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Hypoglycemic potential of *Momordica charantia* Linn in Streptozotocin induced diabetic Albino mice

Rakesh Agarwal¹✉, Yogi Amrit Raj¹ and Dinesh Kumar²

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Abstract

In order to check hypoglycemic property of *Momordica charantia*, experiments were conducted in streptozotocin induced diabetic albino mice. The LD₅₀ of STZ was seen to be 650 mg/kg bw. Significant reduction in blood glucose level was seen within 24 hours after administration of ethanolic extract of MC. On administration of subsequent doses of ethanolic extract, significant decrease has also been seen in serum glucose level, creatinine, ALT, AST, AP of STZ induced albino mice. Thus one or other part of MC can be used in lowering down blood glucose level.

Keywords: Glucose, Kidney function, Liver function, *Momordica charantia*

Introduction

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders causing hyperglycemia, which is due to impaired carbohydrate “glucose” utilization resulting from a defective or deficient insulin secretory response. Along with hyperglycemia, there is also an abnormality in serum lipids (Reaven, 1988). The disease causes morbidity and long-term complications and an important risk factor for cardiovascular diseases (Yeh *et al.*, 2003). To-date there are different groups of oral hypoglycemic drugs and insulin for clinical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing the demand for complementary and alternative medicine with antidiabetic activity and less side effects.

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Numerous herbal preparations have been shown to affect blood glucose levels through various mechanisms, although they are usually limited by toxicity or relative lack of efficacy compared with standard medications. The lack of standardization of ingredients and preparation also causes problems. *Momordica charantia* L. belonging to the family “Cucurbitaceae” is a vegetable indigenous to tropical areas, including India, Asia, South America and Africa, also known as balsam pear. MC is also, used for treating various diseases, one of which is diabetes mellitus (Virdi *et al.*, 2003). The hypoglycemic potential of MC has been demonstrated in normal and diabetic rats (Shibib *et al.*, 1993 and Srivastava *et al.*, 1993). MC can improve glucose metabolism (Welihinda *et al.*, 1986) and the over all condition of persons with diabetes, not only through a direct hypoglycemic effect but also by improving lipid metabolism (Virdi *et al.*, 2003). From reviewing the literature the mechanism(s) whereby MC lower blood glucose remains uncertain. Thus, the aim of the present study is to evaluate the LD₅₀ and biochemical effect of MC ethanolic extract in diabetic mice. This will be a guide to the separation and synthesis of the most active plant

substance for clinical application on one hand and its proper utilization in traditional medicine on the other.

Materials and Method

Plant Material

We purchased 1 kg of unripe fresh fruits of MC from local market in Patna and certified by Botany Department of Patna University.

Extraction of Plant materials

The fruits were cut into small pieces and placed in percolator and then submerged into 95% double distilled Ethanol for 24 hours. After 24 hours ethanol was decanted into conical flask and the extract was stored in deep freezer. The process was repeated for 3 to 4 times so that all the extract from plants came into the ethanol and extract was collected in the same conical flask. The ethanol with plant extract was placed into the Rota vapor (BUCHI-011) under low pressure to separate the Ethanol from plant extract. The temperature of water bath should be maintained between 45° C to 50° C. Ethanol was vaporized and collected into another bottle flask and plant extract was left into the same bottle flask.

Test animals

In the experiments performed, adult albino mice of both sexes weighing 25-30 g were used. Standard diet was provided and water was available on bottle. Following an over night fast, whole blood was obtained without anaesthesia from the retro orbital venous plexus (Madway *et al.*, 1969); All animal procedures were performed after blood collection.

LD₅₀ experiment

The LD₅₀ was determined using mice according to the method described by Karber (1931). The MPD was also determined. The symptoms of acute toxicity and postmortem finding were recorded.

Study design and dosage

The animals were divided into two groups, one normal, while other was rendered diabetic by injection of 100 mg/kg of STZ dissolved in 1 mm citrate buffer at pH 4.5 for 2 consecutive days. Diabetic groups were further divided into 2

subgroups of ten mice each. The first subgroup was not treated with any thing and maintained as control. The second subgroup was treated with 200 mg/kg extracts of MC fruit respectively for 21 days.

Also, the diabetic untreated mice were maintained for further study in parallel to that of diabetic treated one.

Assay

Serum glucose, creatinine, serum alkaline phosphatase, transaminases. AST and ALT, were measured by kits.

Statistical Data

Data are shown as mean $\pm$ SE. The statistical analysis was performed with the analysis of standard deviation. The results obtained at the end of each time phase were compared with those obtained from zero time from the same group.

Results and Discussion

LD₅₀ evaluation

The toxic symptoms of MC alcoholic extract in mice included increased respiratory rate and strong heart beats. After 2 h post injection, the animals suffered from general depression, shallow deep respiration and very weak hearts that ended by death. The LD₅₀ of the ethanolic extract of MC was found to be 650 mg/kg.

Effect of serum glucose level (Fig.1)

The administration of MC ethanolic extract induced a significant decrease in serum glucose levels as compared with its zero time. But there were no reduction shown by control diabetic group in the period of 21 days.

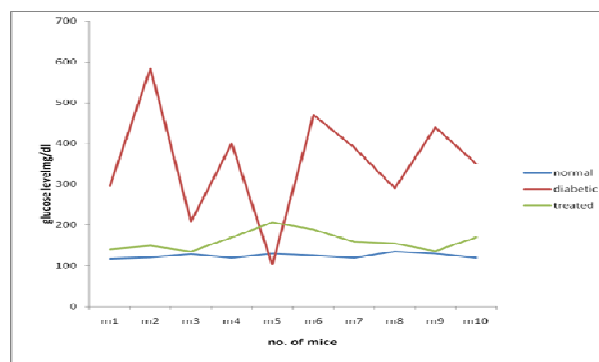
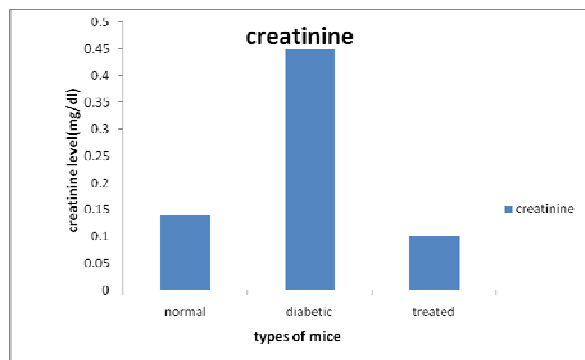


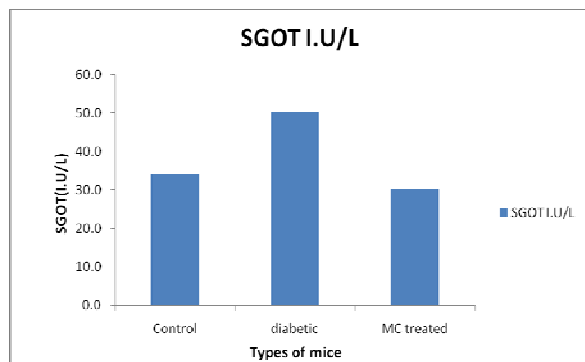
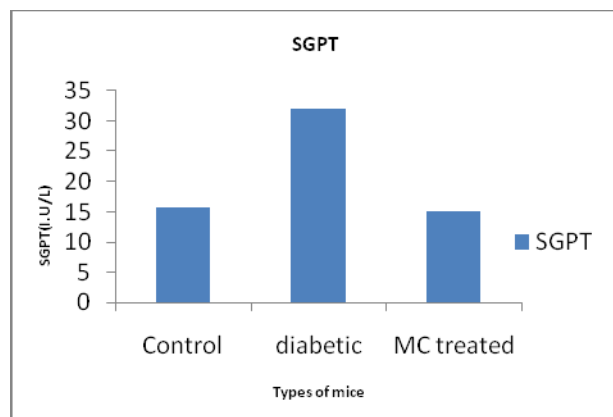
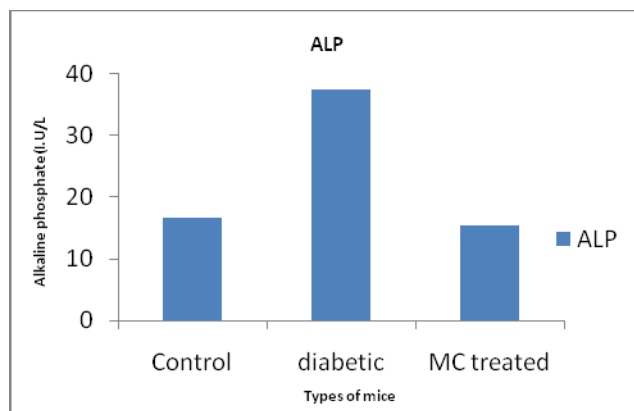
Fig.1: Effect of serum glucose level

Effect on Kidney function (Fig. 2)

Contrary to normal mice, the administration of MC ethanolic extracts exhibited a highly significant effect in serum creatinine levels.

**Fig.2: Effect on Kidney function****Effect on liver function (Fig.3a,b,c)**

Also contrary to normal mice, the administration of MC ethanolic fruit extracts caused a very highly significant effect on enzymes of liver function “ALT, AST and AP” at 21 days of treatment.

**Fig.3a****Fig.3b****Fig.3c**

In the present study, the toxic effect of ethanolic extracts of MC was measured. LD₅₀ was calculated. The post mortem examination revealed congestion of internal organ. The obtained results in the diabetic mice using dose of 200 mg/kg bw/day. On the other hand, data from the present study demonstrated that the administration of MC ethanolic extracts to diabetic mice induced a significant decrease in serum glucose levels. This finding run parallel with that obtained by Ali *et al.* (1993), Srivastava *et al.* (1993), Rao *et al.* (1999), Chen *et al.* (2003), Matsuura *et al.* (2002), Yeh *et al.* (2003) and Miura *et al.* (2004). It was also reported by Chen *et al.* (2003) that MC has been shown to inhibit glucose absorption, promote hepatic glucose utilization (Meir and Yaniv, 1985 and Shibib *et al.*, 1993), possess an insulin-like polypeptide (Khanna *et al.*, 1981), and even to increase insulin positive cell number in the pancreas (Ahmed *et al.*, 1998). Furthermore, an insulin like protein called “insulin p” isolated from MC has been reported to possess hypoglycemic properties when injected subcutaneously (Baldwa *et al.*, 1977, Khanna *et al.*, 1981 and Ng *et al.*, 1986). At the same time the administration of MC ethanolic extracts to diabetic mice revealed significant change in kidney and liver function during the experimental period of 21 days. In STZ-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum creatinine, ALT, AST, AP. After the treatment of diabetic mice with MC ethanolic extracts for a 21days period caused a significant reduction in glucose level, creatinine, ALT, AST and AP. This finding of the present study on kidney and liver function are compatible with many published

results (Rao *et al.*, 1999, Jayasooriya *et al.*, 2000 and Ahmed *et al.*, 2001). Administration of MC ethanolic extract in diabetic mice induced a significant decrease in glucose level, creatinine, ALT, AST and AP. Also, the seed powder of MC has also been shown to have a hypolipidemic effect in diabetic rabbits (Kedar and Chakrabarti, 1982). In conclusion, it is obvious from the present study that MC has beneficial effects on blood glucose level as well as improving kidney & liver function. These results could be used in the medical treatment in case of deficiency of insulin hormone by using a medicinal plant.

Acknowledgement

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Hydrological conditions of River Beas and its fish fauna in Kullu Valley, Himachal Pradesh, India

Ashok Kumar

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Abstract

The present communication highlights the hydrological regime of Beas river in Kullu Valley which was studied at four sites during the whole year of 2003. Flow of current ranged between 378 - 432 cubic feet/sec. Depth of river ranged between 1-4 mts. Width of the river ranged between 40-50 mts. Water temperature ranged between 5.2 °C -17.7 °C. pH showed only little fluctuation. Transparency of the water was high throughout the year except rainy season. Dissolved oxygen was high and it showed an inverse relationship with water temperature. Total alkalinity was also high. Investigation of fish fauna of the river in the valley revealed the presence of 6 species of fishes belonging to 3 orders and 3 families.

Keywords: *Beas*, *fish fauna*, *physico-chemical*, *hydrological*, *fish catch*

Introduction

Kullu district is situated between 31° 58' 00" N latitude and 77° 06' 04" E longitude. The district of Kullu forms a transitional zone between the lesser and the greater Himalayas and presents a typical rugged mountainous terrain with moderate to high relief. The altitude varies from 1300 meters to over 6000 metres from the mean sea level. The high reaches are bestowed with magnificent snow peaks and glaciers. The important glaciers of the district are Kalihen, Beaskund, Saraomga, Tirchu, Parbati, Dibbi and Mantalai.

The river Beas originates from southern slope of Rohtang pass (Beas Kund) at an elevation of 4062 m (msl). The water of Beas and its tributaries remains shallow, rapid cool and clear except during the rainy season. The bed comprises of mainly the boulders, stones and rubble. The river receives a number of tributaries both on right and left banks during its downward drift of over 470

km. Its principal tributaries are Solang, Manalsu, Sujjain, Fojal and Sarvari on the right bank and Alain, Duhagan, Chhaki, Haripur Nalah, Parbati, Tirthan and Sainj on the left bank. The vegetation along the banks consist mainly of the alnus, willow, rubenia and conifers.

The brown trout *Salmo trutta fario* (Linnaeus) was first of all successfully transplanted in the river Beas in the first decade of twentieth century *i.e.*, in 1909 (Howell, 1916). Since after its introduction in the valley its stock is being replenished by stocking trout fry in river Beas and its tributaries. Till date, very limited work has been done on the ecology of River Beas. Shah (1975) reported the food and feeding habits of brown trout in River Beas. Observations were therefore made in the river Beas to study the fish fauna and hydrological conditions in selective stretches. The location of the sampling sites along the River Beas in Kullu district is shown in Fig. 1.

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Materials and Method

In the River Beas, four sampling sites were chosen namely, Bhuntar, Kullu, Patlikulah and Manali. Collections were made from these sites at fixed

time and almost remained uniform throughout as far as possible. The hydrological conditions of the river Beas viz., water current, depth, width, longitude, latitude and altitude. Water current (cubic feet/sec.) was calculated by cork floating method. The flow of current was measured by the following formula: $R = WDaL/T$.

The river depth was calculated with the help of a marked bamboo stick. It was recorded from at least 5-6 points having different depths and the average of all these points was considered as the mean depth. The river width was measured with the help of measuring tape. Longitude, latitude and altitude (msl) were determined with the help of Magellan GPS.

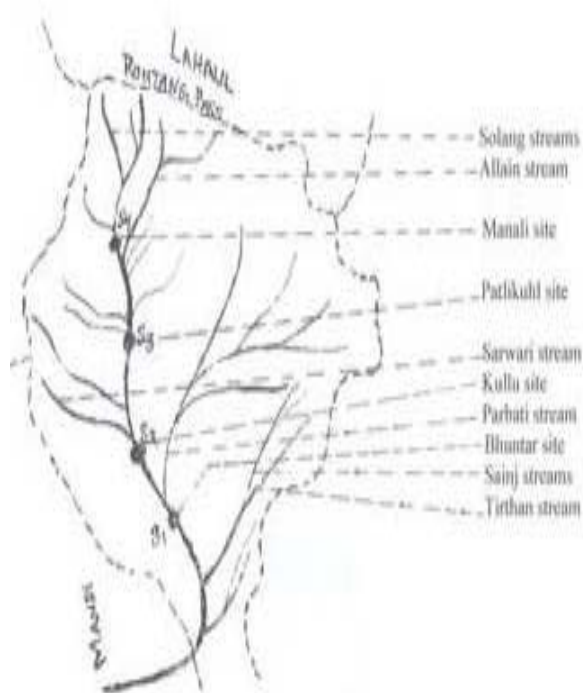


Fig.1: Location of the sampling sites along the river Beas in Kullu District, Himachal Pradesh

The physico-chemical parameters of water of the River Beas viz., water temperature, pH, transparency, dissolved oxygen, total alkalinity were analyzed fortnightly following APHA (1998). Due to shallow depth, stony bottom and fast current, the transparency was measured by bright pin head method (Saha *et al.*, 1971). Hydrogen ion concentration of water was determined by digital pH meter. For dissolved oxygen, unmodified Winkler's method was adopted (Welch, 1948), while carbonate and bicarbonate (total alkalinity) were determined by

titration method with N/50 Sulphuric acid using phenolphthalein and methyl orange as indicators. Fishes were collected regularly by some indigenous methods viz., by bait and hook and by cast net. Collected specimens were preserved in 5% formalin solution with a small abdominal incision. Fishes were identified using key given by Jayaram (1999).

Results and Discussion

The physico-chemical parameters studied in the River Beas includes water temperature, transparency, pH, dissolved oxygen and total alkalinity. Monthly variations in physico-chemical parameters of water are shown in Table-1.

Table.1 Monthly fluctuations in physico chemical factors in River Beas during the year 2003

Months	Water Temperature (°C)	Transparency (cm)	pH	Dissolved Oxygen	Alkalinity (ppm)
January	5.2	47.8	7.6	12.0	70.6
February	7.1	43.3	7.4	11.2	85.0
March	9.2	46.1	7.4	11.2	83.1
April	11.3	51.5	7.3	10.0	80.1
May	10.0	29.3	7.1	10.8	61.2
June	12.8	10.2	7.3	10.2	57.5
July	16.0	6.1	7.5	9.7	59.3
August	17.7	7.7	7.7	9.2	53.7
September	17.1	9.9	7.0	9.9	56.2
October	12.9	29.5	7.0	10.9	67.5
November	8.1	53.2	7.4	11.4	75.6
December	8.2	62.3	7.5	12.8	75.6

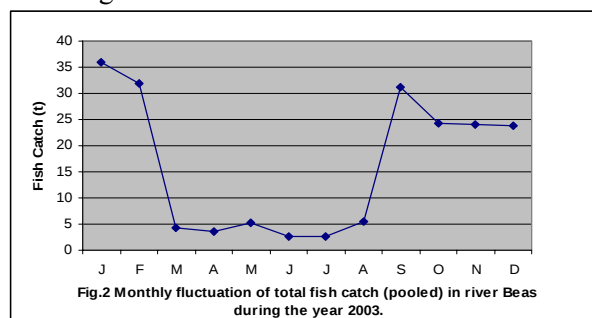
Temperature showed distinct seasonal variation. Water temperature (average) ranged from 5.2 °C (January) to 17.7 °C (August). The pH of the river water was always found to be in the alkaline range (7.0-7.7). River water was clear and transparent during winter and transparency low during monsoon (6.1cm) which was due to fast current and greater inflow of muddy rain water. Total alkalinity was high and the value was maximum in the month of February (85.0 ppm) and minimum (53.7 ppm) in the month of August. Dissolved oxygen was quite high and showed wide fluctuation. Its value was maximum in December

(12.8 ppm) and minimum during August (9.2 ppm). Fish catch in River Beas in Kullu valley and its composition during different months are depicted in Table 2 and graphically shown in Fig. 2. Fish catching was maximum during January (36.0 tons) while minimum in the month of July (2.6 ton).

Table 2: Fish catch in river Beas

Months	Catch(t)
January	36.0
February	31.9
March	4.3
April	3.6
May	5.3
June	2.7
July	2.6
August	5.4
September	31.1
October	24.2
November	24.1
December	23.8

The literature is available on the fish fauna of the River Beas (Tandon and Sharma, 1976; Sharma, 1979 and Tandon and Sharma, 1984; Sehgal, 1970; Shah, 1975). Earlier, during its flow it was a fast flowing typical hill stream with a network of rivulets in the form of khads and ravines and harboured a variety of rich hill stream fish fauna and indigenous fish fauna.



The fish fauna of River Beas include two exotic fishes namely *Salmo trutta fario* L. and *Onchorhynchus myskiss*. According to Jayaram, (1999) it should be *S. gairdnerii gairdnerii richardsonii*) and only one native fish i.e. *Schizothorax richardsonii*. Data is not available on four fish species; hence, their exact status is not evaluated. As *S. trutta fario* feeds voraciously on the young ones of *S. richardsonii*, there is a possibility that in the near future, the population of

S. richardsonii may decline in the river. *Onchorhynchus myskiss* has been reported to feed on insect larvae under natural conditions; therefore, it may not affect the population of *S. richardsonii*.

A survey of fish fauna at different study areas of the River Beas revealed the presence of following Pisces:

Species	Local name
Order: Cypriniformes Family: Cyprinidae	
1. <i>Schizothorax richardsonii</i> (Gray)	Gurgal, Googly
2. <i>Gara gotyla gotyla</i> (Gray)	
3. <i>Nemacheilus rupecola</i> (Mc Clelland)	Natwa
Order: Siluriformes Family: Sisoridae	
4. <i>Glyptothorax indicus</i> (Talwar)/ Syn. <i>G. horii</i> (Show & Shebbeare)	
Order: Salmoniformes Family: Salmonidae	
5. <i>Salmo trutta fario</i> Linn.	Brown trout
6. <i>Onchorhynchus myskiss</i>	Rainbow trout

Further *Onchorhynchus myskiss* is most suitable for culture in high altitude waters, as it is easy to domesticate, accepts artificial feed, withstands low oxygen content of water, shows more resistance to disease especially ferunculosis and exhibits fast growth rate. Hence, its culture in captivity has been undertaken successfully in Kullu region (Johal, 2001). Our observation and the earlier observations have indicated that during the past few years the native fish species have been adversely affected by anthropogenic factors in the upper catchments area of the hill streams. Thus snow trout populations have declined not only due to over fishing, but also by the use of unscientific methods of capture and changes brought about by flash floods, increasing load of silt and hydroelectric projects (Sharma and Singh, 1980; Singh, 1987).

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Effect of paper mill effluent on the amino acid and protein content in liver, blood and gonads of fresh water fish *Mystus vittatus* (Bloch) during the annual breeding cycle

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Abstract

The effects of effluent from the Rayana paper board industries limited (R.P.B.I.L.E) on some biochemical aspects of the fresh water teleost fish, *Mystus vittatus* were studied under experimental conditions through the course of the annual reproductive cycle of these fishes. A quantitative estimation of the amino acids and protein content was made in liver tissues, blood and gonadal tissues. The content of all these nutrient and building materials was observed to be significantly higher during the spawning phase when compared to the other phases of the annual reproductive cycle. The changes produced in these biochemical parameters on account of exposure of the fishes for 96 hr to 0.4 and 0.8 of LC₅₀ – 96 hr of effluent were also studied during all the three phases of the annual reproductive cycle. All the stress induced biochemical alterations were always found to be greater in case of 0.8 than in case of 0.4 effluent both the sublethal concentrations of the annual reproductive cycle of the fish, the amino acid content of liver, blood and gonads was found to be increased, while the protein content was measured to be decrease in liver, blood and gonads. The present study concludes a stress induced metabolic dysfunction in response to effluent toxicity in the fish.

Keywords: *Stress-induced, Spawning phase, Effluent toxicity, Annual reproductive cycle.*

Introduction

Biochemical alterations being intimately associated with all physiological disturbances, their study has found wide usage in chemical diagnosis. Biochemical profiles have also been gaining progressive importance in toxicological research. Some of the biochemical changes have already acquired the status of reliable and sensitive indicators of general specific physiological disturbances under environmental stress. Study of biochemical anomalies now occupies an established place in the universally accepted research framework concerning fish toxicology.

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Various kinds of biochemical alterations in the amino acid and protein metabolism of vital tissues have been observed very frequently in fishes under conditions of environmental stress. Numerous studies have shown significant effects of papermill effluents on the fish populations. Physiological and biochemical changes under the effects of pulp and paper mill effluents on blood and tissues have been described especially of *Onchorhynchus kisutch* (McLeay, 1973, 1977; McLeay and Brown, 1974, 1975, 1979; McLeay and Howard, 1977), *Salmo gairdneri* (McLeay, 1977), *Esox lucius* (Oikari, 1977), *Onchorhynchus mykiss* (Oikari *et al.*, 1983, 1985a), Roach, *Rutilus rutilus* L. (Jeney, *et al.*, 1996; Aarno *et al.*, 1998), Perch *Perca fluviatilis* (Forlin *et al.*, 1995; Aarno *et al.*, 1998), Mosquitofish, *Gambusia holbrooki* (Batty and Lim, 1999; Bortone and Cody, 1999; Parks *et al.*, 2001). Thus, the purpose of the present study was to examine the liver, blood and gonads of *Mystus vittatus* for alterations produced under the stress of

effluent. The biochemical effects of 96-h exposure to sublethal effluent concentrations on the free aminoacids and total protein contents of these tissues were observed through the annual reproductive cycle of the test fish.

Materials and Method

The freshwater teleost, *Mystus vittatus*, was selected for the present work. It is an air breathing as well as annual breeder perch and abundantly present in all fresh water bodies of Northern India. The fishes were collected from a local uncontaminated freshwater pond in Basti city area, located approximately at a distance of 4 km from the research centre. They were brought to the research centre in open containers being filled with sufficient quantity of water so that the stress caused by handling and transportation may be minimized. The fishes having an average body length of 7.6 ± 0.18 cm and body weight of 7.3 ± 0.23 gm were selected for the study. The fishes were acclimatized to laboratory conditions for 3 to 4 week at room temperature in the acclimation tanks, filled with dechlorinated tapwater. The whole effluent was collected from a paper mill industry, Magahar, Santkabeer Nagar, Uttar Pradesh (India) for the study of toxicological responses. The effluent samples were collected from three places during the morning shift when the mill was in its normal course of operation. The samples were mixed thoroughly and brought to the research center in sealed polyethylene containers. The chemical characteristics of effluent analyzed according to the procedures recommended by American Public Health Association (2005), within 12 hr of collection, every month (Table 1). The acute toxicity of effluent to the perch was measured in terms of 96 hr, LC_{50} , by using the static bioassay procedures as outlined by USEPA (1989). For each acute toxicity bioassay, a minimum of 8 concentrations of effluent was used and 20 animals were used for each concentration. Controls were also run separately during experimentation, using normal unpolluted and dechlorinated tapwater. No food was provided to either the control or the test fishes during the period of the toxicity experiments. The experiments were conducted every month and 96 hr, LC_{50} values were determined. The effect on the tissue biochemistry of the perch was studied in individuals exposed for a period of 96 hr with two

sublethal concentrations *i.e.* 0.4 (40%) and 0.8 (80%) of the 96 hr - LC_{50} , determined during the mortality studies. Healthy fishes, netted from the acclimation tank, were divided in 3 groups of 50 individuals each. The first group was exposed to 40% R.P.B.I.L.E, and the second group to 80% effluent. The third group of fishes was kept in unpolluted dechlorinated tapwater, and served as control. The experiment was repeated 5 times. After the expiry of the 96 hr exposure period, the fishes from all the 3 groups were taken out for the sampling of their blood and other tissues. Ten fishes were used for each determination. For obtaining blood samples, the caudal vein was severed and blood was drawn into centrifuge tubes containing the anticoagulant mixture of 1mg% sodium fluoride and potassium oxalate. Serum was obtained by centrifuging the blood samples at 3000 to 4000 rpm for 5 min. The total amount of free aminoacid in blood, liver, ovary and testes was measured using the method of Spies (1957). Ten mg of the tissue was homogenized in 1 ml 96% ethanol (10:1 w/v) in a homogenizer for 5 min and centrifuged at 8000 rpm for 20 min. The supernatant was used for aminoacid estimation. The total protein content in the blood, liver, ovary and testes was estimated according to the method of Lowry *et al.*, (1951), using bovine serum albumin as the standard. The homogenates, obtained by mixing 1 mg tissue per ml of 10% TCA in a homogenizer for 5 min, were centrifuged at 6000 rpm for 20 min and the precipitate was used for the estimation of protein. A photo-colorimeter (systonics) was employed for biochemical estimation. The standard deviation ($\pm$ S.D.), and standard error ($\pm$ S.E.), were calculated and tested for significance according to the statistical methods outlined by Snedecor (1961). To test the significance of the differences between the mean experimental and the corresponding mean control values, the Student's t-test was applied as described by Campbell (1974).

Results and Discussion

The concentration of total protein and free aminoacids estimated in the different tissues of control fish was found to be maximum during the spawning phases when compared to the other two phases of the annual reproductive cycle of the fish (Fig. 1 and 2). The spawning phase incorporates a period of growth and proliferation of gonadal



tissues for the formation of gametes. Hence, an accumulation of the building material, *i.e.* protein, would be expected to occur during this phase; a simultaneous increase in the concentration of free aminoacids would also occur since protein synthesis involves a large pool of free aminoacids (Love, 1980). Liver, being the main organ for protein synthesis in spawning fishes (Wallace, 1985; Revathi *et al.*, 2005), its protein and aminoacid contents are most likely to increase during the spawning period of fishes. A similar increase in protein and aminoacid concentration is also most likely to occur within the gonads of spawning fishes, since a greater amount of proteinaceous material and energy is needed for gonadal growth and gamete formation. Infact, active protein synthesis appears to be a common feature of maturing gonads in fishes (Love, 1980), and more of free aminoacids have been recorded in the gonads of spawning fishes like *Flounders* (Sorvachev and Shatunovskii, 1968), *Gadus callarias* (Maslennikova, 1970) and *Mystus vittatus* (Narain and Pandey, 1989), *Channa punctatus*. The increased protein and aminoacid concentration observed in the liver and gonads of spawning *Mystus vittatus* will, thus, be explained. Apparently, a protein reserve is built up, through active protein synthesis, in the liver which is subsequently translocated to other organs, more so the gonads. Building up of proteins and aminoacid reserve and its translocation to spawning gonads has previously been reported in fishes (Love, 1980; Iles, 1984; Potts and Wooten, 1984).

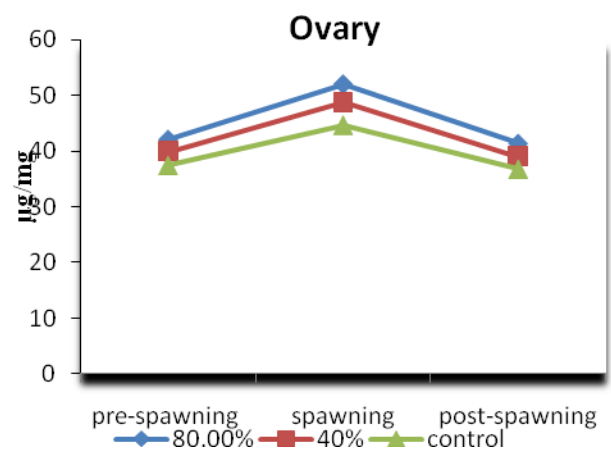
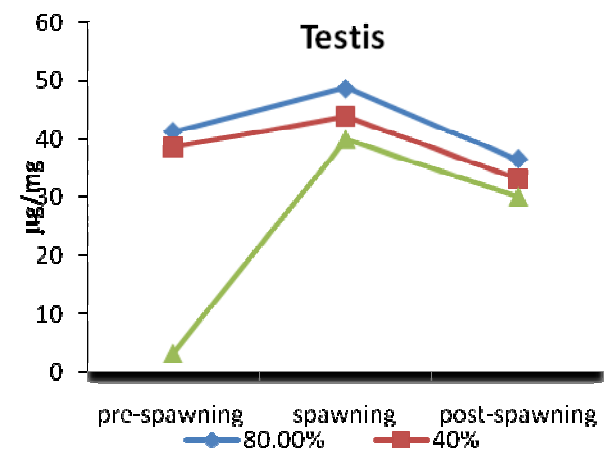
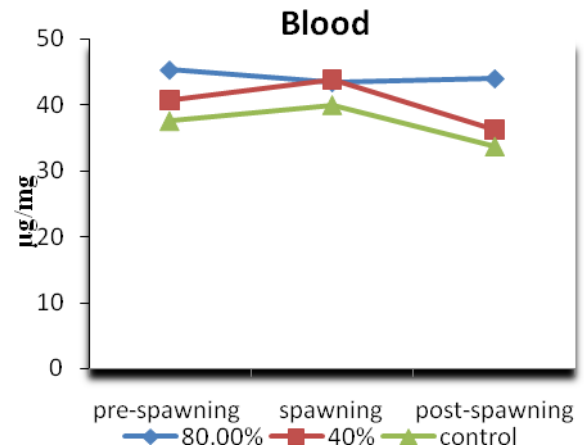
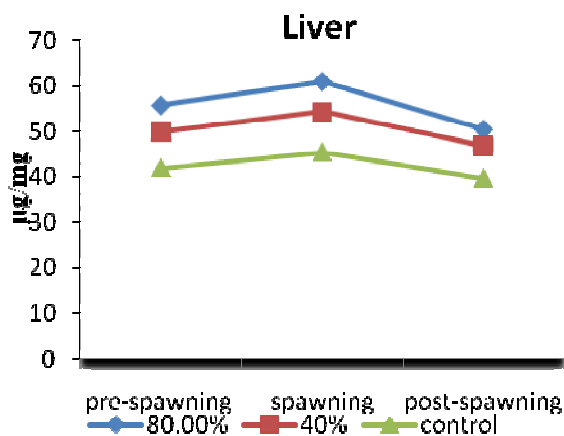


Fig.1: Aminoacids content of control and experimental *M.vittatus* during Pre-Spawning, Spawning and Spawning Phases. All experimental values are significantly different from corresponding control values (<0.05).

Table 1: Physico-chemical characteristics of test effluent (WRPBILE) Magahar, Santkabbeer Nagar, (U.P.) India. Data based on samples taken during the morning shift of the normal course of mill operation at 8 A.M.

Characteristics	Variable constituents through the year (mean values)			Yearly Average $\pm$ S.E.
	Jan-Apr	May-Aug	Sept-Dec	
Color	Dark Brownish	Dark Brownish	Dark Brownish	-
Sodium, Na ⁺ (mg/l)	350	320	351	340 $\pm$ 12.2
Cl ⁻ (mg/l)	420	450	425.7	431.9 $\pm$ 11.3
SO ₄ (mg/l)	1.12	5.8	3.5	3.5 $\pm$ 1.7
Nitrate (mg/l)	7.6	7.3	7.2	7.4 $\pm$ 0.1
Total Nitrogen (mg/l)	1.7	6.3	3.8	3.9 $\pm$ 1.6
PO ₄ (mg/l)	0.77	0.52	0.69	0.7 $\pm$ 0.1
pH	7.3	7.3	7.4	7.3 $\pm$ 0.04
Temp (⁰ C)	23.5	28	26.5	26 $\pm$ 1.6
Suspended solid (mg/l)	5021	4643	4256	4640 $\pm$ 270
Dissolved solid (mg/l)	1111	1222	1013	1115 $\pm$ 74
Total solid (mg/l)	6132	5865	5269	5755 $\pm$ 312
BOD (mg/l)	552	538	498	526 $\pm$ 19
COD (mg/l)	2379	2551	2326	2418 $\pm$ 83
Fe (mg/l)	9.6	13.5	10.4	11.2 $\pm$ 1.5
Mg (mg/l)	1.9	1.65	1.28	1.6 $\pm$ 0.2
K (mg/l)	8.6	6.4	4.4	6.5 $\pm$ 1.5
Cu (mg/l)	0.11	0.14	ND	0.1 $\pm$ 0.1
Total Cr (mg/l)	ND	0.077	0.072	0.07 $\pm$ 0.03
Mn (mg/l)	0.34	0.071	0.53	0.31 $\pm$ 0.12
Co (mg/l)	-	0.001	0.0012	0.001 $\pm$ 0.002
Cd (mg/l)	0.025	0.016	0.018	0.02 $\pm$ 0.004
Zn (mg/l)	0.06	0.08	0.106	0.08 $\pm$ 0.021

The genesis of sex proteins is linked to an increase in the liver RNA of developing fishes (Aida *et al.*, 1973; Love, 1980); this further establishes that proteins related to sex are hepatic in origin. Thus, the present observations corroborate their findings. The acute exposure to effluent was found to produce a noticeable change in the protein metabolism of *Mystus vitatus*. The protein content of liver, blood, ovary and testis was reduced significantly and the difference was significant at

<0.05 and 0.001 (Fig. 2), in comparison to control fishes. The reduction in total protein in stressed fishes may be correlated with the increase in aminoacid, pool of the stressed fishes in various tissues examined. The reduction in protein concentration under stressed conditions has also been reported in different tissue of fishes. For example: in the liver of *Channa punctatus* exposed to cythion (Narain and Sathyanesan, 1985), *Oreochromis mossambicus* exposed to endosulfan



(Ganesan *et al.*, 1989), and in *Gambusia offinis* exposed to tannery effluent (Revathi *et al.*, 2005); in the blood of *Catla catla* exposed to mercury (Rai, 1987), and in *Mystus vittatus* exposed to metasystox and sevin (John. 2007); in the ovary of *Brachydanio rerio* exposed to malathion (Ansari and Kumar, 1987), and in *Gambusia offinis* exposed to tannery effluent (Revathi *et al.*, 2005); in both testis and ovary of *C.fasciata* exposed to arsenic (Shukla and Pandey, 1986). The aminoacid concentration in acute sublethal exposure of test effluent to *M. vittatus* was increased significantly and found significant at $p < 0.05$ and 0.001 (Fig.1). The concentration of aminoacids has also been found to increase, under the influence of stressful conditions in the various tissues of fishes. For example, in the liver of *Tilapia mossambica* exposed to heptachlor (Rao *et al.*, 1990); in the ovary of *Brachydanio rerio* (Ansari and Kumar, 1987) exposed to malathion. Pulp and papermill effluents have also been shown to bring about a reduction in protein concentration of the tissues of fishes like *Onchorhynchus kisutch* (McLeay and Brown, 1974, 1979), *Esox lucius* (Oikari, 1977), and *Rutilus rutilus* (Jeney *et al.*, 1996). For the maintenance of physiological balance in stressed fishes, required additional energy, that gluconeogenesis was stimulated as a supplementary physiological response towards this need and that as a result, proteins are broken down and a rich aminoacid pool created for conversion to glucose. This would largely explain the loss in protein and gain in aminoacid concentration observed in the liver of stressed *C. fasciatus*

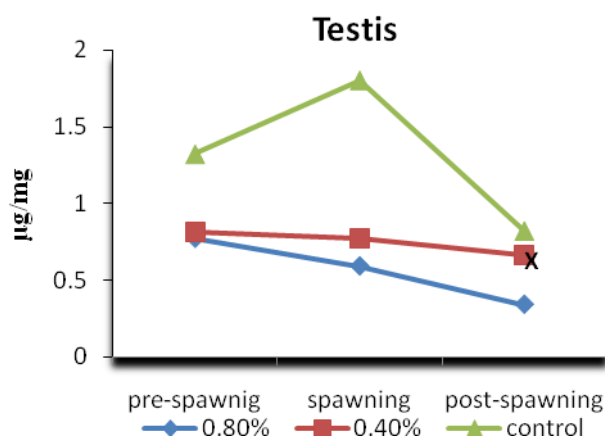
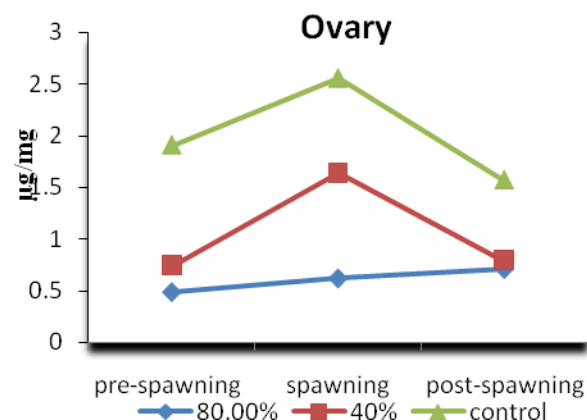
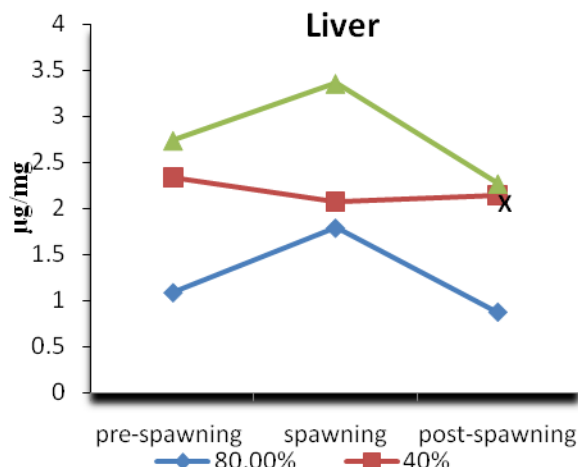
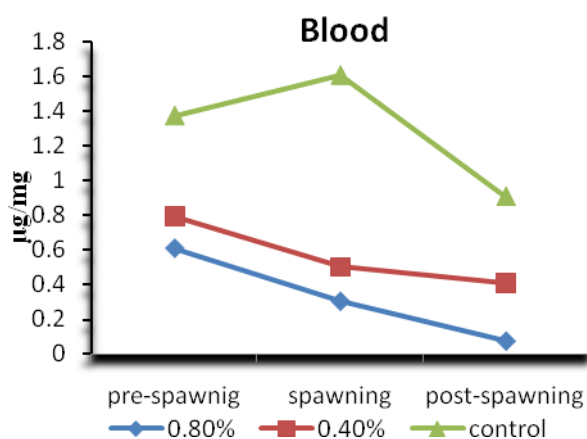


Fig 2: Protein content of the tissues of control and experimental *Mystus vittatus* during pre-spawning spawning and post spawning phases. All experimental values except marked with "X" significantly different from Corresponding control values (<0.05).

because liver is the major gluconeogenetic organ in teleosts (Moon *et al.*, 1985). The protein breakdown observed in the gonads of this stressed fish, may, however, not be explainable on this ground; an alternative explanation would be that the gluconeogenetic breakdown of proteins in the hepatic tissue is supplemented by protein breakdown in other tissue which depresses the protein level and enlarges the aminoacid content of the blood. It has also been reported that gluconeogenesis forms a part of the generalized stress response in vertebrates, whereby a pituitary adrenal excitation tends to promote protein catabolism under the influence of glucocorticoid hormones and that various environmental stress factors are capable of producing similar effects in fishes. Pulp and papermill effluents are also known (McLeay, 1973, 1977; McLeay and Brown, 1974), to elicit such endocrine responses, and increased adrenocorticoid output due to effluent induced stress has been considered an explanation for decreased body protein content of fishes like *Onchorhynchus kisutch* (McLeay and Brown, 1974). So, in *M.vittatus* exposed to papermill effluent, the loss of protein and gain in free aminoacids could also be related to stress-induced gluconeogenetic protein breakdown with a possible involvement of pituitary interrenal hormonal axis. Among other factors contributing to reduction in the tissue protein content of stressed fishes, the histopathological liver damage, if occurring in stressed *M.vittatus*, would be most likely to bring about increased proteolysis leading to lowered protein level in hepatic tissue. Similarly, pancreatic damage would also affect protein metabolism (Matty, 1985).

In view of this, the lowered liver protein and raised aminoacid concentration in the liver tissues of stressed *M. vittatus* may also be related to the damage of insulin secreting Islet cells. Kidney pathology leading to decreased excretory efficiency and liver damage leading to reduced albumin production are also reported (Narain, 1981) causes of hypoproteinemia. In acute conditions, the significant decrease in blood protein in the effluent, stressed fishes reflects the specific effect of the concentrated effluent. Very low blood protein possibly has significance in relation to infectious diseases, kidney damage and nutritional imbalance in fishes (Jeney *et al.*, 1996). The respiratory distress exhibited by the

stressed *Mystus vittatus* and the high BOD and COD values of the tested R.P.B.I.L.E samples point oxygen deficiency; this stressed condition could also be contributing towards the diminution of protein, specially liver and blood, observed in the stressed fishes.

From the present study on *Mystus vittatus*, it can be concluded that the tested effluent with higher concentrations of toxic constituents cause serious disturbances to vital biochemical and physiological functions, hence the fishes suffer from a general stress syndrome in short-term exposure to effluents, which can affect the health status, survival and most importantly the reproductive capacity of the fishes. The findings also revealed that the biochemical parameters like protein and aminoacid metabolism can be used as sensitive and reliable indicators of toxic effects on natural populations of fishes exposed to papermill effluent.

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Traditional use of indigenous plants in Betul district of Madhya Pradesh to cure Diarrhoea and Dysentery

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Abstract

A survey of medicinal plants of different rural and forest area of Betul district was conducted. It was found that the drug preparation of plant origin is commonly used by tribal local inhabitants and folk practitioners for the treatment of diarrhoea and dysentery disease. About 7 plant species belonging to 6 families are described along with the method of drug preparation, mode of administration, probable doses and duration of treatment. The aim to study is not only to prescribe the remedies for disease in human beings but also an endeavour to draw attention for the need of a detailed study on medicinal plants of the area, which could provide better and efficient remedies for diarrhoea, dysentery and many other dreadful diseases. The paper enumerates the traditional uses of more than 50 plants used by tribes inhabiting the Betul district. Information on the medicinal and other traditional uses gathered from the tribal together with their botanical identity is presented. The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of earth due to advent of modern technology and transformation of traditional culture. The collection of information about natural flora, classification, management and use of plants by the people holds importance among the Ethno botanists.

Keywords: *Ethnobotany, Ethnomedicine, Gond, Korku, Bhil, Medicinal Plants*

Introduction

Ethnomedicine is a new branch of ethnobotany, which is mainly based on the ancient knowledge of the plants used in the traditional medicine in the modern world. The traditional Ayurvedic system is mainly based on the knowledge, experience and belief of thousands of years of ancient people living in remote areas. The system makes use of plants products in eradication of common diseases. A vast majority of our population, particularly those living in villages mainly depends on herbal remedies. This knowledge has been acquired and passed from one generation to another. A good number of herbal remedies have been acquired from their knowledge, particularly for the treatment of diarrhoea and dysentery. However, no scientific basis regarding their identity and efficiency was available except that in the treatises

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of Ayurveda, which indicates the importance of plants in human life.

Ayurveda is developed on the basis of this diversity of knowledge utilizing plants as the richest source for health management. National Academy of Ayurveda, Ministry of Health and Family Welfare identified 2159 medicinal plants for various therapeutic purposes (Sharma, 1998). The tribes, who either worked as labourers or cultivated crops inherited rich traditional knowledge about the flora investigated and used to apply this knowledge for making crude phyto-medicines to cure infections as simple as cold to as complicated as cancer. These crude herbal medicines are based not only on traditional knowledge but also on rituals and beliefs.

A large area of Madhya Pradesh is occupied with hills covered by forests. These forests are not only abundant in flora but also it has a wide diversity. District Betul is in ecological zone at a longitude

of 21°22' to 20°24' N and latitude of 77°04' to 78°33' E. Betul is one of the tribal population districts of M.P. This district comes under Satpura plateau and Jawar and Wheat crop zone from the agriculture point of view. Geographical area is 1007.8 thousand hectare out of which 416.7 thousand hectare land is under cultivation. 381.1 thousand hectare is under Kharif and 120.3 thousand hectare under Rabi. The district consists of nearly 1.76 Lakh agricultural families out of which 46% belong to tribe category. Average agricultural land 2.90 thousand hectors under proprietorship. Irrigation area from all sources is 97.7 thousand hector and irrigation percentage is 23 in the district. Crop density is 127%. Average rainfall is 1083.9 mm in the district.

Betul is predominantly a forest and agricultural district, which is surrounded by Chhindwada, Hoshangabad, Harda and Khandwa respectively at West, North, East and South. Betul district is surrounded by Satpura hilly track and two rivers namely Machna and Tapti originated from this place. The total population of Betul district is approximately 13, 94421 millions as per 2001 census and it is tribal dominated area. The total agriculture land consists of 43,300 hectares. Total forest area consist about 4085.043 sq. kms., which is rich in the wild fauna and flora. Recently Government has declared Satpura hills as Biosphere reserve looking to its rich biodiversity. The Betul district is surrounded by dense deciduous forest. 95 % rural population dominated by tribals inhabit in forest areas of Multai, Athner, Chicholi, Bhansdehi and Betul bazaar, Chirapatla, Ghoradongri and Bhimpur. Rest are still living in urban areas. The tribal group like Bhil, Korkou, Banjara and Gond are involved in honeyculture, basket netting, mat making and habituated with the uses of medicinal plants for their ailments. Till date, in this dense forest, these tribes have least medicinal amenities. They have been using herbal medicines for the treatment of diseases like malaria, dengue fever, wounds, dysentery, diarrhoea, cough and cold. They know the rare plants found in the deep forest areas of Betul district, which are used for various treatments.

Materials and Method

The survey work for medicinal plants was performed during winter season I year 2007 to 2008. During the course of present study, survey

was carried out of Betul district of Madhya Pradesh. During the survey work interview were taken from the tribal man, hakeems, vaidyas and the knowledge revealed by them was collected and compiled in the scientific form. It was confined to the adjacent area including some remote tribal places of Betul district. The availability of plants were recorded only when more than one conformation got. Various applications and properties of the plants were known and recorded from the local tribal, rural people including local Hakeem and Ayurvedic practitioner of Betul district. Mostly the male members of tribal community gave the information's and female did not come out due to their social customs. The plants were collected from the places where the local people have been using them for recovering diseases. The medicinal plants were used by tribals for recovering malaria, dengue fever, wounds, dysentery diarrhoea, cough cold and breathing problem. Plants were collected based on tribe's knowledge and folklore. The surveyed plants were identified with the help of local tribal people and the taxonomist. The voucher specimen of each plant is procured in "Herbarium" and maintained at the laboratory. The present study reports more than 50 plants of Betul district to be used in more than 16 diseases by the tribals. In which 7 plants are very much useful to cure diarrhoea and dysentery.

