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Carbon stocks of linear structures of trees out side forest in Kurnool District, Andhra Pradesh, India

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ABSTRACT

In the present study, carbon stocks of linear structures of trees outside forest in Kurnool district were estimated through sampling of 236 0.1 ha plots. A total of 3922 tree individuals belonging to 51 angiosperm species were enumerated in the sampled plots. The mean tree density was 150.50 per ha; mean basal area is 24.54 m^2 ha^{-1}; mean volume of trees with ≥10 cm diameter is 20.07 m^3 ha^{-1}; mean total tree biomass is 176.20 tons ha^{-1} and mean carbon stock is 83.66 tons ha^{-1}. Extrapolated biomass and carbon content for linear structures are calculated as 0.528 Mt and 0.251 Mt respectively. The carbon sequestration potential is estimated as 0.918 Mt CO^2.

1. Introduction

Earth's climate is warming at an unprecedented rate. Burning of fossil fuels and consequent increase of carbon dioxide concentration in the atmosphere is identified as the prime cause for climate change. Due to increased levels of CO_2 concentration, rise of atmospheric temperature by 0.5°C is recorded over the past hundred years and it is projected to rise by 0.6 to 5°C in the next 100 years according to latest report of Intergovernmental Panel on Climate Change (IPCC, 2014). Carbon dioxide levels which was below 300 ppm during the last 600,000 years is now touching 401.30 ppm, average for July, 2015 (Tans and Keeling, 2015).

The main natural carbon sinks are plants, the ocean and soil. The uptake of carbon dioxide (CO_2), one of the principle greenhouse gases, during photosynthesis is the major pathway by which carbon is removed from the atmosphere and this 'capturing and securing of atmospheric carbon in the form of CO_2 during photosynthesis and subsequently to dead organic matter is called as 'carbon sequestration'. Carbon sequestration has been recognized as an effective and low-cost method of mitigating carbon emissions. Vegetation in the form of forests and especially trees plays a pivotal role in sequestration and trees are the largest component of aboveground biomass in terrestrial ecosystems. Apart from forest ecosystems, trees outside forests also have great potential in sequestration of atmospheric carbon (Dhyani *et al.,* 2009).

Trees Outside Forests (from now onwards, abbreviated as TOF) refers to trees found on lands that are not categorized as 'forest' nor 'other wooded land' irrespective of their patch size (FSI, 2009; FAO, 2010). TOF includes agricultural land (including meadows and pastures), built-on land (including settlements and infrastructure) and barren land (including sand dunes and rocky outcroppings), orchards and plantations. In spatial terms they may be scattered on farmland and pasture, or growing continuously in line-plantings along roads, canals and watercourses, around lakes, in towns, or in small aggregates with a spatial continuum such as clumps of trees, sacred woods, urban parks (Alexandrov *et al.*, 1999). TOF offers a range of ecological,

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economic, and social functions including carbon sequestration and offers a win-win land-use strategy for climate change mitigation and adaptation. Despite wide spread distribution, TOF are neglected in terms of their carbon sequestration potential (de Foresta *et al.*, 2013).

Forest Survey of India has started inventory of trees outside forests in the country since 1991 and national level estimates of growing carbon stock were initiated from 2002 (FSI, 2009). Indian Space Research Organisation (ISRO) has initiated National Carbon Project under the auspices of Indian Geosphere Biosphere Programme (IGBP) (Singh and Dadhwal, 2008). Accordingly, TOF are classified into 3 categories: linear, scattered and block; under linear structures, roads, canals, river bunds, rail tracks are included. The major carbon pools in India are estimated based on very coarse resolution data and extrapolation because the primary data for the many regions of the country are non-existed or over-estimated (Dadhwal and Nayak, 1993).

Due to the lack of reliable data on standing biomass and rates of forest degradation, the net carbon emission estimates for India are highly variable (Ravindranath *et al.*, 1997). Precise information on TOF at micro level is lacking and this has become a major hindrance in estimating TOF potential in carbon sequestration. The present study is oriented with this background, to estimate the carbon stocks of linear structures of TOF of Kurnool district, Andhra Pradesh following as a comprehensive format design of Vegetation Carbon pool Assessment (VCP) National Carbon Project (Singh and Dadhwal, 2008). The present study is the first of its kind with reference to estimating carbon stock of TOF at district level through intensive sampling.

2. Study area

Kurnool district is located between 76^0 58′E to79^0 34′E longitudes and 14^0 54′ to 16^0 18′ N Latitudes and has a geographical area of 17,658 km^2. The average elevation varies 300 to 900 m above MSL. The annual average temperature range from 19^0 C to 41.5^0 C and rainfall is about 670 mm. The major soil types are black cotton, red and saline soils. Rivers of major importance are Thungabadra, Krishna, Hundri, Kunderu and Gundlakamma. The district has 19.29% of forest cover to its total geographical area and remaining is under different land use systems. The remaining geographical area after deducting forest area is considered to be the Area outside forest and accounts for 14,251 Sq. km. This area covers roads, canals, railway tracks, cropped area, industrial areas, human settlements etc. There is 10119.31 km road found all over the district including 264 km National highway, 790.24 km State highway, 1966.23 km Major district roads, 815.85 km other district road under the maintenance of Roads and Buildings Department and remaining 6546.99 km road under the maintenance of Zilla Parishad. Railway track in the district accounts for 264.04 km. Canals cover 356.64 km length, of 234.64 km under the name of KC Canal and remaining 122 km as Tungabhadra Lower Level Canal (Anon., 2011).

3. Materials and methods

For the purpose of the present study, field data was collected from randomly laid linear structures outside the forests in Kurnool district. In the present study, a non-destructive approach of above ground biomass estimation was done. A comprehensive format design of Vegetation Carbon pool Assessment (VCP) of Indian Institute of Remote Sensing (IIRS) (Singh and Dadhwal, 2008) was adopted for ground data collection. A total of 236 linear plots of size 100×10 m were laid along the roads, canals, railway tracks in different parts of the district covering varied topographic terrain and different density classes. The geographical co-ordinates for each plot were identified with the help of Global Positioning System. All the tree taxa in the sampled plots were inventoried and identified following regional and local floras. Enumeration of trees was done and girth at breast height (gbh) measurements was taken with measuring tape and height was measured using opti-logic meter.

3.1. Biomass Estimation

In the present study, non-destructive approach of above ground biomass estimation was used. Basal area, volume and specific gravity for trees have been estimated as follows.

Basal area

Basal area of each tree was calculated by using following standard formula:

Basal Area (m^2 ha^{-1}) = πr^2 × area (ha)

Growing Stock (Volume) Estimation

Volume of each tree was estimated using the selected species specific volumetric equation developed and compiled by Forest Survey of India (FSI, 1996).

Specific Gravity

Specific gravity values of different species were selected from literature (Reyes *et al.,* 1992; FRI, 1996; Mani and Parthasarathy, 2007). For stems with unknown specific gravity, the arithmetic mean of all known species was substituted and used in particular sample plot following Brown *et al.,* (1989).

3.2. Estimation of above ground biomass

Bole biomass >10cm diameter

The estimated volume was converted into biomass by multiplying with specific gravity (Rajput *et al*., 1996; Limaye and Sen 1956). Biomass of all the trees was summed to obtain biomass for 1 ha.

Biomass (tons) = Volume (m^3) x Specific gravity

Bole biomass <10 cm diameter

Volume equations for trees <10cm diameter are not available, hence a methodology for trees of this class developed following Singh and Dadhwal (2008) and Patil *et al*. (2011) by relating basal area and biomass. The model developed was Y=3.6808*X+0.264 and used for assessing the AGB of trees <10cm diameter; where, Y= biomass, X= basal area of trees (>10cm diameter and <10cm diameter) and 3.6808 and 0.264 are coefficients

3.3. Estimation of total above ground biomass

The biomass of trees having >10cm diameter and <10cm diameter in each plot were added together to get biomass of 1 ha plot.

3.4. Estimation of below ground biomass

In the present study, 26% of the total above ground biomass was considered as root biomass following Houghton *et al*., (2001) and Ramankutty *et al*., (2007).

3.5. Total biomass

Total biomass for each 1 ha plot was obtained by the addition of total above-ground biomass and below ground biomass. Further the mean was calculated and extrapolated for the whole study area.

3.6. Estimation of carbon stocks

3.6.1. Extrapolation of linear structures of TOF area

Based on the mean biomass estimation of sampled plots, total carbon stock of linear structures of TOF of Kurnool district was estimated by extrapolating the same for the whole district area. For this, tree covered area under each sub category and sub-sub category was determined based on 2011 official statistics of Kurnool district (Anon., 2011).

Estimated area (ha) = Length of linear category (m) x 2 (both sides) x 10 m (transect width)/ 10,000

Tree covered area (ha) = Estimated area x percentage of mean basal area of sampled plots

Considering both sides of the roads, canals and railway track, 1631.6 km length was estimated for approach roads; 3932.46 km for major district roads; 480 km for national highway, 1540.28 km for state highways, 713.28 km length of canals and 528.08 km railway track. As transect width is 10 m, estimated area under each above sub-sub category is calculated my multiplying 10. As the mean basal area of the sampled plots is 11.71 for approach road, the tree covered area is projected for the same at 191 ha. In case of others the projected figures are: major district roads, 1276 ha; national highway, 51.5 ha; state highway, 511 ha; canals 102 ha and railway track, 148 ha.

3.6.2. Estimation of carbon stocks and carbon sequestration potential

Estimation of carbon stocks from the biomass has been calculated by multiplying the total biomass by a conversion factor that represents the average carbon content in biomass. In the present study, the IPCC default of 0.475 carbon fraction (Mc Groddy *et al.,* 2004) has been used.

Carbon (tons) = Biomass (tons) × Carbon %

Carbon sequestration potential of trees was calculated following Eneji et al. (2014) and Chavan and Rasal (2012) through the ratio of CO^2 to C, i.e multiplying carbon content with 3.666.

4. Results and discussion

In the present study, a total of 51 angiosperm species belonging to 43 genera and 23 families were recorded (Table 1). A total of 3922 tree individuals ranging 2 to 43 per sample plot of 0.1 ha were encountered in 236 inventory plots laid all over the district. The mean tree density was 150.50 per ha. The highest values of tree density were observed along major district roads and state highways (Table-2). *Azadirachta indica*, *Pongamia pinnata*, *Tectona grandis*, *Tamarindus indica*, *Albizia lebbeck*, *Albizia saman*, *Dalbergia sissoo*, *Senna siamea* and *Acacia nilotica* are the dominant trees which contribute more than 75% of total number of individuals (TNI) (Table-1).

