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Cover image: *Brachymenium turgidum*

The plant *Brachymenium turgidum* is a small tufts of moss occurred on branches and trunks of trees with corticolous habitat as well as on moist forest ground surface. These plants found mostly in humid areas during rainy seasons where humidity is always higher. The plants generally slender, greenish brown tufts with erect stem. The turgid, sub-pendulous capsules are the key features of the thallus.

Locality: Chikhaldara garden, Gawilgarh fort.
Distribution: Mahabaleshwar, Khandala, Pachgani, Kaas Plateau, Satara, Western Ghats, South India, Gujarat, Lingmala

Photo By: Dr. Wankhede Tushar B.
Assistant Professor, Department of Botany, Shri Shivaji Science College, Amravati, India.

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RESEARCH ARTICLE

Impact of nutrition education on nutritional knowledge, attitude and practices of HIV patients attending ART centre of Susheela Tiwari Hospital, Haldwani, Uttarakhand, India

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ABSTRACT

The study was conducted at ART centre of Susheela Tiwari Hospital, Haldwani, Uttarakhand. The data for the study was collected by personal interview. The impact of nutrition education was assessed by pretested questionnaire before and after imparting nutrition education. The study revealed that majority of the subjects was in the age group 18-60 years. A 10.92 per cent subjects were graduates. The per capita income per month of the subjects was Rs.2125±1512. A significant ($p< 0.05$) improvement in KAP scores was observed in experimental group subjects while no improvement was seen in the subjects of control group. Gain in KAP scores for knowledge, attitude and practices was 4.89, 3.17 and 1.21, respectively in experimental group while in control group, gain in KAP scores was -0.28, 0.3 and 0.12 for knowledge, attitude and practices, respectively. The differences in gain of KAP scores between control and experimental group was comparable at the baseline but a significant ($P<0.05$) improvement was seen in case of experimental group after six months. The quantum of improvement in knowledge, attitude and practices was 1.71, 1.08 and 1.20 times, respectively which signified that nutrition education imparted to experimental group resulted in significant ($P<0.05$) improvement in KAP.

Key words: HIV, socio-demographic, KAP, Nutrition education.

INTRODUCTION

In India, after the first case of HIV was detected in Chennai in 1986, the virus spreads rapidly across the nation in both urban and rural areas. There are around 2.4 million people currently living with HIV and so far 172, 000 people have died with HIV/ AIDS (UNAIDS, 2012). The HIV is transmitted through specific human body fluids. The body fluids that

contain the virus are blood, semen, breast milk, vaginal fluids, and rectal mucosa (www.cdc.gov). In India heterosexual mode of HIV transmission accounts for 88.2 per cent of HIV positive cases (India, NACO, 2012). Many studies found that m icronutrients deficiency aggravates the transmission and progression of HIV infection (Schreck *et al.,* 1991; Piwoz and Preble, 2000). In an independent study carried by Tang *et.al* (1996) and Allavena *et.al* (1995) reported that low or deficient serum concentrations of several micronutrients are associated with low CD4 cell count, advanced HIV related diseases, faster disease progression, or HIV-related mortality (Tang *et al.,* 1996; Allavena *et al.*, 1995).

Beisel and Gershwin (2000) reported that nutritionally acquired immune deficiency syndrome, or NAIDS results in impaired immune functions. Whalen *et al., (*2000) reported that NAIDSnot only contribute to the depletion and dysfunction of CD4 cells but also makes the host susceptible to other infections which increase viral replication and hence quicken HIV progression.

WHO (2008) advocate the importance of nutritional counselling as essential component in the management of HIV/AIDS. Nutritional counselling proved to be helpful in raising awareness about the importance of nutrition (Zambelli *et al*, 1996; de Luis *et al,* 2003) and hygiene in the effective management of HIV care especially in resource limiting settings. Through nutrition counselling improvement is seen not only in dietary intake but also empowers dietary diversity and thus fulfil the micronutrients intake which in turns improves the quality of life by slowing down the progression of HIV to AIDS (Penny et al, 2005). Piwoz *et al.*, (2004) and SCN, (2004) found that good nutrition increases the hostt ability to fight the disease and reduces their vulnerability to opportunistic infections (Piwoz *et.al* (2004) reported that nutrition education counselling (NEC) allows PLHIV to modify their diets, using locally available, nutrient dense and culturally acceptable foods to maintain good health, improve their nutritional statusand SCN (2004) found that NEC improves daily functioning of PLHIV. . ECSA-HC *et.al* (2008) and Bukasuba et.al (2010) reported the importance ofNEC in improving KAP and thus allows PLHIV to utilise the limited resources, modify diets to boost their immunity and improve response to ART and other treatment.

Knowledge means the ability of pursuing and using information, and by understanding, learning experience, and identifying the studying technologies. Attitude indicates the result of making reaction via some ways in some situations, and observes and explains based on the result of reaction or combine into one point of view. Practice indicates what knowledge and habit work together (Lbrahim, 1995). Lack of knowledge and negative attitude and practice towards the disease and treatment was reported to result in inadequate therapeutic outcomes and therefore counselling on knowledge, attitude and practice of patients suffering from chronic diseases is important (Fish and Lung, 2001; Vervoort *et al.,* 2007; Goujard *et al.,* 2003).

HIV/AIDS is one of the pressing public health problems in India. The impact of HIV/AIDS on the economic front is important as it affects mainly the young, who are in the reproductive age group (Anand *et al.,* 1999). The adverse economic impact of HIV and AIDS occurs at three levels: the individual/household, sector, and national or macro-levels (Ojha and Pradhan, 2000). So the present study was planned to assess the impact of nutrition education in KAP of people living with HIV/AIDS in India.

MATERIALS AND METHODS

The present study was carried out at ART centre of Susheela Tiwari Hospital, Haldwani. The study was done in two phases. The baseline survey was conducted from August to September 2013 and after an interval of six months the survey was again conducted from February to April 2014 at ART centre of Susheela Tiwari Hospital, Haldwani.

The sample size of 136 subjects was calculated according to Kish-Leslie (1965) formula. The subjects were equally divided into experimental and control group, 58 subjects in each group. All adults, non-pregnant, non-lactating women and asymptomatic subjects attending ART centre were included for the study. Whereas pregnant and lactating women, symptomatic, unable to give consent for the study and who could not communicate in the study languages were excluded for the study. The subjects who were fulfilled the inclusion criteria during study period registered for the study. By the end of the study 26 subjects dropped from the study and hence excluded from the study and the results have been reported on

110 subjects, 51 in the control and 59 in the experimental group.

The study was approved by the advisory committee of Department of Foods and Nutrition, College of Home Science, GBPUA&T, Pantnagar, Uttarakhand. Permission was taken from the hospital administration of Susheela Tiwari Hospital, Haldwani to carry out the study. For ethical consideration the subjects were well explained the purpose of the study and their confidentiality in participant information sheet. A written consent was obtained from the subjects in participant information sheet for their willingness in participating in the study. The data was collected by:

Personal Interview

Personal interview was carried out by the researcher with the help of pre designed semi structured questionnaire which contained questions regarding socio demographic and KAP.

Knowledge, attitude and practices: The evaluation of nutrition education imparted was done through a composite interview schedule by pre and post test of knowledge, attitude and practices (KAP) of the subjects in experimental and control group. In each section 15 questions were framed on HIV infection, treatment, nutrition, health and hygiene.

Scoring of KAP

In knowledge 1 score was given and 0 for wrong answer. A respondent could score a maximum of 15 and minimum of 0 in knowledge sectioneach For attitude, a score of 1 was given to 'disagree', a score of 2 was given to 'don't know', and a score of 3 was given to 'agree'. So that , a respondent could score a maximum of 45 and a minimum of 15 in the attitude section. For practices, a respondent obtained score 1 for correct answer and 0 score for wrong answer. So that a respondent could score a maximum of 15 and a minimum of 0 in the practice section. The final KAP score was calculated by additing the scores of the knowledge, attitude and practices sections individually. The total KAP score ranged from 15 to 75. The higher the score indicates tbetter respondent's nutrition-related knowledge, attitude, and practices (Anand and Puri, 2013). The scores were also classified into poor, average, and good scores, ranges for which are shown in Table 1.

Response from the subjects were recorded by the researcher by explaining question from each section viz. knowledge, attitude and practices from pre-tested interview schedule developed to assess nutrition relation knowledge, attitude and practices of control and experimental group.

A pretested manual containing varied aspect of HIV infection, health, hygiene and nutrition was designed to impart nutrition education to experimental group. The baseline information from all registered subjects was collected and the nutritional status, nutritional need and nutritional knowledge were assessed. The Nutrition education was imparted to experimental group through individual counselling with personalized diet chart. During counselling the manual was explained to the subject and handed over to them for their reference. The experimental group was followed every month for the period of six months. After six months KAP of both groups were assessed using interview schedule. The impact of nutrition education on KAP was calculated by formulae used by Monga *et.al* (2008) forG-ain in scores and quantum of improvement. .

Grain in scores = Post test score − Pre test score

$$\text{Quantum of improvement} = \frac{\text{Post test score}}{\text{Pre test score}}$$

Table 1: Scoring and classification of KAP

	Knowledge	**Attitude**	**Practices**	**KAP**
Scoring	1 for correct answer 0 for incorrect answer	1=Disagree 2=Don't know 3=Agree	1=Yes 0=No	Knowledge score + Attitude score + Practices score
Range	0-15	15-45	0-15	15-75
Classification	Poor: 0-5 Average: 6-10 Good: 11-15	Poor: 15-25 Average: 26-35 Good: 36-45	Poor: 0-5 Average: 6-10 Good: 11-15	Poor: 15-35 Average: 36-55 Good: 56-75

Statistical Analysis

Data were cleaned, coded, entered and analyzed for Sample size, per cent, central tendency, dispersion and student *t*-test using the Microsoft Excel 2007. . Socio demographic profile and KAP were subjected to per cent, central tendency, dispersion and *t*-test.

RESULTS AND DISCUSSION

The investigation was done to assess the impact of nutrition education on KAP of HIV/AIDS patients attending of ART centre of Susheela Tiwari Hospital, Haldwani. Data related to socio demographic profile of the subjects has been given in Table 2.

Table 2: Socio- demographic profile of the subjects

	Control (*n* 51)		Experimental (*n* 59)		Total (N 110)
	Females (*n* 28)	Males (*n* 23)	Female (*n* 30)	Male (*n* 29)	
Age (years)					
18-30	37.71	13.04	63.33	48.28	41.82
30-60	60.71	82.61	36.67	48.28	55.45
>60	1.58	4.35	0	3.44	2.73
Educational status					
illiterate	28.6	30.4	23.33	10.34	22.72
Primery	21.4	13.05	10	13.79	14.55
Middle	21.4	13.05	20	10.34	16.36
High School	7.1	17.4	20	37.93	20.90
Intermediate	14.3	17.4	16.66	10.34	14.55
Graduate	3.6	8.7	3.33	13.79	7.27
Post graduate	3.6	0	6.68	3.47	3.65
Religion					
Hindu	89.3	69.6	0	41.37	48.18
Muslims	10.7	30.4	80	55.17	45.45
Sikh	0	0	20	3.46	6.37
Marital status					
Married	32.1	69.6	56.67	68.96	56.36
Unmarried	0	8.7	0	27.59	9.09
Widow/widower	64.3	13	43.33	3.4	31.82
Separated	3.6	8.7	0	0	2.73
Type of family					
Nuclear	78.6	73.9	90	79.31	80.90
Joint	21.4	26.1	10	20.69	19.1
Family size					
0-4	71.4	47.8	83.331	75.86	70.90
5-8	28.6	47.8	16.67	24.14	19.09
Above 8	0	4.4	0	0	10.01
Occupation					
House wife	64.3	0	56.66	0	31.81
Private job	10.7	30.4	13.33	58.62	30
Government job	3.6	0	0	3.45	1.81
Labour	7.1	17.4	10	13.79	11.81
Self employed	0	26.1	0	13.79	9.90
Farmer	14.3	21.7	20.34	6.9	15.45
Retired	0	4.4	0	3.45	1.81
Activity					
Sedentary	78.57	39.13	70	58.62	62.7
Moderate	21.43	60.87	30	41.38	37.3
Per capita income (Rs/month) Mean ± SD	1895 ± 1282 (583-5500))	1769±1351 (400-5500)	2650±1830 (666-7500)	2184±1586 (300-6666)	2125±1512 (300-7500)
Location					
Hill	42.86	34.78	50	24.14	39.09
Plain	46.43	60.87	40	65.52	52.73
Bhabhar	10.71	4.35	10	10.34	9(8.18)

Assessment Knowledge, Attitude and Practices

The per cent of male and female subjects were 47.27 and 52.73, respectively. The per cent of the subjects in the age groups 18-30, 30-60 and above 60 years were 41.82, 55.45 and 2.73, respectively.

The literacy level of the subjects revealed that 77.28 per cent of subjects were literate and 22.72 per cent of subjects were illiterate. Among literate only 10.92 per cent were graduates and the majority of the subjects (66.36 per cent) had education up to intermediate. The religion wise distribution shows that 48.18 per cent of subjects were Hindus and 45.45 and 6.37 per cent were Muslim and Sikh respectively. Majority (56.36 per cent) of subjects were married and 9.09, 31.82 and 2.73 per cent were unmarried, widow/widower and separated, respectively. A large per cent of subjects (80.90) were from nuclear family and only 19.1 per cent of subjects were living in joint family. Majority (70.90 per cent) of subjects had small family size (0-4) while 19.09 and 10.01 per cent subjects had family size of 5-8 and above 8 members, respectively.

A 62.7 per cent of subjects were engaged in sedentary activity and rest 37.3 per cent were moderate worker. Per cent of working subjects was 68.19 while non working subjects were 31.81 per cent. Among nonworking all were females and housewives. Among working subjects majority of subjects (30 per cent) had private job and 15.45, 11.81, 9.90, 1.81 and 1.81 per cent subjects were farmers, labourer, self employed, government job and retired respectively. The per capita income per month of the subjects was Rs. 2125±1512, ranged (300-7500). The study shows that the majority (52.73 per cent) were located in plain where as 39.09 and 8.18 per cent of subjects were located in hill and bhabhar region, respectively.

Distribution of KAP scores: Distribution of KAP scores of subjects at baseline and after six months has been presented in Table 2. The knowledge scores of the control group showed that 37.25 and 35.29 per cent subjects obtained poor sores (0-5) at baseline and after six months, respectively. A 35.29 and 37.25 per cent subjects obtained average scores (6-10) at baseline and after six months, respectively. A 27.45 per cent subjects obtained good scores (11-15) at baseline as well as after six months. The knowledge scores of experimental group show that 30.51 and 5.08 per cent subjects obtained poor scores (0-5) at baseline and after six months, respectively. A 57.63 and 22.03 per cent subjects obtained average scores (6-10) at baseline and after six months, respectively.

The subjects in both the group showed to have better attitude as revealed in table that none of the subjects had poor score (15- 25) for attitude. There was no change in attitude of control group after six months and 21.57 per cent subjects obtained average scores (26-35) and 78.43 per cent subjects obtained good scores (36-45). The experimental group show that the 25.42 and 11.86 per cent subjects obtained average scores (26-35) at baseline and after six months, respectively. A 74.58 and 88.14 per cent subjects obtained good scores (36-45) at baseline and after six months, respectively.

Regarding practices, the control group show 41.18 and 27.45 per cent subjects obtained poor scores (0-5) at baseline and after six months, respectively. A 30.51 and 44.07 per cent subjects obtained average scores (6-10) at baseline and after six months, respectively. A 20.34 and 32.20 per cent subjects obtained good scores (11-15) at baseline and after six months, respectively.

Table 3: Distribution of KAP scores of subjects

Section	Score	Control (*n*= 51)		Experimental (*n*= 59)	
		Baseline	6 months	Baseline	6 months
Knowledge	Poor: 0-5	37.25	35.29	30.51	5.08
	Average: 6-10	35.29	37.25	57.63	22.03
	Good: 11-15	27.45	27.45	11.86	472.88
Attitude	Poor: 15-25	0	0	0	0
	Average: 26-35	21.57	21.57	25.42	11.86
	Good: 36-45	78.43	78.43	74.58	88.14
Practices	Poor: 0-5	41.18	27.45	49.15	23.73
	Average: 6-10	45.10	64.71	30.51	44.07
	Good: 11-15	13.73	7.84	20.34	32.20

Table 4 : KAP scores of the subjects

Section	Score	Control (*n* = 51)		*t* value	Experimental (*n*= 59)		*t* value
		Baseline	6 months		Baseline	6 month	
Knowledge	0-15	7.37± 3.89 (1-14)	7.09±3.76 (1-14)	1.85	6.91±3.45 (0-14)	11.80±3.32 (1-15)	18.52*
Attitude	15-45	38.58±3.83 (29-44)	38.88±3.9 (30-44)	1.87	38.09±5.14 (26-45)	41.26±4.39 (29-45)	11.72*
Practices	0-15	6.66±2.86 (2-12)	6.78±2.39 (2-12)	0.63	6.88±3.39 (1-13)	8.09±3.52 (1-14)	8.06*
Total KAP	15-75	50.06±3.1	50.31±2.95		51.88±3.98	61.15±3.73	

Table 5: Impact of nutrition education on KAP

Section	Period	Total		*t* value
		Control (*n* = 51)	Experimental (*n* = 59)	
Knowledge	Baseline	7.37± 3.89	6.91±3.45	0.64
	6 months	7.09±3.76	11.80±3.32	6.34*
Gain in score		-0.28	4.89	
Quantum of improvement		1.03	1.71	
Attitude	Baseline	38.58±3.83	38.09±5.14	0.47
	6 months	38.88±3.9	41.26±4.39	3.16*
Gain in score		0.3	3.17	
Quantum of improvement		1.01	1.08	
Practices	Baseline	6.66±2.86	6.88±3.39	0.35
	6 months	6.78±2.39	8.09±3.52	2.34*
Gain in score		0.12	1.21	
Quantum of improvement		1.02	1.2	

*Significant difference ($p < 0.05$)

The distribution of scores at baseline and after six months revealed that the experimental group subjects shifted from lower to higher scores contrary to control group where subjects shifted from higher to lower scores. Monga *et al.* (2008) also reported that after nutrition education a highly significant (p<0.01) shift of scores from to lower to higher scores.

Mean KAP score of the subjects has been depicted in Table 3 .Control group obtained 7.37±3.89, 38.58 ±3.83 and 6.66 ± 2.86 at baseline and 7.09±3.76, 38.88±3.9 and 6.78±2.39 scores after six month in reference to knowledge, attitude and practices respectively. Experimental group obtained 6.91±3.45, 38.09 ±5.14 and 6.88 ± 3.39 at baseline and 11.80±3.32, 41.26±4.39 and 8.09±3.52 scores after six month in reference to knowledge, attitude and practices, respectively and gained a significant (p< 0.05) change in respective scores.

Impact of nutrition education on knowledge, attitude and practices

A comparative gain in scores by both groups at baseline and after six months has been depicted in Table 4. The table revealed that gain in KAP scores were 4.89, 3.17 and 1.21 in reference to knowledge, attitude and practices, respectively in experimental group , while in case of control group, gain in KAP scores were negligible i.e. -0.28, 0.3 and 0.12, respectively. The differences in gain of KAP scores between control and experimental group were comparable at the baseline but a significant (P<0.05) improvement was seen in case of experimental group after six months It was found that the quantum of improvement in knowledge, attitude and practices was 1.71, 1.08 and 1.20 times, which signified that nutrition education imparted to experimental group resulted in significant (P<0.05) improvement. Whereas in control group quantum of score was only

1.03, 1.01, and 1.02 times in reference to knowledge, attitude and practices, respectively.

At the baseline both group had KAP scores at average range category (36-55) after six months experimental group obtained 61.15 ± 3.73 score and shifted from average range to good score range (56-75) and shows a significant ($p< 0.05$) improvement in KAP scores while non-significant change in case of control group. Mini *et .al* (2010) found a significant (($p< 0.01$) improvement in knowledge attitude and practice after the educational sessions to HIV/AIDS. Through nutrition education significant improvement was noticed in knowledge, attitude and practices but there is need to improve especially nutrition and health related practices. Torheim *et al.* (2004) stated that low socioeconomic status, level of education, personal beliefs, availability of food, and low nutritional knowledge were the reasons for poor dietary practices.

Monga *et al.* (2008) found that the quantum of improvement in knowledge, attitude and practices was 1.40, 1.57 and 1.14 times through nutrition counselling.

CONCLUSION

The subjects of the present study belong to lower socio-economical group with low education level and most of them are engaged in unskilled job. Nutrition education showed to have significant impact on KAP of people living with HIV/AIDS.

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RESEARCH ARTICLE

"*Streptomyces flavomacrosporus,* A multi-metal tolerant potential bioremediation candidate isolated from paddy field irrigated with industrial effluents"

Sunil KCR, Swati K, Bhavya G, Nandhini M, Veedashree M, Prakash HS, Kini KR, and Geetha N*

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ABSTRACT

Prime drive of the research work carried out was to explore heavy metal-tolerant bacteria from rhizospheric soil samples collected from contaminated agricultural lands of southern India. Soil samples of Mysore agricultural lands of Karnataka state, India, irrigated with industrial effluents from several decades, were brought to the laboratory in sterile polythene bags and were screened by plating serially diluted samples on to Minimal Glucose Yeast extract agar media amended with 0.3mM mercury. Morphologically differing cultures were tested for their tolerance to mercury by growing them in the liquid minimal media amended with 0.3, 0.4, 0.5 and 0.6mM concentrations of mercuric chloride and 0.5, 1.0 and 1.5mM of lead nitrate at optimal physiological conditions. Further the isolates were identified using 16s r-DNA PCR amplification. Blast analysis of the sequence results revealed for the first time the tolerance potential of *Streptomyces flavomacrosporus, Bacillus methylotrophicus, Achromobacter xylosoxidans, Bacillus tequilensis, Bacillus pumilus*, and *Bacillus subtilis* and their chances of participation in bioremediation of mercury. Among these isolates the actinomycetes *S. flavomacrosporus*, showed tolerance to multi metals.

Keywords– Heavy Metals, Minimal Glucose Yeast Extract (MGY), Industrial effluents, Agricultural lands, Metal-tolerant bacteria.

INTRODUCTION

To meet dietary needs of growing global population, due to shortage of natural resources, waste water, industrial effluents and municipal sewage wastes are using as sources of irrigation in most of the countries, including India; which enhances the concentration of heavy

metals in soil and aid transportation of metal into the food chain. Industrial effluents containing heavy metals discharged into streams may pose high toxicity risks to aquatic organisms and to human health. Therefore, it is important to understand how to change the amount of effluents with heavy metals discharged from industries into open aquatic ecosystems both for effective management of heavy metals and to foster sustainable ecosystems (Kwon *et al.,* 2014). Naturally occurring hazardous metal mercury exist on earth crust by various anthropogenic means like metal smelting, industrial production and natural means like erosion, volcanic eruptions etc., (Tchounwou *et al.*, 2003). Mercury is unique because of the combination of the extreme toxicity. It is a metal with no known biological function and low vapor pressure of elemental mercury, which is a liquid at room temperature. In contrast, most other heavy metals are needed by the cell, either as cofactors of enzymes or as electron acceptors for anaerobic respiration (Wagner-Döbler, 2003). Lead (Pb) an extremely stable element, toxic to most of the living forms, as discussed in many scientific literatures lead emission of due to anthropogenic means is 100 times higher than natural emission. Lead poisoning retain as a major source for pediatric health problems in India. (Patel *et al.,* 2006). However, existence of lead in top soil is by various anthropogenic means like mining, smelting, recycling of sewage sludge and motor vehicle exhausts. By these various anthropogenic phenomenon, level of Pb is significantly enhanced in surface soil (Reeder and Shapiro, 2003).

Expensive physico-chemical techniques were unsuitable for treatment of bulk effluents with complexing organic and metal contaminations (Malik, 2004). Wide range of aquatic and terrestrial contamination by various anthropogenic means needs special attention from researchers towards developing sustainable biological tool for the promotion and development of better environmental management. (Juwarkar *et al.,* 2010). It is necessary to understand survivability of microbes in contaminated site to understand metabolic capability of native microbes towards utilizing native resources (Ilyina *et al.,* 2003). Diverged properties processed by microbes while interacting against metal ions like metal speciation, toxicity, mobility and mineral dissolution entertained most of the researchers to study interaction of microbial community in contaminated site (Gadd, 2013). This open up to develop clean-up strategies by isolating metal resistant microbes, which is a simple environmental friendly and cost effective and alternative to current treatment technologies (Wagner-Döbler, 2003). It is know that ability of metal tolerance were greatly influenced by the environmental conditions, study of metal-microbe interactions by various extra cellular and intra cellular phenomena will aid scientific community to develop effective clean-up strategies (Gadd and Griffiths, 1977). With this background present work was primely focused on screening of potent bioremediation candidate from the soil samples which can contribute for removal of pollutants by building suitable research strategy in the future work.

MATERIALS AND METHODS

Collection of samples

Soil samples were collected in the mid 2013 from agriculture lands of suburban Mysore, Karnataka state, India, irrigated with industrial effluents. Rhizospheric soil samples were collected from the depth of 0.5-1feet from different location (Fig 1) of the sampling site. Samples were collected in sterile polythene bags and brought to the laboratory for further studies.

Fig. 1: Google earth image showing GPRS location of sampling site in suburban area of Mysuru district.

Isolation of bacteria tolerant to multi-metals

25 g soil samples were serially diluted with 225 mL of 0.9% saline, and six-fold dilutions were plated on to MGY agar (minimal media) (Zhu et al. 2012)

supplemented with 0.3 millimolar concentration of mercuric chloride and MGY agar without mercuric chloride as a control and incubated at 37^0 C for 48 hours and experiments conducted in triplicates.

Morphological and biochemical characterization of metal tolerant bacteria

Gram staining was carried out according to the protocol (Vincent 1970). Results were confirmed by KOH solubility test, also ability of isolates for the production of gelatinase enzyme was performed according to (James and Sherman 1987).

Physiological characterization of metal tolerant isolate

Determination of optimum temperature

The temperature at which the cultures had their maximum growth was considered the optimum temperature. The optimum temperature for each isolates was determined by inoculating them on LB broth at different temperatures like 25^0C, 35^0C, 37^0C and 40^0C in (New Brunswick™ Innova, USA) at 100rpm, growth rate was measured against turbidity of the medium till 96 hours with 24 hours of time intervalsat 600nm using UV spectrophotometer (Beckman coulter DU 700, Germany).

Determination of optimum pH

Optimum pH of metal tolerant isolates was done by growing cultures at different pH conditions, isolates were inoculated against pH 4, 7, 9 and 12. And incubated at their optimal temperature (New Brunswick™ Innova, USA) at 100rpm, growth rate was measured against turbidity of the medium till 96 hours with 24 hours of time intervalsat 600nm using UV spectrophotometer (Beckman coulter DU 700, Germany).

Determination of minimum inhibitory concentration against mercuric chloride

Cultures were inoculated on MGY media amended with mercuric chloride atdifferent concentrations like 0.3, 0.4, 0.5 and 0.6 mM and incubated at optimal conditions, growth rate was measured against turbidity of the medium till 96 hours with 24 hours' time interval by at 600nm using UV spectrophotometer (Beckman coulter DU 700, Germany).

Determination of minimum inhibitory concentration against lead nitrate

Isolates grown in Lead nitrate amended in MGY media at different concentrations of0.5, 1.0 and 1.5 mM were measured to record growth rateat 600nm,using UV spectrophotometer (Beckman coulter DU 700, Germany). After a day of incubation at optimal physiological conditions.

Molecular characterization of metal-tolerant bacterial isolates

DNA isolation

Genomic DNA was isolated by using altered protocol of Doyel and Doyel's method. Five-day-old bacterial cultures grown in LB broth at 35^oC were transferred to 2 ml micro-centrifuge tube and centrifuged at 10000 rpm for 15 min, supernatant was discarded and the pellets were treated with 500µl of extraction buffer with the composition of 100mMTris, 20mM EDTA, 500mM NaCl and 5% SDS, and incubated for 30min at 65^oC. The solution with chloroform and isopropanol was mixed with the ratio of 24:1 till the solution forms slurry. This milky white solution was centrifuged at 12,000 rpm for 10 min. aqueous layer was transferred to sterilized eppendroff and equal volume of chloroform: Isopropanol was added and centrifuged at 12000 rpm to pellet out DNA for about 10 min. To the transferred aqueous solution $1/10^{th}$ volume of sodium acetate was added and incubated at -20^oC for 60 min, after the incubation period it was centrifuged at 12000 rpm for 10 min, the pellets were washed with 70% ethanol which was air-dried and dissolved in 20-40µl of elution buffer and stored at -20^oC (Doyle 1991).

PCR amplification of 16s rDNA

Total DNA from 13 samples were used as template to amplify variable region of bacterial 16s rDNA by PCR using the universal primers 16sF 5'-CCAGACTCCTACGGGAGGCAGC-3' and 16sR 5'-GCTGACGAGAGCCATGCAGCACC-3' (Sigma Aldrich). The PCR reaction system of 50µl included 5µl of 10x Taq buffer,1 µl of 0.2mM dNTPs, 0.15µl of forward and reverse primers(10pmol), 10 µl of DNA dilution 100ng/ µl,1.75 µl of Taq polymerase(1U) and 37.45µl of nuclease free water and the system was programmed with 30 cycles at 94^0C for 4 mins,94^0C for 45secs, 55^0C for 45secs,72^0C for 1min and 74^0C for 10mins (Bio Rad). The results were analyzed by 1.2% agarose gel electrophoresis.

nBlast analysis of nucleotide sequence

All sequences were identified using NCBI nucleotide blast as the selected algorithm, except highly somewhat similar sequences algorithm was chosen to identify the sequence and the Mercury-tolerant bacterial isolates was identified.

Phylogenetic analysis

A phylogenetic tree was constructed based on the aligned sequence of partial 16s rDNA region. Evolutionary analyses were conducted in LIRMM by using Neighbour-Joining (NJ) and Maximum Parsimony (MP) analysis.

RESULTS AND DISCUSSION

Remediation of Alaska coastal line which was contaminated with oil spill of Exxon Valdez in 1989 using bioremediation tools gained the public attention. Most importantly activity of bacteria relies on ability and availability of residual organic materials to serve as energy sources, it is strongly dependent on nature of contamination in the site. Site-specific bioavailability influences bioremediation process positively towards developing effective clean-up strategies (Boopathy, 2000).

Table1- Biochemical characteristics of Mercury tolerant bacteria

Isolates Code	Name of the isolate	Gram's test	KOH solubility test	Gelatin hydrolysis test
UOMGSP001	*Bacillus tequilensis*	+	-	+
UOMGSP002	*Achromobacter xylosoxidans*	-	+	+
UOMGSP003	*Bacillus pumilus*	+	-	-
UOMGSP005	*Bacillus methylotrophicus*	+	-	+
UOMGSP008	*Streptomyces flavomacrosporus*	+	-	-
UOMGSP009	*Bacillus subtilis*	+	-	+

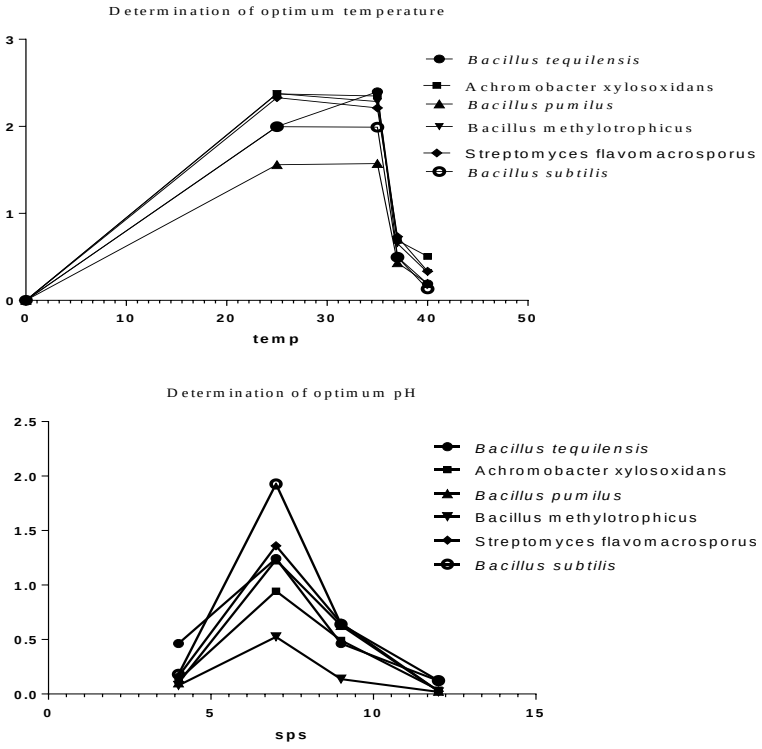

Fig. 2: Physiological characterization of metal tolerant isolate. Determination of optimum physiological conditions of selected mercury resistant isolates

It is known that rhizospheric microbes play a major role in altering physico-chemical characteristics of its surrounding environment, which possess impact on biogeochemical mobility of metals and associated elements (Gadd, 2010).

Our experimental studies on evaluating metals tolerance ability of bacterial isolates isolated from rhizospheric soil samples from the agro-climatic regions of southern Karnataka irrigated with industrial effluents enabled us to isolate twelve bacterial isolates tolerant to mercury and lead, among the twelve isolates *Bacillus methylotrophicus*, *Streptomyces flavomacrosporus, Bacillus subtilis, Bacillus pumilus, Bacillus tequilensis*which were positivetogram's reaction and *Achromobacter xylosoxidans* which

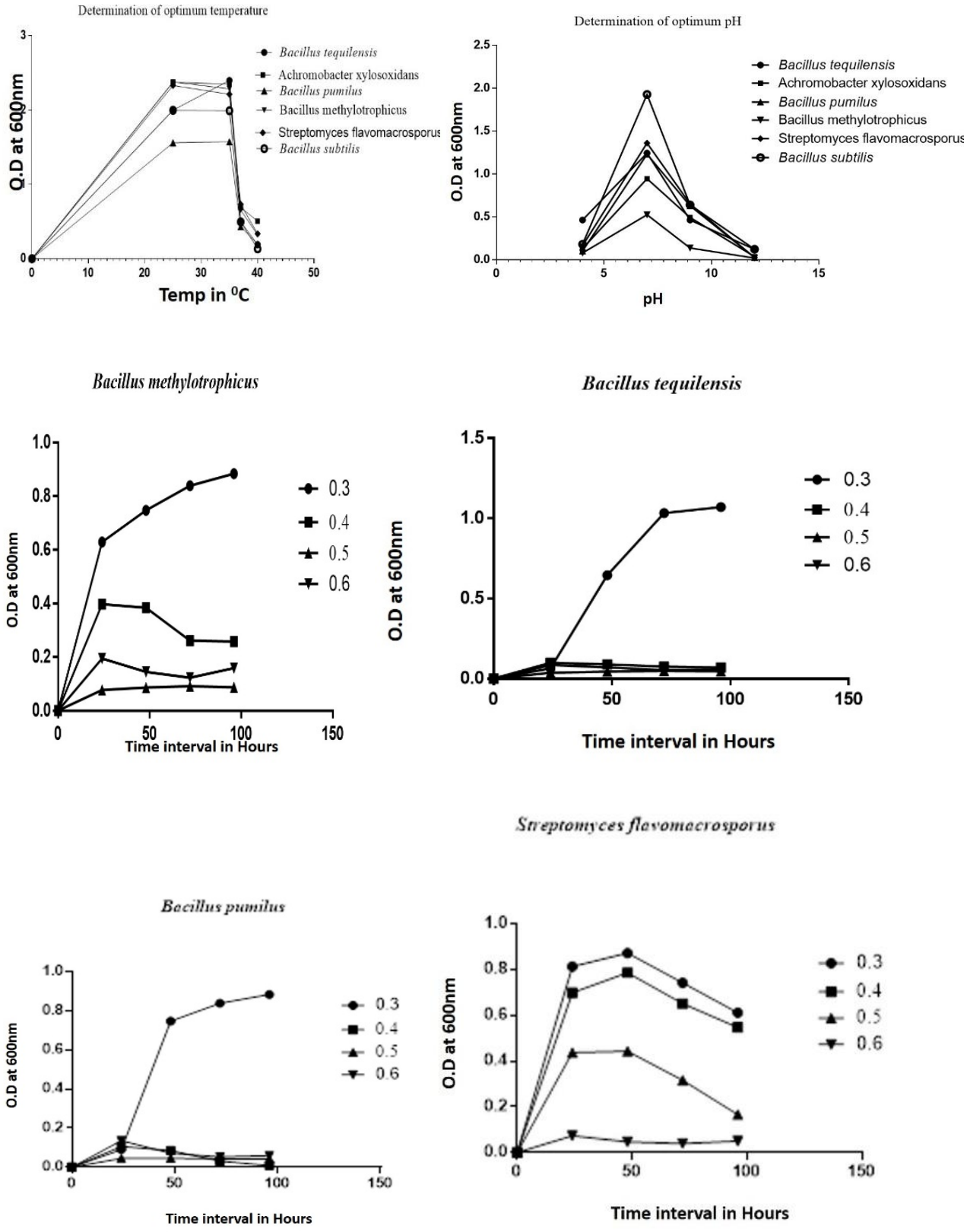

Fig. 3: Determination of MIC of heavy metal salt mercuric chloride against mercury tolerant isolates

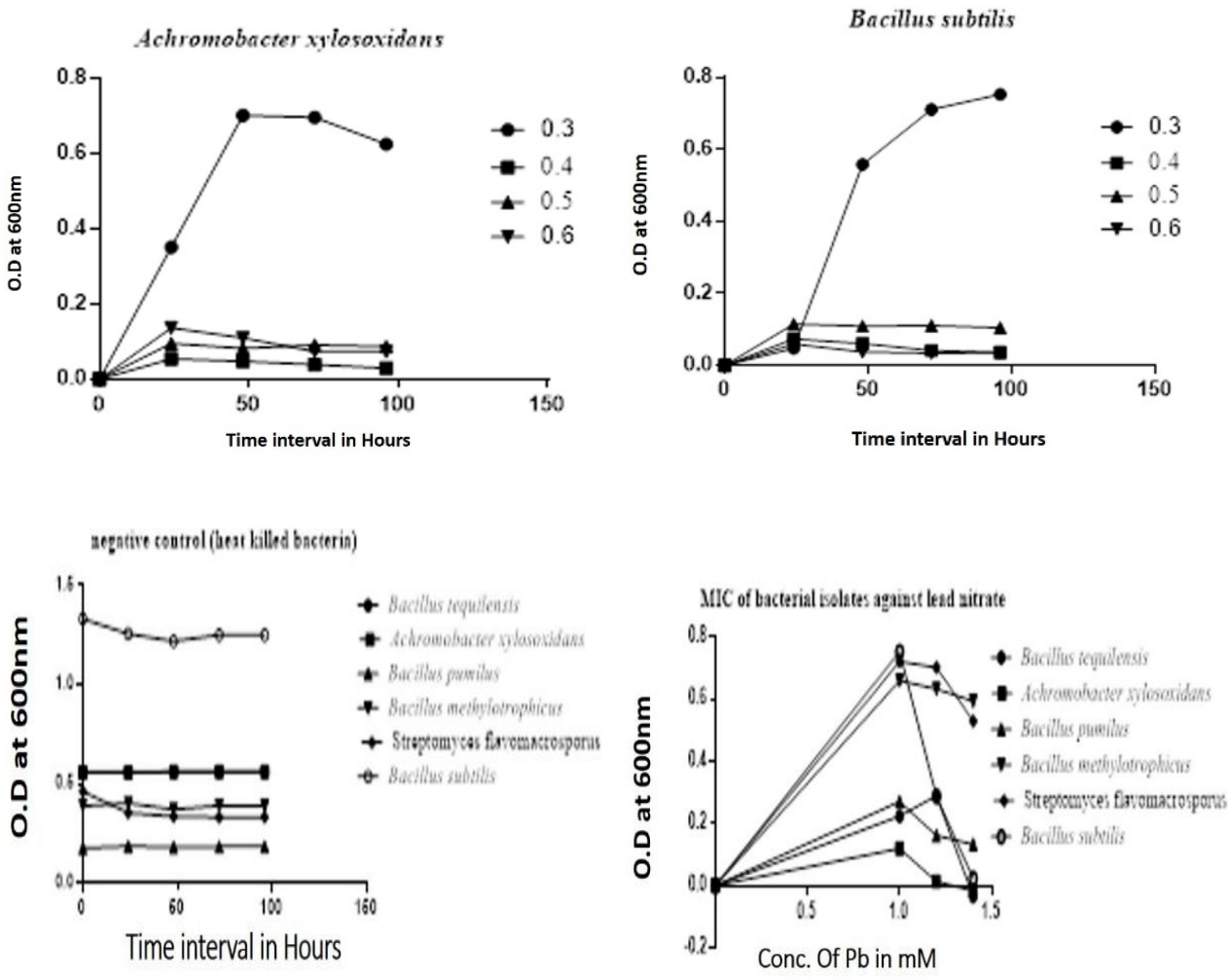

Fig. 4: Determination of MIC of heavy metal salt mercuric chloride against mercury tolerant isolates

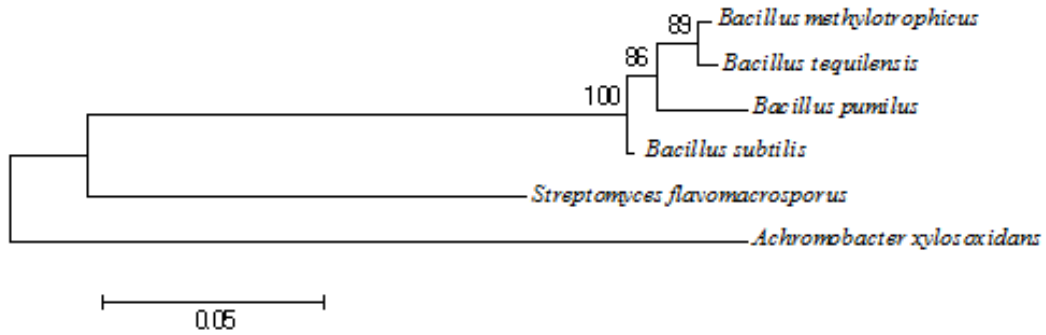

Fig. 5 : Phylogenetic analysis

is negative to gram's reaction. All the selected six isolates were grown at optimal pH 7 and optimal temperature 25^{0}C also shown to be grown equally even at 45^{0}C by exhibiting thermostatic nature, isolates were chosen based on their comparatively higher growth competence and enhanced tolerance for mercury at 0.3, 0.4, 0.5 and 0.6 mM and lead at 0.5, 1.0 and 1.5mM conc. *Bacillus tequilensis, Bacillus pumilus, Achromobacter xylosoxidans* and *Bacillus subtilis* were exhibited higher growth at the concentration of 0.3mM of mercuric chloride concentration after 24 hours and no significant growth were recorded at the higher

concentrations like 0.4, 0.5 and 0.6 mM concentrations respectively. When these isolates were subjected to determine their concentration for minimal inhibition against lead nitrate it is recorded that *Bacillus tequilensis, Bacillus Pumilus* and *Achromobacter xylosoxidans* grown at moderate level not exhibited much resistant at any of the treated concentrations. But isolate *Bacillus subtilis* showed its maximal growth capacity at both the concentration of 0.5 and 1.0mM of lead nitrate.