Results and Discussion

The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of earth due to advent of modern technology and transform of traditional culture. The collection of information about natural flora, classification, management and use of plants by the people holds importance among the ethno botanists. The local people and researchers face the challenging task of not only documenting knowledge on plants, but also applying the results of their studies to biodiversity conservation and community development. With a deep concern and reverence for the vast diversity of flora that our country enjoys and with sense of realization about the invisible therapeutic properties of this phyto diversity, the current research is undertaken.



Total of 50 plants have been collected and their detailed information was proposed on the basis of folklore and their use. It has been observed that there is an inherent transfer of knowledge from one generation to another in some of the families. Generally the villages do not reveal their knowledge and it is very difficult to get the information from them. Still making continuous contacts with them and asking questions on different aspects. Some useful information from them was obtained which has been tabulated in the scientific form as shown in Table. 1. It has been noticed that out of 7 plants belonging to 6 families. The plants were of family Rutaceae, Liliaceae followed by Carssulaceae, Convolvulaceae, Euphorbiaceae, and Moraceae. As for as there part used for the medicinal purposes, leaves of 4 plants, Root of 1 plant, arial part and flower of 1 Plant, fruit of 2 plant and stem, latex, bark, bulbs of 2 plants as listed in Table.1.

The plants on the basis of folklore have been scientifically updated and the list was prepared pertaining their scientific names, vernacular names, parts used, and their family. Following the

method of Jain 1995, the information regarding the use of medicinal plants available in the local area for treating various ailments and disease was collected by directly contacting the elderly villagers, herbal doctors and the persons who have knowledge about this medicinal plants in the tribal community inhabiting the Betul, Chicholi, Bhansdehi, Athner, Betul bazaar and Multai areas which are situated around the Satpura hills. The plant materials were collected and carefully handled for identification by authentic sources. The medicinal value of each plant was enumerated in the following pattern- binomial, family, vernacular names, Parts used and ethnomedicinal uses. Gupta, (1994) has observed the prospects and perspectives of natural plant products in medicines and reported of their different medicinal effects in many ailments. Saxena and Sahu, (2006) have surveyed 22 plants from tribal area of Raisen district M. P. for ethnomedicinal effects. Such kind of studies have also been conducted and reported by Anish *et al.* 2000, Amusan *et al.* 2002, Saxena *et al.* 2001, Dixit and Pandey 1984, Chopra *et al.* 1996, and Tenguria *et al.* 2006..

Table: 1 List of medicinal plants to cure diarrhoea and dysentery

S.No.	Botanical name	Vernacular name	Family	Parts of plants	Medicinal Uses
1.	<i>Aegle marmelos</i> Roxb.	Bel	Rutaceae	Leaf and Fruit	Diarrhoea
2.	<i>Allium cepa</i> Linn.	Pyaz	Liliacea	Bulbs, Leaf	Antibacterial in dysentery, bronchitis, malaria fever, asthma, cough
3.	<i>Asparagus racemosus</i> W.	Satawari	Liliaceae	Roots, Rhizome	Diarrhoea, Dysentery
4.	<i>Bryophyllum calycinum</i> Lam.	Patharchatta	Crassulaceae	Leaf juice	Dysentery, Diarrhoea, Insect bite, Antiseptic
5.	<i>Cuscuta reflexa</i> Linn.	Amarbel	Convolvulaceae	Stem, Leaf	Eczema, Diarrhoea, Gastric troubles
6.	<i>Euphorbia pulcherrima</i> Linn.	Lal dudhi	Euphorbiaceae	Aerial parts, Flower, Latex	Burning, Micturation, Milch Problem
7.	<i>Ficus glomerata</i> Linn.	Gular	Moraceae	Bark, Fruit	Diabetes, Dysentery, Leucoderma

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Seasonal occurrence and mode of damage of *Eusarcocoris capitatus* Distant, a pest of *Ocimum sanctum* L.

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Abstract

Eusarcocoris capitatus Distant (Heteroptera: Pentatomidae), a pentatomid bug, infests valuable medicinal plant, *Ocimum sanctum* L. at Saharanpur District in good number. It is a phytosuccivorous bug, which with the help of their piercing and sucking mouth parts drains out sap from the seeds, flowers and leaves of Tulsi (*Ocimum sanctum*). During this study, it has been observed that *E. capitatus* occur maximum during March to mid of December. Adults of *E. capitatus* undergo hibernation during late December to first week of March to avoid unfavorable cold climatic conditions. Maximum population and infestation of this bug have been recorded during July to October when temperature and moisture content are suitable for its development and reproduction. Studies on mode of damage revealed that all the five nymphal instars and adults suck the sap content from all parts of *O. sanctum* plant. The damaged seeds shrink and loss viability and become unfit for germination. Looking to the medicinal value of Tulsi, control of this bug is urgently needed.

Keywords: *Eusarcocoris capitatus*, Damage, *Ocimum sanctum*, Tulsi, Bug, Medicinal

Introduction

In India, the herb Tulsi or holy basil has been widely known for its health promoting and medicinal value for thousands of years. It is known as “The incomparable one”, “The Mother Medicine of Nature” and “The Queen of Herbs”. It is grown in every pious Hindu home and kitchen garden as it is remedy of many day to day ailments.

Modern agriculture is continuously facing insect problem since its inception. India faces an annual loss of about 1500 crores of rupees due to damage caused by insects to agriculture. Among various insect pests, Heteroptera insects, which are commonly called bugs, inflict good losses in terms of money to the agricultural and horticultural crops as well as medicinal plants. Among this group, pentatomid bugs make a good contribution. *E. capitatus* (Heteroptera: Pentatomidae) infests a valuable medicinal plant, Tulsi (*O. sanctum* L.) at Saharanpur District in large number. Though, on other pentatomid bugs a good piece of work has been carried out by Dhiman (1981, 1983 and 1985)

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Dhiman and Dhiman (1985), Brown (2003), Colazza *et al.* (2004), Dhiman *et al.* (2004), Dhiman and Singh (2005), Nardi and Alves (2005), Chatterjee and Kumar (2006), Dhiman and Kumar (2006), Patel *et al.* (2006), Santosh *et al.* (2006) and Dhiman and Bhardwaj (2008), but on this bug only few observations were made by Dhiman and Jain (2008 a, b). Hence, looking to the medicinal value of its host plant and its pest status, present study has been under taken.

Materials and Method

Tulsi plant (*O. sanctum*) was planted in good number in well manured irrigated soil of earthen pots. These were kept in the open field and some were caged by fine wire mesh. Plants were irrigated at regular interval. *E. capitatus* were reared in laboratory as well as in field in wire gauze cages. At inflorescence and seed setting stage of Tulsi plants, in 5 cages, 2 pairs of newly emerged bugs were released in each and their mode of damage was observed by making close observations. Mode of damage was also observed using a magnifying hand lens (20x) as well as under binocular microscope in reflected light. Rearing in laboratory was carried out in hurricane

glass lantern chimneys covered at top by fine muslin cloth. In each chimney, 5 pairs of bugs were released and fresh food was supplied daily and stale was removed. A cotton swab dipped in water was also placed in the chimney in watch glass for maintaining necessary relative humidity. Population studies on the bug were made in open field on randomly selected plants. Effect of temperature was seen in laboratory by placing the bugs in a muslin cloth covered glass jar in temperature and humidity control cabinet. These were subjected to different temperature levels at different time and their survival was recorded.

Results and Discussion

Mode of damage was observed in field as well as in laboratory which revealed that all five nymphal instars and adults suck the newly set seeds content as well as flowers and sap from the leaves.

Seasonal occurrence

Weather is a composite condition of influence of temperature, light, humidity, rainfall and wind at any given moment in time. It varies continually through days, weeks, months, years and exerts an influence on insect abundance, longevity and development rate and so on, from one year or season to the next. Heteropteran bugs generally hibernate in adult stage on approach of cold weather (Dhiman, 1981, 1983). Similarly *E. capitatus* hibernates in adult stage from December to first week of March (at temp. ranging from 15°C to 24°C and relative humidity 68% to 56%) under fallen leaves, crevices of house wall and tree trunk, under the bark, stones etc. Duration of hibernation varies upon climatic conditions. Temp. and relative humidity. Plays a vital role in longevity of adult insect. Maximum survivality of the bugs was recorded at 30°C and minimum at 40°C (only in hrs). Its population lasts on Tulsi plant from March to November or mid of December. Peak level goes during July to October and being minimum during March to April and November to first week of December. However, fluctuation in population occurs depending upon temperature and relative humidity. of environment (Table. 1).

Mode of damage

The *E. capitatus* is a phytosuccivorous bug and possesses piercing and sucking mouth parts. Prior

to the feeding, suitable feeding site is explored by the sensory setae present at the rostral tip and antennal sensillae present at the terminal clavate segment. Usually a soft feeding site is selected for easy penetration by its stylets.

Table 1: Population fluctuation of *E. capitatus* on Tulsi during 2007 in field

Months	*Average number of bugs per plant	*Average temp. (°C)	*Average R.H. (%)
January	NIL	13.05	79.15
February	NIL	17.08	67.69
March	10	23.49	55.92
April	20	25.08	40.87
May	30	30.01	45.37
June	40	29.62	64.07
July	50	30.46	71.31
August	50	28.58	82.82
September	44	28.40	74.37
October	38	25.38	72.89
November	25	18.87	70.78
December	10	15.62	68.31

*Average has been taken of 10 observations

Prior to piercing operation by stylets, the insect probes the seed, leaf and flower with the proboscis 5 to 6 times. Stylets are able to pierce any part of the host tissue, but generally more woody part is avoided. In *E. capitatus* the mandibular stylets perform the major piercing organ while maxillary stylets in addition to suction, contribute towards the cutting of parenchymal tissue so as to ensure a continuous flow of sap. After probing, a suitable site piercing is done by mandibular and maxillary stylets, using powerful protractor and retractor muscles. Then saliva is poured by powerful salivary pump and the saliva mixed sap is sucked up by cibarial pump. In this bug feeding operation is similar as observed by Dhiman (1985) in *Metacanthus pulchellus* Dall. Feeding generally lasts for 35 to 65 minutes. When the feeding is over, the stylets are withdrawn from the host with a fairly great effort by means of retractor muscle. As soon as stylets are withdrawn, air bubbles are sucked-up into the food canal along with the sap column which ultimately reaches the midgut and



clean proboscis and antennae by antennae and rostrum cleaning device situated at the tibial extremities of fore legs as also described by Dhiman and Dhiman (1985).

Generally, *E. capitatus* (nymphs and adults both) are seed sucker, but some time they also feed upon the leaf and flower. The seeds of Tulsi, *O. sanctum* are dry, rich in amino acids, carbohydrate and fatty contents. The bug pierces the testa so as to reach the stylet to food content. A constant flow of saliva into wound is maintained till the food is well dissolved for sucking. This is the reason; a single penetration by the stylets may last upto 25 to 40 minutes in case of seed feeding. Saliva probably contains amylase and lipase which helps in dissolving the food. Salivary fluid might act as a lubricant also which helps in penetration of stylets. After feeding, labium is withdrawn from the seed and the tip is invariably cleaned between the fore-tarsi having antennae and rostrum cleaner device.

They picked up seed with the help of tibia and tarsi of forelegs and then punctuation occurs with the aid of mandibular and maxillary stylets as described above. They may feed at one spot or carry the seed to some distant places hanging it by stylets. Now, it may take rest or select another feeding site and similarly food content or sap is drained out from the leaves, flowers or newly set seeds. Carbohydrate and protein or lipid diet is taken from seeds while water, minerals and vitamins are taken from leaf sap. In damaged leaf, punctuations and decolorized areas appear when viewed under binocular microscope in reflected light. The chlorophyll content of damaged parts is lost. On a single leaf, many such areas develop after 4 to 6 hours of feeding. Such leaf later on turns brown and wilts up. This reflects the magnitude of damage caused of this bug. Some punctuations on the leaf can be seen without the aid of artificial light after feeding is over. Moreover, damaged seeds lost viability and weight. Only covering of seeds is left while soft cotyledon content is sucked up. The affected seeds of the important host plant *O. sanctum* could not germinate even after a period of 40 days. 99.6 to 99.67 % seeds of Tulsi are damaged in laboratory feeding. On a single Tulsi plant as many as 50 bugs were recorded while on a single inflorescence upto 15 bugs were counted. Thus, all five nymphal instars and adults suck the newly set seeds content as well as of flowers and sap from

the leaves. As a result, the seed setting is greatly affected. *O. sanctum* has high medicinal value and *Eusarcocoris capitatus* occurrence on it in large number at Saharanpur is a new record. Other authors only reported *Monanthia globulifera* its pest (Dhiman and Bhardwaj, 2008). *Monanthia globulifera* population feeds on the leaves while *E. capitatus* mainly feed on inflorescence and seeds. At Saharanpur it occurs during March to November and hibernates during December to first week of March. Dhiman (1983) also recorded hibernation in *Cletus signatus* at Saharanpur. Peak population of *E. capitatus* occur during June to October and minimum from March to May. Dhiman *et al.* (2004) recorded both bug species *Halys dentatus* and *Erthesina fullo* occur throughout the year. The active breeding period of these last from March to October. Dhiman and Singh (2005) stated that *Audinetia spinidens* hibernated in adult stage during late November to mid February. The population increased till June reached its peak in rainy months (July-September) and then declined in October-late November. Dhiman and Kumar (2006) recorded the period of occurrence from March to November, after that *Chrysocoris stoll* under went to hibernation from late November to February. The maximum population occurs in September and minimum in February and November. By desapping habit, *Chrysocoris stoll* population causes damage to these food plants of economic value. Santosh *et al.* (2006) said that *Ocbalus poecilus* hibernates during the coldest months of the year in refuges such as bamboo litter. It left this refuge from middle October to the end of December. Thus, hibernation is found in most bug species in the regions where climatic conditions differ throughout the year.

E. capitatus (nymphs and adults) both are seed sucker, but some time they also feed upon the leaf and flowers. *E. capitatus* caused damage to the growing milky seeds and damage has been recorded on *Ocimum sanctum* from 99.6 to 99.67%. The damage seed loss viability, weight and become unfit for germination. Brown (2003) described the damage caused by stink bugs was eliminated by caging fruits in early July, whereas damage was higher on fruits caged with stink bugs for a 2 week period late July and harvest than on fruits that were not caged. Most stink bug damage occurred from 26 to 60 days before harvest.



Colazza *et al.* (2004) described that the feeding activity of the *Bagrada hilaris* can result in severe damage, potentially capable of causing economic damages. Nardi and Alves (2005) said that infestation of *Euschistus heros* on soyabean plants at the R3-R8 stage, which had significant effects on seed production, number of pods, total number of seeds and total weight, reduced plant height. Chatterjee and Kumar (2006) recorded that *Dolycoris indicus* population caused damage to the growing milky seeds and net damage has been recorded in berseem from 32.02 to 36.73% (maximum, average, 34.65% in May and on *O. sativa* between 59.28% (maximum) and 57.97% (minimum, average 34.65%). Patel *et al.* (2006) recorded that average grain weight were lower in infestation of *Oebalus pugnax pugnax* (Fab.) on rice. *Oryza sativa* L. during anthesis and milky stage and higher infestations during later grain development. Thus percent of damage varies according to the species of pentatomid bug and host plant. Damage caused by them reflects very high magnitude of damage which indicates for the necessary action for the control of these bugs at appropriate time.

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Enumeration and distribution of lichens in Surankote, District Poonch, J & K (India)

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Abstract

An enumeration of 31 species of lichens belonging to 19 genera and 13 families has been done for the first time from Surankote area of Poonch district. The crustose lichens with 20 species exhibit their dominance in the area, followed by foliose lichens represented by 11 species. *Licheinella* species is single fruticose lichen reported from the area. The saxicolous lichens with 18 species show their dominance while the species growing on bark (corticolous) are represented by 13 species.

Keywords: - *Distribution, Enumeration, Lichens, corticolous, Saxicolous*

Introduction

The studies related to lichens are scanty in Jammu region (Sheikh *et al.*, 2006 a, b; Sheikh *et al.*, 2009) and as such no record of lichens is available in literature from Poonch district. In the present communication attempt has been made to enumerate the lichens and also to study their altitudinal distribution from the Surankote tehsil of Poonch district of J&K state. The Surankote town (latitude 74°15' 38" E, longitude 33°38' 30" N and altitude 1400 m above msl) is situated on the left bank of River Suran which drain the entire valley surrounded by lofty peaks. The vegetation of the area is dominated by conifers forming pure patches and also mixed with other trees. *Cedrus deodara*, *Pinus wallinchiana*, *Picea smithiana*, *Platanus orientalis*, *Malus domestica*, *Prunus persica*, *Alnus nitida*, *Morus alba*, *Pyrus pashia*, *Quercus ilex* and *Juglans regia* etc. are the major tree species present in the study area.

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Materials and Method

The lichen collections have been made from different substratum *i.e.* rocks and trees (base to head height of the tree trunks), present in four localities - Surankote town, Bafliaz, Dehra Gali and Noori Chamb, which varies in their location, altitude and vegetation (Table I). The collection has been done for two months *i.e.* May and June 2009 and the details of locality, substratum and altitude have been recorded. The labeled and dried specimens have been lodged in the Lichen herbarium of National Botanical Research Institute, Lucknow and Department of Environmental Sciences, University of Jammu, Jammu. For authentic identification of the samples, morphology (under stereo-zoom binocular microscope), anatomy (temporary mount of free hand sections of thallus and fruiting bodies), colour spot tests of cortex and medulla with chemical reagents (potassium hydroxide, Steiner's stable paraphenylenediamine and aqueous calcium hypochlorite) and thin layer Chromatography (Walker and James, 1980) have been studied. The recent literature of Awasthi (1988, 1991 and 2000), Singh and Upreti (1984), Upreti (1988), Divakar (2001) and Nayaka (2004) has also been consulted for the identification of the specimens.

Results and Discussion

A total of 31 species belonging to 13 families have been recorded from all the sites of the Surankote area of Poonch district (Table.2). Persual of the table reveals that Family Parmeliaceae with 7 species dominate the area. *Lepraria* and *Punctelia* species have been represented in maximum number *i.e.* three each, in the study area. Further, the crustose species dominate the area with 20 species while foliose has been represented by 11 species and one lichen *i.e.* *Licheinella* species is of fruticose growth form. Sheikh *et al.* (2009) has also reported Parmeliaceae to be the dominant family and crustose to be the dominant growth

form of lichens in the J & K state. The area being rocky and mountainous, support maximum number of saxicolous types of lichens *i.e.* 18 species while the corticolous type has been represented by 14 species only which is contrary to the findings of Sheikh *et al.* (2006a). Locality wise analysis of the distribution of different lichen species (Table-3) reveals that maximum numbers of species (21 species belonging to 9 families and 15 genera) of lichens have been recorded from Dehra Gali area. The saxicolous (11 species) and corticolous (10 species) species have almost equal representation at this locality.

Table 1: Location, altitude and dominant vegetation of Collection sites

S.No	Site	Location/Altitude	Dominant vegetation
1	Surankote	Located north west to Jammu on the Jammu – Poonch road at a distance 220 Km on the left bank of the River Surn 1400 m a.s.l	<i>Pinus wallinchiana</i> , <i>Platinus orientalis</i> and <i>Malus domestica</i> etc.
2	Bafliaz	Located on left bank of the river Surn 1800 m (a.s.l)	<i>Prunus persica</i> , <i>Alnus nitida</i> , <i>Morus alba</i> , <i>Pyrus pashia</i> and <i>Prunus persica</i> etc.
3	Dehra Gali	A famous picnic spot of the region, located about 12 km. away from Surankote 3600 m a.s.l	<i>Pinus wallinchiana</i> , <i>Cedrus deodara</i> , <i>Picea smithiana</i> and <i>Juglans regia</i> etc.
4	Noori Chamb	Located 18 km. on Mughal Road from Surankote connecting Surankote to Kashmir. This area has a waterfall known as “Noori Chamb” and is famous tourist spot of the region. 2500 m. a.s.l	<i>Pinus wallinchiana</i> , <i>Cedrus deodara</i> , <i>Corylus cornuta</i> , <i>Pyrus communis</i> and <i>Aesculus indica</i> etc

The other 3 sites Surankote town, Bafliaz and Noori Chamb, have been represented by 9, 13 and 17 species of lichens, respectively. Surankote town, with good diversity of trees, exhibited poor growth of corticolous Lichens represented by two species while 7 rock inhabiting lichens grow luxuriantly in the area. All species recorded from this site have crustose growth form. Heavy anthropogenic pressure *viz.* lopping of trees for fodder and fuel wood, grazing of animals, vehicular movement *etc.* may have contributed for the poor representation of lichens at this site, particularly on the trees.

From Bafliaz locality, 13 crustose lichen species belonging to 6 families have been recorded from rocks (saxicolous). No corticolous species has

been recorded from this site as the collection has been made along the river side where trees are almost absent.

The Noori Chamb locality exhibits occurrence of 17 species of lichens belonging to 14 genera and 7 species grows on bark and 10 on rocks. At this site, 10 species of lichens exhibits crustose type growth form while other 7 species of this site show foliose type of growth.

The construction activity on the Mughal road might have affected the distribution of lichen at this locality. Further analysis of the table reveals that two genera *Lecanora muralis* and *Dermatocarpon vellerum* exhibit wide distribution and have been recorded from all the sites.



Table-2: Lichen species with their growth form and substratum

S. No.	Family	Lichen taxa	Substratum	Growth form
1.	Acarosporaceae	<i>Acarospora</i> sp	R	Cr
2.	Bacidiaceae	<i>Bacidia</i> sp.	R	Cr
3.	Chrysothriceae	<i>Chrysothrix chlorina</i> (Ach).Laundon	R	Cr
4.	Collemataceae	<i>Leptogium denitculatum</i> Nyl. <i>Leptogium burnetial</i> Dodge.	Q R	Cr Fo
5.	Hymenaliaceae	<i>Aspicilia contorta</i> (Hoffm.) Krempeh <i>Aspicilia</i> sp-2	R R	Cr Cr
6.	Lecanoraceae	<i>Lecanora campestris</i> (Schaeree). Hue <i>Lecanora muralis</i> var. <i>muralis</i> (Schreb.) Rabenh	R R	Cr Cr
7.	Lichanaceae	<i>Phylliscum indicum</i> Upreti <i>Lichinella</i> sp	R B	Cr Cr
8.	Lichens imperfecti	<i>Lepraria lobificans</i> Nyl <i>Lepraria</i> sp. I <i>Lepraria</i> sp. II	R Pp R	Cr Cr Cr
9.	Megasporaceae	<i>Aspicilia</i> sp.1	Rd	Fo
10.	Parmeliaceae	<i>Canoparmelia</i> sp. <i>Flavoparmelia caperata</i> (L.) Hale <i>Flovopunctelia flaventior</i> (Stirton) Hale <i>Punctelia borreri</i> (sm.) Krog. <i>Punctelia neutralis</i> (Hale) Krog <i>Punctelia subrudecta</i> (Nyl.) Krog. <i>Xanthoparmelia coreana</i> (Gyelink) Hale	P Rd Cd Rd P Q R	Fo Fo Fo Fo Fo Fo Cr
11.	Physeiaceae	<i>Phaeophyseia hispidula</i> (Ach.) Morberg <i>Phaeophyseia orbicularis</i> (Neck). Morberg	P P	Fo Fo
12.	Teloschistaceae	<i>Caloplaca</i> sp. <i>Caloplaca</i> sp.I <i>Xanthoria candelaria</i> (L.) Arn	R R U	Cr Cr Fo
13.	Verrucariaceae	<i>Dermatocarpon vellerum</i> Zschacke <i>Endocarpon subroseltum</i> A Singh and Upreti <i>Verrucaria acrotella</i> Ach. <i>Staurothele fissa</i> Taylor	R R R R	Cr Cr Cr Cr

Cr – Crustose
B – *Berberis* sp.

F – Foliose
Rd- *Rhododendron*

R – Rock
Cd- *Cedrus deodara*

Pp – *Pyrus pashi*
P-*Pinus wallinchaina*



Table 3: Locality wise distribution of lichens

S. No.	Lichen species	Site.I Surankote	Site.II Bafliaz	Site.III Dehra Gali	Site.IV Noori Chamb
1. 2	<i>Lecanora campestris</i> (Schaeree). Hue <i>Lecanora muralis</i> var. <i>muralis</i> (Schreb.) Rabenh	+ +	+ +	- +	+ +
3 4 5	<i>Lepraria</i> sp. II <i>Lepraria</i> sp. I <i>Lepraria lobificans</i> Nyl	+ + +	- - -	- - -	- - +
6	<i>Caloplaca</i> sp.I	+	-	-	-
7 8	<i>Dermatocarpon vellercum</i> Zschacke <i>Endocarpon subroseltum</i>	+ +	+ +	+ -	+ +
9 10	<i>Phylliscum indicum</i> Upreti Licheniella	+ -	- -	- +	- -
11 12	<i>Verrucaria acrotella</i> Ach. <i>Staurothele fissa</i> Taylor	- -	- +	+ +	+ -
13 14	<i>Aspicilia contorta</i> (Hoffm.) Krempeh <i>Aspicilia</i> sp-2	- -	- +	+ -	+ +
15	<i>Bacidia</i> sp.	-	+	-	-
16	<i>Acarospora</i> sp.	-	+	-	+
17	<i>Xanthoparmelia coreana</i> (Gyelin) Hale	-	-	+	-
18	<i>Chrysothrix chlorine</i> (Ach).Laundon	-	-	+	+
19 20	<i>Caloplaca</i> sp. <i>Xanthoria candelaria</i> (L.) Arn	- -	+ +	- +	- +
21 22 23 24	<i>Punctelia borrieri</i> (sm.) Krog. <i>Canoparmelia</i> sp. <i>Flavoparmelia caperata</i> (L.) Hale <i>Punctelia subrudecta</i> (Nyl.) Krog.	- - - -	- - + -	+ + + +	+ + + -
25	<i>Aspicilia</i> sp.1	-	-	+	+
26	<i>Leptogium denitculatum</i> Nyl.	-	+	+	-
27	<i>Phaeophyseia orbicularis</i> (Neck). Morberg	-	-	+	+
28	<i>Leptogium burnetial</i> Dodge.	-	-	+	-
29 30 31	<i>Flovopunctelia flaventior</i> (Stirton) Hale <i>Punctelia neutralis</i> (Hale) Krog <i>Falvoparmelia caperata</i> (L.) Hale	- - -	- - +	+ + +	+ - -
32	<i>Phaeophyseia hispidula</i> (Ach.) Moberg	-	-	+	-

+ = Present

- = Absent



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Changes in ascorbic acid content of fresh apricot fruits under fungal pathogenesis

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Abstract

The degradation of ascorbic acid content of fresh apricot fruits infected with *Rhizopus stolonifer*, *Aspergillus japonicus*, *A. niger* and *Penicillium citrinum* was studied. The experimental results revealed that there was decrease in vitamin C content of both healthy and infected fruits with an increase in incubation period. Fruits infected with *R. stolonifer*, *A. japonicus*, *A. niger* and *P. citrinum* had lost Vitamin C by $85\% \pm 4.08$, $78.3\% \pm 2.36$, $60\% \pm 4.08$ and $34.97\% \pm 4.08$ respectively after 6 days of pathogenesis whereas the healthy fruits still had $90\% \pm 0.19$ of vitamin C after same period of incubation. The degradation of ascorbic acid was more in infected fruits in comparison to healthy fruits and this may be either due to the production of ascorbic acid degrading enzymes or due to increased respiration rate which may induce rapid oxidation of ascorbic acid in the fruit tissue.

Keywords: *Rhizopus stolonifer*, *Aspergillus japonicus*, *A. niger*, *Penicillium citrinum*, apricot, ascorbic acid

Introduction

Ascorbic acid is widely distributed in fresh fruits and green leafy vegetables. While fruits also contain some dietary fibres, simple sugars, vitamins and minerals, they ought to be consumed fresh to derive maximum benefits as prolonged storage and careless handling can destroy their nutrient content and also affect the texture, colour and flavour (Serrano *et al.*, 2006).

Apricot is one of the most delicious and highly nutritive fruits rich in sugars, minerals and vitamins including ascorbic acid. Ascorbic acid (also known as vitamin C) is an antioxidant and along with other antioxidants, is known for inhibiting reactive oxygen species and their harmful effects (Knekt *et al.*, 1991; Minnunni *et al.*, 1992; Ferraroni *et al.*, 1994). Apricot fruits, fresh as well as dried and their seed kernels serve as dessert and have immense medicinal properties. They are anti-inflammatory, anti-diarrhoeal, antipyretic, emetic, cardiogenic and antiseptic, besides being used in the treatment of respiratory

and digestive ailments (Nyugen and Doan, 1989). However, the fresh apricot fruits, on account of their high moisture content, provide conditions conducive for the growth and multiplication of fungal organisms on the surface that may affect their nutritive value. In view of this, an investigation was undertaken to analyze the changes in ascorbic acid content of apricot fruits under the impact of its 4 most frequently occurring mycopathogens viz., *Rhizopus stolonifer*, *Aspergillus japonicus*, *A. niger* and *Penicillium citrinum*.

Materials and Method

Fully mature, ripe and healthy apricot fruits were collected from the various fruit markets of Jammu, brought to the laboratory in polythene bags and washed thoroughly with sterilized distilled water. These fruits were then artificially inoculated with five days old pure cultures of *Rhizopus stolonifer*, *Aspergillus japonicus*, *A. niger* and *Penicillium citrinum* by pin-prick method and incubated at $25 \pm 2^\circ\text{C}$. The inoculated fruits were then analyzed

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for ascorbic acid content after 2, 4 and 6 days of incubation. Healthy uninoculated fruits were kept as control. The ascorbic acid content was estimated titrimetrically using the indicator dye, 2,6-dichlorophenol indophenol (DCPIP) as followed by Sharma and Sumbali (2000). The dye was first standardized for calculating the quantity of ascorbic acid reacting with 1 ml of dye. For this, 5 mg of standard ascorbic acid was dissolved in 100 ml of 5% metaphosphoric acid. 10 ml of this solution was taken in a beaker and titrated against 0.025% DCPIP solution filled in a microburette. The amount of dye used was recorded when a pink end point persisted for atleast 30 seconds. From this, the quantity of ascorbic acid (in mg) reacting with 1ml of dye was calculated. Thereafter, fruit tissue of healthy fruits and those inoculated with test pathogens was cut into pieces (5 gm each) and crushed in mortar by adding 25 ml of 5% metaphosphoric acid solution to it. In each case, the solution was then filtered through Whatman No.42 filter paper using suction pump and the residue was washed 2-3 times with 5% metaphosphoric acid until the total volume of the filtrate reached 50ml. The filtrate was centrifuged at 4000 rpm for 15 minutes to obtain clear solution. 10 ml of each of this solution was separately titrated against previously standardized DCPIP solution. The volume (in ml) of indophenol solution required for each titration was noted on the basis of three readings. In all cases, a blank correction of the titration value was made as suggested by Frank (1955). The amount of ascorbic acid in mg/100 gm of fruit tissue was calculated using the following formula:

$$\text{Amount of ascorbic acid} = \frac{A \times I \times V}{v \times W} \times 100$$

Where,

A= Quantity of ascorbic acid (in mg) reacting with 1 ml of indophenol reagent

I= Volume of indophenol solution (in ml) required for the completion of the titration with the extract

V= Total volume of extract (50 ml)

v= Volume of extract for each titration (10 ml)

W= Weight of the fruit tissue (5 gm)

Results and Discussion

The average ascorbic acid content of healthy and ripened apricot fruits was observed to be 13.5 mg/100 gm of the fruit tissue. The average ascorbic acid content of the apricot fruits under pathogenesis of various test pathogens viz. *Rhizopus stolonifer*, *Aspergillus japonicus*, *A. niger* and *Penicillium citrinum* showed gradual decline and the results have been summarized in Table 1 and depicted in Fig. 1.

The extent of decline, however, varied with the test pathogen. Out of the four test pathogens, the apricots inoculated with *Rhizopus stolonifer* showed a maximum percentage loss (85.00 %± 4.08) in ascorbic acid content after 6 days of pathogenesis.

Similarly, the percentage loss of ascorbic acid in *Aspergillus japonicus* and *A. niger* inoculated fruits was 78.3 %± 2.36 and 60 %±4.08 respectively after the same duration. On the other hand, loss of ascorbic acid content by *Penicillium citrinum* was comparatively low (34.97 %±4.08) after 6 days of pathogenesis. In the same way, other investigators have also observed rapid decline in ascorbic acid content in other fruits during fungal pathogenesis. Prasad and Bilgrami (1979) showed that with an increase in the incubation period, there was decline in the ascorbic acid content of litchi fruits infected with *Aspergillus flavus*, *A. niger*, *A. variegator*, *A. nidulans* and *A. quadrilineatus*. According to Prasad (1980), the percentage loss of ascorbic acid in plum fruits infected with *Helminthosporium spiciferum* was 92.0%.

The healthy apricot fruits kept as control for 6 days also showed a loss of 10%±0.81 ascorbic acid content. However, this loss was minimal comparatively. A gradual loss in ascorbic acid content of healthy fruits over a period of time may be due to the fact that the ascorbic acid stability in fruits and vegetables is affected by a variety of factors including temperature, light, oxygen, enzymes and pH.

In general, ascorbic acid degradation is very rapid both after harvest and as the storage time increases (Morris, 1947). Similarly, Nunes *et al.* (1998) also noticed a loss of 55-70% of the ascorbic acid content in only 4 days in several uninfected strawberry varieties. Moreover, ascorbic acid is



also known to act as a precursor of tartarate and oxalate in several fruits (Gander, 1982) which may

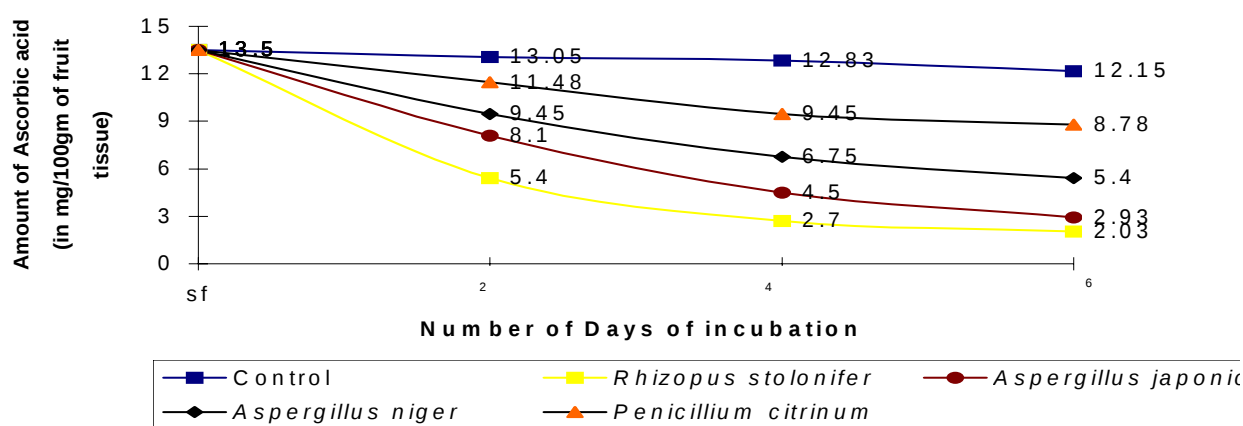
explain its slow but gradual reduction in the healthy fruits over a period.

Table. 1: Post- infection changes in ascorbic acid content (mg/100g of fruit tissue) of apricot fruit due to pathogenesis

Fungal pathogen	Ascorbic acid content (mg/100 g of fruit tissue)				Percent loss of ascorbic acid after 6 days
	Days of incubation				
	Initial	2 day	4 day	6 day	
Control	13.50 ± 1.10*	13.05 ± 0.07	12.83 ± 0.11	12.15 ± 0.11	10.00 ± 0.81
<i>Rhizopus stolonifer</i>	13.50 ± 1.10	5.40 ± 0.55	2.70 ± 0.55	2.03 ± 0.55	85.00 ± 4.08
<i>Aspergillus japonicus</i>	13.50 ± 1.10	8.10 ± 1.10	4.50 ± 0.64	2.93 ± 0.32	78.30 ± 2.36
<i>Aspergillus niger</i>	13.50 ± 1.10	9.45 ± 0.55	6.75 ± 0.55	5.40 ± 0.55	60.00 ± 4.08
<i>Penicillium citrinum</i>	13.50 ± 1.10	11.48 ± 0.95	9.45 ± 0.55	8.78 ± 0.55	34.97 ± 4.08

* Each value denotes the mean, $\pm$ = S.D, n=3

Figure1: Changes in ascorbic acid content of test pathogen inoculated apricot fruits during incubation period.



The decline in ascorbic acid content in fruits under pathogenesis may be attributed to the production of certain ascorbic acid degrading enzymes either by the pathogen or by the host and pathogen interaction (Ghosh *et al.*, 1965; Das, 2007]. In addition, the decline may also be due to increased respiration rate, which may induce rapid oxidation of ascorbic acid. Acceleration of the respiratory

activity has also been found in infected climacteric fruits such as peaches infected with *Monilinia fructicola* (Hall, 1967). Furthermore, the fungal enzymes including polyphenol oxidases, peroxidases and cytochrome oxidases might have played significant role in ascorbic acid degeneration in infected fruit tissue due to the oxidation of ascorbic acid (Ghosh *et al.*, 1966).



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Determination of spectrum of winter migratory birds in Yamuna Nagar district in Haryana (India)

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Abstract

During the three years of study (2005-08), some 58 species of wetland birds were observed in approximately 120 village ponds; out of these, atleast 28 species of wetland birds are winter migratory, coming from far off places located across the Himalayas. During the winter sojourns, these winter migratory birds face multiple threats in rural ponds of Haryana due to cipher attention of conservation authorities and persistent and accelerated utilization of rural ponds by the resident villagers. If the present admixture of interference and disturbance continues, then winter migratory birds like Bar-headed Goose (*Anser indicus*), Greylag Goose (*Anser anser*), Common Coot (*Fulica atra*), Northern Shoveller (*Anas clypeata*), Northern Pintail (*Anas acuta*), Common Teal (*Anas crecca*), Gadwall (*Anas strepera*), Mallard (*Anas platyrhynchos*), Common Pochard (*Aythya ferina*), Tufted Pochard (*Aythya fuligula*), Brahminy Shelduck (*Tadorna ferruginea*) and Red-crested Pochard (*Rhodonessa rufina*) will be wiped out just like Siberian Cranes arrived in Keoladeo National park in Rajasthan in India. The Ministry of Environment and Forests, Government of India should pay timely attention to save migratory birds that visit India every winter in Lakhs and Crores.

Keywords: *Migratory Birds, Rural Ponds, Threats, Yamuna nagar, Haryana.*

Introduction

The present study focusing attention on finding out the diversity of winter migratory birds that come to rural ponds in Yamunanagar district (30.1° N 77.28° E) having an area of 1569 sq. kms and located on the banks of Yamuna river at a distance of 190 kms from Delhi and juxtaposed with Saharanpur District of Uttar Pradesh (35 KMs east of Saharanpur). The avifaunal studies in the Indian subcontinent have been conducted by several scientists like Ali (1962, 1969); Ali and Ripley (1983); Grimmet *et al* (1998); Bhatnagar *et al.* (2008); Higuchi *et al.* (2008); Kasambe (2008); Saikia *et al.* (2008); Sibley and Monroe (1990); Baker and Inglis (1930); Manakadan and Pittie (2001); Kazmierczak (2000); Pandey *et al.* (2008); Kumar *et al.* (2005).

In Haryana, ornithological studies have been carried out by Yadav and Maleywar (1978)

Kalsi (1998), Gupta and Bajaj (1989, 1991a, 1991b, 1996 Gupta and Midha, 1992, 1993, 1994a-d, 1995a-c) Gupta and Goel (1994), Gupta and Kumar (2009) and on wetland birds by Gupta and Bajaj (1997, 1998, 1999, 2000; Gupta *et al.*, 2009, 2010a-c).

It is pertinent to mention that village ponds in Yamunanagar district in Haryana were staffed with winter migratory birds. However, no studies have been carried on the avian biodiversity of village ponds in Yamunanagar District (30.1° N 77.28° E) and hence the present study has been carried out.

Materials and Method

The present studies were conducted for a period of three years (2005-08) during winter seasons (September to April) and approximately 120 rural ponds were visited in 90 villages. Observations were made by covering the shores of each pond foot by foot, and extensive photography was exercised. The camera used was Zenith (Model 1986) with 200 M telelens. The wetland bird's species which were observed were further segregated according to their residential status categories like "Resident"

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“Winter Migratory” “Local Migratory” and “Summer Migratory” by following the technique developed by Kumar *et al.* (2005). Identification of birds was done by following Ali (1996); Ali and Ripley (1983); Grimmet *et al.* (1998); Alfred *et al.* (2001); Besten (2004) and Kumar *et al.* (2005). The nomenclature adopted was Manakadan and Pittie (2001).

Results and Discussion

The present studies reveal that 58 species of wetlands can be generally witnessed in rural ponds in Haryana state (Table.1) and these birds belong to ten orders (Fig.1) and 17 families (Fig.3). The most dominate Order is Charadriiformes (17 species) followed by Anseriformes (13 species) and Ciconiiformes (08 species). The least diversity has been observed in Order like Falconiformes (One Species) and Apodiformes (One Species) closely followed by Podicipediformes (2 species) and Coraciiformes (3 species). Out of 58 species of wetland birds observed in village ponds of Yamunanagar district, 28.49% were winter migratory, 18.21 % were Resident Birds, 10.17% local migratory and 2.3 % were summer migratory. Kumar *et al* (2005) reported 109 residents, while the status is not known for eight species. Kalsi (1998) recorded 161 species of birds at Kalesar National Park in Yamunanagar district in Haryana. Gupta *et al.* (2009) reported 72 species of Wetland Birds belonging to 10 Orders and 19 Families from rural village ponds of Karnal District in Haryana. Out of these, 37 species of birds are winter migratory, 13 are local migratory, 20 are resident. Gupta and Kaushik (2010) reported 66 species of wetland birds from Kurukshetra, out of which 33 species of birds were winter migratory, 21 species resident, 11 species local migratory and 3 species were summer migratory. At the same time, Gupta *et al.*, 2010 recorded a total of 63 species of Wetland birds from village ponds in Kaithal District. Out of 63 species of wetland birds, 31 are winter migratory, 9 local migratory, 3 summers migratory and 20 species are resident birds. It is pertinent to mention large numbers of wetland birds migrate into India annually from far off places located across the Himalayas (Ali and Ripley, 1983, Bird life International 2001)

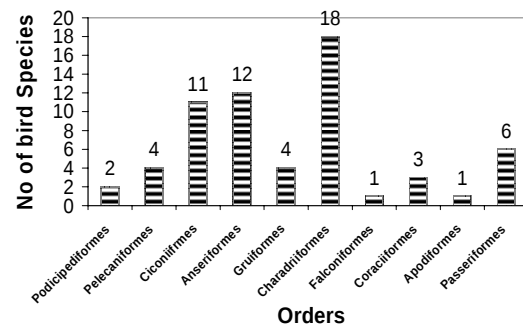


Fig.1 Depiction of incidence of wetland birds in village ponds of Yamuna nagar district in Order-wise manner during the study period (2005-08).

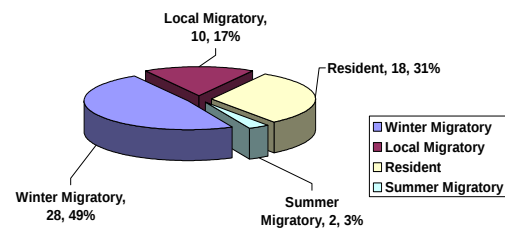


Fig.2 Showing the Residential Status of Wetland birds observed in Yamunanagar district during the study period (2005-08).

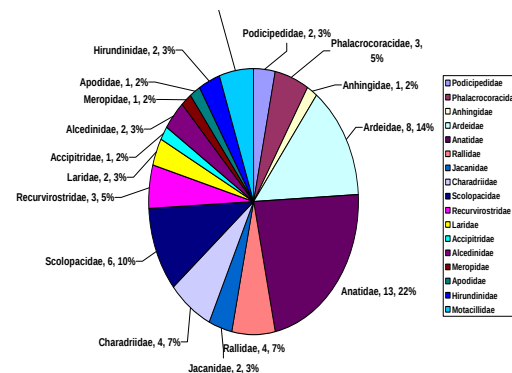


Table 1: Checklist of wetland birds observed in village ponds of Yamuna nagar district in Haryana, India

S. No	Common Name	Scientific Name	RES. STATUS	CHH	JAG	MUS	RAD	SAD	BIL
(I) ORDER-PODICIPEDIFORMES			(i) FAMILY- PODICIPEDIDAE						
1	Little Grebe	<i>Tachybaptus rufficollis</i> (Pallas, 1764)	R	√	√	√	√	√	√
2	Great Crested Grebe	<i>Podiceps cristatus</i> (Linnaeus, 1758)	WM	√	√	x	x	x	x
(II) ORDER-PELECANIFORMES			(i) FAMILY-PHALACROCORACIDAE						
3	Little Cormorant	<i>Phalacrocorax niger</i> (Vieillot, 1817)	R	√	√	√	√	√	√
4	Indian Shag	<i>Phalacrocorax fuscicollis</i> (Stephens, 1826)	LM	√	√	√	√	√	√
5	Great Cormorant	<i>Phalacrocorax carbo</i> (Linnaeus, 1758)	LM	√	√	√	√	√	√
			(ii) ANHINGIDAE						
6	Darter	<i>Anhinga melanogaster</i> (Pennant, 1769)	R	√	√	x	x	√	√
(III) ORDER-CICONIIFORMES			(i) FAMILY- ARDEIDAE						
7	Little Egret	<i>Egretta garzetta</i> (Linnaeus, 1766)	LM	√	√	√	√	√	√
8	Grey Heron	<i>Ardea cinerea</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
9	Purple Heron	<i>Ardea purpurea</i> (Linnaeus, 1766)	LM	√	√	√	√	√	√
10	Large Egret	<i>Casmerodius albus</i> (Linnaeus 1758)	LM	√	√	√	√	√	√
11	Median Egret	<i>Mesophoyx intermedia</i> (Wagler 1829)	LM	√	√	√	√	√	√
12	Cattle Egret	<i>Bubulcus ibis</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
13	Indian Pond-Heron	<i>Ardeola grayii</i> (Sykes, 1832)	R	√	√	√	√	√	√
14	Black-crowned Night Heron	<i>Nycticorax nycticorax</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
(IV) ORDER-ANSERIFORMES			(i) FAMILY- ANATIDAE						
15	Greylag Goose	<i>Anser anser</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
16	Lesser Whistling duck	<i>Dendrocygna javanica</i> (Horsfield, 1821)	SM	√	√	√	√	√	√
17	Brahminy Shelduck	<i>Tadorna ferruginea</i> (Pallas 1764)	WM	√	√	√	X	√	√
18	Bar-headed Goose	<i>Anser indicus</i> (Latham, 1790)	WM	√	√	√	√	√	√
19	Gadwall	<i>Anas strepera</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√



20	Mallard	<i>Anas platyrhynchos</i> (Linnaeus, 1758)	WM	√	√	x	x	x	x
21	Spot-billed Duck	<i>Anas poecilorhyncha</i> (J.R.Forester,(1781)	WM	√	√	√	√	√	√
22	Northern Shoveller	<i>Anas clypeata</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
23	Northern Pintail	<i>Anas acuta</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
24	Common Teal	<i>Anas crecca</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
25	Red-crested Pochard	<i>Rhodonessa rufina</i> (Pallas, 1773)	WM	√	√	x	x	x	x
26	Common Pochard	<i>Aythya ferina</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
27	Tufted Pochard	<i>Aythya fuligula</i> (Linnaeus, 1758)	WM	√	√	x	x	x	x
(V) ORDER-GRUIFORMES (i) FAMILY- RALLIDAE									
28	White-breasted Waterhen	<i>Amaurornis phoenicurus</i> (Pennant, 1769)	R	√	√	√	√	√	√
29	Purple Moorhen	<i>Porphyrio porphyrio</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
30	Common Moorhen	<i>Gallinula chloropus</i> (Linnaeus, 1758)	LM	√	√	√	√	√	√
31	Common Coot	<i>Fulica atra</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
(VI) ORDER-CHARADRIIFORMES (i) FAMILY- JACANIDAE									
32	Pheasant-tailed Jacana	<i>Hydrophasianus chirurgus</i> (Scopoli, 1786)	SM	√	√	√	√	√	√
33	Bronze-winged Jacana	<i>Metopidius indica</i> (Latham, 1790)	R	√	√	x	√	x	x
(ii) FAMILY- CHARADRIIDAE									
34	Yellow-wattled Lapwing	<i>Vanellus malabaricus</i> (Boddaert, 1783)	R	√	√	x	x	x	x
35	River Lapwing	<i>Vanellus duvaucelii</i> (Lesson-1826)	R	√	√	x	√	√	√
36	Red-wattled Lapwing	<i>Vanellus indicus</i> (Boddaert, 1783)	R	√	√	√	√	√	√
37	White-tailed Lapwing	<i>Vanellus leucurus</i> (Lichtenstein, 1823)	WM	√	√	X	x	√	√
(iii) FAMILY- SCOLOPACIDAE									
38	Black-tailed Godwit	<i>Limosa limosa</i> (Linnaeus, 1758)	WM	√	√	x	x	x	x
39	Common Redshank	<i>Tringa totanus</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
40	Common Sandpiper	<i>Actitis hypoleucos</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
41	Ruff	<i>Philomachus pugnax</i> (Linnaeus, 1758)	WM	√	√	√	x	√	√
42	Marsh Sandpiper	<i>Tringa stagnatilis</i> (Bechstein,1803)	WM	√	X	x	x	x	x



43	Wood Sandpiper	<i>Tringa glareola</i> (Linnaeus, 1758)	WM	√	√	x	√	x	x
(iv) FAMILY- RECURVIROSTRIDAE									
44	Black-winged Stilt	<i>Himantopus himantopus</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
45	Pied Avocet	<i>Recurvirostra avosetta</i> (Linnaeus, 1758)	WM	√	x	√	x	√	√
46	River Tern	<i>Sterna aurantia</i> (J.E.Gray, 1831)	LM	√	√	√	√	√	√
(v) FAMILY- LARIDAE									
47	Black-headed Gull	<i>Larus ridibundus</i> (Linnaeus, 1766)		√	√	x	√	x	x
48	Pallas's Gull	<i>Larus ichthyaetus</i> (Pallas, 1773)	WM	x	√	x	X	x	x
VII) ORDER- FALCONIFORMES (i) FAMILY- ACCIPITRIDAE									
49	Brahminy Kite	<i>Haliastur indus</i> (Boddaert)	R	√	√	√	√	√	√
(VIII) ORDER- CORACIIFORMES (i) FAMILY-ALCEDINIDAE									
50	Lesser Pied Kingfisher	<i>Ceryle rudis</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
51	White-breasted Kingfisher	<i>Halcyon smyenensis</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
(ii) FAMILY- MEROPIDAE									
52	Blue-cheeked Bee-eater	<i>Merops persicus</i> (Pallas, 1773)	R	√	√	√	√	√	√
(IX) ORDER- APODIFORMES (i) FAMILY- APODIDAE									
53	House Swift	<i>Apus affinis</i> (J.E.Gray)	R	√	√	√	√	√	√
(X) ORDER- PASSERIFORMES (i) FAMILY-HIRUNDINIDAE									
54	Wire-tailed Swallow	<i>Hirundo smithii</i> (Leach, 1818)	R	√	√	√	√	√	√
55	Common Swallow	<i>Hirundo rustica</i> (Linnaeus, 1758)	R	√	√	√	√	√	√
(ii) FAMILY- MOTACILLIDAE									
56	White Wagtail	<i>Motacilla alba</i> (Linnaeus, 1758)	WM	√	√	√	√	√	√
57	Large Pied Wagtail	<i>Motacilla maderaspatensis</i> (Gmelin, 1789)	LM	√	√	√	√	√	√
58	Citrine Wagtail	<i>Motacilla citreola</i> (Pallas, 1776)	WM	√	√	√	√	√	√

During the winter sojourns, these winter migratory birds face multiple threats in rural ponds of Haryana. While assorting specially the winter migratory birds, it is borne out from the present studies that Haryana rural ponds attract winter migratory birds like Northern Shoveller *Anas clypeata*, Northern Pintail (*Anas acuta*), Common Teal (*Anas crecca*), Gadwall (*Anas strepera*) Bar-headed Goose *Anser indicus*, Common Coot (*Fulica atra*), Common Pochard

(*Aythya ferina*), Tufted Pochard, Red crested Pochard etc. during winter season every year. Further, it is startling to reveal that no attention is given to protect them from varied threats including conversion of traditional rural ponds into fish farming ponds alongwith accentuated interference by villagers linked with cattle bathing, cow dung cakes, pollution of water etc. The present studies also unfold the continued receding of depth of water in centre and across



the shores in practically all the rural ponds. Even if the disappearance of rural ponds persist for next 10 years in a row, then 80% of the rural ponds will die down unnatural death, this directly threatened the very survival of internationally acclaimed winter migratory birds like Bar-headed Goose, Common Coot, Northern Shoveller, Northern Pintail, Common Teal, Gadwall, Lesser Whistling duck, Common Pochard, Mallard, Tufted Pochard and Red crested Pochard. Also, the disappearance of rural ponds will completely wipe out White-tailed Lapwing, Cormorants, Large Egret, Median Egret, Pond Heron *etc.* It is pertinent to mention that the crucial birds like Bar-headed Goose, Mallard, Northern Pintail, Northern Shoveller, Garganey, Common Pochard, Tufted Pochard, Red-crested Pochard, come from across the Himalayas from far off places like Russia (Ali and Ripley, 1983) and stay in winter in Haryana rural ponds which are in extremely dilapidated conditions, pose a threat of an alarming level to the very survival of above mentioned birds of global significance. The present paper wish to draw the requisite attention of Species Survival Commission, United Nations Environmental Programme, World Wide Fund For Nature, Wetland International, Birdlife International, Ministry of Environment and Forest (Government of Haryana and India) to rise up to the situation to save rural ponds and winter migratory birds from a dismal end sooner than later in Northern India (Haryana, Punjab, Uttar Pradesh, Rajasthan and Madhya Pradesh) so as avoid the recurrence of events like Doomed Siberian Crane *vis-à-vis* Keoladeo wetlands in Rajasthan(India).