The mean basal area is 24.54 m^2 ha^{-1} ranging between 0.99 - 189.76 m^2 ha^{-1} across the plots. Lowest values of basal area were recorded in approach road and national highways as many trees in the latter case are planted recently after widening. Mean volume of trees with >10 cm diameter is 20.07 m^3 ha^{-1}. The correlation between basal area and biomass of trees with >10 cm diameter revealed the determination coefficient (R^2) 0.863 (Figure-1).

The mean total tree biomass is 176.20 tons ha^{-1} and varies between 8.16 and 1400.48 tons ha^{-1} across the sampled

Table 1
Inventory of tree species in sampled linear structures in Kurnool district

Name of the Species	Family	No. of individuals in all sampled plots
Acacia holosericea G.Don	Fabaceae - Mimosoideae	1
Acacia nilotica (L.) Delile	Fabaceae - Mimosoideae	198
Adansonia digitata L.	Malvaceae	1
Aegle marmelos (L.) Corrêa	Rutaceae	1
Ailanthus excelsa Roxb.	Simaroubaceae	23
Albizia lebbeck (L.) Benth.	Fabaceae - Mimosoideae	226
Albizia saman (Jacq.) Merr.	Fabaceae - Mimosoideae	178
Annona squamosa L.	Annonaceae	6
Artocarpus heterophyllus Lam.	Moraceae	1
Azadirachta indica A.Juss.	Meliaceae	771
Balanites aegyptiaca (L.) Delile	Zygophyllaceae	50
Bauhinia purpurea L.	Fabaceae - Caesalpinioideae	3
Borassus flabellifer L.	Arecaceae	188
Cocos nucifera L.	Arecaceae	66
Cordia dichotoma G.Forst.	Boraginaceae	2
Dalbergia sissoo DC.	Fabaceae - Faboideae	207
Delonix elata (L.) Gamble	Fabaceae - Caesalpinioideae	24
Delonix regia (Hook.) Raf.	Fabaceae - Caesalpinioideae	89
Eucalyptus camaldulensis Dehnh.	Myrtaceae	42
Ficus benghalensis L.	Moraceae	6
Ficus benjamina L.	Moraceae	4
Ficus glomerata Roxb.	Moraceae	8
Ficus religiosa L.	Moraceae	58
Gyrocarpus americanus Jacq.	Hernandiaceae	1
Hardwickia binata Roxb.	Fabaceae - Caesalpinioideae	5
Kigelia africana (Lam.) Benth	Bignoniaceae	19
Leucaena leucocephala (Lam.) de Wit	Fabaceae - Mimosoideae	98
Limonia acidissima Groff.	Rutaceae	7
Madhuca longifolia var. *latifolia* (Roxb.) A.Chev.	Sapotaceae	4
Mangifera indica L.	Anacardiaceae	14
Morinda pubescens Sm.	Rubiaceae	1
Moringa oleifera Lam.	Moringaceae	2
Parkinsonia aculeata L.	Fabaceae - Caesalpinioideae	1
Peltophorum pterocarpum (DC.) K.Heyne	Fabaceae - Caesalpinioideae	73
Phoenix dactylifera L.	Arecaceae	1
Phoenix sylvestris (L.) Roxb.	Arecaceae	1
Phyllanthus emblica L.	Euphorbiaceae	1
Pithecellobium dulce (Roxb.) Benth.	Fabaceae - Mimosoideae	2

Polyalthia longifolia (Sonn.) Thwaites	Annonaceae	2
Pongamia pinnata (L.) Pierre	Fabaceae - Faboideae	411
Prosopis cineraria (L.) Druce	Fabaceae - Mimosoideae	18
Prosopis chilensis (Molina) Stuntz	Fabaceae - Mimosoideae	19
Santalum album L.	Santalaceae	8
Sapindus emarginatus Vahl	Sapindaceae	4
Senna siamea (Lam.) H.S.Irwin & Barneby	Fabaceae - Caesalpinioideae	263
Simarouba amara Aubl.	Simaroubaceae	3
Syzygium cumini (L.) Skeels	Myrtaceae	2
Tamarindus indica L.	Fabaceae - Caesalpinioideae	397
Tectona grandis L.f.	Verbenaceae	399
Terminalia catappa L.	Combretaceae	5
Ziziphus mauritiana Lam.	Rhamnaceae	8
Total		3922

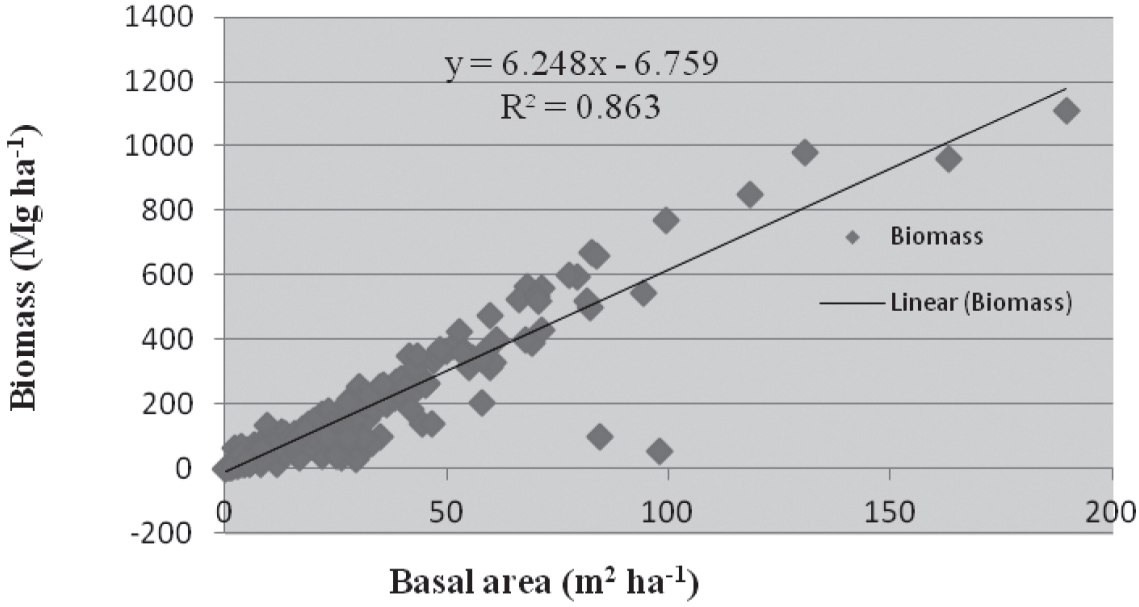

Fig. 1. Correlation between basal area and biomass of trees of >10 cm diameter

plots. The maximum biomass content has been found along major district roads, state highways and canals due to relatively less disturbance and growth of huge mature old trees. The mean carbon stock for linear structures is 83.66 tons ha^{-1} ranging from 3.87 to 665.23 tons C ha^{-1} across the sampled plots. For the total estimated area under all categories of linear structures of trees outside forests in Kurnool district, the projected biomass and carbon content are calculated as 0.528 Mt and 0.251 Mt respectively (Table-2), of which 91.7% was contributed by dominant trees. The carbon sequestration potential is estimated as 0.918 Mt CO^2.

Although studies on different categories of TOF were initiated throughout India data for comparison was available with two districts in Andhra Pradesh. In Prakasam district of Andhra Pradesh linear plots registered a mean of 7.95 ± 9.66 Mg ha^{-1} ranging between 0.10 to 30.84 Mg C ha^{-1} across the sampled plots (Srinivasa Rao et al., 2012a). In Kadapa district, linear plots registered 59.36 ± 121.61 Mg C ha^{-1} ranging between 7.03 to 403.67 Mg C ha^{-1} across the sampled plots (Srinivasa Rao et al., 2012b). Compared to both the districts, Kurnool district has registered more carbon stocks in TOF.

Table-2
Subcategory-wise Linear Structures tree density, basal area, volume, biomass and carbon stocks

Sub category	Sub-sub category	Tree covered area (ha)	Tree density (trees/ha)	Basal area (m^2 ha^{-1})	Volume (m^3 ha^{-1})	Mean Biomass (tons ha^{-1})	Carbon stock (tons ha^{-1})	Extrapolated Biomass (tons)	Carbon stock (tons)
Road	Approach Road	191	140	11.71	8.69	72.74	34.42	13841.77	6574.84
	Major District Road	1276	179	32.45	29.60	259.92	123.46	331657.92	157537.51
	National High way	51.5	128	10.73	7.91	64.93	30.84	3343.89	1588.34
	State High way	511	151	33.24	28.78	253.97	120.57	129778.67	61644.86
	Canal	102	143	30.97	24.91	221.69	105.30	22612.38	10740.88
	Rail track	148	162	28.19	20.57	183.98	87.39	27229.04	12933.79
	Total	2279.5	903	147.29	120.46	1057.18	501.98	528463.67 (0.528Mt)	251020.22 (0.251Mt)
	Mean	150.5	24.54	20.07	176.20	83.66	-	-	

Srinivasa Rao and Ravi Prasad Rao (2015) estimated the carbon stocks of Nallamalais at 26.34 Mt. Since 60% of Nallamalais fall in Kurnool district about 15.78 Mt of Carbon stocks can be projected for forests of Nallamalais. Excluding unknown but minor fraction of carbon stocks of other forests in the district especially Erramalais, TOF shared almost 8% of the total carbon stock of Kurnool district which is near to the general perception that TOF share up to 10% of total carbon stocks of any country.

Conclusion

Evaluation of trees biomass potential in linear structures of Kurnool district highlights the importance of trees outside forests in maintaining recognisable amounts of carbon stocks and their ability in sequestering carbon dioxide. The present work may be considered as a model especially in Andhra Pradesh to understand the potential of TOF in any area. Further this work advocates planting more broad leaf trees outside the forests.