Isolates like *Bacillus methylotrophicus* and *Streptomyces flavomacrosporus* shown to be higher growth competence with respect to all other selected candidates when it is subjected to study their MIC against mercuric chloride at the concentration of 0.3, 0.4, and 0.5mM conc. At fair growth was recorded at 0.6mM conc. and constant growth was recorded even at the concentration of 0.5, 1.0 and 1.5mM of lead nitrate, with respect to *Streptomyces flavomacrosporus* and *Bacillus methylotrophicus* shown to be a good bioremediation candidate by exhibiting its higher growth rate even at 0.3, 0.4 and 0.5mM conc. and lowest growth rate at the conc. 0.6mM with respect to MIC test against mercuric chloride. It has also revealed its capacity to grow at all the test concentrations of lead nitrate of 0.5, 1.0 and 1.5mM of lead nitrate. With results it is concluded that these bacterial isolates may have potential implication cleaving up of detoxifying mercury and lead contaminated sites in the future. Therefore, based on the physiological, biochemical and molecular characterization results, the study reports *Streptomyces flavomacrosporus* for the first exhibiting tolerance to toxic heavy metal Mercury and lead.

CONCLUSION

Though there are several potential metal tolerant bacteria isolated across, it is necessarily important to design the clean strategy with the native species. There is much debate over whether to use natural or genetically engineered microbes in bioremediation. The advantages of naturally-occurring microbes currently outweigh those of GEMs (Boopathy, 2000)

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RESEARCH ARTICLE

Correlative studies on the body weight, testis weight and plasma testosterone of *Pteropus giganteus giganteus* (Brunnich)

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Deshmukh GD and Dhamani AA (2015) Correlative studies on the body weight, testis weight and plasma testosterone of *Pteropus giganteus giganteus* (Brunnich), *Int. J. of Life Sciences*, 3(1): 15-20.

ABSTRACT

In the present study correlation between the body and testis weight with that of mean plasma testosterone concentration was investigated from the pteropid bat, *Pteropus giganteus giganteus*. The cyclic variations in the body and testis weight were found to in consonance with the hormonal fluctuations. The total body and testis weight beginning to rise in July-August which is after 1-2 months after food availability (mango fruiting) in the local study area of this bat. And then, further reach to its peak level during Oct-Nov. which may be correlated with higher mean plasma concentration. Early rise in the body and testis weight which is complemented by the increase in plasma testosterone concentration may be due to initiation of spermatogenesis. Availability of food may act as stimulus for the onset of spermatogenesis.

Key words - Testis**,** plasma, testosterone, spermatogenesis, hormone.

INTRODUCTION

Many mammals are seasonal breeders and respond to annual climatic changes by adaptive alterations in physiological as well as in histoarchitechtural status in anticipation of the coming season. The switching on and off of reproductive functions during the annual breeding cycle of bats is the most striking example of such photoperiodically induced process. (Krutzsch and Crichton, 1990; Gopalkrishna and Badwaik., 1993; Begueliani, et al., 2009). Intraspecific variation has been reported, not just in the timing of reproduction, but also in the periodicity of reproduction in different environments and across the geographic range of the species (Vivier and Van der Merve, 1996). It is therefore often impossible to characterize a specific pattern of reproduction within species with a wide distribution (Bernard and Cumming, 1997).

The occurrence of varied reproductive patterns appears to be generally related to major differences in latitude. (Bernard and Cumming, 1997). A number of studies have addressed seasonal changes in structure and function of the male tract of pteropids (McGuckin and Blackshaw, 1987; 1991). After taking review of pertinent literature it was found that, reproductive pattern of *Pteropus giganteus giganteus* was not undertaken in the study area. It is a unique geographical site, characterized by heavy rainfall and extreme hot summer. This geographical situation and climatic condition in the local area prompt us to undertake reproductive investigation in this ecologically important but neglected pteropid bat.

MATERIALS AND METHODS

The specimens of *Pteropus giganteus giganteus* from roosting site of Mango trees from Bramhapuri forest range, Maharashtra, India. On the same day, these live animals, were bought to the laboratory and specimen anesthetized by petroleum ether and weighted on sensitive spring balance.

Male animal were dissected out to collect the testis from the scrotal sacs. Fixed in alcoholic Bouin's fluid for 24 hrs. Dehydrated in upgrade series of alcohol, infiltrated in paraffin wax and blocks were prepared. Sectioning carried out on rotory microtome for getting ribbon of 5µm thickness. Ribbons having sections of testis and epididymis were affixed on the slides. Staining of sections carried out with the help of double staining of haemotoxyline and eosin. Sections were cleared in xyline and mounted in DPX. Sections were photographed with the help of image capturing device of 'Labomed make' attached to the compound microscope. Microscopic measurements of different parameters were also taken with help of inbuilt software of image capturing device.

RESULTS AND DISCUSSION

The variations in the morphological parameters and plasma testosterone hormonal profile were used to assess the different periods during the annual reproductive cycle of *Pteropus giganteus giganteus* are shown in the Table.1. Table.2. shows correlation coefficient indices for body weight, testis weight and mean plasma testosterone concentration.

On the basis of presence of sperms in the seminiferous tubules and epididymis and mean plasma concentration of testosterone hormone the reproductive cycle was divided into four different periods, preparatory, breeding, post-breeding, and regressed. (Fig. 1 to Fig. 8) The adult testes of *Pteropus giganteus giganteus* shows great variations in their weight during different months annual reproductive cycle. The maximum weight of testis is found during Aug-Oct. (1.67gm ±0.42) which coincides with the mean body weight of the animal which is progressively increases to 750 gm (SEM ±0.83)(Table No.1). After this period the weight of the testis progressively decreases and the minimum weight found during the months of Feb. to April (1.03 gm ±0.36) which is reflected in the drop in the minimum mean body weight to 625 gm (SEM ±0.74) (Table.1).

In the Fig. 9, the annual variations in the mean body weight is correlated with the mean testis weight, which shows that increase in the mean testis weight reflected in the increase in the mean body weight of the animal. The testis of *Pteropus giganteus giganteus* exhibits marked seasonal changes during following periods of annual reproductive cycle as follows.

Table 1: Seasonal variations in body weight, testis weight and mean plasma testosterone

Sr. No.	Parameters	Preparatory (May-July)	SEM (n=5)	Breeding (Aug-Oct)	SEM (n=5)	Post-breeding (Nov-Jan)	SEM (n=5)	Regressed (Feb-April)	SEM (n=5)
1	Body wt.	675 gm	± .78	750 gm	± 0.83	735 gm	± 0.65	625 gm	± 0.74
2	Testis wt.	1.56 gm	±.39	1.67 gm	± 0.42	1.39 gm	± 0.31	1.03 gm	± 0.36
3	Testosterone	6.4 ng/ml	±.26	3.6 ng/ml	± 0.28	9.2 ng/ml	± 0.28	0.46 ng/ml	± 0.23

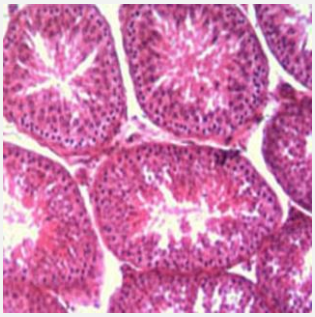
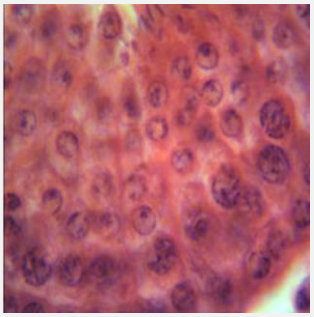
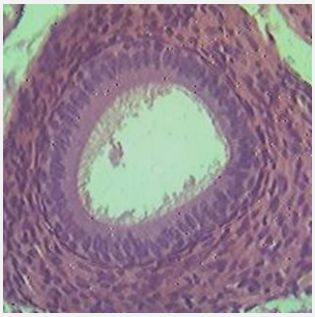
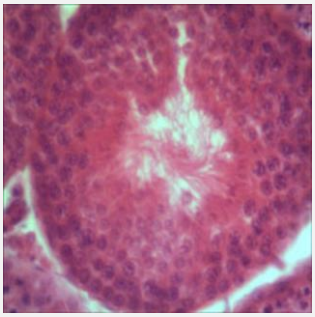





Fig. 1 **Fig. 2** **Fig. 3** **Fig. 4**

Fig. 1: T. S. of testis showing seminiferous tubules and interstitium during the pre-breeding period of *Pteropus giganteus giganteus* (X100)

Fig. 2: T. S. of testis showing seminiferous epithelium having cohorts of spermatogenic cells displaying few spermatozoa towards lumen during the pre-breeding period of *Pteropus giganteus giganteus* (X1000)

Fig. 3: T. S. cauda epididymis showing pseudostratified epithelium surrounded by connective tissue having well developed stericocilia (STC) during the pre-breeding period of *Pteropus giganteus giganteus* (X400)

Fig: 4: T. S. of testis showing seminiferous tubule with epithelium having cohorts of spermatogenic cells and large number of spermatozoa projecting tails towards its lumen during the breeding period of *Pteropus giganteus giganteus* (X400).

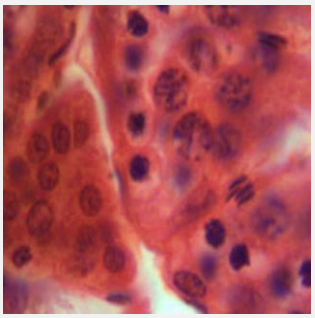
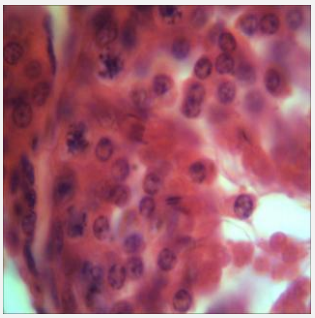
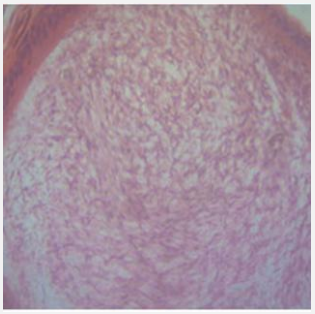
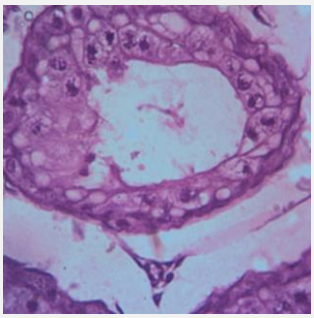





Fig. 5 **Fig. 6** **Fig. 7** **Fig. 8**

Fig: 5: T. S. of testis showing seminiferous epithelium with spermatogenic cells and the Sertoli cells (SC) attached spermatozoa and cluster of Leydig cells (LC) in interstitium during the breeding period of *Pteropus giganteus giganteus* (X400)

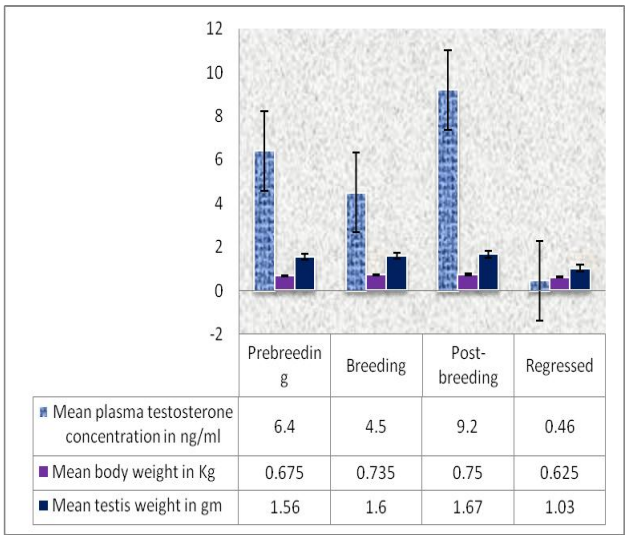
Fig. 6: T. S. of testis showing seminiferous epithelium with Sertoli cell and spermatogenic cells and few sperms (SPZ) released into the lumen also seen few residual bodies (RB) during the post-breeding period of *Pteropus giganteus giganteus* (X1000)

Fig. 7: T. S. cauda epididymis showing large number of spermatozoa entangled into the anastomizing network of well developed long stericocilia (STC) during the post-breeding period of *Pteropus giganteus giganteus* (X1000)

Fig. 8: T. S. of testis showing seminiferous epithelium with vacuoles and large empty lumen (L) separated by spacious interstitium having shrunken Leydig cells during the regressed period of *Pteropus giganteus giganteus* (X400).

Table 2: Correlation coefficient between body weight, testis weight and mean plasma testosterone.

Parameters	Body Wt	Testis Wt	Testosterone
Body Wt	1		
Testis Wt	0.893141	1	
Testosterone	0.790184	0.898001	1



	Prebreeding	Breeding	Post-breeding	Regressed
Mean plasma testosterone concentration in ng/ml	6.4	4.5	9.2	0.46
Mean body weight in Kg	0.675	0.735	0.75	0.625
Mean testis weight in gm	1.56	1.6	1.67	1.03

Fig. 9: Showing correlation between mean plasma testosterone concentration, mean body weight and testis weight

These results in the present investigation expressed in graphical and tabular presentations find its parallel in the similar studies conducted on the number of chiropteran species by many workers. (Bernard., 1986). Adult male grey-headed flying foxes (*Pteropus poliocephalous*) shows well-defined seasonal changes in reproductive parameters in wild (McGuckin and Blackshaw, 1987) and in captivity (McGuckin and Blackshaw, 1991). In some species fluctuation of body weight can indicate times of food abundance and scarcity (Bhasin et al., 1997).

According to McGuckin and Blackshaw (1991), assessment of changes in testicular weight in individuals provided information on the cyclic seasonal changes. The positive correlation between the body weight, testes weight and plasma testosterone concentration has been identified by Singh (1997); Singh and Krishna, (2000) in *Rousettus leschnaulti* which experiences two peaks of testosterone in a single testicular cycle (Sastry and Masram, 2007). The total weight of the body and testes of *Pteropus giganteus giganteus* in the present study beginning to rise in July-August which was about 1-2 months after the higher food availability (Mango fruiting) in the study area of this bat (Fig. 9). Then, the weight of the testes further increases during September and October and reaches to its peak level, these variations in the body weight and testis weight is positively correlated with the mean plasma concentration **(**Fig. 9). The data on steroid concentrations in blood plasma obtained during annual reproductive cycle shows large variations. The higher peak of mean plasma concentration of testosterone during mating period is due to increase in testicular production which is paralleled with similar findings by McGuckin and Blackshaw, 1987. In accordance with the earlier literature, since the biosynthesis of testosterone levels which is a key hormone in the maintenance of body mass and testicular weight, the changes in the testicular and body weights were correlated with the plasma testosterone concentration. (Choudhary and Sastry., 2011).

In the present study attempt has been made to correlate the body weight, testicular weight and testosterone values, since androgen is a potent stimulant of nitrogen retention (Bhasin et al., 1997), causes an increase in the body weight due to an increased serum concentration of potassium (Turner and Bagnara, 1976). In addition, testosterone also modulates growth and metabolism in several peripheral tissues that contain androgen receptors such as skeletal muscle (Sauerwein and Meyer, 1989). In muscular tissue, adrogens stimulate cell hypertrophy to glycolytic white musce cells possibly via reduction in glucocorticoid sensitivity (Sauerwein et al., 1991) and reduced proteolytic enzyme activities. (Blottner et al., 1996). The dramatic variations in plasma concentrations of steroid binding protein observed in the adult male little brown bats also confirm a seasonal variations of testosterone since the steroid binding proteins are the carriers of this hormone (Gustafson and Damassa, 1985; Gustafson, 1987). Physiologic increases or decreases in circulating steroid binding protein levels would be expected to influence the availability and therefore action of androgens (Gustafson and Damassa, 1985).

The data on mean plasma concentrations of *Pteropus giganteus giganteus* during breeding and post-breeding season suggest that the large increase in peripheral testosterone during the mating period is due, at least in part, to increased testicular production. Leydig cells appear fully active, histologically before the peak (October), suggesting that further stimulation occurs resulting in increased production of testosterone. Changes in stimulus could occur in response to many factors including environmental zeitgebers, female pheromones or stimulation of central nervous system associated with mating or territorial behavior (McGuckin and Blackshaw, 1991).

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RESEARCH ARTICLE

Traditional Use of Domestic Animals among Pardhan Tribes of Chhindwara District of Madhya Pradesh, India

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ABSTRACT

Present Ethnozoological study highlights the conservation of domestic animals reared by Pardhan community in Bicchua block of Chhindwara district of Madhya Pradesh. Field study was conducted with Pardhan people with the help of semi structured questionnaire survey. 20 Pardhan people provided information of different ethnomedicinal and other purposes of domestic animals. The results shows that there are 18 domestic animals used in 39 ailments (like weakness, diabetes, cough, cold, tuberculosis, paralysis, fit, asthma, constipation, eye disease, cancer, ulcer, sun stroke, hair growth, drug addiction etc.) and more other purposes. Domestic animals are very important in rural life. Pardhan have rich knowledge of their livestock and emotionally attached with them. In this area there are no any documented literature of their knowledge is available so, this study aims to proper documentation of traditional knowledge of Pardhan community and their livestock.

KEY WORDS: Pardhan community, Domestic, Agriculture, Livestock, Nature, Conservation.

INTRODUCTION

Chhindwara district is located in south eastern part of Satpura plateau between 21 Deg. 28' North Latitude and 78 Deg. 10' East Longitude. In the south of the district lie the plains of Nagpur and Amrawati district in Maharastra, where as Betul and Seoni districts from the western and eastern boundaries.

Since long, humans have always been in intimacy with animal life in their habitats for food, transportation, and medicine through observation and experimentation. However, it can be assumed that concern about animal health only originated after the domestication of

formerly wild animals species for use in transportation, agriculture, medicine, or as direct food source. So our ancestors started converting wild animals into domestic ones for their benefit throughout the world, (Vyas *et al*., 2009).

The healing of human ailments by using therapeutics based on medicines obtained from animals or ultimately derived from them is known as zoo-therapy, (Costa-Neto 2005). Indigenous knowledge of domestic animals or wild animals for therapeutic medicine of tribes is very important. There are no any medical facilities in remote areas, so Pardhan community fulfills this lacuna.

MATERIALS AND METHODS

The field study to acquire information about traditional uses from domestic animals was conducted in Chhindwara district of Madhya Pradesh in the period from January 2010 to June 2010. The study was conducted using questionnaires survey and open interview with *Pardhan* people. Most of the informants were farmers, healers and old peoples. A total of 20 informants including male and female within age group of 45 to 85 years were selected, based on their specialized knowledge, their experience of traditional practices. They were asked about traditional medicines prescribed for various ailments. They were also discussed about the present status of their livestock and steps taken by them for welfare and conservation of their livestock.

The name of animals and the other information related to this study was documented. According to them, their knowledge of traditional medicine was acquired mainly through parental heritage and experience about medicinal value of domestic animals to heal their kin or themselves. Their life is depended upon livestock so they told that it was their moral duty to save the animals.

Pardhan Tribes

Pardhan tribes are dominant in Bicchua block of Chhndwara district. Pardhan economy is based on agriculture. For agricultural and other works of domestic animals like Goat is reared for milk, meat, breeding and selling for their livelihood, Cow is reared for milk, its dung is used as manure and breeding is also done, Buffalo and Oxen for agricultural work and manure too. Apiculture is a common practice. They also rear poultry, pigeons for egg and meat and dogs for guarding their livestocks. They collect forest materials and weeds for their livestock. Pardhan have rich knowledge of zootherapy and other multifarious purposes of domestic animals. So here it is some collected informations from the attempted study of ethno-zoological knowledge of *Pardhan* community of Chhindwara district of Madhya Pradesh.

RESULTS AND DISCUSSION

In this study there are 18 domestic animal species are used by Pardhan community for medicinal, magico-religious, omen indicator, socio-religious, dowry, business, ethno-musical, agriculture, transport, entertainment, industry and food purposes. Out of 18 animal species 39 medicinal, 6 magico-religious, 7 omen indicator, 7 socio-religious, 5 dowry, 2 business, 3 ethno-musical, 9 agriculture, 3 transport, 1 entertainment, 2 industry and 6 food uses are found.

Medicinal and Food values

16 animal species are used for 39 medicinal purposes. Some domestic animals such as *Apis spp*., *Gallus spp*., *Columba spp., Anus spp., Capra spp., Sus scrofa domestica* are the animals with double role among the domestic vertebrates. Mass production of such animal spp. will form important source of ethno-medicine as well as protein supplement to the people and similar kind of use in other tribal group of India, (Lohani 2010; Kakati and Dulo 2002).

Animals in a Magico-religious sphere

Animal parts and products are used for making amulets (Tabij) such as feather of the *Columba spp*., dried placenta of *Equus asinus* and *Felis bengalensis*, amulets protect them diseases and evil spirit. Similar kind of relationship of the Tamang and Jirels people with the animals at the spiritual/cultural level is reported from Nepal and Brasil, (Lohani 2010, 2011; Alves *et al*., 2009, 2012; Alves 2012).

Animals in omen indication

Crossing of the road by cat (*Felis bengalensis*) indicates a bad omen and howling of dog (*Canis familiaris*) predicts bad news for the family of the vicinity. Similarly, crowing of the cock (*Gallus gallus*) at a time

other than morning is bad omen. Similar kind of relationship at spiritual level is found among the Yi people of China (Hong 1990) and Adi tribes of Arunachal Pradesh, (Borang 1996).

Animals in Socio-religious sphere

Only live animals are used in socio religious purposes. On the important festivals it is supposed to give offering of red/white fowl (*Gallus gallus*), *Bubalus bubalis,* and *Capra indica* to Muthua Dev, Mal Dev and Chandi Mata. Sacrificial offerings are made to please the deities or ancestral spirits which are supposed to control the life of these people, also used by Monapa tribes of Arunachal Pradesh, (Solanki and Chutia 2004).

Other multifarious uses

Domestic animals are used by Pardhan community in dowry, business, ethno-musical, transport. These are common practices.

Table 1: Traditional use of Domestic animals by Pardhan tribes of Chhindwara district of Madhya Pradesh

ARTHROPODA			
1.Honey bee	*Apis spp*.	Madhu Makkhi	Bee hive boiled with mustard oil and the extract used to treat cracks and scars**. (Medicinal)**.
			Honey applied on burn area for rapid healing**. (Medicinal).**
			Honey applied in eye to improve eye power. (**Medicinal).**
			Larvae, pupae and eggs are eaten with great taste for its nutritive value. (**Medicinal).**
			Dry hive fume passes through eye part for conjuctivitis**. (Medicinal).**
			Bee is allowed to sting the heart patients for relief**. (Medicinal**).
			Bee is allowed to sting the paralyzed patients. **(Medicinal**)
			Honey is taken orally to be cure weakness. **(Medicinal**).
			Honey is a highly preferred food item. **(Food).**
			Honey bee is worshiped as kuldewta by Gotra- Vithika, Bhor and Bhalavi. **(Socio-religious).**
2.Lac insect	*Lacifer lacca*	Lakh	Lac powder mixed with coconut oil is applied over leprosy and general wound for easy healing. **(Medicinal)**
			Lac powder administered orally for leucorrhea. **(Medicinal)**
			Fume of lac powder especially Pepal tree used for mental disorder. (**Medicinal).**
			Lac fume use for children their general well being and that drive evil spirits away**. (Magico religious).**
			Lac is kept in home to get safe children from bad omen and well being**. (Omen indication).**
3.Silk worm	*Bombyx mori*	Kosa kida	Cocoon grind with water administered orally to children for asthma, cough and cold**. (Medicinal).**
			Ash of larva mixed with honey is applied on chest for pneumonia. **(Medicinal).**
			Silk is obtained from cocoon that is why sericulture has now become a good industry. (**Agriculture and industry)**
AVES			
4.Domestic fowl	*Gallus gallus*	Murgi	Flesh is used for tasty food. (**Food).**
			Egg is used for weakness**. (Medicinal**).
			Fresh gall bladder orally administered for diabetes. **(Medicinal).**
			Fresh egg shakes with milk is administered orally for cough and cold**. (Medicinal**).
			Gizzard of **black** hen is dried, powdered and consumed for 15 days once daily to treat asthma. **(Medicinal**).
			On the festival of **Gyaras, Diwali** and **Amawas** it is supposed to give offering of red/white fowl **to Muthua Dev**, **Mal Dev** and **Chandi Mata. (Socio religious**).

			Both incident such as crowing of the cock at a time other than morning and crowing of hen are though to be bad omen (**Omen indication**).
			Excreta and feather are used as manure. (**Agricultural**).
5.Pigeon	*Columba livia*	Pareva	Flesh is cooked with cumin powder and consumed to treat asthma. **(Medicinal)** Flesh eaten for its nutritive value. (**Food**).
			Fresh blood massaged on affected part for 7 days once a day to cure paralysis**. (Medicinal**).
			To flicker air also use for paralysis**. (Medicinal**).
			It is a common belief amongst the rural folk that pigeons are domesticated it is bad luck in future and predicts the death of person**. (Omen-indication**)
			It is believed that amulets made of feathers of bird have the power to protect the bearer from evil spirits**.(Magico-religious**)
			Egg is a good source of protein for children**. (Food**).
			Excreta and feather are used as manure. **(Agriculture**).
6.Duck	*Anus spp*.	Baduk	Flesh is cooked and eaten**. (Food**).
			Egg is used as nourishing food. **(Food**, **Medicinal**).
			Excreta are used as manure. (**Agriculture**).
MAMMALS			
7.Cow	*Bos indicus*	Gai	Urine used to as an eye drop to relieve eye disease. **(Medicinal**).
			Curd used orally to treat constipation. **(Medicinal**).
			Small piece of abdominal stone (guranjan) used to treat asthma. **(Medicinal).**
			Excreta smeared in the floor of traditional houses**.** (**Socio religious**)
			Skin is used to make Tambura, Dhol, Timki, Kinnari, Mandar, etc. **(Ethno musical).**
			Dried dung is used as source of domestic and Industrial fuel. **(Industrial)**
			Urine and dung is used as manure. **(Agriculture).**
			Cow is used in dowry. **(Dowry).**
			As cow is a sacred animal to hindus. (**Symbolic**)
			Urine of cow is highly valued. It is sprayed in the house and the adjoining areas to sanctify and to disinfect. (**Sanitation).**
			Urine is also used as pesticide and is sprayed in the agriculture field to kill the pest. (**Agriculture).**
8.Bull	*Bos taurus*	Bail	Decayed horn larva (Musca spp.) used for fit. **(Medicinal).**
			Skin is used to make Tambura, Dhol, Timki, etc. **(Ethno musical).**
			Dung cake used for fuel. **(Industrial)**
			Urine and dung is used as manure. **(Agricultural).**
			Bull is used for ploughing (bukkhar) and sowing (nagar). **(Agricultural).**
			Bull is attached to rehangi, khachar, gara for transport work. **(Transportation).**
			Bull is worshiped by farmers in the festival called Pola**. (Socio religious).**
			Bull race is organized in Pola festival. **(Entertainment).**
			Bull is used in dowry. **(Dowry).**
9.Buffalo	*Bubalus bubalis*	Bhais	Fat is used for weakness. **(Medicinal).**
			Buffalo is used for plughing (bukkhar) and sowing (nagar) **(Agricultural).**
			Urine and dung is used as manure. **(Agricultural).**
			Offering is given to god during important festival. **(Socio religious).**
			Bull is used in dowry. **(Dowry).**

			Skin is used in making Dahak**. (Irrigation)**
10.Mithun	*Bos frontalis*	Shand	Penis properly cooked and eaten for breast pain of lactating mother. **(Medicinal)**
			It is worshiped as nandi. **(Socio religious)**
11.Goat	*Capra indica*	Bakri	Meat is cooked and eaten. **(Food).**
			Milk is poured in fingure tips to be protected from sun strock. **(Medicinal)**
			Urine is administered orally for 21 days once daily to cure tuberculosis. **(Medicinal).**
			Dried droppings ground to a smooth paste with water and is applied to relief gout swelling and pain. **(Medicinal).**
			Intestinal juice is used as a remedy for ulcer. **(Medicinal).**
			Liver is cooked and eaten for general weakness and anemia. **(Medicinal).**
			Leg soup is taken to cure asthma and weakness of facilitates delivery. **(Medicinal).**
			Urine and droppings is used as manure. **(Agriculture).**
			Goat is used in dowry. **(Dowry).**
			Goat culture is a source of income. **(Business).**
			Offerings are given to god during important festivals. **(Socio religious**).
12.Mule	*Equus asinus caballus*	Khachar	Animal is used for transportation. **(Transport)**.
13.Ass	*Equus asinus*	Gadha	Milk mixed with wine given to drug addict person to cure it fast. **(Medicinal).**
			Urine is used as eye liner for eye power. **(Medicinal).**
			Milk of **black** ass is used for making surma for strong eye sight. **(Medicinal).**
			Charms are made of dried placenta and it is believed that these drive evil spirit away. **(Magico religious).**
			Ass is used as beast of burden. **(Transport).**
14.Pig	*Sus scrofa domestica*	Sungar	Pork is cooked and eaten. (**Food**).
			Fat oil applied on burns and fractures for rapid healing. (**Medicinal**).
			Cooked liver used to treat cancer. **(Medicinal**).
			Piggery houses present in out side of village made by rural people called 'Basod'. It is a source of income. **(Business**)
			Pig is used in dowry. (**Dowry**).
			Fat oil used as fuel. (**Lighting**).
			Offering is given to god during important festival. **(Socio religious**).
15.Horse	*Equus hemionus*	Ghoda	Sweat mixed with wine and administered orally to cure drug addiction. **(Medicinal**).
			Ash of dung mix with banphool oil, which promotes hair growth. (**Medicinal**).
			Iron nall of toe convert into ring, which are thought to bring strength and vigour to the wearer and frighten the enemy. **(Magico religious**).
			Hair is use making musical instrument. (**Ethno musical**).
16.Dog	*Canis familiaris*	Kukura	Fresh saliva applied on skin to treat skin disease. **(Medicinal**).
			The crying of a dog indicates a bad omen. **(Omen indication**).
17.Cat	*Felis bengalensis*	Majri	Crying of cats is considered as sign of impending bad luck. **(Omen indicator).**
			If cat crosses the road before the onset of journey, it is cancelled because of fear of bad luck in future. It is considered to be bad omen. **(Omen indicator**).
			A black cat is thought to be a symbol of a witch. (**Magico religious**).
			Charms are made out of placenta, these drive evil spirits away. **(Magico religious**).
			If cat gave to birth kitten in any house, it is good indication. **(Omen**

			indicator).
18.Hare	*Lepus nigricollis*	Kharha	Cooked flesh used to treat fever. **(Medicinal**).
			Dry liver is taken orally to cure acidity and dysentery. (**Medicinal**).
			Fume of dropping is taken nasally to cure fit. (**Medicinal**).
			Bone rub with water and use for cough and cold. (**Medicinal**).

CONCLUSION

Documentation of traditional therapeutic knowhow could lead to the discovery of new drugs as well as contribute to the conservation, sustainable management and use of animal resources; therefore, it is very crucial that ethno-zoological surveys be carried out for the preservation of this indigenous knowledge. In order to preserve traditional medicinal knowledge, it is necessary that inventories of domestic animals with therapeutic value are carried out, and the knowledge related to their use documented in systematic studies. These studies can have other values too for society besides conserving traditional knowledge.

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RESEARCH ARTICLE

Biodiversity and conservation assessment of freshwater fishes of Harsi Reservoir, Madhya Pradesh, India

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ABSTRACT

This contribution focuses on the diversity, population and conservation aspects of fishes in one of the large freshwater body of Madhya Pradesh, 'Harsi reservoir'. The extensive survey was conducted from April, 2005 to March, 2007. A total of 51 species were recorded belonging to 33 genera, 16 families and 7 orders. As far as the fishes under different orders are concerned, order Cypriniformes consists of 15 genera belonging to 3 families, Siluriformes of 10 genera to 6 families, Perciformes of 3 genera to 3 families, Osteoglossiformes and Synbranchiformes of 2 genera each to singular family and Clupeiformes and Beloniformes of 1 genus each, to single family. The analysis showed that 07 and 04 fish species, as endangered by two different mode of classification. Apart from the Indian Major Carps, certain threatened species viz., *Chitala chitala*, *Tor tor, Ompok bimaculatus* and *Eutropiichthys vacha* were recorded from the reservoir. A sisorid, *Gagata sexualis* has been reported for the first time from this region. The study confirms that this freshwater body may prove congineal for conservation of regional fish diversity, especially for local and endangered fish species.

Keywords: Conservation Status, Ichthyo-fauna, IUCN categorization, Threats to fish diversity.

INTRODUCTION

Throughout the world, freshwater environments are facing threats as regard to both ecosystem stability, biodiversity and many strategies have been proposed to solve this crisis (Cowx 2002 Suski and Cooke 2006). Stress caused by anthropogenic environment, degradation due to urbanization, construction of dams, abstraction of water bodies for irrigation and power generation and pollution are major constraints towards loss of habitat and thus biodiversity (Lyubov et al., 2011). The biodiversity crisis that we are currently facing requires priority setting at global, regional, and local scales in order to concentrate limited

resources on the most important conservation needs (Darwall and Vie, 2005; Knight et al., 2008;). Myers et al. (2000) identified 18 mega-biodiversity 'hotspot' regions of the world, based on the criterion of exceptional concentration of species and endemism as well as exceptional degree of threats arising out of increased pressures of human intervention, with the possibility of potential extinction of constituent species caused by the latter and they have predicted the possibility of a major extinction spasm impeding in these areas. However, it has been pointed out that if key localities of biotic richness can be identified, conservation priorities could be determined in a more informed and methodological manner (Mittermeier et al., 1999; Myers et al., 2000). The principal drawback, however, remains the lack of basic data, especially of fish species.

India is blessed with a very rich and diverse natural water resource in the form of rivers, streams, estuaries, backwaters, impoundments, mangroves, floodplain wetlands, man-made reservoirs, lakes and ponds. The country is also endowed with a rich fish genetic biodiversity with approximately 2, 200 fish species and ranks 9th in term of freshwater mega biodiversity (Qureshi, 2007). A significant portion of the freshwater fish production in India is still based on the harvest from wild population (Sugunan, 1997). Attempts have been made to assess the freshwater fishes of Madhya Pradesh for their biodiversity and conservation have been done by many scientists including Garg et al. (2007 2010), Saksena (2007), Rao et al. (2007) and Dhakad et al. (2008).

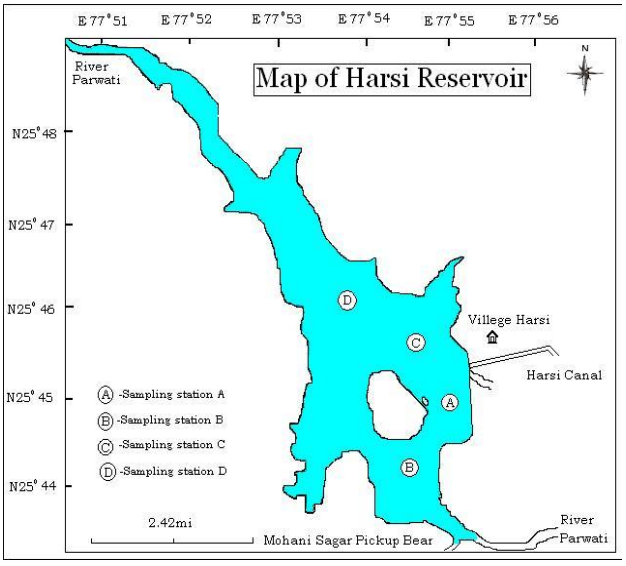

Figure 1: Location map of Harsi reservoir with sampling sites

The first assessment (Anon, 1992-1993) categorized 46 freshwater fish species as threatened in India. In the second assessment, 320 freshwater fishes were included and 43 freshwater fish species were categorized as critically endangered, 90 as endangered and 81 as vulnerable (CAMP,1998) while, a recent assessment for central India (Madhya Pradesh, Chattisgarh and Rajasthan) reported 168 fish species, of which, 41 species (24.40%) were placed as threatened (Sarkar and Lakra 2007). Therefore, In the present study, a detailed survey was conducted in the Harsi reservoir, Gwalior, Madhya Pradesh to ascertain the present scenario of fish diversity within the reservoir.

MATERIALS AND METHODS

Study Area: Harsi is an earthen dyke reservoir constructed on Parwati River which is situated near Harsi village in Bhitarwar Tehsil, District Gwalior, Madhya Pradesh. Geographically, the ordinal points of the reservoir lie at N 25' 47^0 to N 25' 48^0 latitude and E 79'52^0 to E 77' 55^0 longitude (Figure 1). The water spread area of reservoir is 1960 km^2 (at full reservoir level), which is sometimes attained during the peak of the rainy season. Maximum depth of the reservoir was found to be 20.51m , whereas average depth was 10.86±1.08 m during the period of study. The reservoir is being heavily used for fisheries and irrigation of various crops such as wheat, Bengal gram, peas etc. through a canal named Harsi canal.

Samples were collected seasonally from five permanent sites in the Harsi reservoir using a different types of nets including gill net, cast net (Ghagaria jal), dip net and gamchhas. Total water body was divided into five sampling zones covering all representative habitats of the reservoir. Samplings was done after dawn (from 8:00 am to 12:00 noon) and to supplement the above efforts, regular sampling was also done before the dusk (03:00 to 5:00 pm) in order to assess the species diversity found at the study sites. Colour, spots (if any), maximum size and other characters of the fishes caught were recorded and the samples were preserved in 10% formalin solution, while large fishes were gutted for visceral preservation. Systematic identification of the fishes was done with the help of standard keys provided by Talwar and Jhingran (1991), Jayaram (1999) and Srivastava (1968). References to conservation status within this paper are based on IUCN classification as

per CAMP (1998), CAFF (2006) and Sarkar and Lakra (2007).

RESULTS AND DISCUSSION

Madhya Pradesh is the second largest geographic state of the county with an area of 3, 08, 245 km^2. This state has 4, 60, 384 ha of inland waters (Sugunan 1997) and about 138 freshwater fish species recorded, of which nearly 41 species are considered as threatened and 01 species *Hilsa ilisha* as critically endangered (Sarkar and Lakra 2007). Therefore, in order to prioritize freshwater fish species and their conservation action, an urgent need was felt to assess the present status of freshwater fishes of Harsi reservoir.

The ichthyo-faunal diversity of the Harsi reservoir is restricted to 51 species belonging to 33 genera, spread over 16 families. The composition of species and their percent under various orders has shown that 27 species are available under Cypriniformes with 52.94%, 12 species under Siluriformes with 23.53%, 6 species under Perciformes with 11.76%, 2 species each under Synbranchiformes and Osteoglossiformes with 3.92% and 1 species each under Clupeiformes and Beloniformes with 1.96% contribution each (Table 2).

An analysis of the taxonomic composition of fish fauna suggests, that Cyprinidae was the most abundant family with 25 representative species (49.02%) occurring in the study site. Bagridae, second dominant family, has 6 species (9.80%), followed by Channidae with 4 representative species (7.84%), 2 species each to Notopteridae (3.92%), Siluridae (3.92%), Sisoridae (3.92%) and Mastacembelidae (3.92%), whereas, Clupeidae, Cobitidae, Balitoridae, Schilbeidae, Clariidae, Heteropneustidae, Belonidae, Ambassidae and Gobiidae are the families having single species each (1.96%) representation (Table 3).

The ICUN categorization of fish species in the Harsi reservoir has been depicted in (Table 4). An important observation was that 7 species such as *Chitala chitala, Notopterus notopterus, Acanthocobitis botia, Tor tor, Rita rita, Ompok bimaculatus* and *Eutropiichthys vacha*, those were placed under the category of endangered species as per IUCN (CAFF, 2006), were found as stable population and having high conservation significance and enjoying good population in Harsi reservoir. It is worth mentioning here that *Gagata sexualis* belonging to family Sisoridae is a new report from this reservoir and this region. Varied ecological status of the 7 endangered species and *Gagata sexualis* endows uniqueness at Harsi reservoir and therefore, there is an urgent need for conservation of these species by protecting the fauna from over exploitation and habitat destruction etc. Garg et al. (2007 2010) have studied fish fauna of Ramsagar reservoir, Datia, Madhya Pradesh and recorded 42 species of which family Cyprinidae was dominant with 21 (50%) species of the family. In the present investigation, 51 species of fishes were identified in which family Cyprinidae was most abundant with 25 species with 49.02% share which supported the previous fish fauna studies carried out in this region.

In the CAMP (1998), information regarding a total of 166 fishes was compiled for Central region, while in CAFF (2006), a total of 138 fishes were recorded. In these eight years, fish fauna of Madhya Pradesh has declined at a faster rate than the other states and this is a very serious issue for fish scientist towards fish conservation efforts. In the present investigation, we have classified fishes of Harsi reservoir on the basis of CAMP and CAFF as endangered (EN), vulnerable (VU), lower risk near threatened (LRnt), lower risk least concern (LRlc), not evaluated (NE) and Data deficient (DD) with their respective representative fish species were 04, 09, 24, 05, 09, 00 and 07, 10, 23, 06, 01, 04 respectively (Table 4). On the basis of IUCN categorization, we have found the similarity coefficient and distances between the categories and made a cluster diagram using the un-weighted pair group method with arithmetic mean (UPGMA) algorithm using Past software (1.91) which clearly shows that, the LRlc, EN, DD, VU and NE categories are directly correlated with each other while the LRnt (lower risk and near threatened) are not related to the other five categories (Figure 2). Similarly, It has also indicated endangered species (EN) are highly correlated with lower risk least concern (LRlc). It may be assumed that fishes under LRlc go towards the endangered category and therefore, the conservation of fishes categorized as LRlc is extremely desired.