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Antibacterial and phytochemical studies of *Euphorbia hirta* Linn. against respiratory tract pathogens

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Abstract

Euphorbia hirta was evaluated for antibacterial activity against human pathogenic bacterial strains *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* causing respiratory tract infections. Petroleum ether, acetone, methanol and water extracts of *E. hirta* were screened for antibacterial activity by cup-plate method at sample concentration of 200 mg/ml. The result of antibacterial activity revealed that methanolic extracts of the plant exhibited maximum activity as compared to petroleum ether, acetone and water. Minimum inhibitory concentration (MIC) of the methanolic extract of the plant was also calculated against the pathogens. *S. aureus* was the most susceptible bacteria. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, amino acids and steroids. The presence of these bioactive constituents has been linked to the antimicrobial activity of the plant.

Keywords: *Euphorbia hirta*, Antibacterial, Phytochemical, Respiratory tract infections

Introduction

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1985; Chopra *et al.*, 1992; Harborne and Baxter, 1995). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens (Ahmad and Beg, 2001). *Euphorbia hirta* is one of such herbs belonging to the family Euphorbiaceae which is frequently seen occupying open waste spaces and grasslands, road sides and pathways. The plant is used in treatment of asthma and respiratory tract inflammations (Kokwaro, 1993). It is also used for coughs, chronic bronchitis and other pulmonary disorders in

Malagasy (Wong-Ting-Fook, 1980). The medicinal usefulness of the herb has been the subject of numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an antispasmodic, antiasthmatic, expectorant, antidiarrheal and antisyphilitic (Burkill, 1994; Adedapo *et al.*, 2005; Falodun *et al.*, 2006). In the present study, we have selected whole plant of *Euphorbia hirta* to be screened against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

The observed antibacterial activity was believed due to the presence of many biologically active constituents of plant which were identified in the extracts.

Materials and Method

Plant material

The plants were collected from Shivalik range of Himalayas in Haridwar. The plant was identified at Botanical Survey of India, Dehradun,

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Uttarakhand (India). The whole plant was washed by running tap water to remove the adhering unwanted material and cutted into small pieces, dried at room temperature and then powdered by using blender.

Extraction of plant material

The powdered plant material was loaded in soxhlet assembly and extracted in four different solvents *i.e.* petroleum ether, acetone, methanol and water for 72 hours by successive method. At the end of each extract, it was passed through Whatman filter paper No.40 and the filtrates were evaporated under reduced pressure.

Preparation of Plant Extracts

The extracts were prepared by immersing 200 gm of dried powdered material in 600 ml of the solvents *i.e.* petroleum ether, acetone, methanol and water by soxhlet apparatus. After removing the solvents, the crude extracts were stored in sterile bottles at 4°C until further use.

Culture media

Muller Hinton Agar Media No. 173 (Hi Media Pvt. Ltd., Mumbai, India) was used for screening of antimicrobial activity.

Microorganisms used

The bacterial strains *i.e.* *Staphylococcus aureus* (MTCC-1144), *Streptococcus pyogenes* (MTCC-422) and *Streptococcus pneumoniae* (MTCC-655) used in this study are related to respiratory tract infections.

Antibacterial activity

The cup-plate method was used to evaluate the antibacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material (Perez *et al.*, 1990, Ahmad *et al.*, 1998). 0.1 ml of diluted inoculum (10^5 CFU/ml) of tested organism was mixed in Muller Agar Hinton Media, shaken and poured in sterilized petridishes. Wells of 8 mm diameter were punched into the agar medium and filled with 45 µl of plant extracts. All the solvents served as negative control. Each extract was

assayed in triplicate. The plates were incubated at 37°C for 24 hours. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm).

Determination of MIC

The minimum inhibitory concentration of the methanolic extract was determined by using the serial micro dilution method. The sterile Muller Hinton Broth bacterial suspension was added into microtiter plates. The extract in broth was used as negative control and bacterial suspensions were used as positive control. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms. The MIC values were taken as the lowest concentration of the extract in the wells of the microtiter plate that showed no turbidity after 18-24 hours of incubation at 37°C.

Phytochemical screening of the plant

Phytochemical screening was carried out of the plant material for the presence of bioactive components such as alkaloids, flavonoids, glycosides, amino acids, steroids, saponins, tannins and phenolics (Trease and Evans, 1996).

Results and Discussion

The zone of inhibition of the petroleum ether, acetone, methanol and water extracts (200 mg/ml) from the *E. hirta* plant against *S. aureus*, *S. pyogenes* and *S. pneumoniae* are shown in Table 1. The methanolic extract showed the maximum zone of inhibition against all the bacteria followed by water, acetone and petroleum ether. The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent. The zone of inhibition formed by petroleum ether extract is least effective. The methanolic extract was highly active against *S. aureus* (26 mm) followed by *S. pyogenes* (25 mm) and *S. pneumoniae* (22 mm) as compared to others. Water extract showed best activity against *S. aureus* and *S. pyogenes* (24 mm). Acetone is more effective in comparison to petroleum ether. Acetone showed maximum inhibition against *S. aureus* (23 mm) followed by *S. pyogenes* and *S. pneumoniae* (21 mm). Petroleum ether was found most effective against *S.*



pyogenes (20 mm) followed by *S. aureus* (18 mm) and *S. pneumoniae* (17 mm). The basis for their difference in susceptibility might be due to constituents present in the extract. The leaves of *E. hirta* have considerable antibacterial activity against *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Ogbolie *et al.*, 2007).

Table. 1. Antibacterial activity of *Euphorbia hirta* extracts (mm) in different solvents

S.No.	Plant Extract	<i>S.aureus</i>	<i>S.pyogenes</i>	<i>S.pneumoniae</i>
1.	Petroleum ether	18	20	17
2.	Acetone	23	21	21
3.	Methanol	26	25	22
4.	Water	24	24	21

Values represent average of three replicates

The methanolic and aqueous extracts of *E. hirta* showed antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi* and *Proteus mirabilis*. The growth of all the bacteria was inhibited though to varying degree, thus justifying the use of the herb in traditional medicine (El- Mahmood, 2009). The ethanolic extract of *E. hirta* (root) had strong inhibitory effect against *Propionibacterium acnes* (Kumar *et al.*, 2007).

The phytochemical screening of the plant extracts showed the presence of alkaloids, flavonoids, glycosides, amino acids, steroids, saponins, tannins and phenolics (Table-2). Alkaloid was present in acetone and methanolic extracts of the plant whereas absent in petroleum ether and water extracts. Methanolic extract of the plant possess the presence of maximum constituents. Phytochemical screening of the crude extracts of *E. hirta* revealed the presence of tannins, saponins, phenolics, cardiac glycosides, anthraquinones, flavonoids and alkaloids. These compounds have potentially significant application against human pathogens, including those that cause enteric infections (El-Mahmood *et al.*, 2008).

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in extraction procedure. Traditional healers use primarily water as the solvents (Ahmad *et al.*, 1998) but in our studies we found that plant extracts in organic solvents (methanol) provided more consistent antimicrobial activity as compared to those extracted in water.

Table. 2. The phytochemical screening of *Euphorbia hirta*

S.No.	Plant	Alkaloids	Flavonoids	Tannins/ Phenols	Saponins	Glycosides	Amino acids	Steroids
1.	Petroleum ether	-	-	-	-	-	-	+
2.	Acetone	+	-	+	-	-	-	+
3.	Methanol	+	+	+	+	+	+	+
4.	Water	-	+	+	+	-	+	-

+ = present - = Absent

These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the media used in the assay. The observed antibacterial effects on the isolates are believed to be due to the presence of flavonoids, alkaloids and tannins which have been shown to possess antibacterial properties (Ogbolie *et al.*, 2007, Cowan, 1999, Draughan, 2004). Newze *et al.* (2004) have also attributed observed antimicrobial effects of plant extract to the presence of these secondary metabolites. In conclusion, the extract of *E. hirta* plant has high potential as antibacterial agents. This finding has validated the use of these medicinal plants for the treatment of microbial infections. It seems important to recommend that further studies using isolated constituents instead of whole extracts must be done in this field.

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Floristic composition, lifeform classification and biological spectrum of district Jammu, Jammu and Kashmir, North - Western Himalayas

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Abstract

The present study was carried out in the Jammu district which revealed the presence of 395 plant species belonging to 302 genera and 93 families. Fabaceae has been recorded as the largest family (25 genera / 42 species) followed by Asteraceae (24/29), Poaceae (21/23), Labiatae (12/17), Euphorbiaceae (9/16), Verbenaceae (9/10) etc. Thirty five families show monotypic representation in the area. 90.21% of the whole flora is represented by Dicots. The genus-species ratio has been evaluated as 1:30. The species when compared to Raunkiaer's life form system (1934) reveal the preponderance of therophytes (38.23%) followed by phanerophytes (19.50%) thus indicating thero-phanerophytic type of phytoclimate.

Keywords: *Biological spectrum, Floristic composition, Life forms, Phytoclimate, Phanerophytes,*

Introduction

The climate determines the type of plants that can exist in each region and the individuals of each species in a community can be grouped into various lifeforms based upon their physiognomic appearance and growth performance. Biological spectrum is formed when all species of the higher plants of a community are classified into lifeforms and their ratio is expressed in numbers or percentage. Biological spectra are useful in comparing geographically widely separated plant communities and are also regarded as indicators of biotic interaction, climate and habitat deterioration. Since different systems have been devised by many ecologists for the description and classification of plant life forms (Warming, 1909; Dansereau, 1957; Ellenberg and Muller-Dombois, 1974; Box, 1981), the Raunkiaer (1934) system is

the most accepted which is based upon the principle of position and degree of protection of the buds during adverse conditions. The Raunkiaer's Normal spectrum prepared for the phanerogamic flora of the whole world is still widely being used for comparing biological spectra of different regions. Generally, a complete list of plants called floristic composition and their life-forms defines the appearance of plant community. Since structure and rate of change of composition are sensitive indicators of whole environment, it is important to study the floristic composition and lifeforms of different plants to find phytoclimate of the area. In Jammu and Kashmir several workers have studied the floristic composition and biological spectra of different areas. This includes the works of Sapru (1975), Kaul and Sarin (1976), Kapur (1982), Dhar and Kaul (1986), Kumar (1987), Singh and Kachroo (1994), Kumar (1997), Kour (2001), Singh (2002), Kesar (2002), Dutt (2005), Jhangir (2004) etc. However, the work on this aspect for the erstwhile Jammu district has not been done so far.

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Study area

The data has been recorded from the Jammu district (latitude 32°27' to 33°50' N and longitude 74°19' to 75°20' E) covering a total area of 2942 km², comprised of five tehsils including the newly carved out Samba district. Physiographically the area is divided into Jammu foothills comprising low hills embracing features ranging from pine clad slopes to practically bare almost flat topped hillocks and gentle slopes made up of boulder mass supporting dry and thorny vegetation. The level land comprised of Chenab-Ravi plains occupies more than half the geographical area of the region. The area situated in the sub-tropical part has markedly periodic climate characterized by dry and increasingly hot season during May-July, to dry and cold season during December-January. The normal annual rainfall is 1115 mm, 75% of which is received during monsoon months. The area is criss-crossed by a number of seasonal and perennial rivers and streams, the main being River Chenab, River Tawi, Munnawar Tawi, Balole nallah, Basantar nallah etc. The altitude of the area varies from 320 m to 1034m above msl.

Materials and Method

Exhaustive field investigations have been carried out in study area during different seasons for three years for the assessment of plant diversity and floristic composition for angiosperms and gymnosperms only. The plant species were photographed and accordingly identified using local herbaria, flora and relevant literature available for the region. Utmost care was taken during survey and enumeration to avoid disturbance to flora and fauna. Biological spectrum of the flora based on the life forms was prepared by following Raunkiaer (1934) life form classes based upon the location of perennating buds. After having assigned a life form to all plants, Raunkiaer's biological spectrum was calculated as follows:

$$\text{Biological Spectrum} = \frac{\text{No. of sps. falling in particular form class}}{\text{Total no. of all the species for that community /stand}} \times 100$$

Results and Discussion

The forests of Jammu district represent typical subtropical vegetation. The lower altitudinal zonation is dominated mainly by shrubs with a few scattered patches of broad leaved trees. On moderate elevation these shrubs found to be mixed with broad leaved and Chir-pine communities, while at higher elevations are dominated exclusively by Chir-Pine communities. The forests of Jammu district have been divided into three types in accordance with the classification made in revised summary of Forest types of India by Champion and Seth (1968). These are 5B/C2 – Northern dry mixed deciduous forests; 9/C1/DS1 – Himalayan sub-tropical scrub, and 9/C1a – Himalayan sub-tropical pine forests.

Dominant tree species found in the area comprise of *Mallotus philippensis*, *Cassia fistula*, *Acacia modesta*, *Acacia catechu*, *Zizyphus mauritiana*, *Dalbergia sissoo*, *Lannea coromandalica*, *Syzigium cumini*, *Grewia optiva*, *Albizia lebbeck*, *Pinus roxburghii*, *Phyllanthus emblica* etc. The understory is prominently represented by *Lantana camara*, *Carissa opaca*, *Dodonaea viscosa*, *Murraya koenigi*, *Woodfordia fruticosa*, *Colebrookia oppositifolia*, *Punica granatum*, *Justicia adhatoda*, *Rubus ellipticus*, *Mimosa rubicaulis*, *Zizyphus oxyphylla*, *Randia tetrasperma*, *Capparis sepiaria* etc.

Among the climbers, *Phanera vahlii* and *Caesepinia decapatela* the two conspicuous lianas, climb mostly on the broad leaved trees and sometimes even up to the height of 25 metres on chirpine trees. Other climbers typical of these hills are *Tinospora cordifolia*, *Aspidopterys wallichii*, *Abrus precatorius*, *Vallaris solanacea*, *Cryptolepis buechanani*, *Hiptage benghalensis*, *Clematis gouriana*, *Porana paniculata*, *Dregea volubilis*, *Cissampelos pariera*. *Dendrophthoe falcata* and *Cuscuta reflexa* are the only hemiparasite / parasite found in the region.

The analysis of the data further revealed that the ratio of family to genera is 1: 3.24; family to species is 1: 4.24, and, genera to species are 1:1.30. The ratio of genera to species which reflects the floristic pattern in given time and space is lower than that derived for British India 1



: 7 (Hooker, 1872-97); India alone - 1 : 6 (Chatterjee, 1939); Valley of flowers - 1 : 4.3 (Kala and Rawat, 2004); Himachal Pradesh - 1 : 2.93 (Chowdhry and Wadhwa, 1984); Bashar Himalayas - 1 : 2.29 (Aswal and Mehrotra, 1994) Shimla - 1: 20 (Collet, 1902); Great Himalayan National Park - 1 : 1.94 (Singh and Rawat, 2000), Kullu - 1: 1.84 (Dhaliwal and Sharma, 1999),

Kangra - 1 :1.72 (Kapur, 1985), Mussourie - 1: 1.87 (Raizada and Saxena, 1978), Sirmour - 1: 1.65 (Kaur and Sharma, 2004). The ratio tends to match with the flora of Renuka Wildlife Sanctuary - 1 : 1.30 (Subramani *et al.*, 2007); Trikuta Hills - 1 : 1.42 (Kapur and Sarin, 1990), Patnitop and adjoining areas - 1:1.44 (Kumar, 1997) Trikuta Hills - 1:1.42 (Kour, 2001).(Table.1)

Table-1 Comparison of dominant families of District Jammu with adjoining areas of the state and with India

District Jammu (Authors)	Jammu (Sharma and Kachroo, 1981)	Patnitop Hills (Kumar, 1997)	Bhaderwah (Kumar, 1987)	Neeru watershed Bhaderwah (Dutt, 2005)	Trikuta Hills (Kour, 2001)	India (Hooker, 1872-97)
Fabaceae	Fabaceae	Asteraceae	Asteraceae	Asteraceae	Asteraceae	Orchidaceae
Asteraceae	Poaceae	Labiatae	Poaceae	Labiatae	Fabaceae	Fabaceae
Poaceae	Asteraceae	Poaceae	Apiaceae	Apiaceae	Poaceae	Poaceae
Labiatae	Cyperaceae	Fabaceae	Labiatae	Ranunculaceae	Labiatae	Rubiaceae
Euphorbiaceae	Scrophularaceae	Roasaceae	Ranunculaceae	Roasaceae	Euphorbiaceae	Euphorbiaceae
Convolvulaceae	Labiatae	Ranunculaceae	Cruciferae	Cruciferae	Scrophularaceae	Acanthaceae

As per the available records, the percentage of dicots and monocots species in the world flora is 81.3% and 18.7%, respectively. Different studies carried out in the different parts of Jammu by Kour (2001), Kumar (1987), Kumar (1997) and Singh (2002) have reported higher percentage of

dicots. The present study also revealed the higher percentage of dicots (90.21%) from the study area. The ratio of the monocot to dicot families have been recorded as 1: 9.22, of genera 1:7.60 and of species 1:9.37, respectively (Table. 2).

Table-2 Percentage and ratios of the families, genera and species of dicots and monocots (excluding family Pinaceae and genera / species thereof).

Taxa	Dicots		Monocots		Total	Ratio	
	Total number	% age	Total number	% age		Monocots	Dicots
Families	83	90.21	09	9.79	92	1	9.22
Genera	266	88.37	35	11.63	301	1	7.60
Species	356	90.35	38	9.65	394	1	9.37



The number of different life forms has been given for each family on the basis of study of 395 species. The species belonging to different families have been arranged in nine broad life forms as depicted in Table - 3. When compared to Raunkiaer's normal Biological spectrum, the biological spectrum of study area shows that hemicryptophytes, nano phanerophytes, macro phanerophytes and geophytes reveal a lower percentage, whereas therophytes, hemi - cryptophytes and chameophytes showed higher percentage. Rest of the life forms revealed minor deviation from the normal spectrum (Table.4). The higher percentage of therophytes (38.23%) occurring in the area is the characteristics of

subtropics and often related to soil conditions and climate (Subramani *et al.* 2007). Moreover, the predominance of therophytes is also attributed to the introduction of annual weeds, biotic influences like agricultural practices and grazing, deforestation and trampling *etc.* (Sher and Khan, 2007; Mohammad and Sher, 2010). Macro-phanerophytes (19.49%) occupying the second position reveals the predominance of trees as the area falls in Shiwaliks which provides congenial edaphic and climatic conditions for the growth of overstorey. A bit lower percentage of nanophanerophytes (9.37%) may be attributed to ground disturbances like grazing, forest fires, encroachments *etc.*

Table .3: Number of life forms in different families of district Jammu

Family	Ph							Ch	H	Cr		Th
	MMM	MM	M	N	L	P	E			G	HH	
Fabaceae	-	1	3	2	2	-	3	5	3	-	-	26
Asterceae	-	-	-	-	-	-	-	3	1	1	-	23
Poaceae	-	-	-	-	-	-	-	15	3	-	1	5
Labiatae	-	-	-	2	-	-	-	2	5	-	1	7
Euphorbiaceae	-	1	4	4	-	-	-	-	1	-	-	7
Convolvulaceae	-	-	-	-	1	-	3	2	3	-	-	2
Acanthaceae	-	-	1	1	-	-	-	5	-	-	-	3
Solanaceae	-	-	-	1	-	-	-	1	-	-	-	8
Verbanaceae	-	-	3	3	-	-	-	-	-	-	-	5
Mimosaceae	-	4	3	1	-	-	-	-	-	-	-	-
Rubiaceae	-	1	3	2	-	-	-	-	-	-	-	3
Amranthaceae	-	-	-	1	-	-	1	-	3	-	-	4
Caesalpiniaceae	-	-	3	-	3	-	-	-	-	-	-	2
Scrophulariaceae	-	-	-	-	-	-	-	1	-	-	-	8
Moraceae	1	3	4	-	-	-	-	-	-	-	-	-
Cucurbutaceae	-	-	-	-	-	-	2	-	-	-	-	2
Apocynaceae	-	-	2	2	2	-	-	-	-	-	1	-
Malvaceae	-	-	1	2	-	-	-	-	-	-	-	3
Rosaceae	-	-	1	2	-	-	1	1	1	-	-	-
Liliaceae	-	-								4	-	-
Cruciferae	-	-	Floristic composition, lifeform classification							-	-	4
Asclpiadaceae	-	-	-	-	4	-	1	1	-	-	-	-
Boraginaceae	-	-	-	-	-	-	-	1	1	-	-	3



Ranunculaceae	-	-	-	-	-	-	1	-	-	-	-	2
Tiliaceae	-	-	2	-	-	-	-	-	-	-	-	3
Rhamnaceae	-	1	1	1	-	-	-	1	-	-	-	-
Polygonaceae	-	-	-	-	-	-	-	1-	-	-	-	3
Capparidaceae	-	-	-	2	-	-	-	-	-	-	-	1
Flacourtiaceae	-	-	3	-	-	-	-	-	-	-	-	-
Caryophyllaceae	-	-	-	-	-	-	-	-	1	-	-	3
Rutaceae	-	1	1	1	-	-	-	-	-	-	-	-
Celastraceae	-	2	1	-	2	-	-	-	-	-	-	-
Anacardiaceae	-	3	-	-	-	-	-	-	-	-	-	-
Apiaceae	-	-	-	-	-	-	-	-	1	-	-	1
Loganiaceae	-	-	1	-	-	-	-	1	-	-	-	1
Bignoniaceae	-	1	1	-	-	-	-	1	-	-	-	1
Chenopodiaceae	-	-	-	-	-	-	-	-	-	-	-	3
Cyperaceae	-	-	-	-	-	-	-	-	2	-	-	-
Menispermaceae	-	-	-	-	-	-	1	1	-	-	-	-
Nymphaeaceae	-	-	-	-	-	-	-	-	-	-	1	-
Violaceae	-	-	-	-	-	-	-	-	2	-	-	-
Linaceae	-	-	-	-	-	-	-	1	-	-	-	1
Malpighiaceae	-	1	1	-	1	-	-	-	-	-	-	-
Geraniaceae	-	-	-	-	-	-	-	1	-	-	-	-
Oxalidaceae	-	-	-	-	-	-	-	1	-	-	-	-
Meliaceae	-	2	-	-	-	-	-	-	-	-	-	-
Vitaceae	-	-	-	1	-	-	1	-	-	-	-	-
Sapindaceae	-	-	-	1	-	-	1	-	-	-	-	-
Combretaceae	-	2	-	-	-	-	-	-	-	-	-	-
Myrtaceae	-	1	1	-	-	-	-	-	-	-	-	-
Lythraceae	-	-	-	1	-	-	-	-	-	-	1	-
Onagraceae	-	-	-	-	-	-	-	-	-	-	-	2
Primulaceae	-	-	-	-	-	-	-	-	1	-	-	1
Ehretiaceae	-	-	2	-	-	-	-	-	-	-	-	-
Nyctaginaceae	-	-	-	-	-	-	-	1	-	-	-	1
Dioscoreaceae	-	-	-	-	-	-	-	-	-	2	-	-
Commelinaceae	-	-	-	-	-	-	-	-	-	-	-	2
Portulacaceae	-	-	-	-	-	-	-	-	-	-	-	2
Papaveraceae	-	-	-	-	-	-	-	-	-	-	-	1
Fumariaceae	-	-	-	-	-	-	-	-	-	-	-	1
Pittosporaceae	-	1	1	-	-	-	-	-	-	-	-	-



Floristic composition, lifeform classification

Polygalaceae	-	-	-	-	-	-	-	-	-	-	-	1
Bombacaceae	-	1	-	-	-	-	-	-	-	-	-	-
Sterculiaceae	-	-	-	1	-	-	-	-	-	-	-	-
Leeaceae	-	-	1	-	-	-	-	-	-	-	-	-
Moringaceae	-	-	1	-	-	-	-	-	-	-	-	-
Punicaceae	-	-	-	1	-	-	-	-	-	-	-	-
Begoniaceae	-	-	1	-	-	-	-	-	-	-	-	-
Campanulaceae	-	-	-	-	-	-	-	-	-	-	-	1
Sphenocleaceae	-	-	-	-	-	-	-	-	-	-	-	1
Plumbaginaceae	-	-	-	-	-	-	-	-	-	-	-	1
Myrsinaceae	-	-	-	1	-	-	-	-	-	-	-	-
Ebenaceae	-	-	1	-	-	-	-	-	-	-	-	-
Oleaceae	-	-	-	1	-	-	-	-	-	-	-	-
Gentianaceae	-	-	-	-	-	-	-	-	1	-	-	-
Cuscutaceae	-	-	-	-	-	2	-	-	-	-	-	-
Marytyniaceae	-	-	-	-	-	-	-	-	-	-	-	1
Plantaginaceae	-	-	-	-	-	-	-	-	1	-	-	-
Loranthaceae	-	-	-	-	-	2	-	-	-	-	-	-
Ulmaceae	-	-	1	-	-	-	-	-	-	-	-	-
Cannabaceae	-	-	-	-	-	-	-	1	-	-	-	-
Urticaceae	-	-	-	1	-	-	-	-	-	-	-	-
Zingiberaceae	-	-	-	-	-	-	-	-	-	1	-	-
Pontedericeae	-	-	-	-	-	-	-	-	-	-	1	-
Typhaceae	-	-	-	-	-	-	-	1	-	-	-	-
Arecaceae	-	-	1	-	-	-	-	-	-	-	-	-
Potamogetonaceae	-	-	-	-	-	-	-	-	-	-	1	-
Pinaceae	-	1	-	-	-	-	-	-	-	-	-	-
Agavaceae	-	-	-	1	-	-	-	-	-	-	-	-
Zygophyllaceae	-	-	-	-	-	-	-	-	-	-	-	1
Caprifoliaceae	-	-	-	-	-	-	-	-	1	-	-	-
Molluginaceae	-	-	-	-	-	-	-	-	1	-	-	-
Pedaliaceae	-	-	-	-	-	-	-	-	-	-	-	1
Total (395)	1	27	49	37	15	4	16	47	33	8	7	151



Table . 4. Biological spectra and phytoclimate of some parts of North-West Himalayan region based on sub life form classes

Region / Phytoclimate	MMM	MM	M	N	Ch	H	G	HH	Th	L	P	E
Normal Spectrum (Raunkiaer, 1934)	-	-	28.00	15.00	9.00	26.00	4.00	2.00	13.00	-	-	3.00
Trikuta Hills (Kapur and Sarin, 1985) Thero- chameophytic	-	-	9.44	14.45	22.94	13.87	4.62	1.73	31.02	1.55	0.19	0.19
Trikuta Hills (Kour, 2001) Thero- hemicryptophytic	-	-	21.68	11.67	13.30	24.00	7.73	3.09	26.01	1.03	-	3.00
Chamba District (Sharma and Singh, 1995) Thero-cryptophytic	0.30	3.30	4.41	8.12	4.71	15.03	16.94	2.81	39.28	4.11	-	1.00
Renuka Wildlife Sanctuary (Subramani <i>et al</i>, 2007)	0.52	12.34	16.54	19.16	7.61	5.51	2.10	3.41	31.24	0.79	0.52	0.26
Neeru Watershed (Bhaderwah) (Dutt, 2005) Thero- hemicryptophytic	-	-	9.27	7.73	16.49	26.28	7.73	3.09	26.80	0.51	-	2.06
Bhaderwah forests (Kumar ,1987) Thero- hemicryptophytic	-	-	6.35	6.14	11.5	37.10	4.50	3.30	29.30	1.43	-	0.40
Patnitop forests (Kumar ,1997) Thero- hemicryptophytic			10.4	8.5	15.2	26.4	3.5	3.2	29.8	2.6	-	0.2
Kathua district (Jhangir ,2004) Thero- phanerophytic	-	-	16.63	12.79	12.36	15.77	2.55	1.27	32.89	5.11	-	0.63
Jammu district Thero- phanerophytic (Present study)	0.25	6.84	12.40	9.37	11.90	8.35	2.02	1.78	38.23	3.80	1.01	4.05

MMM – Megaphanerophyte, MM – Mesophanerophyte, M – Microphanerophytes, N – Nanophanerophyte, Ch – Chamaephytes, G – Geophyte, HH – Hydrophyte and Halophyte, H – Hemicryptophyte, Th – Therophyte, P – Parasite, L – Liana and E – Epiphyte.



On the basis of the present study phytoclimate of the area, as per the Raunkiaer terminology, may be described as thero-phanerophytic. The therophytes are plants of warm and dry climate and phanerophytes of warm and moist climate. The climate of the study area in general is warm and dry during summers and warm and moist during rainy season, thus confirming the preponderance of therophytes and phanerophytes. Similar studies on phytoclimatic association have also been conducted by Sapru (1975), Kaul and Sarin (1976), Kapur (1982), Dhar and Koul (1986), Kumar (1987), Singh and Kachroo (1994), Kumar (1997), Kour (2001), Singh (2002), Kesar (2002), Jhangir (2004) and Dutt (2005) for Jammu and Kashmir state. The Biological spectra and phytoclimate of some areas of Jammu and Kashmir and other parts of north-west Himalayan region based on sub life form classes have been represented in Table.4.

Conclusion

The forests of Jammu district represent typical subtropical vegetation. During the floristic survey Fabaceae, Asteraceae, Poaceae and Labiatae have been found to be the dominant families in terms of genera to species ratio. On the basis of the present study, the phytoclimate of the area, as per the Raunkiaer terminology, has been described as thero-phanerophytic.

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Assessment of (L_d) day time average noise level at major crossings of Jammu City, J&K (India)

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Abstract

The present study has been carried to assess (L_d) Day Time Average Noise level (7.00 A.M. to 9.00 P.M) at eleven major crossings of Jammu City. The minimum and maximum noise levels were recorded to be 39.9 dB (A) and 109.2 dB (A) respectively. The (L_d) values at all the sites were observed to be above permissible limits prescribed by CPCB. The calculated value of L_d at all the sites were observed to be statistically significant at 0.05 (5%) level of significance ($p=0.000-0.004$).

Keywords: Noise pollution, traffic noise, major crossings, (L_d), day time average noise level

Introduction

Increasing urbanization has led to the problem of noise, introducing into the privacy of urban dwellers, affecting the quality of life. Noise must also be considered a potentially serious health hazard. India is noise loving country *i.e.* from birth to death loud noise, band and orchestra is the part of our culture. It has been rightly pointed out that civilization itself is noise and man's progress through the ages has been accompanied by activities involving ever increasing noise intensities.

Transportation noise is a key problem in the cities of the world today. Noise from road vehicles produces disturbance to more people than from any other source of noise and has been increasing very fast over the last few decades. Traffic noise is created by a continuous flow of auto-vehicles and is generated by engines in jets, motor in trucks and contact of tyres on roads and wheels on rails. This affects not only those persons who live adjacent to roads but also the occupants of the transportation means as well. On any road there is a general noise level as long as any traffic is moving. This general noise level varies with

traffic density and time of the day. Distinctive and regular peaks in the noise level occur in the morning and evening as people travel to and from schools and work places (Ahmad and Khan, 2003). Moreover, no regulation is observed in blowing horns and use of defective silencers. Sometimes, people even use their vehicles without silencer pipes which generate highly intolerable noise. Motor cycles with their exposed engines and inadequately silencing arrangement are notorious noise producers.

The menace of noise pollution is growing not only in developing and underdeveloped countries but the highly civilized and industrialized countries throughout the world are also threatened with the same. The impact of road traffic noise on exposed population can cause annoyance by disturbing sleep, causing speech interference or interrupting daily activities. While there is no risk of long term auditory damage from traffic noise but there was a great deal of evidence that noise could cause adverse health effects on people (Al-Dakhlah and Jadaan, 2005; Chakraborty *et al.*, 1998, 2002; Rao and Rao, 1992; Banerjee and Chakraborty, 2006). Traffic was the dominating source of noise (Skanberg and Ohrstrom, 2002) and was the major source of

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nuisance and annoyance as cited in social surveys (Pandya, 2003).

The present study has been carried out to assess (L_d) Day time average noise level (7.00 A.M. to 9.00 P.M) at major crossings of Jammu city, J&K (India).

Materials and Method

Study area has been divided into XI sites:-

Site-I (Janipur Crossing):- The crossing forms the centre point of Bus Stand-Bantalab, Parade-Janipur Colony, Bus Stand-Kot bhalwal and Bagh-E-Bahu-Janipur Colony traffic routes.

Site-II (Ambphalla Crossing):- This crossing lies on the Jammu-Srinagar National Highway I-A. Various vehicles going to different parts of the city pass through this crossing.

Site-III (Rehari Crossing):- This crossing on National Highway (I-A) lies next to Ambphalla crossing towards Bus Stand. Various public and private vehicles going to Rajpura, Parade, Bantalab, Janipur *etc.* pass through this crossing.

Site-IV (Kachi Chawani Crossing):- This crossing lies in the heart of the city. Kachi Chawani is a hub of various coaching institutes. This zone forms the main focal point of the commercial activity in the Jammu city.

Site-V (Parade Crossing):- This forms the central point of all important traffic routes connecting different parts of the Jammu city such as Kachi Chawani, Bus Stand, Talab Tillo, Gandhi Nagar, Trikuta Nagar, Bagh-e-Bahu Temple, Railway Station *etc.* It is main centre of vegetable market, fruit market and conventional commercial activity.

Site-VI (Indira crossing):- This site is located near Bus Stand. This crossing connects Shalamar, Hari Market, Gumat, Bus Stand and K.C. Chowk.

Site-VII (Jewel Crossing):- It is one of the busiest crossings of Jammu City. The traffic routes to Talab Tillo, Gumat, Bus Stand, Satwari pass through this crossing.

Site-VIII (Vikram Crossing):- This crossing connects old Jammu city with New Jammu city and lies on NH-IA. Muthi-Bagh-E-Bahu Temple, Janipur-Satwari, Ambphalla-Channi, Parade-Railway station, Ambphalla-Airport, Parade-Gandhi Nagar traffic routes pass through this crossing.

Site-IX (Panama Crossing):- This crossing is without commercial activity and lies on outskirts of Jammu. Traffic routes to Railway station,

Channi, Narwal Mandi, Greater Kailash go *via* this crossing.

Site-X (Satwari Crossing):- This crossing lies on NH-IA. Public and private vehicles plying to Pathankot (Samba, Kathua, Gangyal, Digiana and Airport) follow this traffic crossing.

Site-XI (Gole Market crossing):- This lies in Gandhi Nagar commercial area. It is the heart of the well planned part of the Jammu city. It is also regarded as the managed commercial hub of the Jammu city.

The measurement of sound pressure levels at each crossing were carried out at an interval of one hour from 7.00 A.M.-9.00 P.M. hours with the help of Sound Level Meter (Model-8928) at 'A' weightage. During each sampling of noise, 20 readings of SPL were recorded at an interval of 30 seconds in a period of 10 minutes. The minimum and maximum SPL were also recorded. The L_{eq} was calculated by using the formula:-

$$L_{eq} = 10 \log \left(\sum_{i=1}^{n=0} f_i (10^{L_i/10}) \right) \text{ dB (A)}$$

Where,

f_i =fraction of time for which the constant sound level persists

i =time intervals.

n =number of observations.

L_i =sound intensity at a time interval.

L_d (Day time average noise level) was calculated by applying the formula:

$$L_d = 10 \log \{1/15 [15 (10^{L_{eq}/10})]\}$$

Results and Discussion

The analysis of the data revealed that maximum L_d of 90.3 dB (A) was observed at Kachi Chawani crossing (Site-IV) with minimum SPL of 39.9 dB (A) on Sunday and maximum SPL of 107.5 dB (A) on Thursday. Whereas the minimum L_d of 78.2 dB (A) was observed at Rehari crossing (Site III) with minimum SPL of 39.9 dB (A) on Monday, Tuesday, Wednesday and maximum SPL of 104.6 dB (A) on Monday (Table.1 and 2). ANOVA computed data (with SPSS 17.0) of L_d at all the days revealed significant variations in L_d of Sunday versus that of all the working days (Monday, Tuesday, Wednesday, Thursday, Friday and Saturday) at Site-V (Parade crossing), Site-VI (Indira crossing), Site-VII (Jewel crossing)



Table 1. L_d (Day Time Average Noise level) at major crossings of the Jammu City

(L_d) on	(L_d) dB (A)										
	Site-I	Site-II	Site-III	Site-IV	Site-V	Site-VI	Site-VII	Site-VIII	Site-IX	Site-X	Site-XI
Sunday	73.9	76.1	75.7	83.8	77.2	79.0	80.8	77.9	75.3	78.6	80.2
Monday	77.5	79.8	78.6	91.2	87.8	82.2	88.2	82.6	79.5	81.7	84.8
Tuesday	78.0	79.0	79.5	91.4	88.0	82.2	88.0	81.8	79.9	81.8	85.0
Wednesday	78.2	80.2	78.6	91.1	90.2	82.6	87.3	81.4	80.2	81.6	84.7
Thursday	80.6	79.3	76.8	91.9	90.2	82.0	86.7	81.6	80.2	81.2	84.5
Friday	81.6	78.9	78.8	91.5	90.4	82.7	86.8	81.2	80.0	81.2	85.8
Saturday	79.4	78.0	79.4	91.2	90.5	82.3	86.8	80.6	80.0	81.0	84.4
Average L_d	78.5*	78.8*	78.2*	90.3*	87.8*	81.9*	86.4*	81.1*	79.3*	81.1*	84.2*

* = Significant at 0.05 (5%) level.

Table 2. Minimum and Maximum Noise levels (SPL) at major Traffic crossings of the Jammu City(India)

Days	Noise parameter	Traffic crossings (Sites)										
		Site-I	Site-II	Site-III	Site-IV	Site-V	Site-VI	Site-VII	Site-VIII	Site-IX	Site-X	Site-XI
Sunday	Min.	41.2	39.9	40.5	39.9	43.8	40.3	43.2	43.8	39.9	45.2	42.4
	Max.	103.4	101.4	101.6	104.3	102.4	102.8	104.3	102.6	96.4	101.9	104.4
Monday	Min.	47.8	48.4	39.9	41.2	42.6	46.2	45.6	44.5	39.9	46.8	43.2
	Max.	104.3	104.7	104.6	106.3	106.3	104.1	106.2	105.6	101.5	102.4	105.9
Tuesday	Min.	40.4	46.1	39.9	45.4	41.2	49.8	44.7	48.3	42.3	43.6	39.9
	Max.	102.9	103.4	104.3	107.5	106.8	105.3	106.2	104.7	103.1	104.7	105.1
Wednesday	Min.	39.9	45.2	39.9	45.4	43.8	42.7	46.2	42.6	44.3	42.4	39.9
	Max.	101.2	103.3	104.2	106.8	106.2	104.3	105.4	105.1	102.1	101.5	105.4
Thursday	Min.	50.8	46.1	42.0	42.3	43.1	47.2	45.1	45.1	43.8	43.2	40.1
	Max.	106.7	104.1	103.4	108.8	106.2	103.3	105.4	106.9	103.9	102.4	105.2
Friday	Min.	51.6	48.6	41.6	42.6	44.3	40.6	46.1	42.6	42.6	45.1	42.2
	Max.	106.3	102.4	104.2	106.9	109.2	101.4	105.7	104.7	102.4	106.7	105.1
Saturday	Min.	43.9	46.3	42.4	42.3	46.9	45.4	43.6	48.7	43.1	43.1	42.0
	Max.	102.4	104.2	103.1	106.8	106.4	102.4	105.7	104.8	102.4	102.4	105.2

Site-I (Janipur Crossing),
 Site-II (Ambphalla Crossing)
 Site-III (Rehari Crossing)
 Site-IV (Kachi Chawani Crossing)
 Site-V (Parade Crossing)
 Site-VI (Indira Crossing)

Site-VII (Jewel Crossing)
 Site-VIII (Vikram Crossing)
 Site-IX (Panama Crossing)
 Site-X (Satwari Crossing)
 Site-XI (Gole Market Crossing)



and Site-IX (Panama crossing) because these crossings lie nearer to or in a way to schools, colleges and offices and due to non-working day or holiday traffic flow rates were low as compared with that of working days and consequently the noise level (L_d) was observed to be less on Sunday as compared with that of working days. Srivastava and Dhabal (1998) also reported increased noise levels in commercial and residential buildings of Delhi and Calcutta due to traffic noise. Pandya and Srivastava (1999) and Gopalaswamy *et al.* (2002) also reported close relationship between volume of vehicles and noise level. Whereas it revealed insignificant variations in L_d at Sunday versus that of all the working days at Site-III (Rehari crossing), Site-IV (Kachi Chawani crossing), Site-VIII (Vikram crossing), Site-X (Satwari crossing), Site-XI (Gole Market crossing) because in spite of Sunday (a non-working day) these sites (crossings) have high traffic flow and commercial activity as these are junction points from where every vehicle has to cross while going to market or picnic spot or religious place or any working place. So there is no change in traffic flow rate even on Sunday. Site-I (Janipur crossing) exhibited significant variations in L_d of Sunday versus that of Thursday, Friday and Saturday and insignificant variations in L_d of Sunday versus that of Monday, Tuesday and Wednesday. Site-II (Ambphalla crossing) exhibited significant variation in L_d of Sunday versus that Wednesday and insignificant variations in L_d of Sunday versus that of rest of working days.

By applying One-way ANOVA using SPSS-17.0 the calculated value of L_d at all the sites were observed to be statistically significant at 0.05 (5%) level of significance ($p=0.000-0.004$). So it can be concluded that all the crossings vary in the status of noise level due to variation in various attributes like traffic flow rate, degree of jams, commercial activity, location *etc.* which vary from crossing to crossing (Table. 1).

The average L_d at average crossing of Jammu City was calculated to be 82.7 ± 4.2 dB (A) with a range from 78.2-90.3 dB (A) which was observed to be quite high above the permissible limit of 65.0 dB (A).

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Toxicity of tin on nitrogen fixing Cyanobacteria

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Abstract

In the present study the effect of Tin on three nitrogen-fixing cyanobacteria *Nostoc muscorum*, *Anabaena doliolum* and *Aulosira fertilissima* have been analyzed in terms of total growth, total carbohydrate, proteins and amino acids using 5 ppm to 55 ppm concentrations of Tin. Heterocyst frequency is calculated after growth period of 18 days. *Nostoc muscorum* have been found to tolerate a high concentration of the test metal and *Aulosira fertilissima* found to be most sensitive towards Tin. 20 and 30 ppm concentration of Tin found to be toxic for test organisms. Complete growth inhibition occurs at 50 ppm. Heterocyst frequency increases with the increasing concentrations of Tin.

Keywords: *Cyanobacteria, Toxicity, Heavy Metals, purifying alga, nitrogen fixing*

Introduction

Metals including heavy metals are naturally occurring essential components of global ecosystem. These are required by living organisms for various metabolic processes but higher concentrations are toxic to animals, plants including planktonic algae (Cheung *et al.*, 2002; Chang *et al.*, 1996; HazDat, 2003; Fargasova 1994; Martin and Holdich, 1986; Kick *et al.*, 1971). The biological importance of any heavy metal is simply the function of its solubility under physiological conditions. Similarly, its toxicity depends on affinity to sulfur and its interaction with macro-bio-elements. Cyanobacteria (blue green algae) are valuable tools for bioassays of metal toxicity (Fatma and Sultan, 1999; Kapoor *et al.*, 1998a; Angadi *et al.*, 1996; Dubey and Rai, 1987). They are endowed with property to cope up stressful conditions such as water polluted with heavy metals. Although a considerable amount of information is available on metal interaction effects on eukaryotic algae (Strarodub *et al.* 1987; Pabbi and Singha 2006; Sarada and Rengasamy

2006; Chu and Lin 1997) but comparable information on cyanobacteria is lacking. Tin occurs in trace amounts in natural waters. But now a days higher inorganic tin concentrations are getting entry into water by industrial discharges, tributyltin use, disposal of metal cans having tin coatings, smelting and refining processes, waste incineration *etc* (Byrd and Andreae, 1986; Senesi *et al.*, 1999). Although many studies have been done regarding toxicity of Tin on various organisms but limited work has been done on cyanobacteria (Boogaard *et al.*, 2003; White *et al.* 1999; Thomulka and Lange 1996; Han and Cooney, 1995). With such considerations taken into account, a study was proposed to observe the toxic effect of Tin on *Nostoc muscorum*, *Anabaena doliolum* and *Aulosira fertilissima* with reference to total growth, total carbohydrate, proteins, amino acid content and heterocyst frequency.

Materials and Method

Study was done in two stages. At first stage, *Nostoc muscorum*, *Anabaena doliolum* and *Aulosira fertilissima* were grown and maintained as unialgal, clonal and axenic cultures in nitrogen-free Allen and Arnon's culture medium (1955) at

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1800 lux and $28 \pm 2^\circ\text{C}$. At second stage, stock solution of SnCl_2 was prepared and it was then diluted with sterile distilled water to get concentrations ranging between 5 to 55 ppm. Experiments were carried out in triplicate in 125×25 mm test tubes with a total volume of 15 ml (medium plus toxicant). Controls were maintained. The readings were recorded after a growth period of 18 days. Growth was measured by taking optical density of chlorophyll pigments at 630, 645 and 665 nm by UV-VIS spectrophotometer (Systronics-117). Total carbohydrate content was estimated by acid hydrolysis Anthrone reaction method (Plummer, 1979). Total protein content was estimated by Lowry's method (Lowry *et al.*, 1951). Total content of amino acid was estimated by Ninhydrin method (Mahadevan and Sridhar, 1982). Heterocyst frequency of exponentially growing cultures was determined after the growth period of 18 days by calculating an average of 5 fields under microscope. Percentage heterocyst frequency as represented indicates number of heterocyst per 100 vegetative cells. Further analysis of variance, ANOVA, of various growth parameters studied were performed at 5% and 1% level of significance of total growth, total carbohydrate, total proteins, total amino acids and heterocyst frequency which indicated highly significant values (Tables. 1-5).

