atmosphere. It is observed during exploration that- diversity of fern species goes on decreeing as we go from lower side of hills to the top or at high altitudes. Specific ferns were collected at high altitude these include- Tectaria coadunata (Wall.ex.Hook.et Grev) C. Chr., Cheilanthes anceps Blanford., Pteris biaurita Linn., Microsorium membranaceum (D.Don.) Ching. These species were rarely observed or not at all in low areas.

A key for identification of rare and endangered ferns and fern allies in the Western Ghats [8]. Based on the field observations, data from herbarium collections and literature reference, Jenkins and other Indian pteridologists assessed the rare and threatened pteridophytes of India (Chandra et al, 2008).Previous study also confirmed that the most common pteridophyte species viz., Adiantum philippense L., Aleuritopteris anceps Blanf., A. bicolor (Roxb.) Fraser-Jenk.,Asplenium yoshinagae Makino,Christella dentata (Forssk.)Brownsey and Jermy, Christella parasitica (L.) Holttum., Isoetes coromandeliana L. f., I. dixitii Shende, Lepisorus nudus (Hook) Ching., Marsilea minuta L., Microsorum membranaceum (D.Don) Ching., Selaginella delicatula (Desv.exPoir.) Alston., Selaginellaciliaris (Ritz.) Spring, Selaginella tenera (Hook and Grev.) Spring., Ophioglossum costatum R. Br., O. gramineum Willd., O. lucitanicumL., O. nudicaule L., O. petiolatum Hook.,O. reticulatum L., Pityrogramma calomelanos, Pteris biaurita L, P. pellucida C. Presl., P. vittata L., Pyrrosia lanceolata (Wall.) Farw, Salvinia molesta D. Mitch., Selaginella ciliaris (Ritz.) Spring., S. crassipes Spring., S. delicatula (Desv. ex Poir.) Alston., and Tectaria coadunata (Wall. ex Hook. and Grev.) C. Chr., also found growing luxuriantly. Urbanization is a leading cause of habitat loss and biologicalhomogenization [9]. Due to unplanned felling of trees in the forests the members of epiphytic pteridophytes belonging to the families Polypodiaceae, Davalliaceae, Aspleniaceae, Vittariaceae, have been reduced day-by-day. The anthropogenic factors have posed a serious threat, due to which there is complete disappearance od some species. The rapidly shrinking fern cover of Northern Western Ghats prompted to ponder over the issue [10, 11].

IV. CONCLUSION

The maximum diversity was observed at the high altitude zone, high rainfall zone, high atmospheric humidity and low temperature zone. Many pteridophytes were listed in the list of threatened species in one category or the other and yet, effective answers have not been investigated to contest this world-wide problem. As ferns are sensitive to minor changes in climatic change they are becoming rare and endangered. The efforts are required to aware the importance of these species among the local people. Further, these plant species are in great need to have in situ or ex situ conservation.

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Gauno is a good fertilizer from Cynopterus sphinx Frugivorous Bat (MEGACHIROPTERA: PTEROPODIDAE) in Ahmednagar, Maharashtra

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ABSTRACT

Cynopterus sphinx is a frugivorous bat also known as greater short-nosed fruit bat or short-nosed Indian fruit bat known for fruit eating habit. Apart for which its feces called as guano is thought to be rich in nutrient and microbes. Recent research was carried out to study its positive implementation as manure for plants. Results of present study indicate that bat guano is an efficient source of manure which helps to increase plant productivity and improve soil texture.

Key words: Cynopterus sphinx, Guano, Manure.

I. INTRODUCTION

Cynopterus sphinx is a frugivorous bat also known as greater short-nosed fruit bat or short-nosed Indian fruit bat and this species found in Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Malaysia, Myanmar, Pakistan, Sri Lanka, Thailand, and Vietnam (Fujitta and Turtle 1991). Ahmednagar is found to be reach spot of diversity of frugivorus btas. Some distinct species present in Ahmednagar are well known for their fruit consumption. Thses speies are identified as Cynopterus sphinx, Taphozous longimanus, Pteropus giganteus (Kardile V. U. 2009). The use of Bat guano in the improvement of the nutritive value of poor quality roughage fed to ruminants (M. lay, P. M. 2008). Application of farmyard manure (FYM), compost, Green manure and bioinoculants are the most important management practices in farming (Thampan,1993). Improvement of soil fertility through the application of vermicompost is becoming more popular. Guano was somewhat forgotten once chemical fertilizers became the plant food of choice, but it has always been prized by organic growers. Now that the risks of synthetic gardening products are becoming widely known, more and more farmers are realizing that this dark, rich manure is indeed one of nature's treasures. It is an alternative to a shelf full of chemical products, ably serving as plant fertilizer, soil builder, soil cleanser, fungicide, nematocide, and compost activator. Most chemical fertilizers leach out of the soil soon after being bat guano can be safely used as a fertilizer, both indoors and outdoors. Its primary ingredients are roughly 10% nitrogen, 3% phosphorous, and 1% potassium. Besides these three major nutrients, guano contains all of the minor and trace elements necessary for a plant's overall health. Unlike artificial gardening products, guano applied. Guano remains much longer, enhancing the soil and slowly continuing to feed the plant.

Guano "works wonders" as a soil builder and says it can be used year-round to improve soil texture and richness, helping to bind loose soil and lighten heavy soil. Homeowners have reported that the benefits of a single treatment on the lawn can still be seen three or four years later. Because guano is rich in bioremediation microbes, which clean up toxic substances, it is a purifying addition for gardens in transition from chemical to organic practices. These microbes will combat fungus when sprayed directly on a plant's leaves. Bat guano contains powerful decomposing microbes, which help control soil-borne diseases and harmful nematodes and which serve as ideal compost activators, significantly speeding the decomposition process (Keleher, S., 1996).

II. METHODS AND MATERIAL

Selection of Site

Location of Ahmednagar and India coordinates 19.08ºN 74.73ºE at an elevated about 615m above sea level .The study site selected for present investigation is Botanical garden in New Arts, Commerce and Science College, Ahmednagar (See Fig No-1). We are selected five stations on selected site for collection of guano. These stations are Station I – tree species like Delonix regia (Gulmohar) ,Station II- Samanea saman (Rain tree),Station-III- Eucalyptus alba (Nilgiri) ,Station IV- Albizia libbeck (Shirish),Station V- Ficus racemosa (Umbar) (Ingalhalikar, S And S .Barvr) are the roosting sites for a large number of Cynopterus sphinx (about 840) over the past years(See Figure 2) .

Collection and Preservation of sample

Polythene sheets were hanged horizontally to spread collect bat excrement during November 2015 to February 2016(See Fig No 3). Sample is daily collect at afternoon by wearing handglose and mask in paper boxes. Sample is preserved in boxes by sun drying process (See Fig No 4). Amount of sample is crush with the help of mortar and pestle.

Analysis of Guano

Preserved guano at different time intervals the following parameters was used to analysis of guano as follows-

1. P^H

P^H was estimated using P^H meter after preparation of saturated solution by 1:20 dilution w/v with distilled water (Behera, P.K.)

2. Total organic matter

Total organic matter was estimated by weighing 10 Gms sample initially and completely incinerating in furnace above 250ºC and final weight was taken by weighing residue. Organic content was recorded from difference of initial and final weight. Similarly inorganic salts were recorded (Harbone, J. B.)

3. NPK estimation

Sample was given for detection of **N**, **P** and **K** to Shrushtipriya laboratory and environment consultants, Ahmednagar . Following methods was used-

Nitrogen (N): from Kjeldahl method

Phosphorus (P): from Colorimetric method

Potassium (K): Atomic Absorption Spectroscopy.

a) Preparation of different treatment selected

Seven treatments (T1-T7) consisting of loamy soil, bat guano and farm yard manure(FYM)(w/w) were assessed .Red loamy soil (150g) was mixed with bat guano and farm yard manure in different proportion in plastic trays. Sets made for treatment were as follows (See Fig 5: T1 to T7) (Ashwini, KM.et al 2006)-

T1= Soil (Control)

T2 = Soil +Bat Guano (20:10)

T3 = Soil +Bat Guano (20: 5)

T4 = Soil +Bat Guano (20: 2.5)

T5 = Soil +Bat Guano (20: 1)

T6 = Soil +Bat Guano + FYM (20: 2.5:10)

T7 = Soil +FYM (20: 10)

Figure 1. Collection Site

Figure 2. Polythene sheets

Figure 3. Sun Dry sample

(T1: Soil (Control), T2 = Soil +Bat Guano (20:10), T3 = Soil +Bat Guano (20: 5), T4 = Soil +Bat Guano (20: 2.5), T5 = Soil +Bat Guano (20: 1), T6 = Soil +Bat Guano + FYM (20: 2.5:10), T7 = Soil +FYM (20: 10).

b) Selection of seed material and Cultivation

The healthy 100 seeds of Phaseolus aconitifolius (Mataki) were selected per treatment. Plastic trays were sterilized with 0.1% $HgCl_2$ for different sets. Trays were filled with selected ratios of Soil, FYM, and Bat Guano.100 seeds per tray were sown at equidistance and covered by moist filter paper to maintain humidity and avoid water loss.

c) Analysis of plant growth parameter

1. Percentage of germination

After one week of sowing the percentage of germination was determined and recorded (Cook,T).

2. Seedling height

The plastic tray contains the various proportions of soil and guano. After one week of sowing 10 seedlings from each tray was taken and measure the root and shoot length separately and record it. The total of each root and shoot length is seedling height (Cook,T) .

3. Survival of plants

Survival of plants was recorded after four week of germination by counting healthy plants in each tray (Cook,T).

4. Fresh weight and dry weight

The fresh weight was recorded after removing plants from each tray and dry weight was recorded after drying plants at 25°C in hot air oven (Cook,T).

5. NPK uptake

For NPK uptake, dried powder of plant was gives to Shrushtipriya laboratory and environment consultants, Ahmednagar Following methods was used-

Nitrogen (N): from Kjeldahl method

Phosphorus (P): from Colorimetric method

Potassium (K) Absorption Spectroscopy.

6. Statistical Analysis

From recorded data of germination and plant height, Plant Dry Biomass and Nutrient Uptake was statistically analyse.

III. RESULTS

Physicochemical feature

Physicochemical feature of guano is presented in following table. Throughout study it was observe that bolus was acidic in nature (P^H 6.12). Conductivity, Organic matter, Nitrogen, Potassium, Phosphorus, Fungi, was substantially higher than other animal manure.

Table 1. Physicochemical features of guano

Characteristic	Values
P^H	6.12
Total Organic matter (gm)	0.611
Total Nitrogen (mg/lit)	4.1
Phosphorus (mg/lit)	2.3
Potassium (mg/lit)	3.6
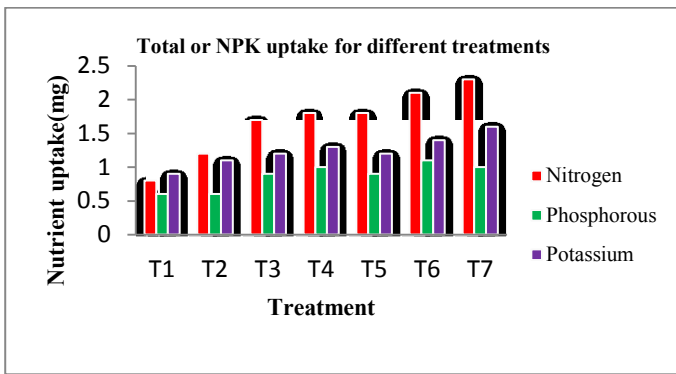
Graph: 1. NPK uptake for different treatments
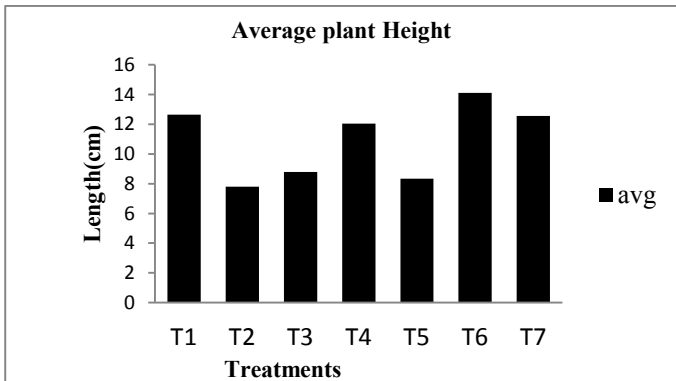
Graph 2. Plant height

Crop growth was better in T1, T6 than T2-T5 and T7. The crop in T6 (soil+guano+FYM) showed the highest growth shoot length, NPK uptake (graph 1). T-test revealed the significant difference ($P<0.05$) between T6 (20:2.5:10) and T1 (Control) as like as T6 and T7 (20:10) in Highest growth, NPK uptake compare to other treatments. The crop in T2 (Soil+ guano) concentration guano is high resulted in wilting of seedling possibly due to high concentration of nutrients. In T6 successfully use as mixing of soil, FYM and guano to increased the growth and nutrient uptake of crop. Manure partially meets the NPK requirement of plantation crop, guano in appropriate ratio may help overcome the deficiencies to improve plant quality.

IV. DISCUSSION

Nitrogenous guano is known to be enhancing crop growth, while phosphorus guano induced root development, shoot budding, multiple branches and flowering. In this study, the uptake of P in treatment T6 indicating the presence of P in available form and importance of bat guano in crop growth. The high P uptake in T6 treatment resulted in increased quality of crops. This indicates the soil amendment with guano at 20:2.5:10 ratios supplied adequate nutrients. Amending soil with high quantities of guano (in T2, T3, T4) resulted in the wilting of seedling possibly due to high concentration of nutrients (Sridhar, K.R et al., 2006).

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4G - Electronic Prototype to Detect Tsunami and Similar Ocean Water Irregularities

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ABSTRACT

"Tsunami" Japanese meaning "harbor wave" is a natural disaster. Tsunami is a series of waves in ocean caused by an earthquake or volcanic eruption and landslide. Tsunami resembles sea waves having far longer wavelength. They resemble a rapidly rising tide, therefore tsunami are often referred to as 'tidal waves'. The Tsunami generates wave heights of tens of meters and their destructive power can be enormous and they can affect entire ocean basins. A typical wave period for a damaging tsunami is about twelve minutes. Meaning thereby the powerful and high velocity sea waves reaches the sea shore after twelve minutes from the instant when the under water earthquake occurs. At present there are 'tsunami warning systems' for the detection of tsunami before it arrives so that the loss of life and property could be minimized. The present systems that are solely dependent on satellite are very expensive, the satellite surveillance fails to detect the under water movement, the present systems are less reliable, also they are not a fully 4G systems for detection of tsunami or similar ocean water irregularities. So, efforts have been made here to put forth a 4G - Electronic prototype which is an embedded system using multi-sensors providing a real time output. This proposed system is a reliable and suitably sensitive system for continuous monitoring of tidal waves. It contains more than one sensor to provide redundancy in case one of the sensor units fails.

Keywords: Tidal waves, multi-sensor, communication, hardware, software

I. BACKGROUND

Tsunami is also termed as tidal waves. It is a series of waves in a water body caused by the displacement of a large volume of water, generally in an ocean. Tsunamis generally consist of a series of high velocity, increased height and powerful waves arriving on the sea shore in twelve to fifteen minutes. Tidal waves of tens of meters can be generated by large events causing enormous destruction of life and property. Although the impact of tsunamis is limited to coastal areas, their destructive power can be massive and they can affect entire ocean basins.

Era demands to serve a brief warning of tsunami to the people on the shore as well as to the entire world so that, the helping hands from the entire world can spread their wings immediately to save the victims of tsunami. The proposed system accomplishes this task of giving early warning. If people on the sea shore are served with a prior 10 to 15 minutes of brief warning then they can survive provided that they immediately run for high ground or seek the upper floors of nearby buildings.

In 2004, a tsunami was caused due to the earthquake in the Indian Ocean whose magnitude was around 9.2 ; it was the deadliest tsunami that killed roughly 2,30,000 people.

In 2006, a tsunami was caused in the java island due to an earthquake in the Indian Ocean whose magnitude was around 7.7.

Basic Tide Gauge

BASIC TIDE GAUGE

Principal cause of Tsunami
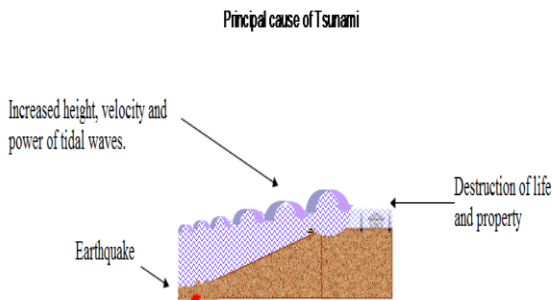
Figure 1

Figure 2

II. METHODOLOGY

Three methods of tidal observations are well known.

1) Placing number of sensors at appropriate depths and places in the ocean - This method is expensive.

2) Satellite Surveillance – This method is unable to detect the under water movement.

3) Therefore, installation of reliable and suitably sensitive system is preferred for continuous monitoring of tide waves. .The float system is the common form of level measuring system. It consists of a basic tide guage consisting of a stilling well with a float unit and a recording drum attachment. The float is attached to a chain. The chain in turn is attached to a counter weight which indicates the level as the float moves up and down.

The figure 2 shows the basic float type tide guage meter.
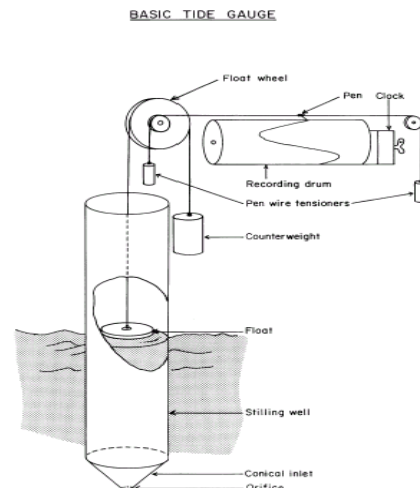
III. PROPOSED SYSTEM

The development tool preferred is Keil micro-vision which was acquired by Arm in the year 2005. It is a popular IDE – originated from Keil software. It is preferred as it includes project management, source code editing, program debugging and flash programming. Along with this it also possesses various other benefits too.

The domain knowledge required for embedded instrument of 4th generation tide guage meter is:

- Physics for sensors and actuators.
- Digital signal processing.
- Communication protocols (TCP/ IP).
- Instrumentation.
- Networking.
- Oceanography.

Figure 3 shows the basic concept of embedded instrument of 4th generation tide guage meter.

Basic Concept of Embedded Instrument of 4th generation TGM
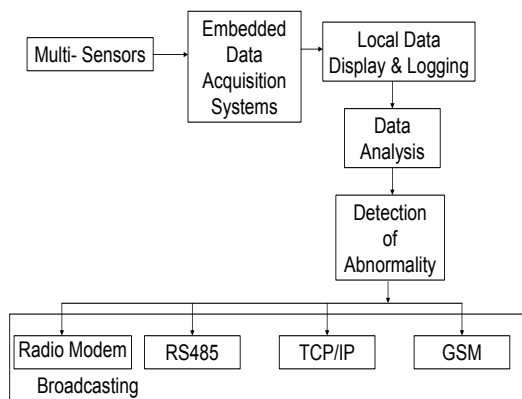
Figure 3

IV. Multi-sensor

Instead of using a single sensor for detection of ocean water irregularities multi-sensors are preferred. The idea behind using a multi-sensor is very genuine. Using more than one sensor provides redundancy in case one of the sensor units fails. Instrumentation is preferred for the measurement and control of the process variables. The process variables used are Level and Pressure.

The following sensors are preferred:

1. Ultrasound Sensor – The principal of operation includes time of flight proportional to the depth of water surface from sensor in air. Now-a-days even there are ultrasound sensors in existence which are capable to work underwater.

Figure 4 shows the ultrasonic sensor

Ultrasonic Sensor
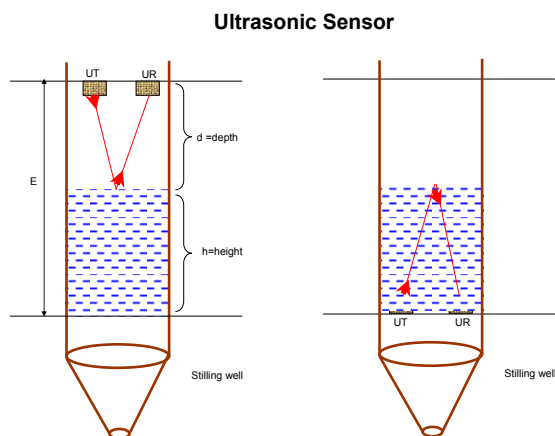
Figure 4

2. Pressure Sensor – The Strain guage type pressure sensor is preferred. The principal of operation includes the input pressure proportional to output voltage. This voltage in turn is proportional to tidal height. Pressure sensor is chosen as they are available in miniature size and high accuracy models capable of 0.05% accuracy. They measure differential pressure as well as extremely low or high pressures.

3. Float / Counter weight System: Absolute Encoding and Incremental encoder are preferred.

Communication

a) GPS

The Global Positioning Satellite enables tide guage for determining location. It provides information related to latitude, longitude and real time tracking.

b) Data transmission modes:

- **RS 485**

  It's works as an electrical interface. It is also known as TIA-485(-A), EIA-485. It is a standard defining the electrical characteristics of drivers and receivers for use in serial communications systems. Digital communications networks implementing this standard can be used effectively over long distances and in electrically noisy environments.

- **Radio Modem**

  Radio modems transfer data wirelessly across a range of up to tens of kilometers. Radio modems is a modern way and it creates Private Radio Networks (PRN). Private radio networks are used to provide real-time data communication. The advantage of radio modems is that it enables the user to be independent of telecommunication or satellite network operators.

- **GSM Mobile**

  A GSM modem acts as a specialized type of modem which accepts a SIM card, and operates over a subscription to a mobile operator, just like a mobile phone. The brief warning becomes available on GSM mobile.

- **Hardware and software TCP/IP stack**

  The TCP/IP Internet protocol suite provides end-to-end data communication specifying how data should be packetized, addressed, transmitted, routed, and received on internet.

  A data network is used as a digital telecommunications network. It allows nodes to share resources. The connections between nodes are established using cable media and wireless media.

Hardware requirements

- **ISAC**

  Intelligent Sensor Actuator Controller from ADI provides a 'system on chip' (SOC). It is programmable. ISAC is also termed as "micro-converter". These components from Analog Devices combine a powerful 8051-family microcontroller core, including flash program and data memory, with a multi-channel 12-bit analogue interface which includes an A/D as well as a D/A converter. ADuC831 is preferred as it has its major features on one silicon chip. Its size is very small around 14.15 mm pin-pin.

- **Wiznet Ethernet module**

  WIZnet is the trend leader of Open Source Hardware. Its unique solution, Hardwired TCP/IP technology, in Arduino's Ethernet Shield has been recognized as standard for IoT. WIZnet is the IoT Device Platform Company. Its unique technology – Hardwired TCP/IP provides better performance and stability than any other software Internet connectivity solutions.

Software Requirements

- Software development in embedded C.
- LabVIEW based GUI

  Laboratory Virtual Instrument Engineering Workbench (LabVIEW) provides a system-design platform and development environment for a visual programming language from National Instruments.

Graph

At the instant tide waves increase in height and start reaching the shore it takes around few minutes to reach the shore. Figure 5 shows the plot of tidal height in centimeter versus time in seconds.
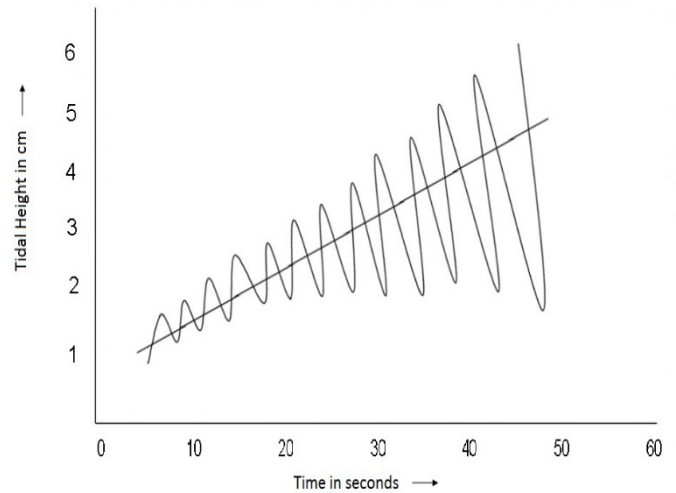
Figure 5

V. CONCLUSION

With the increase in the number of occurrences of the tsunami all over the world, it has become quite essential to develop an efficient tsunami warning system for the detection of tsunami before it arrives. This will ultimately minimize the loss of life and property. As any of the system cannot be hundred percent efficient, so there is always scope to improve the tsunami warning system in terms of speed or accuracy or power consumption, etc. When an earthquake under sea or volcano eruption with landslide is occurring in the sea, then the tsunami warning system can provide sufficient time before the arrival of the tsunami. This proposed work is a step taken to get knowledge of the existing tsunami detection 'the basic tide guage' and to implement a reliable, cost effective and 4th generation technology for detection of Tsunami and similar Ocean water irregularities.

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Antifungal potential of Parthenium hysterophorus and Catharanthus roseus against Aspergillus sp., Candida sp. and Penicillium sp. of aquarium fish

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ABSTRACT

Different type of fungi affects the health of plants and living organisms. Sometimes even in aquarium, fishes get infected with fungal infection due to aquarium conditions or other factors. In severe conditions these infections may be fatal for the fishes. So to prevent such infections fishes get treated with conventional chemicals available in market. We have an excellent substitute to avoid this use of conventional chemicals in aquariums through plants. Number of bioactive molecules, making them an affluent source of variants of medicines. In this study, in-vitro antifungal activity of crude leaves extract of medicinal plants Parthenium hysterophorus and Catharanthus roseuswas evaluated against Candida sp, Penicillium sp, Aspergillus sp which isolated from infected aquarium fish. The solvents used are Ethanol, Methanol, acetone and Chloroform. "The disc diffusion method used to assay the antifungal activity." The Antifungal activity exhibited by cold ethanol extracts and chloroform extracts (leaves) has shown prominent results, also these extracts can be utilized as an option for the antifungal conventional chemicals available in market. These extracts are also ecofriendly, which can help in maintaining good aquarium conditions.

Keywords: Parthenium hysterophorus, Catharanthus roseus, Antifungal activity,Candida sp, Penicillium sp, Aspergillus sp.

I. INTRODUCTION

Aquarium fish are prone to fungal infections which is a high risk to the aquarium business. In addition Aspergillus sp., Candida sp. and Penicillium sp. become more prevalent in fish infections.

Aspergillus sp., Candida sp. and Penicillium sp. genera are prominent for their production of mycotoxins (Simon G. Edwards et.al.2002) which cause severe effects on aquarium fishes. To dispense these infections conventional chemicals are being used like Lotrimin AF, Gyne-Lotrimin, Malachite, etc. These chemicals are known to have hazardous effects on the environment as well as on animals. The plants have shown effective defense system with natural compounds which may provide potential alternative to the utilization of synthetic chemical fungicides. An attempt has been made to use plant extracts, which are considered to be safe with an environmental perspective, as antifungal agent. Many plants has been studied recently for their insecticide, bactericide, fungicide and other effects. The plants selected for this work is Parthenium hysterophorus and Catharanthus roseus.

Parthenium hysterophorus is an unwanted plant i.e. weeds in agriculture. "P. hysterophorus is an annual herb that aggressively colonises perturbed

sites". "It is much-branched with vigorous magnification habit, aromatic, annual (or an ephemeral perennial), an erect, herbaceous plant with a deep taproot. These species reproduces by seed." It grows to 30-90 cm in height (Lorenzi,1982; Kissmann and Groth,1992), can be up to 1.5 m, or even 2.5 m, in exotic situations (Haseler, 1976; Navie et al., 1996).The reason og plant being toxic is 'parthenin' and other phenolic acids such as caffeic, vanillic, anisic, panisic, chlorogenic and parahydroxybenozoic are lethal to human beings and animals. (Mahadevappa, 1977; Oudhia, 1998).Rastogi and Mehrotra (1991) describe Parthenium hysterophorus L. as a medicinal plant because it used in many diseases. Even Parthenium hysterophorus has shown the antimicrobial activity (TerefeTafeseBezuneh .2015).

Catharanthus roseusis anconsequentialmedicinal plant. This perennial plant grows as a herb or a subshrub, spreading along the ground or standing erect up to a meter in height. It has captivating flower in white or pink colour with five petals while the leathery, dark green leaves are arranged in antithesis pairs. "It contains different type of alkaloids and chemotherapeutic agents that are effective in treating various types of infections, diseases, etc." (K. Kabesh et.al.2015).Muhammad et al. (2009) reported that Catharanthus roseus showed the antibacterial potential in crude extracts of different components (viz., leaves, flower, root and stem) against clinically significant bacterial strains. Catharanthus roseus possesses known antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Prajakta J. Patil and Jai S. Ghosh .2010).

Acetone, ethanol, chloroform, methanol and aqueous extract of these plants were assessed for its antifungal activity against Aspergillus sp., Candida sp. and Penicillium sp. This extract can further be analyzed for its active component and be included in the strategy to manage the fungal infections. Parthenium hysterophorus and Catharanthus roseus are the plants which are utilized during the study. These are facilely available.

II. METHODS AND MATERIAL

1) **Extract collection and preservation** : Fresh plant leaves of Parthenium hysterophorus and Catharanthus roseus were amassed from ManjariBudruk, Pune, Maharashtra 411028 (MS) (18.5042° N, 73.9539° E) and washed with distilled water. The samples were homogenized manually with mortar and piston. In order to obtain extract amassment 25ml of solvents (Ethanol, Methanol, chloroform, acetone and dihydrogen monoxide) was integrated with 15g fresh samples. Then the extracts were discretely filtered and amassed from paste by utilizing Whatmann filter paper no.1. Amassed crude extracts were then preserved in the refrigerator at low temperature for further assay.

Table 1. names of selected plants

Local Name	English Name	Scientific Name	Used part
GajarGavat	Carrot grass	Parthenium hysterophorus	Leaves
PandhriSadaphuli	Tiny periwinkle	Catharanthus roseus	Leaves

Figure 1. Parthenium hysterophorus

Figure 2. Catharanthus roseus

2) **Test organisms** :In order to investigate the effects of plants on fungal pathogens of fish fresh fungus isolated from diseased fish from aquarium.

3) **Isolation of fungi:-**

Cotton plug was acclimated to accumulate the fungi from the skin of the infected fish. Then this Cotton plug was introduced to saline water for the further processing. After accumulation of fungi these were introduced to sterile petri dish a PDA media. The petridish was incubated until the occurrence of fungi for about 24 hours at 25˚C.After identification of fungi; they were separated to different petridishes. Subcultivations on petridishes were carried out and utilized for further assay.

4) **Antifungal Assay**:

Antifungal activities of plants were observed by utilizing the disc diffusion method (Kerby Bauer method) on sterile petri dish of PDA media by introducing each fungus in separate petridishes. Then petridishes were incubated for 24 hours at 25˚C.

5) **Investigation of effects of selected plants:**

Culture media and crude extract of the plants were utilized in the disc diffusion method discretely Pathogenic organisms were spread on the surface of the potato dextrose agar plates and discs (Whatmann No.1 filter paper with 9 mm diameter) impregnated with the 10 µl of P.hysterophorus and C. roseus leaves extract. Plants samples were place on the surface individually. The plates were incubated 25°C for

24 h. he antifungal potential of the test samples were quantified by determining the diameter of the zones of inhibition in centimeter.

III. RESULTS AND DISCUSSION

Extract of the Parthenium hysterophorus with cold acetone and chloroform showed the eminent antifungal activity against Penicillium sp.(Figure 5) in virtually double zone of inhibition than that of control. And against Aspergillus (Figure 3) and Candida sp. (Figure 4)effect was good with quite more zone of inhibition than control.

Table 2. Parthenium hysterophorus zone of inhibition (cm)

Fungi		Aspergillus (Figure 3)	Candida (Figure 4)	Penicilium (Figure 5)
Acetone	control	1.0	1.0	1.0
	leaves	1.2	1.4	2.0
Ethanol	Control	1.0	1.0	1.0
	Leaves	1.3	1.5	1.3
Chloroform	Control	0.9	0.9	0.9
	Leaves	0.9	0.0	2.0
Methanol	Control	1.3	0.3	0.0
	leaves	0.9	0.9	0.0
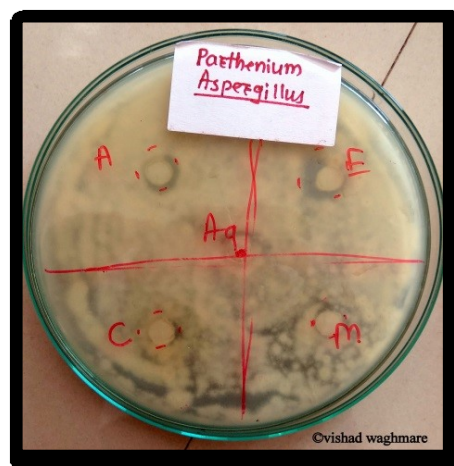
Figure 3
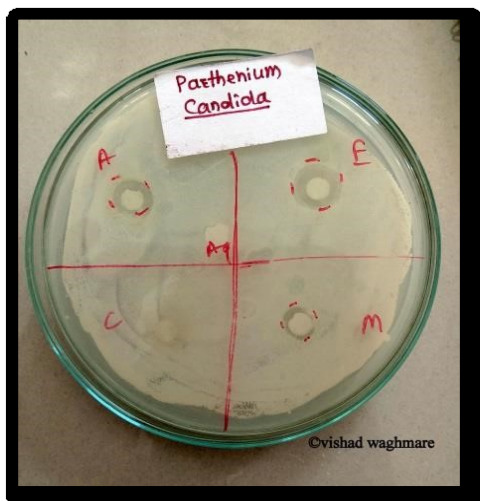
Figure 4
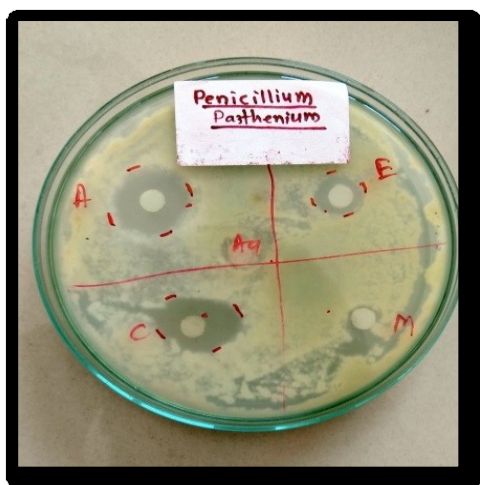
Figure 5

Extract of the Catharanthus roseus with cold ethanol showed a notable antifungal activity against Penicillium sp.(Figure 8) and with cold chloroform it showed activity against Aspergillus sp.andPenicillium sp. (Figure 6) in almost double zone of inhibition than that of control. And against Candida sp. (Figure 7)it showed no activity.

Table 3. Catharanthus roseuszone of inhibition (cm)

Fungi		Aspergillus (figure 6)	Candida (figure 7)	Peniciliu m (figure 8)
Acetone	control	1.0	1.0	1.0
	leaves	1.1	1.3	1.2
Ethanol	Co	1.0	1.0	1.0

	ntrol			
	Leaves	1.3	1.6	2.0
Chloroform	Control	0.9	0.9	0.9
	Leaves	2.7	1.4	2.0
Methanol	Control	1.3	0.3	0.0
	leaves	0.0	0.8	1.0

Figure 6

Figure 7

Figure 8

IV. DISCUSSION

"Antifungal property of P.hysterophorus has been reported against the common fungi that we used on plants and animals" (TerefeTafeseBezuneh 2015). According to A. Devkota and A. Sahu 2016, "methanol crude leaf extract had higher antifungal potential than the distilled water" extract, but in present study it has been observed that P.hysterophorus is more efficacious with acetone and chloroform. In KratikaKumari, Sharmita Gupta 2005 studies C.roseus showed 1.62 cm and 1.5 cm zone of inhibition against Aspergillus sp. and Candida sp. with acetone whereas in current studies we have visually perceived C.roseus showed 1.1 cm and 1.3 cm zone of inhibition respectively. C.roseus has showed good activity against fungal pathogens with ethanol and chloroform with maximum zone of inhibition (Divya Paikaraa1 et.al.2017). The leaf extract of C. roseus is very effective on Candida sp. (Uniyal et al. 2006, Bhadauria and Kumar 2011) Aspergillus sp. and Penicillium sp.

V. CONCLUSION

The present study provides utilizable information regarding the efficiency of plants against fungal infection in fishes. Parthenium hysterophorus and Catharanthus roseus products used virtually both showed better antifungal activity against Aspergillus sp. followed by Candida sp. and Penicillium sp. We have an excellent substitute to avoid use of conventional chemicals in aquariums through Parthenium hysterophorus andCatharanthus roseus.

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Characterization of mycobacterial isolates from clinical samples by using PCR-RFLP assay targeting hsp65 gene region

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ABSTRACT

Mycobacterial have long been recognized as important human and animal pathogens. Mycobacterial diseases such as tuberculosis and leprosy continue to be an important public health problem all over the world. Not only M.tuberculosis and M. leprae are important one, but mycobacteria other than tuberculosis (MOTT) are also pathogens. The immunosuppressed individual infection by HIV infection have become the most significant risk factor for disseminated NTM diseases and of these 95% are due to Mycobacterial avium complex. Thus the rapid and specific diagnosis of tuberculosis is one of most pressing needs in effort to temporary treat and control the disease. Classical identification result for mycobacteria based on culture and biochemical test may take several weeks and test fail sometimes to produce precise identification. PCR- restriction analysis (PRA) is a PCR-RFLP based, very efficient and promising approach for species identification as it gives result within one day. Several targets for PRA analysis of mycobacteria's are available which includes hsp 65kDa. The 65kDa proteins contains epitopes that are unique as well as epitopes that are common to various species of mycobacteria's.PRA is based on amplification of 439bp fragment of hsp65 gene present in all mycobacteria offers an easy rapid an inexpensive procedure to identify several mycobacterial species in single experiment. Thus the current work emphasis on quick, advanced and robust methodology for diagnosis of tuberculosis and other mycobacterial diseases.

Keywords: NTM, hsp, epitopes, PCR

I. INTRODUCTION

Mycobacterium, the genus of family of mycobacteriaceae includes 130 species till now(Euzbey 2008, Katoch 2007). Mycobacteria have long been recognized as important human and animal pathogens. Tuberculosis and leprosy are public health problems all over the world. It is believed that about one third of the world's population is infected with M. tuberculosis (Musser 1995). Tuberculosis has been called as White plague and caption of all the men of death(Ananthnarayan and Pannicker 2001).

Not only M. tuberculosis and M.leprae are important ones, but mycobacterium other than tuberculosis(MOTT) are also pathogens. The range of infection caused by MOTT or opportunistic mycobacteria is quite broad including skin infection(eg .M. marinum), cervical lymphadenitis(eg .M. avium), joint infection(eg. M. avium and M.intracellulare), bacteria in AIDS(eg M.avium) and nosocomnial infection (eg M. fortuitum and M. chelonae).

Various anti-tuberculosis drugs that are effective against infection caused by M.tuberculosis are available. These include Rifampicin, isoniazid,

pyrazinamid, ethambutol, streptomycin etc. In recent years the treatment of tuberculosis is threatened by increasing the number of patient with multi drug resistant tuberculosis especially to rifampicin and isoniazid(Paramsivan et al 1993,94)The rapid and specific diagnosis of mycobacteria is one of the pressing needs in effort to temporary treat and control the disease.

Clinical identification of bacteria based on cultural and biochemical test may take several weeks. Additional techniques such as thin layer chromatography (Marks and Szulga 1965) gas liquid chromatography(Levy Frebault et al 1983) are powerful tools but limited by the need for standardized growth conditions. Several gene probe and gene amplification techniques for detection and identification of pathogenic bacteria from culture as well as directly from the lesion have been reported(Katoch and Sharma 1997).

PCR-restriction analysis(PRA) is a PCR-RFLP based very efficient and promising approach for species identification as it gives result within one day. Several targets for PRA analysis of mycobacteria are available including hsp 65kDa(Telenti et al 1993), rRNA (Dobner et al 1996) 16S-23S rRNA spacer region(Katoch et al 2007).The hsp65 (439bp)gene coding for 65kDa heat shock protein by PCR and restriction enzyme analysis(RFLP)(Bunello et al 2001).The 65kDa protein contains epitopes that are unique as well as common to various species of mycobacteria. (Telenti et al 1993). PRA based on the amplification of 439bp fragment of the hsp65gene present in all mycobacteria offers an easy, rapid and inexpensive procedure to identify several Mycobacterial species in single experiment.(Devallosis er al 1997)

II. METHODS AND MATERIAL

Agarose ,Asparagine, Chloroform, Disodium phosphate EDTA, Ethidium bromide, Hydrochloric acid, Mac Conkey agar, Sodium dodecyl sulphate, Tris base, Lysozyme, Proteinase K Clinical sputum samples were collected and decontamination done by using 4%NaOH. Samples were cultured on LJ medium and incubated for 8 weeks at 37°C(Vestal 1977).Biochemical tests such as Catalase test, Nitrate reduction test, Tween 80 hydrolyis test was done to ensure Mycobacterium tuberculosis infection.DNA isolation(van Embden et al 1993) was done followed by amplification of DNA (Telenti et al 1993). Restriction of amplified DNA was done using HaeIII enzyme.

III. RESULTS AND DISCUSSION

In this study PCR RFLP analysis(PRA)of hsp 65 gene was applied to characterize 17 mycobacterial isolates from sputum samples of pulmonary cases.
The reference strains used were H37Rv(M. tuberculosis), N-2(M. fortuitum), J-7(M. avium), J-28(M. vaccae).

IV. CONCLUSION

The study aims to investigate the suitability of PRA technique targeting hsp 65 gene for the differentiation and identification of rapidly and slowly growing clinical mycobacteria. PCR-RFLP is a universal system of identifying mycobacteria at species and sub species level.Result analysis can be obtained in 1-2 days as compared with 2-3 weeks for biochemical tests.

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At-a-station Hydraulic Geometry of the Mahi River with Special Implication to Annual Maximum Series

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ABSTRACT

Hydraulic geometry is of fundamental importance in flood hydrology and geomorphology. It refers to the rate of change of hydraulic variables, namely width, mean depth, and mean velocity, as discharge increases. An attempt has been made to find out at-a-station hydraulic geometry of the Mahi River with special reference to Annual Maximum Series (AMS).Data regarding hydraulic variables associated with annual peak discharges are available for six sites on the Mahi River and its tributaries. These data have been used to derive at-a-station hydraulic geometry equations. The b/f ratio, m/f ratio and total variance have been computed. The hydraulic geometry exponents (b, f, and m) were plotted on Rhode's ternary diagram. The results of the analysis for all the sites clearly show that the rate of change in mean velocity (m) and mean depth (f) with discharges are greater and the rate of change in width (b) are very slow except one site i.e. Rangeli on the Som River. The rate of change in width (b) with discharge is much slower for Khanpur, Padardi Badi and Mataji sites on the Mahi River which are attributed to nearly box-shaped nature of channels. The rate of change in width (b) with discharge is moderate on the Anas River at Chakaliya and Jakham River at Dhariawad indicating semicircular channel form. However, the rate of change in width (b) with rising discharge is much higher for the Som River at Rangeli. This is attributed to wide open channel of the river. The b/f ratios indicate that the rate of change in width is always lower than the rate of change in mean depth which has important implications for efficiency of the channel since the flood power is directly related to the flow depth. The higher m/f ratios reveal that there is more rapid increase of measured sediment load with increase of discharge. The total variance values for three sites namely Rangeli, Dhariawad and Chakaliya are closer to the theoretical value (0.33). This suggests that the effects of changes in discharge are absorbed equally by all the three variables. However, the total variance values for the remaining three sites namely Khanpur, Padardi Badi, and Mataji are not absorbed equally by all the three variables, but by one or two hydraulic geometry variables. This fact, therefore, suggests that the alluvial river channel of the Mahi River is not a true alluvial channel, which is self-formed through the independent adjustment of the morphological variables. The b-f-m or ternary diagram indicates that three sites fall in sector 6, two sites in sector 2 and a site in sector 3. The sector 6 represents the channel where Froude number and slope-roughness ratio increases and width-depth ratio and velocity-area ratio decreases with increasing discharge.Thesector2 reveals the decrease in width-depth ratio and increase in competence, Froude number, velocity-area ratio, and slope-roughness ratio with rising discharge. Whereas, sector 3 shows the channel characteristics where width-depth ratio, competence, Froude number, and slope-roughness ratio increase and velocity-area ratio decrease with increasing discharge.

Keywords: At-a-station Hydraulic Geometry, Ternary diagram, Mahi River

I. INTRODUCTION

Hydraulic geometry is an account of how the dynamic properties of a river channel responsible with increase in discharge. It may be considered as either at-a-station changes or downstream responses to increasing discharge. At-a-station hydraulic geometry describes the channel characteristics mainly refers to the geometric rate of change of hydraulic variables, specifically width (w), mean depth (d), and mean velocity (v), as discharge (Q) increases. These associations are labelled by the term "hydraulic geometry" [1]. However, the associations have been based almost merely on the numerical similarity of the exponents. The implicit assumptions in such analysis is that channels, as characterized by a particular set of b, f and m values, differ only in their rate of response to changing discharge [2]. Rhodes [3], suggests in his investigations that the hydraulic geometry equations are simple allometric accounts of a set of extremely complex interrelationships. The geometric relationships cannot entirely explain nor describe river systems. Though, investigations of the similarities and differences in the hydraulic geometries of rivers have provided understandings into the operation of fluvial systems. A triangular coordinate system on which the exponents (b, f and m) of hydraulic geometry calculations with their totality by 1.00 for each site are plotted, is a graphical presentation assigned by the name of Ternary diagram or else b-f-m diagram [2]. The objective of this paper, is therefore, to find out at-a-station hydraulic geometry of the Mahi River and its tributaries with special reference to Annual Maximum Series (AMS) and to interpret Rhodes' ternary diagram.

II. STUDY AREA

The Mahi River is the third major west flowing interstate river of the India located in western India (Fig. 1). The river originates near Mindha village of Sardarpur taluka of Dhar district of Madhya Pradesh at an elevation of 500 m ASL. It flows for the distance 538 km. River occupy the total area 34,842 km² and lies between 72° 21' to 75° 19' E and 21° 46' to 24° 30' N. The major right bank tributaries of the Mahi River are the Som and the Jakham and left bank tributaries are the Anas and the Panam. Geographically, the basin bounded by Aravalli hills in the north and northwest, Vindhya in the south and the east and Gulf of Khambhat in the southwest. The lithology comprises metamorphic rocks of Aravalli Super Group, the Deccan Traps Basalt, and the alluvial deposits of Pleistocene and Holocene age. The data of hydraulic parameters of the AMS were available for three sites on the Mahi River and three on its tributaries (Figure 1).

III. DATA AND METHODOLOGY

In order to derive at-a-station hydraulic geometry equations, the values of width, depth and velocity for mean annual discharge data along the river are required. However, data regarding hydraulic variables associated with annual maximum series (AMS) were available for three sites on the Mahi River and three on its major tributaries namely the Jakham, the Som and the Anas. These data have been used to derive at-a-stationhydraulic geometry equations to understand the nature of changes in the
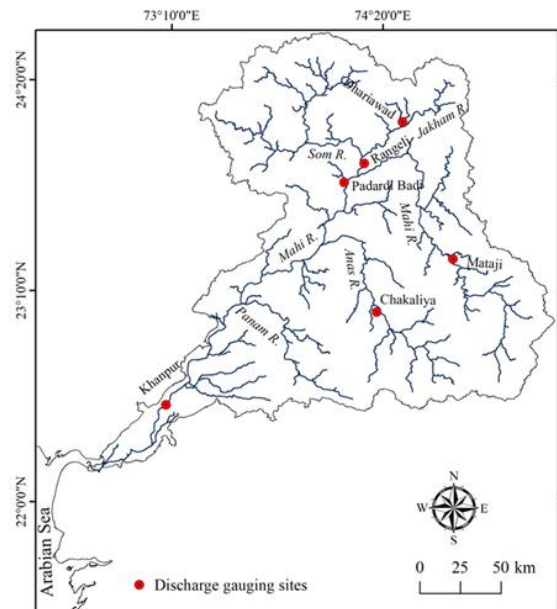
Figure 1. Discharge gauging sites on the Mahi River and itstributaries.

hydraulic variables with discharge. The equations are as under;

$$w =_a Q^b \qquad \text{....Eq.1}$$

$$d =_c Q^f \qquad \text{....Eq.2}$$

$$v =_k Q^m \qquad \text{....Eq.3}$$

Where, w = width; d = mean depth; v = mean velocity;Q = water discharge and a, c, k, b, f and m are numericalconstants.

Above three equations mainly used to express and associate stream channels forms. The changes between discharges as the independent variables and the dependent of width, depth, velocity have often been expressed as simple power-functions [3]. The b/f ratio, m/f ratio and total variance have been computed for understanding of the rate of change in width, mean depth and mean velocity. All the hydraulic geometry exponents (b, f, and m) of the six sites were plotted on Rhode's ternary diagram. This kind of analysis provides values statistically more accurate than those obtained by other methods and offers a unique set of equations. The original presentation of the diagram considered only at-a-station hydraulic geometry exponents [2]. For the graphical data presentation, the divided b-f-m diagram is a tool for the interpretation of the hydraulic geometry [4].