Three species *Notopterus notopterus, Acanthocobitis botia* and *Rita rita* are declared as endangered species in CAFF but were categorized as lower risk near


Fig. 1 *Chitala chitala*


Fig. 2 *Notopterus notopterus*

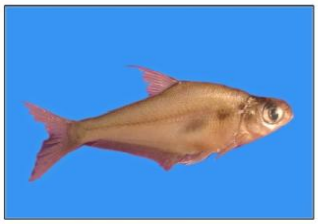
Fig. 15 *Osteobrama cotio cotio*

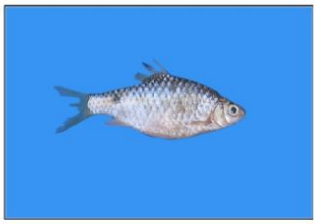
Fig. 16 *Puntius amphibius*

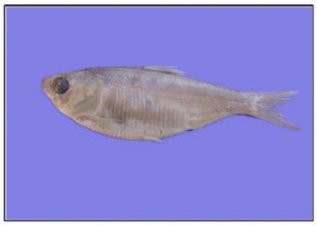
Fig. 3 *Gudusia chapra*

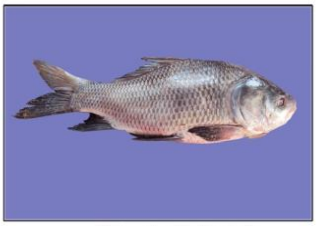
Fig. 4 *Catla catla*

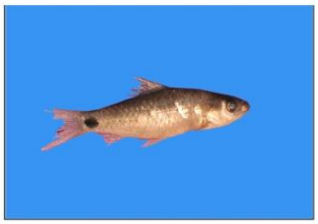
Fig. 17 *Puntius conchonius*


Fig. 18 *Puntius sarana sarana*

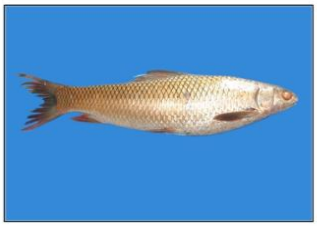
Fig. 5 *Cirrhinus mrigala*

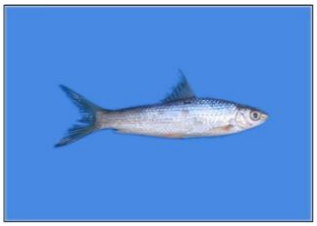
Fig. 6 *Cirrhinus reba*


Fig. 19 *Puntius sophore*


Fig. 20 *Puntius ticto*

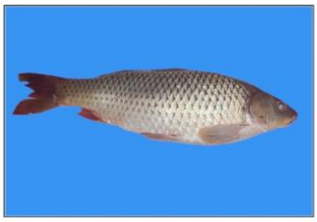
Fig. 7 *Ctenopharyngodon idella*


Fig. 8 *Labeo boggut*

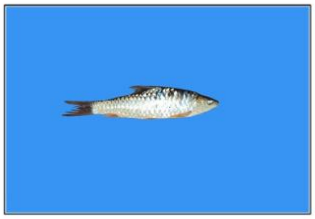
Fig. 21 *Tor tor*

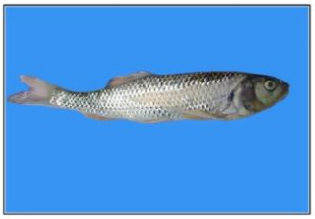
Fig. 22 *Barilius bendelisis*

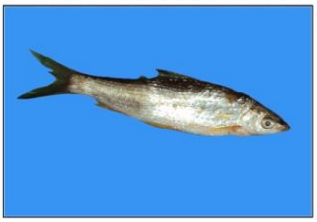
Fig. 9 *Labeo bata*

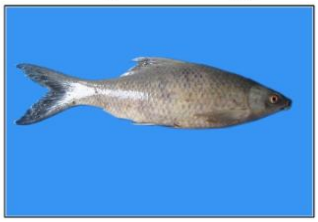
Fig. 10 *Labeo calbasu*

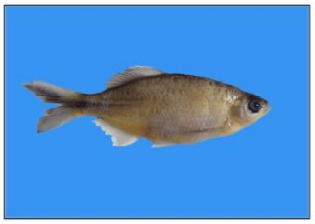
Fig. 23 *Danio devario*

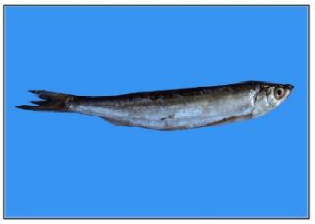
Fig. 24 *Esomus danricus*


Fig. 11 *Labeo dyocheilus*


Fig. 12 *Labeo fimbriatus*


Fig. 25 *Rasbora daniconius*


Fig. 26 *Salmophasia laubuca*

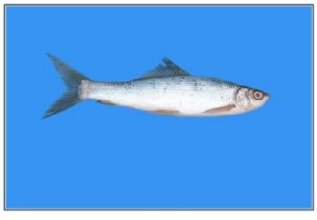
Fig. 13 *Labeo gonius*


Fig. 14 *Labeo rohita*

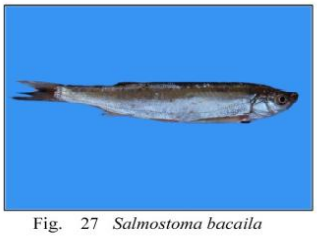
Fig. 27 *Salmostoma bacaila*

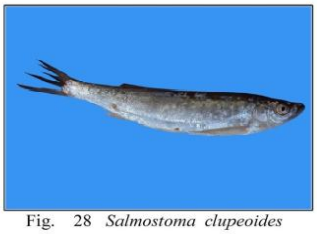
Fig. 28 *Salmostoma clupeoides*

Fig 1 to 28 Showing the various fish species identified in Harsi Reservoir

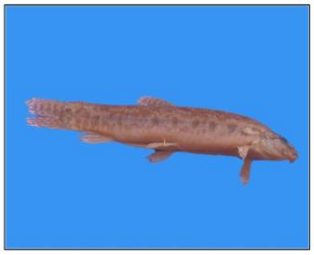
Fig. 29 *Lepidocephalichthys guntea*


Fig. 30 *Acanthocobitis botia*


Fig. 41 *Clarias batrachus*


Fig. 42 *Heteropneustes fossilis*

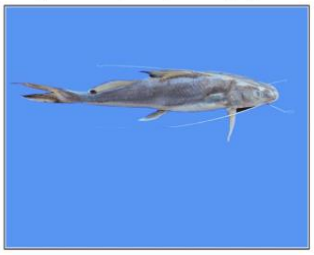
Fig. 31 *Mystus cavasius*


Fig. 32 *Mystus bleekeri*

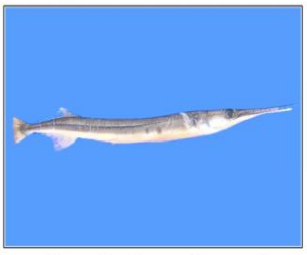
Fig. 43 *Xenentodon cancila*

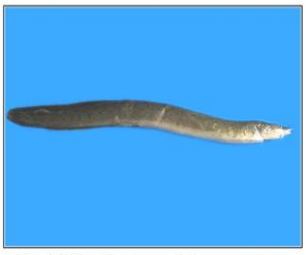
Fig. 9.44 *Mastacembelus armatus*


Fig. 33 *Rita rita*


Fig. 34 *Sperata oar*

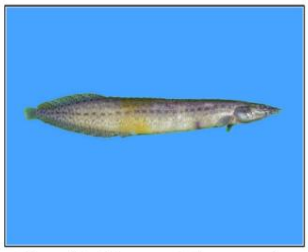
Fig. 45 *Mastacembelus pancalus*


Fig. 46 *Parambassis (Chanda) ranga*

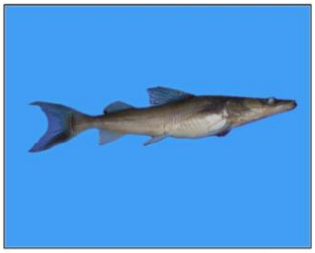
Fig. 35 *Sperata seenghala*

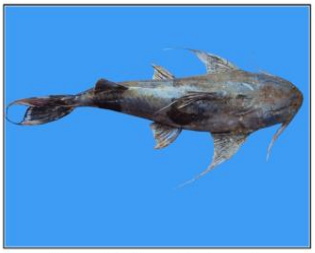
Fig. 36 *Bagarius bagarius*

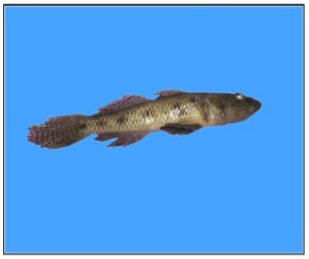
Fig. 47 *Glossogobius giuris giuris*


Fig. 48 *Channa (Ophiocephalus) orientalis*


Fig. 37 *Gagata sexualis*


Fig. 38 *Ompok bimaculatus*


Fig. 49 *Channa (Ophiocephalus) marulius*

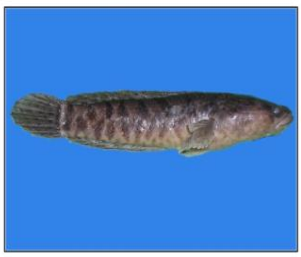
Fig. 50 *Channa (Ophiocephalus) punctatus*


Fig. 39 *Wallago attu*


Fig. 40 *Eutropiichthys vacha*

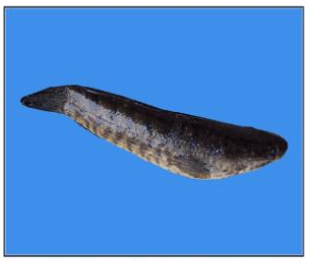
Fig. 51 *Channa(Ophiocephalus) striatus*

Fig 29 to 51 Showing the various fish species identified in Harsi Reservoir

Table 1: Systematic list of fishes of Harsi reservoir along with IUCN categories

Class	Order	Family	S.N.	Name of Fish	Local name	CAMP (1998)	CAFF (2006)
Actinopterygii	Osteoglossiformes	Notopteridae	1.	*Chitala chitala* (HamiltonBuchanan)	Chital	EN	EN
			2.	*Notopterus notopterus* (Pallas)	Patola	LR-nt	EN
	Clupeiformes	Clupeidae	3.	*Gudusia chapra* (Hamilton-Buchanan)	Phulua	LR-lc	LR-lc
	Cypriniformes	Cyprinidae	4.	*Catla catla* (Hamilton-Buchanan)	Catla	VU	LRnt
			5.	*Cirrhinus mrigala* (Hamilton-Buchanan)	Mrigal	LRnt	LRnt
			6.	*Cirrhinus reba* (Hamilton-Buchanan)	Naren	VU	VU
			7.	**Ctenopharyngodon idella* (Valenciennes)	Grass carp	NE	LRnt
			8.	*Labeo boggut* (Sykes)	Boga	NE	LRnt
			9.	*Labeo bata* (Hamilton-Buchanan)	Bata	LRnt	LRnt
			10.	*Labeo calbasu* (Hamilton-Buchanan)	Kariya	LRnt	LRnt
			11.	*Labeo dyocheilus dyocheilus* (Mc Clelland)	Kharont	VU	VU
			12.	*Labeo fimbriatus* (Bloch)	Cut rohu	LRnt	LRnt
			13.	*Labeo gonius* (Hamilton-Buchanan)	Kursa	LRnt	LRnt
			14.	*Labeo rohita* (Hamilton-Buchanan)	Rohu	LRnt	LR-Ic
			15.	*Osteobrama cotio cotio* (Hamilton-Buchanan)	Gudgudi	LRnt	LRnt
			16.	*Puntius amphibius* (Hamilton-Buchanan)	Khadia	NE	DD
			17.	*Puntius conchonius* Hamilton-Buchanan)	Khadia	LRnt	LRnt
			18.	*Puntius sarana sarana* (Hamilton-Buchanan)	Puthia	VU	VU
			19.	*Puntius sophore* (Hamilton-Buchanan)	Khadia	LRnt	LRnt
			20.	*Puntius ticto* (Hamilton-Buchanan)	Khadia	LRnt	LRnt
			21.	*Tor tor* (Hamilton-Buchanan)	Mahaseer	EN	EN
			22.	*Barilius bendelisis* (Hamilton-Buchanan)	Phulua	LRnt	LRnt
			23.	*Danio devario* (Hamilton-Buchanan)	Patukari	LRnt	LRnt
			24.	*Esomus danricus* (Hamilton-Buchanan)	Dendua	LRIc	LRIc
			25.	*Rasbora daniconius* (Hamilton-Buchanan)	Zhanzara	NE	LRIc
			26.	*Salmophasia laubuca* (Hamilton-Buchanan)	Chal	LRIc	LR-IC
			27.	*Salmostomabacaila* (Hamilton-Buchanan)	Chilua	LRIc	DD
			28.	*Salmostoma clupeoides* (Bloch)	Silhani	LRIc	DD
		Cobitidae	29.	*Lepidocephalichthys guntea* (Hamilton-Buchanan)	Bamni	NE	LR-Ic
		Balitori	30.	*Acanthocobitis botia* (Hamilton-Buchanan)	Carri,Natwa	LR-nt	EN

Table 1: Continued...

Class	Order	Family	S.N.	Name of Fish	Local name	CAMP (1998)	CAFF (2006)
Actinopterygii	Siluriformes	Bagridae	31.	*Mystus cavasius* (Hamilton-Buchanan)	Kitua	LRnt	LRnt
			32.	*Mystus bleekeri* (Day)	Kirua	VU	VU
			33.	*Rita rita* (Hamilton-Buchanan)	Gegra	LRnt	EN
			34.	*Sperata oar* (Hamilton-Buchanan)	Tengra	NE	LRnt
			35.	*Sperata seenghala* (Sykes)	Singhara	NE	lRnt
		Sisoridae	36.	*Bagarius bagarius* (Hamilton-Buchanan	Lamra	VU	VU
			37.	*Gagata sexualis* (Tilak)	Buhani/Unknown	NE	NE
		Siluridae	38.	*Ompok bimaculatus* (Bloch)	Pauda	EN	EN
			39.	*Wallago attu* (Block & Schneider)	Lonch	LRnt	LRnt
		Schilbeidae	40.	*Eutropiichthys vacha* (Hamilton-Buchanan)	Bachua	EN	EN
		Clariidae	41.	*Clarias batrachus* (Linnaeus)	Mangur	VU	VU
		Heteropneustidae	42.	*Heteropneustes fossilis* (Bloch)	Singhi	VU	VU
	Beloniformes	Belanidae	43.	*Xenentodon cancila* (Hamilton-Buchanan)	Suja	LR-nt	LRnt
	Synbranchiformes	Mastacembelidae	44.	*Macrognathus armatus* (Lacepede)	Baam	NE	VU
			45.	*Mastacembelus pancalus* (Ham-Buch)	Baam	LRnt	LRnt
	Perciformes	Ambassidae	46.	*Pseudoambassis (Chanda) ranga* (Ham-Buch)	Chanda	NE	VU
		Gobiidae	47.	*Glossogobius giuris giuris (*Ham-Buch)	Patharchita	LRnt	LRnt
		Channidae	48.	*Channa (Ophiocephalus) orientalis* (Ham-Buch)	Sola	VU	DD
			49.	*Channa (Ophiocephalus) marulius* (Ham-Buch)	Sol	LRnt	VU
			50.	*Channa (Ophiocephalus) punctatus* (Bloch)	Gilgonch	LRnt	LRnt
			51.	*Channa(Ophiocephalus) striatus* (Bloch)	Durkasol	LRnt	LRnt

* Exotic fish ; En = Endangered species; VU = Vulnerable; LRnt =Lower risk near threatened;
LRlc = Lower risk least concern; NE = Not evaluated; DD =Data deficient

Table 2: Composition of genera and species under different in orders

S.No.	Order	Genera	% of Genera in order	Species	% of Species in order
1	Osteoglossiformes	02	6.06	02	3.92
2	Clupeiformes	01	3.03	01	1.96
3	Cypriniformes	15	45.45	27	52.94
4	Siluriformes	10	30.30	12	23.53
5	Beloniformes	01	3.03	01	1.96
6	Synbranchiformes	01	3.03	02	3.92
7	Perciformes	03	9.09	06	11.76

Table 3: Composition of genera and species under different in families

S. No.	Families	Genera	% Contribution of Genera to Families	Species	% Contribution of Species to Families
1.	Notopteridae	2	6.06	2	3.92
2.	Clupeidae	1	3.03	1	1.96
3.	Cyprinidae	13	39.39	25	49.02
4.	Cobitidae	1	3.03	1	1.96
5.	Balitoridae	1	3.03	1	1.96
6.	Bagridae	3	9.09	5	9.80
7.	Sisoridae	2	6.06	2	3.92
8.	Siluridae	2	6.06	2	3.92
9.	Schilbeidae	1	3.03	1	1.96
10.	Clariidae	1	3.03	1	1.96
11.	Heteropneustidae	1	3.03	1	1.96
12.	Belanidae	1	3.03	1	1.96
13.	Mastacembelidae	1	3.03	2	3.92
14.	Ambassidae	1	3.03	1	1.96
15.	Gobiidae	1	3.03	1	1.96
16.	Channidae	1	3.03	4	7.84

Table 4: Status of fishes of Harsi Reservoir according to IUCN categorization

S. No.	IUCN categories	Abbreviations	CAMP 1998	CAFF 2006
1.	Endangered	EN	04	07
2.	Vulnerable	VU	09	10
3.	Lower risk near threatened	LRnt	24	23
4.	Lower risk least concern	LRlc	05	06
5.	Not evaluated	NE	09	01
6.	Data deficient	DD	00	04

CAMP, 1998; CAFF, 2006

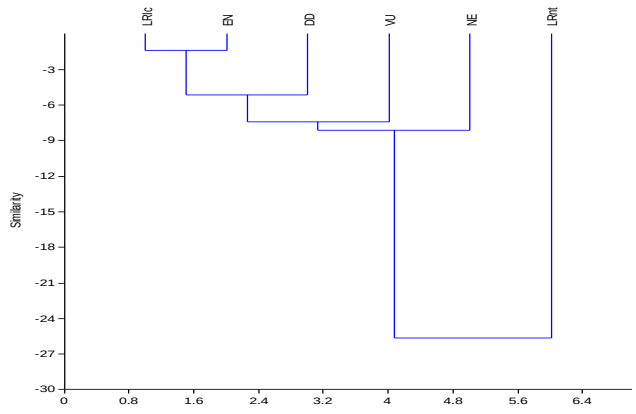

Figure 2: UPGMA clustering (Euclidean) of various IUCN categories using similarity matrix

The overall assessment indicates that a number of species recorded in Harsi reservoir were not observed by Garg et al. (2007, 2010) and Rao *et al*. (2007) in Ramsagar and Tighra reservoirs in this region. It suggested that Harsi reservoir is having a congenial habitat for freshwater fishes of this region. Therefore, it is very much essential to make a conservation management plan for Harsi reservoir, in which it may be possible to replenish the stock of threatened texa with the help of Fisheries and Irrigation Departments, Government of Madhya Pradesh. The contributions of local peoples, fishermen and fisheries societies will also go a long way in the conservation of reservoir and its fish fauna, because no conservation strategy and

safeguarding biodiversity can be successful without the cooperation and involvement of the local communities (Koh and Sodhi 2010; Antons, 2010).

CONCLUSION

Analysis of fish species composition, distribution and ecological status with reference to their conservation status revealed that fish species diversity level in the Harsi reservoir appears to be constant. It is because of the fact that reservoir harbors only one exotic species i.e., grass carp, *Ctenopharyngodon idella*.

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RESEARCH ARTICLE

Diversity and distribution of Sphecoid Wasp in Koradi region Dist. Nagpur, India

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ABSTRACT

Sphecid wasps are a familiar and diverse group of solitary wasp. They are beneficial and harmless insect to human being. The present study was to know the diversity of sphecid wasp around Koradi region, Dist. Nagpur. The study recorded 8 species of sphecid wasp in 5 genera from 2 sub family. The subfamily Ampulicinae recorded 2 species from 2 genera while from subfamily Sphecinae 6 species under 3 genera. The most number of species recorded from the subfamily Sphecinae.

Keywords: Sphecoid wasp, digger wasp

INTRODUCTION

Sphecidae is a cosmopolitan family of wasp with 9716 described species through the world (Pulawaski, 2009). Sphecidae is a diverse group of solitary wasp which may be of different shaped, size and colour. The female dig its nest soil, sand or wood and provisions each nest cell with paralyzed prey and lay egg on it. The wasp larva feed on the provisions while adult feed on nectar, pollen and juice containing high amount of sugar while larva need adults or larva of different insect order and Araneida (Murray, 1940,; Gillott, 2005). The wasps are valuable bio control agent as they control the population of insect pest (Gayubo, 2005; Borror, 1988). The relationship of these insect with man has been unfriendly due to fear from their over rated stinging powers.

Sphecidae are recorded from various part of India. The resent works on Sphecid wasp were done in various state of India. (Gupta,1995; Suresh, et al; 1995; Jonathan et al; 2000,;2003; Kundu et al;2006; Job et al;2014,; Kumar. et al; 2011). In Kerla 47 species of sphecid wasp has been listed (Sudheendrakumar, 1989; 1999; Madhavikutty, 2004).

Present study revealed valuable information about the diversity and distribution of Sphecid wasp around Koradi rejoin, Nagpur district, India.

MATERIALS AND METHODS

The four selected habitats agriculture fields, community gardens, fragmented habitats and residential sites situated at different locations around koradi region dist. Nagpur were selected for collecting the sample. The sample were collected from random ways from August 20013 to May 2014 between 9 AM to 5 PM. The areal net was used for collecting the insect. Aerial nets that were prepared of white meshed material with light weight handle were used for effective collection.

Collection, Preservation and Identification

The insect were collected using hand nets, killed with Acetone, pinned with entomological pins and preserved in insect boxes for identification studies. The collected specimens were identified using Stereo zoom microscope and with the help of literature.

RESULTS AND DISCUSSION

In the present study eight species of sphecid wasp in five genera from two sub family were recorded.

Table 1: Different species of sphecid wasp with their scientific name & food habit collected from Koradi region, Dist Nagpur.

S.no	Common name	Scientific name	Food habit
Sub family- Sphecinae			
1	Yellow and black Mud dauber wasp	*Chalybion bengalense*	Spiders
2	Blue Mud dauber wasp	*Sceliphron madraspatanum*	Spiders
3	Digger wasp	*Sphex sericeus*	Caterpillar, Insect
4	Digger wasp	*Sphex argentatus*	Caterpillar, Insect
5	Digger wasp	*Spex ichneumoneus*	Caterpillar, insect
6	Digger wasp	*Sphex sp.*	Caterpillar, Insect
Sub family- Ampulicinae			
7	Emerald Cockroach wasp	*Ampulex compressa*	Cockroach
8	Cricket hunter wasp	*Chlorion sp.*	Cricket

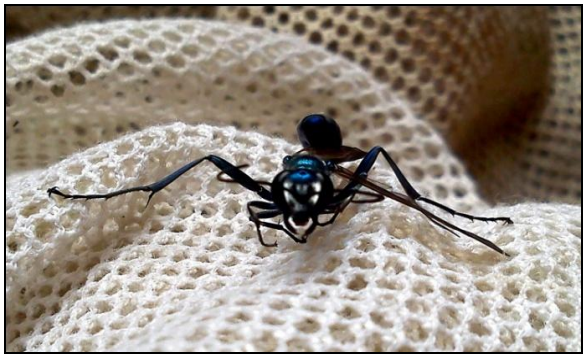

Fig. 1- *Chalybion bengalense*

Fig.2- *Sceliphron madraspatatum*

Fig.3- *Sphex sericus*

Fig.4- *Sphex argentatus*

Fig. 5- Sphex *ichneumoneus*

Fig. 6-*Sphex sp*.

Fig.-7 *Ampulex compressa*

Fig.8- *Cllorion sp*.

The subfamily Ampulicinae recorded two species from two genera while from subfamily *Sphecinae* six species under three genera. Among the Genus *Scelophron* most number of species recorded. One species from *Scelophron* not identified. *Chalybion bengalense* (Fig. 1) are metallic blue mud-dauber wasp. They build their nest by mud or lay egg in mud nest build by other species of wasp. *Sceliphron madraspatatum* (Fig. 2) are Yellow and black Mud dauber wasp they are solitary, female build large multi celled mud nest. The cells are provisioned with mass number of spiders. *Sphex sericus* (Fig. 3) female are golden yellow while male are black and red in colour. *Sphex argentatus* are robust black species (Fig. 4). S*phex ichneumoneus* (Fig. 5) are called grate golden digger wasp it is red and black in colour. *Sphex sp*. (Fig. 6). Genus *Sphex* are called digger wasp because of their dig nest habit. A hole is dug in the soil as a nest with stock of captured insects. The wasp lay their eggs in the provisioned nest, when the larva hatched they feed on the paralyzed insect.

Ampulex compressa (fig.7) are called cockroach wasp. They are metallic green blue colour they used live cockroach (*Periplaneta Americana*) as a host for its larva. *Cllorion sp*. (fig.8) are called cricket hunter wasp. They are metallic blue in colour, they spend most of time to hunt the cricket, and newly hatched larva feed on paralyzed cricket prey.

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RESEARCH ARTICLE

Absence of Endoparasites in Long-Billed Vultures (*Gyps indicus*) in Bundelkhand Region, India

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ABSTRACT

Long-billed Vultures (*Gyps indicus*) are found in many protected and unprotected areas of Bundelkhand Region, but relatively diminutive studies do not illustrate the factors that may pessimistically impact their populations. This species was therefore surveyed for various factors that may influence the health of wild raptors and study of endoparasites was one of these factors. During 2007–2012, carcasses of 16 vultures were found. Out of these 9 dead vultures and 1 live juvenile were examined for endoparasites. Rest of the carcasses were decomposed hence could not be examined. At necropsy, the samples were methodologically observed for the endoparasites in viscera, liver, trachea, heart and other body organs. The faecal matter of the live juvenile was examined microscopically but no protozoan or helminthes infection (trematodes, nematodes and cestodes) was reported. Helminthes were absent in all the carcasses. Death due to helminthiasis can only be in the case of heavy infestation. Moreover the absence of helminthes reflects the feeding habits of vultures i.e. they are scavengers rather than predators and scavenge on dead cattle. The intermediate hosts required for trematodes do not form a part of the vulture food chain, the only doubt being the presence of cestodes and nematodes. No vulture sample represented any clinical signs that could be associated with the presence of parasites, fighting behavior being the main cause of deaths.

Keywords: *Vultures, endoparasites, carcasses, helminthes*

INTRODUCTION

The study of wildlife represents one of the cardinal part of current environmental protection policies, because they are considered as bio-indicators whose presence, abundance and health status is indicative of a particular set of environmental conditions. Birds are an integral part of virtually every ecosystem and it is not surprising that they are commonly found in households and zoos all over the world.

The usefulness of birds as indicators of ecosystem's integrity has been widely discussed (Greenwood, 1977; Bowerman *et al.*, 2000). Given the role that raptors play in the food chain, changes in their health status can have significant effects on the ecosystem integrity.

Birds can harbour a wide variety of endoparasites varying from nematodes, trematodes, cestodes, acanthocephalans, and protozoans (Altman *et al*., 1997; Rupley., 1997; Olsen and Orosz, 2000) Parasites usually cause little or no distress to healthy individuals in the wild, but for birds in captivity parasitic infections are among the most common sanitary problems (Barnes., 1986). Due to an increased risk of exposure, parasites can lead to serious problems or even to death in birds recently brought into captivity, kept for prolonged periods in confined housings, and stressed by injuries, illnesses, or adaptation to new environments (Kronea and Cooper 2002; Lacina and Bird 2000; Smith 1993). Many a times no pathological changes are seen in the avian host when the parasites coexist with them. This study was initiated to gain knowledge of endoparasites in vultures in wild and to determine if vultures sampled from the Bundelkhand Region were infested with endoparasites or not which could further reveal the host parasite relationship.

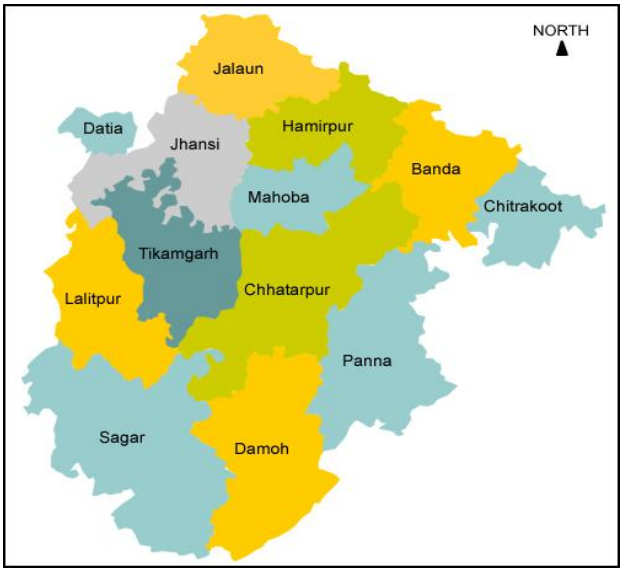

Fig 1: Map of Bundelkhand Region

Source: Land Use Plan for Development of Bundelkhand Region Based on Land and Soil Resources Survey, National Bureau of Soil Survey and Land Use Planning (Indian Council of Agricultural Research), Nagpur: November 1981.

The study was carried out in an area of two States of India, Uttar Pradesh and Madhya Pradesh known as Bundelkhand (Fig.1). The Bundelkhand region within these boundaries has an area of around 70,000 sq. km. The region stretches over districts of Southern Uttar Pradesh and Northern Madhya Pradesh (Bundelkhand Vikas Nidhi, 1990-1991; Bundelkhand Development Authority, 2007). The principal rivers are the Sindh, Betwa, Ken, Bagahin, Tons, Pahuj, Dhasan, and Chambal. Bundelkhand is a hot and semi-humid region. The temperature during summer goes upto 48^0C.

MATERIALS AND METHODS

Since vultures are Schedule I birds as well as Critically Endangered, it was difficult to get samples. A total number of 16 dead vultures were reported during the study period (2007-2012). The post mortal examination of samples for endoparasites was dependent on the condition of carcasses, which was further dependent on the lapse of time until the carcass was post mortem. The carcasses of 9 dead vultures and 1 sick juvenile were examined for endoparasites and further sample were collected for microscopic study. The blood was collected from wing veins of live bird that was about to die, by using 2 ml syringe and needle, feathers in the axillary region were plucked to isolate wing vein and the site was disinfected by 70% methylated ethanol. The blood smears were prepared, air dried, fixed with Methanol, stained with Giemsa and examined for blood parasites (Bennett, 1970). Faecal or intestinal sample were examined for confirmation of parasite infestation on direct smears. Faecal samples were collected in 10% formalin. Small portion of faeces mixed with 1-2 drop of Lugol's iodine was placed on glass slide and microscopically examined. Formalin fixed faeces were also mixed with a supersaturated solution of sugar or salt, filtered and centrifuged then surface supernatant was transferred to a slide and examined for parasite eggs. Endoparasites were also examined by necropsy examination of the dead vultures. These included exposure of the internal organs, removal and separation of the organs such as lungs, liver etc. Viscera were opened and examined properly in normal saline (0.5%).

RESULTS AND DISCUSSION

The faecal matter of the juvenile was examined but no protozoan or helminthes infection was reported. There was no parasitic infestation in trachea, intestines. Helminthes i.e. trematodes, nematodes and cestodes were absent in all the carcasses. Death due to helminthiasis can only be in the case of heavy infestation. The carcasses examined had no tissue damage that may be caused by the parasites as a result of burrowing into the mucosal lining of the mouth, oropharynx, oesophagus, and crop. The samples examined had no diphtheritic membranes extending from the oral cavity to the proventriculus, emaciation, necrosis, oedema, and no inflammation was observed.

Keymer (1972) studied the diseases of birds of prey. Although birds of prey are hosts for a wide range of parasites, there is little evidence of pathogenicity. Blood parasites are known to cause morbidity and mortality in various avifauna (Atkinson, 1991; Forrester, 1991). Hematozoan parasites were not found on blood smears from any of 82 Griffon vultures (*Gyps fulvus*) examined from Spain. Old World vultures have been sampled mostly for haematozoa in Africa. In India no specific research work has been done with haematozoans of vultures. However, Saxena (1967) obtained cestodes from *Neophron percnopterus ginginianus* (Lath.), the smaller scavenger vulture. The tapeworm was described as *Neophronia melanotus* sp. Noy. The genus *Neophronia axena.* 1967, includes three species, viz., *N. Lucknowensis saxena,* 1967, *N. luteus saxena,* 1968 and *N. irregularis Saxena*, 1968. Jairajpuri and Siddiqi (1970a) reported *Porrocaecum inconstrantia* from the intestine of Indian White-backed vulture (*Gyps bengalensis*) located in Laharpur, Sitapur (UP), India. Greiner and Mundy (1979) argued that use of rocky crags as nesting and roosting sites may isolate cape vultures from potential arthropod vectors. Conversely, tree-nesting vulture species showed haematozoa prevalences that varied between 31% in the White-headed vulture and 63% in the Lappet-faced vulture. The samples studied in Bundelkhand Region were of those Long-billed vultures that were breeding in the monuments or cliffs. Thus support the concept of absence of probable arthropod vectors. Alternatively, a well-developed immune system (Ricklefs, 1992) and/or presence of highly host-specific blood parasites in the avian community could explain absence of infections in griffon vultures.

CONCLUSION

In conclusion, the findings suggest that blood parasites were uncommon in *Gyps* vultures occurring in the Bundelkhand region, and they were not demonstrating active infections in the peripheral blood when examined. No vulture sample represented clinical signs that could be associated with the presence of parasites, fighting behavior being the main cause of deaths. Moreover the absence of helminthes reflects the feeding habits of vultures i.e. they are scavengers rather than predators and scavenge on dead cattle. The intermediate hosts required for trematodes do not form a part of the vulture food chain. The vulture colonies were in monuments and cliffs and thus isolated them from the potential arthropod vectors responsible for transmission of protozoan infections. More research is required to ascertain the prevalence of hematozoa in vultures, especially comparative studies on cliff and tree-nesting species that sample nestlings and assess ecological conditions regulating host vector-parasite relationships.

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RESEARCH ARTICLE

Diversity of Zooplankton in some lentic water bodies of Karwar

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ABSTRACT

Zooplanktons are the microscopic animals present in the water bodies. They play a major role in food chain of any ecosystem. The study was carried in Kali River for the period of Oct 2012 to Dec 2013. Kali River was further divided into six sub stations (rivers). In the present study an effort being made to study the diversity of Zooplankton diversity in different selected sites and their relation with hydro biological parameters.

Key words: Zooplankton, Kali River, Diversity, Correlation and lentic.

INTRODUCTION

Zooplanktons are the microscopic animals found in water bodies. They are the main food for many pelagic fishes. The density of zooplankton in any water body is governed by various physic chemical parameters such as light penetration, temperature, nutrient enrichment, toxic substances, mixing of water, parasites, herbivores and heterotrophic microorganism (Reynolds, 1987). Earlier many researchers worked on the fresh water zooplankton in Indian waters. (Ganapati, 1940; Mohan, 1987; Chaudhary & Pillai 2009; Singh & Balasingh 2011; Dakshini & Gupta 1979; Sarwar, 1996, Tiwari & Chauhan 2006, Abbassi et al. 1996 Sugunan, 1980. Organic pollution is one of the major factors that affect the density Moitra and Bhowmik,(1968, Verma and Munshi 1987, Rao and Durve, 1989).

MATERIALS AND METHODS

The zooplankton samples were collected on monthly basis from five stations located between Kinnar to Hinduwada of Kali River (Fig 1). Planktonic samples were collected by filtering 100 litres of water through plankton net made up of bolting silk. The samples were preserved in 5 % formalin.

Table 1: Stations with coordinates

Name of the Place	Distance from Karwar	Geographical position	Study stations	
Kali River	12.5km	14^0-52'-22" N latitude 74^0-12'-07.22" E longitude	1	Kinnar
	17km	14^0-52'-12.74" N latitude 74^0-13'-18.69" E longitude	2	Siddar ITI
	20km	14^0-52'-15.80" N latitude 74^0-14'-57.06" E longitude	3	Vailawada
	33.7	14^0-53'-40.43" N latitude 74^0-15'-24.06" E longitude	4	Kerawadi
	40.2	14^0-54'-11.65" N latitude 74^0-18'-58.46" E longitude	5	Hinduwada

The preserved samples were brought to the laboratory for qualitative and quantitative analysis and the identification was done with the help of methods described by Hustedt (1930), Venkataraman (1939), Cupp (1943), Subrahmanyan (1946), Prescott (1954), Desikachary (1959 and 1987), Hendey (1964), Steidinger and Williams (1970), Davis (1955), Kasturirangan (1963), Wimpenny (1966), Todd and Laverack (1991) and Perumal et al. (1998); Pennak (1953); Arora (1963); Sehgal (1983); Battish (1992); Murugan *et al*.,(1998). Physico-chemical parameters like Air and water temperature, pH, dissolved oxygen, T.D.S, salinity, conductivity, turbidity, colorimetric were recorded at the sampling site using systronics water analyzer (Model 371). Phosphate, Nitrate, Nitrite, silicate were analyzed in the laboratory titrimetric method as per standard methods for examination of water (APHA 1989, Trivedi and Goel 1984).

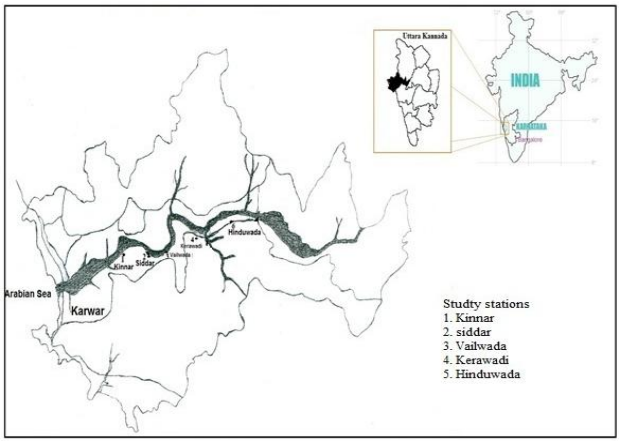

Fig. 1 : Showing Location of study site

RESULTS AND DISCUSSION

Zooplankton community of Kali River comprised of 48 species belonging to 12 groups (Table: 2). Maximum and minimum values of water parameters were given in table 3, 4 and 5. From table 7 it is clear that copepods showed negative correlation with water temperature, turbidity and D.O but positive with pH. Protozoans were positive with water temperature and pH but negative with turbidity and D.O. larval forms showed positive correlation with water temperature and D.O but negative with pH and turbidity.

Table: 2: **Checklist of Zooplanktons**

Sl. No.	Zooplankton Groups	Sl. No.	Zooplankton Groups
1.	**Protozoa**	6.	**Ostracoda**
	Tintinnopsis sp.		*Labidocera* sp.
	Favella sp.		*Oncaea* sp.
	Rhabdonella sp.	**7.**	**Cladocera**
	Globigerina sp.		*Penillia* sp.
	*Acanthometron*sp.		*Evadne*sp.
2.	**Coelenterata**	8.	**Decapoda**
	Obelia sp.		*Lucifera* sp.
	Siphonophora sp.	9.	**Annelida**
3.	**Ctenophora**		Polychaeta:
	Pleurobrachia sp.		*Tomopteris* sp.
4.	**Chaetognatha**		*Spionid* sp.
	Sagittaenflata	10.	**Mollusca(Pteropoda)**
	S. Bedotii		*Creseis acicula*
5.	**Copepoda:**	11.	**Protochordata**
	Acrocalanus sp.		*Doliolum* sp.
	Paracalanus sp.		*Oikopleura* sp.
	*Rhincalanus*sp.		*Salpa* sp.
	Pseudodiaptomus sp.	12	**Larval forms**
	Eucalanus sp.		Copepod nauplius
	*Copilia*sp.		Eupahusidnauplius
	*Macrosetella*sp.		Cirrepedenauplius
	Miocrosetella sp.		Pontellidnauplius
	Undinula sp.		Brachiopod larva
	Acartia sp.		Zoea
	Temora sp.		Cyphonautus larva
	Oithona sp.		Decapod larva
	O. plumifera		Gastropoda
	Euchaeta sp.		Bivalvia
	*Euterpina*sp.		Arachnetcis larva
	Centropages sp.		Fish eggs and larvae

In the present study the concentration of zooplankton was recorded to be minimum in August and maximum in May (2013). Graph (1 to 5). Similar results were noticed by George (1970) and Adoni (1975). Keeping in view the interaction between Zooplankton and their environment, in the present study the total density, seasonal variation in density and correlation with various physico-chemical and biological parameters are dealt and discussed. Among protozoa, *Favella* contributed maximum share and stood first rank in density dominance followed by *Tintinnopsis* whereas minimum density of *Globigerina* was noticed during the study period. *Coelenterata* was comprised by two species (Table: 2) of *Obelia* and *Siphonophora* (0.77 and 0.69/m^3) were contributed less to the total density of the zooplankton. Both the species were absent in the peak southwest monsoon season. The *Pleurobrachia* species belonging to ctenophore group also not contributed much (0.85/m^3) to the total density. In *chaetognata*, *Sagitta enflata* and *S.bedotii*, the latter species showed less density and did not show any marked variation in their standing stock. The copepod was one group which contributed much to the total density of zooplankton and stood second in dominance throughout the study period. Among seventeen species of copepod recorded, the *Euchaeta* has showed minimum density of 0.46/m^3) whereas the species like *Peudocalanus* (103.77/m^3) showed maximum density throughout the study period. Remaining groups did not show any marked variation in density and were found in low density and some of them were completely absent during the southwest monsoon period.