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Biodiversity of soil algae in a tropical endoaquept planted to rice under flooded condition under long-term application of chemical and organic-N sources

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ABSTRACT

Organic N resources are being increasingly used to meet the N demand of the growing rice crop under organic cultivation which could be a win-win option for restoring soil health as well as reducing leakage of reactive N to the environment apart from impact on microbial diversity of rice soils. We studied the diversity of algae and cyanobacterial strains in a field experiment with 9 treatments involving chemical, organic and a combination of chemical and organic-N sources being used for growing rice crop for the last 15 years. A total of 66 species belonging to 33 genera under 4 classes of cyanobacteria, green algae, diatoms and euglenoids were recorded. Highest diversity of species was found for the cyanobacteria (30 species) followed by diatoms (22 species). Among the different treatments, the community structure of soil algae varied with highest diversity being recorded in field plots amended with chemical fertilizer (Shannon diversity index 3.22) and the lowest in the unamended field plots where no N source was added. Results indicate that anthropogenic activities in rice fields including application of organic residues can influence the diversity of soil algae

1. Introduction

Variability in the activity and composition of soil microbial communities may have important implications for the whole gamut of microbially-mediated ecosystem functions upon which agricultural system depends. Soil organic matter is central to sustaining crop production and maintenance of soil health. Flooded rice ecosystem presents a unique microbial ecology that undergoes a cycling of flooded and non-flooded sequences and is characterized by several physicochemical and biological properties (Leisack *et al.,* 2001; Adhya and Rao, 2005). Considerable amounts of biomass are likely to be produced phototrophically in the floodwater (Roger, 1996) and also chemo-autotrophically in the presence of inorganic electron donors and CO_2 (Revsbech *et al.,* 1999).

Soil is the most important non-aqueous habitat for algae and cyanobacteria (Zenova *et al.,* 1995). Cyanobacteria are ubiquitous in nature yet they show great diversity and abundance in rice field ecosystem, as it provides optimum conditions of light, temperature, water and nutrients for their growth and proliferation (Prasanna and Nayak, 2007). Climatic, soil and biotic factors influence the growth and colonization of cyanobacteria and other algae in rice fields that play an important role in C-turnover of this ecosystem. Cyanobacteria or blue green algae comprise a large group of photoautotrophic prokaryotes that contribute to the N-economy of the system, in view of the inherent potential of biological nitrogen fixation by the majority of the group. Soil fertility is generally improved by the organic matter produced by these organisms (Mishra and Pabbi, 2004) apart from the fact that they also secrete diverse growth-promoting substances such as hormones, vitamins, amino acids and organic acids affecting other organisms in many ways (Roger and Reynaud, 1982).

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In the rice-growing regions, application of chemical-N fertilizers is a very common practice to increase rice productivity (Balasubramanian et al., 1999) but is widely acknowledged to affect the soil microbial diversity. Earlier studies have shown that inorganic N-fertilization favours the growth of non-N fixing algae, which inhibit partial or total growth of N_2-fixing cyanobacteria, while N-deficient fields favour growth of N_2-fixing algae. Previous reports have also shown that incorporation of nitrogenous fertilizers in the form of organic and green manures have either inhibitory or favourable effects of algal growth in rice fields (Roger, 1996). In view of the harmful effect of the application of excessive fertilizer-N including release of reactive-N in the environment, application of organic N sources is getting credence. Those organic materials including compost and other organic residues, apart from serving as the source of N and other nutrients, also result in the sequestration of C and build-up of organic matter, apart from maintaining soil fertility and ecosystem resilience.

The objectives of the present research was (i) to evaluate the effects of organic, inorganic and combined nitrogen fertilizers on the diversity of algae and cyanobacterial strains in rice fields; and (ii) to identify the dominant algae and cyanobacterial strains (genera-wise) present through standard light microscopy techniques.

2. Materials and methods

2.1 Site description and field experiment

The study was conducted at the experimental farm of the Central Rice Research Institute, Cuttack, India (85°552 E, 20°252 N; elevation 24 m). Mean annual highest and lowest temperatures are 39.2 and 22.5°C, respectively, and the mean annual temperature is 27.7 °C. Annual precipitation is about ~1500 mm.yr^{-1}, of which 75–80% is received during June to September. The soil is an Aeric Endoaquept with sandy clay loam texture (26.3% clay, 21.4% silt, 52.3% sand), bulk density 1.40 Mgm^{-3}, percolation rate <10 mmd^{-1}, pH (H_2O) 5.26, cation exchange capacity 15 mEq 100 g^{-1}, electrical conductivity 0.5 dSm^{-1}, total organic C 0.38% and total N 0.05%, exchangeable K 120 kg ha^{-1}.

The field experiment on intensive rice cropping under different inorganic, organic, and combined fertilizers as N source was established in 1995 where rice is grown in a rice–rice–fallow sequence. Wet season (July–December) rice was grown under rainfed condition followed by the dry season (January–April) rice grown under irrigated condition. The field was ploughed thoroughly and flooded 2–3 days before transplanting for puddling and levelling. Rice plants (21- day old seedlings of *cv*. Gayatri during the wet season and *cv.* Lalat during the dry season) were transplanted at a spacing of 15 cm x 15 cm with two seedlings per hill in the field plots (5m x 5m) well separated by levees. All the field plots remained continuously flooded to a water depth of 12 ± 7 cm during the entire period of crop growth and were drained 10 days before harvest. The crops were grown following recommended agronomic practices and harvested at maturity.

The experiment was laid out in a randomized block design with three replicates for all the treatments, including control. There were a total of 9 treatment combinations including (i) unamended control without any N addition, (ii) urea added as chemical fertilizer at the rate of 60 kg N.ha^{-1}, (iii) farm yard manure (FYM) at the rate of 60Kg N.ha^{-1}, (iv) Green manuring crop *Sesbania aculeata* at the rate of 60 Kg N.ha^{-1} (v) FYM (30 Kg N.ha^{-1}) + *S. aculeata* (30 Kg N.ha^{-1}) (vi) FYM (30 Kg N.ha^{-1}) + *Azolla* (30 Kg N.ha^{-1}) (vii) *S. aculeata* (30 Kg N.ha^{-1}) + *Azolla* (30 Kg N.ha^{-1}), (viii) rice straw (30 Kg N.ha^{-1}) + *S. aculeata* (30 Kg N.ha^{-1}), (xi) rice straw (30 Kg N.ha^{-1}) + urea (30 Kg N.ha^{-1}). Organic residues like FYM, and rice straw at appropriate quantities were incorporated at the time of field preparation. Fresh biomass of *S. aculeata* and *Azolla* at appropriate quantity was incorporated at the time of puddling and the seedlings were planted 5 days later to allow decomposition of the biomass. Phosphorus (as P_2O_5) and potassium (as muriate of potash) were applied as basal at 40 kg.ha^{-1} at the time of puddling.

2.2 Sampling and morphological observation

Algal samples were collected randomly from each plot in the maximum tillering stage (40 days after transplantation) of rice growth during the wet season of rice crop (rainfed condition). Water samples and soil cores of the top 0.5 cm were taken to determine planktonic and benthic algae and cyanobacteria. Samples were collected using forceps and needle and stored and preserved in pre-sterilized specimen bottles with formaldehyde (4% v/v). A part of each fixed soil suspension was boiled in 10% H-$_2O_2$ solution to remove any organic material and was repeatedly rinsed with distilled water to obtain cleaned diatom frustules (Fujita and Ohtsuka, 2005). Cell measurement was carried out using ocular and stage micrometers and microphotograph of each specimen was taken using a Meiji ML-TH-05 Trinocular research microscope fitted with Nikon Coolpix 4500 digital camera. The organisms were identified following monographs for various algal groups (Desikachary 1959; Hoffman, 1989; Metting, 1981; Patrick and Reimer, 1975; Philipose 1967; Prescott, 1962; Ramanathan 1964; Randhawa 1959; Bhakta, Das and Adhikary, 2010).

2.3 Physicochemical properties of soil

Soil from each field plot was collected at the time of collection of algal samples and analyzed for total organic carbon (%), total N (%), pH and EC following standard protocol of soil analysis (Sparks, 1996) and the values of analysis are given in Table-I. The total organic carbon values were converted into organic matter by multiplying with 1.724 (Pribyl, 2010). Soil pH and electrical conductivity (EC) range were analysed (using soil: water = 1:2.5) using a portable pH meter (Philips model PW 9424) and by a mhos pH meter (Elico, Hyderabad, India) respectively. All the samples were analyzed in triplicate and the values provided are means of observations ± S.D.

2.4 Statistical analysis

All analyses were carried out using samples from replicated field plots for each treatment. Analysis of variance was done to determine the effects of treatment and their interaction using statistical package (IRRISTAT, version 3.1: International Rice Research Institute, Philippines). The indices of soil algal communities were estimated by Shannon species diversity index by $H = \Sigma(p_i)(\log_2 p_i)$, where p_i is the proportion of the individual species (Shannon and Weaver, 1949). Differences were considered to be significant at $p<0.05$ level.

3. Results and discussion

3.1 Algal biodiversity under different chemical and organic-N source amendments

Based on the morphology observed under the light microscope, a total of 66 species were identified in the soils sampled from rice field plots amended with different chemical and organic-N source amendments (Table 1). They belonged to cyanobacteria, green algae, diatoms and euglenoids in 4 classes and 33 genera. Highest diversity of species was found for the cyanobacteria (30 species) which belonged mostly to the genera *Anabaena*, *Oscillatoria* and *Phormidium*, followed by diatoms (22 species) (Tables 1 and 2, Plates 1-7), and a limited presence of green algae mostly predominated by filamentous species. The highest number of taxa was found for the genus *Navicula* (diatoms). Only one instance of euglenoids (*E. sanguinia*) was recorded from field plots amended with chemical N-fertilizer (urea).

Among the different treatments, the community structure of soil algae varied significantly from type to type, with Shannon diversity index ranging from 1.97 to 3.22. The highest index value was measured in the field plots amended with chemical fertilizer and the lowest in the unamended plots where no N source was added. Interestingly, slowly decomposable organic-N sources like FYM and rice straw either in conjunction with green manuring *S. aculeata* or with urea, exhibited nearly similar magnitude of diversity. Algae belonging to cyanobacteria were recorded in a highly diverse pattern, both in the number of species as well as genera, from field plots amended with chemical fertilizer as compared to field plots amended with organic or combined forms of N-fertilizers. Algae belonging to the genera *Aulosira*, *Anabaena*, *Nostoc*, *Oscillatoria*, *Spirulina* and *Spirogyra* were dominant (>20%) in fields amended with chemical fertilizer. Interestingly, algae belonging to the group Bacillariophyta were larger in number in field plots amended with rice straw, especially in conjunction with urea, possibly because of larger supply of silica, from the rice straw, which is an integral part of diatom frustules.

The diversity of soil algal communities is the result of the complex influence of rice plants, soil properties, cropping practices and climatic conditions (Quesada et al. 1995). Occurrence of soil algae in rice paddies also varies with the growth stage of the rice crop (Roger and Reynaud, 1976). At the early growth stage, diatoms and unicellular algae dominate. As the biomass of soil algae increases, there is a shift to filamentous green algae and non-N fixing cyanobacteria just before panicle initiation (Choudhary, 2009), possibly due to crop growth and development of canopy and thus reduction in the available solar irradiation. In the present study, sampling was done at the maximum tillering stage – the most active growth stage of the rice crop and represented a high diversity in the algal community, mostly divided among cyanobacteria and diatoms.