Results and Discussion

The results indicate that the behavior of all the four parameters show a similar trend towards Tin toxicity except heterocyst frequency. These show a considerable increase upto 5 ppm showing stimulatory effect of Tin as a nutrient. Growth responses of *Nostoc muscorum* in presence of Tin from 0 to 55 ppm indicate that metal ion at trace concentrations is not much toxic to organisms. A considerable increase in total growth upto 5 ppm observed shows stimulatory effect of Tin as a nutrient. But a gradual decrease observed and the values found to be more than control upto 20 ppm. Value lower than the control was observed after 25 ppm. Beyond this level growth inhibition of the organism was not so sharp and the trend continued upto 35 ppm of its concentration and inhibition

was significant at 40 ppm which continued upto 55 ppm concentration of the metal (Table.1, Fig.1).

Table. 1 Effects of various concentrations of Sn^{2+} on total growth (ppm)

Concentration (ppm)	<i>Nostoc muscorum</i>	<i>Anabaena doliolum</i>	<i>Aulosira fertilissima</i>
Control	0.420	0.306	0.135
5	0.519	0.490	0.411
10	0.510	0.466	0.292
15	0.497	0.317	0.218
20	0.457	0.292	0.166
25	0.406	0.265	0.106
30	0.345	0.065	0.061
35	0.332	0.041	0.050
40	0.047	0.029	0.022
45	0.034	0.009	0.013
50	0.022	0.007	0.007
55	0.001	0.001	0.003
SEM [±]	0.0043	0.0031	0.0020
CD (5%)	0.0127	0.0091	0.0058
CD (1%)	0.0172	0.0124	0.0078
CV	2.52	2.85	2.78

ANOVA for Total growth (ppm) under the influence of Sn^{2+}

SOV	<i>Nostoc muscorum</i>			<i>Anabaena doliolum</i>			<i>Aulosira fertilissima</i>		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	1.46	0.132584*	11	1.13	0.103047*	11	0.55	0.049696*
Error	24	0.00	0.0000567	24	0.00	0.0000295	24	0.00	0.000118
Total	35			35			35		

(* Significant)

In terms of total carbohydrate content there was significant increase at 5 ppm level but the values were indicative of growth promotion upto 15 ppm. A value lower than control was observed at 20 ppm showing toxic effect of Tin. Beyond this level decrease occurred gradually upto 55 ppm (Table.2). Protein content on the contrary indicated very interesting trend and the protein values were found higher than control upto 25 ppm. Beyond these concentrations, it decreased upto highest concentration studied (Table.3).



Table 2. Effects of various concentrations of Sn^{2+} on Total carbohydrate (ppm)

Concentration (ppm)	<i>Nostoc muscorum</i>	<i>Anabaena doliolum</i>	<i>Aulosira fertilissima</i>
Control	16.62	15.22	12.53
5	19.36	19.44	26.17
10	18.51	18.21	22.91
15	17.84	17.37	21.64
20	11.07	13.07	20.71
25	9.88	14.88	17.66
30	5.07	8.77	6.79
35	3.52	1.36	1.91
40	2.72	0.98	0.06
45	2.79	0.07	0.01
50	0.78	0.01	0.00
55	0.06	0.00	0.00
SEM [±]	0.1622	0.1650	0.2102
CD (5%)	0.4735	0.4815	0.6136
CD (1%)	0.6417	0.6526	0.8315
CV	3.12	3.13	3.35

ANOVA for Total carbohydrate (ppm) under the influence of Sn^{2+}

SOV	<i>Nostoc muscorum</i>			<i>Anabaena doliolum</i>			<i>Aulosira fertilissima</i>		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	1832.21	166.5644*	11	2155.80	195.982*	11	3629.91	329.9917*
Error	24	1.90	0.078962	24	1.96	0.081655	24	3.18	0.132573
Total	35			35			35		

(* Significant)

Table 3. Effects of various concentrations of Sn^{2+} on Total protein (ppm)

Concentration (ppm)	<i>Nostoc muscorum</i>	<i>Anabaena doliolum</i>	<i>Aulosira fertilissima</i>
Control	13.99	13.54	18.73
5	16.43	16.64	47.51
10	16.19	16.12	45.01
15	15.44	16.04	25.09
20	14.54	14.21	22.22
25	14.39	7.08	19.93
30	6.48	3.01	7.22
35	1.29	0.79	1.28
40	0.31	0.48	0.09
45	0.27	0.08	0.01
50	0.06	0.02	0.00
55	0.01	0.00	0.00
SEM [±]	0.1488	0.1432	0.3254
CD (5%)	0.4342	0.4179	0.9498
CD (1%)	0.5885	0.5663	1.2872
CV	3.11	3.38	3.62



ANOVA for Total protein (ppm) under the influence of Sn^{2+}

SOV	<i>Nostoc muscorum</i>			<i>Anabaena doliolum</i>			<i>Aulosira fertilissima</i>		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	1817.19	165.1993*	11	1783.28	162.1165*	11	9882.29	898.3899*
Error	24	1.59	0.066403	24	1.48	0.0615	24	7.62	0.317699
Total	35			35			35		

(* Significant)

Total amino acid content of the organism however, were higher than control upto 20 ppm then declined on gradual basis with lowest values at 45 ppm (Table.4). In *Anabaena doliolum*, growth observed only upto 55 ppm in presence of Tin. Same trend was exhibited where chlorophyll absorbance increase in comparison to control (highest level at 5 ppm) where definitely the increase in absorbance was recorded upto 20 ppm then decreased gradually. In terms of total growth

there was a increase in comparison to control only upto 15 ppm and then reduction continued slowly and significantly as reduction in its contents occurred beyond 35 ppm of Tin (Table-1.). However, total carbohydrate, amino acid and protein content increased in presence of Tin upto 15-20 ppm bearing highest values and beyond that it decreased sharply which continued upto the 55 ppm of its concentration (Table.2, 3 and 4). The growth responses of *Aulosira fertilissima* in

Table 4. Effects of various concentrations of Sn^{2+} on Total amino acids (ppm)

Concentration (mg/l)	<i>Nostoc muscorum</i>	<i>Anabaena doliolum</i>	<i>Aulosira fertilissima</i>
Control	0.38	0.47	1.44
5	0.91	0.63	1.69
10	0.68	0.53	1.59
15	0.59	0.41	1.41
20	0.48	0.49	1.02
25	0.32	0.05	0.42
30	0.09	0.02	0.08
35	0.07	0.01	0.04
40	0.05	0.01	0.02
45	0.01	0.00	0.01
50	0.00	0.00	0.00
55	0.00	0.00	0.00
SEM ⁺	0.0054	0.0045	0.0132
CD (5%)	0.0158	0.0131	0.0387
CD (1%)	0.0214	0.0178	0.0524
CV	3.14	3.56	3.57

ANOVA for Total amino acids (ppm) under the influence of Sn^{2+}

SOV	<i>Nostoc muscorum</i>			<i>Anabaena doliolum</i>			<i>Aulosira fertilissima</i>		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	3.19	0.290217*	11	2.22	0.201419*	11	17.13	1.557175*
Error	24	0.00	0.000088	24	0.00	0.0000605	24	0.01	0.000527
Total	35			35			35		

(* Significant)



presence of different concentrations of Tin showed somewhat similar results. Total growth (Table.1), total proteins (Table.3) and total amino acids (Table.4) content increased upto 20-25 ppm showing high values compared to control (highest values at 5 ppm). Beyond that a rapid decrease was observed upto the last concentration tested. A decrease in heterocyst frequency observed in all the three algae upto 20-25 ppm in comparison to control (Table.5) and then it increases regularly upto the highest concentration studied which indicates heterocyst differentiation due to the higher concentration of heavy metal in the medium but it appears that the growth responses and metabolism of *A. fertilissima* are greatly affected by presence of Tin into the medium. Reduction in growth and content of macromolecules of all three test algae at increasing concentrations of Tin confirms its toxic

characteristics. The authors' findings dealing with tin toxicity are in very good agreement with those of Dubey and Rai (1987) and Rai and Dubey (1989). PawlikSkowronska *et al.* (1997) observed that tin (II) salts inhibited the growth of planktonic cyanobacterium *Synechocystis aquatilis*. Toxicity increased with increasing tin concentrations, time of exposure and pH value of medium in the range 7.0-9.8. At lowest tin (II) concentration of 1ppm, there was a 36-40% decrease in growth and chlorophyll *a* content after 96 hrs at pH 9.8. Wong *et al.* (1982) observed that tin concentration of >5 ppm was toxic to cyanobacterium *Anabaena flos-aquae*. Also concentrations 12ppm and >50 ppm were toxic to green alga *Ankistrodesmus falcatus* and *Scenedesmus quadricauda* respectively. Data on analysis of variance of various growth parameters studied indicated highly significant values at 5 % or 1 % (Tables 1to 5).

Table. 5: Effects of various concentrations of Sn^{2+} on Heterocyst frequency (%)

Concentration (ppm)	<i>Nostoc muscorum</i>	<i>Anabaena doliolum</i>	<i>Aulosira fertilissima</i>
Control	4.95	4.63	3.64
5	3.36	4.18	3.40
10	3.93	4.68	3.43
15	4.07	4.95	3.36
20	4.30	5.16	3.92
25	4.81	4.97	4.01
30	5.02	5.36	4.29
35	5.09	5.87	4.05
40	5.63	5.92	4.12
45	5.89	5.88	4.22
50	6.82	6.07	3.96
55	6.83	6.01	4.17
SEM [±]	0.0668	0.0777	0.0667
CD (5%)	0.1949	0.2269	0.1946
CD (1%)	0.2642	0.3075	0.2637
CV	2.29	2.54	2.98

ANOVA for Heterocyst frequency (%) under the influence of Sn^{2+}

SOV	<i>Nostoc muscorum</i>			<i>Anabaena doliolum</i>			<i>Aulosira fertilissima</i>		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	39.46	3.587287*	11	13.90	1.263631*	11	4.05	0.367784*
Error	24	0.32	0.013381	24	0.44	0.018134	24	0.32	0.013335
Total	35			35			35		

(* Significant)



Toxicity may be manifested either by disruption of the integrity of cell membranes or by inhibition of photosynthetic pigments and key enzymes of nitrogen metabolism, viz. nitrogenase, nitrate reductase and glutamine synthetase of cyanobacteria. Micronutrients may influence the bioavailability and uptake of heavy metals to the micro biota as aquatic environment is comprised of several toxic and non-toxic metallic ions and their inorganic/organic complexes. The exact mechanism has not yet been clearly explored as the toxicity of heavy metals is governed by several factors acting together at one time.

In the present investigation 5 ppm has been found to be the tolerable limit for test organisms. Thereafter all the four parameters showed a declining pattern but a gradual increase can be seen in heterocyst frequency at increasing Tin concentration. Also 20-30 ppm found to be toxic where a reduced growth in terms of total carbohydrate and protein was seen in comparison to control. Complete growth inhibition was seen at 50, 45 and 40 ppm in *N. muscorum*, *A. doliolum* and *A. fertilissima* respectively. So these studies demonstrated that *N. muscorum* can tolerate a high concentration of Tin as compared to other two algae and can be used as a purifying alga in tin polluted water bodies.

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A preliminary phytochemical survey of plant species of Family Apocynaceae

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Abstract

A Phytochemical screening of alkaloids, leucoanthocyanine, steroids or triterpenoids, tannins, flavonoids, saponins, raphides, glycosides etc. was carried out on leaf samples of Family Apocynaceae plants. All plant materials were collected during the period 2008 from Meerut District.

Keywords: *Apocynaceae, Phytochemical, Screening, Flowering, Germination*

Introduction

Therapeutic properties of medicinal plants depend upon the concentration of its active ingredients (Secondary plant metabolites), which is regulated by the ontogenic changes with in the plant and by the local climatic and edaphic factors (Iqbal and Srivastava, 1998). The earliest phyto chemical screening activity was conducted by Arthur (1954) on plants collected in the area of Jesselton (now Kota Kinabalu). Different workers have observed the presence of steroids, alkaloids, tannins, flavonoids and glycosides etc. in *Plumeria acuminata*. Sarraf *et al.* (1994a andb) reported the phytochemical study of some medicinal plants. They worked on the preliminary analysis of the residue which was performed by the phytochemical groups test i.e. alkaloids, flavonoids triterpenoids, steroids, reducing sugars, tannins, saponins and aminoacids. Laily *et al.* (2002) worked out a Preliminary phytochemical survey of plants in crocker Range, Sabah, Malaysia.

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Materials and Method

Sample collection was limited to flowering and / or fruiting plant species for easier identification. In spite of that many collected samples are yet to be identified to species level. The presence of alkaloids was determined using the method (Driver,1960).For steroids or triterpenes and flavonoids test was used (Peach and Tracey,1955), For glycosides test was used (Paris,1963) and test for tannins, saponins, leuconthocyanine, syringin and raphides(Johansen, 1940).

Results and Discussion

Phytochemical are very important for chemical synthesis, spatial orientation, growth, flowering and germination. The present investigation shows that a wide range of variability could be generated through the induced chemical mutagenesis in members of family Apocynaceae. The list of plant species is given in Table 1. A total of 10 flowering plant samples were collected for screening.

The purpose of this paper is to report the screening conducted on plant samples. Alkaloids, raphides, tannins, glycosides, phytosterols, leucoanthocyanine, saponins showed the positive reactions obtained from all leaf samples of family Apocynaceae.

Table. 1. List of species screened for phytochemical spot tests

S.No.	Test Name	Name of species*									
		<i>Alstonia scholaris</i>	<i>Catharanthus roseus</i> (variety pink)	<i>Catharanthus roseus</i> (variety white)	<i>Carissa carandas</i>	<i>Nerium oleander</i> (variety pink)	<i>Nerium oleander</i> (variety white)	<i>Nerium oleander</i> (variety light pink)	<i>Plumeria alba</i>	<i>Tabernaemontana</i> <i>divaricata</i>	<i>Thevetia nerifolia</i>
1.	Raphides Test	+	+	+	+	+	+	+	+	+	+
2.	Isenberg-Buchanan A	-	-	-	-	-	-	-	-	-	-
3.	Isenberg-Buchanan B	-	-	-	-	-	-	-	-	-	-
4.	Leucoanthocyanin	+	+	+	+	+	+	+	+	+	+
5.	Syringin test	-	-	-	-	-	-	-	-	-	-
6.	Maule test	-	-	-	-	-	-	-	-	-	-
7.	Saponin test	+	+	+	+	+	+	+	+	+	+
8.	Tannin test	+	+	+	+	+	+	+	+	+	+
9.	Aurone test	-	-	-	-	-	-	-	-	-	-
10.	Juglon test A	-	-	-	-	-	-	-	-	-	-
11.	Betanins / Betanidins	-	-	-	-	-	-	-	-	-	-
12.	HCN test	-	-	-	-	-	-	-	-	-	-
13.	Borntrager's test	-	-	-	-	-	-	-	-	-	-
14.	Modified Borntrager's test	-	-	-	-	-	-	-	-	-	-
15.	Shinoda test A	-	-	-	-	-	-	-	-	-	-
16.	Shinoda test B	+	+	+	+	+	+	+	+	+	+
17.	Alkaloids	+	+	+	+	+	+	+	+	+	+
18.	Formic acid test	-	-	-	-	-	-	-	-	-	-
19.	Tartaric acid test	-	-	-	-	-	-	-	-	-	-
20.	Oxalic acid test	+	+	+	+	+	+	+	+	+	+
21.	Keller-killiani test	+	+	+	+	+	+	+	+	+	+
22.	Phytosterols test	+	+	+	+	+	+	+	+	+	+

(+) Positive, (-) Negative

* Each Number of different species

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A Seasonal Study Phytoplankton diversity and pollution indicators of Bathi pond near Davangere City, Karnataka (India)

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Abstract

A study was carried out in Bathi pond near Davangere city Karnataka (India) on phytoplankton diversity, density and distribution in different seasons and their correlations with physico-chemical properties of water. A total of 67 phytoplankton species belonging to Chlorococcales, Blue-greens, Desmids, Diatoms and Euglenoids were represented. Relative abundance of phytoplankton showed maximum of Blue-greens (45.61%) followed by Chlorococcales (40.11), Diatoms (13.97), Desmids (0.17%) and Euglenoids (0.13%). The highest density of phytoplankton was recorded during summer season. Chlorococcales varied with peak density (14,134 org/l) during summer and lowest during rainy season (10,333 org/l), Blue-greens recorded 16,361 org/l in summer and least during winter (14,289 org/l). Diatoms were found maximum (5,600 org/l) during summer and minimum with (3,739 org/l) during rainy season, Desmids varied from 76 org/l during summer and lowest during rainy season with 48 org/l and Euglenoids were recorded 57org/l during summer and least during winter with 41 org/l. Our study revealed that the growth of phytoplankton is governed by BOD, Chloride, COD, Conductivity, Potassium and Sodium. During course of study air temperature was found positively correlated with Euglenoids, BOD with Diatoms. Chloride with Desmids and Diatoms. COD showed positive correlation with Blue-green algae, Chlorococcales and Euglenoids while Potassium and sodium were found positively correlated with Desmids. Pollution tolerant species like *Scenedesmus quadricauda*, *Coelastrum* sp., *Tetraodon muticum*, *Closterium* sp., *Euglena* sp., *Phacus* sp., *Trachelomonas* sp., and *Microcystis* sp. were recorded.

Keywords: Bathi pond, Blue-greens, Chlorococcales, Desmids Euglenoids, phytoplankton

Introduction

Environmental pollution is a modern day devil affecting all ecosystems including aquatic ecosystems. Therefore the conservation of freshwater environment and its monitoring is highly essential (Mohapatra and Rengarajan, 1995). Phytoplankton plays an important role in the biosynthesis of organic matter (primary

production) in aquatic systems, which directly or indirectly serve all the living organisms of a water body as food (Anjana and Kanhere, 1998). The planktonic study is a very useful tool for the assessment of water quality in any type of water body and also contributes to understanding of the basic nature and general economy of the lake (Pawar *et al.*, 2006). Unplanned urbanization rapid industrialization and indiscriminate use of artificial chemicals in agriculture are causing heavy and varied pollution in aquatic environments leading deterioration of quality and depletion of aquatic biota (Yeole and Patil, 2005). Due to certain reasons some planktonic population flair-up to dominate water body and ultimately

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blooms form. Unlike other algae all the common bloom forming blue-green algae contain gas vacuoles which can impart positive buoyancy to the algae under certain conditions. Some species of blue-green algae aggregate and make a colony floating over the surface forming the bloom. Water bloom besides imparting color to the water also gives a disagreeable smell and taste to it. Phytoplankton species distribution shows wide spatio-temporal variations due to the differential effect of hydrographical factors on individual species and they serve as good indicators of water quality pollution (Gouda and Panigraphy, 1996). Phytoplankton of pond ecosystems were studied by Hosmani and Bharati (1980), Bhatt and Negi (1985), Saha and Chaudhary (1985), Kant and Raina (1985), Kumar and Dutta (1991), Verma and Mohanty (1995) and several studies on phytoplankton diversity was made in India and abroad on the ponds, lakes and reservoirs (Tiwari and Chauhan, 2006; Tas and Gonulol, 2007; Senthikumar and Sivakumar, 2008). In this paper an attempt has been made to study the seasonal changes and correlation of phytoplankton diversity in relation with physico-chemical parameters in Bathi pond.

Materials and Method

The study was conducted in Bathi pond near Davangere city during Sep. 2003 to Aug. 2005. The pond is situated 3 kms away from Davangere on the way to Harihar. It lies between 14° 28' N latitude and 75° 52' E longitude. Water spread area of this water body is 73 Hectares. Rain water is the main source of water and Bhadra right bank channel is the other source. Water is mainly used for irrigation 0.70 sq. kms which is covered by paddy, sugarcane and groundnut crops.

To evaluate the water characteristics a series of physico-chemical and biological tests were performed during two years period of Sept 2003 to Aug 2005. Water samples were collected in Bathi pond and mixed as per the standard methods of Khanna and Bhutiani (2004). Sampling at each station consists of taking one litre of sample for biological analysis and two litres in polyvinyl carbuoys for physico-chemical analysis.

temperature, pH and DO tests were performed in the field. Alkalinity, chloride, turbidity and hardness were determined. Phosphate, nitrate, nitrite, sulphate and silica were determined using UV-Visible Spectrophotometer. Standard prescribed methods were followed for the physico-chemical analysis of the water sample APHA, (1998) and Khanna and Bhutiani (2004).

For qualitative and quantitative analyses of phytoplankton one liter of composite water samples at surface level were collected at interval of 30 days for 2 years during the period September 2003 to August 2005. One liter of sample was fixed with 20 ml of 1% Lugol's Iodine solution and kept for 24 hours for sedimentation. 100 ml of sample is subjected to centrifugation at 1500 rpm for 20 minutes and used for further investigation. Identification of plankton up to species level was done by referring standard manuals (Philipose, 1967; Fritch, 1945). Quantitative estimation of phytoplankton was done by using Sedgwick Rafter Counting cell. The Pearson correlation coefficient was used to examine the relationships among the different environmental variables including phytoplankton density. Correlation coefficient(r) was calculated to detect the relationship between the various parameters of the water bodies under study.

Results and Discussion

Pearsons correlation matrix of different physico-chemical variables and Phytoplankton taxa recorded are given in Table 1 and 2 while Fig 1 and 2 showed seasonal changes of phytoplankton density and distribution of phytoplankton in percentage (%) in Bathi pond respectively. Total of 66 phytoplankton species were recorded in Bathi pond among which 21 species belong to Chlorococcales, Bluegreen algae, 12 species Diatoms, 15 species, Euglenoids, 9 species and Desmids, 10 species. The major phytoplankton in terms of frequency and abundance were *Crucigenia crucifera*, *Pediastrum duplex*, *Melosira sp.* Seasonal variations in phytoplankton diversity was recorded, although the highest number of species was recorded during summer season.



Table 1. Correlation coefficient calculated among the physico-chemical parameters with the density of the phytoplankton in Bathi pond, Karnataka (India)

Parameters	Blue-green algae	Chlorococcales	Desmids	Diatoms	Euglenoids
Air temp	0.494	0.378	0.350	0.324	0.509
BOD	0.131	0.353	0.445	0.524	0.466
Calcium	0.134	0.330	0.299	0.411	0.118
Chloride	0.069	0.072	0.510	0.584	0.418
COD	0.567	0.536	0.710	0.263	0.737
Conductivity	-0.274	0.168	0.143	0.091	0.101
DO	-0.040	0.225	0.181	0.376	0.222
Carbon dioxide	-0.137	-0.044	-0.235	-0.269	-0.117
Magnesium	-0.365	0.132	-0.012	0.112	-0.106
Nitrate	0.116	0.117	0.361	0.315	0.330
Nitrite	-0.465	-0.331	-0.159	0.091	-0.320
pH	0.132	-0.018	-0.160	-0.196	0.010
Phosphate	0.333	0.474	0.383	0.144	0.238
Potassium	0.178	0.337	0.576	0.418	0.441
Silica	-0.202	-0.244	-0.042	-0.151	0.219
Sodium	0.020	0.346	0.514	0.334	0.236
Sulphate	0.422	0.035	0.075	0.162	0.236
Total alkalinity	-0.159	-0.099	0.192	0.328	0.010
TDS	-0.248	-0.050	0.150	0.129	0.111
Total hardness	-0.200	0.238	0.112	0.251	0.042
Turbidity	0.115	0.084	0.463	0.438	0.483
Water temp	0.450	0.191	0.281	0.248	0.334

Values in the bold letters indicate the significant values

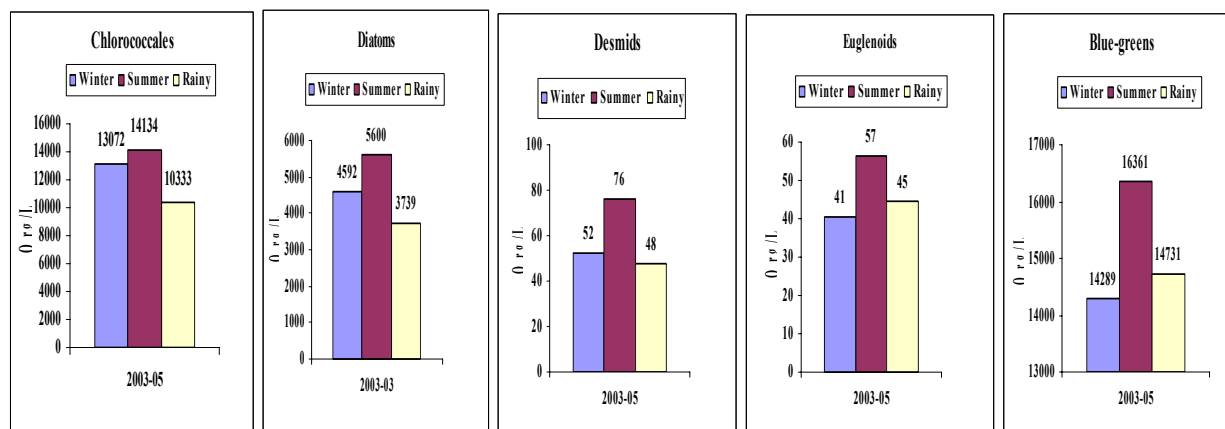
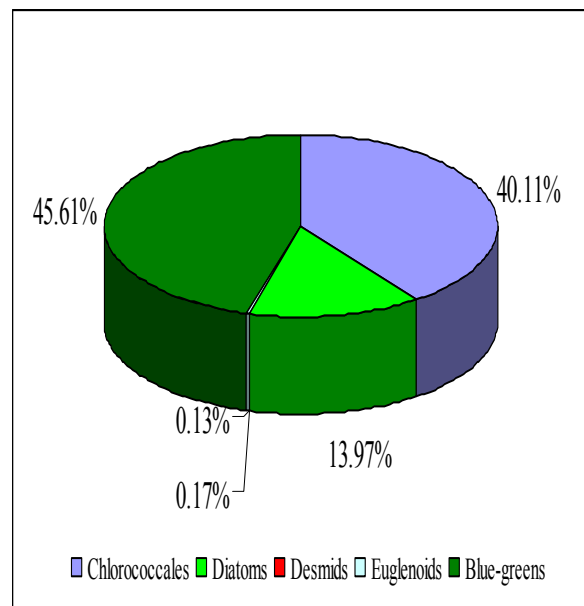
**Fig.1 Seasonal changes of phytoplankton density of Bathi pond**

Table. 2: Phytoplankton taxa record

Chlorococcales			
1	<i>Actinastrum</i> sp.	35	<i>Surirella capronii</i>
2	<i>Ankistrodesmus falcatus</i>	36	<i>Synedra ulna</i>
3	<i>Arthrodesmus</i> sp.	Desmids	
4	<i>Chlorella vulgaris</i>	37	<i>Cosmarium retusifolium</i>
5	<i>Coelastrum</i> sp.	38	<i>Cosmarium subtumidum</i>
6	<i>Crucigenia crucifera</i>	39	<i>Cosmarium depressum</i>
7	<i>Crucigenia retangularis</i>	40	<i>Cosmarium lundelli</i>
8	<i>Kirchneriella lunaris</i>	41	<i>Cosmarium capitulum</i>
9	<i>Pediastrum simplex</i>	42	<i>Closterium lunula</i>
10	<i>Pediastrum duplex</i>	43	<i>Closteriopsis</i> sp.
11	<i>Pediastrum duplex</i> var. <i>tetradon</i>	44	<i>Micrasterias</i> sp.
12	<i>Pediastrum tetras</i>	45	<i>Staurostrum wilde</i>
13	<i>Scenedesmus bijugatus</i>	46	<i>Staurostrum</i> sp.
14	<i>Scenedesmus quadricauda</i>	Euglenoids	
15	<i>Scenedesmus dimorphous</i>	47	<i>Euglena acus</i>
16	<i>Scenedesmus armatus</i>	48	<i>Euglena gracile</i>
17	<i>Scenedesmus acuminatus</i>	49	<i>Euglena accutissima</i>
18	<i>Scenedesmus abundance</i>	50	<i>Euglena</i> sp.
19	<i>Selenastrum gracile</i>	51	<i>Phacus orbicularis</i>
20	<i>Tetradraedron gracile</i>	52	<i>Phacus longicauda</i>
21	<i>Tetradraedron muticum</i>	53	<i>Phacus meson</i>
Diatoms		54	<i>Strombomonas gibberosa</i>
22	<i>Anomoenionis sphaeophora</i>	55	<i>Trachelomonas robusta</i>
23	<i>Closterium</i> sp.	Bluegreens	
24	<i>Cyclotella stelligera</i>	56	<i>Anacystis</i> sp.
25	<i>Gyrosigma gracilis</i>	57	<i>Anabaena aphanizomenoides</i>
26	<i>Gyrosigma spencerii</i>	58	<i>Aphanocapsa</i> sp.
27	<i>Gyrosigma attenuata</i>	59	<i>Chroococcus turgidus</i>
28	<i>Gyrosigma elongata</i>	60	<i>Gloecapsa</i> sp.
29	<i>Melosira granulata</i>	61	<i>Merismopedia glauca</i>
30	<i>Navicula pupula</i>	62	<i>Microcystis aeruginosa</i>
31	<i>Navicula cuspidate</i>	63	<i>Microcystis viridis</i>
32	<i>Navicula pigmea</i>	64	<i>Nostoc microscopium</i>
33	<i>Navicula radiosa</i>	65	<i>Oscillatoria tenuis</i>
34	<i>Pinnularia microstauron</i>	66	<i>Spirulina major</i>

Fig.2 Distribution of phytoplankton percentage in Bathi lake, Karnataka (India)



The class Chlorococcales was represented by highest number of species followed by Diatoms, Blue-greens, Desmids and Euglenoids. In Bathi pond Chlorococcales represents 40.11% of the total phytoplankton population being the second dominated group among the other groups of phytoplankton. This lake supported 10 genera and 18 species of class chlorococcales which *Crucigenia crucifera*, *Pediastrum duplex*, *Scenedesmus dimorphous*, *Kirchneriella* appeared in all the months during the investigation and *Pediastrum tetras* Var. *tetraodon*, *Selenastrum westlii* appeared as rare forms. The genus *Scenedesmus* was represented by seven species, *Pediastrum* by four species, *Crucigenia* and *Tetradron* by two species and other forms like *Actinastrum*, *Ankistrodesmus*, *Chlorella*, *Selenastrum* and *Kirchneriella* were represented by single species.

Diatoms in Bathi pond is represented by 9 genera and 15 species constituting 13.97% of total phytoplankton population. If the diversity of diatoms are considered a genus *Navicula* and *Gyrosigma* represented by four species, *Pinnularia* and the forms like *Anomoenionis*, *Cyclotella*,

Gomphonema, *Melosira*, *Surirella* : Phytoplankton diversity and pollution hardness, turbidity and water temperature is not showing any correlations and COD has shown maximum correlations. Calcium, chloride, conductivity, DO, magnesium, nitrate, nitrite, pH, phosphate, silica, sodium, TDS, total hardness, turbidity and water temperature has not shown any correlations.

The occurrence of Desmids in Bathi pond recorded 5 genera and 10 species constituting of 0.17 % of total phytoplankton population. The diversity of desmids is considered a genus *Cosmarium* represented by 5 species, *Staurastrum* with two species and other genera like *Micrasterias*, *Closterium* and *Closteriopsis* were represented by single species.

Bathi pond favoured more number of Euglenoids with 4 genera and 9 species of Euglenoids comprising 0.13 % of the total phytoplankton. Species diversity of Euglenoids showed *Euglena* represented by four species followed by *Phacus* three species and other genera like *Trachelomonas* and *Strombomonas* represented by single species.

Bathi pond supported 10 genera and 11 species of blue-greens constituting 45.61% of the total plankton population. With regard to their diversity the genus *Microcystis*, *Aphanocapsa* and *Oscillatoria* were represented by two species, *Arthrodesmus*, *Anacystis*, *Anabaena*, *Nostoc*, *Spirulina*, *Chroococcus* and *Merismopedia* were represented by single species.

Some of the pollution tolerant species (Palmer, 1969) identified during the present study are *Scenedesmus quadricauda*, *Coelastrum* sp, *Tetradron muticum*, *Navicula* sp, *Synedra* sp, *Cyclotellasp*, *Pinnularia* sp, *Closterium* sp, *Cosmarium* sp, *Staurastrum* sp, *Euglena* sp, *Phacus* sp, *Trachelomonas* sp, *Oscillatoria* sp, *Microcystis aeruginosa*, *Anabaena* sp., *Microcystis aeruginosa* were recorded from the pond indicates the civic pollution.

Among physico-chemical parameters air temperature was most significantly positively correlated with Euglenoids. BOD was most significantly positively correlated with diatoms. Chloride was most significantly positively correlated with Desmids and Diatoms. COD was most significantly positively correlated with Blue-green algae, Chlorococcales, Desmids and Euglenoids. Potassium and sodium were positively correlated with Desmids. In this calcium, conductivity, DO, carbon dioxide, magnesium, nitrate, nitrite, pH, phosphate, silica, sulphate, total

Bhatt *et al.*, (1999) showed that nitrites and total dissolved solids are negatively correlated with Chlorococcales. Similar observations have also been made in our studies. Tripathi and Pandey (1990) are of the opinion that high temperature favours the abundance of Chlorococcales. Our findings are of conformity with the above said researchers.

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Diversity of Copepods in different water bodies from Lakhani, Maharashtra (India)

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Abstract

The present study reports the Copepods diversity from the different water bodies of Lakhani, District Bhandara, Maharashtra (India). 9 species of Copepods are recorded first time from this region. Out of these 1 species belonged to Diaptomidae family of order Calanoida while remaining 8 from the family Cyclopidae of the order Cyclopoida. The taxonomic notes and key for their identification are appended and their bioindicator value in the aquatic pollution studies is discussed.

Keywords: *Aquaculture, Bioindicator, Calanoida, Copepoda, Cyclopoida, Eutrophication*

Introduction

Among the zooplankton, Copepods constitute about 50-60% of the total number of animals present in a water body. Copepods are of great economic importance. The young fish fry survive on planktonic Copepods in aquaculture. Many small and large freshwater fish feed on copepods. These organisms constitute an essential link in aquatic food chain and form an intermediate trophic level between bacteria, algae and protozoan on the one hand and small and large plankton eaters, mainly fish, on the other (Sehgal, 1983). They are the representative indicators of water pollution (Dzyuban and Kuznetsova (1978), Carter (1971), Patalas (1972), Ringler and Langford (1967). Kurasava (1975), Radhakrishna & Rangareddy (1976) gave an account on Copepods for their indicator value in water pollution. The members of Copepods are extensively studied from India. Gorge, (1966), Baruah *et al.*, (1993) recorded Copepods species varied between 7 and 8.

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Report on a collection of 14 species of Copepoda from Nepal by Henri and Isabella (1977). Sars (1903) gave an account on taxonomy of Copepoda. Due to the inconceivable importance of biodiversity, the studies on collection of base line data related to fauna are important. Otherwise most of the existent organisms may go unrecorded. Present work is primarily based on the work conducted on some water bodies situated at Lakhani region. In the present investigation 9 species of Copepods are reported.

Materials and Method

The zooplankton samples were collected from different sampling stations for a year; the samples were filtered using plankton net with bolting cloth of 25 μ . Then plankton concentrate was preserved in 4% formalin and Copepods were identified according to key from Ward and Whipple (1959) and Sehgal (1983).

Results and Discussion

Following species of Copepods were recorded from Lakhani:

Taxonomic notes: Class: Crustacea, Subclass: Copepoda, Order: Calanoida, Family: Diaptomidae, Sub-family: Diaptominae:

Heliodiaptomus viduus: Ovigerous female with one ovisac attached to the ventral side of the genital somite. General form of the body oval without any segmentation of head and thorax. Antennae with indistinct coxa and basis. Body length: 0.25 mm. Distribution: Assam, West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Kerala.

Order: Cyclopoida, **Family:** Cyclopidae, **Sub-family:** Eucyclopinae.

Ectocyclops phaleratus: Female, body thick widest in the middle. Posterior thoracic segment armed with transverse row of spinules on ventral side. Antennule 9 jointed. Genital segment slightly wider than long receptaculum seminis divided into anterior and posterior parts, Caudal furcal rami of female. Body length: 1.10 mm. Distribution: Punjab, Himachal Pradesh, West Bengal and Deccan Plateau.

Macrocylops distinctus: Female, anterior part of body oval shaped. Caudal rami 2-3 times longer than wide, inner margin of each ramus provided with continuous row of densely hairs. Lateral setae in posterior half of each ramus, distal apical setae longer. Antennule 17 segmented. Body length: 1.70 mm. Distribution: Punjab, Kashmir and Tamil Nadu.

Paracyclops fimbriatus: Female, body flattened dorso-ventrally, anterior end oval. Last thoracic segment extended into wings. Genital segment wider than long. Caudal rami 4-5 times longer than wide, dorsally each ramus provided with transverse row of spinules; distal two apical setae equal in length; outer seta spine like but thicker than inner one. Antennule 9 segmented, hardly reaching to the middle part of cephalothorax. Body length: 0.70 mm. Distribution: Madhya Pradesh, West Bengal and Himachal Pradesh.

Sub-family: Cyclopinae

Cyclops vicinus: Female, body slender. Last thoracic segments extended into posteriorly directed large wing like blades. Abdominal segments carry rows of small posteriorly directed spines. Caudal rami 8-9 times longer than wide.

Antennule 17 segmented almost reaching to the middle of second thoracic segment. Body length: 0.85 mm. Distribution: Kashmir, Deccan Plateau.

Acanthocyclops bicuspidatus: Female, body slender and covered with thick cuticle and small spinules. Caudal rami almost parallel and 6-8 times longer than wide. Each ramus provided with small spinules in the proximal half, inner apical setae longer than outer one. Apical setae thick and well developed. Antennule 15 segmented reaching up to the end of first thoracic segment. Body length: 1.20 mm. Distribution: Deccan Plateau.

Microcyclops bicolor: Female, last segment of the thorax completely rounded on either side and studded with stout seta. Cuticle of body pitted throughout. Furcal rami 3 times longer than wide. Each ramus inner apical distal setae shorter than outer distal seta, median apical seta comparatively short and thick. Antennule 10 segmented hardly reaching to the posterior end of first thoracic segment. Body length: 0.80 mm. Distribution: Orissa, Andhra Pradesh.

Mesocyclops leuckarti: Male, body relatively wide. Caudal rami 2.9-3.2 times as long as wide. Each ramus inner apical seta more than twice the length of outer one; median apical seta long and well developed. Antennule 17 segmented and reaching to the posterior end of second thoracic segment. Body length: 0.85 mm. Distribution: Uttar Pradesh, West Bengal, Orissa, Andhra Pradesh, Tamil Nadu and Kerala.

Mesocyclops Hyalinus: Male, body stout and compact. Caudal rami 2.9 times longer than wide. Each ramus inner apical seta twice the length of outer distal, median apical seta much longer than the outer one. Antennule 17 segmented; distal two segments with hyaline margin. Body length: 0.80 mm. Distribution: Kashmir, Rajasthan, West Bengal and Tamil Nadu.

Copepods are important member of the zooplankton for their role in the tropic dynamics and in energy transfer in the aquatic ecosystem, provide food for fishes in fresh water ponds, lakes and play a major role in fish production (Kamble and Meshram, 2005; Pawar *et al.*, 2003). In spite of this great importance, our knowledge on the taxonomy of freshwater calanoida and cyclopoida



of the country is still very inadequate and is mostly limited to some earlier works. Balamurugan *et al.*, (1999) reported six species of copepods belonging to order Cyclopoida from water body with heavily loaded organic enrichment due to influx of sewage. Species of Cyclops recorded more due to the abundance of diatoms and blue green algae (Meshram, 1996). In the present investigation the Cyclopoids occurred more throughout the year in all ecosystems from Lakhani region. Among Calanoids, *Heliodyptomus viduus* and among Cyclopoids, *Mesocyclops leuckarti* occurred throughout the year. Similar findings were found by Khan (2003) from West Bengal. Kurasawa (1975) noticed the dominance of Copepoda in oligotrophic lakes but Cyclopoid Copepoda were dominant in eutrophic lakes of tropical region. Kotangale (1988) states that the absence of diaptomus sps. or its diminishing significance showed that the water bodies are eutrophic. In the present investigation 1 species of Diaptomus and 8 species of Cyclops disclosed that the water bodies of Lakhani are eutrophic. Further studies on diversity of these species would be helpful in evaluating their bioindicator role.

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Evaluation of immunomodulatory and microbicidal potential of *Thuja occidentalis*

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Abstract

The present paper deals with the investigation for *in vitro* antimicrobial properties and immunomodulatory potential of *Thuja occidentalis*. Its various solvent extracts showed high potency of antimicrobial activities against bacterial pathogens. The growth of the bacteria *Escherichia coli* was found to be inhibited by all plant extracts. *Staphylococcus aureus* was found to be resistant to Hexane extract. While Ethanol extract inhibited the growth of *Staphylococcus aureus*. *Salmonella typhimurium* cultures were inhibited by all extract. Water extracts were more effective against microbial cultures at high concentrations. Rapid rise in total WBC counts in treated organisms proved the plant to have high immunomodulatory property. Plant methanolic extract treated animals showed increased life span than untreated controls. ANOVA analysis of the results showed that the results were significant and reproducible.

Keyword: *Gastrointestinal pathogens, Humoral antibody response, WBC, Thuza occidentalis*

Introduction

Thuja occidentalis L. is indigenous to North America. It has coniferous pyramidal features, with flattened branches and twigs in one plane, bearing small scale-like leaves (British Herbal Pharmacopoeia, 1983). Over the whole year, the leaves are green, with the lower side showing a bright green colour where the resin glands reside. Small, 1–2 cm long green to brown coniferous pines contain the seeds. *Thuja occidentalis* is being used in several indigenous preparations for general health and other diseased condition. Plant metabolites possessing antimicrobial and immunomodulatory properties have been the significant part of traditional medicines (Mathew and Kuttan, 1999; Latha *et al.*, 2000; Belal *et al.*, 2005). Thus, in recent years there has been a high increase in the interest of researchers to explore

the medicinal potential of plants (Chah *et al.*, 2006; Madhuri and Govind, 2009). The present paper deals with the objective of evaluating the immunomodulatory and antimicrobial properties of *Thuja occidentalis* in the present study.

Materials and Method

Thuja occidentalis plants were collected from Chitrakoot region, Uttar Pradesh. Leaves were dried for two days in the hot air oven at 37°C and then grinded in the grinder for three times. Powdered dry plant material was dissolved in different solvents and extracted using Soxlet assembly. Each extract was passed through Whatman Filter Paper No. 1. The extracts were concentrated by using vacuum evaporator at 32°C and stored at 4°C for further use. Antimicrobial activity was assayed by using broth dilution method (McKane and Kadel, 1986; Zahra *et al.*, 2000; Cooposamy and Magwa, 2007). Broth dilution test was used to determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) of plant extracts as well as of standard drugs (Nair and Chanda, 2007).

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The whole plants methanolic extract was used to study immunomodulatory properties. For the preparation of the extracts, dried ground plant material was percolated with 95 % methanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions and their aliquots, which were filter sterilized (0.2 µm) before testing on cell lines. The sample was prepared in double distilled water along with 0.1 % acacia gum. Experimental Swiss mice (weighing 25±3 gm) were obtained from the Central Drug Research Institute (CDRI), Lucknow. The animals were housed in standard environmental conditions.

Preparation of Sheep red blood cells (SRBC) antigen

SRBC were collected aseptically from Jugular vein of sheep, stored in cold sterile Alsever's solution for immunization and challenge, at required time schedule. Stored sheep blood cells were centrifuged and washed three times with pyrogen free sterile normal saline (0.85 % NaCl w/v) and adjusted to a required concentration for immunization (Nelson and Mildenhall, 1967).

Humoral antibody response (Hab) and Delayed type hypersensitivity (DTH-CMI)

Experiments were carried out in different groups (6 mice in each group) bearing cancer. On day 0 the mice were immunized by injecting 0.2 ml of 5×10^9 SRBC ml^{-1} ip and plant extracts were administered orally (100 mg/kg body wt.) for 5 consequent days after immunization. Two parallel controls were run simultaneously. One of them received only normal saline water which was named 'Normal Control', while the other received Levamisole (2.5 mg/kg body wt.) and Cyclophosphamide (250mg/kg body wt. post oral). Blood samples were collected from individual mice by retro-orbital puncture on day +5. Serial two fold dilution of 50 µl of serum sample of an individual mice was done in 50µl of normal saline containing 0.1 % BSA and added 50 µl of 0.1% suspension of SRBC in BSA. After mixing, the erythrocytes were allowed to settle at room temperature for about 60 to 90 minutes. The value of highest serum dilution causing visible haemagglutination was taken as the titre. The

mean titre values of the drug treated groups were compared with the normal control. Doherty's (1981) method was employed to access SRBC induced DTH response in mice. The challenging dose of 20 µl of 5×10^9 SRBC/ml in mice were injected to assess the standard control response for DTH.

Calculation for immune response

$$\text{Immunomodulatory activity} = \{(\text{Test group} - \text{Control}) / \text{Control}\} \times 100$$

WBC Count

Swiss mice (n=6) bearing cancer with EAC were treated daily with *Thuja occidentalis* extract (100 mg/kg) ip for 5 days. Blood was collected by puncturing the retro-orbital plexus. Total WBC and RBC count was determined using a haemocytometer. A normal control group received normal saline (5 mg/kg/ip) and positive control group treated with 5-Fluorouracil (5- FU), an anticancer drug.

Results and Discussion

The results obtained were very interesting shown in Figure.1. Results were repeated thrice and significance of the data was analyzed using appropriate statistical packages. The growth of *Escherichia coli* bacteria was found to be inhibited by all extracts in comparison to control (O.D. 1.56), but ethanol extract (O.D. 1.34) showed more inhibition than other extracts. Water extract (O.D. 1.42) and hexane extract (O.D. 1.43) showed nearly same inhibitory effect. Chloroform extract (O.D. 1.48) showed least inhibitory effect. In the case of *Staphylococcus aureus*, the cultures were found resistant to hexane extract (O.D. 1.83) with respect to control (O.D. 1.83). Chloroform extract (O.D. 1.77) showed a little inhibition of cultures. While ethanol extract (O.D. 1.71) was found inhibiting the growth of *Staphylococcus aureus*. Water extract (O.D. 1.85) had no effect on it. In the case of *Pseudomonas aeruginosa*, water extract (O.D. 1.79) showed resistance towards these bacteria in comparison with control. Ethanol extract (O.D. 1.35) showed more inhibition than other extracts. Chloroform extract (O.D. 1.53)



showed more inhibition than Hexane extract (O.D. 1.59). In the case of *Salmonella typhimurium*, all extract showed good inhibitory effect with respect to control (O.D. 1.70). Maximum inhibition was found in case of water extract (O.D. 1.49). Ethanol extract (O.D. 1.56) also showed good inhibitory effect but showed less inhibition than water extract. Chloroform extract (O.D. 1.67) and Hexane extract (O.D. 1.66) showed very less inhibition. In the case of *Staphylococcus epidermidis* in comparison to control (O.D. 1.70), water extract (O.D. 1.30) have showed more inhibition than other extracts. Ethanol extract (O.D. 1.56) also showed good inhibition. Chloroform extract (O.D. 1.67) was found to be least effective. The effect of different extracts on various gastrointestinal pathogens showed variable results that were in accordance with the earlier studies (Kannan, 1996; Farrukh and Ahmad, 2003; Sharma and Singh, 2002). Antibacterial properties of ethanolic and aqueous extracts might be due to the presence of alkaloids as alkaloids are natural antioxidants. All other extracts were observed to show good microbicidal properties. All observation and statistical data showed a clear-cut view about the activity of different extracts. This might be useful in formulating the antimicrobial potential of *Thuja occidentalis*.

