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Physico-Chemical and Mycological studies of selected soil samples from Simlipal Biosphere Reserve

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Seven composite soil samples were collected at different geographical locations from Simlipal Biosphere Reserve (SBR) and studied for their physico-chemical parameters and fungal diversity. The soil was observed to be acidic and rich with minerals. The organic carbon percentage and N-P-K contents were reported to be very high. In *toto* twenty three fungi belonging to five different genera *viz. Aspergillus, Penicillium, Paecilomyces, Acremonium* and *Curvularia* were isolated. Total fungal load ranged between 10^3 to 10^5 CFU/gm of soil. *Aspergillus* and *Penicillium* sp. accounted for 47.07% and 35.29%, respectively, whereas, *Paecilomyces, Acremonium* and *Curvularia* sp. accounted for 5.8% each. The isolates showed highest growth in PDA followed by RBA and CDA. Approximately 86.95%, 65.2%, 47.2% and 69.56% of the isolates showed extracellular enzymatic activity for amylase, protease, lipase, and phosphatase respectively, by plate assay method.

Key Words : *Simlipal Biosphere Reserve, Soil Fungi, Amylase, Protease, Lipase , Phosphatase Activity*

Introduction

Simlipal is a densely forested hill-range in the heart of Mayurbhanj district of Orissa (India) lying close to the eastern most end of the Eastern ghats. Located in the Mohanadian biogeographical region and within the biotic province of Chhotanagpur plateau, it spreads over an area of 2,750km^2. The whole of the Simlipal-Hill range comes under Simlipal-Tiger Reserve and is located between 20^0 29′ to 22^0 09′ N Latitude and Longitude 86^{0}04′ to 86^{0}37′ E. Natural forest soil provides an unique environment for growth and survival of microbes and to the best of our knowledge this virgin soil has not been explored in terms of its microbial wealth. This prompted us to undertake the study and the issues addressed in this investigation are: i) study of physico-chemical characters of this virgin forest soil, ii) isolation and enumeration of fungi from collected soil samples, iii) characterization and identification of these fungi, iv) study the growth characteristics of the fungi on different media and v) study and evaluation of special features, if any, of these isolates for their biotechnological implications.

Materials and Methods

Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Starch Agar (SA), and Rose Bengal Agar (RBA) media were used for isolation, enumeration and study of the growth characteristics of the isolates. Further, specialized media such as Skimmed Milk Agar (SMA), Starch Agar (SA), Pickovaskaya's Agar (PA) and Tributyrin Agar (TA) were used to study different enzymatic activities of the isolates. All the media were procured from Hi-Media, Mumbai, Pvt. Ltd. and prepared as per manufacturer's instructions.

Seven composite samples were collected randomly following standard soil sampling techniques (Baruah & Barthakur, 1998) from different geographical locations extending from Gudgudia to Chahala through Bareipani. Different physico-chemical parameters such as moisture content, pH and electrical conductivity, percentage of organic Carbon, Nitrogen, Phosphorous and Potassium contents of these samples were studied, following the standard methods.

Isolation of fungi were done by serial dilution followed by pour plate technique (Cruickshank *et*

al., 1972). Selected colonies were picked, transferred into PDA slants, stored at low temperature for further use. The isolates were identified by lactophenol cotton blue staining (Cruickshank *et al.*, 1972; Rath, 1996).

Isolates were grown by spot inoculation on different media *viz.* PDA, RBA, CDA and SA in order to study their growth requirements and growth patterns. Further, all the isolates were studied for different enzymatic activities such as amylase, protease, lipase and phosphatase by plate assay method (Rath, 1996 & 1999).

Results and Discussion

The physico-chemical parameters of the seven soil samples are presented in Table 1. The moisture content of the samples varied from 3.8 to 59.5%. From the pH studies, it was observed that the soil was acidic. However, negligible variability observed with electrical conductivity of the samples indicates the presence of more soluble salts in the soil, confirming the virginity of soil, not being contaminated with inorganic chemical pollutants. The chemical parameter studies revealed the presence of high percentage of organic carbon in the samples. This could be attributable to the microbial degradation of plant and animal materials in these soils. The nitrogen and potassium content was found to be high and medium, was observed respectively. But surprisingly, high concentration of phosphorous was observed in these soils. Only sample No. 5 and No. 7 fall in the medium range of phosphorous contents. This further gave a preliminary idea for the presence of phosphate solubilizing microbes in these virgin soil. Phosphate solubilizing fungi are of great importance in agriculture, as the combined phosphate present in rock and bones are released, solubilized by microorganisms and made available to the plants (Rao, 1977). This prompted us to test the fungal isolates for their phosphate solubilizing ability.

In total twenty three fungi were isolated from the seven samples and assigned lab nos. CRSF-1, to CRSF-23. Seventeen (73.91%) of the isolates could be identified, based on colony morphology and microscopic characters through cotton blue staining. However, six (26.08%) of the isolates could not be identified, with the existing facilities. Out of 17 isolates identified, *Aspergillus* and *Penicillium* sp. accounted for 47.07% and 35.29%, respectively, whereas, *Paecilomyces*, *Aceremonium* and *Curvularia* sp. accounted for 5.8% each. Among *Aspergillus* and *Penicillium* sp., *A. niger* (62.5%) and *P. notatum* (66.66%) dominated the group. Our results corroborates with findings, of several workers (Anisworth and Bisby,1950; Wiclow *et al.*, 1974; Sodestorm, 1978; Azaz and Pekel, 2002; Sridhar and Masia, 2005). One of the interesting characters encountered with strain CRSF-14 was development of tree like, branched sporangiophores with brown

Table-1

Physico-chemical parameters of the soil samples

Sample	Moisture content (percentage)	pH	Electrical conductivity (in mm hom/cm)	Organic carbon (percentage)	Elements (Kg/ha)		
					Nitrogen	Phosphorous	Potassium
1	11.4	5.89	2 x 0.101	0.781	221	13.4	181
2	40.8	5.25	2 x 0.133	0.905	218	16.1	190
3	14.3	6.07	2 x 0.093	0.815	231	15.3	187
4	6.5	5.32	2 x 0.90	0.669	198	13.8	178
5	22.4	5.68	2 x 0.161	0.738	287	12.0	201
6	3.8	6.42	2 x 0.134	0.664	252	14.0	185
7	59.5	4.95	2 x 0.082	0.598	200	11.9	187

powdery spores in the medium. On microscopy it represented thick, aseptate, branched mycelia with rounded double layered spores (Plate 1).

When the isolates were grown on different types of media, the highest growth was observed on PDA followed by RBA and CDA. Very less growth was

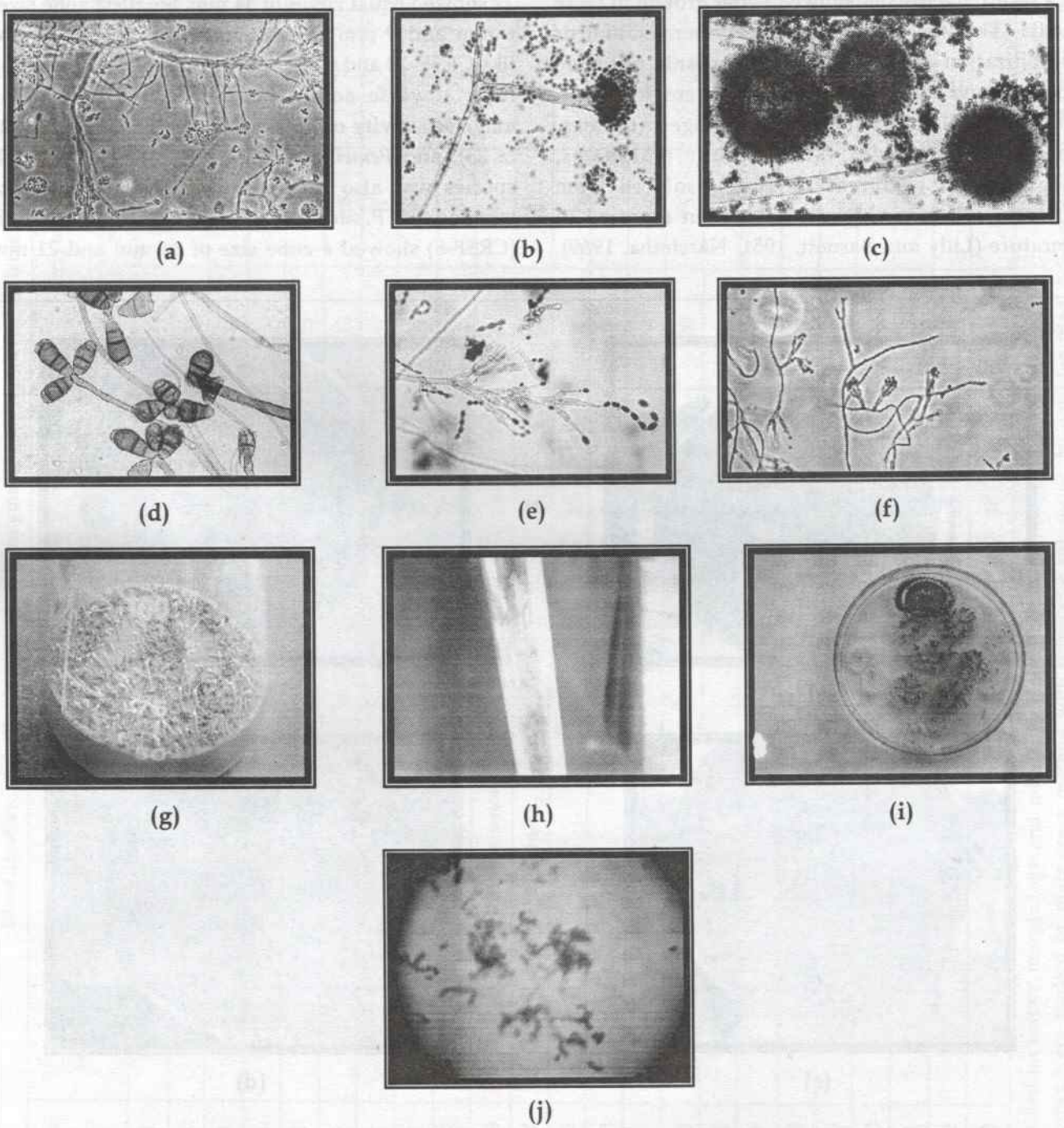

Plate-1: Microphotographs of Fungi : (a) *Acremonium SP* (CRSF-9), (b) *Aspergillus nidulans* (CRSF-6, 15), (c) *A. niger* (CRSF- 1, 4, 7, 16, 21), (d) *Curvalaria lunata* (CRSF-11), (e) *Paecilomyces variotii* (CRSF5), (f) *Penicillum marneffei* (CRSF-8, 20), (g) CRSF-14 on PDA in conical flask (h) CRSF-14 on PDA in test tube (i) CRSF-14 on PDA in petridish (j) CRSF-14 micro photograph

reported on SA, where starch (1% w/v) was the sole source of carbon and minerals (Table 2). In addition to carbohydrates, both CDA and RBA contained mineral sources of nitrate, phosphate and sulphate, as a result, the isolates showed better growth in these media. This further implied the mineralization or solubilization capacity of bound organic nitrogen, phospate and sulphate in these natural environments. Utilization of different natural nitrogen (nitrates, amino acids, amides) and sulphur (sulphates, sulphides etc.) sources by fungi isolated from different soil environments have been reported in literature (Lilly and Barnett, 1951; Narsimha, 1969).

In total twenty (86.95%) of the isolates showed amylase activity when tested on starch agar plate. Strain CRSF-3 showed highest activity (zone size 38 mm) whereas, three strains CRSF-2, CRSF-14, CRSF-22 showed equal zones of 34 mm. Smallest zone sizes 6 mm and 9 mm were observed in case of strains like CRSF-20 and CRSF-13 respectively, representing least amylase activity (Table-2 & Plate-2). The amylase activity of *Aspergillus* (CRSF- 1, 7, 15, 16, 21 & 23), and *Penicillum* (CRSF- 10, 18, 19, 20 & 22) species was also reported here. It is pertinent to mention that *P. variotii* (CRSF-5), *Penicillium marneffei* (CRSF-8) showed a zone size of 31 mm and 21 mm

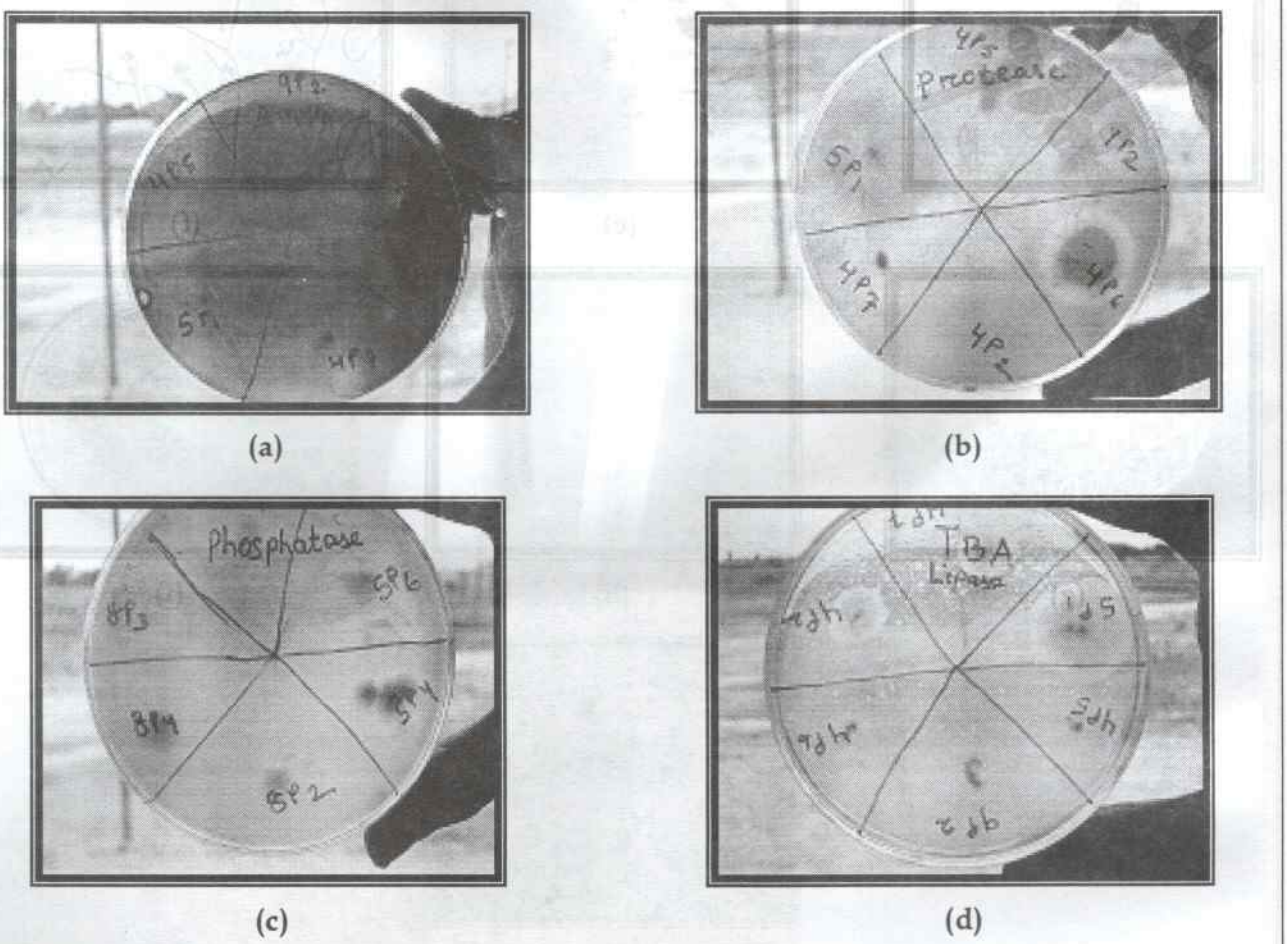

Plate2. Extracellular enzymatic activity of fungi by plate assay method.

(a) Amylase activity on starch agar plate
(b) Protease activity on skimmed milk agar plate
(c) Phospatse activity on Pikovaskay's agar plate
(d) Lipase activity on tributryn agar plate.

Table-2
Identification, growth characters and enzymatic activity of the isolates

Sl. No.	Isolates No.	Soil sample	Fungi*	Growth on different media (Colony size in mm)				Enzymatic activity** of the isolates (Zone size in mm)			
				PDA	CDA	RBA	SA	Amylase	Protease	Lipase	Phosphatase
1	CRSF-1	1	*Aspergillus niger*	18	21	14	4	17	-	5	9
2	CRSF-2	1	NI	50	10	28	NG	34	9	6	-
3	CRSF-3	1	NI	42	35	23	27	38	7	5	10
4	CRSF-4	1	*Aspergillus niger*	22	23	19	3	-	-	6	6
5	CRSF-5	1	*Paecilomyces variotii*	53	23	26	36	31	-	-	8
6	CRSF-6	1	*A. nidulans*	23	18	24	26	13	15	-	6
7	CRSF-7	1	*A. niger*	20	16	19	NG	21	6	4	-
8	CRSF-8	2	*P. marneffei*	21	6	20	9	21	8	-	11
9	CRSF-9	2	*Acremonium. sp.*	26	11	17	9	-	5	-	-
10	CRSF-10	4	*Penicillium notatum*	21	24	16	11	16	-	7	5
11	CRSF-11	4	*Curvularia lunata*	10	6	9	4	22	8	4	-
12	CRSF-12	4	NI	21	17	19	6	-	-	-	-
13	CRSF-13	4	NI	42	19	26	45	9	-	8	12
14	CRSF-14	4	NI	15	6	17	NG	34	14	5	-
15	CRSF-15	6	*A. nidulans*	22	17	19	14	20	15	-	5
16	CRSF-16	6	*A. niger*	21	15	20	8	16	6	-	9
17	CRSF-17	6	NI	14	13	NG	12	11	8	-	7
18	CRSF-18	6	*P. notatum*	16	14	14	10	12	-	-	5
19	CRSF-19	7	*P. notatum*	17	6	15	11	7	-	-	-
20	CRSF-20	7	*Penicillium marneffei*	15	8	7	6	6	7	-	5
21	CRSF-21	7	*A. niger*	19	21	14	11	21	12	-	6
22	CRSF-22	7	*P. notatum*	39	41	61	41	34	20	12	24
23	CRSF-23	7	*A. flavus*	16	23	22	6	16	8	5	6

* Fungi are identified on colony morphology and microscopic observations through LCB staining;

** Enzymatic activities were studied by plate assay method with respective substrates; NI-not identified; NG-no growth; - no enzymatic activity.

respectively. Observation of amylolytic properties of *Penicillium marneffei* and *Paecilomyces variotii* in addition to *Aspergillus* strains could be of commercial interest. Amylase from fungi are used commercially for the production of sugar syrups (Guilbot and Mercier, 1985, Rath and Subramanyam, 1998), and in food industries (Fogarty, 1979).

