Plant Science Research 35 (1&2) : 1-7, 2013

Plant Science Research

ISSN 0972-8546

Degradation of chemically related organochlorine pesticides (γ - hexachlorocyclohexane and vinclozolin) in rice soil, pre-exposed to each other

S. Padhi[Ψ]

Department of Botany, Ravenshaw University, Cuttack-753003, India

ARTICLE INFO

Article history:
Received : 17 December 2012
Received in revised form : 08 October 2013
Accepted : 14 October 2013

Keywords:
Organochlorine compounds,
vinclozolin
γ-hexachlorocyclohexane
enhanced degradation
microorganisms

ABSTRACT

Accelerated degradation of soil-applied pesticides, upon their repeated application, is the result of proliferation of microorganism degrading candidate pesticide and can undermine the efficacy of the pesticide under consideration. In the present study, experiments were conducted both in green house and in laboratory conditions to examine the development of enhanced degradation of vinclozolin in mineral salts medium inoculated with soil suspension from unplanted and planted flooded alluvial soils untreated or pre-treated with commercial HCH and vice versa. Results demonstrated that 15 days after fourth application, approximately 97% of vinclozolin was degraded in the suspension from planted pots. The development of enhanced biodegradation of γ-HCH was examined in a mineral salts medium inoculated with soil suspensions from unplanted or planted flooded alluvial soils untreated or pre-treated with commercial vinclozolin. Fifteen days after third application, only a trace of γ-HCH was recovered from the soil suspension from planted pots. The rice plants played a definite and important role in influencing the development of enhanced degradation of both the pesticides.

1. Introduction

Organochlorine pesticides like DDT, HCH, aldrin, vinclozolin, heptachlor and endosulphan, extensively used for control of agricultural pests and vector born diseases, are of much concern because of their prolonged persistence, lipophilic nature and tendency to accumulate in animal and plant tissues. The use of these pesticides have been banned or restricted in several countries including India. However, neither the ban nor the restricted use has reduced the levels of residues of these compounds in the soil environment (Simonich *et al*., 1995; Cerkvenik *et al*., 2000; Noren *et al*., 2000; Akbar *et al*., 2003). This is especially true for hexachlorocyclohexane (HCH), a highly recalcitrant pesticide, extesively used for control of agricultural pests over the past five decades (Deo and Karanth, 1994), and also for vonclozolin (Vanni *et al*., 2000; Flynn *et al*., 2001; Sandra *et al*., 2001).

The spontaneous or induced microbial degradation is one of the possibilities for decontamination of these highly persistent organochlorine residues. Unfortunately, spontaneous microbial degradation proceeds rather slowly (Johri *et al*.,1996; Lal and Saxena, 1982). Thus addition of naturally occuring microbes to contaminated soils as an alternate strategy, can be assisted to a great extent by exploring the potentials of such isolates.

Manonmani *et al*. (2000) reported that amongst the auxillary carbon-sources, ethanol, benzoate and glucose (at higher concentrations) retarded γ-HCH degradation whereas addition of cellulose, saw dust and low concentration of glucose (< 200 mg/ml) and acetone enhanced the rate of degradation. Enhanced microbial degradation occurred readily in a soil treated with analytical grade carbofuran, chlorpropham, cloethocarb diazinon and fensulfothion (Chapman and Harris, 1990).

The dicarboxymide fungicide vinclozolin [(RS)-(3.5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] is used to control disease caused by *Botrytis* sp., *Alternaria*

[Ψ] *Corresponding author; Email:* san_puri9828@rediffmail.com

sp., *Sclerotinia* sp. and *Monilia* sp. of vegetables and other field crops (Spencer, 1982). The fungicide undergoes hydrolysis and produces several degradation products including 3,5-dichloroaniline (Szeto *et al*., 1989a; Golovleva *et al*., 1991). Golovleva *et al*. (1991) showed that microbial strains utilizing vinclozolin as the sole source of carbon and energy belonged to genera *Pseudomonas* and *Bacillus*. Frederick *et al*. (1994) reported the degradation of vinclozolin in soil, thatch and grass clippings. Pothuluri *et al*. (2000) reported the biotransformation of vinclozolin by the fungus *Cunninghamella elegans*. They reported 93% of transformation of vinclozolin to four major metabolites after 96h incubation.

About 50% of the fungicide is reported to be degraded in aerobic soils within 23 days and the degradation is rather slow in acidic soil (Walker *et al*., 1986). Many a reports regarding the enhanced degradation of fungicide vinclozolin (Walker *et al*., 1986, 1987; Milhomme, 1989; Cork and Krueger, 1991; Golovleva *et al*., 1991; Vega-Palas *et al*., 1992; Cain *et al*., 1996; Mercadear *et al*., 1998) showed the involvement of microorganisms in its degradation. Pirsisi *et al*. (1986) reported the isolation and identification of the major breakdown products of chlozonilate vinclozolin. Cain *et al*. (1996) showed the rapid onset of the accelerated degradation of dicarboximide fungicides including viclozolin in a U.K. soil; with its long history of agrochemical exclusions. Szeto *et al*. (1989b) analysed the kinetics of the hydrolysis of the Dicarboximide fungicide vinclozolin. This report presents the efficiency of degradation of γ-HCH and vinclozolin in rice soil on pre-exposure to each other.

2. Materials and Methods

2.1. Soil

A deltaic alluvial soil from the experimental farm of Central Rice Research Institute, Cuttack, Orissa, was used for the present study. The soil was air-dried in shade and after breaking the clods, sieved through a < 2mm mesh and stored in polyethylene bags at room temperature. The physico-chemical properties of the soil (Table 1) were determined by the following methods (Jackson, 1973). Soil pH was measured by 1:1.25 soils to water ratio using a digital pH meter with Calomel glass electrode assembly. Organic carbon content of the soils was determined and the organic matter was calculated by multiplying the organic carbon values with 1.72. Total nitrogen content of the soils was estimated by Kjeldahl method. The cation exchange capacity (CEC) of soils was determined by 1 N ammonium acetate (pH 7.0) by summation of exchangeable Na, K, Ca, Mg and H. Physical analysis for clay, silt and sand fractions was measured by employing the Bouyoucos hydrometer method (Black, 1965).

2.2. Pesticides

Technical formulation of hexachlorocyclohexane (HCH) (99.1% purity) used in this study were obtained from M/s Lachat chemicals, Mequon, Wisconsin, U.S.A. Commercial formulation of HCH containing 50% active ingredient (γ-HCH) was obtained from M/s Das Enterprise, Calcutta, India. Both commercial formulation of the fungicide vinclozolin (Ronilan 50% w.p.) and analytical grade vinclozolin (99% a.i.) were obtained from M/s BASF, Germany.

2.3. Greenhouse and laboratory experiment

Earthenware pots (25.5 x 9.5 cm dia.) were separately filled with 5 kg of alluvial soils. 30-d old rice seedlings (*cv.* IR-72) were transplanted with 4 hills of one plant each per pot. Phosphorus as single super phosphate (SSP) and potassium as muriate of potash (MOP) at 20 mg/kg soil each were applied to soils of all the pots as basal dressing. Nitrogen (60 mg N/kg soil) was applied as urea to all the pots in three splits with 50% at basal and 25% each at maximum tillering and panicle initiation stages of the crop. There were three uniform replicated pots of each soil for each treatment (HCH-treated or untreated) and amended with commercial formulation of HCH or vinclozolin at 10 mg/g.

First and second application of HCH was made at 10 d and 15 d, respectively after flooding. Fifteen days after each application (before the next application was made) of HCH, duplicate 1g surface soil (1-2 cm) samples collected from HCH treated and untreated pots, were separately shaken with 10ml sterile distilled water in pre-sterilized test tubes (200 x 25 mm) to prepare soil suspensions for use as inoculum for the γ-HCH degradation studies.

Table 1
Physico-chemical characteristics of the soil used in the study

Soil type	Taxonomic group	pH	Organic matter (g/kg)	Total Nitrogen (g/kg)	SO_4 (mg/g)	EC (dS/m)	CEC (cMoles/ 100g soil)	Soil Separates (g/kg)		
								clay	silt	sand
Alluvial	Aeric endoaquept	6.16	8.2	0.9	10.2	0.51	95.5	25.9	21.6	52.5

2.3.1.1. Laboratory methods

A mineral salts (MS) medium [$(NH_4)_2HPO_4$, 0.5 g; $MgSO_4.7H_2O$, 2 g; $FeSO_4.7H_2O$, 0.001 g; K_2HPO_4, 0.1 g; $Ca(NO_3)_2$, 0.01 g and distilled water 1 litre, pH 7.0] was shaken with analytical grade of γ-HCH for 48 hr on a mechanical stirrer and subsequently sterilized by filtration through a Millipore® filter (0.3 mm). 10ml portions of this medium were aseptically dispensed in sterile 100ml Erlenmeyer flasks and later inoculated with 1ml of soil suspension (flooded) from untreated or HCH-treated pots. The un-inoculated medium served as control. The samples were incubated under intermittent shaking (for 4 hr after every 4 hr interval) to provide aerobic conditions. At periodic intervals, 1 ml portion of the inoculated and un-inoculated medium were withdrawn aseptically from duplicate flasks and shaken with 2-4 ml of hexane for 2-3 min for extraction of HCH residues. HCH residues in hexane fraction were analyzed by GLC (Varian, 3400) equipped with a ^{63}Ni detector and a metal column (2m length, 1/8" OD). Column, injector and detector were maintained at 220, 240 and 240^{0}C, respectively with a flow rate of the carrier gas (95% argon in 5% methane) at 20 ml/min.

Accelerated degradation of vinclozolin was studied under greenhouse conditions as described by Bharati *et al*. (1998). The first and second application of vinclozolin was made to the soil in the pots at 10 d and 25 d, respectively after moistening/flooding (unplanted pots) or transplantation (planted pots). Subsequent applications were made at 40 d (50-d after moistening/flooding) and 55 days (65-d after moistening/flooding). At regular intervals, soil samples from pots treated as above were collected as described by Panda *et al.* (1988) and tested for vinclozolin degradation.

2.4. Degradation of γ-HCH or vinclozolin through cross-inoculation

Accelerated degradation of γ-HCH or vinclozolin was measured in soils enriched with commercial formulation of HCH or vinclozolin respectively at concentration mentioned above. Soils were pre-enriched with either HCH or vinclozolin in unplanted or planted pots as described. Flask containing MS medium supplemented with γ-HCH/vinclozolin and inoculated with soil suspension pre-enriched with γ-HCH/ vinclozolin served as positive control for γ-HCH/ vinclozolin degradation.

3. Results and discussion

In most of the experimental studies on the development of xenobiotic-degrading microbial enrichment cultures, the target ecosystem is exposed to repeated application of the candidate insecticide. However, enhanced degradation of many of the pesticides has often been reported wherein the enrichment was developed through the exposure of chemically related pesticide (Racke and Coats, 1990). Both γ-HCH and vinclozolin are being organochlorine compounds, it was thought worthwhile whether any enrichment of vinclozolin-degrading microorganisms developed upon the exposure to γ-HCH or vice versa.

3.1. Degradation of vinclozolin in mineral salts medium inoculated with suspension of soil pretreated with commercial HCH

Accelerated degradation of soil-applied pesticides, upon their repeated application, is the result of proliferation of microorganism degrading candidate pesticide and can undermine the efficacy of the pesticide under consideration (Sethunathan, 1971; Felsot, 1989; Racke, 1990). From the earlier reports (Bhuyan *et al*., 1992, 1993; Sahoo *et al*., 1990) and from the results of present study, it has been established that γ-HCH is degraded faster upon its repeated application to the flooded soil planted to rice. Besides, accelerated degradation of vinclozolin occurred upon repeated application of the fungicide especially under flooded condition. In the present experiment attempts were made to examine the development of enhanced degradation of vinclozolin in mineral salts medium inoculated with soil suspension from unplanted and planted flooded alluvial soils untreated or pre-treated with commercial HCH. The results showed no accelerated degradation after first application of HCH and approximately 60% of vinclozolin was degraded in planted flooded soil after second application. After third application of HCH, vinclozolin degradation was comparatively faster, especially in flooded soils planted to rice. 15 days after fourth application, approximately 97% of vinclozolin was degraded in the suspension from planted pots (Table 2). During the same period, there was some loss of vinclozolin from un-inoculated control and from the medium inoculated with suspensions from untreated soils, possibly due to volatilization/chemical degradation.

3.2. Degradation of γ-HCH in mineral salts medium inoculated with suspension of soil pre-treated with vinclozolin

In a follow up experiment, the development of enhanced biodegradation of γ-HCH was examined in a mineral salts medium inoculated with soil suspensions from unplanted or planted flooded alluvial soils untreated or pre-treated with commercial vinclozolin. In this case, no accelerated degradation of γ-HCH was observed after first and even second application of fungicide to the soil. Comparatively, degradation of γ-HCH was faster after third application of

vinclozolin to the soil. Fifteen days after third application, only a trace of γ-HCH was recovered from the soil suspension from planted pots. Enhanced degradation of γ-HCH became more pronounced after fourth application. Five days after fourth application, 91% of γ-HCH was degraded and the entire amount of γ-HCH was degraded within 10 days (Table 3).

Interestingly, soil planted to rice and maintained under flooded condition exhibited clear-cut enhancement of γ-HCH degradation in the present experiment. It was observed that rice plants play a definite and important role in influencing the development of enhanced degradation of γ-HCH. It is known that flooded soil planted to rice is characterised by an interactive aerobic-anaerobic interface that is dynamic in nature. Such aerobic-anaerobic interface seems to trigger the development of HCH degrading factor in low land rice field upon repeated addition of HCH (Bhuyan *et al*., 1990;1992). As flooded soil planted to rice is microbially more active (Yoshida, 1975), high microbial activity in the flooded soil planted to rice may enhance γ-HCH and/or vinclozolin degradation in these conditions.

Barraga´n-Huerta *et al.* (2007) reported the biodegradation of organochlorine pesticides by bacteria grown in micro-niches of the porous structure of green bean coffee and concluded that defective green bean coffee can be used as both a nutrient source and a support for organochlorine pesticide degrading bacteria in liquid media. Baczynski *et al*. (2010) reported that anaerobic biodegradation of organochlorine pesticides in contaminated soil is significantly influence by the temperature and availability of the pesticide in the soil.

Many reports indicate the inhibitory effect of organochlorine pesticides on their own degradation and their toxic effects on the soil and the microbes responsible for their degradation (Ghadiri *et* al., 1995; Nawab *et al*., 2003; Reddy *et al*., 2012). It has been reported that certain pesticides have inhibitory effects on bacterial growth (Nawab *et al*., 2003). Report suggest that application of endosulfan to a clay soil aged with aldrin, dieldrin, endrin and chlordane significantly reduced the rates of degradation of both aldrin and dieldrin, indicating possible toxic effect of this pesticide on the micro-organisms responsible for the degradation of

Table 2
Degradation of vinclozolin (µg/ ml) in a mineral salts medium inoculated with suspension from unplanted or planted flooded alluvial soil untreated or pretreated with commercial HCH

Incubation (days)	Vinclozolin (µg/ml) recovered soil pretreated with HCH						
	Uninoculated	2nd application		3rd application		4th application	
		Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
0	9.2 ± 0.2	6.6 ± 0.3	5.7 ± 0.5	6.5 ± 0.1	5.4 ± 0.2	6.3 ± 0.7	5.2 ± 0
5	7.4 ± 0.6	5.0 ± 0	3.9 ± 0.3	6.5 ± 0.1	3.5 ± 0.5	2.9 ± 0.2	1.7 ± 0.1
10	6.5 ± 0.1	3.4 ± 0.2	2.7 ± 0.6	3.0 ± 0	1.9 ± 0.1	1.3 ± 0	0.9 ± 0.1
15	5.8 ± 0.5	3.4 ± 0.2	0.9 ± 0.1	1.5 ± 0.5	0.6 ± 0	0.9 ± 0.1	0.2 ± 0

Note: vinclozolin added @ 10 µg/ml mineral salts medium

Table 3
Degradation of γ-HCH in a mineral salts medium inoculated with suspension from unplanted or planted flooded alluvial soil untreated or pretreated with commercial Vinclozolin

IIncubation (days)	HCH (µg/ml) recovered from soil pretreated with Vinclozolin						
	Uninoculated	2nd application		3rd application		4th application	
		Unplanted	Planted	Unplanted		Unplanted	Planted
0	9.7 ± 0.1	6.4 ± 0.2	5.2 ± 0.5	5.7 ± 0.1	4.6 ± 0.5	5.3 ± 0.1	3.8 ± 0.1
5	6.9 ± 0.6	4.5 ± 0	3.7 ± 0.6	3.8 ± 0.2	2.5 ± 0.5	1.7 ± 0.1	0.6 ± 0.1
10	6.0 ± 0	3.6 ± 0	2.1 ± 0.1	1.8 ± 0.2	0.7 ± 0.1	0.8 ± 0	0
15	5.3 ± 0.1	2.1 ± 0.5	0.8 ± 0.1	1.0 ± 0	trace	0.5 ± 0.1	0

Note: vinclozolin added @ 10 µg/ml mineral salts medium

the aged organochlorine pesticides already in the soil (Ghadiri *et al*., 1995). Extensive applications of persistent organochlorine pesticides like endosulfan on cotton led to the contamination of soil and water environments at several sites in India (Reddy *et al*., 2012).

Increasing pesticide usage in agriculture adds to the rise in concern for the environmental contamination (Zhu *et al*., 2004). Pesticides reaching the soil may affect non-target soil microorganisms, thereby disturbing pesticide degradation processes (Pal *et al*., 2006). Adverse effect of pesticidal chemicals on soil microorganisms (Araújo *et al*, 2003), may affect soil fertility (Schuster and Schröder, 1990) becomes a foreign chemicals major issue. Soil microorganisms show an early warning about soil disturbances by foreign chemicals than any other parameters. But the fate and behaviour of these chemicals in soil ecosystem is very important since they are degraded by various factors and have the potential to be in the soil, water etc. So it is indispensable to monitor the persistence, degradation of pesticides in soil and is also necessary to study the effect of pesticide on the soil quality or soil health by in depth studies on soil microbial activity.

Development of enrichment cultures through the exposure of chemically related pesticides have been reported earlier in case of organophosphorous (Sethunathan, 1973) and carbamate pesticides (Racke and Coats, 1990).

The present study indicates that HCH and Vinclozolin, being included in the same organochlorine group, on their repeated applications to the same soil, encouraged an increase in the number of microorganisms capable of degrading the chemical and resulted in more rapid degradation of the chemical compared to untreated soil supporting the assumption that repeated applications of a pesticide may result in an increase in the enzyme activity, but not community size, specifically toward the degradation of the pesticide. Similar mechanism may be in operation resulting into development of organochlorine-degrading enrichment cultures.

It is known that micro-organisms represent the richest repertoire of molecular and biological diversities in nature as they comprise the most diverse forms of life. They are nature's original recyclers, converting toxic organic compounds to harmless end products, offten CO_2 & H_2O. Ever since, it was discovered that microbes have the ability to transform and/or degrade xenobiotics, scientists have been exploring the microbial diversity, particualrly of contaminated areas in search for organism that can degrade a wide range of pollutants. Microbial diversity offers an immense scope of environment friendly options for minerlization of contaminants or their transformation into less harmful hazardous compound. In this context, the development of a broad spectrum of the organochlorine-degrading enrichment cultures as observed and discussed in the present study may have an influencial importance in the decontamination process of the agricultural fields/ lands having the previous history of pesticidal practice and can also be used in the bioremediation programme of xenobiotics in the contaminated sites.

Acknowledgement

The author is thankful to the Director, CRRI, Cuttack for providing facilities for conducting experiments and analysis in Soil Microbiology Laboratory; to the University Grant Commission for the grant of Teacher-Fellowship with financial support.

References

Akbar, N., Aleem, A. and Malik, A. (2003). Determination of organochlorine pesticides in agricultural soil with reference to γ-HCH degradation by *Pseudomonas* strains. Bioresour. Technol. 88: 41-46.

Araujo, A. S. F., Monteiro, R.T.R., and Abarkeli, R.B. (2003). Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52: 799-804.

Baczynski, T. P., Pleissner, D., Grotenhuis, T. (2010). Anaerobic biodegradation of organochlorine pesticides in contaminated soil - significance of temperature and availability. Chemosphere 78(1): 22-8.

Barraga´n-Huerta, B.E., Costa-Pe´rez, C., Peralta-Cruz, J., Barrera-Corte´s, J., Esparza-Garcýa´, F.,. Rodrý´guez-Va´zquez, R. (2007). Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. Int. Biodeterior. Biodegrad. 59: 239–244

Bharati, K., Padhi, S. and Adhya, T.K. (1998). Accelerated biodegradation of γ-hexachlorocyclohexane (γ-HCH) in a flooded alluvial soil retreated with γ-HCH or its metabolite 1,2,4-trichlorobenzene (TCB). Bull. Environ. Contam. Toxicol. 60:858-863.

Bhuyan, S., Sahu, S.K., Adhya, T.K. and Sethunathan, N. (1992). Accelerated aerobic degradation of γ-hexachlorocyclohexane in suspensions of flooded and nonflooded soils, pretreated with hexachlorocyclohexane. Biol. Fertil. Soils 12:279-284.

Bhuyan, S., Sreedharan, B., Adhya, T.K. and Sethunathan, N. (1993). Enhanced degradation of γ-hexachlorocyclohexane (γ-HCH) in HCH (Commercial) acclimatized flooded soil : Factors affecting its development and persistence. Pestic. Sci. 38:49-55.

Black, C.A. (1965). Methods of Soil Aanalysis (Part I and II). Amer. Soc. Agron., Madison, Wisconsin, USA.

Cain, R.B. and Mitchell, J.A. (1996). Enhanced degradation of the fungicide vinclozolin: Isolation and characterization of a responsible organism. Pestic. Sci. 48: 13-23.

Cerkvenik, V., Doganoc, D. Z. and Jan, J. (2000). Evidence of some trace elements, organochlorine pesticides and PCBs in Slovenian cow's milk. Food Technol. Biotechnol. 38: 155-160

Chapman, R.A. and Harris, C.R. (1990). Enhanced degradation of insecticides in soil: factors influencing the development and effect of enhanced microbial activity. In Racke, K.D. and Coats, J.R. (Eds.) Enhanced Biodegrdation of Pesticides in the Environment. American Chemical Society, Washington, D.C. pp. 82-96.

Cork, D.J. and Krueger, J.P. (1991). Microbial transformation of hrbicides and pesticides. Arch. Appl. Microbiol. 36:1-66.

Deo, P.G., Karanth, N.G., Gopalakrishna, N. and Karanth, K. (1994). Biodegradation of hexachlorocyclohexane isomers in soil and food environments. CRC Crit. Rev. Microbiol. 20: 57-78.

Felsot, A.S. (1989). Enhanced biodegradation of insecticides in soil: Implication for agro-ecosystem. Ann. Rev. Entomol. 34: 453-476.

Flynn, K.M., Delclos, K.B., Newbold, R.R. and Ferguson, S.A. (2001). Behavioural responses of rats exposed to long-term dietary vinclozolin. J. Agric. Food Chem. 49: 1658-1665.

Frederick, E.K., Bischoff, M., Throssell, C.S. and Turco, R.F. (1994). Degradation of chlorneb, triadimefon, and vinclozolin in soil, thatch and grass clippings. Bull. Environ. Contam. Toxicol. 53: 536-542.

Ghadiri, H., Rose, C. W. and Connell D. W. (1995). Degradation of organochlorine pesticides in soils under controlled environment and outdoor conditions. J. Environ. Manage. 43(2): 141-151.

Golovleva, L.A., Finkelsteil, Z.I., Polyakova, A.V., Baskonov, B.P. and Nefedoya, M.Y. (1991). Microbial conversion of the fungicide Vincozolin. J. Environ. Sci. Health B26: 293-307.

Jackson, M.L. (1967). Soil Chemical Analysis. Prentice Hall Inc., New Delhi.

Johri, A.K., Dua, M., Tuteja, D., Saxena, R., Saxena, D.M. and Lal, R. (1996). Genetic manipulations of microorganisms for the degradation of hexachlorocyclohexane. FEMS Microbiol. Rev. 19: 69-84.

Lal, R., and Saxena, D. M. (1982). Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. 46: 95-127.

Manonmani, H.K., Chandrachekaraiah, D.H., Reddy, S., Elcey, C.D. and Kunhi, A.A. (2000). Isolation and acclimation of a microbial consortium for improved aerobic degradation of γ-hexachlorocyclohexane. J. Agric. Food Chem. 48: 4341-4351.

Mercadear, C., Vega, D. and Bastide. J. (1998). Chemical and biological transformation of the fungicide vinclozolin. J. Agric. Food Chem, 46: 3817-3822.

Milhomme, H., et al. (1989). Degradation of chloroprophane herbicide in soil: role of the introduction of *P. alkaligenes* and *P. cepacia.* Soil Biol. Biochem. 21: 307-311.

Nawab, A., Aleem, A., Malik, A., (2003). Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation. Bioresour. Technol. 88(1): 41–46.

Noren, K. and Meironyte, D. (2000). Organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. Chemosphere 44: 1111-1123.

Pal, R., Chakrabarti , K., Chakraborty, A.; Chowdhury, A. (2006). Degradation and Effects of Pesticides on Soil Microbiological Parameters- A Review. Int. J. Agric. Res. 1: 240-258.

Panda, S., Sharmila, M., Ramanand, K., Panda, D. and Sethunathan, N. (1988). Persistence of hexachlorocyclohexane isomers and carbofuran applied to surface and subsurface layers of a flooded soil. Pestic. Sci. 23: 199-207.

Pirsisi, F.M. and Meloni M. (1986). Degradation of dicarboximidic fungicides in wine part-II : Isolation and identification of the major breakdown productsof Clozolinate Vinclozolin and Procymidone. Pestic. Sci. 17: 109-118.

Pothuluri, J.V., Freeman, J.P., Heinze, T.M., Beger, R.D. and Cerniglia, C.E. (2000). Biotransfernmation of vinclozolin by the fungus *Cunninghamella elegans*. J. Agric. Food Chem. 48: 6138-6148.

Racke, K.D. (1990). Implications of enhanced biodegradation for the use and study of pesticides in soil environment. In Racke, K.D. and Coats, J.R. (Eds.) Enhanced biodegradation of pesticides in the environment. ACS Symposium series 426, Ameican Chemical Society, Wasington D.C., pp. 269-282.

Racke, K.D. and Coats, J.R. (1990). Enhanced biodegradation of insecticides in mid western corn soils. In Racke, K.D. and Coats, J.R. (Eds.) Enhanced Biodegradation of Pesticides in the Environment. ACS Symposium series 426, American Chemical Society, Washington, D.C. pp. 68-81.

Reddy, B.T., Ramana Reddy, A.V., Rami Reddy, Y. V. (2012). Biodegradation of organochlorine pesticides

endosulfan in soil and water environments. Der. Pharma. Chemica. 4 (3): 1016-1018.

Sahoo, A., Sahu, S.K., Sharmila, M. and Sethunathan, N. (1990). Pesistence of carbamate insecticides, carbosulfan and carbofuran in soils as influenced by temperature and microbial activity. Bull. Environ. Contam. Toxicol. 44: 948-954.

Sandra, P., Tienpont, B., Vercammen, J., Tredoux, A., Sandra, T. and David, F. (2001). Stir bar sorptive extraction applied to the determination of dicarboximide fungicides in wine. J. Chromatogr. A928: 117-126

Schuster, E., Schröder, D. (1990). Side-effects of sequentially applied pesticides on non-target soil microorganisms: field experiments. Soil Biol. Biochem. 22: 367–373.

Sethunathan, N. (1971). Biodegradation of diazinon in a paddy field as a cause of its inefficiency for controlling brown planthoppers in rice fields. PANS 17: 18-19.

Sethunathan, N. and Yoshida, T. (1973). Degradation of chlorinated hydrocarbons by *Clostridium* sp. isolated from lindane amended flooded soil. Plant Soil. 38: 663-666.

Simonich, S.L. and Hites, R.A. (1995a). Organic pollutant accumulation in vegetation. Environ. Sci. Technol. 29: 2905-2914.

Simonich, S.L. and Hites, R.A. (1995b). Global distribution of persistent organochlorine compounds. Science. 269: 1851-1854.

Spencer, E.Y. (1982). Guide to the chemicals used in crop protection, 7th Edn., Publication No. 1043, Agriculture Canada Research Branch, Ottawa, Canada.

Szeto, S.Y., Burlinson, N.E., Rahe, J.E. and Oloffs, P.C. (1989a). Persistence of the fungicide vinclozolin under laboratory conditions. J. Agric. Food Chem. 37: 529-534.

Szeto, S.Y., Burlinson, N.E., Rahe, J.E. and Oloffs, P.C. (1989b). Kinetics of hydrolysis of the dicarboxymide fungicide vinclozolin. J. Agric. Food Chem. 37: 524-529.

Vanni, A., Gamberini, R., Calabria, A. and Pellegrino, V. (2000). Determination of presence of fungicides by their common metabolites 3,5-DCA, in compost. Chemosphere 41: 453-458.

Vega-Palas, M.A., Flores, E. and Herrero, A. (1992). NtcA, a global nitrogen regulator from the cyanobacterium Synechococcus that belongs to the Crp family of bacterial regulators. Mol. Microbiol. 6: 1853-1859.

Walker, A. (1987). Enhanced degradation of iprodione and vinclozolin in soil – a simple colorimetric test for identification of rapid degrading soils. Pestic. Sci. 21: 233-240.

Walker, A., Brown, P.A. and Entwistle, A.R. (1986). Enhanced degradation of iprodione, vinclozolin in soil. Pestic. Sci. 17: 183-193.

Yoshida, T. (1975). Microbial metabolism in flooded soils. In Paul, E.A. and A.D. McLaren, A.D. (Eds.) Soil Biochemistry, Vol. 3, Marcel Dekker, New York. pp. 83-122.

Zhu G., Wu H., Guo J. and Kimaro, F.M.E. (2004). Microbial degradation of fipronil in clay loam soil. Water Air Soil Pollut. 153: 35–44.

Plant Science Research 35 (1&2) : 8-13, 2013

Plant Science Research

ISSN 0972-8546

Allelopathic impact of bark leachate of *Acacia nilotica* (L.) Delile on seed germination and enzymatic activities of *Phaseolus vulgaris* L.

I. Rath[1], N. Dash[2] and S. P. Adhikary[2Ψ]

[1] Assistant Professor, Department of Science and Humanities, College of Engineering, Bhubaneswar, Odisha

[2] Department of Botany, Aska Science College, Aska-761111, Dist. Ganjam, Odisha

ARTICLE INFO

Article history:
Received : 20 November 2012
Received in revised form : 01 March 2013
Accepted : 03 August 2013

Keywords:
Allelopathy
Acacia nilotica
Bark leachate
Green gram
α-amylase
Protease

ABSTRACT

Agro-forestry programme in India has encouraged the farmers to grow different species of *Acacia, Eucalyptus, Albizia* and *Prosposis* in and around their crop fields to fulfil their demands. But recently attention has been focused on allelopathic effect of such trees on germination, growth and yield of the concerned crop. In order to investigate the allelopathic effect of aqueous bark leachate of *Acacia nilotica* on germination and activities of different enzymes concerned with germination of *Phaseolus vulgaris,* different concentrations of bark leachate (2-10%) were taken. Pure line seeds of *P. vulgaris* were germinated in plastic trays (containing cottons) in B.O.D. incubation. Experiments were conducted during 24-144 h for seed germination and 48-144 h for a-amylase and protease activities. Increased concentration of aqueous bark-leachate exhibited a negative correlation with seed germination and biochemical content of germinating seeds. However, a-amylase and protease activities were shown a positive correlation. The bark-leachate of *Acacia nilotica* contains allelochemicals which might have inhibited the seed germination and reduced the biochemical (carbohydrate and protein) content, whereas a-amylase and protease activities of germinating seeds were increased.