Table: 3 Seasonal Variation in Hydrographical parameters of Station 1 and 2

	Min	Max	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
Air temp	3	34	29.2667	7.42069	29	35	30.8	1.82052
Water temp	26	32	29	1.69031	6	32	26.5333	5.91447
pH	7.1	8.5	7.734	0.39122	7.1	8.4	7.5467	0.3852
DO	4	6.9	5.3267	0.88112	4.2	6.3	5.3267	0.58854
salinity	10.2	18.9	13.4667	2.289	10.2	17.6	13.3133	2.42601
TDS	61.5	124	79.44	17.089	63.2	104	77.4067	12.24818
Conductivity	60.2	98.4	72.7693	11.83449	60.2	88.4	69.4487	8.27091
Turbidity	7.1	36.8	17.96	9.24058	7.14	46.8	20.0493	12.17232
Phosphate_P	0.95	65	5.572	16.44183	0.56	124	9.388	31.70809
Nitrate_N	0.48	2.4	1.5327	0.62421	0.4	2.41	1.4607	0.63069
Nitrite_N	0.15	1.18	0.6267	0.29944	0.38	1.08	0.692	0.19807
Silicate_si	144.02	238.1	190.11	27.34331	134.02	205.1	179.72	22.91516

Table:4 Seasonal Variation in Hydrographical parameters of Station 3 and 4

	Min	Max	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation
Air temp	28	33	30.3333	1.34519	28	32	30.4667	1.18723
Water temp	26	30	28	1.25357	26	30	28.3333	1.1127
pH	7	8.3	7.6733	0.40438	7	8.4	7.6607	0.42786
DO	4.8	6.3	5.4533	0.45335	4.5	6.9	5.4933	0.67025
salinity	8.4	15.6	11.334	2.35801	4.5	12.2	9.1067	2.2343
TDS	62.2	99.8	76.3133	11.50148	61.15	100.2	76.0687	13.62432
Conductivity	59.2	85.4	69.528	9.18711	53.2	83.4	66.8353	9.88069
Turbidity	10.12	46.8	21.6913	10.80996	9.2	46.2	20.8653	11.11588
Phosphate_P	0.66	1.86	1.3207	0.30939	0.59	1.46	1.202	0.26247
Nitrate_N	0.54	2.09	1.2573	0.52709	0.46	2.14	1.132	0.41327
Nitrite_N	0.35	1.28	0.7773	0.3154	0.4	1.21	0.8067	0.26199
Silicate_si	135.1	201.1	180.2	20.06889	125.1	199.9	166.58	21.8768

Table: 5 Seasonal Variation in Hydrographical parameters of Station 5

	Min	Max	Mean	Std. Deviation
Air Temp	30	34	31.2	1.14642
Water Temp	27	30	28.5333	0.74322
pH	6.3	709	58.9933	180.73336
DO	4.5	65.9	9.3133	15.66287
Salinity	1.5	62.1	7.92	15.03131
TDS	61.2	112.2	78.3553	16.5951
Conductivity	55.2	98.4	70.734	12.2188
Turbidity	10.2	56.2	24.8907	13.9655
Phosphate_P	0.95	1.98	1.4533	0.28367
Nitrate_N	0.62	2.86	1.39	0.55006
Nitrite_N	0.31	1.28	0.716	0.3233
Silicate_Si	115.1	189.9	153.83	21.10087

Table: 6 Checklist of Zooplankton groups observed during the study period

Species	Seasons			Species	Seasons		
	Pre Monsoon	Monsoon	Pre Monsoon		Pre Monsoon	Monsoon	Pre Monsoon
Protozoa				**Ostracoda**			
Tintinnopsis sp.	+	+	+	*Labidocera* sp.	+	-	+
Favella sp.	+	+	+	*Oncaea* sp.	+	-	+
Rhabdonella sp.	+	+	+	**Cladocera**			
Globigerina sp.	+	+	+	*Penillia* sp.	+	+	+
*Acanthometron*sp.	+	+	+	*Evadne*sp.	+	+	+
Coelenterata				**Decapoda**			
Obelia sp.	+	-	+	*Lucifera* sp.	+	-	+
Siphonophora sp.	+	-	+	**Annelida**			
Ctenophora				Polychaeta:	+	+	+
Pleurobrachia sp.	+	-	+	*Tomopteris* sp.	+	+	+
Chaetognatha				*Spionid* sp.	+	+	+
Sagittaenflata	+	-	+	**Mollusca(Pteropoda)**			
S. Bedotii	+	-	+	*Creseis acicula*	+	-	+
Copepoda:				**Protochordata**			
Acrocalanus sp.	+	+	+	*Doliolum* sp.	+	-	-
Paracalanus sp.	+	+	+	*Oikopleura* sp.	+	-	-
*Rhincalanus*sp.	+	+	+	*Salpa* sp.	+	-	-
Pseudodiaptomus sp.	+	+	+	**Larval forms**			
Eucalanus sp.	+	+	+	Copepod nauplius	+	+	+
*Copilia*sp.	+	+	+	Eupahusidnauplius	+	+	+
*Macrosetella*sp.	+	+	+	Cirrepedenauplius	+	+	+
Miocrosetella sp.	+	+	+	Pontellidnauplius	+	+	+
Undinula sp.	+	+	+	Brachiopod larva	+	+	+
Acartia sp.	+	+	+	Zoea	+	+	+
Temora sp.	+	+	+	Cyphonautus larva	+	+	+
Oithona sp.	+	+	+	Decapod larva	+	+	+
O. plumifera	+	+	+	Gastropoda	+	+	+
Euchaeta sp.	+	+	+	Bivalvia	+	+	+
*Euterpina*sp.	+	+	+	Arachnetcis larva	+	+	+
Centropages sp.	+	+	+	Fish eggs and larvae	+	+	+

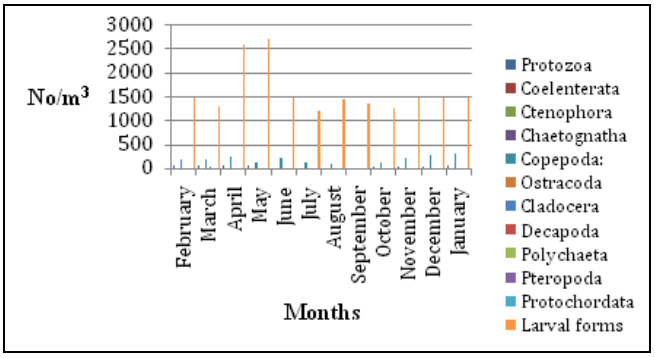

Fig. 2: Seasonal Variation of zooplanton at station I

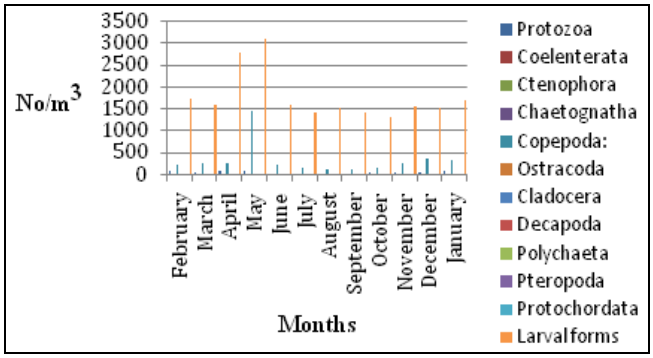

Fig.3:Monthly Variation of Zooplankton at station II

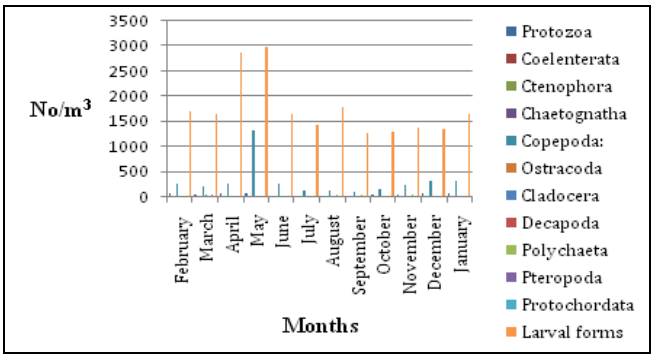

Fig.4:Monthly Variation of Zooplankton at station III

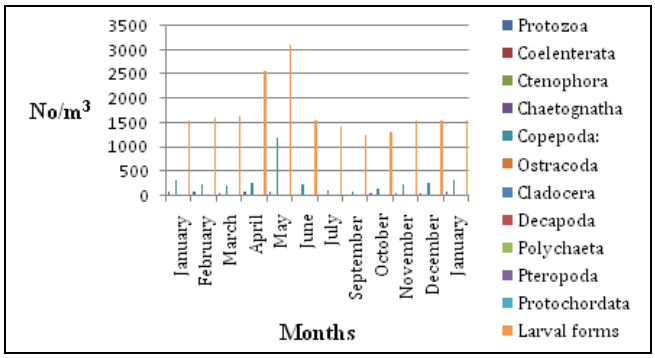

Fig5:Monthly Variation of Zooplankton at station IV

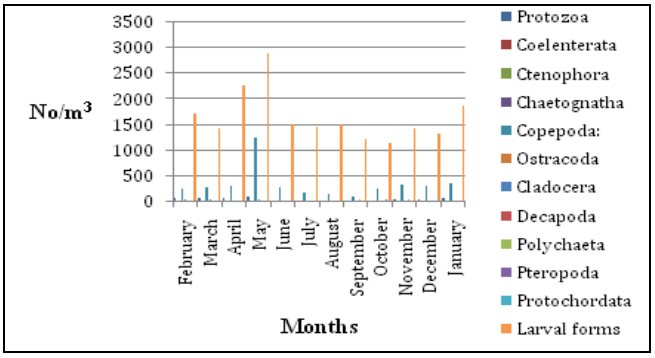

Fig.5: Monthly Variation of Zooplankton at station V

Table: 7 Correlation between abiotic factors and Zooplankton groups

Abiotic/Biotic factors	Copepod	Protozoa	Larval Forms
Water emp	-0.294917	0.48164	0.530464141
pH	0.684274	0.485082	-0.02422473
Turbidity	-0.18596	-0.16872	0.217723
D.O	-0.22505	-0.70938	-0.53832

The largest group which contributed much to the total density of zooplankton was the larval forms This group comprised by different larval forms among which fish egg and larvae and nauplius of copepod and *euphausid* contributed much to the total density of larval as well as zooplankton population. . Among the twelve groups, the larval forms ranked 1st (1264-3067/m^3) followed by copepod (97-1420/m^3) and *protozoa* (41.54/m^3). In all the study stations, the minimum density was observed in the southwest monsoon season whereas maximum peak density was recorded in pre and post monsoon seasons but the former peak was higher than pre monsoon. The larval forms constituted about 83-85% of the total species present in all the stations. *Copepods* constituted 11-13% while *protozoa* constituted only 2-3%.Other groups constituted about 15-17% of the zooplankton diversity. From the study it is clear that the zooplankton population of the study region was found to be dominated by larval forms followed by copepods and protozoans. Therefore it can be concluded that the Kali River has rich biodiversity of zooplankton species.

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RESEARCH ARTICLE

Assessment of trophic status of Ambazari Lake, Nagpur, India with emphasis to Macrozoobenthos as Bioindicator

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ABSTRACT

Assessment of macrozoobenthos and physicochemical parameters of Ambazari Lake of Nagpur from October 2010 to Sept. 2012 helps to reveal the trophic status of the lake. pH of Ambazari lake represented alkaline nature of lake. Macrozoobenthic diversity represented 28 species belonging to Phylum Annelida (22.68%), Arthropoda (21.63%) and Mollusca (55.68 %) represented by oligochaetes, decapodes, odonates, dipterans, gastropods and pelecypodes respectively.
Seasonally, group Mollusca dominated the lake in summer season while winter season revealed the abundance of all the groups in more or less similar trend . Higher values of macrozoobenthic diversity as well as nutrients such as Sulphate, Phosphate and Nitrate values indicated oligomesotrophic status of lake.

Key words: Macrozoobenthos, physico-chemical, bioindicator, trophic status, Ambazari lake, India.

INTRODUCTION

Aquatic Benthic macro-invertebrates are invertebrates that live on the bottom of water body during all or part of their life cycle. They often go unnoticed because of their size and habitat but are an extremely important part of aquatic ecosystems and serve as a link in the food web between decomposing leaves, algae, fish and other vertebrates (Cummins, 1974). They act as the secondary producers and form a part of food web of aquatic ecosystem. They also play an important role in transferring energy from the first trophic level to second trophic level in freshwater ecosystems.

The diversity and abundance of benthic inhabitants of a particular water body is much influenced by physico chemical status of water body which do show seasonal alternations depends on cascade of

events (Kodarkar, 1995). Because of their extended residency period in specific habitats and presence or absence of particular benthic species in a particular environment these can be used as bio-indicators of specific environment and habitat conditions. Aquatic macro-invertebrates are sensitive indicators of environmental changes in streams because they express long-term changes in water and habitat quality rather than instantaneous conditions (Cummins *et al.,* 1984). The proposed study was aimed to assess the trophic status of Ambazari lake with particular emphasis on the structure of macrozoobenthos and physico-chemical environment of the lake.

Study area

Ambazari lake situated to the south west of Nagpur city is almost a natural reservoir formed in the Basin of Nag River (Pathak, 2005). It is located at 21^0 09' N and 79^0 07'E, at an average elevation of 350 m above MSL (Fig.1). The catchment area of the lake is 1551.36 ha (NMC, 2009) which lies in the Northwest of the lake from where storm water feeds the lake.

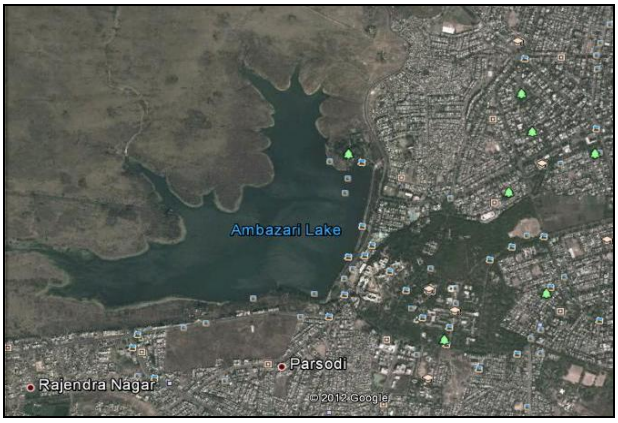

Fig. 1 : Ambazari Lake, Nagpur

Study Stations

Study was carried out collectively at four different sites as per preferably importance. (i) Old pump house- This site is to the East segment of lake. (ii) CRPF Pump house- This site is to the South segment of the lake and is undisturbed. (iii) MIDC Pump house- This site is to the West segment of lake. It is inlet of the lake and good grazing ground for cattle. (iv) Garden- This site is located at the North segment of lake having beautiful garden at its shore. It is tourist attraction during post-monsoon as migratory birds reside here.

MATERIALS AND METHODS

The present study was carried out during October 2010 to Sept 2012. The collection of water samples and biotic fauna was done collectively at four sites in morning hours on monthly basis. Water sample was collected in plastic water samplers of two liter capacity. Analysis of parameters like air temperature, water temperature, pH and D.O. was done on the spot and the rest were determined in the laboratory. Analysis was followed by standard methods (Adoni, 1985; APHA, 1965).

Macrobenthic samples were collected with the use of Ekmans dredge (6''x 6''x 6''). The collected samples were sieved through 500 micron copper sieve. Samples were preserved in formalin, (Hellawell, 1978) and identified with standard keys of Naidu and shrivastav (1979); Needam (1962); Tonapi (1960). However for quantitative analysis species wise individual counting was done on the basis of two year seasonal average. The number of benthos per unit area was calculated as follows.

$$Benthos\ \frac{no}{m2} = \frac{N}{A \times S} \times 100$$

Where, N=Number of organisms collected per sample.

A=Bitting area of samples (15×15 cm)
S=Number of samples taken.

RESULTS AND DISCUSSION

Averages of seasonal variation of physicochemical parameters are represented in Table 1. Air temperature was maximum in summer months, while water temperature was maximum in monsoon months; similar trend was also recorded by Swarnalatha and Rai (1998) in Banjara lake. In ambazari lake the average pH values ranged around 8.2, it represents alkaline nature of lake. Webber and Stumm (1963) have concluded that the pH of raw water sources mostly lies within the range 6.5 to 8.5. pH of inland waters in India lie in the alkaline range without much variation (Ghosh and George 1989).

In Ambazari lake the conductivity was observed in the range of 501 µS/cm to 571 µS/cm. Minimum conductivity (501.9 µS/cm) in rainy season may be due to dilution of salts and its variation in

concentration (Welch, 1952). According to Brown (1971) conductivity of the inland water should range between 150 to 450 µS/cm to flourish good flora and fauna but conductivity in Ambazari lake is slightly high above the range.

Dissolved Oxygen (D.O.) was found to be maximum (5.6 mg/l) in winter and minimum (4.6 mg/l) in summer. Maximum D.O. in winter might be due to low atmospheric temperature and intensive photosynthetic activity while minimum D.O. in summer may be due to high temperature and low solubility of oxygen in water (Kaushik and Saxena 1989).

Total hardness of Ambazari lake was recorded in the range of 172.3 to 245.5 mg/l. According to Sawyer (1960) water with hardness from 150.0 to 300.0 mg/l is considered as hard with hardness thus Ambazari lake water is hard with hardness. Hardness is directly proportional to its ionic balance.

Biological Oxygen Demand (B.O.D) recorded was 9.9 mg/l in summer and minimum 6.3mg/l in monsoon. Maximum B.O.D. in summer may be due to high microbial activities and decline form in monsoon and winter may be due to retard microbial activity. Similiar trend was also observed by Harne (2010) in three lakes of Bhadrawati.

Chemical Oxygen Demand (C.O.D.) recorded was 29.1 mg/l in summer and 21.8 mg/l in monsoon. With increased temperature the oxygen consumption by the living planktonic communities is also increased (Boyd, 1973) and increased organic matter may require more oxygen to oxidize under increased thermal condition as suggested by Ambasht and Sharadendu (1988). The maximum permissible value of C.O.D is 10 mg/l for drinking water (Edward, 1972). C.O.D. value of Ambazari lake exceed the limit.

In the present study the free ions such as Phosphate and Sulphate were notably higher in summer season while lowest in monsoon season. Higher concentration in summer is probably due to activity of biodegradation. Whereas dilution and utilization by aquatic plants gradually brought down the concentration in monsoon, Munawar (1970). Higher concentration of Nitrate observed in monsoon, is due to surface runoff, drainage, siphon runoff, storm water (Seitzniger, 1988).

Phylum Annelida represented *Limnodrillus hoffestry, Nais communis, Aelosoma bengalensis* and *Limnodrillus variegatus* of class Oligochaeta while *glossiphonia sp.* of class Hiudinea. Two year seasonal average distribution was recorded as 450.6 n/m^2 (winter), 186.3 n/m^2 (summer) and 268.6 n/m^2 in monsoon. Maximum number of Oligochaeta diversity was observed in winter and minimum in summer. Similar trend was also observed by Anitha *et al*. (2004) in Mir Alam lake of Hyderabad and Ojha *et al.* (2010).

The most frequently used community to determine the water quality in the streams is the macro invertebrates. (Rosenberg and Resh, 1993).

Table 1: Average of Seasonal Variations of Physico-chemical parameters in Ambazari Lake Oct 2010 to Sep 2012

Sr. No.	Parameters	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Temperature atm.	27.6	±	2.99	38.1	±	4.54	36.4	±	3.67
2	Temperature water	22.5	±	2.55	28.8	±	4.27	30.0	±	2.05
3	Ph	8.2	±	0.12	8.1	±	0.16	8.1	±	0.21
4	Conductivity	511.9	±	11.40	571.0	±	27.87	501.9	±	19.74
5	D.O.	5.6	±	0.25	4.6	±	0.63	5.2	±	0.29
6	Hardness – Total	172.3	±	5.36	245.5	±	26.27	190.3	±	24.54
07	B.O.D.	7.0	±	0.29	9.9	±	1.18	6.3	±	1.11
08	C.O.D.	21.8	±	3.89	29.1	±	2.80	21.8	±	1.49
09	Phosphate	0.5	±	0.22	2.0	±	0.57	1.6	±	0.51
10	Sulphate	14.7	±	3.85	28.1	±	2.64	20.8	±	2.82
11	Nitrate	0.1	±	0.02	0.4	±	0.12	1.0	±	0.23

Table-2: Avearage of Seasonal Variation of Macrozoobenthos in Ambazari Lake during Oct 2010 to Sep 2012

Macrozoobenthos Species		Winter	Summer	Monsoon
		Average	**Average**	**Mean**
Phulum-Annelida		450.8	186.3	268.6
Class-Oligochaeta		446.6	179.3	268
Family-Tubificidae	*Limnodrillus hoffemeistry*	144.6	52.3	88.3
Family-Naididae	*Nais communis*	130.5	52.5	59.1
Family-Aelosmatidae	*Aeolosoma bengalensis*	84.1	47.7	49.7
Family-Lumbricidae	*Lumbricus variegates*	87.4	26.8	70.9
Class-Hirudinea		4.2	7	0.6
Family-Glossiphonidae	*Glossiphonia sp.*	4.2	7	0.6
Phylum-Arthropoda		444.9	209.6	208.7
Class-Arachnida		21.3	2.9	8.3
Family-Hydrachnidiae	*Hydracarina sp.*	21.3	2.9	8.3
Class-Crustacea		14.5	35.5	29.1
Order-Decapoda				
Family-Gelechiidae	*Paratelphusa jaquemonti*	8.5	20.9	17.1
Family-Gelechiidae	*Gelasimus sp.*	6	14.6	12
Class-Insecta		409.1	171.2	171.3
Order-Odonata		12.6	5.4	9.5
Sub order-Anisoptera	*Dragonfly nymphs*	7.2	3.1	5.4
Sub order-Zygoptera	*Damselfly nymphs*	5.4	2.3	4.1
Order-Diptera		393.5	126.3	121.9
Family-Tendipididae	*Chironomous sp.*	188.7	70.5	49
Family-Culicidae	*Anopheles sp.*	15.6	5.7	7
Family-Culicidae	*Culex sp.*	66.5	24.4	29.5
Family-Syrphidae	*Eristalis sp.*	24	-	1.9
Family-Limnoniidae	*Rhapidolabis sp.*	27.1	0.3	8.3
Family-Tabanidae	*Tabanus sp.*	71.6	6.3	21.5
Family-Muscidae	*Musca autumnialis*	-	19.1	4.7
Order-Hemiptera		3	39.5	39.9
Family-Nepidae	*Nepa sp.*	1.7	22.6	22.8
Family-Nepidae	*Ranatra elongate*	1.3	16.9	17.1
Phyllum-Mollusca		490.8	1134.6	596.6
Group-Gastropoda		354.3	829.2	451.7
Family-Viviparidae	*Vivipara bengalensis*	57.9	131.5	55.8
Family-Thiaridae	*Melania striatella tuberculata*	51.1	143	106.9
Family-Thiaridae	*Melania scabra*	64.6	136.7	100.6
Family-Lymnaeidae	*Lymnea lutiola*	65.3	87	67.6
Family-Planorbidae	*Indoplanorbis exustus*	65	145.4	69
Family-Pachilidae	*Faunus ater*	50.4	185.6	51.8
Group-Pelecypoda		136.5	305.4	144.9
Family-Unionidae	*Lamellidens correanus*	52	116.3	55.2
Family-Unionidae	*Lamellidens marginalis*	45.5	101.8	48.3
Family-Unionidae	*Parreysia corrugata nagpoorensis* (Lea)	39	87.3	41.4
Number of Species(S)		**27**	**27**	**28**
Total(N)		**1386.3**	**1530.5**	**1073.7**

These organisms offer valuable information regarding their surrounding conditions and can be used to evaluate the physical, chemical and biological impact and their cumulative effect (Karr and Chu, 1999). Qualitative study of macro benthos was done in Ambazari lake of Nagpur city from Oct. 2010 to Sept. 2012. The study represented 28 species of macro benthos belonging to three phyla viz. Annelida(22.68%), Arthropoda (21.63%) and Mollusca (55.68 %) (Table 3 and Fig.2).

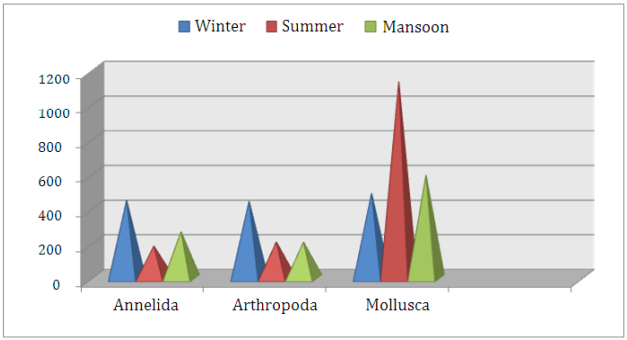

Fig.2: Seasonal average of macrozoobenthos (N0. /m^2) at Ambazari Lake, Nagpur.

Table 3: Percentage wise distribution of Macro-zoobenthos in Ambazari lake

Sr. No.	Group of Macrozoobenthos	Percentage
1	Annelida	22.68%
2	Arthropoda	21.63%
3	Mollusca	55.68 %

The Oligochaeta adapted to every kind of water and are found in algae, aquatic vegetation, floating rotten materials and in bottom mud (Wetzel *et al*., 2000). Many environmental studies have focused on the use of freshwater oligochaetes as indicator of trophic condition (Chapman *et al.,* 1982). Oligochaeta prefer organically rich environment and remain dominant in severally polluted condition (Hawks,1979).

Arthropoda is one of the most widely represented phyla in fresh water habitat and different groups have different ecological adaptations. Ambazari lake represented crustaceans, Insects and Arachnids of phylum Arthropoda. The two year seasonal average value of arthropoda ranged as 444.9 no/m^2 (winter), 209.6 no/m^2 (summer) and 208.7 no/m^2 (monsoon).

Insects are the species which are less to moderate tolerant to the changes in physico-chemical compositions of water and substratum. (Fraser, 1936). Presence of *Chironomous sp.* and *Eristalis sp.* indicate sites affected with anthropogenic activities, washing, cattle grazing and open defecation resulting in the condition of pollution. Chironomus larvae have also been used as pollution indicators by number of workers (Gaufin, 1957; Curry, 1962). In the present study phylum Mollusca represented group Gastropoda and Pelecypoda. The phylum ranged as 490.8 no/m^2 (winter), 1134.6 no/m^2 (summer) and 596.6 no/m^2 (monsoon). They were found dead on the shore in large number during summer.

Throughout the study period Phylum mollusca dominated the lake(55.68%) followed by Annelida (22.68 %) and Arthropoda (21.63%) respectively (Fig 3). winter season shows more or less similar trend of all the macrozoobenthic density while mollusks mostly dominated summer season followed by monsoon (Fig. 2). Higher molluscan density in the summer season might be due to soft and organically rich bottom and alkaline nature of water. Silt matter are known to support thriving populations of macro invertebrates because of reduction in water current as such the substrate trends to make mollusks indistinguishable from their typical lentic habitat. Whitton (1975). Anwar and Siddiqui (1988) recorded peak of benthic invertebrates in summer. In the present study the Molluscan fauna was distributed from shore line to 3 m depth in all type of sediments.

From the results of present study it is clearly evident that the macro-benthic community is dominated by molluscan population. The availability and distribution of chironomous larvae in the lake indicate pollution conditions that have been attributed to be relative to many factors (Bowman, 1976). Higher values of macrozoobenthic diversity as well as nutrients such as Sulphate, Phosphate and Nitrate values indicated oligomesotrophic status of lake.

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RESEARCH ARTICLE

Response of metabolites from culture filtrates of *Alternaria* species against *Triticum aestivum* L

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ABSTRACT

Metabolites are known products of enzyme-catalyzed reactions that occur naturally within cell. The potential of metabolites of two *Alternaria* species, *A. alternata,* and *A. solani* from culture filtrate in Czapek's nutrient broth, was investigated against *Triticum aestivum* L both in laboratory bioassays and in pots. In laboratory bioassays, the potential of culture filtrates of both *Alternaria* species was studied on seed germination and seedling emergence in blotter paper slots. The metabolites from 5-days culture filtrate of both *Alternaria* species enhanced seed germination rate by 9.6 - 10.2% while length of shoot, shoot fresh biomass, length of root and root fresh biomass of wheat seedlings was increased over control by 10-12%; 9-13%; 12-14% and 9-14% respectively. Rate of transformation of germinated seeds to normal seedlings was enhanced over control when treated with 5-days old culture filtrate. The toxicity of culture filtrate increases with longer duration of treatment. The toxicity appeared in 10-25 days old culture filtrates, significantly inhibited seed germination, shoot length, shoot fresh biomass, root length and root fresh biomass over control. In pot trials, foliar application of culture filtrates was made on 1-week and 2-week old wheat seedlings. The seedlings receiving 5-days old metabolites treatment of both *Alternaria* species markedly enhanced the shoot biomass while seedlings emerged from 10-25 days old metabolites treated seeds reduced the shoot biomass. The seedlings of 2-week age old were reported more susceptible to foliar spray than the 1-week old. The reduction in shoot biomass was more significant to 2-week old wheat seedlings when treated with 10-25 days old metabolites. It is revealed that metabolites from 5-days old culture filtrate of both *Alternaria* species resulted enhance effect, may be able to be used as alternative growth promoter while other treatments had inhibitory effects, and may be beneficial for destroying the weeds which reduce the productivity of economical important crops.

Key words: *Alternaria alternata, A. solani, metabolites, growth promoter, toxicity, Triticum aestivum* L.

INTRODUCTION

Pathogenic fungal microbes of diverse group are known to secrete or excrete a variety of a multitude of low molecular weight bioactive organic compounds during a period growth, in infested host tissues, may be either non-toxic or toxic to host cells (Holensein and Stoessi, 2008). The metabolites of non-toxic nature are reported to be beneficial

to host but toxic ones directly act on living host protoplasm creating disturbances in normal cell metabolism to influence the course of disease development or symptom expression (Bhajbhuje, 2013; Bhajbhuje and Pathode, 2014; Kandhare, 2015).

Wheat (*Triticum aestivum* L.), one of the world's main widely planted staple nutritious food crop for more than one third of the world population is grown extensively in all continents around the globe except Antarctica for its amber-coloured, non-dehiscent single seeded caryopsis, as it is proved to be an excellent health-building food and leading source of vegetable protein, minerals, Vit-B and dietary fibre, contributing 20% of all calories and proteins to the world human diet (Wikipedia, 2015). Wheat seed is known for its potential longevity and has multiple applications as whole grain to improve nutrition, boost food security, foster rural development, support sustainable land care and for its value added products (Taylor and Koo, 2011). India is second leading producer of bread wheat on the globe, contributing 14.1% of the World's total annual output. Lion's share of India's production, accounting for over 32.8% of the nation's total output is contributed by Uttar Pradesh followed by Punjab. Whole grain provides 20% of the food calories and mostly used as animal feed as well as raw material for ethanol production, brewing of wheat beer, for cosmetics while white flour from seed endosperm is used for making of bread, preparing zero cholesterol confectionary products, biscuits, pasta, noodles, yeast breads; cakes, cookies, crackers and pastries. Asides from being used as food, wheat has several medicinal virtues including anticancer property (Wikipedia, 2015).

Majority species of *Alternaria* remains as an increasing threat to several crops around the globe causing several diseases including Alternaria leaf blight, damping off of seedlings, producing brown to black leaf spots lead to a reduction of leaf count and rate of photosynthesis. The infected seeds are often shrivelled, reduced in size with a brown discolouration on seed surface and loss seed germination potential that adversely affect annual productivity of vegetables and other crop plants to the extent of 20-30% (Mamgain *et al*., 2013). The pathogen can survive as conidia on seed surface or as mycelium inside seed coat and produced both non-toxic as well as toxic metabolites in storage. Literature survey reveals that fungal metabolites of primary nature enhanced seedlings growth (Sung *et al*., 2011; Chung, 2012; Bhajbhuje, 2013; Bhajbhuje and Pathode, 2014) while secondary metabolites becomes toxic to host cells, damages cell components of actively growing cells to influence the course of symptom expression in host plant (Brakhage and Schroeckh, 2011; Madhavi *et al*., 2012; Bhajbhuje, 2013; Venda Kumari *et al*., 2014; Khandare, 2015). Several researchers have made investigation on role metabolites of *Alternaria* in plant system (Tsuge *et al*., 2013; Bhajbhuje and Pathode, 2014; Bhajbhuje, 2015). Presently response of metabolites secreted in culture filtrate of *A. alternata* and *A. solani* against wheat plant has so far not been reported. It seemed to be worthwhile to study parameters in laboratory and in pot trials concerning to seed germination, length of shoot and root; biomass of fresh shoot and root in using *Alternaria alternata* and *A. solani* metabolites with *Triticum aestivum* L.

MATERIALS AND METHODS

Preparation of cultural filtrates of test fungi

The isolation of leaf blight causing pathogens, *Alternaria alternata* and *A. solani was* made on Czapek's Dox agar nutrient medium from infested seeds of vegetables as an internal seed borne pathogens employing the technique of ISTA (2014). An inoculum (5 mm agar discs) of test isolates from 6 days old culture was transferred aseptically into sterile 35ml Czapek's broth and incubated for a period between 5 to 25 days under static conditions at 25±1^{0}C. Separate sterile broth and distilled water were kept as control. The metabolites were isolated from culture filtrate in different duration following method described earlier (Bhajbhuje, 2014). The cultures were filtered through sterilized muslin cloth followed by Whatman filter paper No.1. These filtrates containing metabolites were preserved at 4 °C in a refrigerator and used for treatment within a period of a week of filtration to avoid chances of any contamination or chemical alteration (Akbar and Javaid, 2010).

Laboratory bioassays

Healthy seeds sterilized with aqueous solution of 0.1% mercuric chloride were soaked for one hour in sterile distilled water to soften seed coat. Hundred water soaked seeds were placed for 3 hours in 5 to 25 days old culture filtrate containing metabolites of *Alternaria*

alternata and *A. solani* in triplicate. After each metabolite treatment, immediately washing of seeds was carried out for 5 consecutive times. The moistened treated and untreated control seeds were transferred to sterile blotter paper folds in slots for germination and seedling growth studies. The slots containing seeds were covered with glass cabinet to avoid spoilage of seeds by any saprophyte contaminants. The moisture content of blotter paper was maintained by addition of sterile distilled water when required. Harvest was taken on 8th day. Data regarding seed germination, root/shoot growth in terms of length and fresh biomass was recorded (Bhajbhuje, 2015).

Pot trials

A pot experiment was conducted in a field using plastic pots of 8 cm diameter and 12 cm deep containing 350 g sandy loam soil supplemented with farm yard manure. Ten seeds of wheat were sown in each pot. After seed germination, pots were divided into two sets to perform the foliar spray on 1-week & 2-weeks old seedlings. Pots containing seeds were watered, when required and kept these pots under natural environmental condition where sufficient light is made available. The culture filtrates of both *Alternaria alternata* and *A. solani* were sprayed 3 times with interval of 5 days on 1-week and 2-weeks old wheat seedlings in triplicates. Plants of the control treatment were sprayed with sterile distilled water. After 30 days growth, plants were carefully uprooted and washed under tap water. Roots were separated from shoots. Result on length as well as dry biomass of shoot and root was recorded (Bhajbhuje, 2015).

RESULTS AND DISCUSSION

Metabolites are intermediate products of metabolism having multifold functions, including fuel, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity, defense, and interactions with other organisms. Metabolites of primary nature are directly involved in normal growth, development, and reproduction while a secondary metabolite usually has several important ecological functions (Wikipedia, 2015). Many new general techniques for both bio-control and for causing enhancement of plant growth have recently been developed. *Trichoderma* spp. possesses innate resistance to most agricultural chemicals, including fungicides, although individual strains differ in their resistance. Majority species of *Alternaria* including *A. alternata* and *A. solani* are known to cause an early blight disease in vegetables producing small, darkened lesions on plant parts that spread into growing black spots of dead tissue, often killing most of the plant in the long run. Seeds infected with the disease may even damp off during germination (Mamgain *et al*., 2013).

Laboratory bioassays

Leaf blight causing pathogens, *Alternaria alternata* and *A. solani* isolated on infested seeds of vegetables were allowed to grow in Czapek's broth nutrient medium for a period between 5 to 25 days in static climate. The metabolites of different duration from culture filtrates of these isolates were tested against *Triticum aestivum*. Results concerning to seed germination, seedlings growth; count of normal seedlings and fresh biomass of treated and untreated control plants is tabulated in Table 1. The metabolites from 5-days old culture filtrates of both the isolates exhibited significant enhancing effect on seed germination, length of shoot and root as well as biomass of fresh shoot and root while 10-25 days old culture filtrate had insignificant effect on these parameters undertaken. The rate of seed germination was confined to enhance by 9.6 – 10.2%; length of shoot and root shoot of seedling by 9.6 – 12.3% and 12.3 – 13.6% while biomass of fresh shoot and root was reported to increase by 8.7 – 13.0% and 9.2 – 14.3% over control for *A. solani* and *A. alternata* respectively with five days old metabolites treatment (Table 1).

The response of metabolites from 10 to 25 days old culture filtrates of test fungal isolates against the parameters understudy was significant. The seed germination rate was declined by 5.7% to 25.5% and 4.5% to 27.4% over control when seeds treated with 10 to 25days old metabolites of *A*. *alternata* and *A. solani* respectively. Control seeds did not express any change. It was noticed that the seedlings growth was suppressed when seeds treated with metabolites of longer duration. Moreover, majority of treated germinating seeds were transformed into abnormal seedlings. The count of normal seedlings declined to the extent of 2.8 – 38.4% and 4.8 – 40.6% while count of abnormal seedlings rose from treated seeds was significantly enhanced (Table 1).

Table 1: Record of per seed viability, length of shoot & roots of metabolite treated and untreated seed of *Triticum aestivum* L in laboratory bioassay.

Duration of treatment (Days)	Seed viability		Seedling height				Biomass of Fresh seedling				Nature of Seedlings			
	Per cent Seed germination		Shoot length (cm)		Root length (cm)		Shoot fresh weight (mg)		Root fresh weight (mg)		Normal seedlings (%)		Abnormal seedlings (%)	
	AA[1]	AS[2]	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS
5	86.5 (+10.2)[3]	86.0 (+9.6)	8.2 (+12.3)	8.0 (+9.6)	9.2 (+13.6)	9.1 (+12.3)	5.2 (+13.0)	5.0 (+8.7)	2.4 (+14.3)	2.3 (+9.2)	89.0 (+13.2)	87.5 (+11.3)	11.0 (-48.6)	12.5 (-41.6)
10	74.0 (-5.7)	75.0 (-4.5)	6.9 (-5.5)	6.7 (-8.2)	7.6 (-6.2)	7.4 (-8.6)	4.2 (-8.7)	4.1 (-10.9)	1.9 (-9.5)	1.8 (-14.3)	84.8 (+7.9)	82.6 (+5.1)	15.2 (-28.9)	17.4 (-18.7)
15	71.5 (-8.9)	71.0 (-9.6)	6.3 (-13.7)	6.1 (-16.4)	7.1 (-12.3)	6.9 (-14.8)	3.8 (-17.4)	3.6 (-21.7)	1.7 (-19.0)	1.6 (-23.8)	76.4 (-2.8)	74.8 (+4.8)	23.6 (+10.8)	25.2 (+17.8)
20	66.0 (-15.9)	65.5 (-16.6)	5.8 (-20.5)	5.7 (-21.9)	6.5 (-19.8)	6.3 (-22.2)	3.5 (-23.9)	3.4 (-26.1)	1.6 (-23.8)	1.6 (-23.8)	62.7 (-20.2)	59.2 (-24.7)	37.3 (+74.3)	40.8 (+90.7)
25	58.5 (-25.5)	57.0 (-27.4)	5.2 (-28.8)	5.0 (-31.5)	5.8 (-28.4)	5.5 (-32.1)	3.1 (-32.6)	2.8 (-39.1)	1.4 (-33.3)	1.3 (-38.1)	48.4 (-38.4)	46.7 (-40.6)	51.6 (+141.1)	53.3 (+149.1)
Czapek's broth	84.0 (+7.0)	84.0 (+7.0)	7.6 (+4.1)	7.6 (+4.1)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)	80.8 (+2.8)	80.8 (+2.8)	19.2 (-10.3)	19.2 (-10.3)
Control (D.W.)	78.5	78.5	7.3	7.3	8.1	8.1	4.6	4.6	2.1	2.1	78.6	78.6	21.4	21.4

1. AA - *Alternaria alternata*; 2. AS – *Alternaria solani;* 3. Values in parenthesis indicate per cent increase or decrease over control

Length of shoot had significant response to metabolites from 10-25 days old culture filtrate, inhibited shoot length by 5.5 to 28.8% and 8.2 to 31.5% over control for *Alternaria alternata* and *A. solani* respectively as compared to control. The effect of these metabolites was significant on shoot biomass. Metabolites of this duration of these two *Alternaria* species declined biomass of fresh shoot over control by 8.7 - 32.6% and 10.9 – 39.1% respectively (Table 1). Length of root exhibited an significant response to these metabolite treatments, significantly inhibited root length to the extent of 6.2 to 28.4% and 8.6 to 32.1% compared to control. The adverse effect of these metabolite treatments on root biomass was significant. Root biomass was reduced to the extent of 9.5 – 33% and 14 – 38% over control (Table 1).

Pot trials

A pot experiment was conducted in a field using plastic pots. Ten seeds of wheat were sown in each pot containing sandy loam soil supplemented with farm yard manure and were allowed to germinate the seeds under natural environmental condition. The culture filtrate of 5-25 days duration of *A. alternata* and *A. solani* was sprayed 3 times on 1-week and 2-weeks old wheat seedlings in triplicates. Plants of the control treatment were sprayed with sterile distilled water. After 30 days growth, result on length as well as dry biomass of shoot and root was recorded (Bhajbhuje, 2015).The effect of metabolites from culture filtrates of shorter duration was reported insignificant and it was significant with metabolites of longer duration. The treatment with metabolites from 5-days old culture filtrates of test fungal isolates enhanced length of shoot of 1-week and 2-week old seedlings by 11.9% and 9.6% respectively (Fig 1). Similar stimulatory effect was recorded for length of root with same metabolite treatment (Fig.2). The root length was enhanced over control by 13.6% and 12.3% for both test fungal isolates respectively (Table 2).

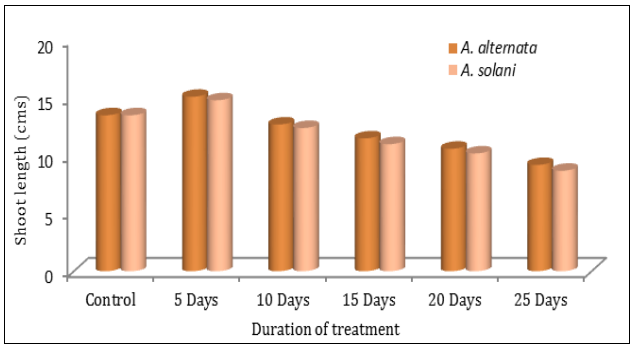

Fig. 1(a) Effect of foliar spray of metabolites on shoot length of 1-week old seedling of wheat

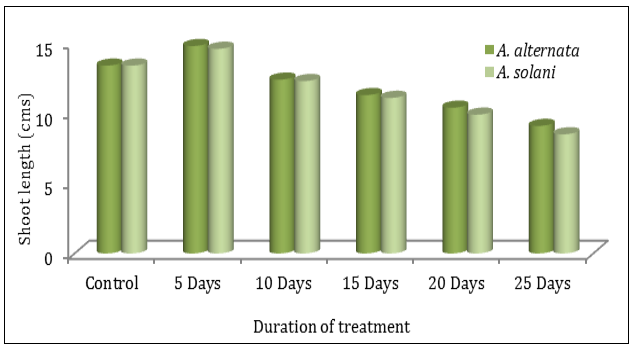

Fig. 1(b) Effect of foliar spray of metabolites on shoot length of 2-week old seedling of wheat

Table 2: Effect of foliar spray of metabolites from culture filtrate of *Alternaria alternata* and *A. solani* on growth of 1- & 2-week old wheat seedlings in pot trials after 30 days growth.

Duration of treatment (Days)	1-week old seedlings								2-week old seedlings							
	Seedling height				Dry Biomass of Seedling				Seedling height				Dry Biomass of Seedling			
	Shoot length (cm)		Root length (cm)		Shoot dry weight (mg)		Root dry weight (mg)		Shoot length (cm)		Root length (cm)		Shoot dry weight (mg)		Root dry weight (mg)	
	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS	AA	AS
5	15.1 (+11.9)	14.8 (+9.6)	9.2 (+13.6)	9.1 (+12.3)	5.2 (+13.0)	5.0 (+8.7)	2.4 (+14.3)	2.3 (+9.2)	14.8 (+9.6)	14.6 (+8.1)	8.9 (+9.9)	8.7 (+7.4)	4.9 (+6.5)	4.8 (+4.3)	2.3 (+9.5)	2.2 (+4.8)
10	12.7 (-5.9)	12.4 (-8.2)	7.6 (-6.2)	7.4 (-8.6)	4.2 (-8.7)	4.1 (-10.9)	1.9 (-9.5)	1.8 (-14.3)	12.4 (-8.1)	12.3 (-8.9)	7.5 (-7.4)	7.3 (-9.9)	4.1 (-10.9)	3.9 (-15.2)	1.7 (-19.0)	1.6 (-23.8)
15	11.5 (-14.8)	11.0 (-18.5)	7.1 (-12.3)	6.9 (-14.8)	3.8 (-17.4)	3.6 (-21.7)	1.7 (-19.0)	1.6 (-23.8)	11.3 (-16.3)	11.1 (-17.8)	6.8 (-16.0)	6.5 (-19.8)	3.5 (-23.9)	3.3 (-28.3)	1.6 (-23.8)	1.4 (-33.3)
20	10.6 (-21.5)	10.2 (-24.4)	6.5 (-19.8)	6.3 (-22.2)	3.5 (-23.9)	3.4 (-26.1)	1.6 (-23.8)	1.6 (-23.8)	10.4 (-22.9)	9.9 (-26.7)	6.2 (-23.5)	5.9 (-27.2)	3.2 (-30.4)	3.0 (-34.8)	1.4 (-33.3)	1.3 (-38.1)
25	9.2 (-31.9)	8.7 (-35.6)	5.8 (-28.4)	5.5 (-32.1)	3.1 (-32.6)	2.8 (-39.1)	1.4 (-33.3)	1.3 (-38.1)	9.1 (-32.6)	8.5 (-37.8)	5.3 (-34.6)	5.1 (-37.0)	2.9 (-36.9)	2.6 (-43.5)	1.2 (-56.1)	1.1 (-47.6)
Czapek's broth	14.2 (+5.2)	14.2 (+5.2)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)	14.2 (+5.2)	14.2 (+5.2)	8.5 (+4.9)	8.5 (+4.9)	4.8 (+4.3)	4.8 (+4.3)	2.2 (+4.8)	2.2 (+4.8)
Control (D.W.)	13.5	13.5	8.1	8.1	4.6	4.6	2.1	2.1	13.5	13.5	8.1	8.1	4.6	4.6	2.1	2.1

1. AA - *Alternaria alternata*; 2. AS – *Alternaria solani;* 3. Values in parenthesis indicate per cent increase or decrease over control

In pot trials, the inhibitory effect of 10 to 25-days old culture filtrates containing metabolites of both *A. alternata* and *A. solani* was confined significant on both 1-week and 2-week old wheat seedlings. Moreover the inhibitory effect of *Alternaria solani* was pronounced against *A. solani* (Table 2). The foliar spray applications of these metabolite treatment reduced length of shoot of 1-week old seedlings over control by 5.9 – 31.9% and 8.2 – 35.6% respectively. The root length of seedlings was declined by 6.2-28.4% and 8.6 – 32.8% with same metabolite treatment (table 2). The shoot biomass was significantly declined by 8.7 – 32.6% and 10.9 – 39.1% in 1-week old seedlings receiving foliar spray of 10-25 days old culture filtrates of *A. alternata* and *A. solani* respectively (fig. 3). The inhibitory effect of culture filtrates of these fungal isolates was insignificant on 2-week old plants. The age of plant was considered important parameter for inducing resistance. The 1-week old seedlings were confined more resistance to foliar spray application where different fungal culture filtrate treatments reduced the root biomass by 32–39% against 47 – 56% declining in 2-week old seedlings (Fig.4).