Various fertilizers that are applied to rice crop at different stages of the crop growth can influence the nutrient dynamics for soil algae. Thus, the dynamics of algal diversity in rice fields are a result of a complex interaction of available solar irradiation, nutrients and crop growth. Earlier reports suggested that chemical N-fertilization favours the growth of non-N fixing algae due to partial or total inhibition of growth of N_2-fixing cyanobacteria. However, in the present study, field plot amended with chemical fertilizer had the highest cyanobacterial diversity with almost equal distribution of nitrogen and non-nitrogen fixers. Further, it is suggested that non-heterocystous cyanobacterial species might become dominant when fertilizers are applied (Jutono, 1973). In the present study, some N-fixing species including those of *Anabaena*, *Gleothece* and *Nostoc* were found in the field plots together with larger proportion of non N-fixing species, such as *Microcystis*, *Oscillatoria* and *Spirulina*.

Table 1
Checklist of abundance of soil algae recorded in flooded field plots planted to rice and amended with inorganic and organic N sources

S. No.	Species	Control	Urea-N	FYM	*S. aculeata*	FYM + *S. aculeata*	FYM + *Azolla*	*S. aculeata* + *Azolla*	Rice straw+ *S. aculeata*	Rice straw + Urea
Cyanophyta/ Cyanoprokaryota										
1	*Anabaena ambigua*	D	D	D	C	C	-	-	-	-
2	*A. constreta*	-	C	R	-	R	R	R	-	-
3	*A. fertilissima*	-	C	-	-	-	-	-	-	C
4	*A. torulosa*	D	D	D	-	C	C	-	-	-
5	*Aphanothece bullosa*	D	-	D	-	D	D	C	-	-
6	*A. gelatenosa*	D	-	C	C	D	C	C	-	-
7	*Aulosira prolifica*	-	D	D	D	-	-	D	C	-
8	*Cylindrospermum catenatum*	-	C	-	-	-	-	-	D	-
9	*C. muscicola*	-	C	-	-	-	-	-	D	D
10	*Glaucocystis duplex*	-	C	C	C	D	D	C	-	-
11	*Gloeothece rupestris*	-	-	-	-	-	-	-	-	D
12	*Gloeotrichia natans*	-	-	-	-	-	-	-	-	C
13	*Microchate tenera*	-	R	-	-	-	-	-	-	-
14	*Microcoleus chthonoplates*	-	-	-	D	D	-	-	C	-
15	*Microcystis wesenbergi*	-	-	D	D	D	D	C	C	-
16	*Myxosarcina spectabilis*	C	-	-	-	-	-	-	-	-
17	*Nostoc paludosum*	C	C	C	D	D	-	-	C	C
18	*Oscillatoria anguina*	-	D	C	-	-	C	C	-	-
19	*O. anguistissima*	-	D	C	-	-	D	C	-	-
20	*O. chlorina*	-	D	C	-	-	D	C	-	-
21	*O. tenuis*	-	D	D	-	-	C	C	-	-
22	*O. terebriforme*	-	-	-	C	C	-	-	D	-
23	*Phormidium boryanum*	D	D	C	-	-	D	C	-	-
24	*P. muscicola*	-	D	D	-	-	-	-	-	-
25	*P. orientalis*	-	C	C	-	-	C	D	-	-
26	*P. rotheanum*	-	-	-	C	-	-	-	D	D
27	*P. tenue*	-	C	-	D	C	-	-	C	-

28	*Rivularia aquatica*	-	-	-	-	-	-	-	-	D
29	*Spirulina laxa*	-	C	-	-	-	-	-	C	-
30	*S. subtillisima*	-	D	D	-	-	C	D	-	-
Chlorophyta										
31	*Bulbochate basispora*	-	-	-	-	-	-	-	C	-
32	*Cosmarium auriculatum*	-	D	-	-	-	-	-	-	-
33	*C. crenatum*	-	-	-	-	-	-	-	-	C
34	*C. nitidulum*	-	-	-	-	-	-	-	-	D
35	*C. pachydermum*	-	-	-	-	-	-	-	-	D
36	*C. pseudocoronatum*	-	D	-	-	-	-	-	-	-
37	*C. supergranatum*	-	-	-	C	-	-	-	D	-
38	*Oocystis elliptica*	-	-	-	R	-	-	-	C	-
39	*Scenedesmus obtusus*	-	-	-	D	-	-	-	D	-
40	*Spirogyra condensata*	C	D	D	-	D	D	D	-	-
41	*S. ghosei*	R	D	D	-	D	D	C	-	-
42	*Zygnema czurdae*	-	D	D	C	D	D	D	-	-
43	*Z. giganteum*	-	-	-	D	-	-	-	C	-
Bacillariophyta										
44	*Achnanthes hungarica*	-	C	-	-	-	-	-	-	D
45	*Frustulia rhmboides*	-	-	-	-	-	-	-	-	D
46	*Gomphonema geminatum*	-	D	-	-	-	-	-	D	D
47	*Navicula cryptocephala*	D	-	-	-	-	-	-	-	-
48	*N. ignota*	-	D	-	-	-	-	-	-	D
49	*N. jurgensii*	-	C	C	C	D	-	D	-	-
50	*N. microspora*	C	-	-	-	-	-	-	-	-
51	*N. notha*	-	C	-	-	-	-	-	-	C
52	*N. protracta*	-	C	C	-	D	C	-	C	C
53	*Nitzschia closterium*	-	D	-	-	-	-	-	-	D
54	*N. nana*	-	D	-	-	-	-	-	-	-
55	*N. vasnii*	-	-	-	C	-	-	-	C	-
56	*Pinnularia acrosphaeria*	D	-	-	-	-	-	-	-	-

57	*P. gibba*	-	C	-	-	-	-	-	-	D
58	*P. obscura*	-	-	-	-	-	-	-	D	D
59	*P. subsimilis*	C	-	-	-	-	-	-	-	-
60	*Pleurosigma normanii*	C	-	-	-	-	-	-	-	-
61	*Stauroneis platystoma*	-	-	-	-	-	-	-	D	D
62	*Surirella tenera* var. *nervosa*	-	-	-	-	-	-	-	C	C
63	*Synedra crystallina*	-	C	C	-	-	C	R	-	-
64	*S. mesolepta*	-	-	-	-	-	-	-	R	R
65	*S. tabulata*	-	C	-	-	-	-	-	-	R
Euglenoids										
66	*Euglena sanguinia*	-	R	-	-	-	-	-	-	-

Abundance: D = Dominant (>20%); C = Common (10-20%); R = Rare (<10%): - = Not found

Table 2.
Summary of the total species (genus) numbers of soil algae revealed in flooded field plots planted to rice and amended with inorganic and organic N sources

Group	Control	Urea-N	FYM	*S. aculeata*	FYM + *S. aculeata*	FYM + *Azolla*	*S. aculeata* + *Azolla*	Rice straw + *S. aculeata*	Rice straw + Urea
Cyanobacteria	7 (5)	19 (10)	16 (9)	10 (9)	11 (8)	13 (7)	13 (8)	10 (8)	7 (7)
Chlorophytes	2 (1)	5 (3)	3 (2)	5 (4)	3 (2)	3 (2)	3 (2)	5 (5)	3 (1)
Diatoms	5 (3)	11 (7)	3 (2)	2 (2)	2 (1)	2 (2)	2 (2)	6 (6)	13 (10)
Euglenoids	-	1 (1)	-	-	-	-	-	-	-
Total	14 (9)	36 (21)	22 (13)	17 (15)	16 (11)	18 (11)	18 (12)	21 (19)	23 (18)

Table 3.
Physicochemical properties* of soils planted to rice and maintained under different treatments

Treatments	pH (soil:water::1:1.25)	EC	Total organic C (%)	Total N (%)	Organic matter (%)
Control	5.3 ± 0.50	0.173 ± 0.02	0.382 ± 0.01	0.053 ± 0.01	0.658 ± 0.04
Urea (60 Kg N. ha^{-1})	5.1 ± 0.20	0.148 ± 0.02	0.581 ± 0.01	0.075 ± 0.02	1.002 ± 0.02
FYM (60 Kg $N.ha^{-1}$)	5.1 ± 0.15	0.167 ± 0.02	0.772 ± 0.03	0.544 ± 0.04	1.331 ± 0.02
Sesbania aculeata (60 Kg $N.ha^{-1}$)	5.3 ± 0.13	0.224 ± 0.02	0.575 ± 0.03	0.470 ± 0.03	0.991 ± 0.02
FYM (30 Kg $N.ha^{-1}$)+ *S.a* (30 Kg $N.ha^{-1}$)	5.1 ± 0.16	0.287 ± 0.01	0.738 ± 0.03	0.506 ± 0.04	1.272 ± 0.02
FYM (30 Kg $N.ha^{-1}$)+ *Azolla* (30 Kg $N.ha^{-1}$)	4.9 ± 0.18	0.201 ± 0.03	0.668 ± 0.03	0.460 ± 0.02	1.152 ± 0.01
S.a (30 Kg $N.ha^{-1}$)+ *Azolla* (30 Kg $N.ha^{-1}$)	5.2 ± 0.18	0.188 ± 0.02	0.659 ± 0.03	0.450 ± 0.04	1.136 ± 0.02
RS (30 Kg $N.ha^{-1}$) + *S.a* (30 Kg $N.ha^{-1}$)	5.1 ± 0.16	0.311 ± 0.03	0.786 ± 0.03	0.468 ± 0.05	1.355 ± 0.01
RS (30 Kg $N.ha^{-1}$) + Urea (30 Kg $N.ha^{-1}$)	5.4 ± 0.16	0.172 ± 0.02	0.750 ± 0.03	0.064 ± 0.01	1.293 ± 0.02