1. Introduction

Till 1970, little information was available on allelopathy in agro forestry system but thereafter, a lot of research works have been carried out. *Acacia* species affect crop growth by competing for various environmental resources than their little interference with the establishment and growth of the adjoining crop plants (Kohli *et al.,* 2006). Different species of *Acacia* release ferulic, vanillic, caffeic, gallic, m-hydroxybenzoic, and m-hydroxyphenyl acetic acids, tannins, flavonoides and gums, including phenolics in their litter. These chemicals may act in many biological processes, such as to suppress the mineral uptake by plants, inhibit cell elongation and cell division, as well as retard photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth (Seigler, 2003; El-Khawas *et al.*, 2005). Carbelleria and Reigosa (1999) have demonstrated that the leachate from *Acacia delbata* showed strong inhibitory effects on the germination and growth of *Lactuca sativa*. Allelopathy has been implicated (Rafiqual-Hoque *et al.,* 2003; El-Khawas and Shehata, 2005). It has been observed that water extracts contains a wide range of chemicals of different *Acacia* species which inhibit germination, root and shoot length and also dry weight of different crops (AI-Wakeel *et al.* 2007; Lorenzo *et al*., 2008).

Phytochemical screening of the stem-bark of *Acacia nilotica* revealed that the plant contains terpenoids, alkaloids, soponins and glycosides (Banso, 2009). Plants, which are rich in alkaloids, tannins and glycosides, have shown to possess antimicrobial activity against a number of organisms. 2-Benzoxazolinone (BOA), a most potent allelochemicals of rye, suppresses the plants growth including crops and weeds (Burgos and Talbent, 2000; Beiz and Hunkle, 2004). The action of allelochemicals in target plant is diverse and affects a large number of biochemical reactions resulting in modifications of different processes. Allelopathic compounds may induce a secondary oxidative stress manifested as enlarged production of reactive oxygen

Ψ *Corresponding author; Email:*adhikarysankarprasad@yahoo.co.in

species (ROS). ROS are known to act as signalling molecules, regulating plant response to biotic and abiotic stresses. Plant growth and development response to stress is controlled by phytohormones. Hormonal signalling transduction depends on ROS production. Hence, a study was therefore planned to assess the allelochemical effects of bark-leachate of *Acacia nilotica* on seed germination and enzymatic activities of (a-amylase and protease) and changes in carbohydrate and protein contents of germinating seeds.

2. Materials and methods

Freshly fallen bark of 10 years old *Acacia nilotica* tree grown in Berhampur University campus (19^0-60' N and 84^0-53'E) were collected at morning time. The barks were washed thoroughly with tap water and then with distilled water and thereafter dried in an incubator at 40±2^{0}C. The dried materials were chopped and then ground to make powder. Two hundred grams of each ground material were leached for 48 hours in one litre of distilled water and filtered separately (Padhy *et al,* 2000).These 20 % concentrated leachates were diluted to get desired concentrations (2-10%) for treatment.

Pure line seeds of *P. vulgaris* were procured from Regional Agricultural Research Station of Odisha University of Agriculture and Technology (O.U.A.T.), Berhampur located at Ratnapur. Healthy seeds of uniform colour, size, and shape were surface sterilised with 0.03% formalin solution for 10 min and then washed thoroughly with tap water followed by distilled water. Then seeds (@ 20 seeds / tray) were allowed to germinate in plastic trays (3 x 9 x 12 cm) having a thin bed of sterilized cotton as per the experimental schedule and containing various concentration of leachate. Incubation was done in dark in a B.O.D. incubator maintained at 30±1^{0}C. The germination (emergence of radicle) was observed at interval up to 6 days.

2.1. Extraction and assay of a-amylase

Germinated seeds from both control and treated sets were collected at random, soaked on blotting paper, weighed 1 g and then were ground with 2-3 ml of citrate buffer (pH 7.0) with a pinch of calcium chloride. The homogenates were centrifuged at 2000g for 15 minutes at 28±1^{0}C and the process was repeated thrice. The supernatants so collected were pooled together and made up to 10 ml with citrate buffer (Malik and Singh, 1980).

For assay of a-amylase activity, a reaction mixture was prepared by adding 1 ml of starch solution (0.15% in 0.04 M KH_2PO_4), 0.5 ml of enzyme extract and 3 ml of Iodine reagent (0.6% iodine in 6% KI solution), allowed to stand for 10 minutes at room temperature and absorbance of the reaction mixture was recorded at 620 nm with help of a spectrophotometer.. The enzymatic activity was expressed in mg of starch hydrolysed per 10 minute per gram fresh weight of seeds from the standard curve prepared with different concentration of starch (15-150 mg/100 ml distilled water).

2.2. Total carbohydrate

The germinated seeds (100 mg) collected from control and treated sets at random were homogenised with 80% ethanol in pre-chilled mortar and pestle and the homogenates were centrifuged at 3000g for 15 minutes at 28±1^{0}C. The supernatants were collected by repeating the procedure thrice. The pooled supernatants were concentrated into 1-2 ml in a water bath maintained at 50±1^{0}C. The slurry was diluted with distilled water to a definite volume and then used for estimation of carbohydrates spectrophotometrically by anthrone method as per Plummer (1979). D-Glucose was taken for preparation of standard curve.

2.3. Extraction and assay of protease

Germinating seeds were collected at random and soaked on blotting paper. One gram of seeds from each treatment were homogenized with 2-3 ml of 0.05 M Tris malate buffers (pH 7.0), equal volume of 40 mM cysteine hydrochloride and 4% sodium chloride solution were added and pH was adjusted to 7.0 with 0.1 N aqueous NaOH solution. The homogenate was centrifuged at 17000g for 20 minutes at 28 ±1°C. The process was repeated thrice and supernatants so collected were pooled together and the volume was made up to 10 ml with tris-malate buffer. Bovine serum albumin (BSA) was used as a substrate. Three millilitre of the assay mixture (1.5 ml of 0.05 M tris-malate buffers (pH 7.0), 0.5 ml of 2% BSA and 1 ml of enzyme extract) were incubated for 2 hours at 40±1^{0}C and the reaction was stopped by adding 1 ml of 20% trichloroacetic acid (TCA). A zero time control was run at the same time with adding enzyme solution immediately before addition of TCA. Then the assay mixture was kept overnight in a refrigerator and centrifuged at 15000g at 4±1^{0}C for 15 minutes. The process was repeated thrice and the supernatants were pooled together and adjusted to pH 5.0 with 0.1 N NaOH. Then 0.5 ml of supernatant was added to 1.0 ml of Ninhydrin reagents and heated for 10 min at 100^{0}C. Four ml of distilled water were added to each sample and the absorbance was measured at 570 nm (Moore and Stein, 1948). Protease activity was expressed in mMole of NH_2/hours.g.fr. wt. of seed.

2.4. Estimation of protein

The pellets left over after extraction of carbohydrates from germinated seed were suspended in 5% (w/v)

Trichloroacetic acid (TCA) at 0°C for 15 minutes and centrifuged at 5000g for 20 minutes. The process was repeated twice and supernatants were discarded. Then 2 ml of 1N NaOH was added to pellets present in centrifuged tubes. The tubes were left as such at room temperature for 30 minutes, then kept in a boiling water bath for 15 minutes, cooled and centrifuged at 5000g for 15 minutes to room temperature. The insoluble protein present in supernatant was estimated by folin-phenol method as described by Lowry *et al.* (1951) taking bovine serum albumin as standard.

3. Result and discussion

3.1. Seed germination

The aqueous bark - leachate of *Acacia nilotica* significantly reduced the germination in green gram. All concentrations of test leachate caused reduction of seed germination and the rate of reduction increased with the concentration of leachate (Table 1). These results are in agreement with those obtained by Duhan and Lakshminarayan (1995), who found that the growth of *Cyamposis tetragonal* growing at distance of 1-2 m from the tree *Acacia nilotica* was inhibited. Inhibition of seed germination with increased leachate concentration was observed by different authors on the other plants viz. maize and kidney bean by leave extract of *Eucalyptus globules* (El-Khawas and Shehata, 2005), mung bean by leaf extract of *Lantana camara* (Maiti *et al*., 2010) and wheat by Eucalyptus (Patil *et al*., 2002). Many other species of genus Acacia, like *A. delbata* (Carbelleria and Reigosa, 1999; Lorenzo *et al*., 2008), *A. confusa* (Chou *et al*., 1998). *A. auriculaeformis*, *A.cunn* (Rafiqual-Hoque *et al*., 2003, Dash 2012) and *A. nilotica* (Al-Wakeel *et al*., 2007) are known to exhibit allelopathic activity.

The effects of allelochemicals have been studied mostly on seed germinations and the mechanisms of inhibition are mostly by disruption of mitochondrial respiration (Abrahim *et al.*, 2000) and interference in normal cell division (Padhy *et al.*, 2005). Effects of allelochemicals on seed germination appear to be mediated through a disruption of normal cellular metabolism rather through damage of organelles. Reserve mobilisation; a process which usually takes place rapidly during early stage of seed germination seems to be delayed under allelopathic stress.

3.2. a-amylase and carbohydrate

The amount of total carbohydrate content in germinating seeds considerably decreased with increase of leachate concentration (Fig.1a). Similarly the a-amylase activity also gradually increased (Fig.1b). Maiti *et al.* (2001) reported that the inhibition of germination behaviour was associated with deceased level of carbohydrate and increase of a-amylase activity in mung bean seeds affected by leaf extracts of *Lantana camara*. The present findings corroborate with report of several workers on the other plants (Maiti *et al.,* 2008; 2010; Das *et al.,* 2012; Dash, 2012).

3.3. Protease and protein

All the concentrations of the leachate considerably exhibited reduction in protein content and increase in the protease activity in germinating seeds (Fig 2 a & b). The present finding corroborate with the findings of different workers in other plants influenced by phytochemicals (Maiti *et al.,* 2008; 2010; Dash, 2012). However, the protease activity in plants influenced by allelochemicals were decreased with increase of leachate concentration reported by other workers (Kohli and Pariana, 1992; Pawar and Chavas, 2004; Gantayat *et al*., 2006).

There is a reduction of protein and carbohydrate content in treated seeds, which possibly played a significant role in the deterioration of the germinating seeds. The activity of a-amylase and protease significantly increased in pre-treated seeds samples than control. These enzymes play a vital role during germination and growth (Maiti *et al.,* 2008). a-amylase activity regulates starch breakdown, necessary

Table 1
Impact of different concentrations of aqueous-bark-leachate of *Acacia nilotica* on germination (%) of green gram seeds at different days after soaking (DAS). Values are means of 5 replicates ± S. E. M.

DAS	Leachate concentration (%)					
	0	2	4	6	8	10
2	20.28 ± 0.53	18.26 ±0.49	13.44±0.33	9.82±0.22	3.20±0.36	0
3	43.82 ±0.29	37.24 ±0.29	31.86±0.27	28.28±0.56	19.22±0.43	11.46±0.26
4	67.44 ±0.24	61.28 ±0.32	55.24±0.21	46.62±0.22	35.26±0.27	25.24±0.24
5	87.28±0.36	79.44±0.41	71.26±0.53	57.24±0.39	43.82±0.21	35.26±0.46
6	95.44±0.42	87.26±0.40	79.24±0.31	65.62±0.33	51.62±0.34	43.28±0.32

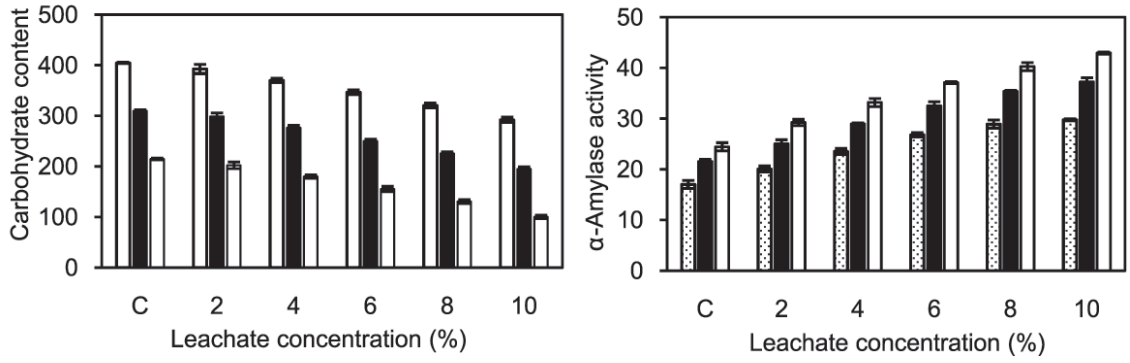

Fig. 1. Changes in total (A) carbohydrate content (mg/g fr. wt) and (B) α-Amylase activity (mg of starch utilised/10 min/g fr. wt.) of germinating green gram seeds at different days after soaking (DAS) influenced by varying concentrations of aqueous-bark-leachate of *Acacia nilotica.* Columns: 2 DAS (variegated); 4 DAS (full); 6 DAS (empty).

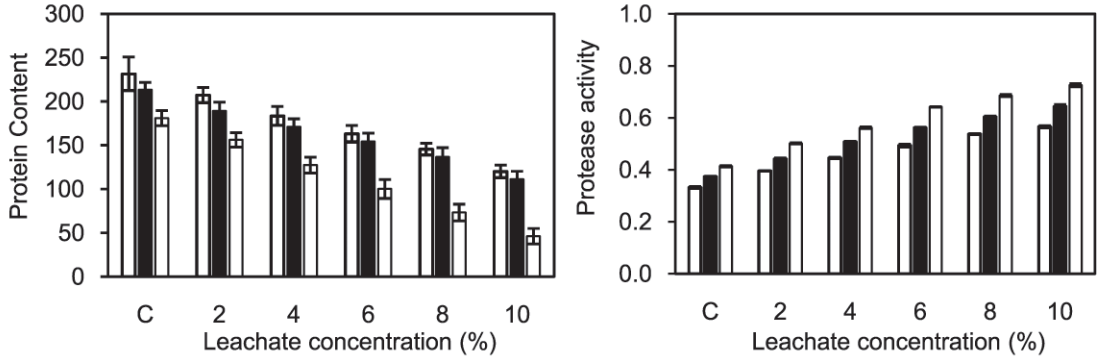

Fig. 2. Changes in (A) protein content (mg / g. fr. wt.) and (B) protease activity (m mole of NH_2 released/g fr. wt.) of germinating green gram seeds at different days after soaking (DAS) influenced by varying concentrations of aqueous-bark-leachate of *Acacia nilotica.* Columns are as for figure 1.

for supplying substrate to respiratory metabolism. Increased activities of a-amylase suggest more utilisation of sugars to meet the increased energy demands of tissues in response to allelochemicals induced stress (Daizy *et al.*, 2006).

Seed germination is a very sophisticated process which required the concerted action and interaction between diverse phytohormones. Seed dormancy and germination are complex traits that are regulated by the antagonistic action of the phytohrmones abscisic acid (ABA) and gibberellins. ABA is considered as a major stress hormone and its accumulation is connected to dehydration related process in plants (Houser *et al.*, 2011). ABA functions as a positive regulator of physiological dormancy while GA and Ethylene negatively regulated dormancy and promote seed germination. Allelochecals might have inhibited the seed germination by suppressing the synthesis of gibberellins and indol acetic acid. Allelopathic compounds may reduce a secondary oxidative stress manifested as enlarged production of reactive oxygen species (ROS). ROS are known to act as signalling molecules, regulating plant response to biotic and abiotic stresses. ROS have been implicated as second messengers in plant hormones response.

It is concluded that the allelochemicals present in bark leachate of *Acacia nilotica* exhibited a negative correlation with biomolecular content (carbohydrate and protein) and increased leachate concentration. Increased a-amylase and protease activities were responsible for breaking down of more carbohydrate and protein to simple form which may serve as respiratory substrate and release large amount of energy required to facing the allelochemical stresses. Allelochemical stress induced redox transformation that ultimately result in the formation of ROS and they imbalance different growth hormones, secondary metabolites and signal molecules which might have reduced the seed germination. Further the studies can be extended regarding the efficacy of these bark leachate under field condition, isolation and identification of allelochemicals responsible for germination, biomolecular content and enzymatic activities in the crop plants.

Acknowledgement

The authors are thankful to Head of the department of Chemistry, Berhampur University, for providing necessary laboratory facilities, Prof. B. Padhy, Retired Prof, of Botany, Berhampur University for his critical review of the script and informative suggestions, Officer-in-charge, Regional Agricultural Research Station of Odisha University of Agriculture and Technology (OUAT), Berhampur for providing pure line seeds of rice cultivars used in this investigation and Principal, Aska Science College, Aska for providing necessary facilities.

References

Abrahim, D., Braguini, W. L., Kelmer-Bracht, A. M. and Ishii-Iwamoto, E. L. (2000). Effects of four monoterpenes on germination, primary root growth and mitochondrial respiration of maize. J. Chem. Ecol. 26: 611-624.

AI-Wakeel, S. A. M., Gabr, M. A., Hamid, A. A. and Abu-El-Soud W. M. (2007). Allelopathic effects of *Acacia nilotica* leaf residue on *Pisum sativum.* Allep. J. 19: 23- 24.

Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J. Med. Plants Res. 3(2): 82-85.

Beiz, R. G. And Hurle, K. (2004). A novel laboratory screening bioassay for crop seedling allelopathy. J. Chem. Ecol. 3: 175-198.

Burgos, N. R. and Talbert, R. E. (2000). Differential activities of allelochemicals from *Secale cereals* in seedling bioassays. Weed Sci. 48: Pp. 302-310.

Carbelleria, A. and Reigosa, M. J. (1999). Effects of natural leachate of *Acacia delbata* link in Galicia (N. W. Spain). Bot. Bull. Acad. Sci. 40: 87-92.

Chou, C. H., Fv, C. Y., Li, S. Y. and Wang, Y. F. (1998). Allelopathic potential of *Acacia confusa* and related species in Taiwan. J. Chem. Ecol. 24: 2131-2150.

Daizy, R. B., Harminder P. Singh, Setia, N., Kaur, S. and Kohli, R. K. (2006). Effect of 2-Benzoxazolinine (BOA) on Seedling Growth and Associated Biochemical Changes in Mung bean (*Phaseolus aureus*). Z Naturforsch. 61(c): 709-714.

Das, C. R., Mondal, N. K., Aditya, P., Dutta, J. K., Banerjee, A. and Das, K. (2012). Allelopathic potential of leachate of leaf litter of some selected tree species on gram seeds under laboratory condition. Asian J. Exp. Biol. Sci. 3(10): 59-65.

Dash, N. (2012). Studies on biochemical changes in chloroplasts of some crop plants influenced by allelochemicals of *Acacia auriculaeformis*. Ph. D., Thesis, Berhampur University, Berhampur, Odisha, India.

Duhan, T. S. and Lakshminarayan, K. (1995). Allelopathic effect of *Acacia nilotica* on cereal and legume crops grown in fields. Allep. J. 2: 93-98.

El- Khawas, S. A. and Shehata, M. N. (2005). The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on Monocot (*Zea mays* L.)and Dicot (*Phaseolus vulgaris* L.) plants. Biotechonology. 4(1): 23-34.

Gantayat, P. K., Padhy, B., Behera, M. and Padhy, S. K. (2006). Impact of aqueous leachate of eucaluptus on germination and seedling growth of red gram seed. Indian J. Environ. Ecoplan. 12 (3): 565-571.

Hauser, F., Wadet, R. and Schroeder, J. (2011). Evolution of absscisic acid synthesis and signaling mechanisms. Curr. Biol. 21(9): 346-355.

Kohli, R. K., Batish, D. R., Singh, H. P. andDogra, K. S. (2006). Invasiveness and Enviromental treats of three tropical Americane invasive weds(*Pathenium hysterophorus* L., *Ageratum coniziodes* L. *and Lantana camara* L.*)* in India. Biol. Inv. 8(7): 1501-1510.

Kohli, R. K. and Pariana, K. (1992). Allelopathic Impact of Sunflower on crops. Allelopathy in India(eds)P. Tauro &S. S. Norwal. ISA Publication: 29-30.

Lowry, O. H., Rosenbrough N. J., Farr, A. L. and Randall R. J. (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265-267.

Lorenzo, P., Pazos- Malvido, E., Gonzilez C. and Reigosa, N. J. (2008). Allelopathic interference of invasive *Acacia delbata*: Physiological effects. Allep. J. 22: 452-462.

Maiti, P. P., Bhakat, R. K. and Bhattacharjee, A. (2008). Allelopathic effects of Lantana*camara* on physiobiochemical parameters *of Mimosa Pudica* seeds. Allep. J. 22: 59-68.

Maiti, P. P., Bhakat, R. K. and Bhattacharjee A. (2010). Evolution of allelopathic potential of an obnoxious weed using mung bean as a bioassay material. Internat. J. Sci. Nature 1(2): 236-241.

Moore, S. and Stein, W. W. (1948). Photometric ninhydrin method for use in the chromatographyof aminoacids. J. Biol. Chem. 176: 376-388.

Padhy, B., Patnaik P. K. and Tripathy, A. K. (2000). Allelopathic potential of Eucalyptus leaf litter- leachate on the germination and seedling growth of finger-millet. Allep. J. 7(1): 69-78.

Padhy, B., Gantayat, P. K., Padhy, S. K. and Sahu, M. D. (2005). Arapid bioassay method for allelopathic studies. Allep. J. 17(1): 105-112.

Patil, R. H., Hunsal, C. S. and Itnal, C. I. (2002). Influence of bund planted Eucalyptus trees rows on winter wheat. Allep. J. 10: 21-28.

Pawer, K. B. and Chavan, P. D. (2004). Influence of leaf

leachate of some tree sps. On carbohydrate metabolism in germinating seeds of *Sorghum bicolor* (L.) Monech. IV. International Congress on Allelopathy in Sustainable Terrestrial and Aquatic Ecosystem. Abst. A.F. 14: 60.

Plummer, T. D. (1979). Estimation of carbohydrate by the anthrone method In: an Introduction to Practical biochemistry, second edition, (Ed. T. D. Plummer), Tata Mc Graw Hill Publishing Company Ltd. 183-184.

Rafiqual-Hoque, A. T. M., Ahmad, R., Uddin, M. B. andHossain, M. K. (2003). Allelopathic effect of different concentration of water extract of *Acacia auriculaeformis* level on some initial growth parameters of live common agricultural crops. Pak. J. Agron. 2:92-100.

Seigler, D. S. (2003). Phytochemistry of *Acacia sensulate*. Biochem. Syst. Ecol. 31: 845-873.

Plant Science Research 35 (1&2) : 14-19, 2013

Plant Science Research

ISSN 0972-8546

Dominant medicinal plants of KBK districts of Odisha with special reference to the strategy of their conservation

B. K. Patnaik[1Ψ] and S. P. Rath[2]

[1] State Environment Impact Assessment Authority, Odisha, Qrt No-5 RF, 2/1, Unit-IX, Bhubaneswar-751022, India

[2] Department of Botany, Utkal University, Vanivihar, Bhubaneswar-751004, India

ARTICLE INFO

Article history:
Received : 14 December 2012
Received in revised form : 08 November 2013
Accepted : 12 November 2013

Keywords:
KBK region
tribal people
forest degradation
medicinal plants
sustainability

ABSTRACT

The KBK region of Odisha, comprising of erstwhile Koraput, Bolangir and Kalahandi districts, are considered to be the poorest and most backward region of the country. It's scanty population over an area of 30.60% landmass of the State, dominated by mostly tribals has attracted the attention of Government. of India to declare it as a special region, which needs to be elevated in all developmental aspects. The present paper depicts as to how those poor, illiterate and ignorant tribal people make best use of plants available around them and take care of their health and day to day medical needs even without the help of a qualified medical professional. Since the over use of the plants has become a threat for the long term sustainability of those medicinal plants found in the wild, some strategy has been adopted for their conservation.

1. Introduction

The KBK region comprising of undivided Koraput (i.e. Koraput, Nawarangpur, Malkangiri and Rayagada), undivided Bolangir (i.e. Subarnapur and Bolangir) and undivided Kalahandi (i.e., Kalahandi and Nuapada) districts is the poorest and most backward region of the country; 89.95 % people of this region still live in villages. The KBK districts account for 19.80% population over 30.60% geographical area of the state. Tribal communities comprising 38.41% of the population dominate this region which include six primitive tribal groups i.e. Bondas, Didayi, Lanjia Saora, Dangria, Kandha, Kutia Kondha and Chuktia Bhunjia. In addition, 16.25% population belong to the scheduled caste (SC) communities as per 2011 census. (Mohanty, 2011).

Because of its very adverse socio-economic and human development indicators, the KBK region has for some time past been attracting the attention of Government of India, National Human Rights Commission (NHRC) and the State Government. The need for long term measures for speedy development of livelihood support has been articulated in early 1990s.

As per the estimates of the 55th round of NSS survey conducted in 1990-2000, (Anonymous, 2006) by Planning & Co-ordination Deptt. Government of Odisha, the incidence of rural poverty in this region is as high as 87.14%. The multifaceted deprivation and backwardness of this region are the result of deep-rooted factors or processes that have emanated from a complex mix of geographical, economic and social factors. The region in general, and undivided Koraput and Kalahandi districts in particular are almost at the bottom of the list of 250 backward districts identified under Backward Regions Grant Fund (BRGF) of India.

The old Koraput and Kalahandi districts and portions of Bolangir districts are mainly hilly. Severe droughts and floods also often visit this region and some areas in quick succession. While many factors like tribal backwardness, hill area backwardness and backwardness due to severe natural calamities haunt this region, many other multifaceted factors are also encountered. All these factors combine to force the local inhabitants to depend more and more on forest and natural resources for eking out their livelihood. Intensive use of forests for sustenance coupled with other anthropogenic factors, continuously lead to forest degradation. In fact, it is observed during the course of

Ψ *Corresponding author; Email:* bijayketanpatnaik@yahoo.co.in

work, that all eight KBK districts are ecologically disturbed and more than 50% of forests of these districts are degraded, which aggravates the problem of poverty in this region.

2. Materials and methods

The plants were collected from different districts under KBK and conserved in the Silviculture research centre, Ghatikia. The medicinal plants were identified with the help of flora (Saxena and Brahmam, 1994) and available literature. The significance of the plants were noted accordingly and the field note books were also maintained in the centre. Photographs were taken with the help of digital canon Kodak 4.0 mega pixes. 35 mm and the photographs were processed in commercial photographic studios. Strategies for conservation were adopted through conventional methods under in situ condition. Important plants under dried condition were stored in the herbarium.

3. Results and discussion

The KBK districts have been historically rich in forest resources. Though the people have been using these forests very intensively and eking out their livelihood from this source, forests of this region have not received adequate investments and management inputs over the time. Intensive use of forests for sustenance coupled with lack of insufficient investments and managerial inputs are, thus, continuously leading to forest degradation and denudation. Although little more than one fourth (12,957 sq km.) of the geographical area (47,016 sq.m.) of this region is recorded as forests which comes to 27.55%, only 12.45 % (5855 sq km.) is actually dense forest (i.e., with crown density over 40%). It has been further ascertained that 9% (4,332 sq.km) forest area is completely devoid of vegetal cover. Another 15.1% (7,102 sq. km) forests are open having crown density more than 10% but less than 40%(Table I).

3.1. Flora and Vegetation of KBK District

Miscellaneous forests of a moist type dominate the tract of 900 meter Koraput plateau which tends towards semievergreen in the valleys even at low altitudes. Most of the high elevation forests, in this area has been lost under shifting cultivation. The prevailing type of vegetation in the Jeypore plateau is sal, which occupies the greater part of the forest area where it sometimes forms almost pure crops. The general panorama of the lower plains of Malkangiri is that of dry miscellaneous forests intensively associated with bamboos.

Deomali hill of erstwhile Koraput district with 1,667 mt. in height is the tallest hill of the state. The hills are inhabited by a number of tribals but Kandha and Savara communities are numerically rich. In the absence of modern medical facilities in these remote areas, they have been using the plants for treatment of various diseases.

The forests of Bolangir district are situated within the dry deciduous zone of Indian vegetation and are of xerophilous nature. According to the revised classification in the forest types of India by Champion and Seth, the forests of Bolangir division can be classified into two major subgroups i.e. Northern tropical dry deciduous forests 5B and Southern tropical dry deciduous forests 5A. (Champion and Seth, 1968) Gandhamardan hills of 'Ramayana epic frame

Table 1
Areas of the districts of KBK showing different types of forest coverage

Name of Erstwhile district	Name of the district after reorganization	Geographical area (km^2)	Forest area coverage (km^2)			Total forest area (km^2)	Forest area (%)
			Very dense forest (>70%)	Moderate dense forest (40 to 70%)	Open forest (10 to 40%)		
Koraput	Koraput	8807	104	718	872	1694	19.23
Koraput	Rayagada	7073	456	891	1769	3116	44.05
Koraput	Malkangiri	5791	157	703	1326	2186	37.74
Koraput	Nawarangpur	5291	188	462	485	1135	21.45
Bolangir	Bolangir	6575	72	222	640	934	14.20
Bolangir	Sonepur	2337	3	200	121	324	13.86
Kalahandi	Kalahandi	7290	370	743	1218	2331	31.97
Kalahandi	Nuapada	3852	85	481	671	1237	32.11
	Total	47016	1435	4420	7102	12957	27.55

Source: Annonymous (2011), Forest Survey of India, Govt. of India.

forms a natural boundary to the north western side of the district. This range consists of several hills, the highest among them being Gandhamardan of 1020 mt. Gandhamardan makes a name in the indigenous medication map of India. Everybody residing in this region uses plants for one element or the other.

The erstwhile district of Kalahandi occupies the south-western portion of Odisha and is situated between $19^{0}.3'$ and $21^{0}.5'$ N. latitude and $82^{0}.20'$- $82^{0}.47'$ E longitude. The principal hill range of the district belong to Eastern Ghats. It covers almost the entire eastern and southern parts of the district. The flora of ravines and valleys in the district is mostly evergreen and where the few small patches and reserve forests occur the crop inside is that of pure sal, where as the adjacent hill sides are covered with grass and mixed forest. Sunabeda plateau of erstwhile Kalahandi district and presently in Nuapada district is famous for its natural beauty endowed with dense forest and a rich depository of medicinal plants. More than 50% plants available in the plateau used for health care of the ethnic people living in and around the locality.

3.2. User Groups and what they use from forest

In Odisha there are 62 tribal communities recognized by Govt. of Odisha. Amongst them the state has the distinction of 13 primitive tribal groups which is highest in the country; out of which 6 primitive groups are found in KBK district only. They are Bonda and Didayi of Malkangiri district, Lanjia Saora and Dangria Kondha from Rayagada district, Kutia Kondha of Kalahandi district and Bhunjia tribes belonging to Nuapada district (Mohanti, 2007). Beside these six primitive tribal groups, other tribal communities found in the district are, Bhottada, Banjara, Gond, Munda, Paraja, Saora, Mirdha, Lodha.

During the investigation, it was observed that the tribal communities, as usual, are mostly dependant on forest and forest products for their sustenance and livelihood. Further, it is established from their day to day life style, that they are mainly centered around shifting cultivation and collection of non-timber forest products including medicinal plants. On our further investigation, the herbal drugs, they use, are mostly procured from the wild. The observation was strengthened by the report of Task Force, Ministry of Environment and Forest, Government of India, (Annonymous, 2000), that almost 90% of plants used in Indian system of medicine like Ayurved, Unani and Siddha are collected from forest and wastelands. They utilized plant parts like root, root bark, tuber, stem bark, fruits, flowers, leaves and some times the whole plants for medicinal purposes. As such, it is nothing but natural that procurement of those raw materials is not sustainable as it causes loss of biodiversity leading to depletion of medicinal plant species, as well as forest cover, which are of greater concern to look for conservation of those species.

3.3. Conservation Strategy

At present, 90% collection of medicinal plants is from the wild i.e.; from common property resources, where access appears to be neither restricted nor regulated. Since 70% of plants collection involves destructive harvesting, many plants are endangered or vulnerable or threatened. In this context harvesting patterns include the parts of the plants used for medicinal purposes, how it is harvested, timing and frequency of harvest, proportion of target material harvested per plant, per season, the geographic concentration or dispersion of the harvest and the methods used to access the harvest area. In fact, harvesting of medicinal plants is a complex issue requiring analysis of multiple dimensions including social and ecological aspects. In view of this sustainable harvesting and management of medicinal plant resources are not possible without promoting participatory local institutions that can oversee, monitor and enforce regulations so as to derive continuous benefit from these resources. (Patnaik, 2005).