IV. RESULTS AND CONCLUSIONS

The results of hydraulic geometry of six sites are shown in Table 1 and Fig. 2, 3 and 4.The results of are not absorbedequally byall the three variables, butby oneor two hydraulic geometry variables [3]. This behavior of the hydraulicvariables can be attributed to the rectangular appearance of the chann-

Table 3. exponent values of at-a-station hydraulic geometry

No.	River	Site	Width (b)	Depth (f)	Velocity (m)	b/f ratio	m/f ratio	Total Variance
1	Mahi	Khanpur	0.04	0.53	0.43	0.08	0.81	0.47
2	Mahi	Padardi Badi	0.02	0.45	0.53	0.04	1.18	0.48
3	Mahi	Mataji	0.13	0.26	0.61	0.50	2.35	0.46
4	Som	Rangeli	0.41	0.26	0.33	1.58	1.27	0.34
5	Jakham	Dhariawad	0.22	0.42	0.37	0.52	0.88	0.36
6	Anas	Chakaliya	0.15	0.45	0.40	0.33	0.89	0.39

the analysis for all the sites clearly show that the rate of change in mean velocity (m) and mean depth (f) with discharges are greater than the rate of change in width (b) except one site i.e. Rangeli on the Som River (Fig. 3b). The rate of change in width with discharge is muchslower for Khanpur, PadardiBadi and Mataji sites on the Mahi River which are attributed to nearly box-shaped nature of channels (Fig. 2a, b and 4a).Therefore, the increase in the discharge is primarily compensated by a remarkable increase in depth. This has important implications for competence of the channel since the flood power is directly related to the flow depth [5]. The rate of change in width (b) with discharge is moderate on the Jakham River at Dhariawad (Fig. 3a) and Anas River at Chakaliya (Fig.4b) indicating semicircular channel form. However, the rate of change in width (b) with rising discharge is much higher for the Som River at Rangeli (Figure 3b). This is attributed to wide open channel of the river.

According to Rhodes [3], hydraulic geometry is linked with Langbein's concept of minimum variance.Hence,the total variance is the sum of the square of thehydraulicgeometry exponents.On the basis calculations of the total variance, values for all the six sites lie between 0.34 and 0.48 (Table 1) and are closer to theoretical minimum total variance, which is 0.333 [3].Nevertheless, the total variance values for the sites viz. Rangeli on the Som River, Dhariawad on the Jakham River and Chakaliya on the Anas River are 0.34, 0.36 and 0.39 respectively and all these values are closer to the theoretical value. In other hand, the total variance values are higher of the sites on Mahi River such as Khanpur, PadardiBadi and Mataji with 0.47, 0.48 and 0.46 respectively. This proposes that at the latter sites, the effects of changes

in discharge el and to the cohesive nature of the bank material of the selected sites. This fact, therefore, suggests that the alluvial river channel of the Mahi River is not a true alluvial channel, which is self-formed through the independent adjustment of the morphological variables [6], [7], [8].

The values of b, f, and m of the six sites were plotted on the ternary diagram (Figure 5).It indicates that three sites fall in sector 6,two sites in sector 2 and a site in sector 3. The sector 6 represents the channel where Froude number and slope-roughness ratio increases and width-depth ratio and velocity-area ratio decreases with increasing discharge. The sector 2 reveals the decrease in width-depth ratio and

a. Jakham River at Dhariawad   b. Som River at Rangeli
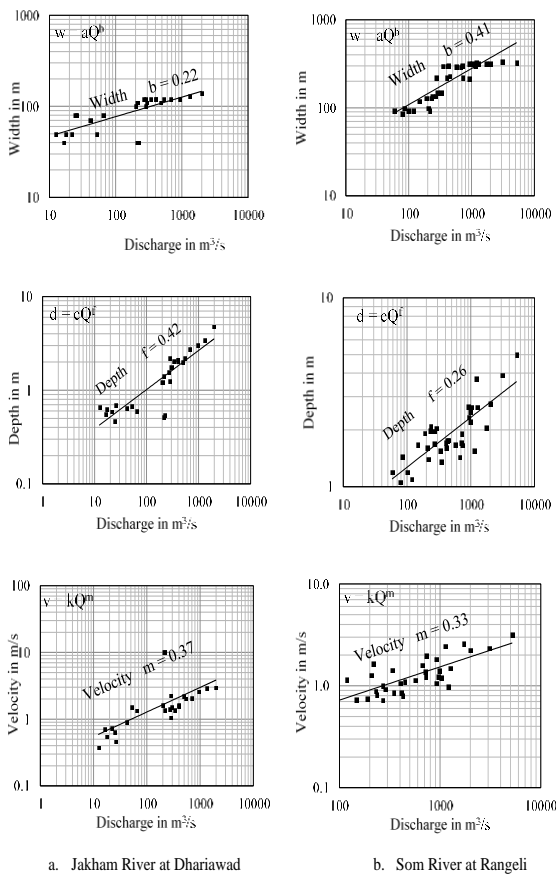
Fig. 3 At-a-Station hydraulic geometry
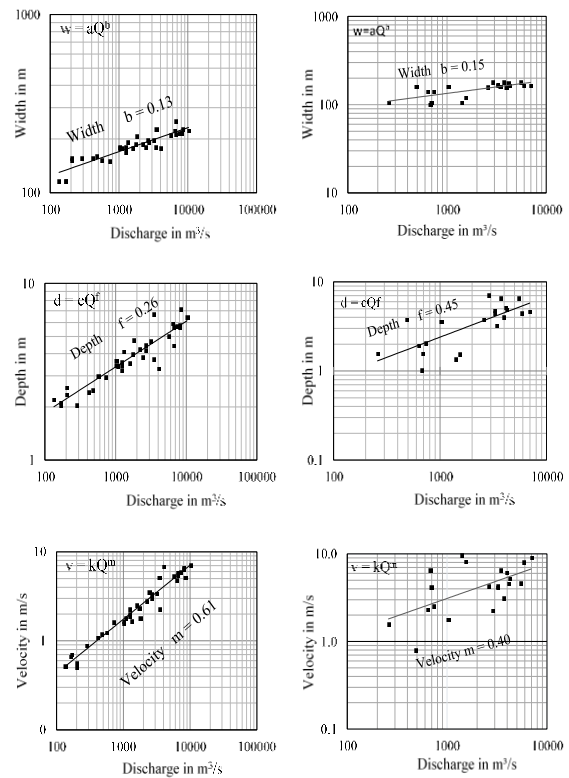
a. Mahi River at Mataji   b. Anas River at Chakaliya

Fig. 4 At-a-station hydraulic geometry

increase in competence, Froude number, velocity-area ratio, and slope-roughness ratio with rising discharge. Whereas, sector 3 shows the channel characteristics where width-depth ratio, competence, Froude number, and slope-roughness ratio increase and velocity-area ratio decrease with increasing discharge.The b-f-m diagram offers a means of grouping and comparing hydraulic geometry of the channels of theMahi River and itstributaries and suggests empirical classification based on hydraulic geometry.
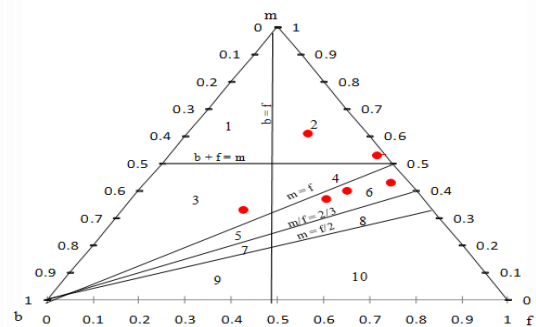
Figure 5. The width-depth-velocity (b-f-m) or Ternary diagram

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Insecticidal Activity and Growth Inhibiting Effects of Three Phases of Tio$_2$ Nanoparticles via Food on First Instar Larvae of *T. Castaneum* (Coleoptera: Tenebrionidae)

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ABSTRACT

Tribolium castaneum (Herbst) is major pest of stored grains .Annual post harvest losses resulting from insect damages, microbial deterioration and other factors are estimated to 10-25 % of worldwide production. Control of these insects depends majorly on many synthetic insecticide and fumigant application. But their widespread use has led to some serious issues. Store grains are found with increased toxic residue. There is increase in application cost also. So, there is an urgent need to provide alternatives which are safe in nature, user friendly & having low cost. Green synthesis of nanoparticles (NPs) by mushroom extract is at present of more interest. NPs synthesis is useful in many biomedical application also eco-friendly in nature. Titanium dioxide (TiO2) has also gained much attention due to many advantages over other oxides thin films. The advantages of TiO2 are it is toxic to insects, stability of hydrogen, optical and piezoelectric behaviours, plasma atmosphere and low price. In present work TiO2 NPs were synthesized using Phellinus mushroom sp. extract. It is one of the useful ceramic materials. TiO2 has many industrial utility and used in day to day life also. When Tribolium castaneum neonates were treated with TiO2 NPs (Sample I, II & III) through diet, the highest mortality was found in Sample-I treated first instar larvae of Tribolium castaneum. The time taken for pupation and adult emergence in treated samples were delayed by 6 to 8 days as compare to control. The percent pupation and percent adult emergence were also affected and were least in TiO2 –I (sample -I, II, &III), as compared to control. It also delayed development to adult stage and affects on fecundity and fertility of treated adults.

Keywords: Tribolium castaneum, first instar larvae, Insecticide, TiO2 Nanoparticles, Toxicity.

I. INTRODUCTION

Insects are one of the highly populated species, with very successful evolution history. Annual post harvest losses resulting from insect damages, microbial deterioration and other factors are estimated around 10-25% percent of world's grain production (10). Many other damages like crop plantation, wood structure are causing serious health and economic issues.

Pest is among the main causes of agriculture losses. Traditional insecticides are commonly used. But its uncontrolled use leads to environmental contamination, human poisoning. There is reduction in the number of insect's natural enemies. Insecticide resistance also limits the effective benefits of traditional pesticides.

However, the excessive use of highly toxic pesticides causes several human health issues like neurological, tumour, cancer and environmental problems. In this scenario, Nano and micro particles have been reaching a prominent position. So, nanoparticles based green pesticides are of special importance in recent years. In the formulations containing insecticides have been prepared in colloidal suspensions or powder in micro or nano scale. It presents several advantages such as increasing stability of active organic compound (UV, thermal, hydrolysis, etc.) foliar setting, reduction in foliar leaching, systemic action synergism, specificity, etc. As consequence of this, the amount of insecticide necessary (dosage), the number of applications, human exposure to insecticides and

environmental impact are reduced. The nano-formulation has been employed not only for synthetic insecticides but also in alternative products to control plague insects such as natural products, herbal extract and entomo-pathogenic micro-organisms.

In order to prepare nano-formulations, several chemical and physical techniques have been developed. In general, they should be prepared by using polymeric material which is biocompatible and biodegradable. The main aim is to avoid the emergence of new environmental and toxicological problems. Titanium dioxide (TiO_2) is one of the most studied compounds in materials science(6).

II. OBJECTIVES

- To find out the effect three different samples of TiO_2 NPs on first instar larvae of *T.castaneum* via food, after interval of 24 hrs.
- To find out the effect of the treatment of TiO_2 NPs on growth and development of *T.castaneum* treated with nanoparticles via food.
- To find out the % survival- mortality ratio of different larval stages of *T.castaneum* treated with NPs via food.
- To find out the effect of the treatment of TiO_2 NPs on fecundity and fertility of adult formed from treated larvae of *T.castaneum*.

III. METHODS AND MATERIAL

- ### Culture of *Tribolium castaneum*
  Tribolium castaneum culture was maintained on diet containing wheat flour and 5% Brewer's yeast, at 29 ± 1^0C and 60% relative humidity. Eggs were collected by sieving (sieve number 40) diet infested with adults. Newly hatched first instar one day old larvae were collected from the sieved eggs.

- ### Synthesis of TiO2 Nanoparticles
  TiO_2 was synthesised by using bio-inspired green method from the extract of mushroom sp. *Phellinus linteus* .In a typical biosynthesis process, mushroom extract was prepared by mushroom powder boiling in 100ml distilled water at 85° C for 15 min. (fig-1)The extract was filtered and stored as a stock solution at 4° C. The 0.15M Ti precursor solution in ethyl alcohol was prepared by using tetraisopropoxide. The
  0.5 ml extract was added drop-wise into Ti precursor. The precipitation was dried and annealed at 500° C, at 300° C& at 700° C for 2hrs. to obtain TiO_2 powder. The structural parameters of TiO_2 are studied using X-ray diffraction (XRD) spectra. The XRD pattern of samples I, II and III are shown (Fig. 1). The presence of diffraction peaks in XRD pattern for samples I and II confirm the anatase phase (JCPDS card no. 21-1272) of TiO_2, while JCPDS card no. 21-1276 confirms rutile phase of TiO_2 for sample III.
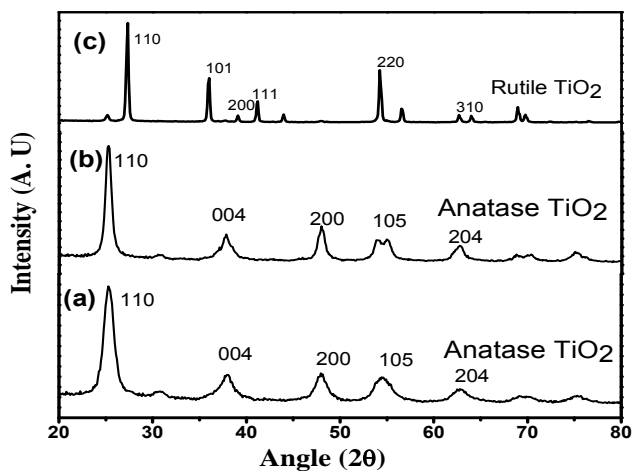
Figure 1. XRD pattern of (a) sample I, (b) sample II and (c) sample III
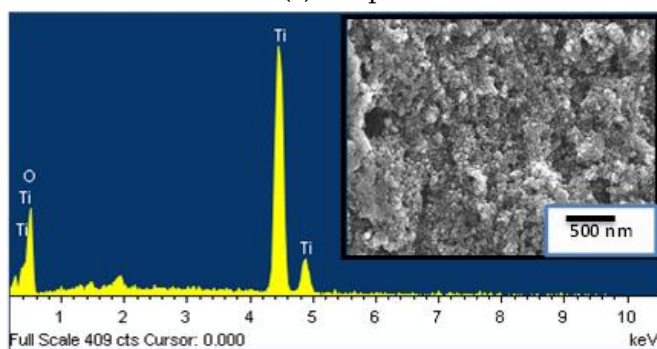
Figure 2. EDAX spectrum of TiO₂ (Inset-FESEM image)

The elemental analysis was performed by investigating EDAX spectrum. (Fig. 2) shows EDAX spectrum of as prepared TiO₂ powder, which confirms the presence of titanium and oxygen in atomic % (O - 73.79 %, Ti - 26.21 %). The inset figure shows the FESEM image of as prepared TiO₂ nanoparticles, agglomerated spherical nanoparticles of TiO₂ powder with 25 nm in size are observed.

- **Bio-assay**

Bioassay for the effect of TiO2 NPs on the first instar larvae of *Tribolium castaneum* were determined by treated wheat flour in different samples. TiO2 was mixed with diet containing wheat flour. The three different samples of TiO₂ with equal volume were thoroughly incorporated in diet of *Tribolium castaneum* (1 mg of TiO₂ in 1gm of wheat flour + 10 larvae) 500° C (TiO₂ sample–I), 300° C (TiO₂ sample –II), 700° C (TiO₂ sample –III) and without any concentration of TiO₂ diet was used as control. The experiments carried out with three replicates. Each of them consisted 10 newly hatched first instar of *Tribolium castaneum*. The mortality count was checked after 24hrs. All the larvae were transferred to fresh diet after 24 hrs and observed further and recorded its mortality on 7th day, 10th day, and 15th day larval stage. Observations were continued till pupal formation and adult emergence. The newly emerged adult from control and treated were also observed for its fertility and fecundity.

IV. RESULTS AND DISCUSSSIONS

Survival of *T. castaneum* first instar larvae to adulthood as well as the fecundity and fertility of these adults was definitely affected by TiO₂ NPs. The dietary treatment of *T. castaneum* larvae with TiO₂ NPs significantly effects the survival of each stage. The maximum mortality of larvae was observed in sample-I, treated larvae. There was significant reduction in all treated stages of TiO₂ NPs as compare to adults (Fig -5).

Figure 4. Larvae of T. castaneum

Furthermore the time taken for pupation and time taken for adult emergence were also affected due to the treatment of TiO₂ NPs, as compare to control. In control, first instar larva turns into pupa in 18- 19 days, while in sample-I it takes 25 to 26 days for pupal formation from first instar larva. Pupa converts into adult in 4 to 5 days in control while in treated

samples pupa turns into adult within 8-10 days. A significant reduction in percent pupation and percent adult emergence were observed in treated samples as compare to control (Table-2). Duration of normal development of *T. castaneum* from first instar larvae to adult was 20 to 22 days, while in dietary treatment with TiO₂ NPs development takes place in 33, 30 & 30 days in sample I, II & III resp. (Table-1).
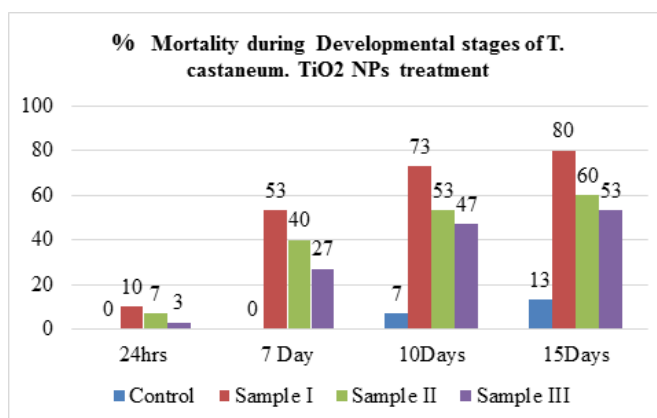
Figure 5.-%mortality during developmental stages of *T. castaneum*

Table 1. Effect of TiO2 NPs on growth & development of *T. castaneum*

Effect of TiO2 NPs on growth & development of *T. castaneum*								
Sample	% Larval survival				% pupation X	Time taken for pupation (Days) X $\pm$ SE(X)	% adult emergence	Time taken for adult emergence (Days) X $\pm$ SE(X)
	24 Hrs.	7th day	10th day	15th day				
Control	100	100	93	87	73%	18 $\pm$ 1	63%	24 $\pm$ 1
Sample I	90	47	27	20	17%	25 $\pm$ 1	10%	33 $\pm$ 2
Sample II	93	60	47	40	37%	23 $\pm$ 1	30%	31 $\pm$ 1
Sample III	97	73	53	47	40%	23 $\pm$ 2	33%	30 $\pm$ 1

ANOVA: Two-Factor with Replication Summary

Control	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	0.00	0.00	2.00	4.00	6.00
Average	0.00	0.00	0.67	1.33	0.50
Variance	0.00	0.00	0.33	0.33	0.45

Sample I	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	3.00	16.00	22.00	24.00	65.00
Average	1.00	5.33	7.33	8.00	5.42
Variance	0.00	0.33	0.33	0.00	8.27
Sample II	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	1.00	12.00	16.00	18.00	47.00
Average	0.33	4.00	5.33	6.00	3.92
Variance	0.33	1.00	0.33	0.00	5.54
Sample III	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	1.00	8.00	14.00	16.00	39.00
Average	0.33	2.67	4.67	5.33	3.25
Variance	0.33	0.33	0.33	0.33	4.39

Total	24 Hrs	7 days	10 days	15 days
Count	12.00	12.00	12.00	12.00
Sum	5.00	36.00	54.00	62.00
Average	0.42	3.00	4.50	5.17
Variance	0.27	4.55	6.64	6.52

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	152.3958	3	50.7986	187.5641	2.22292E-20	2.90112E+00
Columns	159.8958	3	53.2986	196.7949	1.07442E-20	2.90112E+00
Interaction	36.52083	9	4.05787	14.98291	3.71275E-09	2.18877E+00
Within	8.666667	32	0.27083			

Two Way ANOVA Analysis: To test

H01 : α1=α2=α3=α4

H01: There is no significance difference between samples.

H11: There is a significance difference between samples.

H02 : β1=β2=β3=β4

H02: There is no significance difference between block (days) effect.

H12: There is a significance difference between block (days) effect.

H03 : There is no interaction between samples and block (days) effect

H13 : There is a interaction between samples and block (days) effect

For H03 (Interaction)

F value =14.982905982906

F critical value=2.18876576806951

F value > F critical value

Reject H03

There is a interaction between samples and block (days) effect

For H01 (Treatments)

F value = 187.564102564102

F critical value= 2.90111958384084

F value > F critical value

Reject H01

There is a significance difference between treatments.

For H02 (Days effect)

F value =196.794871794872

F critical value=2.90111958384084

F value > F critical value

Reject H02

There is a significance difference between block (days) effect.

V. CONCLUSION

Overall the sample-I was more effective as insecticide. The maximum mortality of larvae was observed in sample-I, treated larvae. It is because the anatase TiO2 (sample-I and II) is more active than the rutile (sample-III). The adults formed from treated first instar larvae, there was no egg laying absolutely of such adults in treated samples. The TiO2 NPs when mixed in diet of T. castaneum and fed for 24 hrs. to newly hatch first instar larvae were shown insecticidal and growth inhibiting effect of that larvae. At all three samples treatment there was no abnormal pupae and adults were observed. The time taken for pupation was 18 to 19 days in control while in sample I it was longest duration for 23 to 27 days. Similarly the effect of Ag doped hollow TiO2 NPs as an effective fungicide against Fusarium solani and Venturia inaequalis phytopathogens (3). Also TiO2 NPs applied to Drosophila melanogaster through food found to toxic as it generate reactive oxygen species which modify multiple signalling pathways and thus can alter the development and behavioural pattern of the fly, were observed. (11)

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Statistical optimization of 2-hydroxyphenazine production by *Microbispora* sp. V2 using design of experiments

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ABSTRACT

The rare actinomycete Microbispora sp. V2 was found out to produce of 2-hydroxy phenazine, an antifungal compound active against fungal plant pathogens like Rhizoctonia solani and Pythium ultimum. Hence studies were focused to enhance the potential of Microbispora sp. V2 as agricultural biocontrol agent by optimizing the 2-hydroxyphenazine production using design of experiments. The student's t-test and pareto chart of the Placket-Burman design revealed that the factors inoculum and carbon source, were significant; pH and temperature were two most important factors. A 5-level Central Composite Design was employed to optimize these factors. The various effects of the factors were studied by student's t-test and F-test for analysis of variance and second order polynomial model was developed. The coefficient of determination (R^2=0.84) justified a good correlation between factors and 2-hydroxyphenazine production and the model fitted well with statistical reliability and significance. The predicted optimum concentration of the factors was carbon source (sesame oil cake): 1.182%; pH: 8.134and temperature: 54.23°C with maximum predicted 2-hydroxyphenazine production of 175.531 mg/l. The experimental 2-hydroxyphenazine production was 150 mg/l. Coefficient of variation and adequate precision were 4.86% and 89.61 respectively which validates the accuracy of the model. The results of experiments proved that these optimized values can be used for large scale production of 2-hydroxyphenazine by Microbispora sp. V2.

Keywords: Microbispora sp. V2, Biocontrol Agent, Antifungal Activity

I. INTRODUCTION

The competitive position and potential profits from a fermentation product are closely tied to the costs of the various components of the production medium. All attempts are made to find an alternate or a substitute low-cost replacement for such a medium component.

In designing a cost-effective medium, it is important to use cheap ingredients. This inevitably leads to the use of raw materials such as oil cakes, corn steep liquor, molasses, etc. which are actually the "Waste Products" of other industries. Hence, the use of an abundantly available cheap agro-industrial waste like edible oil cake serves as good nutrient support rendering both carbon and nitrogen source. It is important to consider the optimization of fermentation medium and process conditions in order

to maximize the profits from fermentation process (Schmidt, 2005). Borrowing, compact replacing, biological mimicry, one-factor-at time, factorial design, placket and burman design, central composite design, response surface methodology, evolutionary operation, fuzzy logic are different well-known and newer optimization method applied in fermentation process (Panda et al., 2007).

In this study, optimization of fermentation media of *Microbispora* sp. V2 was investigated using Response Surface Method to increase the production of phenazines. In the first optimization step, a Plackett-Burman Design was used to determine the likely effects of various concentrations of sesame oil as medium constituent, pH, agitation, inoculum size and source of water on phenazine production. The most effective variables with high significance levels were selected for further optimization while others with lower significance levels or small were omitted in further experiments. In the second step, the Central Composite Design and Response Surface Method were adopted to determine the relationships between variables and responses. Moreover, the optimum of each variable will be obtained by differential approximation. This technique has been widely applied to optimizing parameters and variables for fermentation media for various microorganisms (He et al. 2008, Li et al., 2008 and Su et al., 2010).The optimization was carried out by using Minitab 16.0 software (Yuan et al., 2008; Vuddaraju et al., 2010).

II. METHODS AND MATERIAL

Microorganism, Medium and Shake-Flask Culture Conditions

The microorganism was procured from Dr. Neelu Nawani, D Y Patil, Biotechnology and Bioinformatics Institute, Pune, India. The isolate *Microbispora* sp. V2 was grown on pablum agar. The fermentation was divided into two stages: seed growth and phenazine production. *Microbispora* sp.V2 was inoculated in 100 ml pablum broth and grown on a rotary shaker at

40°C at 180 rpm for 5 days. The broth was centrifuged at 5000 x g for 10 min. The supernatant was discarded and biomass resuspended to reach the absorbance 0.2 for the development of seed inoculum. 100 ml of fermentation medium in 500 ml Erlenmeyer flasks with various combinations for optimization studies were inoculated and incubated for 10 days. The variables studied for optimization were carbon source (%), pH, temperature ($^{\circ}$C), agitation speed (rpm), inoculum size (%) and type of water (distilled or tap).

Analytical Method for 2-Hydroxyphenazine (2-OHPZ) Fermented broth was centrifuged at 4500 x g, for 15 min. Chloroform and supernatant mixed in 1:1 ratio. Organic phase was removed and kept at 40°C for evaporation of chloroform and residue redissolved in 2 ml chloroform. The solvent extract (10 µl) of fermented oil cake medium was applied to precoated preparative silica gel plates (Macherey-Nagel Polygram Sil G/UV$_{254}$). The solvent system used was Butanol: Acetic acid (90:10) (Thomshow et al., 2007). Detection of antimicrobial compounds was done under U.V. light at 365 nm. Phenazine spots were eluted from preparative thin layer plates and redissolved in 2 ml methanol. Extracts were quantized with UV-visible spectroscopy (Maddula et al., 2008). The absorption maxima for PCA and 2-OHPZ were measured at 367 and 484 nm, respectively. The relative amounts of PCA and 2-OHPZ were calculated by dividing the absorption maxima by their standard extinction coefficients (Olson & Richards, 1967). The standard dose response curve was constructed for 2-OHPZ using the standard 2-OHPZ (Colour your enzyme, Bath, Ontario, Canada).

Experimental Design and Statistical Analysis

In the preliminary experiments, physical variables viz., temperature, agitation and pH as well as nutrient source-the oil cake and type of water were evaluated for their suitability to sustain good production of 2-hydroxyphenazine by *Microbispora* sp. V2. The data obtained indicated the major variables affecting the performance of the culture in terms of yields of

phenazine which were chosen for further optimization.

Plackett-Burman Design (PBD)

PBD, an efficient technique for medium component optimization, was used to screen "k" variables in just "k + 1" number of experiments. The PBD was used to evaluate the relative importance of six parameters for production of phenazines by *Microbispora* sp. V2. The variables selected were viz., (A) carbon source (% sesame oil cake), (B) pH, (C) temperature, (D) agitation, (E) inoculum size (%) and (F) type of water. The PBD was based on the following first order model equation:

$\hat{Y} = \beta_0 + \sum \beta_i x_i$, where $\hat{Y}$ is the response (2-OHPZ yield mg/g), β_0 is the model intercepts, β_i is the linear coefficient, and x_i is the level of the independent variable. The effect of each variable on phenazine production was estimated as the difference between average of measurements made at the higher level and at the lower level. The significance of each variable was determined by student's t-test. In all 70 runs were conducted as per the PBD matrix and phenazine production was checked.

Central Composite Designs (CCD) and Response Surface Method (RSM)

CCD was used for investigating the region of the response surface in the neighborhood of the optimum. For six variables, a fractional factorial design with one centre point and 12 star points which allows curvature estimation is typically recommended to have a total number of 54 runs. The star points for this design had a value of 2.366, which can maintain rotability. Using Minitab 16.0, the RSM was obtained which is a second order model. Run Order represents the order of the runs in the experiment when the experiment is performed in random order. Randomization of the run order was done to lessen the effects of variables that were not included in the study, particularly effects that may be time-dependent. Concentrations of phenazines in cell free supernatant were extrapolated

from dose response curve of 2-OHPZ.

Statistical Analysis

A mathematical model describing the relationships among the process dependent variable and the independent variables in a second-order equation was developed. Design-based experimental data were matched according to the following second-order polynomial equation:

$Y = \beta_0 + \sum \beta_i \cdot X_i + \sum \beta_{ii} \cdot X^2_i + \sum \beta_{ij} \cdot X_i \cdot X_j$

The quality of fit of the second order equation was expressed by the coefficient of determination R^2 and its statistical significance was determined by F-test. The significance of each coefficient was determined using student's t-test. The coefficients of the equation and analysis of variance (ANOVA) for the final predictive equation were found out by using MINITAB SOFTWARE (Version 16). The response surface equation was optimized for maximum production of phenazines and the response surfaces were made by the quadratic polynomial equation obtained from the software, holding independent variables with one parameter at a constant value and changing the other two variables. In the RSM, the quality of the fit of the polynomial model is expressed by coefficient of determination (R^2), adjusted coefficient (R^2Adj), predicted coefficient (R^2 Pred), adequate precision (A.P.) and coefficient of variation (C.V.).) (Box et al., 1978; Noordin et al., 2004; Kohli et al., 2011; Mittal et al., 2011 and Bashir et al., 2012).

$$R^2 = 1 - \frac{SS_{Residual}}{SS_{Model} + SS_{Residual}} \qquad \text{Eq. (1)}$$

$$R^2_{Adj} = 1 - \frac{SS_{Residual}/DF_{Residual}}{(SS_{Model} + SS_{Residual}/DF_{Model} + DF_{Residual})} \qquad \text{Eq. (2)}$$

where the terms SS and DF are sum of squares and degrees of freedom, respectively.

$$PRESS = \sum_{i=1}^{n} e^2_{(i)} = \sum_{i=1}^{n} \left[y_i - \hat{y}_{(i)} \right]^2 \qquad \text{Eq. (3)}$$

Adequate Precision (AP) is determined the signal to noise ratio. Adequate precision (A.P.) compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination and can be

used to navigate the design space defined by the CCD. The coefficient of variance (C.V.) is the ratio of the standard error of estimate to the mean value of the observed response and defines reproducibility of the model calculated as:

The mean value is of observed response in CCD matrix of variables.

III. RESULTS AND DISCUSSION

PBD for Screening Important Fermentation Variables for Phenazine Production

The coefficient of determination, R^2 of 2-OHPZ implied that the sample variation of 89.32% for 2-OHPZ acid production was attributed to the independent variables. The coefficient of determination (adjusted R^2) was 89.04% which indicated a good agreement between the experimental and predicted values of biomass production. On the basis of the confidence level, a normal plot chart of the standardized effects of process variables showing the dominance of the individual variables is shown in Fig. 1 represented as pH (confidence level: 100%), temperature (confidence level: 100%) and pH versus temperature (confidence level: 100%) as most important variables whereas inoculum, pH versus inoculum, temperature versus inoculum, carbon source, carbon source versus pH, carbon source versus temperature and aeration versus inoculum were found as significant variables influencing the 2-OHPZ with confidence level more than 90%.

The facts needed for setting priorities on media components and process variables were provided by Pareto charts. Concentrating improvement efforts on these few variables have a greater impact and are most effective on bioprocess development. Hence, the main effect of media components and process variables on production of 2-OHPZ was also studied graphically using Pareto chart.

The variables carbon source versus aeration, carbon source versus inoculum, pH versus aeration and temperature versus aeration were found to have less significance in 2-OHPZ production and hence not taken into account in response surface matrix in 2-OHPZ production.

CCD and RSM

The data shown in table 6 were explained by the following second- order polynomial equation-
$$Y = \beta_o + \sum \beta_{i^*} X_{i^+} + \sum \beta_{ii^*} X^2_{i^+} + \sum \beta_{ij^*} X_{i^*} X_j$$
where Y stands for the predicted response; β_o, β_i, β_{ii} and β_{ij} are the offset term, linear effect, squared effect and interaction effect, respectively; X_i and X_j are the coded independent variables or variables. Response results were analyzed using Minitab 16.0 software. The t- test and P values were used to identify the effect of each variable on production of 2-OHPZ. The highly significant variables were found to be carbon source, pH, temperature, carbon source-pH and carbon source-temperature (P<0.0001) whereas pH-temperature had moderate significance (P<0.05).

The Final Model in Terms of Coded Levels for Production of 2-OHPZ

$$Y \text{ (mg/L)} = B_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$
Therefore, predicted 2-OHPZ production is

= − 391.739 − 104.313A + 124.834B + 0.230353C − 48.8761AB + 8.57187AC − 1.34701BC (Table 1)

= 175.531 mg/l. The Model F-value of 235.65 implies the model is significant. The R^2 coefficient gives the proportion of the total variation in the response predicted by the model and a value close to 1 is desirable and ensures a satisfactory adjustment of the quadratic model to the experimental data. The R^2 coefficient should be at least 0.80 for a good fit of a model. The R^2 coefficient was found to be 0.88, indicating that the regression models explained the production well. The adequate precision was found to have ratio greater than 4, which indicated adequate model discrimination and was used to navigate the design space defined by the CCD. The adequate precision is greater than 4 in the present study. The

lack of fit F-test describes the variation of the data around the fitted model. If the model fits the data well, it indicates insignificant. In this case, lack of fit value was 0.9619. The lack-of-fit test was not significant (very small "Prob > F " would indicate a lack of fit) and hence fit the data well. The coefficient of variation 4.86% indicates response was reproducible.

Table 1. Final model equations for 2-OHPZ in terms of coded levels

Antimicrobial Compound	Quadratic Equations for production of phenazines (mg/ml)
2-OHPZ	$-391.739 - 104.313A + 124.834B + 0.230353C - 48.8761AB + 8.57187AC - 1.34701BC$

The contour plots of 2-OHPZ is shown in Fig. 2. In order to gain a better understanding of the effect of the variables on production of 2-OHPZ, the predicted model was presented as 3D response surface graphs (Fig. 3). The maximum experimental response for 2-OHPZ production was 150 mg/l and the predicted value was 175.531 mg/l. By applying statistical design, production of phenazine was enhanced nearly 7.5 times (150 mg/l) as compared with the initial production medium (20 mg/l) in case of 2-OHPZ (Table 2). Such high increase i.e., eight times in production of antimicrobial compounds in case of actinomyetes has been achieved in case of production of new form of olivanic acid by *Streptomyces olivaceus* MTCC 6820 (Singh et al., 2006).

Table 2. Increase in yields of 2- OHPZ by statistical optimization methodology

2- OHPZ (mg/l)		Fold increase
Preliminary studies	Optimization by RSM	
20	150.0	7.5

IV. CONCLUSION

Statistically based experimental designs proved to be effective for optimizing the fermentation medium and physical factors for production of phenazines by *Microbispora* sp. V2. It was found out that there was a 7.5-fold increase (150 mg/l) in 2-OHPZ production as compared with the original medium (20 mg/l). Validation experiments were also carried out to verify the adequacy and accuracy of the model and the results showed that the predicted value agreed well with the experimental values. The use of sesame oil cake thus proved to be successful for production of phenazine in economic manner.

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An Evaluation of plankton diversity and abundance of Meena River with reference to Pollution

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ABSTRACT

Researches on fresh water bodies such as ponds, lakes, reservoirs, wetlands, rivers, streams have gained much significance in recent years due to their importance. These water bodies harbours broad array of aquatic organisms, in particular Plankton. They form a very significant part of fresh water community and contribute significantly to aquatic productivity. In Present studyPlankton diversity and abundance of Meenariver was assessed before and after pollution. Plankton diversity and abundance is widespread during different seasons, both at non-polluted and polluted sites. A total of 67 Species of phytoplanktons and 27 Species of zooplanktons were found. Myxophycean Species were found to be chief at both the stations. Euglenophyceae have revealed less No. of phytoplankton abundance in both the sites. The studies have exposed that Non-polluted water shows relatively larger abundance of Myxophyceae and zooplanktons as compared to the polluted water. It is concluded from this study that the plankton population of river Meena at Junnar, Pune district is highly influenced by the discharge from different small scale industrial effluents. The shift in the planktonic population structure and dominance of pollution tolerant forms at discharge zone indicated deterioration of water quality in this stretch of the river.

Keywords: Plankton, Diversity, Abundance, Pollution, Meena River.

I. INTRODUCTION

Rivers are most significant systems of aquatic biodiversity and are among the most dynamic ecosystems on the earth because of the favourable environment that supports No. of flora and fauna. River ecosystem is one of the natural source which comes into the service of mankind in many parts of the ecosphere. They play an important role in the productivity as they are beset with varieties of flora and fauna including planktons. Suburbanization, expansion of irrigation and increasing trend ofindustrial development has contributed towards the demand for water. Surface water is the major source of irrigation in rural areas. Most of the fresh water bodies all over the world are getting polluted water, thus reducing the potability of the water [1].The concept of sustainable utilization by maintaining the natural properties of the wetland ecosystem becomes apractical reality only by a appropriate assessment of the relation between the parameters of water with the plankton,understanding its delicate functioning and by creating an cumulative awareness about its ecological value. Several interdependent and influencing abiotic factors along with high crucial productivity have made it a suitable niche formany aquatic forms.The biota of an aquatic system directly reflects condition present in the environment [2] and data produced in the past has been exploited for biological monitoring of the water pollution level. In this regard, scientists have considered the planktons as an index of water quality with respect to industrial,

municipal and domestic pollution [3,4].The present study was carried out on the surface planktons population in the aquatic ecosystem of Meena river water of Pune district in Maharashtra state (Fig.1). The industrial effluents form small scale industries in and around Pune contain numerouspollutants and have entered into the river Meena affecting the water quality. As a consequence, the plankton population of the Meena River has been affected in terms of abundance and diversity. The present investigation is aimed at evaluating the plankton index as the water quality criteria with reference to the fresh water river Meena polluted by small scale industries at Pune.
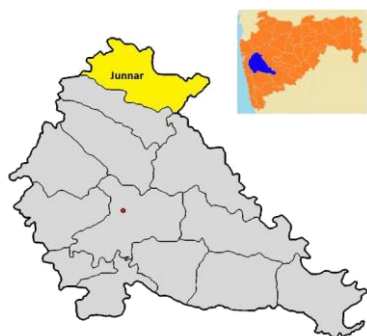
II. MATERIALS AND METHODS:

Figure 1. Study Area: Junnar Tehsil, Maharashtra State, India

The Meena River is located in Junnar of Pune district and flows for distance of more than 30 kms before joining river Ghod river in Pune district. The investigation also examines the effect of village effluent, small scale industries pollutants and assesses the planktonic population in Meena river at Station I (non-polluted) and Station II (polluted). Phytoplankton's were collected using a conical net of bolting nylon of 0.069mm mesh width and mouthring diameter of 35 cm with the help of an outrigger canoe. The net was dragged for ten minutes for surface hauls and the volume of water filtered through it was examined by flow meter attached to it and the net was back washed between the two stations to avoid clogging of meshes. The filtered samples were stored and preserved in 4% formalin with a few drops of Lugol's iodine solution. For the quantitative study of phytoplankton, the settlement method described by Sukhanova [5] was used. Numerical plankton analysis was carried out using an invertedmicroscope. Planktons were identified and enumerated by using the methods described by Hosamani and Bharathi [6]. For qualitative analysis of zooplanktons was done according to the methods given by Edmondson [7], Needham and Needham [8], Pennak [9], and Tonap [10]. Zooplanktons were recognized by usingmonographs of Edmondson [11], Batish [12] and Althof [13].

III. RESULTS AND DISCUSSION

Phytoplankton's been collected from the river water throughout the study period from non-polluted site (Station I) and polluted site (Station II). The results of phytoplankton's counts from each of the selected sites of Meena River are shown in Table 1 and Table 2.

Table 1. Distribution of phytoplankton in station I (Non Polluted Site)

Month	Bacillariophyceae		Desmidaceae		Chlorococcales		Myxophyceae		Euglenophyceae	
	No of Individuals	No of Species	No. of Individuals	No. of Species	No. of Individuals	No. of Species	No of Individuals	No. of Species	No. of Individuals	No. of Species
Jan	400	7	600	8	315	4	60	9	50	5
Feb	415	8	650	8	412	5	100	12	30	4
Mar	300	8	620	7	318	4	50	20	Nil	0
Apr	350	7	518	6	400	4	80	24	40	4
Ma	360	7	545	7	415	4	90	26	20	2
Jun	280	6	612	7	218	3	72	21	30	3
July	415	7	600	6	318	3	102	23	10	2
Aug	450	8	300	4	400	4	68	15	40	3

Sep	389	9	680	7	215	2	94	17	20	2
Oct	400	11	610	6	118	1	180	11	10	1
Nov	250	6	590	5	180	2	104	13	18	1
Dec	180	4	580	5	190	2	84	9	15	2
Total	**4279**	7 Mean	**6705**	6 Mean	**3818**	3 Mean	**4084**	17 Mean	**483**	2 Mean

A detailed microscopic examination of phytoplankton's revealed, the presence of maximum Species of Myxophyceae (19 Species in Station-I and 17Species in Station-II) followed by Bacillariophycean Species (6 Species in Station I and 7Species in Station-II). However, the least No. of Euglenophycean Species (3) and Chloroococcales Species (4) were recorded in Station-I and Station-II respectively. Desmidaceae showed highest No. of Individuals (6908) and euglenoid showed less No. of Individuals (286) in Station-I. Myxophyceae showed highest No. of Individuals and Chlorococcales showed less No. of Individuals in Station-II. pollutants are considered as one of the most important parameters in the aquatic environment which influences the growth, reproduction and metabolic activities of living beings. Distribution of pollutants is mainly based on the season tidal conditions and fresh water flow from land source [14]. In the present investigation a visible change in phytoplankton community with regard to the numerical abundance and Species composition was noticed among the stations studied. A total of 67phytoplanktons taxa were identified.

Table 2. Distribution of phytoplankton in station II (Polluted Site)

Month	Bacillariophyceae		Desmidaceae		Chlorococcales		Myxophyceae		Euglenophyceae	
	No. of Individuals	No. Of Species	No. of Individuals	No. Of Species	No. of Individuals	No. Of Species	No. of Individuals	No. Of Species	No. of Individuals	No. Of Species
Jan	150	6	200	8	70	4	350	9	112	4
Feb	80	4	80	5	28	4	300	13	114	4
Mar	70	4	60	6	35	3	428	18	154	3
Apr	112	5	50	4	48	4	412	26	106	2
May	106	6	30	6	106	3	218	24	180	4
Jun	250	6	116	7	250	3	289	19	189	4
Jul	260	7	106	5	66	2	291	20	192	3
Aug	270	7	180	4	177	4	358	22	106	4
Sep	116	8	90	6	98	2	415	22	88	3
Oct	180	9	70	5	89	1	454	20	95	4
Nov	110	4	60	5	69	2	402	18	108	4
Dec	90	4	48	4	50	1	359	18	160	2
Total	**1794**	6 Mean	**1090**	5 Mean	**1086**	3 Mean	**2276**	19 Mean	**204**	3 Mean

Desmidaceae (8 Species with 6705Individuals) and Bacillariophyceae (11 Species with 4279Individuals) werefound to be leading in non-polluted site. Their population was found to be comparatively less in polluted site. Generic representation of the Euglenophyceae was lowermost throughout the study period, where as the algal population was dominated by Myxophyceae followed by Bacillariophyceae in polluted site. Maximum phytoplankton abundancewas observed throughout the month of February and while lowest No. was recorded in the month of December inStation I. From the analyzed data, it is observed that Speciessymmetry decreased withthe increasing size of algalpopulation. The abundance and

Species composition of phytoplankton wide-ranging strongly at the succeeding months andbetween the stations in the study area. Algal abundance was observed during summer and their No. declined inmonsoon, which was in accordance with Thomas and Prasad [15] who documented similar results in wetlands ofMysore. Abundance of Myxophyceae was observed in the polluted sites during all the seasons. The maximum abundance of Euglenophyceae was recorded in the month of June at polluted site while number of individual of Euglenoids was recorded in March at non-polluted site. Euglenophyceae and/or Chlorophyceae, however, occurred as atransition stage. Such transition stage constantly occurs when intermediate environments of light and rainfall exist [16].Such surroundings are favoring to Euglenophyceae and Chlorophyceae. A similar pattern of phytoplankton Speciessuccession has been previously noted in the lake [17].In the present study four types of Zooplanktons were identified and are shown in Table -3. Rotifera and Crustacea founded the most leading groups in both non-polluted and polluted stations.The most commonly perceived zooplankton Species in the both sites are Asplachna, Cyclops, Daphnia, Mesocyclops, Nauplius, Siphlonurus Species. Arcellasp. Lacane sp., Macrocyclopssp., Tipulasp., Anopheles larvae,andChironomuslarvae are exclusively observed only in polluted site while Carchesiumpolypium, Paramaeciumaurelia, Brachionuscaudatus, Epiphanesmacrourus, Diurella sp., Gastropushyptopus, Keratellaquadrata, Diaphanosoma sp. and Chaoborus sp. are observed in non-polluted site. Although zooplanktons occur under abroad range ofenvironmental conditions, yet numerousSpecies are limited by dissolved oxygen, temperature, salinity and other physico-chemical factors [18]. The supremacy of any Species in the polluted water for one season or more may beconsidered as indicator Species. The natural unpolluted environments are characterized by stable biologicalconditions and contain a great diversity of plants and animals life's with one Species dominating. The greatinstabilities in the quantitative and qualitative composition of the phytoplankton in the different stations over themonths were mostly due to numerous environmental factors, which are variable in different seasons and regions [19].pollutants present in small scale industrial waste water have been identified as the main cause for changing the trophic statusof water body from eutrophictooligotrophic.

Table 3. Distribution of zooplankton in non-polluted site (Station-I) and polluted site (Station- II)

Species	Non-polluted site (Station-I)	Polluted site (Station-II)
Protozoa:		
Amoeba Species	+	+
ArcellaSpecies	-	+
Carchesiumpolypium	+	-
Paramaeciumcaudatum	-	-
Paramaecium Aurelia	+	-
SphaerophysaSpecies	-	-
Rotifera:		
AsplachnaSpecies	+	+
Brachionuscaudatus	+	-
Epiphanesmacrourus	+	-
DiurellaSpecies	+	-
Gastropushyptopus	+	-
Keratellaquadrata	+	-
LacaneSpecies	-	+
MicrocodonSpecies	+	-
Crustacea:		
Cyclops Species	+	+
Daphnia Species	+	+
DiaphanosomaSpecies	+	-
MacrocyclopsSpecies	-	+
MesocyclopsSpecies	+	+
Nupliuslarvae	+	+
NaupliusSpecies	+	+
Zoealarvae	+	+
Insecta:		
Anopheles larvae	+	+
Chironomuslarvae	-	+
ChaoborusSpecies	+	-
SiphlonurusSpecies	+	+
TipulaSpecies	-	+

IV. CONCLUSION

The present study provides vital details on plankton distribution and abundance of Meena River which may unravel the information on the energy turnover

of the river ecosystem. It will serve as a useful tool for further ecological assessment and monitoring of the river ecosystem. The results have shown the need of planktons as index of water quality.

V. AKNOWLEDGEMENT

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Synthesis of Natural Clay Supported Silver Nanoparticles and Its Application in Synthesis of Xanthenes

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ABSTRACT

Synthesis of Natural clay supported silver nanoparticle has been carried out by reduction method. The Natural clay supported nanoparticles were characterized by UV, FE-SEM, EDS and XRD analysis. The catalytic activity of nanoparticle for synthesis of Xanthenes were studied and Progress of reaction was monitored by TLC, products were characterized by ^{1}H-NMR spectroscopy. It was observed that a nanoparticle shows good catalytic activity and good yield of the reaction.

Keywords : Natural Clay, Silver Nanoparticles, Xanthenes, Reduction

I. INTRODUCTION

Xanthenes are important biologically active heterocyclic compounds with anti-bacterial,[1] anti-viral,[2] and anti-inflammatoryactivity.[3]Those compounds are also shows applications in dyes,[4]Laser Technology[5] and pH-sensitive fluorescent materials for visualizationof biomolecules.[6]Several methods have been reported for the synthesis of benzoxanthenes, such as the cyclocondensation reaction of 2-hydroxyaromatic aldehydes and 2-tetralone,[7] the reaction of benzaldehyde and acetophenone[8] and the condensation of β-naphthol with alkyl or aryl aldehydes[9] in acidic condition.

The synthesis of xanthene by use of silver nanoparticle as a catalyst by the condensation of β-naphthol with aryl aldehydes are also reported.[10-11]M. Dabiri et al. gave synthesis of aryl-14H-dibenzo[a,j]xanthene and 1,8-dioxo-octahydroxanthene derivativesusing montmorillonite K10 as reusable eco-friendly catalyst under solvent-free conditions.[12]

There are many methods for preparation of silver nanoparticles are known such asChemical Reduction,[13] reverse micelles process,[14] microwave dielectric heating reduction,[15] ultrasonic irradiation,[16] radiolysis,[17]solvothermal synthesis,[18] electrochemical synthesis,[19] plant mediated synthesis[20-22] etc.

In this report we have synthesized Natural Clay supported Ag Nanoparticles by reduction method and are subjected as a catalyst for the synthesis of Xanthenes from β-naphthol and aryl aldehydes.

II. METHODS AND MATERIAL

Silver nitrate (AgNO₃), Sodium borohydride, Aryl Haide, β-naphthol were supplied by Loba Chem. All chemicals were used as received without any further purification.Double-distilled deionised water was used.Melting points were uncorrected and determined in an open capillary tube.^{1}H NMR spectra were recorded inDMSO-d₆ on a BrukerAvance-III 400 MHz spectrometer using TMS as an internal standard.The residual solvent signals were used as references and

the chemical shifts converted to the TMS scale (DMSO-d6: δ_H = 2.49 ppm).

