ISSN 0972-8546

PLANT SCIENCE RESEARCH

The Orissa Botanical Society

Volume 27 • No. 1 & 2 • December, 2005

PLANT SCIENCE RESEARCH

(Formerly Journal of Orissa Botanical Society)

Official Publication of

THE ORISSA BOTANICAL SOCIETY

Plant Science Research is the official publication of the Orissa Botanical Society and is published twice in a year. Subscription rate for non-members and institutions is Rs.75/- in India and $10/- for overseas per annum. Life members of the Society get the journal free of cost. All communications regarding business matters should be addressed to the Managing Editor, Plant Science Research, P.G. Department of Botany, Utkal University, Bhubaneswar-751 004, India.

Plant Sci. Res. Vol.27 (1&2) : 1-5, 2005

Effect of frequency of irrigation on coir pith amended soil for production of tuber crop - sweet potato

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Coir pith, a fluffy, fibrous carbonaceous waste material obtained from coir fibre extraction units, has the potential to improve the soil physico-chemical properties mainly due to its high water holding capacity, high porosity and presence of some major and minor nutrients. The demand for today's agriculture is to produce more from diminishing land, water and natural resources. Coir pith can play a very important role in this direction. Taking into consideration of its high water holding capacity (734.4%), pot experiments have been conducted in factorial randomized block design with six replications during March to June 2004 to observe the frequency of irrigation for survivability of sweet potato (*Ipomoea batatas* L.) an important tuber crop. Four levels of coir pith were applied to the normal lateritic soil of Bhubaneswar with the recommended level of FYM (10t/ha) which were irrigated at four different time interval (5, 10, 15, 20th day). Constant amount of water was used for irrigation at different time intervals routine wise in the experimental pots. It was observed that the frequency of irrigation can be decreased with increasing doses of coir pith. For an average temperature condition of Maximum 36.67^{0}C and Minimum of 24.64^{0}C plants were found to be alive on coir pith (56, 84, and 280 t/ha) amended soil pots with 15 days irrigation interval. It showed 66% survivability in coir pith amended (280 t/ha) soil even with 20 days irrigation interval. Plants of control (soil only) pots could only tolerate 5days irrigation interval. At a high average temperature of 45 to 46^{0}C, all the plants even in the coir pith amended pots (28, 56, 84, and 280 t/ha) could not survive with irrigation interval of 10 days and above. Plants of control (soil only) could not survive even at 5 days irrigation interval. However coir pith amended plants irrigated at 5days interval could manage to survive even at such high temperature. Results clearly indicate the effectiveness of coir pith as soil conditioner for judicious management of water for production of sweet potato.

Key words: *sweet potato, tuber production, coir pith, water management, soil conditioner.*

Introduction

Sweet potato (*Ipomoea batatas* L.) is an important food crop of tropical and subtropical regions of the world. It grows in a warm humid climate with a mean temperature of about 22°C. Though sensitive to frost, it can also be grown in the hills up to an altitude of 1500-1800 m as a summer crop. Under rainfed conditions the crop requires a fairly well distributed annual rainfall of 75-150 cm. Being a photosensitive crop, sunny days and cool nights are favorable for better tuber development.

India is one of the leading countries of the world in area and production of coconuts. As per the recent estimation, about 15043.2 million nuts are produced every year from an area of 1.70 million hectares in India. Almost all the plant parts of coconut are used except coir pith, which is generated as a waste after extraction of fibre. These are dumped in mounds causing space problem. About 7.5 million tons of coir pith is produced annually. However, coir pith being organic in nature, there are some advantages of its use in agriculture. The unique properties of this waste material are high porosity, high water holding capacity in the range of 400-600% and presence of some amounts of major and micronutrients, which have been found to be highly useful in agriculture as soil conditioner. The high water holding capacity of this waste can be used judiciously for water management in agriculture

(Savithri and Khan, 1994; Mukherjee and Rout, 2000; Mukherjee 2001; Mukherjee et al., 2002)

The present utilization of water for various purposes such as drinking, irrigation industry and energy, etc, is about 750 bcm. The use of water for irrigation constitutes about 84 percent of the water used. With increase in demand of the water for other uses, the share of water used for irrigation is likely to go down to about 73 percent by 2025 AD. In India 93 percent of the total water resources are used by agriculture. Within the next decades, fresh-water scarcity will generate strong pressure to use water for food production more effectively. Thus water resource management in agriculture becomes a vital issue. Management implies the efficient and equitable distribution water resources to the agriculture, avoiding the environmental degradation. Over the last decade three distinct phases of "water management" has evolved viz: (i) the phase of supply management (get more water), (ii) the phase of demand management (get more out of every drop, wherever it is used), (iii) the phase of demand-management by allocation efficiency (get more values out of every drop by allocating it to high-value products.).

In this context, coir pith the organic source with its high water holding capacities can act as substrate for better water management in agriculture. The effectiveness of coir pith as soil conditioner for judicious management of water requirement for production of high energy food crop like sweet potato is discussed in this communication.

Materials & Methods

A pot experiment was carried out to observe the frequency of irrigation for survivability of plants of tuber crops (sweet potato) in soil amended with coir pith at different level irrigated at different time interval. Four levels of coir pith (28, 56, 84, and 280 t/ha) were applied to the normal lateritic soil of Bhubaneswar with the recommended level of NPK (75:50:75) and FYM (10t/ha) which were irrigated at four different time interval (5, 10, 15, 20th day). The experiments were conducted in factorial randomized block design with six replications for each combination.

Initially the cuttings were frequently irrigated for establishment of the plant and then constant amount of water (1litre) was used for irrigation at different time intervals routine wise in the experimental pots. During the experimental period, temperature of the site was strictly recorded.

Treatment details:

T_{1A}: Soil + coir pith mixture (0t/ha) irrigated in 5 days interval

T_{1B}: Soil + coir pith mixture (28t/ha) irrigated in 5 days interval

T_{1C}: Soil + coir pith mixture (56t/ha) irrigated in 5 days interval

T_{1D}: Soil + coir pith mixture (84t/ha) irrigated in 5 days interval

T_{1E}: Soil + coir pith mixture (280t/ha) irrigated in 5 days interval

T_{2A}: Soil + coir pith mixture (0t/ha) irrigated in 10 days interval

T_{2B}: Soil + coir pith mixture (28t/ha) irrigated in 10 days interval

T_{2C}: Soil + coir pith mixture (56t/ha) irrigated in 10 days interval

T_{2D}: Soil + coir pith mixture (84t/ha) irrigated in 10days interval

T_{2E}: Soil + coir pith mixture (280t/ha) irrigated in 10 days interval

T_{3A}: Soil + coir pith mixture (0t/ha) irrigated in 15 days interval

T_{3B}: Soil + coir pith mixture (28t/ha) irrigated in 15 days interval

T_{3C}: Soil + coir pith mixture (56t/ha) irrigated in 15 days interval

T_{3D}: Soil + coir pith mixture (84t/ha) irrigated in 15 days interval

T_{3E}: Soil + coir pith mixture (280t/ha) irrigated in 15 days interval

T_{4A}: Soil + coir pith mixture (0t/ha) irrigated in 20 days interval

T_{4B}: Soil + coir pith mixture (28t/ha) irrigated in 20 days interval

T_{4C}: Soil + coir pith mixture (56t/ha) irrigated in 20 days interval

T_{4D}: Soil + coir pith mixture (84t/ha) irrigated in 20days interval

T_{4E}: Soil + coir pith mixture (280t/ha) irrigated in 20 days interval

The same treatments were duplicated for observation at higher temperature, the original set was kept in partial shade for obtaining yield data in the temperature range of 45-46^{0}C.

Results and discussion

Results of the studies indicate that the frequency of irrigation can be decreased with increasing doses of coir pith. For an average temperature condition of maximum 36.67^{0}C and minimum of 24.64^{0}C, plants were found to be alive on coir pith (56, 84, and 280 t/ha) amended soil pots with 15 days irrigation interval. Moreover it showed 66% survivability in (280 t/ha) coir pith amended soil even with 20 days irrigation interval. However plants of control (soil only) pots could only tolerate 5days irrigation interval.

At a high average temperature of 45 to 46^{0}C, plants could not survive with 10 days irrigation interval even in the coir pith amended pots (28, 56, 84, and 280 t/ha). Plants of control could not survive even at 5 days irrigation interval. However coir pith amended plants irrigated at 5days interval could manage to survive even at such high temperature. Results clearly indicate the effectiveness of coir pith as soil conditioner for judicious management of water for tuber crop production. Increase in yield with increase in doses of coir pith was observed under 5 days irrigation interval. Increase in yield was recorded about 21%, 15%, 11%, 9.6% in soil amended with coir pith @ 280t/ha, 84t/ha, 56t/ha, 28t/ha respectively (Fig. 1). Sweet potato plants when irrigated with 10 days interval, could not survive in control pots. Coir pith amended soils could give reasonable yield of 15t/ha, 7% increase in yield was found when coir pith dose was raised from 28t/ha to 280t/ha. (Fig. 2). When the experimental pots were irrigated at 10 days interval, plants of control and coir pith (@ 28t/ha) amended pots could not survive. However the plants grown in 280t/ha coir pith amended soil could give 9% increase in yield as compared to the plants grown in 56t/ha amended coir pith soil. (Fig. 3). Experimental pots supplemented with 20 days irrigation interval could yield only in the treatment of 280t/ha coir pith amended soil (Fig. 4). At higher temperature range (45 to 46 ^{0}C), plants of control pots could not survive even at 5 days irrigation interval. However under similar irrigation and temperature conditions plants could survive in coir pith amended soil and soil

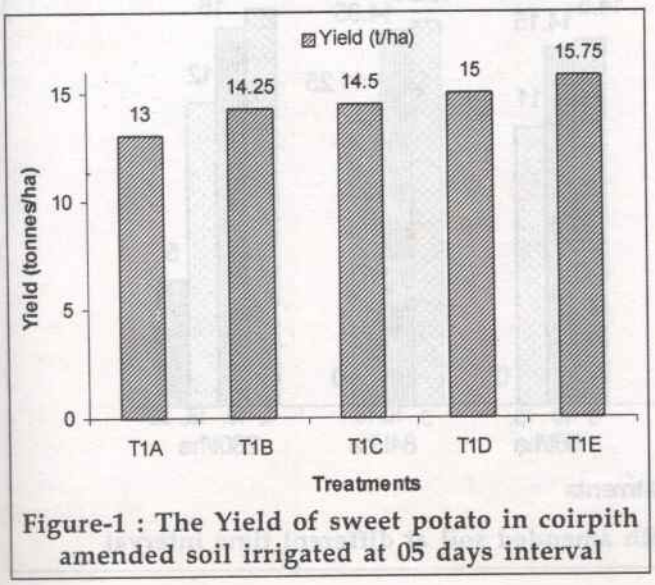

Figure-1 : The Yield of sweet potato in coirpith amended soil irrigated at 05 days interval

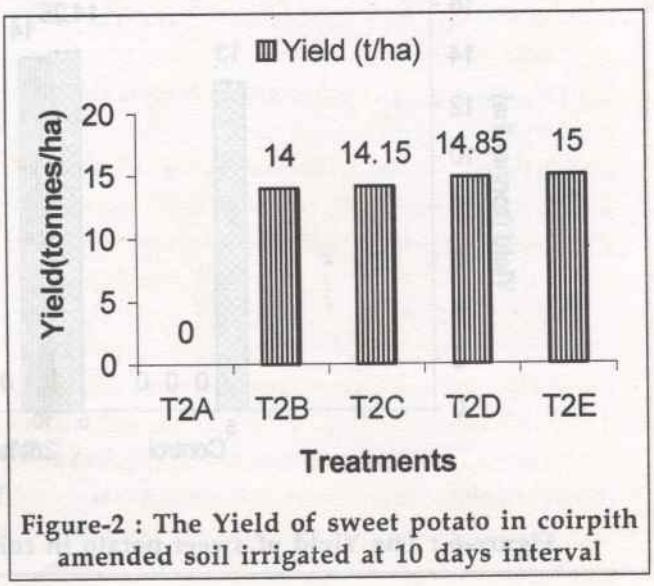

Figure-2 : The Yield of sweet potato in coirpith amended soil irrigated at 10 days interval

amended with 280t/ha coir pith could give remarkable yield of 14.75t/ha (Fig. 5).

The graphical presentation of the yield data of sweet potato (Fig. 6) obtained in different doses of coir pith amended soil in different time interval shows that the yield tends to decrease by increasing the irrigation time interval and increase with the increase of doses of coir pith applied. It is interestingly seen that by applying the dose of 28t/ha of coir pith reasonable yield (14t/ha) can be obtained even if irrigated at 10 days interval as against control plants irrigated at 5 days interval. However, application of 56t/ha of coir pith, with 5days irrigation interval, could give higher yield than the yield obtained in

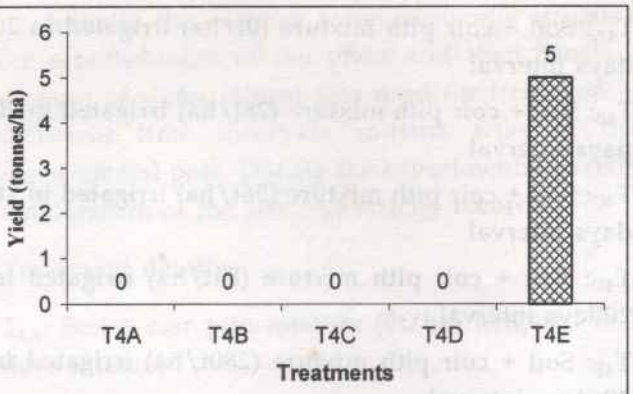

Figure-4 : The Yield of sweet potato in coirpith amended soil at 20 days irrigation interval

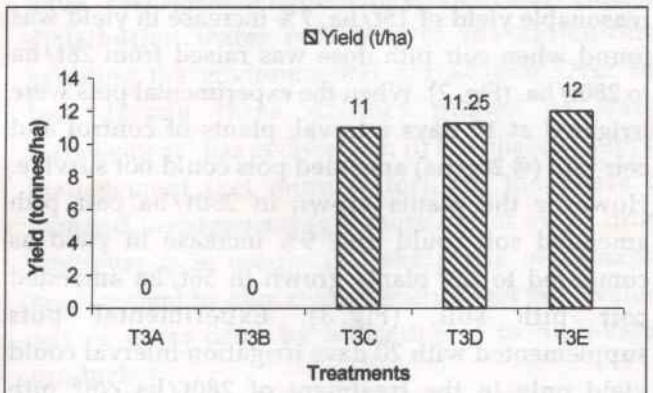

Figure-3 : The Yield of sweet potato in coirpith amended soil at 15 days irrigation interval

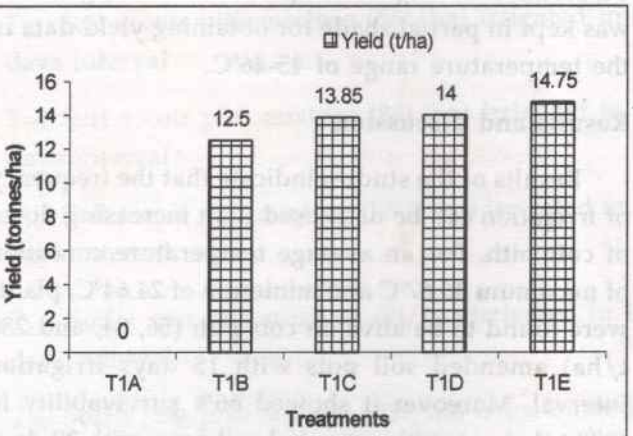

Figure-5 : The Yield of sweet potato in coirpith amended soil at 05 days irrigation interval at a temperature in the range of 45-46°C

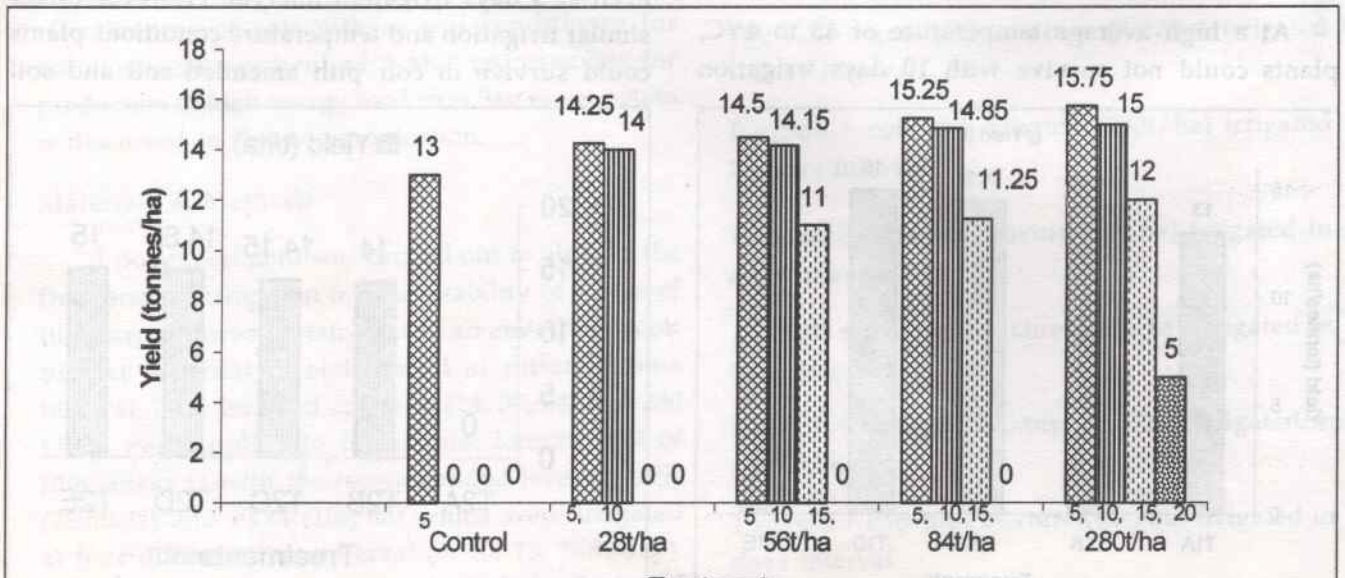

Figure-6 : The Yield of sweet potato in coirpith amended soil at different time interval

28t/ha of coir pith amended soil in 5 days interval. Further the yield was found to be at par with amendment of 84t/ha and 280t/ha coir pith.

Conclusion

From the elaborate experimentation on production of sweet potato in soil amended with different doses of coir pith, under different intervals of irrigation carried out at different temperature ranges revealed that an optimum dose of coir pith @84 t/ha with 10 days irrigation interval gives highest production of sweet potato. Reduction in water requirement is mainly due to high water holding capacity coupled with slow release mechanism associated with coir pith due to its unique structural feature. Application of coir pith was thus found to be quite effective in decreasing the frequency of irrigation and this organic waste coir pith can be utilized as an effective means of water management in different crops.

Acknowledgement

The authors are thankful to Coconut Development Board, Kochi for funding the "Holistic Approach for mitigating pests and diseases affected coconut garden using biocides and coir pith for sustainable agri-horticultural production" project. Authors are also thankful to Dr. Vibhuti N. Mishra, Director, RRL (B), for his valuable suggestions and encouragement and for providing the infrastructure facilities.

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Cytological Effect of Paracetamol and Ofloxacin on Root Meristems of *Allium cepa* L.

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Antipyretic, Analgesic and Antibiotic drugs are commonly used medicines by human beings. To study the effect of these at cellular level an attempt has been made to investigate the cytological effect of Paracetamol,an antipyretic and analgesic and Ofloxacin, an antibiotic on root meristems of *Allium cepa*. The study indicates mitodepressive effect, cellular and chromosomal abnormalities due to the action of Paracetamol and Ofloxacin.

Key words: *Antibiotic, Antipyretic, Analgesic, Mitodepressive, Chromosomal abnormalities.*

Introduction

Paracetamol, an antipyretic and analgesic and Ofloxacin, an antibiotic are the most common medicines used by human. Paracetamol is a paraphenol derivative drug with aspirin which exerts analgesic and antipyretic effects, but it is devoid of significant anti inflammatory effect. This could be due to its weak activity on peripheral prostaglandin synthetase. But it exerts the blocking effect of aspirin on this enzyme in brain. It does not produce acid base imbalance, electrolyte disturbance, gastro intestinal irritation and blood clotting etc (Satoskar and Bhandarkar, 1988). Paracetamol is commonly used to lower the body temperature. It is rapidly absorbed on oral administration within 1/2 to 1h. It is mainly excreted in urine as conjugated products of glucuronic and sulfuric acids, but in infants the capacity of conjugative glucuronic acid and sulfuric acid is poor and so it becomes toxic for them. The adverse reactions of Paracetamol are anemia, methemoglobinaemia and liver damage etc.

Ofloxacin, an antibiotic is used to kill bacterial infection. It hits the bacterial cell wall, cytoplasmic membrane,ribosomes and m-RNA involved in translation of genetic information.

Considering the wide use of Paracetamol and Ofloxacin in human system, the present investigation was aimed to evaluate the effect of these drugs on a plant system at cellular level.

Materials and Methods

Paracetamol (250 mg and 500 mg) and Ofloxacin (200 mg and 400 mg) were used for preparation of solutions. Effects of child dose and adult doses were studied separately. Rooted onion bulbs were kept on tubes containing the solutions of Paracetamol and Ofloxacin. The bulbs were kept for 6, 12 and 18 h for each experiment. One controlled set was maintained keeping the rooted bulbs on distilled water. Samples of roots were pretreated in supersaturated solution of para-dichlorobenzene for 2 h and then were fixed in 1:3 acetic alcohol after 6, 12 & 18 h of treatment. Same procedure was followed for controlled sets. After 24 h of fixation the roots were transferred to 70% alcohol and stored in a refrigerator (4°C). Squashed roots were observed under high power objective and photomicrographs were taken using oil immersion objective.

Mitotic index was calculated by the formula:

MI = Number of dividing cells / Total number of cells X 100

Results

Effect of 250 and 500 mg of Paracetamol was studied on root meristems of *A. cepa* to observe its effect at cellular level by analysing the frequency of dividing cells from mitotic index (Table-1) as it is considered as one of the parameters to know the rhythm of cell division. At 0 hour the mitotic index

was 6.37, but after 6 h of treatment, it was 6.74, 10.60, 6.41, 8.49 and 5.37 with 250 mg Paracetamol, 500 mg Paracetamol, 200 mg Ofloxacin, 400 mg Ofloxacin and control (water) respectively. With increase of duration of treatment i.e. after 12 h the MI was 5.40, 10.84, 5.34, 6.78 and 3.51 with 250 mg Paracetamol, 500 mg Paracetamol, 200 mg Ofloxacin, 400 mg Ofloxacin and control respectively. Similarly after 18 h of treatment the frequency of dividing cells were further decreased to 4.38, 4.78, 3.74, 5.50 and 2.35 with 250 mg Paracetamol, 500 mg Paracetamol, 200 mg Ofloxacin, 400 mg Ofloxacin and control respectively. Mitodepressive effect of both Paracetamol and Ofloxocin was prominent in longer duration of treatment.

Following the treatment with water, 250 mg and 500 mg of Paracetamol for various duration of treatments, many types of abnormalities were observed in the mitotic cells. At 0 hour few cells were only vacuolated (Fig. 1). After different durations many types of abnormalities like clumped chromosomes (Fig. 2), anaphase bridge (Fig. 3), binucleated cells, chromosome breaks (Fig. 4 & 5) and micronuclei were recorded. The frequencies of different types of abnormalities were comparatively more in 6 h of treatment than in 12 h or 18 h. From among the doses 250 mg and 500 mg of Paracetamol, the frequencies of abnormalities were high with 500 mg of Paracetamol (Table-2). The abnormalities like break and bridge formations were probably healed during prolonged treatment. Ofloxacin (200 mg & 400 mg) also exhibited chromosome clumping, vacuolated cells, binucleated cells, elongated cells, unequal condensation of chromosomes (Fig. 6), chromosome breaks and micronuclei. With 6 h of treatment, the frequencies of abnormal cells were more in 400 mg of Ofloxacin than 200 mg. With increase in duration of treatment the frequency of abnormalities were reduced. From the comparative data it is also clear that the frequency of abnormality was minimum at 0 h, maximum in 6 h of treatment and it was reduced in 12 h and maximum reduction was in 18 h of treatment.

Table-1

Effect of Paracetamol and Ofloxacin on mitotic index (MI) in root apical meristems of *Allium cepa* at different time intervals

Type of Treatments	Duration in Hour(s)	MI
CONTROL (WATER)	0	6.37
	6	5.37
	12	3.51
	18	2.35
PARACETAMOL (250 mg)	6	6.74
	12	5.40
	18	4.38
PARACETAMOL (500 mg)	6	10.60
	12	10.84
	18	4.78
OFLOXACIN (200 mg)	6	6.41
	12	5.34
	18	3.74
OFLOXACIN (400 mg)	6	8.49
	12	6.78
	18	5.50

Discussion

At the first step it was tried to assess the effect of Paracetamol and Ofloxacin on progression of cell division in terms of mitotic index. It was observed that maximum mitodepressive effect was in 18 h of duration, the roots might be either immersed in water or Paracetamol or Ofloxcin (Table-1). It was also clearly marked that both Paracetamol and Ofloxacin enhanced the quiescent cells to proliferate in 6 h of treatment against 0 h. This implies that within a short period of 6 h the drugs create stimulating effect for the progression of cell division.

Clumped chromosomes, anaphase bridge, vacuolated cells, binucleated cells, chromosome breaks, elongated cells, micronuclei were the types of abnormalities recorded in both types of treatments. Clumped chromosomes may be due to stickyness of the chromosomes as a result of disturbance in spindle organization. The drugs might act as contact poison which arrest chromosome movement. Darlington

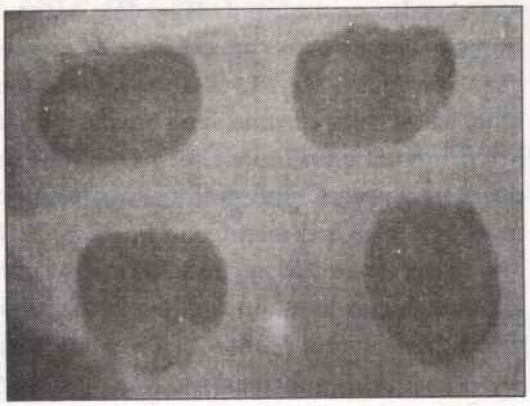

Fig. 1

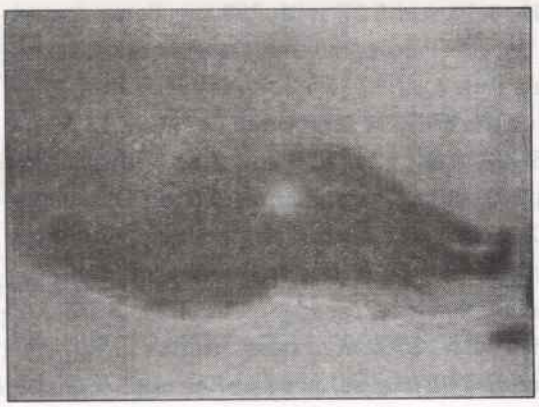

Fig. 2

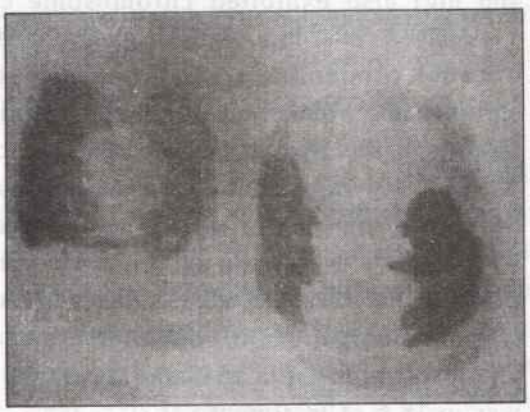

Fig. 3

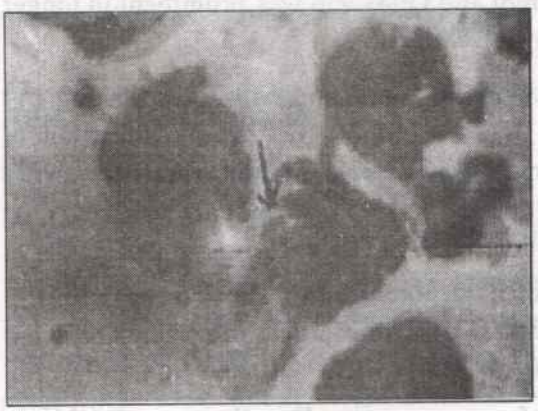

Fig. 4

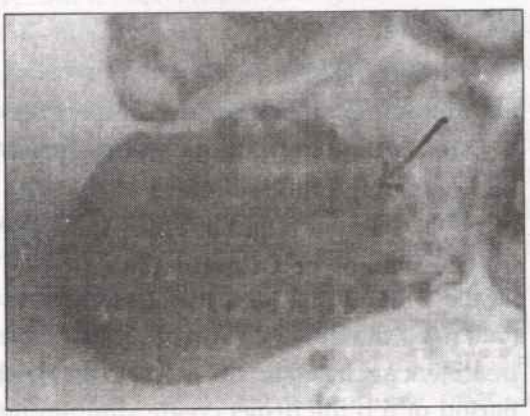

Fig. 5

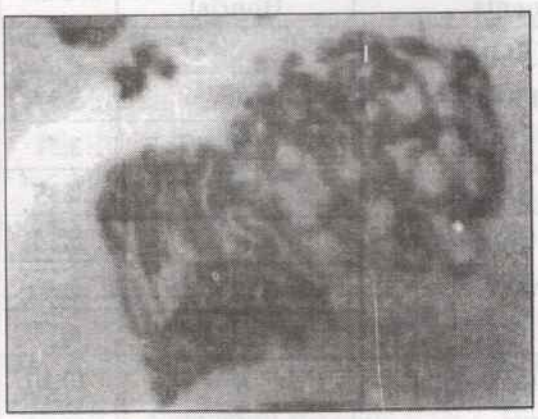

Fig. 6

Fig. 1. Vacuolated cells at 0 hour of treatment in root meristem of *Allium cepa* L.

Fig. 2. Cell Showing clumped chromosomes with 500mg of Paracetamol.

Fig. 3. Cell showing bridge connection during anaphase with 250mg of Paracetamol.

Fig. 4 & 5. Cells showing different grades of chromosome breaks with effect of 500mg of Paracetamol.

Fig. 6. Cells showing unequal condensation of chromosomes with effect of Ofloxacin.

(1942) described stickyness due to disturbance in nucleic acid metabolism. Presence of binucleated cells may be due to inhibition of cell plate formation. Paracetamol and Ofloxacin might inhibit the assembly of proteins and vesicles for cell plate formation.

The frequencies of chromosome breaks were maximum after 6 h of treatment and were reduced after longer duration (Table-2). Production of high frequency of chromsomal break after 6 h and reduction in longer duration may be due to subsequent healing. Anaphase bridges are generally produced due to chromosamal break, joining of broken chromosomal segments, stickyness of chromosomes and disfunctioning of spindle apparatus. Chromosome breaks are regarded as primary lesion for the origin of different types of chromosomal structure changes (Reiger *et al.*, 1976).

Chromotoxic effect of "Pan masala" has been studied by Shukla and Dutta (2002). Many chemical mutagens like alkylating agents are known to cause chromosomal breakage by binding to DNA regions rich in G-C pair (Lawely and Brookes, 1963). But the exact mechanism due to the effect of Paracetamol and Offloxacin is yet to be analysed. The findings of chromosomal and cellular abnormalities with effect of Paracetamol and Ofloxacin resemble to that of mutagen and pesticide treatments (Amar and Ali, 1983; Cortes, 1985 and Rao *et al.*, 1987).

Acknowledgement

The authors wish to thank the Head of the Department of Botany for providing necessary laboratory facilities and Dr. Abhaya Kumar Dalai, Reader in Botany, Ravenshaw College, Cuttack for his valuable suggestions.

Table-2

Chromosomal abnormalities with Paracetamol (250 mg & 500 mg) and Ofloxacin (200 mg & 400 mg) in root meristems of *Allium cepa* L. at different time intervals

Type of Treatments	Duration in Hour(s)	Total No. of Dividing Cells under focus (A)	Total No. of Abnormal Cells under focus (B)	% of Abnormal Cells B/A x 100
CONTROL (WATER)	0	104	02	1.92
	6	87	04	4.54
	12	66	07	10.60
	18	44	08	18.18
PARACETAMOL (250 mg)	6	129	33	25.58
	12	102	13	12.74
	18	80	07	8.75
PARACETAMOL (500 mg)	6	202	123	60.89
	12	171	87	50.87
	18	93	12	12.90
OFLOXACIN (200 mg)	6	133	120	17.69
	12	97	13	13.40
	18	73	05	6.84
OFLOXACIN (400 mg)	6	145	52	35.86
	12	111	23	20.72
	18	88	11	12.50

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Plant Sci. Res. Vol.27 (1&2) : 11-15, 2005

RAPD based molecular characterization of *Phaseolus vulgaris* L. landraces of Uttaranchal Kumaon hills

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Ten morphological variants of landraces of *Phaseolus vulgaris* L. were collected from different locations of Kumaon hills of India and one released variety VL-63 was selected for comparison as a reference to corelate genetic diversity among them. This study in turn will help to identify distinct genotype from the mixture of land races followed by their usages to isolate brassinosteroid like plant growth promotry compounds in order to select the best chemotype. Randomly amplified polymorphic DNA (RAPD) based polymorphism was studied using 12 primers (10mer) which produced 72 polymorphic bands. A dendrogram generated by cluster analysis from data derived from amplified fragments divided these commonly grown bean genotypes into two major clusters A and B. Cluster A has 2 genotypes while cluster B has 8 genotypes which were further characterized into two minor clusters B1 and B2.Collection SHU2012 was found to consist in the major cluster B, VL-63 observed distinct. SHU2014 and SHU 2032 were recognized as the part of B2, which showed maximum similarity (87.5%) with all the genotypes.

Key words: Landraces, *Phaseolus vulgaris*, RAPD

Introduction

Various common beans (*Phaseolus vulgaris* L.) have been popularized as an important leguminous vegetable crop in many parts of world including India. Due to rich protein content (20-23%), these are being widely adapted. Beans are grown in different climatic conditions. The wide dispersion of the beans gives different local names (Tobin and Carpenter 1978) such as rajmash, rajama, snap bean, cran berry, great northerns, haricot kidney, navy, red Mexican and pole bean.

Farmers in Kumaon hills commonly grow and manage landraces of beans since long back. These have been observed phenotypically distinct and genetically diverse on the basis of different seed characters. Deliberate human selection has been imposed on natural selection pressures during past which has resulted a vertical mixture of beans including mixtures of landraces alone or with improved varieties (Wood and Lenne, 1997). These mixtures show diversity of seed colour pattern, shape and size (Martin and Adams, 1987). Farmers use different morphological characters as markers for taste, texture, yields, resistance to environmental stresses and maturity time (Wood and Lenne, 1993). In order to generate accurate information on the amount of diversity present within the gene pools as well as of the spatial distribution of diversity in relation to eco-geographic factors (Waugh, *et al.*, 1992 and Freyre, *et al.*, 1996), present investigation was planned using molecular data to corelate genetic diversity with morphological variations among land races.Different molecular methods including RFLPs and RAPDs analysis have been used to characterize variability in *Phaseolus spp.* Genetic variation among common bean has been revealed in landraces of Middle American origin (Beebe, *et al.*, 2000), northwestern Argentinean cultivars (Galvan, *et al.*, 2001) and swiss common bean cultivars (Eichenberger, *et al.*, 2000) by use of RAPD markers. In the present study, different landraces has been

characterized using RAPD. These are being grown and available with the farmers of Kumaun hills in extreme abiotic stress conditions with a view that these land races may be unique with respect stress tolerant bioorganic like compounds such as brassinosteroids (Yokota, *et al.*, 1990).

Materials and Methods

Source of germplasm

Ten landraces including one variety (VL-63) of *Phaseolus vulgaris* L. were collected using random sampling which were representative of large groups from Kumaon hills at different locations namely Ranikhet, Almora, Nainital, Chamoli and Pithoragarh on the basis of their location at different altitudes in Himalayan region of India. Seeds were characterized preliminary on the basis of colour, presence or absence of mottling, size and shape followed by their classification on the basis of weight of hundred seeds. Descriptors were undertaken on the basis of on visual and quantitative characters developed on variable population available with our programme. Collected material(s) were distinguished on the basis of plant growth and development characters including internode pigmentation, twinning/bushy, colour of flower, early/late flowering.

Molecular analysis

Briefly DNA was extracted from several young leaves from three differents plants randomly from each block after harvesting them from each plant, which was immediately frozen in liquid nitrogen, pulverized and lyophilized. The freeze-dried material was ground to fine powder and stored at -20^0C.

DNA was extracted from the powdered leaf material using SDS method described by Dellaporta (1983), which was quantified by spectrophotometer (Beckman DU 6804) using calibration by adding 2 µL aliquot of extracted DNA dissolved in TE to 98 µL TE buffer. A sub sample was then diluted to a standard concentration of 20 ng μL^{-1} for amplification by the polymerase chain reaction (PCR) as described below for molecular analysis.