Conservation of threatened and endangered germplasm in general and medicinal plants in particular need special attention keeping in view the degradation and denudation of the existing biodiversity. Although the plants are protected in its own way in the in-situ condition, the continuous interference of human population as well as other biotic interference has appeared as a threat to protect those plants. The ethno-pharmacological informations which are widely used in traditional medicine are quite threatening and the plants are vulnerable for extinction. During the present study, although much work could not be taken to conserve the plant under invitro condition like tissue culture and cryo preservation etc. attempts have been taken for storage of seeds and to maintain its viability. All the possible strategies for in-situ and ex-situ conservation has been attempted, for furtherance of the conservation.

3.4. Some Important medicinal plant species found in KBK districts and their uses

(mentioning “F” as name of the family and “L” as local /vernacular name of the plant).

1. *Achyranthes aspera* L. (F:Amaranthaceae; L:Apamarang)

   Found in ‘Mudulipada’ locality of Malkangiri district. Water extract (20-30 ml) of root is given internally to

hasten delivery during labour pains. Dried powered root (6g) is given with water in epileptic condition to regain consciousness.

2. *Adhatoda vasica* Nees. (F: Acanthaceae; L: Basanga)

Found in Manbhang locality of Gndhamardan Hills. All the five constituents of the tree i.e. root trunk, leave, flower and fruit is boiled in water. The quath mixed with honey is taken for relief from cold, cough and asthma.

3. *Andrographis paniculata* (Burm.f.) Wall.ex. Nees. (F:Acanthaceae; L:Bhuinnim)

Found in Manbhang locality of Gandhamardan hills and Mudulipada area of Malkanagiri district. It is a bitter tonic useful for curing malaria fever, worms, dysentery etc. Decoction of 10-12 leaves (250 ml) is given once daily to check diarrhoea.

4. *Asparagus racemosus* Willd. (F:Liliaceae; L:Shatabari)

Found in Mudulipada, Dantipada area of Malkangiri district. Roasted root is given with cow's milk twice a day in post-delivery complaints, like abdominal pain and body ache.

5. *Barringtonia acutangula* Gaertn. (F:Barringtoniaceae; L:Hinjala)

Found in Khuripani and Beherapani area of Gandhamardan hills. Powdered seed with little water is taken internally on empty stomach at 3 hours interval for severe colic pain caused due to liver trouble. Leaf juice 3 ml with honey 3 ml taken to treat amoebic dysentery.

6. *Bauhinia vahlii* Wight & Arn. (F:Caesalpiniaceae; L:Siali leaf)

Found in Chaldhar and Bhitarkhol of Gandhamardan hills and also in Sunabeda plateau of Nuapada district. The decoction of seeds is given twice daily as tonic to children, taken at night as an aphrodisiac. Leaves are used as contraceptive.

7. *Bridelia retusa* (L.) Spreng. (F:Euphorbiaceae; L:Kasi)

Found in Sunabeda plateau of Nuapada district and Mudulipada area of Malkangiri district. Stembark powder given with water to give relief to abdominal pain.

8. *Bryonopsis laciniosa* L. (F:Cacurbitaceae; L:Shivalingi)
Found in common thickets of Batipathar area of Gandhamardan hills. The seed powder with a spoon of honey is taken twice daily to ensure conception and prevent miscarriage.

9. *Bryophyllum pinnatum* (Lam.) Oken.(F:Crassulaceae; L:Amarpoi)

Found in Bainsapada area of Malkangiri district. Leaf paste is applied over stomach to relieve abdominal pain.

10. *Buchanania lanzan* Spreng. (F:Anacardiaceae ; L:Char)

Found in Harisankar, Kapildhar area of Gandhamardan hills. The oil extracted from the kennels mixed with the fruit paste of Sahaj (*Terminalia alata*) is applied externally to get relief from muscular pains, rheumatism, glandular swelling of the neck.

11. *Butea monosperma* (Lam.) Taub. (F:Fabaceae ; L:Palas)
Found in Sunabeda plateau of Nuapada district and Harisankar, Nandapara locality of Gandhamardan hills in Bolangir district.

Seed ash (2 gm) with cold water given once a day for 3 days after menstruation to prevent pregnancy. The red coloured gum, called Bengal Kino are obtained from tree and is valuable for treatment of diarrhoea.

12. *Capparis zeylanica* L. (F:Capparaceae ; L:Asadhua)
Available in Mahadevjharan and Dukura area of Gandhamardan hills. Root extract cures syphilitic disease. The leaf extract is prescribed for external application on swelling, rheumatism and boils.

13. *Cassia histula* L. (F:Caesalpiniaceae ; L:Chakunda)

Available in Mudulipada area of Malkangiri district. Seeds are given as animal feed to check epidemic disease among fowls.

14. *Centella asiatica* (L.) Urb. (F:Apiaceae; L.Thalkudi)

Found in Sileiguda area of Malkangiri district. Quite common in Gandhamardan hills.

Leaf is eaten as vegetable as a tonic. The leaves or the entire plant parts are boiled in water and this decoction is given for treatment of leprosy.

15. *Curculigo orchioides* Gaertn. (F:Liliaceae; L: Talmuli)

Found in Kisanipoda area of Malkangiri district. Paste of rhizomes is bandaged in whitlow.

16. *Datura fastuosa* L. (F:Solanaceae; L: Kaladudura)

Found in Khemaguru area of Malkangiri district Seeds with carum, cardamom, clove and ginger in equal

quantities made into powder and 250 mg given twice a day in joint pain.

17. *Dioscorea bulbifera* L. (F: Diascoreaceae; L:Pita Alu)

Found in Mahadev-jharan locality of Gandhamardan hills. Tubers are edible and nutritious. Eaten in times of famine after boiling and much preparation.

18. *Diospyros melanoxylon* Roxb. (F:Ebenaceae; L:Kendu)

Found in Sunabeda plateau of Nuapada district and Bardaguda area of Malkangiri district. Tender leaf juice taken orally and unripe fruit is eaten for relief from nagging cough.

19. *Emblica officinalis* Gaertn. (F: Euphorbiaceae ; L: Anla)

Found in all the KBK district, specifically in Sunabeda plateau, Gandhamardan hill and Mudulipada area of Malkangiri district. Dried fruit with fruit of Beleric myrobolan made into powder, mixed in equal quantity, given three times a day to check cold. Fruit is eaten raw, as a supplement for Vitamin C.

20. *Erythrina resupinata* Roxb. (F:Fabaceae ; L:Badokanda)

Found in Nrusingnath area of Bolangir district. Root powder (10gm) is prescribed for rheumatism. Root (10 gm) grounded with 1 gm each of 'pipli' (*Piper longum* L.), clove, ginger, black pepper, kalazira and chilies given orally for treatment of leprosy.

21. *Erythrina variegata* L. (F:Fabaceae; L: Rinki)

Found in Mudulipada area of Malkangiri district. Twig is used as tooth brush to reduce toothache.

22. *Ficus hispida* L.f. (F:Moraceae ; L:Pani Dimri)

Found in Bondapoda area of Malkangiri district. Latex is diluted and given internally to check diarrhoea in children.

23. *Flemirgia chhapar* Buch. – Ham. ex. Benth. (F:Zingiberaceae ; L:Banhaldi)

Found in Guptijharan area of Gandhamardan hills. 1-2 drops of juice extracted from fresh seeds are applied on eyes to get relief from catract and related eye troubles.

24. *Helicteres isora* L. (F:Sterculiaceae ; L:Modimodika)

Found very commonly in Balipathar area of Gandhamardan hills and also in Mudulipada area of Malkangiri district. The paste made from the fruit is applied externally on the stomach to get relief from flatulence.

25. *Hemidesmus indicus* (L.) R.Br. (F:Periplocaceae ; L:Ananta mula)

Found in Khandijharan area of Gandhamardan hills. Also in Mudulipada area of Malkangiri district. Ten gm. of powdered root mixed with 3 gm. of stem bark of Jamun (*Syzygium cumini*) is given with water in empty stomach, within 3 days of delivery to increase lactation. Powdered flowers are given for cold, cough and asthma.

26. *Heterostemma tanjorense* Wight & Arn. (F:Asclepiadaceae ; L: Badobhulan)

Found in Harisankar area of Bolangir district. As a witch craft paste applied in forehead to drive away impacts of evil spirit.

27. *Holarrhena antidysenterica* Wall. (F:Apocynaceae; L: Kurei).

Found in Mudulipada area of Malkangiri district and Batipathara, Harisankar area of Gandhamardan hills. Fresh root juice is given internally on empty stomach for deworming in children. The dried bark of the plant constitutes the drug 'Kuruchi'. The chief use of this drug is in curing amoebic dysentery.

28. *Ipomea batatas* L. (F:Convolvulaceae; L:Kandamula)

Found in Bondopada area of Malkanagiri district. Leaf juice is applied locally in snakebite.

29. *Madhuka indica* Gmel. (F:Sapotaceae; L:Mahula)

Found in Sileiguda locality of Malkangiri district. Decoction of bark is used in stomachache.

30. *Mallotus philippinensis* (Lam.) Muell –Arg. (F:Euphorbiaceae ; L:Sindurgundi)

Found in Dantipada locality of Malkangiri district. Fresh leaf juice is given internally in dysmenorrhoea.

31. *Mangifera indica* L. (F:Anacardiaceae; L:Amba)

Found in Khemaguru and Dantipada area of Malkangiri district. Powdered stem bark is given with jaggery once a day in abdominal pain. Bark juice is given to check diarrhoea.

32. *Martynia annua* (L.) (F:Martyniaceae; L:Baghanakhi)

Found in Paikamal locality of Gandhamardan hills in Bolangir district.

The oil extracted from the seed is applied locality on itches, scabies and wounds. Flower paste is also applied locally to prevent skin infection.

33. *Mimosa pudica* L. (F:Mimosaceae ; L: Lajkuli)

Found in Dantipada,, Mudulipada locality of Malkangiri district. Leaf paste is used externally in snakebite. Root powered with water and liquid paste taken twice a day to prevent diarrhoea.

34. *Mucuna nigricans* (Lour.) Steudel, (F:Fabaceae; L:Baidanka)

Found in Panchupandav ghat of Harisankar locality in Gandhamardan hills. The seed paste is used as a local application for the treatment of ulcers of genital organ of both sexes.

35. *Nyctanthes arbortristis* L. (F:Oleaceae; L:Gangasiuli)

Found in Mudulipada locality of Malkangiri district. One tea spoonful leaf decoction is given with honey twice a day for 3 days in intermittent fever.

36. *Sida rhombifolia* L. (F:Malvaceae; L:Bajramuli)

Found in Khemagura area of Malkangiri district and also found in Sunabeda plateau of Nuapada district. Crushed leaves are applied on cuts and wounds. Used as toothbrush in Sunabeda plateau area by Chuktia Bhunjia tribe

37. *Smilax zeylanica* (L.); (F: Smilaceae; L: Mumbarai)

Found in Mudulipada area of Malkangiri district. Twigs are used as tooth brush for dental care. Leaves are used as plates for serving food.

38. *Sterculia urens* Roxb. (F:Sterculiaceae ; L:Genduli)

Found in Bondapada locality of Malkanagiri district. Gum is given with sugar candy for remedy from Diarrhoea & Dyspepsia.

39. *Strychnos nox-vomica* L. (F:Longaniaceae ; L:Kochila)

Found in Koraput district. Tree bark mixed with Neem bark given orally for prevention of dysentery.

40. *Woodfordia fructucosa* (L.) Kurz. (F:lythraceae ; L:Dhatki)

Found in Khemaguree and Mudulipada area of Koraput district. Alro found in Panchapandav ghat and Khandijharan of Gandhamardan hills. Bark is added to the brew to enhance the intoxicating properties of country liquor. Sweet flower juice is sucked by children. Intake of the decoction of the bark is prescribed to treat impotency of man and decoction of root is used for curing ulcers and boils.

Acknowledgement

The authors are thankful to C.C.F. Development, Forest & Environment Deptt., Govt. of Odisha, for providing herbarium facility.

References

Anonymous, (2000). Report of the Task force on conservation and sustainable use of medicinal plants - Planning commission, Govt. of India.

Anonymous, (2006). Resolution of KBK district-Planning & co-ordination department, Govt. of Orissa.

Anonymous, (2011). India State of Forest Report 2011-Forest Survey of India, Ministry of Environment and Forest, Dehradun.

Champion, H.G. and Seth, S.K. (1968). A Revised Survey of Forest types of India. Manager of Publications, Government of India, New Delhi.

Mohanti, K.K. (2007). PTGs and Biodiversity conservation Adivasi, Vol 47 (1&2) June-December 2007; Scheduled castes & Scheduled Tribes Research and Training Institute, Bhubaneswar, Odisha.

Mohanty, L. (2011). Odisha Reference Annual (ed.), Information and Publication Department Govt. of Odisha, Bhubaneswar

Patnaik, B.K. (2005). Conservation and Sustainable Harvesting of Medicinal plants from the wild; In Mohanty, B.K (ed.): Revival of Local Health Traditions and Conservation of Medicinal Plants. Sambandha, Odisha, India; pp 25-30.

Saxena, H.O. and Brahman, M. (1994). The flora of Orissa - Orissa Forest Development Corporation Ltd., Bhubaneswar.

Plant Science Research 35 (1&2) : 20-23, 2013

Plant Science Research

ISSN 0972-8546

Plant diversity in the coastal region of Odisha with special reference to the medicinal plants

R. N. Rath[1], S. L. Sahoo[2] and S. P. Rath[2Ψ]

[1] Department of Botany, Talcher (Autonomous) College, Talcher, Odisha 759107

[2] Departments of Botany, Utkal University, Bhubaneswar, Odisha 751004

ARTICLE INFO

Article history:
Received : 6 December 2012
Received in revised form : 1 November 2013
Accepted : 8 November 2013

Keywords:
Plant diversity
Coastal vegetation
Medicinal plants
Health care

ABSTRACT

India is a country of great diversity in its tradition, culture, language, geography, climate and vegetation. The peninsular India is having a coast line of 5690 Km of which 482 Km long Oidsha coast experiences the sub-humid climate showing strand and estuary vegetation due to the major rivers like; the Subarnarekha, the Budhabalanga, the Baitarani, the Brahmani, the Mahanadi, the Devi and the Rushikulya contributing to the coastal plain of Odisha and making it the "Rice Bowl" of the State.The floristic account in the deltaic region of the coast line comprising of four erstwhile major districts of the state like Baleswar, Cuttack, Puri and Ganjam have revealed 332 species belonging to 200 genera and 82 families. Plants belonging to different habit and habitat and plant parts like stem, root, leaf, flower, bud, fruit and twig etc. were also recorded. The role of the local healers, ayurvedic practitioners, experienced men and women making traditional healthcare were also noted.The importance of the medicinal plants used by the rural and tribal people have also been highlighted during the present investigation.

1. Introduction

India is the second largest populous country in the world. It is a country of great diversity not only because of its vegetation and floristic variation but also because of diversity, both, in anthropogenic and physiographical aspects. So far as the geography is concerned, India is surrounded by the Great Himalayan mountain range in the North, Arabian Sea in the West, Bay of Bengal in the East and Indian ocean in the South, which influence the climatic condition of this great country. According to the geographical location and climatic conditions, the Indian landmass is divided into five different types like the Northern plains, the North East hilly regions, the peninsular plateau, coastal plains and the Thar Desert. However, in this diversified physical scenario, the country is having a coast line of nearly 5690 Km long from East to West (Rao and Sastry, 1974). It is of great concern that the floristic vegetation of the coastline has not yet been properly studied, which needs a thorough investigation emphasizing on the coastal flora of India in general and the State of Odisha, in particular. Since the coastal regions comprise a diverse eco-system and possess many interesting aspects for ecological, physiological and phyto-geographical features, it is necessary to study these aspects in detail. During the present work, concentrating on Odisha, some physiologically specialized and ecologically adapted evolved plant species were found to survive in the saline water as well as in sensitive eco-system. The East coast runs in wide curve, changing direction from North to North East at16^{0}latitude and covers the states of Tamil Nadu, Andhra Pradesh, Odisha and West Bengal (Rao, 1971). The climate along the coastline is relatively uniform throughout and it has been classified into the five climatic groups of which the Odisha coast experiences, mostly, the sub humid climate (Thornthwaite, 1948). Out of the entire length of the coastline of India, Odisha covers 482 km only (Pattnaik *et al.,* 2008) which is nearly 8.4% of the total coastline of India. The vegetation of Odisha coast appears to be peculiar due to wide range of diverse demography and climatic changes.The vegetation

Ψ *Corresponding author; Email:* shibaprasad2001@yahoo.co.uk

of the coastal Odisha seems to be quite mosaic because of the deltaic regions formed at the mouth end of some important rivers like Subarnarekha, Mahanadi, Devi and Rushikulya. Rao and Sastry (1974) were of the opinion that the mixture of coastal sand dunes and inland vegetation divide the coastal vegetation into two subtypes like Strand Vegetation and Estuarine borderland vegetation.

2. Materials and methods

Intensive exploration to collect information from the people of different villages of erstwhile coastal districts of Baleswar, Cuttack, Puri and Ganjam was made. Before the collection of information and data, liaison and survey was made in different areas of the coastal region. The interactions with the people were done, which were on different aspects such as their lifestyle, food habit, socio-economic standard, education and health care practices. Observations on the people residing in the village were also taken concerning their traditional system of herbal healthcare practices based on the plants and plant products. It was also observed at the time of interaction and discussion that they go for this folklore herbal treatment with medicinal plants available in nature.

List of locally available plants used for the treatment and health care, were identified. Photographs of the plants were neatly taken and kept for documentation. The herbaria of the plants were prepared for reference. The botany of the plants was enumerated to ensure the co-relation between the morphology and its medicinal use. The information like name of the plants, their local name, places of availability, plant parts used and name of the diseases etc. were also prepared for furtherance of the work.

3. Result and discussion

During the present investigation it was observed that the deltaic region at the mouth end of the river Subarnarekha, Budhabalanga and the Dhamara exhibited the flora of an estuarine complex (Behuria,1992b) and the important species in the floristic component are *Avicennia marina, A. officinalis, A. alba, Sonneratia apetala, Premna corymbosa, Aegiceras majus, Rhizophora mucronata, Bruguiera gymnorrhiza, Caesalpinia nuga, Excoecaria agallocha, Hibiscus tiliaceus, Clerodendrum inerme* and *Acanthus illicifolius*, The floristic view across the coastline in the deltaic region of the Mahanadi is very rich and varied comprising of 750 genera belonging to 120 families (Behuria,1992c) and widely distributed families are Verbenaceae, Caesalpiniaceae, Fabaceae, Mimosaceae, Poaceae, Euphorbiaceae, Rubiaceae, Asteraceae, Lamiaceae, Moraceae, Arecaceae and Cyperaceae. Among other important families are Dipterocarpaceae, Malvaceae, Meliaceae, Anacardiaceae, Rhizophoraceae, Cucurbitaceae, Combretaceae, Ebenaceae and Apocyanaceae. The important taxa are *Mimosa pudica, Jatropha gossypifolia, Annona squamosa, A. reticulata, Argemone mexicana, Aegle marmelos, Parkinsonia aculaeta, Tridax procumbens, Martynia diandra, Alocasia macrorrhiza, Hyptis suaveolens, Datura metel, Scoparia dulcis, Adhatoda vasica,*etc. Some very common orchard species *Mangifera indica*, *Artocarpus hetrophyllus*, *Psidium guajava,* etc. and the less common species are *Anacardium occidentale, Dillenia indica*, *Citrus sinensis* and *C.aurantifolia*. The palms grown here are *Borassus flabellifer*, *Cocos nucifera*, *Phoenix sylvestris,* etc. Hedges like *Duranta repens*, *Lawsonia inermis*, *Ipomoea carnea,Vitex negundo* and *Bambusa tulda* are widely planted on fence. The common shade trees are *Ficus religiosa* and *Ficus benghalensis*. The common pond species are *Nelumbo nucifera* and *Nymphaea nauchali*. The other wetland and aquatic species like *Myriophyllum indicum*, *Ludwigia adscendens*, *Pistia stratiotes*, *Trapa bispinosa, Nymphoides indica. Hydrilla verticillata, Ottelia alismoides, Ceratophyllum demersum, Vallsineria natans* and, *Eichhornia crassipes* widely grow on ponds, canals and water bodies. However the western hilly region and adjoining forest plains are having a lot of forest trees like; *Shorea robusta, Pterocarpus marsupium, Ougenia oojeinensis, Adina cordifolia, Xylia xylocarpa,Terminalia tomentosa, Anogeissus latifolia, Bombax ceiba, Chloroxylon swietiana, Diospyros melanoxylon* etc found naturally occurring in the forest. Besides the other large number of species like *Dillenia pentagyna, Lannea coromandelica, Garuga pinnata, Protium cerratum,Fagara budrunga, Alstonia scholaris, Anogeissus acuminata, Pongamia pinnata, Albizia lebbeck, Pterospermum heyneanum, Miliusa velutina, M. tomentosa, Lepisanthes tetraphylla, Putranjiva roxburghii, Polyalthia cerasoides, Mallotus philippensis, Streblus taxoides* etc. the herbs, *Rouvolfia serpentina, Hemidesmus indicus, Gymnema sylvestre, Asparagus racemosus* etc. are very common. The region has got sporadic population of different types of Bamboo species and *Bambusa arundinacea* and *Dendrocalamus strictus* are very prominent. The vegetation across the Puri district coastal region is featured with deltaic types of vegetation having beautiful groves of coconut palms, mangoes and raised gardens. Most part of the deltaic region of the district are sandy ridges covered with thorny plants and some places are covered with creepers belonging to the genus *Convolvulus* of the family Convolvulaceae (Senapati and Kuanr, 1977). The deltaic region of the river Devi exhibits the vegetation of mangrove forest. The cultivated plains in between hill region and sea-coast are met with the weeds of rice fields,while the wet lands and the water bodies are filled with the hydrophytes including dangerous water

hyacinth in and around the villages which is a characteristic feature in the flora of the coastal Orissa. The hill region is dominated with dry ever green forest and comprise the forest tree species like *Shorea robusta, Pterocarpus marsupium, Diospyros chloroxylon, Crateva magna, Terminalia alata* etc. Other common plants are bamboos, canes, *Bauhinia vahlii, Milletia auriculata.* The forest vegetation of the Western region of this coastal segment chiefly comprises the *Terminalia tomentosa, T. alata, Eugenia jambos, Diospyros chloroxylon, Bridelia stipularis, Lagerstroemia reginae, Dalbergia sissoo, Careya arborae,Adina cordifolia, Schleichera oleosa*and *Cassia siamea.* Common shrubs species are *Flemingia bracteata*, *Indigofera tinctoria, Oldenlandia corymbosa, Woodfordia fruticosa, Ixora coccinea, Butea monosperma,*etc.and common climber is *Combretum decandrum*. It is interesting to note that most of the medicinal plants, under report, belong to the families as mentioned earlier. The floristic feature of the southern coast is quite rich in view of the forest coverage in the larger part of the erstwhile Ganjam district (Behuria,1992a). The plants in the vegetation are mostly represented by forest plants which are distributed in three forest divisions such as i) Paralakhemundi, ii) Ghumusar North, and iii) Ghumusar south. The sea coast vegetation is inundated by the sea are covered with the xeric psamophytes like *Casuarina equisetifolia*, *Pandanus fascicularis* and some gigantic grasses like *Vetiveria zizanioides*, *Eragrostris coarctata* and *Desmostachya bipinnata* etc. The vegetation pattern in this region changes under variable environmental factors such as the periodic input of forest water which changes according to tidal level.

Out of all the plants collected during this investigation, it is observed that 332 species are having medicinal importance. Of all these plants, about 58 species were found to be common in all coastal districts. Those are *Azadirachta indica, Acanthus illicifolius, Derris trifoliata, Ficus hispida, Hygrophila auriculata, Premna corymbosa, Salvadora persica, Tamarix gallica* and fifty others. The medicinal plant species with their plant parts are important due to their use for healthcare treatment like common cold, fever, dysentery, stomach disorders and some skin diseases. People used to take these traditional plant medicine for their treatment unless otherwise they are forced, under circumstances, to go for any alternative. On the other hand, these types of treatment are not only viable economically, but also, easily available at hand in their surroundings. These plants were also reported earlier by several workers like Subudhi *et al.* (1992), Satpathy and Brahmam (1999), Das *et al.* (2003), Baske and Sur (2010), the Vaidyas and other ethno botanical practitioners. Plants collected during this work were thoroughly scrutinized in the field and through the literature from where some interesting information could be revealed. The leaves of eighty species amounting 24% of the total number of 332 are very useful for different diseases. Similarly the stem, bark, root, flower, fruit, buds, seeds and other plant parts were studied in detailed (Fig.1).

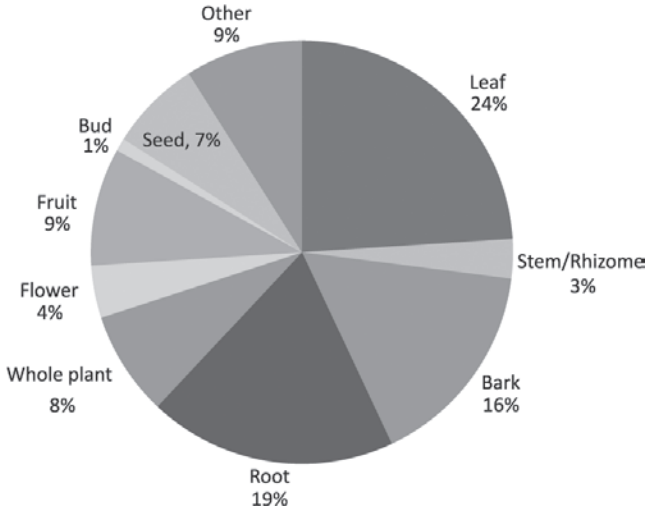

Fig.1 Proportion of the various plant parts used for herbal cure of various diseases in coastal regions of Odisha.

It is revealed from the observation that the leaves, roots and barks of the plants used, appear to be quite high as compared to the other parts. This particular observation substantiates the age old practices of traditional healers and the Vaidyas upholding the views given by several scientists as to why leaves, stem and bark of the plants need to be taken care of.The other plant parts like flower, bud, seed,etc. have also been noticed of their use, which is indisposed due to their significant role for curing some of the important diseases. During the present investigation, an account of diseases cured by these said plants and plant parts were prepared and it was found that nearly 122 of different diseases could be cured. However, in order to signify the diseases and the plant parts used, ten (10) number of plants have been selected which have got maximum use of the plants as a whole or the plant parts to cure those diseases (Fig. 2).

Incidentally, those ten diseases like diarrhea, dysentery, blood dysentery, cough and cold, dental care,and skin diseases etc.were found to be treated by the medicinal plants. On studying the diseases, it could be observed that those diseases are of very common type. It is heartening to note that, the serious diseases like Cancer, Blood Pressure, Diabetes and Cardiovascular disorders etc. have not been significantly treated by medicinal plants, despite the facts, people of ethno medicines, Vaidyas and Ayurvedic practitioners have claimed that those diseases can be cured

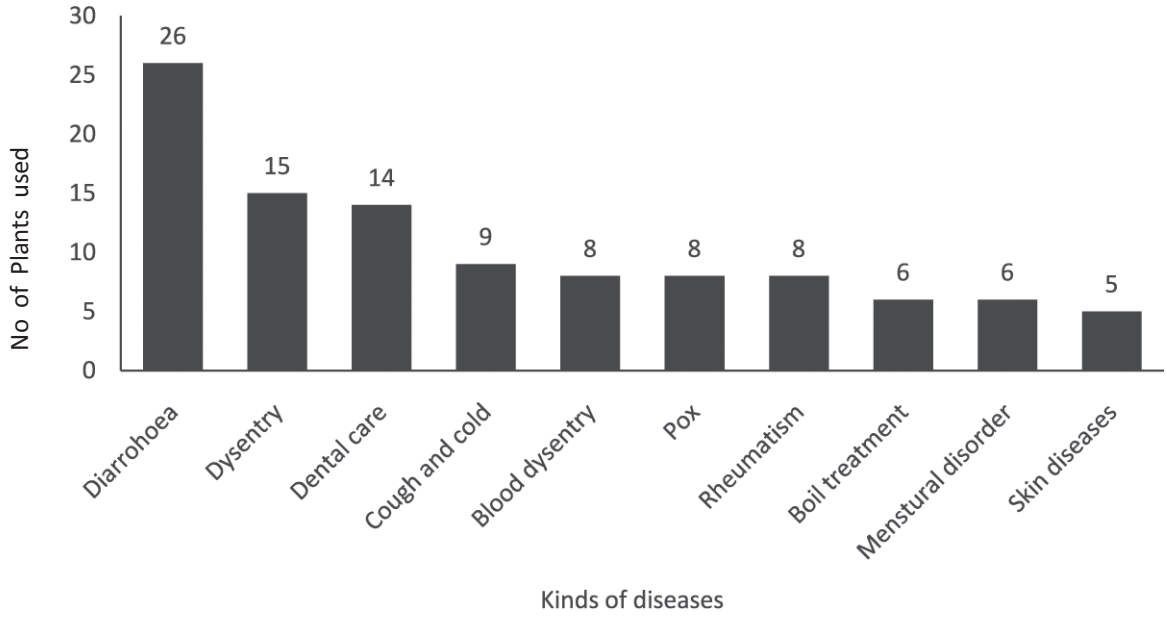

Fig. 2 Number of plants and plant parts used for herbal cure of ten important diseases in coastal regions of Odisha.

by the use of medicinal plants. Now it is an open challenge, faced by the people working on medicinal plants, as to how they can enhance the confidence of common people as regards the use of medicinal plants for those dreaded diseases.

Acknowledgement

The authors express their thanks to the Head of the P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneshwar for providing the facilities to undertake this work. Thanks are due to the Principal, Talcher Autonomous College, Talcher for allowing to pursue this work under study leave.

References

Baske, P. K. and Sur, P. R. (2010). Ethnobotanical study of Gajapati District of Orissa. J. Econ. Taxon. Bot. 34(2):317-322.

Behuria, N. C. (1992a). Orissa District Gazetteer. Ganjam, Gazetteers unit, Department of Revenue, Govt. of Odisha.

Behuria, N. C. (1992b). Orissa District Gazetteer. Baleswar, Gazetteers unit, Department of Revenue, Govt. of Odisha.

Behuria, N. C. (1992c). Orissa District Gazetteers. Cuttack, Gazetteers unit, Department of Revenue, Govt. of Odisha.

Das, S., Dash, S. K. and Padhy, S. N. (2003). Ethno medicinal informations from Orissa state, India, A Review. J. Hum. Ecol. 14(3):165-227

Pattnaik, C., Reddy, C. S. and Dhal, N. K. (2008). Phytomedicinal study of coastal sand dune species of Orissa. Indian J. Traditional Knowledge 7(2):263-268.

Rao, T. A. (1971). Distributional resume of the maritime strand flora of India. Bull. Bot. Surv. India 13(3&4):192-202.

Rao, T. A. and Sastry, A. K. (1974). An outline of coastal vegetation of India. Bull. Bot. Surv. India 16(1-4):101-115.

Satpathy, K. B. and Brahmam, M. (1999). Some intresting phytotherapeutic claims of tribals of Jajpur district (Orissa), India.J. Econ. Taxon. Bot. 34(2): 467-472.

Senapati, N. and Kuanr, D. C. (1977). Orissa District Gazetteers.Puri, Gazetteers unit, Department of Revenue, Govt of Odisha.

Subudhi, H. N., Choudhury, B. P. and Acharya, B. C. (1992). Some Potential Medicinal Plants of Mahanadi Delta in the State of Orissa. J. Econ. Tax. Bot. 16(2): 479-487.