Thuja occidentalis extract was also found to enhance humoral immune responses on 7th day by 18% as compared to the control, Levamisol, which showed 27%. The plant extract showed an enhancement in cell mediated immune responses on 7th day, by 15.37% as compared to control, Levamisol, which showed 23.65%. Total WBC count was also altered in Swiss mice treated with the *Thuja occidentalis* extract (Table 1). The effect of methanolic extract of the plant on the haematological parameters of the tumour bearing mice showed an increase in number of RBCs but a decrease in WBCs compared to the control mice. These data were based on the differential leucocyte count by Leishman staining. Methanolic plant extract treated organisms showed enhanced life span proving the improvement in immunomodulatory potentials (Table 2). Plants are

Table 1: Effect of methanolic extract of *Thuja occidentalis* on haematological parameters

Treatment	Total WBC count ($\times 10^3$) μL^{-1}	Total RBC count ($\times 10^5$) μL^{-1}
Cancer control	6.99 $\pm$ 0.34	3.19 $\pm$ 0.13
Extract treated	5.22 $\pm$ 0.56	3.48 $\pm$ 0.03
5-FU treated	6.11 $\pm$ 0.78	2.97 $\pm$ 0.97
Normal control	3.77 $\pm$ 0.54	5.1 $\pm$ 0.67

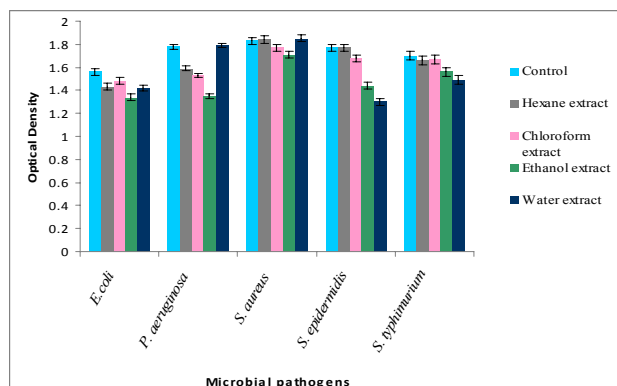


Fig. 1 Antimicrobial activity of different *Thuja occidentalis* extracts against gastrointestinal pathogens. The values represented the mean optical density $\pm$ SE

Table 2: Effect of methanolic extract of *Thuja occidentalis* on life span of tumour induced mice

Treatment	Number of animal with tumour	Number of days survived
Cancer control	6/6	17 $\pm$ 0.4
Extract treated	6/6	23 $\pm$ 0.9
5-FU treated	6/6	25 $\pm$ 1.8

* Values are represented as means $\pm$ SD

the best friend of human being and are big source of natural medicines. *Thuja occidentalis* is one of those plants that are not only being used in traditional medicines but also as ornamental plant, from many years (Chang *et al.*, 2000). The findings in the present study suggested that the plants possess high immunomodulatory and antimicrobial properties that are in accordance with the earlier studies. In folk medicine, *Thuja*

occidentalis has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Shimada, 1956; Baran, 1991; Offergeld *et al.*, 1992). Today, it is mainly used in homeopathy as mother tincture or dilution. In combination with other immunomodulating plants, such as *Echinacea purpurea*, *Echinacea pallida* and *Baptisia tinctoria*, this medicinal plant is also used as evidence-based phytotherapy for acute and chronic infections of the upper respiratory tract and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina, pharyngitis and sinusitis (Von Blumroder, 1985). Thus the plant has a high potential that might be used in several medicinal formulations to make herbal medicines.

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Multivariate analysis of drinking water quality parameters around Gajraula industrial area, India

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Abstract

The present study aims at identifying the ground water contamination problem in the vicinity of Gajraula industrial area at Gajraula (U.P.) India. Ground water samples were collected by hand pumps and bore well, based on the depth of 40 and 120 feet of water table respectively. Analytical techniques were adopted in the standard methods for examination of drinking water quality in ground water samples and results were compared with the standards given by WHO and BIS guidelines for drinking water quality. The physico-chemical parameters like conductivity, TDS, Alkalinity, Ca, Mg-hardness and COD were recorded higher in comparison to standard values. The present study showed that drinking water quality of bore well water found slightly better than water of hand pumps.

Keywords: *Industrial pollution, Ground water, Drinking water quality*

Introduction

Water is an essential and vital component for life support system. Peoples around the world have used ground water as a source of drinking water and even today more than half the world's population depend on ground water for their survival (UNESCO, 2000). Water quality is an index of human health and better hygienic condition of society. Gajraula is a prominent industrial area of western Uttar Pradesh, owes its significance to diverse group of industries, which includes distillery and its associated chemical units, pulp & paper, phosphate fertilizer plant, textiles, pharmaceuticals, dairy and other units. The industrial effluents contain toxic chemicals, hazardous compounds, suspended solids and non-biodegradable materials. The major source of surface and ground water pollution is injudicious discharge of untreated industrial effluents directly into the surface water bodies resulting surface and ground water pollution described by Nasrullah, *et al.*, (2006) and Malik *et al.*, (2009). The ground water quality of hand pumps and bore well around

industrial area has been affected a lot and causes serious disease among the human beings and other livestock population. Most of the labour class society generally depends on ground water of hand pumps for drinking as well as other domestic utilities. Hence, some scientific remedial measures should be taken to improve the drinking water quality in and around of industrial area.

Materials and Method

Gajraula city is approximately 115 Km. away from Delhi on national highway NH-24, Delhi-Lucknow road. Gajraula is well known oldest industrial area of district J.P. Nagar (Uttar Pradesh), situated on globe at a longitude 78° 13'48.75" E and latitude 28° 50'59.26" N at 207 meter sea level. Gajraula industrial area was selected on the basis of existence of large number of industries (chemical units, pulp and paper, phosphate fertilizer plant, textiles, pharmaceuticals, dairy products processing units and others), which discharge a huge amount of wastewater in the form of mixed effluents through different drains, contributed to deteriorate the quality of surface and ground water. Ground water samples were collected by grab sampling method

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from hand pumps and bore well based on water table at depth of 40 feet and 120 feet respectively from different sampling sites of different villages adjoining the industrial zone. The ground water samples (n=120) were collected from each of hand pumps (HP n=5) and bore wells (BW n=5) from each sampling sites during the particular seasons (April-2008 to March-09). The water samples were analyzed by standard methods as APHA, (1998).

Results and Discussion

The ground water resources are now on the verges of threatened status in terms of sustainable quality of drinking water as well as contain various traceable toxic substances. The physico-chemical parameters of ground water of hand pumps and bore well were shown in Table -1 & 2. The minimum and maximum water temperature of bore well and hand pump water was observed as 18.33-27.69 °C during winter and summer seasons respectively. The influence in temperature may be due to different seasonal climatic variations and different sampling time (Parashar, *et al.*, 2008). The values of pH of hand pump and bore well water were recorded in the ranged from 7.26-8.05. The minimum value of pH was observed in bore

well during winter season and maximum value of pH was also observed in bore well water during monsoon season. pH values of hand pumps and bore well water samples were within the permissible limit given by WHO. The minimum value of conductivity (924.97 µmho/cm) was observed in bore well water during monsoon season and maximum value of conductivity (1399.59 µmho/cm) was observed in hand pump water during winter season. The values of conductivity of hand pump and bore well water were recorded higher in comparison to standard value (400 mg/l) given by WHO.

Electrical conductivity with regards to water quality referred to the amount of salts in the water and is a numerical expression of the ability of an aqueous solution to convey an electric current. It is also an approximate indicator of total dissolved ions such as heavy metals and widely used for pollution monitoring (Nasrullah, *et al.*, 2006).

The present study showed TDS values were recorded in the range of 610.48-923.73 ppm. The minimum value of total dissolved solids was observed in bore well water during monsoon season and maximum value of total dissolved solids was observed in hand pump water during winter season. Drinking water with high total

Table-1: Physico-chemical characteristics of ground water of Hand pumps (HP)

Parameters	Summer	Monsoon	Winter	Mean
Temperature	23.41-27.69	23.73-25.95	18.61-23.92	23.75
pH	7.38-7.76	7.55-7.68	7.27-7.41	7.53
Conductivity	1157.66-1266.76	1221.26-1310.56	1174.98-1399.59	1212.20
TDS	764.06-796.42	806.03-864.97	775.48-923.73	797.75
Free-CO ₂	0.680-0.843	0.152-0.247	0.254-0.570	0.392
Alkalinity	325.97-334.89	329.03-339.83	332.11-338.80	333.28
T. Hardness	274.90-282.87	253.62-271.86	269.52-282.35	275.17
Ca	170.67-175.33	152.67-178.27	163.20-183.78	170.43
Mg	102.74-112.20	94.07-113.50	99.10-111.41	104.81
DO	4.09-5.29	4.24-5.37	3.51-6.36	5.00
BOD	9.56-10.48	7.56-10.88	10.36-12.22	39.93
COD	22.43-23.17	22.42-23.71	24.54-25.62	23.68
Chloride	32.10-33.96	26.99-35.95	36.73-43.83	36.78
Potassium	4.94-5.40	5.16-6.08	4.75-5.57	5.42
Nitrate	4.34-5.07	5.81-6.05	6.43-7.24	5.78
Nitrite	1.06-1.13	1.55-1.75	1.87-193	1.56

Note: - All parameters are in ppm, except Temperature (°C) and Conductivity (µmho/cm).



dissolved solids <500 ppm, (WHO, 2004) generally showed inferior potable quality and induced an unfavorable physiological reaction in the transient consumer and gastrointestinal infections (Jain, *et al.*, 2009). Kulshrestha, *et al.*, (2002) also observed the similar range (840-1050 mg/l) of total dissolved solids in ground water Jaipur (Rajasthan) during summer season. The minimum value of alkalinity (260.17 ppm) was observed in bore well water during winter season

and maximum value of alkalinity (339.83 ppm) was observed in hand pump water during monsoon season. Water with more than 200 mg/l value of alkalinity is not permissible for drinking as per WHO. The present values of alkalinity in the water of hand pumps and bore well have shown higher mark on permissible limit for potability of drinking water and contributed for degradation of ground water resources.

The high degree of water hardness can definitely

Table-2: Physico-chemical characteristics of ground water of Bore wells (BW)

Parameters	Summer	Monsoon	Winter	Mean
Temperature	21.78-23.35	23.74-26.58	18.33-20.76	22.87
pH	7.44-7.87	7.38-8.05	7.26-8.04	7.63
Conductivity	925.45-1039.99	924.97-1006.69	949.97-1041.25	976.35
TDS	610.80-686.39	610.48-664.41	626.98-686.57	644.79
Free-CO ₂	0.406-0.669	0.218-0.345	0.110-0.123	0.286
Alkalinity	265.58-282.40	277.57-301.30	260.17-276.31	278.80
T. Hardness	230.64-266.34	238.07-269.01	239.02-259.36	249.92
Ca	129.68-161.13	135.20-166.43	131.54-159.64	151.39
Mg	100.05-101.44	95.43-102.88	98.78-116.81	98.52
DO	4.78-5.25	4.83-6.21	4.71-6.23	5.41
BOD	6.68-8.07	5.61-8.63	5.35-8.20	7.00
COD	12.09-15.53	15.19-16.16	13.99-16.17	15.22
Chloride	19.91-24.41	22.43-23.48	20.23-23.76	21.96
Potassium	3.83-4.04	3.15-3.56	3.27-3.99	3.66
Nitrate	1.85-2.47	2.30-3.10	1.94-2.79	2.54
Nitrite	0.79-1.11	0.97-1.12	1.02-1.07	0.99

Note: - All parameters are in ppm, except Temperature (°C) and Conductivity (µmho/cm).

be attributed to the disposal of untreated and improperly treated sewage and industrial wastes (Shanker, *et al.*, 2008). The minimum value of total hardness (230.64 ppm) was observed in bore well water during summer season and maximum value of total hardness (282.87 ppm) was observed in hand pump water during summer season. All the values of total hardness in hand pump and bore well water were recorded within the standard value (300 ppm) given by BIS. The maximum level of Ca-hardness was recorded 183.78 ppm in hand pump water during winter season and minimum level 129.68 ppm found in bore well water during summer season. The similar trend of calcium hardness occurred in ground water also

reported by Ramakrishnaiah, *et al.*, (2009) and significantly correlated hardness of drinking water with health of human and other livestock population. The minimum value of Mg-hardness (94.07 ppm) was observed in hand pump water during monsoon season and maximum value of Mg-hardness (116.31 ppm) was observed in bore well water during winter season. The values of Mg-hardness were recorded higher in comparison to standard value (30 ppm) given by BIS.

The minimum value of dissolved oxygen (3.51 ppm) was observed in hand pump water during summer season and maximum value of dissolved oxygen (6.36 ppm) was also observed in hand pump water during winter season. The difference



between DO values in hand pump water and bore well water were found due to the depth level of ground water. Bore well water (deep water aquifer) having a least contamination in comparison to hand pump water (shallow water aquifer). The same range of DO (3.9-6.3 ppm) in industrial effluents affected ground water in Kancheepuram, also reported by Balakrishnan, *et al.*, (2008). The minimum value of free- CO_2 was observed 0.110 ppm in bore well water during winter season and maximum value of free- CO_2 was observed 0.843 ppm in hand pump water during summer season. All values of free- CO_2 of hand pumps and bore well water were recorded within the standard value given by WHO.

The BOD values of ground water samples denoted the present status of pollution load. The lowest value of BOD (5.35 ppm) was observed in bore well water during winter season and highest value of BOD (12.22 ppm) was observed in hand pump water during winter season. Biochemical oxygen demand (BOD) is inter-related with the levels of dissolved oxygen in the water. Organic compounds such as carbohydrates, proteins and fats, which can be a result of urban run-off, domestic sewage and industrial effluent are broken down by the micro-organisms present in the water and exerted an oxygen demand in aquatic system (Efe, *et al.*, 2005). The minimum value of COD (12.09 ppm) was observed in bore well water during summer season and maximum value of COD (25.62 ppm) was observed in hand pump water during winter season. All the values of COD in hand pumps and bore well water recorded high comparison than standard value (10 mg/l) given by WHO. The COD have not favored the permissible value of drinking water quality, which existed in ground water of villages located near to industrial area. Hence, the existed drinking water has been contaminated by the leaching industrial effluents and intermixing with ground water in aquifers.

Chloride occurs in all natural bodies in widely varying concentrations. The chloride content normally increases as the mineral contents increases (Dubey, 2003). The chloride levels ranged from 19.91 to 43.83 mg/l in bore well and hand pump water during summer and winter

seasons respectively. Soil porosity and permeability also has a key role in building up to increase chloride concentration in ground water (Ramakrishnaiah, *et al.*, 2009). Excessive chloride in ground water indicated the un-potable characteristics of drinking water to impart bitter taste to water and corrode steel and may cause cardio-vascular problems among human society. The potassium content in both hand pump and bore well water were found in the range of 3.15-6.08 ppm. The minimum value of potassium was observed in bore well water during monsoon season and maximum value of potassium was observed in hand pump water during monsoon season. All the values of potassium in water samples come under the standard value (12 ppm) given by WHO. The minimum value of nitrate (1.85 ppm) was observed in bore well water during summer season and maximum value of nitrate (7.24 ppm) was observed in hand pump water during winter season (Table-1 and 2). Beyond standard value (45 ppm, BIS), it may cause methemoglobinemia or blue baby disease in infants. It may also be carcinogenic in adults (Basappa, 2003). The minimum value of nitrite (0.79 ppm) was observed in bore well water during summer season and maximum value of nitrite (1.93 ppm) was observed in hand pump water during winter season. All the values of nitrate and nitrite come under the standard value given by WHO.

Deleterious level of ground water pollution caused severe diseases in primary stages and slowly created a potential health hazards to the inhabitants of affected industrial area. The present study indicated drinking quality of bore well water observed slightly better than hand pump water. Therefore, the use of ordinary hand pumps should be discouraged for the direct consumption by peoples of industrial area. People dependent on hand pumps water are often to prone to health hazards due to polluted drinking water. The deterioration of ground water quality occurred due leeching processes of surface industrial waste water in water aquifers. At present most of the industries lacking of either independent waste water treatment facilities or common treatment



plant for purification of industrial waste water. The ground water resources must be non-contaminated, purified and treated efficiently on sustainable basis at point and non point sources of pollution level. The drinking water quality should be properly maintained and be available for human society to get healthy longer life.

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An approach to study the effect of automobile exhausts on the leaf epidermal features of some members of Family Apocynaceae

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Abstract

In the present investigation an effect of automobile exhausts on the leaf epidermal features of some members of family Apocynaceae was studied. In the present experimental study the Stomatal Frequency, Stomatal Index, Length, Breadth and Epidermal Frequency for both surfaces i.e. Abaxial (Lower) and Adaxial (Upper) surface for both side i.e. Garden and Road side species were recorded. Selected plant species are *Alstonia scholaris* (L.) R.Br, *Catharanthus roseus* (L.) G.Don Variety (Pink), *Catharanthus roseus* (L.) G.Don Variety (White), *Carissa carandas* (L.), *Nerium oleander* (L.) Variety (Light pink), *Nerium oleander* (L.) Variety (Pink), *Nerium oleander* (L.) Variety (White), *Plumeria alba* (L.), *Tabernaemontana divaricata* (L.) R.Br, *Thevetia neriifolia* Juss.

Keywords: Automobile, Epidermis, Stomata, Stomatal index, frequency, plant species

Introduction

Development of science and technology during the past century has transformed the conditions and qualities of human life. There is a great problem of "Automobile pollution" and the pollution from automobiles is actually coming from only a small group of "grossly polluting". Newer cars, however, as about 10% of cars on the road are accounting for 50% of all harmful emissions put out by automobiles.

Sharma and Roy (1995) described the length and breadth of stomata, stomatal frequency and stomatal index, number and size of epidermal cells of leaves studied in leaf samples from polluted and non polluted atmosphere in rainy, winter and summer seasons. Rangarajan *et al.* (1995) studied the dust deposition on the adaxial surface of the leaves, was found more than on the abaxial surface. Shamnughavel (1995) studied abnormalities such as stomata with a single guard cell, stomata with 4-5 subsidiary cell and giant stomata. Chattopadhyay (1996) studied the effects of air pollution on leaves of twenty three species

in Calcutta. Chandrawat and Raghuvanshi (2007) also studied epidermis of *Delonix regia*. Thakur and Patil (2007) studied epidermal structure in family Euphorbiaceae.

Materials and Method

The plants were collected from August to December for the study of the foliage part of plants i.e. leaf. Plant were identified with the help of flora of gangetic plain (Duthie, 1903-1929), Hutchinson (1959), Hooker (1872-97), Upadhyaya and Singh (1969). Peelings were procured either mechanically from fresh leaves or leaf segments were placed in 1:1 glacial acetic acid and 40 volumes H_2O_2 over night in an oven at 60°C temperature and then the epidermis was gently peeled off. The epidermal peelings were stained either with aqueous saffranin solution or with dalafield haematoxylin and were mounted in 4% glycerol. Stomata were measured by micrometry technique, using Ocular meter and stage micrometer. The numerical data were analysed statistically. The stomatal index was calculated by the following formula.

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$$SI = \frac{S}{e + S} \times 100$$

Where,

SI = Stomatal index

S = Stomata

e = Epidermal cell

Results and Discussion

Automobile pollution is a great problem in the world. In the present experimental study we recorded the effect of automobile pollution on the leaf epidermal features *i.e.* stomatal frequency, stomatal index, length, breadth and epidermal frequency for both surfaces *i.e.* Abaxial (Lower) and Adaxial (Upper) surface for both side *i.e.* Garden and road side species. It has been observed that the leaves respond to pollution and undergo

Table 1. Showing the frequency of stomata, epidermal cells, stomatal index (per mm²) and size of stomata of garden side species.

S.No	Name of Species	Stomata				Epidermal Cell
		S.F.(No.)	S.I (per mm ²)	L.(μ)	B. (μ)	E.F (No.)
1.	<i>A. scholaris</i>	-	-	-	-	1743 ± 58.37
2.	<i>C. roseus</i> (variety Pink)	33.95 ± 16.97	4.00 ± 2.21	240 ± 13.4	218 ± 28.86	812.9 ± 65.88
3.	<i>C. roseus</i> (variety White)	32.57 ± 14.23	3.85 ± 1.85	240 ± 0.00	201 ± 27.00	812.9 ± 65.88
4.	<i>C. carandas</i>	-	-	-	-	982.97 ± 27.77
5.	<i>N. oleander</i> (variety Pink)	-	-	-	-	878.1 ± 71.95
6.	<i>N. oleander</i> (variety White)	-	-	-	-	959.97 ± 102.75
7.	<i>N. oleander</i> (variety Light Pink)	-	-	-	-	930.9 ± 57.03
8.	<i>P. alba</i>	-	-	-	-	1790.3 ± 76.86
9.	<i>T. divaricata</i>	14.16 ± 0.00	1.91 ± 0.105	252 ± 14.69	183 ± 31.32	726.59 ± 49.90
10.	<i>T. nerifolia</i>	-	-	-	-	902.2 ± 47.00

Table 2. Showing the frequency of stomata, epidermal cells, stomatal index (per mm²) and size of stomata of garden side species.

S.No.	Name of Species	Stomata				Epidermal Cell
		S.F.(No.)	S.I (per mm ²)	L. (μ)	B. (μ)	E.F (No.)
1.	<i>A. scholaris</i>	339.95 ± 26.88	17.97 ± 1.60	189 ± 33.0	153 ± 9.00	1550.9 ± 76.87
2.	<i>C. roseus</i> (variety Pink)	246.40 ± 23.8	32.10 ± 3.95	237 ± 09.0	216 ± 26.15	521.2 ± 77.26
3.	<i>C. roseus</i> (variety white)	246.07 ± 22.65	33.08 ± 2.78	231 ± 13.7	177 ± 21.00	509 ± 40.05
4.	<i>C. carandas</i>	385.20 ± 53.6	34.60 ± 4.60	207 ± 09.0	165 ± 20.12	728 ± 78.42
5.	<i>N. oleander</i> (variety Pink)	031.15 ± 16.51	03.54 ± 1.81	195 ± 15.0	143 ± 18.85	846.9 ± 35.13
6.	<i>N. oleander</i> (variety White)	015.93 ± 14.91	01.76 ± 1.65	186 ± 12.0	153 ± 21.00	885.21 ± 96.34
7.	<i>N. oleander</i> (variety Light Pink)	015.57 ± 13.36	01.84 ± 1.42	192 ± 19.8	174 ± 12.00	830.49 ± 31.53
8.	<i>P. alba</i>	315.82 ± 37.50	21.35 ± 2.70	240 ± 18.9	160 ± 14.50	1161.43 ± 106.63
9.	<i>T. divaricata</i>	130.27 ± 28.19	12.89 ± 1.56	246 ± 18.0	159 ± 19.20	1009.8 ± 157.80
10.	<i>T. nerifolia</i>	230.84 ± 27.62	33.08 ± 2.78	207 ± 09.0	153 ± 28.30	1447.5 ± 106.90



Table. 3: Showing the frequency of stomata, epidermal cells, stomatal index (per mm²) and size of stomata in road side species

Adaxial surface

S.No.	Name of Species	Stomata				Epidermal Cell
		S.F.(No.)	S.I (per mm ²)	L. (μ)	B. (μ)	E.F (No.)
1.	<i>A.scholaris</i>	-	-	-	-	1705.3 ±87.59
2.	<i>C. roseus</i> (variety Pink)	33.9 ±14.42	4.16 ± 1.92	234 ± 12.00	201± 27.0	812.9 ±65.8
3.	<i>C. roseus</i> (variety White)	33.98 ± 16.99	3.94 ± 2.14	240± 0.00	198 ± 27.49	827.15± 79.91
4.	<i>C. carandas</i>	-	-	-	-	968.8 ±41.6
5.	<i>N.oleander</i> (variety Pink)	-	-	-	-	875.3 ±73.55
6.	<i>N. oleander</i> (variety White)	-	-	-	-	959.97± 102.75
7.	<i>N.oleander</i> (variety Light Pink)	-	-	-	-	880.97 ± 64.24
8.	<i>P. alba</i>	-	-	-	-	1770.5± 97.5
9.	<i>T. divaricata</i>	14.16± 0.00	1.95± 1.46	249 ± 13.74	168 ±24.0	711.02± 48.17
10.	<i>T. neriiifolia</i>	-	-	-	-	895.12 ±48.99

Table. 4: Showing the frequency of stomata, epidermal cells, stomatal index (per mm²) and size of stomata in road side species.

Abaxial surface

S.No.	Name of Species	Stomata				Epidermal Cell
		S.F.(No.)	S.I (per mm ²)	L. (μ)	B. (μ)	E.F (No.)
1.	<i>A. scholaris</i>	331.4 ±29.86	17.71 ± 1.89	201± 27.0	153 ±9.00	1539.6 ±69.00
2.	<i>C. roseus</i> (variety Pink)	226.5 ±46.9	30.88± .678	231 ± 14.4	180 ±22.15	507.04± 96.8
3.	<i>C. roseus</i> (variety White)	246.41± 23.86	32.70± 3.95	228± 14.69	174 ±22.44	507.04± 57.29
4.	<i>C. carandas</i>	371.08± 40.96	33.08 ±3.92	207 ± 9.0	171 ±19.20	750.67 ±64.00
5.	<i>N. oleander</i> (variety Pink)	25.49 ± 10.59	2.97 ±1.51	198± 14.69	144 ±18.00	831.3 ±65.21
6.	<i>N. oleander</i> (variety White)	14.16 ± 11.56	1.678± 1.10	180 ± 14.14	153.3± 26.24	829.3 ±70.03
7.	<i>N. oleander</i> (variety Light Pink)	12.75 ±14.79	1.50± 1.29	189 ±13.74	171 ± 13.74	835.6± 42.48
8.	<i>P. alba</i>	307.31± 41.57	26.61 ± 2.76	234 ± 12.0	159 ±13.74	1147.2 ±95.01
9.	<i>T. divaricata</i>	123.19 ±27.64	11.05 ±1.70	243 ±21.00	165.0± 20.12	991.45±163.6
10.	<i>T. nerifolia</i>	218.1± 23.86	12.87 ± 2.45	207 ± 9.00	150 ±28.30	1447.5±106.9

S.F. – Stomatal Frequency, E.F = Frequency of epidermal cell, S.I = Stomatal Index, L = Length, B = Breadth
Values are in mean ± S.D.



quantitative changes in varying degree in a number of leaf surface micromorphological characters. Such leaf surface characters can be used as bioindicators and biomonitors of air pollution data indicated that the stomatal frequency, stomatal Index, length and breadth of stomata and epidermal cell increasing in garden

side plant species and decreased in road side plant species.

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Effects of vegetative barriers for channelization of Shiwalik torrent at Sabhawala in Doon Valley (India)

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Abstract

A study under highly fragile ecosystem was conducted at Sabhawala watershed area in Doon Shiwalik hills of Uttarakhand. The Uttarakhand state is particularly sensitive to forest land disturbing activities. Steep slopes, high rainfall and weak geology of the Uttarakhand state accentuate the land degradation and soil erosion process at much faster rate than in the plains. In the head water reaches the sediment is flushed with the high velocity currents. But when it reaches the relatively terrain foothills. Its debris carrying capacity diminishes and the debris starts accumulating on the river bed causing change of river course and flooding its bank. To assess survival percentage of different species for effective vegetative barriers for channelization of Shiwalik torrents, controlling and management of torrent flow, runoff, erosion control, various vegetative barriers species as *Vitex negundo*, *Arundo donax*, *Ipomea carnea*, *Dendrocalamus Strictus* etc were established during the study period. The protective vegetative barrier plant species used as *Ipomea carnea* recorded the height survival percentage as *Ipomea carnea* (90%) having the growth 150 cms followed by *Vitex negundo* (86%) along with 111.0 cms growth *Pennisetum purpureum* (Hybrid Napier) (84 %) with 113.5 growth height, *Jatropha curcas* (83 %) with 110.5 growth height, *Arundo donax* (70%) with 100.0 cms height growth, The protective vegetative barrier measures were provided a base root technology for conservation and management of relative torrential watershed in Doon Shiwalik Himalayan region of India.

Keywords: *Torrent, vegetative barriers, Bio-engineering methods, Shiwalik region, runoff*

Introduction

The Uttarakhand state is particularly sensitive to forest land disturbing activities. Steep slopes, high rainfall and weak geology of the Uttarakhand state accentuate the land degradation and soil erosion process at much faster rate than in the plains. In the head water reaches the sediment is flushed with the high velocity currents. But when it reaches the relatively terrain foothills. Its debris carrying capacity diminishes and the debris starts accumulating on the river bed causing change of river course and flooding its bank. These rivers with flash flows and high sediment loads are known as torrents. Thus, the real problem of torrent formation lies in lesser Himalayan in fact, due to misuse and mismanagement of upstream

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catchments. The problem of torrent menace has been rising in Uttarakhand with the rise, in population pressure and related mismanagement of upstream watersheds.

Torrents or torrential streams have been defined variously, According to FAD; torrential stream /torrent has been defined as small mountain stream which flows with high velocity and flashy, usually carrying large size bedload particles. In defining other subtypes, the source also describes a torrential stream as one which transports heavy load of sediments derived from slides and other unstable areas as a sediment laden torrent or gully washer. Again another type is a small torrent supplied with sediments by a slide area has been described as a flashy slide-fed torrent (FAO, 1981). In an another conservation guide of FAO, torrent has been described as a natural channel or waterway with (a) A small catchments or watershed; (b) Steep and

irregular section of extreme oscillation of the runoff with high flood peak with rain and storm; and (c) High bedload transport, due to intensive erosion and mass movement and sedimentation (Hettinger, 1976).

In the present study, the protective vegetative cover was established in the torrent of Sabhawala watershed area for the identification of effective protective vegetative cover with the help of mechanical measures and appropriate soil and water conservation techniques. Trees and Grasses used for the establishment of the initial vegetative cover by the species as *Dalbergia sissoo*, *Bauhinia purpurea*, *Dendrocalamus Strictus*, *E. hybrid*, *Ipomea carnea*, *Vitex negundo*, *Arundo donax*, *Eulaliopsis binata* (Bhabhar), *Pennisetum purpureum* (Hybrid Napier), *Jatropha curcas*, *Crysopogon fulvus*, *Saccharum munja*, *Pueraria hirsute* (kudzu), *p.antidotale* and *V.zizanioides* etc. The plants used as *Leucaena leucocephala* (Subabool), *Salix tetrasperma*, *Acacia catechu* (Khair) *Cedrela toona*, *Bauhinia* spp. *Erythrina suberosa*, *Lannea grandis*, *Pithecellobium dulce* (Jungle Jalebi) etc has been observed as a effective protective vegetative cover and helping in recharging ground water, channelization of torrent water flow and controlling flood.

Materials and Method

Study area

The study was conducted at Sabhawala watershed in the Doon Shiwalik range of the Uttarakhand (India). The experimented site located at the longitude 77° 48' E and latitude 30° 20' N. Uttarakhand is characterized by two types of climate, sharply differentiated in the plains and the mountainous regions. Rainfall is quite favorable in this area and is more than 1100 mm per annum. However its main concentration is in monsoon season (about 80% of the total precipitation), which is the major cause of erosion in the rainy season. Frost conditions also occur in the winter months. The soils have poor structure and their water retention capacity is quite low. As a result of poor water holding capacity, it supports less biological activities and that is why drought conditions prevail

in the summer months. Due to constant erosion over a period of time, soils have been washed and left with very poor nutrients status.

Methodology

The selected experimental torrent at Sabhawala watershed area originating from Doon Shiwalik foothill regions of Himalaya have been identified and delineated for the experiment. The experimental program was planned taking into account the objectives of the study and the parameters were selected to centralize the aim of sampling to achieve the representativeness and validity of the samples. The study was conducted for two years (April, 2007-March, 2009) and the frequency of sampling was set as per existing conditions during the rainy seasons or flood periods.

Results and Discussion

The primary concern of watershed management is to continuously increase the productive capacity of land, water, vegetation and other resources by selecting and developing the best way of managing the renewable and non-renewable resources in watershed to meet the present and future needs of the community. It requires the collection and analysis of a great deal of information of the physical relationship of vegetation-soil water to land management which would ensure economic and social progress of a region.

In the present study, various plant species effectively served as vegetative barrier and had better soil binding capacity in the layer, which helped in soil aggregation, increased infiltration rates, improve soil moisture level and conserved maximum runoff.

In the present study for the vegetative barrier, live hedges and vegetative spurs, various trees and grasses were planted on bank of the upstream side in the Sabhawala watershed area to identified the best performance of Survival and growth behavior of species like *Dalbergia sissoo*, *Bauhinia purpurea*, *Dendrocalamus strictus*, *E. hybrid*, *Ipomea carnea*, *Vitex negundo*, *Arundo donax*,



Eulaliopsis binata, *Pennisetum purpureum*, *Jatropha curcas* etc.

The best survival and growth was shown species as *Ipomea carnea*, (90%) having the growth 150 cms, *Vitex negund* (86%) along with 111.0 cms growth *Pennisetum purpureum* (Hybrid Napier) (84 %) with 113.5 growth height, *Jatropha curcas* (83 %) with 110.5 growth height, *Arundo donax* (70%) with 100.0 cms height growth, *Dendrocalamus strictus* (76%) with 103.7 cms height, *Eulaliopsis binata* (65%) with 105.0 cms height growth,

Bauhinia purpenea (70%) with 50.0 cms height growth, *Dalbergia sissoo* (64%) with 54.0 cms height growth. Plantation of these vegetative barriers plants species was done in July – August during rainy days. However the best survival and growth was shown by *Ipomea carnea*, (90%) followed by *Vitex negund* and *Pennisetum purpureum* species might be a better choice over fodder species on these freely accessible areas because browsing restricts the establishment of fodder species.

Table. 1: Survival and growth behavior of different vegetative barriers plants species on torrent bed and banks

Vegetative barrier Plant species	Species Survival (%)	Species Growth (cms)		Current species Height	Annual increment Basel dia	Type of vegetation
		Height	Basel dia			
<i>Dalbergia sissoo</i>	64	54.0*	2.5	-	-	Shrub
<i>Bauhinia purpenea</i>	70	50.0	2.0	23.0	0.6	to
<i>Dendrocalamus strictus</i>	76	103.7*	2.2	50.5	0.6	tree
<i>Ipomea carnea</i>	90	150.0	2.5	113.0	0.8	Shrubs
<i>Vitex negund</i>	86	111.0	2.4	77.0	0.7	Shrubs
<i>Arundo donax</i>	70	100.0	2.5	63.0	0.6	Shrubs
<i>Eulaliopsis binata</i>	65	105.0	10.3+	70.0	5.8	Grass
<i>Pennisetum purpureum</i>	84	113.5	3.1	78.5	0.3	Grass
<i>Jatropha curcas</i>	83	110.5	2-6	111.0	0.6	Shrubs

*= Browsed by cattle

+ = Clump diameter

Napier (*P.purpureum*) could be suitable vegetative barrier, where fodder production is a priority while *S. munja* as a barrier is suitable where conservation and crop production is the main objective. Burdak (1982): reported that *S. munja* is recent advances in desert afforestation. Various research works in the rooting behavior of some grasses in the Shiwalik were carried out by Saha *et al.* (1994) on *Saccharum munja*, *Eulaliopsis binata* and *Vetivaria zizanionides* and described at the roots of *S. munja* (Munj) reached the maximum depth. Highest root densities were confined to the plough layer in all the three species, the trend being *E.binata* (89%) > *Vetivaria* (77%) > *S.munja* (48%).

Samra and Sharma (1995) made experiment the soil binding factor for three perennial grasses

(*S.munja*, *S.spontaneum*, *Arundo donax*) in sandy choes at Rei Majra and observed that the *S.spontaneum* had the highest above ground biomass (398 kg) followed by *S. munja* (28.3 kg) and least *A. donax* (3.7 kg). Soil binding capacity in 0-10 cm layer was recorded highest for *S.spontaneum* (1590) followed by *S.munja* (788) and *A.donax* (31).

The selected vegetative barrier plant species effectively served as vegetative barrier and had better soil binding capacity in the torrential soil, which helped in underground water recharges because that increased infiltration rates, improve soil moisture level and conserved maximum runoff and played a significantly contributed to controlled and channelization the degraded torrent channels and protected the agricultural land for the better survival and increase the economy of inhabitants in and around the watershed catchment basin. The vegetative grasses, shrubs and tree plantation on



the earthen embankment of torrent provided the fodder to livestock and fuel of the villagers of Sabhawala

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***Invitro* antimicrobial activity and GCMS analysis of *Anethum graveolens* L.**

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Abstract

Plant based medicine has served mankind for centuries and an impressive number of modern drugs have been isolated from natural sources. The last 20–25 years have witnessed renewed interest in folkloric remedies to fight microbial diseases owing to the emergence of multidrug resistant microorganisms. In present study seed oil of *Anethum graveolens* was investigated against 11 bacterial strains and found that the extract was active against all tested bacterial species.

Keywords: *Multidrug resistant bacteria, Anethum graveolans, Antimicrobial, GCMS, Seed oil*

Introduction

History of essential oils are very ancient, the Egyptians, Hindus, Greeks and Arabs were much familiar with extraction and use of essential oils. In those times distillation was the only method used for their extraction, actual yield of oil extraction and characterization started in early 1920s. Plant essential oils are potential source of antimicrobials of natural origin (Valero and Giner, 2006). Essential oil plants include a wide range of plant species, mainly used in the preparation of perfumes, cosmetics, beverages, medicinal foods, disinfectants, insecticides, fungicides, smoking, chewing, tobacco and condiments. Essential oils are extracted from aromatic plants of many genera, which are distributed worldwide. These oils are found in various parts (seeds, leaves, fruits barks & roots) of aromatic plants. English people name *Anethum graveolens* as dill and in subcontinent it

is called as sowa. It is used as flavoring and preservative agent. Its medicinal uses are as an antispasmodic, carminative, diuretic, stimulant and stomachic (Simon *et al.*, 1984). Some of the earlier studies had shown the antimicrobial activity of *A. graveolens* against *Saccharomyces cerevisia* and *Listeria monocytogenes* (Pascal *et al.*, 2002).

A. graveolens L belongs to family Umbelliferae, known as dill, is an annual herb growing in the Mediterranean region, Europe,

central, southern Asia and it is widely cultured in south eastern region of Iran. The plant is used both medicinally and as an aromatic herb and spice and cookery. Dill has been used traditionally for gastrointestinal ailments such as flatulence, indigestion, stomachache colic and to tract intestinal gas (Hosseinzadeh *et al.*, 2002). The presence of flavonoids, phenolic compounds and essential oil in *A. graveolens* has been reported (Charles *et al.*, 1995; Ishikawa *et al.*, 2002; Justesen and Knuthsen, 2001; Vera and Chane-Ming, 1998). Some pharmacological effects of the plant such as antimicrobial (Chaurasia and Jain, 1978), antispasmodic (Fleming, 2000), anti secretory and mucosal protective effects have also been reported (Hosseinzadeh *et al.*, 2002). The anti-hyper cholesterolaemic and anti-

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hyperlipidaemic activities (TC, TG) of the crude extract have previously been reported (Yazdanparast and Alavi, 2001)

Materials and Method

Collection of plant material

The fresh dried seed of *A. graveolens* were collected from botanical garden of S.G.R.R Degree College Pathribagh Dehradun (Uttarkhand).

The seed of plant were subjected to hydro distillation by Clevenger's apparatus.

Extraction of essential oil

Fresh dried seeds (100g) of *A. graveolens* were crushed and then subjected to hydro distillation for 8-10h in Clevenger's Apparatus to obtain the essential oil. The obtained essential oil was dried over anhydrous sodium sulphate and then kept in refrigerator (3.5%v/w). Then the essential oil studied for its effect on microorganism and chemical composition by GC-MS analysis.

Chemical composition by Gas chromatography Mass spectrometry

This is undoubtedly the most important technique for study of essential oil, since it yields in one operation both qualitative and quantitative analysis.

GC-MS analysis of oil

The GC-MS Analysis of the essential oil of *A. graveolens* was carried out on a Thermo quest Trace GC-2000 coupled with Finnegan mat Polaris spectrometer equipped with a non-polar D.B.5 capillary column (30 x 0.25 μ m). Injection volume was 0.1 μ l in the split mode (split flow rate 40 ml/min). Chromatography conditions were as follow. Helium was used as a carrier gas at a flow rate of 1ml/min; inlet temperatures were 210°C; oven temperature was held at 60-210°C for 10 min. followed by linear temperature. Programming at the rate of 3°C temperature rise min⁻¹. The column was coupled directly to the Polaris mass Spectrometer operated in the electron ionization

mode at 70 ev ion source temperature 200°C. MS transfer line 275°C, scan rate 7-8/sec.

The identification of compounds was made on the basis of mass spectra by matching the mass against the NIST library software and the retention time comparison with the published data of Wiley.

Antibacterial activity

Microorganism tested

The bacterial cultures used in this study are, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus pumilus*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Serratia marcesens* were provided by Department of Biotechnology, S.G.R.R Dehradun (India) and checked for purity by convention biochemical methods. The bacterial cultures were stored on nutrient agar at 4°C

The antibacterial activity of the oil was determined by Bauer *et al.* (1966). The *in-vitro* antibacterial effect of the oil was determined by Disc diffusion method. The bacterial strains were sub cultured in Muller Hinton broth and incubated at 33- 37°C for 24 h. Turbidity of the bacterial suspension was adjusted to the McFarland (0.5) and 100 μ l of inoculum was plated on Muller Hinton agar. Sterile paper disc (6 mm) which is loaded by sample solution (5 μ l) were placed carefully on the surface of the plates. Plates were incubated at 33-37°C for 24 hr. and observed for the zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

MIC of the different concentration of oil was evaluated by Disc diffusion method. Different concentration of the oil *i.e.* 100% (1ml), 50 % (0.5 ml/ml), 25 % (0.25 ml/ml), 12.5 % (0.125 ml/ml), 6.25 % (0.625 ml/ml), 3.125 % (0.0132 ml/ml), were made by two fold serial dilution in DMSO (Dim Ethyl Sulphoxide) as a diluting solvent. 5 μ l of these samples loaded on the disc and discs were placed over seeded agar. The Petri dishes were incubated at 34-37°C for 24 hr. in BOD incubator. Higher zone of inhibition at minimum concentration gave the measure of MIC value.



Results and Discussion

Antimicrobial activity of Dill (seed) oil:

The seed oil were screened for antibacterial activity against various bacterial strains like, *Proteus* sp, *salmonella typhi*, *Serratia rubidaea*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus*, *Bacillus pumilus*, *Serratia marcescens* and *Pseudomonas fluorescens*.

The essential oil of *A. graveolens* L seed was found to be quite effective in inhibiting the growth of various bacterial strains tested, as indicated by zone of inhibition. Maximum zone of inhibition was found against *Escherichia coli* (zone size, 35 mm), followed by *Bacillus pumillus* (30 mm), *Staphylococcus aureus* (29 mm), *Bacillus cereus* (26 mm) and *salmonella typhi* (20 mm) with 5 µl of essential oil. The test solution compared with standard antibiotic viz; Amikacin (250 mg/ml). The results are shown in Table. 1.

MIC of Essential oil

The oil also screened against various pathogen. In different dilution like 100 %, 50 %, 25 %, 12.5 % 6.125 % and in 3.125 % MIC of oil was observed and result are shown in Table 2

Escherichia coli (10 mm) and *Bacillus cereus* (12 mm) are more susceptible strains whereas *Bacillus pumilus* (07 mm) and *Staphylococcus aureus* (07 mm) are more resistant and the remaining bacterial strains showed moderate. Essential oils of dill have been reported to inhibit a broad spectrum of microorganisms (Deans and Ritchie, 1987; Nakatani, 1994). Kaur and Arora (2008) reported the antimicrobial activity of aqueous extract and acetone extract and found that seed extract was active against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

GC-MS analysis of *Anethum graveolens* L essential oil

The various literature reports are available on gas chromatography and mass spectroscopy analysis

of *A. graveolens* L essential oil. There are total 35 constituents, which account for 98.9% of total amounts are reported. The major compound are carvone (55.2%) followed by limonene (16.6%) dillapiole (14.4%), and linalool (3.7%).

In the present study the essential oil of *A. graveolens* L was analyzed by GC-MS for chemical constituents (Fig 1). The major five constituents were found. Carvone (60%), limonene (15.5%), apiole (14.4%), linoleic acid (4.6%) myristicin (1.5%) are the major constituents. The results are shown in Table. 3.

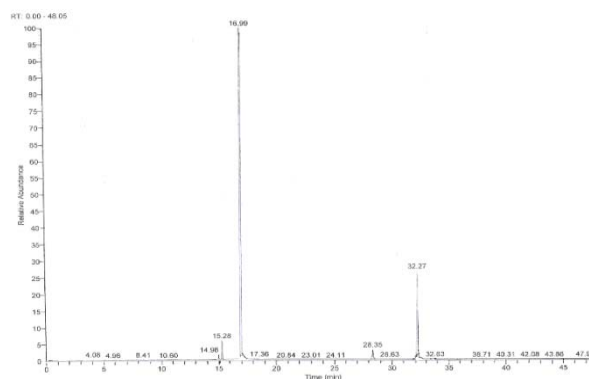
Table.1: Antimicrobial activity of seed oil

S. No		ZONE OF INHIBITION(mm)	
		Standard drug (Amikacin)	<i>Anethum graveolens</i> seed oil
1	<i>Proteus</i> sp	35	15
2	<i>Salmonella typhi</i>	40	20
3	<i>Serratia rubidaea</i>	26	12
4	<i>Proteus mirabilis</i>	28	17
5	<i>Staphylococcus aureus</i>	26	29
6	<i>Klebsiella pneumonia</i>	29	17
7	<i>Escherichia coli</i>	40	35
8	<i>Bacillus pumillus</i>	39	30
9	<i>Bacillus cereus</i>	35	26
10	<i>Serratia marcescens</i>	40	18
11	<i>Pseudomonas fluorescens</i>	42	20

Table 2 Minimum inhibitory concentrations (MIC) of *Anethum graveolens* (Dill) oil against various bacterial strainable 3.

S.No	Bacterial strains	Concentration of Essential oil in percentage					
		100%	50%	25%	12.5%	6.25%	3.125%
1	<i>Proteus</i> sp.	20	15	10	--	--	--
2	<i>Salmonella typhi</i>	10	06	05	--	--	--
3	<i>Serratia rubidaea</i>	25	20	14	10	07	--
4	<i>Proteus mirabilis</i>	--	--	--	--	--	--
5	<i>Staphylococcus aureus</i>	21	20	15	10	07	--
6	<i>Klebsiella pneumoniae</i>	08	05	--	--	--	--
7	<i>Escherichia coli</i>	30	19	12	10	10	--
8	<i>Bacillus pumilus</i>	25	20	14	10	07	--
9	<i>Bacillus cereus</i>	25	20	18	15	12	--
10	<i>Serratia marcescens</i>	21	16	09	07	07	--
11	<i>Pseudomonas fluorescens</i>	17	15	15	10	07	--

S.NO	COMPOUND NAME	PERCENTAGE
1	Carvone	60
2	Limonene	15.5
3	Apiole	14.4
4	Linoleic acid	4.6
5	Myristicin	1.5

Table. 3: GC-MS Analysis of *Anethum graveolens* L essential oil**Fig. 1:** GC-MS Analysis of *Anethum graveolens* L essential oil

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Observation on the collection of zooplankton in Lakhani lake, district Bhandara, Maharashtra (India)

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Abstract

Zooplankton composition of Lakhani Lake, of Bhandara district (India) has been studied for a year. A total of 21 species were identified. 9 of them belonging to Rotifera, 8 to Cladocera, 3 to Copepoda and 01 to Ostracoda. The study of zooplankton diversity would be very useful in preparing the biodiversity atlas of the planktonic species as well as in future environment impact assessment studies.

Keywords: Bioindicator, Cladocera, Eutrophication, Lakhani lake, Rotifera, Zooplankton

Introduction

Bhandara district is popularly known as 'Lake District'. It possesses more than 15000 lakes and reservoirs. Studies on freshwater biodiversity of Bhandara district are scarce. Zooplanktons occupy an important position in the trophic structure and play a major role in the energy transfer of an aquatic ecosystem.

Anthropogenic activities at the basin of lake and nearby agricultural runoff increased the organic load, which considerably alter their physico-chemical characteristics and accelerate the process of eutrophication, which alter food chain sequences leading to production of commercially less valuable higher trophic organisms (Rao, 1982). One of the main difficulties in studying loss of biodiversity due to eutrophication is the absence of previous records of species composition. The present work was undertaken to study the zooplankton composition of Lakhani Lake, Lakhani of Bhandara District. Notable contributions on zooplankton of fresh water

ecosystem are available due to Sharma (1996), Kodarkar (1999) and Kudari *et al.* (2005).

Materials and Method

Lakhani Lake is about 100 year old, situated in the urban area, surrounded by nearby agricultural land. The water body is organically enriched with anthropogenic activities as well as agricultural surface runoff. Samples were taken monthly for a year by plankton net of bolting cloth of 25 μ . The samples were preserved in 4% formaline and observed and identified by using the literature of Ward and Whipple (1959), Micheal and Sharma (1988), Dhanapathi (2000) and Sehgal (1983).

Result and Discussion

Zooplankton recorded from Lakhani Lake, Lakhani belong to Rotifera, Cladocera, Copepoda and Ostracoda. The lake was dominated with Rotifera followed by Cladocera, Copepoda and Ostracoda. A total of 21 species were identified, 9 of them belonging to Rotifera, 8 to Cladocera, 3 to Copepoda and 1 to Ostracoda.

The Lakhani lake is largely colonized by submerged and emerging macrophytes, which

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almost completely occupy its surface area. Littoral rotifers, members of Cladocerns and ostracods favour this kind of environment (Rocha *et al.*, 1995). Out of 9 species of rotifers, 5 species belongs to Brachionidae and one species of Lecanidae and one from Asplanchnidae. Brachionus was the prominent genus represented by 07 species i.e. *Brachionus calyciflorus*, *B. Calyciflous amphiceros*, *B. falcatus*, *B. quadridentatus*, *B. bidentata*, *Platyonus patulus* and *Keratella tropica*. Genus Brachionus is one of the most ancient genus of monogonont rotifers and is represented by 46 species in India (Sharma,

1983). The genus Brachionus is the index of eutrophic waters (Sladeczek, 1983) and its abundance is considered as a biological indicator for eutrophication (Nogueira, 2001). The species *B. calyciflorus* is considered to be a good indicator of eutrophication (Sampaio *et al.*, 2002). According to Liu Fengqui (1996), the small population of Copepods is directly proportional to the large population of the Rotifer species appeared in waters with high eutrophication. The dominance of Rotifer species indicates organic pollution due to direct entry of untreated sewage from catchments area (Arora, 1966).