Twelve random decamer primers (Briand, *et al.*, 1998) supplied by Banglore Genei Pvt. Ltd., India designated as C11 (GGTGATCAGG), G10 (GGGATATCGC), G12 (CCAAGCCTTC), G4 (CAATCGCCGT), G5 (GGAAGCTTGG), G9 (TCTGTGCTGG), G2 (TGCGCCCTTTC), G3 (TGCTCTGCAG), G7 (GGTGACGCAG), C8 (GGAGGGTGTT), T6 (ACGGATCCTG) and T1 (CTGCTGGCAC). Amplification reactions were carried out twice in 25 µL volumes containing reaction buffer (20 mM NaCl), 50 mM Tris-HCl pH 8.3), 1% Triton-X-100, 2.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP (Banglore Genei Pvt. Ltd.), 0.2 µM primer, 1 unit Taq DNA polymerase (Banglore Genei Pvt. Ltd.). Amplification was performed for 45 cycles in a thermal cycler (Biometra). Each cycle consisted of 1 min at 94^0C, 2 min at 36.8^0C and 2.5 min at 72^0C, followed by a final extension time of 7 min at 72^0C. Amplification products were separated by electrophoresis in 2% agarose gels containing 0.5 mg μL^{-1} ethidium bromide.

Data analysis

DNA fragment profiles representing a consensus of two replicates were scored in a binary fashion with 0 indicating absence and 1 indicating presence of band. All monomorphic bands were also scored and included in analysis. Presence or absence of unique and shared polymorphic as well as monomorphic products was used to generate similarity coefficient. A dendrogram was constructed based on the similarity matrix data by applying the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis using a NTSYS computer software. Similarity measures which do not includes negative matches (both isolates that have no bands were also counted) included Jaccard's similarity coefficient (Jaccard, 1908).

Results and discussion

Distinguished morphological characters mainly seed colour, size, shape, mottling, plant height, internode pigmentation flower colour were noticed among individual plants within landraces. These characters were also compared with a popularly grown released variety VL-63 in the region of collected sites as a reference. Variety VL-63 showed similarity with SHU2032 and SHU2024 while

distantly related with SHU2021, SHU2007, SHU2005, SHU2014 and SHU2035on the basis of seed colour. Most of the landraces accessions were similar in regard to seed size including variety VL-63. All the landraces were relatively medium size seeds except SHU2023 and SHU2007. The seeds of most landraces were kidney shaped while SHU2021, SHU2005 and SHU2014 have red, purple and black mottled seeds.

On the basis of plant growth characters, these were grouped into two. The average plant height was found highest (150.3 cm) for SHU2023, while other landraces including variety VL-63 showed varied average plant height (35.2 to 50.2 cm). Inter node pigmentation; twinning/bushy habit, flower colour and flowering habit were also major morphological markers that helped us to group these into two distinct groups. Distinguished morphological markers were decided to compare at molecular level in order to identify a minimum number of easily scorable, reproducible markers identified on the basis of RAPD based DNA fingerprinting of these landraces (Fig. 1). The results showed that two landraces namely SHU2014 and SHU2032 were closely related as indicated by high value of similarity coefficient (0.875). Bean landraces could be divided into two major clusters A and B. Two landraces SHU2021 and SHU 2023 comes under cluster A with 64% similarity while cluster B contains 8 landraces (Fig. 2). Cluster B further divided into two minor subgroups as B1 and B2 with 70% similarity. Subgroup B1 contains three landraces SHU2017, SHU2024 and SHU2035. Out of these SHU2017 and SHU2024 were closely related with 79% similarity and they together showed 74% similarity with SHU2035. Subgroup B2 consist of four landraces namely SHU2007, SHU2005, SHU2032 and SHU2014. Out of these SHU2032 and SHU2014 showed maximum similarity (87.5%) while 81% similarity were observed with SHU2005 and together with SHU2007 showed 74% similarity. SHU2012, however, did not belong to any of the subgroups (B1 and B2). It also showed 65% similarity with one of the landrace of major cluster B. VL-63 was a distinct genotype that was neither part of cluster B nor A. It showed 58% similarity with cluster B and 53% with A. This separation together with phenotypic characters suggests that the two groups may represent two distinct pools of *P. vulgaris*, which were first described by Evans (1976). Recently, different landraces and cultivars of *Phaseolus vulgaris* were studied from south Brazil (Maciel, *et al.*, 2001), Andean cultivars (Franco, *et al.*, 2001) and common Chilean beans (Vera, *et al.*, 1999) on the basis of phenotypic and genetic diversity, which supports this study for occurrence of variation at molecular level among the landraces of Kumaon hills.

Random primers used in the present study produced a number of unique markers. Primers C11, T1, G7, C3, G4 and G10 gave maximum number of unique markers. These primers gave 108 bands out of these 72 were polymorphic. The variation of these

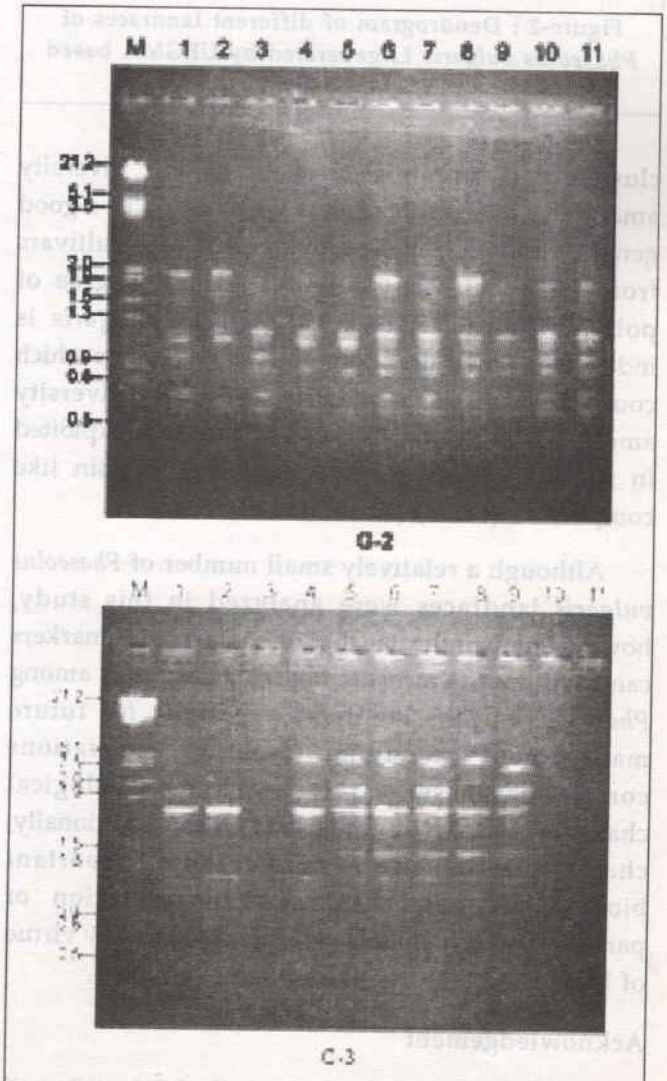

Figure-1 : RAPD profiles of 11 cultivars of *Phaseolus vulgaris* L. using OPG02 and POC03 primers

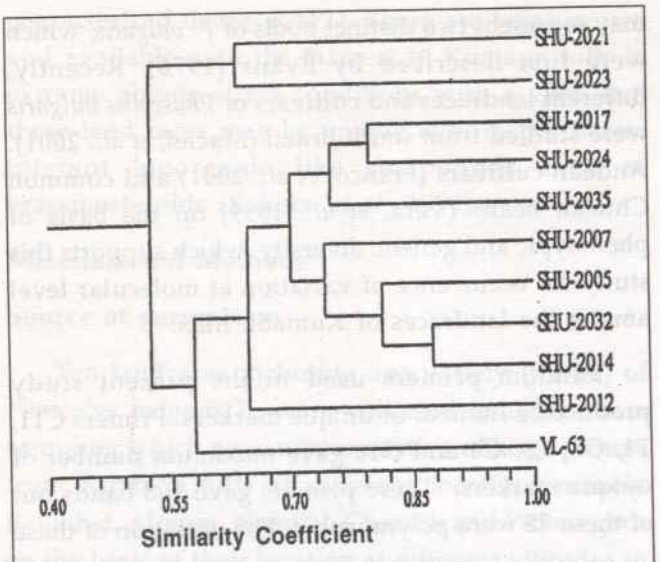

Figure-2 : Dendrogram of different landraces of *Phaseolus vulgaris* L. generated by UPGMA based cluster analysis

clusters demonstrate the genetic genetic diversity among the *Phaseolus* landraces which could be a good genetic resource for development of new cultivars from these cultivated races. The high degree of polymorphism detected in *Phaseolus vulgaris* is indicative of their usefulness as RAPD markers which could be utilized to identify molecular diversity among landraces of such origin and must be exploited in isolating plant growth regulatory brassin like compounds (BLCs) (Yokota, *et al.*, 1990).

Although a relatively small number of *Phaseolus vulgaris* landraces were analyzed in this study, however, data indicates that these molecular markers can be utilized to measure molecular diversity among *Phaseolus vulgaris* landraces especially for future management including breeding operations correlating these markers with morphological characters in order to established QTLs. Additionally, characterization of agriculturally important biomolecules like BLCs and conservation of particularly such valuable genetic resources by virtue of being resistant to environmental stress(s).

Acknowledgement

Authors wish to thank Council of Scientific and Industrial Research (CSIR), Govt. of India for providing a research grant for this study.

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Plant Sci. Res. Vol.27 (1&2) : 16-23, 2005

Interesting medico-botanical claims by Khonds of Nayagarh district of Orissa

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Ethnobotanical study has gained momentum only in the second half of the last century in India. Much has already been said about its importance especially in search of new medicinal plants. Orissa is indeed a haven for such work with abundant natural wealth and ethnobotanical knowledge all over. The present study provides the ethnomedicinal-lore of the aboriginal Khond tribe of Nayagarh district, Orissa. The Khonds are a distinct group of Proto-Australoid stock with considerable Mongoloid admixture and numerically form the largest group among the 62 tribes in Orissa. They constitute 16.20 percent of the total tribal population with a population of 11,40,374 (1991 Census). They are found sporadically throughout the state and they are largely concentrated in the districts of Kandhamal and Rayagada. The Khonds are variously distributed in both plains and hilly areas of Nayagarh district. The information presented in this paper was recorded during the ethnobotanical explorations carried out in various localities of the tribal areas of the district. In this paper information about the traditional uses of 56 angiospermic plant species belonging to 55 genera and 37 families collected through field surveys and personal interviews with the Khond medicine people is reported .The plant species are used for a number of diseases like dysmenorrhoea, rheumatism, bilious derangements, wounds and ulcers, back-pain, piles, stomach cancer, snake-bite, joint-swellings, otitis media, bone-fracture, oedema, stomatitis, conjunctivitis, aphthous ulcers, eczema, fever, asthma, mumps, filariasis, nephritis, headach etc. These ethnomedicinal plants are recommended for pharmacological/ phytochemical investigations.

Key words : *Medico-botanical claims, Khonds, Nayagarh.*

Introduction

The state of Orissa is quite rich in natural resources. More than 40% of its total area is covered with its forests, which offer good opportunity for medico-botanical explorations. The interiors of the forests as well as their outskirts are inhabited by tribal population, still largely dependent on plants for their shelter, food as well as medicine. It provides good scope for ethno-medico-botanical studies. The area selected for the studies i.e. Nayagarh district carved out of the erstwhile Puri district is largely covered with dense forests having rich flora. This area has not received much attention of ethnobotanists in the past.

The area under study includes the entire Forest division of the newly formed Nayagarh district (1993) out of 30 districts of Orissa state. It lies between 20^0-05′ and 20^0-10′ (N) latitude, 85^0-05′ and 85^0-10′ (E) longitude extending over an area of 3939 sq.km. It is bounded on the north and north-east by the newly formed districts of Angul and Cuttack, on the west and south-west by the districts of Boudh and Phulbani and on the south and south-east by the districts of Ganjam and Khurda. Mostly all the important hill ranges of the erstwhile Puri district are seen in Nayagarh district. While the hill system in the state of Orissa has more or less extensive ranges or detached peaks and blocks the Nayagarh Forest division has a continuous fine hill ranges covered with some dense forest pockets. The present Nayagarh district had four feudatory states namely Nayagarh, Khandapara, Ranapur and Daspalla which constitute part of the Eastern ghats. Streching along

the south bank of Mahanadi from Gonia to the border of Boudh broken only near Chamundia forest by the Burtanga river, the range increases its steepness as it passes in to the Satkosia gorge. Out of many hill ranges Langalkhol range between Daspalla and Nayagarh and between Daspalla and Baudh are important.

The vegetation of Nayagarh district is tropical dry and mixed deciduous type with a moist deciduous pockets scattered here and there. Scrub is generally found in the outskirts of these forests and seems to be the resultant of ruthless destruction of the existing forests by man. *Shorea robusta* Gaertn. f. is found in plenty between Nayagarh and Daspalla and Baispalli. *Madhuca indica* Gmel., *Anogeissus latifolia* (Roxb. ex.DC.) Wall. ex Guill. & Perr., *Sterculia urens* Roxb., *Careya arborea* Roxb., *Buchnania lanzan* Spreng. are quite abundant in and around Daspalla and Mahipur ranges. Aquatic and marshy vegetation is found in and around water tanks, reservoirs, streams and rivers and in cultivated fields.

In Nayagarh district mainly three different types of soil are found. Alluvium type- restricted to river banks, Red laterite type found in forests and Coarse alluvial type found in river valleys. Mahanadi, Burtanga and Kusumi are the three rivers besides numerous hill-streams. The general climate of Nayagarh Ghat is a Deccan type, the summers are hotter, rainfall is higher and winter is prolonged. Nayagarh low plains are dry and uncomfortable. There are three main seasons, the hot season from March to May, the Rainy season from mid June to September and the cold season from October to February. The average rainfall of Nayagarh district is 1700 mm. Malaria is prevalent throughout the forest area and sometimes it is more complicated forms like Black water fever and Celebral Malaria which also occur. Diarrhoea, dysentery and rheumatism are very common among the backward population. Scheduled Castes, Scheduled Tribes and other Backward Castes form the bulk of the population. The tribal population is distributed throughout the tract in small villages consisting of five to six families. The principal tribes are Khonds, Saoras etc.

The tribals are a distinct ethnic group who are confined to a definite geographic area, speak a common dialect, culturally homogenous and with unifying social organization. The English word 'tribe' comes from the Latin word 'tribus' designating a particular kind of social and political organization. There are 62 notified as scheduled for the state of Orissa.

The Khonds are numerically the most important tribe of the district. They live in the most remote and inaccessible forest areas. They are variously distributed in both plains and hills but are mainly found in the ex-states of Rampur, Nayagarh and Daspalla. Generally the Khonds are dark in complexion but some fair persons are also found among them. There are 58 sub-divisions of Khonds who speak the 'Kuvi' dialect but all the Khonds speak Oriya. Sometimes a single family lives all alone on top of a hill overlooking its fields. Khonds practice shifting cultivation. They are expert hunters and possess a good knowledge of folk medicine. That the nearest medical-aid of any hue available to some areas visited in the district is even 45 km justifies the exploration.

Though many papers have been published during the last 20 years on ethnobotanical studies of Orissa (Bal, 1942; Saxena and Dutta, 1975; Jain 1970-1971; Jain, *et al.*, 1973; Mudgal and Pal, 1980; Saxena, *et al.*, 1981; Chaudhury, *et. al.*, 1985; Girach, *et al.*, 1987, 1988; Das and Kant, 1988; Hemadri and Rao, 1989; Girach, 1992; Mukherjee and Namhata, 1990; Brahmam and Saxena, 1990; Prusti, 1998; Prusti and Murty, 1999; Misra, 2003; Misra, 2004) the district has remained ethnobotanically underexplored.

Materials and Methods

The ethnobotanical surveys were conducted in the tribal villages of Chadaipalli, Chhamundia, Barakhola, Banigochha, Daspalla-Kuanria, Sappua, Badahamra, Podapanda, Panipoila, Baispalli, Takra, Gonia and also adjoining areas for several times in different seasons from 2000-20005. Information gathered from the tribals (medicine men) of the above areas was documented. The method of study was followed as described by Rao and Hajra in "Manual of Ethnobotany (Jain, 1987). The plants of ethnomedicinal importance were collected and correctly identified with the help of Flora book

(Haines, 1921-25: The Flora of Orissa, 4 vols. Saxena and Brahmam). Matching of voucher specimens was done with the authentic herbarium specimens available at Central Research Institute (Ay.), Bhubaneswar-751009. Relevant information regarding medicinal values, dose, mode of administration, local names, locality and any other ethnobotanical information were documented. The plants with scientific names are arranged in alphabetical order followed by family, local name, available locality, voucher specimen number followed by folk usages. Field number has been given in the name of the collector, Anuja. The Specimens are preserved in the herbarium of P.N. College, Khurda, Orissa.

Enumeration

Achyranthes aspera L.Sp.Pl.204. 1753; Wt.Ic.t. 1777. 1853; FBI 4: 730; H.3:767; G.2: 1176; Saxena & Brahmam, Fl. Orissa 3: 1498.1995.
(Amaranthaceae) 'Apamarang'
Locality: Podapanda, Anuja.228
Roots of 'Apamarang' and 'Lajkuli' (*Mimosa pudica*) are ground to a fine paste and given internally in dysmenorrhoea.

Alangium salvifolium (L.f.)Wang.in Engl. Pflanzenr. 41:9.1910;G.1: Saxena & Brahmam, Fl.Orissa 2:800.1995.
(Alangiaceae), 'Dholanku',
Locality : Daspalla, Anuja 397
Root together with the root of 'Sahada' (*Streblus asper*) and the bark of 'Sajana' (*Moringa oleifera*) are ground to a fine paste and the paste is applied externally to alleviate rheumatic pain.

Alternanthera sessilis (L.)R.Br.ex DC.Cat.Pl.Hort. Monsp. 4: 77.1813; FBI 4: 731; H3:768; M.29; Saxena & Brahmam, Fl.Orissa 3: 1506.1995.
(Amaranthaceae), 'Madaranga'
Locality: Baispalli, Anuja 249
Fresh whole plant is grinded to a fine paste and the paste thus obtained is evenly smeared on the forehead in jaundice.

Amaranthus spinosus L.Sp.Pl.991.1753; Wt.Ic.t.513. 1841; FBI 4: 718; H.3: 761; G.2:1170; Saxena & Brahmam, Fl.Orissa 3: 1510.1995.
(Amaranthaceae), 'Kantamariso'
Locality : Gonia, Anuja 228
Whole plant paste is rubbed well on the forehead thrice daily as a cure for bilious derangements.

Anthocephalus chinensis (Lam.) A.Rich.ex Walp. Repert. 2:491.1843; Saxena & Brahmam, Fl.Orissa 2: 807.1995.
(Rubiaceae); 'Kadamba'
Locality : Phulbani, Anuja 297
Bark and the raw fruit of 'Bahada' (*Terminalia chebula*) is made in to a paste and is applied externally to heal chronic wounds and ulcers.

Ardisia solanacea Roxb.Pl.Cor.t.27.1795; G.2:756; H.2:509; Saxena & Brahmam, Fl.Orissa 2:1003.1995.
(Myrsinaceae), 'Sahajmari'
Locality : Sappua, Anuja 388
Bark together with equal quantities of the bark of 'Chatyana' (*Alstonia scholaris*) and 'Mundica' (*Walsura piscida*) are made in to a decoction. The decoction is given on empty stomach in back-pain for 21 days.

Aristolochia indica L. Sp.Pl.960.1753; FBI 5: 75; H.3:785;G.2:1202;M.59; Saxena & Brahmam, Fl. Orissa3: 1550.1995.
(Aristolochiaceae), 'Pannoairi'
Locality : Barkhola, Anuja 387
Paste of root with black pepper is taken on empty stomach in palpitation. Also the above paste made in to a ' Sherbat' is taken orally in empty stomach to reduce pain due to bleeding piles.

Barleria prionitis L. Sp.Pl.636.1753; Wt.Ic.t.452.1841; FBI4:482; H.2:681; G.2: 1058; Saxena & Brahmam, Fl.Orissa 3: 1333.1995.
(Acanthaceae), 'Daskerinda'
Locality : Daspalla, Anuja 225
Root along with the root of 'Thoba' (*Citrus medica*) and the wholeplant of 'Malang' (*Loranthus longiflorus*) grown on ' Kumbi' (*Careya arborea*) are dried and grinded to powder. The powder is taken in empty stomach for stomach cancer.

Calotropis gigantea R.Br.in Ait.f.Hort.Kew.ed.2.2: 78.1811; FBI 4: 17; G.2:832; H.2:550; Saxena & Brahmam, Fl.Orissa 2: 1083.1995.
(Asclepiadaceae), 'Arka'
Locality : Panipoila, Anuja 290
Milky latex is solidified, made into pills of two grams

each is taken orally in 'Bhagandara,' fistula-in-ano for 20-40 days.

Capparis brevispina DC.Prodr.1: 246.1824; G.1:45; H.1:31; Saxena & Brahmam, Fl.Orissa 1:56.1994.
(Capparaceae), 'Kontikapali'
Locality : Badahamra, Anuja 380
Root together with equal quantity of the root of 'Bhainsa' (**Flacourtia indica**) are made in to a fine paste and the paste thus obtained is taken orally as a cure for 'Katibata' back-ache and in joint swellings.

Caryota urens L. Sp.Pl.1189.1753; FBI 6: 422;H.3: 879; G.3:1560; M.143; Saxena Brahmam, Fl.Orissa 4: 2018, 1996.
(Arecaceae), 'Salappo'
Locality : Badahamra
'Ras' (toddy) of the palm is given internally for 'Mehapoda' (Spermatorrhoea) in the morning for one month.

Cassytha filiformis L.Sp.Pl.35.1753; Wt.Ic.t.1847.1852; FBI 5: 188; H.3:798; G.2: 1241; Saxena & Brahmam, Fl.Orissa 3:1561.1995.
(Lauraceae), 'Nirmuli'
Locality : Barakhola, Anuja 395
Wholeplant with the roots of 'Sambarkai' (***Byttneria herbacea***) and 'Muthuri' (***Smilax zeylanica***) are dried, powdered. The powder (2-3g) is taken internally in every three hours for 40 days in 'Jalodara', ascites.

Cryptolepis buchananii Roem. & Schult. Syst.Veg. 4:409.1819; Wight, Icon.t.494.1841 & III.t.182.f.8.1850; Hook.f.FBI 4: 5.1883; H 2: 549(576). Saxena & Brahmam, Fl.Orissa.2: 1075.1995.
(Periplocaceae), 'Gopakanu'
Locality : Badahamrra, Anuja 297
Latex is used as ear drops in acute 'otitis media'.

Dendropththoe falcata (L.f) Etting.15.Akad.Wiss. Wien.Math.Naturwiss.K1.Denkschr.32 :68.t.13.f.14-15.1872; Saxena & Brahmam, Fl.Orissa 3:1579.1995.
(Loranthaceae), 'Malang,
Locality : Chadaipalli, Anuja 238
Wholeplant grown on mango tree is dried and made into a powder. The powder is taken (5g) orally in empty stomach as a cure for leucorrhoea.

Desmodium triflorum (L.) DC.Prod.2:334.1825; FBI 2 : 173; G.1: 347; H.2:266; Saxena & Brahmam, Fl.Orissa 1: 512.1994. (Fabaceae), 'Luduru'
Locality : Nayagarh, Anuja 382
Root together with the roots of 'Muthura' (***Smilax zeylanica***), 'Sambarkai' (***Byettneria herbacea***), 'Sonargoda' (***Grewia hirsuta***) and 'Hadkankoli' (***Grewia rothii***) are made into paste and the paste is applied externally and bandaged to bone-fracture.

Diplocyclos palmatus (L.) Jeffrey, Kew.Bull. 15(3):352.1962; Saxena & Brahmam, Fl.Orissa 2:743.1995.
(Cucurbitaceae), 'Chitachori,
Locality : Sappua, Anuja 284
Wholeplant is grinded to a fine paste and the paste is applied locally and the root-juice (5-10 ml) given internally in snake-bite.

Elephantopus scaber L. Sp.Pl.814.1753; Wt.Ic.t. 1086.1846; FBI 3:242; G.2:676; H.2:461; Saxena & Brahmam, Fl.Orissa 2:927.1995.
(Asteraceae), 'Mayurchulia'
Locality : Banigochha, Anuja 292
Root ground to a fine paste and the paste thus obtained is applied all over the body for scabies in children.

Evolvulus alsinoides (L.) L.Sp.Pl.(ed.2).392.1762; C.B.Cl.in Hook.f.FBI 4: 220;H 2: 585(614), Saxena & Brahmam. Fl.Orissa 2: 1165.1995.
(Convolvulaceae), 'Krishna onkranti'
Locality : Takra, Anuja 299
Flowers together with equal quantities of flowers of 'Bejibaigan' (***Solanum surattense***) are grounded to a fine paste and the paste is given orally to children above 3 years for cold.

Ficus hispida L.f. Suppl.Pl.442.1781; FBI 5:522; H.3:837; G.3:1367; Saxena & Brahmam, Fl.Orissa 3: 1714.1995.
(Moraceae), 'Dimri'
Locality : Phulbani, Anuja 239
Milky latex collected from the petiole is mixed well with water and given orally to check diarrhoea in infants.

Glycosmis mauritiana. (Retz.)DC. Corr.in Ann.Mus. Hist.Nat.Paris 6: 386.1805; FBI 1 : 449; H.1:163; Saxena & Brahmam, Fl.Orissa 1: 240.1994.
(Rutaceae), 'Chauldua'

Locality : Berbera R.F., Anuja 240
Paste obtained by grinding the root along with the bark of 'Kerua' (***Holarrhena antidysenterica***) and the root of ' Kulthia' (***Tephrosia purpurea)*** together is taken on empty stomach to check severe diarrhoea.

Grewia rothii DC.Prod.1:509.1824; G.118; H.1:95; Saxena & Brahmam, Fl.Orissa 1: 202.1994.
(Tiliaceae), 'Hadkakoli'
Locality : Baispalli, Anuja 286
Root along with equal quantities of the roots of 'Mahakal' (***Trichosanthes tricuspidata***) and 'Sambarkai' (***Byttneria herbacea***) are grounded to paste and the paste is given orally along with black pepper in 'Oedema'.

Gouanialeptostachya DC.Prod.2:40.1825; FBI 1: 643; G.1:225; H:198; Saxena & Brahmam, Fl.Orissa 1: 309.1994.
(Rhamnaceae), Raktapichuli,
Locality : Berbera R.F., Anuja 367
Water-extract of fresh root mixed well with the contents of raw egg and ' Mahuli' (liquor brewed from 'Mahula'– ***Madhuca indica***) is taken orally before bed to remove body pain due to internal injuries.

Holarrhena pubescens (Buch - Ham.) Wall.ex G.Don. Syst.4:78.1837; Saxena & Brahmam, Fl.Orissa 2: 1061.1995. (Apocynaceae), 'Kerua'
Locality : Panipoila, Anuja 381
Bark together with the bark of mango tree (***Mangifera indica***) ground to a fine paste is taken in stomatitis.

Ichnocarpus frutescens (L.) R.Br.Mem.Wern.Nat. Hist.Soc.1: 62.1811; FBI 3: 669; G.2:820; H.2:546; Saxena & Brahmam, Fl.Orissa 2: 1062.1995.
(Apocynaceae), 'Sua noi'
Locality : Gonia, Anuja 303
Exudate of the stem is used as eye drops in conjunctivitis.

Jatropha curcas L. Sp.Pl.1006.1753; FBI 5: 383; H.1: 101; G.2:1340; Saxena & Brahmam, Fl.Orissa 3:1650.1995.
(Euphorbiaceae), 'Lonka joda,
Locality : Panipoila, Anuja 381A
Sap (latex) is applied in aphthous ulcer.

Ludwigia octovalvis (Jacq.) Raven, Kew Bull.15:476. 1962; Rein wardia 6:356.1963, Saxena & Brahmam, Fl.Orissa 2: 728.1995.
(Onagraceae), 'Anicamari'
Locality : Nayagarh, Anuja 249
Paste of wholeplant is applied locally in eczema. Before the application the portion is to be cleared and cleaned with warm water.

Melia azedarach L.Sp.Pl.384.1753; Wt.Ic.t.160.1839; FBI 1: 544; G.1:176;H 1: 177; Saxena & Brahmam, Fl.Orissa 1:276.1994. (Meliaceae), 'Mahalimba'
Locality : Takra, Anuja 294
Water extract of the bark together with the bark of 'Mahula' (***Madhuca indica***) and the root of 'Apamarang' (***Achyranthes aspera***) is taken orally to control fever.

Millettia extensa (Benth.) Baker (Fabaceae), Birchi
Locality : Kuanria, Anuja 343
Extract of root is given in malarial fevers. Powder of leaves is applied externally to heal wounds.

Olax scandens Roxb.Pl.Cor.2: 2.t.102.1799; FBI 1:575; G.1:190; H.1:183; Saxena & Brahmam, Fl.Orissa 1: 288.1994. (Olacaceae), 'Baddaboddolia'
Locality : Badahamra, Anuja 309
Leaves, stem bark and root in equal quantities are ground to a fine paste and the paste thus obtained is applied externally and bandaged in bone-fracture.

Phyllanthus reticulatus Poir. Baill.Etude Euphorb. 613.1874; H.1:129(134), Saxena & Brahmam, Fl.Orissa 3:1674.1995.
(Euphorbiaceae); 'Tejkang'
Locality : Gonia, Anuja 216
Root together with old 'gur' is ground to a fine paste is given internally and the leaf paste is applied externally in filarial swellings.

Smilax zeylanica L. (Smilacaceae), 'Muthuri'
Locality : Chhamundia, Anuja 253
Rhizome is ground to a fine paste and the paste is given internally to check diarrhoea.

Soymida febrifuga (Roxb.)A.Juss., Mem.Mus.Hist. Nat.19: 251.t.11.f.26.1830; FBI 1: 567; G.1:185; H.1:175; Saxena & Brahmam. Fl.Orissa 1: 277. 1994 (Anacardiaceae), 'Suamb'

Locality : Podapanda, Anuja 381
Seeds together with the seeds of 'Onkranti' (*Solanum surattense*) and old paddy are ground to a fine powder and the powder is given orally in asthma.

Streblus asper Lour.Fl.Cochinch.2: 615.1790; FBI 5: 489; H.3: 820; G.:1353; Saxena & Brahmam, Fl.Orissa 3:1729.1995.
(Moraceae), 'Sahada'
Locality : Gonia, Anuja 390

Sap (latex) is applied externally after defecation in piles. *Strychno potatorum* L.f.Suppl.Pl.148.1781; Roxb.Pl.Corom.t.5.1795; Wight,I11. t.156. 1850; CB.Cl.in Hook.f.FBI 4: 90.1883; H 2: 564 (592), Saxena & Brahmam, Fl.Orissa 2:1114.1995.
(Strychnaceae), 'Kataka'
Locality : Kuanria, Anuja 233
Seed is rubbed against a stone and the paste thus obtained is applied locally in conjunctivitis.

Tragia involucrata L. Sp.Pl.980.1753; Hook.f.in Hook.f.FBI 5: 465.1888, pro parte; H 1: 115 (119); Saxena & Brahmam, Fl.Orissa 3:1686-1687.1995.
(Euphorbiaceae), 'Bichuati'
Locality : Ranpur, Anuja 316
Root ground to a fine paste is taken orally along with cow milk in tuberculosis. Also leaves are dried and ground to a fine powder. The powder with 'Gingelly' (**Sesamum orientale**) oil is used as a depilatory.

Urginea indica (Roxb.) Kunth, Enum.Pl.4: 333,1843; FB16: 347;H.3: 1096(1145);M.201; Saxena & Brahmam, 3: 1969.
(Liliaceae), 'Bona piaj', Daspalla.
Bulb is ground to a fine paste and applied to joints in rheumatism.

Ventilago denticulata Willd. Ges.Nat.Fr.Neus.Schr.3: 417.1801; Saxena & Brahmam, Fl.Orissa 1: 317.1994.
(Rhamnaceae), 'Kumbharkani'
Locality : Chhamundia, Anuja 250
Paste of fresh root is added with the powder of black pepper and applied externally in mumps.

Vernonia cinerea (L.)Less. Linnaea 4:291.1829; hook.f.in Hook.f.FBI 3: 233.1881; G,Fl.Madras 2:676(475).1921; H 2: 460 (483); Saxena & Brahmam, Fl.Orissa 2: 966-977.1995.
(Asteraceae), 'Poksunga'
Locality : Chhamundia, Anuja 24
Wholeplant made in to a fine paste along with black peppers is given in 'Raktagada', filariasis.

Vitex pubescens Vahl, Symb.Bot.3: 85.1794; C.B.Cl.in Hook.f.FBI 4: 585.1885; H 2: 711(745), Saxena & Brahmam, Fl.Orissa3: 1431.1995.
(Verbenaceae), 'Madhurgudia'
Locality : Nayagarh, Anuja 383
Water-extract of leaves is taken internally in nephritis.

Zingiber zerumbet (L.) Sm. Exot.Bot.2: 105.t.112.1804; Baker in Hook.f.FBI 6: 247.1892; H 3: 1143 Saxena & Brahmam, Fl.Orissa 3 : 1910 -1911.1995.
(Zingiberaceae), 'Godda'
Locality : Berbera R.F., Anuja 301
Juice of fresh rhizome is given orally as a remedy for snake-bite.

Ziziphus oenoplia (L.) Mill.Gard.Dict.ed.8.n.3.1768; FBI 1: 634; G.1:220; H.1:196; Saxena & Brahmam, Fl.Orissa 1: 322.1994.
(Rhamnaceae), 'Kontai koli'
Locality : Ramapur, Anuja 250A
Paste of fresh leaves with a pinch of salt mixed well is applied on the forehead for relief from headache.

Discussion

The ethnic communities of Orissa have a bulk of traditional knowledge of medicinal use of plants growing around them. This knowledge is handed over from generation to generation verbally and is extensively used for the treatment of common diseases. With the advancement of civilization the ethnobotanical information has been depleting at an alarming rate. Therefore, it is essential to record and document ethnobotanical data for further investigation lending to sustainable utilization of bioresources.

The information presented in this paper was recorded during the ethnobotanical explorations carried out in various localities of the tribal areas of the district through field surveys and personal interviews with the Khond medicine people is reported. The plant species are referred to for a number of diseases like dysmenorrhoea, rheumatism,

bilious derangements, wounds and ulcers, back-pain, piles, Stomach cancer, snake-bite, joint-swellings, otitis media, bone-fracture, oedema, stomatitis, conjunctivitis, aphthous ulcers, eczema, fever, asthma, mumps, filariasis, nephritis and headach etc.

The herbs have been used as natural medicine among the tribes of India since time immemorial. Natural medicine is the oldest and purest system of medicine known to humanity. The medical experts of many developing countries have acknowledged and approved the efficacy of natural medicine. This is to be tested phytochemically/pharmacologically.

Conclusion

Khonds are one of the oldest tribes of India. They are the largest tribal group in Orissa. They mostly live in Phulbani and Rayagada districts and also live in Nayagarh district with large population. The unique feature of Khonds is their habitat which make them conservative and backward. They live on top of the hills and in inaccessible jungle tracts. Agriculture is the mainstay supplemented with hunting and digging roots. Though they are superstitious and practice witchcraft, yet they possess many curious remedies for various ailments they suffer from. Their knowledge of use of plants owes a great deal to the geography of their dwelling. No wonder the claims presented in the paper are mere and interesting as well.

These ethnomedicinal plants are recommended for pharmacological/phytochemical investigations.

Acknowledgements

The authors are thankful to Sri K.S. Murty, Botanist, Central Research Institute (Ay.), Bhubaneswar-751009, Orissa for his help in identification and encouragement. They are also grateful to the D.F.O., Nayagarh Forest Division, for the help provided during survey.

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Plant Sci. Res. Vol.27 (1&2) : 24-32, 2005

Study on Vegetational Pattern and Environmental Quality of Sukinda Chromite Mines Area

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An assessment of vegetational pattern and environmental quality of Sukinda chromite mines areas, South Kaliapani, Orissa was done using quadrat method along with analysis of physico-chemical properties of soil and water (pH, E.C., water holding capacity, organic C, available N and P, Ca, Mg) and microbial activity (general bacteria, total fungi, *Rhizobium, Nitrosomonas,* and *Nitrobacter*). Analyses of soil quality of chromium contaminated effluent water and nearby areas were alkaline showing low E.C. value along with low N, P and organic C content along with poor microbial diversity. All these factors can be correlated to thin vegetational pattern of that area. However, soil contaminated with chromium water harboured a wide variety of plant species though with less frequency, where *Vernonia cinnaria* and *Diectomis fastigiata* constituted the dominant species. On the other hand, a swampy land flooded with chemically treated effluent water showed luxuriant ground vegetation dominated by *Evolvulus numularia* and *Kyllinga monocephala* with abundant occurrence may be due to high nutrient content (organic C, available N and P), microflora, E.C. and water holding capacity. This site though contain luxuriant vegetation, it showed low plant diversity with high frequency and establishment of dominant species. In the present work environmental quality analysis of another uncultivated land indicated a rich microbial population, which was correlated with rich nutrient content and a moderate pH range (7.2). But the population of *Rhizobium* was very less and the soil was mostly dominated by drought resistant species like *Tephrosia purpurea, Cassia tora* and *Croton sparsiflorus*, may be due to low water holding capacity.