Thornthwaite, C. W. (1948). An approach toward a rational classification of climate. Geogr. Rev. 38: 55-94

Plant Science Research 35 (1&2) : 24-28, 2013

Plant Science Research

ISSN 0972-8546

Alterations in the activities of antioxidative enzymes and increase in lipid peroxidation level in germinating mungbean seeds exposed to cobalt stress

Mausumi Nandy, Kabita Behera and S. K. Dey[Ψ]

P.G. Department of Environmental Science, Fakir Mohan University, Vyasa Vihar, Balasore 756020, India

ARTICLE INFO

Article history:
Received : 20 August 2013
Received in revised form : 1 November 2013
Accepted : 4 November 2013

Keywords:
catalase
germination
malondialdehyde
peroxidase
soluble protein
superoxide dismutase

ABSTRACT

Mungbean (*Vigna radiata* (L.) Wilczek) seeds were subjected to germination in presence of $CoCl_2.6H_2O$ (0.5, 1.0 and 10.0 mM) for 48 h and different physiological parameters like soluble protein content, activities of antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) along with the lipid peroxidation level of the embryonic tissues were estimated in order to determined the effect of Co toxicity in germinating seeds. With increase in Co concentrations, the germination percentage decreased by 15% up to 1 mM which further reduced to 25% at 10 mM $CoCl_2.6H_2O$. Soluble protein content increased at 0.5 mM $CoCl_2.6H_2O$, but decreased further and at 10 mM concentration it was decreased by 53%. SOD activity was enhanced which indicated that there was protection against superoxide radical, but at the same time possibility of H_2O_2 accumulation as a dismutation product could not be ruled out. Since there was decrease in the activities of both catalase (CAT) and peroxidase (POX), protection against H_2O_2 was poor. Because of the alterations in the activities of these key enzymes, imposition of oxidative stress was presumed which was in fact observed in the form of increase in lipid peroxidation level in the tissues. The findings of the study indicate that Co at higher concentrations is toxic to the germinating seeds and imposition of oxidative stress might be one of the mechanisms behind such toxicity.

1. Introduction

Cobalt is a natural earth element that occurs in many different chemical forms in our environment. This element is present in trace amounts in soil, plants and also in our diets. In two valence states, i.e., Co II and Co III, it forms a number of organic and inorganic salts. Naturally cobalt occurs in association with other metals such as copper, nickel, manganese and arsenic. Cobalt and its salts are used to make superalloys, as paint drier, as a ground coat for porcelain enameling used on steel bathroom fixtures and large appliances; and also as an ingredient of coloured pigments. In environment, the natural sources of cobalt are soil, dust, seawater, forest fires and volcanic irruptions, burning of coal and petroleum products and also the industrial processes that use cobalt and its compounds in their manufacturing units. Cobaltite, smaltite and erythrite are the minerals in which cobalt occurs (Barceloux, 1999). The soil pollution with Co occurs mainly from mining and smelting activities, use of fertilizers and spreading of sewage sludge (Williams *et al*., 1985; Hamilton, 2000). Even though Co is essential as a micronutrient in trace amount by plants, higher concentrations have been reported to cause toxic effects (Chatterjee and Chatterjee, 2003; Osman *et al*., 2004). Studies on the adverse effects of Co on terrestrial ecosystems are a few. Some reports on the toxicity of Co on soil microbes and invertebrates are, however, available (Lock *et al*., 2004, 2006). Literature regarding toxic effects of Co on higher plants is limited. In this study, therefore, attempts have been made to assess the effect of Co on the antioxidative efficiency of germinating mungbean seeds under laboratory conditions.

2. Materials and methods

Fresh mungbean (*Vigna radiata* (L.) Wilczek) seeds were collected locally and healthy seeds for uniformity of

[Ψ] *Corresponding author; Email:* surjendufmu@rediffmail.com

size were selected. The seeds were then surface sterilized with freshly prepared 3% filtered solution of bleaching powder (calcium oxychloride, $CaOCl_2$) for 30 min followed by several washings with distilled water for another 30 min. Then the seeds were spreaded over filter paper moistened with 10 ml solutions of different concentrations of $CoCl_2.6H_2O$ (0.5, 1.0 and 10.0 mM) in separate Petri dishes. 10 ml of distilled water was taken in another Petri dish as control and 20 seeds were taken in each Petri dish. The seeds were allowed to germinate in dark at 30 ± 2^{0}C for 48 h and after that germinated seeds were collected for analytical studies.

After 48 h of germination, the number of seeds germinated in each case was counted. The embryonic tissues were collected separately (discarding the endosperm portions) and homogenized with sodium phosphate extraction buffer (0.0.5 M) in a mortar and pestle kept in an ice-bath. The pH of buffer was 7.4 for superoxide dismutase (SOD) and 7.5 for catalase (CAT) and peroxidase (POX). The homogenates were centrifuged in a cooling centrifuge (17000*g*) for 10 min at - 4^{0}C and supernatants were collected for assay of enzyme activities after suitable dilutions. Superoxide dismutase (EC 1.15.1.1) activity was assayed following the method of Das *et al*. (2000) and in this method inhibition of superoxide driven nitrite formation from hydroxylamine hydrochloride by SOD was measured spectrophotometrically at 543 nm. The activity was calculated by deducting one from the ratio of absorbance of the control (without enzyme) and sample (with enzyme). The SOD activity was expressed in unit, defined as the amount that inhibits the superoxide driven nitrite formation from hydroxylamine hydrochloride by 50% under the assay conditions. Catalase (EC 1.11.1.6) activity was measured following the method of Aebi (1983) taking H_2O_2 as the substrate and the rate of decreasing concentration of H_2O_2 due to the enzyme was measured spectrophotometrically at 240 nm. The activity (katal) was calculated using the extinction coefficient of 40.0/mM. cm for H_2O_2 at 240 nm. Peroxidase (EC 1.11.1.7) activity was assayed taking H_2O_2 and guaiacol as substrate and reduced co-substrate respectively following the method of Kar and Feierabend (1984). The rate of increase in the colour intensity in reaction mixture due to formation of tetraguaiacol was recorded spectrophotometrically at 470 nm and the activity (katal) was calculated using the extinction coefficient of 26.6/mM. cm due to tetraguaiacol formation.

A portion of the enzyme supernatant, for each sample, was mixed with equal volume of 20% (w/v) trichloroacetic acid (TCA) and kept in refrigerator for over night in order to facilitate the precipitation of buffer soluble protein. The precipitates were washed subsequently with 10% TCA, absolute alcohol, alcohol and chloroform (in a proportion of 3:1), alcohol and ether (in a proportion of 3:1) and finally with ether. After that, the pellets were air dried and re-suspended with 0.3 N NaOH solutions for 16 h at 37^{o}C. Then the samples were centrifuged and supernatants were collected as soluble protein extract. The protein content was estimated following the method of Lowry *et al.* (1951). Soluble protein content of the tissue was calculated from a standard curve drawn with bovine serum albumin as protein.

For determination of lipid peroxidation in the tissues malondialdehyde (MDA) was estimated as thiobarbituric-acid-reactive material from tissue extracts. The extraction was done in 5% (w/v) TCA and MDA content was estimated following the method of Heath and Packer (1968). During this estimation the unspecific turbidity in the reaction mixtures was corrected by subtracting the absorbance at 600 nm, and for absorbance at 532 nm originating from extract after incubation without thiobarbituric acid.

All the experiments were performed at least for three times with three replicates in each time. The mean values are presented and the standard deviations are also indicated.

3. Results and discussion

It has been reported in many studies that the supra-normal doses of different heavy metals cause relatively high toxicity, which are mostly reflected in terms of growth inhibition of plants accompanied by chlorosis of young leaves and other disorders including alterations in the antioxidative efficiency (Daniels *et al*., 1972; Kaer *et al*., 1998; Dey *et al*., 2007; 2009 a, b). In cauliflower it was reported by Chatterjee and Chatterjee (2000) that the activities of several enzymes including that of iron containing enzymes are disturbed by excessive amounts of heavy metals like Co, Cr and Cu. Among these metals Co has been found to readily translocated from old to new tissues (Handrek and Riceman, 1969). Co is usually translocated through xylem sap as divalent cation in plants (Tiffin, 1967).

Germination is an important phase in the growth and development of plant life during which upon absorption of water the tender embryonic tissues in the forms of radicle and plumule emerge out of the seed by rupturing the seed coat. Exposure of the embryonic tissues to any type of stress is expected to have adverse effect not only to the growing seedlings but also to the plants towards the later stages of growth and development. In this study it was found that with increase in Co in the medium, there was decrease in germination percentage and at highest concentration of the metal (i.e., 10 mM $CoCl_2$), 25% decrease

in comparison to the control seeds was recorded (Table 1). Soluble protein content of the embryonic tissues also decreased by 17% and 53% in the tissues exposed to 1.0 mM and 10.0 mM $CoCl_2$ respectively (Table 1), but at 0.5 mM $CoCl_2$, there was about 7.5% increase in comparison to control tissues. This shows that up to 0.5 mM concentration, probably Co was beneficial for the tissues as a micronutrient and beyond that it was toxic. The decrease in the soluble protein content is an indirect indication of the alteration in the activities of enzymes due to Co toxicity.

The antioxidative enzymes play a very important role in preventing the build up of reactive oxygen species (ROS) inside the cell. It has been reported by many workers that the toxicity due to exposure to different heavy metals like Cd (Schutzendubel *et al*., 2001), Pb (Verma and Dubey, 2003), Cd and Pb (Dey *et al*., 2007), Al (Cakmak and Horst, 1991), Cu (Srivastava *et al*., 2006; Dey *et al*., 2009 b) and Cr (Dey *et al.,* 2009 a) have altered the activities of antioxidative enzymes and have consequently imposed oxidative stress in the plants. In this study it was found that SOD activity increased in the embryonic tissues up to 1.0 mM $CoCl_2$ which further declined at 10 mM $CoCl_2$ but it was more than that of the control tissues (Fig. 1). The SOD catalyses the dismutation of superoxide radicals to hydrogen peroxide and oxygen and thus reduces the build up of superoxide radicals in the cell (Halliwell and Gutteridge, 2007). But at the same time, the increased SOD activity also increases the cellular levels of toxic H_2O_2 and therefore, decomposition of H_2O_2 is very vital. Thus, in this study even though protection against superoxide radical was there with increased Co concentration in the medium, but the possibility of increase in H_2O_2 concentration could not be ruled out.

Catalase is the principal enzyme responsible for decomposition of H_2O_2 to H_2O and O_2 and thereby reduces its toxicity (Elstner, 1982; Halliwell and Gutteridge, 2007). Peroxidases are also the antioxidative enzymes that decompose H_2O_2 with co-oxidation of reduced co-substrates (Elstner, 1982). Here in this study, the activities of these two enzymes have been found to reduce with increase in the Co concentration in the medium (Fig. 1). This indicated that the protection against H_2O_2 was poor and there was possibility of build up of H_2O_2 inside the cell. Hydroxyl radicals (·OH) are known to be formed from H_2O_2 in aerobic cells in presence of transition metal ions (Halliwell and Gutteridge, 2007). Further, the elevated steady state levels of H_2O_2 and $O_2^{\cdot}$ can react in presence of transition metal ions to produce hydroxyl radical (·OH), via Haber-Weiss reaction (Elstner, 1982). The ·OH is known to be the most potential toxic species of oxygen and the unsaturated fatty acid components of membrane lipids are highly susceptible to ·OH attack and are peroxidized in presence of it. Therefore, increased lipid peroxidation is considered as a good indicator of prevalence of free radical mediated reactions in aerobic organisms (Kappus, 1985). In this study, the increase in the MDA content in the germinating embryonic tissues in presence of Co (Table 1) indicated the prevalence of oxidative stress in the tissues. Thus, imposition of oxidative stress might be one of the mechanisms behind Co induced toxicity in the germinating seeds.

Cobalt treatment in the soil at low concentration (i.e.,

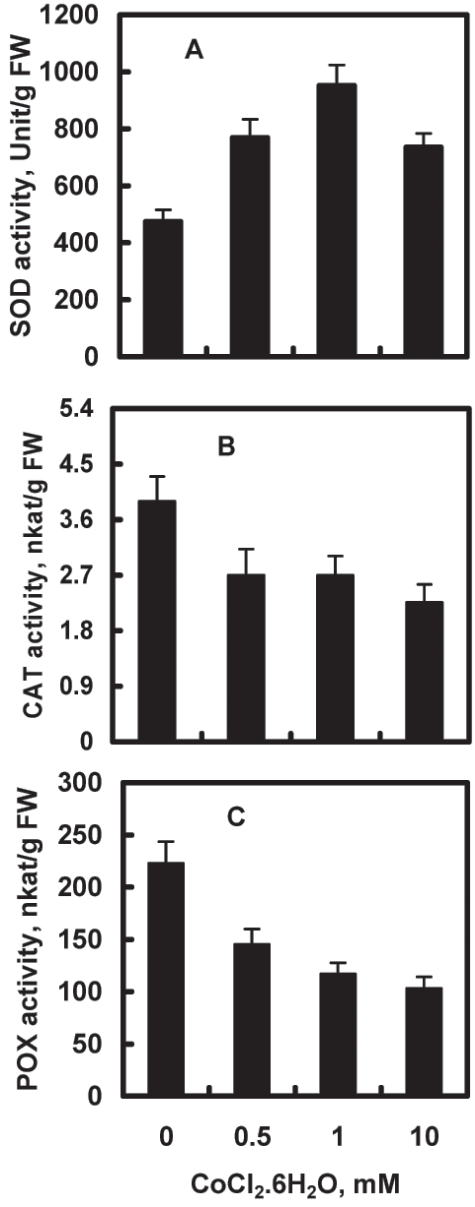

Fig. 1 Changes in the activities of superoxide dismutase (SOD) (A), catalase (CAT) (B) and peroxidase (POX) (C) in embryonic tissues of mungbean seeds germinated in presence of $CoCl_2.6H_2O$ for 48 h.

Table 1
Changes in the germination percentage; soluble protein content and lipid peroxidation level of embryonic tissues of mungbean seeds exposed to cobalt chloride for 48 h

$CoCl_2.6H_2O$ (mM)	Germination (%)	Soluble protein (mg/g FW)	MDA (nmol/g FW)
0	100	17.5 ± 1.2	25 ± 2.4
0.5	85 ± 4	18.8 ± 1.6	33 ± 3.7
1.0	85 ± 3	14.6 ± 1.1	44 ± 4.5
10.0	75 ± 3	8.2 ± 0.9	56 ± 5.2

50 mg/kg soil) is reported to have beneficial effects on the growth of soybean plants (Jayakumar and Abdul Jaleel, 2009). But higher concentrations reduced the growth. According to Stiborova *et al.* (1988), the lower concentration of Co increases the water and nutrient absorption efficiency of roots and thereby enhances the plant growth. In tomato seedlings, treated with 0.5 mM Co, Gopal *et al.* (2003) have observed various morphological as well as physiological anomalies. Along with other physiological anomalies they also found the disturbances in plant phosphorus and sulfur content due to excess Co in the seedlings which affected the carbohydrate and nitrogen metabolisms and that was the main reason behind depressed growth and lowered biomass. In cauliflower, excess of Co was found to inhibit the translocation of nutrients from roots to upper parts as a result there was reduction in the growth (Chatterjee and Chatterjee, 2000). All these findings suggest that Co at higher concentration is toxic to plants which manifest in the forms of different morpho-physiological anomalies. The results of this study suggest that during germination stage, excess of Co can alter the activities of antioxidative enzyme; increase lipid peroxidation level and consequently imposes oxidative stress in the germinating embryonic tissues. Probably imposition of oxidative stress might be one of the mechanisms behind Co induced toxicity in plants. Since during germination stage excess of Co has been found to have toxic effects, prolonged exposure of tender embryonic tissues to Co would definitely pose a threat for the developing seedlings. In fact, Co has also been reported to have toxic effects in different seedlings, as have already been outlined above. Therefore, remediation of soil contaminated with excess of Co is highly essential before sowing seeds or for other plantation programme.

Acknowledgements

The authors are thankful to the Head, P.G. Department of Environmental Science, Fakir Mohan University, Balasore-756020, India for providing necessary facilities to carry out this work.

References

Aebi, H. E. (1983). Catalase. In: Bergmeyer, H.U. (ed) Methods of Enzymatic Analyses, Verlag Chemie, Weinheim. Vol. 3, pp. 273-285.

Barceloux, D.G. (1999). Cobalt. J. Toxicol. Clin. Toxic. 37: 201–216.

Cakmak, I. and Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max*). Physiol. Plant. 83: 463-468.

Chatterjee, J. and Chatterjee, C. (2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. Environ. Pollut. 109: 69-74.

Chatterjee, J. and Chatterjee, C. (2003). Management of phytotoxicity of cobalt in tomato by chemical measures. Plant Sci. 164: 793–801.

Daniels, R. R., Stuckmeyer, B.E. and Peterson, L. A. (1972). Copper toxicity in *Phaseolus vulgaris* L. as influenced by iron nutrition. I. An anatomical study. J. Am. Soc. Hort. Sci. 9: 249–254.

Das, K., Samanta, L. and Chainy, G. B. N. (2000). A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys. 37: 201-204.

Dey, S. K., Dey, J., Patra, S. and Pothal, D. (2007). Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. Braz. J. Plant Physiol. 19(1): 53-60.

Dey, S. K., Jena, P.P. and Kundu, S. (2009 a). Antioxidative efficiency of *Triticum aestivum* L. exposed to chromium stress. J. Environ. Biol. 30(4): 539-544.

Dey, S. K., Sahu, S. and Mohapatra, S. (2009 b). Copper toxicity alters the activities of antioxidative enzymes and increases lipid peroxidation in germinating seeds. Plant Sci. Res. 31: 10-16.

Elstner, E. F. (1982). Oxygen activation and oxygen toxicity. Annu. Rev. Plant Physiol. 33: 73-96.

Gopal, R., Dube, B. K., Sinha, P. and Chatterjee, C. (2003). Cobalt toxicity effects on growth and metabolism of tomato. Commun. Soil Sc. Plant Analysis, 34: 619-628.

Halliwell, B. and Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1-14.

Halliwell, B. and Gutteridge, J. M. C. (2007). Free radicals in biology and medicine. Oxford University Press, New York.

Hamilton, E. I. (2000). Environmental variables in a holistic evaluation of land contaminated by historic mine wastes: a study of multi-element mine wastes in West Devon, England using arsenic as an element of potential concern to human health. Sci. Total Environ. 249: 171–221.

Handrek, K. A. and Riceman, D. S. (1969). Cobalt distribution in several pasture species grown in culture solutions. Aust. J. Agric. Res. 20: 213–226.

Heath, R. L. and Packer, L. (1968). Photooxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.

Jayakumar, K. and Abdul Jaleel, C. (2009). Uptake and accumulation of cobalt in plants: A study based on exogenous cobalt in soybean. Bot. Res. Int. 2: 310-314.

Kaer, C., Petersen, M. B. and Elmegaard, N. (1998). Effects of soil copper on black binweed (*Fallopia convolvulus*) in the laboratory and in the field. Arch. Environ. Contam. Toxicol. 35: 4–10.

Kappus, H. (1985). Lipid peroxidation: Mechanisms, analysis, enzymology and biological relevance. In: Sies, H. (ed) Oxidative Stress, Academic Press, London, pp. 273-310.

Kar, M. and Feierabend, J. (1984). Metabolism of activated oxygen in detached wheat and rye leaves and its relevance to the initiation of senescence. Planta 160: 385-391.

Lock, K., Becaus, S., Criel, P., Van Eeckhout, H. and Janssen, C.R. (2004). Ecotoxicity ofcobalt to the springtail Folsomia candida. Comp. Biochem. Phys. 139: 195–199.

Lock, K., De Schamphelaere, K. A. C., Becaus, S., Criel, P., Van Eeckhout, H. and Janssen, C.R., (2006). Development and validation of an acute biotic ligand model (BLM) predicting cobalt toxicity in soil to the potworm *Enchytraeus albidus.* Soil Biol. Biochem. 38: 1924–1932.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randell, R. J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193: 265-275.

Osman, M. E. H., El-Naggar, A. H., El-Sheekh, M. M. and El-Mazally, E. E., (2004). Differential effects of Co^{2+} and Ni^{2+} on protein metabolism in *Scenedesmus obliquus* and *Nitzschia perminuta*. Environ. Toxicol. Phar. 16: 169–178.

Schutzendubel, A., Schwanz, P., Teichmann, T., Gross, K., Langenfeld-Heyser, R., Godbold, A. and Polle, A. (2001). Cadmium-induced changes in antioxidative systems, H_2O_2 content and differentiation in Scots pine (*Pinus sylvestris*) roots. Plant Physiol. 127: 887-898.

Srivastava, S., Mishra, S., Tripathi, R. D., Dwivedi, S. and Gupta, D. K. (2006). Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. Aquatic Toxicol. 80: 405-415.

Stiborova, M., Ditrichova, M. and Brezinova, A. (1988). Mechanism of action of Cu , Co and Zn on ribulose 1-5 biphosphate carboxylase from barley (*Hordeum vulgare* L.). Photosynthetica, 22: 161-167.

Tiffin, L. O. (1967). Translocation of manganese, iron, cobalt and zinc in tomato. Plant Physiol. 42: 1427–1432.

Verma, S. and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164: 645-655.

Williams, D. E., Vlamis, J. and Pukite, A. H. (1985). Metal movement in sludge-treated soils after six years of sludge addition: 2. Nickel, cobalt, iron, manganese, chromium and mercury. Soil Sci. 140: 120–125.

Plant Science Research 35 (1&2) : 35-43, 2013

Plant Science Research

ISSN 0972-8546

Development and morphological characterization of monosomic alien addition lines (MAALs) from *Oryza brachyantha* A.Chev.et.Roehr to transfer Yellow Stem Borer (YSB) resistance gene(s) on cultivated rice *O. sativa* L.

Aveek Narain and P. Sen[Ψ]

Crop Improvement Division, Central Rice Research Institute, Cuttack, India

ARTICLE INFO

Article history:
Received : 12 December 2012
Received in revised form : 17 November 2013
Accepted : 24 November 2013

Keywords:
O. sativa
O. brachyantha
interspecific hybrids
embryo rescue
MAALs

ABSTRACT

Oryza brachyantha (FF), the African wild rice, which is resistant to Yellow stem borer (YSB), was used to develop Monosomic Alien Addition Lines (MAALs) on cultivated rice. A total of 19, 399 BC_1F_1 spikelets were artificially pollinated with the recurrent parent, out of which 29 BC_2F_1 hybrids were produced through embryo rescue with the crossability efficiency of 0.14%. Embryos collected at 10- 12 days after pollination (DAP) were grown successfully on ¼ MS basal medium with embryo germination of 35.8%. The plantlets with 4-5 healthy roots were acclimatized by direct transfer method and grown. The hybrids were morphologically characterised into 16 different plant types and each plant type was cytologically analyzed. Hybrids resembling with the morphology of primary trisomics of rice and exhibiting 2n+1 (2n=25) chromosome arrangement were designated as MAALs. A total of 8 MAALs were thus identified representing MAAL- 4 (Sterile), MAAL- 5 (Twisted leaf), MAAL- 7 (Narrow leaf), MAAL- 8 (Rolled leaf), MAAL- 9 (Stout), MAAL- 10 (Erect), MAAL- 11 (Pseudo-normal) and MAAL- 12 (Tall).

1. Introduction

The productivity of cultivated rice is affected by several biotic and abiotic stresses. The genetic variability in cultivated rice germplasm in terms of resistance/tolerance to abiotic and abiotic stresses is either limited or the cultivars are becoming susceptible to various biotic and abiotic stresses due to the changed climatic conditions, cropping practises, insect biotypes and/or disease races. It is therefore imperative to broaden the gene pool of rice by introgression of alien gene(s) from wild relatives of rice which are known to be resistant to major biotic and abiotic stresses (Heinrichs *et al*., 1985; Swaminathan, 1986; Sitch, 1990) and can serve as a rich source of variability for rice improvement.

The Yellow stem borer (YSB), *Scirpophaga incertulas* (Walker), a major pest of cultivated rice, causes damage to the rice crop in almost all agro climatic ecosystems and in all stages of growth causing an annual yield loss of 5-10% damaging crops upto 60% in unprotected field conditions (Pathak and Khan, 1994). Though some high yielding rice varieties have been reported to be moderately resistant to YSB (Pathak and Khan, 1994; Maqbool *et al*., 1998), no rice variety truly resistant to YSB has been developed from cultivated rice. It is therefore essential to incorporate alien genes for resistance to YSB from wild species belonging to the secondary gene pool of rice which are reservoirs of such traits. Wild rice germplasm has been screened against YSB and *O. brachyantha*, *O. officianalis*, *O. redleyi* and *Porteresia coarctata* were found to resistant/tolerant to YSB (Padhi and Sen, 2002).

Oryza brachyantha (FF), the wild species widely distributed in Africa belongs to the secondary gene pool of rice. While wild species belonging to AA genome can be easily crossed with *O. sativa*, the more distantly related wild species like *O. brachyantha* and others are difficult to cross due to high genomic incompatibility rendering the F_1 hybrids completely sterile. Introgression of alien genes from these distantly related wild species is possible through the

[Ψ] *Corresponding author; Email:*psencrri@yahoo.com

development of Monosomic Alien Addition Lines (MAALs) employing embryo rescue. The MAALs thus produced characteristically have an extra chromosome (2n=25) ideally from the wild species in addition to a complete chromosome complement of the cultivated species. MAALs representing 6–12 extra chromosomes have been reported in *O. officinalis* (CC), *O. minuta* (BBCC), *O. latifolia* (CCDD), *O. australiensis* (EE), *O. brachyantha* (FF), *O. granulata* (GG), and *O. ridleyi* (HHJJ) (Shin and Katayama, 1979; Jena and Khush, 1989; Brar *et al*., 1991, Brar and Khush, 2002, 2006) which have been characterized based on morphological and cytological characterization, Flourescence- *in situ*-Hybridization (FISH) and molecular markers.

Attempts have been made to develop of MAALs from *O. brachyantha* and introgress BB resistant genes from this species to cultivated rice (Brar *et al*., 1996). In the absence of any rice cultivar truly resistant to YSB, in this study we have attempted to develop a complete set of 12 MAALs from *O. brachyantha* on *O. sativa* cv Savitri with the ultimate goal to introgress gene(s) for resistance for YSB, into cultivated rice.

2. Materials and Methods

Back crossing of BC_1F_1 (*O. sativa* cv Savitri */O. brachyantha//O. sativa* cv Savitri) interspecific backcross hybrids was carried out with the recurrent parent, *O.sativa* cv Savitri following the scheme for the production of monosomic alien addition lines (2*n*=25) suggested by Brar and Khush (1997). The spikelets of BC_1F_1 hybrids were treated with solution A (NAA 25mg/L+ Sucrose 5g/L), sprayed on to the emasculated spikelets before pollination and solution B (GA_3 50mg/L+KN 5mg/L+NAA 5mg/L), sprayed after pollination twice a day till 5 days (Jena and Khush, 1989; Multani *et al*., 1994) to overcome pre- and post-fertilization barriers respectively.

Spikelets with expected fertilized embryos were collected between 7-16 days after pollination (DAP) before embryo abortion. Then they were aseptically excised, treated adopting a protocol suggested by Ko *et al*. (1983) and cultured on ¼ MS medium with 3 different hormonal combinations to confirm an optimum growth medium for embryo rescue and establish an appropriate age of hybrid embryos for rescue. The embryos in culture tubes were incubated in dark at 25±2 ^oC until germination and germinated embryos were then grown in light-dark cycle of 16 hours.

Plantlets at three-leaf stage after 2-4 weeks of growth with 4-5 healthy roots were removed from culture tubes and acclimatized following four different methods (i) Direct transfer method, (ii) Modified Iyer and Govilla (1964) method, (iii) Modified soil: sand method (Niroula *et al*., 2004), and (iv) Peat moss fortified with Hoagland's solution and the responses were recorded to establish an optimum acclimatization protocol for the interspecific hybrids. Methods described by Iyer and Govila (1964) and Niroula *et al.* (2004) were adopted and modified by including the use Hoagland's solution. The surviving plantlets were then transferred to the net house for further growth till maturity.

The surviving BC_2F_1 (*O. sativa* cv. Savitri / *O. brachyantha // O. sativa* cv. Savitri /// *O. sativa* cv. Savitri) progenies, were characterised, at vegetative, mature and flowering stages following a set of qualitative and quantitative morphological characters and categorized into different plant types (PT) based on their morphological features. Each of the plant type was morphologically compared to primary trisomics of rice (Khush *et al*., 1984; Misra *et al*., 1985, Sen and Misra, 1988). More than one plant showing similarities to a particular plant type were grouped accordingly. These hybrids were cytologically characterized to identify their chromosome arrangements. Hybrids resembling with the morphology of primary trisomics of rice and exhibiting 2n+1 (2n=25) chromosome arrangement were designated as MAALs.

3. Results and Discussion

3.1 Development of backcross hybrids

A total of 19,399 BC_1F_1 spikelets were artificially pollinated treating the spikelets with hormonal solutions before and after pollination to overcome strong pre- and post- fertilization barriers between the parent species *O. sativa* and *O. brachyantha* which have also been reported earlier by Sitch and co-workers (1989, 1990). It was observed that treatment with hormonal solutions before and after pollination greatly improved viable embryo formation as they helped in pollen germination, pollen tube development, overcome post- fertilization barriers and embryo abortion. Such observations have also been reported by Jena and Khush (1986, 1989), Multani *et al.* (1994, 2003) and Niroula *et al.*, (2004, 2005). As a result, 5,248 expected fertilized embryos could be harvested, out of which 1256 were viable embryos and rest of the spkikelets were with aborted embryos or filled with watery endosperm. The overall crossability was found to be 0.14% (Table 1). Of the 1256 embryos inoculated in growth media, only 450 embryos germinated in culture thus showing an efficiency of 35.8% (Table 1).

Percentage of embryo survival was evaluated with embryos collected between 7 to 16 days after pollination (DAP) and it was found that embryos collected between 10 to 12 DAP showed maximum rates of survival (Table 2) i.e, 36.3% and 44.4%, thus were the ideal period to harvest

embryos for rescue. Similar observations have been reported in crosses between *O. sativa* and *O. brachyantha* by Sitch *et al*. (1989), Panda and Sen (2006) and Behura *et al.* (2011). Kumari *et al*. (2005) have also pointed out that embryos collected between 8 and 12 DAP were ideal for embryo rescue irrespective of the wide cross combination. Of the three different growth media used for testing embryo germination and plant growth (Table 3), ¼ MS basal medium showed the highest percentage of germination (70%). Several earlier reports by Jena and Khush (1984, 1989, 1990), Yasui and Itawa (1991), Multani *et al*. (1994, 2003) have used ¼ MS basal medium in different cross combinations of *O.sativa* with different wild species. These results suggest that irrespective of any wide cross combination, embryo survival and plant regeneration depend on a combination of cross combination, time of embryo excised and growth medium used.

Out of 4 methods used to acclimatize plantlets before transferring them to net house/green house, the direct transfer method of planting plantlets with 4-5 healthy roots in earthen pots containing sterilized soil supplemented with Hoagland's solution, resulted in 70% survival. The modified method of Iyer and Govila (1964) using Hoagland's solution resulted in 55% survival of the hybrid plantlets whereas the modified sand and soil (1:1) method resulted in 45% plantlet survival. Peat moss method resulted in complete loss of plantlets with no survival. There are limited reports describing hardening methods of embryo rescue regenerated plants. Iyer and Govila (1964) have reported 50% survival rate of plantlets. Jena and Khush (1984) have reported hardening of embryo rescued plants through culture in nutrient solution for 10 days but have not provided details of the nutrient solution used. Though Niroula *et al*. (2004) have reported 100% survival in 3 wild species in their experiments using soil: sand method, in our experiments the method resulted in only 45% survival.