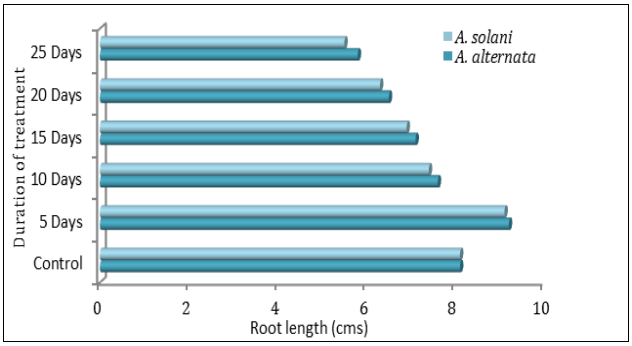

Fig. 2(a) Effect of foliar spray of metabolites on root length of 1-week old wheat seedling

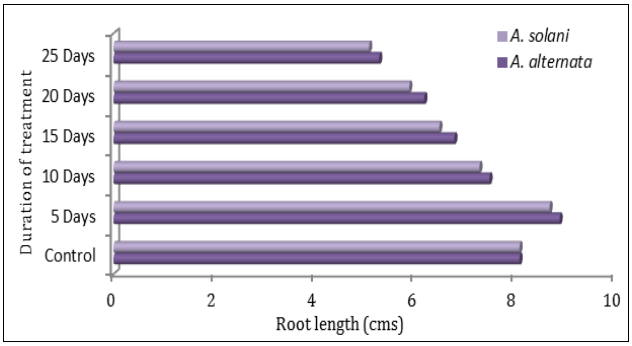

Fig. 2(b) Effect of foliar spray of metabolites on root length of 2-week old wheat seedling

In general the inhibitory effect of foliar spray on root length was much pronounced. Root dry biomass had more pronounced response to foliar spray application over root length. Metabolites from 10-25 days old culture filtrate of test fungal isolates significantly reduced the dry root biomass by 9.5 – 33.3% and 14.3 – 38.1% in 1-week old plants. The effect of these treatments on 2-week old seedlings was much significant. There were 19–56% and 24–48% reduction 2-week old plants, respectively (Fig.4).

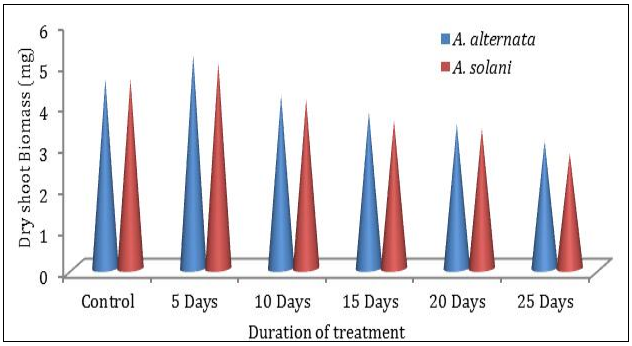

Fig. 3 (a) Effect of foliar spray of dry shoot biomass of 1-week old wheat seedling.

In general the inhibitory effect of foliar spray on root length was much pronounced. Root dry biomass had more pronounced response to foliar spray application over root length. Metabolites from 10-25 days old culture filtrate of test fungal isolates significantly reduced the dry root biomass by 9.5 – 33.3% and 14.3 – 38.1% in 1-week old plants. The effect of these treatments on 2-week old seedlings was much significant. There were 19–56% and 24–48% reduction 2-week old plants, respectively (Fig.4).

In laboratory bioassay, the metabolites from 5-days old culture filtrates of both the isolates exhibited significant effect on rate of seed germination, length of shoot and root as well as biomass of fresh shoot and root while 10-25 days old culture filtrate had insignificant effect on these parameters. The rate of seed germination was confined to enhance over control respectively with five days old metabolites treatment. In pot trials also, the effect of metabolites from culture filtrates of shorter duration was reported insignificant and it was significant with metabolites of longer duration. The treatment with metabolites from 5-days old culture filtrates of *Alternaria alternata* and *A. solani* enhanced length of shoot of 1-week and 2-week old seedlings. Similar stimulatory effect was recorded for other parameters undertaken. It is in agreement with the earlier finding to these parameters involving *Aijung rice* (Islam and Borthakur, 2012); and *Vigna mungo* (Bhajbhuje, 2014) with five to seven days metabolite treatment. Sung *et al*., (2011) reported enhancement in growth of seedling and higher rate of seed germination over control in Canola, cucumber and tomato plants receiving metabolic treatment of culture filtrate of *Shimizuomyces paradoxus*. Bhajbhuje and Pathode (2014) reported enhancement in these parameters over control in wheat seedlings receiving metabolite treatment of *Alternaria triticina*. Moreover, metabolites of *Trichoderma harzianum* induced germination wheat seeds with hard seed coat (Mokhtar and Dehimat, 2013); *Fusarium oxysporum f. sp. lycopersici* and *Alternaria solani* metabolites enhanced seed germination rate of tomato (Bhajbhuje, 2013). Literature survey revealed secretion of metabolites of primary nature and some growth stimulating factors by *A. alternata* and *A. solani* at early growth stages that enhanced the seed germination rate, seedling emergence (Chung, 2012; Bhajbhuje, 2014).

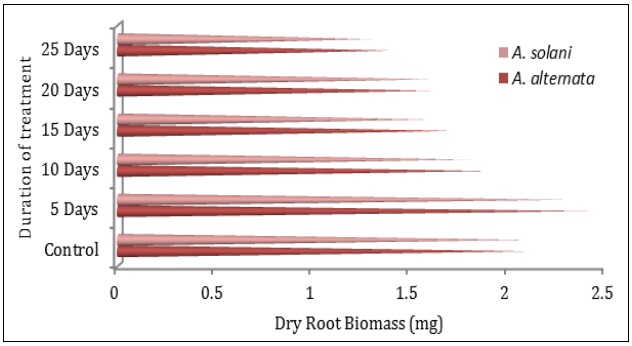

Fig. 4 (a) Effect of foliar spray of metabolites on dry root biomass of 1-week old wheat seedling.

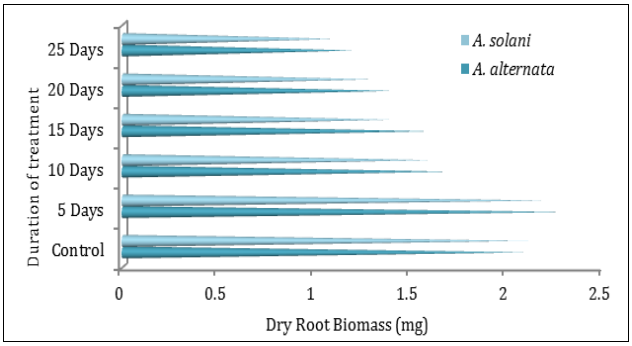

Fig. 4 (b) Effect of foliar spray of metabolites on dry root biomass of 2-week old wheat seedling.

These metabolites of primary nature may serve as growth promoter at low concentration and induced vigorous proliferation by stimulating phosphorylation in the host tissues in association of Ca^{2+} and Mg^{2+} (EFSA, 2011). It is noted that low concentration of these metabolites did not express any phenotypic variation in seedling receiving treatment (Bhajbhuje and Pathode, 2014). Moreover, the biomass of fresh shoot and roots as well as count of normal seedlings were significantly enhanced in seedlings receiving metabolite treatment from 5-days old culture filtrate of both test fungal isolates. A growth stimulating effect in response to seed germination rate and seedling emergence over control in present investigation may be attributed to secretion metabolites of primary nature by test fungal organisms at early stages of their growth that may serve as growth promoters.

In *laboratory bioassay*, the response of metabolites from 10 to 25 days old culture filtrates of test fungal isolates against the parameters understudy was insignificant. The per cent seed germination declined over the control when seeds treated with 10 to 25days old metabolites of *Alternaria alternata* and *A. solani* respectively. The seedlings emergence was suppressed when treated with culture filtrate of longer duration. The metabolite treated germinating seeds did not transform into normal seedlings. The count of normal seedlings was declined while count of abnormal seedling rose from treated seeds was significantly enhanced (Table 1).

Length of shoot had significant response to metabolites from 10-25 days old culture filtrate, declined this growth by 33% and reduced biomass of fresh shoot by 32 -39% over control respectively (Table 1). The root length and biomass exhibited an insignificant response to these metabolite treatments, significantly reduced root length by 28 to 32%; and root biomass to the extent of 33-38 % for *Alternaria alternata* and *A. solani* respectively as compared to control (Table 1).

In pot trials, the inhibitory effect of the metabolites from 10-25 days old culture filtrates of both *Alternaria alternata* and *A. solani* was confined significant in both 1-week and 2-week old plants treatment. Moreover the inhibitory effect of *Alternaria solani* was pronounced against *A. solani.* The foliar spray applications of these metabolite treatment reduced length of shoot, root and dry biomass of seedlings (Fig.1-4). The inhibitory effect of culture filtrates of these fungal isolates was insignificant on 2-week old plants. The 2-week old seedlings were more susceptible to foliar spray for these parameters undertaken.

The results of the present study were confirmed with earlier findings of Madhavi *et al*., (2012) in *Allium cepa* L.; Raithak and Gachande (2013) in *Lycopersicon esculentum* L and Venda Kumari *et al*., (2014) in *Brassica carinata* & *B. braun*; Bhajbhuje (2015) in *Vigna mungo.* Anand *et al*., (2008) confirmed production of nonspecific toxic metabolites in culture filtrate by *Alternaria alternata* and *Colletotrichum capsici* that induced inhibition of seed germination, length of shoot/root and vigour index of the seedlings of chilli, rice, mungbean, maize, cotton, groundnut, okra, eggplant, cucumber and tomato. Savitha *et al*., (2012) isolated toxin of *Alternaria semami* and same was tested on sesamum and tomato and reported greater inhibition of seed germination and length of shoot/ root at 2000 ppm conc. while 50 ppm conc. had least inhibition on these parameters. Wagh *et al* (2013) reported *Alternaria* leaf spot *in vitro* and *in vivo* in plantlets inoculated with *Alternaria alternata* and detached leaves of *Lepidium sativum*. The phenomenon indicates that metabolites are both phytotoxic and mutagenic as far as the present plant material is concerned.

Mycotoxin secretion by several filamentous fungi has been reported in many crops including cereals, vegetables, oil-seed crops and pulses (Holensein and Stoessi, 2008). Host-selective toxins (HSTs) produced by fungal plant pathogens are low-molecular-weight secondary metabolites with a diverse range of structures that function as effectors controlling pathogenicity or virulence in certain plant-pathogen interactions (Tsuge, *et al* 2013). *Alternaria* species can invade crops at the pre- and post-harvest stage and cause considerable losses due to leaf spot, early blight, rotting of fruits and seeds, may results to secretion of a range of mycotoxins as well as other non-toxic metabolites under favourable environment in cereals, mandarins, peppers, apples, sunflower seeds, oilseeds rape, olives, various fruits and vegetable seeds (Wikipedia, 2015). Amongst other species, *Alternaria alternata* (Fr.) Keissler produced several toxic metabolites of major toxicological importance including, HST-toxin, AAL-toxins, tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I (Helambe and Dande, 2012) in artificial nutrient medium during its growth period

provided favourable climatic environment. Alternariol and alternariol monomethyl ether also have been produced by pathogen in artificially mould-infested building materials (Chung, 2012). The pathogen had seven pathogenic variants producing different host-specific toxins (HSTs) and cause diseases on different plants (Helambe and Dande, 2012). HSTs was reported release from germinating conidia of *Alternaria alternata* prior penetration of host cell (Tsuge *et al*., 2013).

CONCLUSION

The present study concludes that culture filtrates of the two tested *Alternaria* species contain beneficial and hazardous chemical constituents. Further studies are required to isolate and identify the potential of these constituents. Once identified, these natural compounds may be used as structural lead for the preparation of ecofriendly pesticides for the management of population weeds that unable to grow and develop crop plant to maturity and ultimately adversely helps to reduce the crop productivity to a greater extent.

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RESEARCH ARTICLE

Antimicrobial Potential of the Moss *Brachymenium turgidum* Broth. ex. Dix. From Melghat Forest

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ABSTRACT

The plant *Brachymenium turgidum* is a small tufts of moss occurred on branches and trunks of trees with corticolous habitat as well as on moist forest ground surface. These plants found mostly in humid areas during rainy seasons where humidity is always higher. The plants generally slender, greenish brown tufts with erect stem. The turgid, sub-pendulous capsules are the key features of the thallus. Since time immemorial to man, the antimicrobial potential of the mosses known to the world especially against wounds, burns and skin infections. In the present investigations, certain preliminary phytochemical tests carried out to trace the presence of alkaloids, tannins, saponins, sterols, terpenoids, flavonoids, glycosides. The antibacterial sensitivity test was elicited out against about seven bacterial and three fungal pathogenic microorganisms. The plant powder extracted in polar and non-polar solvents like water, methanol, ethanol, petroleum ether, chloroform, and acetone to obtain different fractions. Antibacterial effect of these fractions was determined by disc diffusion method The results were compared with the standard antibiotic like, Tetracycline and Nystatin (10 µg/ml). The preliminary phytochemical analysis confirms the presence of alkaloids, flavonoids, glycosides and terpenoids as an important phyto constituent. The antimicrobial activity showed that most of the extract was sensitive to at least one microorganism by exhibiting significant zone of inhibition. Hence, potential antimicrobial activity recorded among the moss and can be found more pronounced in future advanced chemical characterization.

Key words: Moss, Phytochemistry and antimicrobial activity.

INTRODUCTION

Melghat is a prime biodiversity repository of Maharashtra state enriched with diverse forest cover of lower plants like bryophytes. Present study attempted to explore the bryophytic flora of Melghat region along with potential assessment of its *in vitro* antimicrobial screening with chemical analysis. They can grow as epiphytes on bark of trees (Corticolous), leaves (Folicolous), rocks (Rupicolous) on stones

and pebbles (Saxicolous), on fallen logs (Lignicolous), riverbanks and roadside cuts (Terricolous). Since water is inevitable for completing their life cycle, they are known as the "amphibians'' of the plant kingdom (Daniels and Kariyappa, 2007). Bryophytes make a significant contribution to the floral diversity of this "watery planet'' and since its inception constitute an important component of the forest ecosystem being the first colonizers on variety of habitats (Alam et al., 2011). Madsen and Pates (1952) reported first time antibiosis in moss *Sphagnum strictum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Belkin *et al.,* (1952) found that ethanolic extract of the moss *Polytrichum juniperum* possess antitumourogenic activity. McCleary and Walkington (1966) examined 50 species of mosses of which 18 showed strong antibacterial activity while 7 exhibited less but positive activity and rest 25 were inactive. Banerjee and Sen (1979) examined 52 species (40 genera) of the bryophyte for their antimicrobial activity. Out of those species, 29 were active against at least one of the test bacteria. The liverwort *Asterella sanguinea* and *Marchantia paleacea* and the moss *Brachythecium procumbens* showed broad spectrum of antimicrobial activity. Gupta and Singh (1971) have reported antibacterial activity of petroleum ether extracts of mosses *Barbula* and *Timella* against 33 species of bacterial pathogens. Singh *et al.,* (2007) reported antimicrobial activity of ethanolic extracts of 15 Indian mosses like *Sphagnum* sp., *Barbula* sp., *Brachythemium* sp., *Mnium* sp., *Entodon* sp. and found active against 12 micro-organisms. Bodade *et al.*, (2008) described *in-vitro* screening of bryophytes like *Plagiochasma* sp., *Thuidium* sp., *Bryum* sp. and *Rocomitrium* sp. for antimicrobial activity against 10 bacteria and 3 fungi. Ücüncü *et al.*, (2010) recorded antibacterial activity of Turkish moss *Tortula muralis* (Hedw.), *Homalothecium lutescens* (Hedw.), *Hypnum cupressiformae* (Hedw.) and *Pohlia nutans* (Hedw.) against 6 bacteria and 3 fungi. Elibol *et al.*, (2011) reported six Turkish acrocarpic mosses like *Syntrichia* sp., *Grimmia* sp., *Bryum* sp., *Tortella* sp., *Orthotrichum* sp. and *Pleurochaete* sp. showing antibacterial activity. Sharma *et al.,* (2013) reported the antimicrobial activity of the moss *Polytrichum commune* from the Solan region, Himachal Pradesh. against the microorganisms like *S. aureus* and *P. aeruginosa* with promising and significant results.

MATERIALS AND METHODS

The moss thalli collected during rainy season, cleaned carefully and washed under tap water followed by shade dried and powdered in blender. Using Soxhlet apparatus, the powdered samples of plant were extracted in ethanol, methanol, petroleum ether, chloroform and acetone and different solvent fractions were obtained. Dried extracts were stored in labeled sterile wide mouthed screw capped bottles at 4^0C and used for further study (Parekh and Chanda, 2008), (Banerjee and Sen, 1979), (Singh *et al.*, 2006).

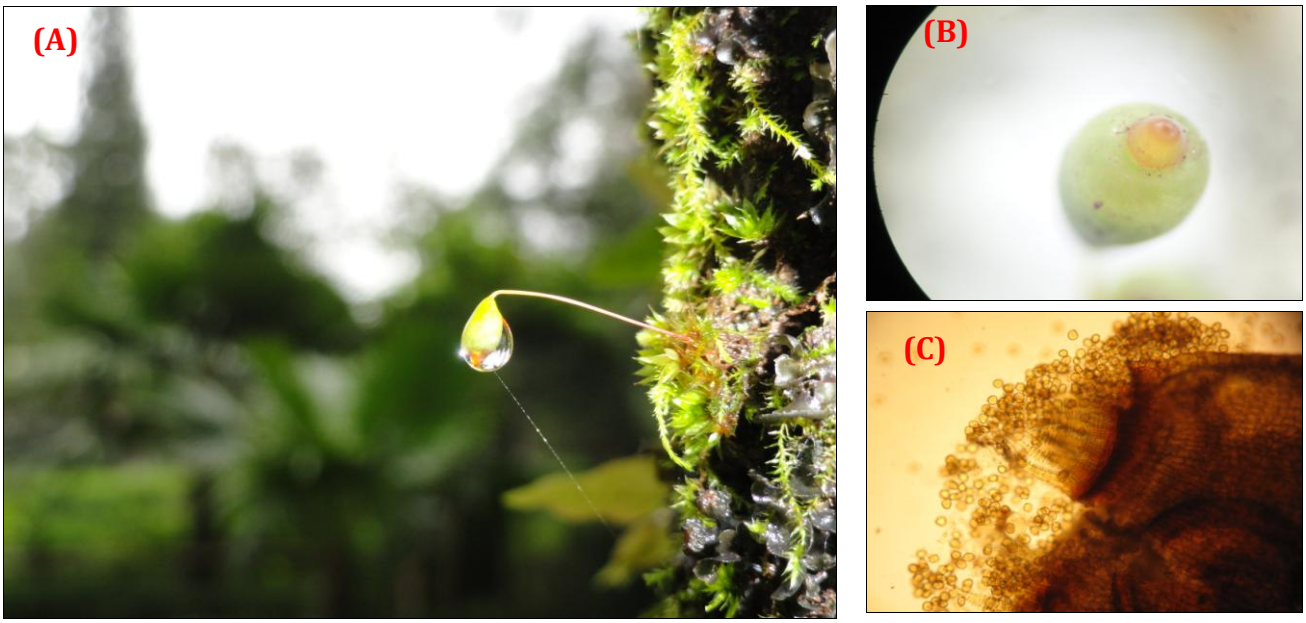

Fig.1: (A) *Brachymenium turgidum* with Sporophyte; **(B)** *B. turgidum* with operculum lid ; **(C)** *B. turgidum* with many spores

The standard pathogenic bacterial and fungal strain cultures procured from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in nutrient broth (Hi-media laboratories, Mumbai, India) at 37^{0}C for 18 hrs and then stored at 4^{0}C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Subcultures were prepared from the stock for bioassay. About 10 different pathogenic microorganisms including gram positive *Staphylococcus aureus* MTCC -96, and gram negative *Escherichia coli* MTCC -729, *Salmonella typhi* MTCC-98, *Klebsiella pneumoniae* MTCC -661, *Proteus vulgar* MTCC - 744, *Pseudomonas aeruginosa* MTCC – 424, and *Shigella flexneri* MTCC- 1457 along with fungus *Aspergillus niger* -343, *Candida albicans*-183, *Rhizopus oryzae*-284 were used with disc diffusion method (NCCLS, 1990). The zone of inhibitions also measured as diameter in mm; the experiment were carried out in triplicate and the averages diameter of zone of inhibition was recorded (Lalitha *et al.,* 1997). The results compared with the standard antibiotic like tetracycline and nystatin (10 µg/ml)

RESULTS AND DISCUSSION

Brachymenium turgidum a moss, extracts obtained in different solvents and tested against various pathogens showing positive results (Table 1.)

The aqueous extract of the plant in distilled water showed least interaction against *E. coli* and *S. aureus* and no activity against other bacterial or fungal test pathogens. The petroleum ether extract showed null effect against all the pathogens under interaction However, the ethanolic extract of the plant was found highly interactive with most of pathogens under study and exhibited sensitivity against microorganisms like *E. coli, P. vulgaris, K. pneumoniae, S. flexneri, S. aureus, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans.* No reaction noticed against the pathogenic fungi *R. oryzae.*

Table 1: Antimicrobial sensitivity test of *Brachymenium turgidum*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RA
Brachymenium turgidum	Aqueous	03	0	0	0	03	0	0	0	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	07	05	04	06	04	0	06	08	07	0
	Chloroform	05	04	06	0	06	0	0	07	06	0
	Methanol	04	05	03	0	06	04	0	04	05	0
	Acetone	0	0	04	03	0	05	06	07	06	0
	Tetracycline	27	24	23	21	22	26	27	-	-	-
	Nystatin	-	-	-	-	-	-	-	27	26	30

* Data represented in mean of three replicates.

***EC =** *Escherichia coli* [MTCC-729], **PV=** *Proteus vulgaris* [MTCC-744], **KP =** *Klebsiella pneumoniae* [MTCC-661], **SF =** *Shigella flexneri* [MTCC-1457], **SA=** *Staphylococcus aureus* [MTCC-96] **, PA=** *Pseudomonas aeruginosa* [MTCC-424], **ST =** *Salmonella typhimurium* [MTCC-98], **AN =** *Aspergillus niger* [MTCC-281] , **CA=** *Candida albicans* [MTCC-227], **RA=** *Rhizopus oryzae* [MTCC-554]

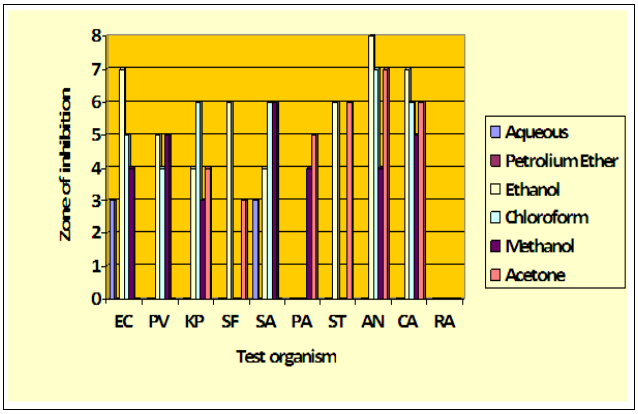

Fig 2: Analysis of antimicrobial sensitivity of *Brachymenium turgidum*

The dark green chloroform extract was found to be effective against the pathogens like *E. coli, P. vulgaris, K. pneumoniae, S. aureus A. niger* and *C. albicans* however, no interaction was noticed in pathogens like *S. flexneri, P. aeruginosa, S. typhimurium* and *R. oryzae.* Subsequently, the methanolic extract of the plant revealed promising results against microorganisms such as *E. coli, P. vulgaris, K. pneumoniae, S. aureus, P. aeruginosa, A. niger* and *C. albicans* while negative results were found against *S. flexneri, S. typhimurium* and *R. oryzae.* The acetone extract showed significant results against pathogen *K. pneumoniae, S. flexneri, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans*

while no specific interaction was observed against bacteria *E coli, P. vulgaris, S. aureus* and the fungus *R. oryzae* Bodade *et al*., (2008) Singh *et al*., (2007).

It is interested and intended to put the results of antimicrobial activity on the canvas of present investigation that during course of activity the aqueous and acetone extracts were less interactive as compared to the other extract like ethanol, chloroform and methanol. The test organism *Rhizopus oryzae* remained negative against all the extracts. The highest zone of inhibition of 8 mm was found in ethanol extract against fungus *Aspergillus niger* and least in aqueous extract against *É. coli* and *C. aureus* with 3 mm and above same as in acetone extract against *S. flexneri*.

CONCLUSION

The phenomenon of antibiosis reported to occur in many bryophytes even though they are at a lower level of evolution as compared to the higher plants. Hence, the occurrence of antimicrobial substances in the thalli of several bryophytes is a key attribute of these novel plants to establish as well as to compete on this earth. During present investigations, *Brachymenium turgidum* found sensitive to various microorganisms in different extracts. The bacterial organisms like *E. coli, P. aeruginosa, K. pneumoniae* and *S. aureus* found most reactive against various extracts of bryophytes. Moreover, the fungal pathogens like *Candida albicans* and *Aspergillus niger* found most reactive against all the extracts which were studied. Hence, it is concluded that all the plant extracts reacted to most of the gram-negative bacteria than gram-positive bacteria. It is observed that all the conventional drugs available today reacts more with gram-positive bacterial strains than gram-negative bacteria. These findings will open new avenues and provide insight to the prospects of medicinal world.

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RESEARCH ARTICLE

New species of genus *Asterina* (Asterinaceae) from Western Ghats, India

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ABSTRACT

The present paper deals with three new species of genus *Asterina* (Asterinaceae) belonging to black mildew fungi, collected on rarely occurring host plants from Mahabaleshwar, Maharashtra, India. These are, *Asterina beilschmiediae sp. nov.* on *Beilschmiedia dalzellii*, *A. hosagoudarii sp. nov.* on *Litsea josephii* and *A. oxyceri sp. nov.* on *Oxyceros rugulosus.* The detail morphological description, colour photographs, line drawings and discussions of these newly described species are provided in this paper.

Key words: Black mildews, fungi, Mahabaleshwar, Maharashtra, taxonomy.

INTRODUCTION

Asterinaceous fungi are inconspicuous, foliicolous, superficial, obligate parasites and host specific black mildews, mostly found in the tropical and subtropical regions of the world (Hosagoudar, 2012). These fungi are characterized by forming thin to dense black colonies on the surface of the host leaves and mycelium, with lateral appressoria forming intracellular haustoria; dimidiate thyriothecia dehisced stellately at the center, at their maturity; asci bitunicate, 4 to 8-spored; ascospores pale to dark brown, transversely uniseptate, smooth or finely ornamented (Hofmann and Piepenbring, 2008; Hosagoudar, 2012).

The genus *Asterina* Lev. is the largest genus of the family Asterinaceae, comprising more than 578 species (Hosagoudar and Abraham, 2000; Hosagoudar, 2012). The literature survey revealed that, the rich diversity of *Asterina* spp. was found on the host of angiosperms families like, Lauraceae (ca. 20 spp.) and Rubiaceae (ca. 25 spp.) from the tropical and subtropical regions of the world (Hosagoudar and

Abraham, 2000; Hosagoudar, 2012; Far and Rossman, 2014). During the exploration of black mildew fungi from Mahabaleshwar and its surrounding area, three undescribed species of *Asterina* are recorded on rarely occurring host plants; of which, *Asterina beilschmiediae sp. nov.* on *Beilschmiedia dalzellii* (Meissn.) Kosterm., *A. hosagoudarii sp. nov.* on *Litsea josephii* S.M. Almeida (= *L. stocksii* (Meissn.) Hook. *f*) from family Lauraceae and *A. oxyceri sp. nov.* on *Oxyceros rugulosus* (Thw.) Tirveng. from family Rubiaceae, are reported here as new species.

MATERIALS AND METHODS

The leaves and twigs of host plants, infected with black mildews were collected during several field trips from study area during winter season (2013–2014). Host plants were identified using the regional flora (Deshpande *et al*., 1995). The specimens were air-dried and preserved in standard size herbarium packets. Both macro and micro-morphological characters are used for taxonomical studies of collected fungi. Microscopic preparations were made in lactophenol, stained with cotton blue and observed under compound light microscope. To study the entire colony, mycelial branching and position of appressoria in its natural condition, a drop of peeling solution (Xylene–Thermocol solution) was applied on selected colonies, and after drying, the film was mounted directly again in the same solution (Bhise *et al*. 2014). Biometric data were based on the minimum to maximum values of 20 measurements of micro-morphological structures; illustrations were prepared with a mirror type Camera Lucida and photographed under Leica DM2000 fluorescence microscope, equipped with digital camera. The fungal specimens were identified by using standard literature (Stevens and Ryan, 1939; Hosagoudar and Abraham, 2000; Hosagoudar, 2012; Far and Rossman, 2014). Type specimens were deposited in Herbarium Cryptogamae Indiae Orientalis (HCIO), IARI, New Delhi (India) for their accession and the detail description and illustration of each newly described species were deposited in MycoBank. The detail taxonomic description, colour photographs, line drawings, comparative account and discussion of each new species are provided in the present paper.

RESULTS AND DISCUSSION

Taxonomy

1. *Asterina beilschmiediae* Bhise, Patil and Salunkhe, *sp. nov.* (Fig. 1)

MycoBank No. MB811704

Type: India, Maharashtra: Mahabaleshwar, on living leaves of *Beilschmiedia dalzellii* (Lauraceae), 17°55'25.6"N, 73°38'16.5"E, elev. 1289 m, 05.02.2014, Bhise M.R., HCIO 51713 (holotype).

***Etymology*:** The specific epithet is based on the host plant genus.

Colonies hypophyllous, dark black, thin, circular to spreading, isolated, up to 10 mm in diameter. Hyphae dark brown, substraight to flexuous, thin, branching opposite to alternate at wide angles, loosely reticulate; cells 21–35 × 6 µm in size. Appressoria alternate to unilateral, moderately placed, unicellular, angular to irregularly lobed, entire, 11–13 × 13–15 µm. Thyriothecia scattered, globose to orbicular, stellately dehisced at the center, inner content not so yellow, up to 217 µm in diameter, margin fimbriate with fringed hyphae. Asci numerous, initially globose, subglobose to ovate at maturity, bitunicate, 8–spored, 54–72 × 45–50 µm. Ascospores oblong, conglobate, uniseptate, constricted at the septum, cells equal, globose at both ends, olivaceous brown, 32–39 × 14–16 µm, wall prominently echinulate.

***Habitat and Distribution*:** Inhabiting living leaves of *Beilschmiedia dalzellii*, a rarely occurring plant in evergreen and semi-evergreen forests along the ghats at Mahabaleshwar, Maharashtra, India.

Notes: About 20 species of *Asterina* have been described on the members of family Lauraceae from the world (Hosagoudar and Abraham, 2000; Hosagoudar, 2012; Far and Rossman, 2014). The present new species can be compared with earlier described species of *Asterina* viz. *A. cinnamomicola* Hansf., *A. munnarensis* Hosag., *A. neolitsiicola* Hosag., C.K. Biju & Abraham, *A. cryptocariicola* Hosag., C.K. Biju & Abraham, *A. litseae* Yates and *A. litseae-ligustrinae* Hosag., Balakr. & Goos known from Australia, India, Philippines and Sri Lanka on hosts of Lauraceae, based on the characters having alternate, unicellular, angular to lobate appressoria and smooth walled to echinulate

ascospores. After comparison, it is revealed that, the present collection differs from related species (*A. munnarensis*, *A. neolitsiicola* and *A. litseae-ligustrinae*) in having thin, hypophyllous, large size colonies with distinctly irregularly lobed appressoria and prominently echinulate ascospores; as well as, larger size of hyphal cells, appressoria, thyriothecia, asci and ascospores. Therefore, based on the host specificity and above distinguishing characters, it is treated as new species. Comparisons between the new species and its morphologically similar species are shown in Table 1.

Table 1: Comparative account of *A. beilschmiediae*, *A. munnarensis*, *A. neolitsiicola* and *A. litseae-ligustrinae*

Sr. No.	Morpho-taxonomic characters	*Asterina beilschmiediae*	*Asterina munnarensis*	*Asterina neolitsiicola*	*Asterina litseae-ligustrinae*
1.	Host Plant	*Beilschmiedia dalzellii*	*Cinnamomum* spp.	*Neolitsea* spp.	*Litsea ligustrina*
2.	Colonies	Hypophyllous, thin, up to 10 mm in diam.	Hypophyllous, thin to subdense, up to 10 mm in diam.	Hypophyllous, thin to subdense, up to 10 mm in diam.	Hypophyllous, up to 5 mm in diam.
3.	Hyphae	Substraight to flexuous, cells 21–35 × 6 µm	Straight to substraight, cells 12–20 × 2–3 µm	Flexuous to crooked, cells 12–18 × 3–5 µm	Straight to substraight, cells 15 – 22 × 3 – 5 µm
4.	Appressoria	Alternate to unilateral, unicellular, angular to irregularly lobed, 11–13× 13–15 µm	Alternate, unicellular, angular to sublobate, 8–12× 7–10 µm	Alternate, unicellular, globose to sublobate, 7–13× 7–8 µm	Alternate to about 15% opposite, unicellular, conoid to sublobate, 9 – 13 × 6 – 10 µm
5.	Thyriothecia	Inner content not so yellow, up to 217 µm in diam.	Inner content golden yellow, up to 65 µm in diam.	Up to 75 µm in diam.	Inner content deep yellow, up to 110 µm in diam.
6.	Asci	Globose to ovate, 54–72 × 45–50 µm	Globose, up to 30 µm in diam.	Globose,12–18 µm in diam.	Globose, 24 – 26 µm in diam.
7.	Ascospores	Conglobate, wall strongly echinulate, 32–39 × 14–16 µm	Conglobate, wall tuberculate, 17–20 × 7–8 µm	Conglobate, wall verruculose, 17–25 × 7–10 µm	Conglobate, wall echinulate, 18–19 × 6–10µm,

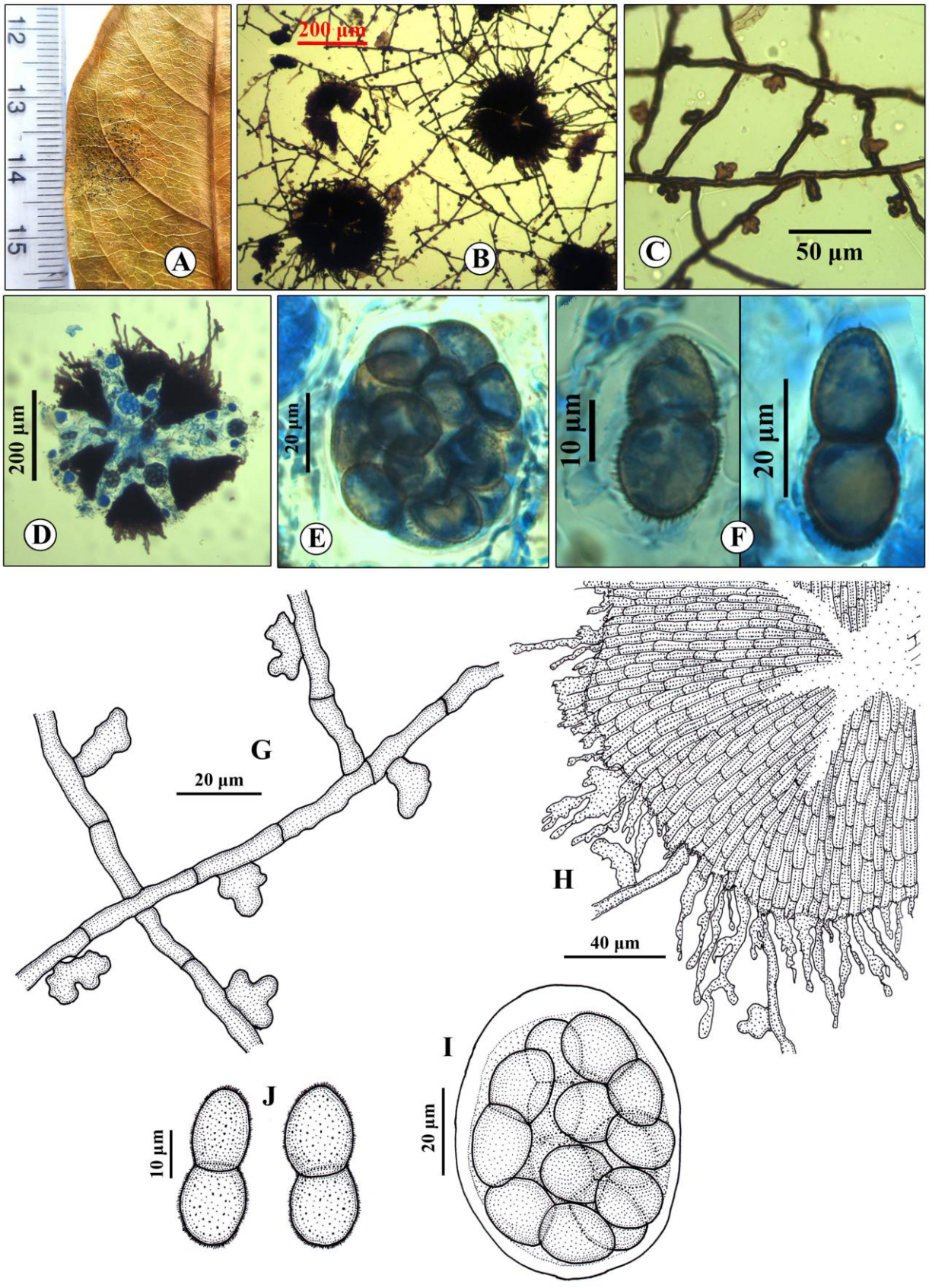

Fig. 1: ***Asterina beilschmiediae*** (holotype). A. Infected leaves; B. Mycelial colony with thyriothecia; C, G. Appressoriate mycelium; D, H. Thyriothecium; E, I. Ascus; F, J. Ascospores.

2. *Asterina hosagoudarii* Bhise, Patil and Salunkhe, *sp. nov.* (Fig. 2)

MycoBank No. MB811705

Type: India, Maharashtra: Mahabaleshwar, Gureghar, on living leaves of *Litsea josephii* (Lauraceae), 17^{0}55'19.23"N, 73^{0}44'22.79"E, elev. 1284m, 19.11.2012, Bhise M.R. HCIO 51658 (holotype); HCIO 51657 (Isotype).

Etymology: Named after Dr. V. B. Hosagoudar, having major contribution in Asterinaceous fungi from India.

Colonies epiphyllous, dark black brown, thin, spreading on entire leaf surface. Hyphae pale brown, straight to undulate, branching opposite to alternate at wide angles, closely reticulate, wall slightly undulate; cells 10 – 23 × 3 – 5 µm in size. Appressoria opposite to alternate, mostly opposite, distantly arranged, unicellular, irregularly lobed to angular, mostly trilobed, 9 – 12 × 7 – 10 µm in size. Thyriothecia scattered, globose to orbicular, stellately dehisced at the center, margin fimbriate with fringed hyphae, inner content not so yellow, up to 168 µm in diam. Asci globose, subglobose to ovate, 4 to 6 in each thyriothecia, 8-spored, 25 – 36 × 20 – 33 µm in size. Ascospores oblong, conglobate, olivaceous brown, 1-septate, constricted at the septum, cells more or less equal, 20 – 23 × 9 – 11 µm, wall smooth.

***Habitat and Distribution*:** Inhabiting living leaves of *Litsea josephii* (Lauraceae) rarely occurring plant in evergreen and semi-evergreen forests along ghats at Mahabaleshwar, Gureghar, Maharashtra, India.

Notes: About 20 species of *Asterina* have been described on the members of family Lauraceae from the world (Hosagoudar and Abraham, 2000; Hosagoudar, 2012; Far and Rossman, 2014). The present new species can be compared with earlier described species *Asterina litseae* Yates and *A. litseae-ligustrinae* Hosag., Balakr. & Goos reported on *Litsea deccanensis* and *L. ligustrina* respectively, from Nilgiris, Tamil Nadu, India (Hosagoudar, 2012), based on the characters having straight to undulate hyphae and alternate to opposite, unicellular, angular to lobate appressoria. However, the present new species differs from former species in having colonies epiphyllous only, appressoria mostly opposite, irregularly lobed or angular, inner content of thyriothecia not so yellow and smooth walled ascospores; also, larger size of thyriothecia, asci and ascospores. Hence, the present species is treated as new to science. Comparisons between the new species and its morphologically similar species are shown in Table 2.

The present species found to be associated with *Meliola litseae* Syd.