Fifteen (65.2%) isolates showed protease activity when tested on skimmed milk agar plates. The isolate CRSF-22 (*P. notatum*) showed better activities with a zone of 20 mm. The least activity was reported with strain CRSF-9 (*Acremonium* sp) and CRSF-6 (*A. niger*) with a zone size of 5 mm and 6 mm respectively.

Only 47.2% of the isolates showed degradation of glycerol tributyrate (at 1% v/v conc.) on TA plates as an indicative of lipase activity. CRSF-22 (*P. notatum*) and CRSF-13, showed highest zone sizes of 12 mm and 8 mm respectively. However, the lipolytic activity of other isolates were observed to be more or less similar, as minor differences observed in terms of zone sizes. The smaller zone sizes on solid agar plates is presumably because of the low amounts of lipase molecules released by colonies (Kouker and Jaeger, 1987). It is established that the logarithm of the lipase activity is linearly related to the zone diameter thereby fulfilling the requirement of a valid agar diffusion assay (Lawerence, *et al.*, 1967).

From Table-2, it is clearly evident that 16 (69.56%) isolates showed phosphatase activity. The strain CRSF-22 (*P. notatum*) showed highest degree of phosphatase activity representing a zone size of 24 mm on Pikovaskaya's agar plate. The least activity was reported with strains CRSF-10 (*P. notatum*), CRSF-15 (*A. nidulans*), CRSF-18 (*P. notatum*), CRSF-20 (*P. marneffei*) representing a zone size of 5 mm only. Tambekar and Bhokare (2003, 2006) isolated *Aspergillus, Penicillum, Fusarium* sp. form saline soil with phosphatase activity with a maximum zone size of 20 mm on solid Pikovaskaya's Agar medium. These observations corroborates with our findings. However, one isolate CRSF-22 (*P. notatum*) showed a zone size of 24 mm in the same medium, in contrast to their studies. Phosphate dissolving soil micro-organisms play an important role in correcting phosphorous balance in crop plants, increase the concentration of soluble phosphates in soil, and made them available in the ecosystem through plants.

It may be concluded that the 23 isolated fungal strains, from SBR soil showed extracellular enzymatic activities. Soil enzymes have proved to be of immense biotechnological importance. The role of the fungal isolates from Simlipal Biosphere Reserve Soil are not only important in degradation and transformation of biomass in this environment but could serve as a novel biocatalyst in various industrial processes.

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Alterations in the activities of antioxidant enzymes and induction of lipid peroxidation in wheat seedlings by fly ash

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Fly ash leachate was prepared with distilled water of three different pH (pH 4.0, 7.0 and 9.0) and wheat seedlings were raised, taking the leachate as the growth medium. In the 7-day old seedlings, the activities of antioxidative enzymes like superoxide dismutase (SOD), catalase and guaiacol peroxidase were assayed to study the effects of fly ash on wheat seedlings. Even though there was no significant effect of the pH of the leaching medium on the growth reduction, the SOD and catalase activities declined significantly in the seedlings grown in presence of fly ash leachate, prepared with distilled water of pH 4.0 and this decline was more prominent in the roots than in the shoots. The peroxidase activity increased in the seedlings grown with the leachate and this increase was most prominent in the shoots of the seedlings, grown in presence of the leachate prepared with distilled water of pH 4.0. Malondialdehyde (MDA) content of the tissues was measured to estimate the level of lipid peroxidation and it was found to be increased in the roots and shoots of the seedlings grown in presence of the fly ash leachate prepared with the distilled water of pH 4.0. The above results indicated the imposition of oxidative stress situation in the seedlings by the fly ash leachate prepared with distilled water of pH 4.0. In this regard the acidic pH (pH 4.0), might have important role in the leaching of heavy metal components of fly ash which might have imposed the oxidative stress in the seedlings and this can further be substantiated by measuring the heavy metal concentration of the leachate and the amount plants have absorbed and accumulated. This would help in drawing any meaningful conclusion regarding the effect of fly ash on the antioxidative system of plants.

Key words: *Triticum aestivum, fly ash, pH, superoxide dismutase, catalase, peroxidase, lipid peroxidation.*

Introduction

Adequate supply of energy is very essential for the progress of a nation. Due to rapid urbanization and industrialization, the demand for energy is increasing at an unexpected rate. Burning of coal for generation of electricity has become a reliable source of energy. Since India has vast reserves of coal, it is expected that it will serve as the prime source of energy during the early part of 21st century. About 34.9 million tones of coal is being burnt every year for electric power generation in India (Fulekar, 1993 and references there in; Fulekar and Dave, 1990). During burning, depending on the type of coal, particle size and combustion conditions, 5-15% of the coal remains as ash of which 3-10% is unburnt organic material. The coal ash is subdivided into fly ash and bottom ash. The fly ash accounts to about 90% of the total ash (Grossman and Nathan, 1988). By the hot combustion gases, this lighter fraction (fly ash) is carried away from the boiler and separated by the electrostatic precipitators. According to Nathan *et al.* (1999), the physical properties of the fly ash, such as moisture content, glass composition, particle mass and unburnt carbon portion are dependent on coal properties, combustion temperature, fuel ratio, air flow, coal pulverization size and the rate of combustion. The size of the fly ash generally ranges from 0.5 to 200 microns. It has a hydrophilic surface and is extremely porous. Usually the mineral matter forms fly ash during combustion and is thermally altered into different minerals. Many of these minerals are chemically very reactive by themselves or can

be chemically activated. During and following its formation, coal is enriched with many elements such as B, Cr, Cu, Ni, Mo, S, V, etc. (Cohen *et al.*, 1999) and therefore, these elements also occur in the fly ash, which can contain 20 to 50 trace elements (Nathan *et al.*, 1999).

In coal fired thermal power plants, the electrostatic precipitators capture 99% of fly ash. Thus burning of coal produces huge quantities of bottom as well as fly ash, the management of which has created a challenge world over. As different management strategies, fly ash is being used for preparation of brick, for land filling, and also in the field as an agricultural amendment. Fly ash as well as bottom ash from coal fired thermal power plants is known to contain several toxic elements like Pb, Cd, Zn, and Cu (Gehrs *et al.*, 1979). Fly ash on coming in contact with water, starts leaching. During this process, different elements beneficial for plants like K, Ca, Mg, S, P, etc., along with other heavy metals like cadmium, chromium, lead etc. are also leached out of the fly ash to the environment. The pH of the medium has a significant role on leaching process (Fulekar, 1993) and heavy metal's leachability drops sharply upon going from pH 4.0 to pH 9.0. Hajarnavis and Bhide (1999) have detected the presence of heavy metals like cadmium, copper, chromium, lead, zinc, etc. in fly ash leachate. Thus, when fly ash is applied in the fields, plants pick up these heavy metals through their roots along with other beneficial elements. The heavy metals are known to cause several physical and physiological anomalies including the imposition of oxidative stress in plants (Verma and Dubey, 2003; Benavides *et al.*, 2005). Therefore, in this study attempts have been made to raise the wheat seedlings with fly ash leachate (prepared with distilled water of different pH), and antioxidative efficiency of these seedlings were analyzed.

Materials and Methods

Fly ash leachate preparation

Fly ash used for the preparation of fly ash leachate was collected from the electrostatic precipitator of a coal-fired power plant of "Birla Tyres", a tyre manufacturing industry, located at Balasore, Orissa. Fly ash leachate preparation was done under laboratory conditions at 30°C. 10g of fly ash was taken in each 500ml of conical flask containing 100ml of distilled water of different pH (pH 4.0, pH 7.0 and pH 9.0) to prepare 3 fly ash slurries. The pH of the distilled water was adjusted by adding dilute HCl or NaOH, as required. The slurries were allowed to stand for 7 days and during this period all the flasks were covered with aluminium foil to avoid evaporative loss of water and were shaken 4 times daily. Then the contents of the flasks were filtered separately and the filtrates were collected as fly ash leachate. These leachates (prepared with distilled water of different pH) were used to raise wheat seedlings to study its effect on the antioxidative efficiency of the wheat seedlings.

Plant material and growth conditions

Wheat (*Triticum aestivum* L.) seeds were selected for the uniformity of the size and then were surface sterilized with 3% solutions of commercial bleaching powder (calcium oxychloride, $CaOCl_2$) for 30 minutes and then washed thoroughly for several times with distilled water. The seeds were then germinated over moist filter paper in Petri dishes in the dark. After 24 h of germination, 20 uniformly germinated seeds were transferred to nylon nets stretched over small plastic glasses containing fly ash leachate, prepared with distilled water of pH 4.0, 7.0 and 9.0. One glass containing distilled water was taken as control. The plants were grown at 8 h light/16 h dark cycle at 30±1°C. The light source was a bank of six 40 W Phillips cool fluorescent tube lights supplemented with 40 W incandescent bulbs.

Growth study

Growth of the wheat seedlings, was studied by collecting the 7-day old seedlings and measuring the root and shoot lengths, separately.

Biochemical analyses

Buffer (50 mM phosphate buffer, pH 7.5) soluble protein and was estimated by the phenol reagent method, as described by Lowry *et al.* (1951). To study the level of lipid peroxidation in the tissues (both in roots and shoots), malondialdehyde (MDA) was extracted with 5% trichloroacetic acid and was

estimated as the thiobarbituric acid reactive substance (TBA-RS) by following the method of Heath and Packer (1968).

Enzyme extraction and assay

Root and shoot tissues of 7-day old wheat seedlings sodium phosphate buffer were taken separately and homogenized under ice-cold conditions in a mortar and pestle using different extraction buffers containing 10% (w/v) insoluble polyvinylpolypyrrolidone. The buffers used for the extraction of enzymes were: 50 mM sodium phosphate buffer, pH 7.5 for catalase and peroxidase; and 50 mM phosphate buffer, pH 7.4 for superoxide dismutase. The homogenates were centrifuged at 17,000g for 10 minutes at -4°C. The resultant supernatants were collected for assaying enzyme activities.

Catalase (EC 1.11.1.6) was assayed by recording the decrease in the absorbance due to H_2O_2 depletion at 240 nm (Aebi, 1983). Peroxidase (EC 1.11.1.7) was assayed by taking H_2O_2 as the substrate and guaiacol as the reduced co-substrate and the increase in the absorbance due to tetraguaiacol formation was recorded at 470 nm as described by Kar and Feierabend (1984). In this study, SOD (EC 1.15.1.1) was assayed by measuring the inhibition of superoxide-driven nitrite formation from hydroxylamine hydrochloride, as formulated by Das *et al.* (2000). The nitrite reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with α-naphthaloamine to produce a red azo compound whose absorbency was recorded in a spectrophotometer at 543 nm. Initially in this reaction, $O_2^{\cdot-}$ was generated by photoreduction of riboflavin.

Presentation of data

All the experiments were carried out at least for three times and the mean values are presented here. Standard deviations are indicated.

Results

The effect of fly ash leachate on the growth of the wheat seedlings was determined by measuring the root and shoot length of 7-day old seedlings and the results are presented in Fig. 1. Both the root and shoot lengths had decreased in the seedlings grown in presence of leachate (prepared with distilled water of pH 4.0, 7.0 and 9.0), in comparison to that of control (seedlings grown with distilled water). However, the pH changes of the leaching medium do not have any significant effect on the changes in the seedling growth. The soluble protein levels also declined in the seedlings grown in presence of fly ash leachate in comparison to the control and it was lowest in the roots and shoots of the seedlings grown in presence of leachate, prepared with distilled water of pH 4.0 (Fig. 1). With the increase in the pH of the

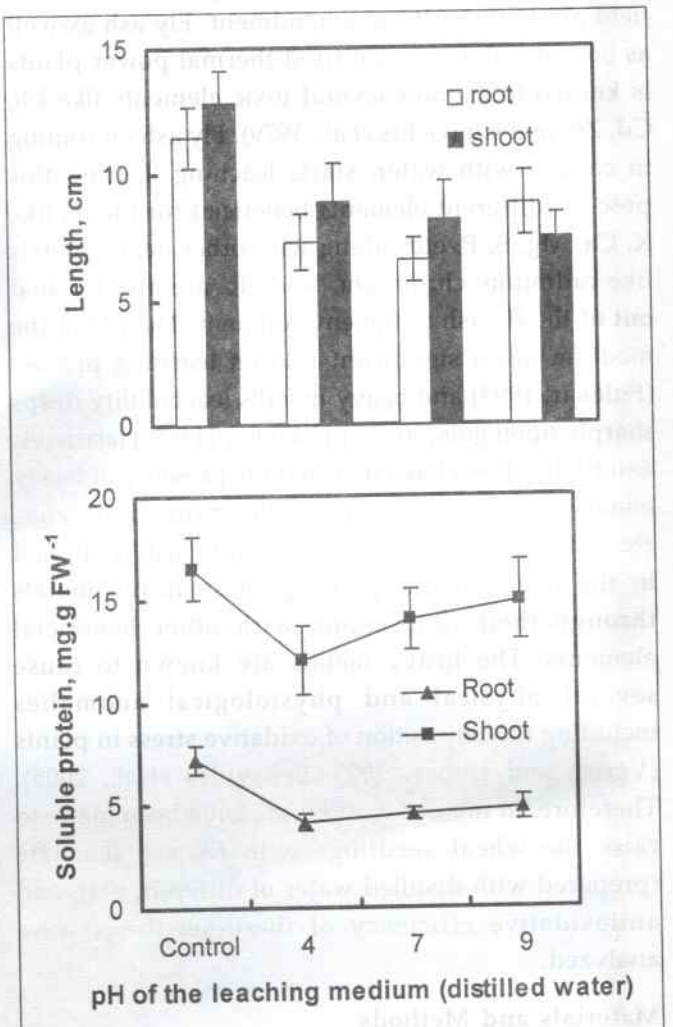

Fig.-1 : Changes in the root and shoot length and soluble protein level in 7-day old wheat seedlings grown in presence of fly ash leachate prepared with distilled water of pH 4.0, 7.0 and 9.0. The control seedlings were raised with distilled water.

growth medium (pH 7.0 and 9.0), there was slight increase in the soluble protein levels both in the roots and shoots, but the levels were always lower than that of the control seedlings.

The enzymes like SOD, catalase and peroxidases are important antioxidative enzymes that protect against oxidative corrosion in the aerobic organisms by scavenging the reactive oxygen species like $O_2^{\cdot-}$ and H_2O_2. The activities of these enzymes were measured in the seedlings grown in presence of fly ash leachate and the results are presented in Fig. 2. The catalase activity in the seedlings grown in presence of leachate (prepared with distilled water of pH 4.0) was significantly low in comparison to that of the control. Both in roots and shoots, the activity increased with the increase in the pH of the leaching medium. At pH 9.0, even the catalase activity in roots was slightly higher than the control, whereas in shoots the activity was slightly lower. Unlike catalase activity, there was increase in the peroxidase activity both in roots and shoots of the seedlings, grown in presence of fly ash leachate (Fig. 2). But this increase was very significant in shoots of the seedlings, grown in presence of fly ash leachate prepared with distilled water of pH 4.0. Increase in the pH of the leaching medium did not significantly change the peroxidase activity in the root tissues. Similar to catalase the wheat seedlings, grown in presence of fly ash leachate, showed decreased SOD activity in comparison to their control. It is important to note that this decline was maximum in the roots and shoots of the seedlings grown in presence of the leachate, prepared with distilled water of pH 4.0.