A. Preparation of Natural Clay supported Ag Nanoparticles.

Natural clay supported Ag nanoparticle catalyst was prepared by stirring 3 gm. of activated clay with Silver Nitrate (0.01mol) in 50 ml water then the 10 ml 0.1M aq. solution of NaBH4 was added drop wise to above mixture and stirred for overnight then filtered and dried at 150⁰C for 1 hr. in hot air oven followed by grinding in morter.

The prepared nanoparticles are characterized by UV spectroscopy, FE-SEM, EDS and XRD.

The UV spectrum(Figure 1) shows the absorption maximum between 400 to 500 nm. The average Particle size from FE-SEM images (Figure 2)is 56.32 nm. EDS spectra (Figure 3) shows the presence of silver and from powder XRD (Figure 4) planes 111, 200, 220 and 311 at38.5°, 44.48°, 64.69° and 77.62° confirm the formation of silver nanoparticles.All the diffraction peaks are well corresponding with the standard diffraction data of JCPDS file No. 04-0783 for face-centered cubic Ag.
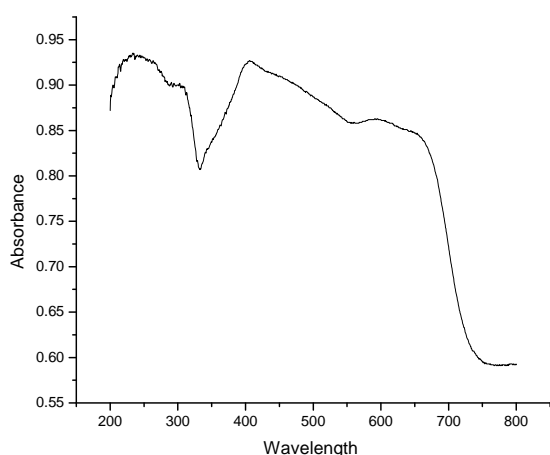
Figure 1. UV Spectrum of Natural Clay – Ag Nanoparticles
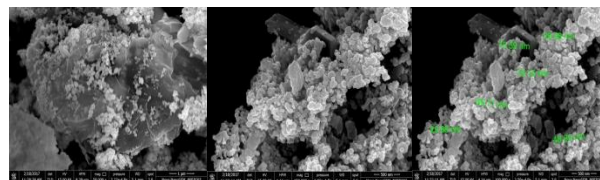
Figure 2.FE-SEM images of Natural Clay – Ag Nanoparticles
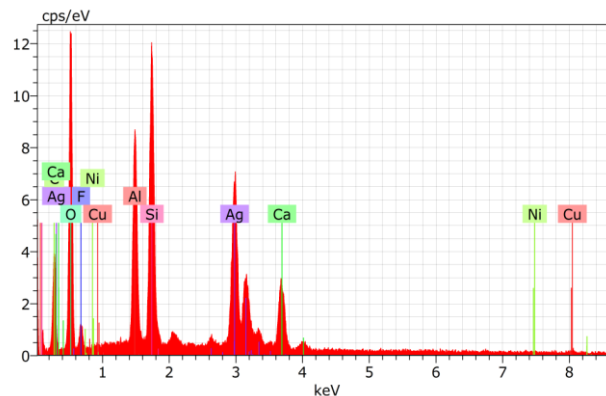
Figure 3. EDS Spectra of Natural Clay – Ag Nanoparticles
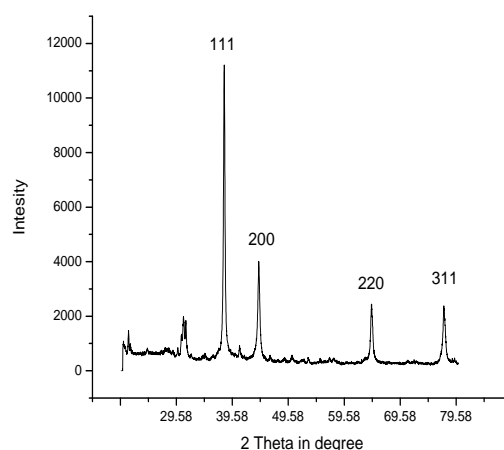
Figure 4. XRD Spectrum of Natural Clay – Ag Nanoparticles

B. General Procedure for Synthesis of Xanthene

A mixture of 2-naphthol (2 mmol), aryl aldehyde (1 mmol) and catalyst (0.100 g) was refluxed in ethanol. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was filtered and washed with diethyl ether (5 mL) to isolate of catalyst. The solvent was evaporated under reduced pressure and the crude product obtained was purified by recrystallization from ethanol.
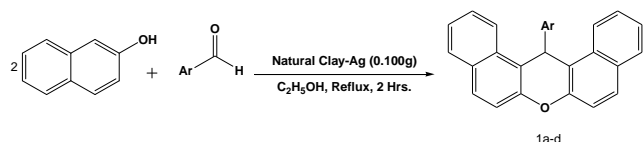
Scheme 1. Synthesis of Xanthene

The product **1a** was isolated as a white solid **Yield :M.P.:** 182-184°C; ¹H NMR (500 MHz, DMSO) δ 8.71 (d, $J = 8.6$ Hz, 2H), 7.93 (dd, $J = 8.4, 3.5$ Hz, 4H), 7.71 – 7.61 (m, 4H), 7.58 (d, $J = 8.9$ Hz, 2H), 7.46 (t, $J = 7.4$ Hz, 2H), 7.15 (t, $J = 7.7$ Hz, 2H), 6.97 (t, $J = 7.4$ Hz, 1H), 6.74 (s, 1H).

The product **1b** was isolated as a white solid **Yield :M.P.:** 288°C; ¹H NMR (500 MHz, DMSO) δ 8.67 (d, $J = 8.5$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 4H), 7.64 (t, $J = 8.9$ Hz, 4H), 7.57 (d, $J = 8.9$ Hz, 2H), 7.48 (t, $J = 7.1$ Hz, 2H), 7.22 (d, $J = 8.6$ Hz, 2H), 6.76 (s, 1H).

The product **1c** was isolated as a yellow solid **Yield :M.P.:** 206°C; ¹H NMR (500 MHz, DMSO) δ 8.66 (d, $J = 8.5$ Hz, 2H), 7.96 – 7.89 (m, 4H), 7.62 (t, $J = 8.1$ Hz, 2H), 7.55 – 7.50 (m, 3H), 7.45 (d, $J = 7.1$ Hz, 2H), 7.05 (d, $J = 8.6$ Hz, 1H), 6.71 – 6.65 (m, 3H), 3.54 (s, 3H).

The product **1d** was isolated as a White solid **Yield :M.P.:** 188-190°C; ¹H NMR (500 MHz, DMSO) δ 8.65 (d, $J = 8.5$ Hz, 2H), 7.90 (dd, $J = 13.5, 8.4$ Hz, 4H), 7.62 (dd, $J = 11.8, 4.6$ Hz, 2H), 7.54 (d, $J = 8.9$ Hz, 2H), 7.44 (t, $J = 7.4$ Hz, 2H), 7.38 (d, $J = 8.8$ Hz, 2H), 6.56 (s, 1H), 6.43 (d, $J = 8.9$ Hz, 2H), 2.62 (s, 6H).

III. RESULTS AND DISCUSSION

The reaction conditions were optimized on the basis of the catalysts, solvents and different temperatures for carbon-carbon and carbon-oxygen bond formations. Thus the model reaction was carried out using benzaldehyde and 2-naphthol as shown in table 1.

Table 1. Optimization of reaction conditions

Entry	Catalyst (gm)	Solvent	Temp.°C	Time	Yield (%)[a]
1	None	none	R. T.	2 Hrs.	00
2	None	EtOH	R. T.	2 Hrs.	00
3	0.100	EtOH	R. T.	2 Hrs.	00
4	0.100	CH₂Cl₂	R. T.	2 Hrs.	00
5	0.100	DMF	R. T.	2 Hrs.	00
6	None	none	Reflux	2 Hrs.	<10
7	0.100	EtOH	Reflux	2 Hrs.	90
8	0.100	CH₂Cl₂	Reflux	2 Hrs.	50
9	0.100	DMF	Reflux	2 Hrs.	55

[a]Isolated Yield

As an outcome of these experiments we found that no conversion was found at room temperature with catalyst entry 3 in table 1. Whereas very less conversion on heating in absence of solvent entry 6 in table 1. Good conversion in presence of DCM and DMF entry 8 and 9 in table 1. Natural Clay supported Ag nanoparticles is the most active catalyst for this cyclization reaction in presence of ethanol as a solvent at reflux temperature entry 7 in table 1.

The activity of catalyst was tested for some different aldehydes such as p-chlorobenzaldehyde, Anisaldehyde and 4-N,N,- dimethyl Benzaldehyde entry 2, 3 and 4 in table 2 with 2-naphthol. The isolated products were characterized by ¹H-NMR spectroscopy.

Table 2. synthesis of xanthenes by using Natural Clay Ag nanoparticles

Entry	Aldehydes	Xanthene	Yield (%)[a]
1	Benzaldehyde	1a	90
2	p-Chlorobenzaldehyde	1b	95
3	Anisaldehyde	1c	70
4	Para-N,N-dimethyl Benzaldehyde	1d	68

Reaction condition: Aldehyde (1mmol), 2-naphthol (2 mmol), Natural Clay Ag Nanoparticles (0.100 gm), EtOH, reflux, 2 Hrs.

Table 3. Scope of Cyclisation between different Aryl Aldehydes and 2- naphthol
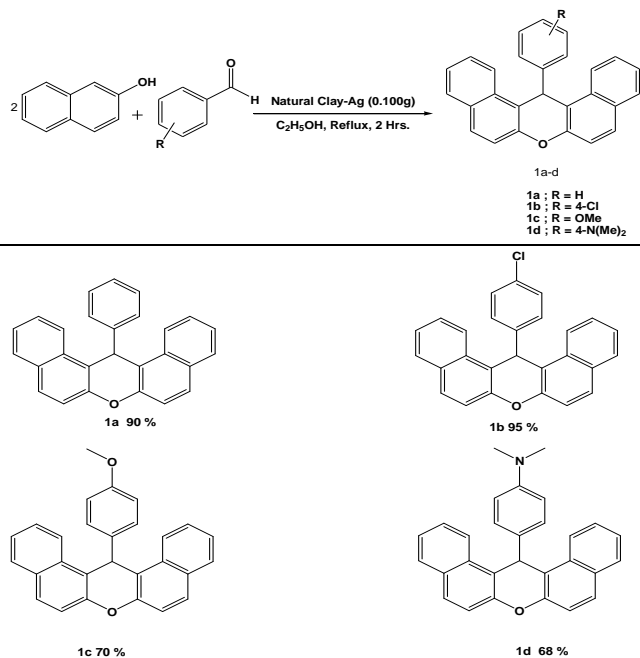
1a ; R = H
1b ; R = 4-Cl
1c ; R = OMe
1d ; R = 4-N(Me)$_2$

1a 90 %

1b 95 %

1c 70 %

1d 68 %

Reaction condition: Aldehyde (1mmol), 2-naphthol (2 mmol), Natural Clay Ag Nanoparticles (0.100 gm), EtOH, reflux, 2 Hrs.

IV.CONCLUSION

In summary, we have developed a mild, highly efficient method for synthesis of biologically active xanthenes in the presence of natural clay supported AgNPs as catalyst. The method requires a simple work-up, is inexpensive, short reaction times and gives good yields.

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In Vitro Response of GA₃ in Caulogenesis of Fiver nut

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ABSTRACT

A protocol was optimized for the caulogenesis of fiver nut. Internodal explant showed immediate response in shoot regeneration and production of callus in in vitro cultures of Caesalpiniabonducella (L.) Roxb.commonly known as fiver nut. MS medium supplemented with 1 to 10 mg/l GA 3 was found to induce callus. The Internodal explant inoculated on MS medium with 6 mg/l GA3 was found to produce shoots after 35 days of inoculation. Maximum amount of pale yellow coloured   friable callus was produced in 7mg/l GA3 of dry weight 1.513± 0.108 g. The method can be used to generate callus and shoot which are natural sources of pharmaceutical compounds without disturbing the natural population of the plant.

Keywords : Fiver Nut, Caulogenesis , Callus.

I. INTRODUCTION

Caesalpiniabonducella (L.)Roxb.is an important medicinal plant belonging to the family Caesalpiniaceae .The entire plant and plant parts like seed ,seed coat , pod ,leaf , stem and roots are used in traditional medicines of India and all over the world. The plant contains biologically active compounds like phenols ,diterpenes , flavonoids ,alkaloids and tannins . Caesalpiniabonducella(L.) Roxb.reported to possess anti-diabetic (SudeepParameshwar et al., 2002) ,anti-filarial (Gaur et al., 2008.),anti- malarial(Sachan NK et al., 2010)and antioxidant (Ogunlana et al., 2012) properties. It is a major constituent of Ayush -64 used against malaria and Female Health by Planet Ayurveda as a supplement to vitamin C . Seed powder is packed and sold under commercial name Kalarchi Kai powder to treat fever. In Ayurveda the plant is reported to balance tri doshas like vata, pitta and kaphadoshas. Unfortunately for this reason the natural habitats of Caesalpiniabonducella (L.) Roxb.were encroached by human beings .The population of fiver nut is drastically reducing for the past two decades.

Recently the plant is placed under endangered category and will extinct if steps are not taken for conservation.

Tissue culture is an efficient tool to increase the number of plants of Caesalpiniabonducella. Santosh Kumar et al. carried out micro propagation of fiver nut. In vitro plant regeneration and acclimatization of plantlet can be done to supply sufficient plants to farmers, environmentalists and common man. It will initiate rapid propagation of fiver nut and we can restore natural population. Even thoughauxins andcytokinins are mostly used for shoot regeneration and callus production of fiver nut   GA₃ is insufficiently utilized. GA₃ is a chemical derivative of gibberllins naturally present in plants.GA₃ has an impact on stem elongation ,organization of shoot primordia and breaking seed dormancy.GA₃ is used to induce callus and shoot regeneration in woody climbers like grapes. Exogenous supply of GA₃ increases the root auxin in transgenic plants .It is also reported to elevate the production of phytogibberllin content in both root and shoot. Therefore our study

aims to conserve the fiver nut plant by providing (1) an alternative source for the production of pharmaceutical compounds through callus (2) an efficient protocol for caulogenesis in fiver nut with GA_3 which is rarely used in in vitro cultures of Caesalpiniabonducella(L.) Roxb.

II. METHODSAND MATERIAL

The plants and the mature seeds of Caesalpiniabonducella(L.)Roxb.were collected from Manjri and authenticated in BSI. The seeds were washed thoroughly and dried. Seeds were sacrificed using Con. HCl for 60 minutes and it was allowed to germinate. After 10 days epicotyl protruded above the soil .Healthy seedlings were established in one month. Three months old seedlings were used as explant source for the experiment.

Leaf, internode and node were used for in vitro cultures. The explant were washed for 30 minutes in running tap water. Then labolene treatment was given for 5 minutes. The treated explant were washed thoroughly. They were kept again in running tap water for 30 minutes. Then it was treated with 1% $HgCl_2$ followed by three times wash using double distilled water in laminar air flow chamber. About 1cm length explants were inoculated in MS medium supplemented with GA ₃ 1 to 10 mg/l. After inoculation the culture were kept in culture room with 25 ± 1^0c temperature, 16 hours 1000 lux light and humidity 50%. Observations were taken after 5, 10 and 15 days. Sub culturing was carried out at 15 days interval. Fresh weight of callus were taken after 35 days using electronic balance. MS basal medium supplemented with 30g sucrose was used as control for the experiments. The pH of the medium was adjusted at 5.8. Statistical analysis were carried out using ANOVA.

III. RESULTS AND DISCUSSION

Phenolic exudation and browning of cut surfaces of explants were reduced significantly in explants collected from three months old seedlings. The shoot was obtained from inter nodal explants after 35 days of inoculation. Subculture of callus and shoots were done at 15 days interval. Green nodular callus was produced in 6 mg/l GA_3 after 15 days of inoculation and shoot after 35 days. Internodal explants produced callus in MS medium supplemented with GA_3 1to 10 mg/l. Callus was initiated in 10days of inoculation in 1mg/l to 4mg/l GA_3.Maximum amount of callus was obtained in 7mg/l GA_3 with dry weight 1.513 ± 0.108g. Beyond 8 mg/l the callus weight was decreased.

Effect of GA_3 in callogenesis and caulogenesis in Caesalpiniabonducella (L.) Roxb.after 35 days.

Table 1

Hormonal Con.(mg/l) GA₃	Morphology of Callus Color Nature		Amount of callus (Dry Wt. in g) Mean ±SE	No. of shoots
1	Green	Friable	0.426 ± 0.059	0
2	Green	Friable	0.533 ± 0.033	0
3	Green	Friable	0.762 ± 0.034	0
4	Green	Friable	0.849 ± 0.042	0
5	Greenish yellow	Flexible	0.773 ± 0.045	1
6	Greenish	Nodular	0.842 ± 0.034	1

	yellow		
7	Pale yellow Friable	1.513 ± 0.108	1
8	Pale yellow Friable	0.654 ± 0.078	0
9	Yellow Compact	0.618 ± 0.248	0
10	Yellow Compact	0.211 ± 0.145	0

Caulogenesis inGA₃ 6mg/l.

A.   B.

Callus in GA₃ 7mg/l   Callus initiation in 7 mg/l GA₃

Callus in GA₃ 8mg/l Callus initiation in GA₃ 4 mg/l

Callus in GA₃ 5 mg/l   Callus initiation in GA₃ 3mg/l

Caesalpiniabonducella (L.) Roxb.requires 7 years for seed germination which is one of the reason for its current status in endangered medicinal plant in nature. Many researchers worked on in vitro propagation of fiver nut using cotyledonary, epicotyledonary and nodal explants (Santosh Kumar et al.,2012).GA₃ is widely used for the micropropagation of woody trees and climbers(Jain et al.,2007).A combination of GA₃ and cytokinins were used for shoot multiplication in tea(Reza et al.,2014) and Lavenduladentata,L. shoot tips (Marilia et al.,2011).To conclude probably first time GA₃ was used in in vitro cultures of fiver nut for the production of callus and shoot. The regenerated shoot can be used for the extraction of bioactive compounds which is very important to conserve the in vivo plants.

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Larvicidal activity of Mentha piperita extract against Musca domestica

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ABSTRACT

The housefly M. domestica L. is a global insect, responsible for vectoring of various diseases. Nowadays Chemical control methods are majorly used against housefly, but they also lead to disadvantages such as harmful side effects to human beings and the environment as well as houseflies resistance. So in order to find an effective and environment friendly botanical control agent, extract of Mentha piperita was evaluated for its larvicidal activity on housefly. The First Instar larvae showed a higher mortality rate than the third Instar larvae when baited with Mentha piperita extract in increasing concentration of 1% to 5 %. At 5% concentration, the first Instar larvae and third instar larvae showed a mortality rate of 96 % and 82% respectively.

Keywords: Mentha piperita, Musca domestica, larvae, Larvicide

I. INTRODUCTION

Houseflies belong to the order Diptera. These are known for their ability to exploit most ecological niches in any biological environment due to their larvae's ability to evolve (West, 1951). The housefly (Musca domestica L) is found worldwide and was first recorded in Hawaii during1869 by Thompson. This common fly originated of the central Asia, but now occurs in many climates from tropical and subtropical regions. (Derek Gammon, 2008). It is medically important insect and one of the major domestic and veterinary pests. The insect is responsible for transmitting infectious diseases and causes public health problems among human communities. Not only houseflies are a nuisance, but they can also be vector of some diseases especially in tropical area (Zhang et al., 2008). It feeds on and breeds in decaying matter, human waste and food, and is considered a mechanical vector for pathogens (Bacteria, protozoa and viruses) to humans and livestock (Olsen et al., 2001, Sangmaneedet et al., 2005). These pathogens may cause food poisoning, diarrhea, cholera, typhoid paratyphoid, shigellosis and anthrax (Banjo et al., 2005, Fasanella et al., 2005, Yap et al., 2008).

Currently, control of housefly largely relies on chemical insecticides. Unfortunately, house fly has developed resistance to most of chemical insecticides (H.A.A. Khan et. al., 2013) and these chemicals also have adverse environment and health effect, threat of persistence and biomagnifications through the food chain(P. Kumar et. al., 2012). Therefore, as a better alternative to synthetic chemicals, the use of botanicals to control housefly is being looked upon as a main source for safer and eco-friendly insecticide. Moreover, botanical insecticides are biodegradable, species specific, no side effects toxic to non target-organisms, human, animal and environment, however, botanical insecticides from plant oils or extracts have been used effective to control insect pests including house fly.(P. P.Sharma et al, 201 6 , C. Regnault et al 20120).

A huge number of plants having medicinal values are available in India. Since plant-based bio-insecticides have insecticidal and/or insect repellent activities, species-specific in action, easy to manufacture and apply, and Mentha piperita are safer for animals and their environments. Mentha piperita have high medicinal value. Plant extract have drawn considerable attention for their uses against various pest species including houseflies [Islam MS et. al. 2013].

II. MATERIAL AND METHOD

Collection and Rearing of Housefly Larvae

The culture (eggs) of houseflies was obtained from National Chemical Laboratory, Entomology Department, Pune. The eggs were incubated in plastic jars (15 × 25 cm) on moist groundnut crush powder in laboratory at 28±2 ° C. The eggs were allowed to develop larvae and into pupae on the same medium. The pupae were transferred to other container (12 × 24 cm) for adult emergence. Emerged adult houseflies were reared in plastic jars covered on top side with muslin cloth. And continued larval culture of housefly. Among the larvae, first instars and third instars were taken to observe difference in mortality rate. The first instars larvae are considered as infantile larvae while third instar larvae are considered as older larvae. The larvae were fed with groundnut crush powder and water.

Plant Material

The experimental plant used in the study was Mentha piperita (Mint). The plant leaves were collected from and around Pune region. Plant leaves were properly washed with water, shade dried for 6-8 days at 30-32°C. Dried leaves were powdered in Mortar and Pestle. Dried leaf powder weighing 50 g was extracted in 833 ml of Methanol solvent in Soxhlet apparatus for 8 hours. The extract was then collected and evaporated to dryness using rotary vaccum evaporator.

Larvicidal Bioassay

The first and third instars larvae obtained from the same batch of eggs, were divided into 5 groups of 15 larvae each. The larval treatment was carried out in transparent plastic jar (5X7 cm). A group of 15 larvae introduced into separate plastic jar using a new camel-haired brush (No.0). It provided with food (groundnut powder and plant extract). The control jar provided with 3 gm groundnut powder and water. In the treated groups the M piperita extract were prepared 1%, 2%, 3%,4%, 5% .(1μL extract mix with 1 ml water to form 1 % solution). The prepared extract offered to experimental larval group with food (3 gm groundnut powder). Each experimental test was repeated six times. The mortality assessed by touching each larva with a paintbrush (no. 0). The number of dead larvae was counted to calculate the mortality percentage.

Table 1. The Mortality percentage of the housefly larvae

Larval instars	Control	1%	2%	3%	4%	5%
1st instar	0	49	60	71	83	96
3rd instar	0	40	49	62	71	82

III. RESULT AND DISCUSSION

In larvicidal bioassay M. Domestica larvae were treated with the leaf extract of M. piperita and result shows the effectiveness of the leaf extract and its potential control of larval population of M. domestica. The different concentrations of extract were used to obtain mortality against first and third instar larvae of housefly. The results obtained from the experiment are as mentioned in the table with larval mortality percentage. In control, the first and third instar larvae remained alive. The first instars larvae showed higher mortality percentage compared to the third instar larvae as the concentration of extract was increased from 1% to 5 % gradually. At 5% concentration, the First Instar larvae showed 96 % while the Third Instar larvae showed 82% of mortality. This shows that the

Third instars show more resistance than the first Instars towards the Mentha piperita extract. Based on the results achieved, the Mentha piperita extract shows larvicidal activity towards Musca domestica larvae.

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Cytoprotective properties of Hemidesmus indicusagainst H_2O_2on Drosophila melanogaster

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ABSTRACT

Reactive oxygen species, including hydrogen peroxide (H_2O_2), singlet oxygen, hydroxyl and superoxide radicals, have positive role in energy production in vivo systems, phagocytosis, intercellular signalling, and regulation of cell growth. At high concentrations, H_2O_2 acts as a toxin which has scavenging properties. Avariety ofdietary plantsincludinggrams,legumes,fruits,vegetables,tea,wine etc.are rich inantioxidants and scavenge the free radicals and reduces the oxidative stress. Hemidesmus indicus (H. indicus) being one of the indigenous plant known for its antioxidant and scavenging properties. Drosophila melanogaster is an excellent model organism to evaluate lethal concentration and the effect of different chemical or bioactive substances due to its short generation time and lifespan. The present study deals with control of H_2O_2 production by H.indicus using drosophila as model organisms. Drosophila species were treated with different concentrations of H_2O_2 and H. indicus. The present study reveals that prolonged exposure to H_2O_2 at higher concentration causes toxicity which in turn affects the hatching and life cycle of drosophila. The study reveals that the supplementation of H. indicus increases the resistance ability against oxidative stress generated by hydrogen peroxide. It acts a cyoprotective molecule thereby significantly reducing the levels of antioxidant enzymes such as catalase and superoxide dismutase generated by hydrogen peroxide in drosophila. Also, it helps to maintain the GSH levels in drosophila both larval and adult forms by restoring the glutathione peroxidase and glutathione reductase levels. Thus, H. indicus is one of accessible source of antioxidant in pharmaceutical industries.

Keywords : Hemidesmus indicus, Hydrogen Peroxide, Drosophila, LC_{50}, Glutathione

I. INTRODUCTION

H_2O_2 (Hydrogen peroxide) in biological system can pass through biological membranes easily hence can react with other biological molecules and generates potent ROS. It target cell externally and internally causes oxidative stress leading to aging. Some of the reactive oxygen species, including hydrogen peroxide, has positive role in energy production in vivo systems, phagocytosis, intercellular signalling, and regulation of cell growth (Packer et al., 2008). Along with that it also affects the DNA and membranes stability by attacking the lipids, proteins, and carbohydrates in cell membrane and tissues and nucleic acid bases (Jung et al., 2009).

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value (Nostro et al., 2000). Hemidesmus indicus one of the indigenous plant also known as ANATMOOL or sativa. It is found in tropical region of India. It is rich in hemidesmol, glycoside, tanin, β-amyrins,flavenmin 1, hemidesmin 2, steroids, 2 – hydroxy, 4-methoxy Benzoic acid,saponins ,

Phenols, and etc. They possess bioactive compound 2 –hydroxy, 4- methyl benzoic acid, acts as antioxidant. Gayatri et al (2008) had shown that H.indicus have hepatoprotective properties in rat. Along with antioxidant properties it also possesses anti venom against viperarusseiiand antimicrobial properties (Baheti et al., 2006). It is use in Ayurveda to cure high blood pressure, Arthritis, increase semen also. The antioxidant present in the H. indicus would act as a potent inhibitor of free radicals generates into the body for example- H_2O_2, $OH^.$, $O_2^.$.The fruit fly Drosophila melanogaster is a eukaryote widely used in genetics. It requires simple facilities, inexpensive culture media, it has a short generation time (appox. 10 days at 25°C), it breeds a large number of individuals per generation, and in vivo assays can be done easily.

In the absence of systematic studies in literature, the present study is aimed to evaluate the role of Hemidesmus indicus root extract in hydrogen peroxide induced oxidative stress in drosophila.

II. METHODS AND MATERIAL

A. Plant Material

The root powder of *Hemidesmus indicus* were directly purchased from Punervasu pharmacy, Pune.

B. Preparation of Plant extract: Different concentrations (0.25 – 7 mg/L) of *Hemidesmus indicus* root powder was prepared in autoclaved distilled water.

C. Preparation of Hydrogen peroxide: Different concentrations (0- 30 mg/L) of hydrogen peroxide (Himedia, USA) were used for the experiment.

D. Drosophila Culture and LC₅₀: The wild-type *Drosophila melanogaster* strain was maintained in the laboratory on a standard cornmeal, yeast, dextrose, and agar medium at 25°C (Ford et al., 2007). Eggs were collected from these flies by shaking them without anesthesia into bottles containing an approximately 2cm layer of fermenting fresh baker's yeast supplemented with sucrose The egg collection bottles were then kept undisturbed in the dark for 8 h at 25°C. After

removing the parental flies, the egg collection bottles were taken back to 25°C where they remained at a relative humidity of 65% for the rest of their development. Three days later, the 72 h larvae were collected by washing them out the bottles with tap water at room temperature through a fine-meshed stainless steel strainer. They were thoroughly washed free of yeast with tap water while still in the strainer.

2.5 Estimation of sub lethal toxicity (LC₅₀): The larvae were transferred to vials (20 larvae/vial) containing 0.5 g of Drosophila Instant Medium (Carolina Biological Supply Co, NC, USA) prepared with the solutions of the test compounds, hydrogen peroxide at 0 to 30 mg/L. Five replications were made for each concentration in five independent experiments for each hydrogen peroxide. The treatment vials were kept at 25°C and at a relative humidity of 65%. The surviving flies were collected from the vials on days 10 to 12 after egg laying and shaken into a flask containing 70% ethanol to quantify mortality. The LC50's for each strain and hydrogen peroxide were calculated using logistic regression with all five replications of every concentration. LC50's obtained from the five experiments were analysed with a two-way ANOVA (one factor being the strain, the other the treatment).

2.6 Measurement of antioxidant enzymes: Catalase (CAT, *EC 1.11.1.6)* activity was determined by the method described by Aebi (1984). Superoxide dismutase (SOD, *EC 1.15.1.1)* was assayed according to Beauchamp and Fridovich (1971). Glutathione peroxidase (GPx, *EC 1.15.1.9)* activity was measured by the method of Lawrence and Burk (1976). Glutathione Reductase (GR, EC 1.8.1.7) activity was determined by the protocol of Goldberg and Spooner (1983).

2.7 Statistical analyses

All experiments were repeated at least five times and data presented is average of these replicates. One-way analysis of variance (ANOVA) test associated

with the Tukey's test was used to determine the statistical significance of the differences among experimental groups. All the statistical analyses were done using SPSS 17.0 software. A logarithmic trend line was used to calculate the LC_{50} values.

III. RESULTS AND DISCUSSION

Drosophila melanogaster is considered to be the best known multicellular eukaryote model organism as we can study the interactions between genes and environmental conditions simultaneously. Recently, there have been successful attempts to use this species to investigate the effect of a certain type of diet on viability and lifespan (Khan et al, 2012; Li et al., 2007; Alberto et al., 2010; Soh et al., 2012).Drosophila use as an excellent model system to evaluate lethal concentration and their effect of different chemical or bioactive substances such as, growth and moulting disruption effects of azadirachatin against drosophila melanogaster (Diptera: Drosophilidae) by RadiaBezzar-Bendjazia et al, 2015. Other studies on Drosophila melanogaster have shown the effect of Asprin and acetaldehyde on longitivity and metamorphosis duration (Duygukeser and AylaKaratas, 2012). Presently 80 percent of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases (Veale et al., 1992). H. indicus serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases, urinary infections, negative emotions and impotence (Jain et al., 2003).

### A. To measure and evaluate the median lethal concentration (LC_{50})

LC_{50} is defined as the lethal concentration at which 50 % of the population if killed in a given period of time. There can be wide range of tolerance to toxic agents among different population of a species which should be taken into account.

In the following experiment the drosophila was treated with different concentrations of hydrogen peroxide ranging from 0 to 30 mg/L. At the different concentrations of H_2O_2 viz 0, 5, 10, 15, 20, 25 and 30 mg/L percent mortality were 3, 14, 28, 37, 52, 69 and 85 respectively. Figure 1 shows the mortality rates and LD50's for hydrogen peroxide was 19.64 mg/L (Figure 1). On the other hand, the ANOVA results show significant differences between control (F = 41.21, p = 0.00003) and hydrogen peroxide interaction (F = 9.62, p = 0.0032). Results revealed an increase in mortality rates directly proportional to increase in different concentrations of hydrogen peroxide (Figure 1). The similar resultwere observed by insecticide beta cyfluthrin on drosophila studeis (GireeshNaada et al., 2005). The crucial element of drosophila ethanol also showed the concurrent result (You et al., 2004).

H. indicus (anatmool) was also used to test its lethal concentration on drosophila with different concentration such as 0.05 to 7 mg/L. At higher (7 mg/L) concentration of H. indicus approximately 25 % mortality was observed.Thus drosophila treated with different concentration ofH. indicus does not showed any significant percent mortality (Table 1).

In the further experiments three concentrations (10, 15, 20 mg/L)of H_2O_2 were used in combination with (0.5 mg/L)H. indicus.
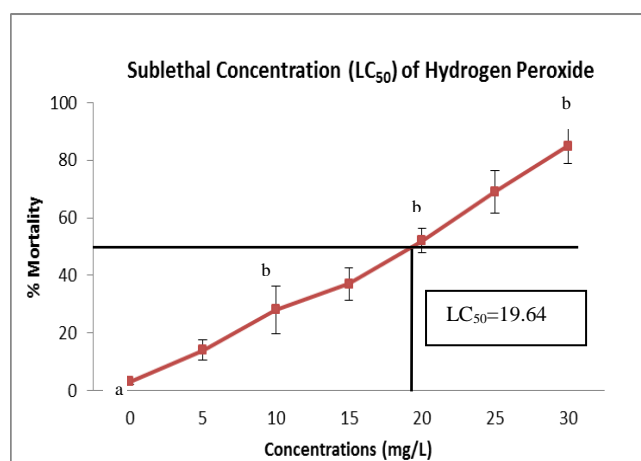
Figure 1. Median Lethal Concentration (LC_{50})at different concentrations of Hydrogen Peroxide

Dissimilar alphabets a and b in superscript in the figure indicate statistically significant difference at 0.05 level.

Table 1. Percent Mortality of flies at different concentrations of H_2O_2 and (0.5 mg/L) of H. indicus

Different Conditions	% Mortality
Control	9 ± 1.2^a
(0.5 mg/L) H. Indicus	14 ± 2.3
(10 mg/L)H_2O_2	14 ± 1.5
(10 mg/L)H_2O_2 + (0.5 mg/L) H. indicus	9 ± 1.1
(15 mg/L)H_2O_2	42 ± 2.3^b
(15 mg/L)H_2O_2 + (0.5 mg/L)of H. indicus	14 ± 1.3^c
(20 mg/L)H_2O_2	52 ± 3.4^b
(20 mg/L)H_2O_2 + (0.5 mg/L) H. indicus	19 ± 1.8^c

Control, H. indicus:Hemidesmus indicus, H_2O_2: Hydrogen peroxide. Dissimilar alphabets a and b in superscript in the figure indicate statistically significant difference at 0.05 level.

B. Morphological Changes in Drosophila Life cycle

Drosophila exposed at different concentrations of H_2O_2 ranging from 5 to 30 mg/L showed significant morphological changes in its life cycle. When drosophila exposed at different concentration of H. indicus no morphological changes were observed in the emerged out larval forms. Following Observations (Slide 1) were made under different treatments:

1. Control and H. indicus (0.5 mg/L) showed no significant change in the morphology and percent mortality.
2. H_2O_2 (15 mg/L and 20 mg/L) showed significant mortality and the size of emerged larva was almost double compared to control larva.
3. In combination (10 mg/L H_2O_2 + 0.5 mg/L and 15 mg/L H_2O_2 + 0.5 mg/L H.indicus) significantly restored the size of larva as control larval forms.

4. At 20 mg/L of H_2O_2 the larval size was almost 3 times than the control larval forms. Also, the delay in the emergence of larval forms was observed compared to control. The addition of extract (0.5 mg/L) significantly restored the larval size of drosophila.

Interestingly at 30 mg/L of H_2O_2 the life cycle of drosophila was ceased at the stage of eggs which was observed till 15 days.

This may be due to inhibitory effect of hydrogen peroxide on gonadal development (Pratt,1980) The inhibition of oviposition is may be a result of imbalanced endocrine system or inhibition of ovarian development or deformities in oviposition organs (Asai et al., 1985). The reduced fecundity rate was observed by the effect of beta -cyfluthrin, (GireeshNadda et al., 2005). Spodopteralittoralis as a sub lethal pyrethroid insecticidal was reported (Radwan 1984) and besides that the life cycle was delayed by 5-6 days as compared to control. i.e prolongation in life cycle. This may be due some cytotoxicity of H_2O_2 or over production of growth hormone.

At the concentration 30 mg/L of H_2O_2 the eggs were not able to hatch from the egg this is may be due to direct impact of H_2O_2 various tissue such as trophocytes, perifollicular tissue, follicular epithelium and oocyte themselves (Kaur et al., 1993) or hormonal imbalance (GireeshNadda, 2005). In the present study it was observed that the egg hatching process and ovicidal action was decreased due to accumulation of H_2O_2 in eggs resulted in their direct death. The eggs which laid but do not hatched, are may be result of inappropriate incorporation of the yolk so that the embryo failed to complete metamorphosis.(Kaur et al., 1993) or may be due antifeedant effect of H_2O_2 resulted in weak and non-viable egg n drosophila species (Moore, 1980; Kumar and chapman, 1984).
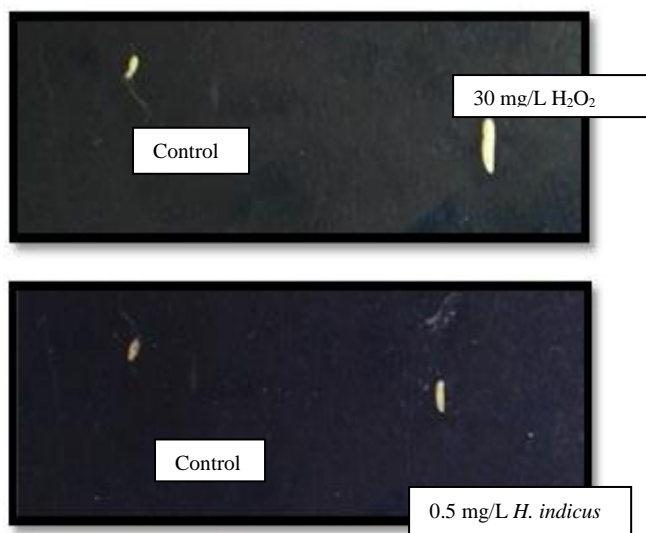
Slide 1. Morphological changes observed in drosophila (3 instar larva) under different conditions

C. Evaluation of Antioxidant Enzymes

The H_2O_2 is one of the potent ROS generator .which induces oxidative stress use in experiment to generate oxidative stress. This oxidative stress can be reduced by bioactive compound obtained from plant (Sinha et al., 2006). Hemidesmus indicus is also one of the plant possess bioactive compounds can act as antioxidant. 2 – hydroxy ,4-methoxy Benzoic acidact as a hepatoprotective compound in rat (Gayatri et al, 2008) i.e act as antioxidant which scavenge the free radical.
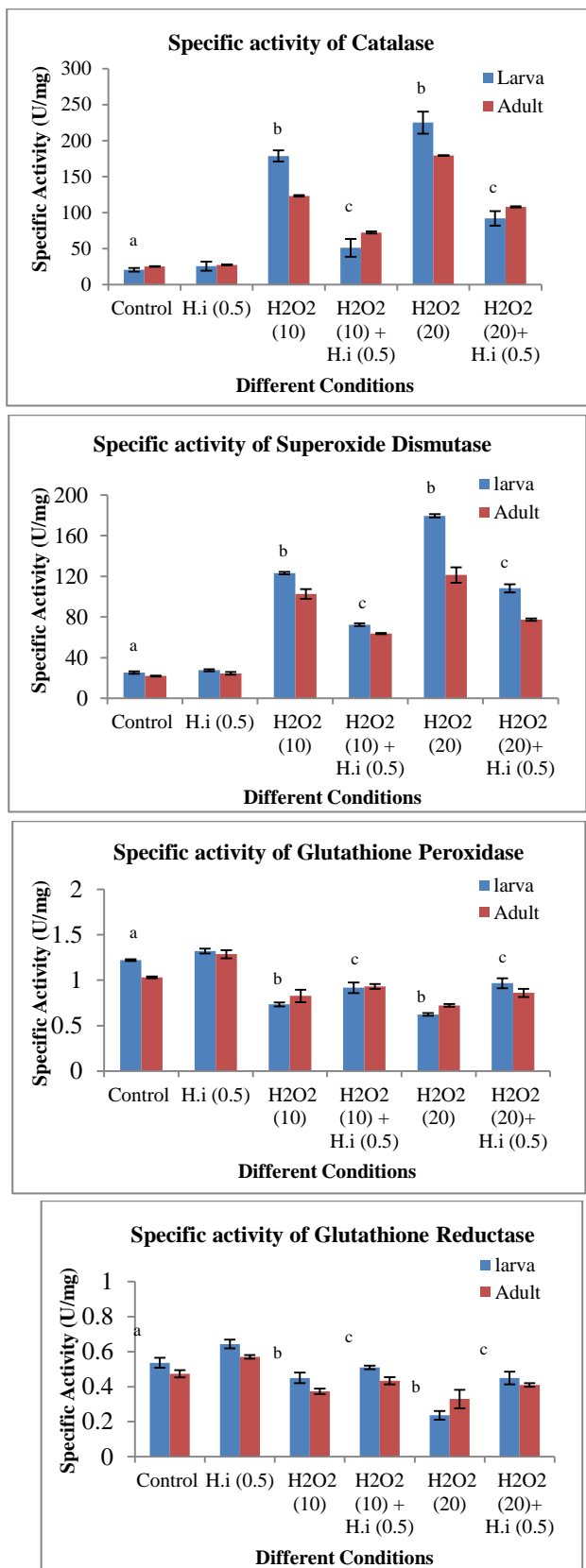
At the concentration of 10 mg/L and 20 mg/L of H_2O_2 the specific activity of catalase was significantly increased in larval (178 ± 0.78 U/mg; 225.2 ± 2.7 U/mg; $p < 0.05$) and adult forms of drosophila (123.2 ± 1.0; 155.7 ± 0.5 U/mg; $p < 0.05$) compare to control larval and adult forms. When the flies were treated on combination with 0.5 mg/L H. indicus the catalase activity was restored back in larva (51.20± 0.9 U/mg; 92.1 ± 2.9 U/mg; $p < 0.05$) and in adult (32.8 ± 1.3 U/mg; 66.1 ± 0.8 U/mg; $p < 0.05$), compared to control adult and larval form (178 ± 0.78 U/mg; 225.2 ± 2.7) (Figure 2).SOD one of the cytoplasmic catalytic enzymes which scavenge the superoxide ions. At the concentration of 10 mg/L and 20 mg/L of H_2O_2 the specific activity of SOD was significantly increased in larval (123.18 ± 1.1 U/mg, 179.56 ±1.5 U/mg; $p < 0.05$) and adult (102 ± 4.778

U/mg; 121.29 ± 7.61 U/mg; $p < 0.05$) forms of drosophila compare to control larval and adult forms. When the flies were treated on combinations with 0.5 mg/L H. indicus the catalase activity was restored back in larva (72.47 ± 1.448; 108.23 ± 4.100U/mg; $p < 0.05$) and in adult (63.71 ± 0.5567 U/mg; 72.76 ± 9.07 U/mg; $p < 0.05$).compare to control larval and adult forms.

Glutathione reductase is an enzyme that reduces glutathione disulphide to sulphydryls from GSH, which is an important cytoplasmic antioxidant activity. At the concentration of 10 mg/L and 20 mg/L of H_2O_2 the specific activity of GPx was significantly decreased in larval (0.83 ±0.07 U/mg; 0.55±0.30 U/mg) and adult (forms of drosophila 0.52 ±0.39; 0.62.±0.02 U/mg) compared to control larval and adult forms. When the flies were treated on combinations with 0.5 mg/L of H. indicus the GPx activity was restored back in larval forms(0.93 ± 0.03 U/mg; 0.86 ± 0.04 U/mg; $p < 0.05$) and in adult (0.92 ± 0.06 U/mg; 0.97 ± 0.06 U/mg; $p < 0.05$) compared to control larval and adult form.At the concentration of 10 mg/L and 20 mg/L of H_2O_2 the specific activity of GR was significantly decreased in larval (0.45 ± 0.03 U/mg; 0.24 ± 0.03 U/mg; $p < 0.05$) and adult (0.37 ± 0.02 U/mg ; 0.33 ±0.05 U/mg ; $p < 0.05$) compare to control larval and adult forms. When the flies were treated on combinations with of 5 mg/L of H. indicus the GR activity was restored back in larva (0.51 ±0.01 U/mg;0.45 ± 0.04 U/mg; $p < 0.05$) and in adult (0.43 ± 0.02 U/mg; 0.41 ± 0.01 U/mg; $p < 0.05$) compare to control larval and adult forms.

The present study when drosophila's were treated with different concentrations of hydrogen peroxide significant increase in the levels of antioxidant enzymes namely CAT, SOD, GPx and GR as observed. Simultaneous treatment with H. indicus protected and restored the levels of these antioxidant enzymes. Concurrent results were observed by N. Mansa and J.S. Ashadevi (2015). This can be due to increase in activities GPx, SOD and CAT which results in increase in longevity. The

relevant results have been reported on Xijnjiang black mulberry fruit on delaying aging (Jiang et al., 2010).

Specific activity of Catalase

Specific activity of Superoxide Dismutase

Specific activity of Glutathione Peroxidase

Specific activity of Glutathione Reductase

Figure 2. Specific activities of different antioxidant enzymes at different conditions

H.i (0.5): 0.5 mg/L of H. indicus; H2O2 (10): 10 mg/L of hydrogen peroxide; H2O2 (10)+ H.i (0.5): 10 mg/L of hydrogen peroxide and 0.5 mg/L of H. indicus ;H2O2 (20): 20 mg/L of hydrogen peroxide; H2O2 (20) + H.i (0.5): 20 mg/L of hydrogen peroxide and 0.5 mg/L of H. indicus. Dissimilar alphabets a, b and c in superscript in the figure indicate statistically significant difference at 0.05 level.

IV. CONCLUSION

The studies reveals that the supplementation of H. indicus increases the resistance ability against oxidative stress generated by hydrogen peroxide and thus being a cytoprotective drug. It reduces the oxidative stress generated by hydrogen peroxide in drosophila. Its acts a cytoprotective molecule by reducing the levels of antioxidant enzymes such as catalase and superoxide dismutase. Also, it helps to maintain the GSH levels in drosophila both larval and adult forms by restoring the glutathione peroxidase and glutathione reductase levels. Thus, H. indicus is one of accessible source of antioxidant in pharmaceutical industries. However, Disruption of growth and development in flies and other insect species under Hydrogen peroxide treatment have yet to be fully investigated.

V. ACKNOWLEDGMENTS

VI. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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New Report of Zyxomma petiolatum (Rambur, 1842) Odonates from Daund Tehsil, MS : India

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ABSTRACT

Morphological similarity often complicates field identification in insects, leading to data analysis on the basis of geographic distribution over planet. Still working on morphometrical data analysis on some endemic or rare species it's really challenging job with conservation status, that's the reason for taxonomical analysis done in August to October 2017 on Zyxomma petiolatum (Rambur, 1842). The Indian dragonfly genus Zyxomma is a difficult group to identify on field due to their extreme occurrence during mild sunny evening time with higher flier conditions, and shortest information on identification keys, geographical distribution and natural history. Zyxomma petiolatum (Rambur, 1842) is belonging from order: Odonata and suborder: Anisoptera commonly known as brown dusk hawk. Primarily based upon the hypothesis that collected specimen may be dragonfly or damselfly as due to showing both characteristic such as, slender abdomen looks like damselfly and spreading of wings, compound eyes, bulgy thorax shown in case of dragonfly. After carefully observing the specimen it showed 8 abdominal segments which were characteristic features of dragonfly.

Keywords: Taxonomy, Morphometry, Diversity, Daund, Dragonfly.

I. INTRODUCTION

The Odonata is one of the primitive and ancient insect orders with beautiful creature on planet. It is highly diverse and is the second largest aquatic insect order. Dragonflies are predaceous and hemimetabolous, which inhabits all kinds of freshwater habitats either permanent or temporary. According to the Silsby in 2001 stated that Odonata were experiences two totally different life styles. In almost all cases, the egg and larval stages are aquatic where as the adults are terrestrial. Generally play an important role in the ecological way in which they are vanishes mostly the harmful insects of crops, orchards and forests and thus has a regulatory impact on the agro forestry. According to Subramanian, 2009 there were 470 species under 139 genera and 19 families were reported in India, (Fraser, 1933, 1934 & 1936; Chhotani *et al.,* 1983; Lahiri and Mitra, 1993). Recently, Mitra (2002) reported 32species from Nicobar Group of Islands. The diagnosis, distribution and systematic position of each species are presented in this paper. Sivaperuman *et al.,* (2011) reported new record of the odonates where they describe studied specimen with three different species from Andaman and Nicobar Iceland. On the basis of hypotheses on natural history, we carefully examined several specimens of same the species and performed quantitative morphometric analysis. In addition, we used citizen science approach to collect spatial data on distribution.

SCIENTIFIC CLASSIFICATION
Kingdom: Animalia

Phylum: Arthropoda
Class: Insecta.
Order: Odonata
Suborder: Anisoptera
Family: Libellulidae
Genus: Zyxomma
Species: petiolatum
SKIMMERS
Family: Libellulidae

The skimmers also report perches and their relatives were belonging from family: Libellulidae, the largest dragonfly family in the world. It is sometimes considered to contain the Corduliidae (Silsby 2001); there still remains a family of over 1000 species. They were worldwide distributed. Many of the members of this genus are brightly colored or have banded wings. The genus Celithemis contains several brightly marked species in the southern United States. Members of the genus Sympetrum are called darters (or meadow hawks in North America) and are found throughout most of the world, except Australia. Several Southern Hemisphere species in the genera Trithemis and Zenithoptera are especially beautiful.

Other common genera include Tramea and Pantala. The libellulids have stout-bodied larvae with the lower lip or labium developed into a mask over the lower part of the face.

II. MATERIAL AND METHODOLOGY

The materials studied in this paper were collected by various areas from Daund taluka and collected specimens are submitted to Zoological Survey of India, Kolkata and Western Regional Station, Z.S.1., Pune. Presently in the National collections of Zoological Survey of India, Kolkata have some species of the *Zyxomma*, based on the morphology they are identified.