Table:1. Diversity of Zooplankton from Lakhani Lake, Maharashtra (India)

Rotifera	Cladocera	Copepoda	Ostracoda
Family: Brachionidae <i>Brachionus calyciflorus</i> <i>B. calyci. amphiceros</i> <i>B. falcatus</i> <i>B. quadridentatus</i> <i>B. bidentata</i> <i>Platyonus patulus</i> <i>Keratella tropica</i>	Family: Sididae <i>Diphanosoma sarsi</i> <i>Diphanosoma excisum</i>	Family: Cyclopidae <i>Mesocyclop</i> spp. <i>Ectocyclops phaleratus</i>	Family: Cyclopidae <i>Cypris</i> spp.
Family: Lecanidae <i>Lecane</i> sps.	Family: Macrothricidae <i>Macrothrix</i> spp. <i>Ilyocryptus</i> spp.	Family: Diaptomidae <i>Heliodiaptomus viduus</i>	
Family: Asplanchnidae <i>Asplanchna brightwelli</i>	Family: Chydoridae <i>Chydorus</i> spp.		
	Family: Daphnidae <i>Ceriodaphnia cornuta</i> <i>Simocephalus</i> spp.		
	Family: Moinidae <i>Moina micrura</i>		

About 600 species of fresh water Cladocerans have been reported (Korovchinsky, 1996) to occur throughout the world and in India 110 species have been recorded (Patil and Goudar, 1989). In the present study 08 Cladoceran species are recorded. According to Uttangi (2001) Cladocerans prefer to live in clear water. In the littoral zone, the Chydorus species usually associate with macrophytes, periphyton or sediment. The Cyclopoids Copepods were recorded during early winter may be due to the water with heavy algal blooms (Meshram, 1996). Ostracod gave very little contribution in the

zooplankton diversity and occurs occasionally. The present study indicates that the water body is highly eutrophic and a sustainable and holistic management planning is necessary for conservation of this lake.

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Nutrient returns through litter fall in age series of rehabilitated limestone mined areas of Mussoorie hills

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Abstract

Litter fall and nutrient return was studied in four different age stands (11years, 8 yrs, 6 yrs and 4yrs old plantation) of Kiyarkuli catchment of Doon Valley of Garhwal Himalaya around Mussoorie hills (30° 25' to 30° 30' N lat. and 78° 0' to 78° 5' E long., 1700 to 1850 m above MSL). Maximum litter fall was estimated in Site I and minimum in Site IV. The trend of annual nutrient return was in order of Ca > N > K > Mg > P for all sites.

Keywords: *Litter fall, Nutrient return, mined ecosystem, age stands, Himalaya*

Introduction

Doon valley in Western Himalaya is known for its natural resources like minerals, soil, water, healthy climate and scenic beauty was also experiencing several environmental problems due to reckless and unscientific extraction of minerals which were in the form of limestone, phosphorite, marble etc. The area affected by mining in the valley is about 6147.43 ha (Soni *et al.*, 1992).

To regain self-sustaining systems, which can maintain the various ecological processes including that of nutrient turnover, restoration of these abandoned mined areas through applying proper measures has become imperative. Litter production and its decomposition play a key role in turnover of nutrients and maintenance of soil fertility and plant productivity. If surface litter is not removed and allowed to decompose and contribute to nutrient cycling, the soil can support higher productivity of trees (Miller, 1981). Ebermayer (1876) has established the significance of litter fall in the nutrient cycle of the forest. The biological cycle of nutrients in an ecosystem is one of the principal processes that support organic matter production. Therefore, a field study was

Conducted to assess the litter production and quantity of nutrients returned to the soil through litter fall in rehabilitated mined ecosystem.

Materials and Method

A field study was conducted for one year in Kiyarkuli catchment of Doon Valley of Garhwal Himalaya around Mussoorie hills. The site is situated near Hathipaon, 37 km north-west from Dehra Dun and 10 km south-west from Mussoorie town. The area falls between 30° 25' to 30° 30' N latitude and 78° 0' to 78° 5' E longitude in an elevation range of 1700 to 1850 m above msl, under Bhitreli reserved forest of Mussoorie Forest Division. The maximum temperature during winter is not more than 18° C. Summer extends from April to June. May and June are the hottest months, when the temperature generally reaches up to 25° to 29° C. The rainy seasons is marked by heavy rains high humidity and 21° to 26° C temperature. The average annual rainfall of the area is 2,225.2 mm.

Litter fall

Litter accretion studies were carried out by litter plots using ground sampling method (Medweeks - Kornas, 1970; Suckachev and Dylis, 1966). The litter accumulated over the month was collected

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and weighed to obtain fresh weight and representative samples were brought to the laboratory for oven dry weight determination at 80⁰ C till the constant weight and then powdered in Thomas Wiley Mill. The powdered samples were used to further chemical analysis (N, P, K, Ca, Mg).

Nitrogen was estimated by using Macro Kjeldahl method (Loomis and Shull, 1937). For analysis of (P, K, Ca, Mg) the stock solutions were made by using wet digestion method. Wet digestion involves digestion of 1 gm of powdered plant samples in a Tri - acid mixture (HNO₃ (10): H₂SO₄ (1): HClO₄ (4) Phosphorus was estimated by the Molybdate blue" method (Vogel, 1961), Magnesium by Thiazole yellow" method (Young

and Gill, 1951) by using Spectrophotometer, whereas Potassium and Calcium were determined by making appropriate dilution of stock solution and taking readings in Flame Photometer after placing the respective filters.

Results and Discussion

Litter in kg/ha along with its percent contribution to the total is shown in Table - 1. Maximum amount of the total annual litter fall was recorded in Site I (11 years old stand) followed by site III (6 years old stand), Site II (8 years old) and least in Site IV (4 years old stand). Thus the trend of litter production (kg ha⁻¹ yr⁻¹) in all these four stands was in order 5286.4 > 1193.2 > 804.0 > 262.0 respectively for Site I, III, II and IV.

Table 1: Monthly litter fall (kg/ha) in four study sites

Sites	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
I	340.0 (6.43)	384.0 (7.26)	404.0 (7.64)	316.0 (5.98)	393.2 (7.44)	289.2 (5.47)	196.0 (3.71)	608.0 (11.50)	820.0 (15.51)	660.0 (12.48)	572.0 (10.82)	304.0 (5.75)	5286.4
II	60.0 (7.46)	44.4 (5.52)	72.4 (9.00)	67.2 (8.36)	172.0 (21.4)	154.4 (19.20)	64.0 (7.96)	12.0 (1.49)	28.0 (3.48)	33.6 (4.18)	80.0 (9.95)	16.0 (1.99)	804.0
III	100.0 (8.38)	60.4 (5.06)	100.0 (8.38)	110.4 (9.25)	152.0 (12.74)	200.0 (16.76)	68.0 (5.70)	108.0 (9.05)	80.0 (6.70)	79.2 (6.64)	75.2 (6.30)	60.0 (5.03)	1193.2
IV	48.0 (18.32)	16.0 (6.11)	24.4 (9.31)	17.2 (6.56)	14.8 (5.65)	5.6 (2.14)	8.0 (3.05)	8.0 (3.05)	20.0 (7.63)	12.0 (4.58)	48.0 (18.32)	40.0 (15.27)	262.0

*Figures in parenthesis refers to percentage contribution

Results presented in Table 2 indicated that the annual nutrient return (Kg/ha) followed the trend in order of Ca > N > K > Mg > P for all experimental sites. The highest annual nutrient returns (kg/ha) was recorded at Site I (283.60) and least by site IV (12.99). Site III has recorded 77.35 kg/ha/yr of nutrients return and Site II 42.33 kg/ha/yr (Table. 2). It was observed that the total

annual return of calcium was highest followed by nitrogen and very small quantity of phosphorus was returned in all the four study sites. The amount of nutrients return through litter fall varies with the amount of litter production and nutrient concentration. Maximum amount of calcium and nitrogen return was also recorded by number of workers in the litter of different climatic zones.



Table 2. Annual nutrients return (Kg/ha) in four study sites

Site	N	P	K	Ca	Mg
I	81.18	3.22	76.62	114.45	8.13
II	12.73	0.36	12.52	15.55	1.174
III	17.40	0.439	17.06	22.92	1.694
IV	3.79	0.080	3.58	5.06	0.478

Similar pattern of annual nutrients return is also reported in restored rock phosphate mined area dominated by *Acacia catechu* and in natural forests characterized by the presence of *Spondias mangifera*, *Toona ciliata*, *Sapium insigne*, *Nyctanthes arbortristis*, *Bauhinia retusa* etc. (Negi, 1998). The pattern of annual nutrient return in the present study was also found to be similar to the various other natural and planted ecosystems. Seth *et al.* (1963) and Negi (1984) have reported a similar order of annual return of nutrients *i.e.* Ca > N > K > Mg > P in semi evergreen Sal forest. Vyas *et al.* (1976) reported same order for deciduous forests and Negi (1984) also found the same order of annual return of nutrients in evergreen Eucalypts. Singh (1975) studied the nutrients return through litter fall in *E. tereticornis* on a unit area basis and found that leaf litter contributed the major portion of total calcium, nitrogen and phosphorus. Gill *et al.* (1987) recorded mean annual concentration of nutrients in the litter of *E. tereticornis* followed the order Ca > N > Mg > K > P. Low phosphorus return through litter in all the study sites may be due to the presence of Calcerite nature of soil of limestone mined area which restricted the availability of phosphorus to the plant. The availability of phosphorus to plant is generally declined above pH of 7.5. Being predominance of CaCO₃ the study sites have pH more than 7.5. Similar results was also drawn by Tyagi (2002) who attributed its restricted availability in lime rich soils. This is in agreement with the findings of Garg (1997).

In the present study all the nutrients returned to the forest floor through litter were found to be increasing with the increase in age of the rehabilitated sites. Similar inference was drawn by Tyagi (2002) that all the nutrients returned were increased with the increase in age of plantation in sodic soils. As an exception, in the present study, the eight year old rehabilitated site accounted less return of nutrients than the six years old rehabilitated site. This anomalous behaviour may be because of differences in vegetation composition and resulting variation in litter production.

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Study on fish diversity and fish production of “Moghat Reservoir Khandwa” (M.P.)

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Abstract

Khandwa is one of the districts of the state M.P. The Moghat reservoir is situated three Kilometers away in the Northwest area of Khandwa town on 21° 49' 36" N latitude and 76° 20' 56" E longitudes. It is a man made reservoir built in 1897. The present study aims to identify the fish fauna presented in reservoir and to give an initial idea about fish production of Moghat reservoir of Khandwa M.P.

Key words: *Fish production, diversity, Reservoir, nutrition*

Introduction

The most important gift for mankind is water which plays a significant role in different vital and structural activities. Water is inevitable for all living organisms as it has a great social and economical value ultimately affecting mans health. It is essentially required for industrial development, fisheries, irrigation, hydro electrical generations and human life survival. Fishes are believed to be the oldest vertebrates and it is thought that all other vertebrates are evolved from them. They have adapted themselves to a wide range of environments. They are found in the icy waters of the Polar Regions on one hand, while they exist miraculously uncooked in the hot desert pools up to a temperature well above 100°F on the other hand. On the whole it can be said that where there is water there are fishes, and three fourth of the earth's surface is covered with water. The logical work in fish and fisheries is to have an exact knowledge of the position of fish fauna in animal kingdom. Proper identification of fishes is of great importance and many workers have done this job on different water bodies in India.

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Limnological studies on the Garhwal Himalayan Rivers were started in 1979 by the work of Badola (1979) who observed the Ichthyofauna of some fresh water resource of Garhwal region. Badola and Singh (1982) made a study on fish and fisheries of river Alakananda, they also studied the hydrobiology of the river of Garhwal Himalaya. Dobriyal and Singh (1981) investigated the diurnal variation in some aspect of limnology of river Mandakini from Garhwal Himalaya. Khanna and Badola (1993) observed Ichthyofauna of the river Ganga at the foothills of Garhwal Himalaya. Dynamics of limnology with respect to fish production were studied by various other scientists also. In view of inland fish production in the world, the position of India is next to China and Russia. The total fish production is 47.89 lakh tones contributed 20.97 lakh tones from inland and 26.92 lakh tones from marine sector respectively in the year 1994-95. Thus fish production from inland sector has great significance. It contributes the major share in the protein rich food for domestic consumption. Kumar (1995) studied pollution in river Mayurkashi; Rao *et al.* (1988) discussed environmental status and fish fauna of Gandhisagar reservoir (M.P.)

Materials and Method

Khandwa is one of the district of Madhya Pradesh which is famous for its historical and holly place Omkareshwar where one of the jyotirlinga of Lord Shiva is situated on the bank of river Narmada. On the other hand the Indirasagar dam is situated on the same river in Khandwa district.

The Moghat reservoir of Khandwa is situated 3 kilometer away in the North West area of the city on 21° 49' 36" N latitude, 76° 20' 56" E longitude and 324.4 meter above from mean sea level. It is a man made reservoir built in 1897 which receives rainwater through two main sources one is called Ajanti canal and other is a local nalaha called barud nalla, from its 23.30 sq. kms catchment's area. The storage area of Moghat reservoir is 2.02 sq.km., which has 5.36 kms shore line surrounded by hills with some large trees shrubs and agricultural land.

Moghat reservoir was initially manufactured to sort the water problem of local people of Khandwa Township but later on this water body is also used for fish production/culture. So far limited knowledge has been available on the fish fauna of Moghat reservoir, therefore the present study was undertaken to collect information regarding the availability and the production of fish fauna of Moghat reservoir at Khandwa. The present study revealed some important information regarding natural fish production at Moghat reservoir and its economics. Jaal, Triangular net, Scoop net, dragnets and Angling were used to collect the fishes, samples and data of production were collected every month during October 2005 to September 2007. After collection, fishes were preserved in 5 percent formalin and identified with the help of keys given by Jhingran (1975), Sehgal (1973), Badola (1979) and Day (1879). Two year data of fish production and the income of fishermen were assessed as per month fish catching and the rate of per kilogram of different species of fishes found in the reservoir according to fish market Khandwa.

Results and Discussion

The fish fauna of the Moghat reservoir was surveyed. As a result of this survey 18 species,

of 4 families have been reported, in which Catla catla showed its dominance among all. Nearly all fishes found in Moghat reservoir are edible and have been an important source of protein.

Table 1: List of Fishes found in Moghat Reservoir Khandwa, M.P. (India)

ORDER	- CYPRINIFORMES
DIVISION	- CYPRINI
FAMILY	- CYPRINIDAE
SUB FAMILY	- CYPRINAE

SCIENTIFIC NAME

<i>Catla catla</i>	Major carp
<i>Labeo rohita</i>	Major carp
<i>Labeo calbasu</i>	Major carp
<i>Labeo bata</i>	Major carp
<i>Labeo fimbriatus</i>	Major carp
<i>Labeo bogut</i>	
<i>Cirrhinus mrigla</i>	Major carp
<i>Cyprinus carpio</i>	

SUB FAMILY	- RASBORINAE
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Rasbora daniconius

DIVISION	- SILURI
FAMILY	- SACCOBRANCHIDAE

Heteropneustas fossilis

FAMILY	- BAGRIDAE
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Mystus vitatus

ORDER	- OPHIOCEPHALIFORMES
FAMILY	- OPHIOCEPHALIDAE

<i>Channa (Ophiocephalus) marulius</i>	
<i>Channa (Ophiocephalus) gachua</i>	
<i>Channa (Ophiocephalus) punctatus</i>	
<i>Channa (Ophiocephalus) striatus</i>	
<i>Ctenopharyngodon idilus</i>	(Grass carp)
<i>Hypothalamichthyes molitrix</i>	(Silver carp)



Today's ever-increasing population with decreasing cultivable land and increasing rate of unproductive land, the world is highly depending on non-vegetable sources of food including fishes which are one of the most important food source. All the fishes which were found in Moghat reservoir during the study period, have economic importance and food value, besides this these fishes have good market and nutritional value.

Natural fish production at Moghat reservoir and its economics

Total Fish production during study period (Oct.2005-Sep.2007) in this reservoir is presented in Table. 2. During course of study it was observed that maximum production is of *Catla*

(148.00 kg/ ha) and minimum production is of *Ophiocephalus punctatus* (12.00 kg/ha). The total income for 2 years will range between Rs. 59,758 - 69,670 and the annual income will range between Rs. 29,879 - 34835 Total income from fish production during study period is given in Table. 2.

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Table 2 Showing the Total Fish production (Oct.2005-Sep.2007) at Moghat Reservoir, Khandwa

S. No.	Fish Species	Total Quantity (kg/ha)	Rate/kg	Total Income (Rs.)
1	<i>Catla catla</i>	148.00	40-45	5920-6660
2	<i>Labeo rohita</i>	133.00	40-55	5320-7315
3	<i>Labeo calbasu</i>	123.00	35-45	4305-5535
4	<i>Labeo bata</i>	90.00	55-60	4950-5400
5	<i>Cirrhinus mrigla</i>	93.00	43-50	3999-4650
6	<i>Labeo fimbriatus</i>	40.00	70-85	2800-3400
7	<i>Cyprinus carpio</i>	35.00	65-70	2275-2450
8	<i>Cyprinocarpus communis</i>	29.00	65-70	1885-2030
9	<i>Labeo boga</i>	80.00	45-55	3600-4400
10	<i>Rasbora daniconius</i>	22.00	70-85	1694-1870
11	<i>Heteropneustas fossilis</i>	38.00	80-100	3040-3800
12	<i>Mystus vitatus</i>	49.00	65-70	3185-3430
13	<i>Ophiocephalus marulius</i>	37.00	45-55	1665-2035
14	<i>Ophiocephalus gachua</i>	29.00	65-70	1885-2030
15	<i>Ophiocephalus punctatus</i>	12.00	55-70	660-840
16	<i>Ophiocephalus striatus</i>	15.00	65-75	975-1125
17	<i>Ctenopharyngodon idilus</i>	80.00	75-80	6000-6400
18	<i>Hypothalamicthyes molitrix</i>	70.00	80-90	5600-6300
Total Income for 2 years				59,758 - 69,670
Annual Income				29,879 - 34835



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Effect of insect growth regulators on the development of *Helicoverpa armigera*

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Abstract

In laboratory study various developmental changes were exhibited by *Helicoverpa armigera* by the treatment of different insect growth regulators (IGRs) i.e. lufenuron, novaluron and UPI-106. Gram pod borer, *H. armigera* is a dreaded enemy of chick pea, pigeon pea, cotton, tomato and many other economically important crops. In the study, the efforts were concentrated in seeking effective toxicant for managing *H. armigera* population. IGRs are newer chemicals having less pollution effect on environment. Lufenuron, novaluron and UPI-106 are chitin synthesis inhibitor (CSI) belonging to acyl urea group. Lufenuron effectively suppressed *H. armigera* populations resulting in significant reduction in crop damage (Gogi, *et al.* (2006)). The insect growth regulators are much more effective than conventional insecticides. IGR inhibits the production of chitin in larval forms and affects the life cycle of *H. armigera*. The different criteria studied were length of body, weight of body, width of body and width of head capsule. Among the different concentration of three IGRs used, the most effective IGR was the treatment of UPI-106 (0.08% conc.) while the least effective was Novaluron (0.025% conc.).

Keywords: *H. armigera*, IGRs, lufenuron, novaluron, UPI-106, chitin synthesis inhibitors

Introduction

The basic need of human is air, water and food. This food is obtained from plants. In India various types of crops like wheat, rice, maize. Sorghum, millet and pulses etc. are grown. Among them pulses hold a prominent place due to losses caused by insect pest. The most damaging insect of gram in our country is *H. armigera*. It is a polyphagous insect and beside pulses found on many economically important crops like tomato, potato, cotton, sunflower, groundnut etc. To control *Harmigera* various techniques has been employed and lots of money is expensed every year. In such cases insect growth regulators are better option due to its

characteristics like they are less toxic to man and domestic animals. They do not persist or accumulate in the environment and are degraded to simple molecules that are unlikely to cause problems of environmental contamination. The insect growth regulators, studies conducted so far, indicate that IGRs are comparatively safer to natural enemies. IGRs are active at very low concentration and effective against target insects. Insect Growth Regulators are also called "Third Generation Insecticides". The use of IGRs is being increased for controlling various insect pest of agriculture, horticulture, stored product and public health pest. The study is therefore, aimed to test the efficacy of IGRs against caterpillar of *H. armigera* so that their population can be managed.

Materials and Method

The larvae of *H. armigera* were collected from the crop field of chick pea. Selection of healthy

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and active larvae was done and transferred into petridishes singly. Incubation of these larvae were done at $25 \pm 20^\circ\text{C}$ with in 2-3 days pupation cell was formed by larvae and pupa were formed. The identified pupae were transferred to rearing jars in ratio 2:4 where 2 male and 4 female were taken. After emergence of moth from pupa, the process of egg laying took place where 200-800 eggs were laid in each rearing jar. After hatching larvae were transferred to semi-synthetic diet. When these larvae reached third instar the tests were conducted on them. The IGRs were obtained as sample from manufacturers and were diluted with ordinary tap water to obtain required concentrations. The application of insect growth regulators was done with the help of micropipette. Treatment dose was prepared by applying following formula

$$\frac{\text{Volume required in ml}}{\text{Strength of formulation}} \times \text{Concentration required in Percent}$$

To prepare 10 ml UPI 106 10% (500 mg/ml) stock solution in Water, the formula is

$$\frac{100}{10} \times 0.4$$

Different concentrations of each IGRs were tested and control were applied with tap water. Every treatment was repeated thrice and averages used for the estimation of Median Lethal Concentrations. Topical application of IGRs was done on insects and allowed to crawl for 1 hour. Observations taken after 24 hour of treatment as -

Living – which could walk normally

Slightly affected (Paralysed) – the trembling ones.

Moribund - showing no other movement except slight movement of head after touching them with brush.

Dead - showing no movement even when repeatedly touched with brush

Throughout the experiment the moribund insects were considered dead. Living and slightly affected were classed as living. The mortality thus obtained was corrected for the mortality observed in the control as per formula given as follows.

$$P = \frac{f - c}{100 - c} \times 100$$

Where,

p = corrected mortality

f = percent kill in treatment

c = percent kill in control

Table.1: Strength of IGR to tested

S.No	Name of IGR	Dose gm.ai/ha	Formulation Dose ml/ha.	% Con C	Volume Of Stock	Volume Of Buffer /Water	Total
					4 ml	16 ml	20
1	UPI 106 10 EC	30	600	0.04	0.4	19.60	20
2	UPI 106 10 EC	50	1000	0.08	0.8	19.92	20
3	UPI 106 10 EC	70	1400	0.16	0.16	19.984	20
4	NOVALURON 10 EC	75	750		0.025	19.975	20
5	LUFENURON 5.4 EC	30	600		0.08	19.980	20
6	LUFENURON 5.4 EC	60	1200		0.016	19.971	20



Results and Discussion

After 24 hours the maximum mortality percent is found by the treatment of UPI-106 (0.08). The least mortality percent (zero) is found by the treatment of UPI-106 (0.016). The above results thus indicate that UPI-106 is the most effective insect growth regulators among all other growth regulators used in experiment. Similar observation have also been reported by Butter *et*

al. (2003), Iain (2007), Kranthi *et al.* (2002), Ma *et al.* (2000), Rao *et al.* (1990) and Whiting *et al.* (2000).

Effect of IGRs was seen on the length, weight, width of body and width head capsule after 24, 48, 72, 96, 120 and 144 hours. The average of all the hours was taken and overall impact was shown in Table. 3.

Table. 2: Treatment of UPI

S.No.	Treatment (%Conc.)	Dead	Moribond	Slightly Affected	Alive	Total	% Mortal
1	UPI – 106 (0.04)	1	1	1	2	5	40
2	UPI – 106 (0.08)	1	2	0	2	5	60
3	UPI – 106 (0.016)	0	0	2	3	5	0
4	NOVALURON (0.025)	0	1	1	3	5	20
5	LUFENURON (0.08)	0	2	1	2	5	40
6	LUFENURON (0.016)	0	1	1	3	5	20
7	CONTROL	0	0	0	5	5	0

Table:3 Average overall percent

S.No.	Treatments	Overall Average Percent			
		Length	Weight	Width	Width Of Head Capsule
1	UPI – 106 (0.04)	96.52	98.69	120.83	128.45
2	UPI – 106 (0.08)	96.42	95.26	126.38	130.89
3	UPI – 106 (0.016)	99.20	99.52	108.33	108.13
4	NOVALURON (0.025)	99.12	99.52	111.11	116.26
5	LUFENURON (0.08)	97.43	98.93	116.66	121.95
6	LUFENURON (0.016)	99.05	99.58	115.27	117.88
7	CONTROL	100	100	100	100

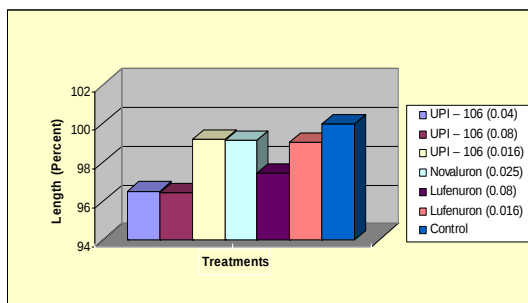


Fig.1. Effect of growth regulators on length of larvae (average percent)

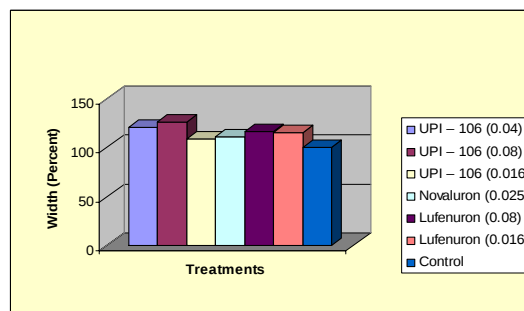


Fig. 3. Effect of growth regulators on the width head capsule of larvae (average percent)

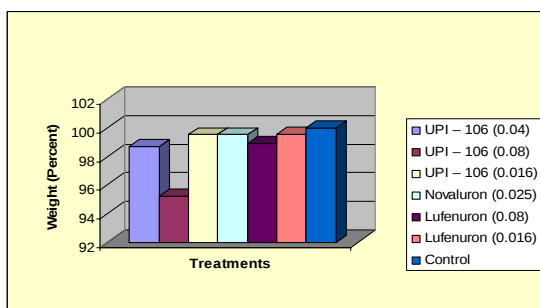


Fig. 2 Effect of growth regulators on weight of larvae (average percent)

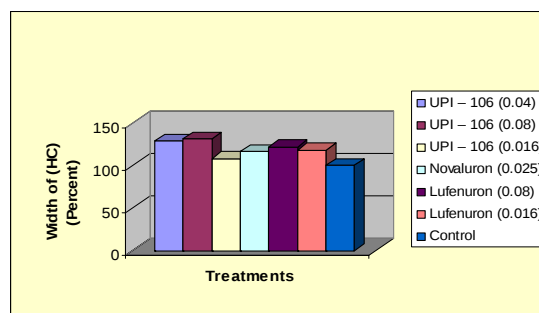


Fig. 4. Effect of insect growth regulators on the width of larvae (average percent)

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Oxidative stress mitigation studies in two pulse crops

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Abstract

Sulphur dioxide is well studied phytotoxic gaseous pollutant. It is one of the main oxidative gas, which is known for the production of various free oxyradicals during its oxidation from unstable form sulphite (SO_3^-) to stable form sulphate (SO_4^-) within the life. The oxyradical are initiated by light and mediated by photosynthesis electron transport chain. The generated free radicals react and cause oxidative damage to various biological molecules and cell organelles.

The mechanism of plant tolerance to air pollutants exposure is probably biological rather than biophysical. Air pollutant have been shown to affect the level of defense of enzymes as well as than of antioxidant biomolecules present within the plant cells. Protection of sensitive plant species against the oxidative stress may be achieved through various means such as coating the leaf surface and providing physical and/or chemical protection, through alteration of plant metabolism etc.

In the present study, an attempt is made to infuse scavenging potential exogenously in cultivars of two pulse crops namely Lentil (*Lens culinaris* L medic) and Mung bean (*Vigna radiate* L.) using certain antioxidants (α – Tocopherol, ascorbic acid and diphenyl amine). Seeds of both the crops and their selected susceptible cultivars were invigorated exogenously with different antioxidant using dry permeation technique. The plantlets generated were subjected to two different SO_2 concentration (655 and 2620 g/m^3) in open top chambers (OCT) and were evaluated for their response through certain physiological and biochemical parameters. Cultivars JM-721 (Mung bean) and SLC-2 (Lentil) appeared to be slightly tolerant than the other respective susceptible cultivars studied (MI-24-91 and Sehore 84 -8). The study trend in general suggests that lower SO_2 concentration was slightly beneficial to both the cultivars of both the crops. All the three antioxidant treatment were comparatively effective in most of the parameters. The treatment affectively however differed for the two cultivars of the same crop. Diphenylamine appeared to be promising in most of the parameters, however most effective was α – Tocopherol followed ascorbic acid.

Keywords : *Oxidative stress, Seed invigoration treatment, Dry Weight, α -Tocopherol, ascorbic acid, Sulphur dioxide Protein*

Introduction

Presence of oxygen in the aerobic cellular environment is necessary for aerobic metabolism. This oxygen status poses a constant oxidative threat to cellular structure and different processes. An inevitable result of chloroplast, mitochondrial and plasma membrane linked electron transport is the leaking of electron on to molecular oxygen in plant cells, with the resultant production of reactive, toxic oxygen species or ROS (Rubinstein and Luster, 1993; Asada, 1992; Fridovich, 1995).

The imposition of biotic and abiotic stress both

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can give rise to further increase in ROS levels (Alscher and Hess, 1993; Foyer and Mullineaux, 1994; Dangl *et al.* 1996). Sulphur dioxide is one of most concerned gaseous pollutants since it is potentially a strong free oxyradical generator with in the plant system. According to Halliwell, 1984 there is some kind of enzymatic and/or non-enzymatic antioxidant mechanism, which present within every organism to prevent oxidation of various cellular components. Each and every plant cell has its own capability of self-defense of antioxidant mechanism to cope with the danger posed by presence of ROS by maintaining high redox potential of glutathione reductase (GR) activities. It seems that the ability of a plant to synthesize and maintain reduced glutathione and

ascorbate may govern its tolerance towards stresses.

Plant cell have embraced the potential of interaction with oxygen for metabolic regulation, (Foyer, 1997). Active oxygen species (AOS) are important metabolites, participating in the metabolism, growth and development of the plant cell. But as the production of this stimulated by environmental stress and since most AOS are highly reactive and lead to perturbation in enzyme activities and membrane damage. They are not compatible with cell function. Also upon further reaction within the cell, can form ROS such as hydroxyl radicals and singlet oxygen, which again frequently are considered to be deleterious and harmful. The study state level of AOS in the cell is determined by the activity of the antioxidant system (Asada 1992, Foyer *et al.*, 1994a) in all situations between AOS the formation consumption is highly controlled. Augmentation of antioxidant defense plays a pivotal role in preventing oxidative stress in plants, (Foyer *et al.*, 1994b; Allen, 1995). Plant's survival against toxic oxygen species depends on the variety of small molecules grouped under the general heading of antioxidants. The scavenging/detoxifying potential of each plant species varies from the other. It mostly involves reduction of harmful oxidizing radicals through protection of antioxidant molecules or stimulation enzymes responsible for enhanced production of antioxidants with in the plant system (Rao, 1990; Kumawat, 1990). The inherent scavenging potential can be boosted by exogenous application/infusion of certain antioxidant molecules (Malviya, 1986; Jain, 1993). In the present study an attempt has been made to ameliorate SO₂ toxicity in two pulse crops namely Lentil and Mung bean through exogenous infusion of naturally occurring antioxidant like diphenylamine (DA). A comparison between the actions of naturally occurring and synthetically available antioxidant was also made during the study.

Materials and Method

Plant Material: Two common pulse crops namely Mung bean and Lentil were taken for the present

study. Seeds of the different cultivars of both the crop were obtained from Government Agriculture College Indore (M.P.) and Rafi Ahmed Kidwai College of Agriculture, Sehore (M.P.) respectively. Two sensitive cultivars each of the two crops were taken for the study. They were; Mung bean: cultivars JM-721 and MI 24-91. Lentil: cultivars SLC -2 and Sehore 84-8.

Experimental setup

Ten healthy seeds of each cultivar of both the crops in their respective growing seasons (Kharif-Mung bean and Rabi-Lentil) were sown in earthen pots separately containing 3kg black cotton soil (clay loam). After 10 days of germination and the plant growth, thinning was carried out and 4 to 5 plantlets were allowed to grow further in each pot. After one month of normal growth the plants were subjected to two concentration of SO₂ (655 and 2620 g/m³) and a control set was also run simultaneously. Each set was run with three replicates.

SO₂ generation and treatment

SO₂ was generated by bubbling dry air into aqueous solution of Sodium Metabisulphite following Sharma and Thakre (1984). Earthen pots (3 replicates) of each cultivar of both the crops were placed separately in fabricated open top polythene chambers (IX IXI m.) for fumigation and SO₂ was supplied into fumigation chamber through Teflon tubes. The desired concentration of SO₂ in the chamber was maintained and checked at various intervals of the study using toxic gas monitor (TGM-555 CEA, USA). Treatment was carried out at the rate of 6 h/day (7 to 10 AM and 4 to 7 PM) for one month. Control set for all cultivars were also run simultaneously. All the past were irrigated timely as required.

Response assay

After one month of SO₂ treatment, different cultivars of both the crops were evaluated for their response against SO₂ toxicity though certain physiological and biochemical parameters following standard procedures as listed below:

1. Stomatal conductance (SC) using steady state porometer Li cor. (1600), USA.
2. Total foliar protein content, following Lowry *et al.*, (1951).
3. Photosynthetic activity using Li cor (6200), USA.



4. Dry weight production study (D.WT.).
5. Seed Invigoration treatment, following dadlani and Agrawal, (1986).

Statistical analysis

The significance of the difference among means was evaluated following Duncan's Multiple Range Test (DMRT). Any value below 95% was rejected.

Results and Discussion

Porometric study stomatal conductance (SC)

All the three antioxidant decrease the SC in case of Mung bean while in lentil an increase was observed, suggesting that an inherent difference

exist between the two crops. DA appeared to be better for cultivar JM -721 of Mung bean even though α -tocopherol and DA were better for other cultivars of lentil and Mung bean. Anova suggest that variable SO_2 in Mung bean was highly significant ($p < 0.01$), whereas variables, antioxidant and variety were significant ($p < 0.05$). In case of Lentil only variable SO_2 was significant. A similar trend was observed in case of transpiration rate in both the crops. Both the porometric parameters do not suggest any regular trend, only a mixed trend of increase and decrease was observed in different cultivars (Table.1-4).

Table-1. Stomatal conductance (cm/s) of two cultivars of Mung bean treated with different SO_2 concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO_2 Concentrations ($\mu g/m^3$)		
		0.00	655.00	2620.00
Only Acetone	JM 721	1.10 $\pm$ 0.56	1.13 $\pm$ 0.56	0.92 $\pm$ 0.47
	MI-24-91	1.06 $\pm$ 0.53	1.16 $\pm$ 0.58	0.83 $\pm$ 0.42
Tocopherol	JM 721	1.19 $\pm$ 0.60	1.34 $\pm$ 0.67	1.08 $\pm$ 0.54
	MI-24-91	1.11 $\pm$ 0.56	1.19 $\pm$ 0.60	0.99 $\pm$ 0.49
Ascorbic Acid	JM 721	1.12 $\pm$ 0.56	1.23 $\pm$ 0.61	0.96 $\pm$ 0.49
	MI-24-91	1.09 $\pm$ 0.54	1.32 $\pm$ 0.66	0.95 $\pm$ 0.48
Diphenyl amine	JM 721	1.06 $\pm$ 0.53	1.24 $\pm$ 0.62	0.98 $\pm$ 0.50
	MI-24-91	1.02 $\pm$ 0.25	1.20 $\pm$ 0.60	0.89 $\pm$ 0.46

Table-2 Analysis of Variance (Split- split-plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.021	0.0107			
SO ₂ (A)	2	0.95	0.4751	94.48**	6.94	18.00
Error (A)	4	0.02	0.005			
Sub Plot Analysis						
Antioxidants (B)	3	0.147	0.0491	4.32*	3.16	5.09
A×B	6	0.037	0.0062	0.54	2.66	4.09
Error (B)	18	0.205	0.0114			
Sub Sub Plot Analysis						
Variety (C)	1	0.39	0.392	5.99*	4.26	7.82
A×C	2	0.008	0.0042	0.64	3.40	5.61
B×C	3	0.035	0.0118	1.81	3.01	4.72
A×B×C	6	0.21	0.0035	0.54	2.51	3.67
Error(C)	24	0.157	0.0065			
Total	71	1.642				
CV(a) = 6.5% : CV(b)= 9.8% : CV(c)=7.4%						

CV(a) = 6.5% ; CV(b)= 9.8% ; CV(c)=7.4%



Table-3- Stomatal conductance (cm/s) of two cultivars of lentil treated with different SO₂ concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO ₂ Concentrations (µg/m ³)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	0.75±0.83	0.74±0.37	0.68±0.34
	Sehore 84-8	0.77±0.39	0.76±0.37	0.71±0.35
α -Tocopherol	SLC-2	0.80±0.41	0.80±0.39	0.70±0.35
	Sehore 84-8	0.77±0.41	0.76±0.38	0.69±0.35
Ascorbic Acid	SLC-2	0.77±0.40	0.77±0.38	0.67±0.33
	Sehore 84-8	0.82±0.42	0.80±0.40	0.74±0.37
Diphenyl amine	SLC-2	0.77±0.39	0.77±0.39	0.67±0.67
	Sehore 84-8	0.82±0.41	0.82±0.41	0.74±0.38

Table-4 Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Som of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.004	0.0022			
SO ₂ (A)	2	0.023	0.0058	8.73*	6.94	18.00
Error (A)	4	0.023	0.0058			
Sub Plot Analysis						
Antioxidants(B)	3	0.011	0.0038	0.82	3.16	5.09
A×B	6	0.002	0.0003	0.07	2.66	4.01
Error (B)	18	0.084	0.0047			
Sub Sub Plot Analysis						
Variety (C)	1	0.013	0.0133	1.78	4.26	7.82
A×C	2	0.002	0.0002	0.11	3.40	5.61
B×C	3	0.019	0.0064	0.86	3.01	4.72
A×B×C	6	0	0	0	2.51	3.67
Error(C)	24	0.18	0.0075			
Total	71	0.44				
CV(a)= 10.0% ; CV(b)= 9.0% ; CV(c)=11.5%						

The Stomatal response to environmental change is important in controlling the observation of pollutants by plant. The reduced stomata aperture resists the entry of pollutant thus preventing their adverse effects on plants (Verma, 2006).

Total foliar protein content

The low SO₂ concentration showed a slightly beneficial effect in Mung bean while the higher SO₂ concentration was deleterious to both the

cultivars of each crop (Table. 5-6). The three antioxidant used, appeared to be slightly effective. According to Anova study in Mung bean, variables SO₂ and variety were highly significant and variety were significant (p > 0.05).

Photosynthetic activity

Lower SO₂ concentration appeared to be beneficial to both the crops but higher concentration resulted in a significant decrease in the photosynthetic



activity. The decrease was more in susceptible cultivars i.e. MI 24-91 of Mung bean (Table .11) and Sehore 84 -8 of Lentil (Table .12). All the three Antioxidant were significantly effective in reducing the harmful effects of SO₂. Tocopherol and AA were slightly better than DA (Table. 11 - 12). The Anova analysis suggest that in Mung bean the SO₂, variety and antioxidant variables along with antioxidant X variety interactions were highly significant but in Lentil only variable SO₂ was significant.

Dry weight study (D. Wt.)

The overall metabolic state of a plant can be judged by its dry matter accumulation over a period of time, In the present study after SO₂ exposure, the dry matter in terms of dry weight of whole plant showed a slight increase at low SO₂ concentration in both cultivars of each crops, but a reverse trend i.e. decrease in dry weight was observed at higher SO₂ concentration (Table. 15-16). The three antioxidants that were infused in the seeds appeared to be effective and resulted in reducing the decreasing in dry weight that was

Table-5- Total protein content (mg/g. D.wt) of two cultivars of Mungbean treated with different SO₂ concentrations and plant protectants using dry permeation method

Treatments	Varieties	SO ₂ Concentrations (µg/m ³)		
		0.00	655.00	2620.00
Only Acetone	JM 721	47.04±0.90	48.26±0.56	36.33±3.31
	MI-24-91	42.91±2.92	41.88±0.43	29.77±12.76
α-Tocopherol	JM 721	47.54±2.03	48.16±0.11	41.01±2.81
	MI-24-91	43.32±1.81	43.50±1.99	34.98±2.43
Ascorbic Acid	JM 721	47.08±4.16	48.36±0.25	40.25±0.18
	MI-24-91	43.74±2.41	44.55±0.13	32.87±0.77
Diphenyl amine	JM 721	47.43±2.03	48.26±0.49	40.72±0.40
	MI-24-91	43.20±2.72	43.76±1.72	32.34±0.02

Table. 6: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	16.19	8.0951			
SO ₂ (A)	2	1457.284	728.6419	133.79**	6.94	18.00
Error (A)	4	21.784	5.446			
Sub Plot Analysis						
Antioxidants(B)	3	45.857	15.2856	1.32	3.16	5.09
A×B	6	40.518	6.753	0.58	2.66	4.01
Error (B)	18	207.922	11.5512			
Sub Sub Plot Analysis						
Variety (C)	1	505.773	505.7734	48.56**	4.26	7.82
A×C	2	30.956	15.4779	1.49	3.40	5.61
B×C	3	2.977	0.9922	0.1	3.01	4.72
A×B×C	6	7.95	1.3251	0.13	2.51	3.67
Error(C)	24	249.948	10.416			
Total	71	2587.195				
CV(a)=5.5%; CV(b)=8.0%; CV(c)=7.6%						



recorded at higher SO₂ treatment (Table. 15-16). The three antioxidants appeared to be equally effective though there was a slight difference between the three and naturally occurring antioxidant α -Tocopherol and AA had a marginal

edge over synthetic protectant DA. The Anova table reveals that SO₂ and variety were highly significant in case of Lentil while only SO₂ variable was highly significant in Mung bean rest other interaction were not very significant.

Table.7: Total (mg/q. D.wt.) of two cultivars of lentil treated with different SO₂ concentration and plant protect ants using dry permeation method

Treatments	Varieties	SO ₂ Concentrations ($\mu\text{g}/\text{m}^3$)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	34.87 $\pm$ 7.79	32.73 $\pm$ 0.20	27.69 $\pm$ 0.45
	Sehore 84-8	33-40 $\pm$ 7.53	32.17 $\pm$ 0.40	24.00 $\pm$ 0.91
α -Tocopherol	SLC-2	39.33 $\pm$ 4.49	37.52 $\pm$ 1.52	34.05 $\pm$ 1.05
	Sehore 84-8	35.83 $\pm$ 6.92	34.79 $\pm$ 1.44	31.60 $\pm$ 1.56
Ascorbic Acid	SLC-2	38.36 $\pm$ 2.10	36.66 $\pm$ 0.60	33.48 $\pm$ 1.51
	Sehore 84-8	31.70 $\pm$ 4.70	30.97 $\pm$ 1.05	26.89 $\pm$ 1.37
Diphenyl amine	SLC-2	35.93 $\pm$ 2.73	34.09 $\pm$ 0.54	29.52 $\pm$ 0.55
	Sehore 84-8	37.52 $\pm$ 14.22	36.41 $\pm$ 0.15	29.80 $\pm$ 0.58

Table. 8: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	11.68	5.8398	37.51**	6.94	18.00
SO ₂ (A)	2	531.398	265.6992			
Error (A)	4	28.336	7.084			
Sub Plot Analysis						
Antioxidants(B)	3	203.27	67.7567	3.23*	3.16	5.09
A×B	6	36.345	6.0574	0.29	2.66	4.01
Error (B)	18	377.464	20.9702			
Sub Sub Plot Analysis						
Variety (C)	1	98.596	98.5964	5.08*	4.26	7.82
A×C	2	4.466	2.2331	0.12	3.40	5.61
B×C	3	147.827	49.2758	2.54	3.01	4.72
A×B×C	6	5.709	0.9515	3.01	2.51	3.67
Error(C)	24	465.982	19.4138			
Total	71	1911.023				
CV(a)= 8.0%; CV(b)=13.8%; CV(c)=13.2%						



Table-9 SH (mole-SH/l. extract) of two cultivars of Mung bean treated with different SO₂ concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO ₂ Concentrations (µg/m ³)		
		0.00	655.00	2620.00
Only Acetone	JM721	0.57±0.16	0.62±0.03	0.93±0.04
	MI-24-91	0.62±0.09	0.68±0.08	1.04±0.07
α -Tocopherol	JM 721	0.58±0.19	0.60±0.05	0.68±0.01
	MI-24-91	0.63±0.22	0.68±0.05	0.87±0.07
Ascorbic Acid	JM 721	0.59±0.11	0.58±0.04	0.70±0.04
	MI-24-91	0.68±0.09	0.74±0.02	0.90±0.04
Diphenyl amine	JM 721	0.62±0.15	0.65±0.03	0.75±0.07
	MI-24-91	0.62±0.09	0.67±0.02	0.84±0.12

Table. 10: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.004	0.002	56.92**		
SO ₂ (A)	2	0.68	0.3402			
Error (A)	4	0.024	0.006			
Sub Plot Analysis						
Antioxidants(B)	3	0.049	0.0161			
A×B	6	0.133	0.0222	2.96		
Error (B)	18	0.099	0.0055	4.05**		
Sub Sub Plot Analysis						
Variety (C)	1	0.156	0.1559	10.85**	4.26	7.82
A×C	2	0.036	0.0179	1.25	3.40	5.61
B×C	3	0.032	0.0107	0.74	3.01	4.72
A×B×C	6	0.004	0.007	0.05	2.51	3.67
Error(C)	24	0.345	0.0144			
Total	71	1.562				
CV(a)=8.0 %; CV(b)=13.8 %; CV(c)=13.2 %						

Table-11 SH (mole-SH/l. extract) of two cultivars of lentil treated with different SO₂ concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO ₂ Concentrations (µg/m ³)		
		0.00	655.00	2620.00
Only Acetone	SLC-2	0.57±0.16	0.65±0.03	0.93±0.03
	Sehore 84-8	0.62±0.09	0.74±0.02	1.99±0.10
α -Tocopherol	SLC-2	0.58±0.19	0.63±0.03	0.81±0.05
	Sehore 84-8	0.63±0.22	0.71±0.05	0.98±0.13
Ascorbic Acid	SLC-2	0.59±0.11	0.64±0.05	0.80±0.04
	Sehore 84-8	0.68±0.09	0.60±0.02	1.04±0.16
Diphenyl amine	SLC-2	0.63±0.15	0.68±0.04	0.93±0.02
	Sehore 84-8	0.62±0.09	0.70±0.03	0.98±0.06



Table. 12: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	0.027	0.0133			
SO ₂ (A)	2	1.538	0.7691	64.82**	6.94	18.00
Error (A)	4	0.047	0.0119			
Sub Plot Analysis						
Antioxidants(B)	3	0.027	0.000	1.75	3.16	5.09
A×B	6	0.05	0.0084	1.63	2.66	4.01
Error (B)	18	0.092	0.0051			
Sub Sub Plot Analysis						
Variety (C)	1	0.117	0.1168	6.47*	4.26	7.82
A×C	2	0.056	0.0279	1.55	3.40	5.61
B×C	3	0.021	0.0069	0.38	3.01	4.72
A×B×C	6	0.029	0.0048	0.26	2.51	3.67
Error(C)	24	0.433	0.018			
Total	71	2.437				
CV(a)=15.4 %; CV(b)=23.2 %; CV(c)=19.4 %						

α-Tocopherol content

α-tocopherol (Vitamin E) probably is the most important antioxidant that is incorporated into the lipid membrane of the cells (Diplock, 1983). Chloroplast contain large amount of α - tocopherol to protect the membrane against oxidative damage, since most of the fatty acids of chloroplast lipids are unsaturated C₁₈ fatty acid (Halliwell, 1984).

It not only, protects against oxygen radicals that might initiate lipid peroxidation of cell membranes, but can also serve as a scavenger of chain propagating free radicals such as lipid peroxy radical (Niki *et al.*, 1984). It does so by donating a hydrogen atom to the lipid peroxy or lipoxyl radical, formatting the corresponding peroxide or alcohol respectively thereby breaking the propagating chain reaction (McCay, 1985). α - tocopherol is metabolized to the α - tocopherol radical, which can either be further oxidized to form the α tocopherol quinone (Boguth and Niemann, 1971) or dimmer (Csallany, 1971) or be reduced to generate α - tocopherol (Pascoe and Reed, 1989). Ascorbate and GSH in combination with α - tocopherol can result in synergistic inhibition of oxidative damage to cell membranes (Niki, 1987), presumably through the regeneration of α - tocopherol. According to Liebler *et al.*, (1986) in bilayer membranes lipid peroxidation

was controlled by the ratio of α - tocopherol and ascorbate. In the present study though the initial α - tocopherol content in all the four cultivars of both the crops respectively had marginal differences among themselves (Table 17-18). However the dry permeation treatment was carried out only in the susceptible cultivars of the crops, Mung bean (JM-721 and M124-91) and Lentil (SLC-2 and Sehore 84-8). Interestingly, with hardly 2 to 3 μg infusion of α-tocopherol through dry permeation could induce reasonably a good amount of tolerance potential in the susceptible test cultivars of both the crops. Though at the end of experiment, in newly set seeds the α - tocopherol content was almost exposed leaves could cause an imbalance between ascorbic acid and dehydro- ascorbic acid resulting in poisoning of specific enzymes and sulphonation of their -SH group.