Key words : *Chromite mine, chromium toxicity, vegetation, microbes, pH, nutrients*

Introduction

Excessive chromite mining activity through open cast mining system is creating serious environmental quality depletion in the Sukinda valley of Orissa, which attributes to 94% of the total chromite deposits of the country (IBM, 2004). Environmental disturbances includes large scale deforestation, removal of top soil, dumping of overburdens along with leaching of toxic heavy metal ions, particularly hexavalent chromium, to the neighbouring crop fields, and degradation of soil quality (Misra *et al.*, 1994) affecting plant growth and human health in this area. It is essential to remove the toxic heavy metal ions from soil and water of mining area, which can be done through the selection of plant and microbes, otherwise known as bioremediation technology. The present work was done to assess the vegetational pattern and environmental quality of chromite mining area of South Kaliapani, Sukinda valley, Jajpur through analysis of physico-chemical properties of soil and water (pH, E.C., water holding capacity, organic C, available N and P, Ca, Mg) and microbial activity (general bacteria, total fungi, *Rhizobium, Nitrosomonas,* and *Nitrobacter*).

Materials and methods

Sample collection from study site and its neighboring area

Soil and water samples were collected from Sukinda chromite mines area of Orissa Mining Corporation (OMC) at South Kaliapani and its neighbouring areas and brought to the laboratory at Post Graduate Department of Botany, Utkal University to study the physico-chemical and microbial properties following standard techniques. The physico-chemical properties include pH, electrical

conductivity, water-holding capacity of soil, organic carbon, available phosphorus, potassium, total nitrogen and heavy metal content of the soil. For water sample parameters viz. pH, electrical conductivity, total dissolved solids etc. were studied. Further, the studies of microbial population related to nutrient (carbon, nitrogen) cycle of the soil sample were also carried out. Microbial analysis of the soil included estimation of population of general bacteria, total fungi, *Rhizobium*, *Nitrosomonas*, *Nitrobacter*, ammonifiers etc. The different samples of soil (S_1, S_2, S_3 and S_4) and water (W_1, W_2 and W_3) are given below.

S_1 was the soil sample collected near the discharge water of chromite mining operation of OMC from where the untreated water containing chromium comes out. S_2 was the soil sample collected from the nearby uncultivated agricultural land selected for the study of phytoremediation of Cr (VI). S_3 was the soil sample collected from a swampy land over which the effluent treated water flows. S_4 was the soil sample collected from Kusumundia village, which was 12 km away from the study site.

W_1 was the water sample collected from the untreated mining water which was contaminated with chromium, W_2 was the water sample collected from the site of swampy land, where the water get treated with chemicals by OMC to reduce the level of contaminated chromium. W_3 was the water sample collected from a pond in Kusumundia village. After collection of samples their physico-chemical properties were analyzed.

Vegetational analysis of the study site and its neighbouring area

A preliminary evaluation of the environmental status of the nearby area along with the vegetational pattern was studied taking the samples from the field sites of South Kaliapani, Sukinda chromite mines, Orissa from where soil samples were collected. Vegetational analysis as well as taxonomic studies was done through quadrat method of an area of 1 X 1 m^2. Various species present in that area were noted down. At the first study site (untreated water pond, S_1) 5 quadrats were plotted and at second (uncultivated agricultural land) and third site (swampy land flooded with treated water) 10 quadrats of 1 X 1 m^2 were laid down. From these, data tables were made and frequency, density, abundance were studied as follows,

$$\text{Frequency (\%)} = \frac{\text{Total no. of quadrats in which the species has occurred}}{\text{Total no. of quadrats studied}} \times 100$$

$$\text{Density} = \frac{\text{Total no. of individuals of the species}}{\text{Total no. of quadrats studied}}$$

$$\text{Abundance} = \frac{\text{Total no. of individuals of the species}}{\text{Total no. of quadrats in which the species has occurred}}$$

Chemical analysis

The chemical analysis performed for all the soil samples were pH, electrical conductivity (E.C.), organic C, available P and N. pH and E.C. were detected in pocket pH and conductivity meter respectively (Hanna make) following the method of Jackson (1967). Organic C was estimated following the method of Ghosh, *et al.* (1983) and available N and P were analysed using the soil and irrigation water kit (Hach make).

Microbiological analysis

Enumeration of soil microflora of all the collected soil samples was carried out following dilution plate technique of Timonin (1940). Microorganisms viz. general bacteria, total fungi, *Rhizobium*, *Nitrosomonas* and *Nitrobacter* were determined by standard dilution plate counting technique of Alexander (1961) using different selective media for different micro-organisms.

Result and Discussion

Vegetation of study sites were studied following quadrat method to get an idea about the vegetational pattern seen in these areas. The site S_1 showed wide varieties of plant species but there was hardly any species dominance. This area, which was contaminated with untreated chromium water, harboured about 24 species belonging to 22 genera (Table-1) with *Vernonia cinnaria* and *Diectomis fastigiata* showing the maximum abundance. *V. cinnaria* belongs to family Asteraceae, is a common weed, having wide range of habitats and not easily taken by cattle,

showed better survival capacity among all the species. This could be due to tolerance of this species to chromium toxicity. But interestingly this plant was absent in S_2 and S_3. Another plant *D. fastigiata* belonging to Cyperaceae family also abundantly seen in this area. Though *V. Cinnaria* was found to be grown one foot away from water source, *D. fastigiata* was seen to be grown in contact with water line. And *D. fastigiata* was found in swampy land, which showed the affinity of this plant to hydrotropic habit. Hence, there was a scope of these two plants viz. *V. Cinnaria* and *D. fastigiata* to be used for remediation of chromium toxicity and *D. fastigiata* could be grown in water line.

Table-1

Vegetational pattern of site contaminated with untreated chromium water from OMC chromite mines at South Kaliapani, Sukinda (S_1)

Sl. No.	Name of the species	Family	Frequency (%)	Density	Abundance
1	*Acacia auriculoformis*	Mimosaceae	80	2	2.5
2	*Aerua lanata*	Amaranthaceae	60	1.2	2
3	*Alysicarpus monilifer*	Fabaceae	60	0.8	1.33
4	*Alysicarpus vaginalis*	Fabaceae	60	0.8	1.33
5	*Atylosia scarabiodes*	Fabaceae	80	2	2.5
6	*Calotropis procera*	Rubiaceae	60	1	1.67
7	*Canscora diffusa*	Gentianaceae	40	0.8	2
8	*Croton sparciflorus*	Teliaceae	20	0.2	1
9	*Crysopogon aciculatus*	Poaceae	100	2.4	2.4
10	*Desmodium gangeticum*	Fabaceae	60	1	6.7
11	*Desmodium trifolium*	Fabaceae	60	1	6.7
12	*Diectomis fastigiata*	Poaceae	80	6.6	8.25
13	*Euphorbia hirta*	Euphorbiaceae	60	0.8	1.33
14	*Evolvulus numularia*	Convulvulaceae	100	5	5
15	*Hedyotis corymbosa*	Rubiaceae	60	1.2	2
16	*Hyptis suaveolens*	Lamiaceae	80	3.2	4
17	*Mimosa pudica*	Mimosaceae	60	1.2	2
18	*Phyllanthus niruri*	Euphorbiaceae	40	0.4	1
19	*Pteris cretica*	Pteridaceae	40	2.2	5.5
20	*Simarouba glauca*	Simaroubaceae	80	2	2.5
21	*Tephrosia purpuria*	Fabaceae	80	1	1.5
22	*Tridax procumbens*	Asteraceae	60	0.8	1.33
23	*Vernonia cinnaria*	Asteraceae	100	7.6	7.6
24	*Ziziphus zuzuba*	Rhmnaceae	40	0.4	1

Unlike S_1, uncultivated barren land (S_2) that showed about 5 species belonging to 5 genera, mostly predominated by the leguminous species viz. *Cassia tora, Tephrosia purpurea* etc. Beside these plants S_2 was also inhabited by *Croton sparciflorus, Chrysopogan.* Though the number of genera was less compared to S_1 but vegetation was thickly populated by these plant species.

Swampy land flooded with treated chromium water (S_3) showed about 13 plant species, dominated by *Kyllinga monocephala* and *Evolvulus numularia. E. numularia* was also found in uncultivated barren land which showed its affinity for hydrotropic habitat. On the other hand *Kyllinga monocephala* was found profusely in S_3 but it did not survive in S_1 and S_2.

Another important observation was that *Tephrosia purpurea* was also found to grow in all the study sites, that grow mostly in terrestrial habitat. *T. purpurea* has better scope for survival under wasteland condition as it is a leguminous species.

Table-2

Vegetational pattern of uncultivated barren land chromium water from OMC chromite mines at South Kaliapani, Sukinda (S_2)

Sl. No.	Name of the species	Family	Frequency (%)	Density	Abundance
1	*Cassia tora*	Fabaceae	80	1.4	1.75
2	*Croton sparciflorus*	Teliaceae	10	6.7	6.7
3	*Crysopogon aciculatus*	Poaceae	100	5.7	5.7
4	*Hedyotis corymbosa*	Rubiaceae	30	0.4	3.33
5	*Tephrosia purpuria*	Fabaceae	90	5.2	5.77

Table-3

Vegetational pattern of swampy land flodded with treated chromium water from OMC chromite mines at South Kaliapani, Sukinda (S_3)

Sl. No.	Name of the species	Family	Frequency (%)	Density	Abundance
1	*Cassia tora*	Fabaceae	70	1.4	2
2	*Chloris barbata*	Poaceae	100	4.6	4.6
3	*Croton sparciflorus*	Teliaceae	40	0.7	1.75
4	*Crysopogon aciculatus*	Poaceae	100	5.7	5.7
5	*Diectomis fastigiata*	Poaceae	100	5.8	5.8
6	*Erogrostis uniloides*	Poaceae	100	5.3	5.3
7	*Euphorbia hirta*	Euphorbiaceae	60	0.8	1.3
8	*Evolvulus numularia*	Convulvulaceae	100	7.0	7
9	*Fuirena ciliaris*	Cyperaceae	100	5.5	5.5
10	*Hygrophila polysperma*	Acanthaceae	60	0.8	1.33
11	*Kyllinga monocephala*	Cyperaceae	100	6.1	6.1
12	*Paspalum scrobiculatum*	Poaceae	100	4.8	4.8
13	*Tephrosia purpuria*	Fabaceae	50	0.9	1.8

Soil quality analysis of study sites and neighbouring area

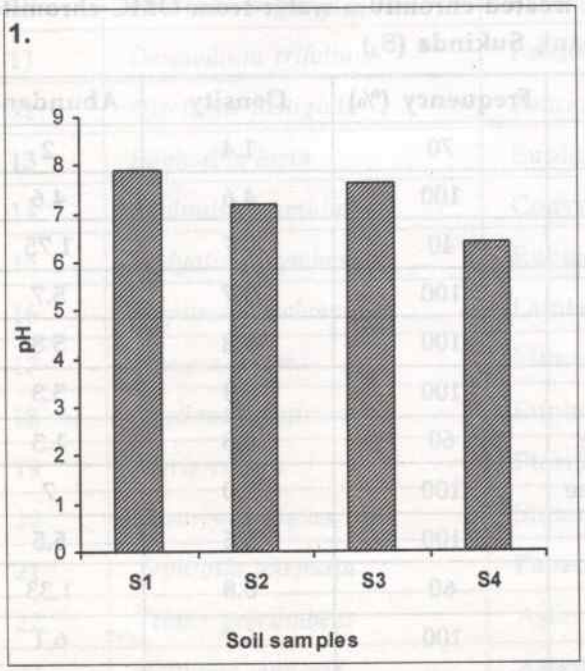
The pH of the S_1 soil sample was found to be more as compared to others (Fig. 4). From the high pH value it was noted that the chromium contaminated soil was alkaline in nature, which was possibly due to the presence of Cr (VI). Baath (1989) has also reported that pH affects the extent of chemical speciation of metals.

Soil pH is of utmost importance for effective metal toxicity. Alkaline condition of soil reveals that S_1 is more contaminated than other types. S_2 was found to be slightly alkaline or neutral in comparison to S_1. These types of soils are sodic soil and often create artificial drought. This occurs because increased salt in the soil solution reduced the ability of the soil water to plants. In extreme cases water can be drawn out of the plant due to osmotic pressure resulting in dehydration and death.

The pH value of S_2 indicated an ideal range of crop production and pH value of S_3 indicated free lime content in soil. pH of Kusumundia village soil which is away from the chromite mining sites indicated slightly acidic solution which may refer to low lime content. This indicated that Cr (VI) could be effectively reduced under acidic condition. This is because of acidic conditions enhance the rate of release of Fe (II) species from soil minerals for reduction with aqueous Cr (VI) species and also increase the Cr reduction by organic matter content (Arnfalk *et al.*, 1996).

Swampy land soil showed the highest electrical conductivity (E.C.) and next is the untreated land soil. Whereas agricultural and Kusumundia soil has the moderate value of electrical conductivity. The high or low E.C. values are accountable for relative acidity and alkalinity of soil and somehow depend upon the availability of salts.

Analysis of soil water holding capacity (WHC) showed a great variation for four types of soil. Extremely low value of W.H.C. was recorded for the agricultural land soil, which may be attributed to the barren condition of the soil. Water holding capacity depends upon the vegetation status of soil. The roots are responsible for holding the water. Vegetation status of S_3 clearly reflects high W.H.C.



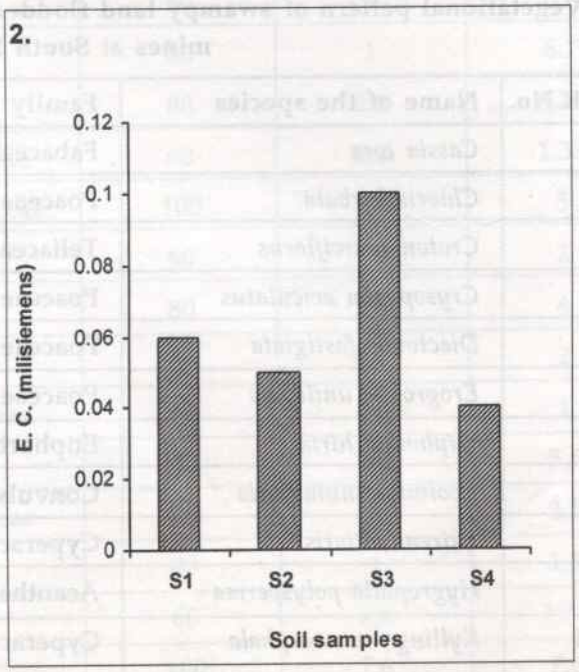


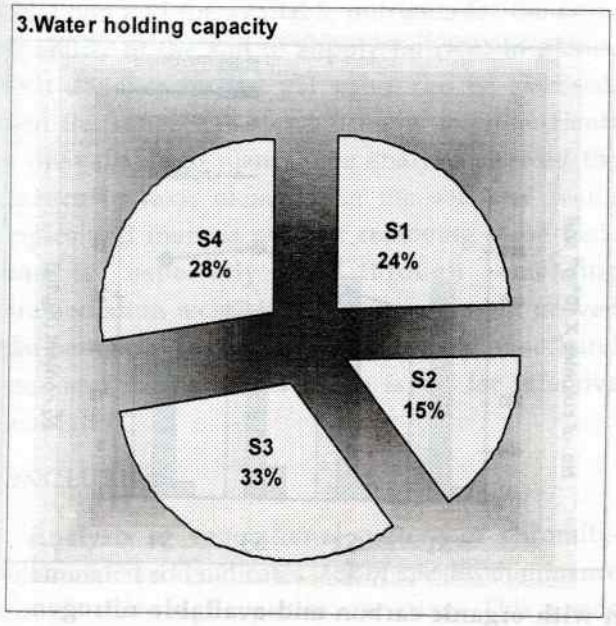
Fig. 1 & 2 : pH and E.C. of different study sites of OMC operated chromite mines of South Kaliapani and its neighbouring areas at Sukinda, Jajpur, Orissa

Available P was generally found to be low in all types of soil. S_2 has a comparatively high P content than others. In S_1 the value of available P may be related to Cr (VI) concentration. As high levels of Cr concentration increases the P content in plants (Hunter and Vergnano, 1953). Due to the uptake of P by plants the soil shows less P amount. Due to high pH values metal complexation is more and such complexes are less toxic (Baath, 1989) and readily available to plants. Low amount of P in S_4 may be attributed to acidic condition of soil.

It was noted that NO_3-N (Nitrate-Nitrogen) content in S_3 was highest among the four types of soil. This is due to fact that the soil contains less chromium and has highest water holding capacity. Nitrate is the most abundant form of soil nitrogen. Of the 16 essential nutrients, nitrogen is the nutrient most likely to be different worldwide. Nitrogen is used by the plant in two forms, ammonium (NH_4^+) and nitrate (NO_3^-). Water percolating through a soil profile leaches and depletes the mobile nitrate from the upper layers to the lower layers. S_1 is the soil with high Cr contamination. N- mineralisation was negatively correlated with pollution level (Baath, 1989). Our result also showed that in S_1 due to increased heavy metal pollution the NO_3-N content was found less. Alternately one can say that low NO_3-N content indicated the metal pollution in the soil. NO_3-N content of Kusumundia soil showed less pollution load. NO_3-Ncontent has a more or less direct correlation with the water holding capacity of soil in all the types of soil.

Organic carbon(C) content of agricultural land soil was found to be more than others. The organic matter of soil helps in reduction of chromium content (Arnfalk *et al.*, 1996, Eary and Rai, 1991). For this reason the soil was slightly alkaline. The S_1 had lowest organic carbon content, which indicated the more toxic and polluted condition of soil. The low value of Kusumundia soil organic 'C' may be attribute to less vegetation.

Enumeration of microbial population reflected comparatively better population of nitrifiers viz. *Nitrosomonas* and *Nitrobacter* than all other microbes viz. general bacteria, total fungi and *Rhizobium* in all the study sites (Fig.6) with low rhizobial population indicating the soil nitrogen source coming from N-mineralisation rather than N_2-fixation. Among all the microbial population, *Rhizobium* was



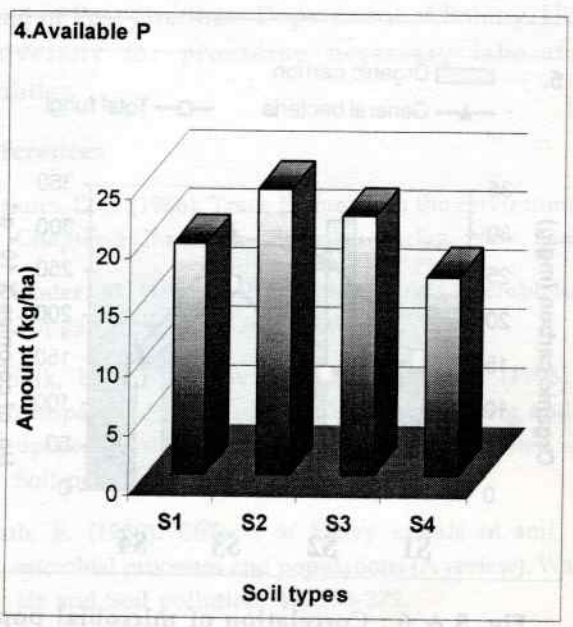


Fig. 3 & 4 : Available phosphorus of different study sites of OMC operated chromite mines of South Kaliapani and its neighbouring areas at Sukinda, Jajpur, Orissa

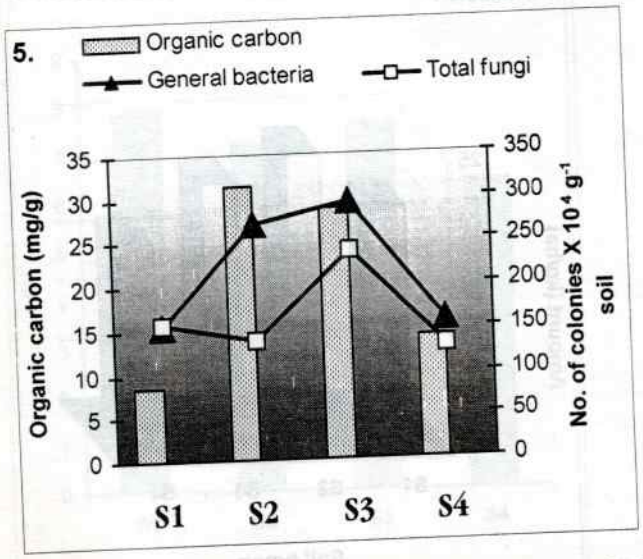
comparatively low than other microbial inhabitants. The low population of *Rhizobium* may be due to heavy metal toxicity as they are highly sensitive to heavy metals (Thatoi, 1994; Patnaik, 1995; Mishra, 2002; Misra *et al.*, 1994; 2004). The percent of organic compounds and N along with pH have also a significant effect on the *Rhizobium* population in soil (Mostajeran *et al.*, 2001). In the present study survival of *Rhizobium* in soil is also correlated with soil pH and E.C. (Yousef *et al.*, 1987). It was also found that S_3 showed comparatively higher rhizobial population, which may be due to high nutrient content along with high E.C. Though S_2 and S_3 showed high amount of organic C, available N and tolerable pH, *Rhizobium* population was relatively low in S_2 than S_3 may be due to sparse occurrence of leguminous plants, which is similar to the observation of Hunter and Fahring (1980).

When microflora of S_1 was compared with S_2 it was seen that except fungi all other microbes were comparatively lower in S_1 than S_2, which may be due to soil alkalinity and chromium toxicity. S_4 with slightly acidic pH (6.4) though situated 12 km away from mining site harboured less number of microbes may be attributed to low organic carbon and available phosphorus content. These observations of our study indicated that, microorganisms were largely regulated by physico-chemical properties of soil (Tiwari *et al.*, 1987; Misra *et al.*, 1994, 2004).

Soil nitrogen content both from N_2 -fixation and biological oxidation of NH_4^+ - N to NO_3^- via NO_2^- with the help of *Nitrosomonas* and *Nitrobacter*, respectively. In our investigation the pH of Kusumundia village is in acidic range, which might be the cause of low population of nitrifiers. In all the study sites the population of *Nitrobacter* was greater than *Nitrosomonas* except S_1. showed the least number of microflora and maximum population was found for S_2 with near neutral pH (7.2). N-mineralisation is regulated by soil acidity, pH (Belser, 1982). The S_1 with alkaline soil pH may be the cause for inhibition of *Nitrobacter* population.

Water analysis of study site and neighbouring area

The three types of water showed alkaline condition and untreated water sample (W_1) has highest alkalinity (Table-4). The alkalinity may be due to the presence of high levels of hexavalent chromium in water. In strongly basic solutions chromate form is predominant. So Cr (VI) has been found to be more in W_1 sample (Data not presented). E.C. is an important factor to determine the relative



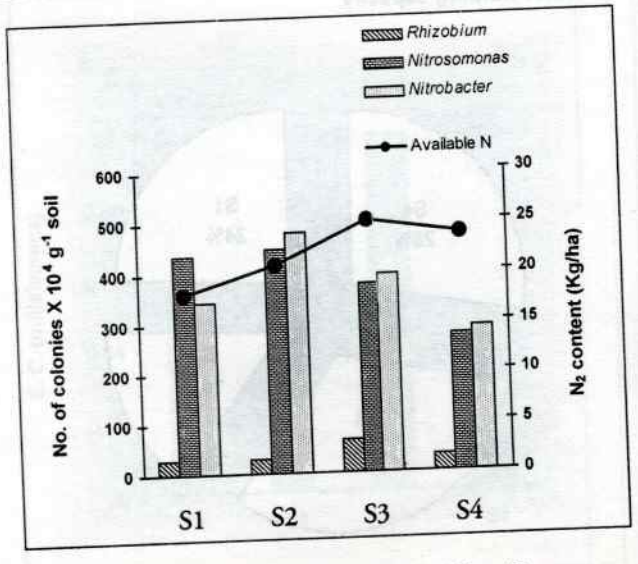


Fig. 5 & 6 : Correlation of microbial population with organic carbon and available nitrogen content of different study sites of OMC operated chromite mines of South Kaliapani and its neighbouring areas at Sukinda, Jajpur, Orissa

Table-4

Water quality analysis of different study sites of South Kaliapani Chromite Mining sites of Sukinda, Orissa

Water samples	pH	E.C. (ms)	P (mg/lit)	NO_3-N(mg/lit)	Ca&Mg (Meq/lit)	PO_4^{-2} (mg/lit)
W_1	8.7	0.19	0.118	1.67	2.2	0.36
W_2	8.0	0.23	0.148	2.33	3.0	0.5
W_3	7.5	0.01	0.129	1.33	0.2	0.4

yield of the crop. From the table it was noted that E.C. value of treated water sample was maximum thus favouring more crop production.

High levels of nitrate and phosphates are found in treated water sample, which are important for crop production. This may result in eutrophication, as there is high PO_4^{3-} content. Due to pollution load the nutrient content (P, NO_3-N) are much less in untreated water sample. Due to less alkaline condition of W_2 it may be considered that PO_4^{3-} competes with chromate for the same absorption site (Adriano, 1986, Barlett and Kimble, 1978).

The above analysis of soil and water are required for our future experiment for assessment of the crop production and the available nutrients for the crop. The ability of the soil to supply nutrient to plants, which depends on the pH value can be assessed. When the soil pH is above 7 many micronutrients are unavailable to plants. The analysis showed the presence of toxic elements in the soil and water samples and there is need of removing these toxic elements especially Cr through emerging bioremediation technology. The experiment proved to be beneficial for assessing the quality of soil and water and further steps to be taken for effective remediation.

CONCLUSION

Analysis of vegetational pattern of chromite-contaminated soil indicates lack of species dominance through with high species variation. This needs selection and screening of hyperaccumulators. *Rhizobium* population is extremely low in the chromite contaminated area which is attributed to : (1) Poor survival of *Rhizobium* under alkaline pH, low phosphorus and low organic carbon content. (2) Screening of *Rhizobium* has to be done taking the above factors in the consideration along with its tolerance to heavy metal toxic ions particularly hexavalent chromium concentration. This assessment of physico-chemical properties of soil and water give us an indication of the environmental status of this area for our future crop production.

Acknowledgements

Financial assistance of Indian Bureau of Mines (IBM), Government of India in the form of a research project to Prof. H.K. Patra and Prof. (Mrs.) A.K. Misra is gratefully acknowledged. We thank Professor and Head of Post-Graduate Department of Botany, Utkal University for providing necessary laboratory facilities.

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Plant Sci. Res. Vol.27 (1&2) : 33-37, 2005

Impact of Salinity on Mangroves of Bhitarakanika

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Mangrove ecosystems are highly fragile and their sustenance mainly depends on a balanced fresh water influx from adjoining rivers and sea. A few sporadic reports are available on the vegetation patterns of mangroves with scant attention for studying the impact of salinity on the distributional pattern. The present study analyses the effect of variation in salinity levels on mangrove vegetation of Bhitarakanika. The water and sediment samples were collected at different locations from upstream (Khola) to the Bay region (Habalikhati) of Brahmani and Baitarani river systems. Salinity index of water samples gradually increases from 3.2 to 28.8 g/l and the sediment salinity varied from 0.1 to 1.2% starting from Khola to Habalikhati region. The vegetation pattern showed that many important species were less abundant and restricted to a particular zone or often absent in some forest blocks. The species *like Avicennia alba, A. marina, Bruguiera cylidrica, Bruguiera parviflora, Ceriops tagal, Lumnitzera racemosa. Rhizophora apiculata, R. mucronata* and *Sonneratia alba* were rarely encountered at Khola, and Bhitarakanika regions, while *Cerbera manghas, Kandelia candel, Sapium indicum* and *Xylocarpus mekongenesis* were hardly observed at the Habalikhati regions. *Avicennia officinnalis* was observed to be the dominant species within the sanctuary area followed by *Heritiera fomes* and *Excoecaria agallocha.* Variation in salinity levels of water and sediment appears to be one of the limiting factors for the existing vegetation pattern in Bhitarakanika sanctuary.

Key words: *mangrove, salinity, sanctuary, distribution pattern*

Introduction

Mangrove ecosystem is ecologically sensitive and provides physical protection for the community and supports tropical, estuarine and coastal food web (Alongi and Christoffersen, 1992). Those are the plants, taxonomically unique and successfully adapted in colonizing inter tidal zone (Kathiresan and Rajendran, 2005) and belonging to very unrelated families, which exhibit different degree of salt tolerance. Mangal vegetations are highly fragile and their sustenance mainly depends on a balanced fresh water influx from adjoining rivers and sea.

Mangroves of India constitute about 7% of the total mangroves of the world (Anonymous, 1986), out of which Orissa sustained 214.85 sq km of mangrove forests as per remote sensing report (1984). This figure has reduced to 195 sq km (Choudhury, 1990) indicating that the mangroves of Orissa are disappearing at an alarming rate. This is attributed to reclamation of mangrove forestland for paddy cultivation and increase in prawn culture, establishment of port and over exploitation of mangroves to meet the human needs.

The coastline of Orissa extending up to 430 km is mainly in the depositional stage with sediment carried down by the river Mahanadi, Brahmani and Baitarani, forming deltas at Bhitarakanika (Choudhury, 1990). Geographically, Bhitarkanika wildlife sanctuary lies between 20° 4′ and 20° 8′N latitude and 86° 45′ and 87° 5′E longitude covering an area of about 650sq km (Anonymous, 1986; Choudhury, 1990). The sanctuary consists of numerous canals, mangrove forests, meandering rivers, innumerable criss-crossed tidal inundated creeks, which makes a unique habitat.

A few sporadic reports on the occurrence and vegetation analysis of the mangroves of Bhitarakanika has been made earlier (Banerjee and Das, 1972; Rao and Sastri, 1974; Choudhury 1984, 1986, 1987; Mishra and Panigrahi, 1987), but little attention was given on the impact of salinity on vegetation. The present study was undertaken to analyze the effect of

variation in salinity levels of water and sediment on the mangroves of Bhitarakanika.

Materials and Methods

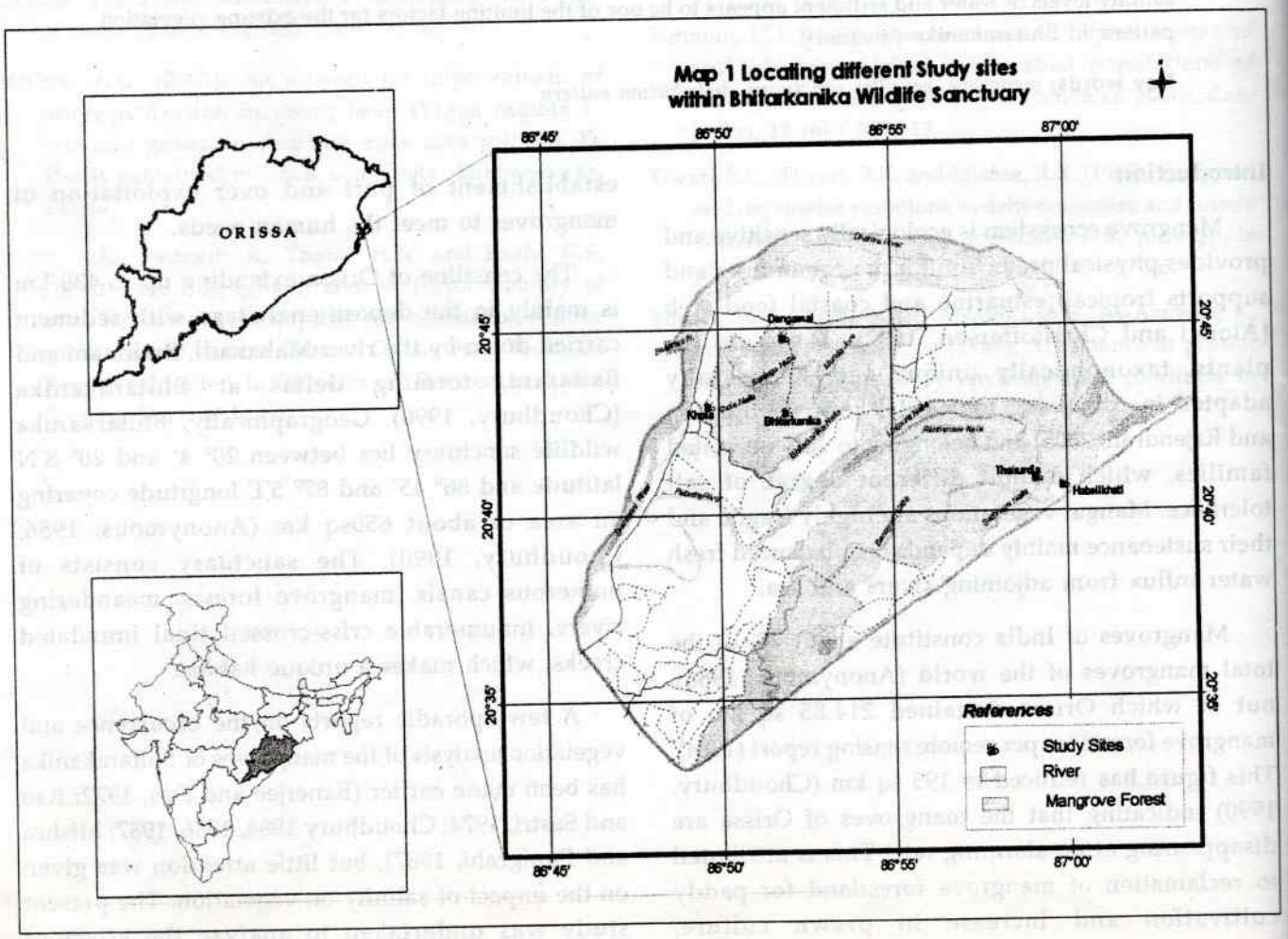
A reconnaissance survey of the vegetation was conducted during January to July 2005 in different forest bits of Bhitarakanika sanctuary. The analytical characters of the plant community were studied in terms of qualitative and quantitative structures by laying down several quadrants in different localities of Khola, Bhitarakanika and Habalikhati (Map-1). The quadrants were randomly placed on 10 points. The sizes of the quadrants for analyzing the true mangroves were 10 X 10 m. The vegetation data were analyzed in terms of frequency, density, abundance, dominance and basal area (Naithani *et al.*, 2004). The important value index (IVI) for the species was expressed as the sum total of the relative frequency, relative density and relative dominance.

The water and sediment samples were collected at different locations from upstream (Khola) to bay region (Habalikhati) of Brahmani and Baitarani river systems during both high and low tide. Salinity values of water and sediments were analyzed followed by APHA (1998) methods.

Results and Discussions

Water and sediment salinity

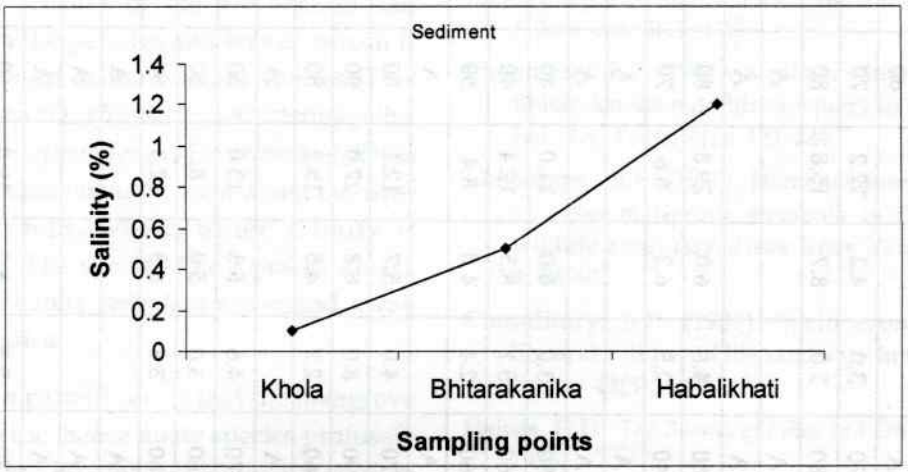
The entire Bhitarakanika wild life sanctuary is intersected by both broad and narrow rivers, channels, creeks, lagoons and marshes. The core area is more or less influenced by fresh water from the river Brahmani and Baitarani and seawater at the mouth of river Dhamara and Patsala. The water salinity (g/l) at different sampling points of the river system varied from 6.41 to 28.83 during high tide and 3.2 to 26.73 during low tide levels (Fig.1). Data showed that water salinity gradually increased from



upstream (Khola) towards the bay region (Habalikhati). The salinity (%) of the sediment varied widely from 0.1 to 1.2 among the sampling points akin to gradual increase as water salinity. The salinity of the sediment depends on the salinity of the tidal water, height of the tide, rainfall of the area and proximity to the fresh water inflow etc. (Bandyopadhya, 1986).