Table 1
Percentage of crossability and germination of BC_1F_1 population

Total SP	Total EFS	Total EC	Total EG	Germination(%)	Total PIC	Total PIP	Crossability(%)
19399	5248	1256	450	35.8	131	29	0.14

SP- spikelets pollinated; EFS- Expected fertilized spikelets; EC- embryos cultured; EG- embryos germinated; PIC- plantlets in culture; PIP- plants in pots

Table 2
Percentage of embryo survival at different days after pollination (DAP)

Days after pollination (DAP)	BC_2F_1 (*O.sativa* cv. Savitri / *O.brachyantha* // *O.sativa* cv. Savitri) population			
	No. of spikelets pollinated	No. of spikelets fertilized	No. of embryos inoculated	Embryos rescued(%)
7	32	15	2	13.3
10	27	11	4	36.3
12	36	9	4	44.4
14	23	8	1	12.5
16	32	8	0	0

Table 3.
Regeneration efficiency of hybrid embryos growing in different growth media

O. sativa cv. Savitri / *O.brachyantha* // *O.sativa* cv Savitri				
Embryo excised at (DAP)	Medium	No. of embryos inoculated	No. of embryos regenerated	Regeneration efficiency (%)
12	¼ MS medium basal + Sucrose (3%) + Agar (0.7%)	10	7	70.0
12	¼ MS medium basal + NAA (0.5mg/l) + KN (2.0 mg/l) + Sucrose (3%) + Agar (0.7%)	10	4	40.0
12	¼ MS medium basal + IAA (0.5mg/l) + KN (1.0 mg/l) + Sucrose (3%) + Agar (0.7%)	10	6	60.0

3.2 Isolation and establishment of Monosomic Alien Addition Lines (MAALs)

Twenty four surviving BC_2F_1 hybrids were studied for their qualitative and quantitative morphological characters and were categorized into 16 plant types (PT) based the observed morphological characters. Two or more plants exhibiting similar morphological characters were grouped into one plant type. Plants exhibiting mixed characters were grouped separately. The detailed characters of each plant type are as follows-

Plant type (PT)- 1: Normal looking, medium height, semi-erect, non-rhizomatous, intermediate tillers; leaves medium in length, intermediate in width, green in colour, intermediate pubescence; leaf sheath base green in colour, junctura light green in colour; flag leaf medium in length, intermediate in width, ligule long, split, whitish in colour, auricles present, white in colour; panicles short, completely exserted, lax type, non- shattering; spikelets short, bold with white apiculus and without awn; The plant is morphologically indistinguishable from disomics. BC_2F_1- 1 and 30 were grouped under PT-1. Spikelet fertility was found to be 85.7±0.8.

Plant type (PT)- 2: Short height, open, very poor growth, non- rhizomatous, low tiller number; leaves narrow and thin, dark green in colour, intermediate pubescence; leaf sheath base green coloured, junctura light green coloured; ligules short, acute, whitish in colour, auricles absent; plant is susceptible to *Cercospora janseana* infection. BC_2F_1- 3 was grouped under PT-2.

Plant type (PT)- 3: Short height, open, non- rhizomatous, slow growing, intermediate tiller number; leaves long, narrow, incurved, dark green in colour, intermediate pubescence; leaf sheath base green in colour, junctura light green in colour; flag leaves long, narrow; ligule short, split and whitish in colour, auricle present, white in colour; panicles short, completely exserted, compact, non-shattering and dense; spikelets short, bold with white apiculus, without awn. BC_2F_1- 4 and 28 were grouped under PT-3. Spikelet fertility was found to be 74.0±0.4.

Plant type (PT)- 4: Medium height, semi-erect, non-rhizomatous, low tiller number; Leaves intermediate in length, narrow, slightly rolled, dark green in colour, intermediate pubescence; flag leaves intermediate in length, narrow; leaf sheath base green in colour, junctura is light green in colour; ligule short, split, whitish in colour, auricles absent; panicles very short, incompletely exerted, compact, non- shattering; spikelets slender, short awns. BC_2F_1- 5 was grouped under PT-4. Spikelet fertility was found to be 45.2±0.0.

Plant type (PT)- 5: Short height, erect, with poor growth, non- rhizomatous, very low tiller number; leaves short, narrow, green in colour, intermediate pubescence, at right angles to the culm; flag leaves short, narrow; leaf sheath base green in colour, junctura is light green in colour; Ligules short, split, whitish in colour, auricles absent; panicles very short, very poor exertion, narrow spikelets without awn. BC_2F_1- 6 was included under PT-2.

Plant type (PT)- 6: Medium tall, semi-spreading, non-rhizomatous, thick (stout) tillers, high tiller number; leaves long, broad, green in colour, boat shaped appearance; flag leaves long and broad; leaf sheath base green in colour, junctura light green in colour; ligules medium, split, whitish, auricles absent; panicles medium, dense, completely exerted, compact, non-shattering; spikelets short, bold, without awn. BC_2F_1- 8 and 36 were grouped under PT-6. Spikelet fertility was found to be 71.2±0.6.

Plant type (PT)- 7: Medium tall, erect, non-rhizomatous, slow growing, intermediate tiller number; Leaves intermediate in length, narrow, thick, slightly folded inwards, dark green in colour, intermediate pubescence; flag leaves intermediate, narrow; leaf sheath base green in colour, junctura is light green in colour; ligules short, acute, whitish, auricles white coloured, hairy; panicles very short, completely exserted, lax type, non- shattering; spikelets short slender, without awn. BC_2F_1- 10 and 32 were grouped under PT-7. Spikelet fertility was found to be 82.0±0.4.

Plant type (PT)- 8: Very short height, semi-spreading, non-rhizomatous, very poor growth, very low tiller number; leaves very short, narrow, thin, rolled, green in colour, intermediate pubescence, at right angles with the culm; leaf sheath base green in colour, junctura light green in colour; ligules are short, acute, whitish in colour, auricles;.BC_2F_1- 12 was included under PT-8.

Plant type (PT)- 9: Medium height, open, appears bushy, non- rhizomatous, medium high tiller number; leaves long, intermediate width, twisted, light green in colour, intermediate pubescence; flag leaves long, intermediate width; leaf sheath base colour green, junctura light green in colour; ligules short, acute, whitish in colour, auricles present, white in colour; panicles very short, incompletely exserted, lax type, low shattering, spikelets slender without awn. BC_2F_1- 13, 33, 34 and 37 were grouped under PT-9. Spikelet fertility was found to be 62.4±0.9.

Plant type (PT)- 10: Medium height, erect, non- rhizomatous, low tiller number; leaves intermediate in length, narrow, thick, glabrous, dark green in colour, intermediate flag leaves; leaf sheath base green in colour, junctura is light green in colour; ligules medium in length, split, whitish, auricles

absent; panicles very short, incompletely exserted, compact, non- shattering; spikelets short without awn. BC_2F_1- 27 was included under PT-10. Spikelet fertility was found to be 0.0.

Plant type (PT)- 11: Tallest among all plants, slightly open, non-rhizomatous, robust tillers, intermediate tiller number; leaves long, broad, green in colour, pubescent; flag leaves long, broad; leaf sheath base green in colour, junctura light green in colour; ligules medium in length, with fringe of hairs, whitish in colour, auricles absent; panicles medium in length, completely exserted, compact, non- shattering; spikelets long, bold, with tip awn. BC_2F_1- 31 was included under PT-11. Spikelet fertility was found to be 94.7±0.0.

Plant type (PT)- 12: Medium height, semi-erect, non-rhizomatous, low tiller number; leaves long, intermediate width, green in colour, intermediate pubescence; flag leaves long, intermediate; leaf sheath base colour green, junctura light green; ligules short, split, whitish in colour, hairy auricles present; panicles moderately short, incompletely exerted, compact and non-shattering; spikelets slender with tip awn. BC_2F_1- 29 was included under PT-12.

Plant type (PT)- 13: Medium height, appears erect, non- rhizomatous, intermediate tiller number; leaves long, narrow, slightly folded, dark green in colour, intermediate pubescence; leaf sheath base green in colour, junctura is light green in colour; ligule short, split, and whitish, hairy auricle present; panicles very short, incompletely exerted, compact, moderately shattering; spikelets slender, without awn. BC_2F_1- 35 was included under PT-13.

Plant type (PT)- 14: Medium height, appears semi-erect, very slow growth, non- rhizomatous, low tiller number; leaves are short, intermediate width, thick, dark green in colour, pubescent; leaf sheath base colour green, junctura light green in colour; ligules medium in length, split, and whitish, hairy auricles are present; panicles very short, incompletely exerted, lax type, moderately shattering; spikelets short, without awn. BC_2F_1- 39 was included under PT-14.

Plant type (PT)- 15: Very short height, semi- erect appearance, non- rhizomatous, very slow growth, low tiller number; leaves short, narrow, thick, folded, dark green in colour, pubescent; leaf sheath base green in colour, junctura light green in colour; ligule medium, split, whitish, auricles absent. BC_2F_1- 40 was included under PT-15.

Plant type (PT)- 16: Medium height, appears semi-spreading, non- rhizomatous, very slow growth, low tiller number; leaves intermediate in length, broad, broad, dark green in colour, leaves, intermediate pubescence; leaf sheath base colour green in colour, junctura light green in colour; ligule short, split, whitish, small auricle present; panicles very short, partly exerted, lax type, non- shattering; spikelets short, some spikelets have tip awn. BC_2F_1- 41 was included under PT-16.

Plant types were morphologically compared with the primary trisomics of rice. One or more than one plant showing similarity to a particular trisomic was grouped into the respective group (Table 4). PT-1 (BC_2F_1s- 1 and 30) resembled Triplo- 11, PT-3 (BC_2F_1s- 4 and 28) resembled Triplo-8, PT-4 (BC_2F_1- 5) resembled Triplo-7, PT-6 (BC_2F_1s- 8 and 36) resembled Triplo-9, PT-7 (BC_2F_1s- 10 and 32) resembled

Table 4
Morphological grouping of BC_2F_1 hybrids

Plant types	BC_2F_1 hybrids	Morphological resemblance with Rice primary trisomic
PT-1	BC_2F_1- 1	Triplo 11 (Pseudonormal)
	BC_2F_1-30	
PT-2	BC_2F_1-3	Did not survive
PT-3	BC_2F_1- 4	Triplo 8 (Rolled leaf)
	BC_2F_1- 7	
	BC_2F_1- 28	
PT-4	BC_2F_1- 5	Triplo 7 (Narrow leaf)
PT-5	BC_2F_1-6	Did not survive
PT-6	BC_2F_1- 8,	Triplo 9 (Stout)
	BC_2F_1-36	
PT-7	BC_2F_1- 10	Triplo 10 (Short grain)
	BC_2F_1-32	
PT-8	BC_2F_1-12	Did not survive
PT-9	BC_2F_1-13	Triplo 5 (Twisted leaf)
	BC_2F_1-33	
	BC_2F_1-34	
	BC_2F_1-37	
PT-10	BC_2F_1- 27	Triplo 4 (Sterile)
PT-11	BC_2F_1-31	Triplo 12 (Tall)
PT-12	BC_2F_1-29	Triplo 12+ Triplo 10
PT-13	BC_2F_1- 35	Triplo 4+ Triplo 10
PT-14	BC_2F_1-39	Triplo 4+ Triplo 9
PT-15	BC_2F_1-40	Triplo 4+ Triplo 9 (Did not survive)
PT-16	BC_2F_1-41	Triplo 5+ Triplo 7+ Triplo 9
Total	24	

Triplo- 10, PT-9 (BC_2F_1s- 13, 33, 34 and 37) resembled Triplo-5, PT-10 (BC_2F_1- 27) resembled Triplo-4 and PT-11 (BC_2F_1-31) resembled Triplo- 12. Plant types 2, 5, 8 and 15 could not be compared to any primary trisomic because of non-survival of the plants and plant types 11, 13, 14, and 16 exhibited mixed characters of 2-3 primary trisomics. Cytological analysis of the hybrids was carried out and plants exhibiting a typical trisomic chromosome arrangement of 2n+1 (2n=25 chromosomes) were identified. As per the cytological observation, out of 24 plants, 16 plants (BC_2F_1 hybrids) with the chromosome arrangement of 2n+1 and displaying morphological resemblance to primary trisomics of rice were designated as MAALs. Thus 16 plants represented 8 MAALs with addition of an extra chromosome from the wild rice *O. brachyantha*. The 8 MAALs identified are as follows- MAAL- 4 (Sterile), MAAL- 5 (Twisted leaf), MAAL- 7 (Narrow leaf), MAAL- 8 (Rolled leaf), MAAL- 9 (Stout), MAAL- 10 (Short grain), MAAL- 11 (Pseudo-normal) and MAAL- 12 (Tall). The detailed comparative morphological data of the 8 MAALs are presented in Table 5 and photographs of different MAALs are presented in Fig 1 (a-h). Shin and Katayama (1979), Jena and Khush (1985, 1989, 1990), Brar *et al.* (1996) Multani *et al*. (1994, 2003), and Yasui and Itawa (1991) have similarly isolated MAALs from different wild species and characterized their respective MAALs by comparing the observed morphological characters with that of primary trisomics of rice.

Table 5
Comparative morphological characters of MAALs

Characters	MAALs							
	MAAL4	MAAL5	MAAL7	MAAL8	MAAL9	MAAL10	MAAL11	MAAL12
Stem	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh
Plant height (cm)	72.0	100.0	61.0	20.0	80.0	127.0	72.0	138.0
EBT	9.0	19.0	18.0	15.0	12.0	14.0	13.0	13.0
Leaf colour	DG	LG	DG	DG	DG	G	DG	G
Blade pubescence	NP	MP	MP	MP	P	P	MP	P
Flag leaf L (cm)	20.0	23.0	20.0	19.0	45.0	30.0	22.0	24.0
Flag leaf B (cm)	0.5	1.4	0.5	0.5	1.8	1.0	0.5	1.0
LSB colour	W	W	W	W	W	W	W	W
Ligule colour	W	W	W	W	LG	W	W	W
Ligule shape	S	S	S	S	S	S	S	S
Ligule length (cm)	0.8	1.2	0.2	0.4	0.6	0.3	1.0	0.9
Auricle colour	A	W	A	W	A	A	W	W
Junctura colour	LG	LG	LG	LG	LG	LG	LG	LG
Panicle type	C	L	C	C	C	L	C	C
Panicle exsertion	IE	IE	IE	E	E	E	E	E
Panicle length (cm)	11.0	24.0	11.0	11.0	15.0	28.0	17.0	20.0
Spikelet L (cm)	1.0	0.7	0.5	0.6	1.0	0.8	0.9	0.9
Spikelet B (cm)	0.5	0.2	0.2	0.4	0.8	0.3	0.3	0.4
Spikelet fertility (%)	0.0	62.4±0.9	45.2±0.0	74.0±0.4	71.2±0.6	82.0±0.4	85.7±0.8	94.7±0.0
Apiculus colour	W	W	W	W	W	W	W	W
Awn length (cm)	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.2
Stigma colour	P	P	P	P	P	P	P	P
Stigma shape	B	B	B	B	B	B	B	B

Nrh- non- rhizomatous; W- white; G- green; LG- light green; DG- dark green; GW- greenish white; P- pubescent; MP- medium pubescent; NP- non-pubescent; LSB- leaf sheath base; S- split; NS- non-split; A- absent; C- compact; L- lax; IE- incomplete exsertion; E- exserted; P- purple; B-bifid.

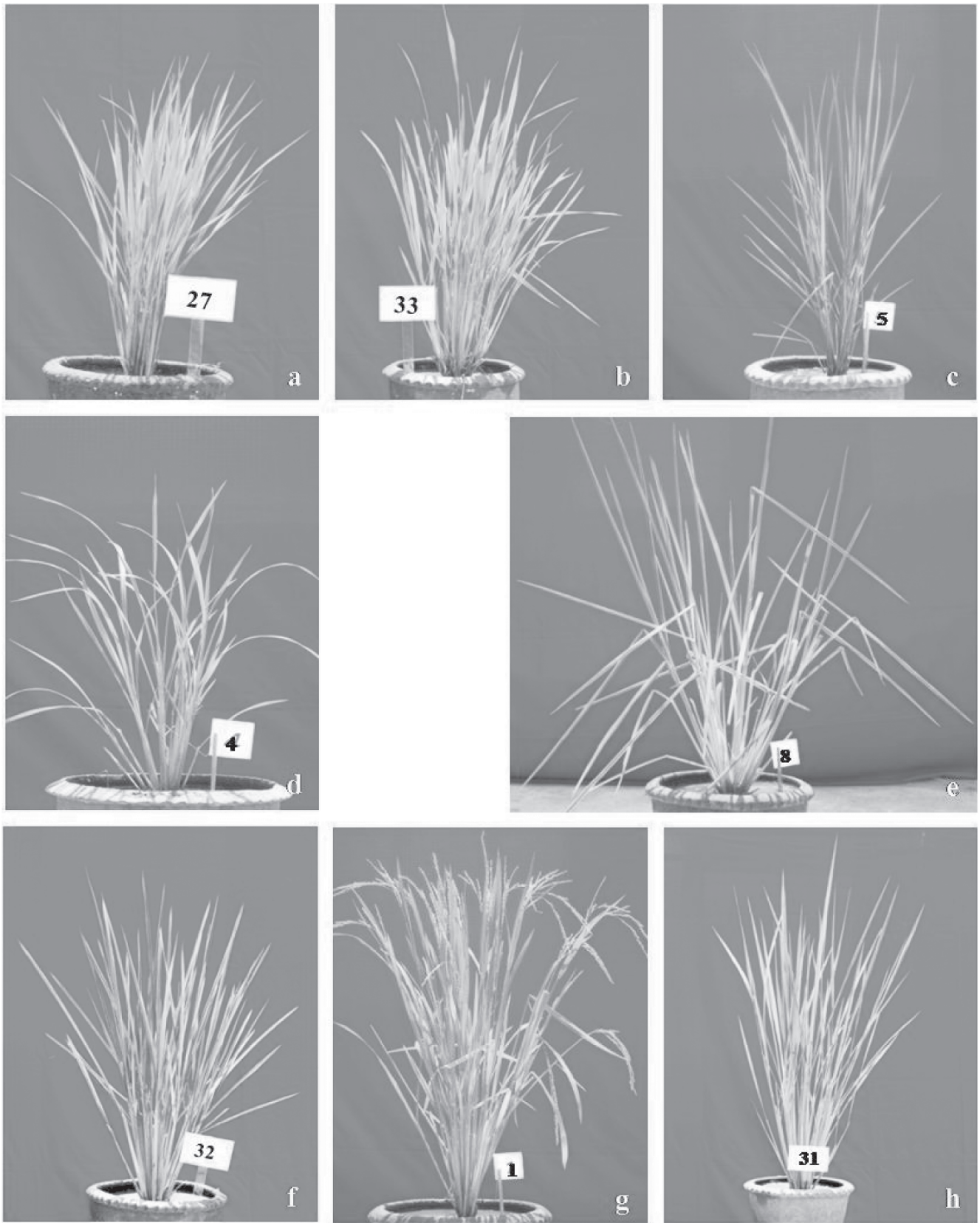

Fig. 1. (a) BC_2F_1- 27 representing MAAL- 4, (b) BC_2F_1- 13 representing MAAL- 5, (c) BC_2F_1- 5 representing MAAL- 7, (d) BC_2F_1- 4 representing MAAL- 8, (e) BC_2F_1- 8 representing MAAL- 9, (f) $BC_2F_1$1- 32 representing MAAL- 10, (g) BC_2F_1- 1 representing MAAL- 11, (h) BC_2F_1- 31 representing MAAL- 12

4. Conclusion

Oryza brachyantha is an African wild species of rice representing FF genome and important source of resistance to BB, Blast, and YSB which can be transferred to cultivated rice for crop improvement. While BB resistance from *O. brachyantha* has already been transferred to cultivated rice, transfer of YSB resistance is imminent since it causes wide spread damage to the rice crop in all agro-climatic conditions. During wide hybridization between the two species *O.brachyantha* (FF) and *O. sativa* (AA), low germination and crossability percentages were observed which suggest strong hybridization barriers between them and emphasize the importance of hormonal treatment and embryo rescue technique when working with rice species so genetically apart. Apart from embryo rescue, the effectiveness of morphological characterization in identifying and establishing MAALs from wild species to cultivated species is also demonstrated. In the present study 8 MAALs from *O. brachyantha* could be identified. Work is in progress to identify the remaining 4 MAALs to produce a complete set of 12 MAALs. Further use of cytological investigations and advanced techniques like Fluorescent-*in situ*- hybridization (FISH) and its variant Genomic-*in situ*-hybridization (GISH) and molecular markers will help to confirm the introgression of alien genes resistance to YSB into cultivated rice more effectively. These MAALs can be used to identify gene(s) for resistance to YSB and develop introgressed lines from *O. brachyantha*, which can be used as pre-breeding lines to develop a rice variety with inbuilt resistance to YSB. As a result the production and productivity of rice will be enhanced.

Acknowledgement

Financial support for the research work by ICAR, New Delhi is deeply acknowledged.

References

Behura, N., Sen, P. and Kar, M.K. (2011). Introgression of yellow stem borer (*Scirpophaga incertulas*) resistance genes into cultivated rice from wild species. Indian J. Agri. Sci. 81(4): 359-362.

Bouharmont, J. (1961). Embryo culture of rice on sterile medium. Euphytica 10:283- 293.

Brar, D.S. and Khush, G.S. (1997). Alien introgression in rice. Plant Mol. Biol. 35: 35–47.

Brar, D.S. and Khush, G.S. (2002). Transferring genes from wild species into rice. Quantitative genetics, genomics and plant breeding. CABI, Wallingford, UK.

Brar, D.S. and Khush, G.S. (2006). Cytogenetic manipulation and germplasm enhancement of rice (*Oryza sativa* L.). Genetic resources, chromosome engineering and crop improvement. CRC, FL, USA.

Brar, D.S., Dalmacio, R., Elloran, R., Aggarwal, R., Angeles, R. and Khush, G.S. (1996)Gene transfer and molecular characterization of introgression from wild *Oryza* species into rice. Rice Genet. III: 477-486. IRRI, Philippines.

Brar, D.S., Elloran, R. and Khush, G.S. (1991). Interspecific hybrids produced through embryo rescue between cultivated and eight wild species of rice. Rice Genet. Newsl. 8:91–93.

Heinrichs, E.A., Medranof, G. and Rapusash, R. (1985). Genetic evaluation for insect resistance in rice. IRRI, Philippines.

Iyer, R.D. and Govila, O.P. (1964). Embryo culture of interspecific hybrids in the genus *Oryza*. Indian J. Genet. Plant. Breed. 24(2):116-121.

Jena, K.K. and Khush, G.S. (1984). Embryo rescue of interspecific hybrids and its scope in rice improvement. Rice Genet. Newsl. 1: 133– 134.

Jena, K.K. and Khush, G.S. (1985). Production of Monosomic Alien Addition Lines of *Oryza sativa* having a single chromosome of *O.officinalis.* Rice Genet. I: 199-211. IRRI, Philippines.

Jena, K.K. and Khush, G.S. (1989). Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behaviour. Genome 32: 449 -455.

Jena, K.K. and Khush, G.S. (1990). Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice *O. sativa* L. Theor. Appl. Genet. 80:737–745.

Khush, G.S., Singh, R.J., Sur, S.C. and Librojo, A.L. (1984). Primary trisomics of rice: origin, morphology, cytology and use in linkage mapping. Genetics 107:141– 163.

Ko, S.W., Wong, C.K. and Woo, S.C. (1983). A simplified method of embryo rescue in rice of *Oryza sativa* L. Bot. Bull. Academia Sinica 24: 94-101.

Kumari, H.M.P.S., Abayarathne, W.M., Abeysiriwardana, D.S.DE Z. (2005). Development of a protocol for Embryo Rescue in wide hybridization in Rice. Annals Sri Lanka Department Agric. 7: 157- 164.

Maqbool SB, Husnain T, Riazuddin S, *et al*. (1998) Effective control of yellow stem borer and rice leaf folder in transgenic rice indica varieties Basmati 370 and M 7 using the novel ä endotoxin cry2A *Bacillus thuringiensis* gene. Mol. Breed. 4(6): 501- 507.

Misra, R.N., Jena, K.K., and Sen, P. (1985). Cytogenetics of Trisomics in Indica Rice. Rice Genet. I: 173-183. IRRI, Philippines.

Multani, D.S., Jena, K.K., Brar, D.S., delos Reyes, B.C., Angeles, E.R. and Khush, G.S. (1994). Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice O. sativa L. Theor. Appl.Genet. 88: 102- 109.

Multani, D.S., Khush, G.S., Delos Reyes, B.G. and Brar, D.S. (2003). Alien genes introgression and development of monosomic alien additional lines from *Oryza latifolia* Desv. to rice. Theor. Appl. Genet. 107:395- 405.

Niroula, R.K., Subedi, L.P., Sharma, R.C. and Upadhyay, M.P. (2004). Embryo Culture for Interspecific Hybridization in the Genus *Oryza*: A Simplified Technique of Hardening Improves the Field Plant Establishment. Agricultural Research for Enhancing Livelihood of Nepalese People. Proceedings of 2nd SAS-N Convention, 30 July 2003, Kathmandu.

Niroula, R.K., Subedi, L.P., Sharma, R.C. and Upadhyay, M.P. (2005). Interspecific hybrid plants recovered from in vitro embryo rescue in rice. Scientific World 3(3): 90- 94.

Padhi, G. and Sen, P. (2002). Evaluation of wild rice species against Yellow Stem Borer (*Scripophaga incertulas*, Walker). J. Appl. Zoo. Res. 11(2 & 3): 103- 104.

Panda, B., and Sen, P. (2007). Application of embryo rescue technique for development of interspecific hybrids in rice resistance to yellow stem borer. J. Cytol. Genet. 8 (NS): 57-63.

Pathak, M.D. and Khan, Z.R. (1994). Insect pests of rice. IRRI, Philippines.

Sen, P. and Misra, R.N. (1988). A primary trisomic series in rice, cv. Sona. J. Cytol. Genet. (Suppl.). 1: 1-7.

Shin, Y.B. and Katayama, T. (1979). Cytogenetical studies on the genus *Oryza*. XI. Alien addition lines of *O. sativa* with single chromosomes of *O.officianalis*. Jpn. J.Genet. 54: 1- 10.

Sitch, L.A. (1990). Incompatibility barriers operating in crosses of Oryza sativa with related species and genera. Gene manipulation in plant improvement II. Plenum Press, New York.

Sitch, L.A., Romero, G.O. and Dalmacio, R.D. (1989). Prefertilization incompatibility barriers in interspecific and intergeneric crosses involving *Oryza sativ*a. Int. Rice Res. Newsl. 14: 5-6.

Swaminathan, S. (1986). Plant research and world agriculture. Plant Mol. Biol. Rep. 4: 1- 17.

Virmani, S.S., Virktamath. B.C., Casal. C.L., Toledo. R.S., Lopez, M.T., and Manalo, J.O. (1997). Hybrid Rice Breeding Manual. IRRI, Philippines.

Yasui, H. and Iwata, N. (1991). Production of monosomic alien addition lines of *Oryza sativa* having a single *O. punctata* chromosome. Rice Genetics II: 147-155. IRRI, Philippines.

Plant Science Research 35 (1&2) : 44-48, 2013

Plant Science Research

ISSN 0972-8546

Callus mediated shoot proliferation from internode explant of *Paederia foetida* L.

Nupur Naik[1], B. R. Behera[1], S. K. Naik and D. P. Barik[Ψ]

Department of Botany, Ravenshaw University, Cuttack-753 003, Odisha, India

ARTICLE INFO

Article history:
Received : 30 October 2013
Received in revised form : 14 November 2013
Accepted : 6 December 2013

Keywords:
Callus culture
internode explant
medicinal plant
Paederia foetida

ABSTRACT

The present study describes the establishment of callus culture and subsequent plant regeneration using *in vitro* internode explants of *Paederia foetida.* Four different types of explants were evaluated for callus culture, of which *in vitro* internode explant was found to be the most suitable. MS basal medium supplemented with 15 different combinations of growth regulators were assessed for callus initiation and proliferation. MS supplemented with 1.5 mg/l N^6-benzyladenine (BA) and 0.1 mg/l naphthalene acetic acid (NAA) was found to be the most suitable for establishment of callus culture irrespective of explants used. The highest callus induction (100% and 93.75%) was observed in *in vitro* internode and *in vivo* internode explants respectively. The maximum percentage as well as number of shoot regeneration (33.3%; 2) was observed from the callus derived from *in vitro* internode explants inoculated on MS fortified with 1.0 mg/l BA and 0.5mg/l indole 3-butyric acid (IBA) medium.

1. Introduction

Paederia foetida L. commonly known as Gandhaprasarini (Hindi) and Pasaruni (Odiya), belongs to the family *Rubiaceae*, is an important medicinal plant. It is native to eastern and southern Asia (Wagner *et al.,* 1999). It is also found in India (Himalayas from Dehradun eastwards upto an altitude of 1800m and also in Assam, Bihar, Odisha and Andhra Pradesh), China and Philippine Island. In Peninsular Malaysia, it grows wild in open places, scrambling over trees and bushes.

The plant is well known for its use in Ayurvedic medicines of India (Mishra *et al.,* 2004). It is also widely used for the treatment of asthma, rheumatism, bowel problems, diarrhea, diabetes and seminal weakness (Blatter *et al.,* 1981; Nandkoni, 2002). It is considered as an antispasmodic, diaphoretic, expectoretant and stomachic. The leaves are boiled, mashed and applied to abdomen for urinary retention. The leaves are eaten to aid digestion and to expel gas. Fresh leaves are reported to have antioxidant properties (Osman *et al.,* 2009). Bark decoction is used as emnetic, piles and liver inflammation, decoction of whole plant is used for abdominal pain, abscesses and arthritis. Roots has been claimed to be an amollient and a caraminative. The plant has anti-inflammatory effect (Srivastava *et al.,* 1973; De *et al.,* 1994), relief from gastro-intestinal disorder by helminthic infections (Roychoudhury *et al.,* 1970); antidiarrheal effects (Afroz *et al.,* 2006). The fruit is used for toothache (Ghani, 1998). *Paederia foetida* also contains many useful chemical constituents such as iridoid glucoside, asperuloside, scandoside and peaderoside. The plant also contains alkaloids, b-paederin and essential oils. The leaves and stems contain ursolic acid, epifriedelinol, friedelin, sitosterol, stigmasterol and campestrol. Embelin is isolated from aerial parts. Leaves contain mixture of fatty acids-capric, myristic, arachnidic and palmitic acids (Alam *et al.,* 2010).

Conventional propagation of *Paederia foetida* is primarily through seeds and stem cuttings is inadequate to meet commercial demands. Besides, the plant is also facing danger of extinction (Srivastava and Srivastava, 2004). So, development of an *in vitro* plant regeneration protocol is

[Ψ] *Corresponding author; Email:* barikdp@yahoo.com
[1] Both the authors have equal contribution

essential to conserve the plant. An efficient *in vitro* propagation method may play an important role in rapid multiplication and germplasm conservation of this medicinally important herb. To our knowledge, during the past years, there have been a few reports regarding *in vitro* clonal propagation of *Paederia foetida* L. using different methods of plant tissue culture (Srivastava *et al.,* 1973; Alam *et al.,* 2010). However, an efficient callus mediated organogenesis from internode or leaf has not yet been reported for this plant species. Therefore, our objective was to develop a rapid, effective and reproducible callus mediated plant regeneration protocol for *Paederia foetida* using internode or leaf explant.

2. Materials and methods

Healthy plants were selected from the medicinal plant garden, Department of Botany, Ravenshaw University, Cuttack, Odisha. Young leaves, internodes and nodal explants were collected from healthy plants of *Paederia foetida*. Explants were washed under running tap water for 30 min followed by 8 min treatment with 5% (v/v) aqueous solution of Teepol (Reckitt Benckiser Ltd., HP, India) and 5 min rinse with double distilled water. Then the explants were surface sterilized with 0.1% (w/v) aqueous solution of $HgCl_2$ for 3 min (leaf explants) and 5 min (internode & node explants). Finally, explants were washed thoroughly 5 times with sterilized double distilled water.

Sterilized mature leaf and internode explants (*in vivo* explants) were inoculated on Murashige and Skoog's (1962) (MS) medium alone or MS supplemented with a range of growth regulators i.e. BA (1.0- 4.0 mg/l) + NAA (0.1- 2.0 mg/l) for callus initiation and proliferation. Mature nodal explants were used to develop *in vitro* shoots. These established shoots were used as the explant source for *in vitro* leaf and internode (*in vitro* explants). The *in vitro* leaf and internode explants were inoculated on the same callus initiation and proliferation medium as mentioned earlier. The calli after establishment were repeatedly sub-cultured on the same medium at an interval of 20 days. The pH of all the media were adjusted to 5.8 ± 0.1. Irrespective of media, 0.8% agar (Himedia, India) and 0.3% sucrose were used.

Green, semi hard calli were transferred to MS medium augmented with BA or Kin 1.0-4.0 mg/l and combinations of BA (1.0 mg/l) and IBA (0.1 -1.5 mg/l) for shoot regeneration. *In vitro* generated shoots were excised and transferred to rooting media comprising of one fourth strength MS (¼ MS), half strength MS (½ MS) and MS supplemented with IBA 0.05-2.0 mg/l.