Lipid peroxidation is a good indicator of prevalence of oxidative stress in aerobic cells, since it is the consequence of free radical mediated reactions (Kappus, 1985). In this study, the level of lipid peroxidation was measured by estimating malondialdehyde (MDA) which is a decomposition product of peroxidized membrane lipid. In root tissues of seedlings grown with fly ash leachate, there were higher levels of MDA and maximum MDA content was recorded in the roots of the seedlings grown in presence of leachate prepared with distilled water of pH 4.0 (Fig. 3). However in shoot tissues of pH 4.0 leachate, although there was slightly higher level of MDA than the control shoots, it further declined with the increase in the pH of the leaching medium. At pH 7.0 and 9.0 leachate, the level of

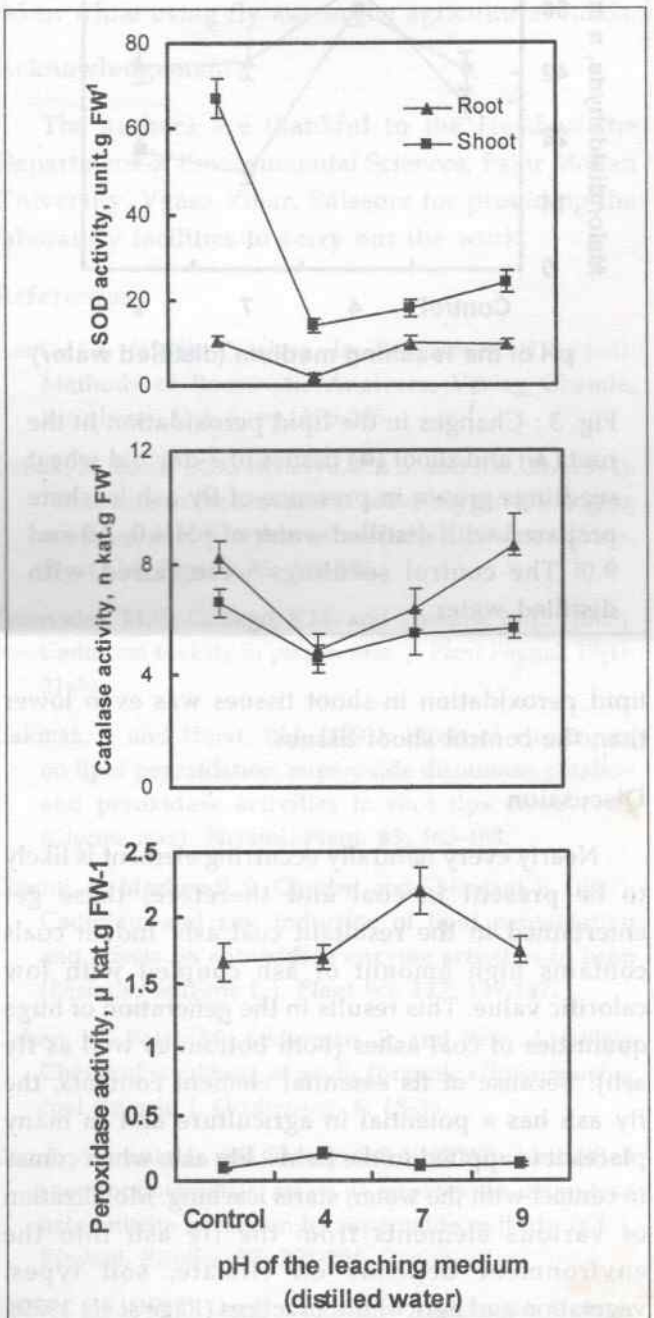

Fig. 2.: Changes in the activities of SOD, catalase and peroxidase in the root (▲) and shoot (■) tissues of 7-day old wheat seedlings grown in presence of fly ash leachate prepared with distilled water of pH 4.0, 7.0 and 9.0. The control seedlings were raised with distilled water.

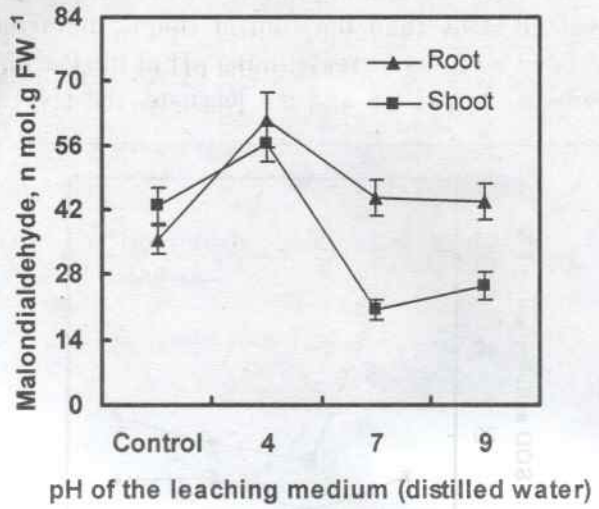

Fig. 3 : Changes in the lipid peroxidation in the root (▲) and shoot (■) tissues of 7-day old wheat seedlings grown in presence of fly ash leachate prepared with distilled water of pH 4.0, 7.0 and 9.0. The control seedlings were raised with distilled water.

lipid peroxidation in shoot tissues was even lower than the control shoot tissues.

Discussion

Nearly every naturally occurring element is likely to be present in coal and therefore, these get entertained in the resultant coal ash. Indian coals contains high amount of ash coupled with low calorific value. This results in the generation of huge quantities of coal ashes (both bottom as well as fly ash). Because of its essential element contents, the fly ash has a potential in agriculture and in many places it is applied in the fields. Fly ash, when comes in contact with the water, starts leaching. Mobilization of various elements from the fly ash into the environment depends on climate, soil types, vegetation and agriculture practices (Page *et al.*, 1979). Among the different elements, the heavy metal components are matter of serious environmental concern. The most important aspects of leaching mechanism is the effect of the pH of the water on leaching process. The lower pH is found to be efficient mobilizer of heavy metals in fly ash leaching (Fulekar, 1993). In the present study among the different fly ash leachate, the leachate prepared with distilled water of pH 4.0, had significant effects in altering the activities of important antioxidative enzymes like SOD, catalase and peroxidase in the wheat seedlings (Fig. 2). Even though there was decrease in the root and shoot lengths of the seedlings in comparison to their control (Fig. 1), pH as a function of the leaching medium does not have significant effect on the growth. The lower levels of soluble protein in the root tissues of the seedlings grown in presence of fly ash leachate in comparison to their control (Fig. 1), suggests that probably there might have higher level of accumulation of heavy metals in the roots than in shoots, which might have reduced the protein levels.

SOD, catalase and peroxidase are three important antioxidative enzymes in the cells that prevent the accumulation of different reactive oxygen species. SOD dismutates $O_2^{\cdot-}$ to H_2O_2 and O_2 while catalase and peroxidase decompose H_2O_2. This causes the prevention of the formation of $^{\cdot}OH$ since this highly toxic oxygen radical is formed by the interaction of $O_2^{\cdot-}$ and H_2O_2, the reaction being catalyzed by transition metal ions (Elstner, 1982). In the present study, the activities of SOD and catalase had declined both in the root and shoot tissues and the root tissues of the seedlings grown in presence of the leachate prepared with distilled water of pH 4.0 had the lowest catalase and SOD activities (Fig. 2). This has resulted in the higher build up of $O_2^{\cdot-}$ and H_2O_2 in the tissues. Even though there was elevation in the peroxidase activity in the roots as well in shoots of the treated seedlings in comparison to the control, this higher activity can not be attributed to the efficient H_2O_2 scavenging capacity in the tissues. This is because peroxidases are widely accepted as stress enzymes and induction in peroxidase activity has been reported under a variety of stressful situations like water stress, salt stress, heavy metal stress etc. (Zhang and Kirkham, 1994; Baisak *et al.*, 1994; Mittal and Dubey, 1991; Cakmak and Horst, 1991). Thus here the increase in the peroxidase activity in the seedlings grown in presence of the fly ash leachate indicates the prevalence of oxidative stress in the tissues. This increase in the peroxidase activity might be possibly due to the increased release of guaiacol peroxidase, localized in the cell wall.

Decline in the activities of SOD and catalase favours the accumulation of $O_2^{\cdot-}$ and H_2O_2 in the tissues. In presence of transition metal ions, these two reactive oxygen species interact to form ·OH, the most toxic oxygen species. In the aerobic cells, lipid peroxidation is a good indicator of the prevalence of oxygen free radical mediated reactions since unsaturated fatty acid components of membrane lipids are highly susceptible to ·OH attack and are peroxidized in the presence of ·OH (Elstner, 1982). In this study, the roots as well as the shoot tissues of seedlings grown in presence of fly ash leachate prepared with distilled water of pH 4.0 had higher levels of MDA (Fig. 3), the decomposition product of peroxidized membrane lipids. This indicates the higher level of lipid peroxidation in those seedlings. However, the shoot tissues of seedlings grown in presence of fly ash leachate prepared with distilled water of pH 7.0 and 9.0 had lower levels of lipid peroxidation than the control, which might be due to the presence of fewer amounts of heavy metals and more amounts of essential nutrients in that leachate.

The results of this study indicate that among the three different fly ash leachate, the one prepared with distilled water of pH 4.0 had significant effect in decreasing the SOD and catalase activities and increasing the peroxidase activity in the wheat seedlings and these alterations are more pronounced in the roots than in shoots. Because of these alterations, an oxidative stress situation had imposed in the seedlings and this was indicated by the higher levels of lipid peroxidation. As reported by Fulekar (1993), at acidic pH (pH 4.0), significant amount of different heavy metals are leached from fly ash. The heavy metals like lead (Verma and Dubey, 2003), cadmium and zinc (Chaoui *et al.*, 1997) are known to induce the lipid peroxidation and alteration in the activities of key antioxidative enzymes. Therefore, in this study, it is presumed that probably the seedlings grown in presence of fly ash leachate prepared with distilled water of pH 4.0 might have accumulated higher level of heavy metals from the medium, which have imposed the oxidative stress situation in the seedlings. But for this, measurement of the different heavy metal concentration in different leachate and the amount the plants has accumulated in roots and in shoots is very essential to draw any meaningful conclusion. However, these preliminary findings suggest that enough precautions should be taken while using fly ash in the agricultural fields.

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A Glimpse of the Vegetation of Ansupa Lake, Orissa

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An account of the aquatic, semi-aquatic and marshland plants of Ansupa lake of Orissa has been presented in the paper. The flora of this wetland is represented by 150 angiospermic plant species under 105 genera and 46 families. The dominant families are Poaceae (29) followed by Cyperaceae (17), Scrophulariaceae (11) and Asteraceae (8). The dicot and monocot ratio is 1:1. The conservation strategies and restoration of the lake have been discussed in brief.

Introduction

Orissa harbours a rich and varied vegetable wealth due to its peculiar geographic location and topography. Different vegetational types are metwith in the state of Orissa, of which the aquatic/wetland vegetation deserve special mention. The aquatic flora of Orissa is very rich and diverse. In addition to these, there are two lakes namely Chilika and Ansupa. Ansupa the largest fresh water lake had rich aquatic flora. But this is depleting significantly in the course of time due to severe anthropogenic pressure coupled with other environmental factors. As a result, many species have been disappeared and some are on the verge of extinction. In recent times wetlands have attracted the attention of scientific populace for their conservation and sustainable utilisation. Realising the importance of wetlands, a survey programme was undertaken. In this programme, a total of 150 angiospermic species have been collected, identified with the help of modern floras and preserved as herbarium specimens in the Herbarium of Ravenshaw College, Cuttack. The species have been preserved following standard procedures (Jain and Rao, 1977).

Location

Ansupa, the largest freshwater lake of Orissa lies between 85^0 35' - 85^0 37' E longitude and 20^0 25' - 20^0 30' N latitude in the Banki subdivision of Cuttack district. Ansupa, the so called sister lake of Chilika once was looking like a heavenly picture gallery with small fishing boat on the blue waters and hills on the banks, birds of different colours and above all lotuses and lilies. The lake is bounded by Saranda hills on its western side, Bishnupur hills on its north east side and surrounded by villages like Subarnapur, Kadalibadi, etc. The lake is connected with river Mahanadi on its south-east side by a channel known as Kabuli Nallah. Ansupa extends over an areas of 8.0 sq. Kms. having length and breadth of 4 Kms. and 2 Kms. respectively. The water depth is 10-15 mt.

Soil and Climate

The soil of the lake consists of silt loam or clay loam. The margin of the lake is sandy in nature often mixed with clay. The climate of the lake is tropical and monsoonal i.e. Summer, Rainy and Winter. June is the hottest month with a temperature of 104^0F and December is the coldest month with minimum temperature of 55^0F. The annual rainfall is 1241.96mm.

Review of Literature

Haines (1921-25), the pioneer plant explorer for Bihar and Orissa collected plants from Bihar and Orissa and compiled his treatise "The Botany of Bihar and Orissa", but he did not collect plants from hill terrains as well as river estuaries. Realizing this, Mooney (1950) made extensive survey of plants from Western Orissa and many river estuaries. But, both the workers did not make any special mention of aquatic flora although wetland vegetation is very important and rich in Orissa.

After this, Patnaik (1956) and Patnaik and Chyau-Patnaik (1956) reports some weeds and hydrophytes of Cuttack. Then floristic studies in general and aquatic flora in particular remained arrested for a long time. Later on Panda and Choudhury (1984) and Rath *et al.* (1979) reported grasses and sedges of Cuttack city and Orissa respectively including many aquatic species. Realising the unexploredness of aquatic plants, Mohanty and Choudhury (1984) reported some hydrophytes of Cuttack. But the information is very scanty. After this, Subudhi (1991) and Panda (1992) compiled the flora of Cuttack and Puri districts respectively, where they have made exhaustive collection of aquatic plants including mangroves. Das *et al.* (1994), Choudhury and Choudhury (1996), Swain *et al.* (2002) surveyed the wetland flora of east coast of Orissa. Besides this Subudhi *et al.* (2002) reported 76 aquatic weeds from Cuttack district of Orissa.

It is evident that there is no systematic survey of aquatic plants of Orissa in general and Ansupa lake in particular, which is the only freshwater lake and grand repository of aquatic plant wealth.

Statistical Analysis

During this survey, a total of 150 angiospermic species under 105 genera belonging to 46 families were collected and identified and deposited as herbarium specimens in Ravenshaw College. The dominant families are Poaceae (29) followed by Cyperaceae (17), Scrophulariaceae (11), Asteraceae (8), Fabaceae (7) etc. The total dicot and monocot species are 76 and 74 respectively and the ratio is 1:1, out of 74 monocot species, 46 are grasses and sedges (Table-1).

Table-1

Group	Family		Genera		Species	
	No	(%)	No	(%)	No	(%)
Dicotyledones	32	69.6	53	50.5	76	50.6
Monocotyledones	14	30.4	52	49.5	74	49.4
Total	46		105		150	

Vegetational Analysis

The vegetation of Ansupa lake is mostly of aquatic/semi-aquatic type. Some marshy plants are also met with during the dry season (November-May). Usually, the following types of vegetation are very common in this lake. These are free-floating, emergent, submerged, amphibious, sedges and grasses and marshland vegetation.

Free-Floating

The species *viz. Nymphoides indicum, Nymphoides hydrophylla, Wolffia globosa, Ipomoea aquatica, Ludwigia adscendens, Neptunia oleracea, Pistia stratiotes, Trapa natans var. bispinosa, Myriophyllum tetrandrum, Spirodella polyrhiza* etc. are very common. Pure formation of *Eichhornia crassipes* is met with very luxuriantly floating on the water surface of the lake.

Emergent

The species of this category are *Nymphaea nouchali, Nymphaea stellata, Nelumbo nucifera, Sagittaria sagittifolia, Monochoria hastata, Monochoria vaginalis, Ottelia alismoides, Aponogeton natans, Sagittaria guayanensis ssp. lappula.*

Sub-merged

The species like *Ceratophyllum demersum, Hydrilla verticillata, Ottelia alismoides, Blyxa echinosperma, Utricularia inflexa var. stellaris, Vallisneria natans, Nechamandra alternifolia, Najas faveolata, Potamogeton nodosus, Potamogeton pectinatus, Enydra fluctuans, Utricularia aurea* are very common in this category.

Amphibious Forms

After November, the water area gradually decreases. Some of these areas are being cultivated with paddy. In this area, some marshy plants usually occur during December-May. The common plants are *Polygonum hydropiper, Aschynomene aspera, Aschynomene indica, Limnophila indica, Caesulia axillaris, Phyla nudiflora, Dopatrium junceum.* In the dry season, *Bacopa monieri, Fimbristylis miliacea, Rotala indica, Ammania baccifera* etc. are very common. In the marshy places, the species viz *Limnophyton obtusifolium, Polygonum plebeium, Rumex maritimus, Ludwigia octovalvis, Centella asiatica, Blumea lacera, Caesulia*

axillaris, Sphenoclea zeylanica, Hoppea dichotoma, Hydrolea zeylanica, Lindernia parviflora, Verbascum chinense, Lippia javanica, Boerhavia diffusa etc. are commonly met with.

Sedges and Grasses

Usually in the dry season, more number of sedges and grasses are seen. The common sedges are *Schoenoplectus articulatus, Mariscus compactus, Fimbristylis ferruginea, Fimbristylis miliacea, Cyperus platystylis, Cyperus corymbosus, Fuirena ciliaris, Lipocarpha chinense* etc. The common grasses are *Echinochloa crusgalli, Echinochloa stagnina, Hygroryza aristata, Isachne globosa, Oryza rufipogon, Panicum repens, Apluda mutica, Arundinella pumila, Brachiaria ramosa, Chloris barbata, Elytrophorus plicatus, Eragrostris gangetica etc.*

Conservation Strategies

In the remote past, Ansupa was endowed with very rich and diverse wetland vegetation which has gradually been depleted. Although the lake harbours a rich vegetable wealth but due attention was not given for exploration, conservation and restoration of the lake. So, it is high time to document the information and conserve the flora for future use. Due to heavy siltation of the lake and erosion in the catchment area, the lake is going to be converted to a fallow land. The water area is also decreasing. People are also cultivating paddy in this area. Hills around the lake are devoid of vegetation which has resulted in siltation of the lake. Plantation of tree/ shrub should be taken up in the peripherial area to check soil erosion. The mouth of the lake should be opened to river Mahanadi to allow permanent flow of water to check pollution of water. Now the lake is full of *Eichhornia crassipes* and *Pistia stratiotes*. These should be cleaned totally and boating facility and pisciculture should be encouraged in the lake, so that local people will be benifited economically. It should be declared as tourist spot and find place in the tourist map of Orissa. As it is the only fresh water lake in Orissa it will attract the birds from other far off places during winter season and like Chilika lake it could be the home land of several species of birds.