Vegetational Characteristics

Quantitative assessment of true mangroves of Bhitarakanika wild life sanctuary was studied in three forest blocks viz. Khola, Bhitarakanika and Habalikhati which is presented in Table-1. Out of the 65 species of mangroves including associates reported by various researchers (Banerjee, 1984; Chodhury, 1990; Haines, 1921-25; Mooney, 1950; Saxena and Brahmam, 1994), 29 species of true mangroves were found to be present during the study. The different study sites like Khola, Bhitarakanika and Habalikhati form one tidal zone from the upstream river system towards the costal region. From the above study it is found that many important species are less abundant and restricted to a particular locality or often absent in some forest blocks. The species like *Avicennia alba, A. marina, Bruguiera cylidrica, Bruguiera parviflora, Ceriops tagal, Lumnitzera racemosa. Rhizophora apiculata, R. mucronata* and *Sonneratia alba* were rarely encountered at Khola, and Bhitarakanika regions, while *Cerbera manghas, Kandelia candel, Sapium indicum* and *Xylocarpus mekongenesis* were hardly seen at Habalikhati region.



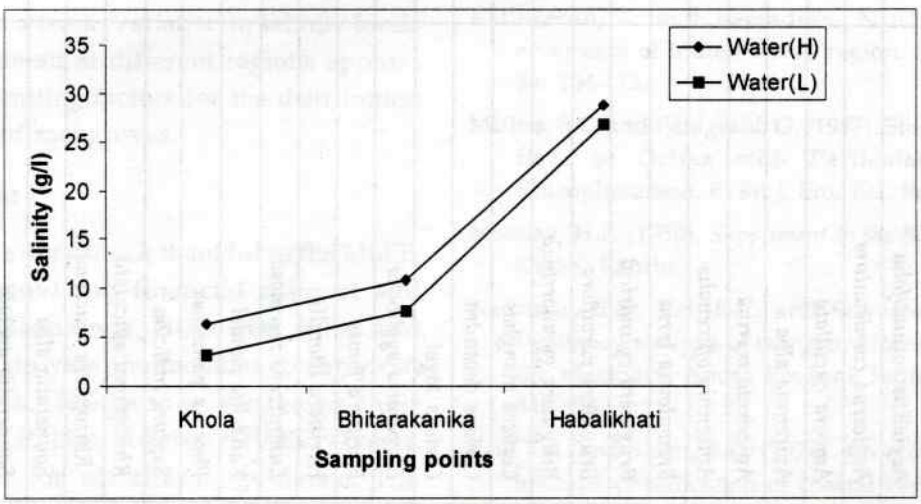


Figure-1 : Variation in salinity of water and sediment at different locations of Brahmani and Baitarani river system.

Table-1

Quantitative structure of true mangroves present at different forest blocks of Bhitarakanika wildlife sanctuary

Sl. No.	SPECIES	KHOLA				BHITARAKANIKA				HABALIKHATI			
		F	D	Ab	IVI	F	D	Ab	IVI	F	D	Ab	IVI
1	*Acanthus ilicifolius*	70	5.2	7.2	12.1	50	2.5	5.0	7.3	50	2.8	5.6	3.4
2	*Aegialitis rotundifolia*	A				60	3.0	5.0	9.0	70	5.5	7.8	10.0
3	*Aegicerus cornicuatum*	70	3.0	4.2	12.2	70	4.5	6.4	14.6	80	6.5	8.1	14.5
4	*Amoora cucullata*	80	7.0	8.7	15.8	80	5.5	6.8	13.9	70	3.2	4.5	7.9
5	*Avicennia alba*	A				A				80	5.1	6.3	17.7
6	*Avicennia marina*	A				A				80	7.2	9.0	12.0
7	*Avicennia officenalis*	80	4.8	6.0	29.8	80	6.5	8.1	32.7	70	4.0	5.7	19.2
8	*Brownlowia tersa*	40	2.5	6.2	6.6	70	3.2	4.5	9.9	50	2.0	4.0	5.4
9	*Bruguiera cylindrical*	A				A				A			
10	*Bruguiera parviflora*	A				A				60	3.0	5.0	8.3
11	*Bruguiera gymnorrhiza*	50	3.0	6.0	11.0	40	2.5	6.2	9.5	60	3.6	6.0	10.5
12	*Cerbera manghas*	60	5.1	8.5	12.4	40	1.2	3.0	6.5	A			
13	*Ceriops decandra*	60	2.4	4.0	8.4	50	2.8	4.8	10.5	90	6.8	7.5	13.3
14	*Ceriops tagal*	A				A				60	3.5	5.8	7.6
15	*Excoecaria agallocha*	70	4.0	5.7	12.1	80	6.0	7.5	16.4	90	8.0	8.8	15.3
16	*Heritiera fomes*	90	8.0	8.8	27.0	90	7.5	8.3	24.0	60	5.8	9.6	17.9
17	*Heritiera litoralis*	60	2.7	4.5	15.2	80	4.4	5.5	18.5	50	2.	4.0	9.6
18	*Lumnitzera recemosa*	A				A				70	7.0	10.0	16.4
19	*Kandellia candel*	70	4.6	6.5	13.0	50	3.0	6.0	9.7	A			
20	*Phoenix palludosa*	60	3.0	5.0	8.	50	3.8	7.6	9.2	50	2.3	4.6	3.5
21	*Sapium indicum*	60	3.8	6.3	12.3	60	2.4	4.0	10.7	A			
22	*Rhizophpra apiculata*	A				A				70	4.7	6.7	11.3
23	*Rhizophora mucronata*	A				A				80	5.2	6.5	12.3
24	*Sonneratia alba*	A				A				70	4.9	7.0	12.1
25	*Sonneratia apetala*	70	5.8	8.2	19.0	60	4.2	7.0	15.9	60	4.3	7.1	14.9
26	*Sonneratia caseolaris*	80	7.0	8.7	24.3	70	5.1	7.2	21.1	60	4.0	6.6	14.2
27	*Xylocarpus granatum*	70	2.8	4.0	22.8	50	2.2	4.4	21.9	60	2.4	4.0	12.9
28	*Xylocarpus moluccensis*	80	3.8	4.7	21.5	60	3.0	5.0	20.6	50	2.1	4.2	11.6
29	*Xylocarpus mekongenesis*	40	1.2	3.0	13.4	60	2.8	4.6	18.5	A			

F= frequency (%), D= density, Ab= abundance, IVI= Important value index and A= absent

Avicennia officinnalis is observed to be most dominant species within the sanctuary followed by *Heritiera fomes, Excoecaria agallocha, Sonneratia caseolaris and Xyllocarpus granatum*. The mangrove community is subjected to regular/occasional inundation; different mangrove species exhibit different distinctive morphological peculiarity to adopt wide salinity variations. It was observed that the pneumatophores of different species like *Rhizophora spp, Heritiera fomes, Avicennia officinnalis, Sonneratia apetala,* and *Kandelia candel* are structurally different from one another. Besides the pneutophores, other types of root system are found in some species like Stilt roots of *Rhizophora mucronata,* Knee roots of *Ceriops decandra* and *Bruguiera spp* etc. and the differences in their size also varies with tidal fluctuations. The bay region is dominated by *Avicennia marina* adapted to higher salinity condition because of the presence of salt excretory glands in the petioles and leaves, which is considered as the most saline tolerant species. Tree species like *Avicennia officinalis, Avicennia alba, Sonneratia apetala, Heritiera fomes, Excoecaria agallocha* and *Xyllocarpus granatum* usually occur along the river bank and near creeks, where water salinity is comparatively less. The species like *Kandelia candel, Brownlowia tersa, Phoenix palludosa* are found away from the water bodies.

The distribution pattern revels that the mangrove habitations are specific, hence many species profusely found in the upstream become less abundant or often absent in the down stream. Variation in salinity levels of water and sediment at different regions appears to be one of the limiting factors for the distribution and composition of mangroves.

Acknowledgement

The authors are very much thankful to the MoEF, New Delhi for providing financial support and Director, RRL, Bhubaneswar, HOD, HM & EM and NPD divisions to provide the facilities to carry out the research work. The author expresses their gratitude to Dr. B.P. Das, Adviser SPARC (Trust), and Bhubaneswar for his sincere guidance. The Divisional Forest Officer, Rajnagar, Orissa is duly acknowledged for his kind help and cooperation during the field study.

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Plant Sci. Res. Vol.27 (1&2) : 38-45, 2005

Floristic Survey of Motijharan Hill, Sambalpur, Orissa

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An exhaustive floristic survey of Motijharan Hill of Sambalpur was carried out during January to December, 2004. During the present survey, a total of 69 angiospermic species under 64 genera belonging to 38 families have been reported. The approximate ratio of monocotyledons to dicotyledons is 1:4.3. So, it indicates that the dicotyledons are represented by four times than monocotyledons in Motijharan Hill of Sambalpur. Among the dicots, Rubiaceae are the dominant family, followed by Acanthaceae, Verbenaceae and Euphorbiaceae, while among the monocots, Poaceae represent the dominant flora, followed by Zingiberaceae and Commelinaceae. The decline in vegetation may be assigned to various anthropological activities in this area. Hence, general awareness regarding socio-economic aspects of the plants having economic value should be generated among the common people so as to check further genetic erosion.

Key Words: *Flora, vegetation, floristic analysis, Motijharan Hill.*

Introduction

Orissa has a rich and diversified flora due to its unique topography and variable climatic conditions. Out of 30 districts of Orissa, Sambalpur, which lies between 20^0 30′ - 22^0 30′ N Latitude and 83^0E - 85^0 1′E longitude, has a wide diversity of vegetation due to various environmental and biological conditions. It has a mean elevation of 486 ft to 513 ft above sea level (Sambalpur Master Plan, 1981-2001AD). Haines (1921-1925) mostly collected plants from Bihar and few from Orissa. However, many species are devoid of precise locality of collection and some hilly areas like Motijharan of Sambalpur have not been fully botanised as indicated by Haines in the introductory chapter of his treaties. Mooney (1950) launched an extensive botanical exploration of the hilly terrains of Western Orissa and made representative collection. The bunglow at Motijharan used by Mooney during his stay at Sambalpur bear the testimony of his work at Motijharan.

Motijharan, present at the Northern part of Sambalpur town covers 156.60 acres of land and it is surrounded by Sarla Mouza in east, military lines in the west, Sakhigopinath Mouza in north and Sunapali Mouza in south. In the mean time i.e. 1950 to 2004, this area has experienced devastation of flora due to cutting of trees for fuels and timber, for construction of forest offices, bunglow, Doordarsan station, schools, microwave station, etc. As a result, many species are becoming vulnerable and others are on the threat of extinction. Hence, information regarding present plant wealth is highly essential for their conservation and judicious utilization. In view of this fact, a floristic survey programme was undertaken to catalogue the plants occurring in Motijharan Hill, before further genetic erosion of economically important plants occur.

Materials and Methods

To keep a proper record of the vegetation of Motijharan, the area was intensively studied and collections were made in different seasons by regular field trips to the different parts of the site during 2004. The detailed field notes were maintained on the spot and the specimens were identified in the laboratory. After the specimen is pressed, dried and poisoned by mercuric chloride, it is affixed on a herbarium sheet.

As far as possible, the correct names of the species have been mentioned first in accordance with the articles and recommendations laid down by the International Code of Botanical Nomenclature. The

correct name is followed by basionym (if any) and synonym occurring in 'The Flora of Orissa' by Saxena and Brahmam (1995-1996) and 'Flora of Sambalpur' by Panda and Das (2004). References to other recent floras/monographs in support of the correct names have been cited. The identified herbarium sheets were sorted out family, genus and species wise. All the specimens have been housed in the herbarium of Post-Graduate Department of Botany, Gangadhar Meher College, Sambalpur according to modified Bentham and Hooker's system of classifications (1862-1883).

Results and Discussions

During the present survey, a total of 69 angiospermic species under 65 genera belonging to 38 families have been collected during January 2004 to December 2004. Out of 69 species, 56 species of dicots are distributed under 53 genera belonging to 32 families while 13 monocot species spread over 11 genera under 6 families. The approximate ratio of monocot to dicot species is 1:4.3. So, it indicates that dicotyledons are represented by four times than monocotyledons in Motijharan of Sambalpur (Table-1). Out of the total 38 families, 21 families are represented by single species. Rubiaceae are the dominant family, followed by Acanthaceae, Verbenaceae, Euphorbiaceae among the dicotyledons, while Poaceae are the dominant family among monocotyledons, followed by Zingiberaceae and Commelinaceae.

Systematic Enumeration

DICOTYLEDONS

Menispermaceae *A. L. Juss. nom. cons.*

1. ***Cissampelos pareira*** L. Var. hirsuta (Buch. -Ham. ex DC.) Forman, Kew Bull. 22:356.1988. C. Pareira L. Sp. Pl. 1031.1753, Pro parte; Hook. f. & Thoms. in Hook.f.Fl. Brit. India 1:103.1872; Gamble, Fl. Madras 1:30(21). 1915; Haines, Bot. Bihar & Orissa1: 16(17). 1921; Fl.Orissa I: 33. 1994.

F.N. - BOTG 13738

Table-1

Number of species found in different families

	Dicot	Monocot	Total
Family	32	6	38
Genera	53	11	64
Species	56	13	69

Capparaceae A. L. Juss. nom. cons.

2. ***Cleome viscosa*** L., Sp.Pl.672.1753; Hook. f. & Thoms., *l.c.* 170.1872; Haines, *l.c.* 29.1921; Sharma *et al., l.c.* 2: 317. 1993; Panda & Das 43. 2004.

F.N.- BOTG 13716

Violaceae Batsch. nom. cons.

3. ***Hybanthus enneaspermus*** (L.) F.V.Muell. Fragm. 10:81.1876; Jacobs & Moore in Steenis, Fl. Males. I. 7:197.f.6.1971; Banerjee & Pramanik, Fasc. Fl. India 12:2. f. 1-6. 1983; Fl. Orissa I: 70. 1994.*Viola enneasperma* L. Sp. Pl. 937. 1753.*Viola suffruticosum* L. Sp. Pl. 937. 1753. *Ionidium suffruticosum* (L.) Ging.ex DC. Prod. 1:311. 1834;Wight, Ill. t. 19. 1840. & Icon. t. 308. 1840; Hook. f. & Thoms. in Hook. f. Fl. Brit. India 1:185. 1872; Gamble Fl. Madras 1:49(35). 1915; Haines, Bot. Bihar & Orissa 1:33(34). 1921.

F.N. - BOTG 13717

Dipterocarpaceae Bl. nom. cons.

4. ***Shorea robusta*** Gaertn. Fruct. 3:48.t.186.1805; Roxb. Pl. Corom. t. 212.1811; Bedd. Fl. Sylv. t. 4.1869; Dyer in Hook. f. Fl. Brit. India 1:306.1874; Gamble, Fl. Madras 1:83(60). 1915; Haines, Bot. Bihar & Orissa 1:56(58). 1921. Tewary & Sarkar, J. Econ. Tax. Bot. 11.108.t.3.1987; Fl. Orissa I: 122. 1994.

F.N. - BOTG 13711

Malvaceae A. L. Juss. *nom. cons.*

5. ***Sida acuta*** Burm. f. Fl. Ind. 147.1768, emend. K. Schum. in C. Martius, Fl. Bras. 12.3:326.1891; Wight, Icon. t. 95.1838; Gamble, Fl. Madras 1: 90(64). 1915; Haines, Bot. Bihar & Orissa. 1:61(63). 1921; Borssum, Blumea 14:187.1966: Paul & Nayar, Fasc. Fl. India 19:202. t. 49.1988; Fl. Orissa I: 159. 1994.

F.N. - BOTG 13756

6. ***Sida cordifolia*** L., Sp. Pl. 684. 1753; Mast., *l.c.*324. 1874; Haines, *l.c.* 59.1921; Sharma *et al., l.c.* 3:285.1993; Panda & Das 58.2004.

F.N. - BOTG 13757

7. ***Urena lobata*** L. ssp. sinuata (L.) Borss. Var. sinuata: Paul & Nayar in Nayar *et al.*, Fasc. Fl. India 19:230. 1988; Sharma *et al.*, Fl. India 3:382. 1993. *U. sinuata* L., Sp. Pl. 692. 1753; Mast. in Hook. f., Fl. Brit. India 1:329. 1874; Haines, Bot. Bihar & Orissa Pt. II: 63. 1921; Panda & Das 62.2004.

F.N. - BOTG 13762

Sterculiaceae B. Artl. *nom. cons.*

8. ***Helicteres isora*** L. Sp. Pl. 963. 1753; Wight, Icon. t. 180.1839; Bedd. Fl. Sylv. t. 5. f1.1871; Mast. in Hook. f. Fl. Brit. India. 1: 365. 1874; Gamble, Fl. Madras 1: 107 (77). 1915; Haines, Bot. Bihar and Orissa 1: 78 (82). 1921; Fl. Orissa I: 178.1994.

F.N. - BOTG 13727

9. ***Melochia corchorifolia*** L., Sp. Pl. 675. 1753; Mast. in Hook.f., Fl. Brit. India I: 374. 1874; Haines, Bot. Bihar & Orissa Pt. II: 82. 1921; Sharma *et al.*, Fl. India 3:441. 1993. *Riedleia corchorifolia* (L.) DC., Prodr. 1:491. 1824; Panda & Das 65.2004.

F.N. - BOTG 13754

Tiliaceae A. L. Juss. *nom. cons.*

10. ***Corchorus aestuans*** L., Syst. Nat. ed. 10. 1079. 1759; Sharma *et al.*, Fl. India 3:485. 1993. *C. acutangulus* Lam., Encycl. 2: 104. 1786; Mast. in Hook. f., Fl. Brit. India 1: 398. 1874; Haines, Bot. Bihar & Orissa Pt. II: 87.1921; Panda & Das 68.2004.

F.N. - BOTG 13759

Rutaceae A. L. Juss. *nom. cons.*

11. ***Aegle marmelos*** (L.) Corr. Trans. Linn. Soc. London 5:223.1800; Roxb. Pl. Corom. t. 143.1800; Wight, Icon.t.16.1838; Bedd. Fl. Sylv. t. 161.1871; Hook. f. in Hook. f. Fl. Brit India 1:516.1875; Gamble, Fl. Madras 1:161(115). 1915; Haines, Bot. Bihar & Orissa 1: 167(173). 1921; stone in dassan & Fosberg, Rev. Handb. Fl. Ceylon 5:414.1985; Fl. Orissa I: 228.1994.

Meliaceae A. L. Juss. *nom. cons.*

12. ***Chloroxylon swietenia*** DC., Prodr. 1:625. 1824; Hiern. in Hook. f., Fl. Brit. India 1:569. 1875; Haines, Bot. Bihar & Orissa Pt. II: 173. 1921; Hajra *et al.*, Fl. India 4: 355. 1997; Panda & Das 77.2004.

F.N. - BOTG 13702

Olacaceae Mirbel.ex DC. *nom. cons.*

13. ***Olax scandens*** Roxb. Pl. Corom. t.102.1799 & Fl. Ind. 1:163.1820; Mast. in Hook. f. Fl. Brit. India 1:575. 1875; Glamble, Fl. Madras 1:190(136). 1915; Haines, Bot. Bihar Orissa 1:183 (189). 1921; sleumer, Blumea 26: 157.1980 & in steenis, Fl. Males. I. 10:7.1984;Fl. Orissa I: 288. 1994.

F.N. - BOTG 13742

Rhamnaceae A. L. Juss. *nom. cons.*

14. ***Ziziphus oenoplia*** (L.) Mill.Gard.Dict.ed.8.3.1768; Lowson in Hook. f. Fl. Brit. India 1:634.1875; Gamble, Fl. Madras 1:220(158). 1988; Haines, Bot. Bihar & Orissa 1:196(203). 1921; Bhandari & Bhans. Fasc. Fl. India 20:103.1990; Fl. Orissa I: 322.1994. *Rhamnus oenoplia* L. Sp. Pl.194.1753.

Anacardiaceae Lindl. *nom. cons.*

15. ***Anacardium occidentale*** L.Sp.Pl.383.1753; Bedd. Fl. Sylv.t. 163.1871; Hook. f. Fl. Brit. India 2:20.1876; Gamble, Fl. Madras 1:260(185). 1918; Haines, Bot. Bihar & Orissa 1:220(228). 1922; Ding Hai in steenis, Fl. Males. I. 8: 421.t.6.1978; Fl. Orissa I: 359.1994.

F.N. - BOTG 13710

16. ***Semecarpus anacardium*** L.f., Suppl. 182. 1781; Hook. f. in Hook. f., F1. Brit. India 2:30.1876; Haines. Bot. Bihar & Orissa Pt. II: 222.1921. Panda & Das 89.2004.

F.N. - BOTG 13714

Fabaceae Lindl. *nom. cons.*

17. ***Butea monosperma*** *(Lam.)* Taub, in Engl.&Prantl. Pflanzenf. 3 (3): 366.1894; Anon., Draw. Ind. pl. (Icon.Roxb.67) 5:t.25.1971. *Erythrina monosperma* Lam. Encycl. 1:391.1785. *Butea frondosa* Koen.ex Roxb. Asiat. Res. 3:369. 1792 & Pl. Corom. t. 21. 1795; Bedd.Fl.Sylv.t.176.1872; Baker in Hook.f. Fl. Brit. India 2:194. 1876; Gamble, Fl. Madras 1:357 (252). 1918; Haines, Bot. Bihar & Orissa 2:270 (292). 1922; Mooney, Suppl. Bot. Bihar & Orissa 51.1950. Fl. Orissa I: 460.1994.

18. ***Pongamia pinnata*** (L.) Pierre, Fl. For. Cochinch. t. 385. 1899; Mooney, Suppl. Bot. Bihar & Orissa 56. 1950. *Cytisus pinnatus* L., Sp. Pl. 741. 1753. *Pongamia*

glabra Vent., Jard. Malm. 1:28. t. 28. 1803; Baker in Hook.f., Fl. Brit. India 2:240.1876; Haines, Bot. Bihar & Orissa Pt. III : 299. 1922; Panda & Das 113.2004.

F.N. - BOTG 13709

19. ***Tephrosia tinctoria*** Pers. Syn. 2:329. 1801; Wight, Icon. t. 388. 1840; Baker in Hook. f. Fl. Brit. India 2:111. 1876; Gamble, Fl. Madras 1:319(225). 1918; Haines, Bot. Bihar & Orissa 2:243(254). 1922; Mooney, Suppl. Bot. Bihar & Orissa 48. 1950; Fl. Orissa I: 602.1994.

F.N. - BOTG 13763

Caesalpiniaceae R.Br. *nom. cons.*

20. ***Cassia absus*** L., Sp. Pl.376.1753; Baker in Hook.f., Fl. Brit. India 2:265. 1878; Haines, Bot. Bihar & Orissa Pt. III: 305. 1922; Mooney, Suppl. Bot. Bihar & Orissa 57.1950; Fl. *Eugenia jambolana* Lam., Enc. Meth. Bot.3: 198. 1789; Duthie in Hook.f., Fl. Brit. India 2:499. 1879; Haines, Bot. Bihar & Orissa Pt. III:360. 1922; Panda & Das 142. 2004. Fl.Orissa I: 385.1994.

F. N. - BOTG 13732

21. ***Cassia fistula*** L. Sp. Pl. 377.1753; Baker in Hook. f. Fl. Brit. India 2:261.1878; Gamble, Fl. Madras 1:400(283). 1919; Haines, Bot. Bihar & Orissa 2:302(315). 1922; Fl. Orissa I: 386.1994.

Myrtaceae Juss. *nom. Cons.*

22. ***Syzygium cumini*** (L.) Skeels in U.S. Dept. Agric. Bur. Pl. Ind. Bull. 248.25. 1912; Mooney, Suppl. Bot. Bihar & Orissa 65. 1950. *Myrtus cumini* L., Sp. Pl. 471. 1753.

F.N. - BOTG 13705

Lythraceae Jaume Saint- Hilaire *nom. cons.*

23. ***Woodfordia fruticosa*** (L.) Kurz, j. Asiat. Soc. Beng. 40:56.1871; Gamble, Fl. Madras 1:511(361).1919; Haines, Bot. Bihar & Orissa 2:374 (390). 1922; Mooney, Suppl. Bot. Bihar and Orissa 67.1950; Fl. Orissa II: 722.1995.

F.N. - BOTG 13713

Onagraceae Juss. *nom. cons.*

24. ***Ludwigia perennis*** L., Sp. Pl. 1:119. 1753. L. *praviflora* Roxb., Fl Ind. 1:440. 1820; Clarke, *l.c.* 588. 1879; Haines, *l.c.* 382. 1922; Panda & Das 153.2004.

F.N. - BOTG 13760

Rubiaceae A. L. Juss. *nom. cons.*

25. ***Anthocephalus chinensis*** (Lamk.) A.Rich. ex Wall. Rep. 2:491.1843; Manilal *et* Sivaranjan, Fl. Calicut 133.1982. *Cephalanthus chinensis* Lamk. Encycl. Meth. Bot. 1: 678.1785. *Anthocephalus cadamba* (Roxb.) Miq. Pl. Ind. Bat. 2:135.1856; Hook. f. in Hook. f. Fl. Brit. India 3:23.1880; Haines, Bot. Bihar & Orissa 2:441. 1961.

26. ***Borreria articularis*** (L.f.) F.N. Will. in Bull. Herb. Boiss, Ser. 2, 5:956. 1905. *Spermacoce articularis* L.f. Suppl. 199. 1781. excl. syn. Rumph. *S. hispida* L., Sp. Pl. 102. 1753; Hook.f. in Hook.f., Fl. Brit. India 3:200. 1881; Haines, Bot. Bihar & Orissa Pt. IV: 45. 1922; Panda & Das 167.2004.

27. ***Borreria pusilla*** (Wall) DC., Prodr. 4: 543. 1830. *Spermacoce pusilla* Wall. In Roxb., Fl. Ind.1: 379.1820. *Spermacoce stricta* L. f. Suppl. 120. 1781; Hook.f. *l.c.* 200.1881; Haines, *l.c.* 450. 1922; Panda & Das 168.2004.

F.N. - BOTG 13764

28. ***Gardenia latifolia*** Aiton in Hort.Kew.1: 294.1789; Hook.f. *l.c.*116.1880; Haines, *l.c.*431. 1922; Panda & Das 169.2004.

F.N. - BOTG 13706

29. ***Morinda pubescens*** Son.in Rees, Cyclop.24 n.3. 1813. *M. tinctoria* Roxb., Fl. Ind.ed.Carey & wall.1: 543.1820; Hook.f., *l.c.* 156.1880; Haines, Bot. Bihar & Orissa Pt.IV: 423.1922, non Noronha, 1790; Panda & Das 176.2004.

F.N - BOTG 13707

30. ***Mitragyna parvifolia*** (Roxb.) Korth.,Obs. Naucl. Ind. 19. 1839; Haines, Bot. Bihar & Orissa Pt. IV: 422. 1922. *Nauclea parvifolia* Roxb., Pl. Corom. 1:40, t.52. 1795. *Stephegyne parvifolia* Korth.,Verh. Ges. Bot. 161. 1840; Hook.f. in Hook.f., Fl. Brit. India 3:25. 1880; Panda & Das 175.2004.

F.N. - BOTG 13741

Oleaceae Hoffm. *nom. cons.*

31. ***Jasminum flexile*** Vahl, Symb. Bot.3:1. 1794; Wight, Icon. t. 1253. 1848; C.B.Cl. in Hook. f. Fl. Brit. India 3:601. 1882; Haines, Bot. Bihar & Orissa 2:526(552). 1922; Gamble, Fl. Madras 2:791(556). 1923; Green in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 6:261.

1987. Fl. Orissa II: 722.1995. *J. azoricum sensu* Burm. F. Fl. Ind. 6. 1768, pro parte, non L.

F.N.- BOTG 13735

Apocynaceae A. L. Juss. *nom. cons.*

32. ***Holarrhena pubescens*** (Buch.-Ham.) Wallich ex G.Don, Gen.Hist. 4:78.1837. Plate-64g. *Echites pubescens* Buch.-Ham., Trans. Linn. Soc. London 13:521. 1821. *Holarrhena antidysenterica* Wallich ex A.DC. in DC., Prodr. 8:413.1844; Hook.f. in Hook.f.,Fl. Brit. India 3:644. 1882; Haines, Bot. Bihar & Orissa Pt.IV: 538.1922. Panda & Das 210.2004.

F.N.- BOTG 13701

33. ***Ichnocarpus frutescens*** (L.) R. Br. Mem. Wern. Nat. Hist. Soc. 1:62.1811 (Preprint 1810) & in Ait. f. Hort. Kew. 2:69.1811; Wight, Icon. t. 430. 1841; Hook f. in Hook. f. Fl. Brit. India 3:669.1882; Gamble, Fl. Madras 2:820(577).1923; Haines, Bot. Bihar & Orissa 2:546(573).1922; Huber in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 4:71.1973. Fl. Orissa II: 1062.1995

F.N.- BOTG 13739

Convolvulaceae A. L. Juss. *nom. cons.*

34. ***Erycibe paniculata*** Roxb. Pl. Corom. t. 159. 1802; Wight, Ill. 2:t.180.1850; C.B.Cl. in Hook. f.Fl. Brit. India 4:180.1883; Haines, Bot. Bihar & Orissa 2:605(634).1922; Gamble, Fl. Madras 2:930(653).1923; Austin in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 1:308.1980. Fl. Orissa II: 1164.1995

F.N.- BOTG 13708

35. ***Evolvulus alsinoides*** Linn. Sp. Pl. (ed. 2). 392.1762; C.B.Cl. in Hook. f. Fl. Brit. India 4:220.1883; Haines, Bot. Bihar & Orissa 2:585(614).1922; Gamble, Fl. Madras 2:923(648).1923; ooststr. In steenis, Fl. Males. I. 4:395.1953; Austin in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 1:309.1980. Fl. Orissa II: 1165.1995

F.N. - BOTG 13725

36. ***Merremia tridentata*** (L.) Hallier f.,Bot.Jahrb. Syst.16:552.1893. *Convolvulus tridentatus* L., Sp.pl.157 1753. *Ipomoea tridentata* Roth ex Roem. et Schult.,Arch. Bot. 1(3):38.1798; Clarke, l.c.205.1883; Haines, *l.c.* 595.1992. Panda & Das 240.2004.

F.N. - BOTG 13726

Cuscutaceae Dumort. *nom. cons.*

37. ***Cuscuta reflexa*** Roxb. Pl. Corom. t. 104.1799; C.B.Cl. in Hook. f. Fl. Brit. India 4:225.1883; Haines, Bot. Bihar & Orissa 2:606(635).1922; Gamble, Fl. Madras 2:931(654).1923; ooststr. in steenis, Fl. Males. I. 4:393.1953; Austin in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 1:306.1980. Fl. Orissa II: 1203.1995

F.N. - BOTG 13744

Bignoniaceae A. L. Juss. *nom. cons.*

38. ***Kigelia africana*** (Lam.) Benth. In Hook. Niger Fl. 463. 1849; Steenis in Steenis, Fl. Males. I. 8:183.1977. *Bignonia africana* Lam. Encycl. 1: 424. 1785. *Kigelia pinnata* DC. Prod. 9:247.1845; Haines, Bot. Bihar & Orissa 2:660(692). 1922; Gamble, Fl. Madras 2:1000(703). 1924. Fl. Orissa II: 1308.1995

Pedaliaceae R.Br. *nom. cons.*

39. ***Sesamum orientale*** L., Sp. Pl. 634.1753. *S. indicum* L., Sp. Pl. 634.1753; Clarke in Hook. f. Fl.Brit. India 4:387.1884; Haines, Bot. Bihar & Orissa Pt. IV: 661. 1992. Panda & Das 264.2004.

F.N. - BOTG 13733

Martyniaceae Stapf.

40. ***Martynia annuna*** L. Sp. Pl. 618. 1753. *M. diandra* Glox., Obs. Bot. 14, t. 1. 1785; Clarke in Hook. f., Fl. Brit. India 4:386. 1884; Haines, Bot. Bihar & Orissa Pt.IV: 662. 1922. Panda & Das 265.2004.

Acanthaceae A. L. Juss. *nom. cons.*

41. ***Andrographis paniculata*** (Burm.f). Wall. ex Nees in Wall. Pl. As Rar.3:116.1832; Wight, Icon.t.518.1842; C.B.Cl.in Hook.f. Fl.Brit. India 4:501.1884; Haines, Bot. Bihar & Orissa 2:699(733).1922; Gamble, Fl. Brit. India 2:1048(734).1924; Mooney, Suppl. Bot. Bihar & Orissa 118.1950. Fl. Orissa III: 1328.1995.

F.N. - BOTG 13730

42. ***Justicia quinqueangularis*** Koen. *ex* Roxb., Fl. Ind.1: 133.1832; Clarke, *l.c.* 536. 1885; Haines, *l.c.* 692.1922; Mooney, *l.c.* 117.1950. Panda & Das 279. 2004.

F.N. - BOTG 13746

43. ***Ruellia tuberosa*** L., Sp. Pl. 635. 1753; Haines, Bot. Bihar & Orissa Pt. IV: 675. 1922; Mooney, Suppl. Bot. Bihar & Orissa 109. 1950; Panda & Das 283. 2004.

F.N. - BOTG 13703

44. ***Rungia pectinata*** (L.) Nees in DC., Prodr. 11:469.1847. *Justicia pectinata* L. in Torner, Cent.II P1.3.1756. *Rungia parviflora* (Retz.) Nees var. *pectinata* (L.) Clarke in Hook.f., F1.Brit.India 4:550. 1985; Haines, Bot. Bihar & Orissa Pt. IV: 690.1922. Panda & Das 284.2004.

F.N. - BOTG 13712

Verbenaceae Jaume Saint - Hillare. *nom. cons.*

45. ***Lantana camara*** L. var. *aculeata* (L.) Mold. Torreya 34.9.1934; Mold. & A.L. Mold. in Dassan. & Fosberg, rev. Handb. Fl. Ceylon 4:225.1983. Fl. Orissa III: 1413.1995. L. *camara* sensu Haines, Bot. Bihar and Orissa 2:705(739). 1922; Mooney, Suppl. Bot. Bihar & Orissa 120.1950.

F.N. - BOTG 13749

46. ***Lippia javanica*** (Burm.f.) Spreng., Syst. 2:752.1825. *Verbena javanica* Burm.f. Fl. Ind.12. t.6, f.2. 1768. *Lippia geminata* H.B.K., Nov.Gen.et Sp. 2:266. 1818; Clarke in Hook.f. Fl. Brit. India 4:563.1885;Haines, Bot.Bihar & Orissa Pt.IV: 706.1992; Mooney, Suppl. Bot. Bihar & Orissa 120.1950. Panda & Das 290.2004.

F.N. - BOTG 13724

47. ***Stachytarpheta jamaicensis*** (L.) Vahl, Enum. Pl. 1:206. 1804; Mold. & A.L. Mold. in Dassan. & Fosberg, Rev. Handb. Fl.Ceylon 4: 253.1983. Fl. Orissa III: 1424.1995. *Verbena jamaicensis* L. Sp. Pl. 19.1753. *Stachytarpheta indica* auct. non (L.) Vahl: C.B.Cl. in Hook. f. Fl. Brit. India 4: 564. 1885; Haines, Bot. Bihar & Orissa 2: 707(741). 1922; Gamble, Fl. Madras 2: 1090(763). 1924; Mooney, Suppl. Bot. Bihar & Orissa 120.1950.

F.N. - BOTG 13715

48. ***Symphorema polyandrum*** Wight, Icon.t. 363. 1840; C.B.Cl. in Hook.f. Fl. Brit. India 4:599.1885; Haines, Bot. Bihar & Orissa 2:724(759). 1924; Gamble, Fl. Madras 2:1104(773). 1924. Fl. Orissa III: 1425.1995

F.N. - BOTG 13740

Lamiaceae Lindl. *nom. cons.*

49. ***Anisomeles indica*** (L.) Kuntze, Rev. Gen. Pl. 2:512.1891; Gamble, Fl. Madras 2: 1140(797). 1924; Haines, Bot. Bihar & Orissa 2: 745 (782). 1924; Mukerjee, Rec. Bot. Surv. India 14:152.1940; Keng in Steenis, Fl. Males. I. 8: 329.1978; Cramer in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 3: 176. 1981. Fl. Orissa III: 1441.1995. *Nepeta indica* L. Sp. Pl. 571. 1753. *Anisomeles ovata* Ait. F. Hort. Kew. 3:364. 1811; Wight, Icon. t. 865. 1844-45; Hook f. in Hook. f. Fl. Brit. India 4: 672. 1885.