III. RESULT AND DISCUSSION

Taxonomical features

Eyes metallic green, face olivaceous, thorax chocolate brown, legs ferruginous, wings hyaline with brown tips, abdomen red brown with black circle at each segment and swollen at upper half then abruptly thin.

BROWN DUSK HAWK

Figure 1. Zyxomma petiolatum (♂)

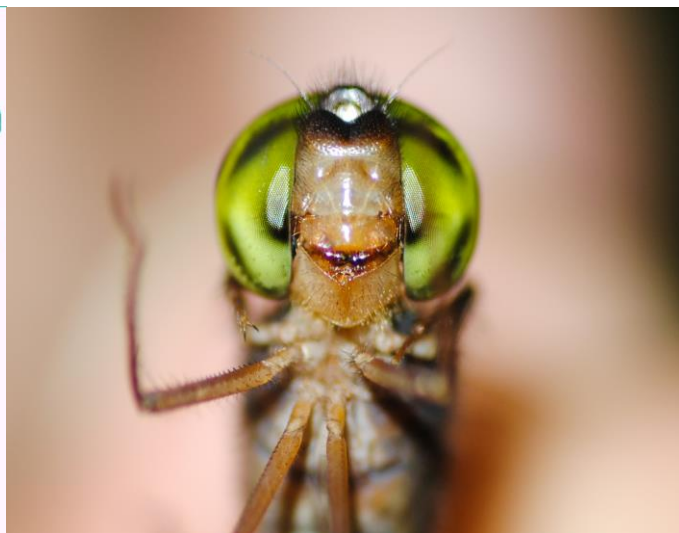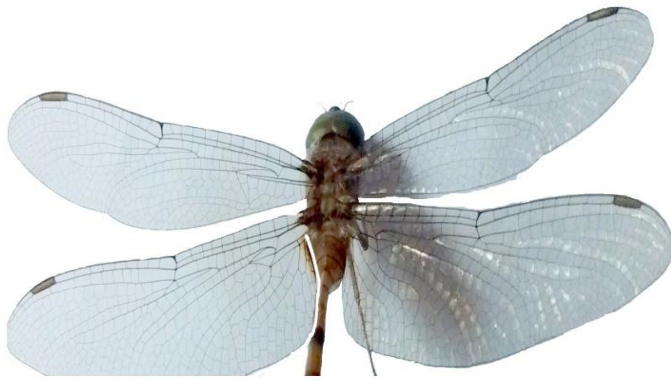Figure 2. Zyxomma petiolatum head (♂)

Figure 3. Zyxomma petiolatum wings (♂)   **Figure 4.** Zyxomma petiolatum lateral (♂)

Table 1. Morphometry of Zyxomma petiolatum (Rambur, 1842)

(♂)/(♀)	Abdomen Length	Hindwing	Wings spot	Eyes	Nodal index			
					Left Fore wing (mm)	Right fore wing (mm)	Left hind wing (mm)	Right hind wing (mm)
Male (♂)	36- 42mm	32-35 mm	Blackish Emerald Green	Emerald Olive green color	13-8	11-8	10-9	9-9
Female (♀)	37- 42mm	32-38 mm	Blackish Dull green.	Olive dull green color	13-9	11-7	9-9	8-9

Taxonomic keys to the species of Zyxomma petiolatum (Rambur, 1842)

1. Male (♂):

Large brown dragonfly with extremely thin abdomen and brown-tipped wings. Head: labium pale yellow; labium pale ochreous ; face and frons pale olivaceous. and usually dark reddish-brown margined with bright golden yellow below in front ; eyes are emerald-green in day time. occiput very small. Prothorax and thorax chocolate-brown, paling at sides, unmarked. Legs: pale reddish-brown or ochreous. The base of wings is variably dark reddish brown. The first antenodal nervure except in the basal space which is hyline. Abdomen: Reddish brown of variable shades, darkening to black at end of segments sides of segments 1 to 3 pale brown with sutures finely outlined in dark brown to black. Anal appendages: Reddish-brown in color. Superiors anal appendages changing from brown to black at apices end.

2. Female (♀):

Head: Labrum- Small black point over labrum. Other part of the head is   palest brown. Prothorax & thorax are Olivaceous brown on dorsum side with bronzed black colour markings. Sexual characters wings usually more broadly dark reddish-brown at apices. Habit/ Habitats: Around shrubby plants, near pools. Distribution: Barkuda Island, Debrigarh, Fraser (1934). Behaviour: Largely crepuscular, but also active during overcast days, in extremely rapid flight low over water bodies, hawking midges.

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Study of Cash Crop Production In Dhule District

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ABSTRACT

The paper deals with the study of two cash crops that is cotton and sugarcane from Dhule district. In the present study data of cash crops production and area under cultivation for the ten years is taken in to consideration .The secondary data for the study is collected from department of agriculture of Dhule district and different websites and libraries.

Keywords : Cotton, Sugarcane, Dhule, District, Cash Crop

I. INTRODUCTION

Agriculture plays an essential role in the economic development of less developed country like India. Besides providing food to nation, agriculture release labour, provides saving , contributes to market of industrial goods and earns foreign exchange (Amarnath Tripathi, A.R. Prasad 2009). Maharashtra is considered as one of the progressive states in the country.The state has achieved the massive development in the field of agriculture particularly after the green revolution (Sharmishtha Balwan ,Abhijit Kurve). Due to unavailability of required water for agricultural activities Indian agriculture faces low productivity problem.(Dr. Sanjeeva Ryudu, T. C., Dr. krishnaiah Y. V. 2011).Cash crops are those crops which are grown for sale in the market either in raw form or in the proceed form. Thus cash crops have special characteristic of earning cash for farmer (D.R.Khullar 2012).

Study Area:-

Dhule district is a district of Maharashtra state in western region of India .Dhule city is the administrative headquarters of the districts. In this district occurs tribal population. It is the part of historical region of Khandesh.It's extends 20 degree 38 minute North latitude to 21 degree 61 minute North latitude and 73 degree 50 minute East longitude to 75 degree 11 minute East longitude . Total area of the district is 8063 sq km (3113 sq mile). The district has four tahsil like as Dhule , Shirpur, shindkheda and sakri.This district bounded by Gujarat state ,Madhyapradesh state ,Nandurbar district ,Jalgaon district and Nasik district .

Objectives of the Study:-

1) To compare the cash crop production during the year 2006-07 to 2015-16 .
2) To study the fluctuation of cash crop production in different years .
3) Analysis of cash crop production in Dhule district.

Methodology:-

The study is entirely based upon secondary data. Data regarding the Dhule district area and production of cash crops is taken from Department of Agriculture. Data includes yearwise production and cultivation of cotton and sugarcane crop, area in hectare .Data and charts were prepared by author on the basis of

information available from secondary source and different websites and libraries. Various simple statistical techniques like as calculate average.

Result and discussion:-

Compared to the cultivation area of sugarcane and cotton it seems that the sugarcane regions fluctuate appears large scales. The cotton cultivation area does not show much fluctuation in ten years. Only a slight downward trend on 2008-09 and 2015-16. The cotton cultivation areas seems to have increase in the remaining years. When compared production, it seems that there is a large fluctuation in the production of both crops.

Dhule district cultivation area and production of cash crops (A-Area in hectare, P-Production in Million Tones)

Table 1

Year	Sugarcane		Cotton	
	A	P	A	P
2006-07	97	6277	835	1240
2007-08	128	8201	1151	2523
2008-09	79	4428	1107	731
2009-10	46	3504	1293	1977
2010-11	53	4426	1331	3359
2011-12	52	3596	1562	2701
2012-13	53	4341	1605	2602
2013-14	65	5369	1732	4327
2014-	70	4384	2099	2582
15				
2015-16	19	1558	1885	686
Avg	66.2	4608.4	1460	2272.8

Studies of the sugarcane cultivation area and production have shown that in 2006-07, the production and cultivation area of sugarcane was highest in the last ten years. The lowest cultivation area and production of sugarcane was in 2015-16. Sugarcane average cultivation area and production of last ten years was 66.2 hectare and 4608.4 million tones respectively.

Considering the cultivation area and production of cotton, there is not much change except for a couple of years. Cotton cultivation areas in 2014-15 were highest in the last ten years and the highest production occur in 2010-11. The lowest cotton planting area was in 2006-07 and the lowest production was on the 2015-16 of the last ten years. The average planting area of last ten years was 1460 hectares and the average production was 2272.8 million tones.
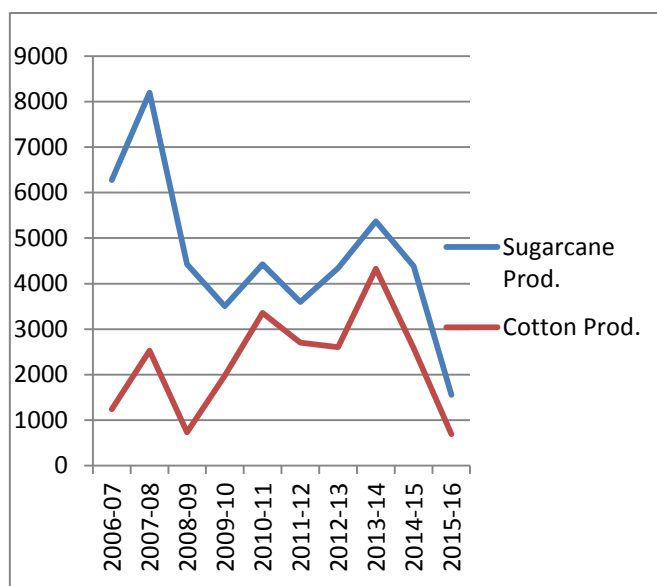
Ghrap 1

II. CONCLUSIONS

From the present study fluctuations are observed in the area under cultivation and production of cash crop sugarcane and cotton crop from the year 2006-07 to 2015-16. But according to time not increase production due to several problems like as low rainfall, irrigation, shortage of good quality seeds, wrong agriculture methods etc. After solving these problems production will be definitely increase.

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Economic Development and Life Style in Pune District, Maharashtra : 1991-2011

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ABSTRACT

This paper is an attempt to study the impact of economic development on the life style of people in Pune district of Maharashtra in recent period. Economic growth or development is an important factor affecting on the life style of people in a specific area in a specific period of time, whether change is positive or negative. Economic activities i.e. Primary, Secondary, Tertiary or Quaternary provide different level of income to people. The life style of people in any area is directly related to the income of people. Pune district is the second highest district in terms of population in the state of Maharashtra. Pune district is one of the most urbanized districts having 61 % of its population in urban areas. But still in the western parts of the district there is existence of tribal people. The district is advanced in terms of industry, commerce, agriculture, transport, etc. This area is become a growth pole and centre of assimilation of migrants from all over the India and within the state. Western parts of the district are mainly hilly and economically backward, where mostly tribal people resides. While eastern part of the district is plateau and main economic activity is agriculture, some of the areas are urban which are industrially and economically developed. If we compare the life style of people in these three distinct localities in Pune district, we can find that life style of these people is different in terms of housing, education, public amenities, health, etc. due to different level of economic development. The economic development which in turn is affected by physiography, climate and industrialization.

Keywords: Economic Development, Urbanization, Industrialization, Human Development Index, Public Amenities.

I. INTRODUCTION

Now-a-days the focus of the study of geography is shifted towards the Man. In earlier period, Geography was supposed to be mainly study of natural environment. It mainly deals with the physical factors, processes and changes on earth surface. 'Geography is concerned to provide accurate, orderly and rational description and interpretation of variable characters on the earth surface.'(Hartshorne,1959). Geographers mainly study the impact of environment on human beings. In early 20th century, thanks to the Vidal-de-la-Blache, Jean Brunhes and others, the man himself becomes the center of geographical study. Human geography mainly deals with the use of natural environment surrounding the man for the betterment of himself.(Fredrick Ratzel,1905) .In other words, it is a study of changing relationship between unresting man & unstable earth.(Miss. Ellen Semple,1912). Geography is the study of areal differentiation in all its aspects. Such regional studies are known as idiographic studies. The work of Vidal de la Blache has served as model in studying regional geography for half a century.

Human geography touches all aspects of human life e.g. agriculture, Settlement, life style, transport, industry, social & cultural aspects, etc. Many branches have evolved for studying these different aspects. But man, his population, its activities,

economic development & life style itself are very important aspects of study. From that point of view, a special branch has evolved in geography i.e. Economic Geography. Development is a dynamic concept. It has different meaning for different people. Some people say it means increase in income, others emphasis on employment, income, quality of life, happiness and so on. Economic Development has been defined as, "a process of growth, expansion or realization of potential, bringing regional resources into full productive use" (Majid Husain). The roots of the concept of human development can be traced to early periods in human history. Aristotle wrote, "wealth is evidently not the good we are seeking, it is merely useful and for the sake of something else." The human development approach in geography became popular by French geographers Febvre and Vidal de la Blache. Subsequently, Hartshorne, Kirk and Tuan along with William Petty, Gregory King emphasis on human development.

The concept of Human Development Index (HDI) is used to measure the development of any region. It was developed & applied for first time in 1990. It is a device to measure a country's or region's achievements in the enhancement of human capabilities. The 1996 Human development Repot (HDR) published by the United Nations Development Programme (UNDP) states:" Human development is end, economic growth a means." The basic objective of development is to enlarge the choices of people primarily by providing them with education, health, nutrition, employment opportunities and social security.

II. METHODS AND MATERIAL

This research paper is based on secondary data. The data is collected from various governmental and non-governmental sources. The authentic data from Pune district censes handbook is used in this study. The present study is mainly based on secondary data collected from various sources. Analytical method is used to assess the trends, reasons and factors of

economic development and their impact on the life style of people in different parts of the Pune district. After collecting the secondary data, it has been tabulated and presented with the help of various statistical techniques. Various maps and diagrams have been prepared to show different types of information. The analysis and interpretation of data has been done from the geographical point of view.

III. RESULTS AND DISCUSSION

A.Study Region

The study region i.e. Pune district is an important district in Maharashtra state. Pune district is advanced in agriculture, industry, urbanization, education, and culture. Pune district is fourth populous district in India with about 94,26,959 population ,according to 2011 censes. Pune district has the sex ratio of 915 females per 1000 males comparing to state 927 per 1000 males. The population density of the district is 603/sq.km. Comparing to 365/sq.km. of the state. Pune district has an area of about 15643 sq.km. which is divided in 15 tehsils and 13 panchayats. The tehsils are Junnar, Ambegaon, Khed, Maval, Mulshi, Velhe, Bhor, Haveli, Purandar, Indapur, Daund, Baramati, Shirur, Puny city and Pimpri-chinchwad city. There are around 1866 villages in Pune district. It is bounded by Thane district to the northwest, Raigad district to the west, Satara district to the south , Solapur district to the southeast and Ahmednagar district to the north and northeast.

Figure 1. Location Of Pune District In Maharashtra

The district has average rainfall of 60-70 cm. During monsoon season June-Oct. Summers are hot and dry during March –May. Temperature ranges from 20˚c to 38˚c , sometimes may reach beyond 40 ˚c. During winter season November to February,temperature hovers around 9˚c to 14˚c, sometimes drops up to 3˚c. Pune district has many rivers originating in western ghat or Sahayadri ranges, like Mula, Mutha, Pavana, Nira, Bheema, Indrayani, etc. make it water rich district. Beside that district has very fertile black cotton soil. This make Pune district most suitable for sugar production.

B. Demography of Pune District

Pune district has the second highest number of population in Maharashtra. Pune district is one of the most urbanized districts with 61 % urban population. Pune district has literacy rate of 86.2% which is higher than state rate of 82.3%. Fursungi village in Haveli tehsil is highest population of 66062 persons while Padalghar village in Mulshi tehsil has only 07 persons. Bawada village in Indapur taluka has area of 8097 hectors while Ahire village in Haveli taluka has only 20.25 hectors area among 1877 villages in district. District has total population of 94,29,408 (2011). As Pune district is advanced in agriculture (sugarcane, fruits, vegetables), industries (automobile, engineering, sugar) and services sector and good health facilities population of Pune district is ever increasing.

Table 1.Population Trends In Pune District: 1901To 2011

Year	Urban		Rural		Total	
	Population	Sex ratio	Population	Sex ratio	Population	Sex ratio
1901	224291	926	871567	994	1095858	979
1911	230384	872	946854	1004	1177238	977
1921	251412	853	853602	990	1105014	957
1931	306465	838	969417	992	1275882	952
1941	400160	841	1072812	992	1472972	948
1951	834723	870	1116253	994	1950976	939
1961	939906	872	1526974	992	2466880	944
1971	1329774	868	1848255	983	3178029	933
1981	1971082	883	2193388	988	4164470	937
1991	2807029	904	2725503	964	5532532	933
2001	4200837	899	3031718	947	7232555	919
2011	5751182	904	3678226	932	9429408	915

Source: Census Handbook

Pune district population has continuously rising from year 1901 to 2011.There are many reasons for that besides fall in mortality rate and increase in standard of life, major reason is migration of population from surrounding district & states to the Pune district. Especially, the population in urban areas i. e. in cities and town has increased tremendously due to urbanization. Overall density of population in Pune district was 354/sq.km. in 1991, increased to 603/sq.km. in 2011. It is 248/sq.km. in rural areas while 7046/sq.km.in 2011. Sex ratio in both urban and rural area has continuously decreasing from 1901 to 2011. Especially in urban areas it dropped to 904/1000 males in 2011 census.

C. Economic Development of Pune District

Pune district is most economically developed district in Maharashtra after Mumbai. All economic activities from primary to quaternary are carried out in district, but these are concentrating in specific regions. Economic development can be seen in agriculture, industry and services. According to Maharashtra

Human Development Report (2012), Per Capita income of Pune district was Rs.34,358 in 2001, which increase to Rs.1,40,570 in 2011. Human Development Index (HDI) was 0.722 in 2001 which raze to 0.814 in 2011. It indicates the economic development of Pune district.

Table 2. Percentage of workers in economic activities in Pune

Sr.No.	Categories	1991		2011	
		Male	Female	Male	Female
1	Cultivators	25.76	44.68	17.82	31.15
2	Agriculture Labours	8.88	26.15	6.98	17.62
3	Livestock, fishing, etc.	1.85	1.01	1.50	0.84
4	Mining & quarrying	0.19	0.11	0.48	0.35
5	Manufacturing & processing In Household Industry	1.46	1.60	2.76	5.44
6	-//- Other than Household Ind.	21.32	4.47	27.10	18.17
7	Construction	6.45	3.14	7.85	6.11
8	Trade & Commerce	12.11	4.35	14.20	5.82
9	Transport & communication	6.54	0.65	9.80	3.96
10	Other Services	15.44	13.84	10.22	9.67

Source: Labour Department

In 1991, the percentage of total workers engaged in agriculture was highest, while workers in industries & services are low. It indicated that comparing to 2011 in 1991 the level of economic development in Pune district was low. Most of the people were engaged in primary activities like agriculture, livestock, fishing etc. Participation of women workers in agriculture was high but in other sectors was very low. It indicated the low rate of education & opportunities for women. So the life style of people especially in rural and tribal areas was not so good. After the introduction of new economic policy in 1991, industrialization in Pune district has boost. Maharashtra government setup MIDC in various locations e.g. Ranjangaon, Jejuri, Talegaon, Baramati, Kurkumbh, Chakan etc. so there is large amount of skilled & unskilled labour migration in Pune district. Around 2000 A.D. Information and Technology (IT) industry was also concentrated around Pune in Hinjewadi, Magarpatta City, Talawade etc. This trend is also manifested in figures of 2011 census. The number of workers in industry, trade and services has increased tremendously. There are 21763 micro units, 5818 small units, 102 medium units and 639 large units working in the district which generates employment for more than 2,20,878 persons (MSME Report 2012). Pune district is developed in agriculture sector too. Due to construction of dams in different part of district like Ujani, Khadakwasla, Veer, Bhatghar, Panshet, etc. various cash crops are now cultivated in the district. Especially, sugar industry has boom with more than 15 in number. Along with it many Agro-based industries are set in Pune district. Horticulture has developed in Junnar, Ambegaon, Khed, Purander tehsils. But western part of the district including Mulshi, Maval, Bhor,Velhe is still not developed comparing to other parts of Pune district. This area has mountain region, high rainfall, sparse population and lack of transport network. This is mainly tribal area where Katkari, Bhil, Mahadev Koli tribes resides. They are engaged in primary activities like food gathering, animal husbandry, fishing wood cutting and primitive agriculture. The level of economic development is very low in this region.

D. Public Amenities in Pune District

Public amenities are the facilities provide by the government to common people. These include education, health, safe drinking water, electricity supply, transport and communication, etc. These facilities need huge investment by government; it depends on economic development of that region, so that government can raise capital for investment in infrastructure. We can see the variation among the distribution of these facilities in Pune district according to different economic level.

I) Education Facilities:

Pune district has 1844 villages. In 1991, 1796 villages have primary education facility that is 97.40% of total rural population. Many villages have school up to secondary level. Pune city and urban areas have 100% education facility. Khed, Shirur, Daund Indapur, Baramati and Maval tehsils have 95% education facility. In 2011, education facility is available to 99.95% villages. But in western part of district, 49 tribal villages have no schools. People in 16 villages have this facility within 5 km. while 32 villages have to travel 5-10 km. for education facility. There are 4641 primary schools, 1727 middle or secondary schools and 80 degree colleges in Pune district (2012).

II) Medical Facilities:

In 1991, only 491 villages out of total 1844 villages had medical facilities, which was 27% of total. Tehsilwise it was lowest with 6.25% in Velhe tehsils which was tribal region, while it was 100% in Pune City. People from 1353 villages had to travel distance of 5 km. for medical facility. The residents of 230 villages had to cover more than 10 km. distance for medical facility. In urban areas there were on an average 3 beds were available per 1000 population. In 2011, medical facilities are now available to 1188 village i.e. 64.15% of total villages and 90.60% of total population in Pune district. But there are regional disparities, only 112 villages can avail this facility within 5 km. Tribal people in western part of district have to travel 5-10 km.

Table 3. Distribution Of Amenities Within District

Sr.No	Name of Tehsil	No.of Villages	Education	Medical Facilities	Drinking Water
1	Junnar	181	181 (100)	134(74.03)	181(100)
2	Ambegaon	143	143(100)	94(65.73)	143(100)
3	Khed	185	185(100)	122(65.95)	185(100)
4	Mawal	181	181(100)	98(54.14)	180(99.45)
5	Velhe	124	123(99.23)	33(26.61)	122(98.3)
6	Shirur	115	115(100)	99(86.09)	115(100)
7	Mulshi	143	143(100)	54(37.76)	143(100)
8	Haveli	118	118(100)	89(75.42)	118(100)
9	Pune	1	1(100)	1(100)	1(100)
10	Daund	102	102(100)	93(91.18)	102(100)
11	Purander	107	107(100)	73(68.22)	107()100
12	Bhor	194	194(100)	76(39.18)	194(100)
13	Baramati	116	116(100)	104(89.66)	116(100)
14	Indapur	142	142(100)	118(83.10)	142(100)

Source: District Collectorate & Census Book
(figures in bracket is % to total of villages in tehsils)

III) Drinking Water Facility:

Availability of safe drinking water for people is essential. Fortunately, from 1991 all villages and towns in the district have availability of safe drinking water. Out of total villages in the district, 1583 villages have open wells, 529 villages have taps as main source of drinking water. River is source of

water for 447 villages while 101 villages have tanks to supply water.

IV) Postal Facilities:

Post office provides effective mode of communication to common people in rural areas. In 1991, post & telegraph facility was available to 560 i.e. 30.37% villages in district. On an average there are 16 post offices for 1 lakh population. In Baramati tehsil 54.46% villages had post offices while in tribal tehsil Velhe had only 10.94% villages had post offices. In 2011, 828 villages in district i.e. 44.71% villages have post offices which covers 76.18% population of Pune district. But there are striking disparities among urban ,rural and tribal areas. Postal facility is available to 100% population in Pune city and urban areas, while 45% rural population has post offices, but in Remote and tribal Velhe tehsil only 15.325 villages have postal facility. There are 793 post offices and 310 telephone centres in Pune district (2011).

V) Electricity Supply:

Electricity is basic need for economic development. Activities like industries, transport, services and even agriculture needs it. The proportion of electrified villages in 1991 in the district was impressive. All 34 towns are 100% electricity while 1811 i.e.98.21% villages were electrified. In 2011, fortunately all villages and rural areas have 100% electricity in Pune district.

VI) Approach Road:

Roads act as a pull factor for development and upliftment of an area. In 1989, length of roads in district was 9440 km. Out of that 23 km. were cement roads, 1909 km. were tar roads while 3094 km. roads were water bound macadam and 4414km. other roads. The national highway no.4 Pune-Bangalore and Pune-Hyderabad passes through district. Out of total 1018 villages were pucca roads. Baramati tehsil had 72.32% villages with pucca road while tribal and remote Velhe tehsil had only 17.19% villages with pucca road. In 2011, 92.39% villages connected with pucca roads which serve about 94.51 % population of district. Agriculturally and industrially developed Baramati tehsil has heist 98.28% road connectivity while Velhe tehsil has only 71.77% villages connected with pucca road.

IV. CONCLUSION

The study is carried out to find the connection between economic development and life style of people i.e. the amenities available to common people in Pune district. The findings are as below;

1) The demography of Pune district has striking variation in the sense of population distribution, rural-urban ratio, literacy and sex ratio in urban, rural and tribal areas.

2) The level of economic development is high in urban areas of the district due to industrialization and availability of transport & trade facilities.

3) The people in urban areas in district like Pune city, Pimpri-chinchwad city and other towns have almost 100% access to all amenities like education, medical, drinking water, electricity and pucca roads.

4) The people in rural areas in Baramati, Shirur, Daund, Purander, Junnar, khed, Indapur tehsils which are agriculturally developed have moderate availability of these amenities.

5) The tribal people in Bhor, Velhe and Mulshi tehsils are still lagging behind in accessing the facilities like education, medical, post offices and pucca roads. The main reason is that these areas are mountain and remote, where there is limitation for development of industries, trade and services.

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Detection of Changes in the Annual Rainfall over theTapi Basin of Central-Western India

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ABSTRACT

It is widely accepted that various elements of climate, especially rainfall, is extremely sensitive to climate change phenomena and it is reflected through the variability in its amount and intensity. In this paper an attempt has been made been made to evaluate the long-term changes/trends in the annual rainfall over the Tapi Basin from Central-Western India by using the test of Mann-Kendall. The rainfall data were procured from India Meteorological Department (IMD) for fifty six raingaugestations located at the tahsilheadquartersin the basinfor the 20th century. It is obvious that the scrutiny of various stations in the basin will underline visible long-term changes/trends in the rainfall at few stations. However, summarizing the rainfall trends obtained at various stations in the basin, it is evident that the low rainfall stations in the basin shows a significant increasing rainfall trend. The stations having rainfall close to the average rainfall of the basin does not present increasing or decreasing rainfall trend. Most importantly, the Tapi Basin, as a whole, does not show any significant rainfall trend over the period of a century. Since, the rainfall over the Tapi Basin not had undergone large variations, which can indicate any specific trend of it, these observations, therefore, provide a weak support to the general view that the rainfall in the basin is progressively decreasing.

Keywords : Tapi Basin, Annual Rainfall, Rainfall Trend

I. INTRODUCTION

A question of prime importance to rainfall studies in India is whether the monsoon rainfall has changed over the last few decades and whether a change is likely to occur in future. Although it is difficult to recognize the likely future trend of rainfall, it is possible to detect the nature of changes that have occurred in the past. Determining the trends or changes in the rainfall are extremely important because studies of hydro-meteorological conditions caused them is useful to detect climatic changes[1]. Even though most of the investigations in the last few decades have revealed secular variations in the Indian monsoon rainfall [2]-[5] However, the studies of

rainfall to determine long-term trends/changes on river basin scale are limited. In the present paper an attempt has been made to analyze the annual rainfall data of the Tapi Basin to study the long-term fluctuations in the rainfall. The data analyzed in this paper consists of long-term annual rainfall series available for 56 representative rain gauge stations in the basin. Almost all the stations have long records of rainfall and hence are suitable for identifying long-term trends that might have resulted from long-term changes in the rainfall. The length of data for most of the stations is about over 100 years which is reasonably suitable for long-range studies of rainfall. The objective of this paper is, therefore, to analyze the
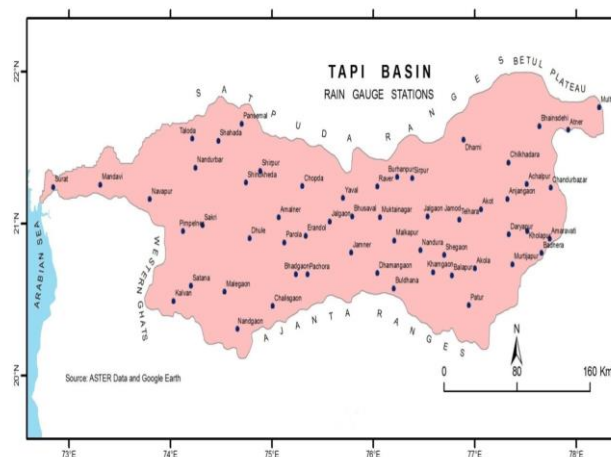
annual rainfall records and to detect changes/trends in the annual rainfall of the basin.

There are many studies carried out to detect the rainfall trend on all India or regional scales. According to the type and record length of the rainfall data used for analysis there are varied results regarding the trend of rainfall in the country.Rupa Kumar et al. [6] found increasing trend of monsoon rainfall over central peninsula and western coastal area and decreasing trend over northeast peninsula and northwest peninsula. Pattanaik[7] concluded that the monsoon rainfall over the central and northwest India during 1941–2002 presents decreasing trend. The annual rainfall for the first six decades of India has been analyzed by Parthasarthy and Dhar[8] and noticed an increasing trend over central India and decreasing trend over eastern India. However, Kumar et al. [9] found that the analysis of fairly long-term rainfall data of India for the period 1871-2005 does not show any visible trend on country level. Although less in number, a few studies examined the rainfall data of some major river basins of India to determine the trend of rainfall on the basin scale. The study of Mirza et al. [10] revealed that the rainfall over Ganges, Brahmaputra and Meghna Basins does not present any significant trend. The trend analysis of rainfall over the Mahanadi Basin carried out by Rao[11] also does not show any clear trend. However, the analysis of rainfall after 1960 over major river basins in the central India by Singh et al.[12] exhibit varying results. They found an increasing trend of rainfall over Indus, Brahmaputra, Ganga, Cauvery and Krishna Basins and decreasing trend over Narmada, Tapi, Sabarmati, Mahi, Godavari and Mahanadi Basins.

II. THE STUDY AREA

The Tapi Basin is an important interstate river basin of central-western India (Fig.1). The Tapi River is one of the major rivers of peninsular India. The river rises in the eastern Satpura Range at an elevation of 730 m ASL near Multai in the Betul District of Madhya Pradesh. The river flows almost east to west and it is the second longest west flowing river of India after Narmada. With a total length of 724 km, the river drains a catchment of 65145 km^2 which is nearly 2% of the total geographical area of India. Flowing through Madhya Pradesh (9804 km^2), Maharashtra (51504 km^2) and Gujarat (3837 km^2), the river discharges into the GulfofKhambhat of the Arabian Sea near Surat city in Gujarat State. The average annual rainfall of the basin is 814 mm received in average 44 rainy days. The basin is located within the zone of severe rainstorms. Thus, the occasional heavy rains results from incursion of cyclonic storms and depressions originating over the Bay of Bengal or adjoining land.

Figure 1. Map of the TapiBasin and location of raingauge stations

III. DATA AND METHODOLOGY

The principal objective of the present study is to analyze the annual rainfall records and to detect changes/trends in the annual rainfall of the Tapi Basin. Therefore, average annual rainfall data were obtained for rain gauge stations located at 56 talukaheadquarters within the basin from India Meteorological Department (IMD), Pune. The data have been obtained for the period of 1901-2004 i.e. mainly for the 20th century. The data analyzed in this paper consists of long-term annual rainfall series

available for 56 rain gauge stations in the basin. Almost all the stations have long records of rainfall of about 100 years and hence are suitable for identifying long-term trends that might have resulted from long-term changes in the rainfall. To evaluate the long-term changes/trends in the annual rainfall records of the Tapi Basin, Mann-Kendall test [13] is used. Mann-Kendall test is a powerful statistical technique for randomness against trend [14].

IV. RESULTS AND DISCUSSION

A. *Detection of changes in the annual rainfall*

Use of Mann-Kendall test in trend analysis of meteorological parameters, particularly of rainfall has been reported by several workers. Srivastava et al. [15] utilized Mann-Kendall test for finding the trend in rainfall over India for the period 1901-1992. Krishnakumar et al. [16] determined the long-term changes in seasonal and annual rainfall over Kerala with the help of Mann-Kendall trend test. This non-parametric method has also been used by several workers to quantify the direction and magnitude of trends in the streamflow and rainfall records [17]-[24]. Suresh et al. [25] also applied this test for identification of the nature of changes in the rainfall of the small regions or stations.

The Mann-Kendall's Tau (τ) has been obtained by following equation;

$$\tau = \frac{Actual\ total\ of\ scores}{Maximum\ possible\ total} \ldots Eq.\ 1$$

The actual total of scores (ATS) is achieved by procedure outlined in Table 1, which is just a demonstration. The scores obtained by this procedure are not used in final results of the study. The annual rainfall data of the Tapi Basin for twelve years have been selected as a representative for calculation of actual total of scores, maximum possible total, and for calculation of τ.

The annual rainfall (AR) of the first year i.e. 1901 is compared with the AR of the subsequent years i.e. 1902, 1903, 1904 and so on. If the values (AR) of the subsequent years (1902, 1903, 1904 and so on) are greater than the value (AR) of the first year (1901) then the scores are +1 and if the values subsequent are smaller than the value of the first year then the scores are −1. For instance, AR of the year 1902 (677 mm) is smaller than AR of the year 1901 (721 mm), therefore, the score is -1; and AR of the year 1903 (828 mm) is greater than AR of the year 1901 (721 mm), thus, the score is +1. In this manner, the AR value of the first year is compared with the AR values of all the years and the scores are obtained. Then sum of scores is calculated, which in this illustration is -3. For the next comparison, the first year's (1901) AR value is dropped and the second year's (1902) value is compared with the rainfall values of the subsequent years (i.e. 1903, 1904, 1905 and so on) and the sum(s) scores are calculated for it. The actual total of scores (ATS) is the total of all sum(s), which in this illustration is -2.

Table 1. Scores for calculating Mann-Kendall's τ for the Tapi Basin (An illustration)

YEAR	AR											
1901	721											
1902	677	-1										
1903	828	1	1									
1904	600	-1	-1	-1								
1905	622	-1	-1	-1	1							
1906	863	1	1	1	1	1						
1907	620	-1	-1	-1	1	-1	-1					

Year	Value												
1908	710	-1	1	-1	1	1	-1	1					
1909	757	1	1	-1	1	1	-1	1	1				
1910	911	1	1	1	1	1	1	1	1	1			
1911	549	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1		
1912	625	-1	-1	-1	1	1	-1	1	-1	-1	-1	1	ATS
	Sum	-3	0	-5	6	3	-4	3	0	-1	-2	1	-2

The maximum possible total has been obtained with following equation;

Maximum possible total $= N(N-1)/2$ ... Eq. 2

Where; N = Number of observations (for this illustration, N=12).

Therefore, the Mann-Kendall's τ is obtained by putting values in Eq. 1;

$$\tau = \frac{-2}{66} = -0.030 \qquad \ldots \text{Eq. 3}$$

The positive (negative) sign of τ indicates increasing (decreasing) trend. Therefore, the negative value of τ for the given illustration suggests that the rainfall trend for the given period is decreasing. It to be noted that the scores obtained for this example are not used in the study.

B. *Testing the significance of Tau (τ)*

For the large N, the method outlined for testing the significance of τ becomes extremely cumbersome. However, Kendall [26] has shown that when N is larger than 8, the theoretical distribution of all possible values of τ approaches the normal distribution. The τ may be transformed into a normal standard deviate as follows;

$$z = \frac{\tau}{\sqrt{2(2N+5)/9N(N-1)}} \qquad \ldots \text{Eq. 4}$$

Substituting the calculated value of τ, the value of the z can be obtained. For large number of observations ($N > 30$), z value has to be greater than 2.32 at 0.01 level and 1.64 at 0.05 level for the sample to be statistically significant.

It is obvious that different stations in the basin cannot present similar trend of rainfall. Therefore, to check the rainfall trend at a station, three representative stations viz. Chikhaldara, SakriandJamner are selected. The basis to choose these specific stations is that these stations represent different zones of the basin as well as they are having varying annual rainfall amounts. Chikhaldara, situated in the eastern hilly areas, is the highest rainfall receiving station in the basin. Sakri, located to the west, is the lowest rainfall receiving station whereas, Jamner, having almost similar rainfall to that of the average rainfall of the basin, represents to the central area of the basin. This exercise predominantly intended to observe the change/trend over the basin scale, therefore, Mann-Kendall's τ and z scores are obtained for the whole basin besides the above-mentioned three representative stations and the results are given in Table 2.

The application of this non-parametric test to the annual rainfall data of the basin designate varied results for different stations. Significant decreasing trend at 0.05 level is observed for Chikhaldara, a highest rainfall receiving station in the basin. Conversely, Sakri, the lowest rainfall receiving station in the basin present significant increasing trend at 0.05 level(Table 2). The test, however, does not exhibit any clear trend in the annual rainfall for Jamner, a rainfall station with average annual rainfall close to the basin's average rainfall, located in the central part of the basin (Table 2). It is very important to note here that the Tapi Basin does not show any significant trend of the rainfall (Table 2). Therefore, it can be stated that the annual rainfall is decreasing with respect to time at high rainfall

stations in the basin. Conversely, low rainfall stations in the basin present an increasing trend of rainfall. However, the stations having similar rainfall amount with the basin does not indicate any considerable trend.

The Tapi Basin is an extensive in area, therefore, before generalizing the results of rainfall trend given in Table 2, and to validate these results, trend analysis has been further carried out for three more stations with same procedure. These stations are Multai (high rainfall station), Jalgaon (a station with annual rainfall close to the average annual rainfall of the basin) and Satana (low rainfall station) representing to east, central and west zones of the basin respectively. It is found that no specific rainfall trend has been noticed at Multai. Similarly, Jalgaon also does not designate any specific rainfall trend. However, Satana, a low rainfall station indicate a statistically significant increasing rainfall trend at 0.01 level (z score = 3.80).

Table 2. Nature of changes/trends in annual rainfall records based on Mann-Kendall test

Station	Period	N	Tau (τ)	$z$score	Trend/change
Chikhaldara	1901 – 2004	104	-0.118	-1.78	Decreasing* trend
Sakri	1901 – 2004	104	0.110	1.66	Increasing* trend
Jamner	1901 – 2004	104	0.022	0.33	No specific trend
Tapi Basin	1901 – 2004	104	0.270	0.27	**No specific trend**

See Figure 1 for location of stations; N= number of observations; * = statistically significant at 0.05 level

V. CONCLUSIONS

Summarizig the rainfall trends obtained at above-mentioned stations in the basin, it is evident that the low rainfall receiving stations in the basin shows a significant increasing rainfall trend. The stations having rainfall close to the average rainfall of the basin does not present increasing or decreasing rainfall trend. Most importantly, the Tapi Basin, as a whole, does not show any significant rainfall trend over the period of a century. It is obvious that the scrutiny of various stations in the basin will underline visible long-term changes/trends in the rainfall at few stations.However, certainly the rainfall over the Tapi Basin not had undergone large variations, which can indicate any specific trend of it. These observations, therefore, provide a weak support to the general view that the rainfall in the basin is progressively decreasing.

Most of the investigations for larger areas (all-India scale) during last few decades have given similar results. These studies clearly specified that the monsoon rainfall, particularly on all-India scale, is trendless and is predominantly random in nature over a long period of time.Mooley and Parthasarathy [2], Gregory[27]observed that there is no significant linear trend in the monsoon rainfall of any of the ten macro-regions that he recognized. However, though not at particular locations, the presence of some pockets of significant long-term rainfall changes are also reported by some workers [6], [28], [29].

VI. ACKNOWLEDGEMENTS

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Flood Frequency Analysis of the Par River : Western India

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ABSTRACT

An evaluation of the effectiveness of flows depends on the magnitude and frequency of the events than mean discharges. Magnitude-frequency analysis is one method that identifies the hydrological and geomorphological importance of these events quantitatively, particularly the frequency of flood events of various magnitudes. Therefore, an attempt has been made to understand the magnitude and frequency characteristics of floods on the Par River on the basis of available annual peak discharges and field data. The Annual Maximum Series (AMS) data were available for the Nanivahial site on the Par River for 49 years. To estimate discharges of a given return period, frequency distribution is compiled from a data series of extreme events. By using Gumbel Extreme Value type I (GEVI) probability distribution, peak flows have been estimated for different return periods. The distribution has also been employed to estimate the recurrence interval of mean annual peak discharge, large flood and actually observed maximum annual peak discharge. The magnitude-frequency analysis based on GEVI distribution reveals that the mean annual peak flood has a recurrence interval of 2.33 years, large flood has 6.93 years and maximum peak discharge has 185 years. Two general conclusions emerge from the analyses. First, the river displays extraordinary hydrologic characteristics of a flood-dominated river. Second, large floods are relatively frequent. This fact suggests that large-magnitude events have an important role to play in the bedrock channel morphology of the Par River.

Keywords: Annual Maximum Series, return period, Gumbel Extreme Value type I, recurrence interval.

I. INTRODUCTION

According to Leopoldet al.[1] and Schumm[2] the channel form and the processes of erosion and transportation in a river are closely associated with the river regimes specifically to the flows which they transmit. The regional hydro-climatic regime conditions strongly control the river regime [3]. Numerous case studies in the last six decades have showed that the geomorphic effects of a discharge of a given magnitude and frequency differ from one regime to another [4] For instance, Wolman and Miller [5] revealed that the frequently occurring low and moderate flows largely determine the transfer of sediments and the channel size under humid

temperate regime. On the contrary, infrequent large magnitude floods maintain and control the channel size of rivers in arid tropical regime [6]. In semi-arid tropics the channel morphologic properties are not directed by a particular discharge but by a series of discharges taking place at different intervals [7]. Similar conclusion has been proposed by Gupta [8]. He suggested that in seasonal tropics the rivers are not only controlled by the seasonality of discharge but also high-magnitude floods. Hire [4] opines for the Tapi River that the low- or moderate-magnitude flows transport most of the fine-grained sediment (clay, silt and sand) and modify the channel bedforms to some extent. However, the channel size and shape is maintained by large-magnitude floods that occur at

long intervals. Considerable attention has been given to morphology of bedrock channels and dynamics and to fluvial erosional processes in recent years [9]. These studies, therefore, point out that a systematic understanding of the main features of the fluvial and flood regime of a river is essential for the estimation of the pattern of geomorphic work. In the present study, hence, an attempt has been made to inspect the magnitude frequency analysis of the AMS data.

II. GEOMORPHIC, GEOLOGIC AND CLIMATIC SETTINGS OFTHE PAR RIVER

The Par River from western India has been selected for study of flood frequency analysis (Fig. 1). It has its source near Harantekadi at an elevation of 982 m ASL. Physiographically, upper Par River and its tributaries flow on the Jawhar Plateau whereas at lower reaches river flows on the Kokan Plains. The Par Basin is bordered by, roughly east-west trending, Surgana and Peth Ranges to north and south respectively and by Western Ghats to the East. The altitude of Surgana and Peth Hills ranges from 450 to 750 m ASL. The Western Ghats (>900 m ASL) is higher in altitude than Surgana and Peth ranges. The basin relief, i.e. Kem Hill (1177 m), is located as offshoot of Western Ghats.

The river flows to the west through Maharashtra (46.45% area) and Gujarat (53.55% area) States and drains into the Arabian Sea near Umarsadi in the Gujarat State. The length of the river is 142 km. The Nar River, with the length of 87 km, is the major tributary of the Par River and joins from the north. Other major tributaries of the Par River are the Manmora, the Keng, the Vajri, and the Bhimtas. The Par Basin extends over an area of 1664 km². The entire basin is underlain by horizontally bedded Cretaceous-Eocene Deccan Trap basalts. The river has single, sinuous, and well-defined channel, incised into bedrock. The channel floor is either of bedrock or covered by pebbly/cobbly material or boulders. The Par River and its tributaries are south-west summer monsoon fed (June to September). The average

annual rainfall of the basin is 2076 mm and 93% of the annual rainfall occurs during south-west monsoon season. The basin occasionally receives heavy rains due to cyclonic storms and depressions originating over the Bay of Bengal or adjoining land and the Arabian Sea.
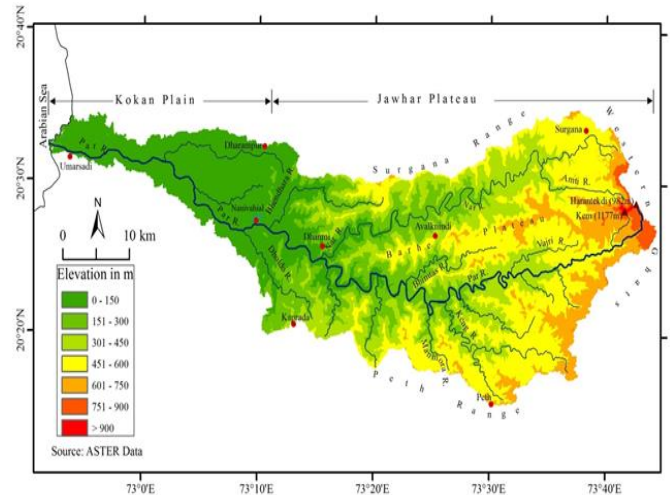

Figure 1. Geomorphic setting of the Par River

III. METHODOLOGY

The Par River, similar to other monsoonal rivers, also subjected to high-magnitude floods at regular intervals. Thus, it is of paramount significant to know the hydrologic characteristics of floods in terms of magnitude, frequency and distribution. Therefore, flood frequency analysis has been carried out for the Par River. FFA necessitates a good quality, long and continuous records. Typically the AMS data have been more frequently used for the analysis. In case of the study area, the AMS data of flood stage and magnitude are available for Nanivahial site (Fig. 1) on the Par River for the last 49 years (since 1961). This data have been used for magnitude-frequency analysis. In order to estimate discharges of a given return period, a frequency distribution is compiled from a data series of extreme events. By using Gumbel extreme value type I (GEVI) probability distribution, peak flows have been estimated for different return periods such as 2, 5, 10, 25, 50, and 100 years. The distribution has also been employed to estimate the recurrence interval of mean annual peak

discharge (Qm), large flood (Qlf) and actually observed maximum annual peak discharge (Qmax). A visual inspection of the fit of the frequency distribution is possibly the best way in determining how fine an individual distribution fits the AMS dataset or which distribution fits "best" [10]. Therefore, flood frequency of the Nanivahial site is represented graphically (Fig. 2) which fairly represents the Par Basin.

A. Gumbel Extreme Value Type I (GEVI) Distribution

Assuming the GEVI distribution for the AMS data of the selected site, an estimate of flows for a desired recurrence interval were obtained by using the following equation [11].

$$Q_T = Qm + [K(T) * \sigma Q] \quad \dots\text{Eq. 1}$$

where, Q_T = discharge of required return period, Qm = mean annual peak discharge, σQ = standard deviation of AMS, and K(T) = frequency factor and is the function of the return period T. K(T) values were obtained from tables provided in the standards books on Applied Hydrology.

The recurrence intervals (T) of given discharges (X), such as mean annual peak discharge (Qm), large flood (Qlf) and peak on record (Qmax), have been estimated by applying the following equation [11].
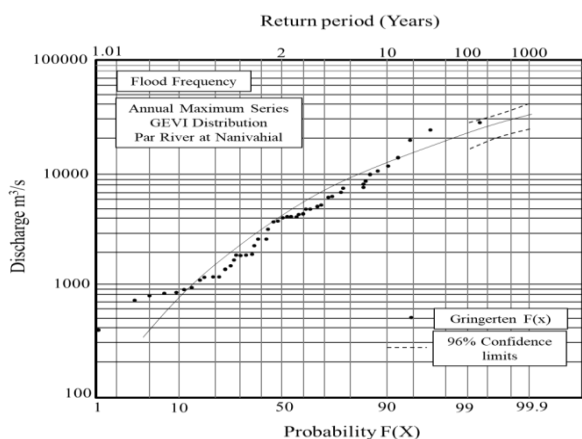
Figure 2. Annual Maximum Series, GEVI distribution, Nanivahial, Par River

$$\frac{1}{T} = 1 - F(X) = 1 - \exp[-e^{-b(X-a)}] \quad \dots\text{Eq. 2}$$

where, T = recurrence interval for a given discharge, F(X) = probability of an annual maximum Q $\leq$ X, and a and b are two parameters related to the moments of population of Q values. The parameters a and b were determined by the following equations.

$$a = Qm - \frac{\gamma}{b} \qquad (\gamma = 0.5772) \quad \dots\text{Eq. 3}$$

$$b = \frac{\pi}{\sigma Q \sqrt{6}} \qquad \dots\text{Eq. 4}$$

where, Qm = mean annual peak discharge, and σQ = standard deviation of annual peak discharge. The return periods of required discharges have been calculated by applying Equation 3.

In the GEVI analysis, the observed annual peak discharges have been plotted against the return period or F(X) values (plotting positions) on the Gumbel graph paper, designed for GEVI probability distribution. Several formulae have been used to calculate plotting positions, however, of the several formulae in use, the best is due to Gringorten since the outliers fall into line better than other plotting positions [11]. The F(X) values have been calculated as follows;

$$P(X) = 1 - F(X) = \frac{r - 0.44}{N + 0.12} \qquad \dots\text{Eq. 5}$$

where, r = flood magnitude rank and N = the number of years of records.

A line can be drawn by eye to fit the scatter, especially using the Gringorten plotting positions. However, it is sensible to draw the line mathematically. Additionally, since most of the AMS data are available for short period of time, it is essential to construct confidence limits about the fitted line relationship between the AMS and the linearized probability variable [11]. Shaw[11] has given procedure to fit the line mathematically and to

$$P(X) = 1 - F(X) = \frac{r - 0.44}{N + 0.12} \qquad \text{....Eq. 5}$$

construct the confidence limits. The same procedure has been followed in this study.