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In vitro Propagation of *Desmodium gangeticum* : A medicinal plant

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Cotyledon segments from *in vitro* grown seedlings of *Desmodium gangeticum* were cultured on to Murashige and Skoog (MS) medium supplemented with different concentration and combination of plant growth regulators. Highest regenerative response was observed in the medium containing 0.5mg/l BA (N^6-benzyl adenine). Regenerated shootlets were cultured on to MS medium containing different concentration of IBA (Indole-3-butyric acid) and IAA (Indole-3-acetic acid) for rooting.

Key words: cotyledon segments, growth regulators and shoot buds induction

Introduction

Desmodium gangeticum (L.) belonging to the family Fabaceae commonly known as Salaparni or Prishniparni (Hindi & Sanskrit), have multiple uses in the Indian system of medicine, particularly in the Ayurvedic system. The plant grows mainly in South East Asia and Northen Australia. Prishniparni is used by vaidyas of Travancore and Cochin (Pillai *et al.*, 1981). As a single drug it's effect has been described on pregnancy with reference to abortions (Pillai *et al.*, 1981). This plant is widely distributed in India ascending to 5000ft in the Himalayas. It contains indole alkaloids in its green parts. The plant shows anthelmintic, aphrodisiac, astringent, diuretic properties. It is used in general anasarca, consumption, cough, diarrhea, dysentery, chronic fever including enteric fever, piles, respiratory disorder, vomiting, worms, asthma, snake bite and scorpion sting (Kurup *et al.*, 1979; Anonymous, 1952). Kirtikar and Basu (1975) have listed various uses of roots of *D. gangeticum* and have reported that the seeds contain significant amount of b-carbaline alkaloids. Roots are chewed daily for the cure of typhoid and pneumonia. Boiled root extract of several plants including *D. gangeticum* is orally administered to overcome weakness. Paste prepared from the root mixed with water and sugar candy when taken orally in empty stomach in the morning daily for about a week prevents spermatorhoea. It was found that *Desmodium gangeticum*, mostly found in sal forest, shows annual seed set (in the month of March-May) and low rate of multiplication in the natural condition. Due to its high medicinal value, there is an urgency to develop appropriate technique for mass propagation of this valuable species and to domesticate it for future use. In the present study a protocol for regeneration and mass propagation of *D. gangeticum* has been established using cotyledon from invitro grown seedling explants.

Materials and Methods

Explant Preparation

Ripe and dried pods *Desmodium gangeticum* were collected. Seeds were removed and were initially rinsed thoroughly in running tap water and agitated in a liquid detergent solution (2% Labolene; Qualigen, India), for 15-20 minutes followed by agitation in 0.1% $HgCl_2$ for 7-8 minutes for surface sterilization. Finally the seeds were thoroughly rinsed in sterile double distilled water for at least four times under aseptic conditions and the sterile seeds were inoculated on to basal Murashige & Skoog (MS) medium (Murashige and Skoog, 1962) containing Kn 0.1 mg/l and incubated in an incubator for germination. Segments of cotyledon (0.5 to 1.0 cm) were aseptically excised from 10 days old aseptically grown seedlings and cultured on MS medium containing different plant growth regulators. All

operations during inoculation including surface sterilization were carried out inside the laminar airflow cabinet (Thermadyne, India).

Media preparation and culture condition

MS salt composition, sucrose (30 g/l) and bacteriological grade agar-agar (8 g/l, bacteriological grade; Qualigens, India) were used throughout the study. For shoot induction, growth regulators BA (N^6-benzyl adenine), IAA (Indole-3-acetic acid) and Kn (Kinetin) alone or in combination were added to the MS basal medium. For root induction MS basal medium supplemented with either IAA or IBA (Indole-3-butyric acid) were incorporated and the pH was adjusted 5.8. All the explants were incubated in a culture room maintained at 25 ± 2°C under 16 h/8 h light/dark photoperiod, 45 mmol m^{-2} s^{-1} irradiance level provided by cool white fluorescent tubes (Philips, India) and with 55-60% RH. Each treatment consisted of 10 replicates and repeated thrice.All basal salts were procured from Qualigen Fine Chemicals, India. And these growth regulators were procured from M/s Sigma Chemicals, USA.

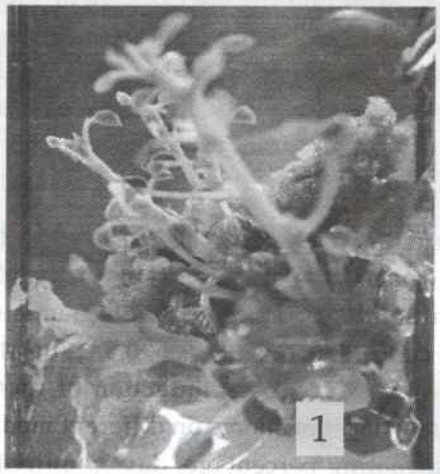
Results

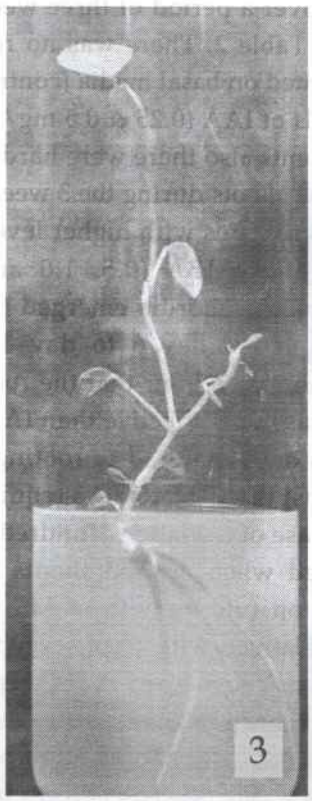
Desmodium gangeticum seeds cultured on plane MS media for germination were not responded. Then trial was taken in MS medium supplemented with 0.1 mg/l Kn. Hundred percent of the *D.gangeticum* seeds germinated within 4-5 days of inoculation in a medium containing Kn 0.1 mg/l After two-three weeks of germinations the cotyledon segments were excised and transferred to media containing different hormone concentrations. The explants failed to show any morphogenetic response on growth regulator free MS medium. Explants cultured on MS medium supplemented with growth regulators showed swellings at different points along the cut surface within the first week of inoculation and continued to form highly compact green and brown structures. In the subsequent 1-2 weeks some of the green structures developed into shoot buds while the rest brown and green structures enters into callusing (Fig. 1). Both the callus and shoot buds continued to grow simultaneously and at a later stage of the culture explants covered with fast growing callus as well as shoots growing in length. Some of the cotyledon segments showed either callusing or only shoot buds or both callusing and shoot buds. Among the different concentrations and combinations tested, MS medium enriched with BA 0.5mg/l stimulated the best response in terms of shoot formation in which each cotyledon produced 68.3 ± 0.333 shoots in an average with shoot length of 3.3 ± 0.5cm (Fig. 2).

When regenerated shoots attained a length of about 3 cms or more, they were excised and planted on semi solid basal medium supplemented with varying concentrations (0, 0.25, 0.5, 1.0 or 1.5 mg/l) of either IAA or IBA for induction of rooting. The rooting responses of shoots on different media, which included rooting percentage, days required for root initiation, mean number of roots/shoot and mean root growth over a period of three weeks have been presented in Table 2. There was no rooting in case of shoots planted on basal media (control). Similarly, at lower levels of IAA (0.25 & 0.5 mg/l) or IBA (0.25 mg/l) treatments also there were hardly any rooting in the cultured shoots during the 3 weeks observation period. In all cultures with higher levels of IAA (1.0 and 1.5 mg/l) or IBA (0.5, 1.0 and 1.5 mg/l) treatments, root primordia emerged from the shoot base starting from day 6 to day 16 after shoot inoculation and soon after that the root growth was rapid. IBA was more effective than IAA in induction of rooting as days required to rooting was only 6-8 days as against the 12 to 16 days required for similar response in case of the latter. Hundred percent result were obtained when excised shoots 2 cm or more were rooted on full strength of MS with 1.5 mg/l IBA (Fig. 3). Plantlets with six or seven fully expanded leaves and well developed roots were successfully hardened in a growth chamber on vermiculite for 4 week and subsequently transferred to a mixture of sand: soil (1: 1) (Fig. 4). The percent of survival was 100%. So starting with a single cotyledon explant one could get about 50-70 shoots could be obtained

Discussion

The dependence of cultured explants on plant growth regulators for bud break and shoot multiplication was extensively discussed (George and Sherrington, 1984). This has also been recently reported in case of micropropagation of many





***In vitro* propagation of *Desmodium gangeticum* through direct shoot regeneration from cotyledon explants**

1. Formation of shoot buds and callus from cut surface (4 weeks old)
2. Cluster of shoots (8 weeks old)
3. A single shootlet rooted on MS basal media supplemented with IBA1.5mg/l.
4. A hardened plant (5 weeks old)

Table-1

Effects of various mediums on shoot bud regeneration of *in vitro* grown cotyledon explants of *Desmodium gangeticum*

MS with growth regulators (Mg/l)	Treatments	COTYLEDON		
	BA	% culture respond	Number of Shoots	Shoot length
	0.25	91.6±0.33c	61.6±0.33c	1.8±0.02e
	0.5	99.8±0.16*a	68.3±0.33a	3.3±0.05a
	1	99.3±0.33*a,b	64.3±0.66b	2.7±0.03b
	1.5	98.2±0.17a,b	61.3±0.88c	2.3±0.044e
	Kn			
	0.25	60.0±0.73e	58.3±0.33d	2.4±0.03d,e
	0.5	80.0±1.15d	56.0±0.57e	2.5±0.03c,d
	1	98.0±0.57a,b	59.3±0.24d	2.4±0.01
	1.5	89.6±0.88c	51.1±0.16f	2.6±0.02b,c
	BA0.25 & IAA 0.25	97.0±1.00b	58.3±0.33d	2.70±0. 03b

Data (mean ± SE) of three independent experiments each with 20 replicates. Means followed by the same letter within the column are not significantly different ($p<0.05$) as tested by the multiple range test of Duncan (1955). (*) –Some explants responded to callus formation. Shoots measuring < 0.5 cm not taken into account for calculation of shoot length.

Table-2

Root formations of *Desmodium gangeticum* shoots cultured on semi solid MS medium supplemented with various concentrations of IAA and ABA

MEDIUM (mg/l)	PERCENT RESPONSE	DAYS TO ROOTING	NUMBER OF ROOT	LENGTH OF ROOT
CONTROL	NR	NR	NR	NR
IAA				
0.25	0*	0	0	0
0.5	0*	0	0	0
1	31±0.577*c	14.33 ±1.201a	1.35 ±0.017e	1.68 ±0.005d
1.5	51±0.577*b	14.33±1.452a	1.63±0.033d	1.57±0.008e
IBA				
0.25	0	0	0	0
0.5	51±0.577b	9.00±0.577b	2.64±0.026c	1.74±0.026c
1	100±0.000a	7.00±0.577b	4.60±0.0577a	3.37±0.120a
1.5	100±0.000a	7.66±0.881b	4.11±0.008b	3.31±0.005b

Data (mean ± SE) of three Independent experiments each with 20 replicates. Means followed by the same letter within the column are not significantly different ($P<0.05$) as tested by the multiple range test of Duncan (1955). (*) –Callus formation.

medicinal plants like *Hemidesmus indicus* (Patnaik and Debata, 1996). Another interesting point to be noted that, BA along with auxin IAA gave better response. Shoot growth in such combinations were better than the shoot growth observed in the medium with BA alone. This is in corroboration with results obtained in cotyledon (Hossain *et al.*, 1994) and hypocotyl (Hossain *et al.*, 1995) explants of *Aegle marmelos*. The results of the present study indicated that auxin along with cytokinin is essential for enhanced sprouting response. A review of literatures indicate that addition of either of IAA or NAA in the culture medium improved the response in a number of species in terms of shoot growth. It has been reported that nodal cultures of *Acacia senegal* responded better when BAP (4.0 mg/l) and NAA (0.5 mg/l) were added to the culture medium (Kaur *et al.*, 1998). A similar response was observed in *Prosopis juliflora* cultures (Nandwani and Ramawat, 1991).

Concentration of auxin in the medium was found to be the critical factor in producing healthy roots. A range of concentration of IAA and IBA was tested (0.25 -1.5 mg/l) for rooting in the present studies. But the overall rooting response with IBA at higher concentration (0.5mg/l or more) was better, while IAA responded better between 1.0 -1.5 mg/l. Pradhan *et al.* (1998) reported that the rooting of *in vitro* shootlets of *D. latifolia* was better at 2.0-mg/l ofIBA.

The present results represent a three-step protocol for efficient plant regeneration system through direct organogenesis from cotyledon explants.

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In vitro propagation of *Adhatoda vasica* Nees. : A potential medicinal plant

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In vitro propagation of *Adhatoda vasica* Nees. was achieved through multiple shoot induction from nodal explants derived from *in vitro* grown shoots. The explants were cultured in MS medium supplemented with various concentrations of cytokinins (BAP or Kinetin). Among the two cytokinins tested BAP was found more effective than Kinetin for multiple shoot induction. Maximum percentage of shoot was observed in the MS medium supplemented with BAP (1.0 mg/l). The micro-shoots were elongated and successfully rooted in MS medium devoid of any phytohormones. Addition of auxin into MS medium had no significant effect on rooting. The micro shoots also rooted in the medium containing lower concentration of auxin (0.1-1.0 mg/l) but with increase in auxin concentration callusing was formed in the cut end. Rooted plantlets were successfully established in the soil with 85% of survival.

Key Words: *Adhatoda vasica, multiple shoot, medicinal plants.*

Introduction

Adhatoda vasica Nees. (Vasak) a member of family Acanthaceae is an important medicinal plant and commonly grown as a hedge plant. The plant is highly valued for its bronchodialator effect which is due to its two alkaloids content viz. Vasicine and Vasicinine. All parts of the plant are used for herbal medicines. The leaf extract form an important constituent of many ayurvedic drugs for curing cough, bronchitis, asthma, rheumatism, diarrhoea and dysentery. The root is a valuable antiseptic, antiperiodic and antihelmintic. The fresh flowers are used in Ophthalmia and jaundice (Kirtikar & Basu, 1935). Due to its high medicinal value the plant is highly exploited. The plant rarely produces seeds and is mainly propagated by vegetative means. The alkaloid content of plant also varies with genotype therefore vegetative method of propagation is highly recommended (Dastur, 1985). Plant tissue culture acts as an alternative method for rapid clonal propagation and conservation of medicinal plants.

Micro propagation in *Adhatoda vasica* was earlier studied through nodal explant culture (Anand *et al.*, 2002; Azad *et al.*, 2003; Nath and Buragohain, 2005) and through shoot tips (Abhyankar and Reddy, 2007); regeneration of plantlets from leaf derived callus culture (Amin *et al.*, 1997) and regeneration of plantlets from inter nodal explants (Azad *et al.*, 1998).

The present study was carried out to establish the micropropagation of *A. vasica* through axillary bud culture by manipulating the growth regulators.

Materials and Methods

The terminal buds of *A. vasica* with 1-2 nodes were collected from healthy plants grown in medicinal plant garden of P.G. Dept. of Botany, Utkal University and were taken as explants. The explants were thoroughly washed with water to remove dust particles. Surface sterilization was carried out by treating the explants with 5% (v/v) teepol for 10 minutes and by keeping them under running tap water for 30 minutes. The explants were further surface sterilized with 0.1% (w/v) mercuric chloride (HgCl2) solution for 5 minutes under laminar air flow cabinet. The explants were cut into smaller segment with one node and inoculated in the cultured flask containing Murasighe & Skoog (MS) basal medium (1962) supplemented with various

concentration (0.5-2.5 mg/l) of cytokinin (BAP or Kinetin). Effect of Gibberellic acid (GA_3) on multiple shoot formation was also tested by adding 0.5mg/l GA_3 to the above mediums. The medium was fortified with 3% sucrose and 0.8% agar, P^H of the medium was adjusted to 5.8 prior to autoclaving at 104 kpa and 121°C for 20 minutes, 5 replicates were maintained for each experiment and each was repeated thrice. The cultures were maintained in culture room at 25°±2°C with light intensity of 2000 lux provided by cool white fluorescent tubes with alternating periods of 16 hrs photo-periods at 55-60% relative humidity.

In order to avoid the deficiency of nutrient and for maintaining the growth of the explants, repeated sub-culture into fresh-medium was done periodically at an interval of 2-3 weeks. The micro shoots were successfully elongated and transferred to rooting MS medium devoid of phyto-hormones. To study effect of auxins on percentage of rooting MS medium supplemented with lower concentration (0.1-1.0 mg/l) of auxins (NAA or IBA) were also tested. Rooted plantlets were successfully acclimatized and transferred to field condition.