Table 2: Comparative account of *Asterina hosagoudarii, A. litseae* and *A. litseae-ligustrinae*

Sr. No.	Morpho–taxonomic characters	*Asterina hosagoudarii*	*A. litseae*	*A. litseae-ligustrinae*
1.	Host Plant	*Litsea josephii* (*L. stocksii*)	*L. deccanensis*	*L. ligustrina*
2.	Colonies	Epiphyllous, spreading on entire leaf.	Amphigenous, up to 5 mm diam.	Hypophyllous, up to 5 mm diam., rarely confluent.
3.	Hyphae	Straight to undulate, cells 10 – 23 × 3 – 5µm	Straight to substraight, cells 12 – 22 × 3 – 5 µm	Straight to substraight, cells 15 – 22 × 3 – 5 µm
4.	Appressoria	Mostly opposite, irregularly lobed to angular, 9 – 12 × 7 – 10µm	Alternate, conoid to ampulliform, 6 – 13 × 5 – 7 µm	Alternate, entire to variously sublobate, 9 – 13 × 6 – 10 µm
5.	Thyriothecia	Inner content not so yellow, up to 168 µm in diam.	Inner content deep yellow, up to 125 µm in diam.	Inner content deep yellow, up to 110 µm in diam.
6.	Asci	Globose to ovate, 25 – 36 × 20 – 33 µm	Globose, 18 – 25 µm in Diam.	Globose, 24 – 26 µm in Diam.
7.	Ascospores	20 – 23 × 9 – 11µm, wall smooth	18 – 19 × 8 – 10 µm, wall smooth	18 – 19 × 6 – 10µm, wall echinulate

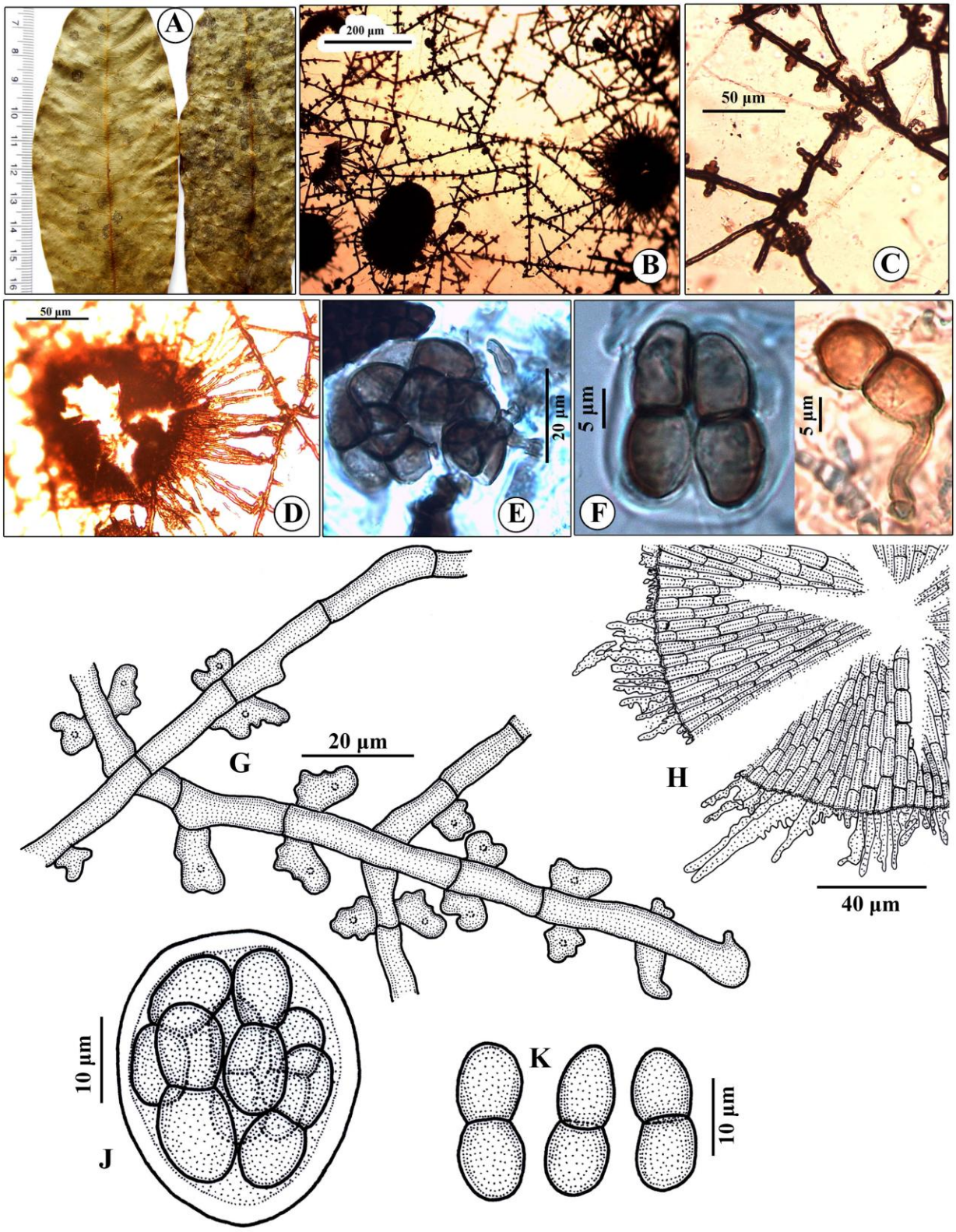

Fig. 2: ***Asterina hosagoudarii*** (holotype). A. Infected leaves; B. Mycelial colony with thyriothecia; C, G. Appressoriate mycelium; D, H. Thyriothecium; E, J. Ascus; F, K. Ascospores.

3. *Asterina oxyceri* Bhise, Patil and Salunkhe, *sp. nov.* (Fig. 3)

MycoBank No. MB811706

Type: India, Maharashtra: Mahabaleshwar, Hatlote, on living leaves of *Oxyceros rugulosus*, 17°51'43.6"N, 73°35'33.8"E, elev. 742 m, 06.02.2014, Bhise M.R., HCIO 51719 (holotype).

***Etymology*:** The specific epithet is based on the host plant genus.

Colonies amphigenous, brownish black, thin, circular to spreading, rarely confluent, up to 5 mm in diameter. Hyphae dark black to brown, straight to substraight, branching opposite to alternate at acute to wide angles, loosely reticulate; cells 25–32 × 6–8 µm in size. Appressoria opposite to rarely subopposite, closely arranged, antrorse to subantrorse, straight to curved, unicellular, conoid to obclavate, entire, 11–14 × 7 µm. Thyriothecia scattered, globose to orbicular, stellately dehisced at the center, up to 342 µm in diameter, margin fimbriate with fringed hyphae. Asci few, initially globose, subglobose to ovate at maturity, 8–spored, 50–70 × 36–46 µm. Ascospores oblong, conglobate, olivaceous brown, uniseptate, constricted at the septum, cells equal, 31–34 × 12–14 µm, smooth walled.

***Habitat and Distribution*:** Inhabiting living leaves of *Oxyceros rugulosus*, a rarely occurring plant in semi-evergreen and moist deciduous forests at Hatlote, Mahabaleshwar, Maharashtra, India.

Notes: About 25 species of *Asterina* have been described on the members of family Rubiaceae from the world (Hosagoudar and Abraham, 2000; Far and Rossman, 2014; Hosagoudar, 2012). The literature survey revealed that, the present species is close to *A. canthii* Yates, *A. canthii-dicocci* Hosag. and *A. psychotriicola* Hosag. & Archana described from Philippines and India (Stevens and Ryan, 1939; Hosagoudar, 2012), based on the characters in having opposite to alternate and unicellular appressoria. However, *Asterina oxyceri* is differs from the related species in having opposite to rarely subopposite, conoid to obclavate appressoria; larger size of thyriothecia, asci and ascospores. Therefore, based on the host specificity and above distinguishing characters it is treated as new species. Comparisons between the new species and its morphologically similar species are shown in Table 3.

Table 3: Comparative account of *Asterina oxyceri*, *A. canthii*, *A. canthii-dicocci* and *A. psychotriicola*

Sr. No	Morpho-taxonomic characters	*Asterina oxyceri*	*A. canthii*	*A. canthii-dicocci*	*A. psychotriicola*
1.	Host Plant	*Oxyceros rugulosus*	*Canthium* sp.	*Canthium dicoccum*	*Psychotria* sp.
2.	Colonies	Amphigenous, thin, up to 5 mm in diam.	Amphigenous	Amphigenous, up to 2 mm in diam.	Epiphyllous, up to 3 mm in diam.
3.	Hyphae	Straight to substraight, cells 25–32 × 6–8 µm	Straight, cells 12–20 × 2–3 µm	Straight, cells 32–36 × 5–7 µm	Flexuous, cells 9–35 × 3–5 µm
4.	Appressoria	Opposite to rarely subopposite, unicellular, conoid to obclavate, 11–14 × 7 µm	Opposite, unicellular, oblong to cylindrical, 4–5× 5 µm	Alternate, ovate, oblong, cylindrical, often attenuated at the apex, 11–16 × 8–10 µm	Alternate, ovate to clavate, often attenuated at the apex, 8–13 × 6–11 µm
5.	Thyriothecia	Up to 342 µm in diam.	Up to 175 µm in diam.	Up to 160 µm in diam.	Up to 400 µm in diam.
6.	Asci	Globose to ovate, 50–70 × 36–46 µm	Globose, 30–40 × 8–10 µm	Globose, up to 35 µm in diam.	Globose, up to 30 µm in diam.
7.	Ascospores	Conglobate, wall smooth, 31–34 × 12–14 µm	Conglobate, wall smooth, 10–12 × 3–4 µm	Conglobate, wall smooth, 20–22 ×11–13 µm	Conglobate, wall strongly tuberculate, 20–24 ×9–13 µm

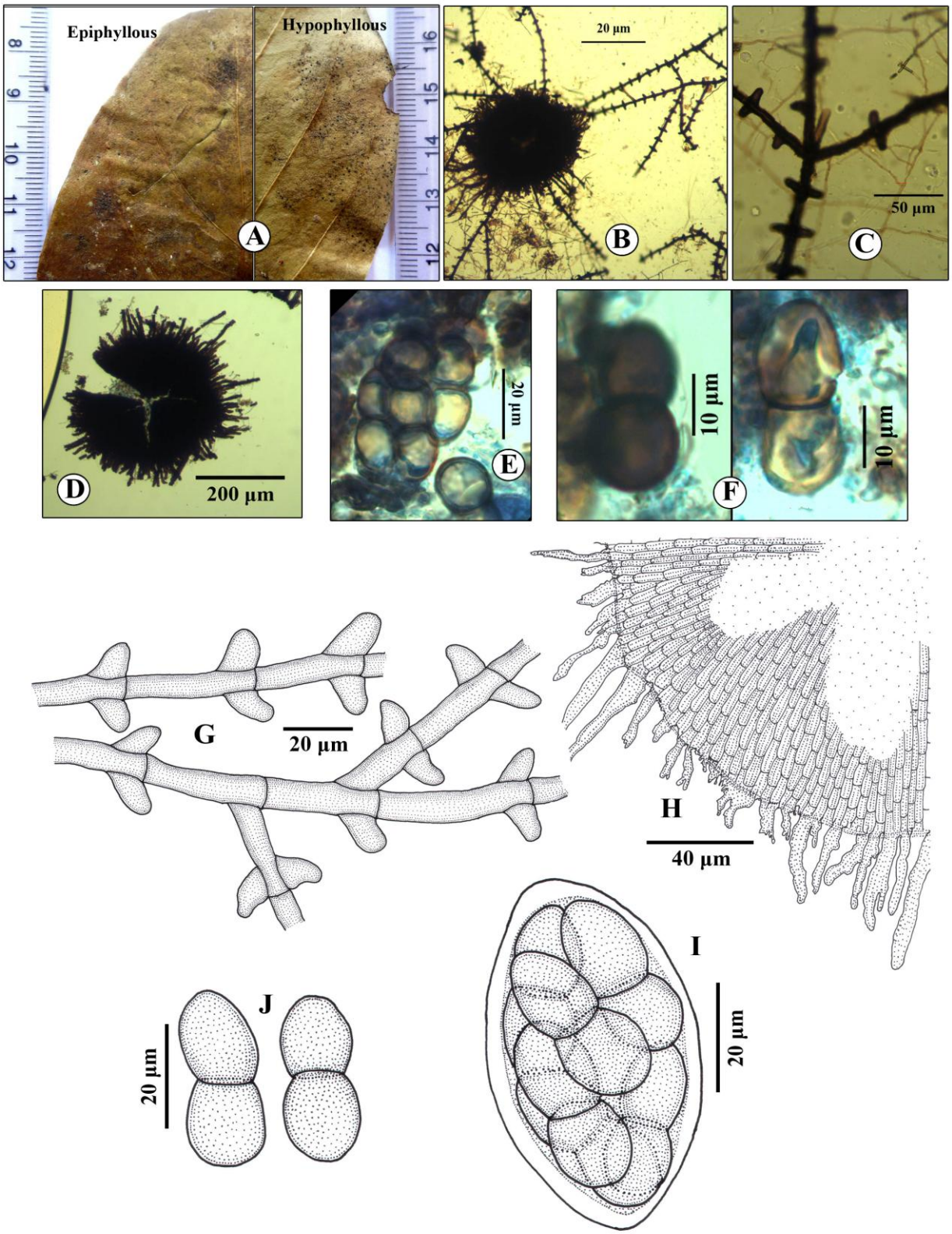

Fig. 3: ***Asterina oxyceri*** (holotype). A. Infected leaves; B. Colony with thyriothecia; C, G. Appressoriate mycelium; D, H. Thyriothecium; E, I. Ascus; F, J. Ascospores.

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RESEARCH ARTICLE

Quantitative analysis of diversity during seasonal variations of Sanjay Gandhi National Park (SGNP) by Quadrat Method

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ABSTRACT

The Study intends to identify changes in biotic factors, especially in floristic diversity during different seasons with help of quadrat study in Shilonda trail of SGNP that is protected by Park Management. The study further aims at drawing a conclusion in terms of differences in Floristic Diversity of the Site calculated by Simpson's Reciprocal Index and Shannon – Weiner Index during monsoon, post – monsoon and winter seasons. On calculation, Simpson's Reciprocal Index for the Site during monsoon was estimated to be 29.82 while Shannon – Weiner Index was recorded to be 40.253. During post – monsoon the diversity expressed by the Simpson's Reciprocal Index was 29.1 and 38.0 by Shannon – Weiner Index. Similarly for winter, Simpson's Reciprocal Index was calculated to be 12.75 and Shannon – Weiner Index was 14.839 Through the study it has been observed that Shilonda Trail houses species identified under IUCN Red Data List of Threatened Plants, namely, Gloriosa superba L.; enlisted as threatened. Dipcadi saxorum Blatt. and Chlorophytum borivilianum Santapau & R.R.Fern., both endemic and endangered species have also been recorded in the Site, during the monsoon season. It can be concluded that seasonal variations have a profound effect on species and genetic diversity of the Site under study.

Keywords: Seasonal variations, quadrat study, diversity, Shannon–Weiner Index (eH'), Simpson's Reciprocal Index, IUCN Red Data List.

INTRODUCTION

Sanjay Gandhi National Park (SGNP) also popularly known as the Borivali National Park is encircled by the Thane City and Mumbai Metropolis. It is divided into an outer recreational zone, a buffer zone

and a core zone. SGNP is an example of one of the least represented biographic zones – the Malabar Coast of the Western Ghats which forms only 0.4% of the Protected Area network. The official area of the park has expanded by five times since pre – independence era and presently covers around 104 sq. km., housing many rare, threatened and beautiful species (Monga, 2000).

Since the vegetation pattern and diversity depends majorly on the climatic conditions of the area, the study intended to identify the changes in the biotic factors, especially in the floristic diversity during different seasons with an objective to quantitatively estimate diversity by applying quadrat sampling and further analyzing data by employing various diversity indices. Differences hence achieved by use of indices will aid in developing comparisons between seasonal variations in the chosen site.

MATERIALS AND METHODS

Some areas inside the park at the Borivali entrance are manned by park officials who restrict the entry of visitors without appropriate permission from the Nature Information Center one of which is the Shilonda trail and access to this part of the park is strictly for educational and research purpose. To get complete representation of flora in different seasons, quadrats were laid in three different seasons viz., Monsoons: June to August; Post Monsoon: October to November and Winter: January to March. 30 quadrats of 1m x 1m were laid along the Shilonda trail during the three seasons. Flora was identified with aid of the Bombay Natural History Society (BNHS), Agharkar Research Institute (Pune) and Blatter Herbaria. The values of flora obtained from quadrats and tally marks were further expressed by use of indices like Relative Frequency, Relative Density, Relative Abundance, Index of Dominance and diversity indices like the Simpson's Reciprocal Index, the Shannon – Weiner Index, Species Richness and Species Evenness.

***Simpson's Reciprocal Index*:**

In Simpson's Diversity Index, value of **D** (Diversity) ranges from 0 to 1. With this index, 0 represents infinite diversity and 1 represents no diversity, i.e., the bigger the value the lower the diversity. Since values obtained by Simpson's Diversity Index would not appropriately represent the raw data for comparative analysis of disturbed and undisturbed habitats, floral diversity was quantified using *Simpson's Reciprocal Index*. In Simpson's Reciprocal Index, a high value of D suggests a stable ecosystem and a low value of D suggests degraded ecosystem or habitat (Rutherford, 2009).

***Shannon – Weiner Index*:**

The uniqueness of floral species in sampled areas was incorporated by using index of evenness (equitability) called *Shannon's Index* or *Shannon – Weiner Index.* Main objective of the Index is to try to measure the amount of *order* (or disorder) contained in a system (Margalef, 1958). The index provides a measure of the amount of disorder in a system, such that communities with more unique species have higher H while system with lower H may be perfectly ordered but has no diversity (Bradshaw & Brook, 2010).

***Species Richness (SR)*:**

This is the oldest and simplest concept of species diversity i.e., number of species in the community or region. McIntosh (1967) coined the name species richness to describe this concept. It is therefore the base currency used for most biodiversity assessments (Krebs, 2013).

***Evenness Index*:**

It measures the relative abundance of various populations present in an ecosystem. A community in which each species is equally abundant has high evenness; a community in which species differ widely in abundance has low evenness (Heip Carlos et al., 1998).

Relative Frequency (RF), Relative Density (RD) and Relative Abundancewere calculated for each plant species using the respective the following formulae (Misra, 1968; Ambasht*et* al,, 1984; Dalvi *et al.*, 2012):

Data obtained from transects was further quantified for dominance by use of *Index of Dominance*. The relation between diversity and dominance lies in the fact that low dominance indicates high diversity whereas high dominance indicates low diversity (Bradshaw & Brook, 2010).

RESULTS AND DISCUSSION

Monsoon Season (June to August):

The monsoon season is marked by the growth and proliferation of a myriad of diverse floral species. Water is a key and also a limiting factor that regulates the growth and development of plants. Hence, the onset of rains in Mumbai in June, results in a significant increase in the floristic diversity of the park. From the values obtained after quantitative analysis by quadrat study during the monsoon season, it has been observed that the *Geissaspis cristata Wight & Arn.* showed the highest values of RD, RF and RA, which were at 8.1855, 4.6812 and 0.0819. *Smithia sensitiva Aiton* also showed high values of RD calculated to be 6.9944, RF to be 4.000 and RA to be 0.0699. The species which showed the lowest distribution in the site were *Tirchosanthes cucumerina L.*, *Aeschynomene indica L.* and *Pedilanthus tithymaloides (L.) Poit.*. The RD, RF and RA values calculated for all the three species were 0.0253, 0.0145 and 0.0003 respectively. Also species like *Breynia retusa (Dennst.) Alston*, *Gloriosa superba L.*, *Lindernia crustacea (L.) F.Muell.* and *Smilax ovalifolia Roxb. ex D.Don* were species with lower RD, RF and RA values as compared to other species except. The values of RD, RF and RA for these species were recorded to be 0.0507, 0.0290 and 0.0005 respectively**.**

Post – monsoon Season (October to November):

The monsoon season is followed by a period of warmer climate during the post – monsoon months of October to November. This results in decline of the species diversity and abundance due to fall in the favorable conditions that follow post the rains. From the values obtained, it was noticed that *Neuracanthus sphaerostachys* was recorded with the highest values of RD, RF and RA, which were at 6.5808, 2.5536 and 0.0658. *Alternanthera sessilis (L) R.Br. ex Dc.* also showed high values of RD calculated to be 5.0621, RF to be 1.9643 and RA to be 0.0506. The species which showed the lowest distribution were *Gloriosa superba L., Lindernia crustacea (L.) F.Muell.* and *Pedilanthus tithymaloides (L.) Poit.*. The RD, RF and RA values calculated for all the three species were 0.0460, 0.0179 and 0.0005 respectively. Also species like *Carrisa congesta*, *Hemidesmus indicus (L) R.Br. ex Schult.*, *Eupatorium odorantum, Breynia retusa (Dennst.) Alston* and *Smilax ovalifolia Roxb. ex D.Don* were species with lower RD, RF and RA values as compared to other species except for the former. The values of RD, RF and RA for these species were 0.0920, 0.0357 and 0.0009 respectively.

Winter (January to March):

The season of winter is the most unfavorable season for the growth and development of plants as an important factor like water is a scarce resource during this time. Due to this, there is a drastic shift of diversity of species from being the maximum during the monsoons, gradually declining through the post – monsoons and further declining during winter. *Urena lobata L.* had been recorded as the species with the highest values of RD, RF and RA. The values of the three parameters for the species were calculated to be 14.9068, 2.2857 and 0.1491 respectively. *Sida acuta Burm.f.* was yet another species found to have high values of RD, RF and RA, viz., 11.8012, 1.8095 and 0.1180 respectively. On the other hand species like *Eranthemum roseum*, *Breynia retusa (Dennst.) Alston* and *Smilax ovalifolia Roxb. ex D.Don* had the lowest and identical RD, RF and RA values which when calculated were 0.6211, 0.0952 and 0.0062 in that order . *Achyranthes aspera L.* was yet another species with low distribution with RD of 0.9317, 0.1429 and 0.0093.

During monsoon, the Shilonda Trail, regarded as an area of high species and genetic diversity illustrated an astounding 69 species of plants belonging to 33 families. Some species that have been noted in the IUCN Red Data list of Threatened Plants have been observed in this trail, namely, *Gloriosa superba L.;* enlisted as threatened and *Dipcadi saxorum Blatt.* and *Chlorophytum borivilianum Santapau & R.R.Fern.,* both endemic and endangered species have been recorded only during the monsoon in this site. During the post monsoon season the abundance of vegetation differed as compared to that found during the monsoon season where 56 species belonging to 29 families were recorded at the Site. On analyzing the data obtained during the winter season i.e. between January and March, it can be observed that number of species have fairly declined at the Site, i.e. only 21 species belonging to 13 families were recorded. For the sake of convenience the values of the important species and of significant values are discussed above.

Index of Dominance, Species Richness and Evenness:

The values of various parameters are represented in Table 1 and Fig 1.

Table 1:Species Dominance, Richness and Evenness recorded at the Shilonda Trail during monsoon, post-monsoon and winter season by quadrat method.

Season	Index of dominance	Species Richness	Species Evenness
Monsoon	0.0338	4.2341	0.8727
Post – monsoon	0.031	4.0254	0.9037
Winter	0.0813	3.0445	0.8859

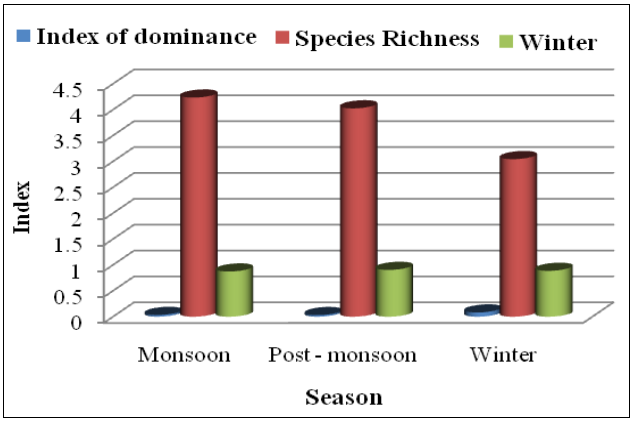

Fig. 1: Presentation of calculated values of indices for species recorded at the Shilonda Trail during the three seasons by quadrat method.

Diversity measurements such as Index of Dominance, Species Richness and Species Evenness form an integral part in the study of biodiversity of an area. The relation between Index of Dominance and biodiversity lies in the fact that an area with *low dominance indicates high diversity while that with high dominance will have less diversity*. As for the diversity indices calculated for the Site, the Index of Dominance was computed to be 0.0338. The Site showed a Species Richness of 4.2341 and Species Evenness of 0.8727. As for the diversity indices calculated during the post – monsoon season, the Index of Dominance was computed to be 0.031. The Site showed a Species Richness of 4.0254 and Species Evenness of 0.9037. The diversity index studies during the winter season showed that the Index of Dominance was 0.0813, while Species Richness and Species Evenness were recorded to be 3.0445 and 0.8859 respectively.

Diversity Indices:

The various indices calculated during the study are represented in Table 2 and Fig 2.

Table 2 : Diversity indices for species recorded at the Shilonda Trail during monsoon, post-monsoon and winter season by quadrat method

Season	Shannon – Weiner Index	Shannon – Weiner Index as eH'	Simpson's Reciprocal Index
Monsoon	3.6952	40.253	29.82
Post – monsoon	3.6376	38.0006	29.1
Winter	2.6972	14.8385	12.75

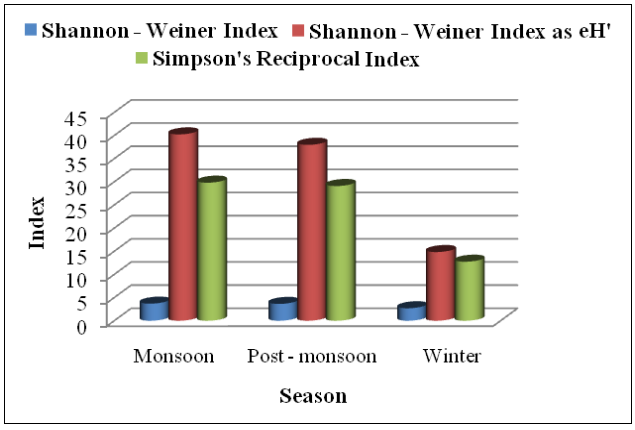

Fig.2: Presentation of calculated values of diversity indices for species recorded at the Shilonda Trail during the three seasons by quadrat method

During the monsoon season, the Shannon Weiner Index is 3.6952 and when expressed as eH' was at 40.2530, while the Simpson's Reciprocal Index was at 29.82. The Shannon Weiner Index during the post – monsoon season was recorded to be 3.6376 and when expressed as eH' was at 38.0006, whereas the Simpson's Reciprocal Index was at 29.1. While the diversity indices during the winter were recorded as: The Shannon – Weiner Index and as expresses in eH' were calculated to be 2.6972 and 14.8385. The Simpson's Reciprocal Index showed a value of 12.75.

CONCLUSION

Fig.1 and 2 clearly depict how seasonal variations have a proportional and exponential change in growth and development of flora. During monsoon, the Shilonda

Trail, regarded as an area of high species and genetic diversity illustrated an astounding 69 species of plants belonging to 33 families. Some species that have been noted in the IUCN Red Data list of Threatened Plants have been observed in this trail, namely, *Gloriosa superba L.;* enlisted as threatened and *Dipcadi saxorum Blatt.* and *Chlorophytum borivilianum Santapau & R.R.Fern.,* both endemic and endangered species have been recorded only during the monsoon in this site. During the post monsoon season the abundance of vegetation differed as compared to that found during the monsoon season where 56 species belonging to 29 families were recorded at the Site. On analyzing the data obtained during the winter season i.e. between January and March, it can be observed that number of species have fairly declined at the Site, i.e. only 21 species belonging to 13 families were recorded. Thus, it can be reiterated that the diversity of the park depends on seasons and hence variations in species composition with variation in season can be observed from Figures 1 and 2. In conclusion, therefore, the protection of the Shilonda Trail is vital for conservation of heterogeneity and the inimitable flora thriving in it. Furthermore, the diversity has immensely changed from the monsoon season to the winter season, marking the fact that seasonal and climatic conditions have a profound effect on the diversity of the area.

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RESEARCH ARTICLE

Response of Black gram *Vigna mungo* (L.Hepper) to Biofertilizer

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ABSTRACT

Biofertilizers are commonly called microbial inoculants which are capable of stoping important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. For the last one-decade, biofertilizers are used in large quantity as an eco-friendly approach to reduce the use of chemical fertilizers, improve soil fertility status and for improvement of crop production by their biological activity in the rhizosphere. The seeds of Vigna mungo were treated with bio-fertilizers for 45 days as compared to untreated. It was observed that the plants treated with experimental bio-fertilizer *Rhizobium* showed excellent result in the morphological and bio-chemical parameters.

Key Words- Biofertilizer, *Rhizobium japonicum, Vigna mungo* (L.hepper).

INTRODUCTION

Biofertilizer like *Rhizobium, Azotobacter, Azospirillum and blue green algae (BGA)* are in use since long time ago. *Rhizobium* inoculants is used for leguminous crops.azotobactor is used on crops like wheat, maize, mustard, cotton, potato and other vegetable crops. Azospirillum inoculants are recommended mainly for sorghum, millets, maize, sugarcane and wheat. Nostoc genera represents blue green algae, while the atmospheric nitrogen are fixed by *Anabaena, Tolypothrix and Aulosira* and these are used for the growth of paddy crop of both upland and lowland condition. Water fern azolla is associated with anabaena, it helps in contributing 60kg/ha/season ans its usefull in enriching soil with organic matter and bacteria which are usefull so-called phosphate solubilizing bacteria like Pantoea agglomerans strain P5 and Pseudomonas putida strain P13. These bacteria solubalize the insoluble phosphate source. Some phosphates are not mobile due to mineral ions such as Fe, Al and Ca or organic acids, the rate of available phosphate (Pi) in soil is well below plant needs.

Biofertilizers are mostly named as microbial inoculants which are capable of controlling some of the nutritional element in the soil from useless to usefull by the crop plant from their biological processes. Since many years biofertilizers are used as in large amount as an eco-friendly process which has reduced the use of chemical fertilizer. This improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere.Vast researches were carried out on the use of bacteria, (*Azotobacter, Azospirillum, Rhizobium, phosphobacteria*) vam fungi as biofertilizers supply nitrogen and phosphorous improves the growth of several crop plants was observed (Marwaha, 1995). Dual inoculation of VAM and bacteria biofertilizers proved more effective in increasing the growth of different crop plants (Panwar, 1993). In recent years, biofertilizers have started on large scale as a promising component of integrating nutrient supply system in agriculture. Our whole system of agriculture depends in many important ways, on microbial activities and there appears to be a tremendous potential for making use of microorganisms in increasing crop production. Some of the small or microbe fertilizers or biofertilizers are important part of our environment for sustainable agricultures practices (Bloemberg 2000). Main biofertilizers are nitrogen fixing bacteria, phosphate solubalizing and plant growth promoting microorganism (Goel 1999). Most of the biofertilizers benefiting the crop production such as *azotobactor , azospirillum blue green algae (BGA)* and *Rhizhobium*(Hegde1999). Many experiments were conducted to study the effect of biofertilizers alone or in combination with other chemical fertilizers (Patel et al. ,1992).Pulses play a vital role in Indian agriculture. Pulses are important sources of food. They are very rich in protein, particularly to the vegetarian who constitute the bulk of population in India. Blackgram is an annual food legume. It is very nutritious and is recommended for diabetics. Biofertilizers are small microbes which can be created which contain living cells of nitrogen fixing and phosphate solubalizing microorganism for treatment of seed or soil. They are organic product which contain living cells of various types of microorganism, which are capable of converting important elements fro unavailable to available from through biological processes (Vessey et al., 2003). They are converting te area with the objective of increasing such microorganisms and accelerate microbial process to augment to extend of the availability of the nutrient in a form which can easily assimilated by plant (Subba-Rao, et al, 1986The findings of previous studies in the field show that the biofertilizers are widely used in several countries with proven results in all kinds of plants and trees. (Victor and(Ruben, 2002). Nitrogen is an essential nutrient for the growth of different crops; its application is beset with economic burdens and environmental risks. Biological nitrogen fixation not only improves plant growth but also helps to minimize the use of chemical nitrogen fertilizers, so that the cost of production and environmental risks are reduced.

MATERIALS AND METHODS

Seeds of *Vigna mungo (L.Hepper)* were treated with experimental *Rhizobium japonicam* as follows.

Seed treatment with *Rhizobium japonicum:*

Rhizobium japonicum was mixed with rice starch in a container to form a slurry. *Vigna mungo* seeds were soaked in the slurry and kept overnight for germination.

Inoculation of seeds treated with biofertilizers

Nearly 100 undamaged healthy seeds of were selected for experiment . After selection, the seeds were sowed at equal depth in 10 pots with soil. 10 control pots were also maintained by sowing untreated seeds. The plants were watered at regular interval and the growth parameters were recorded after 45 days of sowing. The morphological parameters such as number of leaves, length of leaves, breath of leaves, length of plant, shoot and root length were measured. The bio chemical parameters such as the total chlorophyll , total protein content and total carbohydrate content were analysed.

RESULTS AND DISCUSSION

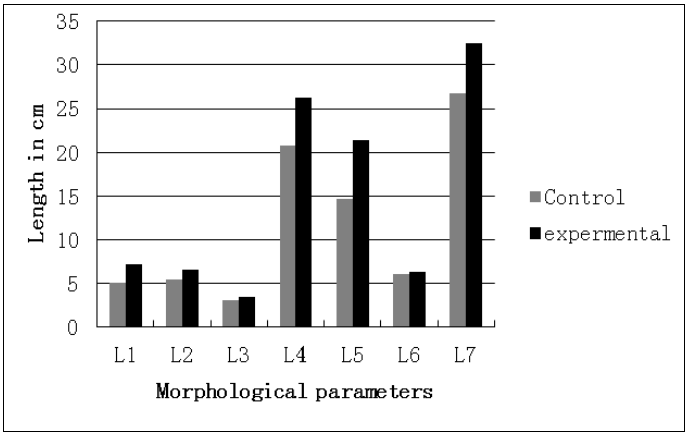
It was observed that when the biofertilizer *Rhizobium japonicum* was applied to *Vigna mungo (L.hepper),* the plant showed excellent growth as compared to control. In general all plants treated with bio fertilizers showed significant improvement in the parameters like, number of leaves, length of leaves, breadth of leaves, length of plant, shoot length and root length (Table 1).

Table 1: Effect on morphological parameters of black gram plants treated with Biofertilizer

TREATMENT	(A) Number of leaves/plant (cm)	(B) Length of leaves (cm)	(C) Breadth of leaves (cm)	(D) Length of plant (cm) (above ground)	(E) Shoot length (cm)	(F) Root length' (cm) (below ground)	(D+F) Total length of plant (cm)
CONTROL	5.0	5.4	3.0	20.7	14.7	6.0	26.7
EXPERIMENTAL	7.2	6.5	3.4	26.2	21.4	6.3	32.5

Table.2 Effect on biochemical parameters of *Vigna mungo (L.hepper) treated* with *bacterial Biofertilizer*

Sample	Total Carbohydrate Content	Total Chlorophyll Content	Total Protein Content
Control	2.25	0.821	2.8
Experimental	2.35	0.885	3.0



L1-Number of leaves/plant, L2-Length of leaves, L3-Breadth of leaves, L4-Length ofplantL5- Shoot length, L6-Root length, L7-Total length of plant.

Fig. 1: Effects on morphological parameters of black gram plants treated with biofertilizer

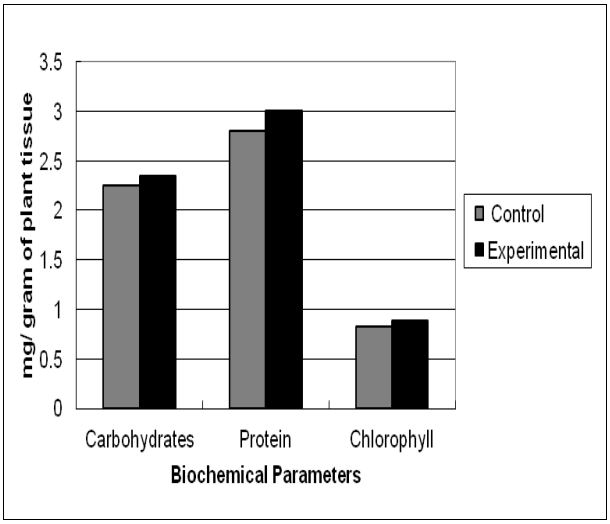


Fig. 2: Effect on biochemical parameters of *Vigna mungo (L.hepper),* plants treated with Biofertilizer

The total chlorophyll content levels of inoculated plants were significantly higher than the un-inoculated plants. The same results were observed in carbohydrate and protein content (Table 2).

CONCLUSION

The seeds treated with bacterial biofertilizer *Rhizobium japonicum* showed significant increase in growth of the plant- *Vigna mungo (L.hepper).* The morphological parameters such as number. of leaves, length of leaves, breadth of leaves, length of plant, shoot length, root length, and total length of plant showed significant increase. The effect was also seen in the bio chemical parameter such as carbohydrate content, protein content, and chlorophyll content. The results prove that plants treated with *Rhizobium japonicum* showed excellent performance in both morphological as well as biochemical parameters. Hence the use of biofertilizers should be encouraged by the Agricultural Institutions of Government of Maharashtra India because it is cost effective and helps in keeping the environment pollution free.

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RESEARCH ARTICLE

Synergistic response of *Azadirachta spp.* and *Syzygium spp.* on some fungi due to immunomodulators

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ABSTRACT

Medicinal herbs act as immunomodulators supporting the body's immunity system to deal with allergens. Since Neem and Clove are great source of traditional medicines, they were taken up as tool for our study to evaluate their lethal effects on three fungi- *Aspergillus, Candida* and *Penicillium spps*. using ethanolic and acetone solvent extracts through Disc Diffusion method. It was clearly observed that Neem had better efficacy as compared to Clove since it showed appreciably higher potency in both the extracts. However the results of the comparative potential of Neem in three fungi was found to be most in *Penicillium* followed by *Aspergillus* and least in *Candida*. Another method was carried out using Well Diffusion method where three different ratio concentrations of Clove : Neem (1:2, 1:1, 2:1) were taken using ethanolic and acetone extracts separately and tried on three organisms at individual level. The result noted showed best antifungal response in 1: 1 followed by 1 : 2 and minimum effect by 2 : 1 which hinted the possibility of the enhanced effect of Clove when paired with Neem. The above experiments proved Neem to be a better fungicide and also yielded enhanced effect when in mixture with Clove.

The Spectrophotometric readings for the presence of Flavonoids, phenols, and sterols provided us with the information which showed the concentration of phenols to be more than the others which lead us to believe that in all probability phenols is the active principle behind the better efficiency of *Azadirachta* as compared to Clove as a fungicide and thus can be pursued as a source of alternative medicine.

Keywords: Neem, Clove, fungicide, well diffusion method, disc diffusion method, Synergistic.

INTRODUCTION

The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, Flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones, Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective (Verkerk and Weight, 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (National Research Council, 1992).

Ayurveda is complete health care system, medicinal herbs used in it act as immune-modulators this means it controls infections and enhances body's immune system. Antioxidants are the bio molecules which has potential to overcome various diseases in human body. These carry out free radical scavenging activities and act against reacting oxygen species.

Azadirachta indica commonly called as neem is an ever green tree. The major active constituents of the tree are nimbin, nimbidin and nimbinene (National Research Council, 1992; Biswas *et al*., 2002). The leaves yield quercetin (Flavonoid) and nimbosterol (β-sitosteriol) as well as a number of liminoids (Jacobson, 1990). The trunk bark contains nimbin (0.04%), nimbinene (0.001%), tannins (6.0%), while, the stem bark contains tannins (12-16%) and non-tannins (8-11%) (Biswas *et al*., 2002). The oil extracted from the seeds contains nimbosterol and flavonoids (Biswas *et al*., 2002). *Syzygium* species (Fam. Myrtaceae) have been reported to possess antibacterial (Shafi *et al*, 2002) and anti-inflammatory activity (Muruganadan *et al*, 2001). It was reported that the buds of *Syzygium aromaticum* (L.) Merr. & Perry (clove) were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity (Boulos, 1983). The antimicrobial activity of the essential oils from clove and rosemary (*Rosmarinus officinalis* L.) has been tested alone and in combination (Fu *et al*, 2007). The immature unopened flower bud of *Syzygium aromaticum* commonly called clove showing rust brown in colour is a tropical tree. The clove consists of free Eugenol, Oleanolic acid, gallotannic acid and methyl-n-amyl ketone. Studies of Thoroski et al(1989) showed that Clove is found to active against most pathogenic and non-pathogenic bacteria and fungi.

The phytochemicals, flavonoids, phenols and sterols possess antifungal, antibacterial, antiviral, antipyretic, anti-inflammatory and analgesic effects. The world around is retuning back to the basics of ayurvedic medicines ayurvedic treatment is non invasive and non-toxic, so it can be used safely as an alternative therapy or along with conventional therapy thus increasing research interest in natural antifungal activities observed in plants. Considering all these aspects, the present study aims at detecting certain antioxidants which may be responsible for the antifungal activities of *A. indica* and *S. aromaticum* against fungi *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum*.

MATERIALS AND METHODS

***Collection of Neem leaves and Clove buds*:** Fresh green Neem leaves were selected for the experiment were washed thoroughly to remove dirt and immediately crushed in mortar and pestle to make a paste. Dried clove flowers were powdered in mortar and pestle for experiment.

***Solvent Systems*:** Four solvent systems were used for experiments to obtain different types of bio-molecules in extract. Ethanol, Methanol, Acetone and Water were used as solvent in experiment

***Media used*:** P.D.A. (Potato Dextrose Agar) Composition: Water: 1lit, Potato: 200g, Dextrose: 20g, Agar powder: 20g

***Preparation of Inoculums for test organism*:** Three different fungi *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum* were taken from pure cultures and inoculated separately on different PDA plates by 'Streak Plate' method for well diffusion method. For disc diffusion method loop full of culture from culture stock was bulk seeded in culture media.

Methods used for analysis:

Diffusion method: Standard disc diffusion method and well diffusion method were used to study effects of extracts on three different fungi.

***Preparation of Neem and Clove extracts*:** Clove buds and fresh leaves of Neem were individually soaked in four different solvents- Acetone, Ethanol, Methanol and Water in two different flasks for 30-40 min. each

and crushed to get a smooth paste which was filtered with muslin cloth. The collected extracts of clove and Neem were concentrated by evaporation under room temperature.

Sterilized whatman paper discs were dipped in antifungal agent 2% ketoconazole (Effective against all three fungi) was taken in ratio 1:2(determined by trial and error method) with distilled water.

***Disc diffusion method*:** Disc diffusion method was used to study effect of extracts individually on selected fungi. Discs (Diameter:10mm) of clove and Neem extracts and one positive control disc were kept in plates streaked with *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum* separately and incubated at 37^0 C for 48 hrs.

***Well diffusion method*:** Well diffusion method was used to study synergistic effect of Neem and Clove.

***Preparation well and inoculation*:** In this method the agar plates were allowed to set and equidistance wells of 8mm diameter were made with sterile borer. Three fungi were inoculated on different Petri plates using streak-plate method. 100µ each extract was propelled directly into the wells of the agar plate. Three different ratios of Clove: Neem extracts viz. 1:2, 1:1, 2:1 were used to check synergistic effect as well as their individual performance. The plates were allowed to stand for 1hr. For diffusion of the extract in to the agar and incubated at 37^0 C for 48hrs.

***Estimation of phenols, Flavonoids and sterols*:** Concentration of total phenols, total flavonoids and total sterols were estimated from standard methods- 'Folin Ciocalteau method', and 'Aluminium chloride method' and 'Liebermann-Burchard' method respectively.

RESULTS AND DISCUSSION

Chemical Analysis:

Spectrophotometric Estimation of phenols, Flavonoids and sterols shows that the concentration of active principle phenol is more in both extracts as compared to others (Table 3).

Estimation of Flavonoids is carried out by standard aluminium chloride method (Bansod and Mahendra, 2008). Concentration of Flavonoids in ethanolic extracts of Neem is found to be 192.6µg/ml. where as it is found to be 217µg/ml in Acetone extract on Neem. For clove concentration of Flavonoids is less as compare to Neem; in ethanolic extract it is found to be 183.6µg/ml and in acetone extract it is found to be 207.0µg/ml.

Estimation of sterols is carried out by Liebermann-Burchard method. It is found to be 27µg/ml and 34µg/ml in Ethanolic and Acetone extract respectively, while Clove shoes 25µg/ml and 18µg/ml concentration of Sterols in Ethanolic and Acetone extract respectively.

The concentration of phenolic content in both the extracts was determined using spectro-photometric method (Quettier, 2000). Phenolic concentrations in both Neem and Clove are found to be maximum i.e. 375µg/ml.