The culture were maintained at 25 ± 1^0C under 16 h photoperiod of 35 µmol/ m^2s^1 photon flux density provided by cool white fluorescent tubes (Philips, India) with relative humidity 55-60%. Photographs were taken on Sony digital (7.2 megapixel) camera. Mean percentage of explants forming callus, mean % of callus showing shoot bud formation, mean number of shoots / callus were calculated. Mean data pooled from a total 4 no of replications each comprising of 4 culture tubes containing 2 explants per tube for callus experiments whereas, for shoot regeneration mean data pooled from a total 3 no of replications each comprising of 5 culture tubes containing 1 explant per tube. For rooting experiments mean data pooled from a total 3 no of replications each comprising of 4 culture tubes containing 1 explant per tube. Data were analyzed using analysis of variance (ANOVA) for a completely randomized design (CRD). Duncan's New Multiple Range Test (DMRT) was used to separate the means for significant effect (Gomez and Gomez, 1984).

3. Results and discussion

MS medium devoid of growth regulators failed to initiate callus. Calli were successfully initiated from irrespective of the explants (*in vivo* and *in vitro* leaf and internode explants) cultured on MS media supplemented with cytokinin (BA) + auxin (NAA). The response of *in vivo* and *in vitro* explants for callus initiation and proliferation was different in different hormonal combination (Table 1). Callus initiation was on the margins of the cut ends of the explants proceeding towards the center. The callus proliferation frequency % was higher in *in vitro* explants than *in vivo* explants. Least days required for callus initiation was recorded as 21 and 18 days for *in vivo* as well as *in vitro* leaf and internode explants respectively. Similar types of observations on different explant types on callus induction were observed in *Tylophora indica* (Thomas, 2007) and *Centella asiatica* (Mohapatra *et al.,* 2008). Different tissues may have different levels of endogenous hormones, and therefore, the type of explants source in most of the cases have a crucial impact on the callus initiation and its regeneration success (Das *et al.,* 2013).

The maximum callus induction frequency was found to be different in different explants type. Optimal medium for callus induction was found to be MS + 1.5 mg/l BA + 0.1 mg/l NAA. Of the four different explants evaluated the *in vitro* internode explants showed maximum (100%) callus induction followed by *in vivo* internode (93.75%) (Fig 1A, B). Both the source of leaf explants (*in vivo* leaf, 84.37 %; *in vitro* leaf, 90.6 %) showed inferior response for callus initiation in compare to internode explants (Table 1). Combination of BA and NAA are also found suitable for callus induction in *Adhatoda vasica* (Azad and Amin, 1998), *Cucurma longa* (Salvi *et al*, 2006), *Ophiorrhiza prostate*

Table1
Effect of various concentrations and combinations of growth regulators for callus formation of different explants of *Peaderia foetida* L.

MS + Growth regulators (mg/l)		% of callus proliferation				Average days for callus initiation			
BA	NAA	LEAF		INTERNODE		LEAF		INTERNODE	
		In vivo	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*
MS		0	0	0	0	0	0	0	0
1.0	0.1	78.12	84.37	90.62	96.87	21	21	18	18
	0.5	81.25	81.25	84.37	87.5	21	21	18	18
	1.0	62.5	59.3	84.37	81.25	38	28	27	18
	2.0	62.5	62.5	81.25	84.37	43	32	32	28
1.5	0.1	84.37	90.6	93.75	100	21	21	18	18
	0.5	78.12	87.5	90.62	96.87	21	21	18	18
2.0	0.1	78.12	81.25	84.37	96.87	28	21	20	18
	0.5	59.37	62.5	81.25	87.5	26	21	21	18
3.0	0.1	59.37	56.25	75	81.25	32	28	28	24
	0.5	53.12	56.25	78.12	87.5	32	32	28	28
4.0	0.1	62.5	68.75	75	71.87	32	32	28	28
	0.5	65.62	68.75	81.25	84.37	32	32	28	28
CD at 5%		1.32	1.21	1.41	1.36				

Mean data pooled from a total 4 no of replications each comprising of 4 culture tubes containing 2 explants per tube (4 x 4 x 2 = 32). CD at 5%, Duncan's New Multiple Range Test

(Beegum *et al,* 2007) and *Rauwolfia serpentine* (Salma *et al*, 2008).

Greenish calli were observed in most of the combination of BA and NAA. However, white friable callus was recorded when a comparatively high concentration of NAA (2.0 mg/l) was used along with 1.0 mg/l BA. Shoot regeneration was found only in green callus. MS basal medium fortified with kinetin resulted in no shoot bud differentiation from callus. Regeneration of only a single shoot was observed in all the concentration of BA. Four to six shoot buds appeared on green calli after 20 days of transfer to the optimum shoot regeneration medium (1.0 mg/ l BA + 0.5 mg/l IBA) (Fig. 1 C). Of the shoot buds only one or two shoot bud(s) elongated to shoots. The highest shoot regeneration (33.3%) with 2 shoots / callus was observed on calli derived from *in vitro* internode explants inoculated on shoot regeneration medium at day 45 of culture (Table 2, Fig. 1 D). Combination of BA and IBA was also recorded to have optimum shoot regeneration capacity in *Aristolochia indica* (Soniya and Sujitha, 2006), *Centella asiatica* (Hossain *et al.,* 2000; Martin, 2004; Mohapatra *et al.,* 2008), *Ocimum basilicum* (Sahoo *et al.,* 1997), and *Vitex trifolia* (Hiregoudar

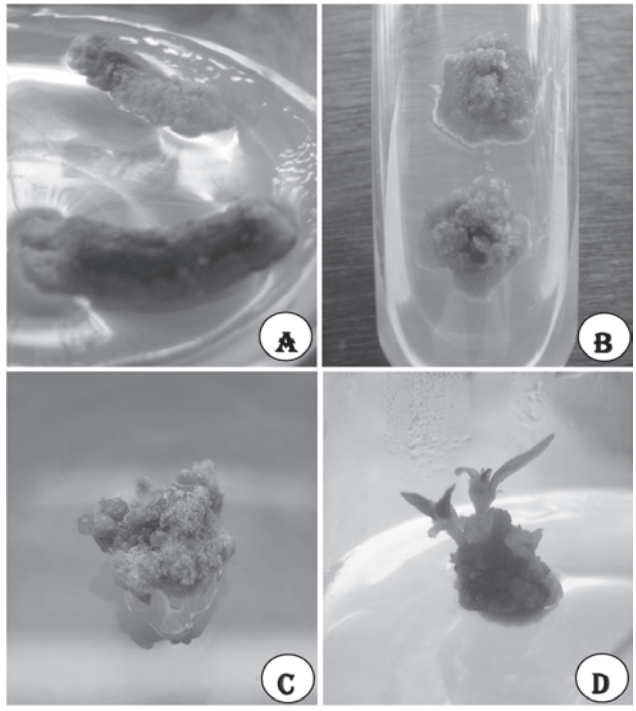

Fig. 1 (A) *In vivo* internode and **(B)** *In vitro* internode derived callus on MS + 1.5 mg/l BA + 0.1 mg/l NAA at day 40 **(C)** Shoot bud formation from *in vitro* internode derived callus on MS + 1.0 mg/ l BA + 0.5 mg/l IBA at day 20 **(D)** Shoot elongation on MS + 1.0 mg/l BA + 0.5 mg/l IBA at day 45.

Table 2

Effect of different concentrations of cytokinin alone or in combination with auxin on shoot regeneration from *in vitro* internode derived callus of *Paederia foetida*.

MS + Growth regulators (mg/l)			Cultures responded (%)	Average number of shoots/callus	Average shoot length (cm)
BA	IBA	Kin			
	MS		-	-	-
1.0	-	-	26.0[e]	1	1.9
2.0	-	-	25.3[e]	1	2.1
3.0	-	-	26.0[e]	1	2.3
4.0	-	-	19.3[f]	1	1.8
1.0	0.1	-	28.6[d]	1	2.1
1.0	0.5	-	33.3[a]	2	2.1
1.0	1.0	-	31.8[b]	2	2.3
1.0	1.5	-	30.3[c]	1.5	2.3
—	—	1.0	—	—	—
—	—	2.0	—	—	—
—	—	3.0	—	—	—
—	—	4.0	—	—	—

— = Nil/ No response

Mean data pooled from a total 3 no of replications each comprising of 5 culture tubes containing 1 explant per tube (3 x 5 x 1 = 15). Mean values within column with same superscripts are not significantly different ($p < 0.05$; Duncan's New Multiple Range Test)

et al., 2006). Rooting experiment is under progress and results of rooting experiment are yet to be documented.

References

Afroz, S., Alamgir, M., Khan, M. T. H., Jabber, S., Nahar, N. and Choudhuri, M. S. K. (2006). Antidiarrhoel activity of the ethanolic extract of *Paederia foetida* Linn. (Rubiaceae). J. Ethnopharmacol. 105(1-2): 125-130.

Alam, M., Azam, F. M. S., Karim, M. M., Rehana, F., Sharmin, N., Kalpana, M. K., Jahan, R., Rahmatullah, M. and Khatun, M. A. (2010). *In vitro* regeneration of *Paederia foetida* : a widely used medicinal vine in Bangladesh. American-Eurasian J. Sustain. Agric. 4(2) : 164-169.

Azad, M. A. K. and Amin, M. N. (1998). *In vitro* regeneration of plantlets from internode explants of *Adhatoda vasica* Nees. Plant Tiss. Cult. 8(1) : 27-34.

Beegum, A. S., Martin, K. P., Zhang, C., Nishitha, I. K., Ligimol, Slater A. and Madhusuoodanan, P. V. (2007). Organogenesis from leaf and internode explants of *Ophiorrhiza prostate*, an anticancer drug (camptothecin) producing plant. Elect. J. Biotechnol. 10 (1): 115-123.

Blatter, E., Caius, J. F. and Mhaskar, K. S. (1981). Indian Medicinal Plants 2: 1297-1299.

Das, J., Mao, A. A. and Handique, P. J. (2013). Callus-mediated organogenesis and effect of growth regulators on production of different valepotriates in Indian valerian (*Valeriana jatamansi* Jones.). Acta Physiol. Plant. 35 : 55–63

De, S., Ravishankar, B. and Bhavsar, G. C. (1994). Investigation of the anti-inflammatory effects of *Paederia foetida*. J. Ethnopharmacol. 43(1): 31-38.

Ghani, A. (1998). Medicinal plants of Bangladesh: Chemical constituents and Uses. Monographs: *Elaeocarpus serratus* Linn. Asiatic. Soc. Bangladesh Publ. 253-254.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedure for Agricultural Research. John Wiley & Sons, New York.

Hiregoudar, L.V., Murthy, H.N., Bhat, J.G., Nayeem, A., Hema, B.P., Hahn, E.J. and Paek, K.Y. (2006). Rapid clonal propagation of *Vitex trifolia*. Biol. Plant. 50: 291-294.

Hossain, S.N., Rahman, S., Joydhar, A., Islam, S. and Hossain, M. (2000). *In vitro* propagation of thankuni (*Centella asiatica* L.). Plant Tiss. Cult. 10: 17-23.

Martin, K.P. (2004). Plant regeneration through somatic embryogenesis in medicinally important *Centella asiatica* L. *In Vitro* Cell. Dev. Biol. Plant. 40: 586-591.

Mishra, B. P., Tripathi, O. P., Tripathi, R. S. and Pandey, H. N. (2004). Effects of anthropogenic disturbance on plant diversity and community structure of a sacred grove in Meghalaya, northeast India. Biodiv. Conserv. 13(2): 421-436.

Mohapatra, H., Barik, D.P. and Rath S.P. (2008). *In vitro* regeneration of medicinal plant *Centella asiatica* L. Biol. Plant. 52 (2): 339-342.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nandkoni, K.M. (2002). Indian Materia Medica. Bombay Popular Prakashan, Mumbai, pp: 892.

Osman, H., Rahim, A. A., Isa, N. M. and Bakhir, N. M. (2009). Antioxidant Activity and Phenolic Content of *Paederia foetida* and *Syzygium aqueum*. Molecules 14(3): 970-978.

Roychoudhury, G. K., Chakrabarty, A. K. and Dutta, B. (1970). A preliminary observation on the effects of *Paederia foetida* on gastro-intestinal helminths in bovines. Ind. Vet. J. 47(9) : 767-769.

Sahoo, Y., Pattnaik, S.K. and Chand, P.K. (1997). *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. *In Vitro* Cell. Dev. Biol. Plant. 33: 293-296.

Salma, U., Rahaman, M. S. M., Haque, N., Jubair, T. A., Haque, A. K. M. F. and Mukti, I .J. (2008). The influence of different hormone concentration and combimation on callus induction and regeneration of *Rauwolfia serpentia* L. Benth. Pak. J. Biol. Sci. 11(12) : 1638-1641.

Salvi, N. D., George, L. and Eaphen, S. (2006). Plant regeneration for leaf base callus of turmeric and random polymorphic DNA analysis of regenerated plants. Plant Cell Tiss. Organ Cult. 66(2): 113-119.

Soniya, E.V. and Sujitha, M. (2006). An efficient *in vitro* propagation of *Aristolochia indica*. Biol. Plant. 50 (2): 272-274.

Srivastava, M. C., Tewari, J. P. and Kant, V. (1973). Anti-inflammatory activity of an indigenous plant *Paederia foetida* (Gandhali). Ind. J. Medical Sci. 27(3): 231-234.

Srivastava, S. K. and Srivastava, N. (2004). *In vitro* multiplication of *Paederia foetida* L.-A rare medicinal plant. J. Plant Biochemistry and Biotechnol. 13: 89- 91.

Thomas, T.D. (2007). Pretreatment in thidiazuron improves the *in vitro* shoot induction from leaves in *Curculigo orchioides* Gaertn., an endangered medicinal plant. Acta. Physiol. Plant. 29: 455–461.

Wagner, W.L., Herbst, D. R. and Sohmer, S. H. (1999). *Manual of the Flowering Plants of Hawai'i*. 2 vols. Bishop Museum Special Publication 83, University of Hawai'i and Bishop Museum Press, Honolulu, HI.

Plant Science Research 35 (1&2) : 49-55, 2013

Plant Science Research

ISSN 0972-8546

Validation of tribal claims on *Dioscorea pentaphylla* L. through phytochemical screening and evaluation of antibacterial activity

S. Kumar, S. P. Behera and P. K. Jena[Ψ]

Department of Botany, Ravenshaw University, Cuttack-753 003, Odisha, India

ARTICLE INFO

Article history:
Received : 9 October 2013
Received in revised form : 24 November 2013
Accepted : 7 December 2013

Keywords:
tribal claims
Dioscorea pentaphylla L.,
antibacterial activity
validation

ABSTRACT

The present study elucidates the ethnobotanical uses of various parts of *Dioscorea pentaphylla* L. by the rural and tribal communities of Similipal Biosphere Reserve, Odisha and its adjoining areas. Further, it validates the tribal claims using phytochemical screening and antibacterial activity. Acetone, Methanol and aqueous extracts showed relevant zone of inhibition against two Gram positive bacteria *Streptococcus mutans* (MTCC*497) and *Streptococcus pyogenes* (MTCC1926); three Gram-negative bacteria *Vibrio cholera* (MTCC3906), *Shigella flexneri* (MTCC1457) and *Salmonella entericatyphi* (MTCC1252).

1. Introduction

Research evidences of the recent years have shown the urgent need for new antimicrobial agents to replenish, the arsenal of anti-infective agents or drugs on the light of antibiotic resistance reported in pathogenic microbes. It is reported that, on an average, two or three antibiotics are launched each year (Osbourn, 1996). After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that, the effective life span of any antibiotics is limited (Eisenberg *et al.*, 1993; Alper, 1998; Wise, 2011). Therefore, it is necessary to know more about antimicrobial resistance (AMR), the mechanisms of action and screening of secondary metabolites from bio-wealth.

Plant materials are of wide use in traditional systems of medicine, and in several communities of the developing world (Fischbash and Walsh, 2009). They are the only resources available for the treatment of different microbial infections among many rural and tribal communities. Most of the common plants have been reported to have antimicrobial activity, still a number of unexplored wild plants are available in the forests having good ethnic values as traditional medicine (Dianella, 2012). There is need for the screening of bioactive compounds present in such plants and the antimicrobial activities of their extracts (Ginsburg and Deharo, 2011). Among those unexplored plants wealth, genus *Dioscorea* is very common, found abundant in wild forests of Odisha (Kumar and Satpathy, 2011; Kumar *et al.,* 2012; Misra *et al.,* 2013). There are 11 wild species of this genus found in Odisha (Kumar *et al.,* 2012). Among them, *Dioscorea pentaphylla* L. (Plate-1: A, B and C) is easily available (Sinha and Lakra, 2005; Kumar *et al.,* 2012).

D. pentaphylla L. (Dioscoreaceae; local name- Panja Sanga) is a tuberous monocot vine bearing aerial bulbils (Fig. 1). It is left twining much more slender climber, more or less prickly below, 3-5 foliate leaves. Leaves are glabrous/ pubescent beneath. Leaflets of lower leaves rarely larger, centre ones obovate or elliptic suddenly cuspidate or acuminate, cuneate towards base. Axillary racemes and sometimes terminaly panicled, or the axilary racemes sometime branched. Spike of female flower is long and solitary or 2-nate rarely panicled. Capsule large oblong and deflexed. Elongate obpyriform bulbils. Tubers are oblong or clavate, proceeding direct from the base of the aerial stem and thickening downwards (Saxena and Brahmam, 1995).

Tribal communities use its part as food and against microbial infections. The Hill-Kharia tribe, Mankirdia tribe, Santhal tribe, Ho tribe, Kolha tribe, Munda tribe and Bhumij tribe of Odisha use rhizome of *D. pentaphylla* against skin

[Ψ] *Corresponding author; Email:*padan_jena@rediffmail.com

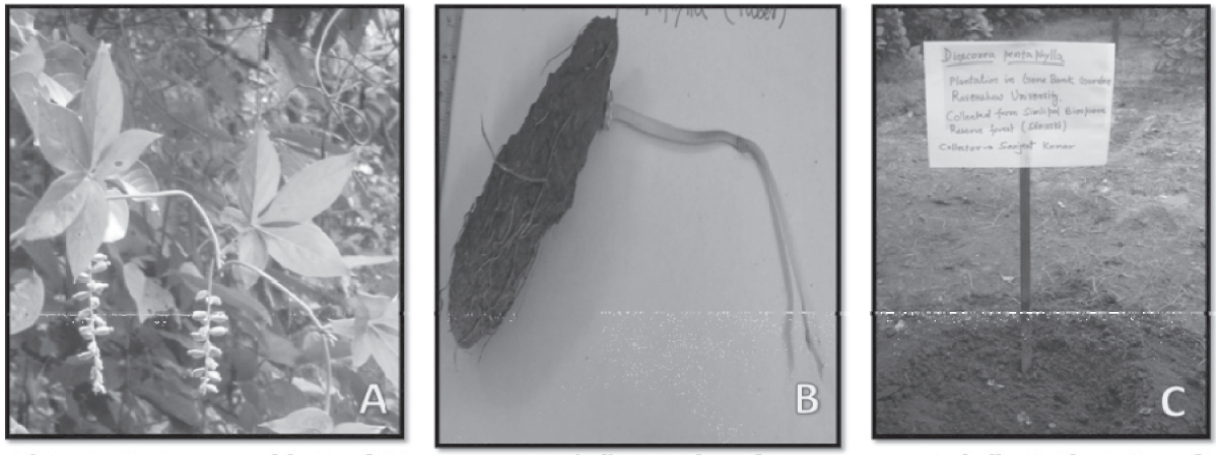

Plate-1: A- Leaves and fruits of *Dioscorea pentaphylla*; B- Tuber of *Dioscorea pentaphylla*; C- Plantation of *Dioscorea pentaphylla* at Dept. of Botany, Ravenshaw University, Cuttack

infections (Misra *et al.,* 2013). All these evidences make a sound platform for the study on antibacterial activity of plant extracts. In this study, an attempt has been made to validate the tribal claims (ethnobotanical values) to evaluate the bioactive compounds present in *Dioscorea pentaphylla* L.

2. Materials and Methods

2.1 Assessment of ethnobotanical data

The field work was conducted with the rural and tribal communities of adjoining areas (Jashipur, Karanjia, Bisoi, Kendumundi and Padampur) of Similipal Biosphere Reserve (SBR) during 2010-2013. The methodological framework was followed as per standard technique of ethno-biological approaches of Christian and Brigitte (2004). The information on plant used as traditional medicine against different pathogens and disorders were collected through questioners with different rural and tribal communities (Lohar, Mankirdia, Ho, Santhal, Kolha. Hill-Kharia and Munda). The pharmacological and medicinal properties of *Dioscorea pentaphylla* L. were confirmed by cross check with informants. Plant species was confirmed using standard flora (Saxena and Brahmam, 1995).

2.2 Collection of plant material

Tubers of *D. pentaphylla* L. were collected from the village Padampur of district Mayurbhanj. Collected tubers were planted in the experimental garden of Botany Department, Ravenshaw University, Cuttack. Plant parts were collected from mature plant in the garden. They were washed properly and were cut into small pieces and left for air drying. The dried materials were crushed to powder with mechanical device and were kept it in air tight container for phytochemical screening and antibacterial activity.

2.3 Extraction and detection of bioactive compounds

As per polarity index three solvents (acetone, methanol and aqueous) were selected for extraction. Extraction was done using soxhlet apparatus following the protocol of Tiwari *et al.* (2011). The powder of tuber was poured in the thimble at the ratio of 20 g per 250 ml of solvents at the temperature of 60° C (Methanol), 50 ° C (Acetone) and 100 ° C (Aqueous). Collected residue was then dried at room temperature. Qualitative detection of the bioactive compounds was done on n-Hexane, acetone, methanol, and aqueous extracts of different parts standard procedure (Sofowora, 1993).

2.4 Antibacterial activity

The extracts of *D. pentaphylla* were screened for antibacterial activity against two Gram positive bacteria *Streptococcus mutans* (MTCC *497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella enterica typhi* (MTCC 1252) collected from Institute of Microbial Technology (IMTECH), Chandigarh. Nutrient broth was used to maintain broth cultures. Antimicrobial activity was done using Agar Well Diffusion assay (Allen *et al.,* 1991) with slight modification. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (dimethyl sulfoxide; Sigma) and twofold serial dilutions were made in amount of 100 µl per well ranged from 0.5, 1.0 and 2.0 mg / ml. Samples (100 µl) were added by sterile syringes into the wells and allowed to diffuse at room temperature for 2 h. Only the solvent (DMSO) was poured into the wells in another set of plates as part of negative control (Amanda *et al.,* 2012). The positive control set consisted of standard antibiotics Kanamycin with disc potency 10µg in place of samples. Plates were

incubated at 35 ± 2°C for 18-24 h. after which the zones of inhibition were measured to determine the antibacterial activity.

2.5 Data analysis

Triplicates were maintained and the experiment was repeated thrice. Mean and SD (standard deviation) was performed to evaluate triplicate values of zone of inhibition (cm) of samples.

3. Results and discussion

Field survey revealed that *Dioscorea pentaphylla* L. is very popular as food and medicine among the tribal communities of SBR and its adjoining areas (Table 1). Tubers are bitter in taste but after traditional preparation such as continuous washing overnight and boiling, these are consumed as vegetables. Tuber paste is also used against skin infections and other diseases.

The qualitative analysis of phytochemical screening has shown the medicinal potential of the plant. Terpenoids, Tannin, Saponin, Glycosides, Reducing sugar, Flavonoids and Phenolic componunds are major bioactive compounds present in the tuber, leaf and bulbils of *D. pentaphylla* (Table 2). Presence of these bioactive compounds is thought to be effective against different bacterial and fungal infections. The antibacterial activity of acetone, methanol and aqueous extracts of tuber showed significant zone of inhibition (cm) against *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457), *Salmonella enteric typhi* (MTCC 1252), *Streptococcus pyogenes* (MTCC 1926) and *Streptococcus mutans* (MTCC *497). Aqueous extract of tuber showed excellent activity against MTCC 1926 (Table 3; Fig. 2). It has been observed that methanol and acetone extract were found to be active against Gram-negative bacteria *Vibrio cholera* (MTCC 3906). Therefore it is presumed that these compounds can be used for formulation of new drugs which may be effective against cholera and can also fight against antimicrobial resistance. The extracts also showed good growth inhibition against Gram-positive bacteria *Streptococcus mutans* (MTCC *497).

Table 1
Ethnobotanical values of *Dioscorea pentaphylla* L. among the tribal communities (adjoining areas of SBR)

Plant Part(s)	Collection site(s)	Races	Medicinal Use(s)	Mode of use(s)	Other use(s)
Tubers	Padampur	Santhal	Skin Infections	Macerated tuber paste is applied externally on lesions	Edible
	Jashipur haat	Mankardia	Cold	Approx 250 gm tuber is boiled with about 1 lit of water and juice is prepared. One cup of juice with salt is taken thrice a day to remove cough.	Edible
	Bisoi	Ho	Constipation	One year old tubers are left overnight in running water and this tubers are used as chips to cure stomach pain and constipation problems.	Edible
	Karanjia	Santhal	Poor appetite	After successive boiling, the tubers are eaten as vegetables and to reduce poor appetite twice a week.	Edible
	Haatibaadi	Santhal	Against cut	Approx 200 gm of fresh tuber is crushed with water and made into paste, which is used externally on cut and other similar wounds thrice a day till cure.	Edible
Leaves	Padampur	Munda	Against joint pain	Leaves paste made with Karanja oil (*Pongamia pinnata*) and is rubbed on joint	

Table 2
Qualitative analysis of bioactive compounds in plant parts of *Dioscorea pentaphylla* L.

Plant part(s)	Solvent used	Bioactive compound(s) detected
Tubers	n-Hexane	Terpenoids
	Acetone	Tannin, Flavonoids, Glycosides and Reducing sugar
	Methanol	Tannin, Phenolic compounds and Steroids
	Water	Saponin, Reducing sugar and Glycosides
Leaves	n-Hexane	No bioactive compounds detected
	Acetone	Tannin, Saponin, Flavonoids, Terpenoids, Glycosides and Reducing sugar
	Methanol	Tannin, Saponin, Phenolic compounds and Glycosides
	Water	Tannin, Saponin, Flavonoids, Reducing sugar and Phenolic compounds
Bulbils	n-Hexane	Terpenoids
	Acetone	Tannin, Flavonoids, Glycosides and Reducing sugar
	Methanol	Tannin, Phenolic compounds and Steroids
	Water	Saponin, Reducing sugar and Glycosides

The compounds present in this extract might be playing active role against primary etiologic agents of coronal caries and root caries for oral diseases such as dental and periodontal caused by *Streptococcus mutans* (Hay *et al.,* 1994), *Streptococcus sobrinus* (Ellen *et al.,* 1985), *Streptococcus oralis* (Sansone *et al.,* 1993) etc. The extracts also showed relevant results against Gram-negative bacteria *Salmonella enterica typhi* (MTCC 1252).

Salmonella enterica typhi is considered as deadly bacteria causing typhoid fever and being responsible for the dead of more than 6 lakh people annually all over the world (Falkaw *et al.,* 2004). Strains were tested for their vulnerability using chloramphenicol, trimethoprim and amoxicillin and found all strains are resistance (Philippa *et al.,* 1998) to all these formulation. The tuber extracts being effective against this fatal strain, can be used in new drug formulation against *Salmonella enterica typhi* (MTCC 1252).

The present study validates the tribal claims as reported for medicinal uses of the plant parts. The santhal tribe of Padampur reported that the tuber of *Dioscorea pentaphylla* L. used against different types of skin infections, which is correct as tannin presence in methanol extract and saponin presence in aqueous extract (Okwe and Okwe, 2004; Aderotimi and Samuel, 2006; Mohan and Kalidas, 2010) created zone of inhibition against *Streptococcus poyogenes* (Table 3 & 4; Fig 2). At concentration 500 µg/ml, aqueous extract showed higher zone of inhibition (1.20 cm) than acetone (0.92 cm) and methanol extract (1.00 cm) against *Streptococcus polygenes (*MTCC 1926).

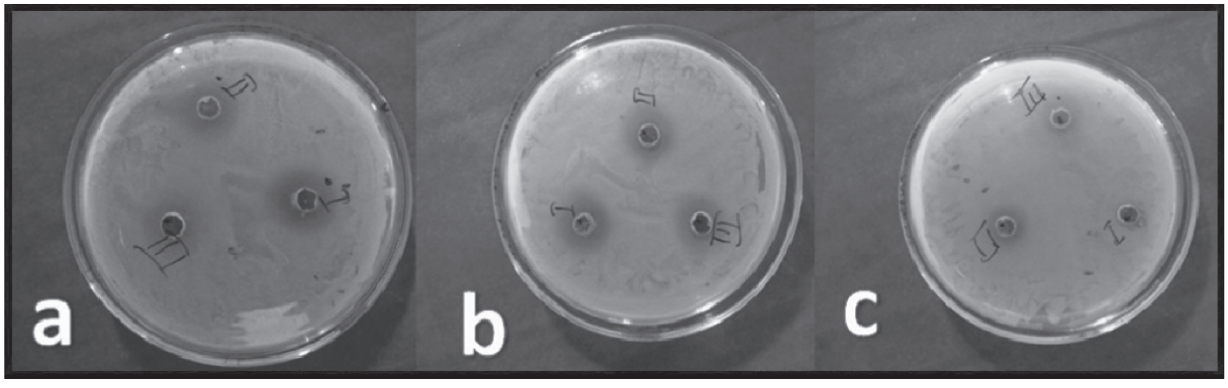

Fig. 2. Zone of inhibition of aqueous extract against MTCC 1926 (a), MTCC 1457 (b), and MTCC 3906 (c). I: 2000 µg/ml; II: 1000 µg/ml; I: 500 µg/ml.

Table 3
Antibacterial activity of *Dioscorea pentaphylla* tuber extracts with standard.

Strain(s)	Zone of inhibition (cm)			Extract / Standard
	500 µg/ml	1000 µg/ml	2000 µg/ml	
MTCC 3906	0.55 ± 0.07	1.05 ± 0.07	1.00 ± 0.14	Methanol Extract
MTCC 1252	0.85 ± 0.07	1.10 ± 0.00	1.45 ± 0.07	
MTCC 1457	1.20 ± 0.14	1.35 ± 0.21	1.50 ± 0.14	
MTCC 1926	1.00 ± 0.14	1.40 ± 0.21	1.50 ± 0.00	
MTCC *497	0.85 ± 0.21	1.20 ± 0.41	1.40 ± 0.14	
MTCC 3906	1.05 ± 0.07	1.27 ± 0.03	1.41 ± 0.01	Acetone Extract
MTCC 1252	0.95 ± 0.07	1.37 ± 0.03	1.72 ± 0.10	
MTCC 1457	0.87 ± 0.03	1.19 ± 0.01	1.44 ± 0.07	
MTCC 1926	0.92 ± 0.10	1.29 ± 0.01	1.77 ± 0.03	
MTCC *497	0.92 ± 0.10	1.2 ± 0.14	1.49 ± 0.01	
MTCC 3906	1.10 ± 0.14	1.10 ± 0.14	1.30 ± 0.14	Aqueous Extract
MTCC 1252	0.70 ± 0.14	0.85 ± 0.07	1.05 ± 0.07	
MTCC 1457	1.10 ± 0.14	1.15 ± 0.21	1.45 ± 0.07	
MTCC 1926	1.20 ± 0.14	1.50 ± 0.14	1.60 ± 0.14	
MTCC *497	0.85 ± 0.21	1.20 ± 0.00‘	1.47 ± 0.03	
MTCC 3906		1.63 ± 1.53		Kanamycin
MTCC 1252		1.56 ± 0.58		
MTCC 1457		1.73 ± 0.58		
MTCC 1926		1.76 ± 0.58		
MTCC *497		1.73 ± 0.58		

Note: MTCC 3906- *Vibrio cholera*; MTCC 1252- *Salmonella enterica typhi*; MTCC 1457- *Shigella flexneri*; MTCC 1926- *Streptococcus pyogenes*; MTCC *497- *Streptococcus mutans*.

Table 4
Correlation of Tribal Claims with bioactive compounds & antibacterial activity of *Dioscorea pentaphylla* L. tuber extracts for validation.