* Mean of three replicate values ± standard deviation

FYM = Farm yard manure; *S.a* = *Sesbania aculeata*; RS = rice straw

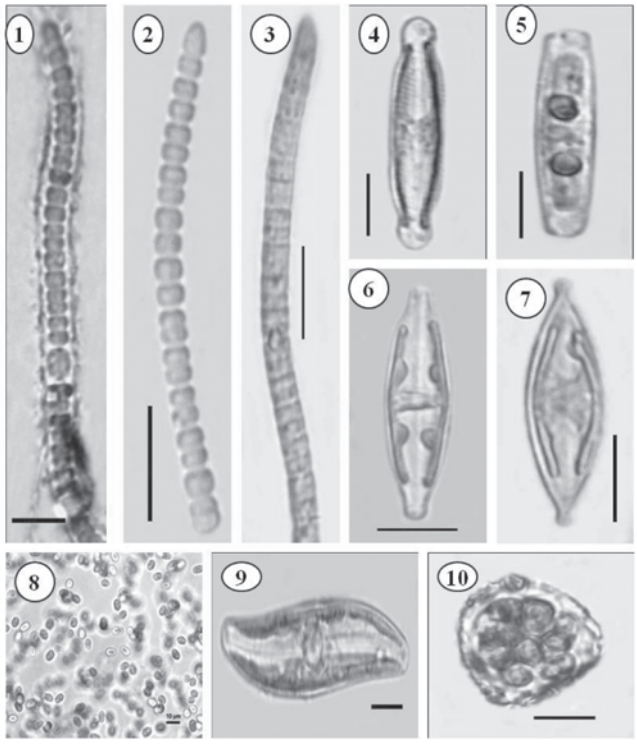

PLATE-1 (Control Field)
Figs. 1-10: 1- *Anabaena ambigua, 2- Anabaena torulosa, 3- Phormidium boryanum, 4- Aphanothece gelatenosa, 5- Pinnularia acrosphaeria, 6- Myxosarcina spectabilis, 7- Navicula microspora, 8- Pinnularia subsimilis, 9- Pleurosigma normanii, 10- Navicula cryptocephala.* Scale = 10µm

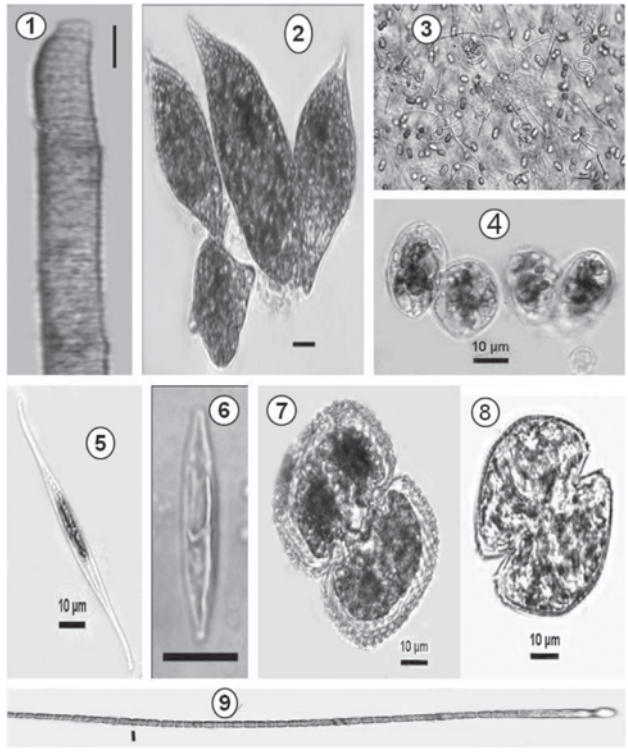

PLATE-2 [Inorganic nitrogen fertilizer (Urea)]
Figs. 1-9: 1- *Oscillatoria anguina, 2- Euglena sanguinia, 3- Phormidium muscicola, 4- Glaucocystis duplex, 5- Nitzschia closterium,* 6- *Microchate tenera,* 7- *Cosmarium pseudocoronatum, 8- Cosmarium auriculatum,* 9- *Navicula notha.* Scale = 10µm

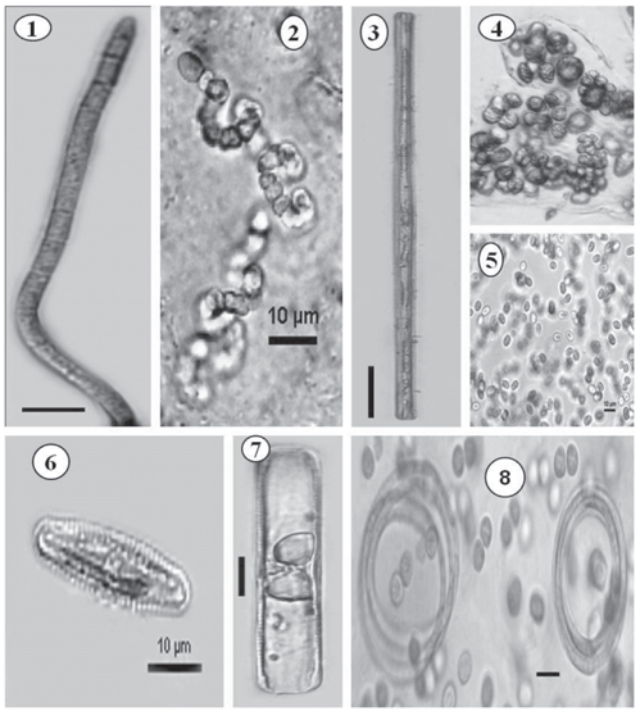

Plate-3 (Farm Yard Manure)
Figs. 1-8: *1- Oscillatoria chlorina, 2- Nostoc paludosum, 3- Synedra crystallina, 4- Microcystis wesenbergi, 5- Aphanothece gelatenosa, 6- Navicula protracta, 7- Navicula jurgensii, 8- Aphanothece bullosa* + *Phormidium orientalis.* Scale = 10µm

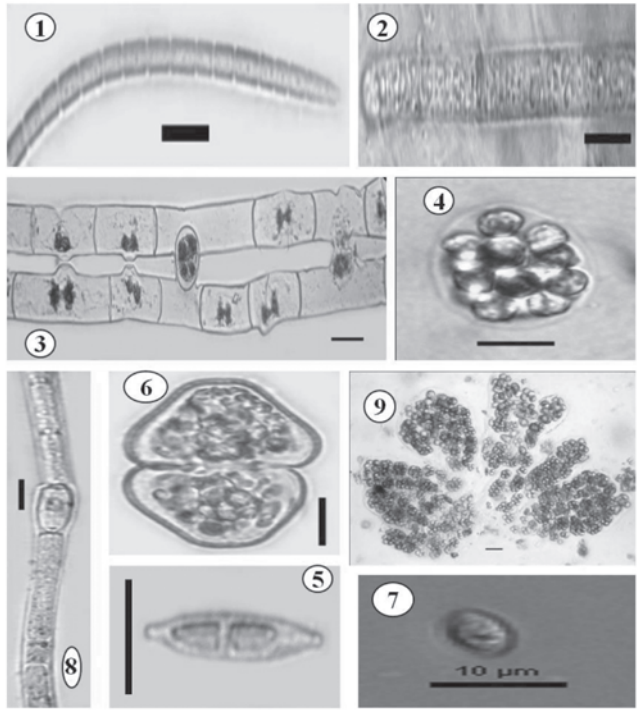

Plate-4 (*Sesbania aculeata)*
Figs. 1-9: 1- *Phormidium tenue, 2- Phormidium rotheanum 3- Zygnema giganteum,* 4- *Oocystis elliptica* 5- *Nitzschia vasnii,* 6- *Cosmarium supergranatum, 7- Scenedesmus obtusus,* 8- *Aulosira prolifica,* 9 - *Microcystis wesenbergi.* Scale = 10µm

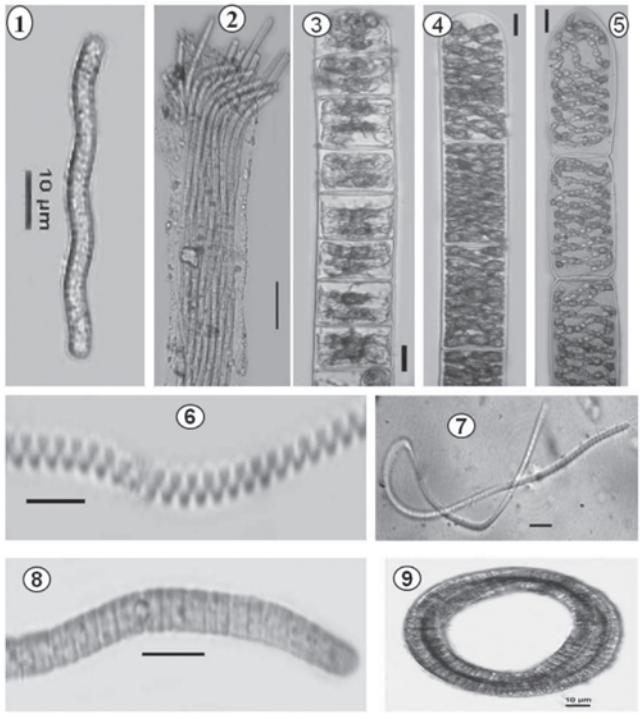

Plate 5 (Farm Yard Manure + *Sesbania aculeata)*
Figs. 1-5: *1- Oscillatoria terebriforme 2- Microcoleus chthonoplates, 3- Zygnema czurdae, 4- Spirogyra condensata, 5- Spirogyra ghosei.* Scale = 10µm
Plate 5 (Farm Yard Manure + *Azolla)*
Figs. 6-9 : 6- *Spirulina subtillisima* 7- *Oscillatoria anguistissima,* 8- *Phormidium boryanum,* 9- *Oscillatoria tenuis.* Scale = 10µm

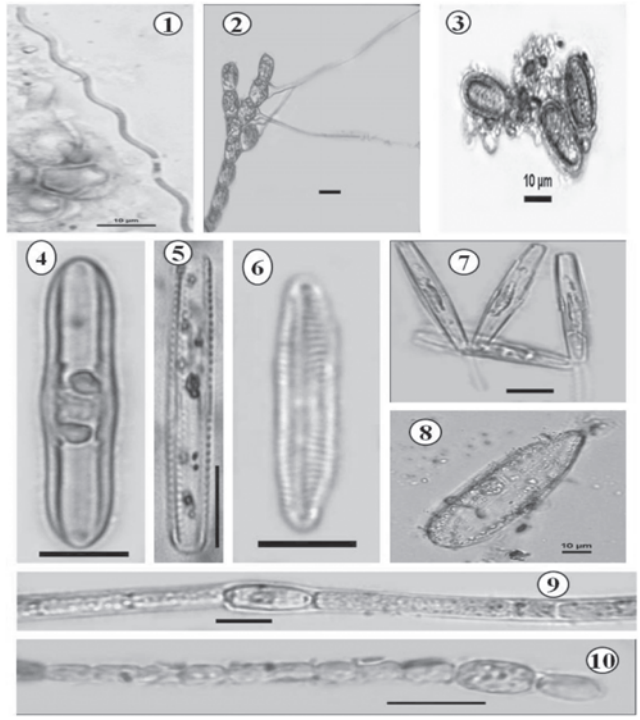

Plate-6 (Rice straw + *Sesbania aculeata*)
Figs. 1-10: 1- *Spirulina laxa,* 2- *Bulbochate basispora, 3- Cylindrospermum muscicola,* 4- *Synedra mesolepta* 5- *Stauroneis platystoma,* 6- *Pinnularia obscura,* 7- *Gomphonema geminata, 8- Surirella tenera* var. *nervosa,* 9- *Aulosira prolifica* 10- *Cylindrospermum catenatum* . Scale = 10µm