In the present study the ascorbic acid content initially in seeds of all the cultivars of both the crops was marginally different (Table 17-18). But in the selected susceptible cultivars of both crops Mung bean and Lentil, following dry permeation, an increase in ascorbic acid content in the range of 2 to 3 μg was recorded. The trend of foliar increase at different intervals (30 and 45 days) Was



almost same as that was found in α -tocopherol. Also the ascorbic acid content in the newly set seed was almost same as was in the start of the study. Ascorbic acid/ascorbate is perhaps the most important antioxidant in the plants, with fundamental role in the removal of H_2O_2 (foyer,1993). Oxidation of ascorbate occurs in two sequential steps, first producing mono-dehydro-ascorbate and if not rapidly re-reduced to ascorbate, the mono-dehydro-ascorbate disproportionates to ascorbate

and dehydro-ascorbate (Asada, 1994). Ascorbate is not only a potent antioxidant, but is implicated in the pH-mediated modulation of P.S.II activity and its down-regulation associated with zeaxanthin formation (Naubauer and Yamamoto, 1992), which is a potent mechanism for preventing photo-oxidation. In the present study, may be the initial increase in ascorbic acid might have added to the inherent antioxidant at least in the juvenile stages of the corps.

Table.13: Photosynthetic rate (μ mol/m²/s) of two cultivars of Mung treated with different SO₂ concentrations and plant protectants using dry permeation method.

Treatments	Varieties	SO ₂ Concentrations (μ g/m ³)		
		0.00	655.00	2620.00
Only Acetone	JM 721	26.31 $\pm$ 0.78	26.95 $\pm$ 0.78	20.94 $\pm$ 0.65
	MI-24-91	23.38 $\pm$ 0.22	24.06 $\pm$ 0.27	17.25 $\pm$ 0.84
α -Tocopherol	JM 721	27.07 $\pm$ 1.24	27.94 $\pm$ 1.25	22.85 $\pm$ 0.60
	MI-24-91	24.73 $\pm$ 1.28	25.40 $\pm$ 1.58	18.95 $\pm$ 1.04
Ascorbic Acid	JM 721	26.70 $\pm$ 1.43	27.46 $\pm$ 1.15	22.06 $\pm$ 1.53
	MI-24-91	22.62 $\pm$ 1.00	23.21 $\pm$ 1.05	17.46 $\pm$ 0.68
Diphenyl amine	JM 721	27.75 $\pm$ 1.38	28.50 $\pm$ 1.71	23.12 $\pm$ 0.66
	MI-24-91	23.45 $\pm$ 1.55	24.09 $\pm$ 0.89	18.25 $\pm$ 0.18

Table. 14: Analysis of Variance (Split Split-Plot Design)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F- Value	Tabular F-Value	
					5%	1%
Main Plot Analysis						
Replication	2	7.089	3.5443			
SO ₂ (A)	2	529.971	264.9857	90.52**	6.94	18.00
Error (A)	4	11.7	2.9274			
Sub Plot Analysis						
Antioxidants(B)	3	40.611	13.537	7.33*	3.16	5.09
A×B	6	8.678	1.4463	0.78	2.66	4.01
Error (B)	18	33.242	1.8468			
Sub Sub Plot Analysis						
Variety (C)	1	218.326	218.3264	186.84**	4.26	7.82
A×C	2	6.757	3.3785	2.89	3.40	5.61
B×C	3	19.316	6.4387	5.51**	3.01	4.72
A×B×C	6	9.017	1.5029	1.29	2.51	3.67
Error(C)	24	26.044	1.1685			
Total	71	912.762				
CV(a)=8.0 %; CV(b)=13.8 %; CV(c)=13.2 %						



Table.15: Photosynthetic rate ($\mu\text{ mol/m}^2/\text{s}$) of two cultivars of Mung treated with different

Treatment	Varieties	SO ₂ Concentrations		
		0.00	655.00	2620.00
Only Acetone	SLC-2	13.31 2.73	13.53 0.80	10.04 0.25
	Sehore 84-8	13.41 2.33	13.57 1.13	9.49 0.55
α -Tocophenol	SCL-2	12.71 0.55	12.81 1.36	10.52 1.52
	Sehore 84-8	13.14 2.13	13.27 0.23	10.41 0.13
Ascorbic Acid	SLC-2	13.53 1.05	13.77 0.55	11.12 0.43
	Sehore 84-8	13.53 1.32	13.63 0.53	10.60 0.63
Diphenyl amine	SLC-2	13.41 1.83	13.69 0.17	10.82 0.75
	Sehore 84-8	12.26 1.69	12.36 0.16	5.99 0.61

SO₂ concentrations and plant protectants using dry permeation method

Table. 16: Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F -values	
					5 %	1 %
Main plot analysis						
Replication	2	0.054	0.027			
SO ₂ (A)	2	142.872	71.4359		66.96	6.94
Error (A)	4	4.268	1.0669			
Sub plot analysis						
Antioxidants (B)	3	4.762	15.875	1.35	3.36	5.09
A× B	6	5.688	0.978	0.83	2.66	4.01
Error (B)	18	21.21	1.1783			
Sub plot analysis						
Variety (C)	1	2.538	2.5375	1.84	4.26	7.82
A×C	2	1.203	0.6017	0.44	3.40	5.61
B×C	3	4.96	1.6533	1.2	3.01	4.72
A×B×C	6	1.018	0.1697	0.12	2.51	3.67
Error (C)	24	33.149	1.3812			
Total	71	221.901				

cv (a) = 15.40 %; cv (b) = 23.20 %; cv (c) = 19.40 %

Table.17: Dry weight (g) of two cultivars of Mung bean treated with different SO₂ concentrations and plant protectants using dry permeation method

Treatment	Varieties	SO ₂ Concentrations		
		0.00	655.00	2620.00
Only Acetone	JM 721	2.64 0.40	2.76 0.06	2.16 0.06
	MI- 24-91	2.61 0.57	2.75 0.06	2.05 0.09
α -Tocophenol	JM 721	2.71 0.63	2.81 0.19	2.38 0.15
	MI- 24-91	2.67 0.53	2.80 0.42	2.20 0.21
Ascorbic Acid	JM 721	2.74 0.21	2.87 0.12	2.42 0.36
	MI- 24-91	2.63 0.43	2.76 0.31	2.18 0.19
Diphenyl amine	JM 721	2.75 0.62	2.86 0.25	2.39 0.01
	MI- 24-91	2.65 0.44	2.78 0.06	2.20 0.15



Table. 18: Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F – values	
					5 %	1 %
Main plot analysis						
Replication	2	0.145	0.0726			
SO2 (A)	2	3.864	1.9319	18.18	6.94	18.00
Error (A)	4	0.425	1.1063			
Sub plot analysis						
Antioxidants (B)	3	0.134	0.0447	0.56	3.16	5.09
A×B	6	0.07	0.0116	0.15	2.66	4.01
Error (B)	18	1.44	0.08			
Sub sub plot analysis						
Variety (C)	1	0.222	0.224	0.88	4.26	7.82
A×C	2	0.046	0.023	0.09	3.40	5.61
B×C	3	0.41	0.0136	0.05	3.01	4.72
A×B×C	6	0.015	0.0025	0.01	2.51	3.67
Error (C)	24	6.039	0.2516			
Total	71	12.441				

cv (a) = 8.00 %; cv (b) = 13.80 %; cv (c) = 13.2 %

Table.19: Dry weight (g) of two cultivars of Lentil treated with different SO₂ concentrations and plant protectants using dry permeation method

Treatment	Varieties	SO ₂ Concentrations		
		0.00	655.00	2620.00
Only Acetone	SLC-2	1.76 0.32	1.80 0.05	1.32 0.08
	Sehore 84-8	1.49 0.19	1.52 0.07	1.02 0.08
α -Tocophenol	SCL-2	1.82 0.11	1.85 0.07	1.50 0.08
	Sehore 84-8	1.41 0.14	1.43 0.07	1.13 0.03
Ascorbic Acid	SLC-2	1.78 0.09	1.81 0.04	1.46 0.04
	Sehore 84-8	1.40 0.19	1.42 0.13	1.12 0.09
Diphenyl amine	SLC-2	1.68 0.48	1.70 0.06	1.38 0.08
	Sehore 84-8	1.51 0.09	1.53 0.08	1.19 0.05

Table. 20: Analysis of Variance (Split Split-Plot Design)

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F – value	Tabular F – values	
					5 %	1 %
Main plot analysis						
Replication	2	0.019	0.0094			
SO2 (A)	2	1.96	0.9801	25.65	6.94	18.00
Error (A)	4	0.153	0.0382			
Sub plot analysis						
Antioxidants (B)	3	0.022	0.0073	0.28	3.16	5.09
A×B	6	0.087	0.0145	0.56	2.66	4.01
Error (B)	18	0.464	0.0258			
Sub sub plot analysis						
Variety (C)	1	1.623	1.623	42.52	4.26	7.82
A×C	2	0.004	0.0018	0.05	3.40	5.61
B×C	3	0.12	0.0399	1.04	3.01	4.72
A×B×C	6	0.01	0.0017	0.01	2.51	3.67
Error (C)	24	0.916	0.0382	0.04		
Total	71	5.377				

cv (a) = 15.40 %; cv (b) = 23.20 %; cv (c) = 19.40 %

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Microbiological method for determination of cyanocobalamin in pharmaceutical formulation

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Abstract

A simple and precise microbiological method has been developed for the quantitative estimation of Cyanocobalamin in pharmaceutical formulations. After employing different microorganisms for achieving best results, *Escherichia coli* 113-3 ATCC No. 11105 was found to be the most suitable. In the method zone of exhibition achieved by using the aforesaid organism were measured from which content of Cyanocobalamin in pharmaceutical formulation was determined. The results were good in most of the formulations available in the market. Cyanocobalamin is added in 100% overages in the pharmaceutical formulations which were shown during its estimation. Moreover there is no interference of other vitamins present in the formulations by using this culture and method. The method employed is economical and simple and can be used in the quality control of bulk manufacturing and also in pharmaceutical formulations

Key words: Microbiological, vitamin, pharmaceutical, capsule, syrup

Introduction

Vitamin B₁₂ is the name generally used for a group of related cobalt containing compounds, also known as cobalamins, of which Cyanocobalamin and Hydroxocobalamin are the principal forms in clinical use. Cyanocobalamin is an especially common vitamer of the B₁₂ vitamin family. It is the most famous vitamer of the family because it is chemically the most air stable, and it is the easiest to crystallize and therefore easiest to purify after it is produced by bacterial fermentation. The cyanide is added to the molecule by activated charcoal columns in purification. Thus the use of this form of B₁₂ is the most wide spread.

Vitamin B₁₂ preparations are used in the treatment and prevention of Vitamin B₁₂ deficiency. Deficiency of Vitamin B₁₂ leads to development of megaloblastic anaemias, demyelination and other neurological damage. A specific anaemia known as perinious anaemia develops in patients with an absence of intrinsic factor necessary for good

absorption of the vitamin from dietary sources. Cyanocobalamin and Hydroxocobalamin are generally administered by intramuscular route, although Cyanocobalamin may be given orally or intranasally.

1. Drugs Testing Laboratory, A-20, Lawrence Road Industrial Area, Delhi

2. Central Drugs Testing Laboratory, 37, Naval Hospital Road, Periamet, Chennai

Preparations: B-complex capsules/tablets/ syrup
The objective of this work is to develop analytical microbiological procedure which would serve as a rapid and reliable method for Cyanocobalamin in B-complex capsules/ tablets/syrup.

B-complex capsules/tablets/syrup contains many Vitamins and excipients which may interfere with microbiological analysis but this method gives good zone of exhibition of Cyanocobalamin in most of the available formulations in the market.

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Experimental Organism used: *Escherichia coli* 113-3 ATCC No. 11105

Maintenance medium: Following ingredients were dissolved in 1000ml of distilled water:

Ingredients	Grams/litre
Casein enzymic hydrolysate	5.00
Yeast extract	5.00
Monopotassium phosphate	0.50
Magnesium sulphate	0.20
Sodium chloride	0.10
Sucrose	12.0
Liver extract	0.05
Ferrous sulphate	0.001
Agar	15.0

Final pH was 7.0 ± 0.2

The medium was completely dissolved by boiling on a steam bath. Approximately 10 ml portions of hot solution in test tubes were placed and plugged with cotton. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled and make slants were made.

Maintenance of the culture:

Three or more of the slants were inoculated by pure culture of *Escherichia coli* 113-3 ATCC No.11105. Incubated for 16-24 hrs at temperature of $35 \pm 2^\circ \text{C}$ and finally stored in a refrigerator.

Assay medium: Following ingredients were dissolved in 1000ml of distilled water:

Ingredients	Grams/litre
Potassium dihydrogen phosphate	6.00
Di-potassium hydrogen phosphate	14.00
Sodium citrate hydrated	1.00
Magnesium sulphate hydrated	0.20
DL-Asparagine hydrated	8.00
L-Arginine hydrochloride	0.20
L-Glutamic acid	0.20
Glycine	0.20
L-Histidine	0.20
L-Tryptophan	0.20
L-Proline	0.20
Ammonium sulphate	10.00
Glucose (added aseptically)	10.00
Agar	15.00

Final pH was 7.0 ± 0.2

Boiled to dissolve the medium completely on a steam bath. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Preparation of standard solution:

The dilutions of standard in distilled water were prepared so as to make a final dilution containing 0.1 mcg/ml & 0.05mcg/ml of Cyanocobalamin.

Preparation of test solution:

The dilutions of test sample were prepared in distilled water so as to make a final dilution containing 0.1 mcg/ml & 0.05mcg/ml of Cyanocobalamin.

Assay

The assay of Cyanocobalamin is done by using cylinder plate method or cup plate method. To the assay medium at 45°C 0.1 ml of culture suspension obtained from fresh slants was added. Mix it properly and poured it into sterile petri plates. Ensure that the layers of the medium are uniform in thickness, by placing the plate on a levelled surface. After solidification holes 5 to 8 mm in diameter were bored in the medium with a sterile borer. Now 0.1 ml of standard and test solutions into different cups on the plate was added. The plates were incubated at 37°C for 24 hrs to measure the zones of exhibition.

The assay was calculated according to the following formula

$$\% \text{Potency} = \text{antilog} (2 + a \times \log I)$$

Where ,

$$a = \frac{(T_2 + T_1) - (S_2 + S_1)}{(T_2 - T_1) - (S_2 - S_1)}$$

T_1 and T_2 zone readings of test solutions

S_1 and S_2 are zone readings of standard solutions.

a may have a positive or negative value and should be used alzebrically.

I = is the ratio of dilutions.

$$\text{Potency of sample} = \frac{\% \text{Potency} \times \text{assumed potency of sample}}{100}$$

The values for the assay are given in Table.1



Method validation

Accuracy and Specificity: Spiking of Cyanocobalamin in pre analysed test preparation was done and analysed. Recovery studies showed accuracy and reproducibility (Table-2). The % RSD of replicate standard solution area was less than 1%.

Linearity: Linearity was checked by preparing standard solution at five different concentration levels ranging from 0.025 to 0.40 mcg/ml.

Limit of Detection and quantification: The detection limit for Cyanocobalamin was around 0.005 mcg/ml. and the limit of quantification was 0.0125 mcg/ml.

Application: The developed method is applied for the analysis of Cyanocobalamin in real drugs samples obtained from market (Table.1).

Recovery Studies

To study the accuracy, reproducibility and precision of the proposed method, recovery experiments were carried out. A fixed amount of pre analysed sample was taken and standard drug was added at four different levels to confirm the

absence of positive and negative interference from excipients.

Results and Discussion

To develop a suitable and robust microbiological method for determination of Cyanocobalamin, different microbial cultures and mediums were employed to achieve the best estimation. British Pharmacopoeia (2009), *Bergey's Manual of Systemic Bacteriology*, (1984), Indian Pharmacopoeia (2007), *The United States Pharmacopoeia* (2009). The culture organism of *Escherichia coli* 113-3 ATCC No. 11105 was found to be an appropriate culture allowing adequate estimation of Cyanocobalamin without any interference from other vitamins present in the pharmaceutical formulations. To ascertain the effectiveness of the method recovery studies and accuracy were carried out.

Linearity

The plot of zone of exhibition of *Escherichia coli* Vs Concentration of Cyanocobalamin was found to be almost linear in the range of 0.0125 to 0.40 mcg/ml. The mean recovery of Cyanocobalamin by the proposed method is 98.75%, as shown in Table.2.

Table-1 Results of analysis of Cyanocobalamin in real drugs samples (Capsules/Syrup) obtained from market and results of Microbiological Assay

Formulation	Label Claim Cyanocobalamin	Quantity found mcg/capsule or 5 ml of syrup	Quantity % of label Claim (overages to the tune of 100% are present in pharmaceutical formulations)
A (Capsule)	15 mcg	28.3 mcg	188.67
	15 mcg	28.6 mcg	190.67
	15 mcg	28.0 mcg	186.67
	15 mcg	28.5 mcg	190.00
	15 mcg	28.8 mcg	192.00
B (Syrup)	15 mcg	22.5 mcg	150.00
	15 mcg	22.9 mcg	152.67
	15 mcg	22.1 mcg	147.33
	15 mcg	22.7 mcg	151.33
	15 mcg	22.5 mcg	150.00



Table.2 Results of Recovery Studies

S.No.	Component Recovery	Conc. of added drug in Pre analysed formulation (mcg/ml)	Recovery of added drug (mcg/ml)	% Found
1.	Cyanocobalamin	0.5	0.497	99.40
2.		1.0	0.988	98.80
3.		1.5	1.480	98.66
4.		2.0	1.960	98.15
				Mean : 98.75%

Conclusion

The developed method is fast, simple and precise. The mean amount of Cyanocobalamin found on analysis by the above proposed method was 22.54 mcg/5 ml of syrup & 28.44 mcg/cap. The low value of RSD (1.2 %) indicates that the method is precise and accurate. The mean recovery value is 98.75 % which shows that the method is almost free from interference of the other vitamins and excipients used in the formulation. The developed method for quantitative assay of Cyanocobalamin showed good results.

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Estimated energy content of solid waste at commercial area of Udhampur, Jammu and Kashmir

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Abstract

The present study is an attempt to analyze the percentage generation of solid waste in commercial area of Udhampur (J&K) and to estimate the generation potentiality of energy through combustion of solid waste. The waste to energy industry has proven itself to be an environmentally friendly solution to the disposal of municipal solid waste and the production of energy. The estimation of energy content of commercial solid waste of Udhampur town is discussed in this paper. A regression equation was used for estimating the energy content of commercial solid waste. The estimation of energy content of municipal solid waste (MSW) is normally done by the use of Modified Dulong Equation (MDE). The MDE requires changing all MSW components to percentages of carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and sulfur (S), which is very time-intensive effort. An easier-to-use and more practical new equation is used, which directly uses the percentages of MSW components. The observations made during the course of investigation shows that, the minimum is the percentage by weight of organic waste (vegetable and fruit waste) maximum is the energy content (kJ/kg) of the waste.

Keywords: *Udhampur, Municipal solid waste, Regression equation, Modified Dulong equation (MDE), energy content*

Introduction

With the fast depletion of the conventional resources, the growing awareness and concern regarding the environmental effects of their utilization, there has been a major thrust in the recent past to identify and develop alternate energy resources. India generates vast amounts of MSW in hundreds of tons which when converted to fuel fluff/pellets have appreciable calorific value and hence, are over the country and totally renewable. The cost of the fuel is also much lesser than that of conventional fuels. Besides that advantages MSW based project is also eco-friendly as it does not add to the environmental pollution and rather it would help cleaner environment. Keeping the above factor in focus, the Ministry of Non-Conventional energy sources (MNES), Govt. of India, has been aggressively

promoting the concept of biomass/MSW based power plants in the 5-15 MW range. The national program on biomass/MSW based power generation had extended capital subsidies for first few demonstration projects (Kumar *et al.*, 2010).

In this context, solid waste appears to be the most promising energy resource as these are abundantly available, very inexpensive and renewable. Over 1000 million tones of solid waste are produced annually, which could be converted into solid, liquid and gaseous fuel to cater the needs of principal sectors *viz*; transport household, industry and agriculture. So, recycling of waste and their conversion into wealth become very vital for the development of the country. Energy recovery from the solid waste is on the upswing with a growing market share for the mass burning of fossil fuel (Agarwal *et al.*, 2004). Ramakrishna and Babu (2010) studied Electric Power Generation (EPG) by firing MSW as fuel in the boiler-steam-turbine system. The results show that the EPG is directly

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proportional to the calorific value of MSW fired. Khan and Gharah (1991) developed a linear regression equation for predicting energy content values correctly and hence this is adequate for estimating energy content for Indian cities. The estimation of energy content of municipal solid waste (MSW) is normally done by the use of Modified Dulong Equation (MDE). The MDE requires changing all MSW components to percentages of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S), which is a very time-intensive effort. An easier-to-use and more practical new equation is presented which directly uses the percentages of MSW components to calculate the energy content (kj/kg).

This paper is an attempt made to estimate the quantity of solid waste that can be generated in the commercial area of Udhampur town (Jammu and Kashmir) and to estimate the generation potentiality of energy through combustion of solid waste. The waste to energy industry has proven itself to be an environment friendly solution to the disposal of municipal solid waste and the production of energy. The study area is a Commercial area of Udhampur town and extends from 32° 53'N to 32° 56'N and 75° 03'E to 75° 09'E at an average elevation of 756 meters above the sea level, comprised mostly of fruit/vegetable shops and karyana shops. So, bulk of waste is of combustible and compostable nature and has great potential for energy recovery. The conversion of waste to energy is a step forward to cleaner environment with added advantage of providing some energy. Shah (1994) recommended the use of solid waste for energy generation to mitigate environmental problems. Roy (1998) suggested recycling of municipal waste as a technique to create renewable source of energy and to solve disposal problem. Qudais and Qdais (2000) analysed energy content of municipal solid waste and its potential utilization in Jordan and reported that the energy content of MSW generated in Jordan accounts for 6% of the annual imported oil consumption of the country and may result in annual saving of US\$ 24 million in case of utilization. Gupta and Manhas (2008) analysed percentage generation and energy content (kj/kg)

of municipal solid waste and recommended it as a best alternative source of energy *viz a-viz* waste minimization. Thus, in the present study, an attempt has been made to explore the potential recovery of the energy available in the commercial solid waste generated in the township of Udhampur (J&K).

Materials and Method

The study area was divided into four sites for the purpose of collecting data. Shops in the commercial area were divided into different types *i.e.* karyana, tea stall and meat shops *etc.* Two samples per month of solid waste generated were taken from each types of shop. During each sampling, different types of biodegradable, non-biodegradable and inert solid waste were collected for analysis and the components of waste identified in the analysis were paper, polythene, organic *etc.* as shown in the Table. 1. The percentage by weight of different components was calculated and then the solid waste energy content (kj/kg) was calculated with the help of a regression equation which uses the percentages by weight of MSW components directly in it as follows:

$$EC = 37.658 + 241.054(PR) + 55.153(HF) + 174.874(PC)$$

Where,

EC = Energy content of waste (kj/kg).

PR = Percentage weight of plastic and other synthetic materials.

HF = Percentage weight of hay, straw, food waste and wood.

PC = Percentage weight of paper and cardboard.

The standardized co-efficient (241.054) is high for plastic and other synthetic materials which showed that plastic and other synthetic materials generate maximum energy from the waste.

Results and Discussion

In the course of present study on solid waste generated in the commercial area of Udhampur (J&K), it has been observed that at Site-I (Bus stand area) energy content (kj/kg) was found to be



maximum during January-March (9681.16 kj/kg) and a minimum during July–September (8816.91 kj/kg). The Site-II (Court road area) showed the maximum value of energy content during January-March (10630.85kj/kg) and minimum during July–September (9680.679 kj/kg).The value at Site-III (Mukherjibazar area) was found to be the maximum during October - December (8926.355 kj/kg) and minimum during July–september (8506.78 kj/kg).The Energy content (kj/kg) at Site-IV (Goal market area) was maximum during July-September (9634.812 kj/kg) and a minimum

during October-December (9077.08 kj/kg).Among all the four sites of present studies (Table.1), the maximum organic waste was found at Site-III during July-September (65.67%) where the energy content was minimum (8506.78 kj/kg) whereas energy content was maximum (1063.85 kj/kg) during January-March at Site-II where organic waste was minimum (39.58 %). The observation reveals that the minimum is the percentage by weight of organic waste (vegetable and fruit waste) maximum is the energy content (kj/kg)

Table-1: Percentage by weight of municipal solid waste components generated at for four sites of commercial area of Udhampur (J&K) and their computed energy contents (kj/kg)

Site-I (Bus stand Area)

TYPES OF WASTES	Paper/ cardboard	Jute/ textile	Wood waste	Organic waste	Plastic waste	Polythene waste	Thermocol waste	Other wastes	Energy content
JAN-MAR	20.22	1.53	4.1	54.75	3.93	5.7	1.67	0.97	9681.16
APR-JUNE	18.48	1.31	3.74	58.03	4.56	4.03	1.56	0.71	9234.24
JULY-SEPT	16.06	1.21	3.02	63.91	4.21	3.36	1.41	0.87	8816.91
OCT-DEC	19.26	1.39	2.94	58.67	4.04	5.01	1.48	0.67	9455.62
AVERAGE	18.51	1.36	3.45	58.84	4.19	4.53	1.53	0.81	9296.98

Site-II (Court road Area)

TYPES OF WASTES	Paper/ cardboard	Jute/ textile	Wood waste	Organic waste	Plastic waste	Polythene waste	Thermocol waste	Other wastes	Energy content
JAN-MAR	25.76	2.41	2.66	39.58	5.77	5.47	3.55	1.1	10630.85
APR-JUNE	22.45	1.96	2.72	49.7	5.85	4.63	2.31	0.64	10081.18
JULY-SEPT	20.34	1.55	2.23	54.99	5.71	3.71	2.26	0.53	9680.68
OCT-DEC	25.23	2.1	2.81	44.49	5.98	4.25	2.87	1.04	10389.45
AVERAGE	23.45	2.01	2.61	47.19	5.83	4.52	2.75	0.83	10195.54

Site-III (Mukherji Bazar Area)

TYPES OF WASTES	Paper/ cardboard	Jute/ textile	Wood waste	Organic waste	Plastic waste	Polythene waste	Thermocol waste	Other wastes	Energy content
JAN-MAR	15.9	1.92	1.59	58.47	3.34	5.82	1.76	0.64	8904.15
APR-JUNE	15.07	0.82	1.58	64.82	3.17	4.61	1.19	0.41	8565.26
JULY-SEPT	14	0.84	1.55	65.67	3.36	4.28	1.66	0.46	8506.78
OCT-DEC	15.74	1	1.58	60.36	3.29	5.97	1.66	0.59	8926.36
AVERAGE	15.18	1.15	1.58	62.33	3.29	5.17	1.57	0.53	8725.64

Site-IV (Goal Market Area)

TYPES OF WASTES	Paper/ cardboard	Jute/ textile	Wood waste	Organic waste	Plastic waste	Polythene waste	Thermocol waste	Otherwastes	Energy content
JAN-MAR	17.56	1.02	2.15	56.05	4.57	4.38	2.35	0.65	9134.37
APR-JUNE	19.94	1.1	1.45	52.82	5.48	4.57	2.47	0.46	9621.83
JULY-SEPT	19.08	1.07	2.1	54.77	5.49	4.62	2.47	0.59	9634.82
OCT-DEC	16.56	0.99	2.28	56.18	4.78	4.63	2.3	0.76	9077.08
AVERAGE	18.29	1.05	2	54.96	5.08	4.55	2.4	0.62	9367.02

of the waste. This shows that synthetic waste such as plastic and polythene and wood, paper and textile waste contain more energy content (kj/kg) compared to organic waste (vegetable and fruit waste). Among various types of waste generated during the course of study, organic waste (percentage by weight) exhibited the maximum percentage in all the study sites during all seasons *i.e.* January-March, April-June, July-September and October- December. A critical evaluation of Table 2, Fig.1 showed that the average solid waste (kg/day/site) generation and energy content do not follow a set pattern of increase or decrease at different sites and in different seasons. Average solid waste observed to

be 66.52 kg with maximum value of 77.96 kg at Site-I comprise 9296.98kj/kg of energy content followed by 63.63kg of waste with 8727.64kj/kg of energy at site III and then by site II which exhibit the average value of 62.38 kg comprising 10195.54 kj/kg of energy. The average energy content was found to be maximum during January-March (9587.63 kj/kg) followed by October-December (9462.13 kj); April-June (9375.63 kj/kg) and then by July-September (9159.8 kj/kg). Site-II exhibited the maximum energy content (10195.54 kj/kg) among all study sites whereas the minimum energy content (8725.64 kj/kg) was recorded at Site-III. The overall average energy content was found to be 9396.29 kj/kg.

Table. 2: Seasonal variation in average solid waste (kg/day) and average energy content (kj/kg) at four different study sites of commercial area, Udhampur (J&K).

Month / Sites	Average solid waste					Average energy content				
	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Average	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Average
Site I	67.08	81.23	92.47	71.06	77.96	9681.16	9234.24	8816.91	9455.62	9296.98
Site II	48.23	73.77	73.68	53.85	62.38	10630.85	10081.18	9680.68	10389.45	10195.54
Site III	53.53	73.77	70.79	56.41	63.63	8904.15	8565.26	8506.78	8926.35	8725.64
Site IV	47.41	73.77	70.79	56.41	62.1	9134.37	9621.83	9634.82	9077.08	9367.02
Average	54.06	75.64	76.93	59.43	66.52	9587.63	9375.63	9159.80	9462.13	9396.29

A critical observation of the study area revealed that the Municipality has not provided dustbin/metallic bins in commercial area for the collection of waste generated. Open dumping is the regular practice as a result of which all the waste generated in the area finds its way into drains and finally into Devika, the sacred Ganga of Udhampur, thereby polluting it heavily. The biodegradable waste components of solid waste disposed off in the vacant plots or area serve as breeding ground for the flies, insects, fungus and other disease causing agents. Besides this, it gives an ugly look to the area and unpleasant odour to the residents of adjoining area. Solid waste generated at Site-I is dumped off in an open area near roadside, thereby, exposing the population to various health hazards. The waste is then transported for its final disposal at the confluence

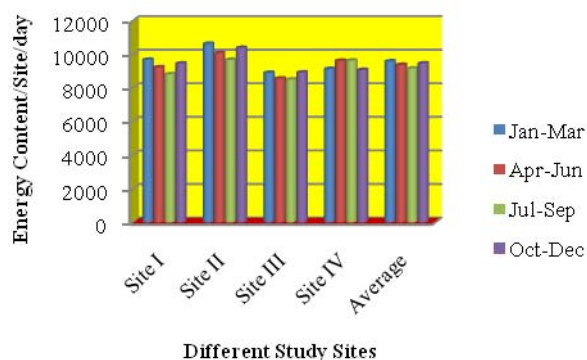


Fig. 1: Seasonal Variation in energy content values at four study Sites

of Dudder nallah and the river Tawi. Similarly, the solid waste from Site-IV is dumped off near Govt. (Boys) Hr. Sec. School located in the study area. At the study site- II and III, most of the waste is

thrown by shopkeepers directly into drains. So, in order to prevent the harmful effects of improper solid waste disposal on the inhabitants of the area, proper hand planed scientific management should be done to maintain the beauty of the area. One of the beneficial methods of proper solid waste disposal on the inhabitants of the area is the conversion of waste to energy. It is a step towards cleaner environment with added advantage of providing some energy.

Uchendu (2008) reported 5.9 million tonnes per year generation of municipal solid waste in Nigeria and discussed the problems of waste disposal with its short and long-term effects. He also suggested a sustainable system for waste disposal. Gupta and Manhas (2008) analyzed the percentage generation and energy content (kj/kg) of municipal area of Janipur, Jammu and highlighted that it has good potential of energy and this can be utilized for the production of energy in various forms. Tripathi *et al.*, 2006, suggested vermicomposting for scientific disposal of solid waste.

Conclusion

From the present study, it is concluded that waste generated in the commercial area of Udhampur (J&K) has good potential of energy and this can be used for the production of energy in various forms. The conversion of waste to energy is one of the best ways to dispose solid waste on proper and scientific lines.

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Comparison of α -amylase from bacterial and plant sources

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Abstract

14 plant and bacterial species were extensively investigated for α -amylase enzyme present in them. Comparison was made on the basis of optimum pH, optimum temperature, Km, Vmax, pH stability range and thermal stability of enzyme. It was found that the plant enzyme is equally good than the bacterial enzyme in terms of characteristics.

Keywords: *Dolichos biflorus*, *Phaseolus vulgaris*, α -amylase, enzyme

Introduction

The α -amylase industry generally focuses on bacterial species for production of the enzyme. Among plants, the Family Poaceae is considered for enzyme production in food industry to a certain extent. The present research focuses on two new sources of α -amylase from the family leguminosae. It is of considerable interest that if a comparison is made between the properties of α -amylase obtained from bacterial sources and that from leguminous sources, then the industrial feasibility of α -amylase of leguminous plants can be explored to a certain extent. In the present paper studies on two 14 α -amylase sources have been compared.

Materials and Method

Kinetic characteristics of *Dolichos biflorus* and *Phaseolus vulgaris* were studied according to standard protocols adopted by Goyal and Dobriyal, 2010. Optimum pH, optimum temperature, pH stability, thermal stability, Km and Vmax were studied according to standard protocol of Miller (1959). Studies on *Eleusine coracana* Indaf- 15 were done according to

Nirmala and Muralikrishna (2006). *Sorghum bicolor* was studied according to Nour and Yaqoub (2010); *Triticum aestivum* according to Mohammed *et al.* (2009); *Glycine max* according to Kumar *et al.* (2010) and *Zea mays* according to Biazus (2009). Among bacteria, the *Bacillus subtilis* (2 strains) and *Bacillus amyloliquefaciens* strain F, α -amylase were investigated according to Welker and Campbell (1967), Gangadharan *et al.* (2009), Femi-Ola and Olowe (2011). *Bacillus cereus* α -amylase was studied according to Anto *et al.* (2006); *Thermomonospora curvata* was studied according to Glymph and Stutzenberger (1977); *Xanthomonas campestris* K-11151 according to Abe *et al.* (1994) and *Streptococcus bovis* JB1 was studied according to Freer, 1993.

Results and Discussion

α -amylase of *Dolichos biflorus* and *Phaseolus vulgaris* (Family Leguminosae) showed complete instability in highly alkaline pH range. Optimum pH of Rajma came out to be 6.5 while that of Kulath came out to be 6.1. These were completely unstable above pH 7.5. However, these were compared with α -amylase of the family Poaceae, which were stable in acidic pH range only, and become completely unstable above pH

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7. *Eleusine coracana* Indaf-15, *Sorghum bicolor*, *Zea mays* and *Triticum aestivum* were studied. All these have pH stability range and optimum pH below 7 (Table 1). Among bacteria, 6 bacterial strains were studied, of which 3 belonged to the genus *Bacillus*. All *Bacillus* species have an optimum pH below 6.5, and are unstable in alkaline range. *Thermomonospora curvata* and *Streptococcus* had showed slight stability in alkaline range, only up to pH 8.5. It was thus concluded that that optimum pH of almost all bacterial and plant amylases range between pH 4 to 8.

The thermal stability of all amylases is not the same. It was deduced that the legume family has a high thermal stability, and the amylase is stable almost up to 80°C. However, the grass family has a relatively low thermal stability with amylase stability up to a temperature of 50°C. Among bacteria, the thermal stability is varying. Strains of *Bacillus* spp. are highly thermo-stable, while *Streptococcus* and *Xanthomonas* have low thermal stability. Generally, the optimum temperature ranges between 40-55°C (Table 2).

Table.1: Table showing comparison between temperature and pH for plant amylases

S.No	Property α -amylase source	Common Name	pH stability range	Optimum pH	Thermal Stability (°C)	Optimum temperature (°C)
1	<i>Dolichos biflorus</i>	Kulath	5.5 -7	6.1	40-75	45
2	<i>Phaseolus vulgaris</i>	Rajma	4 -7.5	6.5	30-80	50
3	<i>Glycine max</i>	Soyabean	4-6	5.5	25-85	50
4	<i>Eleusine coracana</i> Indaf-15	Ragi or Finger millet	5-5.5	5.3	45-50	47
5	<i>Sorghum bicolor</i>	Jowar	4.5-6	5.5	30-70	70
6	<i>Triticum aestivum</i> (5 isozymes)	Wheat	5.5-7	5.5-7	40-50	50
7	<i>Zea mays</i>	Corn	4-6.5	5.5	50-90	55

Table 2: Table showing comparison between temperature and pH for bacterial amylases

S.No	Property α -amylase source	pH stability range	Optimum pH	Thermal Stability (°C)	Optimum temperature (°C)
1	<i>Bacillus amyloliquefaciens</i> strain F	5.5 -6	5.9	0-70	65
2	<i>Bacillus subtilis</i> Strains (W23 and BS5)	5.7 -6	6.3	0-70	65
3	<i>Bacillus cereus</i>	4-6	5	35-75	55
4	<i>Thermomonospora curvata</i>	6 -8	7.5	40-70	53
5	<i>Xanthomonas campestris</i> K-11151	4.5-5	4.5	45-55	45
6	<i>Streptococcus bovis</i> JB1	5.5-8.5	5-6	Below 50	Below 50

Further comparison was made on the basis of size. Among plants, it was found that the plant amylases are small in size in comparison to bacterial amylases. Such small sized proteins are beneficial in food industry. Recent researches conducted by food industries proved that small protein molecules are easily digestible in the small intestine, and provide very high surface area for reaction (Table.3 and 4). Table 5 gives a very close comparison of km value between plant and bacterial enzyme. Low km values of plant alpha amylases studied indicate high affinity of the enzyme for starch substrates, like some bacterial strains. Not all workers have stated the Vmax values in their work owing to the fact that, Vmax values of different enzymes are difficult to compare as they depend on the substrate used and the reaction conditions.

The investigation simply proves that the kinetic characteristics of plant and bacterial amylases are similar. It was finally concluded that the plant enzyme is as good as bacterial enzyme in terms of characteristics. The study also proves that the enzyme can be better produced commercially by legume sources.

Table 3: Molecular weight of amylase from Bacterial sources

Bacterial Source	Molecular weight (KDa)	Reference
<i>B. stearothermophilus</i>	43 to 46	Yutani (1973)
<i>Thermomonospora curvata</i>	62	Glymph and Stutzenberger (1977)
<i>Bacillus subtilis</i> AX20	149	Najafi et al. (2005)
<i>Bacillus subtilis</i> BS5	63	
<i>Xanthomonas campestris</i> K-11151	55	Abe et al. (1994)
<i>Streptococcus bovis</i> JB1	77	Freer 2010

Table 4: Molecular weight of amylase from Plant sources

S. No	Plant Source	Molecular weight (KDa)	Reference
1.	<i>Dolichos biflorus</i>	Below 30	Goyal and Dobriyal, 2010
2	<i>Phaseolus vulgaris</i>	Below 30	Goyal and Dobriyal, 2010
3	<i>Eleusine coracana</i> Indaf-15	22	Nirmala and Muralikrishna, G (2003)

Table 5: Comparison of Km value of α -amylase from plant and bacterial sources

S.No	Source of α -amylase	Km value (mg/ml)
1	<i>Dolichos biflorus</i>	1.95
2	<i>Triticum aestivum</i> (5 isozymes)	1.42-1.7
3	<i>Bacillus amyloliquefaciens</i> strain F	3.076
4	<i>Bacillus subtilis</i> BS5	16.67
5	<i>Thermomonospora curvata</i>	0.39
6	<i>Streptococcus bovis</i> JB1	0.88

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Role of social stimuli in the photoperiodic induction

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Abstract

Two experiments were performed to study of the effects of social interaction on photoperiodic induction in house sparrows. In the first experiment, short-day pretreated birds were exposed to stimulatory long day lengths (16L: 8D) for 4 weeks. The first set had a group of male and female birds kept individually in cages so that they could not see to each other. The second was similar to the first but the cages were separated by a transparent partition so that birds could see each other. In third four male and four female were kept together in the same cage. The second experiment differed from the experiment 1 in the sense that it housed individual birds and also included another variable, the noise. Birds were disturbed by the sound of a ringing bell for 15 minutes at three times of the day, ZT4, ZT8 and ZT12. Observations on body mass and gonad size were made at the beginning and end of the experiment. The pairing in the environment appears to affect the gonadal growth in females but not in males.

Keywords: *Social stimuli, Day length, Gonadal growth, Body mass, Zeitgeber time*

Introduction

The daily photoperiod has been widely investigated as the most potent 'primary timer' (Immelmann, 1971) or 'the initial predictive information' (Wingfield, 1983), which brings birds in a state of breeding readiness so that the actual reproductive effort could begin. This could be because the photoperiod can easily be manipulated and studied experimentally. Other social and environmental factors might also potentially affect the timing of actual reproduction. For instance, female birds do not respond to photoperiod as dramatically as males do, suggesting the importance of factors other than photoperiod contributing to the successful reproduction. It is evidenced that behavioral interactions act as strong force in regulating the

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timing of nesting in individual birds. Reciprocal interaction provides a secondary stimulus in female canaries: a female-male, but not female-female, pairing leads to quick gonadal growth and intense nesting activity (Hinde and Steel, 1978). Successful reproductive behaviors associated with male-male competition and courtship, as well as the production of gametes. By using predictive cues, such as photoperiod, to stimulate gonadal recrudescence, individuals can coordinate the physiological, behavioral, and neural changes necessary for the timely expression of reproductive behavior (Wingfield, 1983). Social attachment between mating partners is widely distributed among vertebrates. Reproductive pairing is most prevalent in birds, which may pair for the breeding seasons or longer. House sparrow is a social species. However, the underlying physiological mechanisms that mediate pair formation and maintenance are unknown in any bird species.

Social interactions have been shown to influence sex steroids, neurobiology (Francis *et al.*, 1993; Hartman and Crews, 1996; Tramontin *et al.*, 1999), and reproductive physiology (Brzoska and Obert, 1980; Delville, *et al.*, 1984; Cheng, 1986; McComb, 1987; Gudermuth, *et al.*, 1992; Rissman, 1992).

Circadian and seasonal rhythms are dependent on environmental temporal cues, or zeitgebers, for steady entrainment to a 24-h cycle. Although the most ubiquitous zeitgeber is photoperiod, a number of nonphotic stimuli have been found to influence circadian rhythms by resetting the circadian clock, entraining rhythms, or facilitating re-entrainment following phase shifts of the light cycle, such as those experienced with transmeridian jet travel (Mrosovsky *et al.*, 1989; Turek, 1989; Antle and Mittleberger, 2000; Aschoff *et al.*, 1971; Goel and Lee, 1995a, 1995b; Amir and Stewart, 1996). Social influences on circadian timing might function to tightly organize the social group, thereby decreasing the chances of predation and increasing the likelihood of mating. Governale and Lee (2001) routed odors from a housing unit containing entrained donor animals to a box containing phase-shifting animals. Recovery from the phase shift was equivalent to earlier experiments housing the donor in the same cage as the shifting animal. It is unclear whether male's apparent inability to accelerate re-entrainment when exposed to social cues is due to an absolute incapacity for social cue responsiveness or a decreased sensitivity to olfactory social cues relative to females. For some species, social cues can serve to synchronize circadian rhythms in the absence of other time cues or to amplify ambiguous light cues. This has been demonstrated to various degrees in fruit flies, fish, birds, bats and humans; however, studies in rats and hamsters have shown that social cues are less salient time cues for these species. Songbirds highlight the powerful influence of social cues on motor production during learning more generally, and reveal that motor output during learning may underestimate the actual progress of competency (Kojima and Doupe, 2010). Birdsong is a complex learned vocal behavior developed by motor practice in early life, with many striking

parallels to human speech (Brenowitz *et al.*, 2010). Social influences on circadian timing might function to tightly organize the social group, thereby decreasing the chances of predation and increasing the likelihood of mating (Davidson and Menaker, 2003).

A large field of study in avian behavior is centered on the evolution and maintenance of different mating systems (Andersson, 1994). Although the distinction between monogamy and other mating systems in birds has blurred due to the discovery of extra-pair fertilizations found in presumed monogamous species (Mbller and Birkhead, 1992), general trends between mating pattern and certain behavioral repertoires remain. For example, polygamous systems often feature high levels of male-male aggression, and strongly monogamous species have high levels of paternal care (Ketterson and Nolan, 1994; Mbller and Birkhead, 1993; Schwagmeyer *et al.*, 1999). The biological process addressed in this study is the control of seasonal reproduction in an adult subtropical avian species, the house sparrow by photoperiod.

In this study, we analyzed the effects of seasonal responses in the house sparrow birds of environmental disturbance. Birds exposed to stimulatory LD cycles were disturbed to assess the effect of disturbance on the physiological response. The other goal of this study was to address upon the question as to whether male-male or male-female interaction will influence the magnitude of photoperiodic response. In stimulatory LD cycles, birds were kept such that they could/ could not interact with the other individuals.

Materials and Method

Two experiments were performed on the adult male and female house sparrows (*Passer domesticus*) which were procured locally (~29° N). They were then acclimatized to laboratory conditions for about 2 weeks.

Experiment 1: This experiment began in June 2004 to examine the effects of pairing of male and female (social interaction) birds. They were exposed to short day lengths (8L:16D) for 4 weeks ensuring their sensitivity to photoperiods before exposure to experimental long day lengths



(16L:8D). They were grouped in three sets. In the first set, a group (N = 4 each) of male and female birds were kept in separate cages in the same photoperiodic box but separated by a plywood partition so that they could not see each other. The second set had a group of male and female birds kept in separate cages in the same photoperiodic box but separated by a transparent partition so that they could see each other. In third set four males and four females were kept together in the same cage.

Experiment 2: This experiment began in December 2004, to determine the effects of disturbances on the seasonal responses. Birds exposed to stimulatory LD cycles were disturbed by noise to assess their physiological responses. At the beginning of the experiment, all birds had normal body weight and unstimulated testes and ovaries. This time birds were untreated with short days since at this time of the year they are found photosensitive. This experiment differed from the first experiment in being housed individually in the cage and in having experienced an additional cue, i.e. timed disturbance. Birds were exposed to stimulatory long day lengths (16L:8D) in three sets. In the first set, male and female birds were kept individually in cages that were separated by a plywood partition so that they could not see each other. The second set had male and female birds kept individually in cages but separated by a transparent partition so that they could see each other. In third set a male and a female were kept together in the same cage. In addition to this, birds were disturbed by sound of a ringing bell at three times of the day for 15 minutes at ZT 4, 8 and 12 (ZT0; zeitgeber time = time of light onset).

All experimental birds were maintained under uniform husbandry conditions. Birds caged in groups of 4 wire-mesh cages (size-45 x 30 x 30 cm) were placed in the photoperiodic box (size- 75 x 70 x 60 cm); 2 cages were thus in one box. Birds under NDL were also similarly housed and kept in a room that received unrestricted environmental light and air from large windows. Food (seeds of kakuni, *Setaria italica* and paddy, *Oryza sativa*) and water were available *ad libitum*, and replenished once daily during daytime. Once every month, birds

received in water glucose (Glucon-D, Heinz India Private Limited), vitamins (Vimeral, Glaxo-Smithkline Pharmaceutical Limited, Mumbai, India) and antibiotics (Tetracycline hydrochloride, Hoechst Roussel Vet. Ltd). Artificial light was provided by 14 watt fluorescent tubes (CFL) at an intensity of ~500 lux at the perch level, and the timing of light and dark was regulated by an automatic Muller electronic timer.

The photoperiodic effects, assessed as changes in body mass and testis volume or follicle diameter, were measured at the beginning (day 0) and end (day 30) of the experiment. Body mass was recorded using a top pan balance to an accuracy of 0.1g. The testicular growth was recorded by laparotomy performed under local anesthesia as per procedure described by Kumar *et al.*, 2001. Briefly, a small incision was made between last two ribs on the left flank, and the left testis was located within the abdominal cavity with the help of a spatula. The dimensions of the left testis were recorded and testis volume was calculated from $\frac{4}{3} \pi ab^2$, where a and b denote half of the long and short axis, respectively. In females, an ovarian follicle of a small or regressed ovary with indistinct follicles was scored as 0.1 mm in order to make the data statistically comparable. Data generated from experiments are presented as mean $\pm$ SE. They were further analyzed statistically to produce more meaningful results. One-way analysis of variance was employed to examine the effect of a photoperiodic treatment over a period of time. In these experiments, before and after means of the same group were compared using paired t-test. Significance was always taken at $P < 0.05$.

Results and Discussion

Experiment 1: The mean body mass among the three groups of male and female birds was not significantly different (fig. 1a, c). Male birds of set 1, set 2, and set 3 showed a significant gain in testicular volume (day 0 vs day 30: $P < 0.05$, paired t-test). Testes were not significantly different among the three groups of male birds ($F_{2,11} = 0.9268$, $P = 0.4246$; 1-way ANOVA). Female birds of set 1, set 2, and set 3 showed a significant gain in



follicle diameter (day 0 vs. day 30: $P < 0.05$, paired t-test). The follicle diameter were not significantly different among the three groups of male birds ($F_{2,11} = 0.7143$, $P = 0.5129$; 1- way ANOVA).

Experiment 2: The mean body mass among the three groups of male and female birds was not significantly different (fig. 2a, c). Male birds of set 1, set 2, and set 3 showed a significant gain in testicular volume (day 0 vs. day 30: $P < 0.05$, paired t-test). Testes were not significantly different among the three groups of male birds ($F_{2,11} = 0.8690$, $P = 0.4518$; 1-way ANOVA). The follicle diameter of set 3 birds was significantly greater than the set 1 and 2 birds ($F_{2,11} = 8.561$, $P = 0.0083$; 1- way ANOVA).