F.N. - BOTG 13761

50. ***Hyptis suaviolens*** L. Poit. Ann. Mus. Natl. Hist. Nat. 7:472.t.29.f.2.1806; Hook. f. in Hook. f. Fl. Brit. India 4:630.1885; Gamble, Fl. Madras 2:1129 (789). 1924; Haines, Bot. Bihar & Orissa 2:736(772). 1924; Mukerjee, Rec. Bot. Surv. India 14:63.1940; Keng in steenis, Fl. Males, I. 8:371.1978; Cramer in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 3:155.1981. Fl. Orissa III: 1451.1995. *Ballota suaveolens* L. Syst. Nat. (ed.10) 1100. 1759

F.N. - BOTG 13728

Nyctaginaceae A. L. Juss. *nom. cons.*

51. ***Boerhavia diffusa*** L. Sp. Pl. 3.1753; Haines, Bot. Bihar and Orissa 2:757(795). 1922; Gamble, Fl. Madras 2:1162(814). 1925; Stemm. in Steenis, Fl. Males. I. 6:454.1964. Fl. Orissa III: 1492.1995

F.N. - BOTG 13752

Amaranthaceae A. L. Juss. *nom. cons.*

52. ***Allmania nodiflora*** (L) R.Br. ex Wt. in Hook., J. Bot. 1:226.1834; Hook. f. in Hook. f., Fl. Brit. India 4:716.1885; Haines, Bot. Bihar & Orissa Pt. V: 759. 1924; Mooney, Suppl. Bot. Bihar & Orissa 127.1950. *Celosia nodiflora* L., Sp. Pl. 205.1753. Panda & Das 306.2004.

F.N. - BOTG 13734

Polygonaceae A. L. Juss. *nom. cons.*

53. ***Antigonon leptopus*** Hook. & Arn. Bot. Deechey Voy. 308.t. 69.1841; Haines, Bot. Bihar & Orissa 2:783(821). 1924; Gamble, Fl. Madras 2: 1129 (835). 1925. Fl. Orissa III: 1531.1995

F.N. - BOTG 13755

Euphorbiaceae A. L. Juss.

54. ***Antidesma ghaesembilla*** Gaertn., Fruct.1:189.1788; Hook.f., *l.c.*357.1887; Haines, *l.c.* 139.1921. Panda & Das 325.2004.

F.N. - BOTG 13743

55. ***Sebastiania chamaelea*** (L.) Muell. - Arg. in DC. Prod. 15:1175.1866; Hook. f. in Hook. f. Fl. Brit. India 5:475.1888; Haines, Bot. Bihar & Orissa 1:118(122). 1921; Gamble, Fl. Madras 2:1344(940).1925; Airy Shaw, kew Bull.26:339.1972. Fl. Orissa III: 1682.1995

F.N. - BOTG 13731

56. ***Suregada multiflora*** (Juss.) Baill. Etude. Gen. Euphorb. 396. 1858; Airy Show, Kew Bull. 36:349. 1981. Fl. Orissa III: 1685.1995. *Gelonium muiltiflorum* Juss. Euphorb. Gen. Tent. 3:t.10.f.31A. 1824; Hook.f. in Hook.f. Fl. Brit. India 5:459. 1887; Haines, Bot. Bihar & Orissa 1:114(118). 1921; Gamble, Fl. Madras 2:1343(940). 1925.

F.N. - BOTG 13704

MONOCOTYLEDONS

Zingiberaceae Lindl. *nom. cons.*

1. ***Costus speciosus*** (Koenig) Sm. Trans. Linn. Soc. London 1: 249. 1791; Wight, Icon. t. 2014. 1853; Baker in Hook.f. Fl. Brit. India 6: 249. 1892; Haines, Bot. Bihar & Orissa 3: 1145 (1196). 1924; Fischer in Gamble, Fl. Madras 3: 1490(1041). 1928; Mooney, Suppl. Bot. Bihar & Orissa 205. 1950; A.S. Rao & Verma, Bull. Bot. Surv. India 14: 138. 1972; Burtt & Sm. in Dassan. & Fosberg, Rev. Handb. Fl. Ceylon 4: 491. 1983. Fl. Orissa III: 1893.1995. *Banksea speciosa* Koenig in Retz. Obs. Bot. 3: 75. 1783.

F.N. - BOTG 13737

2. ***Zingiber capitatum*** Roxb. Asiat. Res. 11: 348. 1810; Baker in Hook. f. Fl. Brit. India 6: 248.1892; Haines, Bot. Bihar & Orissa 3: 1144 (1195). 1924; A.S. Rao & Verma, Bull. ; Bot. Surv. India 14: 138. 1972. Fl. Orissa III: 1908.1995.

F.N. - BOTG 13736

Dioscoreaceae R.Br. *nom. cons.*

3. ***Dioscorea glabra*** Roxb. (Hort. Beng. 72. 1814, *nom. nud.*), Fl. Ind. ed. 2, 3:804. 1832; Hook.f., *l.c.* 294.1892; Haines, *l.c.* 1119. 1924. Panda & Das 368.2004.

F.N. - BOTG 13723

4. ***Dioscorea pentaphylla*** L., Sp.Pl.1032. 1753; Hook. f., *l.c.* 289.1892; Haines, *l.c.* 1123.1924. Panda & Das 369.2004.

F.N. - BOTG 13729

Liliaceae A. L. Juss. *nom. cons.*

5. ***Gloriosa superba*** L., Sp. Pl. 305. 1753; Hook.f. in Hook.f., Fl. Brit. India 6:358. 1892; Haines, Bot. Bihar & Orissa Pt. VI: 1093. 1924. Panda & Das 370.2004.

F.N. - BOTG 13751

Commelinaceae R.Br. *nom. cons.*

6. ***Murdannia nudiflora*** (L.) Brenan in Kew Bull. 7:189. 1952. *Commelina nudiflora* L., Sp. Pl. 41. 1753, pro typ. Parte. *Aneilema nudiflorum* (L.) R.Br., Prodr. 271.1810; Hook.f., *l.c.* 387. 1892; Haines, *l.c.* 1080. 1924. Panda & Das 380.2004.

F.N. - BOTG 13748

7. ***Tonningia axillaris*** (L.) Kuntze, Rev. Gen. Pl.2: 721.1891. Fl. Orissa III: 2000.1995. *Commelina axillaris* L.Sp.Pl.42.1753. *Cyanotis axillaris* (L.) Schult. & Schult.f. Syst.Veg. 7:1154. 1830; Hook.f.in Hook.f. Fl. Brit. India 6:388. 1892; Haines, Bot. Bihar & Orissa 3:1081(1130). 1924; Fischer in Gamble, Fl. Madras 3:1550(1082). 1928.

Tradescantia axillaris Roxb. Pl.Corom.t. 107. 1799.

Amischophacelus axillaris (L.) R.S. Rao & Kamm. J.Linn. Soc. Bot. 59:306.1966.

F.N. - BOTG 13753

Cyperaceae A. L. Juss. nom. cons.

8. ***Cyperus paniceus*** (Rottb.) Boeck. Linnaea 36:381.1870, pro parte; Kern in Steenis, Fl. Males. I. 7:643.974. *Kyllinga panicea* Rottb. Descr. Icon. Rar. 15.t.4.f.1. 1773. *Mariscus paniceus* (Rottb.) Vahl, Enum. Pl.2:373. 1806; C.B.Cl. in Hook. f. Fl. Brit. India 6:620. 1893; C.B.Cl. Ill. Cyp. t.22.f. 1-2. 1909; Haines, Bot. Bihar & Orissa3: 1250(1304). 1924; Fischer in Gamble, Fl. Madras 3:1644(1143). 1931; Choudhury & Patnaik, J. Orissa Bot. Soc. 2:23. 1980; T. Koyama in Dassan.

& Fosberg, Rev. Handb. Fl. Ceylon 5:235.1985. Fl. Orissa IV: 2140.1996

F.N. - BOTG 13718

9. ***Cyperus triceps*** (Rottb.) Endl., Cat. Hort.Vindob. 1:94.1842. *Kyllinga triceps* Rottb., Descr. et *Ic.* 14, t. 4. f. 6. 1773, *nom.superfl.*; Clarke, *l.c.* 587.1893; Haines, *l.c.*907. 1924; Mooney, *l.c.* 146. 1950; Panda & Das 402.2004.

F.N. - BOTG 13747

Poaceae Barhhart. *nom. cons.*

10. ***Alloteropsis cimicina*** (L.) Stapf. In Prain, Fl. Trop. Africa 9:487. 1919; Haines, Bot. Bihar & Orissa Pt. V:1009. 1924; Mooney, Suppl. Bot. Bihar & Orissa 172. 1950; Bor, Grass. Bur. Cey. Ind. & Pak. 276. 1960, *Milium cimicinum* L., Mant. Alt. 184. 1771. *Axonopus cimicina* (L.) Beauv., Ess. Agrost. 12 .1812; Hook.f. in Hook.f., Fl. Brit. India 7:64. 1896; Panda & Das 412.2004.

F.N. - BOTG 13721

11. ***Chrysopogon fulvus*** (Spreng.) Chiov. Fl. Somala 1:327. 1929; Bor, Grass. Burma Ceylon India Pakistaan 116. 1960; Fl. Orissa IV: 2292.1996. *Pollinia fulva* Spreng. Pl. Minus Cognit. Pug. 2: 10. 1815. *Chrysopogon montanus* Trin.ex Spreng. Neue Entdeck. 2:93. 1821; Haines, Bot. Bihar &Orissa 1037(1084). 1924, pro parte; Fischer in gamble, Fl. Madras 3:1739(1205). 1934 pro parte. *Andropogon monticola* Roem. & Schult. Syst. Veg. 2:665. 1827; Hook. f. in Hook. f. Fl. Brit. India 7: 192.1896, pro parte.

F. N. - BOTG 13765

12. ***Panicum auritum*** Presl. ex Nees, Agrost. Bras. 176. 1829; Hook. f. in Hook f. Fl. Brit. India 7: 40. 1896; Haines, Bot. Bihar & Orissa 3: 996 (1042). 1924; Mooney, Suppl. Bot. Bihar & Orissa 174. 1950; Bor, Grass. Burma Ceylon India Pakistan 324. 1960; Majumdar, Bull. Bot. Soc. Bengal. 27:53. 1973. Fl. Orissa IV: 2431.1996.

F. N. - BOTG 13766

13. ***Paspalum scrobiculatum*** L., Mant. 1:29.1767; Hook. f. in Hook. f., Fl. Brit. India 7:10. 1896; Haines, Bot. Bihar & Orissa Pt. V:1000. 1924; Mooney, Suppl. Bot. Bihar & Orissa 176. 1950; Bor, Grass. Bur. Cey. Ind. & Pak. 340 1960. *P. orbiculare G.* Forster, Fl. Insul. Austr. Prodr. 7. 1786; Bor, *l.c.*340. 1960.*P. commersonii* Lam.; Tab.Ency. Meth. Bot. 1:175.1791; Bor, *l.c.*335. 1960. *P. longifolium* Roxb., Fl. Ind. 1:283. 1820; Bor, *l.c.*339. 1960; Panda & Das 435.2004.

F.N.- BOTG 13720

Many angiosperms are found to be economically important, but due to lack of adequate knowledge about this, they are destroyed indiscriminately. Hence adequate measures should be taken to conserve the germplasm of the endangered/vulnerable species. Awareness should be generated among common people regarding the importance of the flora of Motijharan and their judicious utilization.

Acknowledgement

The authors wish to thank Head of the Department of Botany and Principal, G. M. College (Autonomous) for providing laboratory facilities.

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Plant Sci. Res. Vol.27 (1&2) : 46-49, 2005

Biodegradation of lignocellulosic agro-wastes by fruiting body of *Pleurotus sajor-caju*

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Abundant availability of agro wastes cause problem of disposal and pollution. Biodegradation of these renewal wastes would solve pollution problems and also the organic wastes will be available for soil enrichment. The biodegradation of various agro and agro-industrial wastes was studied incubating with the extract of *Pleurotus sajor-caju* mushroom's fruiting body, which was grown on 50% paddy straw + 50% cashew wood powder. Results demonstrated that the enzymes present in the fruiting body help in degradation of coconut coir (between 16.5% and 31.5%) and cane sugar wastes (between 41.5% and 67%). This paper's discussion is mainly on the degradation of agro wastes by mycelium and fruiting body of mushrooms.

Key words: *Mushroom, Biodegradation*

Introduction

In nature, both edible and non-edible mushrooms are available. They grow on soil and wood. Experimentally we can grow them on different substrates like cereal straw, wood dust powder, different combinations with varied requirements of nutritional and environmental conditions (Chang and Hayes, 1978; Swamy *et al.*, 2003). Mushrooms have the capabilities to biodegrade the lignocellulosic wastes and their bioconversition to edible biomass. The plant wastes contain 25 to 35% cellulose, 15 to 25% hemicellulose, and 15 to 25% lignin. The lignin content is degraded mostly by mushroom mycellium growth phase and cellulose is degraded during frutification (Rajarathnam *et al*, 1987b; Buswell *et al.*, 1996). The capacities and capabilities of mycelium to biodegrade the substrate are enormous and multitudinal (Swamy, 2000). On the other hand, the chemical, biological, and biochemical properties of the fruiting bodies are numerous (Rajarathnam *et al*, 1998). For an understanding of biological and biochemical properties of the fruiting body, in the present study, the degradation capacity of Pleurotus *sajor-caju* mushroom's fruiting body extract was investigated.

Materials and Methods

Pleurotus sajor-caju strain was obtained from National Center for Mushroom Research and Training (NCMRT), Solan, Haryana, India. Standard methods of subculture and growth of mushroom were used (Aneja, 2001). Substrate for mushroom (*Pleurotus sajor-caju*) was prepared with 50% paddy straw + 50% cashew wood powder. Fruiting body was cut, washed with sterile distilled water, and extracted gently with sterile citrate buffer (pH 7.2) in a mortar and pestle. For degradation studies 10 gms of extract was mixed with 2 gms of material (coconut coir or cane sugar) and incubated at 25^{0}C in a Remi B.O.D. Incubator. Weight of mixture was observed at different time intervals (7 days, 14 days, 28 days and 48 days).

$$\text{Computing of dry material loss rate} = \frac{\textit{Net Wt. loss of dry material}}{\textit{Total dry Wt. of original material}} \times 100$$

Results and Discussion

In order to observe the variability of degradation capability of *Pleurotus sajor-caju*, it was grown on 50% paddy straw + 50% cashew wood powder beds.

The morphology of mushroom was with semi wrinkled edged fruiting body (Fig.1). The extract of fruiting body in citrate buffer (pH 7.2) was taken for the biodegradation experiments. The results of Biodegradation analyses of fruiting body extract of *Pleurotus sojar-caju* mushroom grown on 50% paddy straw + 50% cashew wood powder are presented in Table 1 and 2. The extract of semi wrinkled edged morphological mushroom *Pleurotus sojar-caju* (Fig.1) was mixed with agro waste fine ground pieces of coconut coir and cane sugar waste and was incubated. The data reported in Table-1 indicated increased percentage of degradation of coconut coir after incubating with the extract of fruiting body. The degradation was between 16.5% and 31.5% up to 42 days of time. However, the percentage of degradation was rapid up to 14 days, and from there it was inclined towards saturation. The increase in percentage of degradation indicated that fruiting body proteins play an important role for degradation. Intracellular enzymes data in both *Chlorophyllum molybditis* and *Cortinarius mellionlens* suggested that total amylase, alpha- amylase, proteinase, lipase, peroxidase, catalase, and polyphenol oxidase activities were increased with maturity of fruiting bodies than in very young and immature ones (Kadiri and Fasidi, 1994). All the enzymes showed greater activity in the pilei than in the stipes, which in turn may be different for different mushrooms. Like in *Chlorophyllum molybditis* and *Continarius mellionlens*, *Pleurotus sajor-caju* must be having the intracellular proteins and enzymes in fruiting body. In addition, a serine proteinase and 2-metalloendopeptidase were purified from the homogenized fruiting body of *Pleurotus ostreatus*. These two enzymes are having the amylolytic and esterolytic activities besides

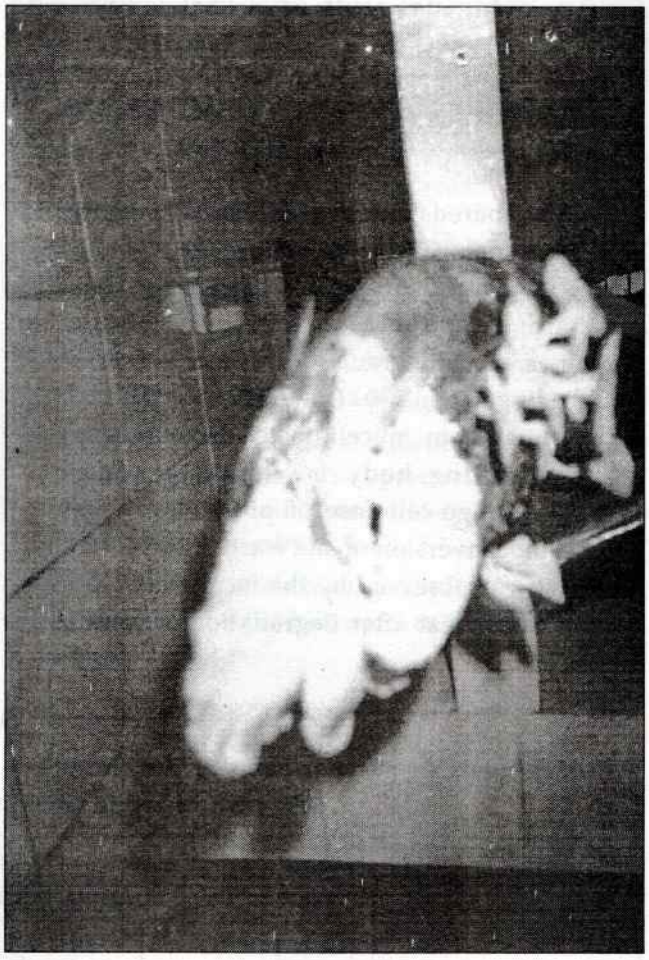

Figure-1 : *Pleurotus sajor-caju* mushroom grown on 50% paddy straw + 50% cashew wood powder

Table - 1

Biodegradation of coconut coir by the extract of *Pleurotus sajor-caju* (grown on 50% paddy straw + 50% cashew wood powder) mushroom's fruiting body

	Initial Wt.	Net Wt. Loss of dry material*/time in days				%age of Degradation/ time in days			
	(Gms.)	7	14	28	42	7	14	28	42
Material	02	-	-	-	-	-	-	-	-
Material + Buffer	12	2.90	5.07	7.90	9.93	-	-	-	-
Material + Extract	12	3.23	5.64	8.53	9.62	16.5	28.5	31.5	-

* Evaporation Factor subtracted then considered.

proteolytic activity (Dohmae *et al.*, 1995). It further may suggest presence of degradation enzymes in fruiting body, which will help in degradation of lignocellulosic agro-waste coconut coir. The same fact is supported by the results of incubation of fruiting body extract with cane sugar waste (Table-2). However, the percentage of degradation was high (41.5% to 67%) between 7 and 14 days of incubation time when compared to coconut coir.

As compared with degradation of substrate by extracellular enzymes of mycelium (data not shown), it is very unique that fruiting body plays an important role with its biological activities showing the capacities of biodegradation of agro waste, which contain lingo-cellulose. In addition to the clean up job of mushroom mycelium by decomposing agro wastes, fruiting body may further add up in degrading lingo-cellulose of plant waste, and will help in bioconversion of the waste into ***bio-manure***. This fact was observed by the farmers of Gunupur, South Orissa, that after degradation of compost by mycelial extracellular enzymes, the cut and extracted fruiting bodies mixture enhanced further degradation of compost, which in turn, became as a best ***bio-manure*** for the higher yield of vegetables.

The "biodegradation", a catabolic process of mushroom metabolism can be exploited further to reveal the mushroom biotechnology (Tripathy, 2001) for a large scale metabolic products and degradation enzymes. Research for search of different enzymes useful for degradation from fruiting body of mushroom is in progress in our laboratory. In association with chemical engineering branch by using bubble column reactor and air lift reactor, it is possible to get degradatory enzymes from different sources and to know their applications in biodegradation of agro wastes to get ***bio-manure.***

Acknowledgements

The authors express thanks to Chairman, Secretary and Principal, G.I.E.T, Gunupur, for their financial support and facilities provided for this work.

Table- 2

Biodegradation of sugar cane by the extract of *Pleurotus sajor-caju* (grown on 50% paddy straw + 50% cashew wood powder) mushroom's fruiting body

	Initial Wt.	Net Wt. Loss of dry material*/time in days				%age of Degradation/ time in days			
	(Gms.)	7	14	28	42	7	14	28	42
Material	02	-	-	-	-	-	-	-	-
Material + Buffer	12	2.12	3.83	7.16	10.50	-	-	-	-
Material + Extract	12	2.95	5.17	7.61	10.34	41.5	67.0	-	-

* Evaporation Factor subtracted then considered.

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Plant Sci. Res. Vol.27 (1&2) : 50-54, 2005

Effect of Crusher Dust and Fly Ash on the Growth Parameters of Medicinal Plants *(Artemisia nilagirica)*

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In order to evaluate the relative influence of crusher dust along, Fly ash alone and in combination with soil on growth of medicinal plant *Artemisia nilagirica*, pot culture experiments were undertaken. The results indicated that various growth parameters of the medicinal plant. *A. nilagirica* were altered by crusher dust, Fly ash and soil combinations. Plant height, number of leaves per plant, length of roots and fresh and dry herb yield were significantly more in the treatment receiving Fly ash: Soil-1:1 ratio. But Number of roots was more in the treatment receiving crusher dust : Fly ash: Soil in 1:1:1 ratio. It is thus inferred that crusher dust and Fly ash have positive agricultural value.

Key words: *Crusher dust / Fly-ash / Soil growth: parameters, Artimisia nilagirical*

Introduction

Crusher Dust is generated by grinding rocks and stones in the stone crushing units. Deposits of crusher dust has adverse effect on vegetation like decrease in chlorophyll content [Chl. A, chl. Band carotenoids], lowering of photosynthesis rate and solar heat stress with the tissues. Fly ash is generated from burning of finely powered coal in thermal power station. Due to resemblance of fly ash to soil with regards to some physical and chemical properties, it was assumed that fly ash can be used for agricultural purposes. It was decided to test the effect of crusher dust and fly-ash on the growth parameters of medicinal plant *Artemisia nilagirica.*

Materials and Methods

The plant material i.e. juvenile plants of *A. nilagirica* of 5-6cm height was collected from the garden of P.G. Department of Botany, Utkal University, Orissa. Crusher dust was collected from a stone-crushing unit of Tamando area of Khurdha district near N.H.-5, Bhubaneswar and bulk-sample of fly-ash from the ash pond of National Aluminium Company, Angul, Orissa. Garden soil was taken from the Botanical garden of P.G. Department of Botany, Vani Vihar, Orissa.

A pot culture experiment was conducted in the Botanical garden of P.G. Department of Botany with six different crusher dust, fly as and soil combinations viz. Soil alone (T_1), Fly ash alone (T_2), Crusher dust alone (T_3), Soil: Fly ash (T_4) 1:1, Soil: crusher dust (T_5) 1:1, and Soil : Fly ash: Crusher dust (T_6) 1:1:1 as the first set. The second set of experiment has five crusher dust and soil combinations Viz. Crusher dust : Soil (C_1- 1:1; C_2- 2:1, C_3- 3:1, C_4-4:1, C_5- 5:1) and five Fly ash : Soil combinations (F_1-1:1, F_2- 2:1, F_3- 3:1, F_4- 4:1, F_5- 5:1) which were replicated three time in randomized Block design.

In earthen shallow pots of 25cm diameter calculated quantities of crusher dust, fly ash and soil as per treatment were filled after thorough mixing. Each replication consisted of labeled earthen pots in which 6 plants of *A. nilagirica* were planted about equidistant from each other and irrigated regularly. The periodic growth was recorded after regular intervals and data obtained was subjected to statistical analysis.

Results and Discussion

1. Effect in height of the plant

The data given in Table-1 revealed that in case of *A. nilagirica* plant height exhibited an increase in

Table-1

Impact of Crusher dust, fly ash and their combinations with soil on herb yield of *A. nilagirica*.

Treatments	Weeks after planting				
	2	4	6	8	10
Height of plant in cm (mean)					
T_1	5.58±0.02	8.03±0.11	9.00±0.60	10.35±0.27	10.92±0.38
T_2	6.55±0.31	8.25±0.21	8.95±06	10.40±0.29	11.05±0.21
T_3	6.65±0.33	7.45±0.33	8.72±0.07	9.86±0.31	10.63±0.66
T_4	8.85±0.23	1.33±0.41	15.09±0.28	18.37±0.56	19.25±0.45
T_5	7.65±0.41	8.24±0.26	9.20±0.12	10.95±0.12	12.29±0.48
T_6	7.60±0.38	8.55±0.33	9.35±0.33	11.23±0.23	12.56±0.23
Number of leaves / plant (mean ± S.E.)					
T_1	7.75±0.34	9.25±0.49	10.50±0.41	11.32±0.12	12.10±0.09
T_2	5.75±0.10	8.87±0.35	8.90±0.09	10.25±0.15	11.79±0.06
T_3	6.25±0.11	7.25±0.38	8.03±0.06	10.32±031	11.58±0.14
T_4	6.38±0.20	9.31±0.51	13.1±0.15	15.59±0.34	16.25±0.41
T_5	6.50±0.08	8.20±0.26	8.85±0.03	9.45±0.10	10.51±0.49
T_6	6.28±0.09	8.33±0.38	9.00±0.07	9.93±0.11	10.30±0.32
Number of roots / plants (mean ± S.E.)					
T_1	11.86±0.56	13.37±0.46	16.69±0.25	19.75±0.58	21.90±0.66
T_2	15.52±0.23	18.76±0.50	23.82±0.39	26.46±0.42	29.09±0.78
T_3	10.11±0.12	12.46±0.29	15.04±0.36	18.28±0.42	21.35±0.53
T_4	11.25±0.41	159.59±0.37	23.33±0.25	26.41±0.59	28.82±0.67
T_5	13.75±0.85	17.61±0.92	18.66±0.79	25.25±0.86	30.05±1.01
T_6	14.00±0.26	22.39±0.36	26.82±0.59	28.01±1.00	33.31±1.02
Length of roots in cm (mean ± S.E.)					
T_1	6.71±0.15	9.72±0.08	11.24±0.20	12.35±0.36	13.03±0.35
T_2	5.77±0.21	8.21±0.10	11.38±0.16	14.97±0.18	15.56±0.39
T_3	4.55±0.35	8.22±0.12	9.89±0.41	11.35±0.19	12.56±0.25
T_4	7.25±0.41	9.50±0.22	13.00±0.21	15.82±0.19	19.55±0.52
T_5	8.62±0.15	10.63±0.81	12.62±0.33	14.35±0.29	16.63±0.63
T_6	8.56±0.22	9.93±0.37	11.59±0.52	13.36±0.48	15.39±0.56

C.D at 5% =

T_1- soil, T_2- fly ash, T_3- crusher dust, T_4- fly ash: soil-1:1, T_5- crusher dust: soil-1:1 T_6- soil: fly ash : crusher dust - 1:1:1.

the treatments receiving Crusher dust and Fly-ash over control treatment. Significant rise, around 76.28% in plant height was observed in treatment T_4- Fly ash: Soil - 1:1 ratio.

The mean plant height in *A. nilagirica* in different crusher dust and soil combination (C_1, C_2, C_3, C_4, C_5) was in between 5.50 ± 0.03 cm and 10.32 cm ± 0.09 (Table-2). Increase in plant height was maximum in treatment C_2 receiving crusher dust and soil in 2:2 ratio showing maximum height of 10.32 cm ± 0.09 after ten weeks. The data in Table-3 revealed that among different Fly ash: Soil combinations (F_1, F_2, F_3, F_4, F_5) maximum height (12.82 ± 0.21) of *A. nilagirica* was recorded in treatment F_3, Fly ash : Soil - 3:1 ratio. However F_2 combination showed nearly same result.

2. Effect on number of leaves / Plant

Table-1 showed that crusher dust and Fly ash did not record any significant increase in leaf number

Table-2

Impact of Crusher dust, Soil combinations on the growth parameters of *A. nilagirica*.

Treatments	Weeks after planting				
	2	4	6	8	10
Height of plant in cm (mean)					
C_1	5.50±0.03	6.31±0.02	6.95±0.01	7.35±0.02	7.98±0.10
C_2	6.73±0.02	7.84±0.04	8.72±0.03	9.85±0.08	10.32±0.09
C_3	6.08±0.03	7.38±0.09	8.51±0.21	9.18±0.06	9.94±0.18
C_4	6.39±0.01	7.01±0.04	7.48±0.01	8.12±0.15	9.02±0.05
C_5	6.55±0.02	7.63±0.03	8.88±0.14	9.10±0.03	9.95±0.05
Number of leaves / plant (mean ± S.E.)					
C_1	4.35±0.01	7.69±0.07	8.56±0.04	9.6±0.18	9.84±0.21
C_2	5.77±0.03	8.91±0.09	10.73±0.15	11.90±0.09	12.08±0.16
C_3	4.67±0.12	5.03±0.01	6.13±0.11	6.51±0.02	7.19±0.31
C_4	3.68±0.02	9.61±0.18	10.38±0.05	11.21±0.28	12.11±0.66
C_5	6.08±0.19	7.98±0.04	8.56±0.02	9.94±0.18	10.82±0.49
Number of roots / plants (mean ± S.E.)					
C_1	9.66±0.25	15.01±0.27	18.54±0.56	20.33±0.46	25.64±1.62
C_2	13.33±0.31	19.66±0.48	25.67±0.36	32.66±0.66	39.01±1.12
C_3	12.82±0.24	17.58±0.72	18.54±0.56	25.00±0.87	28.33±0.92
C_4	9.67±0.18	18.67±0.82	25.67±0.36	29.50±0.91	31.82±1.26
C_5	8.00±0.10	10.39±0.35	22.33±0.34	31.00±1.05	34.45±1.34
Length of roots in cm (mean ± S.E.)					
C_1	4.94±0.01	5.73±0.03	7.09±0.04	8.63±0.06	9.87±0.33
C_2	5.58±0.02	7.98±0.10	9.01±0.03	11.15±0.07	11.40±0.35
C_3	6.07±0.13	8.97±0.41	11.26±0.50	12.71±0.81	13.09±0.11
C_4	5.10±0.10	6.85±0.21	8.05±0.25	10.39±0.38	11.58±0.20
C_5	5.98±0.20	7.49±0.16	9.00±0.33	11.02±0.72	12.14±0.18

C.D at 5% = 2.78

C_1-Crusher dust: soil- I:1, C_2- Crusher dust :Soil-2:1, C_3- crusher dust: Soil-3:1, C_4- Crusher dust: soil-4:1, C_5- crusher dust: soil-5:1.

in *A. nilagirica* over control except treatment T_4 that exhibited an increase of 34.29% over control i.e. soil alone.

However, maximum number of leaves (12.08 ± 0.16) was obtained in the treatment C_2 (crusher dust; soil-2:1 ; and C_4 also recorded nearly same number of leaves (Table-2). Better result was obtained by the plants grown in different combinations of Fly ash and soil. Maximum were 22.50 ± 0.75 and 22.30 ± 0.82 in F2 and F3 respectively after ten weeks (Table-3).

3. Effect on number of roots / plant

In *A. nilagirica* the mean number of roots/plants was in between 10.11 ± 0.12 and 33.31 ± 1.02 (Table-1) indicating maximum in T_6 having crusher dust, Fly ash and soil in equal proportions but T_2 Fly ash alone and T_4, Fly ash; Soil - 1:1 ratio also exhibited significant increase in number of roots/plants.

Maximum number of roots/plants (39.01 ± 1.12) was recorded in C_2; C_4 and C_5 also showed good number of roots/plants, 31.82 ± 1.26 and 34.45 ± 1.34 respectively (Table-2). Table-3 revealed better

Table-3

Impact of Fly ash:, Soil combinations on the growth parameters of *A. nilagirica*

Treatments	Weeks after planting				
	2	4	6	8	10
Height of plant in cm (mean)					
F_1	6.86±0.31	7.64±0.02	8.34±0.02	8.94±0.11	9.53±0.28
F_2	7.93±0.39	9.11±0.06	9.93±0.04	0.50±0.49	11.07±0.66
F_3	8.43±0.38	9.44±0.18	10.47±0.11	11.66±0.28	12.82±0.21
F_4	6.56±0.02	7.15±0.01	7.91±0.28	8.54±0.14	9.05±0.42
F_5	6.50±0.05	7.02±0.39	7.59±0.26	8.12±0.13	8.68±0.36
Number of leaves / plant (mean ± S.E.)					
F_1	9.69±0.14	10.64±0.29	12.57±0.13	13.25±0.74	13.66±0.49
F_2	11.33±0.28	12.03±0.31	20.50±0.91	21.98±0.59	22.50±0.75
F_3	11.66±0.31	12.55±0.16	20.92±0.63	22.07±0.87	22.33±0.82
F_4	9.68±0.18	10.34±0.25	13.69±0.66	15.56±0.71	15.66±0.62
F_5	6.59±0.40	9.31±0.10	11.49±0.86	12.87±0.28	13.33±0.25
Number of roots / plants (mean ± S.E.)					
F_1	9.66±0.66	19.39±0.49	28.98±0.91	39.21±1.23	44.33±2.12
F_2	14.02±0.21	26.37±0.65	41.52±1.44	53.75±3.23	60.50±2.92
F_3	14.67±0.25	30.48±1.01	43.85±2.52	56.81±3.84	75.33±3.12
F_4	11.33±0.16	23.53±0.72	38.29±2.12	44.38±2.27	52.66±2.16
F_5	8.34±0.33	19.46±0.29	26.31±0.94	32.88±2.33	48.39±1.82
Length of roots in cm (mean ± S.E.)					
F_1	4.55±0.03	5.73±0.32	7.89±0.41	9.46±0.58	10.66±0.76
F_2	6.45±0.14	8.26±0.05	11.01±0.39	14.09±0.71	16.91±0.87
F_3	6.50±0.22	8.36±0.16	12.28±0.32	15.72±0.81	17.98±0.79
F_4	5.88±0.15	6.47±0.20	8.45±0.21	11.09±0.33	13.91±0.95
F_5	4.63±0.01	5.98±0.31	7.93±0.62	10.35±0.88	12.56±0.52

C.D at 5% = 2.78

F_1-fly ash: soil- I:1, F_2- fly ash :Soil-2:1, F_3- fly ash: Soil-3:1, F_4- fly ash: soil-4:1, F_5- fly ash: soil-5:1.

root growth of *A. nilagirica* in fly ash and soil combinations. Maximum value (75.33 ± 3.12) was obtained in F_3 with Fly ash : Soil in 3:1 ratio.

4. Effect on length of roots (cm)

Highest length of roots in *A. Nilagirica* was observed in treatment T_4 (19.55 cm ± 0.52), however T_5 and T_6 also showed significant in length of roots from planting till 10th weeks. Among the five combinations of crusher dust and soil treatment C_3 and C_5 had maximum length of roots (13.09 cm ± 0.11 and 12.4 cm ± 0.18 respectively) at tenth week of planting (Table-2). But treatment of F_3 (Fly ash: Soil - 3:1) exhibited better increase in length of root.

5. Effect on Fresh and dry herb yield

Impact of crusher dust alone, fly ash alone, soil alone and in combination on fresh and dry matter production of *A. nilagirica* is cited in Table-4. Highest values of Fresh shoot and root weight (5.230 gm ± 0.19 and 3.054 gm ± 0.05) and dry shoot weight (1.021 gm ±0.01) was obtained in T_4 (Fly ash: soil - 1:1). Similarly treatment F_3 yielded highest fresh shoot and root weight, 4.790 gm ± 0.37 and 2.410 gm ±0.27 respectively and dry shoot and root weight of 1.335 gm ± 0.16 and 0.863 gm ± 0.20. There was 69.20% increase in fresh and dry herb yield in F_3 over control (soil). However, T_5 and T_6 also showed significant fresh dry herb yield. Among the crusher dust and soil combinations, C_2 exhibited maximum fresh root and shoot weight (0.995 gm ± 0.14, 3.232 gm ± 0.13) but top dry weight was more in C_1 and C_4 (1.147 gm ± 0.06 and 1.249 gm ± 0.06).

In the present study attempt has been made to use the two pollutants, crusher dust and Fly ash for growing medicinal plants in order to minimize pollution, make the waste lands green and to develop new agro techniques. In *A. nilagirica* height of plant, number of leaves and length of roots increased significantly in T_4 Fly ash: soil - 1:1 ratio. Similar effect of Fly ash amended soil on growth of *Spinach* was reported by Rees and Sedrak[6] (1956) and Malewar[3] *et al.* (1999) and in *Bacopa monnieri* by Pradhan[5] *et al* (2002).

In *A nilagirica* F_3 (Fly ash: soil - 3:1) was best for the fresh and dry herb yield. An increase in yield off seed and dry matter was reported with application of fly ash @ 10t/ha in sunflower and cotton by Malewar[3] *et al.* (1999), in yield of crop by Matte and Kene[4] (1995). Crusher dust : Soil combination in 2:1 ratio showed maximum herb yield in *A. nilagirica*. This is perhaps due to higher percentage of Silica in Crusher dust, as uptake of Silica excretes hydroxyl ions (OH^-) which tends to raise soil pH (Selvakumari[7] *et al.*, 2000) and increase in pH increases the nutrient availability, water holding capacity of the soil.

Addition of Fly ash to soil would have facilitated better N absorption, increase in Ca, P, K, Mg, S uptake by plant due to presence of appreciable amount of these in Fly ash Kene *et al.* (1999), (Kuchanwar[2] *et al.* 1997) this mighty have enhanced growth of *A. nilagirica* in soil amended with crusher dust and Fly-ash.