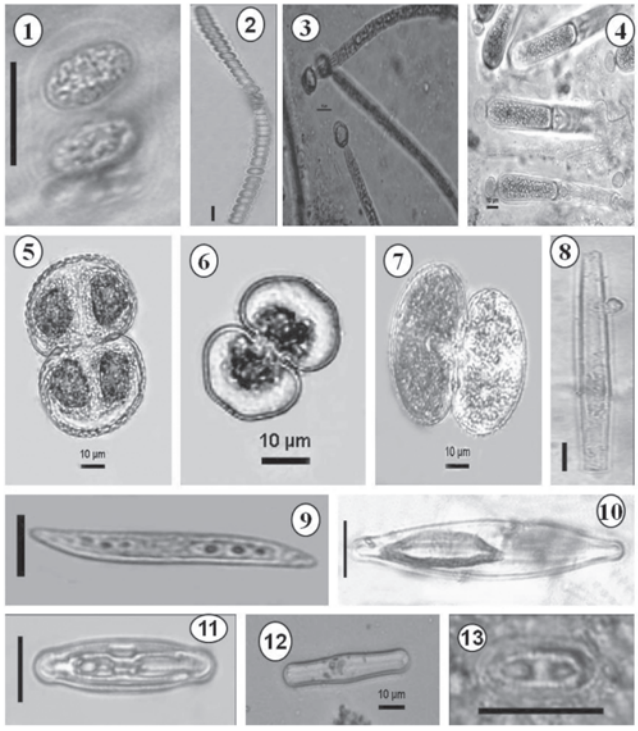

Plate-7 (Rice Straw + Urea)
Figs. 1-13 : 1- *Gloeothece rupestris,* 3- *Anabaena fertilissima, 3-Rivularia aquatica,* 4- *Gloeotrichia natans,* 5- *Cosmarium crenatum,* 6- *Cosmarium nitidulum,* 7- *Cosmarium pachydermum,,* 8 - *Synedra tabulata,* 9- *Nitzschia nana,* 10- *Frustulia rhmboides,* 11- *Navicula ignota,* 12- *Pinnularia gibba,* 13- *Achnanthes hungarica.* Scale = 10µm

3.2 Physicochemical properties of soil

The field plots indicated a marginal or almost no change in the soil pH and salinity under the influence of different types of fertilization (Table 3). The pH of the soils remained slightly acidic ranging from 4.9 to 5.4. As expected, application of organic residues resulted in an increase in the organic C content, especially in field plots amended with FYM either alone or in combination with other organic sources, as FYM is considered to be a slow degrading organic residue. FYM amended plots also show large diversity next only to urea-N amended plots.

4. Conclusion

Rice fields are places of intense farming activities including tillage and field preparation, application of fertilizers and other agrochemicals including pesticides and herbicides. Such activities are known to influence the diversity of soil microflora including that of algae. Nitrogen is the major nutrient limiting rice crop growth and amendment of fertilizer-N is a standard practice to increase the crop yield. However, intensive application of chemical fertilizer to the crop are reported to have several adverse impact on ecosystem functioning. Currently, there are efforts to grow crops without the influence of agrochemicals, the so called organic agriculture. Organic agriculture practices insist on using organic resources for plant nutrition. Our studies reveal that application of organic residues apart from resulting into increase in organic-C content of the soil also influences the algal flora.

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An efficient method of *in vitro* propagation of *Gloriosa superba* L. – an endangered medicinal plant

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ABSTRACT

Gloriosa superba L. is an important medicinal plant of India. It is an important source of pharmaceutical compound known as colchicine. This plant has become endangered in our country. To protect this species we adopted *in vitro* methodology to develop simple protocols for shoot multiplication as well as *in vitro* tuberization. Maximum shoots (15.67±0.34) were multiplied from tuber explants on MS medium fortified with 5.0 mg/l BAP. The elongated shoots with healthy tubers were subcultured for rooting and best response was on MS medium supplemented with 1.0 mg/l IBA. The *in vitro* raised plantlets were acclimatized in green house and successfully transplanted to natural condition with 75% survival rate. The regenerated plants were cytologically and phenotypically stable.

1. Introduction

Gloriosa superba (Glory lily) an important species of the family Colchicaceae, is a perennial tuberous climbing herb having attractive wavy edged flowers. (Amano et al., 2008). It is known as 'Malabar glory lily' in English, in Hindi as 'Kalihari', in Sanskrit as 'Agnisikha' having its trade name as 'Glory lily'. It is widely scattered in tropical and sub-tropical parts of Africa and Southeast Asia. In India, it is usually found in the Himalayan foot-hills, Tamil Nadu, Andhra Pradesh and West Bengal. Its flower is the national flower of Zimbabwe and also the state flower of Tamil Nadu in India due to its high ornamental value (Jana and Shekhawat, 2011).

Gloriosa superba L is one of the seven Upanishads in the Indian medicine, which cured many ailments, but may prove fatal on misuse (Joshi, 1993). It is an important medicinal plant and is a source of important pharmaceutical compound known as colchicine. Colchicine has been used in medicine for a long time. Colchicine has been effectively used in the treatment of several inflammatory conditions, such as gouty attacks, serositis related to familial Mediterranean fever, Behcet syndrome, and more recently also in acute and recurrent pericarditis. Growing evidence has shown that the drug may be useful to treat an acute attack and may be a way to cope with the prevention of pericarditis in acute and recurrent cases and after cardiac surgery (Ghosh *et al.,* 2002, 2007, Imazio *et al.,* 2009).

Gloriosa superba L. an industrially important medicinal crop of India, with high colchicine content, is still collected from wild. It is mainly vegetatively propagated but the rate is very low (Krause, 1986) as only 2 tubers are produced per year. The medicinal properties of the plant has led to it's over exploitation further inadequate cultivation and unsatisfactory attempts for its replacement has brought marked depletion of its wild resources. So, it has been affirmed as endangered plant by the IUCN Red Data Book (IUCN, 2010; Lal and Mishra, 2011; Sivakumar and Krishnamurthy, 2004, Contu 2013).

Its conventional propagation cannot meet the increasing demand and will ultimately lead to extinction if no attention is given to its conservation and propagation. *In vitro* methods

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of propagation provide an alternate and effective means for rapid multiplication of species through continuous production to meet the demand for commercial exploitation. The objective of the present study was to develop a simple efficient protocol for large scale plant production of *Gloriosa superba* through tuber culture and to assess genetic stability of regenerants by chromosome analysis.

2. Materials and methods

2.1. Plant material

Gloriosa superba L. were collected from various locations of West Bengal, India and were grown in the experimental garden of RKMVC College, Rahara, Kolkata, India. Healthy tubers were collected from six months old plants used as explants.

2.2. Explants disinfection and implantation

Tubers were cut into round slices with each of them possessing axillary buds. They were washed thoroughly under running tap water for 15 min and then surface sterilized with 2% w/v solution of Bavistin® (systemic fungicide) for 20 min followed by 5% v/v solution of Tween 20 (liquid detergent) for 10 min. Then the explants were thoroughly washed with fresh water to remove the detergent. Finally, the explants were surface sterilized with freshly prepared 0.1% w/v solution of mercuric chloride ($HgCl_2$) for 15 min, rinsed in sterilized distilled water for 3 times to remove traces of $HgCl_2$ under the sterile condition. Then the explants were placed vertically onto sterilized MS media with and without Plant growth regulators.

2.3. Culture media

MS basal medium (Murashige and Skoog, 1962) with 3% sucrose was used for breaking the dormancy of shoot buds as well as for proliferation. The pH of the medium was adjusted to 5.6 ±0.2 using 0.1 N KOH or 0.1 N HCl as and when required and 0.8% (w/v) agar (Merck, India) before autoclaving. Medium was dispensed into each culture tube (25×150 mm), conical flask (250 ml) and jam bottle, plugged with non-absorbent cotton. The tubes were then wrapped in one layer of cheese-cloth and steam sterilized at 15 lb $inch^{-2}$ for 18 min.

2.4. Culture conditions

All the cultures were incubated at 24 ±2 °C temperature and 55 ±5% relative humidity under 16 h photoperiod at 55 ìmol $m^{-2}s^{-1}$ irradiance, supplied by Philips (Trulite 5 star 36 w/82 2700ÚK G84, made in India) fluorescent tubes.

2.5. In vitro shoot multiplication

The excised tuber explants were inoculated vertically on to MS medium supplemented with different concentration of BAP, Kn, 2iP, NAA individually and in combination and replicates thrice of each experiment. The multiple shoots were subcultured at every two weeks for 60 days. A control culture (only basal medium) was maintained to record the frequency of response.

2.6. Shoot elongation

After 40 days induced multiple shoots were excised individually and subcultured on to MS medium supplemented with BAP (1.0 mg/l) and varying concentrations of gibberellic acid (GA_3) (0.2, 0.4, 0.6, 0.8, and 1.0 mg/l) for elongation of shoots. A control culture (basal medium only) was also maintained.

2.7. In vitro root induction

Micro-shoots (1- 3 cm) were excised from the culture and transferred to full- strength MS medium augmented with different concentrations auxins viz., IBA, IAA and NAA (0.2, 0.5, 1.0 and 2.0 mg/l) and 2% sucrose (w/v, Merck, India) for root initiation. Micro-shoots were cultured in one tube each under the same culture conditions as described above. After 4 weeks the percentage of shoots forming roots, the number of roots per shoot and root length were assessed. One culture set was inoculated on basal MS medium without any plant growth regulator and considered as control.

2.8. Acclimatization

For hardening, at first the tissue culture derived healthy rooted plantlets were placed at room temperature for 7-10 days. Then the plantlets were removed from the agar medium, washed thoroughly under running tap water and transferred to earthen pots containing soilrite (Keltech Energies Ltd, Bangalore, India). To preserve moisture, the potted plantlets were covered with transparent polythene bag and the pots were placed on a plastic tray containing water under diffused light (16 h, photoperiod) in the poly-house for 30-35 days, thereafter the acclimatized plants were transferred to field condition under full sunlight.