Result and Discussion

Multiple shoot induction

Apical buds were cultured in the MS medium supplemented with various concentrations of BAP and Kinetin taken separately and along with GA_3. The apical shoots elongated and gave rise additional leaves within 2-3 weeks and produced established shoots (Fig. 1). The nodal explants from this apical shoot culture were used as explants for multiple shoot induction. The medium for multiple shoot induction were fortified with various concentrations (0.5-2.5 mg/l) of cytokinins (BAP and Kinetin) with or without GA_3. Shoot growth was observed within 1-2 weeks of culture. The maximum percentage of shoot growth was observed in MS medium containing 6-BAP (1 mg/l) (Fig. 2) which decreased with further increase in BAP concentration. BAP is found to be more effective than Kinetin in multiple shoot induction. This finding is agreed with Ajad *et al.* (2003). Addition of auxin into the medium had no

Table-1

Induction of multiple shoots from nodal explants by using different concentration of Cytokinins

Concentration of Cytokinin (mg/lit)	Response (%)	No. of multiple shoots/Culture (Mean ±SE)
BAP		
0.5	95	7.2 ± 0.37
1.0	100	11.2 ± 0.58
1.5	100	8.4 ± 0.24
2.0	80	6.6 ± 0.24
2.5	80	2.2 ± 0.37
Kinetin		
0.5	100	8.4 ± 0.24
1.0	95	9.4 ± 0.5
1.5	100	5.2 ± 0.37
2.0	90	4.6 ± 0.4
2.5	70	2.0 ± 0.31

*Mean data based on 5 replicates and each expt. repeated thrice.

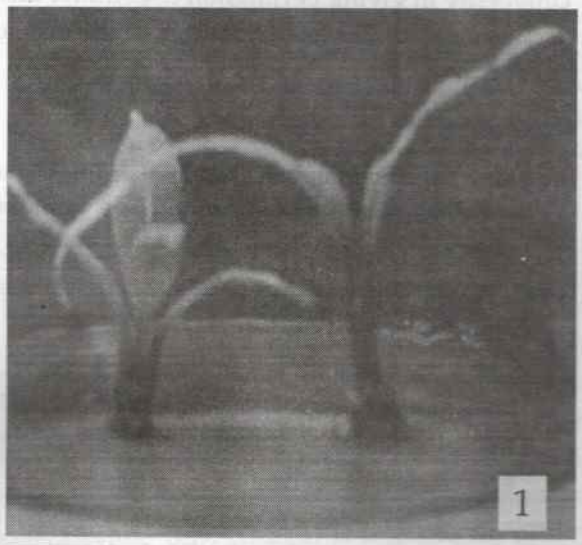
Table-2

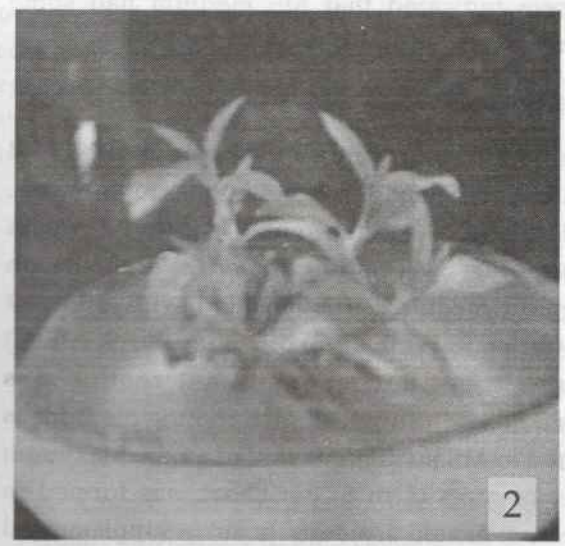
Effect of different concentration of auxins on induction of rooting from micro-shoots of *Adhatoda vasica*

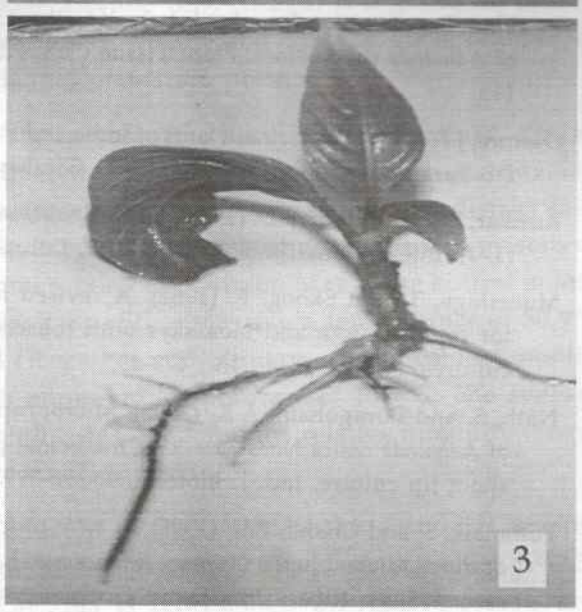
MS + auxin Conc. (mg/l)	Response (%)	Av. No. of Roots/ shoot (Mean ± SE)
MS (controlled)	100	5.8± 0.1
NAA		
0.1	95	1.2± 0.37
0.3	95	2.2±0.5
0.5	100	4.0±0.06
0.7	80	3.0±0.24
1.0	80	0.8±0.37
IBA		
0.1	95	1.4±0.24
0.3	95	2.3±0.5
0.5	100	3.8±0.10
0.7	90	4.1±0.4
1.0	70	1.5±0.1

*Mean data based on 5 replicates and each expt. repeated thrice.

significant effect on shoot growth and it was found that cytokinin was alone sufficient for multiple shoot induction. This result is contradictory to the findings of Amin *et al.* (1997) and Azad *et al.* (2003) who had proposed that addition of auxin has promotory effect on multiple shoot formation. With addition of auxin (IBA or NAA) in the concentration of 0.1-1.0mg/l into the culture medium, callusing was obtained at the cut end. With the increase of auxin concentration callusing was more which resulted stunted growth of axillary buds and retardation of multiple shoot growth. Though addition of GA_3 (0.5mg/l) to shooting medium enhanced multiple bud formation in certain medicinal plants like *Saussurea lappa* (Arora and Bhojwani, 1989), *Ocimum sanctum* and *Ocimum canum* (Pattnaik *et. al.*, 1996), in the present study addition of GA_3 resulted high frequency of callusing from cut end which were fragile and only elongated bud was formed and there was inhibition of multiple shoot formation.







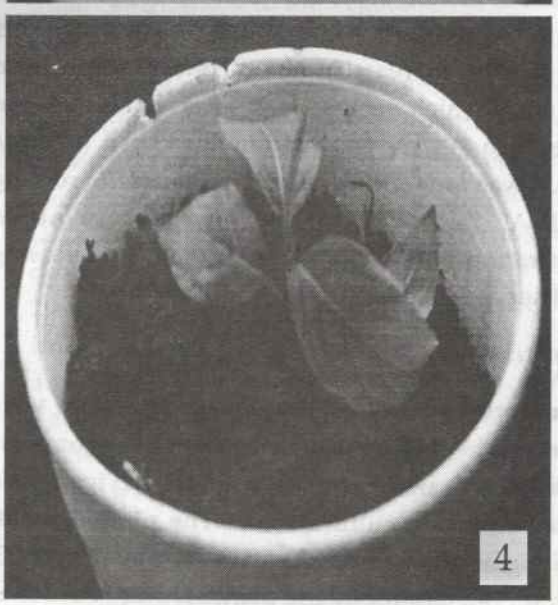


Fig. 1-4 : Micropropagation of *Adhatoda vasica.* Fig. 1. Establishment of Apical shoot in MS+BAP (0.5mg/l), Fig. 2. Multiple shoot induction in MS+BAP (1mg/l), Fig. 3. Induction of roots on MS medium without growth regulator. Fig. 4. Acclimatization of *in vitro* grown plant.

Percentage of shoot growth and multiple shoot induction was increased with increase of cytokinin concentration and declined above the optimal level. Maximum numbers of multiple shoots 9 to 12 were observed within 60 days of culture on MS medium containing 1mg/l BAP. With increase of BAP concentration (2.0 mg/l) in the culture medium, the growth of multiple shoot became reduced. There was no inhibiting effect on shoot growth of MS medium devoid of growth regulator. This finding is contradictory to the report of Anand *et al.* (2002). They have indicated that MS medium had some inhibitory effect on multiple shoot induction.

In vitro rooting

One month old micro-shoots were excised and inoculated in MS medium devoid of phytohormones. The micro shoots become rooted within 2-3 weeks of culture (Fig. 3). To study the effect of auxins on rooting MS medium supplemented with different concentration (0.1-1.0 mg/l) of auxins (NAA & IBA) were also tried. But no significant rooting was observed in auxin supplemented rooting medium as compared to MS medium devoid of auxin. Maximum number of roots (5 to 6) per shoot was formed in MS medium devoid of auxins. In auxin supplemented medium increase in percentage of rooting was observed with increase in auxin concentration from 0.1-0.5mg/l which decline there after. Maximum no. of roots (3 to 4) were observed in MS +NAA (0.5mg/lt). NAA was found more effective for *in vitro* rooting than IBA. But with further increase in auxin concentration from 0.5mg/lt to 1.0mg/lt, callusing was observed at the cut end of microshoots.

Acclimitization

The rooted plantlets from culture medium were transferred to small pots filled with vermi-compost, pure soil and sand in the ratio of 1:2:1 (Fig. 4) and maintained in the culture room. The plotted plants were irrigated with half MS salt solution at every 3-days interval. Gradually the plantlets were hardened by lowering salt concentration in irrigating solution and percentage of humidity. Then the plants were transferred to normal field condition with 85% of survival rate. The regenerated plants were identical in their morphological characteristics to the donor plants.

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Some Potential Medicinal Plants of Anugul District of Orissa and its conservation

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Anugul, one of the centrally located districts of Orissa, lies between 20°37' to 21°10'N latitude and 84°53' to 85°28'E longitude in the Indian subcontinent. This district is endowed with rich vegetable wealth, of which the medicinal plants deserve special mention. There is large depletion of the plant wealth due to rapid industrialization and anthropogenic interferences as well. Hence, the urgent need is to conserve the plants from the threat of extinction. Realising this the present paper deals with 40 angiospermic plant species along with their medicinal utility and family and their conservation.

Introduction

Anugul is one of the most industrially developed and centrally located districts of Orissa State in Indian sub-continent. This district had very rich and diverse vegetable wealth in the remote past but now-a-days the forests are depleting at an alarming rate due to the rapid march of industrialization and urbanization as well. Despite this, Anugul is a grand repository of vegetable wealth and quite a good number of medicinal plants are present.

The southern and northern area of Anugul district is covered with hills. The hill slopes are covered with forests and scrub vegetation. The district falls under tropical climate with three distinct seasons of summer, rainy and winter. May is the hottest month of the year with a maximum temperature of 50.9°C and December and January are the coldest months with minimum temperature of 10.9°C. The average rainfall is 141.4mm and the relative humidity ranges between 25% to 85%.

Most of the landmass of this district is covered by dense forests of 3509.59 sq. kms, of which 1742.59 sq.km area is represented by reserve forests and 1431.15 sq.km., 334.56 sq.km and 1.29 sq.km is covered by un-demarcated, protected and unclassified forests (Nayak and Dash, 2000) respectively. The total population of the district is 9,61,037.

Though this district had rich and diverse floristic composition, no systematic floristic studies have been made till today. Only sporadic reports have been made by Haines (1921-25) the pioneer plant explorer for the states of Bihar and Orissa and Mooney (1950), the subsequent worker. Later on, Singh and Verma (1964) made some contribution to the forest botany of Anugul and Patra and Choudhury (1992) made some contribution to the vegetation of Satakosia wild life sanctuary of Anugul district.

Realising the species richness and diversity of medicinal plants, 178 medicinal plants collected from different areas of the district have been identified and housed in the herbarium of P.G. Dept. of Botany, Utkal University, Bhubaneswar. The present paper deals with 40 species belonging to 39 genera under 29 families. The medicinal properties are also given in consultation of standard literatures of Agarwal and Ghosh (1985), Chopra *et al.* (1956), Kirtikar and Basu (1935), Satyavati *et al.* (1987) and Warrier *et al.* (1994-1996). The species are arranged alphabetically along with their family and uses in a tabular form.

Conservation

Man has always been dependant on the vegetable world to cater his various needs. According to Charak, the premier herbalist, each and every plant has some therapeutic value. Anugul is a grand repository of potential vegetable wealth, of which

the medicinal plants of different categories deserve special mention.

Anugul had very rich forests in the remote past which are now started degrading at an alarming rate due to interferences of various factors. These are rapid march of industrialization, urbanization and cutting down of tree species for various requirements etc. So conservation of the medicinal plants is the need of the hour as they play a pivotal role in health care. Protection of medicinal plants from the verge of extinction documentation of the informations pertinent to the utilitarian value of the drug plants are highly essential.

The general plants are conserved in in-situ and the endangered and threatened are undergone *ex-situ* way of conservation. By the *ex-situ* way the germplasm of the medicinal plants are conserved and the genetic erosion is checked. Hence, in future judicious use of medicinal ingredients should be adopted.

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Table : List of medicinal plants of Angul District, Orissa

Sl.No.	Name of the Species	Family	Parts used	Uses
1.	*Abrus precatorius* L.	Fabaceae	Seed, Root	Abortion, Colic, Eye complaints
2.	*Adhatoda zeylanica* Medic.	Acanthaceae	Leaf, Flower	Asthma, Cough, Cold fever
3.	*Alangium salvifolium* (Linn.f.) Wanger.	Alangiaceae	Leaves, Shoots	Boils
4.	*Anisomeles indica* (L.) Kuntze	Lamiaceae	Whole plant	Colic
5.	*Aristolochia indica* L.	Aristolochiaceae	Root	Snakebite, Fever
6.	*Asparagus racemosus* Wild.	Liliaceae	Root, Leaves	Weakness of semen, diseases of kidney and liver
7.	*Asteracantha longifolia* L.	Acanthaceae	Root, Leaves, Seeds	Jaundice, Rheumatism
8.	*Atylosia scaraboides* L.	Fabaceae	Whole plant	Diarrhoea
9.	*Azadirachta indica* A. Juss	Meliaceae	Leaves	Ulcers
10.	*Barleria strigosa* Wild.	Acanthaceae	Leaves, Roots	Cough, inflammation
11.	*Blumea lacera* (Burm. f.)	Asteraceae	Roots	Diarrhoea
12.	*Canavalia virosa* (Roxb.) Baker	Fabaceae	Fruits	Hernia, Colic
13.	*Celosia argentea* L.	Amaranathaceae	Seeds	Diarrhoea
14.	*Crotalaria prostrata* Rottl. *ex* Wild.	Fabaceae	Root	Stomach pain, Diarrhoea
15.	*Cucumis trigonous* Roxb.	Cucurbitaceae	Root, Stem	Snake bite
16.	*Dioscorea wallichi* Hook f.	Diocoreaceae	Tuber	Joint pain
17.	*Diospyros embryopteris* Pers.	Ebenaceae	Fruit	Antibacterial
18.	*Eryngium foetidium* L.	Apiaceae	Whole plant	Stomachic
19.	*Erythrina variegata* L.	Fabaceae	Leaves	Increasing blood level during pregnancy
20.	*Flacourtia indica* (Burm. f.) Merr.	Flacourtiaceae	Leaves	Rheumatism

Sl.No.	Name of the Species	Family	Parts used	Uses
21.	*Gloriosa superba* L.	Liliaceae	Rhizome	Ulcers, Leprosy, dyspepsia, expulsion of the placenta.
22.	*Helecteres isora* Linn.	Sterculiaceae	Fruit	Fever, Cough, cold
23.	*Hemidesmus indicus* (L.) R.Br.	Asclepiadaceae	Root	Leucoderma, Leprosy, Rheumatism and fever
24.	*Holarrhena antidysenterica* Wall.	Apocynaceae	Bark, Seed	Constipating, Expectorant
25.	*Holarrhena pubescens* (Buch. Ham.) Wall. *ex.* G. Don.	Apocynaceae	Bark	Dysentery, Rheumatism
26.	*Knoxia sumatrensis* (Retz.) Dc.	Rubiaceae	Whole plant	Bubo, stomach troubles
27.	*Lannea coromandelica* (Houtt.) Merr.	Anacardiaceae	Bark	Asthma
28.	*Mimosops elengi* L.	Sapotaceae	Bark	Diarrhoea, Dysentery
29.	*Ocimum basilicum* L.	Lamiaceae	Seeds, leaves	Gonorrhoea, Cough
30.	*Physalis minima* L.	Solanaceae	Oil	Spleen disorder
31.	*Rauvolfia serpentina* (L.) Benth.	Apocyanaceae	Root	Expelling intestinal worms
32.	*Ricinus communis* Linn.	Euphorbiaceae	Bark	Easy abortion.
33.	*Schleichera oleosa* Lour	Sapindaceae	Bark	Malaria
34.	*Sesamum orientale* Linn.	Pedaliaceae	Seeds	Bubo, Eczema
35.	*Solanum nigrum* Linn.	Solanaceae	Fruit	Fever, Liver disorder
36.	*Strychnus nux - vomica* L.	Loganiaceae	Bark, leaves, Fruit	Cholera, Chronic, Wounds, Paralysis, Anemia, Asthama, Bronchitis.
37.	*Terminalia belerica* Roxb.	Combretaceae	Bark	Anaemia, Leucoderma
38.	Vanda tessellata (Roxb.) Hook. *ex.* G. Don.	Orchidaceae	Leaves	Rheumatism, curing pus formation in ear.
39.	*Woodfordia fruticosa* (L.) Kurz.	Lythraceae	Fruit	Menorrhagia, burning sensation.
40.	*Wrightia arborea* (Denst.) Mabb.	Apocynaceae	Milky latex	Haemorrhage

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Identification and *in-vitro* Antimicrobial activity of a compound produced by fluorescent pseudomonas isolated from *Taxus baccata* rhizosphere

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Rhizosphere micro flora has received significant attention because of the role played by these microorganisms in plant growth and health. Yet little is currently known about their Antimicrobial activity against human pathogenic microorganisms. In the present investigation a bacterium identified as *Pseudomonas fluorescence* was isolated from *Taxus baccata* rhizosphere. Ethyl acetate extracts from its culture filtrate yield an active Antimicrobial compound. The active metabolites was resolved by column chromatography on silica gel (60-120 mesh) using ethyl acetate and petroleum ether as a solvent system in the ratio 1:2 and further characterized on the basis of spectral data (UV, IR and ^{1}H-NMR), which indicates the presence of aromatic ring and phenolic functionality. The compound showed significant Antimicrobial activity against two-gram positive bacteria *(Bacillus subtilis* and *Staphylococcus aureus)* four-gram negative bacteria *(Escherichia coli, Klebsiella pneumoniae, Shigella flexneri* and *Pseudomonas aeruginosa)* and one pathogenic fungus *(Coletrotrichum albicans)*. The minimum inhibitory concentration (MIC) of the compound determined against the test pathogens was found to be between 600-75 µg/ml.