IV. RESULTS AND CONCLUSIONS

By using GEVI probability distributions, peak flows have been estimated for different return periods such as 2, 5, 10, 25, 50, and 100 years. The estimated discharges are given in Table 1.

Table 1. Estimated discharges in m³/s for different return periods for Nanivahialsite on the Par River (Based on GEVI distribution)

Record length	Return period (years)					
	2	5	10	25	50	100
49	4200	8767	11777	15618	18576	21327

See Figure 1 for location of site

The distribution has also been employed to estimate the recurrence interval of mean annual peak discharge (Q_m), large flood (Q_{lf}) and actually observed maximum annual peak discharge (Q_{max}) (Table 2).

Table 2. Return period of Qm, Qlf and Qmax for Nanivahialsite on the Par River (Based on GEVI)

Record length	Q m³/s	Return period (yr)
49	Qm = 5030	2.33
	Qlf = 10220	6.93
	Qmax = 23820	185.47

Qm = mean annual peak discharge; Qlf = large flood; Qmax = maximum annual peak discharge; GEVI = Gumbel Extreme Value Type I; See Fig. 1 for location of site

In the GEVI analysis, the observed annual peak discharges have been plotted against the return period or F(X) values (plotting positions) on the Gumbel graph paper, designed for GEVI probability distribution. The plotted graph is shown in Fig. 2 which show that, the fitted lines are fairly close to the most of the data points and, therefore, can be reliably and conveniently used to read the recurrence intervals for a given magnitude and vice versa. Interestingly, in plot of GEVI distribution, the actually observed peak on record (Qmax) falls well close to the fitted lines. This means the return period of Qmax of Nanivahial station predicted by GEVI distribution are likely to be quite reliable.

Two general conclusions emerge from the analyses. First, the river displays extraordinary hydrologic characteristics of a flood-dominated river. Second, large floods are relatively frequent. This fact suggests that large-magnitude events have an important role to play in the bedrock channel morphology of the Par River.

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Fluvial Soil Textural Characteristics in upper Ghod Basin using GIS and GPS Techniques

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ABSTRACT

The present research paper deals a fluvial soil textural characteristics in upper watershed. GIS and GPS are essential tools for understanding the soil texture. With the help of GPS soil sample location are demarked and various maps are prepared using in GIS. The analysis of soil samples revealed that the proportion of clay (< 0.002 mm diameter) in the sample is varying between 0.25 to 49 percent, of silt and very fine sand (below 0.1 mm diameter) from 5 percent to 65 percent, of sand (0.1 to 0.2 mm diameter) from 5.54 percent and 59 percent and coarse material (above 0.2 mm diameter) from 3 percent to 75percent. On the basis of textural characteristics soil, we find out six textural class of soil. The soil texture control permeability, fertility, erodibility of soil.

Keywords : Soil texture, GIS, GPS

I.   INTRODUCTION

Soil is the mixture of minerals, organic matter, gases, liquids, and the myriad of organisms that together support plant life. The addition of organic matter, water, gases and time causes the soil of a certain texture to develop into a larger soil structure called an aggregate. At that point a soil can be said to be developed, and can be described further in terms of color, porosity, consistency, reaction etc. At that point a soil can be said to be developed, and can be described further in terms of color, porosity, consistency, reaction etc.

STUDY AREA

The study area located Ambegoan, Junner, Shirur tehsil in Pune district, Maharashtra. Ghod River is a tributary of Bhima River. The Ghod River originates on Auhpe Village, the eastern slope of the western Ghat at 1029 meters (3580ft) an above sea level. before the confluence of Bhima. The geographical location of the Study area can be expressed from 18^0 46' 36'' to 19^0 15' 08''N latitude and 73^0 31'58'' to 74^0 18' 52''logitude. The study area selects to study origin of river to confluence with Kukadi River. The length of the Ghod River is 126.0 km between Origen to Ghod and Kukadi river confluence.

Location Map of Ghod River Basin
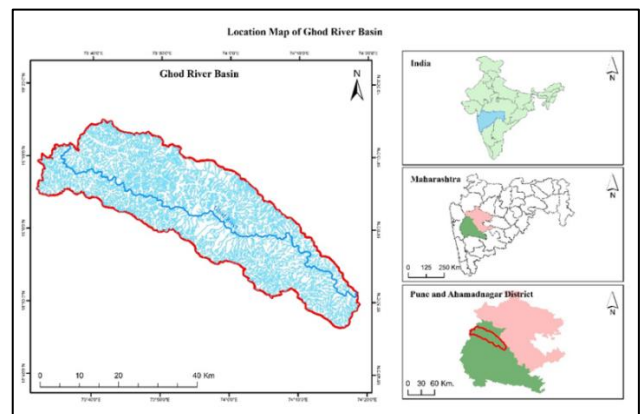
OBJECTIVE

- To Assess Textural characteristics of soil

Identify distribution soil texture in study area

METHODOLOGY

Soil samples are collected through field survey. Random sample method are used for sampling of soil. Soil samples were analyzed in the laboratory to find out texture, structure, permeability and organic matter content. Textural analysis was done using 64 International Pipette Method and the proportion of coarse material, sand, fine sand, silt, and clay was measured. Results were verified with the reports obtained from Government Soil Testing Laboratory, Government of Maharashtra.

II. RESULTS AND DISCUSSION

Soil samples were collected from Sixty Six sites. The care was taken so that they are widely distributed in the study area covering most of the geomorphic units from ridge to valley and from source to mouth. Soil samples were collected from top 5 cm (2 inches) layer of soil.
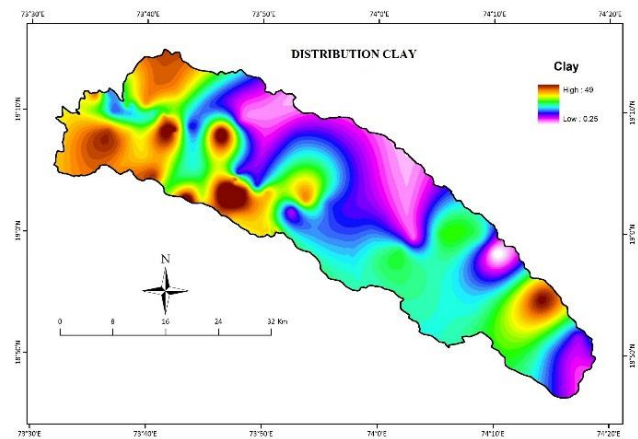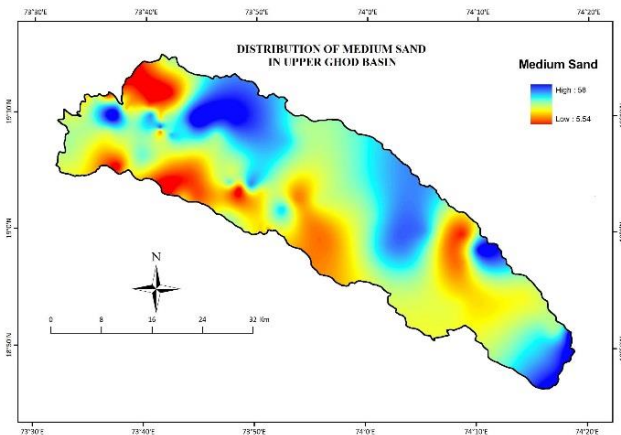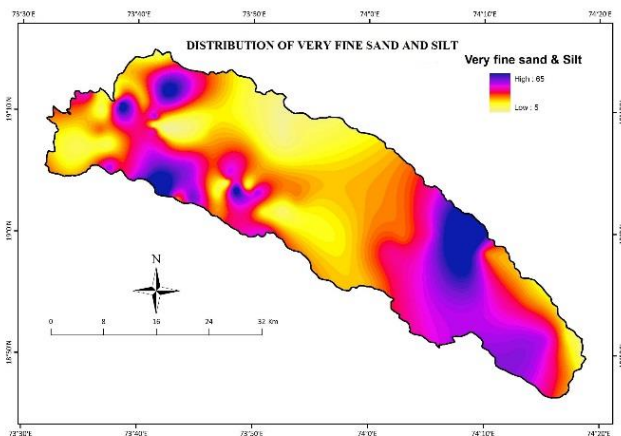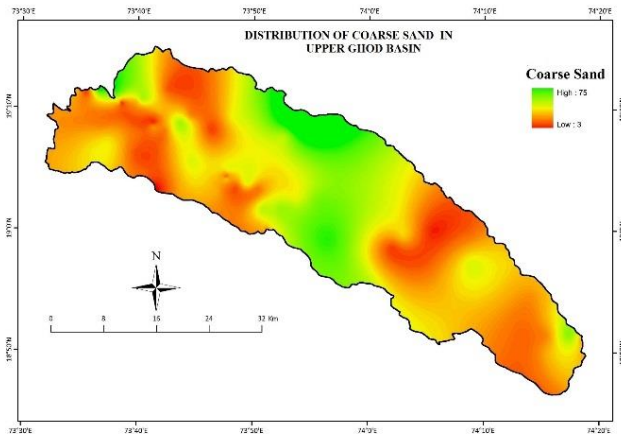
Soil profiles in Ghod basin were studied and soil samples were analyzed at 66 locations widely distributed through geomorphic units within the study area. The basin exhibits six types of textural classes of soils viz., clay, clay loam, loam, sandy clay loam, sandy loam and silt clay loam.

Table 1. Soil Texture Parameter

Soil Series	Textural Class	Coarse Sand %	Sand %	Silt & V. Fine sand	Clay %
1	Sandy Silt Clay	25.33	26.05	28.77	19.69
2	Silt Clay Loam	17.94	17.39	29.08	35.43
3	Sandy Loam	38.1	24.39	12.42	25.02
4	Sandy Clay	36.81	31.61	11.44	17.87
5	Silt Clay Loam	13.45	23.98	32.34	30.1
6	Sandy Clay	32.63	42.64	10.36	11.87
7	Clay	19.86	19.14	11.29	48.72
8	Silt	10.23	8.06	44.94	36.68
9	Sandy Loam	29.51	27.49	29.56	11.33
10	Sandy Clay	23.6	35.98	6.67	30.21
11	Sandy Silt Clay	32.56	16.75	31.53	19
12	Clay	12.74	31.05	20.83	35.28
13	Clay	7.74	32.97	15.26	42.92
14	Sandy Clay	17.04	56.84	9.43	13.82
15	Sandy clay	17.5	35.43	5.12	40.06
16	Sandy Clay	41.17	39.5	5.07	11.9
17	Sandy Silt clay	21.62	29.3	24.65	24.32
18	Sandy Clay	15.19	40.24	7.5	34.64
19	Silt Clay Loam	16.31	22.84	33.52	31.6
20	Sandy Silt Clay	17.65	42.68	21.62	18.84
21	Sandy Clay	39.46	13.09	16.51	30.19
22	Clay	22.56	22.23	25.83	31.05
23	Sandy clay Loam	5.77	46.92	9.05	36.01
24	Silt Clay Loam	16.81	16	27.55	39.53
25	Clay	11.65	30.71	22.86	35.04
26	Clay	20.85	21.73	25.77	28.12
27	Silt Clay Loam	7.89	29.3	28.72	33.46
28	Sandy Clay	59.41	11.38	13.65	15.48
29	Silt Clay Loam	51.9	22.51	13.7	22.06
30	Silt Clay Loam	24.78	20.07	31.92	23.16
31	Sandy Clay	17.04	57.89	9.43	13.82
32	Silt	5.91	24.96	51.4	16.3
33	Clay	15.86	31.16	22.15	27.84
34	Sandy Clay	28.59	24.08	8.82	35.36
35	Clay	16.26	28.5	19.62	35.47
36	Clay	9.99	34.15	26.24	29.44
37	Silt Clay Loam	26.64	9.35	31.83	32.07
38	Clay	22.38	20.55	23.85	32.8
39	Silt Clay Loam	11.15	15.31	37.14	36.29
40	Clay	3.14	5.54	64.97	23.34
41	Clay	17.67	20.41	19.03	42.65
42	Clay	15.28	30.79	22.38	29.42
43	Silt Clay Loam	22.04	12.86	36.07	26.69
44	Sandy clay	42.03	38.35	7.55	8.28
45	Clay	30.67	16.32	17.07	32.22
46	Sandy Silt Clay	50.86	13.11	21.82	14
47	Sandy clay	45.77	25.13	14.5	14.46
48	Sandy clay Loam	39.33	39.83	5.12	11.93
49	Sandy Clay	28.35	50.8	13.59	6.9
50	Clay	10.68	10.84	45.21	30.99
51	Clay	21.44	26.64	13.33	35.17
52	Sandy Clay	74.98	32.37	7.17	9.17
53	Sandy Silt Clay	5.42	42.44	27.84	21.3
54	Sandy clay Loam	25.25	46.21	20.28	5.06
55	Sandy Clay	36.02	33.41	18.09	9.4
56	Sandy Clay	55.12	15.05	12.98	14.46
57	Sandy Clay	37.86	25.85	16.6	18.5

58	Sandy Silt Clay	7.96	42.98	24.92	20.26
59	Sandy silt	32.46	17.8	41.75	9.16
60	Sandy Silt Clay	24.2	24.42	32.3	17.67
61	Sandy clay Loam	23.7	54.42	7.17	11.96
62	Sandy clay	44.55	32.36	11.76	8.66
63	Clay	17.12	24.41	17.58	38.73
64	Sandy Silt Clay	24.94	52.36	20.86	0.25
65	Silt	12.53	11.43	56.98	18.63
66	Sandy Silt Clay	17.53	35.51	29.65	15.45

DISTRIBUTION OF COARSE SAND IN UPPER GHOD BASIN

DISTRIBUTION OF VERY FINE SAND AND SILT

DISTRIBUTION OF MEDIUM SAND IN UPPER GHOD BASIN

DISTRIBUTION CLAY

The basin exhibits six types of textural classes of soils viz., clay, clay loam, loam, sandy clay loam, sandy loam and silt clay loam. The analysis of soil samples revealed that the proportion of clay (< 0.002 mm diameter) in the sample is varying between 0.25 to 49 percent, of silt and very fine sand (below 0.1 mm diameter) from 5 percent to 65 percent, of sand (0.1 to 0.2 mm diameter) from 5.54 percent and 59 percent and coarse material (above 0.2 mm diameter) from 3 percent to 75percent. The soil texture is determined by the relative proportions of sand, silt, and clay in the soil. The addition of organic matter, water, gases and time causes the soil of a certain texture to develop into a larger soil structure called an aggregate.

III. CONCLUSION

Water may infiltrate into very coarse-textured soils or well-aggregated soils so readily that none is lost in runoff even under the heaviest downpour. In contrast the surface layer of a bare clay soil may soak up the first moisture that falls; then it may swell and become a dense waterproof layer that sheds the remainder of the water. The soil texture is determined by the relative proportions of sand, silt, and clay in the soil. The above maps shows distribution of Coarse sand, medium sand, silt and clay in Ghod watershed. The highest 71 percent coarse sand lowest 3 percent coarse sand found in study area.

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Management of Collar Rot of Groundnut (Arachis hypogaea L.) by biocontrol agents

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ABSTRACT

Groundnut is an economic important edible oilseed crop. Groundnut suffers seed, soil and foliar diseases. Among the groundnut diseases, collar rot is one of the economic important disease. Collar rot damaged regularly due to its seeds and soil borne nature. Collar rot of groundnut is caused by Aspergillus niger Van Tieghem. It is a soil-borne pathogen, usually the occurrence of collar rot disease during the early stages of crop growth and results in high seedling mortality. Collar rot disease of groundnut is one of the most serious, destructive diseases in and yield losses range from 13 to 52% and can be as high as 93.6% in some areas. Seven isolates of Trichoderma spp. belonging to viz., viren, viride, harzianum species groups were screened for their modes of biocontrol ability against Aspergillus niger van Teighem the causal agents of collar rot of groundnut. It was observed that T. viride 60 inhibited maximum (86.2%) growth of test fungus, followed by T. harzianum 2A (80.4%). However, filed evaluation of these isolates should be under taken to evaluate their efficiency against the soil borne pathogen of groundnut.

Keywords : Aspergillus Niger, Trichoderma Spp., Groundnut, Collar Rot

I. INTRODUCTION

Out of nine oilseed crops grown in India, groundnut accounts for 35% of the total area cropped under oilseed and 40% of the total oilseed production. Though, India is the largest producer of groundnut, its average productivity levels are very low as compared to USA and China. In India, the major groundnut growing states are Gujarat, Andhra Pradesh, Rajasthan, Tamil Nadu and Punjab [1]. A major limiting factor in profitable cultivation of this crop is the attack of several diseases mainly caused by fungi, which takes heavy loss of the crop at all the stages of growth right from sowing to harvest and storage. An important factor contributing to low yield are diseases [2]. Grover [3] listed more than 55 pathogens in groundnut crop. Only a few, such as early leaf spot (Phaeoisariopsis arichidicola) late leaf spot (Phaeoisariopsis personata), rust (Puccinia arichidis), collar rot (Aspergillus niger van Tieghem), stem rot (Sclerotium rolfsii Sacc.), root rot (Macrophomina phaseolina), and aflaroot (Aspergillus flavus) are economically important in India. Nematode diseases like root knot, and viral diseases like peanut bud and stem necrosis, groundnut mottle and clump [4] are major diseases that limit groundnut production and productivity. In addition, the pre- and post-harvest aflatoxin contamination in the kernels and meal also reduces groundnut quality as well as export value.

Economic importance and occurrence of disease

Collar rot disease in groundnut prominently is distributed in countries with tropical and sub-tropical climates where high temperature prevails during the rainy season. Collar rot disease of groundnut (Arachis hypogaea L.) caused by Aspergillus niger van Tiegham

is an important disease in several temperate countries [5]. However, in India, at Nagpur first reported the Aspergillus blight of groundnut caused by A. niger and worked out the morphology of the pathogen [6]. The primary source of the inoculum of collar rot pathogen has been shown to be mycelium and spores carried on the seeds and organic debris in the soil [7].

Figure 1. Groundnut plants showing typical collar rot symptom

Collar rot causes heavy losses in pod and fodder yield of groundnut. Most of the varieties of groundnut are susceptible to this disease. Many seed dressing fungicides are reported to be effective against collar rot of groundnut [8]. But limited work has been done on successful exploitation of bio-control agents, for the management of collar rot disease through induced resistance. The above method is very needed to keep the disease below the economic threshold level without damaging the agro-ecosystem in soil [9]. Trichoderma have been used as biological control agents against soil-borne plant pathological fungi [10]. The main objective of the present study was to find an, in vitro Trichoderma strain that will act as the best bio-control agent for effectively inhibiting the growth of A. niger (as all Trichoderma strains do not work equally against a specific disease).

II. METHODS AND MATERIAL

Isolation and maintenance of microbes

Groundnut seedlings which showed typical symptoms of collar rot, were cut into small bits using a sterilized blade. The pure pathogen culture (A. niger) was made by the hyphal tip isolation method [11] on the solidified PDA medium in petri plates. A typical black mycelium (conidia) growth of A. niger was observed after 72 h of incubation, at $28\pm2°C$, in an incubator.

Three spp. of Trichoderma viz., T. virens, T. viride and T. harzianum were used in the present investigations. All the biocontrol agents were obtained from culture bank of Agharkar Research Institute, Pune (Maharashtra). Both the microbes were maintained throughout the study by periodical transfers on PDA media under aseptic conditions, to keep the culture fresh and viable.

Test of antagonistic potential:

Three spp. of Trichoderma viz., T. virens, T. viride and T. harzianum were used for antagonistic test by dual culture technique using 20 ml of Potato dextrose agar medium in 90 mm culture plates. Potato dextrose agar medium in the culture plates was seeded with the Trichoderma species and test pathogen (5 mm culture discs of three days old culture) opposite each other near the periphery of petri plates. The medium inoculated with the pathogen alone served as control. The experiment was conducted in four replications for each antagonist. All the inoculated plates were incubated at a temperature of $30\pm1°C$. After six days, the plates were observed for growth of antagonist and test fungus. Index of antagonism as per cent growth inhibition of A. niger, was determined by following the method of Watanabe [12].

III. RESULTS AND DISCUSSION

In vitro antagonism between bio-agent Trichoderma and pathogen – A. niger

Growth inhibition of A. niger during in vitro interaction with bio-control agents Trichoderma, at 6 days after inoculation (DAI), was depicted in Fig. 2. Per cent growth inhibition of pathogen (A. niger) was significantly higher in T_3 (86.2%) antagonist, followed by T_5 (80.4%), T_1 (60.9%) and T_2 (50.6%) at 6 DAI. Non significant differences were observed between

antagonists T6 (32.4%) and T4 (28.8%). However, T7 antagonists were recorded with a below 30% growth inhibition of fungal pathogen. Thus, it was observed that T3 antagonist (i.e. interaction between Trichoderma viride 60 and pathogen (A. niger) have a better growth inhibition of test fungus A. niger, compared to the other bio-control agents.
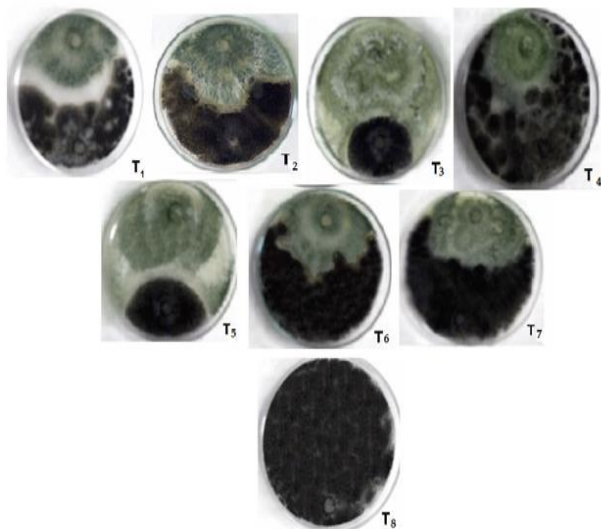
Figure 2. Antagonism between Trichoderma isolates and A. niger

T1 – T. viren X AN; T2 – T. viridie 54 X AN; T3 – T. viridie 60 X AN; T4 – T. viridie 62 X AN; T5 – T. harzianum 2A X AN; T6 – T. harzianum 4A X AN; T7 – T. harzianum 5A X AN;T8 - Control A. niger (AN).

Groundnut is an economically important crop but the collar rot disease was affecting its growth. The present experiment was initiated to study the comparative efficacy of the bio-control agents Trichoderma on different susceptibilities of groundnut varieties against A. niger causing collar rot at the pre emergence phase. An antagonistic effect of fungal bio-control agents against the test pathogen fungus (A. niger) was observed. T. viride 60 (T3) showed maximum reduction in growth of test fungus showed maximum reduction in growth of test fungus followed by T. harzianum 2A (T5). These results are in confirmation with the finding, who reported that the T. viride and T. harzianum were found to be effective in reducing the radial growth of A. niger in vitro [13]. The bio-control agent T. viride had a greater inhibition on A. niger than T. harzianum [14].

IV. CONCLUSION

Collar rot of groundnut caused by Aspergillus niger (Van Teighem) is one of economic important seed borne disease. The disease is expressing their symptoms in pre and post emergence phases. During experiment results of the present study, confirmed clearly that Trichoderma species inhibited the growth of pathogens remarkably well. The competence shown by Trichoderma species to inhibit the growth of the tested pathogens in vitro.

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Effect of Lead Acetate Toxicity on Experimental Chick Embryo

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ABSTRACT

Heavy metals are natural components of the Earth's crust and are important environmental pollutants which are mostly toxic even at very low concentrations. Lead is a common industrial and environment pollutant which is carcinogenic in animals. This study was conducted to understand the toxic effects of lead acetate on antioxidant enzymes and its bioaccumulation in liver and brain using a chick embryo as model. Chick embryos were divided into three groups, first group represented the healthy control and remaining two groups were injected as single dose of 20μl and 40μl/egg of lead acetate respectively, on 7th day of incubation with 1mg. concentration of lead acetate. On 11th and 14th day of incubation, control and treated chick embryos were sacrificed to collect liver and brain for biochemical assays. Different doses of lead acetate caused an increase or decrease of the activity of all investigated Enzymes, with a variable values, at almost all developmental stages. The lead that entered into the body is accumulated in organs and modifies the function of organ by changing the structure of cells of organs biochemical modifications in cells and functional variations of many of enzymes. Results of the present study clearly suggest that lead acetate induce toxic effects on the chick embryo.

Keywords : Lead Acetate, Antioxidant Enzymes, Liver, Brain

I. INTRODUCTION

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. They are a unique class of toxicants, cannot be degraded because of their bioaccumulation. Heavy metal toxicity can result in damage to blood composition, lungs, kidneys, liver and other vital organs and some metals or their compounds may even cause cancer (International Occupational Safety and Health Information Centre 1999). Lead is the most common heavy metal pollutants listed by the Environment Protection Agency (EPA). Important sources of environmental lead contamination include mining, smelting, manufacturing and recycling activities, chemical fertilizers, and canned foods, foods grown around industrial areas, newsprint and colored advertisements. In addition, heavy rains may cause lead in surface soil to migrate into ground water and eventually into water systems, subsequently transferred to humans through the food chain.

Lead was categorized as toxic element, do not play any metabolic function but can be harmful for humans, even at low concentrations, when ingested over a long time period. Lead is absorbed into the body via inhalation and ingestion and to a limited extent, through the skin. Once absorbed, lead is distributed to blood plasma, the nervous system and soft tissues. Lead is bound to red cells (erythrocytes) in the bloodstream. Absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in body organs. The available data on the carcinogenicity of lead following ingestion by laboratory animals indicate that lead is carcinogenic. Lead compounds do

not appear to cause genetic damage directly, but may do so through several indirect mechanisms.

The avian embryo is a long-standing model for developmental biology research. Chick embryo has contributed enormously to experimental embryology and there is a vast amount of literature describing the development and their use as model system (Freeman and Vince 1974; Patten 1961).

II. MATERIAL AND METHODS

Materials

Lead Acetate(PbA), Copper Sulphate (CuSO4), Sodium Carbonate, Sodium potassium carbonate, Phenyl Methyl sulfonyl Fluroid(PMSF), Hydrogen peroxide (H2O2), Amino-antipyrine(A.A.P), Tris HCL Buffer, Phosphate Buffer, Acetate Buffer, Triton 100, Formalin, Eosin etc. All other reagents used were of high quality analytical grade and was procured from the local companies.

Methodology

Source of Fertilized Eggs and Incubation Conditions

Freshly laid zero day old fertilized eggs were purchased from Venketashwara hatcheries Pvt. Ltd. Pune, Maharashtra. The eggs were incubated horizontally and rotated (3h intervals) at $37.5 \pm 0.5°C$ with a relative humidity of 65% in an egg incubator.

Experimental Design

Fertile eggs were divided into three groups: group A, Group B and Group C. Two groups A &B were administered as single dose of 20µL and 40µL/egg of lead acetate respectively, on day7 of incubation. Group C received no lead acetate and served as healthy control.Eggs were set-up in an upright position with the blunt end at the top.

On day 7, each egg was sterilized with 70% ethanol and egg shell was opened to obtain access to the air cell, where all the test samples were injected directly on to the inner shell membrane. The hole was covered by a parafine wax to ensure the embryo's health until tissue collection and blood sampling takes place. The eggs were placed back into the humidified incubator. The eggs were further incubated until the date of examination. Eggs were injected by the air sac method according to Blankenship et al., (2003), on day 7 of incubation. On day 11th and 14th of incubation, the egg shell was broken at the air chamber and embryos were pulled out, from which liver and brain were collected. Tissue samples were washed in ice cold normal saline solution to remove blood and fat debris.

Tissue was minced with scissors and homogenized in 50mM Tris-HCl buffer pH 8.0 containing 0.25M sucrose and 1mM PMSF using a potter homogenizer centrifuged (3000xg for 10min). The resulting clear supernatant was used as enzyme source for antioxidant enzyme assays.

A. Alkaline phosphatase assay:

Buffer, 1 ml. (pH 10 for alkaline), is added to 1 ml. substrate(M / 100 Na2-phenyl phosphate), warmed at 37° C for three min. 0.1 ml. plasma added and mixed. The solution is incubated at 37° C for 15 min. for alkaline phosphatase. Then 0.8 ml. N/2 NaOH is added for alkaline phosphatase and 1.2 ml. M/2 NaHCO3 added for alkaline phosphatase. Then 1 ml. 0.6% A.A.P. is added and mixed. Finally 1 ml. 2.4% K3Fe (CN) 6 is added and mixed. Take the readings at 405nm.

B. Acid phosphtase assay:

First add 0.2 ml of the enzyme solutions to 1.2ml of the acetate buffer and 0.1 ml of Triton X-100 and add 0.5ml of the substrate solution. Incubate for 10 min and 20 min and stop the reaction by adding 2ml of the alkaline tris buffer. Read extinction at 405 nm and calculate the enzymatic activity by reference to a standard curve of p-Nitrophenol.

C. Assay of catalase:

Catalase activity was measured by the method of Aebi (1974).

Catalase in tissues with relatively high activity, such as liver and brain, can be determined spectrophotometrically if complete lysis of all organelles and clear (or only slightly colored) solutions or extracts can be obtained. Normally, catalase activity of tissue samples is expressed on a milligram wet weight or milligram total N basis. A convenient method for the measurement of catalase activity in tissue extracts. Assay Conditions Wavelength, 240 nm; light path, 10 mm; final volume, 3.00 ml. Read the sample containing, 2.00 ml enzyme solution or hemolysate and 1 ml H_2O_2 at 20 ° (- room temperature) against a blank containing, 1 ml phosphate buffer instead of substrate and 2 ml enzyme solution or hemolysate. The reaction is started by addition of H_2O_2. The initial absorbance should be approximately A = 0.500. Mix well with a plastic paddle and follow the decrease in absorbance with a recorder for about 30 sec.

III. OBSERVATIONS:

The observations show the various changes in chick embryo. As days pass mortality and change in weight of eggs were observed. Due to treatment of lead acetate change in size of control and treated chick embryo and its tissues were seen. Morphological deformities like blood clot on head region, patches on liver and absence of feathers were observed.

A. Normal Vs Affected.
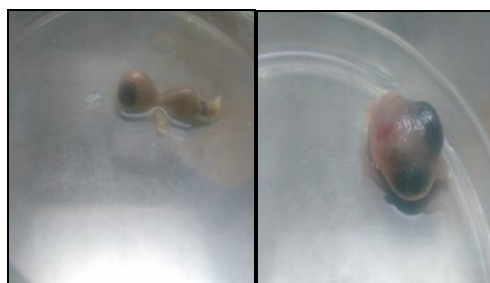

14d developed embryo   20µl PbA treated embryo

14d developed liver   20µl PbA treated 14d Liver

14d developed Brain   20µl PbA treated 14d Brain

A.   Gross Abnormalities:

40µl PbA treated 14d   Blood clot in 14d 40µl PbA
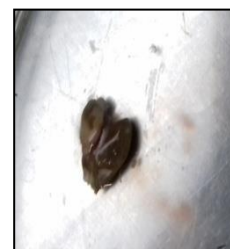Embryo   Treated embryo

Figure 9 absence of feather 11d Figure 10. Patches on liver 11 d

20µl PbA treated embryo   PbA treated embryo

IV. RESULT AND DISCUSSION

Tables and Graphs showed changes in the activity of various enzymes in liver and brain tissues of the chicken embryo exposed to lead acetate.
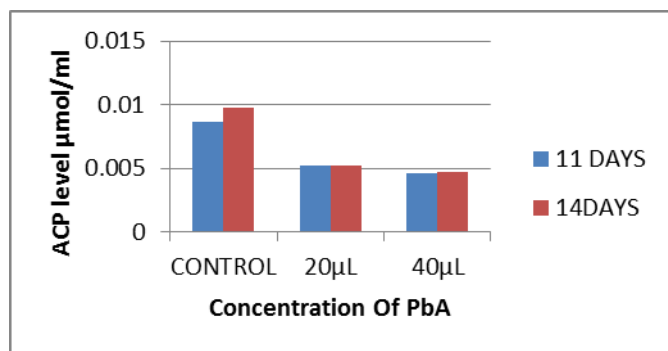
A. Alkaline phosphatse:

The alkaline phosphates content in (μmol/ml) brain tissue of chick embryo after treating with lead acetate gradually decreases as days of treatment increases when compare with control.

Table 1&2 and Graph 1&2: Alkaline phosphatase activity in chick embryonic brain and liver exposed to lead acetate

BRAIN

Exposure period (Days)	Control ±SD	Experimental (20μL) ±SD	Experimental (40μL) ±SD
12	0.145±0.0087	0.211±0.0052	0.128±0.0046
14	0.205±0.0098	0.284±0.0052	0.284±0.0047

Table 1 and Graph 1: Alkaline phosphatase activity in chick embryonic brain exposed to lead acetate

LIVER

Exposure period (Days)	Control ±SD	Experimental (20μL) ±SD	Experimental (40μL) ±SD
12	0.232±0.0060	0.243±0.0047	0.257±0.0051
14	0.208±0.0085	0.232±0.0060	0.267±0.0046
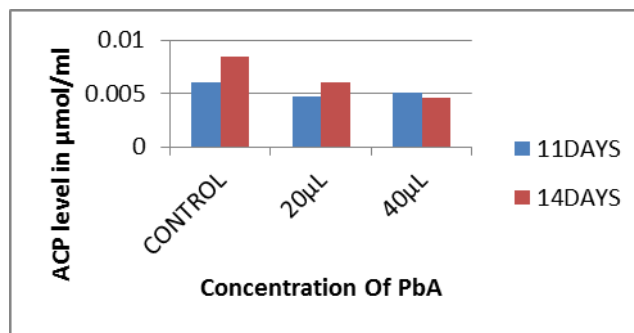
Table 2 and Graph 2: **Alkaline phosphatase activity in chick embryonic liver exposed to lead acetate**

B. Acid phosphatase:

The acid phosphatase content in (μmol/ml) different tissues of chich embryo after treating with lead acetate in comparison with control shows gradually increase as days of treatment increases.

Table 3&4and Graph3&4: Acid phosphatase activity in chick embryonic brain and liver exposed to lead acetate.

BRAIN

Exposure period (Days)	Control ±SD	Experimental (20μL) ±SD	Experimental (40μL) ±SD
12	0.291±0.0115	0.358±0.0117	0.366±0.0118
14	0.299±0.0115	0.325±0.0353	0.466±0.0235
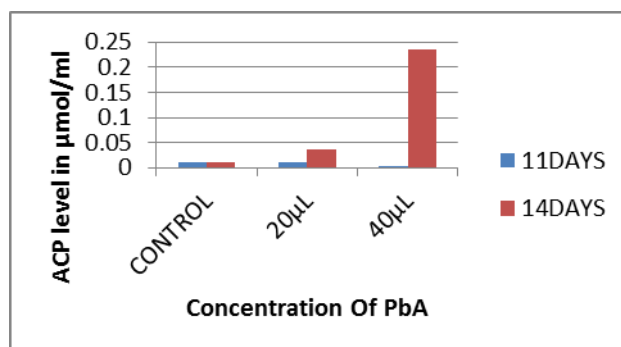
Table 3 and Graph 3: Acid phosphatase activity in chick embryonic brain exposed to lead acetate

LIVER

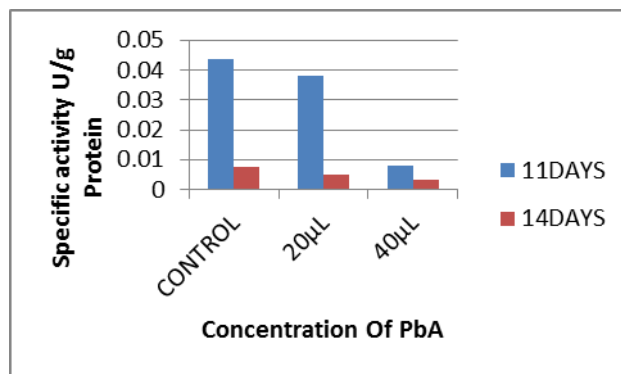| Exposure period (Days)	Control ±SD	Experimental (20µL) ±SD	Experimental (40µL) ±SD
12	0.400±0.0203	0.408±0.0235	0.466±0.0318
14	0.491±0.0118	0.456±0.0235	0.566±0.0311
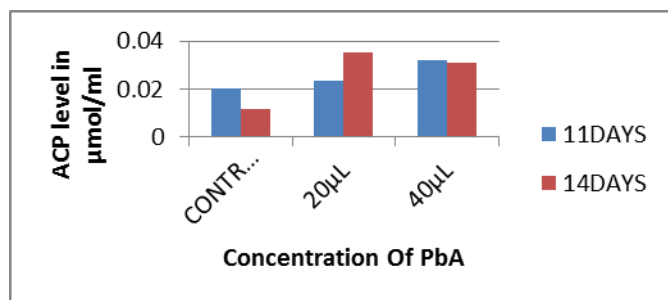
Liver

Table 4 and Graph 4: Acid phosphatase activity in chick embryonic liver exposed to lead acetate

C. CATALASE ACTIVITY:

The effect of lead acetate on catalase activity of chick embryo liver was shown in graph 3. The catalase activity was significantly ($p<0.05$) decreased in 20µL and 40µL PbA treatment compared to control group.
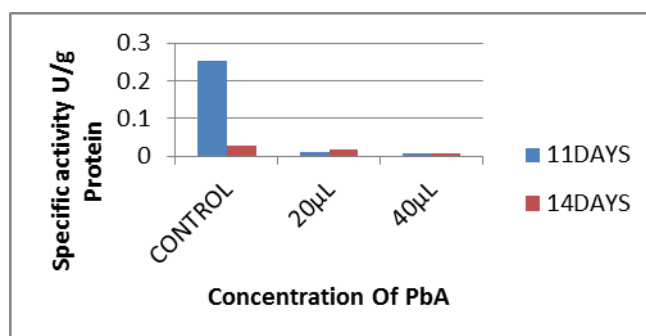
BRAIN

Exposure period (Days)	Control ±SD	Experimental (20µL) ±SD	Experimental (40µL) ±SD
12	0.87325±0.0075	0.75425±0.00506	0.5365±0.00332
14	2.1635±0.04356	1.90525±0.0379	0.5345±0.00785

Table 5 and Graph 5: Catalase activity in chick embryonic liver exposed to lead acetate

LIVER

Exposure period (Days)	Control ±SD	Experimental (20µL) ±SD	Experimental (40µL) ±SD
12	1.33825±0.2521	0.98275±0.01176	0.59175±0.00665
14	0.895±0.02768	0.82425±0.01628	0.26425±0.00723

Table 6 and Graph 6: **Effect of lead acetate on catalase activity of 12d and 14 day old chick Embryonic Brain.**

V. DISSCUSION

Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with a number of body functions and is primarily affecting the central nervous, hematopoietic, hepatic and renal system producing serious disorders.

Phosphatases are nonspecific phosphomonoestarse having pH specifically, which hydrolyze various phosphate esters and liberate phosphate from the substrate. Acid phosphatase (ACP) hydrolyses the phosphorous ester in acidic medium and autolysis process of the cell after its death. The employment of biochemical markers in monitoring pollution resulting from heavy metals has significantly shown the sensitivity of the tools and the potential risks facing man at the upper trophic level along the food chain . the present investigation shows toxicity of heavy metals on biosystems. Acid and alkaline phosphatase activities of control embryos are gradually changes as development proceeds from the 11th to 14th day of incubation. This findings are in accord with those of Romanoff (1988) and King and Liu (1974 a and b).

Catalase is one of the important antioxidant enzymes which help in converting the hydrogen peroxide to water and oxygen. Catalase is an efficient decomposer of H2O2 and is known to be susceptible to lead toxicity. (Sandhir and Gill 1995). The obtained results have displayed that treatment of chick embryo with lead acetate caused a reduction of liver catalase (Graph:4 and 5).Reduction in CAT impairs scavenging of hydrogen peroxide radicals. The reduction of catalase in liver and brain by Pb must be due to Pb induced inhibition of heme biosynthesis and since heme is a basic prosthetic molecule available with catalase for catalysis of peroxy radicals. Therefore the present results are in accordance with the earlier reports (Sandhir and Gill 1995).

VI. CONCLUSION

The environmental contamination by lead generated from human activities has become an evident problem during the last decades. Lead can penetrate to the human or animal organisms by inhalation, ingestion and by skin (Ryan and Terry, 1988;El-Fekiet al., 2000). The adverse effects of lead on health and productivity have been studied relatively little in poultry, particularly in layers, in comparison with other farm animals. Berget al.,(1980) stated that contamination of the environment with lead has reached such a level that can affect the growth, productivity, and health of poultry as the toxicity of these metals (cadmium, lead) relies on binding the metallic cations with sulphydryl, amino and carboxylic groups of enzymes thus inhibiting enzymatic activities and disturbing energy metabolism.

In the present study, treatment with Pb-acetate has resulted in altered activity of alkaline phosphatase and acid phosphatase and the significant decrease of CAT levels. It shows changes in proteins content. It leads to excessive liver and brain damage and shows the tendency of bioaccumulation in the chick embryo. According to the results obtained in the present study, it appears that lead acetate administration to chick embryo would increase the toxicity and causing damage to vital tissue.

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Alpha Diversity And Species Assemblage of Odonata from Different Habitats in Pune-Chindchwad Municipal Corporation (PCMC) Area, Pune, Maharashtra

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ABSTRACT

The present work is aimed to study diversity and abundance of dragonflies and damselflies of Pimpri-Chinchwad Municipality in Pune city of Maharashtra in Northern western Ghats. This study has been carried out for one year from June 2016 to March 2017. A total number of 32 species under 6 families was observed. The most abundant family was Libellulidae comprising 20 species followed by Coenagrionidae comprising 6 species, Chlorocyphidae comprising 2 species, Lestidae with 2 species, Aeshnidae and Platycnemidae comprising of 1species. Assemblage of different species in relation to their habitat diversity and diversity indices were also reported.

Keywords : Alpha Diversity, Species Assemblage, Bio-Indicators, Habitat Diversity

I. INTRODUCTION

Dragonflies and damselflies (Order – Odonata) are colorful predatory insects of freshwater habitat and characterized by their elongate body, extended wings and large eyes. These are carnivorous and a number of species are predators. They prefer to live in freshwater, non polluted and well oxygenated habitats. Therefore Dragonflies and Damselflies are precious bio-indicators for environmental contamination studies (Needham, et al., 2000).

Yet, there is no detailed report on Diversity and abundance of Dragonflies and Damselflies of PCMC area in Pune district of Maharashtra state. The present investigation was, therefore, done to put together the study on Diversity and abundance of Dragonflies and Damselflies of PCMC range in Pune district.

Biodiversity has number of function like maintaining balance of the ecosystems.; provisions of biological resources and food; social benefits; human health, cultural developement etc. Countries are facing threats due to activities like ruthless cutting of mangrove trees, overexploitation of fishery resources, ruthless mining of coral reefs, widespread degradation of habitats due to urbanization, industrialization, and increase in pollution level in estuaries, mangroves, backwaters and seas. During the last few years, the necessity for the conservation of biological resources and biodiversity assessment has promptly increased; taxonomic diversity studies are being encouraged now. Thus biodiversity and its measurement have a grave importance especially if we look into the present scenario.

Worldwide, 29 Orders of class Insecta occurs which comprise of 1,004,898 species (Foottit &Alder, 2009). It is still not known that how many insect species are there as many are still to be described. Currently, it is essential to improve taxonomic

knowledge on insect groups that would be truly beneficial for the point of conservation.

The Western Ghats is one of the world's most heavily populated biodiversity hotspot supporting about 400 million people through water, food and resources to sustain livelihood (Molur et al., 2011). Today these are being rapidly degraded due to various land use changes that have occurred in the recent past. Apart from the traditional impacts from farming, grazing and fire there are newer changes in land-use that are leading to biodiversity losses. This includes deforestation due to mining, construction of roads, dams, townships and industrialization. Urbanization and industrialization in Maharashtra, Gujarat and Goa has altered the natural ecological attributes over the last several decades. Thus there is a special requirements of sustainable development in areas that are ecologically fragile,

Maharashtra is one of the states of the Northern Western Ghats. The state of Maharashtra lies between 15°32' to 22°02' N latitude and 72°36' to 80°54' E longitude and is the third largest state of the country with an area of 3,07,714 sq km constituting 9.36% of India's total geographic area. The Ghats spread over 10 districts which includes 67 tehsils, Pune being one of them and covering a total of 527 km² (Jagtap and Singh, 2002).

According to the recent updated list, Odonata has a worldwide distribution of 5,952 species of which 474 species in 142 genera and 18 families exists in India (Subramanian, 2014). The Western Ghats is especially diverse with 174 species, comprising 56 endemics to the region (Subramanian et al. 2011). Based on morphology, the Order Odonata is divided into three groups, viz. damselflies (Zygoptera), Anisozygoptera and dragonflies (Anisoptera). The suborder Anisoptera consists of 7 families worldwide, suborder Zygoptera with 11 families and a single family under suborder Anisozygoptera.

A voluminous literature contributed by several authors is being available relating to Odonata taxonomy and bio-ecology. Tillyard (1917) provided one of the earliest account of dragonfly biology, a book still of great value, since he emphasized functional morphology and anatomy. The biology, physiology and ecology of Odonates have also been well studied by Philip S. Corbet (1962) in his book "A Biology of Dragonflies". Prasad, (1996) studied Odonata from Maharashtra state and published in Records of Zoological Survey , where he presented a list of all the 83 species known from Maharashtra state till then. Koparde et al.,(2014) reports seven new records from Kolhapur district. However, very few studies have looked into Odonata fauna of North Western Ghats of Maharashtra (Fraser 1924, 1933, 1934, 1936; Kulkarni & Subramanian, 2013). Saha and Gaikwad, (2012) reported 27 species of Odonata from Tamhini, a sacred grove in Pune District. Saha and Gaikwad, (2014) studied the diversity and abundance of Odonates in parks and gardens of Pune city and recorded a total of 33 species. They also illustrated the importance of human-managed urban parks and gardens in supporting their diversity and abundance. Saha and Gaikwad, (2015) studied the Odonata assemblage at a very small marshy land in the heart of Pune city, which was documented to support 17 species of Odonata and thus can be considered as species rich diversity site in a purely urban backdrop. Though the Odonata of Pune District has been documented by some workers since the beginning of the 20th century (Fraser 1933–36; Prasad 1996), they were mostly species checklists without any details of habitat and seasonal distribution.

Odonata is sensitive to habitat structure and is an excellent indicator of changes in habitat structure (Clausnitzer, 2004). The group constitutes a valuable tool for various types of bio-assessment and bio-monitoring of aquatic habitats (Oertli, 2008). Odonates have been already in use as biomonitoring tools in countries like South Africa, U.S.A, Europe, Japan (Clark and Samways, 1996; Samways, 1996)

Therefore the importance of this group both ecological and economical. The current investigation aims at generating a baseline data on habitat distribution, seasonal variations and diversity of

Odonata of PCMC are in Pune district in Northern Western Ghats .

II. METHODS AND MATERIAL

1) Study area:

The area under Pimpri-Chinwchwad Municipal Corporation (PCMC) jurisdiction was selected as the study area. For selection of study sites, the center was geographically located, and the sampling was done from different localities. The selection of study sites was based on the availability of different habitat of odonates like urban and agricultural habitat. The urban habitat was again categorized based on the level of pollution. The highly polluted sites were specifically sampled. These sites are located at the vicinity of factories or dense human habitation. A total of localities were sampled during premonsoon and post monsoon seasons from July2016 to March 2017. Collection sites was mainly divided under three land-use categories which forms different habitats for Odonates. These are as follows:1)Agricultural   habitat, 2)Polluted Urban habitat, 3) Non-polluted Urban habitat

Methodology:

The present study was done for one year from July 2016 to March 2017. Specimens of Dragonflies and damselflies were observed in field with careful note on their habitats. Repeated visits to field have been made in morning, afternoon and evening. For diversity and abundance, specimen number of each species has been counted by visual observations. The specimens were identified in the field by using field guides of Subramanian (2005, 2009) and the Handbook of Common Odonates of Central India by Andrew,et al (2009).

During survey standard Transect method was used. Different transects of 1 km length was drawn in different parts of the present area and the breadth of each transect was 20 feet. Results were recorded by visualizing the specimens through-out transect and after that summarization of all transects were done.

Preparation of data sheet:

In order to record systematically all the above-mentioned ecological details regarding the study sites and also about the species, data sheets were prepared. This data sheet helped to keep a systematic record of all the details for every species during every visit. Diversity indices was measured using the data collected during repeated field visits.

III. RESULTS AND DISCUSSION

Systematic account of Order Odonata.