2.9. Cytological study

Young and healthy root tips of *G. superba* were excised from the source plant as well regenerated plants in order to analyze the chromosome numbers. The root tips were pretreated with saturated solution of PDB for 6 hrs at 14^0C fixed in 1:3 acetic acid:dehydrated ethanol. Aceto-orcein staining technique was adopted. Chromosome plates were observed in Leica DM750 microscope and photographed with Leica DFC 295 camera. Minimum of 5 metaphase plates from each root tip were analyzed to determine the somatic chromosome number at the metaphase stage.

2.10. Statistical analysis

All the experiments were conducted under controlled conditions with three replications. Means and standard errors were carried out for each experiment and data was analyzed using one way Analysis of Variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Tukey's multiple range test at a 5% probability level. Data analysis was performed using SPSS v 16.0 software.

3. Results and Discussion

3.1. Role of plant growth regulators on in vitro *shoot induction and multiplication*

A number of experiments were conducted with a view of finding out optimum culture condition for maximum shoot multiplication from the culture of tuber explants. Multiple shoots developed from the tuber explants when they were cultured on to MS medium supplemented with different combination and concentration of BAP, Kn, 2iP alone and combination with NAA.

It was observed that multiple shoot buds induced from tubers when cultured on MS medium (Murashige and Skoog, 1962) containing cytokinins (BAP, Kn and 2ip) and auxin (NAA) (Table-1). Among the different cytokinins it was found that MS media supplemented with BAP was most effective in shoot induction and proliferation than others. Within 10 days of inoculation, explants swelling were observed. First appearance of buds was observed within 5-7 days. Multiple shoots commenced to emerge from the cut ends in case of BAP and Kn supplemented media. In MS media supplemented with NAA, poor multiplication was observed and a callus like structure was formed. A maximum response of 85% was recorded in MS media supplemented with 5.0 mg/ l BAP alone, where also the number of shoots induced per explants was highest 15.67 ± 0.34 with an average length of 6.56 ±0.03.cm (Fig.1a). It was also reported that BAP was the best cytokinin for *Aegle marmelos* (Nayak *et al.,* 2007). MS medium supplemented with Kn and 2iP showed comparatively less response. MS medium containing 1.0 mg/l Kn showed maximum response as compared to the five concentrations of Kn (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l) assessed and showed maximum 57% sprouting frequency and an average of 5.66 ±0.30 number of shoots per explant segment. MS medium suplimented with 0.5 mg/ l 2iP showed maximum response of 44% as compared with other five concentrations of 2iP (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l). Different combination and concentration among any two types of cytokinins (BAP + Kn, BAP + 2iP, Kn +2iP) or cytokinin with NAA (BAP+NAA, Kn+ NAA, 2iP +NAA), in MS medium failed to exhibit better shoot induction than single cytokinin (Table-1). Some stunted shoots were induced but their multiplication rate was significantly low. Similar results were also observed in *Curcuma* sp. (Yasuda *et al.,* 1987); *Kaempferia galanga* (Shirin *et al.,* 2000) and *Alpinia calcarata* (Amin *et al.,* 2001).

3.2. Shoot elongation

Excised shoots were cultured on to the MS basal medium consisting of 1.0 mg/l BAP in the presence of GA_3 at different concentrations for 2 weeks and were evaluated for shoot length. The result suggest that small amounts of GA_3 (0.2 mg/l) with 1.0 mg/l BAP were effective in stimulating *G. superba* shoot elongation. Shoots attained a maximum height of 10.2 cm during GA_3 treatments (Table-2). Similar nature of response was also found during *in vitro* culture of *Camella sinensis* (Gonbad *et al.,* 2014) and *Plumbago zeylanica* (Chatterjee and Ghosh, 2015).

3.3. Induction of roots from in vitro grown micro shoots

To develop a successful and consistent micropropagation protocol, easy and high frequency rooting from micro shoots is very important. Production of plantlets with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil. To induced rooting, elongated shoots (2.0 – 4.0 cm) were cultured on to MS medium supplemented with different types of auxins at different concentration viz., IBA (0.5, 1.0, 1.5, 2.0, 2.5 mg/l), IAA (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) and NAA (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) individually. A control set up was also maintained using basal MS medium. Among the three different auxins tested, number of roots and root length varied. Maximum number of excised micro shoot induced roots within 25-30 days of culture. Among different types of auxin used in the present experiment, IBA was found to be the most effective at different concentrations tested for producing roots from the base of micro- shoots. Among different concentrations of IBA, 0.5 mg/l was found to be best concentration auxin for proper rooting of *G. superba* in which 85% shoots rooted within four weeks of culture (Table-3). The similar results were also reported in *Kaempferia galanga* (Shirin *et al.,* 2000), *Tylophora indica* (Haque and Ghosh 2013a) *Luffa acutangula* (Saha and Ghosh, 2014) *Plumbago zeylanica* (Chatterjee and Ghosh, 2015). The present findings are in agreement with those observed in similar rhizomatous plant species such as *Zingiber officinale* (Haque *et al.,* 1999); *Alpinia calcarata* (Amin *et al.,* 2001).

3.4 Acclimatization

The successfully rooted plantlets were transferred to small earthen pots containing soilrite and covered with

Table-1
Influence of different cytokines on shoot bud formation and multiplication from tuber explants of *G. superba*

Cytokinin	Concentration (mgl^{-1})	% of explants showing shoot bud induction	Mean No. of shoots/explants	Mean shoot length (cm)
BAP	0.5	62± 2.0^{c}	7.22±0.33^{de}	3.86±0.08^{f}
	1.0	68± 3.0^{b}	8.67±0.43^{b}	4.97±0.03^{b}
	2.0	72± 1.1^{b}	9.0±0.57^{a}	5.13±0.03^{a}
	3.0	78± 2.0^{a}	14.01±0.50^{c}	5.23±0.08^{d}
	5.0	85± 3.0^{a}	15.67±0.34^{hi}	6.56±0.03^{gh}
Kinetin	0.5	55± 1.0^{bc}	5.34±0.32^{fghi}	3.36±0.06^{i}
	1.0	57± 1.2^{b}	5.66±0.30^{d}	3.30±0.03^{g}
	2.0	45± 0.5^{cd}	4.66±0.35^{c}	2.33±0.05^{d}
	3.0	42± 1.0^{c}	3.34±0.58^{def}	2.03±0.03^{f}
	5.0	41± 1.0^{bc}	3.00±0.32^{ghi}	1.60±0.05^{g}
2iP	0.5	44± 1.3^{c}	3.67±0.34^{efgh}	1.33±0.03^{ih}
	1.0	42± 1.5^{cd}	3.32±0.33^{c}	3.83±0.03^{c}
	2.0	38± 0.1^{f}	2.34±0.56^{d}	2.83±0.03^{e}
	3.0	30± 2.0^{c}	2.00±0.58^{efg}	1.53±0.02^{gh}
	5.0	25± 1.3^{bc}	1.32±0.32^{i}	1.23±0.04^{i}
NAA	0.5	25± 1.0^{c}	1.33±0.44^{ef}	1.03±0.14^{f}
	1.0	20± 1.0^{ef}	1.02±04^{gh}	1.00±0.2^{i}
	2.0	Callus	Callus	Callus
	3.0	Callus	Callus	Callus
	5.0	Callus	Callus	Callus
BAP+Kn	0.5+0.5	57± 1.3^{c}	5.6±0.23^{a}	3.68±0.69^{g}
	1.0+0.5	44± 1.0^{cd}	4.2±0.37^{ij}	4.50±0.58^{cd}
	3.0+0.5	32± 1.1^{e}	3.36±1.02^{c}	3.02±0.51^{bc}
BAP+2iP	0.5+0.5	75± 0.3^{f}	3.30±0.65^{ab}	3.60±0.57^{i}
	1.0+0.5	79± 0.5^{fg}	2.20±0.26^{gh}	3.00±1.03^{e}
	3.0+0.5	75± 1.0^{gh}	1.05±0.95^{fg}	3.40±0.87^{a}
Kn+2iP	0.5+0.5	62± 1.2^{ghi}	2.20±0.91^{ghi}	3.30±0.44^{fg}
	1.0+0.5	70± 1.3^{cd}	2.23±0.65^{ab}	2.80±0.51^{e}
	3.0+0.5	60± 0.1^{cd}	1.20±1.20^{i}	3.70±1.02^{fg}
BAP + NAA	0.5+1.0	50± 1.0^{ghi}	2.0±1.2^{c}	3.12±1.3^{b}
	2.0+1.0	31± 1.0^{f}	1.12± 0.4^{d}	2.0±1.90^{cd}
	3.0+1.0	Callus	Callus	Callus
Kn + NAA	0.5+1.0	29± 1.0^{fg}	2.10±0.32^{cd}	1.79±2.1.2^{bc}
	2.0+1.0	20± 1.1^{gh}	1.17±0.12^{ef}	1.45±2.1^{cd}
	3.0+1.0	Callus	Callus	Callus
2iP + NAA	0.5+1.0	23± 0.2^{cd}	2.0±1.0^{def}	1.20±0.32^{def}
	2.0+1.0	16± 1.0^{gh}	1.0±.2^{efg}	0.07±0.21^{ghi}
	3.0+1.0	Callus	Callus	Callus

(Each value represents the mean ± SD of 10 replicates and each experiment was repeated thrice)

Table 2
Effect of GA_3 on shoot elongation of *G. superba* when cultured on MS medium supplemented with GA_3 and BAP (1.0 mg/l). (Data collected after 14 days of culture.)

BAP (mg/l)	GA_3 (mg/l)	Response for shoot elongation (%)	Mean length of shoot (cm)
1.0	0.2	85± 2.0^{a}	10.2 ± 1.0^{c}
1.0	0.4	82± 1.7^{c}	9.5 ± 0.5^{b}
1.0	0.6	80± 1.2^{b}	9.2 ± 1.5^{a}
1.0	0.8	75± 0.4^{c}	7.0 ± 1.2^{ab}
1.0	1.0	65± 1.1^{b}	5.0 ± 0.2^{ef}

Table -3
In vitro rooting of *G. superba* by using different types of auxins. (Data collected after 30 days of culture).