Plant Parts	Tribal claims	Correlation with bioactive compounds	Correlation with antibacterial activity	Reference
Tubers	Skin infections	Tannin presence in methanol extract and saponin presence of aqueous extract of *Dioscorea pentaphylla* L. tuber may be responsible for curing skin infections.	Antibacterial activity of aqueous extract against *Streptococcus polygenes*	Okwe and Okwe, 2004; Mohan and Kalidas, 2010; Aderotimi and Samuel, 2006
	Cold	Presence of Terpenoids in n-Hexane extract might be responsible		Musa *et al.,* 2009
	Cut and wounds	Presence of flavonoids in acetone extract might be responsible		Musa *et al.,* 2009
Leaves	Joint pain	Presence of flavonoids in acetone and methanol extract might be responsible.		Majumadar *et al.,* 2008
Bulbils	Not bitter in taste	No indication of Saponin in aqueous may be indicated less bitterness properties.		Magdolena, 2009

4. Conclusion

Dioscorea pentaphylla L. a wild tuber crop abundant in Similipal Biosphere Reserve forest and its adjoining areas possesses wide ethnobotanical values among the rural and tribal communities of the study areas. Presence of bioactive compounds has shown the potential antibacterial and pharmacological action of this wild tuber. Present study might be useful to supplement scientific information to establish tribal claims for the presence of phytocompounds and medicinal values. Further research is needed to document the detail bioactive compounds present in such wild plants predominant in Odisha and their probable use against known pathogens and antibiotic resistance as well.

Acknowledgement

The authors are grateful to the Field Director, Similipal Biosphere Reserve, HOD, Department of Botany, Ravenshaw University for providing the facilities for this work. The authors wish to thank the traditional tribal healers of Similipal Biosphere Reserve and its adjoining areas and also thank to Dr. R.C. Misra, National Bureau of Plant Genetic Resource, Base Centre, Cuttack.

References

Aderotimi, B. and Samuel, A. (2006). Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum, Bacopa monnifera* and *Datura stramonium*. Biokemistri. 18 (1): 39-44.

Allen, K.L., Molan, P.C. and Reid, G.M. (1991). A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmac. 43: 817–822.

Alper, J. (1998). Effort to combat microbial resistance lags. Amer. Sci. Microb. News. 64: 440-441.

Amanda, J.D., Bhat, N., Ruth, A.K., Katherine, L.O. and David, R.M. (2012). Disc Diffusion Bioassays for the detection of antibiotic activity in body fluids: applications for the pneumonia etiology research for child health project. Clin. Inf. Dise. 54: 159-164.

Christian, R.V. and Brigitte, V.L. (2004). Tools and methods for data collection in ethnobotanical studies of homegardens. Field Method. 16 (3): 285-306.

Dianella, S. (2012). Plant derived antimicrobial compounds: alternatives to antibiotic. Future Microbiol. 7 (8): 979-990.

Eisenberg, D.M., Kessler, R.C., Foster, C., Norlock, F.E., Calkins, D.R. and Delbanco, T.L. (1993). Unconvential medicine in the United States: prevalence, costs and patterns of use. New Eng. J. Med. 328: 246-252.

Ellen, R.P., Banning, D.W. and Fillery, E.D. (1985). Longitudinal microbiological investigation of a hospitalized population of older adults with a high root surface caries risk. J. Dent. Res. 64:1377-1381.

Falkow, S., Monack, D. M. and Mueller, A. (2004). Persistent bacterial infections: the interface of the pathogen and the host immune system. Nature Rev. Micribiol. 2: 747-765.

Fischbash, M.A. and Walsh, C.T. (2009). Antibiotics for emerging pathogens. Sci. 325: 1089-1093.

Ginsburg, H. and Deharo, E. (2011). A cell for using natural compounds in the development of new antimalarial treatments- an introduction. Malarial J. 10: 1-11.

Hay, D.I., Ahern, J.M., Schluckebier, S.K. and Schlesinger, D.H. (1994). Human salivary acidic proline-rich protein polymorphisms and biosynthesis studied by highperformance liquid chromatography. J. Dent. Res. 73: 1717-1726.

Kumar, S. and Satapthy, M. K. (2011). Medicinal plants in an urban environment; herbaceous medicinal flora from the campus of Regional Institute of Education, Bhubaneswar, Odisha. Int. J. Pharm. Lif. Sci. 2 (11): 1206-1210.

Kumar, S., Jena, P.K. and Tripathy, P.K. (2012). Study of wild edible plants among tribal groups of Simlipal Biosphere Reserve forest, Odisha, India; with special reference to *Dioscorea* species. Int. J. Biol. Tech. 3 (1): 11-19.

Magdolena, K. (2009). Chelation of Cu (II), Zn (II) and Fe (II) by tannin constituents of selected edible nuts. Int. J. Mol. Sci. 10: 5485-5497.

Majumdar, S.H., Chakarborty, G.S. and Kulkarni, K.S. (2008). Medicinal potential of *Semecarpus anacardium* nut: a review. J. Herb. Med. Toxicol. 2 (2): 9-13.

Misra, R.C., Sahoo, M.K., Pani, D.R. and Bhandari, D.C. (2013). Genetic resources of wild tuberous food plants traditionally used in Simlipal Biosphere Reserve, Odisha, India. Genet. Resour. Crop Evol. DOI 10. 1007/ s10722-013-9971-6.

Mohan, V.R. and Kalidas, C. (2010). Nutritional and antinutritional evaluation of some unconventional wild edible plants. Trop. Subtrop. Agroecosys. 12: 494-506.

Musa, A.M. Aliyu, A.B., Yaro, A.H., Magaji, M.G., Hassan, H.S. and Abdullahi, M. (2009). Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extract of *Anisopus mannii* in rodents. African J. Pharm. Pharmac. 3(8): 374-378.

Okwe, D.E. and Okwe, M.E. (2004). Chemical composition of *Spondias mombin* Linn., plant parts. J. Sustain. Agric. Env. 6: 140-147.

Osbourn, A.E. (1996). Performed antimicrobial compounds and plant defence against fungal attack. Plant Cell. 8: 1821-1831.

Philippa, M.A., Shanahan, J., Mary, V., Thomson, C. J. and Sebastia, G. B. (1998). Molecular analysis of and identification of antibiotic resistance genes in clinical

isolates of *Salmonella typhi* from India. A. Soc. Microbiol. 36: 1595–1600.

Sansone, C., Van, H., Joshipura, K., Kent, R. and Margolis, H.C. (1993). The association of *mutans streptococci* and non-mutans *streptococci* capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. Int. J. Dent. Res. 72: 508-516.

Saxena, H.O. and Brahmam, M. (1995). The Flora of Orissa. Orissa Forest Development Corporation Ltd. And Regional Research Laboratory, Bhubaneshwar. 3: 1940-1956.

Sinha, R. and Lakra V. (2005). Wild tribal food plants of Orissa. Ind. J. Trad. Knowl. 4(3): 246 252.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books limited. Ibadan.

Wise, R. (2011). Regenerating antibacterial drug discovery and development, the urgent need for antibacterial agents. J. Antimicrob Chemo. 66: 1939-1940.

Plant Science Research 35 (1&2) : 56-60, 2013

Plant Science Research

ISSN 0972-8546

Desmid diversity in Khanajan - a manmade channel linking Deepor beel Ramsar site to Brahmaputra River (India)

P. P. Baruah[Ψ] and Rosaballe Baruah

Department of Botany, Gauhati University, Guwahati -781014, Assam, India

ARTICLE INFO

Article history:
Received : 17 November 2013
Accepted : 12 December 2013

Keywords:
Desmids
Khanajan
Seasonality
Brahmaputra River
Deepor beel

ABSTRACT

For compilation of desmid flora, the authors studied the species diversity of planktonic desmids collected from Khanajan water channel regularly at the interval of one month from November 2011 to December 2012. A total of 22 taxa of desmids have been identified belonging to seven genera, that are *Closterium* Nitzsch (5 species), *Euastrum* Ehrenberg (1 species), *Micrasterias* C.A.Agardh (2 species), *Cosmarium* Corda ex Ralfs (9 species), *Anthrodesmus* Ehrenberg (1 species), *Staurastrum* Meyen (3 species) and *Desmidium* C.A.Agardh (1 species). All the taxa are taxonomically enlisted. The annual mean values of the total cell density and biomass were 84.02×10^2 cells /L and 0.048 mg/L respectively. Higher occurrence of desmids with 17 taxa in post-monsoon season had attributed to heavy rainfall and back flow of Brahmaputra water. *Cosmarium was* the most abundant genus particularly in monsoon and pre-monsoon season.

1. Introduction

Desmids are the freshwater phytoplankton belonging to the green algal order Zygnematales. They may be unicellular or filamentous, comprising of two semicells which are identical in shape. They are often found in water bodies where the conductivity and nutrient concentrations are very low (Ngearapat and Peerapornpisal, 2007). They are more common and diverse in oligotrophic water ecosystems (Gerrath, 1993) and have been playing an important role in aquatic ecosystem as one of the pioneer groups in food chain. High sensitivity to the changes in the environmental parameters makes them one of the important microbial bio-indicators of any water body (Coesel, 2001).

Knowledge regarding the diversity of desmids in India can be found from the works of Prasad and Misra (1992), Sindhu and Panikkar (1995), Misra and Srivastava (2003), Dwivedi *et al*. (2004), Misra *et al*. (2006), Dwivedi *et al*. (2009). A little work has so far been done in North East India in general and Brahmaputra river valley in particular (Deka *et al*., 2011; Yasmin *et al.*, 2011). In order to understand the basic nature of water body in changing environment, an attempt has been made to study the diversity of desmid flora in the Khanajan channel, which carries excess water of Deepor beel Ramsar site during winter and pre-monsoon seasons and takes part in recharging the beel during monsoon to post-monsoon when river Brahmaputra flows above the normal water level. The aim was also to construct the baseline data on desmids for future study. Being a bioindicator of oligotrophic condition, desmids of this discharge and recharge channel of Deepor beel is supposed to tell the health of the Ramsar site.

2. Materials and methods

2.1 Study site

The present study was carried out in a man-made channel that links the Deepor beel Ramsar site to the mighty Brahmaputra River. The Deepor beel, which is located within the co-ordination of 91°35' E to 91°43' E and 26°05' N to 26°11' N on 165-186 feet above MSL (Saikia and Bhattacharjee, 1987) has been considered of riverine origin and lies on the southern bank of river Brahmaputra covering

[Ψ] *Corresponding author; Email:* ppbaruah@gauhati.ac.in

an area of about 40 km^2. It is a large natural wetland having great biological and ecological importance besides being the only major storm water storage basin for the Guwahati city, the gateway of North East India. The beel is endowed with rich floral and faunal diversity. Deepor beel is characterised by a humid, tropical, prolonged monsoon season from May to September, a short post-monsoon period in October and November, a relatively dry, cool winter starting from December till February and a pre-monsoon period from March to May with occasional storms. The Khanajan, around 5 km long channel with an average width of 65 m was constructed by a local King in the mid seventeenth century to make the Deepor beel a hide out for military boats (Bhuyan, 1989) and thereafter, it has been taking part in natural recharge and discharge of storm and flood water to and from the beel. The channel has thus been performing one crucial role in maintenance of Deepor beel Ramsar site.

2.2 Sampling technique

Samples were collected monthly from November 2011 to December 2012. Collections were made with the help of planktonic mesh net (pore size 50µm) and were fixed in 4% formalin (aqueous solution of formaldehyde). Specimens were preserved in the Department of Botany, Gauhati University, Assam. These were microscopically examined (Magnus LXi microscope) and identified using standard literatures (Prasad and Misra, 1992; Perumal and Anand, 2009; Yamagishi, 2010).

The samples for total cell and volume analysis were concentrated into 30 ml after 24 h sedimentation. The procedure of Lund *et al*. (1958) was followed for counting precision. The cell counts were converted into biomass values using the formula as proposed by Yinxin and Minjuan (2005).

3. Results and discussion

3.1 Species composition and seasonal fluctuation

A total number of 22 species of desmids belonging to seven genera had been identified during the study period. *Cosmarium* was found to be the dominant genus comprising of 9 species (41%) of the total desmids identified (Fig.1). Other genera are *Closterium* (5 species, 23%), *Euastrum* (1 species, 4%), *Micrasterias* (2 species, 9%), *Anthrodesmus* (1 species, 4%), *Staurastrum* (3 species, 14%) and *Desmidium* (1 species, 4%). Majority of the taxa are unicellular, with the exceptions of *Micrasterias foliacea* and *Desmidium bengalicum*, which are filamentous in nature. All species of desmids were, of course, reported earlier from the state of Assam in different explorations in different occasions but seasonal composition was different. The dominant species were different in different seasons. No single species was found during every collecting date (Table-1) indicating that there is no single species dominance in

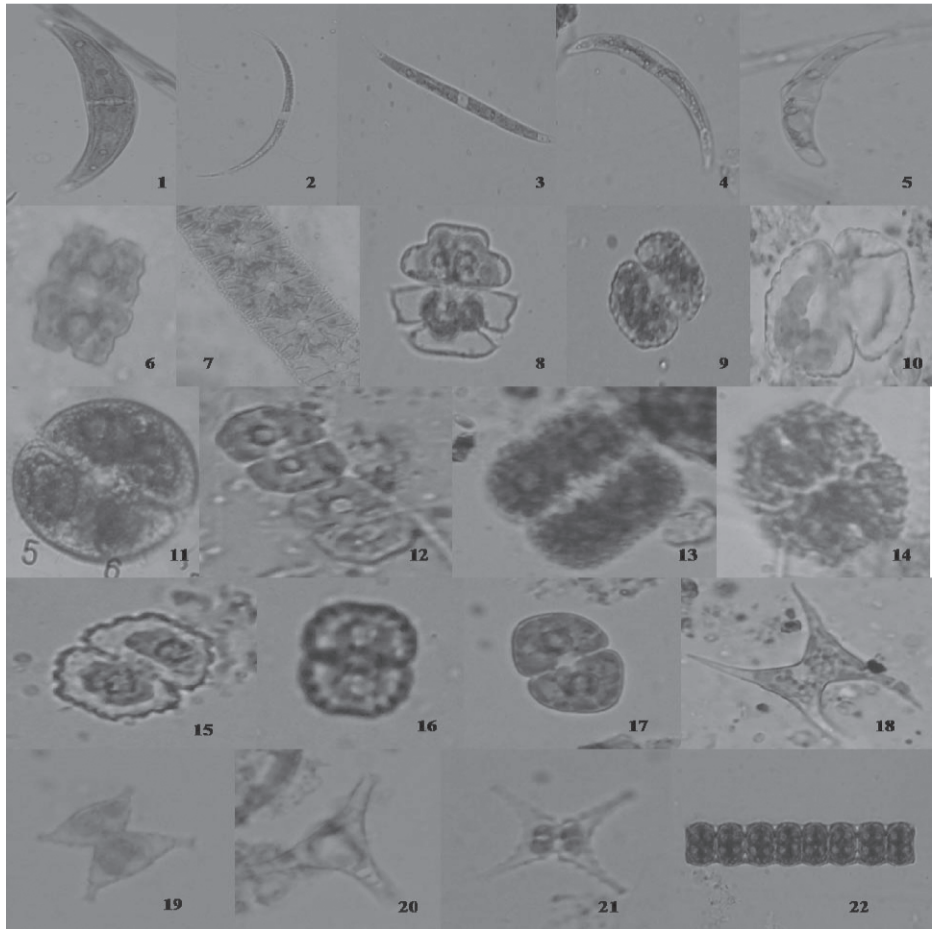

Fig. 1. 1.*Closterium acerosum* (Sch.) Ehrenb. X 1000 2.*Closterium acutum* (Lyngb.) Breb. X 1000 3.*Closterium decorum* Breb. X 800 4.*Closterium dianae* Ehrenberg X 800 5.*Closterium leibleinii* Kuetz. X 1000 6.*Euastrum coralloides* Josh X 400 7.*Micrasterias foliacea* Bail. X 800 8.*Micrasterias zeylanica* Fritsch X 800 9.*Cosmarium formulosum* Hoffmann X 800 10.*Cosmarium granatum* Breb. X 1200 11.*Cosmarium miscellum* Skuja. X 1200 12.*Cosmarium obsoletum* (Hantzsch) Reinsch. X 400 13.*Cosmarium pseudoretusum* F. Ducell. X 1000 14.*Cosmarium quadrum* Lund. X 1000 15.*Cosmarium sexnotatum* Gutw. X 1000 16.*Cosmarium subcrenatum* Hantzsch X 1000 17.*Cosmarium subprotumidum* Nordst. X 1000 18.*Anthrodesmus incus* (Breb.) Hass. X 1000 19.*Staurastrum crenulatum* (Näg.) Delp. X 800 20.*Staurastrum gracile* Ralfs. X 1000 21.*Staurastrum tetracerum* (Kütz) Ralfs. X 1000 *22.Desmidium bengalicum* C.A. Agardh. X 400.

the water body. This is an interesting finding that might be attributed to change of water qualities during recharging and discharging of water either from Brahmaputra river to Deepor beel, or vice versa. Besides such characteristic of the water body also showed that there is no danger of the appearance of algal bloom. All the species reported in the channel are common and distributed worldwide.

The species number and species composition of phytoplanktonic desmids were different in different seasons (Table 1). Maximum was 17 taxa in post-monsoon season followed by monsoon (10 species) and pre-monsoon (6 species) and minimum was 4 taxa during winter months.

3.2 Taxonomic enumeration

The systematic positions of the taxa were assigned according to the classification system of Fritsch (1935).

Order: Zygematales

Family: Desmidiaceae

Genus: *Closterium* Nitzsch 1817

1. *Closterium acerosum* (Sch.) Ehrenb. (p. I, f. 1)
   Collection number and date- KJR/192, 18-07-2012.
2. *Closterium acutum* (Lyngb.) Breb. (p. I, f. 2)
   Collection number and date- KJR / 045, 14-02-2012.
3. *Closterium decorum* Breb. (p. I, f. 3)
   Collection number and date: KJR /221, 16-10-2012.
4. *Closterium dianae* Ehrenberg (p. I, f. 4)
   Collection number and date: KJR /229, 16-10-2012..
5. *Closterium leibleinii* Kuetz. (p. I, f. 5)
   Collection number and date: KJR / 292, 14-12-2012.

Genus: *Euastrum* Ehrenberg 1832

1. *Euastrum coralloides* Josh (p. I, f. 6)
   Collection number and date: KJR / 036, 06-01-2012.

Genus: *Micrasterias* C.A. Agardh

1. *Micrasterias foliacea* Bail. (p. I, f. 7)
   Collection number and date: KJR / 009, 25-11-2011.
2. *Micrasterias zeylanica* Fritsch (p. I, f. 8)
   Collection number and date: KJR / 223, 16-10-2012.

Genus: *Cosmarium* Corda ex Ralfs 1848

1. *Cosmarium formulosum* Hoffmann (p. I, f. 9)
   Collection number and date: KJR / 165, 25-06-2012.
2. *Cosmarium granatum* Breb. (p. I, f. 10)
   Collection number and date: KJR / 235 16-10-2012.
3. *Cosmarium miscellum* Skuja (p. I, f. 11)
   Collection number and date: KJR / 276, 25-10-2012.
4. *Cosmarium obsoletum* (Hantzsch) Reinsch (p. I, f. 12)
   Collection number and date: KJR / 238, 16-10-2012.
5. *Cosmarium pseudoretusum* F. Ducell. (p. I, f. 13)
   Collection number and date: KJR / 244, 16-10-2012.
6. *Cosmarium quadrum* Lund (p. I, f. 14)
   Collection number and date: KJR / 180, 06-07-2012.
7. *Cosmarium sexnotatum* Gutw. (p. I, f. 15)
   Collection number and date: KJR / 184, 06-07-2012.
8. *Cosmarium subcrenatum* Hantzsch (p. I, f. 16)
   Collection number and date: KJR / 182, 06-07-2012..
9. *Cosmarium subprotumidum* Nordst. (p. I, f. 17)
   Collection number and date: KJR /278, 25-10-2012.

Genus: *Anthrodesmus* Ehrenberg 1838

1. *Anthrodesmus incus* (Breb.) Hass. (p. I, f. 18)
   Collection number and date: KJR /115, 25-04-2012.

Genus: *Staurastrum* Meyen 1829

1. *Staurastrum crenulatum* (Näg.) Delp. (p. I, f. 19)
   Collection number and date KJR / 078, 14-03-2012.
2. *Staurastrum gracile* Ralfs (p. I, f. 20)
   Collection number and date: KJR / 119, 23-03-2012.
3. *Staurastrum tetracerum* (Kütz) Ralfs. (p. I, f. 21)
   Collection number and date: KJR / 121, 23-03-2012.

Genus: *Desmidium* C.A. Agardh 1824

1. *Desmidium bengalicum* Turner (p. I, f. 22)
   Collection number and date: KJR / 279, 25-10-2012.

3.3 Seasonal fluctuation of cell density and biomass

The annual mean values of the total cell density and biomass were 84.02×10^2 cells /L and 0.048 mg/L respectively on the basis of average values recorded. The seasonal fluctuation trends of phytoplanktonic desmids were approximately similar in entire extent of the Khanajan channel. The maximum values on cell density were observed in Monsoon (683.34×10^2 cells /L) and on biomass in postmonsoon (0.078 mg/L). The minimum values for both were in premonsoon and the values were 19.56×10^2 cells / L and 0.013 mg/L respectively.

This study provides a useful baseline data of the desmidian flora of the state of the Assam belongs to Eastern Himalaya which would certainly help in the further studies based on the algal floristic account of the ecologically

Table 1
Seasonal occurrence of the desmids taxa in Khanajan during the study period

Sl. No.	Name of the taxa	Seasons			
		Winter	Pre Monsoon	Monsoon	Post monsoon
1.	*Closterium acerosum*	-	A	A	A
2.	*C. acutum*	-	C	-	C
3.	*C. decorum*	-	-	-	O
4.	*C. dianae*	-	-	-	O
5.	*C. leibleinii*	C	-	-	-
6.	*Euastrum coralloides*	O	-	C	C
7.	*Micrasterias foliacea*	-	-	-	C
8.	*M. zeylanica*	O	-	-	O
9.	*Cosmarium formulosum*	-	-	A	A
10.	*C. granatum*	-	-	C	C
11.	*C. miscellum*	-	-	C	A
12.	*C. obsoletum*	-	-	-	A
13.	*C. pseudoretusum*	-	-	-	C
14.	*C. quadrum*	-	-	C	C
15.	*C. sexnotatum*	-	-	A	A
16.	*C. subcrenatum*	-	-	O	A
17.	*C. subprotumidum*	-	-	-	C
18.	*Anthrodesmus incus*	O	O	-	-
19.	*Staurastrum crenulatum*	-	C	C	-
20.	*S. gracile*	-	C	-	-
21.	*S. tetracerum*	-	C	C	-
22.	*Desmidium bengalicum*	-	-	-	A

Note: According to the number of desmids under microscope in each view field: abundant = A; Common = C; occasional = O.

sensitive Eastern Himalaya. Out of the total 22 species identified from the Khanajan channel, which was totally unexplored along with Deepor beel Ramsar site, 9 species were belongs to the Genus *Cosmarium*, an oligotrophic plankton as substantiated by Reynolds (2006) and Dwivedi *et al.* (2009) particularly during monsoon and post monsoon season. Absence of the genus during winter was the indication of change of water quality once the incoming of river water from the Brahmaputra stopped till the onsetting of monsoon. The study thus, revealed that the water quality of the Khanajan channel in monsoon to post monsoon is so far in good condition harbouring the ecologically important desmidian flora. The majority of the desmids enlisted here seemed to be remarkably sensitive to environmental conditions, especially to water chemistry of the Khanajan and the Deepor beel and were suitable indicator of seasonal eutrophication. The result thus indicated that back flowing of river water from mighty Brahmaputra might be a boon for maintenance of Khanajan channels and Deepor beel Ramsar site too.

Acknowledgements

Authors are thankful to University Grant Commission (New Delhi) for financial assistance. The Head, Department of Botany, Gauhati University is also acknowledged for his constant encouragement.

References

Bhuyan, S.K. (1989). Asom Buranjir Katha (Aspects of Assam History), Purbanchal Prakash, Guwahati, pp. 132.

Coesel, P. F. M. (2001). A method for quantifying conservation value in lentic freshwater habitats using desmids as indicator organisms. Biodiv. Conserv. 10: 177-178.

Deka, S. J., Sarma, G. C. and Deka, S. P. (2011). Preliminary checklist of desmids of Urpad Beel (Wetland), Goalpara district, Assam, India. Asian J. Exp. Biol. Sci. 2(3): 391-398.

Dwivedi, S., Misra, P. K. and Susheela, M. R. (2004). Some Desmids from Central and Western Uttar Pradesh, India. Phytotaxonomy. 4: 64-73.

Dwivedi, R. K., Shukla, C. P., Misra, P. K., Shukla, S. K. and Seth, M.K. (2009). On Desmids of Southern Himachal Pradesh of Indo-Western Himalaya. Feddes Repert. 120 (3-4): 236-249.

Fritsch, F.E. (1935). The Structure and Reproduction of Algae Vol. I., Cambridge University Press, London, pp. 791.

Gerrath, J. F. (1993). The Biology of Desmids a Biological Approach. In. Round F. E. and Chapman, D. J. (eds.). Progress in Phycological Research, Biopress Ltd., Bristol, pp. 79-192.

Lund, J. W. G., Kipling, C. and Cren, LE. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimating by counting. Hydrobiologia 11: 143-170.

Misra, P. K. and Srivastava, A. K. (2003). Some desmids (chlorophyceae) from North-Eastern Uttar Pradesh, India. J. Ind. Bot. Soc. 82: 85-92.

Misra, P. K., Dwivedi, R. K. and Shukla, C. P. (2006). Some freshwater desmids from district Mandi, Himachal Pradesh. Phytotaxonomy 6: 12-125.

Ngearapat, N. and Peerapornpisal, Y. (2007). Application of desmid diversity in accessing the water quality of 12 freshwater resources in Thailand. J. Appl. Phycol. 19: 667-674.

Perumal, G.M. and Anand, N. (2009). Manual of Freshwater Algae of Tamil Nadu, Bishen Singh Mahendra Pal Singh, Dehradun, pp. 41-85.

Prasad, B. N. and Misra P. K. (1992). Fresh water algal flora of Andaman and Nicobar Islands, Vol. II., Bishen Singh Mahendra Pal Singh, Dehradun, pp 1- 248.

Reynolds, C. S. (2006). Ecology of phytoplankton, Cambridge Univ. Press, London, pp 1- 535

Saikia, P. K. and Bhattacharjee, P.C. (1987). A study of the Avifauna of Deepor Beel : A Potential Bird Sanctuary in Assam, Asian Wetland Bureau/ IWRB, Kuala Lumpur.

Sindhu, P. and Panikkar, M. V. N. (1995). Desmids new to Kerala, India-I. Feddes Repert. 106(3-4): 317-323.

Yamagishi, T. (2010). Plankton Algae of Southeast Asia, Bishen Singh Mahendra Pal Singh, Dehradun, pp.1-161

Yasmin, F., Buragohain, B. B. and Medhi, K. K. (2011). Planktonic desmid flora of south of the Eastern Himalayas: A Systematic Approach on Algae-I. Int. J. Bot. 7(2): 154-161.

Yinxin, W. and Minjuan, Y.U. (2005). Phytoplanktonic desmids community in Donghu Lake, Wuhan, China. Chinese J. Oceanol. Limnol. 23 (1): 91-97.

Plant Science Research 35 (1&2) : 67-71, 2013

Plant Science Research

ISSN 0972-8546

Evaluation of antimicrobial activity of *Ichnocarpus frutescens* (L.) R.Br. against human pathogens

K. Sahoo[Ψ], S. Das and N. K. Dhal

CSIR-Institute of Minerals and Materials Technology, Bhubaneswar-753013, Odisha, India

ARTICLE INFO

Article history:
Received : 3 November 2013
Received in revised form : 19 November 2013
Accepted : 24 November 2013

Keywords:
Ichnocarpus frutescens,
Gahamakhunti,
swamnai, antimicrobial activity

ABSTRACT

Leaves of *Ichnocarpus frutescens* (L.) R.Br. were collected from the Gahamakhunti of Dhenkanal districts, Odisha, commonly called as *swamnai*. The leaves were extracted with hexane, chloroform, diethyl ether, methanol and water for the *in vitro* study of antimicrobial property. The chloroform extract was found to be more active against *Pseudomonas putida* and *Aspergillus fumigatus*. Methanol extract was found to possess maximum antimicrobial activity against most of the test pathogens but maximum effect was found against *Bacillus sphaericus* (15 mm), *Bacillus polymyxa* (14 mm), *Aspergillus niger* (25 mm) and *A. fumigatus* (22 mm) and minimum activity was found against *Bacillus circulans.* Negative response was found against *Escherichia coli* and *Pseudomonas putida*. In case of chloroform extract of *I. frutescens,* maximum inhibition (21 mm) was found against *Pseudomonas putida* whereas minimum activity (5 mm) was found against *Bacillus circulans*. The diethyl ether extract did not show any response against the test pathogens except *Bacillus sphaericus* (15 mm) and *Bacillus circulans* (11 mm). A maximum inhibition zone of 17 mm and minimum of 8 mm was found against *Bacillus polymyxa* and *Bacillus sphaericus,* respectively. The present screening result demonstrated that the methanol and chloroform extracts of leaf of the fiber yielding plant *I. frutescens* has potent antibacterial as well as antifungal activity and the studied plant may be a new source for novel antimicrobial compound discovery for treating disease causing human pathogens.

1. Introduction

The World Health Organization reported recently that at least 75 - 95% of the world populations of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents (Robinson, 2011). Traditional medicine usage is a common practice in developed countries at the primary healthcare level (Essawi and Srour, 2000). Presently thousands of plants are yielding valuable medicines of great use to man and are commonly known as medicinal and drug plants (Bradshaw, 1992). Herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost. The use of plant extracts and phytochemical, with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Behera and Mishra, 2005; Govindraj *et al.,* 2006). These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as tannins.

India is a vast repository of medicinal plants that are used in medical treatments (Bradshaw, 1992), which also forms a rich source of knowledge (Nadkarni, 1984). Thus according to Ganesan *et al.* (2013) medicinal plant constitutes a group of industrially important plants which bring appreciable income to the country by way of export. With the eventual development, the mankind and industries led to the sole dependences of the human being on manufactured medicines.

[Ψ] *Corresponding author; Email:*kalpanactc@gmail.com

The elemental analysis of plant parts of *Ichnocarpus frutescens* was carried out by EDX (Energy Dispersive X-Ray spectroscopy) analysis which showed the presence of Ca, Si, Mg, Cl, K, C in ethanolic extract using $CaCO_3$, SiO_2, MgO, KCl, K-MAD, Cawollastonite as the standards. The EDX analysis showed root possesses all the tested elements. The weight % of carbon was found to be maximum i.e. 51.60 % in leaf part in comparison with the root and stem. The percentage of essential element was higher in root in compared to leaf and stem *Ichnocarpus frutescens* (L.) RBr. (Apocynaceae) have been used as folk medicine and as an ingredient in Ayurvedic and Unani preparations against diseases of blood, skin, headache, snake bite and inflammation (Starlin *et al.,* 2012). *I. frutescens* is rich in polyphenols and flavonoids. Distribution of various flavonoids and phenolic acids in the leaves of *I. frutescens* has been systematically studied. The root portion of this plant was much more used in traditional as well as in modern era. It has shown the presence of phenylpropanoids, phenolic acids, coumarines, flavonoids, sterols and pentacyclic triterpenoids. Pharmacological study revealed hepatoprotective, antioxidant, anti-inflammatory, and analgesic, anti diabetic and anti-tumor activity (Mishra *et al.,* 2009). In the present study realizing the medicinal values *in vitro* screening of leaf extracts of *I. frutescens* for antimicrobial activity of the proposed plant with different organic solvents has been carried out.

2. Materials and Methods

2.1 Botany of Ichnocarpus frutescens (L.) R.Br.

Ichnocarpus frutescens (L.) R.Br. (Apocynaceae; common name-swamnai) is an evergreen plant with white, small, 7.5mm diameter flowers It is a large, evergreen, lactiferous, woody creeper with red appearance found almost throughout India, ascending up to an altitude of 4000 ft. The roots of the plants are used in the medicine as a substitute for Indian Sarsaparilla (*Hemidesmus indicus*) and are often mixed with the latter; neither their therapeutic properties nor their suitability for use as "sarsaparilla" substitute have been established.