Order Odonata Fabricius, 1793
Suborder Zygoptera Selys, 1854
I. Family: Lestidae Calvert, 1907
1. Genus: Lestes Leach, 1815
    1. Lestes elatus Hagen in Selys, 1862
    2. Lestes viridulus Rambur, 1842
Family: Chlorocyphidae Cowley, 1937
2. Genus: Heliocypha Fraser, 1949
    3. Heliocypha bisignata (Hagen in Selys, 1853)
    Superfamily Coenagrionidea Kirby, 1890

III. Family: Coenagrionidae Kirby, 1890
3.Genus: Agriocnemis Selys, 1877
    4. Agriocnemis pygmaea (Rambur, 1842)
4. Genus: **Ceriagrion** Selys, 1876
    5. Ceriagrion coromandelianum (Fabricius, 1798)

5. Genus:**Ischnura** Charpentier, 1840
    6. Ischnura aurora (Brauer, 1865)
    7. Ischnura senegalensis (Rambur, 1842)
6. Genus: **Pseudagrion** Selys, 1876
    8. Pseudagrion decorum (Rambur, 1842)
    9. Pseudagrion rubriceps Selys, 1876
Family: Platycnemididae Yakobson & Bainchi, 1905
7 . Genus: **Copera** Kirby, 1890
    10. Copera vittata deccanensis Laidlaw, 1917

8. Genus: **Disparoneura** Selys, 1860

    11. Disparoneura quadrimaculata (Rambur, 1842)

Suborder Anisoptera Selys, 1854

Superfamily Aeshnoidea Leach, 1815

III. Family: Aeshnidae Leach, 1815

9. Genus:Anax Leach, 1815

    12. Anax immaculifrons Rambur, 184

Superfamily Libelluloidea Leach, 1815

IV. Family: Libellulidae Leach, 1815

10.Genus:Acisoma Rambur, 1842

    13. Acisoma panorpoides Rambur, 1842

11. Genus:Brachythemis Brauer, 1868

    14.Brachythemis contaminata (Fabricius,1793)

12. Genus:Bradinopyga Kirby, 1893

    15. Bradinopyga geminata (Rambur, 1842)

13. Genus:Crocothemis Brauer, 1868

    16. Crocothemis servilia (Drury, 1770)

14.Genus:Diplacodes Kirby, 1889

    17. Diplacodes trivialis (Rambur,1842)

15. Genus:Neurothemis Brauer, 1867

    18. Neurothemis intermedia intermedia (Rambur, 1842)

    19. Neurothemis tullia (Drury, 1773)

16.Genus:Orthetrum Newman, 1833

    20. Orthetrum luzonicum (Brauer, 1868)

    21. Orthetrum pruinosum (Burmeister, 1839)

    22. Orthetrum sabina (Drury, 1770)

    23. Orthetrum taeniolatum (Schneider,1845)

17. Genus:Pantala Hagen, 1861

    24. Pantala flavescens (Fabricius, 1798)

18.Genus:Tholymis Hagen, 1867

    25. Tholymis tillarga (Fabricius, 1798)

19. Genus:Tramea Hagen, 1861

    26. Tramea basilaris (Palisot de Beauvois, 1805)

    27. Tramea limbata similata (Rambur, 1842)

20. Genus: Rhyothemis Hagen, 1867

    28. Rhyothemis variegata (Linnaeus, 1763)

21. Genus:Trithemis Brauer, 1868

    29. Trithemis aurora (Burmeister, 1839)

    30. Trithemis festiva (Rambur, 1842)

    31. Trithemis pallidinervis (Kirby, 1889)

22. Genus: Zyxomma Rambur, 1842

    32. Zyxomma petiolatum Rambur, 1842

Species Diversity in the study area:

In present investigation a total number of 363 individuals belonging to 32 species under 22 genera, 6 families and two suborders were recorded. The families are Libellulidae, Coenagrionidae, Ashnidae, Chlorocyphidae, Lestidae and Platycnemidae. The most dominant family in present study is Libellulidae which comprises 13 genera (61% of total genera) and 22 species (58.45% of total species).

The abundance of sub-order Anisoptera was more in comparison to Zygoptera .since dragonflies are strong flier and can easily get adapted to environmental variation on the other hand damselflies are week fliers and more sensitive to environmental disturbances.

Species collected from agricultural area mainly comprised of Ceriagrion coromandelianum, Diplceodes trivialis, Neurothemis tullia, Crocothemis servillia, Trithemis aurora, Orthetrum sabina etc. They are predators for crop pests like moths and beetles.

Species observed in polluted urban area were Brachythemis contaminata, Pantala flavesance, Orthetrum sabina ,Trithemis festiva. The larvae of these species can tolerate a wide variation in pH, temparature and chemical constitute of water.

Species assemblage in non -polluted urban areas like well maintain park and garden river side etc. is dominated by Acisoma paneropoides, Orthetrum luzonicum, Trithemis pallinidervis, etc.

Heliocypha bisignata is particularly found in very clear streams since they are sensitive to pollutants.

Table 1. The diversity indices of Odonates from all the localities

Localities	Taxa_S	Individuals	Dominance_D	Simpson_1-D	Shannon_H	Margalef	Fisher_alpha
S1	22	55	0.0843	0.9157	2.762	5.24	13.59
S2	10	17	0.1349	0.8651	2.15	3.177	10.19
S3	14	34	0.0934	0.9066	2.484	3.687	8.902
S4	10	19	0.1191	0.8809	2.205	3.057	8.541
S5	11	22	0.1405	0.8595	2.158	3.235	8.755
S6	9	20	0.205	0.795	1.851	2.67	6.296
S7	11	20	0.145	0.855	2.181	3.338	10.03
S8	10	23	0.2628	0.7372	1.808	3.338	6.733
S9	7	27	0.2702	0.7298	1.566	1.82	3.066
S10	8	15	0.1911	0.8089	1.859	2.585	6.966
S11	11	26	0.1479	0.8521	2.138	3.069	7.193
S12	11	16	0.1094	0.8906	2.307	3.607	15.54
S13	9	17	0.1765	0.8235	1.952	2.824	7.753
S14	9	12	0.1667	0.8333	2.023	3.219	16.36
S15	6	9	0.2099	0.7901	1.677	2.276	7.867
S16	7	12	0.1944	0.8056	1.792	2.415	7.028
S71	12	21	0.1247	0.8753	2.281	2.415	11.64

Table 1 denotes the diversity indices of Odonates from all the localities. The site S1 (Hinjewadi) is a urban locality which shows highest diversity indices (2.762). This is due to the large pieces of marshy land that still exist. But the rapid urbanization is posing a great threat to this existing diversity. The least diverse sites are S15, S16 etc which are rather polluted due to presence of nearby factories or industries.

Fig. 1 Family abundance in the study area
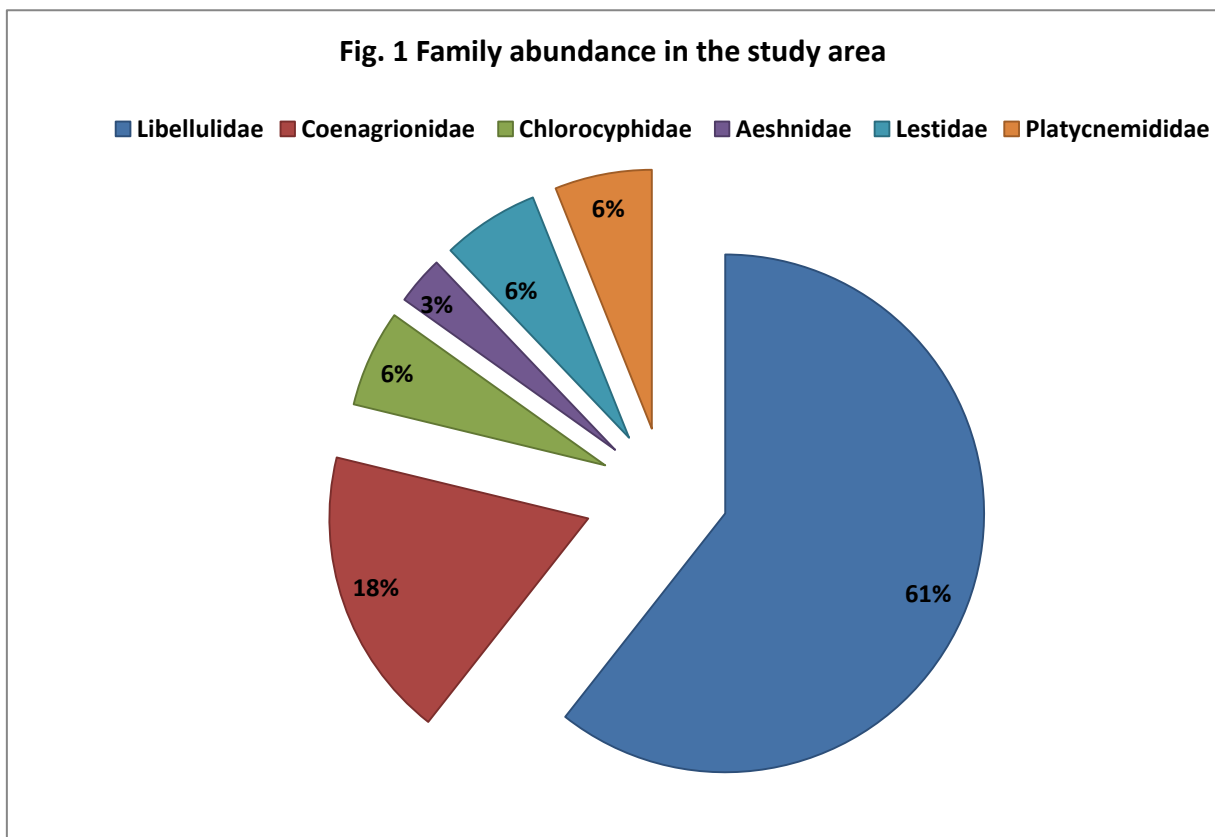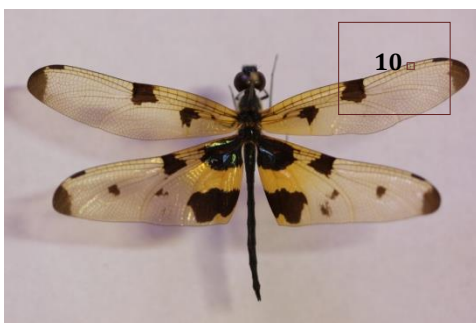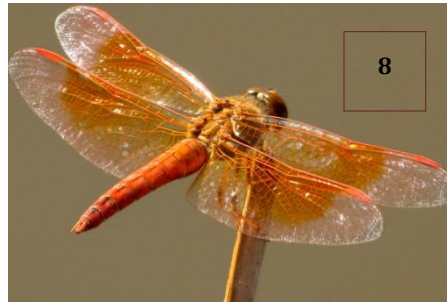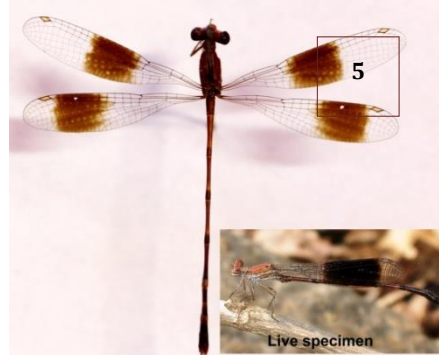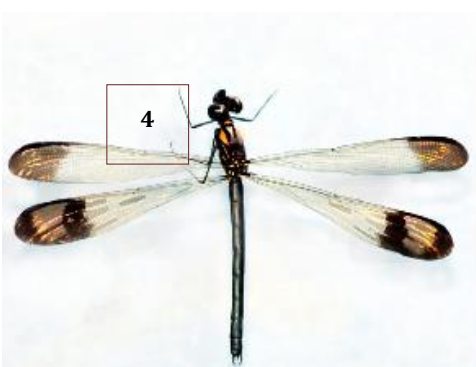
Fig. 2. Abandance of Odonates in the study area
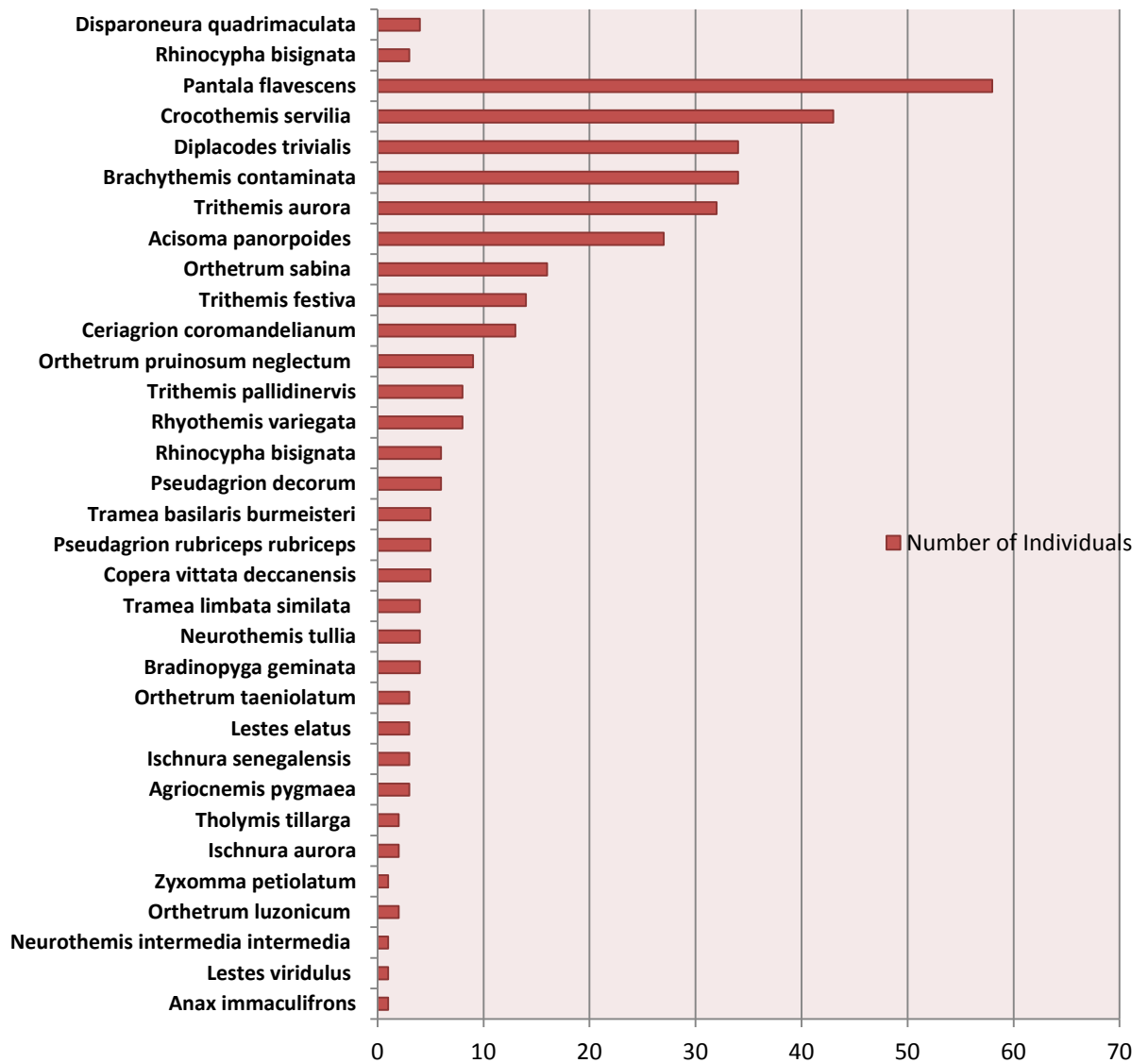
Fig. 1. Site (polluted-urban) 2. Site (agricultural) 3. Site (unpolluted urban). 4. *Heliocypha bisignata* (Hagen in Selys, 1853) 5. *Disparoneura quadrimaculata* (Rambur, 1842) 6. *Ceriagrion coromandelianum* (Fabricius, 1798) 7. *Acisoma panorpoides* Rambur, 1842 8. *Brachythemis contaminata* (Fabricius,1793) 9. *Pantala flavescens* (Fabricius, 1798) 10. *Rhyothemis variegata* (Linnaeus, 1763) 11. *Orthetrum luzonicum* (Brauer, 1868) 12. *Trithemis festiva* (Rambur, 1842)

IV.CONCLUSION

This study highlights how the different types of habitat influence Odonate assemblage and the threatening effect of pollution because of urbanization and industrialization on the same. Similar type of species assemblages were recorded from specific habitats. Information on diversity and distribution of various taxa and their habitat is the key to diversity conservation. In this context, the present study on Odonate diversity, abundance and distribution in Pune district of Maharashtra which falls under Northern Western part has been initiated to fill in the above mentioned lacunae.

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Biochemical Characterization of Lipase from Fagopyrum Esculentum Seeds and Its Application

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ABSTRACT

Enzymes are biocatalysts, providing opportunity for economically sustainable industrial applications. Immobilization of enzyme further enhances its reusability. Lipase a hydrolytic enzyme, owing to its enantioselectivity and regioselectivity finds varied industrial applications. Lipases are used in detergent, food and various industries. In the present study, lipase was purified from the seeds of *Fagopyrum esculentum* (buckwheat). The enzyme was immobilized on chitosan beads. Optimization of different parameters viz. use of different cross linkers, concentration of enzyme, etc. for effective enzyme immobilization was carried out. Biochemical characterization of both the native and the immobilized enzyme was studied. Further, the reusability of the immobilized enzyme was also evaluated.

Keywords : Lipase, enzyme immobilization, buckwheat

I. INTRODUCTION

Fagopyrum esculentum commonly known as buckwheat belongs to *Polygonaceae* family and is a common staple food grain in many countries in Asia, viz. Japan, Korea, and China. It is also cultivated and used as a fasting food in north Indian states with its Indian name kuttu. The proteins in buckwheat have significant biological effects and have balanced amino acid composition with high percent of the essential amino acids such as lysine and isoleucine [1].

Lipases are triacylglycerol acylhydrolases (E.C. 3.1.1.3). They catalyze the hydrolysis of carboxylester linkage of triacylglycerol to release glycerol and free fatty acids. Lipase activity plays an important role in quality deterioration of buckwheat flour [2]. The catalytic power of lipases increases significantly at the lipid-water interface, thus showing the phenomenon of "interfacial activation". In water-restricted environment, lipases catalyze reverse reactions, catalyzing various reactions like like interesterification, transesterification, hydrolysis, alcoholysis, acidolysis, or esterification reactions. Thus, immobilization of lipase presents a wide scope in various industries [3].

Immobilization is the key to optimize enzymatic processes in industries. Immobilization of enzymes renders the Industrial processes economically cheap and reusable. Moreover, it aids in the development of continuous processes and the termination of industrial processes becomes easy [4]. Chitosan has shown it versatile use in its shape and immobilization of different substances [5].

II. MATERIALS AND METHODS

A. Isolation of semi-purified lipase

Buckwheat seeds used as the source of enzyme was procured from local market of north Indian states. Lipase from 100g of Fagopyrum esculentum seed meal was extracted in 10mM Tris buffer pH 7.2 with EDTA and PMSF [6] at constant stirring with a magnetic

stirrer for 4 hours at 20°C. The latter was centrifuged at 10,000 rpm at 4°C for 30 mins. The supernatant was then subjected to ammonium sulphate precipitation, which was then passed onto a Hydrophobic Interaction matrix of 6FF phenyl sepharose (sigma) column for further purification. The extract was dialysed as and when required. This semipurified enzyme was used for immobilization and for its biochemical characterization.

B. Preparation of Chitosan beads

4 % chitosan (low molecular weight, obtained from SRL) solution was made in 2.0% aqueous acetic acid. The chitosan solution was dropped into an aqueous 2M NaOH solution using a syringe to form chitosan gelatinous beads. The chitosan beads were thoroughly washed with distilled water until neutrality was reached [7].

C. Activity measurement for free and immobilized enzyme

Lipase activity was assayed essentially as described by Winkler and Stuckmann [8] using para-nitrophenyl palmitate (pNPP) as substrate as follows: 0.3 ml of enzyme extract and 0.4 ml of Tris buffer (10 mM, pH 7.2) were pre-incubated at 30 °C for 5 mins. 0.3 ml of the substrate solution was added and the mixture was incubated at 30 °C for 20 mins. The reaction was arrested by adding 200 mM Tris buffer pH 9. The intensity of the yellow colour due to p-nitrophenol obtained due to lipase action was measured at 410 nm. For activity measurement for immobilized enzyme 0.3 ml was replaced with 5 beads. The substrate solution consisted of 1 ml of pNPP solution (0.3% in isopropanol) and 9.0 ml of tris buffer[10 mM, pH 7.2, containing 0.4% (v/v) Triton –X-100]. One unit of enzyme activity is defined as the amount of lipase liberating 1 μmol of p- nitrophenol per min.

D. Optimization of immobilization of beads

i. Crosslinking with glutaraldehyde and epichlorohydrin: The beads were stirred on a magnetic stirrer for 1 h with 1%, 3%, 5% and 10% crosslinkers viz glutaraldehyde and epichlorohydrin. The crosslinked beads were washed with distilled water to remove excess crosslinker followed by 3 washings with the 10mM Tris buffer (pH 7.2).

ii. Immobilization of lipase on crosslinked beads: 5 g crosslinked beads were stirred for varying time of 1h, 2h, 3h, 4h and 5h; followed by washings with buffer to remove unbound protein. The activity of the immobilized enzyme was measured with the same lipase assay.

iii. Effect of temperature: The immobilized and free enzyme activity was measured at different temperature 10, 20, 30, 40, 50 and 60°C by incubating the assay at these temperatures.

iv. Temperature stability: The stability of both free and immobilized enzyme was measured at different temperature 10, 20, 30, 40, 50 and 60°C by pre-incubating the enzyme in buffer at different temperature for 1 h and then the activity was measured by the lipase assay.

v. Effect of pH: The immobilized and free enzyme activity was measured at different pH by carrying out the assay with different pH buffer viz. 3, 4, 5, 6, 7, 8, 9 and 10.

vi. pH stability: The stability of both free and immobilized enzyme was measured at different pH by pre-incubating the enzyme in different pH buffer viz. 3, 4, 5, 6, 7, 8, 9 and 10 for 1 h and then the activity was measured by the lipase assay.

vii. Effect of metal ions: The enzymes were pre-incubated with 5 mM metal solutions viz. Fe^{3+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, Sn^{2+}, Al^{3+}, Cu^+, K^+, Ni^{2+}, Cd^{2+}, Na^+, Co^{2+} and Zn^{2+} for an hour then the assay was performed to check the residual activity of the enzymes. All the required controls and blanks with and without metal solutions were maintained.

viii. Reusability of immobilized enzyme: The activity of the beads were measured at optimum temperature and pH once, then the beads were washed with buffer until all yellow colored pNP

product is removed to perform next cycle to check the reusability.

III. RESULTS AND DISCUSSION

A. Effect of different crosslinkers
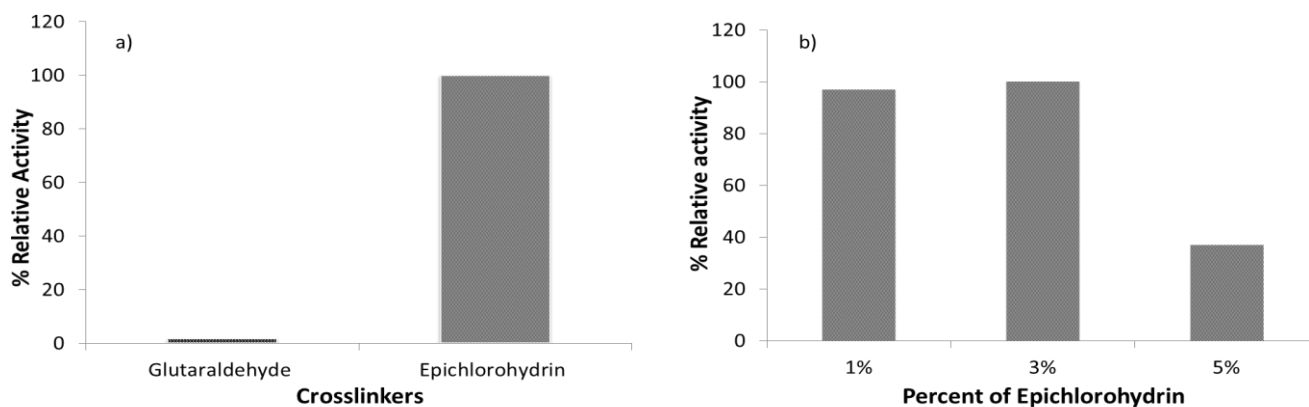
Figure 1. a) % Relative activity with different crosslinkers b) % Relative activity with different % of epichlorohydrin

Crosslinking with glutaraldehyde and epichlorohydrin at different percentages was carried out. 3% Epichlorohydrin shows maximum lipase activity in immobilized enzyme as shown in Fig. 1. This reflects the bonding of carbon atoms, disturbing the epoxide ring and the removal chlorine atom [9], compared to chitosan immobilization of peroxidase [7].

B. Effect of temperature on lipase activity and stability
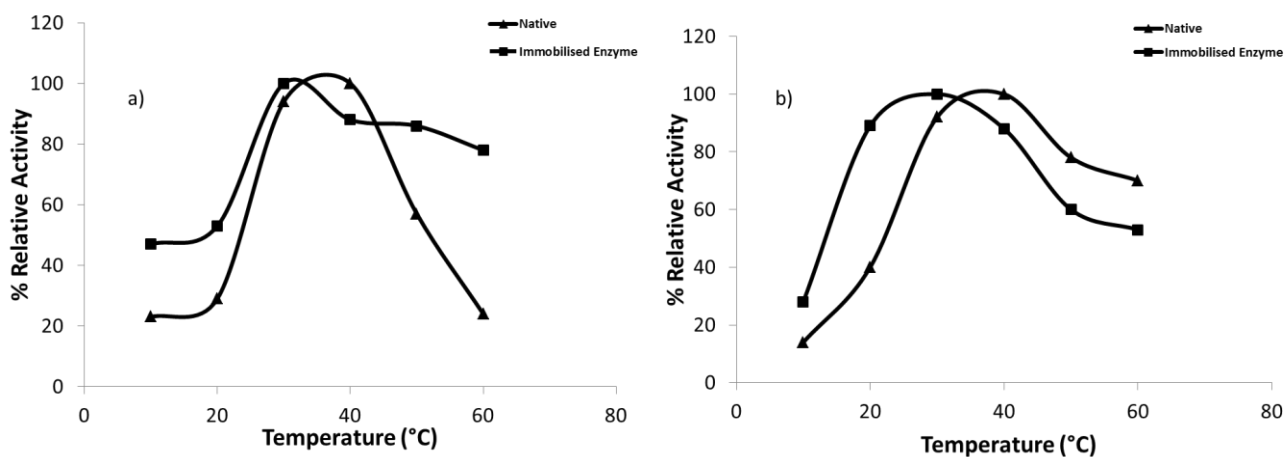
Figure 2. A) Effect Of Temperature On Native And Immobilized Lipase Activity B) Temperature Stability Of Native And Immobilized Of Lipase

As seen in the above Figure 2 a) shows the optimum temperature of native enzyme as 37°C and optimum temperature of immobilized enzyme as 30°C. As seen in the graph the immobilized enzyme has more than 80% relative activity at temperature higher than 30°C. As seen in b) the immobilized enzyme is stable in the range of 25-40°C and the native enzyme is stable in the temperature range of 30-45°C.

C. Effect of pH on lipase activity and stability
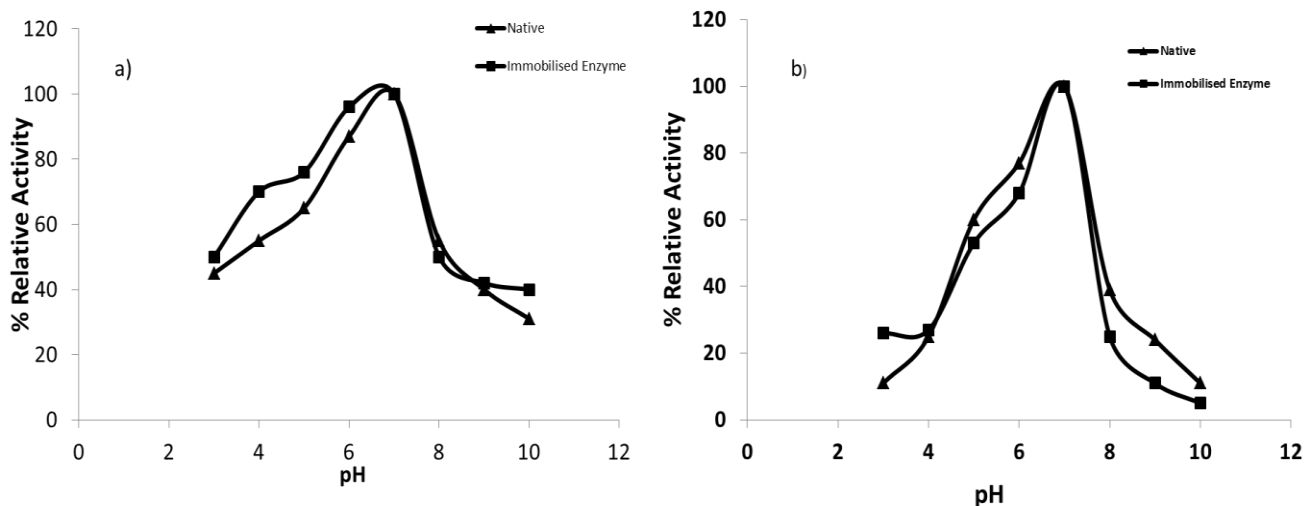
Figure 3 a) Effect of ph on on native and immobilized lipase activity b) ph stability of native and immobilized of lipase

As seen in the above figure 3 a) & b) the effect of different pH and stability of enzyme in terms of their relative activity shows the optimum pH and maximum stability for both immobilized and native enzyme is pH 7.

D. Effect of different metal ions on lipase activity
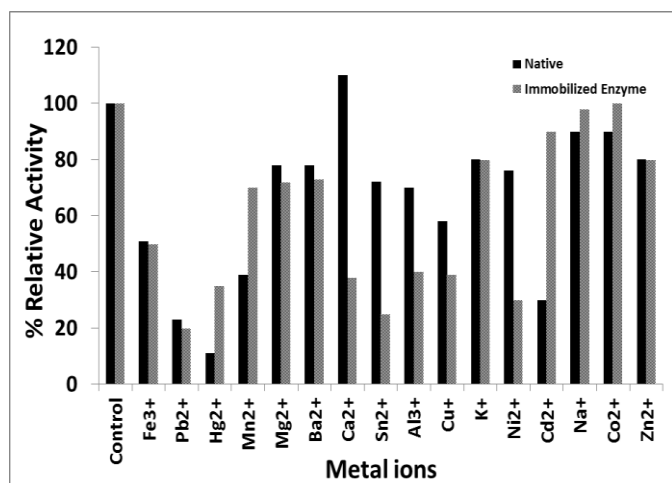
Figure 4. Effect of metal ions

As seen in the figure 4 the activity of native enzyme was maximally inhibited by Hg^{2+} ions, whereas the immobilized enzyme was maximally inhibited by Pb^{2+} ions.

E. Reusability of immobilized lipase on chitosan beads
Reusability is the most significant parameter to evaluate the application of immobilized enzymes in industries [10].
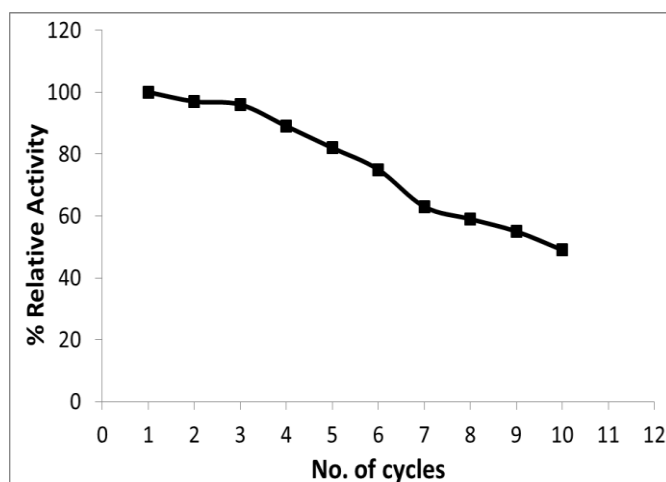
Figure 5. Reusability of the immobilized enzyme

Figure 5 shows that the immobilized enzyme retains almost 80% the lipase activity till 4-5 cycles of reuse of the same beads.

IV. CONCLUSION

The present study showed that the immobilization of semi-purified lipase enzyme on chitosan beads was successful in adsorbing and reusing the enzymes for repeated cycles with limited leaching with 3% epichlorohydrin.

Also, the inhibition of enzyme activity in presence of metal ions opens avenues for development of a biosensor based on this immobilized enzyme for sensing of lead and mercury toxicity studies.

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Biochemical Studies and Characterization of Immobilized Alkaline Phosphatases on Carboxyl-Functionalised Carbon Nanotubes

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ABSTRACT

Alkaline phosphatase has been isolated from the seeds of 'Arachis hypoghaea' by ammonium sulphate fractionation (30-80%) and ion exchange chromatography. The isolated enzyme was studied for its biochemical properties viz. optimum pH and temperature. The purified enzyme was immobilized using carboxyl-functionalised carbon nanotubes (CNTs) by cross linking with epichlorohydrin (ECH) as well as a matrix of ethyl cellulose. The resulting two matrices were characterized by FTIR and SEM to obtain structural and morphological properties. By both methods the immobilised enzyme showed good activity comparable with the native enzyme. Reusability of the immobilised enzyme was demonstrated up to 8-10 cycles. The immobilized enzyme showed   higher temperature stability. The investigation of cutting-edge nanomaterial with the help of enzyme immobilisation using exceptional techniques makes nanobiocatalyst of promising thirst for knowledge for biosensor applications.

Keywords: Alkaline phosphatase, Immobilization, CNTs, Biochemical studies

I. INTRODUCTION

Immobilisation of enzymes have been well-defined as enzymes which are actually confined or localised, although without a losing their catalytic activity, and can be used repetitively and continuously [1]. Immobilisation of enzymes on a matrix offers significant cost benefits for industrial and clinical processes, since it enables enzyme recycling, enables enhancements in thermo-stability which ultimately reduces enzyme inactivation and permits for better control of enzyme activity [1, 2]. Likewise, Nanobiocatalysis is also a speedily increasing research ground which increases to the application of enzymes immobilized materials. A Nano material contributes some benefits over the bulk solid materials, specifically the high surface area which can lead to higher enzyme loading, the nanoscale dispersion and the ease of surface functionalization [3]. Carbon nanotubes (CNTs) have fascinated significant benefits among nanostructured materials for their unique mechanical, thermal and electrical properties as well as their biocompability [4].

In this present work, Alkaline phosphatase has been isolated from the seeds of '*Arachis hypoghaea'* and was then immobilised onto the carboxyl functionalised multi-walled carbon nanotubes (MWNTs) supports using various cross linkers viz. Epichlorohydrin (ECH) as well as a matrix of Ethyl cellulose. The biopolymer may also serve as a potential cheap and easily available biosorbent for environmentally harmful metal ions [5–9]. The enzymatic activity was then measured according to the typical process. Structural and morphological

changes for immobilized enzyme were analysed using various spectroscopy techniques. The catalytic efficiency of the immobilised alkaline phosphatase, in terms of thermal stability and reusability, was also studied.

II. METHODS AND MATERIAL

A. Isolation and purification of Alkaline phosphatase

'*Arachis Hypoghaea*' seed meal (100 g) was extracted with 500 ml of physiological saline (0.145 M NaCl) for 4 hours at 4 °C. The extract was centrifuged at 16,000 rpm and subjected to fractional precipitation with ammonium sulphate. The proteins precipitating between 30% and 80% saturation of ammonium sulphate were collected by centrifugation, dissolved in minimum amount of distilled water, extensively dialysed against distilled water and finally against Tris buffer (pH 8.0, 0.1 M). The dialyzed protein solution, clarified by centrifugation (Fraction A) was used to isolate alkaline phosphatase by ion exchange chromatography on a UNOsphere-Q column an anion exchanger.

The optimum pH of the enzyme was determined as follows; a test system containing 200 μl Tris buffer (0.1 M pH 6-10) + 200 μl substrate (*p*-nitrophenyl phosphate) and 200 μl enzyme was incubated for 30 minutes. The reaction was arrested by adding 1 ml 0.5 M NaOH. The pH stability of the enzyme was determined by pre-incubating 200 μl of enzyme with different buffers (200 μl) for 8 hrs. along with corresponding control. The amount of p-nitrophenol liberated was estimated at 410 nm.

To study effect of temperature on the enzyme activity, the reaction was carried out at temperatures ranging from room temperature 20 °C to 90 °C all for 30 min. To determine the temperature stability of the enzyme, the pre-incubated enzyme (200 μl) in buffer (200 μl) was incubated at varying temperature (20-80 °C) each

for 30 min. Then 200 μl of substrate was added to it and the reaction mixture was incubated at room temperature for 8 hrs.

Effect of metal ions on enzyme activity was checked by preincubating the demetallised enzyme with 5 mM metal ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, K^+, Fe^{3+}, $Mn^{2+,}$ Ba^{2+}, Mo^{5+}, Ni^{2+}, Al^{3+}, Na^+, Cu^{2+}, Co^{2+}, Be^{2+}, V^{5+},) for 30 minutes at 60 °C. The enzyme activity was checked after arresting reaction. Corresponding controls without metal ions were run simultaneously.

B. Immobilization of Alkaline phosphatase on MWCNTs by various cross linkers.

Alkaline phosphatase was immobilized on commercially available carboxyl functionalized multi walled carbon nanotubes MWCNTs by cross-linking with various cross linkers viz. Epichlorohydrin (ECH), Citric acid, Glutaraldehyde and 1-Ethyl -3-(3-dimethyl amino propyl) carbodiimide (EDC) as coupling agents.

C. Immobilization of Alkaline phosphatase on MWCNTs by various cross linkers and Ethyl cellulose sponge matrix.

Also, the immobilized enzyme activity studies were also studied using ECH-Ethyl cellulose Matrix and MWCNTs. For this, Ethyl cellulose is dissolved in minimum required Ethanol and then continuously mixed with ECH with constant stirring followed by MWCNTs.

The optimum pH, pH stability, optimum temperature, temperature stability and effect of metal ions also studied for both types of immobilized enzyme. The effect of concentration of enzyme and composite matrix was also studies.

III. RESULTS AND DISCUSSION

A. Biochemical studies of native and immobilized enzyme

The effect of pH on alkaline phosphatase activity from *'Arachis Hypoghaea'* seeds is shown in Figure 1.
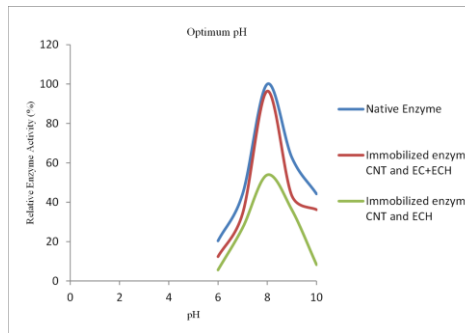

Figure 1. Optimum pH for Native and Immobilized enzyme

The optimum pH for alkaline phosphatase activity found to be 8.0. Epichlorohydrin (ECH) as cross linker is finalised based on the results of enzyme activity for 8-10 cycles when compared to other cross linking agents such as citric acid, glutaraldehyde and EDC with the 0.3M concentration. Figure 2 depicts the effect of temperature on activity of native and immobilized enzyme. The optimum temperature for alkaline found to be 60^0C for native as well as immobilized enzyme. For all studies, the reusability of enzyme showed up to 8-10 cycles.
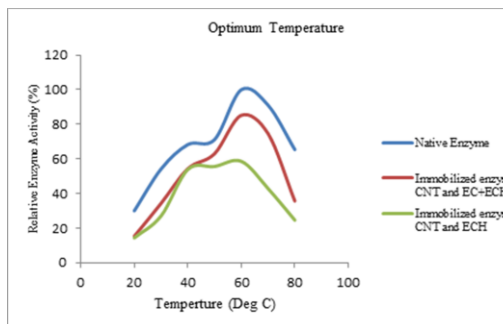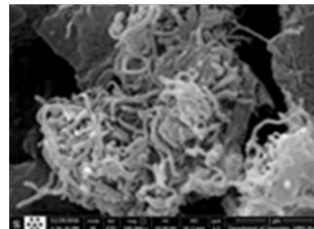

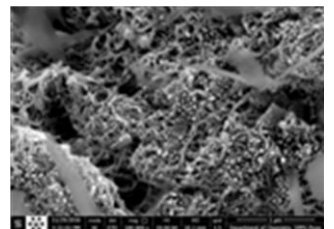Figure 2: Effect of Temperature on Native and Immobilized Enzyme
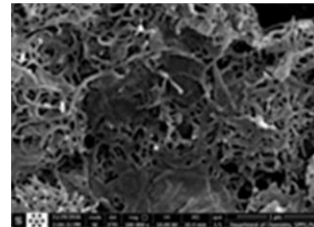
Figure 3a: MWCNT-ECH Matrix without enzyme
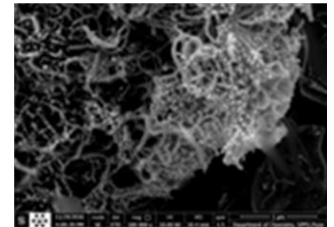
Figure 3b: MWCNT-ECH Matrix with enzyme

Figure 3c: MWCNT-ECH and Cellulose Matrix without enzyme

Figure 3d: MWCNT-ECH and cellulose Matrix with enzyme

FESEM images of MWCNT-ECH matrix and MWCNT-ECH-Cellulose sponge matrix with and without immobilized enzyme clearly shows as in Figure 3a, 3b, 3c, 3d that MWCNT-ECH-Cellulose sponge matrix exhibited randomly distributed micro-porous structures and highly 3D porous networks. As shown in Figure 3c and 3d this sponge matrix exhibits an interconnected porous structure with the pore sizes ranging from quite a few to one hundred microns which allows more free surface area for enzyme binding.
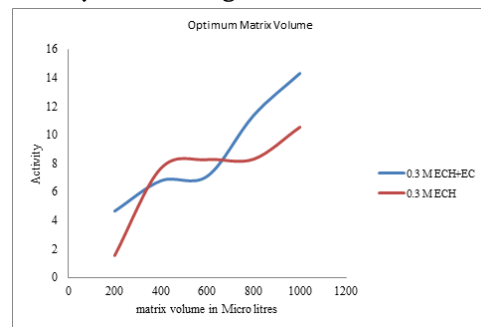
Figure 4: Effect of volume of matrix on Enzyme activity
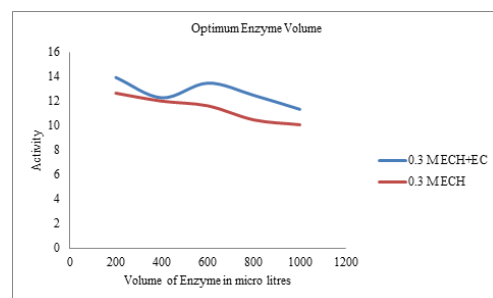
Figure 5: Effect of volume of enzyme on Enzyme activity

Superhydrophobicethyl cellulose (SEC) sponges were prepared by cross-linking EC with epichlorohydrin (ECH) and complexing with Multiwall carbon nanotubes (MWCNTs) with ratio 1: 1. Due to the presence of residual hydroxyl groups in EC backbone, EC can be cross-linked by ECH in alkaline conditions. Chemical cross-linking can increase the mechanical properties of the Sponges [10]. Also cellulose favours the high light transparency [11].

Based the enzyme activity results shown in Figure 5 and Figure 6 we finalized optimum Matrix Volume 1000 micro litre and optimum Enzyme Volume 1000 micro litre. Mixing time of MWCNT, ECH and enzyme has optimised for 2 hrs.
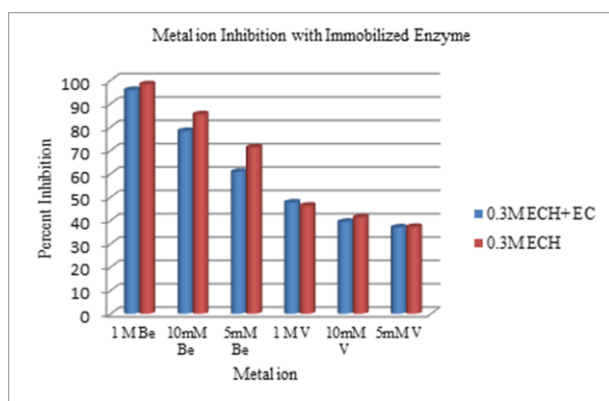

Figure 6b: Effect of Different metal ions on Immobilized enzyme activity

As seen in Figure 6a and 6b, remetallization of *'Arachis hypogaea'* alkaline phosphatase with 5 mM Be^{2+} resulted in restoration of more than 100% of the enzyme activity. Al^{3+}, K^+, Ca^{2+} and Ni^{2+} had no effect on the enzyme activity. Alkaline phosphatase was found to be strongly inhibited by V^{5+} whereas moderate inhibition was observed with Mn^{2+}, Ba^{2+}, Mo^{5+}. Considering harmful effects of Beryllium and Vanadium, we carried out enzyme activity studies for these metal ions for immobilized enzyme which depicts similar results.

IV. CONCLUSION

In this report, we explored the immobilizations of Alkaline Phosphatase from 'Arachis hypogaea' on MWNTs under several different conditions.

The characterization studies have revealed that the enzyme immobilization took place more efficiently at even 60 ^{0}C temperature ionic liquid as the medium for the better dispersion of carbon nanotubes, and the resultant immobilized enzyme displayed a good performance like Native enzyme. Further purification is in progress.

'Arachis hypogaea' alkaline phosphatase can be a used as sensor for vanadium detection since its immobilization increases its reusability without affecting enzyme activity.

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Isolation and study of Starch and Starch composite Film from source Eleusine coracana

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ABSTRACT

Starch was extracted from the seeds of Ragi (*Eleusine coracana)*. Starch was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimeter (DSC), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD). Starches, Starch -glycerol, Starch- glycerol-PVA composite film were prepared. The surface morphology was checked by SEM. Biodegradation of starch and starch composite film was studied by enzymatic hydrolysis also by soil degradation, which shows within 8 days the starch film was degraded completely.

Keywords: Biodegradation, Starch based biopolymer, Enzymatic degradation, Composting

I. INTRODUCTION

Starch is the second most abundant natural polymer on earth. It is a renewable, inexpensive and widely available resource [1]. Starch has received considerable attention because of its totally biodegradable nature. Starch has a wide range of industrial applications .These include food, textile, and pharmaceutical, paper as well as in synthetic polymer industries [2]. Starch also plays a prominent role in technology development. The modification of starch can be done by physical and chemical means as well as enzymatically. Amongst naturally occurring biopolymers, starches have the greatest potential to produce biodegradable films by different processing techniques such as casting, injection or blow molding and so on [3]. Several studies have reported the use of starches from different sources as a raw material for forming films and coating with different properties showing the potential of this carbohydrate in different industrial applications [4]. In food industry the use of biodegradable starch based films is continuously increasing [5].

Biodegradation is the process by which organic substances are broken down by microorganism aerobically or anaerobically. Microorganisms such as bacteria and fungi are involved in the degradation of natural as well as synthetic polymers. Generally, an increase in the molecular weight of polymer results in a decline in its rate of degradation. Plastic material, being non-biodegradable is a significant source of environmental pollution [6].

II. METHODS AND MALERIALS

2.1 Materials

Eleusine coracana and seeds were procured from the local market, Pune, India, for isolation of starch respectively. Glycerol (99.5%purity) methanol were obtained from Merck chemicals Pvt.Ltd. Mumbai. Polyvinyl alcohol (with Mol. wt. 1.0×10^5 and degree of hydrolysis 98%) and toluene were obtained from Himedia Chemicals Pvt. Ltd. Mumbai.

2.2 Isolation of starch

Starch was isolated from ragi seeds (Eleusine coracana) essentially as described by Adkins and Green wood (1966) with slight modifications. 100g E. coracana seeds were soaked in water for 10 h followed by washing of the steeped material with water to remove the seed coat and then mixed with 500ml water. The mixture was homogenized in a blender to form slurry

which was sieved through a sieve of 85 micron opening. The residue was again dispersed in water, and the above process repeated. The slurry containing starch was pooled and centrifuged, at 10, 000 g for 10 minutes and then washed with mild alkali (0.1 N) saline (0.145M) and finally toluene to remove the contaminating proteins and pigments. Finally the isolated starch was air dried and preserved for further use at Room temperature (R.T) [7].

2.3 Characterization of E. coracana starch
2.3.1 Total reducing sugar content
Total reducing sugar content after mild hydrolysis was determined by Dintro salicylic acid (DNSA) method using maltose as standard [8].

2.3.2 Differential Scanning calorimeter
The gelatinization temperature and enthalpy of starch was determined by using Differential Scanning Calorimeter (Perkin Elmer). Native starch sample (1 mg) was hermetically sealed in a large volume high pressure aluminum DSC pan, equilibrated at room temperature for about 1 h prior to experiment and scanned in the differential scanning calorimeter to record the calorigram. The instrument was calibrated using Indium as standard material and was programmed to rise 10 °C/min. The thermal transition of starch in terms of temperature of onset (T_o), peak temperature (T_p) and endset temperature (T_e) were recorded from calorigrams automatically. Based on the area of triangle of the calorigram, the ΔH (enthalpy) associated with gelatinization of starch was calculated [9].

2.3.3 Fourier Transform Infrared (FTIR)
FTIR spectrum of starch was recorded using Tensor 37 spectrometer between 4000 to 400 cm^{-1} (16 times per sample) with a resolution of 4.The spectrum was Fourier transformed and recorded in absorption mode, single beam spectra of the sample were obtained and corrected against the background spectrum of sample holder. IR solution software was employed for getting the spectrum.

2.3.4 Scanning Electron Microscopy (SEM)
For scanning electron microscopy, the samples were fixed on aluminum stub using double adhesive carbon tape and to make the sample conductive a thin layer of platinum (4-5 nm) was vacuum deposited on it in sputter coater (Quarum technologies).Samples were visualized for surface and cross section morphology with field emission scanning electron microscope (FESEM –Nova nanosem 450, FEI Netherland). All samples were examined using accelerating voltage of 15kv.

2.3.5 X- ray diffraction (XRD) study of E.coracana starch:
Isolated E. coracana starch was examined by X-ray diffraction to check its crystallinity, preferred orientation and average crystalline size. XRD was performed using an X-ray diffractometer (Philips PW1710, Holland) with CuKα radiation λ= 1.5405Å over wide range of Bragg angle 10-90°C, for identification of different phases. XRD analysis gives inter- planer spacing "d" (which is calculated using the Bragg law nλ =2d sinθ, where, λ is the wavelength of CuKα radiation, n is the order of diffraction and θ is the angle between the incident beam and the diffraction plane) [10,11].

2.4 Preparation of starch composite films
2.4.1 Starch film
E.coracana starch was processed into a biodegradable film as follows. An aqueous solution of starch (5%, 40 ml) was heated at 90° C under stirring for 30 minutes to get a viscous solution. The hot solution was then poured in a polystyrene petri dish and air dried at room temperature (25 ± 3°C) for 24 h to form film. The film thus obtained was carefully removed from the petri plate and used for further studies.

2.4.2 Starch Glycerol film
Starch glycerol films were prepared as follows: To 39.2 ml starch solution (5% w/v), 0.8 ml glycerol (2% v/v) was added. The hot homogeneous mixture was then spread over the polystyrene petri dish and air dried at

room temperature (25 ± 3°C) for 24 h to form film. Glycerol was used as a plasticizer.