Key words: *Taxus baccata, Pseudomonas fluorescence,* Antimicrobial activity, aromatic ring, phenolic functionality.

Introduction

Taxus baccata, commonly known as Himalayan yew is an important medicinal plant. This plant is found distributed in the temperate region of Eastern Himalayas of Arunachal Pradesh, India and has recently assumed tremendous commercial importance because of its leaves and bark yield Taxol - a proven anticancer metabolites.[27] In the recent years *Taxus* sp. worldwide gained importance due to isolation of an endophytic fungus, *Taxomyces andreanae* from Pacific yew *(Taxus brevifolid),* which has been reported to produce Taxol *in vitro*.[28] Moreover many other fungal endophytes were isolated from different *Taxus* sp. of the world that has been reported to produce important bioactive metabolites.[26,30,33,36] It is assumed that other *Taxus* sp. around the world may harbour rare and interesting microbes that may produce important bioactive molecules useful for the mankind.[29] With this aim we studied rhizospheric and endophytic microorganisms associated with Himalayan yew *(Taxus baccata)* of Arunachal Pradesh and screened for their bioactive metabolites.

Rhizosphere microflora has received significant attention because of the role played by these microorganisms in plant growth and health.[8,10,16] Indeed, some bacterial populations are pathogenic whereas others are beneficial. Beneficial rhizobacteria include symbiotic and free-living microorganism. Among the latter, special attention has been given to the *fluorescent pseudomonas* group. *Fluorescent pseudomonads* are ubiquitous bacteria that are common inhabitant of the rhizosphere and are the most studied group within the genus *Pseudomonas*. Positive effects on plant growth and health of inoculation of bacterial strains belonging to this bacterial group have been reported since the late '70s.[4,5,7,12,35] Strains of *Pseudomonas* spp. worldwide origin have shown their abilities to suppress a wide variety of fungal root pathogen in many different agricultural crops around the world.[12,14,17,32,34,25] In most cases the biocontrol

ability of those strain is directly correlated with the production of antibiotic such as 2-4-diacethylphloroglucinol (DAPG), Phenazines (Phz), Pyrrolnitrin (PRN), Pyoluteorin (PLT) and hydrogen cyanine (HCN).[1,4,14,20,23,32] Production of antibiotic in several strains of *Fluorescent pseudomonas* has been recognized as a major factor in suppressive of root pathogens. Antibiotics from *fluorescent pseudomonas* have been reported to exhibit antifungal, antibacterial, antihelmenthic and phytotoxic activity.[3,4,7,13,17,23,24,25] Most of these antibiotics have been used as a biocontrol agent against phytopathogenic bacteria and fungi.[17,34,35] Yet little is currently known about their antimicrobial activity against human pathogenic microorganisms. Only pyrrolnitrin antibiotic produced by many *fluorescent pseudomonads* has been primarily used as a clinical antifungal agent for treatment of skin mycoses against dermatophytic fungi, particularly member of the genus *Trichophyton*.[1,6] Therefore in the present study we report an antimicrobial activity of a compound produced by *florescent pseudomonas* against some human pathogens extracted from ethyl acetate nutrient broth medium.

Materials and Methods

Collection and Isolation

Rhizosphere soil was collected from *Taxus baccata* growing on two different locations, namely Tawang and Dibang valley of Arunachal Pradesh, India. The two sites are located at an altitude of 3250m and 2600m respectively. Soil temperature (17°C) and soil pH (6.8) was recorded at the time of collection. The soil samples was collected in sterile polybags and brought immediately to the laboratory, 10g of the soil closely adhering to the root surface was suspended in a flask containing 100ml of sterile distilled water. The flasks were placed on a rotary shaker at 200 rpm for 120 minutes. Microorganisms were isolated by dilution plate technique. For isolation of bacteria serial dilution of the mixture were plated on Nutrient agar (NA) (per liter: 5g peptone, 5g sodium chloride, 3g beef extract, 2% agar powder, pH- 7.2) and incubated at 37±1°C for 24 hours. An antagonist bacterial strain having antimicrobial activity was isolated by the above methods and was send to Institute of Microbial Technology (IMTECH), Chandigarh, India for identification.

Growth medium and optimum culture for production of metabolites

For the production of active metabolites the bacterial isolate was grown on Nutrient broth (NB) medium, Himedia (per liter: 10g peptic digest of animal tissue, 12g sodium chloride, 10g beef extract, pH-7.3). Activity of the liquid culture was tested against *Bacillus subtilis* and *E. coli* by agar cup diffusion assay methods.[10] On the production of antibiotic various parameters like effect of temperature (20, 25, 30, 35, 40 and 45°C), incubation period (24h, 36h, 48h and 60h) and pH values (4, 5, 6, 7, 8 and 9) were studied against *Bacillus subtilis* by agar cup diffusion method.[10]

Extraction and characterization of the metabolites

The organism was grown on Nutrient both media at 32°C for 30h to yield maximum metabolites. The liquid broth was extracted with equal volume of ethyl acetate in separating funnel by vigorously shaking for 10 minutes. The cell mass were separated and discarded and the organic solvent so obtained was collected, pooled and dried in a rotary evaporator. The crude metabolite so obtained was resolved by using thin layer chromatography (TLC) with eluents of different polarity. The Rf values of the major products with eluent, ethyl acetate and petroleum ether in the ratio 1:1, 1:2, and 1:3 were 0.80, 0.40 and 0.27 respectively. The active metabolite was further purified by column chromatography using silica gel (60-120 mesh) with ethyl acetate and petroleum ether in the ratio 1:2. The pure antimicrobial metabolite was characterized on the basis of spectral data (UV, IR and ^{1}H-NMR).

Antimicrobial screening

The antimicrobial activity of the active metabolite was tested by agar cup diffusion assay.[10] The compound was dissolved in Dimethylsulphoxide (DMSO) at a concentration of 1g/ml and tested against two gram- positive bacteria *(B. subtilis* and *S. aureus)*, four-gram negative bacteria *(E. coli, K. pneumoniae, S. flexneri* and *P. aeruginosa)* and one

pathogenic fungus *(C. albicans)*. Muller Hinton agar (MHA) plates were inoculated with 0.2ml of overnight culture of each test bacteria containing 1.0 x 10^9 cells. Similarly, the test fungus was inoculated on Sabouraud's agar plate. The plates were evenly spread out with sterile cotton swab. Agar cup were prepared on the plates pre-inoculated with the microorganisms. Each cup were then loaded with 200µl of the metabolites and incubated at 37±1°C for 24hrs and examined. The diameter of the zone of complete inhibition was measured to the nearest millimeter. As a positive control standard antibiotic disc-Streptomycin (Hi-media, 10µg) was used (Table-1).

Determination of minimum inhibitory concentration (MIC)

The MIC of the compound was determined by serial dilution method.[11] In this method a series of Mueller Hinton broth (for bacteria) and Sabouraud's broth (for fungus) containing different concentration of the compound was prepared in test tubes and inoculated with standard numbers of the test organisms. The tubes were incubated at 37±1°C for 24hrs and examined. MIC was determined as the lowest concentration of the compound that reduced and completely inhibited the growth of the microorganisms (Table-2).

Results and Discussion

A total of 31 bacterial strains were isolated from *Taxus baccata* rhizosphere soil of Arunachal Pradesh. These bacteria were screened for their ability to produce antimicrobial compound. An antagonist organism was isolated among these bacterial strains,

Table-1

Antimicrobial activity of the compound

Test organisms	Zone of inhibition (mm)	
	Compound (1g/ml)	Streptomycin 10µg/disc)
Bacillus subtilis	25	30
Staphylococcus aureus	21	29
Escherichia coli	15	30
Klebsiella pneumoniae	18	25
Shigella flexneri	19	28
Pseudomonas aeruginosa	10	30
Candida albicans	22	ND

ND : Standard positive control was not determined.

Table-2

Minimum inhibitory concentration of the compound (antibiotic)

Test organism	Concentration of the compound (mg/ml)						
	1200	600	300	150	75	35	10
Gram positive							
Bacillus subtilis	-	-	-	-	+	-	-
Staphylococcus aureus	-	-	-	-	+	-	-
Gram negative							
Escherichia coli	-	-	+	+	-	-	-
Klebsiella pneumoniae	-	-	-	+	-	-	-
Shigella flexneri	-	-	-	+	-	-	-
Pseudomonas aeruginosa	-	+	+	-	-	-	-
Pathogenic fungus							
Candida albicans	-	-	-	-	+	-	-

+ indicates MIC value

which exhibited good antimicrobial activity against some human pathogen. The organism was identified as *Pseudomonas fluorescence* at the Microbial Type Culture Collection Centre and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The authentic culture of the organism has been deposited there with an accession no. MTCC, 6732. Different parameters were tested for maximum production of the active metabolites. It was found that Nutrient broth medium at 32±1°C for 30hrs and pH at 7.0 was most suitable for production of active metabolites. The active compound was recovered by solvent extraction method using ethyl acetate as solvent. The crude metabolites was subjected to thin layer chromatography (TLC) with eluents of different polarity. The Rf values of the major products with eluent, ethyl acetate and petroleum ether in the ratio 1:1, 1:2 and 1:3 were 0.80, 0.40 and 0.27 respectively. The active metabolite was further purified by column chromatography using silica gel (60-120 mesh) with ethyl acetate and petroleum ether in the ratio 1:2. The pure antimicrobial metabolite was characterized on the basis of spectral data (UV, IR and ^{1}H-NMR). The IR spectrum of the purified product showed two strong signal at 2854 and 2924 cm^{-1} indicating the presence of aromatic ring. A band at 3319 cm^{-1} indicates the presence of either N-H of O-H group. Signal between 6 to 7 ppm in the ^{1}H-NMR indicates the presence of aromatic proton. The sharp singlet at 9.5ppm indicates the presence of phenolic functionality. The ^{1}H-NMR signal along with the IR band at 3319 cm^{-1} gives an impression that the major component may be a phenolic. The sharp singlet counting nearly 9 protons at 3.4 ppm gives an indication of the presence of O-alkyl group that is probably a tert-butyl one.

The antimicrobial activity of the active compound was determined against two gram-positive bacteria *(B. subtilis* and *S. aureus),* four gram negative bacteria *(E. coli, K. pneumoniae, S. flexneri* and *P. aeruginosa)* and one pathogenic fungus *(C. albicans).* The compound showed moderate to strong antimicrobial activity against the test pathogens (Table-1). The result indicates that, gram-positive organism both *B. subtilis* (25mm) and *S. aureus* (21mm) showed