2.2 Collection of sample

The fresh leaves of *I. frutescens* free from disease were collected from the Gahamakhunti of Dhenkanal districts, Odisha. The specimen was deposited at the herbarium, Institute of Minerals and Materials Technology (IMMT), Bhubaneswar, Orissa for authentification and identified following Flora of Orissa (Saxena and Brahmam, 1994-96). Specimen was labeled, numbered, annotated with the date of collection, the locality and medicinal uses were recorded.

2.3 Solvent extraction

The leaves were collected, washed thoroughly (2-3 times) with running tap water followed by distilled water and dried under sterile blotting paper. These were then dispersed in a tray and kept under shade for 8-10 days for air drying at room temperature. After drying the leaves were powdered with the help of electric blender and stored in a zipper polythene bag. The powdered material (15-20 g) was filled in the thimble and extracted successively with different organic solvents such as methanol, chloroform, hexane, petroleum ether and water in Soxhlet extractor for 24-48 hr (Bradshaw, 1992). The collected extracts were filtered using standard filter paper and concentrated to dryness under reduced pressure below 60^0C under a rotary flash evaporator and stored at 4^0C in an air tight bottle for further use.

2.4 Growth and maintenance of test organisms

For antimicrobial study different human pathogenic bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas putida, Bacillus polymyxa, B. sphaericus,* and *B. circulans* from SUM hospital and fungal cultures such as *Aspergillus fumigatus* and *Aspergillus niger* obtained from OUAT, Bhubaneswar, Orissa were used for the present study. The bacteria were maintained in Nutrient agar (NA) slants at 37^0C and fungi were maintained in Potato Dextrose Agar (PDB) medium at 28^0C.

2.5 Antibacterial assay

In vitro antibacterial screening was carried out by disc diffusion method (Bauer *et al.,* 1996). The test organisms were inoculated into nutrient broth (NB) and Potato Dextrose Broth (PDB) and incubated at 37^0C for 18-24 hrs. The inoculum of individual pathogens was swabbed with the help of a sterile cotton swab on Nutrient Agar (NA) plates in duplicates. Filter paper discs loaded with extracts (20 µl) of each solvent extract were placed in the surface of growth media at equidistant points and then were incubated at 37^0C±0.5^0C for 18-24 hrs after which the diameter of inhibition zones formed around the discs were recorded. Control and standard set of experiments were also carried out with different solvents and standard antibiotics (streptomycin, gentamycin, penicillin, ampicillin and amikacin. Minimum Inhibitory Concentration (MIC) was determined by standard test procedures using disc diffusion method.

2.6 Antifungal assay

Inhibition of mycelial growth using the agar well diffusion technique (Groover and Moore, 1962; Shahi *et al.,* 1999). Potato Dextrose Agar (PDA, Dehydrated culture media) was

autoclaved and then maintained in water bath at 40 °C. The extracts or the solvent fractions were added to sterile molten PDA to obtain final concentration of 250 µg/ml. The media was poured into sterile petridishes, solidified and fungal discs with seven day old mycelium of the pathogen were placed in the centre of the plate. Plates were incubated for 2-3 days at 28-30^{0}C. The diameter of inhibition zone formed around the discs were recorded. Control and standard set of experiments were also carried out with different solvents and standard antibiotic (nystatin).

3. Results

Results obtained in the present study revealed that the tested leaf part of *I. frutescens* showed considerable antimicrobial activity against the studied microorganisms. Different extracts of the plants exhibited antibacterial and antifungal activity in a concentration dependant manner. Methanol extract was found to possess maximum antimicrobial activity against most of the test pathogens but maximum response was found against *B. sphaericus* (15 mm) and *B .polymyxa* (14 mm) and minimum (12 mm) was found against *B. circulans* whereas negative response was found against *E. coli* and *P. putida*. In case of chloroform extract of *I. frutiscens* maximum inhibition (21 mm) was found against *P. putida* whereas minimum activity (5 mm) was found against *B. circulans*. The diethyl ether extract did not show any response against the test pathogens except *B. sphaericus* (15 mm) and *B. circulans* (11 mm). A maximum activity (17 mm) and minimum activity (8 mm) was found against *B. polymyxa* and *B. sphericus* respectively whereas a positive result was also found against *B. sphaericus, B. circulans, B. polymyxa, K. pneumoniae, P. vulgaris* and *P. putida* in hexane extract (Fig.1). In case of standards penicillin showed maximum inhibition (33 mm) against *P. putida* whereas ampicillin showed maximum activity (24 mm) against *E. faecalis*. Gentamycin showed maximum activity (22 mm) against *E. coli* and *Proteus mirabilis*. Streptomycin showed maximum activity (19 mm) against *B. sphaericus* and *E. faecalis* whereas amikacin was showing maximum activity (24 mm) against *B. circulans* and *B. sphaericus* (Fig. 1).

Antifungal activity of different solvent extracts of leaves of *I. frutiscens* showed significant activity. Maximum activity i.e. 25 mm and 23 mm was observed in hexane extract whereas minimum i.e. 8 mm and 15 mm was found in chloroform extract against *Aspergillus niger* and *Aspergillus fumigatus* respectively. Aquous extract did not show any activity against both the strains. In case of standard nystatin was showing 24 mm and 27 mm against *A. niger* and *A. fumigatus,* recpectively (Fig. 2).

The MIC value of methanol extract against *B. sphaericus* and *K. pneumoniae* was found to be 30 µg/ml and 40 µg/ml whereas in case of chloroform extract MIC value was found to be 5 µg/ml against *P.putida*. In case of hexane extract it was found to be 30 µg/ml against *B.*

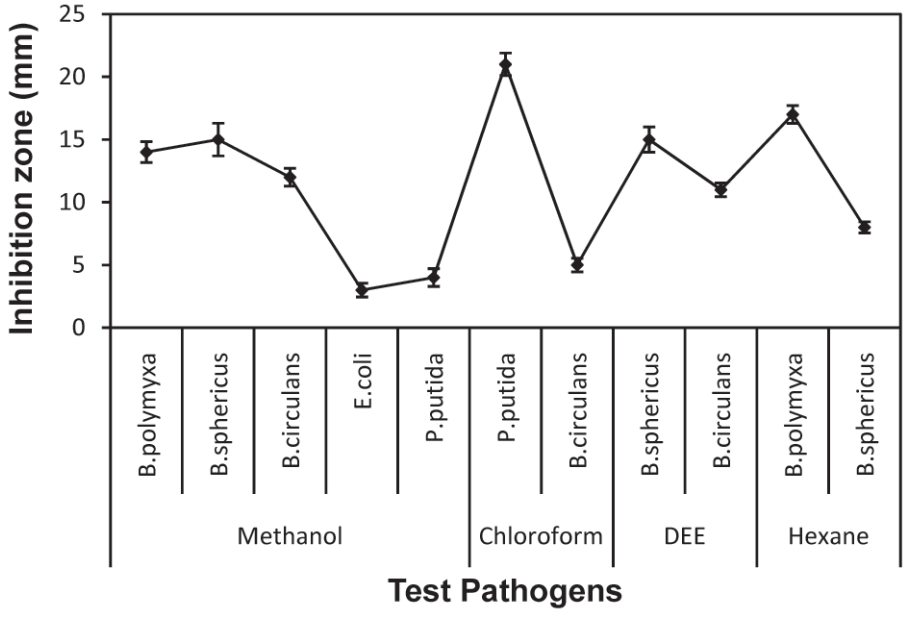

Fig.1 Antibacterial activity of leaf extracts of *Ichnocarpus frutescens*

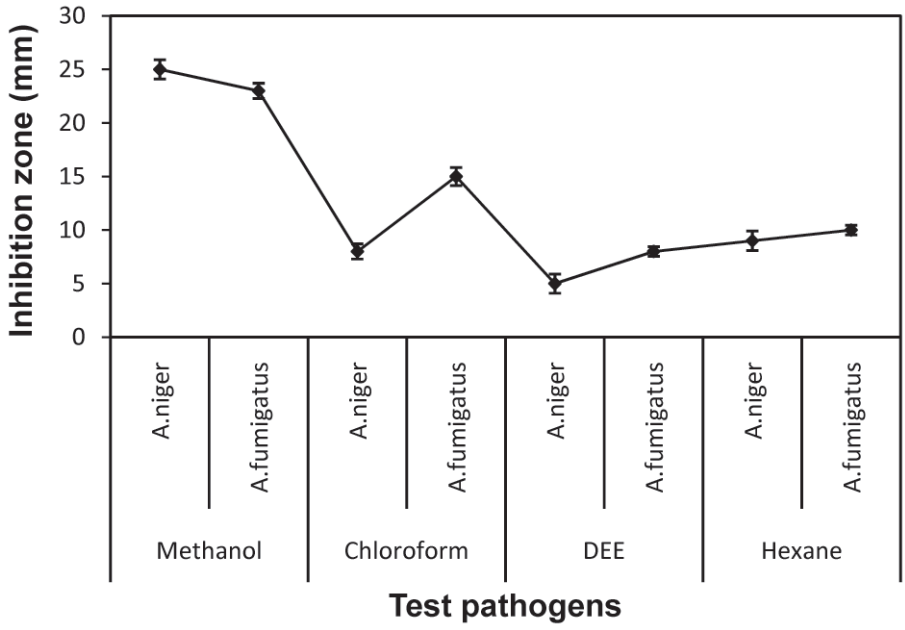

Fig.2 Antifungal activity of leaf extracts of *Ichnocarpus frutescens*

polymyxa whereas in diethyl extract it was 20 µg/ml against *B. sphaericus*. In methanol extract, the MIC was 35 µg/ml against *A. niger*.

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, as a result plants are one of the bed rocks for modern medicine to attain new principle (Evans *et al.,* 2002; Saxena and Brahmam, 1996; Bonjar, 2004; Mahesh and Satish, 2008). In the present study methanol, hexane, and chloroform extracts of the *I. frutescens* showed greater antibacterial as well as antifungal activity than the corresponding water extracts. This finding is interesting, because in the traditional method of treating a bacterial infection, decoction of the plant parts in water was employed. Whereas, according to present study preparing an extract with an organic solvent was shown to provide a better antimicrobial activity, in accordance with the results obtained by Nair *et al.* (2005). These observations may be attributed to two reasons: Firstly, the nature of biological active components whose activity can be enhanced in the presence of methanol, hexane and chloroform; secondly, the stronger extraction capacity of the above solvent could have produced greater number of active constituents responsible for antimicrobial activity. So, *I. frutiscens* plant can be used to discover bioactive natural products that will lead to the development of new pharmaceuticals. Such screening of various natural organic compounds and identifications of active agents must be considered as a fruitful approach in the search of new herbal drugs. Moreover, plant extracts are far more economical because of being freely available around in nature. This can result in control of disease at the required concentration of extracts. Needless to say that it would be great service to mankind if the scientific community can create awareness among people about the judicious use of this gift of natural resources.

The antimicrobial activity can be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in preventing the administration of inappropriate concentrations, a common practice among many traditional medical practitioners.

Acknowledgement

The authors are thankful to the Director, CSIR-IMMT, Bhubaneswar for providing necessary facilities to carry out the research work.

References

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turek, M. (1996). Antibiotic susceptibility testing by a standard single disc method. Amer. Jor. Clin. Pathol. 36: 493-496.

Behera, S. K. and Misra, M. K. (2005). Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa. India. J. Ethnopharmacol. 102: 319-325.

Bonjar, G. H. S. (2004). Evaluation of antibacterial properties of Iranian medicinal-plants against *Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae* and

Bordetella bronchoseptica. Asian J. Plant Sci. 3 (1): 82-86.

Bradshaw, L. J. (1992). Laboratory Microbiology. Fourth Edition, Saunders College Publishing. Fort Worth.

Essawi, T. and Srour, M. (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 70: 343-349.

Evans, C. E., Banso, A. and Samuel, O. A. (2002). Efficacy of some nine medicinal plants against *Salmonella typhi*: an in vitro study. J. Ethnophrmacol. 80: 25-35.

Ganesan, R., Desingu, K., Chinnasamy, R. and Devarajan, N. (2013). Screening of antibacterial and phytochemical analysis of leaf and leaf derived callus extracts of *Sebastiania chamelelea* (L.) MUELL. ARG. Indo Am. J. Pharma. Res. 8019-8025

Govindarajan, R., Vijayakumar, M., Singh, M., Rao, C. H. V., Shirwaikar, A., Rawat, A. K. S. and Pushpangadan, P. (2006). Antiulcer and antimicrobial activity of *Anogeissus latifolia.* J. Ethnopharmacol. 106: 57-61

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci. 4: 839-843.

Mishra, A., Pradhan, D. K., Mishra, M. R., Dalai, M. K., Jha, S. and Meher, A. (2009). Energy Dispersive X-Ray Spectroscopy (EDX) Analysis of *Ichnocarpus frutescens* plant parts. Drug Inventory 1(1): 1-2.

Nadkarni, C.K. (1984). Ethnomedico-botanical investigation in Kerela I. Some primitive tribals of Western Ghats and their herbal medicine. J. Ethnopharmacol. 11: 59-77.

Nair, R., Kalariya, T. and Sumitra, C. (2005). Antibacterial activity of some selected Indian medicinal flora. Turk J. Biol., 29: 41-47.

Robinson, M.M. (2011). Classifications, Terminology and Standards, WHO, Geneva: Xiaorui Zhang Traditional Medicines, WHO. Traditional medicines: global situation, issues and challenges. 3rd Edition.

Saxena, H.O. and Brahmam, M. (1994-96). Flora of Orissa, Orissa Forest Development Corporation, Bhubaneswar, India.

Starlin, T., Ragavendran, P., Arul Raj, C.A., Perumal, P.C. and Gopalakrishnan, V.K. (2012). Element and functional group analysis of *Ichnocarpus frutescens* R.Br. Int. J. Pharm. Pharmceut. Sci. 4(5): 343-345.

Plant Science Research

ISSN 0972-8546

Orthosiphon aristatus (Lamiaceae): A new record for the flora of Odisha, India

A. K. Nayak[1], B. R. Dash[1], T. Kar[1Ψ] and K. K. Mandal[2]

[1] Similipal Tiger Reserve, Bhanjpur, Baripada 757002, Odisha, India

[2] P. G. Department of Botany, North Orissa University, Baripada 757003, Odisha, India

ARTICLE INFO

Article history:
Received : 29 October 2013
Accepted : 6 November 2013

Keywords:
Orthosiphon aristatus
New record
Odisha
Similipal

ABSTRACT

Orthosiphon aristatus (Blume) Miq. (Lamiaceae) is reported for the first time from Odisha state from Similipal Biosphere Reserve. Brief botanical description, nomenclature, distribution and ecological notes of the taxon has been provided in the present paper.

The genus *Orthosiphon* Benth. belongs to the tribe Ocimoideae of the family Lamiaceae and is comprised of about 40 species. The species of the genus occur mostly in the Old World tropics, South Africa, Madagascar and tropical and sub-tropical Asia (Mabberley, 2008). The genus is represented by 8 species in India (Hooker, 1885) and only 3 species are known to occur wild in Odisha (Saxena and Brahmam, 1995). Similipal, the largest Biosphere Reserve in Eastern India, lies between 20^0 17' to 22^0 34' N latitude and 85^0 40' to 87^0 10' E longitude. The flora of Similipal is quite rich in terms of species diversity and also interesting from phytogeographical point of view. During botanical exploration in Similipal, few interesting specimens belonging to the family Lamiaceae have been collected. Critical study of specimens of the genus *Orthosiphon* and perusal of relevant literature (Hooker, 1885; Haines, 1921-25; Kanjilal *et al*., 1939; Saxena and Brahmam, 1995; Singh *et al*., 2001) revealed the identity of the specimens as *Orthosiphon aristatus* (Blume) Miq. The occurrence of the species in Similipal Biosphere Reserve is a new distributional record for the state of Odisha. The identity of the specimen has been confirmed by comparing with the specimens available in Central National Herbarium (CAL), Howrah, India. A detailed description with photographs, notes on phenology, distribution and ecology have been provided to ascertain identity of the species. The specimens have been preserved as herbarium specimens in the Herbarium of Similipal Tiger Reserve, Baripada, Odisha, India.

Orthosiphon aristatus (Blume) Miq., Fl. Ned. Ind. 2 : 943.1858; Kanjilal *et al*., Fl. Assam 3: 502. 1939; Mukh. in Rec. Bot. Surv. India 14(1): 26. 1940; *Ocimum aristatum* Blume, Bijdr. 14: 833. 1826. *Orthosiphon stamineus* Benth. in Wall., Pl. Asiat. Rar. 2: 15. 1830; Hook. f. Fl. Brit. India 4: 615. 1885; Fischer. in Rec. Bot. Surv. India 12(2) : 125.1938. *Orthosiphon grandiflorus* Haines, Bot. Bihar Orissa 731.1922 (Repr. ed. 2:731, 1988). (Fig. 1.)

Erect undershrub, up to 2 m high; stems quadrangular, sparsely pubescent. Leaves simple, opposite, decussate, ovate, 3-10.5 x 2-5 cm, chartaceous, base cuneate, margins coarsely dentate, apex acuminate, glandular punctate beneath. Petioles 1-4 cm long. Verticillasters laxly-flowered, terminal, up to 15 cm long racemes; bracts broadly ovate, *ca* 3 mm long, ciliolate. Flowers bisexual, zygomorphic. Calyx campanulate, 3-6 mm long, upper lip broad, orbicular, two lower lip subulate. Corolla bluish purple, tube upto 1.5 cm

Ψ *Corresponding author; Email:* truptirekha86@gmail.com

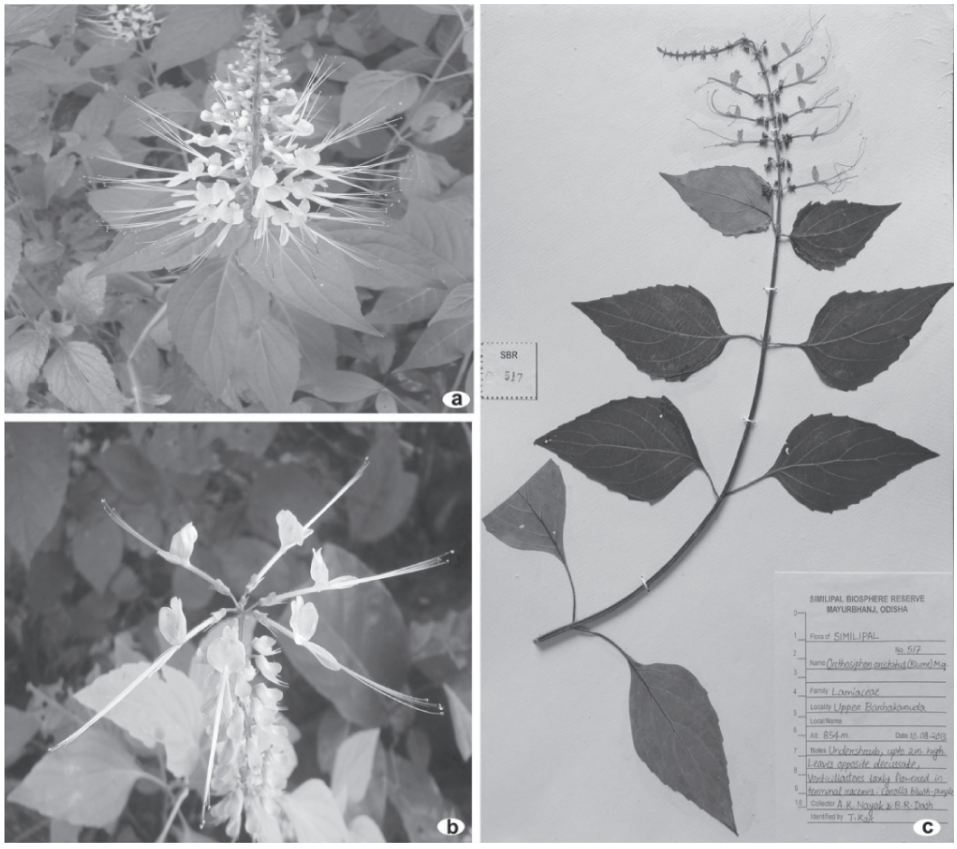

Fig.1. Orthosiphon aristatus (Blume) Miq.: a. Habit; b. Close-up view of flowers; c. Herbarium image

long, slender, puberulous outside, distinctly bilipped; upper lip shallowly 4 lobed, recurved; lower lip horizontal and concave. Stamens 4, long exserted, declinate. Ovary superior, 4-partite; ovules one in each cell; style gynobasic, 4-5 cm long; stigma club-shaped and shortly bifid. Nutlets ovoid-oblong, upto 3 mm long, compressed, rugulose.

Flowering and Fruiting.: August-October.

Specimens collected: Upper Barhakamuda (Similipal), A.K. Nayak and B.R. Dash, SBR 517, 10th August, 2013..

Habitat: The species is found in shady places of moist deciduous forests; rare at an altitude of 854 m.

Distribution: India (Assam, Andaman and Nicobar Islands, Andhra Pradesh, Jharkhand, Manipur, Mizoram, Odisha), Australia, Thailand, Myanmar, Vietnam, Malesia, Indonesia, Philippines.

Specimens Examined: Visakhapatnam Dist. (A.P.)**:** Balakrishnan, 785, 24.09.1961 (CAL); Arong Car Nicobor: Nair, 4583, 06.10.1976 (CAL); Sungri, Assam: Panigrahi, 14335, 24.07.58 (CAL); Eastern Manipur: Prajer 184, 12.07.1890 (CAL); Singbhum, Bihar: H. H. Haines 194, September, 1899 (CAL); Mount Stuart Anamalais: CEC Fischer; 3455, 12.07.1912 (CAL).

Acknowledgements: The authors are grateful to the Director, Botanical Survey of India, Kolkata for granting permission to consult herbaria and also Mr. Gopal Krishna of CNH, Howrah for his help in confirmation of identification.

References:

Haines, H. H. (1921-25). The Botany of Bihar and Orissa, 6 Parts, Adlard and Sons, London

Hooker, J. D. (1885). The flora of British India Vol. 4, L. Reeve and Co., London.

Kanjilal, U. N., Das, A., Kanjilal, P. C. and De, R. N. (1939). Flora of Assam. Vol.3. Govt. of Assam, Shillong, India.

Mabberley, D. J. (2008). The Plant-Book. A portable dictionary of plants, their classifications and uses. Third Edition. Cambridge University Press, Cambridge, England.

Saxena, H. O. and Brahmam, M. (1995). Flora of Orissa. Vol. 3. Regional Research Laboratory and Orissa Forest Development Corporation, Bhubaneswar.

Singh, N. P., Mudgal, V., Khanna, K. K., Srivastava, S. C., Sahoo, A. K., Bandopadhyay, S., Aziz, A., Das, M., Bhattacharya, R. P and Hajra, P. K. (2001). Flora of Bihar: Analysis. Botanical Survey of India, Calcutta.

Plant Science Research

ISSN 0972-8546

Three new plant records for the state of Odisha

P. C. Panda[Ψ], P. K. Acharya, S. K. Kar and A. K. Mahapatra

Taxonomy and Conservation Division, Regional Plant Resource Centre, Bhubaneswar 751 015, India

ARTICLE INFO

Article history:
Received : 2 December 2013
Accepted : 5 December 2013

Keywords:
Homalium tomentosum,
Melinis repens,
Vahlia digyna,
new report, Odisha

ABSTRACT

Three angiospermic plant species namely, *Homalium tomentosum, Melinis repens* and *Vahlia digyna* are reported here as new distributional records for Odisha state.

During the course of floristic inventories, survey of wild economic plants and quantitative assessment of plant resources in different parts of Odisha, three angiospermic plant species were collected, which after critical examination, could be identified as *Homalium tomentosum, Melinis repens* and *Vahlia digyna*. Perusal of literature on flora of Odisha revealed that wild occurrence of these three species has so far not been reported from the State and thus turned out to be new distributional records. Correct botanical name with synonym (s), botanical description, notes of taxonomy, ecology, phenology and distribution of these species are provided in the present paper. The herbarium specimens have been deposited in the Herbarium of Regional Plant Resource Centre (RPRC), Bhubaneswar.

Homalium tomentosum (Vent.) Benth. J. Proc. Linn. Soc. Bot.4: 34. 1859; Gamble, Fl. Presid. Madras 1:522.1915. *Blackwellia tomentosa* Vent. Choix de Plantes t.57.1803. (FLACOURTIACEAE) Fig. 1 (a)

Common name: Burma Lancewood, Moulmein Lancewood

Deciduous, medium-sized tree, 20-25 m high, sometimes with buttresses, bark thin, white, smooth. Leaves broadly obovate to obovate-oblong, 10-15 cm long, rounded at apex, obtuse or apiculate, cuneate to the base, rounded at the base, crenate, sub-coriaceous, nerves glabrascent. Inflorescence pendulous, about 30 cm long. Flowers small, greenish-white, 5-6 merous; in 3-5 flowered glomerules. Calyx tomentose, segments linear-oblong. Petals similar to calyx segments. Stamens solitary, opposite to petals. Ovary 1-celled. Fruit capsule, coriaceous, valvular at apex. Seeds few, angular-oblong.

Fls: June - November; *Frts*.: September- January.

Very rare, only few trees are found in mixed deciduous forest with calcareous soils.

Distribution: Myanmar, Cambodia, Bangladesh, Sri Lanka, Indonesia, Laos, Thailand, India (North-East India and Western Ghats)

Specimens examined: Brahmanipadar, Ganjam district, P. K. Acharya & S. C. Jena 18096, 19.10.2012.

Note: The accrescent calyx and corolla often form a parachute after anthesis. Thus seed dispersal is mainly by wind. Wood is said to be used for shoots of heavy carts, furniture, planking, electrical transmission poles, spars etc. in South East Asia.

The genus *Homalium* is represented by 180 species in the world (Mabberley, 1997). In Odisha, *H. nepanense* is the

[Ψ] *Corresponding author; Email:*pcpanda2001@yahoo.co.in

most common species and occur in a wide range of habitats. *H. tomentosum* varies from *H. nepalense* by having large tomentose leaves, flowers in 5-6-flowered glomerules borne on drooping racemes. Though Gamble (1915) reported its occurrence from Northern Circas (Ganjam), the available herbarium specimens are from outside the geographical boundary of present day Odisha. Therefore, it does not find a place in Flora of Orissa (Saxena & Brahmam, 1994).

Melinis repens (Willd.) Zizka, Biblioth. Bot. 138: 55. 1988. *Saccharum repens* Willd. Sp. Pl. (ed 4) 1: 322. 1797. *Rhynchelytrum repens* (Willd.) C. E. Hubb., Bull. Misc. Inform. Kew. 1934: 110. 1934; Bor, Grasses Burma Ceyl. Ind. Pak. 355. 1960; Hsu, Fl. Taiwan 5: 596. 1978. *Erianthus repens* (Willd.) P. Beauv. Ess. Agrost. 162: 177. 1812. (POACEAE) Fig 1 (b)

Common name: Natal grass, Rose Natal grass, Ruby grass

Annual or loosely tufted short-lived perennial. Culms geniculately ascending, often rooting at lower nodes, up to 150 cm tall. Leaf sheaths loose, usually with tubercle-based hairs; leaf blades linear, up to 20 cm long. Panicle silvery-pink or purple, ovate to oblong, 8–20 cm, fluffy; branches capillary; pedicels with a few long hairs. Spikelets ovate, 2–4 mm, densely villous, hairs up to 6 mm; lower glume narrowly oblong, 1-veined, with stiff short hairs, separated from the upper by a short internode; upper glume 5-veined, gibbous below middle, tapering upward into a glabrous membranous beak, emarginate, mucronate or with short awn up to 1 mm; lower floret staminate, lemma similar to upper glume but narrower and less gibbous, palea keels ciliate; upper floret whitish, thinly cartilaginous, smooth, shining, ca. 2 mm. Caryopsis ovate, flattened, about 1.3 mm long, light brown in colour.

Fls & Frts.: June–November; most part of the year at suitable localities.

Locally abundant in waste places, roadsides and open grassy fields.

Distribution: Native grass of tropical South Africa, introduced into most warm countries for soil conservation and as a forage crop. Very widely naturalised in Australia, southern USA, Pacific islands, parts of the Mediterranean, India, Taiwan, Pakistan

Specimens examined: RPRC campus, Bhubaneswar, Khurda district, S. C. Jena18861, 04.11.2013.

Note: This is a polymorphic, pantropical weed, recognizable by its pink, fluffy panicles. These flower spikelets are densely covered with silky hairs that are initially reddish or purplish in colour. These hairs tend to lose their colour as the seed-heads mature and often end up a pinkish or silvery colour. These light and fluffy seeds are often wind-dispersed and may also become lodged in clothing, vehicles and animals. Seeds can also be dispersed alongwith agricultural produce (*i.e.* fodder and pasture seed).

Vahlia digyna (Retz.) Kuntze, Revis. Gen. PI. 1: 227. 1891; Anon., Draw. Ind. PI. (Icon. Roxb. No. 592) 6: t. 20.1973; Bridson in Kew Bull. 30: 177. 1975. *Oldenlandia digyna* Retz. Obs. Bot. 4: 23. 1786. *Bistella digyna* (Retz.) Bullock in Acta Bot. NeerI. 15: 84 & 85. 1966. *Vahlia viscosa* Roxb. FI. India 2: 89. 1832; Hook. f. Fl. Brit. India 2: 399.1878; Gamble, Fl. Madras Presidency 1: 447.1919. (VAHLIACEAE) Fig. 1 (c)

Erect sparsely to much-branched annual herb, 10-20 cm tall; stems covered with patent or crisped, often glandular, hairs. Leaves ovate to ovate-lanceolate, acute at apex, tapering or rounded at base, usually pubescent. Flowers sessile or subsessile. Calyx-tube copular, 1–2 mm. long, sparsely covered in patent or shorter crisped hairs; lobes 5, ovate, acute, glabrous or sparsely pubescent outside. Petals 5, yellow, fading to white, always shorter than sepals, round to ovate, usually distinctly apiculate, narrowed to base, entire to finely or irregularly crenate towards apex, with mid-vein alone distinct. Filaments translucent with vein apparent, with a small membranous hairy scale-like appendage at the base. Style-bases distinctly thickened, styles 2, up to 1 mm long, glabrous. Capsule 1.5–2.5 mm. long, sparsely pubescent. Seeds straw-coloured, ovoid-cylindrical, with indistinct longitudinal ridges.

Fls. & Frts.: February- April.

Occassional, in moist sandy soils along rivers, lakes and in harvested rice fields.

Distribution: W. Pakistan, Iran, Egypt, tropical Africa, Madagascar and India.

Specimens examined: Rambha, sandy shore of Chilika lagoon, Ganjam district, P. C. Panda 10375, 27.02.2013; Purohitpur Sasan, bank of Dhanua river, Khurda district, S, C, Jena & S. K. Kar 9374, 19.02.2012.

Note: The genus *Vahlia* is represented by five species in the world (Mabberley, 1997) and two species are reported to occur in Odisha. *V. digyna* differs from *V. dictotoma* by having sessile flowers borne in axillary pairs. The flowers of *V. dictotoma* are pedicellate and peduncled. Though flower colour of *V. dictotoma* is described as either white or yellow elsewhere, plants collected from Odisha invariably bear white flowers. However, *V. digyna* always have yellow flowers. Haines (1921-25) apprehended the occurrence of this species

Fig.1: (a) *Homalium tomentosum* (b) *Melinis repens* (c) *Vahlia digyna*

from the then Bihar and Orissa, but did not provide any precise locality, nor does this species find a place in Flora of Orissa (Saxena & Brahmam, 1994).

Acknowledgements

Financial support from Chilika Development Authority, Bhubaneswar under Integrated Coastal Zone Management (ICZM) Project and also Department of Biotechnology, Govt. of India is gratefully acknowledged.

References

Gamble, J. S. (1915). Flora of the Presidency of Madras, Part-1. Adlard and Sons, London.

Haines, H. H. (1925). The Botany of Bihar and Orissa, Part-1. Adlard and Sons, London.

Mabberley, D. J. (1997). The Plant Book- A Portable Dictionary of the Vascular Plants. Second Edition, Cambridge University Press, U.K.

Saxena, H. O. & Brahmam, M. (1994). Flora of Orissa, Vol. 1. Regional Research Laboratory & Orissa Forest Development Corporation, Bhubaneswar.