2.4.3 Starch Glycerol Polyvinyl alcohol film

Starch glycerol polyvinyl alcohol (PVA) film was prepared as follows: 2 g PVA was dissolved in 10ml of water at 80-90 °C. To this E. coracana starch solution (2g in 20 ml distilled water) was added and the mixture heated at 80°C- 90°C. Then to the above mixture 0.8ml glycerol (2%) and 9.2 ml of distilled water were added to have a final volume 40 ml. The above slurry thus obtained containing 5% starch and 5 % PVA was stirred for 1 h at R.T .The hot homogeneous mixture was then spread over polystyrene petri dish and air dried at R.T (25 ± 3°C) for 24 h to form film. The film thus obtained was carefully removed from the petri plate and used for further studies.

2.5 Enzymatic hydrolysis of films

The biodegradation of the above film was evaluated by performing enzymatic hydrolysis using the enzymes α amylase (pancreatic) , glucoamylase (Rhizopus) β amylase (Barley) and fungal amylase (Aspergillus niger) at 37°C as follows:1x1 cm film (70 mg)was incubated with enzyme solution (0.2 mg/ml ,1 ml) and 1ml phosphate buffer pH (7.0, 10 mM)for different time intervals (0,2,4,6,8,12,24,48,72,96 h)After incubation, the reaction was terminated and the reducing sugar content determined by DNSA method [8]. Corresponding controls without enzymes were run simultaneously.

2.6 Soil degradation by soil burial

The biodegradation of the above film was also evaluated by soil burial for different time intervals upto 60 days at R.T .For this, film (2.6g) was cut into pieces and the pieces were buried under 400 gm of a 1:1mixture of   soil and compost containing organic matter for different time intervals. The pH of compost was fixed within range of 7.0-8.0. From time to time 20ml tap water was added to maintain humidity to

enable aerobic conditions. After incubation, the films were washed, air dried and weighed to check the biodegradation of films [12] .The weight loss was calculated as follows:

$$\text{Weight Loss (\%)} = \frac{Wi - Wd}{Wi} \times 100$$

(Equation 1)

Wd= Dry weight of film after washing with distilled water

Wi= Initial weight of film.

III RESULTS AND DISCUSSION

In the present work starch was isolated from the seeds of Eleusine coracana followed by its characterization and evaluation of its efficacy as an inert biodegradable support for immobilization of peroxidase.

3.1 Characterization of Starch

Starch was isolated from the seeds of E. coracana. The yield of starch was 49g / 100g seeds. The starch was characterized by DSC, FTIR spectra , FESEM and XRD.

3.1.1 DSC studies: DSC provides a quantitative measurement of enthalpy (ΔH) and facilitates measuring the energy required for gelatinization of starch. [13]. Table 1 shows the thermal properties of E. coracana starch.

Table 1. Thermal properties of E. coracana starch.

S. No	Parameter	Result
1	Onset temperature(T_o)°C	48.41
2	Peak temperature(T_{op})°C	84.47
3	End set temperature(T_e)°C	131.23
4	Gelatinization temperature(T_e-T_o)°C	82.82
5	Heat Enthalpy(ΔH)(kJ/g)	79.43

3.1.2 FTIR

In the IR spectra of the ragi starch (Fig.1) the peaks obtained at low wave numbers correspond to the skeletal mode vibrations of the glucose pyranose ring, whereas the band at 929cm^{-1} was due to the glycosidic linkages in starch. The vibrational band related to the C and H atom was observed between1500–1300cm^{-1}. Water adsorbed in the amorphous region of starch was identified as a broad band at 1640 cm^{-1} while the C-H and O-H stretching mode showed bands between 2800-3000 cm^{-1} and 3000-3600 cm^{-1} respectively [14].
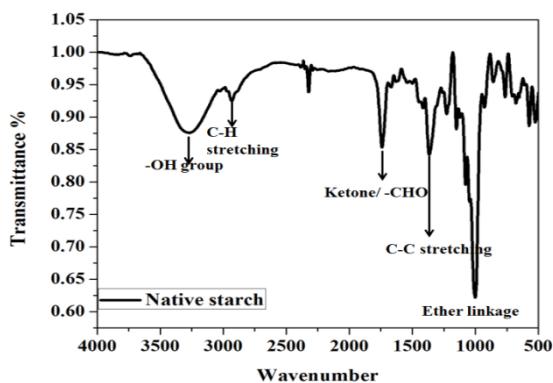

Figure 1. FTIR spectra of starch

3.1.3 Scanning Electron Microscopic studies

Figure 2 shows the scanning electron micrograph of native starch As seen in the micrograph, most of the native starch granules were of polygonal and irregular shape with sizes between 3 -7 μm [13].
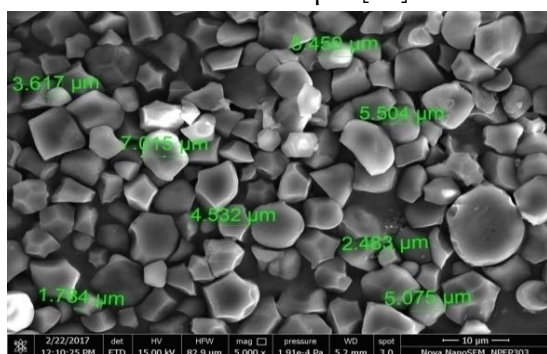

Figure 2. SEM of starch.

3.1.4 XRD studies: Scattering angle 2θ at which diffraction intensity was observed and the spacing was used to discriminate the plane of different sites on ragi starch granules. X ray diffractogram of native starch showed a typical A type of diffraction pattern with strong reflection at15& 23°C & degree of crystallinity of native starch was 30.09% [13].
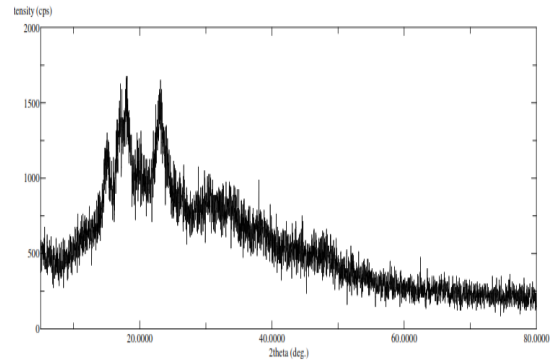
Figure 3. XRD of starch.

3.2 Biodegradation of Starch and starch composite films

All the films were degraded by different enzyme amylases within 24 h. After 24 h there was no change in degradation pattern.As seen in figure 4, the degradation of starch and starch glycerol films was more as compared to the starch glycerol PVA films (15).
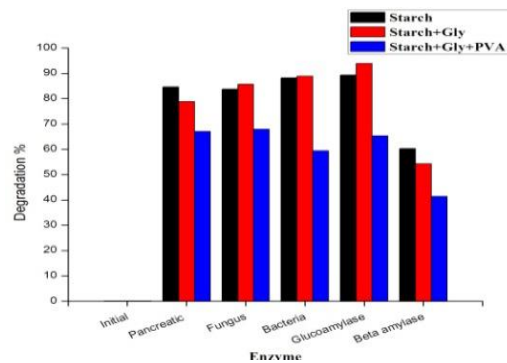
Figure 4. Degradation of starch and composite film by DNSA for 24 h.

3.3 Soil degradation

The weight loss rate during the soil burial compost tests of all starch based films was higher than the weight loss rate of the cellulose film used as a control. Most of the degradation was observed within the first 8 days due to composting (Table 2). It has been suggested that composting leads to an increase in the temperature which results in rapid microbial activity. As compost is rich in organic matter aerobic degradation is observed. Overall the degradation of starch, starch glycerol film was faster compared to starch -glycerol-PVOH composite film. The highest

percentage of weight loss was observed for ragi starch film and starch glycerol composite film within 8 days, whereas the lowest percentage of weight loss was observed for starch glycerol polyvinyl alcohol within 60 days (15).
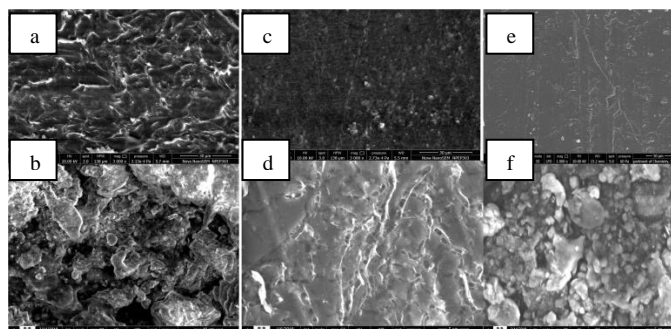
Figure 5. SEM images of a.Starch film(Before degradation) b.Starch film(After degradation) c. Starch glycerol film(Before degradation) d. Starch –glycerol film (After degradation) e. Starch glycerol PVA film(before degradation) f. Starch glycerol PVA film(after degradation).
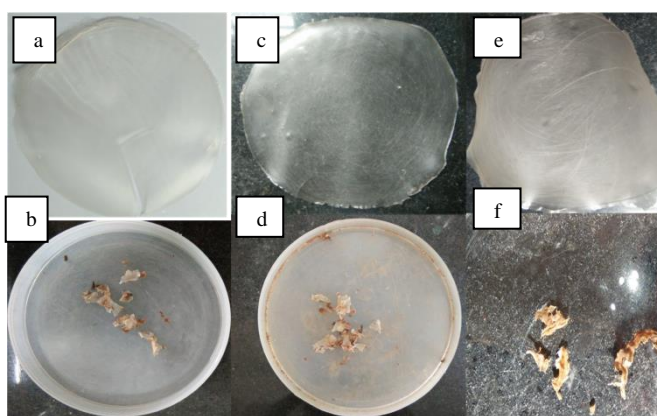
Figure 6. images of a.Starch film(Before degradation) b.Starch film(After degradation) c. Starch glycerol film(Before degradation) d. Starch –glycerol film (After degradation) e. Starch glycerol PVA film(before degradation) f. Starch glycerol PVA film(after degradation).

Tabel 2. Degradation of different starch and starch composite film in soil.

Film	Degradation %(w/w)	No of days
Starch (5 %)	74.7%	8
Starch glycerol (5%,2%)	85.4%	8
Starch gly PVA composite (5%,2%, 5%)	93.23%	60

IV. CONCLUSION

New processing techniques and the current demands of biodegradable and renewable resources have highlighted the versatility of starch and introduced it to new markets. Today starch has moved from its traditional role as food to being an indespersible safe, effective tool in industry, food and medicine, due to its adhesive thickening, gelling, swelling and film forming properties.

In the present work, starch from *E. coracana* seeds has been isolated and some of its properties have been studied. Biodegradable Starch, Starch glycerol, starch glycerol films were prepared and their biodegration were checked by Enzymatic degradation and soil buried, Compost environment is the ideal environment for degradation due to microbial environment and their diverse enzyme. Enzymatic method of is rapid method to check biodegradation.

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Biochemical Studies on Peroxidase from the Seeds of Macrotyloma uniflorum

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ABSTRACT

Peroxidases are the heme containing enzymes that catalyse oxidation of substrates like phenolic compounds by reducing hydrogen peroxide concurrently. They play a key role in a number of metabolic processes such as regulation of cell elongation, lignification of cell wall, phenolic oxidation and defence against stress. Peroxidase was isolated from 4 days old seedlings of Macrotyloma uniflorum followed by germination study. The enzyme showed optimum pH 6 and optimum temperature 30℃. Peroxidase showed a stability over a broad pH range (4 to 9). Enzyme also was found to be stable up to 70℃. Kinetic studies were also performed.

Keywords : Peroxidase, Macrotyloma Uniflorum, Seedlings, Hydrogen Peroxide.

I. INTRODUCTION

Peroxidase (E.C. 1.11.1.7) is an oxidoreductase enzyme which reduces H_2O_2 to water and oxidizing variety of phenolic compound like diphenol, polyphenol, aminophenol and much more which act as proton donor in the reaction.(1) The majority of reactions catalysed by the peroxidase can be express as follows:

$$H_2O_2 + 2RH \xrightarrow{\text{Peroxidase}} 2H_2O + 2R\cdot$$
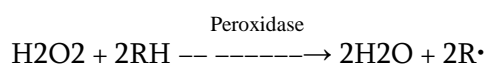
Peroxidase is a heme containing glycoprotein which is widely distributed in living organism including microorganism, plants, and animals (2). In plant kingdom it is widely distributed. Extraxction of peroxidase from several plants also reported like Horseradish (Armoracia rusticana), peach (Prunus persica), turnip (Brassica campestre rapifera), Caribbean plant (Euphorbia continifolia), Date palm leaves (Phoenix dactylifera L.), sweet potato (Ipomoea batatas ex L., Lam) etc (3).

Plant peroxidases play various physiological roles in plant growth and devolopment. It is mainly involved in rigidification and lignification of cell walls, synthesis of indole acetic acid (IAA), organogenesis, phenol oxidation, plant defence system during pathogenic attacks , wound healing , leaf senescence, ethylene biosynthesis , scavenging of peroxides and various environmental stress conditions etc. (4)

Peroxidases are having a prominent position in biochemical research and biotechnology (enzymology, biochemistry, physiology, histo chemistry, medicine and genetics) (5). Commercially peroxidases are used for production of secondary antibody in different process. Horseradish peroxidase (HRP) has been used in ELISA experiment as an important reagent. It is also used in various diagnostic kits. (8) Because of its wide substrate specificity peroxidase is used in waste water treatment, such as removal of phenolic compounds and in dye degradation process. (9)

Construction of biosensor using immobilised enzyme on different matrices is also an important emerging application of peroxidase.(10-14) The ability of enzyme to perform oxidation of H_2O_2 is used for developing reliable methods for detection of hydrogen peroxide generated in various biological as well as industrial processes.(15,16)

Although presence of peroxidase has been reported in a variety of plant sources, purification of peroxidase from seedlings of Macrotyloma uniflurom is not reported. The main purpose of this study is to determine different biochemical properties such as temperature and pH optima, stability of enzyme at various temperature and pH, kinetic studies and its purification.

II. METHODS AND MATERIAL

Chemicals:

Guaiacol, Sodium Chloride, Sodium Phosphate monobasic, Sodium Phosphate dibasic, Ammonium sulphate, hydrogen Peroxide from SRL.

Purification of peroxidase:

Peroxidase was isolated from 4 days old seedlings of Macrotyloma unifluroum. The seedlings were grown from 100gm of seeds under controlled conditions which was followed by checking enzyme activity after different time intervals. The 4 days old seedlings were washed with distilled water and homogenised with 500 ml of physiological saline (0.145M, 0.85% NaCl) for 4 h at 4°C. The extract was filtered through cheese cloth and supernatant was clarified by centrifugation at 4°C (8000 rpm 10 min) and subjected to ammonium sulphate fractional precipitation (60-80%). The obtained pellet from 60-80% saturation was dissolved in minimum amount of distilled water and dialysed extensively against distilled water and finally against phosphate buffer (pH 7, 10mM). The dialyzed protein (Fraction A) solution clarified by centrifugation and applied on an ion exchange (UNO Sphere) column equilibrated with phosphate buffer (pH 7, 10mM) followed by washing of the column with equilibrating buffer till protein absorbance at 280nm was ≤ 0.02. The adsorbed proteins were eluted with a discontinuous gradient of NaCl in the same buffer. Fraction of 5ml each were collected at flow rate of 15ml/h and monitored for its protein content by taking the absorbance at 280nm and peroxidase activity were checked.

Enzyme assay:

Peroxidase activity was estimated by George's method at room temperature as follows (17). The 1.5 ml reaction mixture contain 0.5 ml of phosphate buffer pH 6.0(10mM); 0.5 ml of 20 mM guaiacol (2-methoxyphenol); 0.250 ml of 10mM H_2O_2; and 0.250 ml of enzyme extract. The reaction was carried out at room temperature and time required for change in optical density bye 0.1 observed at 470nm (ϵ = 26.6 cm.$^{-1}$ mM $^{-1}$). The amount of enzyme catalysing the oxidation of 1 mmol of guaiacol in 1 min represents one units of peroxidase activity (18).

Biochemical characterisation
Effect of pH

Optimum pH of the enzyme was determined as follows: The 0.250 ml peroxidase was incubated at 30 °C with 0.5 ml of different buffers in the presence of 0.250 ml guaiacoal (20 mM). Buffers used were sodium citrate buffer (20 mM, pH 3.0), sodium acetate buffer (20 mM, pH 4.0 -5.0), phosphate buffer (pH 6.0 – 8.0), Tris-HCl buffer (20 mM, pH 9.0), Glycine-NaOH buffer (20 mM, pH 10.0). The peroxidase activity was determined as described earlier.

pH stability of peroxidase:

The pH stability of peroxidase was determined by incubating 0.250 mL of enzyme with different buffers (0.5 mL) for 1 h at 30 °C. After incubation, pH was adjusted to 7.0 using acid or alkali and the peroxidase activity was determined using 0.5 mL guaiacoal in presence of 0.250ml H_2O_2 (10 mM)   as described above.

Effect of temperature on Peroxidase activity

The temperature optima of the peroxidase were determine as follows : The 0.250 ml of enzyme were pre-incubate with 0.5ml of phosphate buffer (20 mM, pH 7) and 0.250ml guaiacol (20 mM) for 10 min for different temperatures . The reaction was carried out at temperatures ranging from 10 – 80 °C followed by addition of H_2O_2 (10 mM) for same temperature. The peroxidase activity was determined as described earlier.

Temperature stability of peroxidase:

The effect of temperature on peroxidase stability was determined by incubating 0.250 ml of peroxidase and 0.5 ml of buffer at varying temperatures (range 10 – 80 °C) each for 1 h. After pre-incubation with 0.250 ml Guaiacol the residual peroxidase activity was determined by addition of 0.250 ml of H_2O_2 (10 mM) at 30 °C as described above.

Kinetics studies:

Effect of substrate concentration on enzyme activity:

The rate of peroxidase activity was determined by varying concentrations of H_2O_2 (10, 20, 30, 40, 50, 60, 70, 80, 90 and100 mM/ml) at fix saturating concentration of Guaiacol. The same method was used to determine kinetic behavior of enzyme with keeping saturating concentration of H_2O_2 constant and varying concentration of Guaiacol (10,20, 30, 40, 50, 60, 70, 80, 90 and100mM/ml). The reaction was carried out as descried earlier. The peroxidase activity was determined and a double reciprocal plot was drawn according to the method of Lineweaver and Burke. The K_m and V_{max} of the enzyme were determined.

III. RESULTS AND DISCUSSION

The peroxidase isolated from seedlings of Macrotyloma uniflorum was purified with ammonium sulphate fractional precipitation (60-80%) followed by ion exchange chromatography. The column was pre equilibrate with phosphate buffer (pH 6, 10 mM). The dialysed clear protein solution applied on Cation exchanger column Uno-Sphere. The adsorbed proteins were eluted with a discontinuous gradient of sodium chloride (0.1, 0.2, 0.3, 0.5 and 1 M) in same buffer. Fractions of 3 mL each were collected at a flow rate of 20 mL/h and monitored for protein content by spectrophotometer at 280 nm. As seen in the elution profile (Fig 1), peroxidase was eluted as a major peak at 0.1 M NaCl concentration. These peroxidase rich fractions were used for further studies.
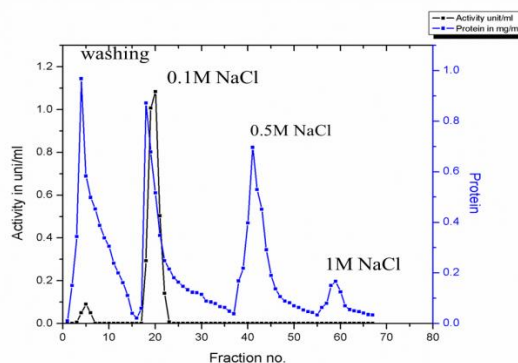
Figure 1. Elution profile of UNOsphere ion exchange chromatography

Table 1. Purification Table

Purification steps	Protein conc. mg/ml	Enzyme activity (u/ml)	Specific activity (u/mg)	Fold purification
Saline Extract	29.56	1.8	0.060	1
Ammonium Sulphate precipitation	22.7	2.811	0.19	2.08
Ion exchange chromatography	1.09	3.484	3.19	53.61

Effect of pH on peroxidase activity:

As shown in the figure 2, purified peroxidase enzyme showed optimum activity at pH 6 and purified peroxidase was found to be stable over a broad pH

range i.e. 3.0- 9.0 same results also reported in Caribbean plant: Euphorbia cotinifolia (4)
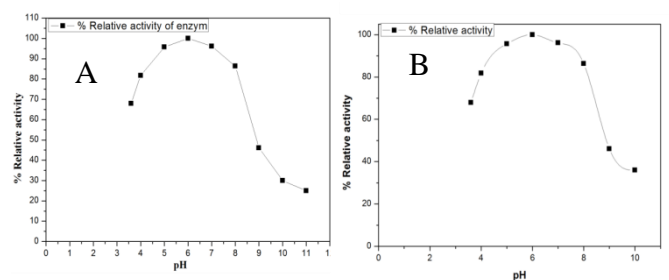
Figure 2. A) pH optima B) pH stability of peroxidase

Effect of temprature on peroxidase activity:

Purified peroxidase showed maximum activity temperature at 30°C and it was found to be stable in the range of 10–70°C. The maximum activity of enyzme betwwen 25-30°C also reported for the same soure in the tissue culture study. (19) Where as in leaves of Copaifera langsdorffii it was found 35°C. (**3**)
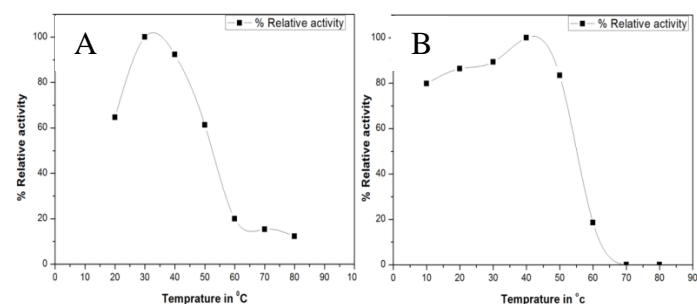
Figure 3. A) Temperature optima B) Temperature stability of peroxidase

Kinetic studies

In the figure4 (A) shows Michalies –Menten curves using guaiacol as variable concentration at saturating substrate concentration of H_2O_2 (10mM). While fig (B) represented Lineweaver Burk plot with km value of guaiacol is 40mM. In case of Daucus carota it was found 1300μM. (20)
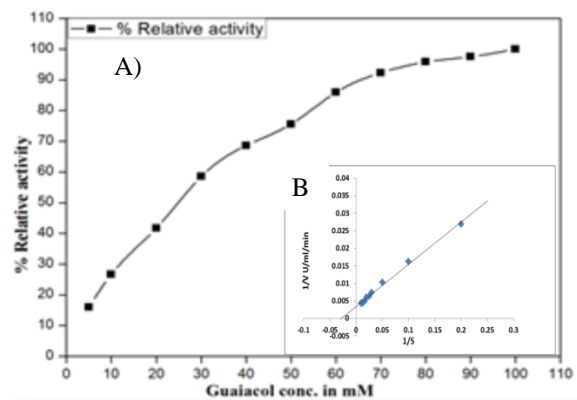
Figure 4. A) Michalies –Menten curves using guaiacol as variable concentration at saturating substrate concentration of H_2O_2. B) Lineweaver Burk plot

Same process was applied to found Km value of H_2O_2 by Michalies –Menten equation using H_2O_2 as variable concentration at saturating substrate concentration of guaiacol (20 mm). Fig5 (A) represented Michalies – Menten curves Fig5 (B) shows Lineweaver Burk plot with km value of H_2O_2 is 2mM. In case of Daucus carota it was found Km valu for guaiacol 1300μMand for H_2O_2 50μM (20).
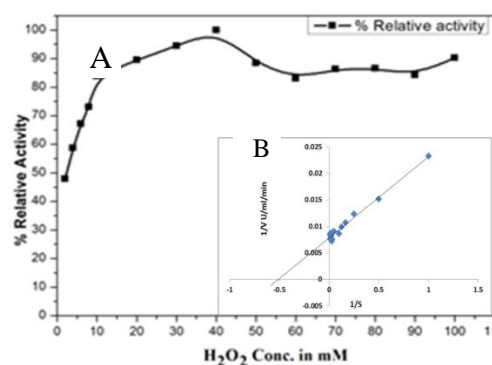
Figure 5. A) Michalies –Menten curves using as H_2O_2 variable concentration at saturating substrate concentration of guaiacol. B) Lineweaver Burk plot

IV. CONCLUSION

Peroxidase isolated from Macrotyloma uniflorum purified by cation-exchange chromatography successively with fold purification 53. The purified peroxidase shows broad pH and temperature range respectively 3-9 , 10-70 ° C. the optimum pH of

enzyme found at pH 6 and temperature optima observed at 30°C. In the kinetic study the Km value for guaiacol was found 40mM and for H_2O_2 2mM.

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Urban Mushroom Farming

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ABSTRACT

Nowadays, mushrooms are gaining popularity in cities due to its high nutritional value and awareness about health. There are several varieties of edible Mushrooms under cultivation but still Button Mushroom' is dominating in terms of production and consumption. People are interested to cultivate button mushrooms on their own but the constraint is heavy initial investment and more space needed for compost making. In the present study, button mushroom composting is carried out by many Mushroom units across India. But only few sells spawned ready to grow compost bags to the urban population for its growing at their houses. Mushroom Club, Pune has initiated this unique kind of concept – 'Urban Mushroom Farming' in Maharashtra in recent years. Necessary training is provided to them so as to acquaint them with the technology. It was found that from a bag of 10 Kg compost which costs Rs.100/- to the grower and in return they were able to harvest around 2 Kgs of fresh Button Mushrooms in just two months time period and earn Rs.260/-. One grower can easily place 300 bags in 300 sq.ft. room.   There is a tremendous potential for button mushroom farming in urban areas especially in Societies provided if they can get ready to grow Compost bags through centralized composting facility made to be available in nearby areas. It is concluded that the unemployed youth can raise 'Centralized Composting Unit' (CCU) through Prime Minister Mudra loan scheme and provide compost bags to the urban population to fulfill their need of quality protein rich food and earn handsome income.
Keywords : Button Mushrooms, CCU, Spawned bags.

I.   INTRODUCTION

Mushroom is an excellent source of Proteins, vitamins, minerals, antioxidants, fibers and Folic acid. Mushrooms especially button mushroom is gaining popularity due to its more self life and public awareness. In India Cultivation of Button mushrooms under controlled condition is of recent origin. Earlier its cultivation was restricted to hilly regions of Ooty, Solan, Jammu Kashmir, Dehradun etc. as seasonal growing.

Now a day, button mushrooms are commercially cultivated throughout the year in various states like Maharashtra, Tamil nadu, AP, MP, UP, Delhi and Gujarat using controlled climatic conditions and modern technology to fulfill the market demand. Mushroom cultivation has now become a household name in almost all regions in India Mehta et.al. (2011). Urban Farming is gaining popularity in India for growing varieties of organic and exotic vegetables in densely populated cities. Growing relatively expensive vegetables on open terrace, balcony and at unused space saves money as well as on bartering of produce. Urban people generally spend a substantial part of their income (50 – 70%) on food and growing own food can help to save household expenditures. Urban farming is a profitable option when producing products are perishable and with high demand.

Growing vegetables is comparatively cheaper than cultivation of Mushrooms. Attempts were made before to cultivate Oyster mushrooms at home on large scale but lack of market for its production declined. On the other hand, for Button mushrooms, market is at door step. But the difficulty in urban area is space and investment for the preparation of compost. Considering this, exclusive CCU (Centralized Compost Unit), nearby city area can cater as a facilitator among City Growers. Demand of pasteurized and synthetic compost for white button mushroom is increasing day by day near the towns because of urban people accepted mushroom cultivation as kitchen garden. People who have not the facilities of pasteurization want to cultivate mushroom. Establishment of synthetic compost or pasteurized compost preparation unit could be a choice for entrepreneur or self-employment generation Kumar et.al. (2013).

II. MATERIALS AND METHODS

During experiment in the years 2015-2016, composting was carried out at Mushroom Division, Agricultural College, Pune whereas growers were carried out Button Mushroom Growing at their premises located at Mahableshewar, Panchgani and Pune City. Standard Compost Bags of 10 Kg each mixed with 100 gm spawn (Seed) were distributed to the individual grower, Women Self Help Groups and Farmers. Proper onsite training was given to them by Mushroom Club, Pune to improve mushroom quality and yield. Different compost batches were made and monitored on a regular basis by our technical team and mushroom yield was measured time to time.

Compost Batch:
Raw materials used - Wheat Straw, Chicken Manure, Gypsum and De Oiled Cake (DOC). All these raw materials were procured locally.

Compost Preparation:
Formulation –Wheat Straw 6.5 Tons, Chicken Manure 3.5 Tons, Gypsum 500 Kgs and DOC 500 Kgs.
Formulation Nitrogen = 1.75 % and C: N Ratio = 24: 1

Phase I – Composting
1 to 5 days- **Pre Wetting**, Mixing and Heap Making, Turnings while Heap Shifting etc. were made on a concrete platform using Goody Pit water (recycled). Manual process are used to avoid expensive machineries like JCB, Mixing and Turning machines due to a trial and smaller volume batch. During phase I days, Compost Heap Moisture (60%) and the Temperatures (50^0C,) were monitored and maintained to built up microbial mass.

6 to 15 days - **Bunkering**. Partially decomposed compost was filled into a specially built open type Bunkers having an aeration system underneath. After every 3 days, manual shifting of compost made from one Bunker to another. Total 2-3 shifting is necessary to get better caramelization of compost. During bunkering period, Compost Moisture (75%) and temperature (80^0C,) were closely monitored and controlled.

Phase II – Pasteurization.
On 16th Day, after all the quality parameters check at QC Lab, fully caramelized compost from Bunker is filled into a Pasteurization Chamber (Tunnel). Different temperatures at various stages like Leveling, Warming Up, Pasteurization, Conditioning, Cooling down were maintained strictly in a 7 days cycle. Major part of Phase II – 'Pasteurization' is done at 58 to $60^0C$for about 8 to 10 hours depending on the disease level of the farm.

Spawning: Pasteurized compost is being mixed with about 1% spawn (strain-Sylvan A15 Intermediate hybrid) at a very hygienic place and condition. Spawn is made available locally from a Hind Mushroom Spawn Lab. Rajgurunagar. About 10 Kgs compost is filled in a specially designed HD bags having few small holes to avoid water condensation due to its self heat process.

Growing: Spawn mixed bags were shifted to the cleaned Mushroom growing units with proper handling method. Special care always was taken that; compost temperature should not cross beyond 30^0C.Button Mushroom Growing is total 60 days cycle

and the stages were Spawn Run (15 days at 27^{0}C Temp), Case Run (8 days at 25^{0}C.Temp.),Flushing/Pinning (12 days at 20^{0}C. Temp.) and later Mushrooms Harvesting (20 days at 18^{0}C Temp.). Rest 4-5 days needed for the surplus operations like delayed stages and rooms fumigation etc.

During cropping period, regular watering practices were carried out as per the QC standards. Also there was a Pest and Disease management schedule, Post Harvest management practices like packing, cold storage, transport etc.

Button Mushroom Production: Button mushroom production is totally depends on the quality of compost, Spawn and the growing parameters. Here we have conducted two trials using same quality compost and spawn. Only the growing climatic conditions were differs like - **1)** Women Self Help Groups and the Farmers from Mahableshewar and Panchgani grown mushrooms using natural environment without using air coolers and AC's. Whereas **2)**City grower, Dr. Swati and Mr. Mahesh Kulkarni from Pune have successfully grown good quality button mushrooms using Air Conditioner to control climate throughout crop cycle.

III. RESULTS AND DISCUSSION

Nearly One to Two Kg button Mushrooms were harvested from a single compost bag. Hilly areas like Mahableshewar and Panchgani growers were obtained average 1.47 kg mushroom per bag with low cost growing method i.e. without any cooling arrangement. Whereas grower from Pune Dr. Swati and Mr. Mahesh Kulkarni obtained average 1.95 Kg Fresh mushrooms/bag from 100 bags as cooling facility was available in growing room during fruiting. It is found that commercial button mushroom production is possible with low investment if spawned compost bags are readily available. It was also found that transportation of compost bag is possible immediately after spawning without affecting quality and quantity of the produce.

Urban Mushroom farming is possible through centralized composting units. In India under National Horticulture Mission, financial assistance up to Rs.20 lac is available for Centralized composting unit and it is 100% of the cost to public sector and in case of private sector, 50% of cost, as credit linked back ended subsidy. Mushroom cultivation through centralized composting unit can provide income opportunities to urban youth. Similar findings are reported by Easin et.al. (2017) while studying Mushroom Cultivation as a small-scale family enterprise for the alternative income generation in rural Bangladesh. Celik and Peker (2009), reported that, price determination is the most important input in the mushroom production mainly the "Compost". Producers face various problems in making good quality and homogeneous compost. To eliminate this deficiency from mushroom cultivation, establishment of Centralized Composting Unit is of paramount importance.

In this present study, the cost of compost bag of 10 kg. is considered around Rs.100/- whereas cost of 3 kg casing soil is Rs.20. Total cost of the bag was around Rs.140/- including transportation cost from Centralized Composting Unit. One bag produced around 2 Kg of fresh button mushrooms. The mushrooms were sold by the growers at a wholesale rate of Rs.120/- per Kg. and the growers can earn Rs.100/- per bag. Apart from this, spent compost (waste compost at the end of crop) is also converted into organic fertilizer and sold at retail rate of Rs.10/- per Kg. Roy et.al (2015), reported that use of spent compost as a bio fertilizer and improvement in the growth of Capsicum annuum L. crop.

It was also found that in a room of 100 sq.ft. area, one can accommodate 100 bags and earn Rs.12,000 in a month. Zhang et.al. (2014), describes the rapid growth of mushroom cultivation and its contribution to food

security and rural sustainable development and also examined the roles of bio-innovation, technological dissemination, and marketing.

IV. CONCLUSION

Button mushroom cultivation is possible without heavy initial investment in urban areas if centralized composting facility is available in nearby area. It was also found that, if someone provides ready to grow compost bags to the city growers, they can easily grow even better quality button mushrooms provided if split AC is available to maintain temperature during fruiting. It was also recorded that transportation of compost bags immediately after spawning in possible without any reduction in yield.

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Tourism Potential of Forts in Pune District with the help of Geospatial Technology

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ABSTRACT

The total information regarding natural resources viz. physiography, climate, soil, water, natural vegetation (pattern and type of flora), animals (fauna) etc. as well as human factors like population (distribution and growth), settlement pattern, transport and communication facilities, festivals and other cultural activities is difficult to get from a single body and in short time for the above task. A database for an area, if generated will be useful for the planners, government, economists, echo-tourists, researchers and common man. Pune district is highly rich by its scenery, spiritual base and its culture. In this present study try to provide an integrated plan for tourist places forts in the Pune district with some special case studies.

Keywords: Tourist Information System (TIS), sustainable planning

I. INTRODUCTION

Tourism is considered as one of the world's largest industry. India is a developing nation. The Government and their agencies as well as private sector units and individuals are taking various measures to promote tourism. Promotion of tourism can contribute immensely to our economy. Many years tourism was neglected at various levels but now a day's concentrated effort are being made to improve the position and standard of tourism and for also the social benefit of the people. Pune district is highly rich by its scenery, spiritual base and its culture. Tourism industry will change the future of the district and will improve the social, cultural, economic status of the district; this was the main motive behind selection of this study area.

Maharashtra is a land of forts with its 350 odd forts. The ASI, an agency controlled by the union government, controls 29 of the important forts. The state archaeological department controls 39 other forts and 99 forts are unprotected forts. The remaining 183 forts are either controlled by the revenue department, which knows little about archeology or are privately owned. This means that most forts are at the mercy of those who know nothing about conservation. What's more, there is no record of the ownership of some forts. The 350 odd forts in Maharashtra were constructed since the time of some of its early ruling dynasties like the Satvahanas, the Rashtrakutas, the Chalukyas, the Siddhis, the Marathas, the Peshva, the British, etc. They were a primary defense mechanism against enemy invasions and were called 'Killa' in local language. Konkan has various forts built by many rulers like Portugese, British, French, Dutch, Siddhis and Marathas.

Pune district lies in the Western Ghats or Sahyadri mountain range and it extends on to the Deccan plateau on the east. With its physiography it has abundance of forts and fortress located in it. With the long-standing bastion of the maratha empire and home to the legendary King Shivaji, the Pune district

is marked by magnificent land forts, testimony to its glorious past. Every taluka in Pune district has significant forts located in the district. It is necessary to preserve and conserve them, as well as it can be great potential for creating job opportunities for the local people. Most of the forts in Maharashtra are in dilapidated condition. It represents history, they must be preserved. The study will help to aware people to conserve these sites. Tourism is the major subject of jobs and other means of subsistence for the local people at fort sites increasing number of tourists will surely be beneficial to the local people. They can earn some amount by providing meals and other things needed by the visitors. Business and jobs for locals will be created due to conservation of forts.

II. STUDY AREA

The study area, Pune district, usually termed as the cultural capital of Maharashtra. It is situated at 559 m ASL and lies between 17º 54' to 19º 24' N Latitude and 73º 33' to 75º 10' E Longitude. The total geographical area of the district is 15643 sq.km. The district headquarters or the district place is Pune. The district consists of 14 tahasils. The area of district is surrounded by Thane district in the north and northwest, Raigarh district in the west, Satara district in south, Solapur district in the south and southeast and Ahmadnagar district to the east.
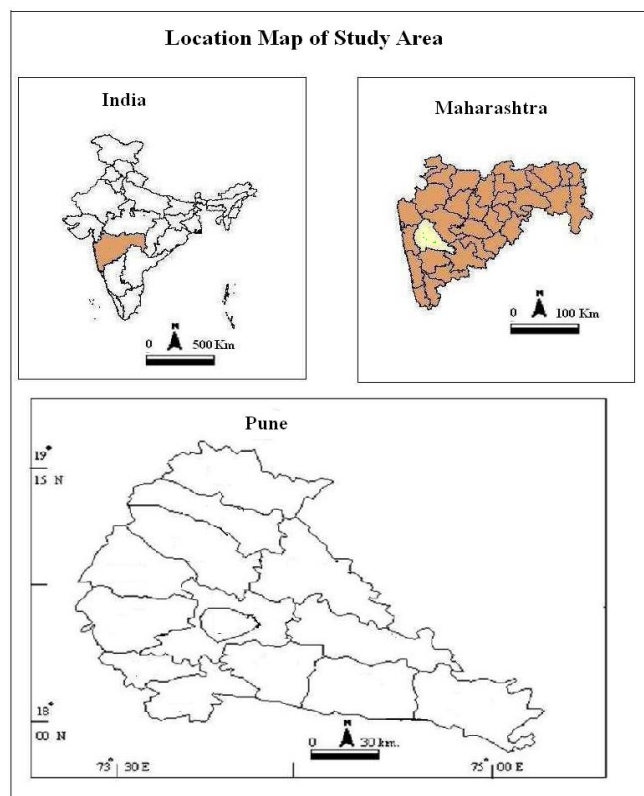
Figure 1. Location Map of Study Area

III. OBJECTIVES

The broad objectives of the proposed study are:
1. Assessment of site and situation of forts in Pune district.
2. Assessment of present day fort tourism in Pune district.
3. To identify and examine the tourism potential of the forts in Pune district.

IV. METHODOLOGY AND DATABASE

In order to understand for tourism potential of forts in Pune district the methodology adopted for the present study is divided into three phases are namely pre-field work phase, field work phase and post field work phase.

In the first phase i.e. pre-field work phase literature review i.e. previous work carried out by other researchers are obtained from various journals, internet, visit to the MTDC resort to know about annual tourist flow, collection of survey of India

toposheet (SOI) having scale 1:250000 (47/E,47/F,47/I,47/K,47/M,47/N,47/O), Atlas, Gazetteers, District Census Handbook, Tourist maps, etc. use for collection of information, District Resource map of Pune district published by Geological Society of India, Government published map of Pune district P.W.D. map, and other were completed with help of S.O.I. toposheets.

In the second phase i.e. fieldwork phase extensive field surveys were undertaken, to existed tourist places and newly found tourist places. The questionnaire filled in this phase. During this field surveys tourist facilities regarding destination photographs, GPS reading altitude and the related information is noted which were also useful to site suitability study.

In the third phase i.e. Laboratory worked is carried out. Government published map then digitization to generate thematic layer i.e. point layer- tourist places, tahasil headquarter, line layer- roads, railways, rivers and polygon layer- dams, reservoirs etc. and map were georeference same time. Integrating all above information and maps with help of GIS software. Finally these thematic layers are analyzed to demarcate tourism potential zones.

V. TOURISM POTENTIAL OF FORTS IN PUNE DISTRICT

The term potential means something existing but not yet fully exploited. There are various criteria to selecting new potential suitable sites i.e. on the basis of phsiography, climate, and purpose of visit, on the basis of natural and cultural resources. We classified potential suitable sites forts on the basis of natural and cultural resources, i.e. Pune, Haveli, Junnar and Baramati. Potential tourist centers are selected in this manner they represent the entire Pune and same problem found in similar type of tourist centers in throughout the district. Potential forts are very rich in natural and cultural resources, these tourism resources

are not fully utilized because inadequate tourist facility, lack of information regarding this centers. New potential suitable sites forts can provide more scope for a generation of employment opportunities due to increases tourist facilities in terms of different socio economic and marketing indicators also bring money and reducing the regional disparities in Pune district. New potential suitable sites forts will be reducing the pressure on existed tourist centers and also reducing the migration of local peoples towards the Pune city. There are 32 forts in Pune district. Every fort has its own importance and uniqueness. The forts are selected according elevation controlled sampling (Table 1).

Table 1. Forts Selected For Study

Sr. No.	Tahashil	Fort and Height (m)	Major Forts Selected for study
1	Maval	Induri 595, Anghai 612, Tung 964, Tikona988, Lohgad1016, Visapur 1045, Morgiri 1052	Tung, Tikona, Lohgad
2	Bhor	Kavlya 597, Kenjalgad 1267 Raireshwar 1375, Rohida 1095	Raireshwar
3	Khed	Chakan 618, Bhorgiri 735	Chakan, Bhorgiri
4	Mulshi	Rajmachi 692, Korigad 929, Kailasgad 944, Ghangad 982	Rajmachi, Ghangad
5	Purandar	Daulatmangal 815, Sonori 953, Vajragad 1290,	Purandar

		Purandar 1320	
6	Junnar	Narayangad 820, Jivdhan 898, Nimgiri 980, Junnar 998, Shivneri 998, Chavand 1010, Sindhola 1043, Hadsar 1431	Nimgiri, Shivneri
7	Velhe	Rajgad 1307, Torna 1358	Rajgad, Torna
8	Haveli	Sinhgad 1308	Sinhgad
	Total	**32**	**14**

I. ANALYSIS OF FORTS: SHIVNERI

A. Location

Shivneri is situated in Junnar tahasil and 93 km away from Pune. Shivneri is located at $19^0 13'$ north latitude and $73^0 49'$ east longitudes. Junnar is nearest town to Shivneri which is approximately 1km away. The altitude of Shivneri is 998 m. The area of the Junnar is 2.65km^2 (*Figure 3, 4*).

Figure 2. Tourist information system, Shivneri Fort

Figure 3. Google Image, Shivneri Fort

A. **B. Geology**

The Shivneri fort is the part of Deccan Plateau and hence rock formation is associated with the flows of basic lava. There are two types of traps, viz. Massive and Zeolitic. Massive trap is found on the large scale as compared to Zeolitic trap in the Shivaneri according to the Geological Survey Department.

C. **Historical Background**

The Shivneri Fort was built during the time of Satavahanas. After the Satavahanas, the Shivneri Fort was occupied by the Shilaharas, the Yadavas, and the Bahamanis. In 1599 AD the hill fort was granted to Shivaji's grandfather, Maloji Bhosale and passed down to Shahaji. Jijabai (Shivaji's mother) spent the days of her pregnancy at this fort and this is the birth place of Shivaji, the famous Maratha warrior who was born in 1630 AD. Shivaji spent his childhood in Shivneri Fort and also undertook his military training here.

There are two routes to top of the fort. One path takes you via stone steps dug out from the natural strata. The second is a rough pathway that was extremely hazardous with the help of a parapet and chain barricades. The fort has a total of seven doors Maha Darwaja, Parvangicha Darwaja, Hatt Darwaja, Peer Darwaja, Shipai Darwaja, Patak Darwaja, and Kulambkat Darwaja, The fort tapers to one side. Its northern face is called Kadeloat. To reach the fort, climb 300 m steep slope where to the northern face a huge lake is constructed named Badami Talav. Around

the lake are remnants of quarters and bungalows. There is much for the tourist to see. An arch shows the Mughal influence, towering over a water tank that is heavily reminiscent. In the Shivneri fort most importance tourist places are the Ganga – jamuna reservoir and the Idgah at the central square of the fort and Shri Shivai Mandir. Shivneri has number of under ground caves. The fort is unique as it has 50 caves at the middle and also covered by three hills ranges having 150 caves. Shivneri is surrounded on all sides by fort and dams. Until 1925, this fort too, was left to the vagaries of nature and was in a state of utter decline. After the formation of the state of Maharashtra, a good motorable road was made that reached right up to the main door of the fort. Every year, the birth celebrations of Chhatrapati Shivaji Maharaj take on colorful hue here; this is an excellent occasion to visit.

D. Drainage

The tributary of the Kukadi River are originated at Shivaneri fort. These tributary are seasonal during, rainy season they ave enough water in their path otherwise through the year they are dry. Shivneri is surrounded on all sides by dams. The Ganga – jamuna reservoir and Badami Talav is another water tank provides water facilties on the fort.

E. Climate

The Shivneri fort is situated on hillock and hence the various parameters of climate become the resource of tourist activity. Shivneri offer more prospects with its combination of cool pleasant summer climate along with its enchanting natural settings. The area can be characterized by rainy, winter and summer season. The mean annual temperatur of Shivneri is 32℃. Month of May is generally hottest month of the year. The average rainfall received by the fort is 721.70mm. Natural Vegetation: The factors like relief, soil and precipitation affect upon the natural vegetation. Mainly the distribution of the rainfall controls the type of natural vegetation. The natures of soil and climatic condition have a direct impact on the growth

of vegetation. This Shivneri fort is owned by the Forest Department therefore, trees and forest have been preserved. The natural vegetation is mainly of low scattered trees occurring along the border of the study area. The hot and dry climate has resulted mixed thorny trees, stunted grass and scanty vegetation.

Accommodation: There are a quite number of hotels, restaurants and lodges available for accommodation in Junnar. Other than several private hotels there is a PWD and Forest Dept. rest house available for accommodation in Junnar.

F. Transportation

State highway No. 52 and Main District highway No. 3 and 1 connect the Junnar which provides excellent road network so that tourist can reach easily to the Junnar by metal road. MSRTC buses that operate on the Pune – Junnar routes, will take Shivneri. There is no state transport for the last 3 km, but local transport will reach to the foot hill of the Shivneri also, helipad facility available on Shivneri.

Mobile network services are available at the regions of Shivneri and Junnar.

G. Water supply

Shivneri is surrounded on all sides by fort and dams. To scale the fort, you will have to negotiate a climb of 300m to the northern face a huge lake is constructed- Badami Talav. Around the lake are remnants of ancient monuments. There is much for the tourist to see. An arch shows the Mughal influence, towering over a water tank that is heavily reminiscent. The Ganga – jamuna reservoir is another water tank provides water facilties on the fort.

H. Population

In the year 2001 the total population of Junnar town was 21416. The Junnar town has population of 25315 of which 13066 are males while 12249 are females as per report released by Census 2011. There are about

5637 houses in Junnar town. The density of the population in 2011 was 4119km². The decadal growth rate of population is 2.32%.

Nature of occupation and economic development are associated with each other. Primary, secondary and tertiary are the three types of occupations. The population of the Junnar has been working mainly in the agricultural sector and hence there is dominance of proportion of workers in the primary sector. The proportion of workers in agriculture is 75% while the persons engaged in the secondary occupation in the tahsil 16%. The percentage of workers engaged in the tertiary occupation in the tahsil 9% is also less than that in the district (14.96%). The data has also shown graphically (Fig. 2.8). All these account shows that the Junnar tahsil is economically backward than the district.

I. Present Status

The present status of Shivneri as a tourist centre come under developing category. Shivneri has a great potential for tourism development. Archeological Department of India, Government of Maharashtra and MTDC give more attention to provide funds, donation and subsidy for infrastructure development on Shivneri forts. However serious attempts are needed to develop tourism in this region.

J. Important Tourist Centers in and around Shivneri

The Shivneri fort was the birth place of Maratha Empire Shri Chhatrapati Shivaji Maharaj. Shivneri has number of under ground caves. In the upper and lower scarps are two irregular lines of Buddhist caves all of them small and some more like dwellings of vultures than of monks. In the Shivneri fort most important tourist places are the birth place of Shivaji, statues of Shivaji and Matoshri Jijabai, the Ganga – Jamuna reservoir, and the Idgah at the central square of the fort and Shri Shivai temple also, newly created lawn and garden etc. A week would be ideal to cover these interesting tourist centers.

II. CONCLUSION

Present study tries to provide an integrated plan for tourist places forts in the Pune district with some special case studies. If someone wants to know the forts along the Mumbai-Pune Express way map can be displayed. Also encouraged and facilitated youth geographers to travel and foster national integration. As far as potential places are concerned, after field work it is observed that few people only visited these forts who know about the places, but other have no any information about the places which should be published or advertised or marketing and the information should be reached to the tourists and they can visit the places. Also take an account of domestic needs of the Pune district by giving information such as cheap accommodation, easy way to transportation.