Studies have shown that paternity rates (proportion of young fathered by the male parent) are relatively high in species where males make relatively large contributions to incubation and nestling care (Ketterson and Nolan, 1994; Mbller and Birkhead, 1993; Schwagmeyer *et al.*, 1999).

This correlation between mating system and male parental care can be further expanded by adding a

third dimension, seasonal male testosterone (T) profile. Wingfield *et al.*, (1990) found that annual T peaks are short-lived in males of strongly monogamous and paternal species compared to the peaks in males of polygamous species. However, hormone data are rare for species that exhibit high levels of both paternity and male parental care. Male woodpeckers both incubate and feed nestlings at similar rates as females (Winkler *et al.*, 1995). Few data are available on extra-pair fertilizations (EPFs) in woodpecker species; however, existing studies have found very low frequencies of EPFs, even among the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*; Haig *et al.*, 1994; Michalek, 1998). Acorn woodpeckers (*Melanerpes formicivorus*) also maintain genetic monogamy when nests are cared for by a single male-female pair (Dickinson *et al.*, 1995). In accord with these behavioral patterns, limited hormone data show that male woodpeckers have low circulating levels of T during breeding periods relative to species with greater extra-pair paternity and less male parental care (Khan *et al.*, 2001).

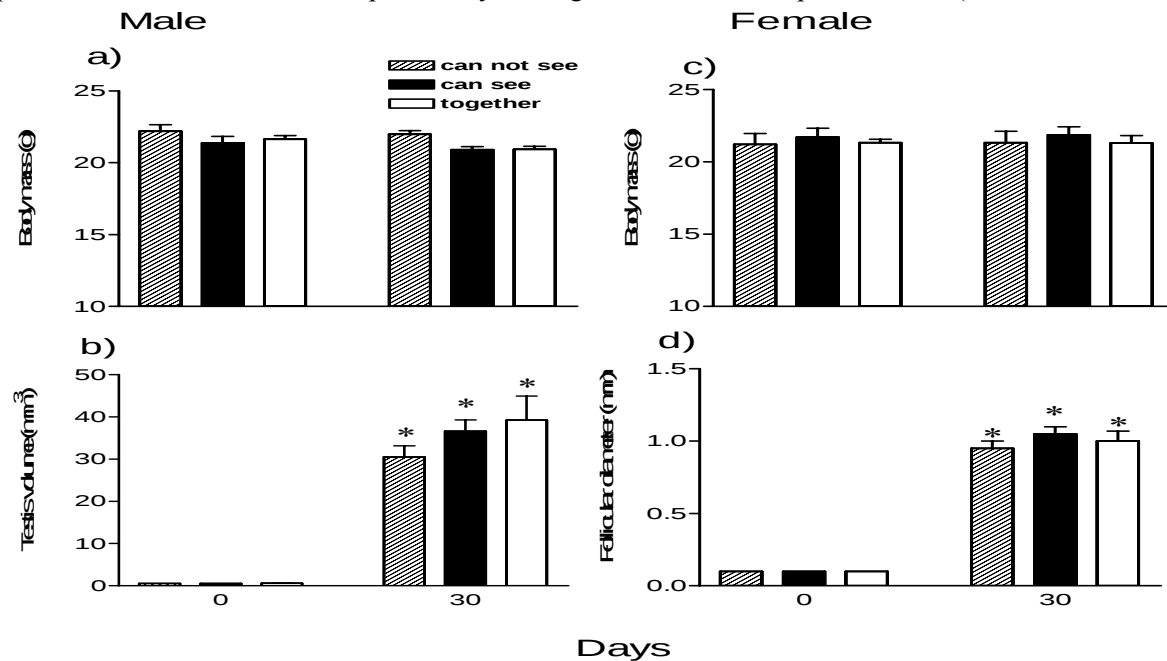


Figure 1. A group ($n=4$ each) of male or female birds were kept individually in cages separated by a plywood partition so that they could not see each other (Set 1) or kept individually in cages but separated by a transparent partition so that they could see each other (Set 2) or kept together in the same cage (Set 3). Each point symbol represents mean ($\pm$ SEM) response in male (a, b; left panel) and female (c, d; right panel).

Wingfield *et al.*, (1990) classified species into three groups according to whether male showed low, moderate, or high levels of aggression during the breeding season. Males in species with low levels of aggression rarely interacted and tended to have high levels of parental care. They also had relatively low levels of T throughout the year. Males in species with moderate levels of aggression interacted frequently before the parental phase, but the frequency of interactions dropped after nestlings hatched. Those males, on average, showed seasonal T peaks approximately five times the breeding baseline levels. Wingfield *et al.*, 1990 and Hirschenhauser *et al.*, 2003 showed that males had a greater hormonal response to social challenges than males with different combinations of life history traits. Therefore, male house sparrows show

a considerable increase in testis size in all groups. Wingfield (1994) showed that the sexes in sexually monomorphic species have similar T levels (Archawaranon *et al.*, 1991; Hegner and Wingfield, 1987; Wikelski *et al.*, 2000; Wingfield *et al.*, 1982, 1989). In males in monogamous but sexually dimorphic species often have T concentrations three times higher than females (Schwabl and Sonnenschein, 1992). The reason for this pattern is not certain, but Wittingham and Schwabl (2002) showed that circulating T in females during the breeding season is correlated with the rate of competitive interactions among females. As we showed here, house sparrow females, which are together and can see the male bird showed greater response, male birds showed equal response against all three schedules.

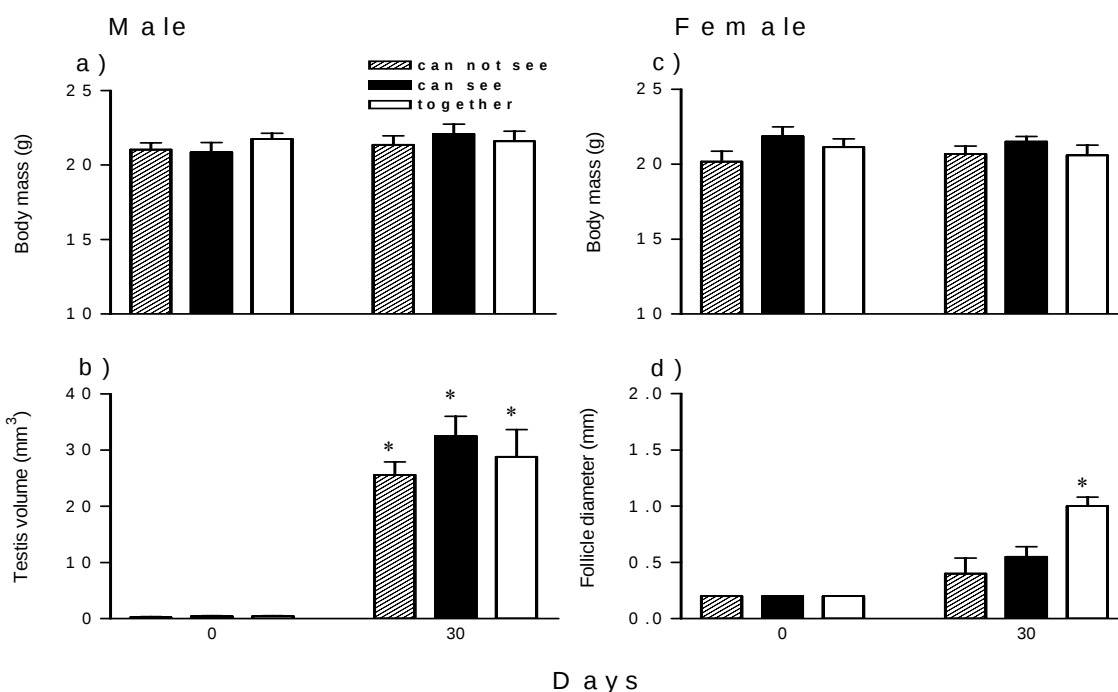


Figure 2. Individual male and female birds were kept individually in cages separated by a plywood partition so that they could not see each other (Set 1) or kept individually in cages but separated by a transparent partition so that they could see each other (Set 2) or kept together in the same cage (Set 3). In addition to this birds were disturbed by sound of a ringing bell at three times of the day for 15 minutes at ZT 4, 8 and 12 (ZT0; zeitgeber time = time of light onset). Each point symbol represents mean ($\pm$ SEM) response in male (a, b; left panel; N = 4) and female (c, d; right panel; N = 4). Asterisk (*) indicates significance of difference at $P < 0.05$.

Wingfield *et al.*, (1990) hypothesized that increases in T level between the non-breeding and breeding baselines facilitate sexual interactions. While individuals of many species of birds do not have the opportunity to interact sexually with a former or future mate during non-breeding periods, individuals of other species do. With increased association frequency, other more courtship like behaviors such as solicitation poses and the exchange of visual, vocal, and acoustic signals could occur. Our results revealed no significant relationship in males and females, who cannot see each other, they can contact vocally. But because of sound disturbance, their signals were not clear. Another two groups, which had visual contact, they were less effective by sound disturbance. Social cues are of the product of interactions between individuals, and in such cases it can be difficult differentiate the effect of the reception of cues from the production of responses (Burmeister and Wilczynski, 2000). Social interactions, especially competitive ones, are often stressful (Fox *et al.*, 1997) and may be especially so in cases where the individual is unable to exert control. The interaction between the auditory system and vocal production pathway may be related to acoustically evoked mate calling. Not surprisingly, many of these same brain regions that are involved in reception and production of social signals are also responsive to circulating sex steroids (Kelley *et al.*, 1975; Morrell *et al.*, 1975; Kelley, 1981). Social relationships between males are likely to go beyond neighbours with whom territory boundaries are shared (Naguib, 2006). The pairing in the environment appears to affects the gonadal growth in females but not in males. All male groups whether they were separated or remained together with females had almost similar testicular volume but females when put in company of males had grown significantly ($P < 0.05$; 1-way ANOVA) larger follicles. This is adaptive since females this way can limit the reproduction when the chances of survival are greatest. Males on the other hand cannot adapt to such social cues since testicular growth and development to when there is full spermatogenesis requires a period of at least 6 to 8 months. The

present findings are therefore significant in showing the importance of pairing between males and females.

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Freshwater: Resources and Pollution

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Abstract

Today with the rapid increase in the population, the gap between demand and supply of resources has been doubled. To overcome this gap increase in industrial revolution came into existence causing the severe damage to nature and its resources. Today our aim is not only to overcome the gap between demand and supply but also to manage these resources so that our future generations can also use them. Water, everyone's need is the major resource which is severely affected and being polluted enormously. In context of India if we look towards our biggest river basin i.e. River Ganga, nourishing the agricultural fields, providing water for domestic and commercial purposes is majorly polluted due to industrial effluents and chemicals. We still have our resources plenty in amount but only if we manage them properly with new management techniques. We are the caretakers of these resources, not owners; all these resources belong to our future generations.

Keywords: *Water, Management, Resources, Pollution*

Introduction

Water, like other primordial elements was revered and worshipped since Vedic times. The Rig Veda says: "Agni and water are givers and sustainers of life, they are affectionate mothers, givers of all givers of life, they have healing powers". If it was true then, it is truer now as never before.

Through our history water has been a natural resource critical to human survival. Water a substance which is flowing, swirling and seeping, constantly moving from sea to land and back again. It not only shape's the earth but moderate our climate also. If we see our body composition, we found 60% of our body is water. Water dissolve nutrients and distributes them to cells, regulates body temperature, support body structure and removes waste products. Water is the only medium in which all living processes occur.

The great rivers, like mothers, nourished civilizations such as Indus Valley, the Sumerian or

the Nile, in fact, evolution of human cultures and civilization has revolved around river systems, in other words human history can generally be considered to be water-centered. The main reason of the development of civilization and habitats along the banks of rivers was easy availability of water for drinking, farming, and transportation was an important requirement for survival. In other words human history can, in fact, be written in terms of interactions and interrelations between humans and water.

World's water resources consist of fresh water found on and under the Earth's surface, and the immense salt-water reserves of the oceans. Since fresh water is used to meet most of our current needs it has become the focus of immediate concern. With population growth and industrial development on the increase, demand on limited supplies is likely to intensify still further. Both the Millennium Development Goals and the Johannesburg Summit on Sustainable Development recognize the need to provide greater access to clean fresh water, to improve

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sanitation, and to produce integrated plans for the development of this universal resource.

Fortune magazine stated that, in a world fleeing the vagaries of technology stocks, the water industry is the best investment for the century. The World Bank places the value of the current water market at close to \$1 trillion. With only 5 percent of the world's population currently getting its water from corporations, the profit potential is unlimited. Water from a fundamental right and necessity for survival has become a product of profit in the market.

The total amount of water available on earth has been estimated at 1.4 billion cubic kilometers, enough to cover the planet with a layer of about 3 km deep. About 95% of the earth's water is in the oceans, which is unfit for human consumption and other use because of its high salt content; about 4% is locked in the polar ice caps; and the remaining 1% constitutes all the freshwater in hydrological cycle including ground water reserves. Only 0.1% is available in as fresh water in rivers, lakes and streams, which is suitable for human consumption. This shows that at what level we need the water for the survival of life on earth. The above statistics of availability are devoid of

the influences of contamination, pollution and other human contributions shrinking the supply of drinkable water further.

The decreasing supply of water arises from the meager absolute natural endowments of water the earth is blessed with as well as from the meager natural endowments relative to increasing growth of population, and their increasing demands for use and misuse. During the last fifty years, human population has increased by 240 percent to 6 billion people. By 2050, human population is estimated to be at 9 billion people. India with 1 billion people at present is projected to become the most populace country before the middle of next century. Global consumption of water is doubling every 20 years, more than twice the rate of human population growth. According to the United Nations, more than one billion people on earth already lack access to fresh drinking water. By 2025, if the current trends persist, the demand for freshwater is expected to rise to 56 percent above the amount currently available.

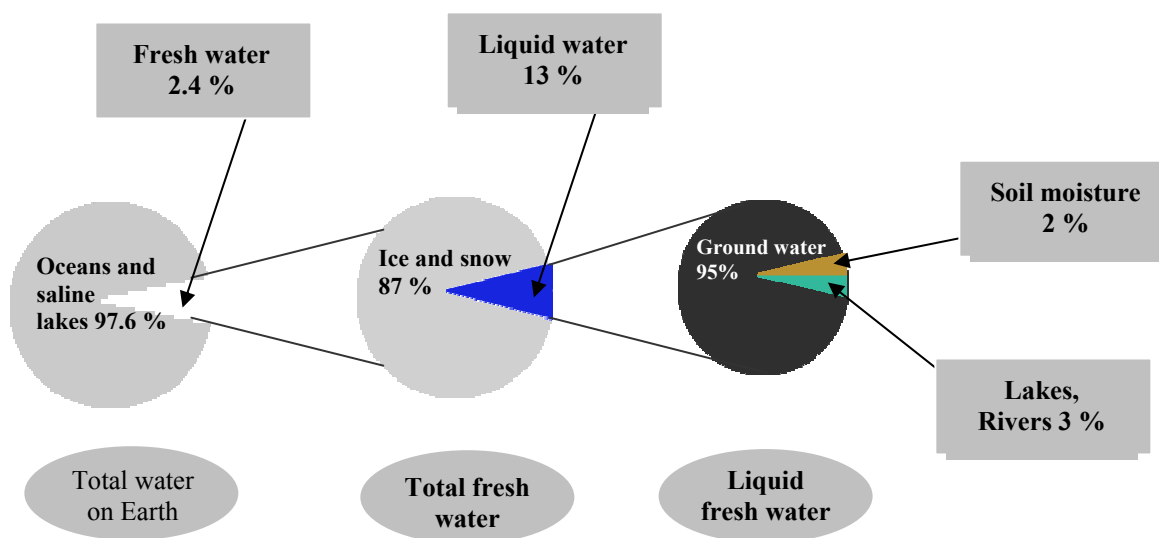


Figure 1: Distribution of water in different forms

According to National Water Policy, 2002 “Water is a prime natural resource, a basic human need and a precious national asset. Planning development and management of water resources need to be governed by national perspectives”. A combined study conducted by United Nations International Children Education Fund (UNICEF) and the World Wide Fund (WWF) for Nature revealed the alarming situation of fresh water depletion in the country. The main cause was found of the decline of the quality and quantity of the presently available water resources is due to: (1) increase in the population (2) improper resource management (3) pollution in water sources (4) Shortcomings in the design and implementation of legislation and regulations, which address these problems.

The demand for water needed for the mere purposes of survival is compounded by the need of water for industries, agriculture, livestock maintenance and other activities. Thus, with water being the essence for human survival and a necessity for carrying out all human activities, there can be no decrease in the demand for water, not even a slump but only an ever-increasing demand. This holds sadly true with the running down of its finite supply. In other words, scarcity of water is continuously increasing. ‘Scarcity’ is a term often associated with the developing world, be it food, medicines, schools *etc.* But when it is a matter of water scarcity, the developed world and the developing world equally suffer. North America (New Jersey, Carolinas, Texas) is facing the problems of water shortages just as the people of Asia, Africa and Latin America. The scarcity arises in both the developed and the developing world, not just because of decreasing water availability from the sources of water supply, but also due to the inefficient working of the water supply system. The water distribution system remains inefficient, more so in the developing world. Further with intensive urbanization, deforestation, water diversion and industrial farming, water available in cities and villages suffer from lack of quality and irregular supply.

Continuously increasing scarcity, defines the status of the global water situation. In short, water is becoming globally scarce in availability and where available, further scarce through misuse, poor water management and inefficient distribution system.

According to latest census, India’s population is about 1020 million, which is projected to go up to 1333 million by A.D. 2025 and further to 1640 million by A.D. 2050 for feeding a population of 1.64 billion, nearly 450 million tones of food grains would be required by the year 2050, production of which would be a gigantic task considering the constraints being faced in the irrigation sector, including irrigation water resources. If we see the situation in India as regards availability of water is paradox. The country accounts for 2.45 per cent of the total land area and 4 per cent of the water resources of the world. Nevertheless, water is a scarce national resource with demands on it increasing on account of a growing population of over one billion. Much of the available surface water and ground water estimated at 1869 billion cubic metre is presently unable to be harnessed for use on account of topographical and other constraints. India is at the 133rd position among 180 countries and as regards the quality of the water available, it is 120th among 122 countries. Of the present water usage in India, 92 per cent is devoted to agriculture; around 3 per cent is used by industries and only 5 per cent for domestic purposes like drinking water and sanitation. The picture gets complicated by the other constraints. 40 million hectare of land in the country is flood prone and an average, floods affect an area of around 7.5 million hectare per year. One-sixth area of the country is drought prone. Water pollution is a serious problem with 70 per cent of India’s surface water resources and an increasing number of its ground water reserves standing contaminated by biological, toxic organic and inorganic pollutants. The National Water Policy advocates a participatory approach to management of water resources and non-conventional methods for utilization of water like



artificial recharge of ground water and traditional water conservation practices like rain water harvesting. With water being a scarce resource, its sharing and distribution requires a regulatory framework which is brought about through not only written laws but also traditional and customary practices. At this stage it's too important to develop the educational level of water management, sustainable use of water resources.

Major water resources

Distribution of water compartments in which water is present are in different forms. Nearly all the world's water is in the oceans. Oceans play a crucial role in moderating the earth's temperature, and over 90 percent of the world's living biomass is contained in the oceans. What we mainly need though is fresh water. Of the 2.4 % which is locked up in ground water and huge glaciers in various parts of the earth. Accessible water for human beings and to other organism is 0.1% of the total freshwater present on earth.

Ground water resources

In India ground water contributes 70-80% of agricultural produce in India, about four fifth of the domestic water supply in rural areas and about 50% of urban and industrial uses. In the most developing countries as ours, most of the water sources of freshwater, especially in outskirts of large cities and villages are polluted. Continuous flow of industrial effluents and sewage in the ground the water has become the store house of various kinds of bacteria's and viruses also.

Assessment of ground water resources has been made at 431.9 BCM by the Central Ground Water Board through a large volume of hydrologic and related data. This is the sum total of potential due to natural recharge from rainfall and due to recharge contributions from canal irrigation. The utilizable ground water resources have been assessed at 395.6 BCM (70.0 BCM for domestic and industrial uses and 325.6 BCM for irrigation).

A management approach is said to be perfect, if the demand side of the elements of interest balance with its supply-side inputs. In case of groundwater management, the elements are: (i) hydrogeologic

and socio-economic conditions of the system (ii) regulatory provisions (iii) regulatory interventions; (iv) costs and benefits of management activities and interventions. Some of the issues which will emerge in groundwater development and management are: (i) the protection of ground water against pollution and aquifer remediation (ii) the ground water depletion requiring practicing of artificial recharge using different methods to utilize monsoon runoff and wastewater from different sources; (iii) the management of trans boundary aquifers; (iv) regulate the ground water development and formulation of an exclusive National Groundwater Policy defining the custodianship of natural and recharge groundwater; (v) the management of water logged and inland Salinity areas; (vi) updating the technologies of tackling the groundwater quality problems of fluoride, arsenic, nitrate and selenium. With stimulated models and optimization methods as the new methods for the management of ground water management which finally produces a single programme that optimizes management objective while meeting physical and technical constraints on ground water behaviour, we can not only pre-judge the results of our management techniques used but also save the time and money, which leads to sustainable development.

Table 1: Earth's Major Water Compartments

Compartments	Volume (1,000 km ³)	% of total water
Total	1,386,000	100
Oceans	1,338,000	96.5
Ice and snow	24,364	1.76
Saline groundwater	12,870	0.93
Freshwater	10,530	0.76
Fresh lakes	91	0.007
Saline lakes	85	0.006
Soil moisture	16.5	0.001
Atmosphere	12.9	0.001
Marshes, wetlands	11.5	0.001
Rivers, streams	2.12	0.0002
Living organisms	1.12	0.0001

Source: UNEP, 2002



Surface water

Fresh, flowing surface water is one of our most precious resources. Rivers, lakes, wetlands are the main sources of surface water. Rivers flowing from various mountain ranges having a minute amount of water level of water in rivers depends upon the precipitation and on the icecaps which melts in summer thus increasing the water level in the rivers. The sixteen largest rivers in the world carry nearly half of all surface runoff on the earth, and a large fraction of that occurs in a single river, the Amazon, which carries ten times as much water as the Mississippi.

The main river systems of the country are the Indus and the Ganga-Brahmaputra-Meghna (Barak) system. The Ganga rising from the snow capped Himalayan mountains, flows through the great indo-gangetic plains. The Brahmaputra rises in Tibet where it is known as the Tsangpo and runs a long distance until it crosses over into India in Arunachal Pradesh under the name of Siang or Dihang. The Ganges and the Brahmaputra join inside Bangladesh and continue to flow under the name Padma forming the Sunderban delta.

Holy River Ganga, along with its tributaries forms the largest river basin of India is a symbol of purity. But with rapid growth in population and pollution is severely polluted, that is in utter disregard to its sanctity. Not only River Ganga other river of the country also facing the same problem at different level. Many cities of the country like Kanpur situated near River Ganga and Delhi situated near Yamuna are facing drinking problems. People living near to these water bodies are found to be affected by many diseases like malaria, cholera *etc.*

In spite of being the life line of the country, and having much dependency of peoples from industries to domestic use these major fresh water bodies are severely being polluted. The rapid pace of industrialization and urbanization has posed a serious threat to these vast varieties of water resources. Effluents from various industries with having excessive concentration of nitrates and phosphates causes well known lake eutrophication, disrupting the whole aquatic environment.

Water is an indispensable need of life. It is necessary to develop a suitable and sustainable technique to protect and manage the quality and resources not only from biological and biodegradable pollutants but also from toxic inorganic compounds and non-biodegradable wastes. Hence the whole water system should be examined and proper management methods should be developed with latest technologies like models to preserve and maintain the water quality of the surface water.

Table 2: World's ten largest river

River	Location	Annual Discharge (m ³ / Second)*
Amazon	Brazil, Peru	175,000
Orinoco	Venezuela, Colombia	45,300
Congo	Congo	39,200
Yangtze	Tibet, China	28,000
Brahmaputra	India	19,000
Mississippi	United states	18,400
Mekong	Southeast Asia	18,300
Parana	Paraguay, Argentina	18,000
Yenisey	Russia	17,200
Lena	Russia	16,000
* 1 m ³ = 264 gal.		
Source: Data from World Resource Institute.		

Water pollution

Today human activities are constantly adding industrial, domestic and agricultural wastes to water bodies at an alarming rate. The main sources of pollution in Indian freshwater bodies are through effluent discharge from industries, pesticide leaching from agricultural fields, oil spills from oil tankers and boats, sewage and waste disposal and retting of coconut husk along estuaries and backwaters.



According to reports more than 90% of the industries in India do not have adequate facilities to treat the effluents and as a result huge quantities of untreated effluents containing chemicals and wastes are discharged into the aquatic environment leading to serious consequences in the environment.

The availability of water, both in quality and quantity, is one of the prime factors in deciding the growth of towns and cities as well as industries. For industries, the available of water must be as near as possible to the factory site and should also be soft otherwise the manufacturing cost will increase.

Pollution from factories, power plants, sewage treatment plants, underground coal mines and oil wells are classified as point sources of pollution, being discharged from specific locations. With this kind of pollution sources it is possible to treat the effluents before they enter into the water bodies. Pollution from soil erosion, chemical runoff, and animal waste pollution are all examples of non-point source pollution. Non-point source pollution refers to pollutants that come from a widespread area and cannot be tracked to a single point or source. Non-point source pollution is major water quality problem by sheer volume and in terms of current and future economic costs because it not only affects the surface water quality but also decline the productivity of agriculture fields also.

During the past two decades, the public has become increasingly concerned with hazardous and toxic materials discharged into the aquatic environment. Due to the development of more industries, increased urbanization and reclamation of areas, the environment is under severe stress and these create an unfavourable situation for aquatic organisms to live. The pollutants can weaken the organisms making it susceptible to disease or they cause disease directly. Toxicants or other stressful situations may enhance the disease. A proper monitoring of water resources pollution and enforcement of strict measures to control pollution would help enhancing the productivity of the aquatic environment.

Water management

According to World Bank report today, about 700 million people live in countries experiencing water stress or scarcity. By 2035, it is projected that 3 billion people will be living in conditions of severe water stress. Many countries with limited water availability depend on shared water resources, increasing the risk of conflict over these scarce resources.

Water resource management is the integrating concept for a number of water sub-sectors. Use of an integrated water resources perspective ensures that social, economic, environmental, and technical dimensions are taken into account in the management and development of surface waters (rivers, lakes, and wetlands) and groundwater.

The lack of water resources experienced in different parts of the world has now been recognized and analyzed by different international organizations such as WHO, the World Bank *etc.* Recently published documents from the UN Environment Programme confirms that severe water shortage affects 400 million people today and will affect 4 billion people by 2050 (Thomas and Durham, 2003).

IWRM was previously mentioned in the Millennium Development Declaration of the UN, (Nations, 2000) article 23 "To stop the unsustainable exploitation of water resources by developing water management strategies at the regional, national and local levels, which promote both equitable access and adequate supplies." This approach includes the:

- Development of alternative water resource;
- Protection of water resource to stabilize and improve its quality and quantity;
- Demand management implemented at the level of each river basin.

If we are agreeing to consume water despite the recognition that there is a growing crisis of scarcity then we have to decide *who* gets to make decisions about that process? Is it the role of government, in which case we should discuss mechanisms and bureaucratic modalities for water governance or, is it up to the individual as



regulated by civil society? Writers considering sustainable development (Chambers, 1997; World Bank, 2003), common pool resource management (Korten, 1986; Berkes, 1989; Poffenberger, 1990; Western and Wright 1994; Ostrom, *et al.* 2002; Deitz, *et al.* 2003), and global norms in water management policy (WMO, 1992; OECD, 1998; Kemper, *et al.* 2003) all have come to point in the same direction: (1) all resources, especially water resources, need to be managed, (2) decision making across multiple levels fosters sustainability, and (3) communities, especially rural communities, have a greater interest in managing resources sustainably than state or corporate managers. Communities are more aware of local environmental processes, and thus can be mobilized to manage the resource within multi-level natural resource use regimes. In the water sector, these assumptions codified in the Dublin Accords are reflected in attempts at complementing centralized physical infrastructure with lower cost community-scale systems, decentralized and open decision making, water markets and equitable pricing, application of efficient technology, and environmental protection (Gleick 2003). The four 'Dublin Principles' read :

Principle No. 1—Fresh water is a finite and vulnerable resource, essential to sustain life, development and the environment.

Principle No. 2—Water development and management should be based on a participatory approach, involving users, planners and policy makers at all levels.

Principle No. 3—Women play a central part in the provision, management and safeguarding of water.

Principle No. 4—Water has an economic value in all its competing uses and should be recognized as an economic good.

Unfortunately, the development path that countries in Southeast Asia have taken is one that is highly disruptive to the hydrological cycles. Aquifers,

which store precious ground water, have lost their water holding capacities due to massive deforestation such as those experienced in Indonesia and the Philippines. Over-exploitation of water sources for industrial and domestic purposes has contributed to the rapid depletion of already limited freshwater resources. These disruptions have severely compromised the ability of the forests to “catch” and then “shed” the water into streams, rivers and reservoirs, as well as the capacity of aquifers to recharge.

The extremely rich biodiversity of Southeast Asia is a testament to the abundance of freshwater systems and high rainfall that support life. The Ganga river of India, Mekong River of Mainland Southeast Asia, Chao Phraya of Thailand, and the other rivers and lakes of the region are important sources of food, water for various uses, medicines, energy, minerals, *etc.* More importantly, these surface waters as well as the underground aquifers provide communities with drinking water.

Water management and conservation are also economically and environmentally sound ways to prevent and overcome the problems like floods and drought damages and store water for future use than building huge dams and reservoirs. A series of small dams on tributary streams can hold back water before it becomes a flood. Ponds formed by these dams provide useful wildlife habitat and stock-watering facilities. They also catch soil where it could be returned to the fields. Small dams can be built with simple equipment and local labour, eliminating the need for massive construction projects and huge dams.

Another important tool is the development of country water resources assistance strategies (listed on the left), which describe what the Bank can and will do to help improve water resources management in a country. These strategies can improve the strategic focus of water-related activities, stimulate coordination across water-related sectors within the Bank and the country, and engage regional and global water knowledge.

To mitigate the adverse impacts of water pollution, suitable measures are to be evolved through comprehensive water management studies. The change in human attitude towards water resources



is also important as the resources are not unlimited and moreover they must be preserved for future generations. Steps like interlinking of the flowing rivers flowing within the Indian Territory. States with flowing rivers must behave with a level of maturity and the Centre should act as a mediator. In this way water could reach through out the country and droughts can be prevented.

Water management or conservation is not a single handed process nor fulfilled without the participation of local people, it's a collective effort of the community and legal system. Community should initiate the resource management in every part of the micro-watershed or even at the macro-watershed level this will increase the public awareness, conducting research, providing basic infrastructure (waste disposal systems), technical assistance whenever needed and last but not least they can help us to predict more accurately what will the output of our techniques as the local people are more aware of the past and present condition.

Conclusion

Water is needed in all aspects of life. The general objective is to make certain that adequate supplies of water of good quality are maintained for the entire population of this planet, while preserving the hydrological, biological and chemical functions of ecosystems, adapting human activities within the capacity limits of nature and combating vectors of water-related diseases. Innovative technologies, including the improvement of indigenous technologies, are needed to fully utilize limited water resources and to safeguard those resources against pollution.

Looking at the Water Framework Directive from India's perspective, it is particularly relevant to note that this philosophy of water management, despite its roots in a society that pre-dates modern society by over fifteen centuries, is based on science and technology. Modern water law and policy cannot exist without active participation from science. Yet, science cannot make judicial and policy decisions. In the larger scheme of democratic governance, science, scientists, and scientific institutions have an obligation to see that

the best available scientific knowledge is brought to bear on the creation of water laws and policies, as well as their implementation. Science has to learn to address a different type of knowledge that lies outside its traditional boundaries.

As scientific knowledge grows, there comes the need for social responsibility. Further, such knowledge growth becomes available for adaptation in differing situations. There is an immediate need of nationwide consensus to adapt to river and water resources management. We believe that this is achievable through pursuance of scientific studies, 'correct' perception of scientific outputs and, through persuasion of planners and policy makers to develop suitable policies. With reference to coastal systems management in India, bridging natural sciences, social sciences and policies is imperative to further endorse the truism that 'coasts protect those who protect coasts'.

If we are to protect our valuable water resources, changes have to be made in the way we see and treat our environment. The real solutions to protect water quality must begin at the sources of pollution. One such effective and lasting measure is the implementation of clean production processes. By eliminating the use of toxics from the very first step of production by which pollution of water sources can be effectively prevented.

Our constant exposure to polluted fresh water sources—clogged, or foul smelling river and lakes, as well as contaminated groundwater—have made water pollution a reality which we have learned to accept. Thus, we focussed on technology meant to 'clean up' pollution rather than to prevent it, and drafted laws that merely regulate the extent of toxicity in water, rather than prohibit it completely.

We must learn to unlearn this 'reality.' Clean water is the given we must protect if we are to ensure that our water will continue to sustain life well into the future.

Beau
Bacongus, Toxics
Campaigner, Greenpeace
Southeast



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Seasonal hill River problems and their bio-engineering remedial measures in Shiwalik Himalaya

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Abstract

Doon valley in lower Shiwalik hills of Uttarakhand is infested with large number of seasonal hill rivers originated from Shiwalik hills. The flash flood in the torrent as the peak storm during rainy season caused severe main problems as land erosion and the flood water often inundated the household properties, death of livestock and flooding in agricultural fields. In the study, Sabhawala watershed area land damaged by uncontrolled torrent flow during monsoon period which is very severe to watershed in particular affecting 257.78 ha out of total 1173.6 ha (21.96 %) watershed area. The present paper conclude that bio-engineering measures are essential for the protection and rehabilitation of seasonal rivers affected agricultural area for sustainable conservation of agriculture land resource

Key words: *Torrent, Torrential watershed, Bioengineering measures, Shiwalik region*

Introduction

The Uttarakhand state is particularly sensitive to natural land disturbing activities. Steep slopes, high rainfall and weak geology of the Uttarakhand state make prominent land degradation and soil erosion process at much faster rate than in the plains due to fragile ecosystem. In the head water reaches the sediment is flushed with the high velocity currents and reached the relatively flat foothills along with debris sediment starts accumulating on the river bed causing change of river course and flooding its bank. These rivers with flash flows and high sediment loads are known as torrents. Torrents in Shiwalik Himalaya have been created constant threat to the natural resources as well as human settlements. The formation of torrents though due to geological and climatic factors and its ecological problems due to mismanagement of watersheds of their origin in the headwater reaches. Torrent affected

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erosion is due to the high run-off down the hill slopes with heavy gravel, sand and silt loads that fill up the channel beds thereby reducing their capacity to carry the run-off and sediment and as a consequence, the flow shifts from the original course. Moreover, the torrents have meandering nature *i.e.* they often change their course and cause damage of adjoining land, life and property. Torrents are causing vast area submergence and damage to life, property and infrastructure almost every year. This is the most common problem in, Himalayas spread over the northern states of India. In the foothills of Shiwalik Himalaya, torrents are the prominent land features. The torrents are seasonal in nature and characterized by high sediment laden flash flow during monsoon period. These torrents have low banks and thus the flow frequently over tops the banks and cause floods. Torrent have mostly taken as small mountainous streams rushing down to the slopes with flashy floods and often loaded with sediments. On rushing the milder slopes such as

foothills, they develop into channels with large volumes of debris and low velocity of flow (Tiwari, 2004). Torrent effected states include Uttarakhand, Punjab, Harayana, Jammu & Kashmir, Himanchal Pradesh, Assam and the north eastern states (Juyal *et al.*, 2005 a,b). Torrent training works are basically aimed to control meandering tendency of the torrent by restricting it in a defined and narrow course. If this is achieved, problems of overtopping and undermining of banks and debris deposition in the flow channel can be eliminated. A study was taken up to evaluate the costs of engineering structures and vegetative covers to find out the best economical rehabilitation technology for the management of torrents in Sabhawala watershed on the basis of relative efficacies of different mechanical and vegetative control measures in terms of their effects on the flow, sediment transportation and scour or deposition patterns and also in terms of structural stability and longevity.

Materials and Method

The study was conducted in the Doon Shiwalik range of the Uttarakhand at Sabhawala torrential watershed area. The experimented site located at the longitude 77° 48' E and latitude 30° 20' N in Tehsil Vikasnagar, Block Sahaspur of District Dehradun. Rainfall is quite favorable in this area and is more than 1100 mm per annum. However its main concentration is in monsoon season (about 80% of the total precipitation), which is the major cause of erosion in the rainy season. As a result of poor water holding capacity, it supports less biological activities and that is why drought conditions prevail in the summer months. Due to constant erosion over a period of time, soils have been washed and left with very poor nutrient status. The temperature in summer goes more than 45° C and while in winter it goes sometimes below 0°C. The selected experimental torrent at Sabhawala watershed area originating from Doon Shiwalik foothill regions of Himalaya have been identified and delineated for the experiment. The experimental program was planned taking into account the objectives of the study and the parameters were selected to centralize the aim of

sampling to achieve the representativeness and validity of the samples. The study was conducted for two years (April, 2007-March, 2009) and the frequency of sampling was set as per existing conditions during the rainy seasons or flood periods.

Results and Discussion

In the Sabhawala watershed land,damaged by uncontrolled torrent flow during monsoon period is very severe in the area in general and watershed in particular affecting 257.78 ha out of total 1173.6 ha (21.96 %) watershed area. The forest zone consisted 921.1 ha of total area in upper region of watershed in which, the area affected by torrent was found approximately 125.25 ha with 43.01 %. In forest zone, maximum area (20.72 ha) effected by torrent observed in upper reaches of forest zone in transition region of forest and agriculture pasture land. The total area effected by torrent in agriculture land was recorded as 94.18 ha (75.34 %) and played a significant contribution to the flood during the rainy season. Torrent affected area under habitation was recorded as 13.5 ha (27.0 %) out of total 50.0 ha habitational area. The flood plain basin consisting about 77.50 ha out of which 24.85 ha (32.06 %) was affected by torrent (Table.1 and Fig.1).In the upper reach the area affected by torrent is less since the flow is discharged rapidly due to steep slopes. However, in the lower reach area, which used for cultivation and habitation the flood flow spreads along the torrent banks due to sudden reduction in the land slope and caused severe damages affecting over half of the area. It is clear from the recorded data that where the slope is relatively steeper, the flood damage is less compared to the lower reaches where due to flatter slope, the torrent water floods the adjoining lands and affects over half of the total land. This area is most vulnerable to torrent flood hazards the entire cultivation and habitation. The problem has been further aggregated by heavy encroachment of land along the torrent bed for cultivation. About 22 % of the watershed area was affected by the torrent flow hence torrent treatment works was taken up in the lower reaches.



Table -1 Area affected by torrent under Sabhawala watershed

S.No	Particulars	Total area (ha)	Area affected (ha)	Affected area (%)
1	Forest area (upper reaches)	121.0	20.72	17.13
	Forest area (middle reaches)	345.5	41.44	12.00
	Forest area (lower reaches)	454.6	63.09	13.88
2	Agriculture	125.0	94.18	75.34
3	Habitation	50.00	13.50	27.00
4	Flood plain basin	77.50	24.85	32.06
	Total	1173.6	257.78	21.96

In the present study, the torrent treating measures adopted by different developed departments, research organization; NGO's and local communities in selected areas were studied. Comparative per sq/cu meter work cost of different torrent treating measures at Sabhawala watershed was calculated for the identification of effective protective vegetative cover. The cost for the construction of Gabion protection wall of 28.125 cu m volume having the size as length 15 m bottom width 1.5 m, top width 1 m, height- 1.5 m was calculated Rs. 784.71 per cu m work. As the cost for construction of Gabion spur of 5.625 cu m volume having the size as length 3 m bottom width 1.5 m, top width 1 m, height- 1.5 m was calculated Rs. 784.00 per cu m work. The cost for construction of katta crate spurs of 5.625 cu m volume having the size as length 3 m bottom width 1.5 m, top width 1 m, height- 1.5 m was calculated Rs. 624.00 per cu m work. Coir Geonet cost and bioengineering measures for degraded slope of 45 sq m volume having the size as length 15 m width 3 m was calculated Rs. 45.00 per sq. m work. The construction of earthen embankment of 28.125 cu m volume having size as length 15 m bottom width 1.5 m, top width 1 m, height- 1.5 m cost was calculated Rs. 40.00 per cu m work. The cost of planting live vegetative barrier of 45 sq m volume having the size as length 15 m width 3 m was calculated Rs. 20.00 per sq. m work.

The cost of dry vegetative hedge row of 45 sq m volume having the size as length 15 m width 3 m

was calculated Rs. 20.00 per sq. m work.. The cost of seed showing of 45 sq m volume having the size as length 15 m width 3 m was calculated Rs. 10.55 per sq. m work Table.2 and Fig. 2. For best economical torrent treating measure various existing torrent control measures was evaluated on the basis of their comparative cost of having the almost same size.

The performance of spur was evaluated on comparative construction cost basis as smaller patches were built on bank of torrent along with short spur about 3 m in length and width angle 45⁰ projecting downstream (attracting type) spaced at 4 times the length, suitable for flow guidance.

A long spur (15 m) provided at lesser angle (30⁰) also performance well and is suitable for protecting for house or other vital installation near the torrent bank spur caused deposition of sediment behind them which consequently helped in stabilization of torrent bank.

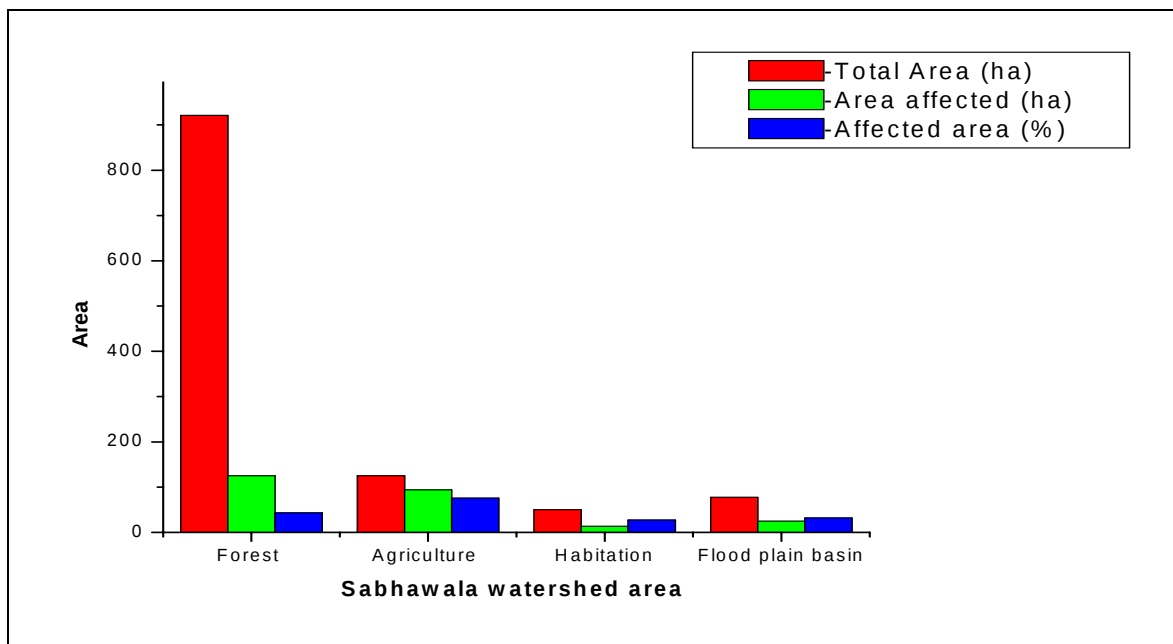
The gabion protection wall requires big size stone which often have to be transported from long distance involving high cost. In order to use the available torrent bed material, which has much smaller in size "Katta Crate" technology was successfully used for construction of torrent training structure. Mortar was filled in empty gunny bags (katta).

These bags were laid as per the design Cement, sand and torrent bed material were mixed in the ratio 1: 6:10 with water and the enclosing them in wire mesh or without it as per the site condition.



Table -2 Comparative cost evaluation of different torrent treating measures for the construction of same size structure at Sabhawala watershed

S.No	Particular	Length	Base /width	Height m	Volume	Amount (Rs.)	Cost of per sq/cu m work
1.	Gabion protection wall	15m	1.25m	1.50m	28.125cu m	22070.00	784.71
2.	Gabion spur	3m	1.25m	1.50m	5.625cu m	4412.00	784.00
3.	Katta crate spurs	3m	1.25m	1.50m	5.625cu m	3509.00	624.00
4.	Coir Geonet	15m	3m	-	45 sq m	2025.00	45.00
5.	Earthen embankment	15m	1.25m	1.50m	28.125cu m	1125.00	40.00
6.	Live vegetative barrier	15m	3m	-	45 sq m	900.00	20.00
7.	Dry vegetative hedgerow	15 m	3m	-	45 sq m	900.00	20.00
8.	Seed sowing	15m	3m	-	45 sq m	475.00	10.55

**Figure 1 Area affected by torrent under Sabhawala watershed**

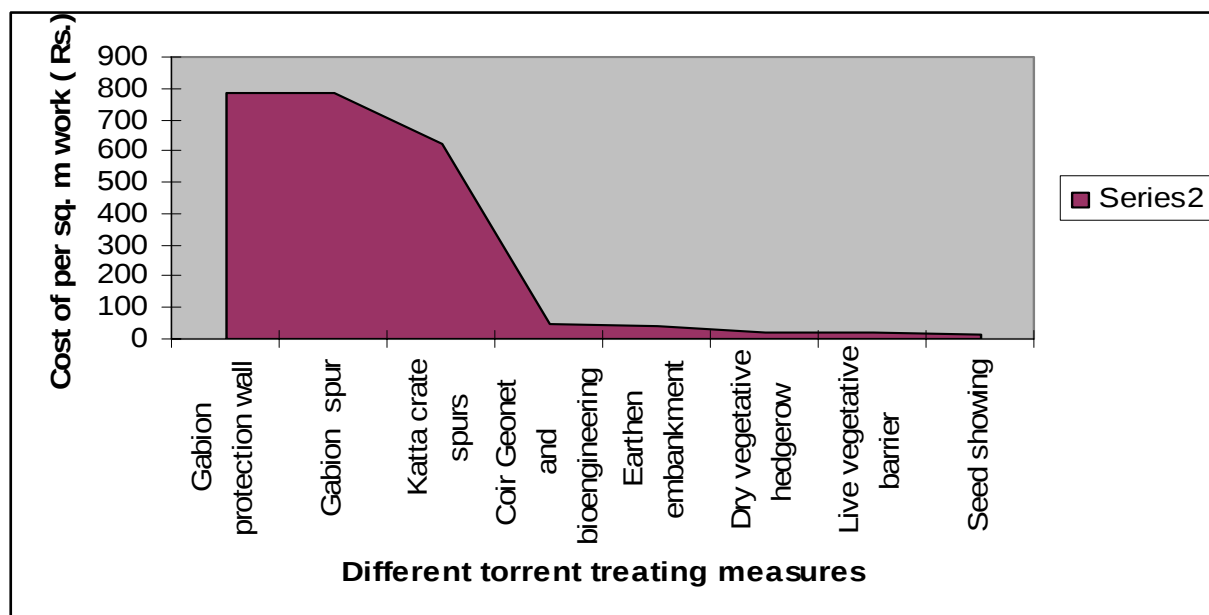


Figure 2 Comparative cost evaluation (of per sq. m/cu m work) of different torrent treating measures

The Katta Crate structures functions well and were 32 % (with wire mesh) and 58% (without wire mesh) cheaper than the conventional gabion protection wall structure respectively. Vegetative barriers and live hedges are used for erosion control purpose in agricultural lands as well as non-arable lands. Vegetative barriers established on farmer's field are expected to perform as erosion diminishing mechanism and also provide fodder, fruits and other minor products. Spurs are structures used for torrent training work. In the present study, various plant species effectively served as vegetative barrier and had better soil binding capacity in the layer, which helped in soil aggregation, increased infiltration rates, improve soil moisture level and conserved maximum runoff. In the present study for the vegetative barrier, live hedges and vegetative spurs, various trees and grasses were planted on bank of the upstream side in the Sabhawala watershed area to identified the best performance of Survival and growth behavior of species like *Dalbergia sissoo*, *Bauhinia purpurea*, *Dendrocalamus Strictus*, *E.hybrid*, *Ipomea carnea*, *Vitex negundo*, *Arundo*

donax, *Eulaliopsis binata* and *Pennisetum purpureum*. Samra and Sharma (1995) made experiment for the soil binding factor for three perennial grasses (*S.munja*, *S.spontaneum*, *Arundo donax*) in sandy choes at Rei Majra and observed that the *S.spontaneum* had the highest above ground biomass (398 kg) followed by *S. munja* (28.3 kg) and least *A. donax* (3.7 kg). Soil binding capacity in 0-10 cm layer was recorded highest for *S.spontaneum* (1590) followed by *S.munja* (788) and *A.donax* (31).

Conclusion

The present study conclude that bio-engineering measures are essential for the protection and rehabilitation of torrential agricultural area for sustainable conservation of agriculture land resource and checked out the losses in habitation area in Sabhawala watershed region. Vegetative measures are cheaper but difficult to establish. Engineering measures provide greater efficiency and stability but are very costly. Combination of both types of measures can be the best option for the torrent control. Proper and planned

combination of engineering and biological remedial measures provides better torrent control than any individual measures used along. The combination of above measures reduced the cost of torrent treatment by ten to twelve times, when compared with the cost of treatment by construction of engineering measures alone at Sabhawala watershed area.

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