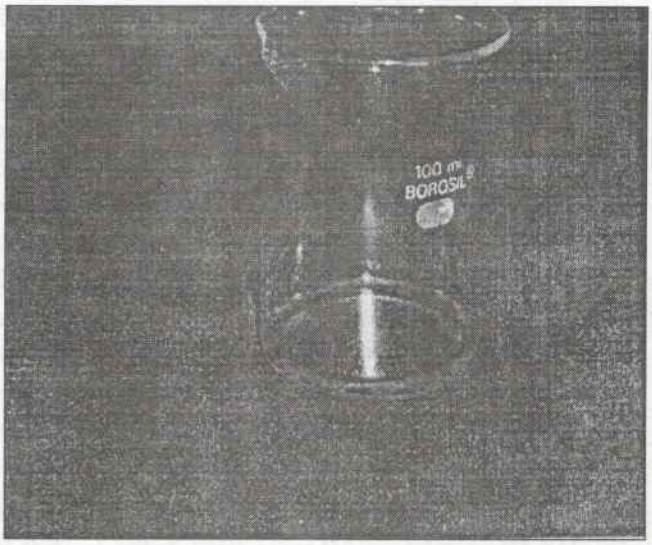

Plate-1 : Extracted crude metabolites

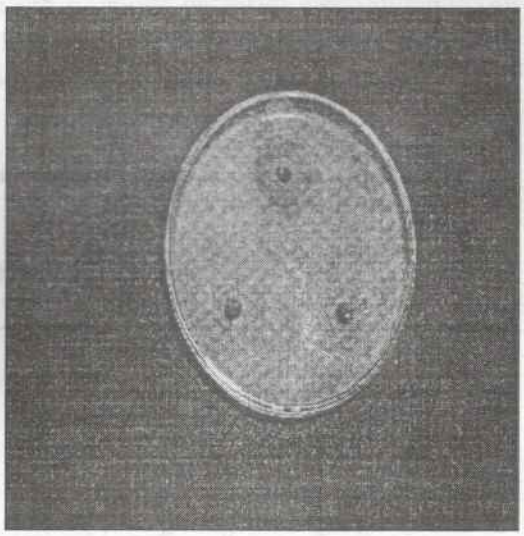

Plate-2 : Activity against *Bacillus subtilis*

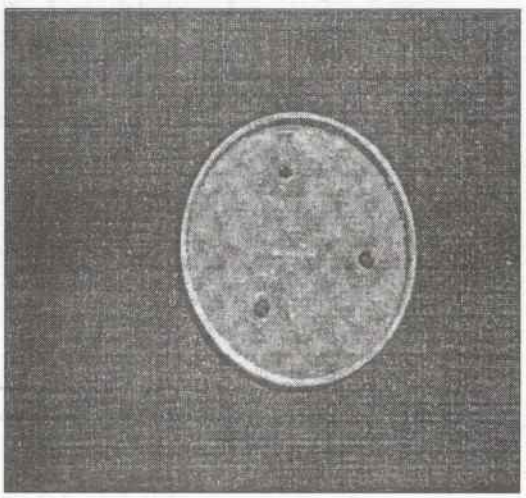

Plate-3 : Activity against *Klebsiella pnemoniae*

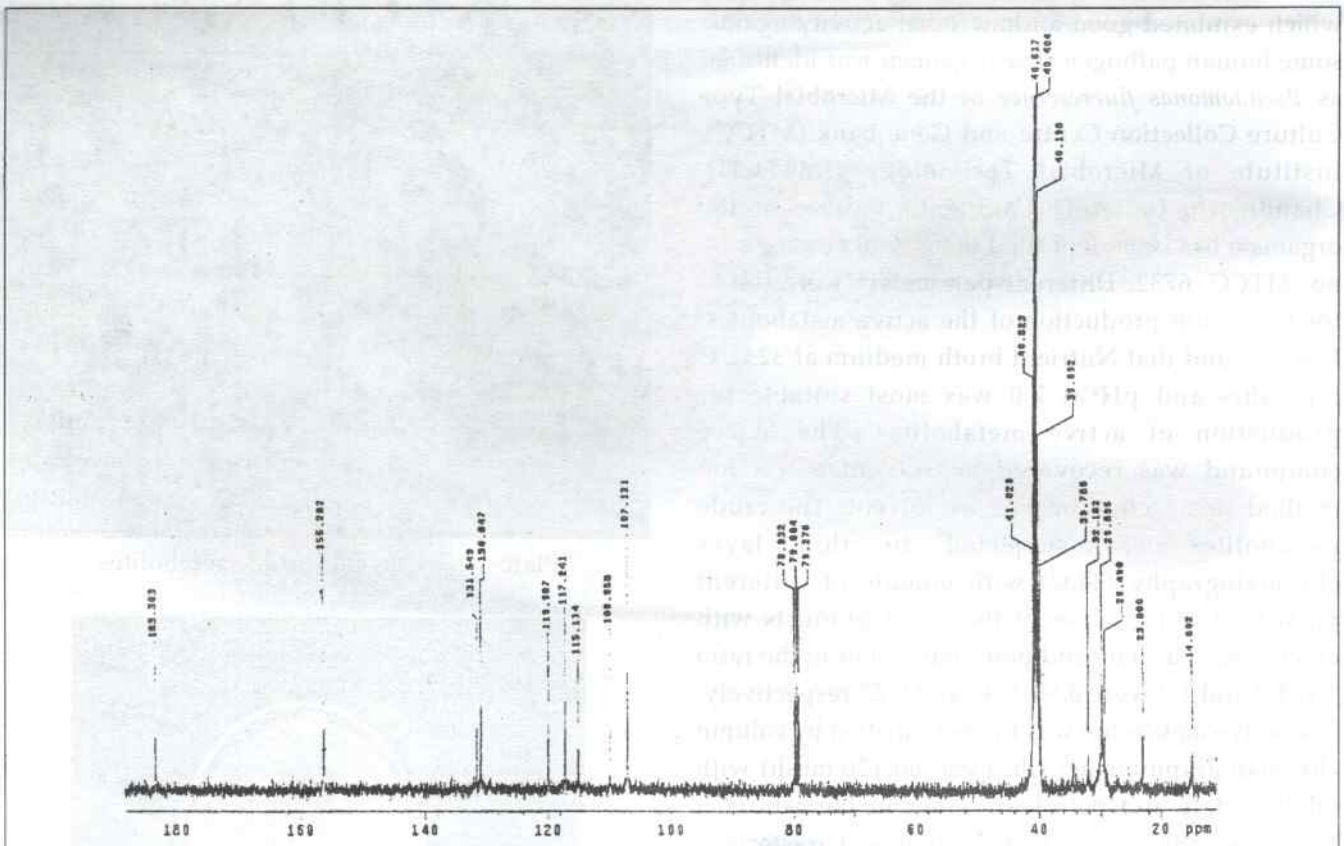

Fig. 1 : IR Spectrum of the active metabolite

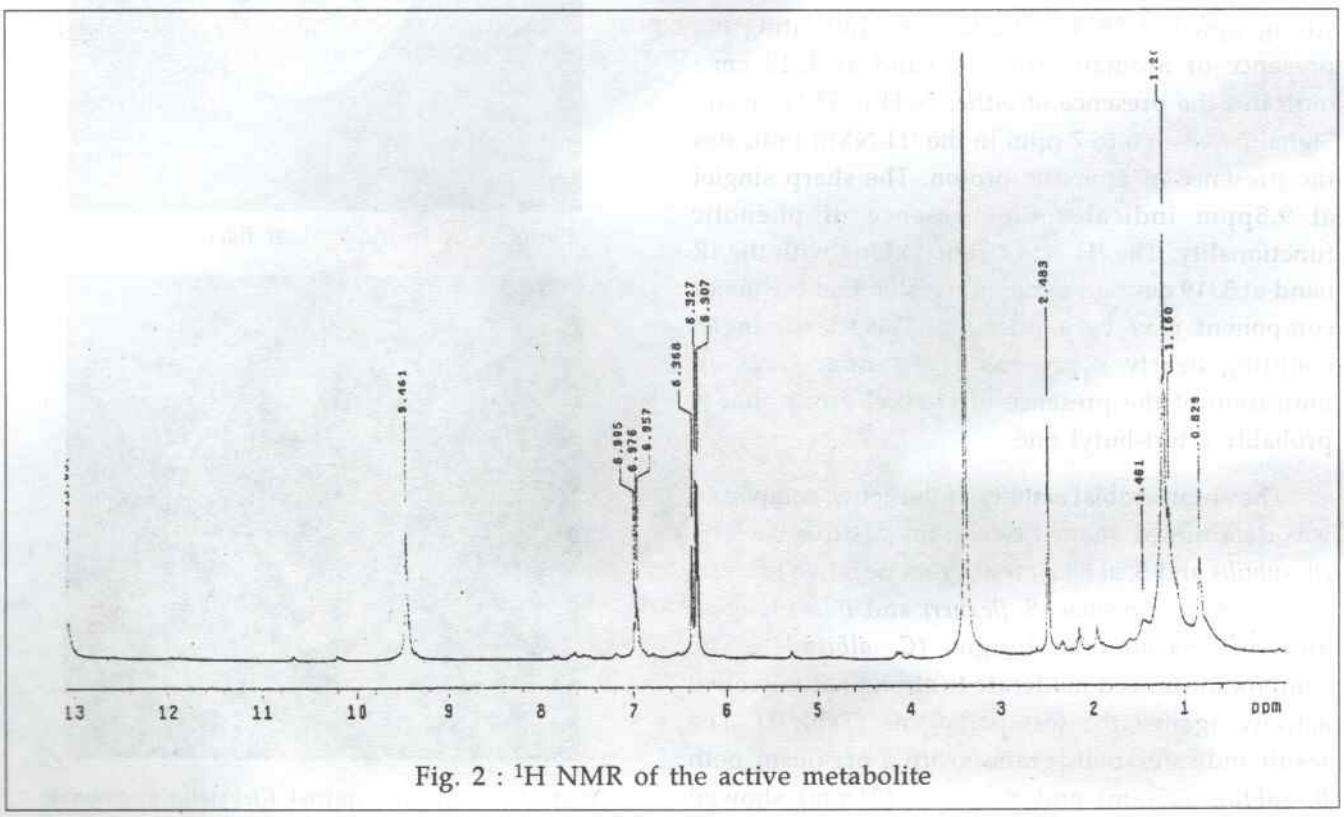

Fig. 2 : ^{1}H NMR of the active metabolite

strong antibacterial activity. The compound also showed strong antifungal activity against *C. albicans* (22mm). Moderate antibacterial activity was observed against gram-negative bacteria *E. coli* (15mm), *K. pneumoniae* (18mm) and *S. flexneri* (19). However, *P. aeruginosa* (10mm) exhibited low antibacterial activity when compared with the other organisms. The Minimum Inhibitory Concentration (MIC) of the compound was 75 µg/ml against *B. subtilis, S. aureus,* and *C. albicans*. It was found to be 125µg/ml against *K. pneumoniae* and *S. flexneri*. The MIC value against *E. coli* and *P. aeruginosa* was 300µg/ml and 600g/ml respectively (Table-2).

The compound showed significant antimicrobial activity against all the test organisms. However *P. aeruginosa* exhibited low antibacterial activity and higher MIC value. This may be due to the reason that related organisms produce similar compound, which may not have much inhibitory effect. Strong antibacterial activity against *B. subtilis* (25mm) and *S. aureus* (21mm) indicate that the compound to be more effective to gram positive bacteria than gram negative bacteria which could be ascribed to the morphological differences between these micro-organisms in their cell wall component. The compound also showed strong antifungal activity against *C. albicans* (22mm). This indicates the active metabolite to be a broad spectrum one. *Pseudomonas fluorescence* have been highly exploited as biocontrol agent against wide variety of phytopathogenic fungi and bacteria.[17,34,35] The biocontrol mechanism has been explained due to production of siderophores such as pyoverdins[16,18] and by producing secondary metabolites with antibiotic such as 2-4-diacethyl-phloroglucinol (DAPG), Phenazines (Phz), Pyrrolnitrin (PRN), Pyoluteorin (PLT) and hydrogen cyanine (HCN).[1,2,4,14,20,23,32] Phloroglucinol antibiotics are important phenolic bacterial metabolites because of its ability to exhibit antifungal, antibacterial, antihelmenthic and phytotoxic activities.[13,14,20,23] Of great importance and particular interest is 2-4-diacethylphloroglucinol (DAPG) because of its

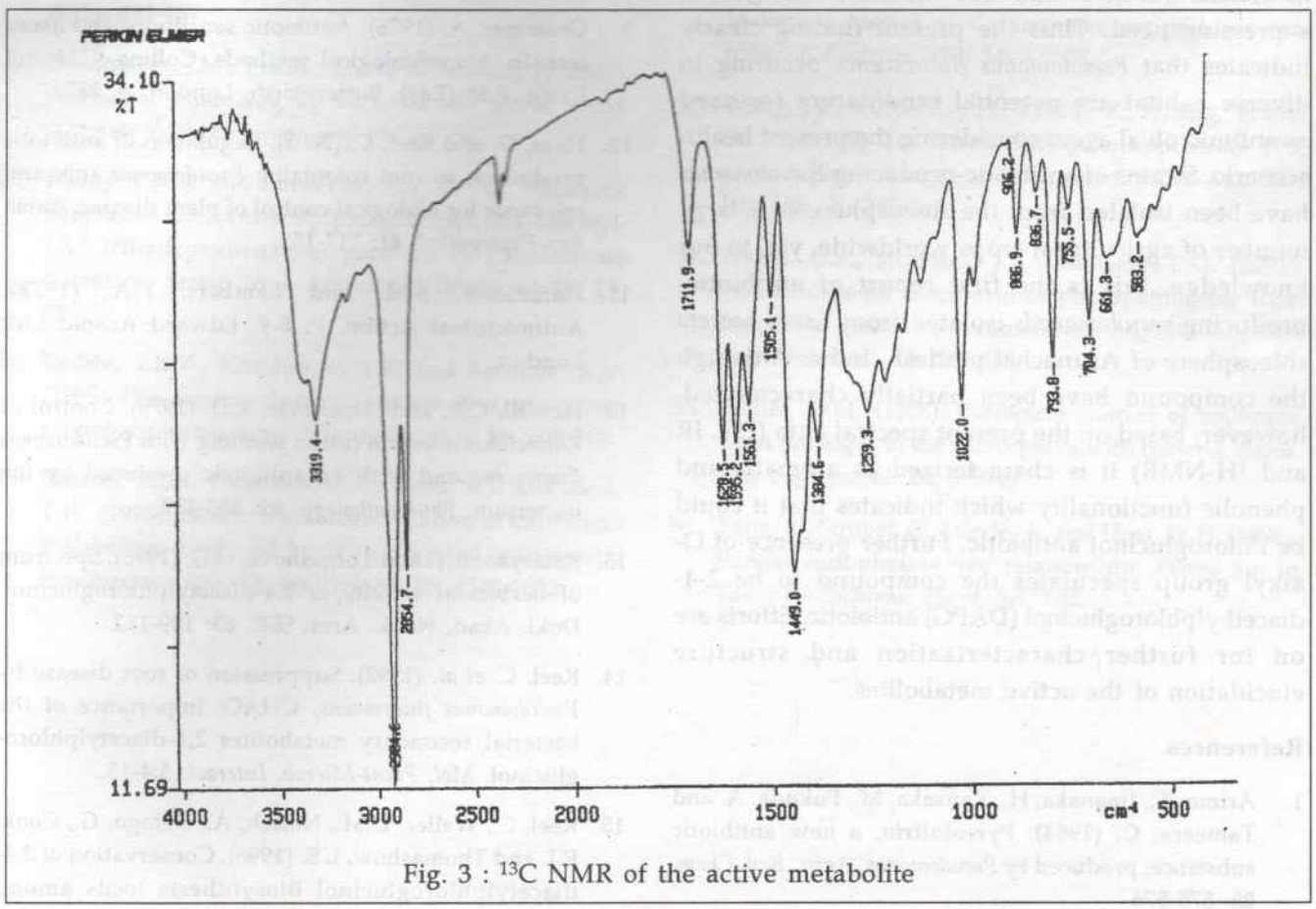

Fig. 3 : ^{13}C NMR of the active metabolite

production by *fluorescent pseudomonas* spp. of world wide origin.[21] Several distinct groups of DAPG-producing *Pseudomonas* have been identified from strains of various origins.[15] This indicates the genetic diversity of this strain and its ability to produce antibiotic of different nature. Studies on antibiotic from *Pseudomonas fluorescene* for used as clinical agent are very limited. Only pyrrolnitrin antibiotic produced by many *fluorescent pseudomonads* and non-fluorecsent strains has been primarily used as a clinical antifungal agent for treatment of skin mycoses against dermatophytic fungi, particularly member of the genus *Trichophyton*.[1, 6] In the present study we demonstrated active metabolites produced by *Pseudomonas fluorescence* from *Taxus baccata* rhizosphere displayed considerable antimicrobial activity against some human pathogens. This indicates the great potential of these strains for used as antibiotic for clinical agent. In the recent year pathogenic microorganisms are gaining resistance to existing antimicrobial agents hence the search for new, safe and more effective antimicrobial agent is a pressing need. Thus the present finding clearly indicates that *Pseudomonas fluorescence* occuring in diverse habitat are potential candidature for used as antimicrobial agent considering the present health scenerio. Strains of antibiotic-producing *Pseudomonas* have been isolated from the rhizosphere of a large number of agricultural crops worldwide, yet, to our knowledge, this is the first report of antibiotic-producing *pseudomonads* isolated from *Taxus baccata* rhizosphere of Arunachal pradesh, India. Although the compound have been partially characterized, however, based on the present spectral data (UV, IR and ^{1}H-NMR) it is characterized as aromatic and phenolic functionality which indicates that it could be Phloroglucinol antibiotic. Further presence of O-alkyl group speculates the compound to be 2-4-diacethylphloroglucinol (DAPG) antibiotic. Efforts are on for further characterization and structure elucidation of the active metabolites.

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Effect of Zinc and Nitrogen on growth, yield and nutrient uptake in scented rice

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An experiment was conducted to study the effect of two sources of zinc ($ZnSO_4$ $7H_2O$ and Zn-EDTA) and two methods of application (soil and foliar) at four different levels of nitrogen (0, 30, 60 and 90kg/ha) on the growth, yield and nutrient uptake in scented rice variety KASTURI. Plant height, leaf area index and dry matter production increased significantly with zinc application. The combined application of zinc and nitrogen showed higher grain and straw yield as compared to that of nitrogen alone. The zinc sulphate showed significant increase in growth, yield and nutrient uptake over Zn-EDTA. The soil application of zinc was superior than foliar application.

Key words: *Scented rice, zinc sulphate, Zn-EDTA and nitrogen*

Introduction

Intensive cropping with modern high yielding variety resulted in depletion of reserve of micronutrient in the soil. The micronutrient plays a significant role in growth and development of rice plant and determines the grain yield. Among the nutrients zinc is an important nutrient along with nitrogen and phosphorous limit the growth and yield of rice. Zinc is the major component and activator of several enzymes involved in the metabolic process like auxin and protein synthesis, nuclic acid and carbohydrate metabolism and utilisation of nitrogen and phosphorus for seed production. There is a synergetic effect between zinc and nitrogen in rice yield. But sufficient information about zinc and nitrogen interaction is lacking in scented rice. Keeping this in view an experiment was conducted to study the interaction effect between zinc and nitrogen on growth, yield and nutrient uptake in scented rice.

Materials and methods

An experiment was conducted at Central Rice Research Institute, Cuttack during dry season of 2006. The scented rice variety Kasturi (125 days) was taken for experimental purpose. Twenty treatment combination consisting of two forms of zinc ($ZnSO_4$ $7H_2O$ and Zn-EDTA), two methods of application (soil and foliar application) and four levels of nitrogen (0, 30, 60, 90kg/ha) along with nitrogen levels alone (without Zinc) were tested in a randomised block design with three replications. For soil application $ZnSO_4$ $7H_2O$ (22%Zn@25Kg/ha and Zn-EDTA (Chelamin 12%Zn) @ 500g/ha were applied at basal. Zinc sulphate at 4% and Zn-EDTA at 0.05% were spread foliar at 30 and 40 days after transplanting. The soil of the experimental site was sandy loam having P^H 6.7 and available N 100kg/ha, P 14kg/ha and K 90kg/ha. It had a sub optimal Zn status (0.8 ppm, DTPA extractable Zn). Nitrogen was applied in three splits (25% basal, 50% at tillering and 25% at panicle initiation) while P and K each at 40kg/ha were applied during transplanting.

The growth parameters like plant height, leaf area index and dry matter production, yield contributing characters like panicle/m^2, grains/panicle and thousand grain weight, grain yield and straw yield were taken. Estimation of zinc and nitrogen uptake were done as per standard procedures.

Result and Discussion

The growth parameters like plant height, leaf area index and dry matter production were increased significantly with increase in different doses of

nitrogen (Table-1). This supports the findings of Khanda *et al.* (1997). The above growth parameters were also increased significantly by the application of zinc possibly due to action of auxin a type of growth hormone which promotes the stem elongation and cell enlargement in presence of zinc. The growth parameters were higher during combined application of zinc and nitrogen rather than nitrogen alone. Of the two sources of zinc application $ZnSO_4$ performed better than Zn-EDTA. Soil application of zinc was superior than foliar application. Katyal and Agrawal (1982) found that the soil application is superior than foliar due to continuous supply of zinc in the rhizosphere through mass flow.

Yield attributing characters like panicle/m^2, grains/panicle and thousand grain weight were increased significantly with the increase in nitrogen levels. These characteristics were also higher during combined application of zinc and nitrogen rather than nitrogen alone. These characters performed better by application of $ZnSO_4$ than Zn-EDTA. Similar trend was observed in soil application than foliar application of zinc.

The grain and straw yield in KASTURI were significantly increased with increase in different levels of nitrogen (Table-1). Khanda *et al.* (1997) also observed similar type of results which supports our

Table-1

Growth, yield and yield attributes of scented rice as influenced by zinc and nitrogen fertilization

Treatment	Plant height (cm)	Leaf area index	Dry matter (g)	Panicles m^{-2}	Grains panicle^{-1}	1000-grain weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
N levels (kg ha^{-1})								
0	104.00	2.86	18.13	318	65	19.00	3.50	4.22
30	106.25	3.01	21.08	347	76	20.15	4.12	5.16
60	107.25	3.35	22.10	383	86	20.35	4.45	5.72
90	108.15	4.03	22.18	406	95	21.27	4.80	6.12
CD (5%)	0.12	0.09	0.20	3	1	0.48	0.08	0.13
Zn and Nitrogen combination								
Nitrogen alone	105.20	3.06	19.82	345	70	19.85	3.95	4.78
Zinc+Nitrogen	106.30	3.22	20.65	362	81	20.25	4.24	5.36
CD (5%)	0.10	0.09	0.12	3	2	0.32	0.08	0.08
Zn sources								
Zn SO_4	106.10	3.27	21.13	373	85	20.25	4.28	5.50
Zn-EDTA	105.15	3.20	20.83	364	78	20.00	4.12	5.18
CD (5%)	0.10	0.08	0.14	2	2	NS	0.06	0.09
Method of Zn application								
Soil application	106.00	3.35	21.81	370	83	20.25	4.25	5.40
Foliar application	105.35	3.17	21.76	362	77	20.05	4.18	5.27
CD (5%)	0.10	0.08	0.18	2	2	0.30	0.07	0.09

Plant height, leaf area index and dry matter were measured 90 days after transplanting, NS = Not significant.

findings. The maximum grain and straw yield (4.80 and 6.12t/ha) were obtained in 90kg N/ha which were 37.2, 16.5, 7.2% more for grain yield and 45.1, 18.6, 7.0% more for straw yield at 0, 30, 60kg/ha nitrogen level respectively. Hayami (1983) observed that the increase in nitrogen application increased the rate of photosynthesis. So more carbohydrates were accumulated in the plant resulted in increasing the grain and straw yield.