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Immobilisation and Applications of a Thermostable Lipase from the Seeds of Macrotylomauniflorum

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ABSTRACT

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) have proved to be one of the most important industrial enzymes as they are able to perform in both aqueous and water-restricted environments catalyzing stereospecific hydrolysis and transesterification reactions respectively. Lipases from plant sources have interesting features for application in different fields. In the present study, a lipase from seeds of Macrotylomauniflorum was isolated and purified using hydrophobic interaction chromatography. The enzyme was found to be thermoactive, having maximum hydrolytic activity at 60° C and stability over the range of 20 to 70° C. The optimum pH for the enzyme is 8.6 and is stable in alkaline range upto pH 10. The enzyme was inhibited by Cd^{2+}, Hg^{2+}, tween 20, tween 40 as well as pesticides malathion, methyl parathion and chlorpyrifos. Immobilization of enzyme on calcium alginate beads has been done and its application in carrying out transesterification reactions in non-polar solvents has been investigated.

Keywords: Lipase, Macrotylomauniflorum, Thermostable, Immobilization.

I. INTRODUCTION

Enzymes are extensively being used in many industries for biotechnological, pharmaceutical and food applications on account of their unique characteristics. The limiting factors in widespread applicability of enzymes are their substantially unstable nature and requirement of stringent conditions of pH and temperature. Also, the enzyme once used in a reaction, cannot be recovered for repeated use. Immobilisation of enzyme by different methods is the key to improve enzyme stability and reusability[1,2].

Lipases (EC 3.1.1.3)are the biocatalysts that have inherent ability to hydrolysetriacylglycerols into free fatty acids and glycerol. The unique competence of lipases to catalyse stereospecific transesterification reactionsin water-restricted environments has made them one of the most favourable candidates in organic biosynthesis [3].To date, a large number of lipases have been profoundly studied from the bacterial and fungal sources whereas;the knowledge about plant lipases isstill very limited regardless of their biocompatibility and low production cost[4].

Macrotylomauniflorum (Lam.) Verdc. commonly known as horse gram or Kulithis one of the lesser known legumes. It is mainly used as cattle fodder though it is also consumed as cooked food in some parts of India. It has been reported to have hepatoprotective, hypercholesteramic and antioxidant activities [5].

In the present study, a lipase enzyme was isolated and partially purified from the seeds of Macrotylomauniflorum. The partially purified lipase was immobilised by entrapment in calcium alginate beads [6] in order tocatalyse the transesterfication reaction to form Ethyl propanoate.

II. METHODS AND MATERIAL

Materials

The seeds of Macrotylomauniflorum were purchased from local market. P-nitrophenylpalmitate was purchased from Sigma-Aldrich while sodium alginate and other chemicals were procured from Sisco Research Laboratories Pvt. Ltd. (India).

Protein estimation

Protein in samples was estimated by Lowry's Method [7] using Bovine Serum albumin as a standard.

Lipase Assay

Lipase activity was determined with slight modifications to Winkler and Stuckmann method [8] using p-Nitrophenyl Palmitate as a substrate. p-Nitrophenyl Palmitate was dissolved (3mg/ml) in isopropanol and then 1 part of it was added dropwise to 9 parts of Tris-HCl buffer, pH 8.6, 50mM solution containing 0.4% Triton x-100. The assay was performed by adding 0.7 ml of the substrate solution to 0.3 ml enzyme in Tris-HCl buffer. After incubating the reaction mixture for 20 minutes, absorbance was measured at 410nm. The μmoles of p-nitrophenol released was determined by using molar extinction coefficient of it ($0.012750\ \mu m^{-1}cm^{-1}$). One unit of enzyme activity is defined as the amount of enzyme liberating one micromole of p-Nitrophenol in one minute [9].

Partial Purification of Lipase from Macrotylomauniflorum

M. uniflorum seeds were allowed to germinate for 36 hours, extracted in four volumes of physiological saline followed by 30-80% ammonium sulphate precipitation. The precipitated protein was collected after centrifugation, dissolved in minimum amount of distilled water and dialysed against distilled water and finally against Tris buffer.The dialysed protein was subjected to Hydrophobic Interaction Chromatography using a column packed with phenyl sepharose matrix equilibrated in phosphate buffer pH 7.0, 50mM containing 1M ammonium Sulphate. The column was washed with discontinuous gradient of ammonium sulphate in buffer and finally with distilled water. Sephadex G-100 gel filtration column was used to further purify HIC eluted lipase.

Determination of Optimum pH and temperature

The pH optimum of the semi-purified lipasewas determined by performing standard lipase assay at different pH values ranging from 5.0 to 10.0 in appropriate buffers. For determination of optimum temperature, semi-purified lipase and substrate solution were incubated at different temperatures ranging from 20 to 90 ^{0}C under standard assay conditions.

Thermostability and pH stability of the enzyme

Thermostability of the enzyme was assessed by pre incubating the enzyme at various temperatures (20-90 ^{0}C) for 1 hour and then determining residual enzyme activity at standard assay conditions. The pH stability of the enzyme was determined by measuring residual enzyme activity after incubating the enzyme at different pH ranging from 3.0 to 10.0 for 1 hour at room temperature.

Effect of Metal ions on lipase activity

The semi-purified lipase was preincubated with 5mM solutions of metal ions (K^+, Na^+, Ag^+, Cu^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Cd^{2+}, Hg^{2+}, Fe^{3+}, Pb^{2+}, Co^{2+}, Sn^{2+}) for 1 hour at room temperature and then residual enzyme activity of each enzyme solution was determined at standard assay conditions.

Effect of detergents on activity of Lipase

The effect of detergents on lipase activity was assessed by incubating the lipase in the presence of surfactants viz. Triton X-100, Tween 20, Tween 40, Sodium taurocholate and four commercial detergents (Tide, Surf excel, Wheel and Rin) at 1% W/V or 1% V/V for 1 hour at room temperature.The residual enzyme activity was determined at pH 8.6 and temperature 60 ^{0}C for 20 minutes.

Effect of Pesticides on Lipase activity

The commonly used organophosphorous pesticides, Malathion, Methyl Parathion and Chlorpyrifos were incubated at 10 mM concentration with the M. uniflorum.Lipase for 1 hour at room Temperature. The lipase activity after incubation was determined at standard assay conditions.

Immobilisation of Lipase

For immobilisation of lipase, equal volumes of lipase were mixed with sodium alginate solutions so as to keep effective concentration of alginate in beads ranging from 6- 12 %. The lipase - Na alginate mixed solutions were added with syringe; dropwise into 50 ml of Calcium Chloride solutions (0.2M) while stirring on a magnetic stirrer to form enzyme entrapped beads. Corresponding control beads were also prepared without adding lipase. After 30 minutes of hardening, beads were separated from $CaCl_2$ solutions and washed three times with Tris-Cl buffer (pH 8.6). The protein content and lipase activity in filtered $CaCl_2$ solutions and three washings was determined so as to determine Loading efficiency [6].

$$\text{Loading efficiency (\%)} = \frac{CiVi - CfVf}{CiVi} \times 100$$

Where, Ci- Initial protein Concentration, Vi- initial volume of the enzyme solution, Cf- Protein concentration in total filtrate and washings and Vf- Total volume of the filtrate and washings.

Hydrolytic activity of lipase entrapped beads was determined by standard lipase assay using 20 mg beads instead of 0.3 ml enzyme.Immobilisation Yield was calculated as follows:

$$\text{Immobilisation yield (\%)} = \frac{A\ immb}{A\ free}$$

Where Aimmb- Specific activity of immobilised enzyme and A free- Specific activity of free enzyme [6]

Synthesis of ethyl propionate by immobilised enzyme

Immobilised lipase was used for synthesis of ethyl propionate through transesterification reaction [10]. Reaction mixture (10 ml) contained 50 mg of lipase entrapped beads, 100 mM each of ethanol and propionic acid in n-octane in a glass stoppered bottle. This reaction mixture was incubated at 60 ^{0}C in a shaking incubator for 12 hours. Synthesis of ethyl propionate was confirmed by TLC.

III. RESULTS AND DISCUSSION

Isolation and Partial purification Lipase from the seeds of *Macrotyloma uniflorum*

It is well kown that the activity of lipase is high during germination [11, 12]. The lipase isolated after 36 hours germination was partially purified by salting out, dialysis and column chromatography. As shown in elution profile of hydrophobic interaction chromatography (Fig. 1), lipase was eluted out of phenyl sepharose column with distilled water. Gel filtration further purified the enzyme to near homogeneity.
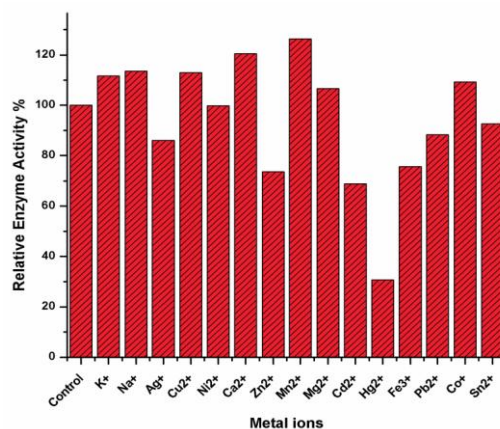
Effect of pH on Enzyme activity

Optimum pH of the enzyme was found to be 8.6. The enzyme was stable in the alkaline range up to pH 10. The effect of pH is as per shown in Fig. 2 a) and c). Optimum pH for almond seed lipase and rice bran Lipase were found to be 8.5 and 11.0 [13,14]

Effect of temperature on Enzyme activity

The effect of temperature on enzyme activity is shown in Fig 2 b) and d). It is a thermoactiveenzyme showing maximum activity at 60^{0} C. The enzyme is stable over the temperature range 20-70^{0} C. similar results were obtained for almond seed lipase (65^{0} C) and rice bran lipase (80^{0} C) [13,14]
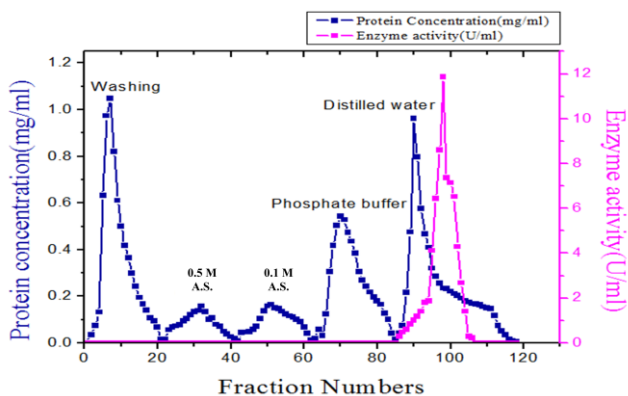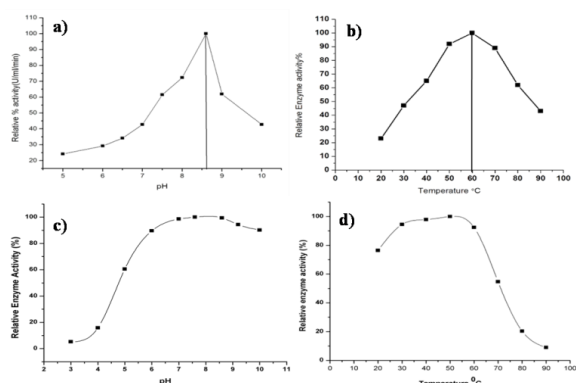
Figure 1. Elution profile of Lipase on Phenyl Sepharosehydrophobic interaction chromatography (A.S.= Ammonium Sulphate in Phosphate Buffer)
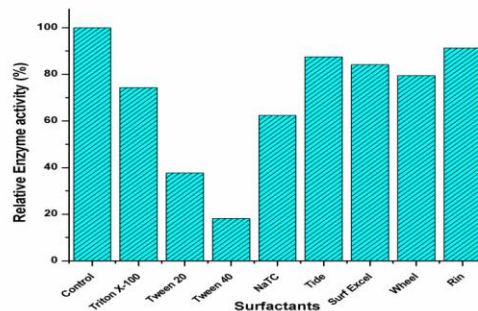

Figure 2. a)Effect of pH on enzyme activity. Optimum pH was found to be 8.6. b) Effect of temperature on enzyme activity. Maximum enzyme activity was obtained at 60 °C. c) pH stability of enzyme d) Temperature stability of enzyme.

Effect of Metal ionson lipase activity:

It is well known fact that metal ions alter the enzyme activity [15]. The effect of monovalent, divalent and trivalent metal ions on lipase activity is as shown in Fig. 3. A notably lower activity of the enzyme was observed in presence of Ag^+, Hg^{2+}, Cd^{2+} and Zn^{2+}. whereas Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, and Na^+ were found to activate enzyme. Bhardwaj et al. have reported an inhibition effect of divalent cations like Mg^{2+}, Zn^{2+}, Cu^{2+}, and Cd^{2+} on rice bran lipase activity [14].Nishio et al. have reported a stimulatory effect of Ca^{2+} and Mg^{2+} on *Pseudomonas fragi* lipase activity [16].

Figure 3. Effect of metal ions on Lipase activity

Effect of detergents on activity of Lipase

A relative lipase activity was observed in presence of surfactants and commercial detergents. The enzyme was strongly inhibited by Tween 20 and Tween 40 while it retains more than 80% of its lipolytic activity with commercial detergents.The results of commercial detergents are in accordance with Polizeli et al. and Weerasooriya et al.[17, 18]

Figure 4. Effect of Surfactants and detergents

Effect of Pesticides on Lipase activity

All the three pesticides inhibited the enzyme profoundly. The results are shown in Fig. 5.Kartalet al. andGangadharaReddy et al. have done similar work in order to design a biosensor for pesticide detection.
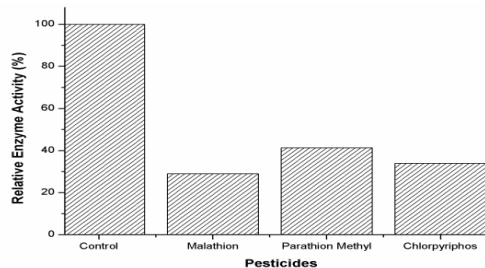

Figure 5. Effect of pesticides on enzyme activity

Immobilisation of Enzyme

M. uniflorum lipase was immobilised by entrapment in Calcium alginate gel beads. The beads retained lipolytic activityeven after repeated usage proving its reusability.

Gelation is the effect of crosslinking between alginate and calcium and thus have substantial effects on immobilisation efficiency. Therefore, effect of alginate concentration on loading efficiency and immobilisation yield was investigated. Alginate concentration was increased from 6% to 12 % keeping other parameters constant. Loading efficiency increased with increasing alginate concentration while immobilisation yield decreased as shown in Fig. 6. As crosslinking is expected to increase with alginate concentration, lipase will not leak from the beads. While higher crosslinking may affect conformation of enzyme leading to decrease in immobilisation yield. Similar results were observed by Keehoon et al. with *Candida rugosa* lipase [6]. Lipase entrapped beads were used for synthesis of ethyl propionate by transesterification which are confirmed by TLC.
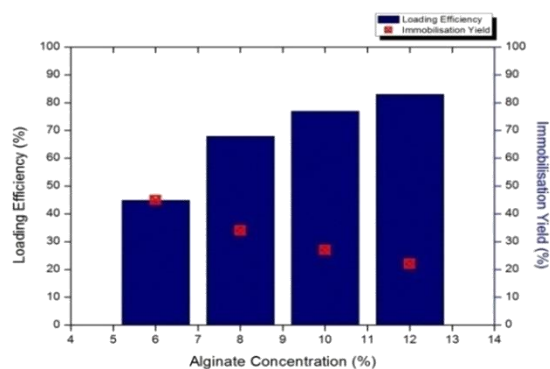
Figure 6. Effect of alginate Concentrationon Immobilization

IV. CONCLUSION

Lipase was isolated and partially purified from *Macrotylomauniflorum* seeds. The enzyme was found to be thermoactive showing maximum activity at pH 8.6 and 60⁰C temperature. Stability of the lipase enzyme at high temperature and alkaline pH makes it suitable for industrial applications. The lipase was strongly inhibited by heavy metals ions like mercury and cadmium as well as surfactants tween 20 and tween 40. Lipase retains more than 80% activity in the presence of commercial detergents andhydrolytic activity was enhanced in presence of Ca^{2+} and Mn^{2+}. Immobilisation of lipase was done by entrapment in Ca alginate beads and effect of alginate concentration on loading efficiency and immobilisation yield was analysed. At higher concentration of alginate immobilisation yield decreased suggesting need to determine optimum alginate concentration for entrapment. The immobilised lipase can be used to catalyse transesterification reactions in organic solvents so as to synthesize industrially important biocompatible compounds at cheaper cost.

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A Study of Relief Feature in Shirur Tehsil, Pune District, Maharashtra State, India

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ABSTRACT

The study of economic development was viewed differently by geographers and economists while geographers have tended to emphasize the role of physical environment (Munton R.J.C.1969). Land is the basic resource of human society. Agriculture is the most important primary economic activity which is closely related to physical environment relief. In any scientific and viable inquiry into agricultural phenomena, it is perquisite to pay attention to the basic relation between these physical determinants and agriculture (Singh and Dhillon, 1994). In the country like India, where the rainfall is both inadequate and unpredictable, it affects badly on agricultural productivity. The study of development process has found a limited place in geographic literature in the past (Gilbert A.1971). It is therefore, necessary to evaluate the agricultural land use of the study region needs to unfold the nature of ecology of the Shirur Tahsil. This paper covers the profile of the tehsil, i.e. relief, geomorphology.

Keywords : Physical Environment, Relief, Geomorphology

I. INTRODUCTION

Agriculture is the most important primary economic activity which is closely related to physical environment relief, climate and soils. In any scientific and viable inquiry into agricultural phenomena, it is perquisite to pay attention to the basic relation between these physical determinants and agriculture (Singh and Dhillon, 1994).

Figure 1

Land is the basic resource of human society. Its utilization shows a reciprocal relationship between ecological conditions of region and man. This chapter covers the profile of the Shirur Tehsil, i.e. relief, geomorphology.

The Shirur Tahsil lies in the eastern part of Pune district of Maharashtra. The absolute geographical location of study area can be expressed as from 18º.49'.00" N to19º.34'.00"N latitude and74º.22'.00" E to 75º.03'.00" E longitude. The area of Shirur tahsil extent form north to south 24 km and 50 km from east to west. The study area is included in Survey of India Topographic Index Numbers 47J/1, 47J/2, 47J/5, 47J/6, 47J/10 and 47J/11on 1: 50,000. Its total area occupied was1552 sq.km.
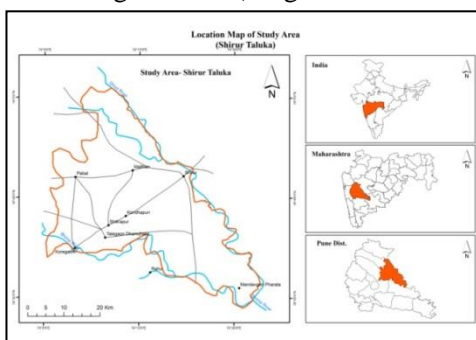
Objectives-

1. To study the physical background of the Shirur tehsil
2. To study the relief feature in Shirur Tehsil .

II. METHODS AND MATERIAL

The secondary SOI toposheet used for data preparation of different thematic maps. With the help of Arc GIS software Maps are prepared.

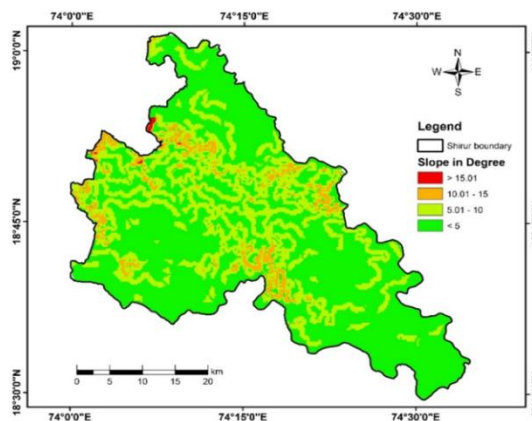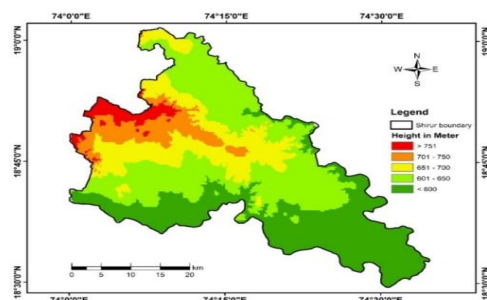## III. RESULTS AND DISCUSSION

Physiographically Shirur Tahsil represents plateau and plain region. The western part of the study area is having an altitude of more than 751 m, the central part has an altitude between 601 to 750 m and the eastern part has less than 560 m elevation.

Plain area is distributed along the bank of Bhima River that is the southernmost part of the Shirur tahsil and also at the confluence of Bhima and Ghod River in the south-eastern part of the Shirur Tahsil. The height is ranging between 540 to 560 meters above the mean sea level. The maximum proportion of Vadgaon Rasai circle is occupied by plain area. The plateaus of 600 -750 m elevation are known as region of middle level plateaus. Such plateaus are located in the central and northern part of the study area. It is distributed in the northern part of Talegaon Dhamdhere circle.

Slope:

SLOPE MAP OF SHIRUR TAHSIL(Source: SOI Toposheets)

Slope of a land is one of the important physiographic aspects that influence the overall suitability of the natural elements. The southern and south-eastward slope of the study area has gentle and it is less than 5^0. The central part has occupied by the eastern offshoots of the Sahyadri ranges and this area has 5^0 to 10^0 slope with undulating topography. The eastern part of Shirur circle, southern and western part of the Takali Haji circle and the eastern part of Nahavra circle have 10^0 to 15^0 slopes.

IV. CONCLUSION

The Shirur Tahsil represents plateau and plain region. The western part of the study area is having an altitude of more than 751 m, the central part has an altitude between 601 to 750 m and the eastern part has less than 560 m elevation.

Plain area is distributed along the bank of Bhima River that is the south-eastern part of the Shirur Tahsil. The height is ranging between 540 to 560 meters above the mean sea level. The maximum proportion of Vadgaon Rasai circle is occupied by plain area. The plateaus of 600 -750 m elevation are known as region of middle level plateaus. Such plateaus are located in the central and northern part of the study area.

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Acclimatization of medicinal fernCheilanthes farinosa Kaulf

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ABSTRACT

Pteridophytes are one of the important components of any mountainous flora which is one of the successful plant groups on the earth. They almost distributed in Western Ghats, Himalaya. These are considered as lower vascular plants. Fern acclimatization and conservation can be considered as a part of conservation biology. Various techniques are established for conservation of medicinal , rare, endangered ferns. Whole ecosystems and biodiversity is considered because of various conservation programmes. Various medicinal ferns also acclimatized under Pune condition and they are conserved. Pteridophytes are vascular cryptogams and form a neglected group of plants in biodiversity. Many fern and fern allies are growing on Himalayan slopes and are used by rural population and many tribal communities for treatment of various diseases. About 110 genera and 600 species are fund in India (Sukumaran et.al.2009). Many taxa of Pteridophytes have been lost or eradicated from Western Ghats due to the present pace of rapid industrialization and exploitation of natural resources.

Exhaustive systematic survey of pteridophytic localities for many years by Mahabale (1987) has revealed the occurrence of 59 species from 35 genera in Western Ghats of Maharashtra. Blatter and Almeida (1922) have described 57 species occurring in Bombay Presidency. On the whole, Maharashtra is quite rich in pteridophytes, there are about 55-60 ferns and 11 fern allies known, so far (Mahabale, 1987). The medicinal uses of some ferns and pteridophytes of India have also been described by Caius (1935 b) and Nair (1959). The medicinal uses of 61 different fern and fern allies have been described by Benjamin and Manikam (2007).

Chilenthes farinosa Kaulf. Commonly called silver fern. It belongs to family Sinopteridaceae. The paste of fronds and rhizomes along with turmeric is applied for skin diseases. Roots are used in eczema and eaten in stomachache as well as veterinary larval infection. The roots are also applied on the wounds. Fronds are utilized in menstrual disorders. Fresh fern has antibacillus activity. The anti-inflammatory and anti-nociceptive activities of Cheilanthes farinosa (Forsk.) Kaulf (Adianthaceae), used in many parts of Ethiopia to treat inflammatory skin disorders, were studied using in vivo models of inflammation and pain.

Considering all these medicinal values and other uses of Cheilanthes farinosa Kaulf different methods were applied for conservation and tried for it's acclimatization under Pune conditions. Cheilanthes farinosa Kaulf is collected from Mahabaleshwar. The acclimatized ferns have been successfully maintained in the house garden.

Keywords : Cheilanthes farinosa Kaulf, Pteriophytes, acclimatization, conservation, medicinal uses

I. INTRODUCTION

Pteridophytes are one of the most ancient plants. These are non flowering, vascular and spore bearing plants which include ferns and fern allies. India is among twelve mega diversity centers.One can get rich pteridophytic flora in Eastern Himalayas and Western Ghats . The fern grow luxuriantly in moist tropical and temperate forests . They occurre in different eco-geographically threatened regions from sea level to the highest mountains are of much interest.Plants are global resource. Human societies are using plants and

various plant parts for various purposes like medicinal, ornamental, commercial etc. The pteridophytes constitute the primitive vascular plant group and found scattered all over the world having important contributuion to the plant diversity.It is one of the major groups which are used for medicinal purpose as well as for other purpse like ornamental etc. . Actually not more attention is given towards the uses of ferns yet it possesses equal economic importance including medicinal uses (Mannan et al., 2008).

Most of the ferns are rare with medicinal value so it should be conserved.The most of the ferns and fern allies are noticed growing luxuriously.But because of human activities there is threat to fern species.Some steps are desired to be taken for their conservation for pteridophytic taxa.Indian fern flora is endemic to country and so it needs specialattention conservation. Caius (1935) is supposed to be first man who described medicinal uses of some ferns of India.There are some ferns which are from western India used in folk remedies to cure varouus diseases.(Puri & Arora in 1961,1970).

The previous work indicate that the medicinal plants specially the flowering plants are given the more importance in these contents, non-flowering plants particularly fern and fern allies are ignored by scientists. Hence, in present investigation it was decided to conduct a survey of list of the pteridophytes growing in Mahabaleshwar forest area as well as to study their habitats and try to conserve **Cheilanthes farinosa Kaulf** species under Pune climatic conditions from Mahabaleshwar.

Mahabaleshwar is located in Satara District at 17.92°N 73.67°E. It has an average elevation of 1,353 metres (4,439 ft) and located about 120 km southwest of Pune and 285 km from Mumbai. Mahabaleshwar is a vast plateau measuring 150 km2, bound by valleys on all sides. It reaches a height of 1,438 m at its highest peak above sea level, known as Sunrise Point. Mahabaleshwar distrusted in main villages in addition

to main city; these are Malcolm Peth, Old "Kshetra" Mahabaleshwar and part of the Shindola village. The major vegetation types are tropical evergreen forests, moist deciduous forests, scrub jungles. The annual rainfall is 5000 mm. The rivers venna, koyna, flows during rainy and winter seasons. The tropical climate with high humidity, moderate temperature and red lateritic soil provide suitable conditions for luxuriant growth of ferns. Hence, it was thought worthwhile to take up the survey of the forest for pteridophytic diversity. The present investigation was carried out to fill the gap in the knowledge of fern and fern allies flora of Mahabaleshwar with the following specific

To understand the mechanisms behind acclimatization of ferns in new environment and to suggest conservation means, it is crucial to unravel local and regional dynamics of fern populations. A microclimatic and demographic study of fern populations would give the best information needed for successful conservation of ferns (Menges and Gordon, 1996) and enable the development of realistic spatially open models, allowing for the characterization of local population dynamics and the regional dynamics of the species (Münzbergová et al., 2005; Milden et al., 2006).

For the acclimatization of medicinal fern **Cheilanthes farinosa Kaulf various methods** were tried under pune climatic conditions.

II. METHODS AND MATERIAL

Samples were collected from Mahabaleshwar forest area these were compared with herbaria and confirmed from the authorities of BSI, Pune. Based on medicinal use *Cheilanthes farinosa* Kaulfwas selected . The habit of selected ferns was marked in Mahabaleshwar forest to study microclimatic conditions and soil properties.

Microclimatic conditionsof *Cheilanthes farinosa* **Kaulf.-**

Well in exposed dry situations along forest margins, roadside and bridle paths. Grow better on slightly acidic substratum with pH 5.4-5.8

III. RESULTS AND DISCUSSION

Cheilanathes farinosa Forssk (Kaulf)

Cheilanthes farinosa **Kaulf**

Methods For Acclimatization of *Cheilanthes farinosa* **Kaulf**
In present investigation,methodshods were designed based on criteria of microclimatic conditions and substratum (rhizosphere soil) observed in their habitats at Mahabaleshwar.

Earthen or cement/plastic pots were used for acclimatization .

The pot mixture was prepared by mixing coarse sand, brick pieces, shredded moss, rhizosphere soil, garden soil and compost. The proportion was 1:1:1:10.5:0.5:1. The content was mixed thoroughly and pot was filled with pot mixture. The rhizomes of selected ferns (5 numbers) were planted in each pot in such way that the growing points of the rhizomes will remain open.

The pots were distributed in three sets and each set has minimum 5 pots. The pots were maintained in conditions as mentioned in three different methods. These conditions are:

1. Under shade near the northern side of wall.
2. Pots were kept in big sized perforated polythene bags.
3. Pots were kept near humidifier.

A humidifier, constructed of PVC pipe was used to maintain humidity near the culture site. To construct a humidifier a perforated 3-inch diameter 100 cm height pipe was filled with brick pieces, fitted in container containing water, and used to maintain the humidity near the pots. Different parameters like humidity, temperature and light intensity were recorded regularly near the pots to maintain the required climatic conditions at conservation site. The pots were kept on northern side of the wall to avoid direct sunlight. Pots were watered regularly to maintain the humidity. The sprouting of rhizome was noted regularly. The ferns grown under these methods were observed for their growth and percent survival was determined for each fern species.

IV. CONCLUSION

This medicinal fern species selected for acclimatization and multiplication under Pune climatic conditions was observed for it's habitats in Mahabaleshwar forest. The observations noted from different locations.

The fern species selected for acclimatization was growing luxuriantly under shady condition, In open places, these were observed only on northern and eastern side of sharp hilly slopes, near water streams or on moist rocks / boulders in the areas where direct sunlight is not reaching or in some other places where only morning or evening sunrays are preponderate.

Almost all the habitats where ferns are growing have been found out of direct noon bright sunlight.

Table 1. Table showing sprouting & survival of
Cheilanthes farinosa **Kaulf**

Cheilanthes farinosa Kaulf	Method I Shade + Bricks	Method II Shade +Bag	Method III Shade + Humidifier
sprouting(%)	60.22	78.08	70.08
survival (%)	52.22	68.08	70.08

From table 1.1Cheilanthes farinosa Kaulf showed maximum sprouting in method II and survival in method-III, minimum sprouting and survival in method-I and moderate sprouting and survival in method-III.

Cheilanthes farinose Kaulf grow in exposed areas under partial shady situations.

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Long Term Fluctuations and Global Teleconnections in the Monsoonal Rainfall and Associated Floods of the Mahi Basin : Western India

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ABSTRACT

This paper examines the long term fluctuations and global teleconnections in the monsoonal rainfall and associated floods of the Mahi Basin from western India. In order to analyze rainfall and associated floods, the rainfall data for monsoon season of the Mahi Basin have been obtained for last 149 years (1857-2005) from India Meteorological Department (IMD), Pune. The discharge data of the Wanakbori site, procured from Kadana Dam flood control cell, have been used to show its association with the monsoonal rainfall. Time series analysis, percent departure from mean, Normalized Accumulated Departure from Mean (NADM), Mann-Kendall test and conditional probability technique for El Nino and Southern Oscillation (ENSO) have been used for the analyses of monsoonal rainfall data of the Mahi Basin and its effect on floods. The results indicate year to year variations in the monsoon rainfall with significant departure from mean rainfall of the Mahi Basin. It is observed that majority of the floods are associated with positive departure from mean rainfall. The NADM shows epochal behavior of high and low monsoon rainfall of the basin and the Mann-Kendall test reveals statistically significant decreasing trend in the monsoon rainfall of the basin. The analysis of ENSO reveals that the probability of the occurrence of the floods in the Mahi basin is high during cold (La Nina) event than warm (El Nino) event.

Keywords : Mahi Basin, monsoonal rainfall, global teleconnection, rainfall trend, floods

I. INTRODUCTION

The Indian monsoon is very complex phenomena. The intensity, amount and pattern of rainfall, therefore floods, over the India show significant variations. Therefore, analysis of long term fluctuation in the monsoon rainfall and associated floods in the river basins of the Indian subcontinent is one of the major aspects of investigation in the field of flood-hydrometeorology. Several studies have been carried out on the monsoon rainfall trend at India or regional level but very few at basin scale. Since, it is not possible to predict the exact future trend of rainfall and frequency and magnitude of floods, but change detection can be done on the basis of past records.

According to Kale [1] high magnitude floods are associated with the increased precipitation in the basin. . Mooley and Parthasarthy [2], Parthasarthy et.al [3], and Kripalani and Kulkarni [4] identified significant variation in the monsoon conditions over the Indian territory during last few decades. It is well known fact that the Indian monsoon has significant teleconnection with the sea surface temperature (SST) anomalies of the eastern and central equatorial Pacific Ocean and El Nino. Rasmussen and Carpenter [5], and Shukla and Paolino [6] noticed that during warm El

Nino phase Indian Summer Monsoon Rainfall (ISMR) over Indian Peninsula is suppressed. The rainfall trends and floods over various river basins of India have been studied by Kale [1], Mirza et.al. [7], and Rao [8]. According to investigation by Singh et. al. [9] rainfall over the Indus, Brahmaputra, Ganga, Cauvery and Krishna Basins increasing and decreasing over Narmada, Tapi, Mahi, Sabarmati, Godavari and Mahanadi Basins. The main objective of the paper is, therefore, to find out long term fluctuations and global teleconnections of the monsoon rainfall and their association with the floods in the Mahi Basin.

II. DATA AND METHODOLOGY

The Mahi River is the third major west flowing interstate river of India located in Western India. It originates from north of Vindhyas near the village Mindha in Sardarpur tehsil in Dhar district of Madhya Pradesh at an elevation of 500 m above ASL. It flows for a total length of 583 km. The average gradient of the river is 0.00086. It flows northward through Madhya Pradesh state and turns northwest to enter Rajasthan state. Subsequently, it abruptly changes its course in southwest direction to flow through Gujarat state. It drains into a wide estuary at the Gulf of Khambhat; a part of Arabian Sea. Mahi Basin extends over a catchment area of 34,842 km². The basin is located in the heart of the classical Indian summer monsoon which regulates the river regime and flood characteristics. The basin receives more than 90% of the rainfall during monsoon season (June-September). The mean monsoon rainfall of the basin is 687 mm.

III. METHODS AND MATERIAL

The major objective of this investigation is to understand the long term fluctuations and global teleconnection in the monsoon rainfall and their association with floods in the Mahi Basin. Therefore, monsoon (June-September) rainfall data were obtained from India Meteorological Department (IMD), Pune. Besides, data on sea surface temperature

(SST) anomaly averaged over the central and eastern Pacific Ocean, published by Wright [10] for the period (1901-1982) and for 1983 onwards were obtained from Climate Predication Centre (CPC) of National Oceanic and Atmospheric Administration (NOAA) to understand the global teleconnection with the monsoon rainfall and floods in the Mahi Basin. The peak discharge data for Wanakbori site have been obtained from Kadana Dam flood control cell. The data of major flood events have been collected through several field surveys and published research articles. The monsoon rainfall data for the period of about 150 years (1857-2005) are suitable for understanding the long-term trends over the Mahi basin. To appraise the long term fluctuations in the monsoon rainfall and associated floods, time series plot, percent departure from mean, normalized accumulated departure from mean (NADM), Mann-Kendall test are used. In addition to this, index of ENSO has been applied understand global teleconnection with monsoon rainfall and floods in the basin.

IV. RESULTS AND DISCUSSION

A. Inter-monsoon rainfall variability and associated floods

The interannual and inter seasonal variation in rainfall are significant characteristics of Indian monsoon. A spatio-temporal variation in the floods of the Indian River basins is mainly significant due to differences in the distributional pattern of monsoon over the Indian subcontinent during the south-west monsoon. Similar to other monsoon dominated rivers of India, Mahi River also shows significant inter seasonal variation in the rainfall and flood events. Figure 1 shows that prior to 1900s rainfall was above average, but inter-monsoonal variability was low. The period from 1900 to 1930 experienced greater fluctuations and the rainfall appears to be below average for many years. Inter monsoon variability was characterized by increased frequency and magnitude of floods on the Mahi River mainly after 1930s.

The pattern of variation in the monsoon rainfall is also depicted by the plots of departure from mean expressed as percentage of mean, for the Mahi Basin (Figure 2). The plot reveals that the rainfall sometimes varies by as much as about ±90%. Most of the floods in the basin were occurred when monsoon rainfall was positive from mean and very few years experienced floods during negative departure from mean. The analysis indicates that out of 22 major floods of the Mahi Basin 18 were occurred when rainfall was above average and only four floods were observed when rainfall was below average. The peak flood on record i.e. 1973 flood had occurred when the when monsoon rainfall was +68% from the mean. It is, therefore, concluded that above-average monsoonal rainfall in the basin produces large floods.
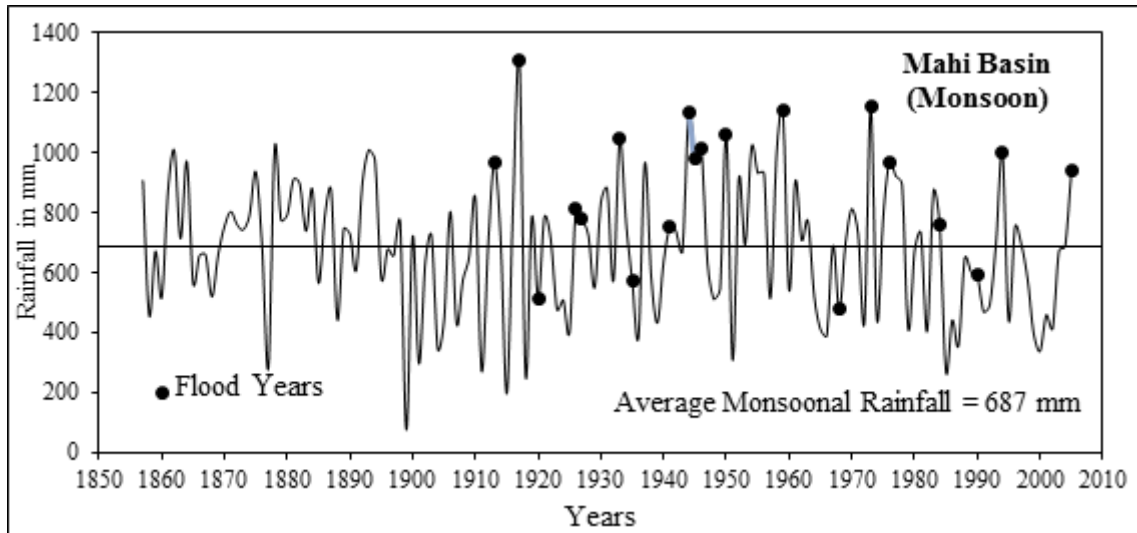

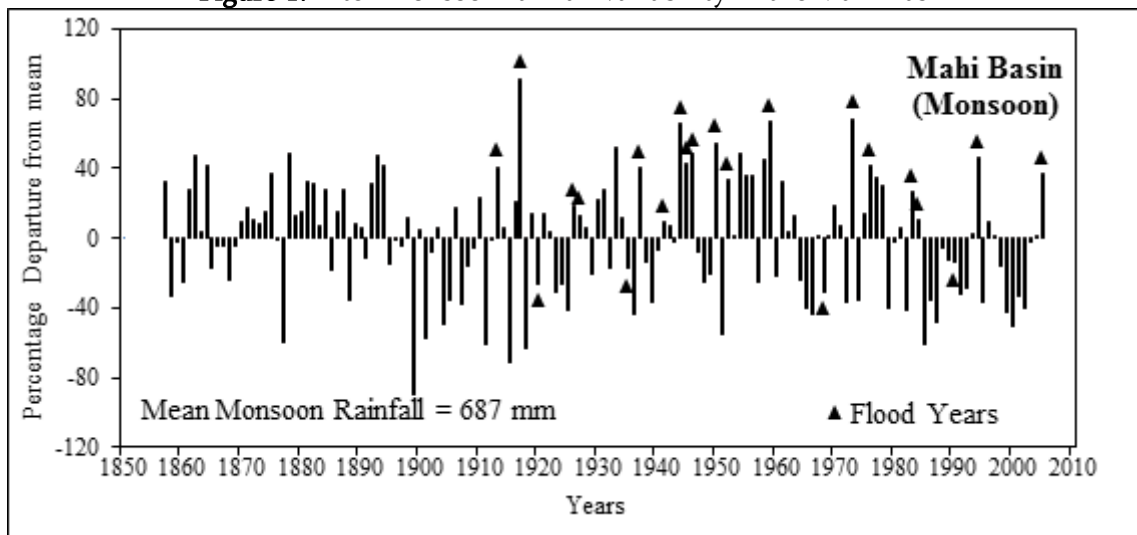**Figure 1.** Inter-monsoon rainfall variability in the Mahi Basin

Figure 2. Percentage departures from mean rainfall of the Mahi Basin

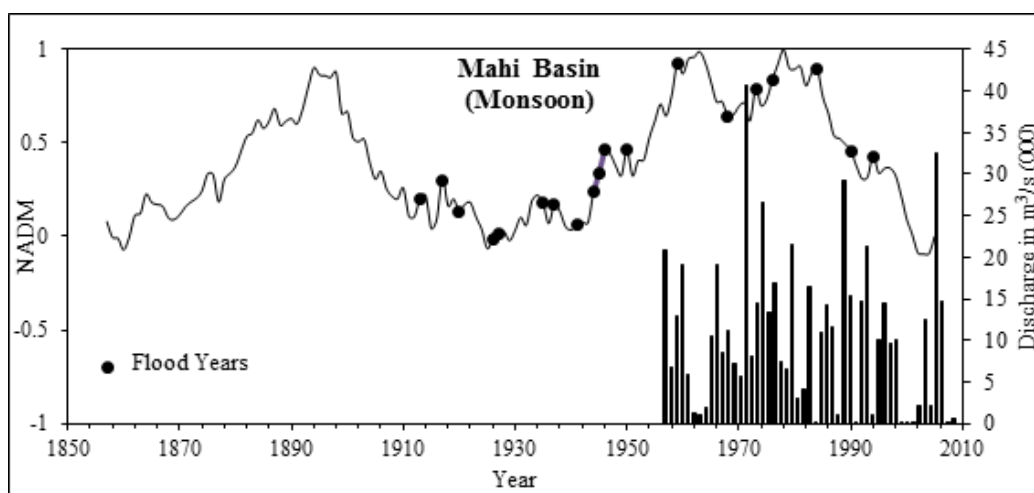## B. Normalized Accumulated Departure from Mean (NADM) and Floods

In order to highlight and emphasis the long-term trends and identify periods of high and low rainfall, the normalized accumulated departure from mean (NADM) method has been applied. The NADM is the accumulated departure from arithmetic mean, divided by the largest absolute number in order to plot between -1 and +1 [1]. The NADM plot shows above average rainfall conditions characterized by positive slopes (rising limbs) and the periods of below-average

rainfall are represented by negative slopes (declining limbs). The analysis of NADM indicates epochal behaviour of the monsoon over the Mahi Basin. The major epochs of monsoon rainfall fluctuation are 1857-1898 wet, 1899-1930 dry, 1931-1978 wet and 1979-2005 dry (Figure 3).

The NADM graph clearly illustrates that most of the floods had occurred when rainfall was above average (rising limb) and very few floods were experienced during below average (declining limb) rainfall. A comparison of the NADM graph with the plot of large floods in Mahi Basin clarifies that the period of below average monsoon rainfall was associated with low frequency of floods and above average monsoon rainfall (1930s to 1990s) was associated with high frequency and large magnitude of floods in the Mahi Basin.

Figure 3. Normalized Accumulated Departure from Mean (NADM) and Discharge

C. Detection of changes in the monsoon rainfall and floods

It apparently appears that the frequency and magnitude of large floods has increased in recent decades. In order to verify whether the trend reflected in the monsoonal rainfall, the non-parametric Mann-Kendall test has been applied to the monsoon rainfall data of the Mahi Basin (1857- 2005). Mann-Kendall's τ and z scores are obtained for the Mahi Basin and have been summarized in Table 1. The analyses based on of monsoon rainfall trend/change over the Mahi Basin show decreasing trend for about last 150 years i.e. is from 1857 to 2005. However, it is not statistically significant at 0.05 levels.

Table 1. Nature Of Changes/Trends In Annual Rainfall Records Based On Mann-Kendall Test

Station	Period	N	Tau (τ)	Z Score	Trend/Change
Mahi	1857-	14	-	-	Decreasing*

Basin	2005	9	0.09378	1.70	

N = number of observations; * = Statistically significant at 0.05 level

D. El Nino and Southern Oscillation (ENSO) and floods in the Mahi Basin

It is very well known fact that variations in the Indian summer monsoon rainfall (ISMR) are linked with El Nino and Southern Oscillation (ENSO) phenomena [11], [12]. Therefore, it is necessary to understand connection between ENSO and rainfall while studying floods in the Mahi Basin. The technique adopted by Eltahir for the Nile River was used. The index is averaged over the monsoon season (June - October). The data on the basis of SST (-0.5°C and +0.5°C) as advocated by Eltahir [13] and Patil [14] were categorized into cold, warm and normal conditions. Figure 4 gives the categories of the annual rainfall of the Mahi Basin and ENSO index. Table 2 indicates that the probability of having high monsoon rainfall

in the Mahi Basin is more during cold and normal conditions (40% to 44%) and very low during warm ENSO conditions (48%).

Table 2. Conditional Probability Of The Monsoon Rainfall Over Mahi Basin Given The SST Index Of ENSO (N = 149 Years)

Basin		Cold	Normal	Warm
Mahi Basin	High	0.44	0.4	0.2
	Normal	0.19	0.22	0.32
	Low	0.37	0.38	0.48

Low < 10% and High > 10

Table 3 shows that the frequency of occurrence of floods is generally high during the cold and normal rainfall years. The result of the conditional probability and occurrence of floods clearly indicates that 17 floods in the Mahi Basin have been occurred during normal and La Nina conditions and seven during warm ENSO conditions. Therefore, it can be stated that monsoon rainfall and associated floods of the Mahi Basin have significant teleconnection with ENSO events.
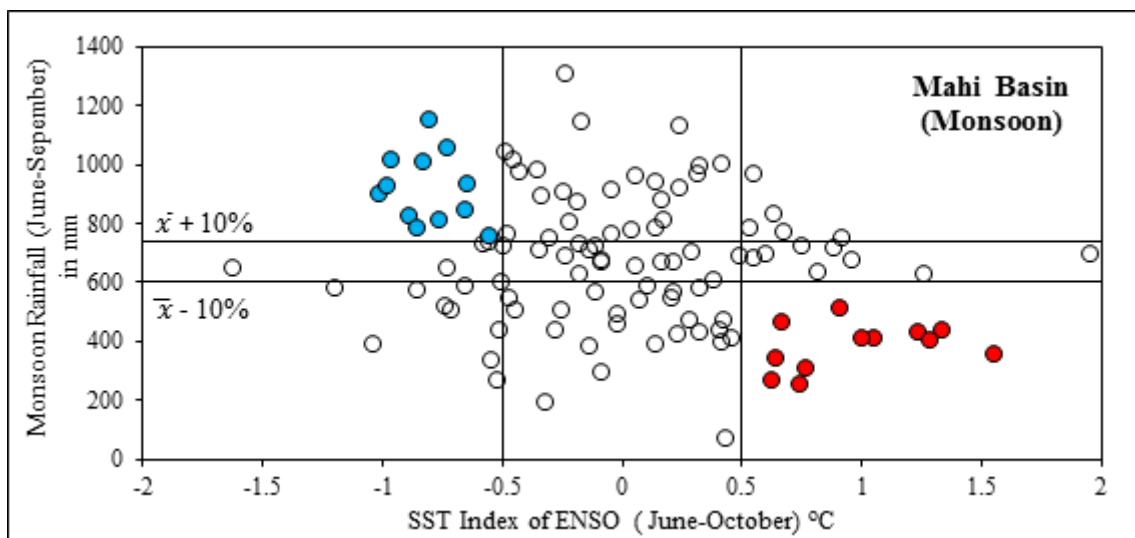
Figure 4. Categories of annual rainfall and ENSO index of the Mahi Basin; Data source: IMD

Table 3. Occurrence of floods and its relation with the annual rainfall and SST index of ENSO.

Basin	Rainfall	Cold	Normal	Warm
Mahi Bain	Above Normal	1944, 1945, 1946,1950, 1959,1973, 1983	1913, 1917, 1919, 1926, 1927, 1933, 1937, 1944, 1945,	1930, 1941, 1952, 1976, 1994, 2006
	Normal		1943	-
	Below Normal		1935, 1990	1968

Below-normal < 10% and above-normal >10%

V. CONCLUSIONS

The time series of monsoon rainfall reveal remarkable inter-monsoonal variability in rainfall. Long term

temporal variations in the monsoon rainfall indicates some notable years when monsoon rainfall was above (high) and below (low) average rainfall of the basin.The analysis of percent departure from mean indicates that out of 22 major floods of the Mahi Basin 18 were occurred when rainfall was above average and only four floods were observed when rainfall was below average. The peak flood on record i.e. 1973 flood had occurred when the when monsoon rainfall was +68% from the mean. It is, therefore, concluded that above-average monsoonal rainfall in the basin produces large floods.

The major epochs of monsoon rainfall fluctuation are: 1857-1898 wet, 1899-1930 dry, 1931-1978 wet and 1979-2005 dry. The NADM graph clearly illustrates

most of the floods had occurred when rainfall was above average (rising limb) and very few floods were experienced during below average (declining limb) rainfall.

The analysis of monsoon rainfall over the Mahi Basin based non-parametric Mann-Kendall test, show decreasing trend for about last 150 years i.e. is from 1857 to 2005. Therefore, it can be stated that monsoon rainfall and associated floods of the Mahi Basin have significant teleconnection with ENSO events.

VI. ACKNOWLEDGEMENTS

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