Antifungal Activity:

A. ***Disc diffusion method:***

In disc diffusion method acetone extracts of Neem shown potential inhibition of all three fungi (*A. niger, P. chrysogenum and C. albicans*) *P. chrysogenum* is eminently affected by Acetone extract of Neem (A.O.I. :314.2mm^2) followed by *A.niger* (A.O.I.: 153.9mm^2) and *C. albicans* (A.O.I.: 113.1mm^2)

Ethanolic extract of Neem also showed inhibition of all the three fungi to the lesser extent as compare to acetone extracts of Neem. In contrast to acetone extract *A. niger* is inhibited to greater extent (A.O.I.: 98.5mm^2). *P. chrysogenum and C. albicans* showed similar area of inhibition (A.O.I.: 95mm^2). Methanolic and water extract has no visible inhibitory effect on test fungi.

In disc diffusion method for Clove; Acetone extract shown pronounced effect on all the three fungi (A.O.I.:132.7mm^2). While Ethanolic, Methanolic and water extracts has no visible inhibitory effect on any fungi

B. Well diffusion method:

In well diffusion method three different solvent ratios of Neem and Clove were used (1:2, 1:1, 2:1). Acetone extract was found to be the most effective as compared to other three solvents.

Table 1: Disc diffusion method for Neem Extract and Clove Extract

Fungi/Extracts	Area of Inhibition (mm²)							
	Neem Extract				Clove Extract			
	Acetone	Ethanol	Methanol	Water	Acetone	Ethanol	Methanol	Water
A. niger	153.9	98.5	0	0	132.7	98.5	0	0
P. chrysogenum	314.2	95	0	0	132.7	95	0	0
C. albicans	113.1	95	0	0	132.7	95	0	0

Table 2: Well diffusion method for Neem and Clove Extract:

Fungi/Extracts	Area of Inhibition (mm²)											
	Acetone			Ethanol			Methanol			Water		
	1:2	1:1	2:1	1:2	1:1	2:1	1:2	1:1	2:1	1:2	1:1	2:1
A. niger	452.4	1256.6	78.54	0	78.54	0	0	0	0	0	0	0
P. chrysogenum	581.1	1809.5	78.54	0	78.54	0	0	0	0	0	0	0
C. albicans	514.7	1398.67	78.54	0	78.54	0	0	0	0	0	0	0

Table 3: Estimation of Flavonoids & Sterols

Sample Extracts	Flavonoids:				Sterols			
	Neem		Clove		Neem		Clove	
	O.D.	Concentration	O.D.	Concentration	O.D.	Concentration	O.D.	Concentration
Ethanol	1.07	192.6µg/ml	1.02	183.6µ/ml	3.0	375µg/ml	3.0	375µg/ml
Acetone	1.21	217.8µg/ml	1.15	207.0µg/ml	3.0	3.75µg/ml	3.0	375µg/ml

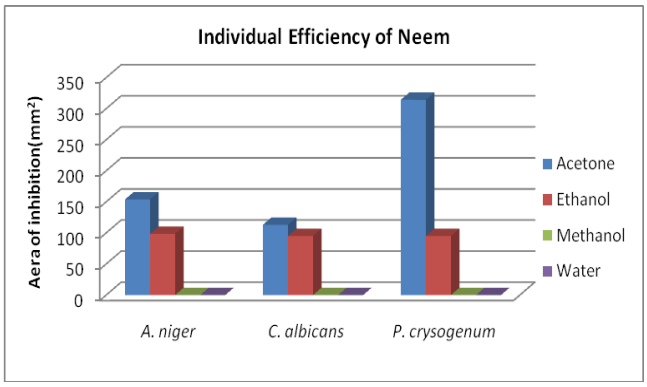

Fig. 1: Inhibition of Neem extracts on three fungi

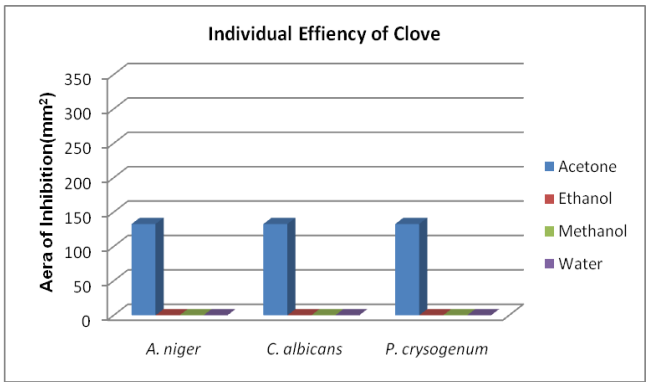

Fig. 2: Inhibition of Clove extracts on three fungi

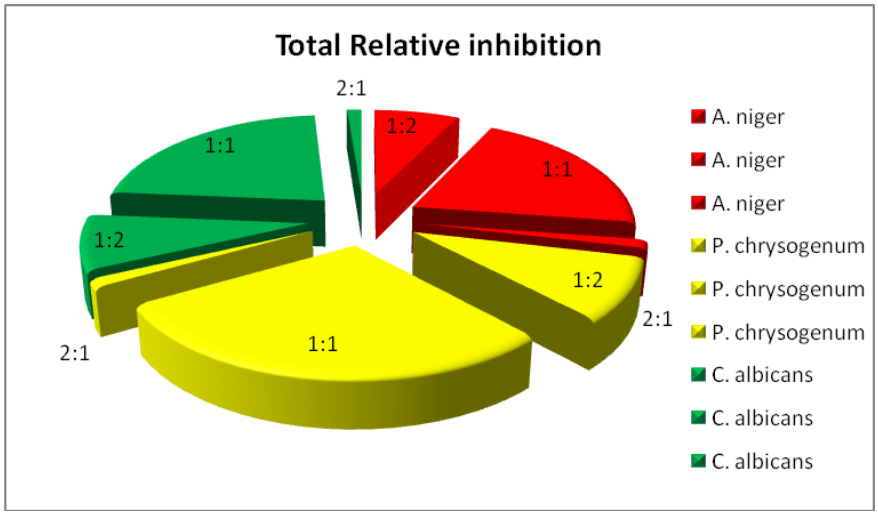

Fig. 3: Relative inhibition of Neem and Clove extracts on three fungi

1:1 ratio of Clove: Neem showed maximum inhibitory effect on all the three fungi i.e. *P. chrysogenum* (A.O.I.:1809.5mm^2) followed by *A.niger* (A.O.I.: 1256.6mm^2) and *C. albicans* (A.O.I.: 1398.67mm2).

1:2 ratio of Clove: Neem also showed inhibition of all the three fungi but to the lesser extent as that of 1:1 ratio *P. chrysogenum* (A.O.I.:452.4mm^2) followed by *A.niger* (A.O.I.: 581.1mm^2) and *C. albicans* (A.O.I.: 514.7mm^2).

A ratio of 2:1 showed minimum inhibitory response in *P. chrysogenum* (A.O.I. :78.54mm^2), *A.niger* (A.O.I.: 78.54mm^2) and *C. albicans* (A.O.I.: 78.54mm^2) in comparison with test ratios 1:1, 1:2.

Ethanolic extracts also showed notable inhibition of all the three fungi but only with ratio 1:1 *P. chrysogenum* (A.O.I.:78.54mm^2) followed by *A.niger* (A.O.I.: 78.54mm^2) and *C. albicans* (A.O.I.: 78.54mm^2).

Methanol and water extracts have not shown any visible inhibitory effect on any of the test fungi for all three ratios.

From the result of the current study it is clear that Neem has greater antifungal activity as compared to Clove. Effectiveness of Neem oil as a fungicide has earlier been reported by several worker (Lokhande et. al 1998).

The differences in the toxicity of different extracts could be attributed to the presence of the active principles that are extracted by different solvents, which may be influenced by several factors such as age of plant, method of extraction and type of extracting solvent (Nicolls 1969).

Qualitative analysis of both Neem and Clove extracts showed abundance of Phenolic components followed by Flavonoids and Sterols. Acetone extract showed maximum concentration of components/ml of extracts.

From table 1 and 2 it is clear that fungi *P. chrysogenum* is most affected by both the Neem and Clove extract followed by *A. niger* and *C. albicans*.

Ethanolic extracts of Neem also showed notable inhibitory effect on all the test fungi. Madali et al. (2009) also used Ethanolic extract of Neem for retarding the growth of Aspergillus species.

From the observation of inhibitory effect of extracts of Neem and Clove it is clear that in current studies higher inhibitory principle is released from acetone extract.

In well diffusion method acetone extract of both the Neem and Clove taken individually showed pronounced inhibition. Interestingly 1:1 Acetone extract of Neem and Clove not only showed greater inhibition as a mixture but also showed enhanced inhibitory response as compare to above two plants studied separately; thus indicating synergistic action of Neem and Clove. Thoroski et al (1989) Shown that when Acetone extracts when used in equal amount (1:1) Clove was found to be active against most pathogenic and non-pathogenic fungi.

However 1:2 of Clove: Neem shown comparatively less antifungal response while 2:1 ratio of Clove: Neem showed least antifungal response amongst all test ratios in acetone. Ethanolic extract also showed considerable anti fungal response in test ratio (1:1).

Both aqueous and Methanolic extracts showed no visible antifungal activity on test fungi for any of the test ratio (1:2, 1:1, 2:1).

The further analysis of clove is required since it also shown considerable inhibitory effect against all the three fungi. Bansod and Mahendra (2008) showed that The MIC of *Azadirachta indica* was found to be higher than MIC of clove which is in accordance with our studies. Hence *A. indica* can be considered as alternative of medicine due to its higher potential.

Further investigations are required to isolate and identify the other active principles for both qualitative and quantitative and assessment and their mechanisms of the activity.

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RESEARCH ARTICLE

Phytochemical screening of *E. chaetaria,* (Roem. & Schult.), Cyperaceae Bhandara District of Maharashtra

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ABSTRACT

*E. chaetaria, (Roem. &Schult.)*is less known plant species of *Eleocharis*, in India, even in Maharashtra? These plants are economically and biologically insignificant. But traditionallymore important, because some traditional people used such a plant in medicinal purposes. The present study investigate, the qualitative and quantitative phytochemical analysis of the major bioactive constituent in *E. chaetaria, (Roem. &Schult.)*.The plant where found to contain, Alkaloid, Tannin, Flavonoids, Saponin, Steroid, Terpene. The importance of the distribution of these chemical constituent discuss with respect to biologically most active form.

Key word: - E. chaetaria, *Eleocharis*, Phytochemical analysis.

INTRODUCTION

E. chaetaria, (Roem. & Schult.) is species of genus *Eleocharis*, family Cyperaceae, verified by Clark C. B. 04-1887 it is a combination to *E. retroflexa*.*E.chaetaria* are amphibious species fresh water usually C3and C4, carboxylation plant. Culms- sometime solitary, strongly compressed in cross section, Leaves- basal 2 per culm, Bract- absent rarely proximal scale of spikelet resembling short bract. Flower- bisexual, bristles straight or curved, Stamens- 1-3, Style- longer, 2-3 fid, base persistent. Exiccata- Betekar Bothali water margin of Lake, Mohadi Tehsil of Bhandara District Maharashtra, India.

MATERIALS AND METHODS

Collection and identification of plant materials:

The whole plant of *Eleocharisacutangulawhere* collected from uncultivated farmland located near wet environment of lake BapheraTumsar Tehsil. The plant sample identified by authors.

The voucher specimen where deposited. The plant samples were air dried and ground into uniform powder. The aqueous extract of sample prepared by soaking 100g of dried powder sample in 200 of distilled water for 12 h. The extract were filtered using whatman filter paper no. 42 (125m.m.)

Phytochemical Screening:

Chemical test were carried out on the aqueous extract using standard procedure to identify the constitute as described by Harborne (1992 ;1998),; Kokate(1994); Ablude (2001; 2007).

Fig.1. E.chaetaria, (Roem. &Schult.)

Alkaloid Determination:

0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl warmed & filtered. 2 ml of the filtrate were treated separately with both reagent (Maeyer's & Drangendorff's reagent) after which it was observed whether the alkaloids were present or absent in the turbidity. Yellow or reddish brown precipitation formation represent Alkaloid present (Harborne 1992)

Carbohydrate Determination:

Fehling test- 5cm3 of mixture of equal volumes of Fehling A and B was added to 2cm3 of each extract in a test tube. The resultant mixture was boiled for 2 minute. A brick red precipitation of copper oxide was observed. (Ablude 2001)

Tannin and Phenol Determination:

Two drop of 5persent fecl3 was added to 1cm3 of extract. A blue dirty green precipitate was observed in each extract presence of tannin and phenol respectively (Ablude 2007).

Flavonoids Determination:

5 ml of dilute ammonia solution where added to a portion of the aqueous filtrate of plant rhizome extract followed by addition of conc. H_2SO_4. A yellow colour observed in extract indicated the presence of flavonoids. The yellow colouration disappeared on standing then add few drop of 1% aluminum solution of filtrate further yellow colour obtained indicating the presence of flavonoids. (Safowara 1993, Harborne 1993).

Gum and resin Determination:

About 10ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicate the presence of Gum and Resins. (Harborne 1993)

Fixed oil and Fat determination:

A drop of concentrated extract was passed in between two filter paper and kept undisturbed. Oil stained on the paper indicate the presence of Oil and Fats. (Harborne 1993)

Saponin Determination:

About 1ml of the extract was dissolve in 20ml of water and shake in graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicate the presence of saponin. (Kokate 1994)

Phytosterol Determination:

Two ml of acetic anhydride was added to 0.5g ethanolic extract of sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some sample indicating the presence. (Harborne 1993)

Terpenoids Determination:

Five ml of each extract was mixed in 2ml of chloroform and conc. H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive result for the presence of terpenoids. (Ablude 2001)

Glycosides Determination:

10cm3 of 50persent H2So4 was added to 1cm3 of each extract in a test tube. The mixture was heated in boiling water for 5 minute. 10cm3 of Fehling solution (5cm3 of each solution A and B) was added and boiled. A brick red precipitated indicating presence of Glycoside. (Ablude 2001)

Quantitative Phytochemical Analysis

By standard procedure applied for Alkaloids, Carbohydred, Tannin, Phenol, Flavonoids, Saponin, and Terpen.

Alkaloid

5 g. of the sample was weighed into 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hour. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated Ammonium hydroxide was added dropwise to the extract until the precipitation was completed. This whole solution allow to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed. (Harborne 1993)

Carbohydrates

5g. of sample was weighed and added to 4.5ml of alcohol. The mixture was shaken for 10 minute and centrifuged to obtained precipitate. The precipitate was dissolve in 0.5ml of 0.1N H_2So_4. This reconstituted solution was transfer to glass stoppered tubes and then hydrolyse in a water bath at 100^{0c} for 1 hour and weighed. (Goel et.al.1985, kokate 1994).

Tannin

500mg of the sample in each case was taken in a plastic bottle and 50ml of distilled water was added. Then it was shaken in a mechanical shaker for 1hour and filtered in a 50ml volumetric flask made up to the mark. 5ml of the filtrate was pipetted out in to the test tube and mixed with 2ml of 0.1M. $FeCl_3$ in 0.1N. HCl and 0.001M. $K_4Fe\ (CN)_6$ (Potassium Ferrocyanide). The absorbance was measured at nm with in 10minute. (Van Burden and Robinson 1981, Ablude 2001).

Phenol

The fat free sample was boiled with 50ml of ether for extraction of phenolic component for 15minute. The extract pipetted out in 50ml conical flask to added 10ml distilled water and 2ml ammonium hydroxide solution and 5ml concentrated amyl alcohol were also added. The sample were made to mark and left to react for 30minute for colour development. This was measured at nm. (Harborne 1993)

Flavonoids

10g. of plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. (Bohm and kocipal, Abyazan 1994)

Saponin

20g. of each grounded sample was put into a conical flask and $100cm^3$of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 hour with constant stirring at about 55^{0c}. The mixture was then filtered and the residue was again extracted with another 200ml 20% ethanol. The combined extract was reduced to 40ml on a hot water bath at about 90^{0c}. The concentrate was transferred into a 250ml separatary funnel, added 20ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-Butanol was added. The combined n-Butanol extract where washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in oven, weighed, and saponin content was calculated as percentage. (Obadoni and Ochuko, 2001; Abulude, 2001).

Terpene

100g. of plant powder were taken separately and soaked in alcoholic solution for 24 hours then filtrate. The filtrated extract treated with petroleum ether.

Then estimate the total extract for terpene (Kokate, 1994; Ferguson, 1996).

RESULTS AND DISCUSSION

The present study carried out on the plant samples revealed the presence of medicinally active constituent. The phytochemical character of the E. chaetaria plant investigated are summarized in table-1, Alkaloid, Corbohydrate, Tannin and Phenol, Flavonoids, Saponin, found present in all plant part. Except phytosterol present in Aerial part, but absent in underground part of rhizomes. Quantitative estimation of the percentage crude chemical constituent in E.chaetaria studied is summarized in table-2. Rich amount of Alkaloid, Corbohydrate, Flavonoids, Saponin, present up to one percentage to Tannin and Phenol.

Table 1 Preliminary phytochemical screening of *E.chaetaria (Roem&Schult),*Aerial and underground part of plant.

Sr. No.	Plant Part	Alk.	Cor.	Ta & Fe	Flv.	Gum & Resin	Fixed Oil & Fats	Sap.	Pste.	Terp.	Glyc.
1	Arial Stem	+	+	+	+	-	-	+	+	-	-
2	Inflorence Fruiting Body	+	+	+	+	-	+	+	+	-	-
3	Underground rhizome	+	-	+	+	-	-	+	-	-	-

+ sign present, - sing absent, Alk- Alkaloids, Cor- Corbohydrates, Ta & Fe- Tanin&Fenol, Flv- Flavonoids, Sap- Saponin, Pste- Phytosterol, Terp- Terpene, Glyc- Glycocide.

Table 2: Percentage of proximate chemical composition of *E.chaetaria* (Roem & Schult).

Sr. No.	Plant Part	Alk.	Cor.	Flv.	Sap.	Ta.	Fe.	Terp.
1	Arial Stem	0.10	0.82	0.86	1.08	0.02	0.04	-
2	Inflorence Fruiting Body	0.49	0.07	0.59	2.09	0.16	0.09	-
3	Underground rhizome	0.31	-	0.86	0.71	0.05	1.00	-

The phytochemical analysis of quantitative estimation of percentage yield of crud chemical constituent studied show that the Aerial and Underground plant part rich in Alkaloids, Carbohydrates, Flavonoids, Saponin. They were known to show medicinal activity as well as exhibiting physiological activity. They are also widely employed as livestock and poultry feed, steroidal compound are of importance and interest in pharmacy due to their relationship with such a compound as sex hormones. Plant show the some activity of useful drugs. As claimed by traditional healers. Because of bioactive compound found in E.chaetaria plant.

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RESEARCH ARTICLE

Application of certain homoeopathic medicines used against fruit rot of apple caused by *Penicillium expansum* Link

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ABSTRACT

Blue mold of apple caused by *Penicillium expansum* is one of the most important serious destructive post harvest disease in India. Twenty three isolates of *Penicillium expansum* were isolated from rotted fruits of apple collected from APMC fruit market of Vashi, Navi Mumbai. Their sensitivity was tested against carbendazim. It was found that Pe-9 was sensitive while pe-15 resistant. MIC values ranged from 750.6-970.3µg/ml. Sensitive isolate was selected for further studies and treated with chemical and physical mutagens and resistant mutant of *P.expansum* (Pe-EMS-10) was found (4850.6g/ml). Of 13 homoeopathic medicines were used for the management of carbendazim resistant mutant of *Penicillium expansum* (EMS-Pe-10). *Sepia officinale* was more effective PCE value (40.42) when used individually and in mixture with carbendazim PCE value was increased as compared to individual (53.25) and followed by *Arsenicum album, Tabacum, Cynopodium, Baptisia tinctoria, Ustilago maydis, Iris versicolor, Zincum metallicum* and *Argentum metallicum.*

Key words: Apple, Blue mould, *Penicillium expansum*, Homoeopathic medicines, Carbendazim.

INTRODUCTION

Blue mould of apple (*Pyrus malus* L.) caused by *Penicillium expansum* is one of the most important post harvest disease. Apple plays a vital role in human diet by supplying the necessary nutritional components such as vitamins and minerals that can help to keep a good state of health. It contain high level of sugar, minerals and nutrient elements and their low pH value make them susceptible for fungal attack and are being rotten (Singh and Sharma, 2007). Fungi not only cause rot to a number

of fruits but also reduce their market values (Arya, 2004). Some fungal pathogens viz. *Colletotrichum acutatum, Venturia inaequalis, Monilinia fructicola, Botrytis cinerea, Alternaria alternata, Aspergillus fumigates A. flavus, Sclerotina fructigena, Rhizopus stolonifer, Mucor piriformis* and *Penicillium expansum* on apple was reported during the transportation and storage condition. Among the pathogens *Penicillium expansum* was more serious and dominant in the store houses of local and central fruit market of Navi Mumbai, APMC Fruit Market, Vashi in packing boxes noticed damages of apple. 20-25% losses of the post harvested fruits are decayed by certain fungal pathogens during post harvest handling even in developed countries (Al-Hindi *et al.,* 2011).

Carbendazim is recommended to manage various fruit rot of pathogens during post harvest. Fungicides resistance few cases have been reported in India and abroad (Chander and Thind, 1995; Gangawane and Reddy, 1987; Gangawane, 2008). Apple growers rely heavily on the use of fungicides for control of fruit rot of apple. Excessive use of carbendazim was harmful to apple fruit as well as *Penicillium expansum*. Therefore, substitute for carbendazim presently suggested that the use of homeopathic medicines to control various pathogens was highly effective and safe for fruit and environment. Inhibitory effect of homoeopathic drugs such as *Lycopodium, Thuja, Arsenicum, Zincum* etc. against *Alternaria alternata, Fusarium moniliforme, Gloeosporium psidii, Colletotrichum gloeosporioides* and *Pestalotia sp*. and certain fruit rot pathogens have been reported by(Khanna and Chandra, 1989 and 1992; Chandra *et. al*., 1981; Wilson *et. al*.,1991). The present investigation showed that the effect of homoeopathic medicines i.e. *sepia officinale* was fruitful PCE (40.42) value individually and in mixture with carbendazim PCE value increased upto 53.25.

MATERIALS AND METHODS

Homoeopathic medicines viz; *Belladonna, Tabacum, Thuja occidentalis, Argentum metallicum, Sepia officinale, Lycopodium clavatum, Ustilago maydis, Iris versicolor, Cynopodium, Zincum metallicum, Arsenicum album, Baptisia tinctoria* and *Teucrium marum verum* etc. was purchased from wholesale market of Vashi. Potency (200) of all these medicines was used. The antifungal homoeopathic medicines were tested individually and in mixture with carbendazim (970.3µg/ml) against mycelial growth of carbendazim resistant mutant (Pe-EMS-10) of *Penicillium expansum* using potato dextrose agar (PDA) medium by food poisoning method (Nene and Thapliyal, 1982). Percentage Control Efficacy (PCE) was determined using formula.

$$PCE = \frac{C - T}{T} \quad X\ 100$$

Where, C - Mycelial Growth in Control
T - Mycelial Growth in Treated

RESULTS AND DISCUSSION

Results are present in (Table.1) observed that thirteen homoeopathic medicines were used for the management of carbendazim resistant mutant (Pe-EMS-10) of *Penicillium expansum.* It was seen that all homoeopathic medicines were inhibitory against *Penicillium expansum*. *Sepia officinale* showed significantly increased PCE (40.42) individually and followed by *Arsenicum album* (38.75), *Tabacum* (38.56), *Cynopodium* (38.00), *Baptisia tinctoria* (36.58), *Ustilago maydis* (36.56), *Iris versicolor* (36.32), *Zincum metallicum* (34.80), *Argentum metallicum* (32.58) and four homoeopathic medicines showed PCE 20.72-30.58 individually. In other hand all 13 homoeopathic medicines were mixed with carbendazim PCE against *penicillium expansum* was increased. *Sepia officinale* mix with carbendazim the PCE (53.25) value increased as compared to individual PCE value. The lowest PCE (35.85) was observed in *Belladonna* and followed by other homoeopathic medicines which showed values of PCE more than 52.65. There are few reports on the use of homoeopathic medicines against plant pathogens correlate with other researcher. (Dahiwale and Suryawanshi, 2010) observed that fruit rot of pomegranate caused by *Alternaria alternata* is one of the most important post harvest diseases. It was revealed that certain homoeopathic medicines were inhibitory against *A. alternata* (Dahiwale and Suryawanshi, 2014) also revealed that the control of grey mould of grape caused by *Botrytis cinerea* using homoeopathic medicine. Fruit rot of strawberry caused by *Alternaria alternata* control using homoeopathic medicines. *Nux vomica* shows higher PCE (50) when used individually while Sulphur 30 CH was effective showing maximum PCE (84.45) when

Table 1: Percentage Control Efficacy (PCE) of carbendazim individually and in mixture with homoeopathic medicines against resistant mutant of *Penicillium expansum* on PDA medium.

Sr. No.	Homoeopathic medicines	Percentage Control Efficacy *	
		PCE individual	PCE mixture With Carbendazim
1.	*Belladonna*	20.72	35.85
2.	*Tabacum*	38.56	52.52
3.	*Thuja occidentalis*	28.45	43.55
4.	*Argentum metallicum*	32.58	45.75
5.	*Sepia officinale*	40.42	53.25
6.	*Lycopodium clavatum*	30.58	45.38
7.	*Ustilago maydis*	36.56	49.95
8.	*Iris versicolor*	36.32	51.50
9.	*Cynopodium*	38.00	52.58
10.	*Zincum metallicum*	34.80	49.46
11.	*Arsenicum album*	38.75	51.00
12.	*Baptisia tinctoria*	36.58	52.65
13.	*Teucrium marum verum*	28.50	43.78
14.	Carbendazim (970.3µg/ml)	51.00	---
	SE	1.929	1.716
	CD at 0.05	4.008	3.581
	at 0.05	4.733	4.240

** Values are replicates.*

used in mixture with mancozeb and followed by *Cina, Rhus toxicodendron, Arnica montana, Sanguinaria canadensis, Tarentula hispana* and *Selenium* (Patil and Suryawanshi , 2014).

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RESEARCH ARTICLE

Screening of Antibacterial Activity of Rose Varieties against Bacterial Pathogens

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ABSTRACT

Rose is an ornamental aromatic plant belong to the family Rosaceae. In all 22 varieties from 2 different rose species viz Hybrid Tea and Floribundas (F) were used in this study. Out of these 19 rose petals varieties were moderately to strongly antibacterial against all the screened bacteria and the remaining 3 varieties demonstrated weak antibacterial activity. Pseudomonas aeruginosa was found to be strongly sensitive to all the 22 rose varieties with maximum zone of inhibition (32mm). Bacillus coagulans, Escherichia coli, Staphylococcus epidermidis, Shigella flexeneri and Salmonella typhi were moderately sensitive while Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus and Salmonella typhimurium were weakly sensitive. Pseudomonas aeruginosa was resistant to most of the standard antibiotics. Enterococcus faecalis was resistant to 2 varieties namely Koppies and Zorina. Staphylococcus aureus was resistant to 2 varieties namely Vimala and Zorina. Proteus vulgaris was resistant to 3 varieties namely Charleston, Viamala and Zorina where as Klebisella pneumoniae was resistant to 2 varieties namely Viamala and Zorina and Bacillus coagulans was resistant to 1 variety namely Zorina. These rose varieties may act as good alternatives to reduce the excess use of antibiotics to some extent, without side effect. Further studies are required for the screening of volatile and non-volatile active antimicrobial chemical constituents of rose petals to utilize them in pharmaceutical industries.

Key words: Rose petals, bacterial pathogens, antibacterial activities.

INTRODUCTION

In this long struggle to achieve mastery over powerful forces of nature, man has always turned to plants for help. This is especially so when he was struck with ailments both physically and mentally. “Arkprakash” one of the Vedas of Ayurveda gives the evidences of use of rose petals for medicine.

One of the major problems that concerns human health is bacterial resistant against antibiotics (Karlowsky *et al*., 2012; Gayatri *et al*., 2013). Therefore, researchers have been screening natural sources for an undiscovered antimicrobial agents (Mahesh *et al*., 2008).

The floral petals of higher plants are known to possess antibacterial activity (Darokar *et al*., 1998). The aroma in flower is due to essential oil secreted in the papillae from epidermal cells. The rose oil contains genaniol, citronellol, ethanol, rose oxide, linalool, nerol, eugenol, etc (Sharma, 2003).

The petals of rose species possess antibacterial activity against different pathogenic bacteria reveals presence of several different volatile and non-volatile chemical constituents in the petals of different varieties leading to bacterial inhibition (Mankar and Tambekar, 2006). Efforts are thus directed to identify plant product, which have broad spectrum antimicrobial property and no ill effect (Farnsworth, 1998). *Rosa damascena* was used in ancient medicine in the effective treatment of abdominal pain, digestive problems, skin problems and headaches (Foster and Duke, 1990).

R. damascena act as an anti – diabetic by reducing blood glucose level there by acting as potent of α – glucosidase enzyme inhibitor (Gholamhoseinian *et al*., 2008). *R. damascena* has potential to increase heart rate and contraction. The mechanisms of these effects are unknown (Boskabady *et al*., 2011). *R. damascena* has mild excitatory effect on ileum contraction and this aqueous fraction may be useful as a mild laxative agent (Dolati *et al*., 2013).

So the aim of present investigation was to evaluate the antimicrobial profile of rose petals varieties against bacterial pathogens.

MATERIALS AND METHODS

Plant Material

Rose Petals were collected from 22 rose varieties maintained in the Amravati Garden Club, (M.S.), India. The Hybrid Tea and Floribandas varieties were used. The rose varieties were collected in the morning between 7.30 am to 9.30 am and stored in refrigerator until further use. All the rose varieties were repeated for 3 times during rainy to winter season.

Using a metal borer, 10 mm diameter discs were cut from the rose petals and then sterilized by distilled water and then with 0.1% mercury chloride for 2 min. and again resterilized with distilled water for 5 to 6 times.

Anitimicrobial Susceptibility Testing

Standard antibiotic susceptibility and antibacterial activity of rose petals were screened for these bacteria by a standardized single disc method (Bauer *et al*., 1966). Broth cultures of both Gram - positive bacteria *Bacillus coagulans* (MTCC 2302), *Enterococcus faecalis* (MTCC 439), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435) and Gram - negative bacteria *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 390), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426), *Shigella flexeneri* (MTCC 1457), *Salmonella typhimurium* (MTCC 98), *Salmonella typhi* (MTCC 733). The bacterial lawns were prepared by spreading of 0.1ml over night grown bacterial culture (approximately 10^8 CFU/ml) on nutrient agar plates. The petal discs were placed on seeded nutrient agar and incubated at 37^0C for 24 h. All the bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The results were recorded as zone of inhibition in mm.

RESULTS AND DISCUSSION

In the present study, *P. aeruginosa* was found to be highly sensitive against all 22 rose varieties with zone of inhibition in between 21 to 32mm. *B.coagulans, E. coli, S. epidermidis, S. flexeneri, S. aureus, E. aerogenes, S. typhi* and *S. typhimurium* were moderately sensitive while *E. faecalis, K. pneumoniae, P. vulgaris,* were weakly sensitive (Table 1). *P. aeruginosa*, which was resistant to most of the standard antibiotics, found sensitive to almost all varieties of rose petals while *K. pneumoniae*, was sensitive to all of the standard antibiotics, found weakly sensitive to almost all varieties of rose petals (Fig. 1). The pattern of antibacterial activity was measured in terms of zone of inhibition and compared with standard antibiotics such as, ampicillin, cephalothin, co-trimoxazole, gentamicin, nalidixic acid, nitrofurantioin, norfloxacin and tetracycline. It was observed that the antibacterial activity of rose varieties was similar to antibiotics in terms of zone of inhibition. (Fig. 2).

Table1: Antibacterial activity in the floral petals of different varieties of roses against bacterial pathogens.

Name of Rose Varieties	(zone of inhibition in mm)											
	B. coagulans MTCC2302	*E.aerogenes* MTCC 111	*E. coli* MTCC 390	*E. faecalis* MTCC 439	*K. Pneumoniae* MTCC 109	*P.aeraginosa* MTCC 424	*P. vulgaris* MTCC 426	*S. aureus* MTCC 96	*S.epidermidis* MTCC 435	*S. flexeneri* MTCC 1457	*S.typhimurim* MTCC 98	*S. typhi* MTCC 733
Azure Sea (HT)	16	15	16	14	15	30	13	14	20	16	16	16
Alabama (HT)	17	18	18	16	12	28	17	20	16	21	16	18
Ace of Heart (HT)	16	16	18	18	14	26	15	15	23	14	14	20
Barkarole (HT)	16	15	14	20	13	22	14	15	17	25	14	14
Century Two (HT)	15	13	21	12	14	21	12	13	20	22	18	16
Catalonia (HT)	21	12	14	17	16	30	14	16	14	21	15	13
Cabaret (HT)	20	21	20	18	17	32	25	21	26	18	15	23
Charlestron (F)	20	15	26	15	15	21	-	15	15	20	15	22
Deep purple (F)	20	15	14	16	14	25	16	20	20	20	13	18
Disco Dancer (F)	15	14	20	17	13	20	16	17	22	16	18	17
Garden Delight(HT)	18	15	17	13	16	23	17	14	16	17	12	18
Garden Medaillon (HT)	16	13	12	13	15	27	12	15	18	20	19	14
Koppies (HT)	21	14	18	-	12	26	16	12	16	21	13	13
Lustige (HT)	18	16	16	18	13	21	16	17	19	19	14	19
Madame violet (HT)	24	18	17	16	18	30	20	18	21	22	16	15
Perfume Delight (HT)	17	13	15	14	16	29	16	20	18	20	18	16
Summer Holiday (HT)	16	16	18	15	20	30	28	17	18	24	21	18
Summer snow (F)	20	14	20	14	15	24	16	17	17	22	16	21
Simplicity (F)	20	17	20	20	16	23	12	20	21	18	18	23
Valentine (F)	15	18	20	13	17	25	17	19	25	26	18	20
Viamala (HT)	-	12	17	13	-	20	-	-	16	13	15	14
Zorina (F)	15	14	19	-	-	22	-	-	12	20	-	-

Zone of inhibition : 12mm – 14mm (weakly sensitive), 15mm – 20m (moderately sensitive), 21mm – 32mm (highly sensitive), - = no zone of inhibition

HT = Hybrid Tea, F = Floribanda

Table 2 : Antibacterial activity of Standard antibiotic against bacterial pathogens

Antibiotics	(Zone of inhibition in mm)											
	B. coagulans MTCC2302	*E.aerogenes* MTCC 111	*E. coli* MTCC 390	*E. faecalis* MTCC 439	*K. Pneumoniae* MTCC 109	*P.aeraginosa* MTCC 424	*P. vulgaris* MTCC 426	*S. aureus* MTCC 96	*S.epidermidis* MTCC 435	*S. flexeneri* MTCC 1457	*S.typhimurim* MTCC 98	*S. typhi* MTCC 733
Ampicillin	14	15	17	16	13	-	14	26	29	13	15	13
Cephalothin	17	16	16	14	16	-	12	26	29	15	14	16
Co-trimoxazole	14	14	23	15	15	-	14	23	23	15	14	15
Gentamicin	15	12	19	17	14	16	12	18	19	12	12	12
Nalidixic acid	15	16	22	14	17	-	12	-	-	16	18	17
Nitrofurantioin	16	15	20	15	16	-	13	20	20	15	12	15
Norfloxacin	13	14	24	14	16	21	16	21	19	16	15	14
Tetracycline	18	13	20	15	12	-	15	24	25	16	13	12

– = no zone of inhibition

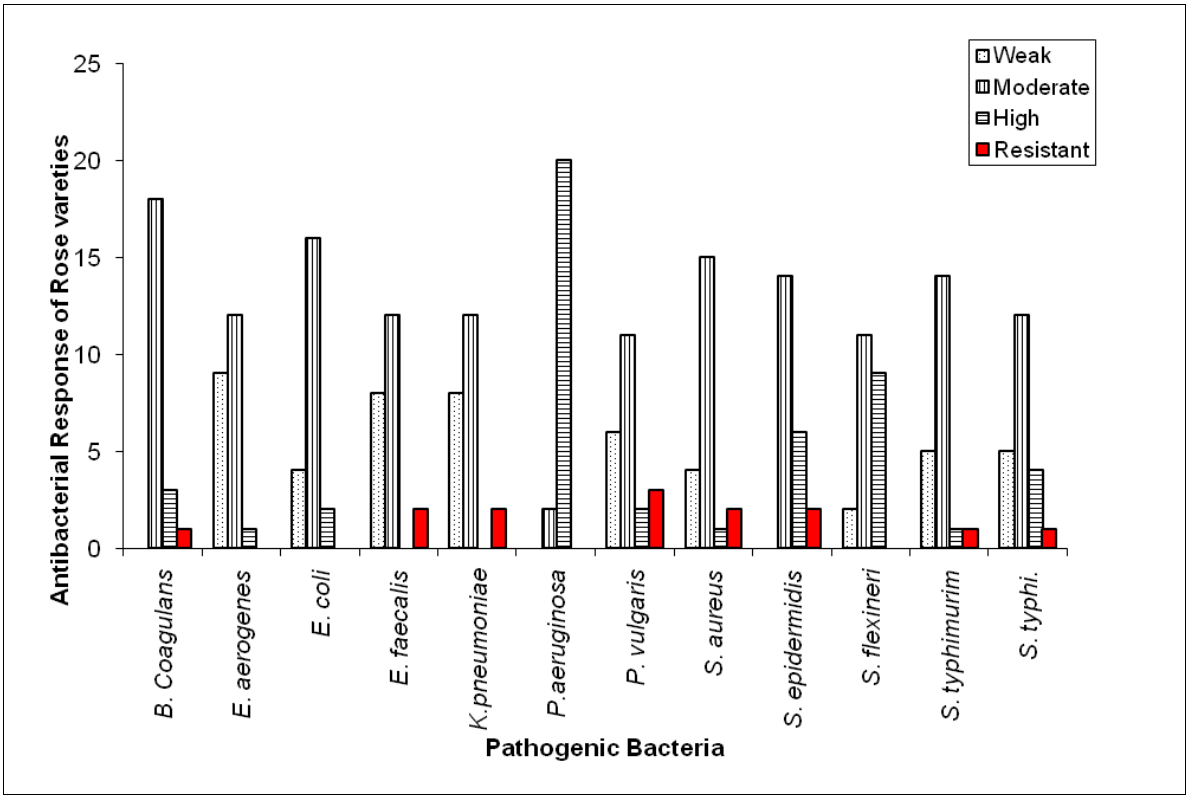

Fig. 1 : Antibacterial response of rose petals to pathogenic bacteria.

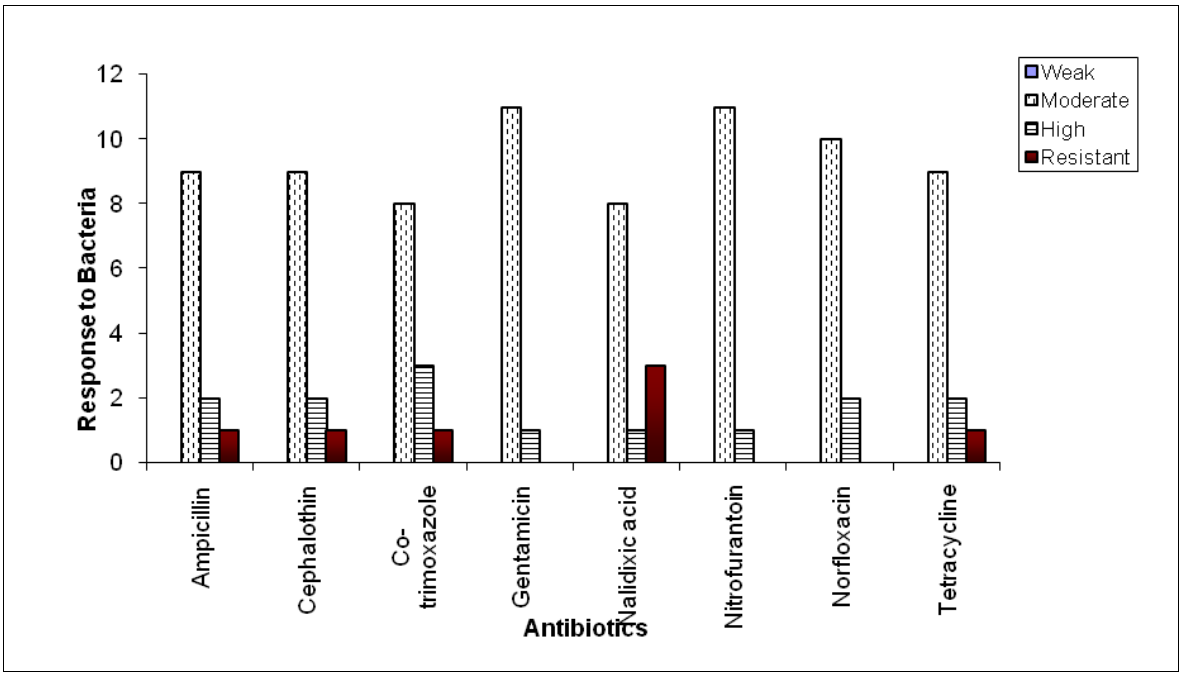

Fig. 2 : Antibacterial response of antibiotics to pathogenic bacteria.

The rose varieties Cabaret, Summer holiday, Valentine, Simplicity, Madame violet and Lustige were highly antimicrobial against Garm positive bacteria such as *B. coagulans, E. faecalis, S. aureus, S. epidermidis* and Gram negative bacteria such as *K. pneumoniae, P. aeruginosa, P. vulgaris, E. coli, E. aerogenes, S. flexeneri, S. typhi* and *S. typhimurium* (Fig. 3).

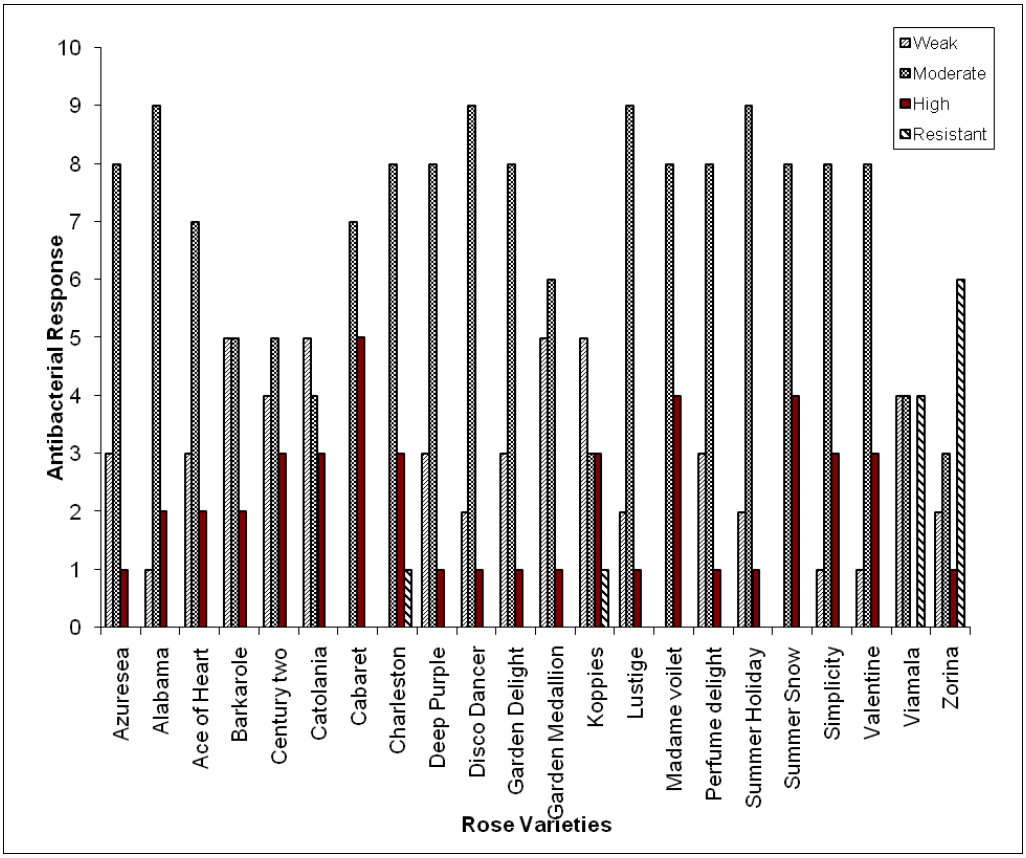

Fig. 3: Antibacterial activities of rose petals against pathogenic bacteria.