The grain and straw yield was significantly more during application of zinc over no zinc application. The combined application of zinc and nitrogen increased the grain yield 7.4% and straw yield 12.1% over nitrogen alone. Vyas *et al.* (1990) also observed similar type of coordinated action between zinc and nitrogen. Of the two sources of zinc application $ZnSO_4$ recorded 3.9% grain yield and 6.2% straw yield higher than Zn-EDTA. Findings of Saravanan and Ramanathan (1988) and Khanda *et al.* (1997) corroborates with present study. There was increase in 1.7 and 2.5% for grain and straw yield respectively when zinc applied to soil rather than foliar application.

Uptake of nitrogen Kasturi increased significantly with increase in level of nitrogen (Table-2). Maximum nitrogen (106.98kg/ha) was uptaken by the plant at 90kg N/ha level. Application of zinc also enhanced the nitrogen uptake. The combined application of zinc and nitrogen increased the nitrogen uptake by 13.7% over that of the nitrogen alone. Application of $ZnSO_4$ source of zinc had higher (7.1%) nitrogen uptake than Zn-EDTA source which was conformed by the findings of Srivastava *et al.* (1992). The total nitrogen uptake was 4% higher in soil than foliar

Table-2

Uptake of nitrogen and zinc in rice as influenced by zinc and nitrogen fertilization

Treatment	N uptake (kg ha^{-1})			Zn uptake (kg ha^{-1})		
	Grain	Straw	Total	Grain	Straw	Total
N levels (kg ha^{-1})						
0	22.06	33.31	55.37	0.122	0.187	0.309
30	31.15	44.01	75.16	0.170	0.276	0.446
60	37.66	52.62	90.28	0.205	0.369	0.574
90	46.98	60.00	106.98	0.232	0.465	0.697
CD (5%)	0.58	0.72	0.58	0.012	0.008	0.010
Zn and Nitrogen combination						
Nitrogen alone	29.15	42.18	71.33	0.140	0.186	0.326
Zinc + Nitrogen	34.03	47.08	81.11	0.181	0.314	0.495
CD (5%)	0.48	0.60	0.48	0.010	0.007	0.009
Zn sources						
Zn SO_4	35.12	48.70	83.82	0.184	0.330	0.514
Zn-EDTA	32.66	45.60	78.26	0.176	0.303	0.479
CD (5%)	0.42	0.52	0.44	0.009	0.006	0.008
Method of Zn application						
Soil application	34.68	48.12	82.80	0.186	0.322	0.508
Foliar application	33.40	46.25	79.65	0.180	0.307	0.487
CD (5%)	0.42	0.52	0.42	0.009	0.007	0.007

application which is similar in agreement with Sahu (1989). The combined application of zinc and nitrogen increased the zinc uptake by 52% over than that of nitrogen alone. The application of $ZnSO_4$ source of zinc have significantly higher (7.3%) zinc uptake than that of Zn-EDTA. The total zinc uptake was significantly higher in soil application than foliar application.

It was apparent from the experiment that increase in the graded levels of nitrogen increase the grain yield. Application of zinc along with nitrogen also enhance the grain yield over that of nitrogen alone. Soil application of zinc is a superior method than foliar application and zinc sulphate source is better than Zn-EDTA for better yield in scented rice.

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In-Vitro propagation of *Asparagus racemosus* with reference to its therapeutic properties in Cardio-Vascular abnormalities

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Studies on *In vitro* propagation of *Asparagus racemosus* carried out by using leaf, internodes and shoot spear segments as explants gave the response 10-80% in MS medium. Maximum response observed with shoot spear i.e. 80% after 15 days of inoculation medium supplied with NAA (2.0mg/l) +BAP (1.0mg/l) shown better callusing in comparison to MS medium augmented with BAP (1.0mg/l) +2, 4-D (0.04mg/l).

Keywords: Shoot spear segments, Callusing; MS medium; *Asparagus racemosus*.

Introduction

Asparagus racemosus is a representative of the family Lilliaceae is popularly known as Satawari. Botany of the species reveals that it is straggling branched, perrnial, spinose, under shrub, rootstock fusiform, tuberous, stem fasciculated, tri-quetrous with pointed or curved spines, leaves scaly, triangular, acuminate, reduced to sub-erect or sub-curved spines, flowers white in axillary, solitary or fascicled, simple or branched racemes terminated by flowers, born in a compact bunch, fleshy and spindle shaped, pilus 5-15cm in length & 2cm in thickness, silvery white or light ash colored externally and white internally. Lack a well-marked odor, but are sweet & bitter in taste. It is found in sal and mixed forest of Madhya Pradesh, Chhatisgarh and Jharkhand.

Four types of shatavarin (shatavarin - I-IV) were identified in the root stock. They have a common steroidal nucleus but differ in attached sugar moieties (Ravikumar, 1987). Besides these shatavarin, asparagamine, a polycyclic alkaloid (Sekine, 1995) and racemosol, a cyclic hydrocarbon (Sekine, 1997) are also present in *Asparagus racemosus* Wild.

Asparagus racemosus Wild. cures gastrointestinal disorders and it has galactogogue effects. It is used in solving the problems of uterus. It is used as immuno-modulator. It has also some anti-hepatotoxic, anti-neoplastic activities. It is used in curing cardiovascular disorders. It has effects on respiratory and central nervous system problems. It is used as a diuretic, hypoglycemic and anti-amoebic effect (Goyal *et al.*, 2003).

Materials and methods

Samples collected from the P.G. Department of Botany, Utkal University, VaniVihar, Bhubaneswar and viz. five different types of explants, root segments, inter-node, nodal segments, leaves and shoot spear segments (ca, 3-5mm) were used for callus induction and proliferation. The explants were taken and washed thoroughly under running tap water for 30 min. Then the plant materials were transferred to an aqueous solution of 5% (v/v) teepol for 10 min and rinsed with autoclaved double distilled water, 0.1% $Hgcl_2$ (BDH, India) for 5min and rinsed 5-6 times with a autoclaved double distilled water.

Murashige & Skoog's medium (1974) gelled with 0.8% agar (w/v) was used as the basal medium; its pH was adjusted to 5.8 before autoclaving. Different explants of 3-5 mm were taken and inoculated on the MS medium with various combinations of growth regulators and nutrients. Media like Ms+(0.1-0.5mg/l), BAP+(0.0-5.0mg/l) 2,4-D; Ms+(0.0-2.0mg/l)

NAA+(0.0-2.0mg/l) BAP; Ms+(0.1-1.0mg/l) BAP+ (0.2-0.6mg/l) GA3 were used in all cases.

Then the cultures were incubated inside a culture room and maintained at 25±1°C, 55-60% relative humidity, 16 hr photo period with illumination provided by cool white fluorescent tubes giving a photon flux density 50 micro mol m^{-2} s^{-1} at rack level. The resultant primary calli & shoot buds were sub-cultured on the medium.

Result and Discussion

The morphogenetic as well as the nutrient status of the medium regulated the response of the explants employed. Out of the four explants used during present investigation freshly sprouted shoot spear segments showed the highest percentage of callus induction as well as proliferation.

The composition of culture medium is an important parameter, which must be optimized in order to achieve successful callus-mediated plant regeneration. In this study the basal media MS was found suitable. MS media had been used successfully to induce callus in several other Asparagus species (Kar & Sen, 1985). In order to develop a reproducible and quick responding medium to stimulate de-differentiation and to elicite callusing response, the MS basal medium was supplemented with various combinations and concentrations of growth regulators and their response was evaluated. A combination of lower concentration of an auxin (NAA) and a higher concentration of cytokinin produced green, compact and regenerative calli.

The morphogenetic response was related to culture age as evident from the present observations in *Asparagus racemosus* Wild. There was an inverse relationship between callus age and differentiation (Table-1). Younger calli were superior in morphogenic performance, when calli from mature explants were transferred to differentiated media they respond favorably. Maximum shoot was developed from shoot spear segments (Table-2).

Augmenting the basal Ms Medium with a cytokinin + auxin was essential to induce high frequency bud break and multiple shoot formation in nodal segments of *Asparagus racemosus* Wild (Table-3). High frequency of axillary shoot proliferation coupled with the maximum number of shoots per explant as well as longer shoot were recorded with Ms+BAP (2mg/l)+GA_3 (0.5mg/l) in *Asparagus racemosus* but in both these cases bud break and multiple shoot formation takes place from the nodal segments obtained from callus originated shoot.

Monocots are generally recalcitrant. By certain modification differentiation can be achieved through organogenesis & embryogenesis. In essence, the results from the present investigations on evaluation and optimization of several factors influencing bud break followed by multiple shoot proliferation in *Asparagus racemosus* Wild. could be very useful for developing a reproducible protocol towards rapid mass propagation as well as the formulation of an efficient strategy for *ex-situ* conservation of this important medicinal plant species.

Table-1

Morphogenic Response of various Explants to the MS medium in *Asparagus racemosus*

Types of Explants	Percentage of Response	Time Interval
LEAF	0%	3DAYS
	0%	7DAYS
	10%	15DAYS
	10%	30DAYS
INTERNODAL EXPLANTS	0%	3DAYS
	11%	7DAYS
	14%	15DAYS
	13%	30DAYS
SHOOT SPEAR SEGMENTS	10%	3DAYS
	40%	7DAYS
	80%	15DAYS
	60%	30DAYS
NODAL EXPLANTS	0%	3DAYS
	10%	7DAYS
	12%	15DAYS
	10%	30DAYS

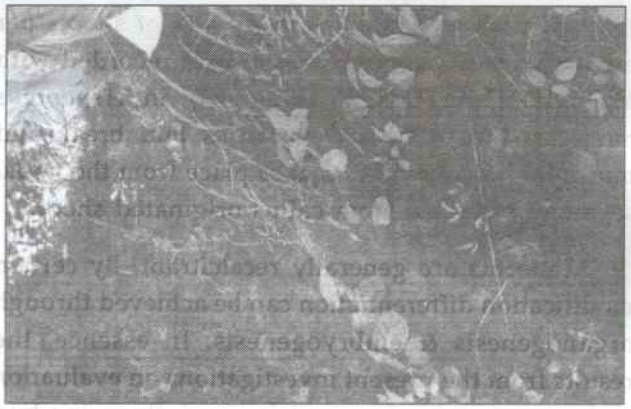

Fig. 1: *Asparagus* racemosus plant in nature.

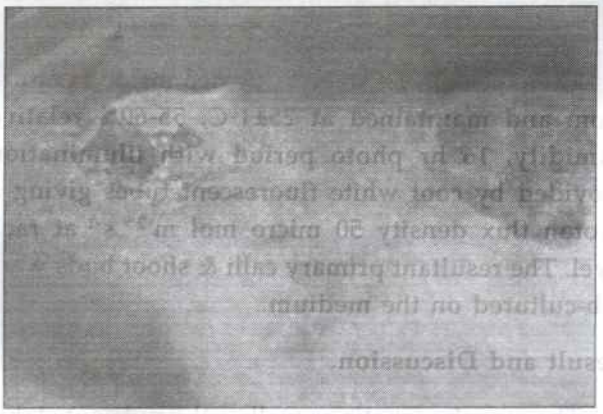

Fig. 2: Callus after 2 months of inoculation.

Fig. 3: Callus showing multiple shoot formation.

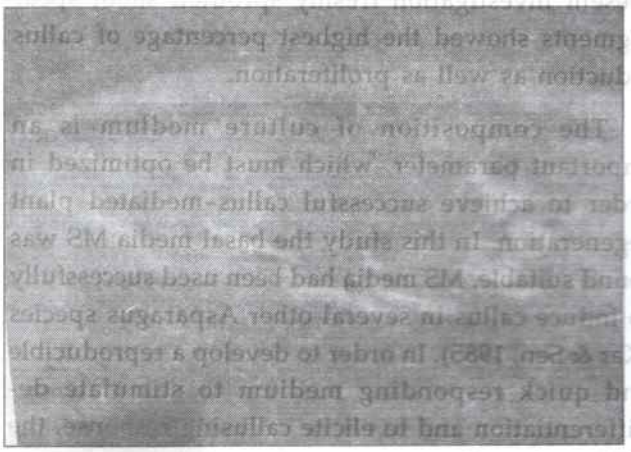

Fig. 4: Sub culturing of Callus after 3 months of inoculation.

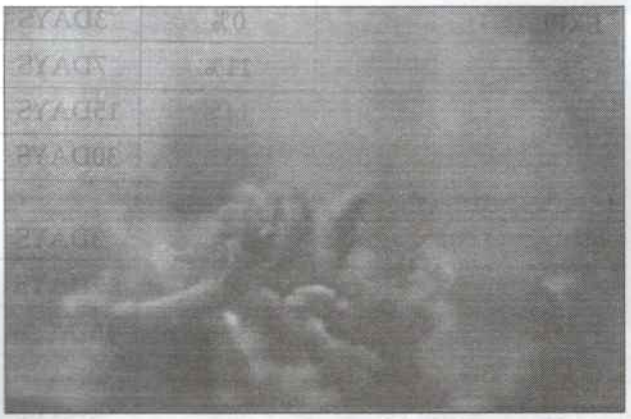

Fig. 5: Inter-nodal segments showing multiple shoot formation.

Fig. 6: Callus showing *in-vitro* rooting and Somaclonal variation.

Table-2

Response of shoot segments to 6-BAP+2, 4-D to form callus in *Asparagus racemosus*

Concentration in mg/l		No. of explants survive to respond	Percentage of respond
BAP	2,4-D		
0.0	5.0	Nil	Nil
0.1	0.04	8	88 ± 0.06
0.2	0.04	5	55.5 ± 0.33
0.3	0.04	3	33.3 ± 0.57
0.5	0.04	1	11.1 ± 0.05

Table-3

Response of shoot segment to auxin + cytokinin combination to form callus in *Asparagus racemosus*

Concentration in mg/l		No. of explants survive to respond	% of response
NAA	BAP		
0.0	0.5	Nil	Nil
0.0	1.0	Nil	Nil
1.0	0.0	Nil	Nil
2.0	0.0	Nil	Nil
0.2	0.5	8	88 ± 0.34
1.0	2.0	5	55.5 ± 0.66
2.0	1.0	9	100 ± 0.33

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Impact of Cyclone on Fungal Flora of Orissa : Retrospect and Prospect

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Cyclone is a regular feature in the coastal areas. Super cyclone is occasional. Both of them leave trail of devastation behind them. The greatest destruction is done to tall trees, which stand unprotected at the time of cyclone or super cyclone. Trees are not only the home of birds but are also a place where thousands of microbes grow, reproduce and thrive. This includes a large number of fungi also. It is natural that when a tree is destroyed, by any means, many microbes also die, destroyed or damaged. The life cycle of microbes is also affected. If some microbes are specific to some plant species the destruction of the plant species endangered the life of those microbe/which survive on them.

Keywords: Cyclone, fungi, damage

Introduction

After the super cyclone of October 1999, and subsequent cyclones, a few qualitative observations on plant and fungi lives have been made. One of them was that the regeneration process of some trees was faster and sometime the flowering was earlier than the annual season. This might lead to unavailability of suitable host surface at the seasonal arrival of microbial spores or particularly fungal spores. In the present study, it was observed that one species of *Marasmius* which is dependent on specific host Katimba (*Anthocephalous kadama mig*) for its survival was limited in occurrence due to destruction of leaves of Kadamba. Some fungi are associated with bark tree trunks and roots. Though we do not have the exact number of fungi so far reported from Orissa, yet it seems that due to regular unusual cyclonic conditions their habitat must be disturbed. This study is based on general observation of flowering plants just after cyclones within one month and subsequently for one year for the occurrence of some of the species of fungal flora on different plants and plant products. The powdery mildews caused by *Erysiphe* and *Uncinula* was observed on a new host; the Bel (*Aegel marmelos*) leaf which is not normally attacked by thsi fungus. It may be due to the reason that many of its original hosts have been defoliated. Some myconihizal fungi and leaf letter fungi were also studied. The ecological importance of fungi was observed.

Materials and Methods

Leaves and twigs of different plant species were collected after each cyclone and after heavy rains throughout the year. Leaf litters were collected when the rainy season was over. Larger fungi were collected individually or in groups, some time with either the host or the substrate. All leafy materials were preserved with substrates or hosts in the laboratory. Larger fungi were preserved in formalin acetic acid alcohol (FAA) solution. Morphological characters were recorded after careful observations. Anatomical characters were studied under the compound microscope (40x and 100x). Identification of fungal specimens were done with monographs. Photographs were taken both in the field and in the laboratory. Data on the reports of fungi were compared with earlier reports from Orissa. The fungal damage estimate was done on the basis of data available.

Result and Discussion

The above estimate includes all types of trees, but as far as the matter of fungal flora is concerned, three aspects may be pointed out, number one the

Marasmius on Kadamba leaves

The cyclone impact

debris will increase the number of fusarium which will block the water channel in the long run deteriorating the health of trees as it happened in the case of Sissoo (*Dalbergia sissoo*). Number two, many of the mycorrhizal fungi will gradually vanish from the rhizosphere of the soil where these trees use to grow. Number three, the larger fungi like *Russula* species are diminishing in number because they are mycorrhizal fungi on Casuarina (*Casurina emisetifolia*) and sal trees (*shorea robusta*). Almost all birds have nests in the tree. It is certain that the nests and birds must be affected due to super cyclone and cyclone when trees are damaged or destroyed. The fungal flora of feather and hair was studied earlier. It must have also been affected but a study is required. The law of over production is also followed by fungi but due rate of survival diminishes. The disturbance in fungal ecology may disturb the total ecology of the state.

The fungal flora of the state of Orissa was recorded earlier, the compilation of second phase of record is under process. After this the prospect of fungal flora in the state may be ascertained. In the year two thousand seven, Orissa was spared by a super cyclone.

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