Conclusion

It is thus inferred that crusher dust and Fly ash have positive agricultural value. Crusher dust and Fly ash can be used for upgrading waste lands particularly in industrial belts thereby providing relief to social and environmental hazards. These pollutants can be better used for vast cultivation of medicinal plants.

Acknowledgement

Financial support from the UGC supported SAP-DRS Programme is greatly acknowledged.

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Plant Sci. Res. Vol.27 (1&2) : 55-61, 2005

In vitro Multiplication of a Medicinal Plant *Calotropis procera.* (Ait) R.Br.

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Protocol for rapid propagation of a medicinally important plant species *Calotropis procera.* (Ait) R.Br. (Dead sea apple) through in-vitro cultures is described. Verification of the germination requirements of the seeds indicated that higher concentration of osmotically active sugar as well as high salt concentration inhibited the germination percentage as well as the vitality of the seedling. The presence of seed coat was also found to hinder germination. Callusing from leaf and stem explants were best obtained on MS medium supplemented with 1 mg/1 6-benzy adenine Purine and 2 mg/l Naphthalene Acetic Acid. Bud break and development of multiple shoots from nodal explants occurred and the optimum concentration 2 mg/l of 6-benzyl adenine Purine. Synergistic effect on the number of multiple shoot formation and shoot length was found when Gibberrelic $Acid_3$ was used along with 6-benzyl adenine Purine. By repeated sub culturing of nodal segments harvested from the newly formed axenic shoots, prolific shoot-culture, free from proximal callusing and showing a high frequency of multiplication rate were established within three month. The percentage of shoot multiplication, as well as the number of shoots per node were the highest value (100%, 9 shoots per node) during the first two culture passages; beyond this there was gradual decline in shoot bud differentiation. Rooting of the excised shoots from secondary or subsequent culture was best on half strength Murashige and Skoog's medium containing 1.5 mg/l Indole butyric acid. Vermicompost was the most suitable planting substrate for hardening. The use of vermicompost ensured high frequency of survival (96%) of regenerated plantlets prior to outdoor transfer, albeit with the use of presumed exogenous elicitors of phytoalexins in plants. The micropropagated plants were uniform and identical to the donor plants in respect of vegetative and floral morphology.

Introduction

Calotropis procera (Ait) R.Br. is a medicinal plant belonging to the family Asclepiadaceae. In English it is known as Swallow-Wort, Sodom Apple and Dead Sea Apple. In Sanskrit it is known as Arka or Alarka and in Hindi as Ak. It is an erect, tall, large and much branched perennial shrub reaching height of about 5.4 metres. It produces milky latex throughout the plant body. It has a pubescent texture and soft and corky bark. Leaves are sub-sessile, opposite and pubescent. Flowers are borne in umbellate cymes. Follicles bear ovate, flattened seeds with a silky coma. It has myriad uses since early times as a herb of profuse medicinal value. The leaves were used in Vedic times in sun worship. It is considered as a sacred plant and its flowers are offered to Lord Shiba. Ancient Arabs also used the roots religiously. Hindu physicians used the secretions from the root bark to treat skin diseases and enlargements of abdominal viscera, the roots used for cough and root bark for elephantiasis treatment. The juice is used for toothache. The plants also is used for treating leprosy, hepatic and splenic enlargements. The leaf powder is used for wound healing. Realising the immense utilitarian value of this plant, the Hindus worship the plant. It is found in most parts of the world in dry, sandy and alkaline soils and warm climates. In India, it is found mostly in wastelands and grows abundantly from Punjab and Rajasthan to Assam and Kanyakumari up to an altitude of 1050 metres.

The crude extract has fibrinolytic and anticoagulant activity on human and mouse plasma. The alcoholic extract of leaves and roots were found to have anticancerous activity against human

epidermal carcinoma of the nasopharynx. Aqueous extract has mild diuretic effect on rat. The aqueous extract contains many useful chemicals. Mudarine is one of the important components. The other chemicals are Caloropin D-I, Calotropin D-II, Calotropin F-I and Calatropin F-II.

Thus the plant is in great demand for the production of traditional and modern medicines in India. Owing to the large scale and unprohibited exploitation of this natural resource, the wild stock of this medicinal plant is being depleted. Conservation through tissue culture is being attempted because the plant is not amenable to vegetative propagation through cuttings and propagation through seeds resulting in variation.

During the last few years the application of in vitro culture of endangered medicinal plants through clonal multiplication has been considerably emphasized (Arora & Bhojwani, 1989; Sharma *et al.*, 1991; and Sudha & Seeni, 1994). Re-generation of complete plantlets in vitro from apical and axillary buds have been reported from number of medicinal species (Bajaj *et al.*, 1988 and Purohit *et al.*, 1994). The present investigation on the evaluation of various factors influencing micropropagation and rapid clonal multiplication of this important species has been carried out.

Materials and Methods

The plant materials used for tissue culture of *Calotropis procera.* (Ait). R.Br. were seeds, healthy leaves and nodal explants (0.3 to 0.7 cms.). The explants were collected from the experimental garden of the Post-Graduate Department of Botany, Utkal University. The explants were washed thoroughly under running tap water for 25 minutes. These were treated with solution of 5% (v/v) Laboline (Qualigens, India) and 7% (v/v Sodium Hypochlorite (BDH, India) for 3 minutes and there after washed 5 to 6 times with double distilled water. The explants were then surface-disinfected with 0.1% (v/v) aqueous mercury chloride solution for 5 minutes and finally rinsed with autoclaved double distilled water (5 to 7 changes).

The culture medium used for present study was Murashige & Skoog (1962) medium (MS) supplemented with 100 mg per/l (w/v) Myoinositol and 3% (w/v) sucrose. Depending on the type of explants to be used, changes were made in the MS medium by withholding some constituent or supplementing some others constituents. The pH of the medium was adjusted to 5.8 before gelling it with 0.8% (w/v) agar (Hi-Media, India). All chemicals used were analytical grade (British Drug House, Sigma, E.Merck & Hi-Media). Thirty-five millilitre of molten media was dispensed in to 200 ml screw capped glass jars (Excel Glasses Ltd. Allepey, India) or 150 ml Erlenmeyer flasks (Borosil, India). The flasks were capped with aluminum foils. The culture vessels containing the media were autoclaved at 104 KPa and 121°C for 20 minutes. The surface disinfected explants were implanted on the culture medium (4 ± 1 explants per jar/flask). All cultures were maintained at 25 ± 1°C, under 16 hr photoperiod of 35-50 μ mol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes (Philips, India) and with 60-65% relative humidity. The surface disinfected seeds were inoculated in MS medium devoid of any phyto-hormones. Some of the seeds were inoculated with seed coats intact and others with seed coats removed. The sucrose concentration in the MS medium was graded from 0% to 3% through 1% and 2% (w/v). Some seeds were inoculated in MS medium with mannose instead of sucrose. Some seeds were all so inoculated in MS medium with lower salt strengths i.e. half salt strength and quarter salt strength to observe there effects on germination of seeds. The leaf and stem (nodal) explants collected directly from the garden as well as those derived from seedlings were inoculated in MS medium fortified with 0.5 to 2mg./l. B.A.P. and 0.5 to 3mg./l. N.A.A. Subsequent sub-cultures were made in the same medium at periodic intervals of 3 weeks. The nodal explants were inoculated in 0.5 to 4 mg./l. B.A.P. $G.A._3$ (0.1 - 1 mg/l) was added to observe synergistic effect if any. The callii were inoculated on MS medium supplemented with 0.25 - 4 mg./l. 2,4 and 0.5 - 4 mg./l. B.A.P.

For root induction, the shoots of 5-7 cm height with 5 to 7 leaves were harvested from secondary

cultures and transferred to half salt strength MS (½ MS) containing 2% (w/v) Sucrose and 0.7% (w/v) agar. The medium was further supplemented with 0.25 to 2 mg/l I.A.A., I.B.A. or N.A.A. individually. The same medium was used to inoculate callii to induce them to root. After 30 days, rooted shoots were transferred to ½ MS medium without growth regulators for root elongation.

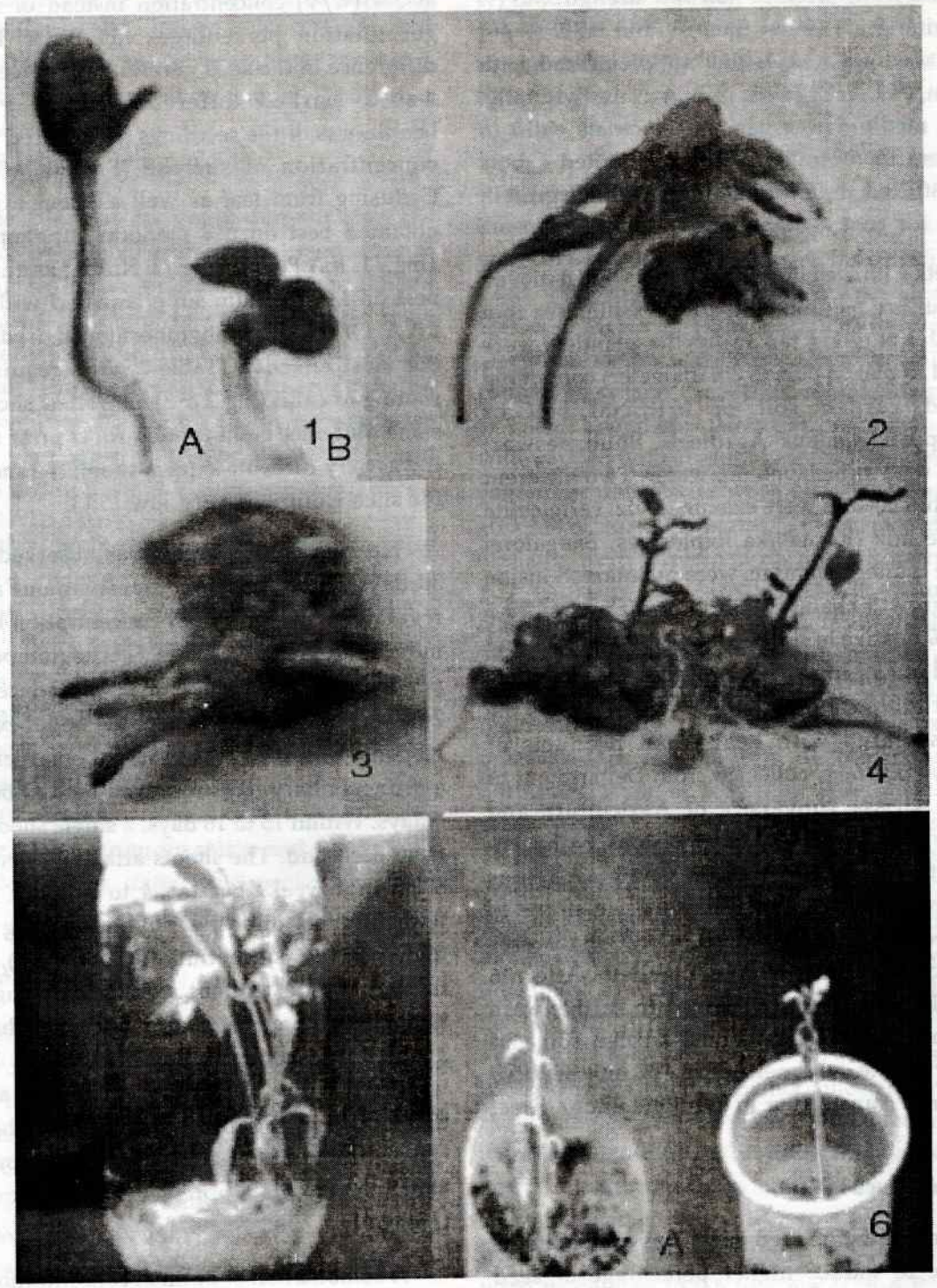
Plantlets with well developed roots were removed from the culture medium and after washing the root gently under running tap water the plantlets were transferred to plastic pots (5 cm diameter) containing autoclaved garden soil or artificial soil or vermicompost (Ranjans Agrotech, Bhubaneswar) moistened with autoclaved tap water. Two different types of artificial soils were examined viz. vermiculite and soilrite mix (Karnataka Explosives, Bangalore, India). The potted plantlets were maintained inside the plant growth chamber (SICO, India) set at 25 ± 1°C, 80-85% relative humidity and 16 hrs photoperiod of 50 μ Mol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes (Philips, India). After 30 days the plantlets smeared with heat-killed products of *Phytophthora infestans*, collected from Department of Pathology, Orissa University of Agriculture and Technology, Bhubaneswar. The innocuous, pathogen-derived extracts was smeared on young as well as fully expanded leaves and internodes in order to test the effectiveness of the extract to prevent diseases in the nascent, culture-derived plantlets. After 36 days the plantlets were transferred to earthenware pots (15 cm diameter) containing garden soil and kept under shade in a net house for another two weeks prior to transferring to outdoors under direct sunlight.

Results and Discussion

The removal of the seed coats was an inevitable prerequisite to induce higher percentage of seed germination. The percentage seed germination with intact seed coats was merely 5%. The germination percentage on MS medium with 3% (w/v) sucrose was 25% for coatless seeds. On reducing the concentration of sucrose to 2%, 1% and 0% (w/v), the seed germination percentage increased to 51%, 79% and 100% respectively. When mannose was used at 3% (w/v) concentration instead of sucrose, the germination percentage was 100%. Besides the difference in the seed germination percentage, there was a marked difference in the vitality and healthiness of the seedlings germinated on different concentration of sucrose (Fig.1 A, B & Fig 7). Callusing from leaf as well as stem explants were obtained best on MS medium supplemented with 1mg./1. B.A.P. and 2mg./l. N.A.A. Rooting occurred best on MS medium supplemented with 1.5 mg./l. I.B.A. Of the three phytohormones used, I.B.A. was the most effective (Table-2). The requirements for rooting of callii (Fig 2 & 3) as well as shoots was the same. But once callii formed roots prior to shooting, it was very difficult to form shoot; the shooting rates for such callii was very low (5%).

No sign of bud break was observed even after 30 days on MS basal medium without any growth regulators. The optimum concentration of B.A.P. to induce but break was 2mg./l. The number of shoots per explant increased and B.A.P. concentration of 2mg./l. induced with 79% of the nodal explants. The response observed was in initial enlargement of the existing axillary buds followed by bud break in 7 to 9 days. Within 15 to 16 days, a single shoot elongated from each bud. The shoots attained a length of 5 to 6 cm in 4 weeks bearing 4 to 8 nodes, each node with bifoliar, opposite phyllotaxy (Fig 4 & 5). At higher B.A.P. concentration (5.0 mg./l.), the shoots formed rosette without significant elongation. The bud break percentage also declined sharply after about B.A.P. concentration of 2 mg./l. When GA_3 was used with MS medium, there was a mere bud break of 14 to 18% in 18 to 25 days at the optimum concentration of GA_3 (0.3 mg/l). GA_3 concentration above 0.3 mg./l. suppressed bud break completely (Table 1).

A combination of optimal concentration of B.A.P. (2 mg/l) and GA_3 (0.3 mg/l) in the culture medium, lowered the time of bud break to 4 days and increased the bud break percentage to 95%. It also had a synergistic effect on shoot length (7-9 cm) by enhancing internode length. K.I.N. (2 mg./l.) and G.A_3 (0.3 mg./l.) also exhibited synergistic effect, although this effect was lower than BAP.-GA_3



Figs. 1-6

Fig.1 A-Seed germinated on sucrose-free medium. B-seed germinated on 3% sucrose (w/v) medium

Fig.2 & Fig.3 - Rooting of Callus

Fig.4 - Multiple Shooting of Callus

Fig.5 - An in vitro raised plantlet with a well-developed root system.

Fig.6 - A - Plantlet after treatment with Phytoalexin elicitors & B-Plantlet without elicitor treatment

synergy. Of the four substrates used, vermicompost was found to be the most favourable with a survival of 96% (Table-3).

Even after successful acclimatization, the plants after transfer outdoors were found to be highly susceptible to diseases. Thus, the final survival declined. The plants treated with heat-killed *Phytophthora infestans* extracts survived and did not contract any disease until flowering. On the other hand untreated plants fell prey to some debilitating disease (nature not studied elaborately) (Fig.6 A, B).

The seed germination shows that *Calotropis procera* (Ait). R.Br. has hardwired its physiology to its ecological conditions and needs. The delay in germination caused by the presence of the seed coat and the high solute concentration (high negative water potential) in the vicinity of the seed ensures that adequate amount of water was present in the soil to enable a completion of its life cycle. These are in consonance with the arid and semi-arid locales that the plant inhabits.

The stimulatory effect of a singular supplement of B.A.P. on bud break and multiple shoot formation in *Calotropis procera* was similar to that reported earlier in other medicinal plant species including *Curcuma longa* and *Zingiber officinale* (Balachandran *et al.*, 1990), *Chlorophytum borivilianum* (Purohit *et*

Table-1

Morphogenic Response of Nodal Explants of *Calotropis procera* to different concentrations of Cytokinins (Kin, BAP) and/or GA_3

Growth Regulator (mg/1)	% Shoot Development	Shoot Number Explant	Shoot Length (cm)
0.0			
KIN			
0.25	27.86 ± 1.76	1.00 ± 0.00	1.23 ± 0.07
0.5	44.47 ± 1.32	1.17 ± 0.11	1.76 ± 0.04
1.0	58.16 ± 1.44	1.50 ± 0.13	1.71 ± 0.03
2.0	61.87 ± 1.11	1.62 ± 0.12	2.32 ± 0.07
5.0	39.44 ± 1.46	1.35 ± 0.06	b
BAP			
0.25	41.10 ± 1.47	1.45 ± 0.11	1.32 ± 0.11
0.5	67.48 ± 1.22	1.87 ± 0.18	2.17 ± 0.15
1.0	84.34 ± 1.71	4.22 ± 0.11	3.16 ± 0.18
2.0	76.46 ± 1.79	3.14 ± 0.11	2.20 ± 0.08
5.0	30.14 ± 1.43	1.11 ± 0.01	b
KIN + GA_3			
2.0 + 0.2	68.37 ± 1.25	2.18 ± 0.07	4.16 ± 0.12
2.0 + 0.3	76.54 ± 1.33	2.85 ± 0.10	5.27 ± 0.18
2.0 + 0.4	72.60 ± 1.45	2.72 ± 0.13	5.88 ± 0.17
BAP + GA_3			
2.0 + 0.2	85.40 ± 1.25	3.28 ± 0.13	5.44 ± 0.13
2.0 + 0.3	93.87 ± 1.73	4.45 ± 0.12	6.16 ± 0.14
2.0 + 0.4	91.64 ± 1.64	4.27 ± 0.11	6.86 ± 0.37

a) Data (X ± SE) collected after 30 d from three independent experiments each with 15 replicates; b) Shoot length could not be measured due to resettle form of shoot clumps.

al., 1994) and *Ocimum basilicum*. (Sahoo *et al.*, 1997). Our observations on the suppression of sprouting at higher B.A.P. concentrations were in consonance with those of Ahuja *et al.* (1982) in *Ocimum gratissimum* and *O. viride*. In another species of *Ocimum* viz. *O. basilicum*, although the nodal segments required the presence of B.A.P. at 2.0 mg./l. at the initial stage of bud break for their further growth and proliferation demanded transfer to a medium containing BAP at a relatively low concentration (0.25 mg./l.) (Sahoo *et al.*, 1997). In the present investigation, a B.A.P.-GA_3 coupling had a noticeable synergistic influence on multiple shoot formation. This promotive effect has been reported earlier in other perennial medicinal herbs including *Saussurea lappa* (Arora & Bhojwani, 1989), *Ocimum americanum, O. sanctum* and *O. basillicum)* (Sahoo *et al.*, 1997). On the contrary, GA_3 has been shown to suppress shoot bud differentiation in *Plumbago indica* (Nitsch, K. & Nitsch, J.P.,1967). Thus, the role of gibberellic acid with respect to shoot bud induction in medicinal plant species remains controversial.

Table-2

Influence of different Auxins on Rhizogenesis of invitro formed shoots of *Calotropis procera*

Auxins (mg/l)	Rooting (%)	Roots/Shoot	Root Length (cm)
Zero Auxin	-	-	-
IAA			
0.5	-	-	-
1.0	16.50 ± 1.11	1.11 ± 0.09	1.50 ± 0.16
2.0	36.00 ± 1.50	1.51 ± 0.17	2.51 ± 0.10
NAA			
0.5	bc	-	-
1.0	bc	-	-
2.0	51.47 ± 1.54	5.41 ± 0.06	5.55 ± 0.17
IBA			
0.5	60.11 ± 1.17	5.23 ± 0.09	4.04 ± 0.14
1.0	93.17 ± 1.54	7.20 ± 0.15	5.11 ± 0.19
1.5	97.11 ± 1.13	8.01 ± 0.19	7.10 ± 0.13
2.0	77.11 ± 1.22	6.34 ± 0.15	4.90 ± 0.11

a) Data (X ± SE) collected after 30 d from three independent experiments each with 15 replicates; bc. Basal callusing; - No root formation.

Table-3

Evaluation of different planting substrates for acclimatization of invitro shoots

Planting Substrate	No. of Plants Transferred	No. of Plants Survived	Survival (%)	Shoot Height (cm)	Leaves/ Plantlet	Root Length (cm)
Vermiculite	50	32	64	5.90 + 1.11	5 + 2	7.11 + 0.11
Soilrite mix	50	41	81	10.48 + 1.71	10 + 2	6.17 + 0.14
Vermicompost	50	48	96	16.90 + 1.81	21 + 2	10.12 + 0.18
Garden soil	50	31	61	7.11 + 1.45	9 + 2	9.03 + 0.12

(Data (X + SE) recorded after 30 d of culture on planting substrates.

After successful acclimatization, the survival of plants was hindered by some disease attack. The disease has not been characterised but, an attempt was made to provide resistance to the plants. This was attempted at by trying to harness the potential of plants to produce Phytoalexins. Phytoalexins, one of the important group of secondary metabolites produced naturally due to the effect of some exogenous factors. They are known to provide broad-spectrum resistance to fungal pathogens. The production of Phytoalexins needs an exogenous elicitor in the form of wound, virus or inactivated pathogen. This is presumed to trigger an endogenous elicitor. The endogenous elicitor in most cases is a fully mature cell wall product. The endogenous elicitor stimulates Shikimic acid pathway or Mevalonic acid pathway to produce phenols or terpenoids which act as Phytoalexins.

In the present work, the products, left after *Phytophthora infestans,* was killed by autoclaving, were smeared on young and mature parts of separate plants. Those plants treated on mature parts were found to gain resistance to any further disease attacks. The untreated plants, as well as those whose young parts were smeared with the innocuous pathogen - product, were found to be still susceptible to diseases. This observation brings to light the role of Phytoalexin elicitation in offering disease resistance to tissue culture - derived plants and strengthen the process of hardening.

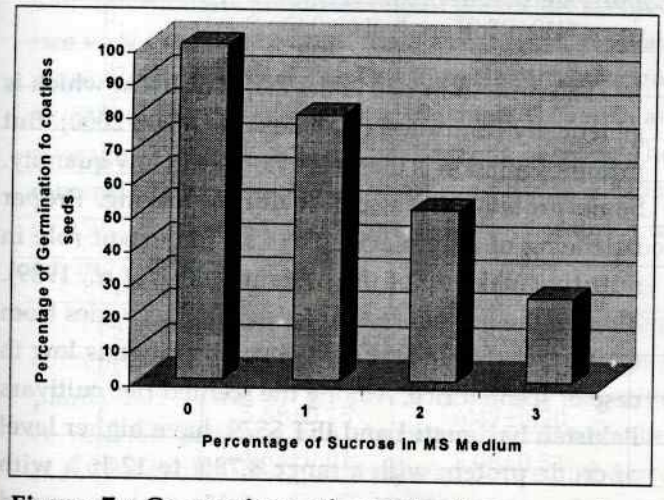


Figure-7 : Comparison of germination percentage of coatless seeds.

In conclusion, the present investigation has resulted in a protocol, which could be used for ex situ conservation and true-to-type mass propagation of this herb of immense pharmaceutical importance.

Acknowledgement

The authors are thankful to UGC for funding in the form of DRS and UGC fellowship to the first author. We are also thankful to Department of Science and Technology, Government of India for providing this FIST programme to the Department of Botany, Utkal University for providing facility to conduct the experiment.

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Plant Sci. Res. Vol.27 (1&2) : 62-65, 2005

Biochemical Composition of Scented Rice

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The Biochemical composition of twenty scented rice varieties were analysed. The crude protein content varied from 8.78% to 12.16% and soluble protein 4.35% to 6.68% on dry weight basis. The soluble protein fraction albumin globulin, prolamine and glutelin were present in proportion of 13:15:6:66 of the total extracted protein. Significant variations were observed in proteins, total soluble sugars, amylose, amylopectin and mineral content among the scented rice cultivars. A positive correlation was observed between grain crude protein content and grain yield and negative correlation between crude protein and glutelin.

Key words : *Scented rice, crude protein, starch grain yield*

Scented rice are highly preferred for its scent, cooking characteristics and palatability in international market. The scented rice shares a majour portion of total agricultural export and earns a good premium of foreign exchange. The export potential of scented rice mainly depends upon the quality of rice which is directly influenced by its chemical composition. Carbohydrate is the major component and next to it is protein. So an attempt has been made to evaluate chemical composition of some scented rice varieties and to identify superior parents which can be used in the cross breeding programme for transfering such quality traits to high yielding variety in order to improve the nutritional quality along with yield in scented rice.

MATERIALS AND METHODS

Twenty scented rice varieties were grown at Central Rice Research Institute Cuttack in rabi season 2004. All package of practices were taken in crop growth period with uniform land as well as crop management condition. The grains were dehusked manually. After defatting the grains in petrolium benzene for two hours the samples were washed in distilled water and dried in oven at 40°C for 24 hours. The samples were ground in a Willy mill to pass through 60 mesh sieve. The crude protein content was determined by using Micro kjeldahl method (AOAC 1965). Soluble protein was extracted by using the method of Singh, *et al.* (1978) and estimated as per the method of Lowry, *et al.* (1951). Protein fractions were extracted as per the method of Osborne (1924) modified by Gloria, *et al.* (1966) and protein content of different solubility fractions were estimated (Lowry *et al.*, 1951). Total soluble sugar was determined by using Anthrone method (Yem and Wills, 1954) and reducing sugar by Miller (1959), starch content was estimated as per the method of Chopra and Konwar (1976), amylose by method of Sowbhagya and Bhattacharya (1979), phasphorous content by Fiske and Subbarow (1925), iron by Wong (1928) and calcium by Flame photometer. All the extractions were carried out in duplicate and means were taken for analysis.

The protein in rice is the major nutrient which is genetically controlled (Matsue and Ogata, 2000). But majour limitation is that it is available in low quantity. Some proteins are also lost during milling. Proper balancing of amino acid plays an important role in nutritive make up of the protein (Sikka, *et al.*, 1989). Though the protein range of rice varieties varies from 5% to 17% (Juliano, *et al.*, 1968), this range is low in case of scented rice. Among the scented rice cultivars Pakistan basumati I and IET 8579 have higher level of crude protein with a range 8.78% to 12.16% with average of 10.48%. The total soluble protein ranged from 4.35% to 6.68% with a mean 5.34%. The average

glutelin is 65.84%, then albumin have 13.05%, glutelin 15.42% and prolamine have 5.67% of total extracted protein (Table 1). The higher levels of soluble protein are more digestable than other protein. Among the four soluble protein fractions glutelin is the most abundant. Storage protein have more lysine and metheonine content in rice seed and is nutritionaly better protein. Albumin content is next to glutelin. The prolamine is nutritionaly poor protein due to low lysine content and with low biological value (Lasztity, 1984). Therefore it is essential to improve the nutrionally superior cented rice and to evolve higher grain yield with higher glutelin and low prolamine content.

In addition to starch rice contents small quantity of soluble carbohydrate which is generally known as total soluble sugar. It includes both reducing and non reducing sugar. The total soluble sugar of the twenty scented rice variety ranged from 0.465% (CRM 8-30) to 0.875% (Musk budhi) with a mean value of 0.65%. The reducing sugar ranged from 0.102% to 0.168% with a mean 0.136%. The starch contain varied from 65.55% to 79.0% with a mean 73.25%. The photosynthetic activities of different stages of growth and translocation and utilization of phatosynthetic products effects the starch content (Singh, 1989).

The amylose content of the scented rice varieties ranged from 16.68% (Basmati 370) to 22.42%(Pakistan basmati-1) with an average of 18.78% of total starch extracted indicating its intermediate class. Scent along with intermidiate amylose content makes the scented rice vary valuable because these rice does not spread more during cooking. The amylopectine varied from 80.20% (Gyanbhog) to 83.32% (Basmati 370) with an average value 81.20% of total starch. Among the mineral content studied phosporous range 0.220% to 0.362% calcium 0.015% to 0.030% and iron 1.5 to 4.8 mg/100 gm^{-1}.

The correlation study revealed that crude protein content is positively correlated with grain yield (gm/plant) but Taira, *et al.* (1973) reported negative correlation between two characters but Watanabe, *et al.* (2004) obtained no correlation between the two characters. A negative correlation was observed between glutelin % and crude protein content but a contrasting result was obtained by Kandai and Borah (1994) and Lasztity (1984). Glutelin % was negatively correlated with startch content which was supported by Novoa and Loomis (1984) because the glutelin contents mostly lycin and synthesis of lycin requires more amount of energy which is obtained from sugar thus it limits the synthesis of startch.

From the above analysis we conclude that the scented rice are nutritionally poor due to low protein content. The scented rice can be hybridised with higher protein content rice varieties in order to get nutritionally better exportable scented rice along with grain yield.

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Table-1

Biochemical components of scented rice varieties (on dry weight basis)

	Grain crude protein (%) (%N×5.95)	Soluble protein (%)	Protein fractions (% of extracted proteins)				Total soluble Sugar (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Starch (%)	Amylose (%)	Amylo Pectin (%)	Phospo-rous (%)	Calcium (%)	Iron (mg100 gm^{-1})	Grain yield/ Plant (gm)
			Albumin	Globulin	Pralone	Glutelin										
CRM 8-30	9.15	4.54	11.26	13.25	5.25	70.24	0.465	0.124	0.341	72.65	18.24	81.76	0.325	0.018	1.5	9.97
IET 2057	8.78	4.73	12.65	14.53	5.63	67.19	0.542	0.152	0.390	76.32	17.07	82.93	0.315	0.016	1.8	9.27
HKR 226	10.25	5.25	13.25	15.68	6.26	64.81	0.686	0.143	0.543	74.55	17.47	82.53	0.275	0.021	2.5	8.28
HKR 238	11.05	4.68	14.16	16.65	5.86	63.33	0.752	0.102	0.650	68.36	18.24	81.76	0.220	0.015	2.6	7.46
HKR 241	11.95	5.54	12.68	15.36	4.95	67.01	0.862	0.125	0.737	67.50	18.05	81.95	0.245	0.022	3.1	10.12
IET 113480	9.65	6.15	11.96	13.26	5.26	69.52	0.523	0.142	0.381	65.55	19.59	80.41	0.315	0.019	2.8	8.76
IET 12016	10.28	5.35	11.68	14.15	6.13	68.04	0.498	0.124	0.374	72.36	19.48	80.52	0.282	0.025	1.6	10.35
IET 8579	12.03	4.46	12.15	14.26	6.43	67.16	0.613	0.136	0.477	68.53	18.46	81.54	0.310	0.030	1.8	12.72
Ksturi	11.95	5.55	14.83	16.87	5.63	62.67	0.525	0.156	0.369	73.26	17.17	82.83	0.278	0.018	2.9	14.39
Basmati 370	10.48	4.35	15.16	18.05	4.90	61.89	0.638	0.162	0.476	77.53	16.68	83.32	0.240	0.016	3.2	12.76
Basmati 385	9.68	5.68	12.78	14.26	5.16	67.80	0.751	0.151	0.600	76.55	19.32	80.68	0.245	0.021	4.6	10.43
Pusa Basmati 1	10.63	6.35	13.59	15.78	6.13	64.50	0.653	0.108	0.545	68.26	22.42	77.58	0.275	0.015	4.2	8.89
Pakistan Basmati1	12.16	4.38	14.68	16.25	5.44	63.63	0.558	0.132	0.426	78.36	19.47	80.53	0.312	0.030	3.2	6.14
Karnal local	10.95	4.96	12.35	15.85	4.98	66.82	0.657	0.125	0.532	77.26	19.44	80.56	0.344	0.024	2.5	8.65
Kalimochi	8.90	5.76	14.45	18.01	6.15	61.39	0.768	0.112	0.656	66.35	21.28	78.72	0.298	0.021	1.9	10.15
Gourav	9.18	6.43	11.89	14.25	5.62	68.24	0.840	0.121	0.719	71.25	17.38	82.62	0.362	0.018	3.1	12.00
Basmati Bahar	10.65	6.56	12.52	15.26	6.00	66.22	0.635	0.148	0.487	76.85	17.07	82.43	0.271	0.016	2.9	11.89
Musk budhi	11.65	6.68	13.15	16.25	6.32	64.28	0.875	0.152	0.723	79.00	18.68	81.32	0.302	0.019	3.6	12.79
Laxmi bhog	10.53	4.55	12.85	15.86	6.03	65.26	0.653	0.142	0.511	78.25	20.23	79.77	0.285	0.024	3.8	8.84
Gyan bhog	9.68	4.76	12.96	14.65	5.42	66.97	0.612	0.168	0.444	76.32	19.80	80.20	0.309	0.022	4.8	10.89
Mean	10.479	5.335	13.05	15.424	5.677	65.848	0.655	0.136	0.519	73.253	18.777	81.198	0.290	0.021	2.92	10.238
+- S.E.	0.121	0.081	0.124	0.153	0.055	0.271	0.132	0.002	0.014	0.489	0.167	0.164	0.004	0.001	0.001	0.230

Table-2
Correlation of component characters in scented rice

Crude protein/Grain yield	0.103
Crude protein/Glutelin	-0.342
Glutelin/Starch	-0.109

Monteitn and Colin Webb (Eds) Martinus Nijhoff Dr. N. Jonk Pub. pp. 191-197.

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Plant Sci. Res. Vol.27 (1&2) : 66-70, 2005

Effects of ionic and chelated Cr(III) complexes on mung bean seedlings during early phases of plant growth

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The present study showed relatively less toxic effect of chromium (Cr^{+3}) (with and without chelating agents) during early phases of seedling growth as compared to Cr(VI) observed in earlier studies. The effect of trivalent chromium (Cr^{+3}) was found to alter the growth parameters of growing mung bean seedlings. Growth parameters like root length decreased markedly with increase in Cr^{+3} concentrations but changes in shoot length was unaffected at higher concentrations. Among the seedlings treated with chelating agents, the root length was found maximum in Cr^{+3} (10μM)-EDTA (10μM) and the shoot length elongation was maximum in Cr^{+3} (10μM) treated seedlings. Similarly from all six types of treatment, Cr^{+3} (10μM)-EDTA (10μM) was found to induce maximum root fresh weight as compared to less shoot fresh weight. Among all the treatments, maximum dry matter of shoot was found in control and minimum in Cr^{+3} (10μM)-EDTA (10μM) treatment. The dry matter of the roots of the seedling was found highest grown under Cr^{+3} (10μM)-EDDHA(10μM) and minimum in Cr^{+3} (100μM) treatments. The total chlorophyll content of the seedlings was found minimum under Cr^{+3} (100μM) where it was maximum in the seedlings of control. The catalase activity was maximum both is roots and shoots in Cr^{+3} (10μM)-DTPA (10μM) treated seedlings and minimum in Cr^{+3} (100μM).

Key Words: *Chromium (III), Chelating agents, Chlorophyll, Catalase and mung bean seedlings*

Introduction

Chromium (Cr) is the seventh most abundant element on earth (Katz and Salem, 1994). It is a transition metal and placed in group VI-B of the periodic table. It occurs in several oxidation states ranging from Cr^{-2} to Cr^{6+}, with the trivalent, Cr(III) and the hexavalent, Cr(VI) states being the most stable and common in terrestrial environments. The hexavalent form of the metal, Cr(VI) is considered a potent, extremely toxic, carcinogen than the relatively innocuous and less toxic Cr(III) form (Panda and Patra, 1997; WHO, 1988). Cr(VI), as chromate permeates the cell membrane through the sulfate transport channel, where as Cr(III) complexes enter the cell membrane through passive transport and it form complexes with several legands and thereby alters the physiological functions (Debetto and Luciani, 1988). Very little informations have available from the work of different researchers regarding physiological responses of plants to Cr(III) (Zayed and Terry, 2003; Patra *et al.*, 2004, Sayed *et al.*, 2005). The paper is designed to compare the effect of Cr(III) on growth parameters, chlorophyll and catalase activity in presence or absence of chelating agents. Another recent avenue in the discovery of modern science is that certain chelating agents like EDTA, DTPA in soil amendments greatly facilitate Cr uptake by soil-grown plants (Jena *et al.*, 2004).