E. faecalis was found resistant against two rose varieties namely, Koppies and Zorina. *S. aureus* was found resistant to two rose varieties namely, Viamala and Zorina. *B.coagulans* was found resistant to Viamala only. *P. vulgaris* was found resistant to three rose varieties namely, Charleston, Viamala and Zorina. *K. pneumoniae* was found resistant to Viamala and Zorina where as *S. typhimurium* and *S. typhi* were resistant to Zorina only.

In the study of antibacterial activity petals of rose varieties the results showed that *P. aeruginosa* was proved to be the most sensitive to rose petals where as in case of Gram positive bacteria *S. epidermidis, S. aureus, E. faecalis* were moderately sensitive to rose varieties. The Gram negative bacteria *E. coli, E. aerogenes* were more resistant to rose petals as compared to *K. pneumoniae, S. typhimurium* and *P. aeruginosa* (Darokar *et al*., 1999). However, in present study we get similar result in case of *P. aeruginosa* which was highly sensitive to rose petals and Gram positive bacteria *S. epidermidis, S. aureus, E. faecalis* were moderately sensitive to rose varieties, but in case of Gram negative bacteria we found contrast result base on our study it showed that *K. pneumoniae* was weakly sensitive to rose varieties as compared to *E. coli, E. aerogenes, S. typhimurium* and *P. aeruginosa.*

Today, the realization that many pathogenic microorganisms are becoming resistant to antibiotics, that increases the need for the screening of new sources of antibacterial agents.

The use of our traditional medicinal aromatic plants thus may become a good alternative to synthetic drugs in future, The rose, which is known as queen of Flowers is one of them. Roses are ornamental aromatic flowers and differ in their fragrance with volatile constituents. The rose petals tissues, may possess antibacterial activity as a natural protection system for reproduction and further perpetuation through seed formation (Fabry *et al*., 1998).

The plants are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agents such as viruses, bacteria, fungi and other parasites that are specific to them (Williams *et al*., 1989). The presence of antibacterial compounds of wide specificity in the

petals of rose plants appears to vary in the different varieties. It was found that petals of poorly fragrant varieties were possess stronger antibacterial activity than highly fragrant varieties like *R. damascena.* The antibacterial profile of rose petals against the various bacteria indicated the presence of such antibacterial compounds of wide specificity and antibacterial activities which was found equivalent to streptomycin (Darokar *et al*., 1999).

The broad spectrum antimicrobial activity of the petals of *R. damascena* extracts use in folklore medicine. The petal extract of *R. damascena* may be used for the treatment of skin infections and for the throat infections (Shohayeb *et al*., 2014). The petals of rose varieties may be used against infection caused by pathogenic bacteria that may decrease the use of antibiotics and its ill effects (Mankar and Tambekar, 2006).

This study shows that rose petals may possess some chemical constituents which are responsible for antimicrobial activity that can partially minimize the use of antibiotics to some extent. However, further studies are required for screening of the volatile and non-volatile active antimicrobial constituents of rose petals to utilize them in pharmaceutical industries.

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RESEARCH ARTICLE

Phytochemical screening of *E. acutnagula*, (Cyperaceae) Bhandara district of Maharashtra, India

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ABSTRACT

The present study designed for phytochemical screening. Different extracts of *E. acutangula* (Roxb.)Schult aerial and underground part of plant where screened for the presence of chemically active compound by slandered method. The result revealed the presence of steroids, terpenoide and resins in petroleum ether extract, Flavanoids and resins in chloroform extract, carbohydrates in methanolic extract, the water extract show the presence of Saponins, Tannin.

In India even in Maharashtra less information available about phytochemical analysis of Eleocharis species. Therefore I have chosen to investigate phytochemistry in *E. acutangula* (Roxb.)Schult, and study about isolation characterization of the various chemical active substance.

Keyword: Phytochemical screening, Eleocharis, *E. acutangula* (Roxb.) Schult.

INTRODUCTION

The genus Eleocharis R.Br. family Cyperaceae include about 200 species, occurring in wet environments like swamps, lake and river margins. There aerial part are formed by simple ramified stalks that end in a spiciform inflorance formed by numerous inconspicuous flower. There subterraneous part are formed by root and stem called rhizome or stolon. Data carried litterature[2] of E. dulci (Trin)] E. colorodoensis (Britt.), E. acuta (R.Br.) by Gills L.S. (1992), published by (Ruiz, A. L. T. G. et. all. 2006). E. acutangula found in Baphera in an irrigated area of Bhandara District of Maharashtra India. This species wildly distributed in temperate zone and are fully aquatic 20-40 inch in tall.

MATERIALS AND METHODS

Collection and identification of plant materials: The whole plant of *Eleocharis acutangula where* collected from uncultivated farmland located near wet environment of lake Baphera Tumsar Tehsil. The plant sample identified by authors. The voucher specimen where deposited. The plant samples were air dried and ground into uniform powder. The aqueous extract of sample prepared by soaking 100g of dried powder sample in 200 of distilled water for 12 h. The extract were filtered using whatman filter paper no. 42 (125m.m.).

Fig. 1: ***Eleocharis acutangula***

Phytochemical Screening: Chemical test were carried out on the aqueous extract using standard procedure to identify the constitute as described by Harborne, 1992; 1998, Kokate, 1994, Ablude 2001; 2007.

Extraction: One hundred and fifty centimeter of water was added to 20gm of ground sample in a conical flasks. The mixture was covered and allow to stand for three hours with occasional stirring. The mixture was filtered with a Watman No. 2 filter paper. This filtrate was stored in plastic container and kept in ambient temperature prior to analysis.

Alkaloid Determination: 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1 % HCl warmed and filtered. 2 ml of the filtrate were treated separately with both reagent (Maeyer's & Drangendorff's reagent) after which it was observed weather the alkaloids were present or absent in the turbidity. Yellow or reddish brown precipitation formation represent alkaloid present (Harborne 1992).

Carbohydrate Determination: Fehling test- 5cm^3 of mixture of equal volumes of Fehling A and B was added to 2cm^3 of each extract in a test tube. The resultant mixture was boiled for 2 minute. A brick red precipitation of copper oxide was observed. (Ablude 2001).

Tannin and Phenol Determination: Two drop of 5% $FeCl_3$ was added to 1cm^3 of extract. A blue dirty green precipitate was observed in each extract presence of tannin and phenol respectively. (Ablude 2007).

Flavonoids Determination: 5 ml of dilute ammonia solution where added to a portion of the aqueous filtrate of plant rhizome extract followed by addition of conc. H_2SO_4. A yellow colour observed in extract indicated the presence of flavonoids. The yellow colouration disappeared on standing then add few drop of 1% aluminum solution of filtrate further yellow colour obtained indicating the presence of flavonoids. (Safowara 1993, Harborne 1993).

Gum and resin Determination: About 10ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicate the presence of Gum and Resins. (Harborne 1993)

Fixed oil and Fat determination: A drop of concentrated extract was passed in between two filter paper and kept undisturbed. Oil stained on the paper indicate the presence of Oil and Fats. (Harborne 1993)

Saponin Determination: About 1ml of the extract was dissolve in 20ml of water and shake in graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicate the presence of saponin. (Kokate 1994)

Phytosterol Determination: Two ml of acetic anhydride was added to 0.5g ethanolic extract of sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some sample indicating the presence (Harborne 1993)

Terpenoids Determination: Five ml of each extract was mixed in 2ml of chloroform and conc. H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive result for the presence of terpenoids. (Ablude 2001)

Glycosides Determination: 10cm^3 of 50% H_2SO_4 was added to 1cm^3 of each extract in a test tube. The mixture was heated in boiling water for 5 minute. 10cm^3 of Fehling solution (5cm^3 of each solution A and B) was added and boiled. A brick red precipitated indicating presence of Glycoside. (Ablude 2001)

RESULTS AND DISCUSSION

Table 1: Preliminary phytochemical screening of *E. acutangula* Aerial and underground part of plant.

Sr. No.	Plant Part	Alk.	Cor.	Ta & Fe	Flv.	Gum & Resin	Fixed Oil & Fats	Sap.	Pste.	Terp.	Glyc.
1	Arial Stem	+	+	-	+	-	-	+	+	+	-
2	Inflorence Fruiting Body	+	+	+	+	-	+	+	+	-	-
3	Underground rhizome	+	+	+	+	+	-	+	+	-	-

+ sign present, - sing absent, Alk- Alkaloids, Cor- Corbohydrates, Ta & Fe- Tanin & Fenol, Flv- Flavonoids, Sap- Saponin, Pste- Phytosterol, Terp- Terpene, Glyc- Glycocide.

The phytochemical screening analysis, the extract show that presence of alkaloid, carbohydrate, flavonoids, saponin, phytosterol, is the active component which is found present in Aerial and underground part of plant. But tannin & phenol, terpene, gum and resin, fixed oil and fats are absent in Aerial stem and gum and resin, terpene, glycoside absent in inflorescences. Fixed oil and fats, terpene, glycosides absent in underground Rhizomes.

CONCLUSION:

The most of the active compound like alkaloid, carbohydrates, tannin, saponin, phytosterol, are pharmaceutically important are found present in *E. acutangula*. The plant contains Stem and Rhizome are free from fixed oil and fats, gum and resins and Glycosides.

Such active components found in *E. acutangula*. So this unusual and biologically significant plant is to known our civilize society.

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RESEARCH ARTICLE

Phytochemical analysis of some plant latex

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ABSTRACT

Phytochemical analysis of plant latex was carried out from ten plant species from Nanded region for presence of secondary metabolites such as alkaloids, flavonoids, phenols, tannin, saponin, terpenoids, steroids and glycosides. Alkaloids were found in all plant latex except *F. benghalensis* & *C. papaya,* while flavonoids and phenols revealed the presence in all latex samples except *Nerium oleander* & C*arica papaya*. *Calotropis procera, Jatropa curcas, J. gossypifolia, N. oleander*, *C. papaya* showed presence of Saponin but absence of tannins. Terpenoids are absent in *Euphorbia tirucalli*, *Ficus benghalensis, F. religiosa*. Steroids are present in *E. tirucalli*, *C. procera, F. benghalensis,* while glycosides were absent in *E. tirucalli*, *N .oleander* and *F. religiosa.*

Key word: Latex, Phytochemical analysis, Secondary metabolities.

INTRODUCTION

About 10% of flowering plants produce latex and are found in over 40 families including Euphorbiaceae, Apocynaceae, Caricaceae, Moraceae, Asclepidaceae. (Agrawal and Konno, 2009) Latex is milky fluid secreted by ducts of laticirerous tissue (Hagal *et. al.,* 2008) and flow inside laticifers including leaves, stems, fruits & roots of some flowering plants. (Pickare, 2008) Latex is a complex mixture of secondary metabolites (Santos *et al.,* 2011) contains various biologically active compounds and antimicrobial activities. (Siritaperawee *et al.,* 2012, Kanokwiroon *et al.,* 2008) In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents (Balandrin *et al.,* 1985). Known constituents of latex are proteins, alkaloids, tannins, terpens, starch, sugars, oils, resins, gums and enzymes. (Pandey, 2001) Plant latex has wider ethno pharmacological application as it is used by tribal communities. *E. hirta* latex is traditionally used as ear drops and in the treatment of boils, sore and wound healing. (Igoli *et. al.,* 2005) Jatropha latex has some ehnomedicinal use like wound healing, coagulant activities of blood. (Om *et al.,* 2008) Hence the present study is aimed to find out phytochemical constituents of latex.

MATERIALS AND METHODS

Plant Species:

Euphorbia hirta L., *Euphorbia tirucalli* L. *Jatropha curcas* L, *Jatropha gossypifolia* L. (Euphorbiaceae), *Plumeria rubra* L. *Nerium oleander* L., (Apocynaceae), *Calotropis procera* (Ait) R.Br (Asclepidaceae)., *Ficus benghalensis* L., *Ficus religiosa* Linn, (Moraceae) *Carica Papaya* L. (Caricaceae). Their identification was confirmed using the 'Flora of Marathwada' (Naik, 1998).

Collection of Latex:

Latex samples were collected early in the morning from each plant species by nipping the leaves or by incisions of the branches of the plant, allowing to drain in the sterile glass tube separately.The samples were brought to the laboratory, kept in refrigerator at 4˚C until use. Latex was homogenized in a homogenizer and filtered through four folds of muslin cloth and used for photochemical analysis.

Phytochemical Screening of the Latex:

Latex samples from each plant used in this study were screened for identification of their phytochemical contents using standard procedures. (Kokate, 1999; Harborne, 1998).

RESULTS AND DISCUSSION

This study was focused on investigating phytochemical properties of latex. Pytochemical analysis of latex is represented in Table: 1. Alkaloid, flavonoid, phenols, tannin, saponin, terpenoids, steroids, glycosides were widely distributed in most of the plant latex. The crude latex of *E. hirta* L. & *P. rubra* L. revealed the presence of all phytochemicals except saponins and steroids. The present investigation agrees with work of Mallesha (2012). *E. tirucalli* latex showed the presence of alkaloid, flavonoid, phenols, tannin & steroids. Anti-inflammatory and analgesic activities and phytochemical constituents of *E. tirucalli* latex have been reported by Prabha *et al.,* (2008).

Table 1 : Phytochemical analysis of some plant latex

Sr. No	Plant sp.	Pytochemicals							
		Alkaloids	Flavonoids	Phenols	Tannins	Saponins	Terpenoids	Steroids	Glycosides
1.	*Euphorbia hirta* L.	+	+	+	+	-	+	-	+
2.	*Plumeria rubra* L.	+	+	+	+	-	+	-	+
3.	*Euphorbia tirucalli* L.	+	+	+	+	-	-	+	-
4.	*Calotropis procera* (Ait) R.Br.	+	+	+	-	+	+	+	+
5.	*Jatropha curcas* L.	+	+	+	-	+	+	-	+
6.	*Jatropha gossypifolia* L.	+	+	+	-	+	+	-	+
7.	*Ficus benghalensis* L.	-	+	+	+	-	-	+	+
8.	*Nerium oleander* L.	+	-	-	-	+	+	-	-
9.	*Ficus religiosa* L.	+	+	+	+	-	-	-	-
10.	*Carica papaya* L.	-	-	-	-	+	+	-	+

Screening of C*. procera* latex revealed presence of all phytochemical constituents except tannin. Goyal and Mathur (2011) reported the antimicrobial potential and pytochemical analysis of *C. procera* latex. Latex of *J. curcas* & *J. gossypifolia* revealed the presence of all phytochemicals except tannins & steroids. These results support the findings of Patil & Borase (2012). *F. benghalensis* showed the presence of flavonoid, phenols, tannin, steroids, and glycosides. *N. oleander* revealed the presence of Alkaloid, saponin, terpenoid, while *F. religiosa* showed the presence of Alkaloid, flavonoid, phenols, tannin, and *C. Papaya* showed presence of saponin, terpenoid, glycosides, Sibi *et al.,* (2013) have reported the phytochemical analysis & antimicrobial activity of various solvent latex extracts.

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RESEARCH ARTICLE

Effect of *Sapindus mukorossi* and *Balanites aegyptiaca* on colour fastness properties of Silk dyed with *Butea monosperma*

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ABSTRACT

The paper reveals the study of Sapindus mukorossi and *Balanites aegyptiaca* as natural soap for degumming of silk was carried out. Three different concentrations 2, 4 and 8 % on weight of fabric were taken and degumming time 30 and 45 minutes was maintained during process. Silk Degummed with sapindus mukorossi and *Balanites aegyptiaca* were mordanted with potash alum and stannous chloride as metal mordent. Butea monosperma flowers were used for dyeing degummed silk, and M:L ratio was kept 1:50. Dyeing was carried out with 60^0C for 60 minutes .Colour fastness properties of sample degummed with sapindus mukorossi and *Balanites aegyptiaca* and dyed with butea monosperma were assessed. It was observed that when increased in soap concentration reetha and hinganbet showed improved wash fastness rated as very good. In case of sunlight fastness there is slight difference was found reetha showed better fastness, where as no difference found among the sample degummed with hinganbet.

Keywords: Sapindus mukorossi, *Balanites aegyptiaca*, Butea monosperma, Silk, Degumming and Fastness properties.

INTRODUCTION

Silk is universally accepted as a luxury fiber. The international Silk Association of the United States emphasizes this by its slogan “Only silk is silk.” Silk has a combination of properties not possessed by any other fiber: It has a dry tactile hand, unique natural luster, good moisture absobtion, lively suppleness and draping qualities, and high strngth (Kadolph, 2006). Silk has been considered as one of the elegant of fiber. it is popularly known as queen of fiber (Satheesh *et al*., 2005) The process of degumming“ Removel of sericin from raw silk fabric is knwon as degumming process Ghembad (2014) Upadhyay and Singh (2012). The sapindus mukorossi (Gaertn) is a fairly large, deciduous tree with a straight trunk up to 12 meters in height. Flowers are about 5 mm across.

The fruit is valves for the saponins (10.1%) present in the pericarp and constitutes upto 56.5 of the drape known for inhibiting tumour cell growth. *Balanites aegyptiaca* (L) Delile is a deciduous tree found native to much of Africa and part of Middle East. This tree reaches 10m (33 ft) in height the yellow single seeded fruit is edible, but bitter (Dubey *et al*., 2011).

Butea monosperma (Lam) is commonly known as flame of forest, belong to the family fabaceae. It is locally knwon as palas. Gneraly it grows gregariously on open grasslands and cattered in mix forest. It is an erect tree 12-15 m height and cattered in mix forest. It is an erect tree 12-15 m high with crooked trunkand irregular branches,bark,rough,ash coloured,young parts downy. and Deshwal, 2011). The plant of this genus are well known for their colouring matters. Flowers of butea monosperma exhibited anticonvulsant activity. (Sharma and Deshwal, 2011).

MATERIALS AND METHODS

Materials:
100% pure silk was used. Silk being a natural protein fibres, only the silk of moth caterpillers has been used for textile manufacturing. hence preferred safe route of using reetha and Hinganbet for degumming. *buteamonosperma* flowers extract was used as a source of natural dye. two metal mordants *potash alum* (Alum) and *Stannous chloride (*Tin) were used.

Experimental Methods:

Degumming with *sapindus mukorossi* (reetha) and *balanites aegyptiaca* (hinganbet):
To make the degumming process eco friendly natural soaps were used for degumming. Optimization and soap concentrations were optimized, Three different concentrations 2, 4, and 8 % of reetha and hinganbbet on weight of fabric were taken, with two different time period. Degumming was carried out at 60^0C 1:50 M: L ratio was maintained during the degumming process. Optimum time for degumming was determined at 60^0C with 1:50 M: L ratio. Time intervals for degumming were 30 and 45 minutes respectively.

Aqueous extraction of *butea monosperma* flowers:
Dye extract was prepared with 60% dye material concentration (OWF) keeping M:L ratio 1:50 extraction was carried out for 30 minutes, temperature was kept 90^0C maintaining the level of extract in the container throughout. The extract was then allowed to cool at room temperature and filtered to remove residual part to get clear solution was then transferred to open bath for exhaust dyeing.

Mordanting:
Mordanting of degummed silk sample was carried out with 10% alum in combination with tin in 9:1proportion (OWF) was taken in to make the process more ecofriendly. M:L ratio of mordanting bath was kept as 1:50. Initial temperature of the mordanting bath was 60^0C and it was slowly raised up to 90^0C. mordanting was carried out for 45 minutes with constant fabric liquor movement. Mordanting bath was allowed to cool. Mordanting was carried out separately for each mordent and experimental sample.

Dyeing:
Mordanted sample was entered into the previously prepared dye bath. The dye bath was set with 1:50 M:L at 60^0C. temperature was slowly raised up to 90^0C. Dyeing was carried out for 60 minutes with continuous fabric liquor movement. The dye bath was allowed to cool for minutes. The dyed sample was removed, washed thoroughly and shade dried. The procedure was repeated for each experimental sample.

Assessment of fastness properties:
Wash fastness (ISO2) (IS:3361-1979)
Sunlight fastness (IS:686-1985)

RESULTS AND DISCUSSION

Table - 1 reveals the Silk sample were subjected for washing fastness 100% silk was treated with 3 different soap concentration for two different time periods in the degumming process. Two natural sources Reetha and Hinganbet were used . Samples degummed using 2% Reetha powder for 30 minutes showed good colour fastness rated 4 on grey scale. Similar results was obtained for sample degummed for 45 minutes. When silk was treated with 4% soap solution for 30 and 45 minutes showed no significant difference in washing fastness which rated 4 as good colour fastness. for 8% Slight decrease in fastness was noted for dyed sample treated for 30 and 45 minutes showed fairly good results(fastness).

Table 1: Washing fastness of Silk Degummed with Reetha and Hinganbet Dyed with Palas

Soap Concentration	Degumming Time	Reetha		Hinganbet	
		C.S	C.C	C.S	C.C
2%	30	5	4	5	4
	45	5	4	5	4
4%	30	5	4	5	4/5
	45	5	4	5	4/5
8%	30	5	3/4	5	4/5
	45	5	3	5	4/5

Table 2: Sunlight fastness of Silk Degummed with Reetha and Dyed with Palas extract

Soap Concentration	Degumming Time	Reetha C.C	Higanbet C.C
2%	30	4	4
	45	4	4.5
4%	30	4.5	4.5
	45	4.5	4.5
8%	30	4.5	4.5
	45	4.5	4.5

It can be said over can discussion increase in soap concentration slight decrease in colour fastness when the sample were degummed with Reetha as a source of natural soap. Table also depicts rating for sample degummed with hinganbet powder it can be seen from the table when degumming time was kept 30 minutes and degummed with 2% Hinganbet powder dyed with palas extract when subjected for washing test it rated good fastness that is(4) 45 minutes degummed time showed no significant difference on fastness which also rated 4 as good fastness. Increased in soap concentration showed improved wash fastness which rated very good wash fastness rated 4/5 increased in degumming time showed no significant difference and noted similar wash fastness. Further increase in soap concentration during degumming time made absolutely similar results with very good wash fastness (4/5).

Kumaresan *et al*. (2011) have studied the application of eco-friendly natural dye on silk using combination of mordants. The colour fastness properties of the flower of Cordia Sebestena dyed on silk were studied using combination of mordants such as myrobolan:nickel sulphate myrobolan: aluminium sulphate, myrobolan: potassium dichromate, myrobolan: ferrous sulphate, myrobolan:stannous chloride in the ratio of 1:3, 1:1, 3:1. The washing, rubbing, light and perspiration fastness of the dyed samples was also evaluated, giving fair to excellent fastness grades.

It is clear from the table 2 that reetha powder was used as source of natural soaps in three different concentrations for two different time periods. It was observed that when 2 % reetha soap was used for degumming and time allotted for degumming was 30 minutes. When dyed with palas extract. Good sunlight fastness that is 4 sunlight fastness. Similarly good fastness was noted for dyed sample which was degummed with hinganbet for 30 minutes. Using 2 % soap concentration for 45 minutes for degumming with reetha silk when dyed similarly good fastness were rated 4. Whereas for same concentration and time period using hinganbet and dyed noted improved sunlight fastness which was rated 4/5 as very good fastness. It was further noted that increase in soap concentration that is 4%for degumming and 8% with increased time period of degumming from 30 minutes to 45 showed very good sunlight fastness which rated 4/5 on grey scale for all the samples.

Where it can be said that there is slight difference between reetha showed better fastness properties, where as no difference was found among the sample degummed with hingan.

CONCLUSION

From the result it can be concluded that when degumming with *balanites aegyptiaca* as a natural soap found better performance compared to reetha.

Metal mordant helps in improving colour fastness properties degumming with *balanites aegyptiaca* natural soap imparted better fastness properties compared to Sapindus mukorossi.

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RESEARCH ARTICLE

Amino acid analysis of three accessions of *Physalis philadelphica*

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ABSTRACT

Physalis philadelphica commonly called as Mexican husk tomato is widely grown as a minor crop and extensively used in western countries as it responds positively to any type of soil and climate. The fruits are used for the preparation of curries, jams, sauces and jellies and the taste is similar to tomato. A steroid Physalin extracted from leaves and roots has prophylactic and therapeutic properties for treating infections caused by protozoans. Also it is used in autoimmune diseases and it acts as anti-leukemic agent. The Mexican people use decoction of calyces for diabetes. This species attracts considerable attention because of its economic as well as medicinal properties. Information relating to the biochemical work on species of *Physalis* is meagre. Therefore with a view to study the biochemical variability, three accessions of *Physalis philadelphica* from different sources were collected and to employ these three accession in biochemical investigations to study qualitative and quantitative analysis of free and protein amino acids and their effect on the morphology of the three accessions.

Keywords: Accession, Free Amino acid, *Physalis*, Protein Amino acid

INTRODUCTION

Physalis philadelphica commonly called as Mexican husk tomato is a native of Mexico belongs to family Solanaceae. This plant is widely planted as a minor crop and extremely used in western countries as it responds positively to any type of soil and climate. The fruits are used for the preparation of sauce, jams and jellies and the taste is similar to tomato. A steroid Physalin extracted from leaves and roots has prophylactic and therapeutic properties for treating infections, auto immune diseases and it also acts as anti leukemic agent. It attracts considerable attention because of its economic as well as medicinal properties. Keeping in view, three accessions of *Physalis philadelphica* were collected from different sources. All these three accessions had a significant difference in pollen fertility of flower. On the basis of their

pollen fertility they were named as P1, P2, P3. In view of the variations in the pollen fertility, the flowers of these three accessions were studied for free and protein amino acids by using AUTOMATIC AMINO ACID ANALYSER.

MATERIALS AND METHODS

The seeds of different accessions of *Physalis pheladelphica* was collected from different sources were given in the Table :1

Table 1:

S.No	Accession	Source	Collected by
1	P1	U.S.A. E.C.No.291558	George, A White Plant introduction officer, Maryland.
2	P2	U.S.A. E.C. No.291559	-do-
3.	P3	U.S.A. E.C.No.270459	Director, Royal Botanical Garden, KEW.

Method The fresh flowers of P1, P2 & P3 accessions were collected for the estimation of free and protein amino acids by using Automatic Amino Acid Analyser.

RESULTS AND DISCUSSION

Seed germination and survival rate of *Physalis philadelphica* were slightly less in P3 compared to P1 and P2. In contrast to P1 and P2, in P3 the flowering was one week delayed and pollen fertility and seed set were significantly reduced.

In view of significant variation in their pollen fertility, the flowers of the three accessions were studied for free and protein amino acids by Amino Acid Analyser. Free and protein amino acids and their quantities in the fresh flowers of the three accessions were compared, each accession presented a different pattern, as can be expected from the fact that they were affecting different morphological parameters. In addition to the amino acids present in the standard aminogram some peaks were present in the aminograms of three accessions representing unknown amino acids.

Amino acids aspartic acid, cystine and methionine were present in the flowers of the P1 and P2 and were in traces in P3. On the other hand amino acids leucine, threonine and serine were present in the flowers of the P3 and were in traces in the flowers of P1 and P2.

Table 2: Free and protein amino acid composition (micromoles/gram) of fresh flowers of (P_1,P_2 & P_3) *Physalis philadelphica*.

Amino acid	P1		P2		P3	
	Free amino acid	Protein amino acid	Free amino acid	Protein amino acid	Free amino acid	Protein amino acid
Aspartic acid	1.5	2.5	0.50	0.02	*	0.001
Throenine	0.001	0.5	0.001	0.80	3.50	*
Serine	0.01	2.0	*	0.01	0.13	1.9
Glutamic acid	*	8.0	1.10	6.50	4.10	*
Proline	*	106.6	*	108.0	*	29.2
Glysine	2.7	25.3	0.50	23.4	*	*
Alanine	*	*	0.10	*	*	72.1
Cystine	1.5	103.0	1.20	102.0	*	10.0
Valine	*	62.7	1.10	60.5	0.20	21.2
Methionine	0.01	2.8	0.80	7.90	0.001	7.60
Isoleucine	0.01	29.2	0.30	28.5	0.01	38.1
Leucine	0.01	8.5	0.001	6.50	0.06	19.3
Tyrosine	0.025	18.4	0.01	19.1	0.001	6.50
Phenylalanine	0.32	25.7	1.11	27.1	0.01	47.9
Histadine	0.15	18.3	0.16	16.8	0.80	25.8
Lysine	0.006	13.8	0.01	12.8	*	26.1
Arginine	*	*	*	*	*	18.7
Total	**6.242**	**427.30**	**6.892**	**418.93**	**8.813**	**344.20**

Amino acid composition of the flowers of the P3 permits several kinds of comparisons. In protein amino acids, glutamic acid was missing in P3 while it was present in some quantity in P1 and P2. This amino acid accumulated as free acids in the flowers of the P3. Indeed these are the only two amino acids to accumulate as free acids while others present in traces. Secondly, glycine was absent either as free or protein amino acid in the P3, while it was present in considerable quantity among the free and protein amino acids of the P1 and P2. Thirdly, P_3 has greatly reduced quantities of proline , cystine, valine and tyrosine. Fourthly, the only amino acid that was significantly in excess of the P_3 was arginine which was lacking in the P1 and P2 either as free or as a protein acid. All these features point out a gross deficiency of total proteins and a significantly altered protein composition of the P_3 accession.

Amino acids, the initial products of nitrogen assimilation are building blocks of proteins. Characteristic differences in some bound and free amino acid fractions have been detected between the flowers of the three accessions. Such differences in the amino acid patterns of the free and bound fractions were also reported in the species and species hybrids of *Gossypium* (Sarvella and Stojanovic, 1968).

In the present investigation the observed deficiency or increase in the quantity of all free amino acids in these three accessions may be explained due to a partial or complete block of synthesis of one or more specific proteins. Patterson et al. (1986) recorded an increase in the total quantity of free amino acids in the male steriles of *Gossypium* over their non-segregating male fertile and they attributed this phenomenon to a partial block of protein synthesis.

The P3 accession in the present study was by and large partial pollen sterile and it differ in its degree compared to P1 and P2 accessions, which were highly pollen fertile. Among the amino acids, proline is an important source of nitrogen in plant metabolism (Britikov et al., 1970). Besides, it is an important component of many biochemical substances produced during the course of sexual differentiation (Kaul, 1997) and is generally correlated with fertility of seed or pollen (Duvick, 1965). On the other hand glutamic acid is considered to be a precursor of proline in plants (Coleman and Hagerly, 1957 Vogel and Bonner, 1954). The lack of proline in plants is due to the retardation in the conversion of glutamic acid to proline (Fukasawa, 1962). Some of the amino acids already mentioned above regulate the growth and development of plant parts as well as the pollen or seed fertility.

In the present investigation also some of the amino acids proline ,glutamic acid and glycine are deficient in P_3 compared to P1 and P_2. Thus an overall reduction in quantity of above said amino acids may be are of the factors responsible for reduced pollen fertility and seed set in P_3. It is generally known that accumulation of free amino acid followed by deficiencies in the protein fraction causes sterility. In the present study also total accumulation of free amino acids followed by deficiency of the protein fraction causes pollen sterility and such situation was encountered in the species hybrids of *Gossypium* (Sarvella and Stojanovic, 1968).

The factors that cause accumulation or depletion of certain free amino acids in the flower may be manifold. The differences recorded in amino acid composition of sample materials of P3 accessions may not be due to environmental influence because all the plants were grown under the same environmental conditions, but probably due to gene or gene cytoplasmic interactions.

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REVIEW ARTICLE

Role of Phytochemicals in Neutralizing the Adverse Effects of Ozone Depletion

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ABSTRACT

Ozone layer, a natural sunscreen of earth, shields human beings from harmful ultraviolet radiation of the Sun. Ozone is a toxic, unstable gas and naturally present in the stratosphere zone of atmosphere. Ozone layer absorbs ultraviolet radiation and thus prevent from reaching on the Earth's surface. When destruction of stratospheric ozone increases with respect to its production, this phenomenon alters ozone balance and aggravates ozone depletion.Natural phenomena and man-made compounds such as chlorofluorocarbons cause ozone depletion and thus allow ultraviolet radiation to arrive at earth. Small amounts of ultraviolet radiations are essential in the production of vitamin D but excess exposure develops oxidative stress by generating reactive oxygen species (ROS). These reactive oxygen species has genotoxic and carcinogenic effects and produces various organ disorders. The adverse effect of ultraviolet radiation can be neutralized by phytochemicals. Phytochemicals are non-nutritive plant chemicals (viz. lycopene in tomatoes, isoflavones in soy and flavanoids in fruits) that act as antioxidant and neutralize the free radicals generated during exposure of ultraviolet radiation.

Key Words: Ozone depletion, phytochemicals, reactive oxygen species, ultraviolet radiation.

INTRODUCTION

Ozone denoted as O_3, is a gas naturally present in atmosphere. Ozone is found primarily in two regions of the atmosphere viz. troposphere (10%); the region closest to Earth (from the surface to about 10-16

kilometres) and the remaining ozone (about 90%) resides in the stratosphere between the top of the troposphere and about 50 kilometres altitude. The high concentration of ozone in the stratosphere is referred as *ozone layer*. The ozone layer was discovered in 1913 by the French physicists Charles Fabry and Henri Buisson (ozone.unep.org).

Ozone formation is a multistep chemical process that requires sunlight. In the stratosphere, ozone formation begins with degradation of an oxygen molecule (O_2) by ultraviolet radiation of the sun. In the troposphere ozone is formed by a different set of chemical reactions that involve naturally occurring gases and those from pollution sources.

Ozone formed at Earth's surface in excess of natural amounts is considered as *bad ozone* because of its harmful effect to human, plants, and animals whereas, stratospheric ozone absorbs about 95-99% of destructive ultraviolet radiation emitted by the Sun and thus considered as *good ozone*. But nowadays, depletion of stratospheric ozone is a global environmental problem, increasing incidence of various disorders. For prevention of the adverse effect of ozone depletion, reducing excessive exposure to solar radiation is desirable but this approach is unavoidable. Some novel strategies like use of phytochemicals may be a good approach in minimizing the destructive effect of UV radiation. Phytochemicals are photoprotective in nature because of their potential antioxidant properties.

Ozone depletion

Industrial processes and consumer products result in the emission of ozone depleting substances (ODSs) to the atmosphere. The main ozone depleting substances are chlorofluorocarbons (CFCs), hydrochlorofluor carbons (HCFCs), carbon tetrachloride, halons (brominated fluorocarbons) and methyl chloroform. The chlorofluorocarbons (CFCs) are used in almost all refrigeration and air conditioning systems, and the halons are used in fire extinguishers. Halons can destroy up to 10 times as much ozone as CFCs can. A 1% decrease in the ozone layer is estimated to increase UV-B radiation by 2% (www.enviropedia.org).

The phenomenon of ozone depletion starts with emission of chlorine and bromine containing gases at Earth's surface. These nonreactive gases accumulate in the troposphere and transported to stratosphere in super reactive form by natural air motions and thus destroy ozone layer. Rain and snow removes some reactive chlorine and bromine gases from Earth's atmosphere (www.enviropedia.org).

Stratospheric ozone which absorbs the solar ultraviolet radiation is a basic bio-protective filter and its degradation leads to increase of UV radiation level in environment that constitutes a danger for health of the whole human population. Small amounts of UV radiations are essential in the production of vitamin D and thus beneficial for human being. UV radiation is also used to treat several diseases, including rickets, psoriasis, eczema and jaundice. The adverse health effects associated with stratospheric ozone depletion are primarily related to the pulmonary organ, skin, eyes, and immune system (Armstrong, 1994).

Genotoxic effect

The genotoxic potential of ozone is caused by the reaction of ozone and its reactive intermediaries (ORI) with cellular macromolecules. When ozone is dissolved in biological fluids it decomposes and reacts instantly with unsaturated fatty acids in cell membranes generating ORI such as hydrogen peroxide (H_2O_2), aldehydes, ozonides and lipid peroxides. Proteins in plasma and cell membranes are oxidised, mainly in thiol groups. Nucleic acids are unlikely to be damaged by ozone itself, but the cascade of ORI that is generated may have genotoxic effects (Diaz-Llera *et al.*2002).

Carcinogenic effect

In humans, chronic exposure to ultraviolet (UV) radiation induces nonmelanoma skin cancer (NMSC). Wavelength UV-B (290-320 nm) is more harmful as during exposure, it is easily absorbed into skin which results in development of erythema, burns and skin cancer. In UV induced skin carcinogenesis, DNA damage occurs which facilitates incorporation of wrong bases into the genetic material, leading to disruption in cellular processes. This result in mutation which leads to inappropriate expression of affected genes (Ananthaswamy, 2001).

Skin cancer may develop due to mutation in p^{53} protein as it is a tumor suppressor gene and also

involved in apoptosis. The p^{53} protein functions as a guardian of the genome by aiding DNA repair of cells with excessive DNA damage (Jankowski and Cader, 1997).

Acute effects of UV radiation include photokeratitis, photoconjunctivitis, DNA damage, lipid peroxidation and protein crosslinking that lead to erythema, sunburn and immunosuppression. Chronic effects include cataract, Pterygium, squamous cell carcinoma of the cornea or conjunctiva and skin cancer (Jankowski and Cader, 1997; www.cheneysd.org).

Effect on pulmonary organ

Ozone is a powerful oxidant and toxic gaseous pollutant. The primary target tissue of ozone is the lung and breathing elevated concentrations of ozone results in a range of respiratory symptoms viz. decreased lung function, increased airway hyper-reactivity, asthma and chronic obstructive pulmonary disease (COPD)(Kosmider *et al.*2010).

After inhalation ozone first reaches to the lung lining fluid compartment, where it reacts with other substrates such as proteins or lipids and thus secondary oxidation products arises which transmit the toxic signals to the underlying pulmonary epithelium followed by initiation of a number of cellular responses. These responses include cytokine generation, adhesion molecule expression and tight junction modification. Together, these responses lead to the influx of inflammatory cells to the lung in the absence of a pathogenic challenge. Moreover, lung permeability is increased and oedema develops (Mudway *et al.,* 2000; Bocci, 2006).

Effect on eyes

UV radiation damages the human eye lens, hastening the deterioration that leads to age related cataract. The effectiveness of UV radiation, causing damage to ocular tissue is wavelength dependent as UV-B radiation at 280-320 nm is an important risk factor for cortical cataract. Additionally, UV-A wavelengths (320-400 nm) and visible radiation have also been implicated in the aetiology of cortical cataract in humans (www.epa.gov).

Effect on skin

Exposure of human skin to UV-B radiation results in excessive generation of reactive oxygen species (ROS) that overwhelms the antioxidant defence system resulting in oxidative stress. Skin related disorders such as photoaging and photocarcinogenesis are mediated by the generation of ROS (Ichihashi, 2009).

Suppression of immune system

UV radiation appears to diminish the effectiveness of the immune system by changing the activity and distribution of the cells responsible for triggering immune responses. Immunosuppression can cause reactivation of the viruses like herpes simplex virus in the lip (Norval *et al*., 2007).

Approaches to combat adverse effects of ozone depletion

For combating the adverse effects of ozone depletion, there is need to reduce the emission of ozone depleting substances and also, adapt the approaches to neutralize them.

Hydrofluorocarbons (HFCs) are strong greenhouse gases and used as substitute for chlorofluorocarbon (CFCs) and hydrochlorofluorocarbons (HCFCs). CFCs and HCFCs are used in vehicle air conditioning and theses gases are powerful destructive factors of ozone (www.enviropedia.org).

Use of Phytochemicals

Phytochemicals are non-nutritive plant chemicals viz. lycopene in tomatoes, isoflavones in soy and flavanoids in fruits, have protective or disease preventive properties. Phytochemicals acts as an antioxidant and thus helps in reducing the free radicals generated during exposure to UV radiation.

Lycopene

Lycopene is a pigment, gives red colour to many fruits and vegetables and also act as an antioxidant, thus prevents destructive effects of free radicals. The lycopene in tomatoes may reduce sun damage by 35 % (www.lycopene.com).

Oral lycopene have a protective effect against UV skin damage. Like sunscreens which provide external protection from UV radiation, lycopene in the diet might provide internal protection from free radicals. Since, lycopene is a lipid, for better absorption, it should be consumed with oil.

Flavonoids

Flavonoids are pigments present in different indispensable components of human diet viz. fruits, vegetables, nuts and beverages. Flavonoids possess antioxidative, anti-cancer, anti-inflammatory and anti-viral properties. Flavonoids are small organic compounds which have been normally absorbed by the human body for long time and thus can be used as safest non-immunogenic drugs. They reduce ozone induced toxicity by working as enzyme inhibitors, antioxidants, hormones or immune modulators (Lee *et al.* 2007).

Isoflavones

Isoflavones are polyphenolic compounds that are capable of exerting estrogen-like effects. Isoflavones have potent antioxidant properties, comparable to that of the well known antioxidant vitamin E. Genistein is the most potent antioxidant among the soy isoflavons, followed by daidzein. It reduces the long term risk of skin cancer by preventing the free radical damage to DNA (Wei *et al.* 1995; 2003). Isoflavones have an anti-aging effect on the UV-damaged hairless mice model, which is partly due to the inhibitory effects on UV-induced MMP-1 (metalloproteinases-1) expression and the subsequent collagen degradation (Kim *et al.* 2004).

Polyphenols

Polyphenols viz. epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) are present in green tea. All of these polyphenols act as potent antioxidants and can scavenge ROS, such as lipid-free radicals, superoxide radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen. EGCG has been considered to be the main compound responsible for these effects, constituting approximately 40% of the total polyphenolic mixture (Katiyar *et al.* 2001; Afaq and Mukhtar, 2006).

Polyphenolic compounds viz. anthocyanidins and hydrolysable tannins possess strong antioxidant and anti-inflammatory properties. Pomegranate is a rich source of two types of polyphenols (Afaq *et al.* 2005).

A polyphenolic phytoalexin, resveratrol (trans-3, 4', 5-trihydroxystilbene) is found in the skin and seeds of grapes, nuts, fruits, and red wine. It is a potent antioxidant with anti-inflammatory and antiproliferative properties (Adhami *et al.,* 2003; Afaq *et al.,* 2003). It potentially inhibits generation of hydrogen peroxide, infiltration of leukocytes and skin oedema which occurs due to exposure of UV radiation (Afaq *et al.* 2003).

Other nutrients

Nutrients viz. vitamin E, vitamin C, ratinoids and linoleic acid act as antioxidant and suppresses the effect of UV radiation induced effects in human beings.

CONCLUSION

Thus, many phytochemicals, with antioxidant properties exert anti-inflammatory, cancer-preventive and anti-photoaging effects on the skin. This suggests the possibility of these phytochemicals for the prevention and treatment of a variety of human skin disorders. Theses phytochemicals acts as free radical scavenger that will reduce the adverse effects of UV radiation induced by ozone depletion.

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