Materials and Methods

Graded dry seeds of mung bean (*Vigna radiata* L. Wilczek. Var k-851) were obtained from 'National Seed Corporation', Bhubaneswar. The seeds were stored in dark and cool place for experimental use. Uniform seeds were selected and surface sterilized by soaking in 0.1% mercuric chloride ($HgCl_2$) solution for about five minutes and then were washed several times with tap water followed by distilled water. The surface sterilized seeds were placed in sterilized petriplates over saturated cotton pads for

germination. The seeds were germinated at 25^{0}C in darkness for two days. Different chelated chromium compounds *i.e.*, Cr^{+3} (10μM)-EDTA (10μM), Cr^{+3} (10μM)-DTPA (10μM), Cr^{+3} (10μM)-EDDHA (10μM) were prepared each in equimolar ratio.

After two days of germination, the healthy seeds were transferred to well-aerated half strength Hoagland nutrient solution (without chromium) in glass culture vessels. Treatment with different concentrations of free chromium and chelated chromium compounds in other culture vessels were also run side by side. The seedlings were grown in growth chamber with 12 hr light period. The white light was provided by filtered cool white fluorescence tubes (36W Philips TLD) with a photon flux density of 52μE m^{-2} s^{-1} (PAR). The nutrient culture solution of the growing mung bean seedlings were changed everyday with freshly prepared solution. Five days old seedlings were harvested for measurement of root and shoot length (cms). Fresh and dry matter content of both control and treated samples were measured using electronic balance. They dry matter of the seedlings were taken by keeping the seedlings in oven at 80^{0}C for a period of three days or more till constant dry weights were determined.

The growth parameters like root length, shoot length, fresh matter and dry matter of five days old mung bean seedlings were used for study. Different Cr^{+3} concentrations (10μM, 100μM) without and with chelating agents such as EDTA, DTPA, EDDHA were used during growth parameter study.

Chlorophyll content was extracted by using the methods of Nadler *et al.*, (1972) with slight modifications. A control was run simultaneously in which the enzyme activity was stopped at zero time. One unit of catalase activity was calculated as that amount of enzyme which breaks down one micro (μ) mol of H_2O_2/min under the assay condition (Patra *et al.*, 1978).

The data presented in the figures and table are mean of three independent experiments. The data were analyzed statistically and standard errors of mean (SEM) were given.

Results

Treatment of different chromium (III) concentrations (10μM, 100μM) and chelating agents (EDTA, DTPA, EDDHA) showed marked changes in the different growth parameters of five days old mung bean seedlings (Table 1). A decrease in root length was seen in chromium treated plants growing in different chelating agents and the order was EDTA>EDDHA> DTPA and it was clear that the root length decreased at higher chromium concentration *i.e.*, at 100μM. The shoot length markedly decreased in the seedlings supplemented with EDTA, DTPA and EDDHA as compared to control. The shoot length elongation was found maximum in Cr^{+3} (10μM) treated seedlings and minimum in Cr^{+3} (10μM)-DTPA (10μM) treatment (Figure 1).

A marked decrease in root fresh weight was observed in the seedlings treated with Cr^{+3}(100μM) as compared to all other treated seedlings and maximum root fresh weight was found in Cr^{+3}(10μM)-EDTA (10μM). A marked decrease in shoot fresh matter was observed in the seedlings treated with Cr^{+3}(10μM)-EDTA (10μM) as compared to other chromium treated seedlings. The shoot fresh mater was found to be increased with increase in chromium concentration from 10μM to 100μM (Table 1). However, the control seedlings had the highest shoot fresh matter values as compared to other treated seedlings.

The dry matter of the roots of the seedlings were found to be highest grown in Cr^{+3}(10μM)-EDDHA(10μM) treated seedlings and minimum in Cr^{+3}(100μM). The dry matter of roots of the seedlings was found to decrease with increase in chromium concentration from 10μM to 100μM. However, the reverse values were found in shoots *i.e.*, the dry matter of shoots of the seedlings was found to increase with increase in chromium concentration from 10μM to 100μM. Maximum dry weight of shoot was found in control and minimum in Cr^{+3} (10μM)-EDTA (10μM) treated seedlings (Fig. 2).

An increase in chlorophyll content was also noted in Cr^{+3} treated plants growing in presence or absence

of chelating agents. The total chlorophyll content of the seedlings was found to be minimum under Cr^{+3}(100μM) where as the total chlorophyll was maximum in the seedlings treated with chelating agent *i.e.*, at Cr^{+3} (10μM)-DTPA (10μM) (Fig. 3). Catalase activity was found stimulatory in all seedlings treated with chelated complexes but it had lower activity in the seedlings treated with higher concentration of chromium *i.e.* at 100μM (Fig.4).

Table-1

Effects of Cr(III) and chelating agents on root length, shoot length, fresh weight, and dry weight of 5-days old mung bean seedlings grown in hydroponic condition

Growth Paratmeters	Control	Cr(III) Treatments (μM)		Cr(III) 10mM + Chelating agents (10mM) (1:1)		
		10	100	Cr^{+3}-EDTA	Cr^{+3}-DTPA	Cr^{+3}-EDDHA
Root Length (cm)	10.9 ± 0.088	8.66 ± 0.141	5.42 ± 0.060	12.61 ± 0.180	10.52 ± 0.292	10.98 ± 0.195
Shoot Length (cm)	18.6 ± 0.115	20.64 ± 0.178	20.12 ± 0.216	17.9 ± 0.291	16.42 ± 0.064	18.1 ± 0.120
Root Fresh Weight (mg)	423 ± 1.154	363 ± 1.155	322 ± 2.645	487 ± 1.855	410 ± 2.604	425 ± 0.881
Shoot Fresh Weight (mg)	267 ± 2.081	224 ± 0.881	248 ± 0.882	204 ± 0.577	263 ± 0.578	222 ± 0.823
Root Dry Weight (mg)	12 ± 0.057	12.3 ± 0.176	11 ± 1.202	13 ± 0.881	14 ± 0.578	15 ± 0.577
Shoot Dry Weight (mg)	162 ± 0.881	151 ± 1.155	158 ± 1.203	122 ± 1.856	160 ± 1.452	124 ± 0.882

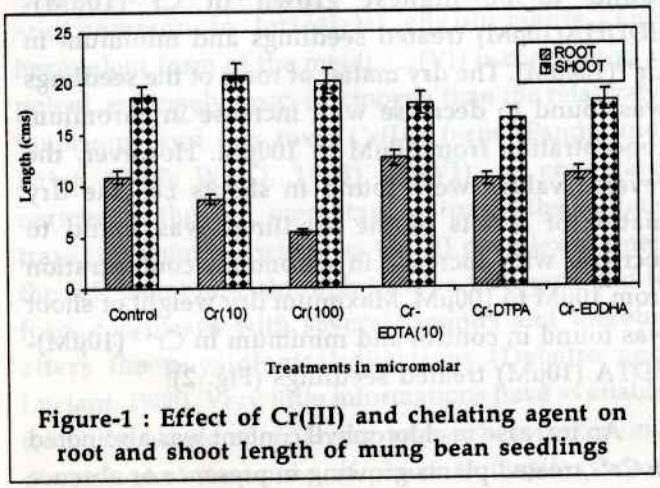

Figure-1 : Effect of Cr(III) and chelating agent on root and shoot length of mung bean seedlings

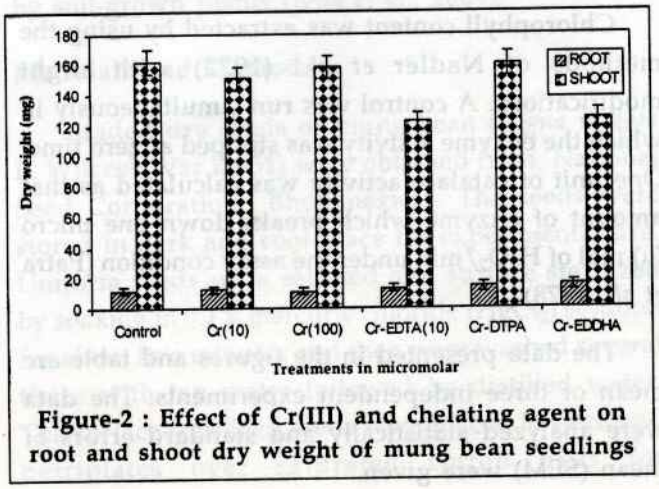

Figure-2 : Effect of Cr(III) and chelating agent on root and shoot dry weight of mung bean seedlings

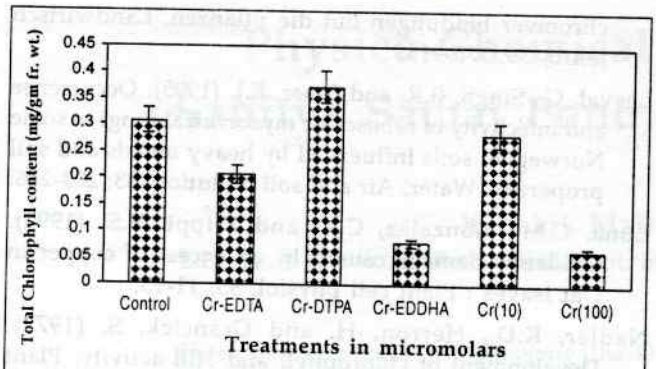

Figure-3 : Effect of Cr(III) and chelating agent on total chlorophyll content of mung bean seedlings

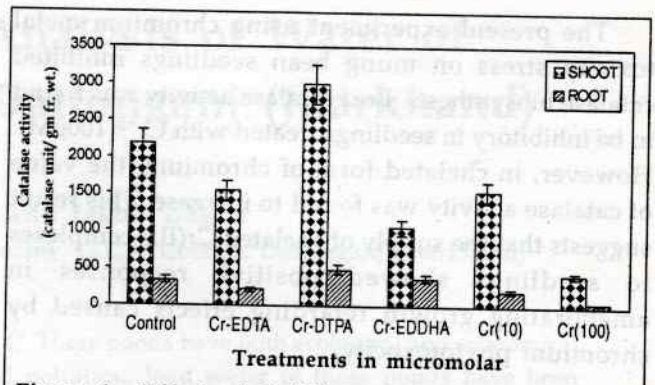

Figure-4 : Effect of Cr(III) and chelating agents on catalase activity of shoot and root mung bean seedlings

Discussion

Among all types of stresses which a plant face, heavy metal stress is the least studied phenomenon. Chromium, one of the toxic heavy metal exists in two forms *i.e.*, Cr(III) and Cr(VI) and, Cr(VI) is more toxic to plants than Cr(III) (Zayed and Terry, 2003). Hauschild (1993) examined chromium toxicity in barley and rape plants as affected by chromium studied in hydroponic condition. He found that the chromium accumulation caused root growth reduction, reduced shoot growth and leaf chlorosis.

The present study revealed some of the less toxic effects of trivalent chromium (Cr^{+3}) on the growth and development of mung bean seedlings. The effects of interactions of Cr(III) with different chelating agents in the developing seedlings were studied.

Although growth stimulating effects by the addition of the lower concentrations of chromium were found in presence or absence of chelating agents, the increased yield have also been observed (Bonet *et al.*, 1991 and Terry, 1981).

Chromium (III) was found to alter the growth parameters of developing mung bean seedlings growing with or without of chelating agents being supplemented with nutrient culture. An increase in the root length was observed in the seedlings grown under chelated Cr(III) but a reverse trend of shoot growth was observed. There was an increase in shoot length of the seedlings grown in the medium containing chromium without chelating agents i.e., in Cr^{+3}(10 μM) and Cr^{+3}(100μM). The reason might be due to the induction of some of the biosynthetic enzymes or some of the phytohormones by the trivalent metal cation, which resulted in increased seedling growth. However, a decrease in both fresh and dry matter of root was seen in chromium treated seedlings alone. But, among all the treatments (without and with chelating agents), Cr^{+3}(10μM)-DTPA(10μM) treated seedlings had showed highest value of shoot fresh weight and dry weight except control. It might be due to the joint action of chelating agent and chromium interference at hormonal level. Leyval *et al.*, (1995) and Turnau (1998) reported that low toxicity chelating agent such as EDTA are used to enhance the bio-availability of heavy metals for bioaccumulation.

From all the treatments, the total chlorophyll content was observed maximum in the presence of chelating agent such as Cr^{+3}(10μM)-DTPA(10μM) which might be due to the inhibition of chlorophyllase activity of lower Cr(III) level. The increase in Chl-a, Chl-b and total chlorophyll content in nitrate supplied seedlings treated with Cr(VI) was also observed in wheat seedlings (Panda and Patra, 2000a). Panda and Patra (2000b) also observed similar results in exised wheat leaves treated with Cr(III). Jena and Patra (2004) also observed high chlorophyll biosynthesis at higher concentration of Cr(VI) supply (at 100μM) which had resulted stunted growth of the seedlings. The Cr(III) might be stimulating some of the steps of chlorophyll biosynthesis and retarding senescence of the primary leaves of five days old mung bean seedlings. Similar type of result have also obtained with Cr^{2+} and Zn^{2+} ions by earlier workers (Luna *et al.*, 1994; Nagalakshmi and Prasad, 1998).

The present experiment using chromium metal toxicity stress on mung bean seedlings inhibited catalase biosynthesis. Leaf catalase activity was found to be inhibitory in seedlings treated with Cr^{+3}(100μM). However, in chelated form of chromium, the value of catalase activity was found to increase. This result suggests that the supply of chelated Cr(III) complexes to seedlings showed positive responses in ameliorating growth retarding effects caused by chromium phytotoxocity.

Acknowledgements

The authors are thankful to the Head, P.G. Department of Botany, Utkal University, Bhubaneswar for providing necessary laboratory facilities to undertake this investigation. The financial assistance from UGC-DRS(SAP) programme is greatly acknowledged.

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Plant Sci. Res. Vol.27 (1&2) : 71-75, 2005

Physico-Chemical Analysis of Water of Sahitya Samaj Pond, Daltonganj (Jharkhand)

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There are many ponds in Daltonjang (Jharkhand). These ponds have both ecological and economical importance. But due to population explosion and pollution, load water of these ponds have been contaminated. The present study deals with the physico-chemical characteristics of one of the important ponds of Daltonganj, namely Sahitya Samaj Pond during a period of 12 months, 2002-03. The results indicated degradation of water quality which might affect public health.

Key Words: *Physico-Chemical, water, ecosystem, pond, water quality.*

Introduction

Importance of Ponds has been established in view of ecology and economy of a society. Every pond is a typical example of fresh water ecosystem. They have distinct functional and structural identity. Salient features of ponds are area specific and are influenced very much by local environmental factors. The abiotic or non-living parts of a fresh water pond include the water, dissolved oxygen, CO_2, inorganic salts such as phosphates, nitrates and chlorides of Na, K and Ca and a multitude of organic compounds such as amino acids, humic acids, etc. The biotic or living parts of fresh water pond can be sub-divided according to the function of the organisms, i.e. what they contribute towards keeping the ecosystem operating as a stable, interacting whole. Moreover urban water bodies are also subject to pollution from the discharge of industrial effluents, domestic sewages and run-off water from agricultural field, that contain inorganic fertilizer and pesticides. As most of the water bodies are located in and around the villages and township, the water quality should be maintained at the level of satisfaction. The accumulation of pollutants may have deleterious effects not only on plants life but also on human life.

Chaudhari (1987) studied the physico-chemical factors in a fresh water pond of Darbhanga, similar studies have been made by Dudani (1987) in fishpond of Darbhanga, Saha (1987) in a perennial pond of Bhagalpur, Singh *et al.* (1991) in a lake of Muzaffarpur and Singh and Ahmad (1990) in a pond and river water at Patna, respectively. Other significant contributions pertaining to the physico-chemical characteristics of water of inland lakes, ponds and reservoirs have been made by Gulati (1980), Jana (1980), Michael (1980), Jampani (1987), Chatterjee *et al.* (2003) in Asansol, Jena *et al.* (2003) in Sunderbans and Sivakumar *et al.* (2003) in Coimbatore district in Tamil Nadu. Work to improve water quality through photosynthetic bacteria has been carried out by Dwivedi *et al.* (2003).

Materials and Methods

Water samples were collected from the 5 different sites monthly from March 2002 to February 2003. Water samples were collected in washed and dried polythene bottle of 1 liter. The parameters like temperature of water and air, light penetration, pH, DO and free CO_2 were detected on the spot where as other parameters were analyzed in the laboratory. The physico-chemical analyses were done following the standard methods of A.P.H.A. (1985). Finally the numerical calculation of collected data was carried out in computer to establish different statistical relationship.

Results and Discussion

The details of the physico-chemical characteristics of water of Sahitya Samaj Pond have been presented

in Table-1. Data obtained from the investigation show that the water temperature is lower than air temperature in the months during March to November where as it is reverse during December to February. Ganapati (1973), Hussainy (1967), Sreenivasan (1970) and Singh (1991) also observed similar results while studying different freshwater bodies. The higher temperature values were observed from April to September. This may be possibly due to the highlight intensity and longer photoperiod. The lower values of water temperature were observed during winter season, which could be attributed to the effect of cold wind and low light intensity. Similar findings were also reported by George (1964) and

Table-1

Variations in Physico-Chemical Characteristics of Water

Months→ Parameters ↓	Mar 02	Apr 02	May 02	June 02	July 02	Aug 02	Sept 02	Oct 02	Nov 02	Dec 02	Jan 03	Feb 03
Air Temp.	26	32	36	39	36	34	32	30	25	16	11	14
Water Temp.	20	27	29	30	28	27	24	22	20	19	18	19
T.D.S.	430 ±.77	410 ±.45	410 ±.55	500 ±.60	610 ±.73	725 ±1.5	620 ±.95	590 ±.66	510 ±.45	470 ±1.2	425 ±.44	480 ±.52
Turbidity	18.5 ±.86	18.5 ±.46	15.4 ±.71	16.2 ±.49	16.8 ±.58	17.4 ±.50	17.9 ±.86	18.5 ±2.3	16.2 ±.81	16.0 ±1.5	14.8 ±.55	14.5 ±.74
pH	7.2 ±.77	7.4 ±.44	7.3 ±.81	7.6 ±1.3	7.5 ±.75	7.9 ±1.2	8.2 ±1.3	8.1 ±2.3	7.6 ±.45	7.5 ±1.6	7.2 ±.66	7.4 ±.63
D.O.	9.6 ±.55	8.2 ±.75	7.4 ±1.4	4.8 ±.81	4.2 ±.55	3.6 ±.75	3.0 ±.75	4.6 ±.55	5.8 ±.55	7.2 ±.99	11.4 ±.41	11.8 ±.50
B.O.D.	11.6 ±.16	12 ±.67	12.7 ±.86	13.1 ±.52	13.7 ±.41	13.4 ±.44	12.6 ±.46	11.6 ±.63	11.2 ±.74	10.9 ±.63	11 ±.86	11.4 ±.55
C.O.D.	96 ±.22	97 ±.77	98 ±.16	92 ±.22	87 ±.63	88 ±.19	92 ±.44	93 ±.44	93 ±.15	95 ±.12	96 ±.74	96 ±.12
Chloride	256 ±.63	385 ±.88	392 ±1.6	402 ±.66	424 ±.43	380 ±.55	267 ±.56	245 ±.71	253 ±.45	236 ±.76	206 ±.42	245 ±.47
Calcium	168 ±1.5	174 ±1.2	179 ±.45	182 ±.71	145 ±.71	129 ±.60	100 ±.79	156 ±.61	160 ±.61	163 ±2.1	165 ±1.1	142 ±.80
Nitrate	0.10 ±.56	0.13 ±.95	0.20 ±.05	0.36 ±.50	0.31 ±.66	0.36 ±1.9	0.35 ±.52	0.18 ±.72	0.08 ±.64	0.08 ±.74	0.05 ±.75	0.20 ±.66
Phosphate	0.042 ±.74	0.047 ±2.2	0.048 ±.47	0.051 ±1.9	0.038 ±.47	0.038 43	0.039 ±.66	0.040 ±.36	0.040 ±.40	0.039 ±.52	0.041 ±.64	0.041 ±.51
Sulphate	18.21 ±.49	20.72 ±.74	21.11 ±.59	22.14 ±.70	18.71 ±.49	13.21 ±.77	14.74 ±.48	15.95 49	15.94 ±.66	16.21 ±.60	16.89 ±.33	17.32 ±.74
Carbonate	16.8 ±.47	17.2 ±.41	18.6 ±.46	21.6 ±3.3	24.6 ±.71	25.2 ±.85	25.0 ±1.6	22.6 ±.66	23.4 ±.50	20.2 ±.69	18.5 ±.79	21.2 ±.70
Bi-carbonate	123.2 ±.50	118.6 ±.55	114.2 ±.48	100.4 ±.65	91.7 ±.41	82.2 ±.61	62.1 ±.60	70.4 ±1.7	68.2 ±.81	90.4 ±.85	111.7 ±2.0	100.1 ±.43

Temperature = 0C
Turbidity = JTU
Light penetration = c.m
Others except pH = ppm

Verma (1967). The analysis reveals a minimum value of transparency and higher value of total dissolve solids during monsoon season. This might be due to increase in the suspended colloidal organic matter and detritus. Similar observations were also reported by George (1966) and Singh (1991). The pH of water of the pond was alkaline throughout the year. It was low during monsoon, which could be attributed to the surplus amount of free CO_2 found in water. The pH values were high during winter. This might be due to the dense population of Phytoplankton. The photosynthetic process of these aquatic plants removed CO_2 from the water during day time thereby increasing DO and pH. Thus the high pH value in the pond water was the indication of greater photosynthetic activities and dominance over the respiratory activities of heterotrophic organisms. Increase in pH by photosynthesis was also reported by Wong (1969). The study depicts that concentration of DO was low during summer. This could be attributable to the speedy decay of organic matter due to high temperature. Similar results were observed by Michael (1973) and Bohra (1977). During monsoon there observed a decrease in bicarbonate value. This could be attributed to the rainfall, which dilute the concentration of bicarbonates. Pearsall (1930) and Zafar (1966) had reported that pH of watger depends on relative quantity of Calcium carbonate and bi-carbonate. In the present study a direct co-relation has been observed between the carbonate content and pH and a reverse co-relation between pH and bi-carbonate. It is evident from the present study that there is high value of Chloride, Calcium, Nitrate, Phosphate and Sulphate during summer. This might be due to increased rate of decomposition of organic matter due to high temperature and concentrated sewage discharge from its surrounding settlement. Many earlier workers have also reported similar findings like Hussainy (1967), Munawar (1970), Mohanty (1981) and Singh (1991).

Table-2 shows the Coefficient of Correlation values for two combinations of different physico-chemical parameters. The two parameters were considered as two variables as X and Y and coefficient of correlations (r) were determined following Karl Pearsons's method.

$$r = \Sigma dx.\, dy / \sqrt{\Sigma dx^2 . \Sigma dy^2}$$

Correlation of Coefficients of some physico-chemical parameters of river Thungabhadra have

Table-2

Correlation Coefficient of different parameters

	Temperature	Turbidity	pH	Chloride	D.O.
T.D.S.	.85 ±.10	.65 ±.05	.77 ±.08	.58 ±.07	.63 ±.02
Calcium	(-) .63 ±.05	.78 ±.05	(-) .70 ±.10	.52 ±.04	.55 ±.04
Nitrate	.45 ±.10	.55 ±.05	.65 ±.02	.63 ±.05	.55 ±.08
Phosphate	(-) .82 ±.03	.60 ±.02	(-) .70 ±.04	.78 ±.02	.65 ±.07
Sulphate	.65 ±.04	.70 ±.02	.56 ±.05	.79 ±.02	.67 ±.10
Carbonate	.82 ±.07	.85 ±.05	.58 ±.01	.52 ±.10	.69 ±.08
Bi-carbonate	.66 ±.05	.76 ±.05	.51 ±.04	.74 ±.04	.80 ±.10

been carried out by Arvinda *et al.* (1998) and significant linear relationship among some water quality parameters were observed. Gupta *et al.* (2001) obtained positive correlation values due to brick industry pollution on tropical deciduous plants. Statistical analysis in various designs was done by Snedecor and Cochran (1971) and Mishra and Mishra (1988). Linear correlations coefficient values among physico chemical parameters of fresh water Malyanta Pond at Laxmisagar has been done by Verma and Mohanty (1995).

Positive correlation has been observed in the study among all most all the parameters except that of Calcium & Temperature, pH & Calcium, Phosphate & Temperature and Phosphate & pH. Positive correlation of pH was observed with chloride, dissolved oxygen and conductivity by Verma and Mohanty (1995). Ali *et al.* (1983) observed positive correlation of pH with conductivity and chlorides. Freiser and Fernando (1966) reported positive correlation of pH with alkalinity. Mohanty (1981) had reported negative correlation of pH with calcium in some water bodies of Bhubaneswar. Atkin and Harris (1924) have reported negative correlations of pH with magnesium and calcium. The negative correlation was probable due to loss of some Ca^{2+} and/or Mg^{2+} in to the sediments in the form of precipitate resulting in low Ca^{2+} and Mg^{2+} content at extremely high pH (Atkin and Harris, 1924). Freiser and Fernando (1966) had reported positive correlation between pH calcium and pH alkalinity. Muni and Mohanty (1985) oberved positive correlation between temperature and dissolved oxygen in Vani Vihar lake.

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Plant Sci. Res. Vol.27 (1&2) : 76-80, 2005

Changes of Chlorophyll Fluorescence and CO_2 Photo-assimilation in Rice Cultivars during Submergence and Subsequent Re-aeration

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The effect of submergence on chlorophyll a fluorescence and CO_2 photo-assimilation rate was compared in four *Indica* rice (*O. sativa* L.) cultivars namely, FR13A and Kalaputia (tolerant) and IR42 and Sarala (susceptible). Twenty one days old seedlings were completely submerged for 8 days. Chlorophyll a fluorescence, CO_2 photo-assimilation rate and chlorophyll content were measured in 3 different treatments viz. 8 days' submergence, 24 hour after re-aeration along with air grown control plants. Tolerant cultivars maintained higher chlorophyll content vis-a-vis photosynthetic activities during submergence and re-aeration. Quantification and de-convolution of chlorophyll fluorescence transient into several phenomenological and bio-physical parameters (JIP test) revealed that there were large cultivar differences in the response of PS II to submergence. Among them potential quantum yield of primary photochemistry (Fv/Fm), area above the fluorescence curve between Fo and Fm, performance index (Pi), electron transport per cross section (ETo/CS), reaction center per cross section (RC/CS) reduced due to submergence and on subsequent re-aeration for 24 h. The decrease was more in the susceptible cultivars (e.g. IR42 and Sarala) compared to the tolerant (e.g. FR13A and Kalaputia) cultivars.

Key words: *Rice, chlorophyll fluorescence, photosynthesis, submergence tolerance*

Introduction

Out of 42 biotic and abiotic stresses listed for rain fed low land rice areas submergence considered the third most important limitation to rice production Hossain and Laborte (1996). Complete submergence of low land rice crops occurs during flash flood in wide areas of south-east Asia resulting in an increase mortality and low grain yield. Factors influencing rice plant survival during submergence are limitation to gas diffusion (deficiency of oxygen and carbon dioxide) under water, reduced irradiance that cause several visible injuries damages photosynthetic apparatus and impair photosynthesis Sarkar *et al.* (2001).

The photosynthetic apparatus especially PS II is well known to be very sensitive to different stresses. Chlorophyll a fluorescence is corresponding only a small fraction of dissipated energy from the photosynthetic apparatus is widely accepted to study its structure and function Stresser and Tsimilli-Michael (2001). The photochemistry of PS II has been extensively studied using chlorophyll fluorescence emission and a sensitive indicator of photosynthetic energy conversion Pereira *et al.* (2000). A dark adapted leaf shows a characteristic change in the chlorophyll fluorescence intensity known as Kautsky effect. This Kautsky effect has become one of the most important tools in applied and basic photosynthetic research. Based on it Stresser and Stresser (1995) developed a test known as O-J-I-P or JIP-test to know the structural and functional characteristics of photosynthetic apparatus. The analysis of fluorescence transient according to the JIP test leads to the calculation of several phenomenological and biophysical expressions Stresser and Stresser (1995). In the present investigation we employed this approach which has

not been used so far in rice under submergence stress.

The main goal of the current investigation was to assess the impact of submergence and subsequent re-aeration on chlorophyll fluorescence and CO_2 photo assimilation in rice cultivars differing in submergence tolerance.

Materials and methods

Plant material and growth conditions

The experiment was conducted on four *Indica* rice cultivars [*Oryza sativa* (L.)] namely, FR 13A & Kalaputia (tolerant to submergence) and IR 42 & Sarala (susceptible to submergence). All the cultivars were sown directly in earthen pots containing two kg of farm soil and farmyard manure in a 3:1 ratio. Each pot was supplied with 80mg urea, 192mg single super phosphate (P_2O_5) and 70mg murate of potash (K_2O). Ten days after germination, seedlings were thinned and five plants per pot were maintained. Twenty one-day-old seedlings were completely submerged for 8 days in a concrete tank under 110cm depth of water. Measurements of different parameters were taken in different treatment such as under 8 days complete submergence (S), 24 h after submergence (R) and in non-submerged controlled plants (C).

Carbon dioxide photo-assimilation and chlorophyll

Measurements of CO_2 photo-assimilation were made on the fully expanded leaves of five different plants within 30 minutes at the end of submergence treatment using an open system photosynthetic gas analyzer (PP Systems, USA) under normal environmental conditions. The second and third leaf from the top was selected and kept inside the chamber under natural irradiance until stable reading was documented.

For chlorophyll estimation, 100mg of finely chopped fresh leaves were placed in a 25ml capped measuring tube containing 25ml of 80% acetone, and kept inside a refrigerator (4°C) for 28 h (Sarkar, 1998). The chlorophyll was measured spectrophotometrically following Porra (2002).

Chlorophyll fluorescence

After measuring the CO_2 photo-assimilation rate, the same leaves were used for the measurement of chlorophyll fluorescence using a Plant Efficiency Analyzer, Handy PEA (Hansatech Instruments Ltd., Norfolk, UK) and recorded from 10 µs up to 1 s, with a data acquisition every 10 µs for the first 300 µs, then 100 µs up to 3 ms and after that 1 ms. The signal resolution was 12 bits (0 to 4000). For each treatment, the Chl a fluorescence transients of 12 individual leaves were measured. All measurements were done on fully dark-adapted attached leaves. From the first O-J-I-P transients, several bioenergetic parameters were derived according to the equations of the J-I-P test using the program BIOLYSER (R. M. Rodriguez, Bioenergetic Laboratory, University of Geneva; available at http://www.unige.ch/sciences/biologie/bioen).

Results and Discussion

The potential photochemical efficiency of PS II was estimated by Fv/Fm ratio was higher under control conditions (Fig. 1A). Submergence decreased the Fv/Fm ratio. During re-aeration, Fv/Fm ratio did not change much compared to the submerged conditions. The tolerant cultivar FR 13A and Kalaputia maintain higher values of it during submergence and 24 h of re-aeration than the susceptible cultivar. Decrease of this parameter is a reliable sign of photo inhibition Krause and Weis (1991) and it indicated the structural damage of PS II Waldhoff, *et al.* (2002).

The area above the fluorescence curve between minimal (Fo) and maximal (Fm) fluorescence is proportional to the pool size of the electron acceptor Q_A on reducing side of PS II was highly sensitive to submergence (Fig. 1B). The fall in area was maximum in Sarala (77%), followed by IR 42 (75%), FR 13 A (68%) and Kalaputia (49%) compared to the respective control values. The decrease of this value was due to the fall in the concentration of acting electron acceptor of PS II and highly suitable for ranking the genotypes to submergence tolerance. Comparison of Fv/Fm and area suggests that area is more sensitive to submergence than the former. The performance index (Pi) calculated based on the overall utilization

of the fallen light energy decreased on the imposition of submergence in all the cultivars. The rate of decrease was maximum in Sarala followed by IR 42. In Kalaputia, the Pi was more or less same under control, submerged and re-aeration periods, whereas the increase in the values of Pi during re-aeration was greater in FR 13A compared to the other cultivars (Fig. 1C).

The phenomenological energy fluxes per excited cross section (CS) give ideas how plant is capable to absorb the fallen energy and efficient enough to transport this energy towards photosynthesis. The phenomenological energy fluxes like the electron transport (ET/CS) was decreased under submergence in all the cultivars but this decrease was more in IR 42 and Sarala than FR 13A and Kalaputia (Fig. 1D).

The active reaction centre (RC/CS) was more or less same in control conditions among the cultivars but under submergence the number of active RCs decreased greatly especially in susceptible cultivars (Fig. 2A). During re-aeration the number of RCs further decreased both in susceptible and tolerant cultivars, yet the tolerant cultivars maintained higher number of it compared to the susceptible cultivars. The tolerant cultivars maintained higher chlorophyll content than susceptible cultivars both under submergence and air-adapted conditions (Fig. 2B). Submergence decreased the chlorophyll content Sarkar, *et al.* (1996). In general the degradation of chlorophyll was more in susceptible cultivars (60-69%) than tolerant cultivars (11-29%). On subsequent re-aeration for 24 h, chlorophyll content further decreased both in tolerant and susceptible cultivars but the tolerant cultivars maintained higher quantities of it compared to the susceptible cultivars, resulted better maintenance of PS II as evident by different chlorophyll fluorescence parameters (Fig. 1 and 2).

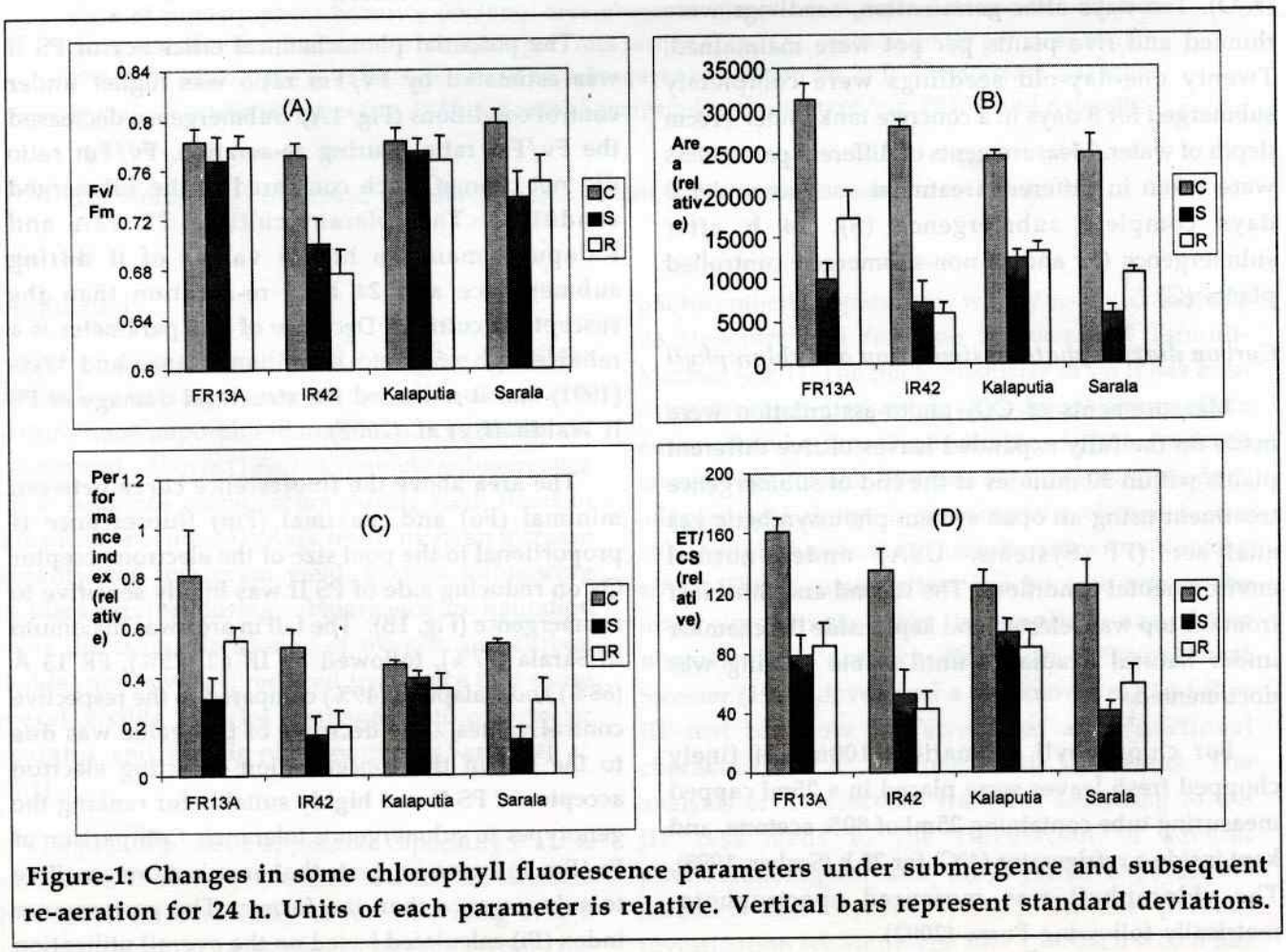

Figure-1: Changes in some chlorophyll fluorescence parameters under submergence and subsequent re-aeration for 24 h. Units of each parameter is relative. Vertical bars represent standard deviations.

Under control conditions the CO_2 photo-assimilation rate was more in the cv. Sarala (Fig.2C). Submergence decreased the CO_2 photo-assimilation. The decrease in photosynthetic activities was maximum in Sarala (99%), followed by IR 42 (95%), Kalaputia (82%) and FR 13A (75%). Photosynthetic activities have shown to be significantly inhibited in flooding intolerant plant (Mustroph and Albrecht, 2003). After going to air significant increase of photosynthetic activity was observed in the cv. Kalaputia compared to the submerged conditions. After 24 h of air-adaptation the photosynthetic

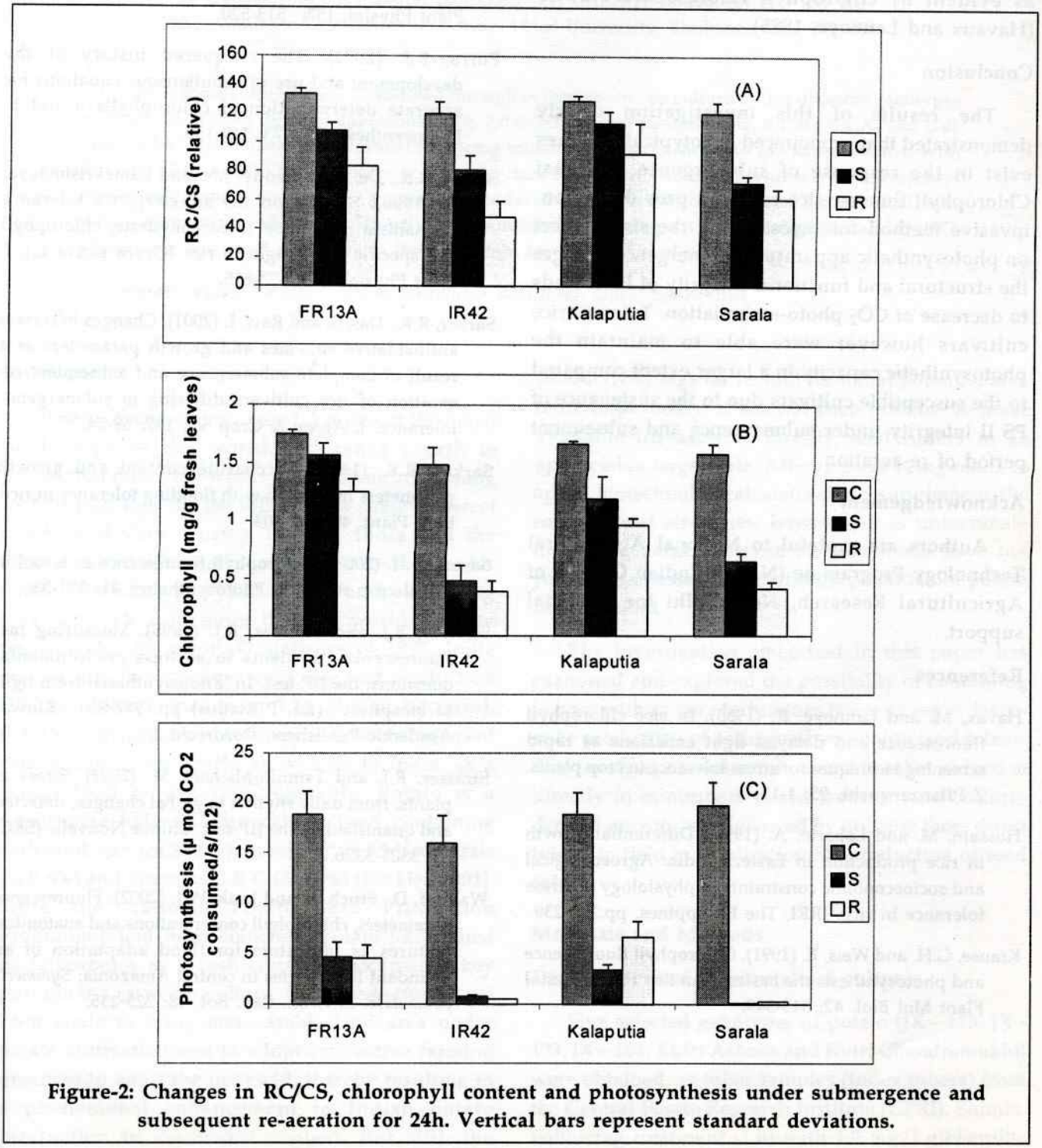

Figure-2: Changes in RC/CS, chlorophyll content and photosynthesis under submergence and subsequent re-aeration for 24h. Vertical bars represent standard deviations.

activities were more in Kalaputia (6.7 μ mol CO_2 s^{-1} m^{-2}), followed by FR 13A (4.6 μ mol CO_2 s^{-1} m^{-2}). In susceptible cultivars the photosynthetic activity was almost absent. One of the reasons for the decrease of photosynthesis under submergence was probably due to the damage to the photosynthetic apparatus as evident by chlorophyll fluorescence studies (Havaux and Lannoye, 1985).

Conclusion

The results of this investigation clearly demonstrated that pronounced genotypic differences exist in the response of submergence. The fast Chlorophyll fluorescence transient provides a non-invasive method for investigating the stress effect on photosynthetic apparatus. Submergence changes the structural and functional integrity of PS II leads to decrease of CO_2 photo-assimilation. Tolerant rice cultivars however were able to maintain the photosynthetic capacity in a larger extent compared to the susceptible cultivars due to the sustenance of PS II integrity under submergence and subsequent period of re-aeration.

Acknowledgement

Authors are grateful to National Agricultural Technology Programme (NATP), Indian Council of Agricultural Research, New Delhi for financial support.

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Plant Sci. Res. Vol.27 (1&2) : 81-88, 2005

Direct *Ex vitro* Tuber Formation in Minitubers of Potato (*Solanum tuberosum* L.) Raised through *in vitro* Culture

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Axenic shoot cultures were established through *in vitro* sprout-tip culture of five different genotypes of potato in MS salt medium augmented with 2.0 mg/l calcium pantothenate, 0.1 mg/l GA_3 and 0.01 mg/l NAA. Microtubers were formed following transfer of *in vitro* shoots to MS supplemented with 8-10 mg/l BA and 50-120 g/l grocery sugar under dark incubation. Plants which grew from microtubers in fertilized natural soil inside a glasshouse produced minitubers within 95 days. Several small size tubers were developed *ex vitro* directly from minitubers following a 3 week storage in dark at 18^{0}C. These were harvested and sown in the open field for production of seed tubers.

Key words : *Potato, In vitro culture, Microtuber, Minituber, Direct tuberization*

Introduction

Potato (*Solanum tuberosum* L.) is one of the major food crops of the world and ranks fourth in production following wheat, rice and maize. Among root crops it tops the list followed by cassava, sweet potato and yam. During 1961-97, India had the highest record of annual compound growth rates for potato production and yield in the world, amounting to 19 and 2.6 fold more than its world average respectively. As per FAO statistics of 1998, India ranked fifth in potato growing area and production (19.24 million tonnes from 1.14 million hectares). Potato is prized as a culinary classic being used extensively in the world as well as in India, as a staple food or a major vegetable. Potato is a wholesome balanced low-calorie food containing carbohydrates (*ca.*22.6%), protein (*ca.*1.6%), minerals (*ca.*0.6%) and vitamins B & C (*ca.*17%) (Ezekiel, 2001). Since the National Potato Seed Production Programme had been launched, potato has gained its importance as a cash crop and occupied a key position in Indian agriculture. Sustained efforts have been made to bring more arable land area under potato cultivation and to adopt innovative farming practices to boost the net yield, thereby resulting in a phenomenal enhancement in Indian potato production in the world context. But still, the productivity lags far behind its actual potential and, more importantly, it has not been possible to make available disease-free quality seed tubers in an appreciably large scale. Attempts are being made to apply biotechnological methods to supplement the conventional strategies; however, it is unfortunate that the general merits of such methods have not yet been harnessed to their fullest potential for potato improvement.

The investigation embodied in this paper has examined and explored the possibility of combining *in vitro* with *ex vitro* techniques towards rapid large-scale production of pathogen-free quality seed tubers. This involved a novel *ex vitro* tuber-to-tuber induction directly in minitubers raised from *in vitro* culture-derived microtubers, followed by utilizing these direct tubers in field multiplication for production of seed tubers.

Materials and Methods

Development of *in vitro* shoot cultures

Five selected genotypes of potato (JX - 115, JX - 123, JX - 161, Kufri Ashoka and Kufri Chandramukhi) were obtained as tuber samples (Index tubers) from the Central Potato Research Institute (CPRI), Shimla. Following treatment (1 h) with 1.0 mg/l gibberellic

acid (GA_3) + 1.0% thiourea solution and storage in diffused light to break dormancy, tubers were sprouted and sprouts proliferated for 5-8 days. Sprout-tips (0.4 -0.6 mm) were excised, disinfected first with 70% ethyl alcohol (30 s) and then in 0.1% mercuric chloride solution (4 min). Explants were thoroughly rinsed with autoclaved distilled water (5 changes) and each inoculated in a culture tube (30 ml, Borosil, India) containing 10 ml agar (8.0 g/l) - gelled Murashige and Skoog (1962) medium (MS) containing 3% sucrose and augmented with various concentrations and combinations of growth regulators. The cotton-plugged culture tubes were incubated at 24 ± 1^{0}C under 16 h photoperiod regime with a photon flux density of 50 µmol m^{-2} s^{-1}.

In vitro microtuberization and harvest of minitubers

Single nodal segments (ca. 10 mm) from 40 day-old *in vitro* shoots were placed on Whatman paper bridge projecting out of partial immersion in 60 ml culture tubes (Borosil, India) each containing 15 ml liquid MS medium with supplements as above. After 20 days the medium was gently decanted off and replaced by liquid tuber-inducing medium composed of MS salts augmented with BA (2.0 - 10.0 mg/l) and grocery sugar (20 -150 g/l). Cultures were incubated in dark at 18^{0}C. After the microtubers matured following 2.5 months of culture, the culture tubes were exposed to artificial light and temperature conditions (as described above) for greening of the microtubers. Green microtubers were sown in earthenware pots (3-5 microtubers per 30 cm diam pot) containing fertilized soil (75% soil + 25% FYM + N : P : K :: 60 : 80 : 100 kg/ha). Following germination, plants were allowed to grow further inside the glasshouse for producing minitubers.

Direct tuberization in minitubers

Minitubers were harvested, treated in 0.2%, solution of Dethane M-45 for surface-disinfection and stored in incubator at 18^{0}C in dark for a period of 3-4 weeks. Small size tubers which were initiated after 20 days were harvested after 2 months.

Experimental design and Statistical analysis

The experiments with five genotypes in three replications were conducted in a randomized block design (RBD). Each genotype was represented 4 times in a replication with two plants per culture tube. Different biometrical observations viz. number of tubers/kg of mother tubers, yield (g)/kg of mother tubers, % shoot emergence and seed tuber yield (kg)/kg of direct tubers.

Field multiplication using direct tubers for production of seed tubers

Experimental land earmarked for potato cultivation at the Horticultural Research Station of the Orissa University of Agriculture & Technology (OUAT) near Khandagiri (Orissa) was prepared by repeated ploughing to make the soil fine tilt for planting of direct tubers. The soil was fortified with farm yard manure (FYM) and half-dose N (60 kg/ha) and full-dose of P & K (80 kg/ha & 100 kg/ha respectively). The fertilizers were thoroughly mixed with the soil and layout made by ridges and furrows maintaining a line-length of 3.0 m with a distance of 60 cm between lines and 20 cm between plants. Direct tubers (20-45 g size) were planted in randomized block design (RBD) with 4 replications. The plot size was maintained at 3.0 m x 2.0 m. The inter-row and intra-row (inter-plant) spacings were maintained at 60 cm and 20 cm respectively throughout. Irrigations were applied as appropriate. The first top dressing (1/4 N) and second top dressing (1/4 N) were done at 25 days interval. When the crop was 80 day-old, the seed tubers were harvested. Some important quantitative characters like tuber number, weight and yield parameters were recorded.

Results

Apices from 5–8 day old sprouts proliferated each as a single shoot within 25 days of culture in semi-solid MS medium with supplements (Fig. 1 a,b). A growth regulator combination of 0.01 mg/l NAA with 0.1 mg/l GA_3 along with an additional vitamin, calcium pantothenate, at an optimal concentration of 2.0 mg/l was most effective when augmented to MS salts in promoting shoot proliferation. Complete plantlets (rooted shoots) were obtained within 45 days. The filter-bridge technique facilitated considerably further culture of nodal segments from *in vitro* shoots on the medium with nutrient

composition as aforesaid minus the gelling agent. Shoots developed from nodes in 10–15 days and they rooted within 20 days (Fig. 1 c). By successive sub-culturing of nodal segments from the newly formed shoots an average multiplication rate of 3–5 fold per culture was established in 2–3 months.

Microtubers were produced in axenic shoot cultures in MS salt medium lacking growth regulators but the frequency was very low (< 20%) and the number of microtubers never exceeded two per plantlet despite 30 days of culture. On the other hand, microtuberization occurred (99–100%, 1–6 microtubers/plantlet) within 12–15 days of transfer of rooted shoots to the tuber-inducing medium containing MS nutrients fortified with BA and grocery sugar. Of a number of permutations and combinations tested, BA at 8.0 mg/l coupled with for 50 g/l grocery sugar was most effective regardless of genotypes examined, though microtubers were initiated in all such supplemented media. The more responsive genotypes were Kufri Chandramukhi, JX-123 and JX-161. The maximum number of microtubers per plantlet (5.5) and highest yield (1270 mg/plantlet) were recorded in Kufri Chandramukhi while JX-123 produced the heaviest microtubers averaging 450 mg.

During initiation stage, microtubers first appeared as small, whitish globular structures ranging 1–3 mm in diameter and formed mostly on the aerial parts of the stem (stolon) or occasionally on the stolons adjacent to the culture medium. As they matured over a period of 75 days, their diameter increased to appx. 5–12 mm and they turned creamish-white to pale yellow, while some changed their shape to become ellipsoidal (Fig. 1 d). Incubation was attempted using a range of photoperiods (8-16 h) of a low light intensity (20 μmol m^{-2} s^{-1}) at a day/night temperature of 20 ± 2°C/18 ± 2°C. Better tuberizing effect was scored with shorter photoperiods (8–10 h) compared to longer ones (12–16 h). The microtuber formation was considerably facilitated in dark incubation while being delayed under illumination. Dark-incubated microtubers were more in number per plant resulting in a higher net yield. There was no marked difference between the average number of eyes per microtuber induced under light or dark incubation. After a week following transfer to artificial light the matured microtubers turned green.

Minitubers were harvested after 95 days of planting of microtubers in the glasshouse (Fig. 1 e,f). Among the five genotypes examined there was a significant variation with regards to percentage emergence (sprouting), number of minitubers per plant and yield (g) per plant (Table 1). Microtubers from Kufri Chandramukhi showed the highest emergence (98.25%) while JX-161 had the lowest (76.75%). Compared to the genotype JX-123 which resulted in the maximum number of minitubers/plant (14.5), JX-115 produced the minimum (10.5 minitubers/plant). The heaviest minituber (maximum 46.98 g, average 18.8 g) was recorded for JX-123 while Kufri Ashoka had the lighest minitubers averaging 9.93 g. It was JX-123 which also exhibited the highest yield averaging 272.6 g/plant against 119.2 g/plant as for Kufri Ashoka.

Interestingly, several small size tubers (5–20 mm diam) were developed directly on minitubers following storage at 18°C in dark for a period of 3 weeks (Fig. 2 a). As many as about 40–45 small direct tubers were obtained from a single minituber of JX-123 (Fig. 2 b). Among the five genotypes, there was a significant difference in the number of tubers generated per minituber. The average number of direct tubers per kg of minitubers was the highest in JX-123 (345) followed by Kufri Ashoka (340), JX-161 (334), Kufri Chandramukhi (330) and JX-115 (325) (Fig. 3). There was a significant variation in the yield (g) of direct tubers per kg minitubers; JX-123 had the highest yield (182.8 g) while JX-115 had the lowest (82.5 g). The direct tuber yield for Kufri Chandramukhi, Kufri Ashoka and JX-161 were 134.5, 124.4 and 120.6 g/kg minitubers respectively. There was no significant difference between genotypes with respect to emergence percentage; the highest (75%) and the lowest (65%) sprouting were recorded in JX-161 and JX-115 respectively. As regards yield of seed tubers in direct tuber-derived plants in the field (Fig. 2c), JX-161 had the highest average (8.5 kg/kg direct tubers) while JX-115 showed the lowest yield averaging 5.8 kg/kg direct tubers. The remaining genotypes Kufri Chandramukhi, Kufri Ashoka and

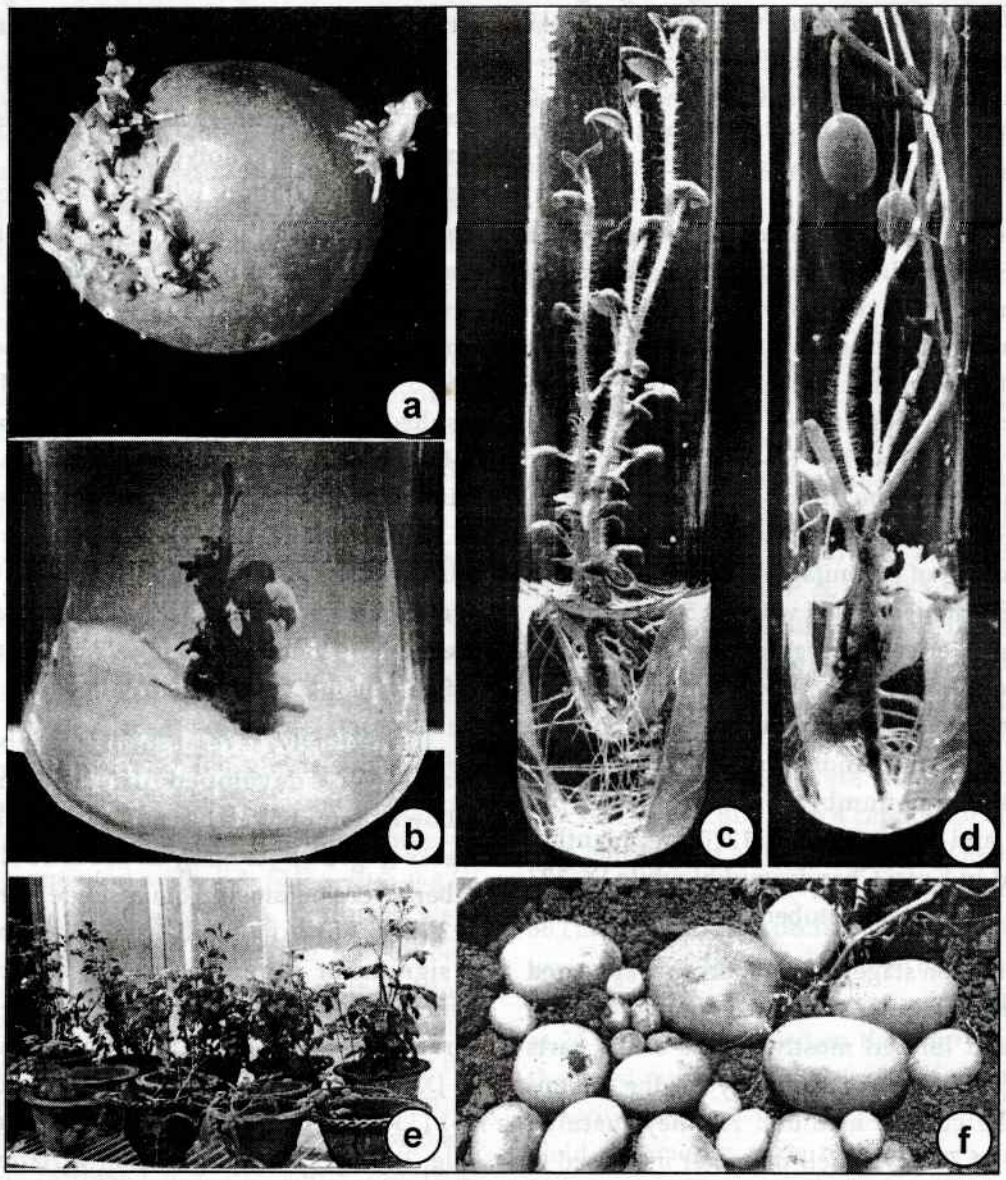

Fig. 1: Production of minitubers via *in vitro* induction of microtubers

(a) A mother tuber bearing a number of healthy sprouts

(b) Shoot proliferation from a sprout-tip on MS augmented with 2.0 mg/l calcium pantothenate + 0.01 mg/l NAA + 0.1 mg/l GA_3

(c) Well-rooted plantlets raised from nodal segments cultured on a filter paper bridge in MS liquid medium with composition as (b), ready for transfer to microtuberization medium (d).

(d) *In vitro* induction of microtubers at day 12 following transfer of axenic shoots to microtuberization medium (MS+8.0 mg/l BA + 50 g/l grocery sugar).

(e) Microtuber-derived plants grown inside the glasshouse

(f) Minitubers harvested from glasshouse plants grown from microtubers

Table-1

Production of minitubers in microtuber-derived plants

Genotype	% Emergence ($\bar{x}$)	Minitubers/ Plant ($\bar{x}$)	Weight of minitubers(g) ($\bar{x}$)	Yield/plant (g) ($\bar{x}$)
JX-115	80.00	10.50	16.20	170.1
JX-123	77.75	14.50	18.80	272.6
JX-161	76.75	11.75	18.59	218.5
Kufri Ashoka	80.75	12.00	9.93	119.2
Kufri Chandramukhi	98.25	13.00	16.80	218.4
	S	S	NS	S
SE (m)	2.85	1.07	-	24.36
SE (d)	2.01	0.76	-	17.23
SD	4.03	1.52	-	34.45
CV %	4.87	12.31	-	15.96
CD	6.57	2.47	-	56.15

Data pooled from three experiments each with four replicates. **S** : Significant, **NS** : Not Significant.

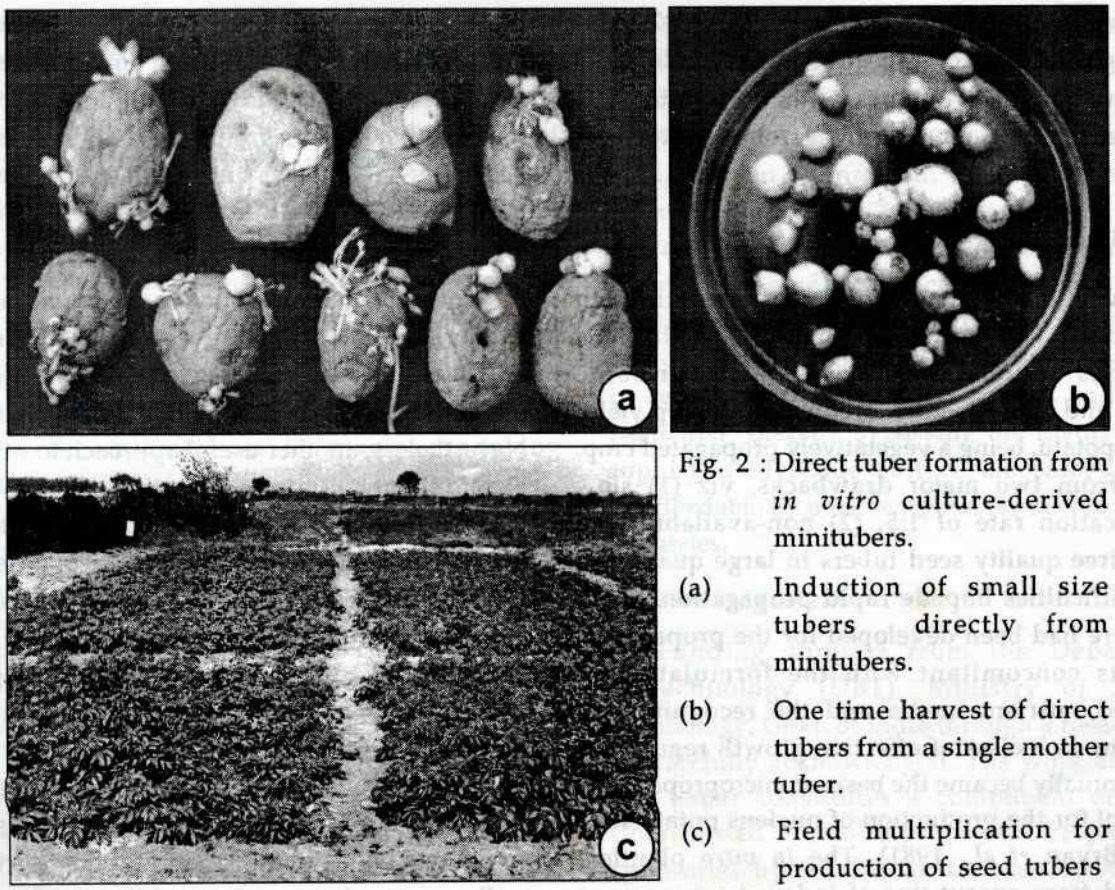

Fig. 2 : Direct tuber formation from *in vitro* culture-derived minitubers.

(a) Induction of small size tubers directly from minitubers.

(b) One time harvest of direct tubers from a single mother tuber.

(c) Field multiplication for production of seed tubers

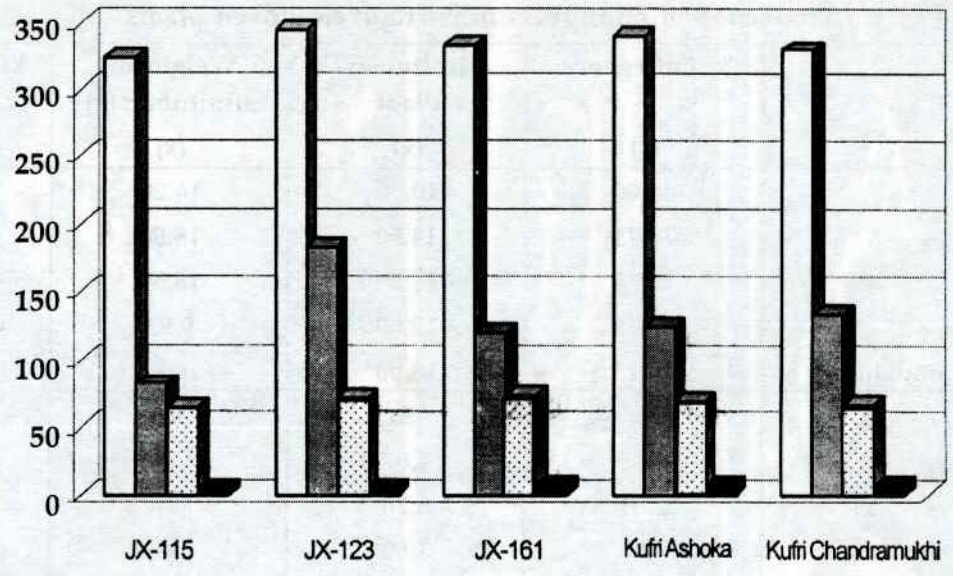

Fig. 3 : Variation among genotypes in respect of direct tuber formation and their field performance.
No. of tubers / kg of minitubers. Yield (g)/kg of minitubers. Percentage emergence.
Seed tuber yield (kg) / kg of direct tubers.

JX-123 had intermediate yield figures i.e. 7.8, 7.5 and 6.0 kg/kg direct tubers respectively. Nevetheless, the variations among genotypes with respect to seed tuber yield in field plants grown from direct tubers was not significant.

Discussion

Conventionally, potato is propagated through seed tubers. This cultivation method results in a continuous accumulation of various tuber-borne diseases and consequently a reduction in crop yield. Besides, potato, being a vegetatively propagated crop, suffers from two major drawbacks, viz (1) slow multiplication rate of 1:5, (2) non-availability of disease free quality seed tubers in large quantities. These difficulties impede rapid propagation. Shoot tip culture had been developed for the propagation of plants concomitant with the formulation of improved nutrient media and the recognition of cytokinins as a class of effective growth regulators. This eventually became the basis of micropropagation and a tool for the production of nucleus potato seed stocks (Bryan *et al.*, 1981). The *in vitro* plantlets produced from sprout-tips of indexed tubers were the basic starting material which were multiplied on solid/liquid medium using single node cuttings. Here, the method followed with a view to availing the base material for production of potato seed stocks has all the advantages of breaking the cycle of tuber-borne disease-causal organisms and thus obtaining pathogen free plants.

The propagation of potato by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange and conservation (Roca *et al.*, 1979; Ranalli *et al.*, 1994). Nevertheless, another useful approach to utilize these axenic shoot cultures is induction of microtubers (*in vitro* microtuberization). Conceptually, microtubers are very small tubers induced in plantlets raised through *in vitro* culture when incubated under suitable conditions (Wang & Hu, 1982; Estrade *et al.*, 1986). The microtubers which can be produced round the year would be supposedly free from pathogens and viruses. Besides, these can be stored for a reasonably long period and also conventionally transported. In addition, microtubers would considerably facilitate germplasm conservation as well as germplasm exchange between laboratories.

Work on microtuberization in potato has mainly focussed on the use of growth regulators (Palmer & Smith, 1969; Wang & Hu, 1982, Estrada *et al.*, 1986, Vecchio *et al.*, 1994), though a range of factors including sucrose concentration, temperature, photoperiod, light intensity and the genotype have been implicated (Hussey & Stacey, 1984; Garner & Blake, 1989 ; Gopal *et al.*, 1998). In the present study, grocery sugar was used in MS medium instead of purified (analytical grade) sucrose from a branded scientific manufacturer keeping in view that it could reduce significantly the cost of microtuber production. Sixteen different combinations of BA and grocery sugar in MS were examined for *in vitro* microtuberization. Although microtubers were induced in all of these media, MS + 8 mg/l BA + 50 g/l grocery sugar proved to be the most efficient. The percentage of shoots experiencing microtuberization was upto 99-100%, producing the highest average of 6 microtubers/plantlets with the highest average yield of 1270 mg/plantlet. These observations appear to be considerably superior and certainly more encouraging compared to the previous reports including the recent one as that of Gopal *et al.*, (1998). The five genotypes chosen for study were found to differ significantly with regards to microtuber number, weight and yield. The significance of genotype x culture conditions interaction, however, indicates the need of developing genotype-specific protocols to maximize microtuberization.

The microtubers, being small in size, had relatively a low survival percentage when grown directly in the open field. Therefore, it was necessary to grow the microtubers to plants producing minitubers under controlled glasshouse conditions. Further, it could also be rewarding to grow minitubers in the field to produce seed tubers which could form the breeders' stock of desired quality.

An interesting observation during the present investigation was the direct tuber development in minitubers under controlled *ex vitro* incubation condition. All the five genotypes tested were capable of direct tuberization with some variations with respect to tuber number and weight. The genotype JX-123 produced the maximum number of direct tubers and resulted in the highest tuber yield whereas JX-161 showed the maximum percentage of shoot emergence and resulted in the highest yield of seed tubers from such direct tuber-derived plants. The direct tuber production in minitubers without growing them into plants and without any additional input barring the healthy minituber itself is a very simple and effective method. In the present study 20-100 small size tubers each weighing 0.1-10 g were produced per month on a single minituber.

The mechanism underlying tuber-from-tuber formation may be ascribed to conversion of sucrose to starch under dark incubation and the deposition of latter as reserve material in the stolons. By virtue of this technique 15,000-25,000 small tubers can be produced in one m^3 space and 2-3 crops of such small tubers can be harvested at an interval of 20-25 days. The technique of large-scale production of direct tubers within an exceptionally short period can evolve as an efficient agro-industry. This will have a considerable potential for production of quality seeds with less disease infestation compared to those derived from the traditional cultivation system as the former had not been exposed to environment where disease transmitting agents could be plentiful. Small direct tubers can be packed, transported and distributed to the farmers so that there would be absolutely no requirement for them to establish an infrastructure for *in vitro* cultures and hence the demand for high-cost media and labour input would be nil. In essence, such a short-cut *ex vitro* multiplication strategy could considerably reduce the cost of production of disease-free quality seed tubers, in case of tuber crops in general and potato in particular, thereby bypassing the involvement of a sophisticated tissue culture laboratory, presently unaffordable by most marginal farmers in developing countries.

Acknowledgement

Funding support from the Department of Biotechnology (DBT), Ministry of Science & Technology, Govt. of India through a research project is gratefully acknowledged. The work embodied in this paper constitutes a component of All India Coordinated Potato Improvement Project (AICPIP) sponsored by the Indian Council of Agricultural Research (ICAR) New Delhi.

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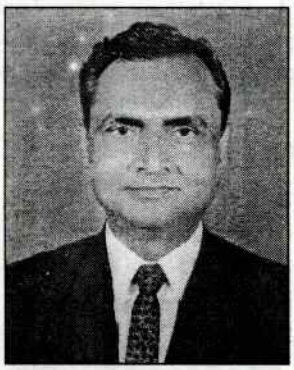
OBITUARY



GOPINATH PANIGRAHI

Born : 27.02.1924 • Died : 15.12.2004

Born on 27th February 1924, Gopinath had his early education in his village school Baikunthapur near Basudebpur of Bhadrak district. A very bright student from his childhood, Gopinath secured First Class First position in whole of the Patna University (spanning over the present day Bihar, Jharkhand and Orissa State). A rare feat by a village lad of Orissa when the whole country was under the spell of "Quit India" movement. At that time he joined Ravenshaw College and completed I.Sc. and B.Sc. (Hons.) with rank. Since there was no post-graduate teaching facility in Orissa at that time, he joined Allahabad University and successfully completed M.Sc. course. In July 1948, he joined Ravenshaw College as a faculty member. Like many others, he also started his research work under the able guidance of Prof. Samantarai and co-authored a paper on "Mechanism of photoperiodism on *Amaranthus giganteus* var *tristis*". Eventually in 1952 he joined Prof. Irwin Manton, FRS of Leeds University, UK, from where he obtained his Ph.D. Degree and joined back his *alma mater* in 1954. But with interest and choice he opted to work as a Taxonomist in the newly formed Botanical Survey of India (BSI) and joined there directly as Regional Botanist in 1956 and continued there till his superannuation in 1982. But his love for taxonomy forced him to be associated with BSI from 1982-87 as Emeritus Scientist.

As a teacher and researcher Dr. Panigrahi received several awards and ovations by National and International bodies as a front line Taxonomist of the world being an authority on "Plant Nomenclature".

Dr. Panigrahi had all along been an out-and-out patron and acted sincerely to strengthen the Orissa Botanical Society since its very inception. It was he who always stood by at hours of financial crisis of the Society and generously supported the publication of the Society journal 'Plant Science Research'. Of his kind contribution, with a view to encouraging the young plant science researchers of the State, the Society has been able to institute **"Sarojini G. Panigrahi Young Scientist Award"** for the best oral presentation during the Annual Conference of the Society every year in his honour and of his beloved wife. Again, his generous donation only a year ago has enabled the Society to institute **"Dr. Gopinath Panigrahi Memorial Award in Plant Taxonomy"** being given away biannually to the best taxonomist of the State.

He is no more, but his colleagues, students and disciples still cherish the dedication, hard work, discipline and perseverence he had nurtured to uphold taxonomy as a master area in the discipline of Plant Science.

With his demise the country has lost one of its proud son who brought laurels to his country in International scenario. The Orissa Botanical Society has lost its best friend, philosopher and guide.

In this moment of despair we pray Almighty to give strength and courage to his family members to sustain this loss.

Let his soul rest in peace.

OBITUARY

HARIHAR PATTNAIK

Born : 20.08.1924 • Died : 22.11.2005

Born on 20th August 1924, in the village Talagarh of Balarampur Panchayat in the then Cuttack district, Harihar Pattnaik had his early education in village school after which he joined Ravenshaw College for higher studies. He graduated from Ravenshaw College, Cuttack in the year 1946 and subsequently moved to Allahabad to pursue his higher studies as there was no facility for M.Sc. in Botany in Orissa. He joined Ravenshaw College as a faculty member in 1948 and imparted teaching in Plant Morphology. However, he left Ravenshaw College in 1962 to work under the famous algologist Prof. G.E. Fogg, FRS at the Westfield College of London University and returned to his *alma mater* in 1964 having awarded Ph.D. degree. Then, the only referred taxonomist Harihar in the State became a famous algal physiologist. He pioneered the establishment of the branch of Algology in Orissa as Professor and Head of the newly formed Botany Department of Berhampur University. That was the year 1969, just two years after the formation of this University. It was his stewardship and unparalleled leadership which gave him a recognition as a person of dedication, commitment and achievement. Since then he never looked back. Not only he earned name as a distinguished academician by publishing a number of original papers both in national and international journals of repute as well as by producing several Ph.D. and M.Phil awardees but also he achieved the apex as the Vice-Chancellor of Berhampur University. To add to his feather as an administrator, he was also the first Chairman of Railway Service Commission of the country. He was the recipient of several awards and honours.

His prolific writing in Oriya literature brought laurels to him and as an active member of Orissa Bigyan Prachar Samitee he dedicated himself for popularisation of science till his death.

With his sad demise, the scientific world has lost one of the front line researcher in the field of Algal Physiology. In Orissa we missed a star Botanist, a lovable teacher with excellent oratory and a great patron for the Orissa Botanical Society.

Let Almighty give strength and courage to his bereaved family members to bear this irreparable loss.

Let his soul rest in peace.

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