MSO + Auxins	% of rooting	No. of root / shoot	Root length (cm)
Control	-	-	-
IBA			
0.2	67±0.7^{c}	3.7±1.2^{c}	4.3±0.2^{b}
0.5	85±0.3^{a}	5.8±3.5^{a}	6.3±2.2^{a}
1.0	78±1.1^{b}	4.3±0.7^{c}	4.9±1.1^{c}
2.0	45±0.2^{c}	2.8±1.2^{e}	3.2±0.4^{bc}
IAA			
0.2	53±3.2^{f}	3.8±1.8^{cd}	3.9±2.3^{b}
0.5	65±2.1^{cd}	4.8±3.0^{a}	4.8±2.0^{a}
1.0	48±0.7^{e}	4.0±2.5^{b}	3.1±1.5^{c}
2.0	32±0.4^{c}	2.3±0.5^{e}	2.1±0.5^{bc}
NAA			
0.2	38±0.3^{fg}	3.5±0.5^{f}	3.2±0.2^{f}
0.5	31±0.1^{h}	2.7±1.0^{e}	2.1±0.7^{e}
1.0	23±0.1^{g}	1.8±0.7^{c}	1.2±0.1^{e}
2.0	callus	callus	Callus

transparent poly bags for hardening. All the plantlets were maintained in the culture room (25± 1^{0}C) conditions initially for 6-7 weeks and then transferred to normal laboratory in room temperature conditions and maintained for about 4- 5 weeks. Finally the plantlets were transferred to Poly House and maintained 3- 4 weeks and then were transferred to the experimental field condition (Fig.1c). It was observed that 75% ± 5 of the acclimatized plants survived in field condition. There was no noticeable variation among the acclimatized plants with respect to morphological and growth characteristics. All the micropropagated plants were free from external disease.

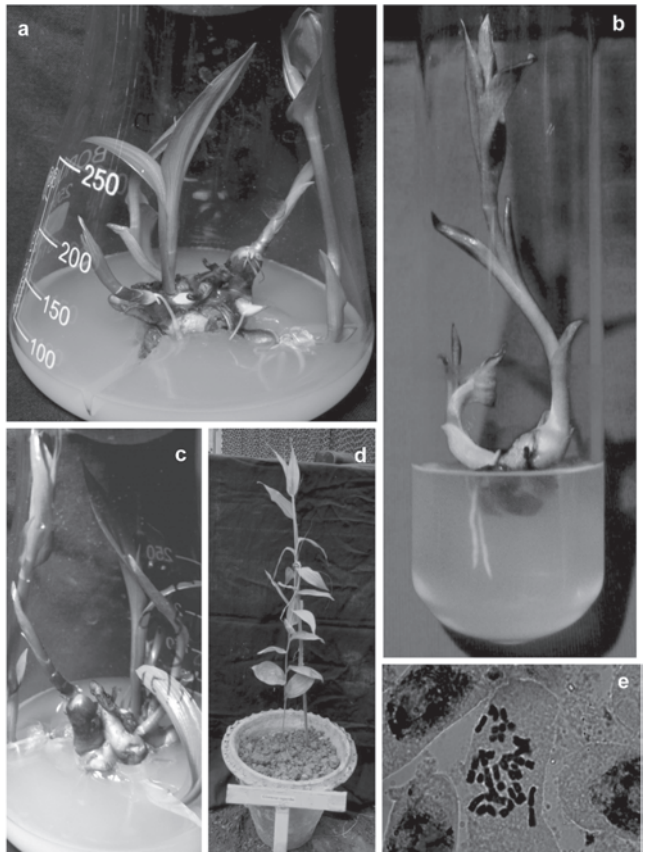

Fig-1. *In vitro* propagated plant of *Gloriosa superba.* (a). Multiplication of shoots from tuber, (b & c) Single complete plantlet, (d) ex-vitro growing tissue culture raised plant, (e) Root tip cell of ex-vitro growing plants showing metaphase stage with (2n= 22) chromosomes.

3.5 Chromosomal study

Chromosomal analysis in our present studies from randomly selected root tips from the source plant showed 2n= 22 chromosomes and cytological preparations from the *in vitro* derived 15 plantlets showed diploid number (2n= 22) of chromosomes (Fig. 1d). Thus the somatic chromosome complements of *in vitro* generated *G. superb* plants remained stable even after passing through three cycles of multiplication. Such mode of propagation can be utilized in other medicinal and economically important plants. A similar cytogenetically stable plant was also observed in *Asparagus cooperi* (Ghosh and Sen, 1992, 1994); *Gloriosa superba* (Ghosh *et al.,* 2007); *Aloe vera* (Haque and Ghosh, 2013b) and *Plumbago zeylanica* (Chatterjee and Ghosh, 2015).

Our studies provide a simple and effective protocol of *in vitro* mass propagation for *in vitro* storage of *G. superba*. In this study we also established genetic stability of regenerants through chromosome analysis reveling diploid chromosome number. The present protocol of *in vitro* propagation of *Gloriosa superba* may be highly useful for

raising quality planting material for commercial and off-season cultivation and also for genetic stock restoration.

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Comparison of antibacterial activities of some selected wild cucurbits collected from Similipal Biosphere Reserve

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ABSTRACT

Similipal Biosphere Reserve (SBR) is situated in the district of Mayurbhanj, Odisha. It forms the major part of Eastern Ghats having rich floral diversity. SBR is inhabited by many tribal communities too. They are in habit of using the wild phytoresources against different microbial infections. Wild cucurbits are prime components of the phytoresources of SBR. Keeping this in view, four wild cucurbits (*Trichosanthes tricuspidata, Diplocyclos palmatus, Cucumis melo, Trichosanthes cucumerina*) were collected from SBR and experimented for their antibacterial activities against five selected bacterial strains (*Streptococcus mutans* - MTCC 497, *Streptococcus pyogenes* - MTCC 1926, *Vibrio cholerae* - MTCC 3906, *Shigella flexneri* - MTCC 1457 and *Salmonella typhi* - MTCC 1252). The MIC (minimum inhibitory concentration) of the extracts was determined using broth dilution assay. Results revealed that the methanol extract of *T. tricuspidata* fruits showed lowest MIC values against *S. pyogenes* whereas the acetone and methanol extract of *C. melo* fruits showed lowest MIC values against *S. mutans*. The paper highlights a comparative account of antibacterial potential of wild cucurbits collected from SBR and activity of the extracts against specific bacterial species.

1. Introduction

Similipal Biosphere Reserve (SBR) is situated in the central part of Mayurbhanj district in the state of Odisha (Bhakta *et al.,* 2014). It lies between 21° 10′ to 22° 12′ N latitude and 85° 58′ to 86° 42′ E longitude, ranging between 300 m to 1,180 m above sea level (Das and Das, 2008). The name "Similipal" has been derived from the pre-dominant floral species Semul, the red silk cotton (*Bombax ceiba* L., Malvaceae) which bloom abundantly in this area (Mishra *et al.,* 2008; Rout and Thatoi, 2009; Misra *et al.,* 2013). This biosphere reserve is unique for its varied topography, geologic formation, and excellent biodiversity along with many inhabited tribal communities (Rout and Panda, 2010; Panda *et al.,* 2010). It has mixed vegetation such as Orissa semi evergreen forest, Tropical moist broadleaf forest, Tropical moist deciduous forest, Dry deciduous hill forest, High level Sal forest with grassland and Savanna (Mishra, 2010; Misra *et al.,* 2011; Kumar *et al.,* 2012; Misra *et al.,* 2013; Tripathy *et al.,* 2014). The diverse vegetation here provides rich diversity of wild medicinal plants. The rural and tribal communities of SBR are in habit of using these medicinal plants for cure against microbial infections (Thatoi *et al.,* 2008; Rath *et al.,* 2009; Panda *et al.,* 2010; Padhi *et al.,* 2011; Panda *et al.,* 2012; Kumar *et al.,* 2013; Kumar *et al.,* 2014). Large number of medicinal plants belonging to different families are available in SBR which are used by the locals against various diseases. Among them, wild cucurbits are quite popular due to their easy availability in the forest edges of SBR. The most common wild cucurbits are *Cucumis melo, Trichosanthes cucumerina, Trichosanthes tricuspidata, Diplocyclos palmatus, Solena* spp, *Mukia maderaspatana* etc. These species posses wide ethnobotanical uses against microbial infections throughout the study area (Tripathy *et al.,* 2013). The available reports also support the use of these plants against some common

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diseases (Tang *et al.,* 2010; Jain *et al.,* 2012; Yuvarajan *et al.,* 2015; Castillon *et al.,* 2012; Giday and Teklehaymanot, 2013; Teklay *et al.,* 2013; Maroyi, 2013; Megersa *et al.,* 2013; Rai *et al.,* 2013; Natarajan and Dhas, 2013; Ma *et al.,* 2014; Yaseen *et al.,* 2015; Agarwal and Varma, 2015). Murthy *et al.* (2013) reported the traditional medicinal uses of some wild cucurbits from the Eastern Ghats of Odisha. The authors previously have reported the preliminary antibacterial activity (disc diffusion and agar well diffusion assay) of some common wild cucurbits (Tripathy *et al.,* 2014a; Tripathy *et al.,* 2014b; Tripathy *et al.,* 2014c) of SBR. Keeping all these in view, an attempt has been made in the present study to assess the antimicrobial activity of some common selected wild cucurbits (*Trichosanthes tricuspidata, Diplocyclos palmatus, Cucumis melo* and *Trichosanthes cucumerina* (Fig. 1) available in SBR and to compare their antibacterial potential through determination of MIC. It also aims at drawing the attention of pharmacological scientists / researchers for screening of new antimicrobial compounds present in these plant species, for their successful use in formulation of new antimicrobial drugs.

2. Materials and Methods

2.1. Collection of wild cucurbits for experimental work

The plant samples were collected from the Padampur, Sanuski and Kalikaparsad village of SBR during late autumn and early winter season and are kept in poly bags tagged with the botanical name. They were sorted out as per standard sampling procedure and passport description (Koppar, 1998). The collected germplasm of experimental plants were propagated and grown in the field gene bank of Department of Botany, Ravenshaw University, Cuttack.

2.2. Preparation of plant extracts

Soxhlet method was adopted to obtain the plant extracts (Tiwari *et al.,* 2011). The plant parts (leaf, fruit and root) of experimental plants were collected and dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out as 1:10 ratio (solute: solvent) with n-butanol, methanol, acetone and aqueous using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored for further experimental work.

2.3. Determination of MIC

The extracts of experimental plant parts were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252). These microbes were taken for study since very less reports are available on the effect of plant extracts on these common human pathogens. All used MTCC (Microbial Type Culture Collection)

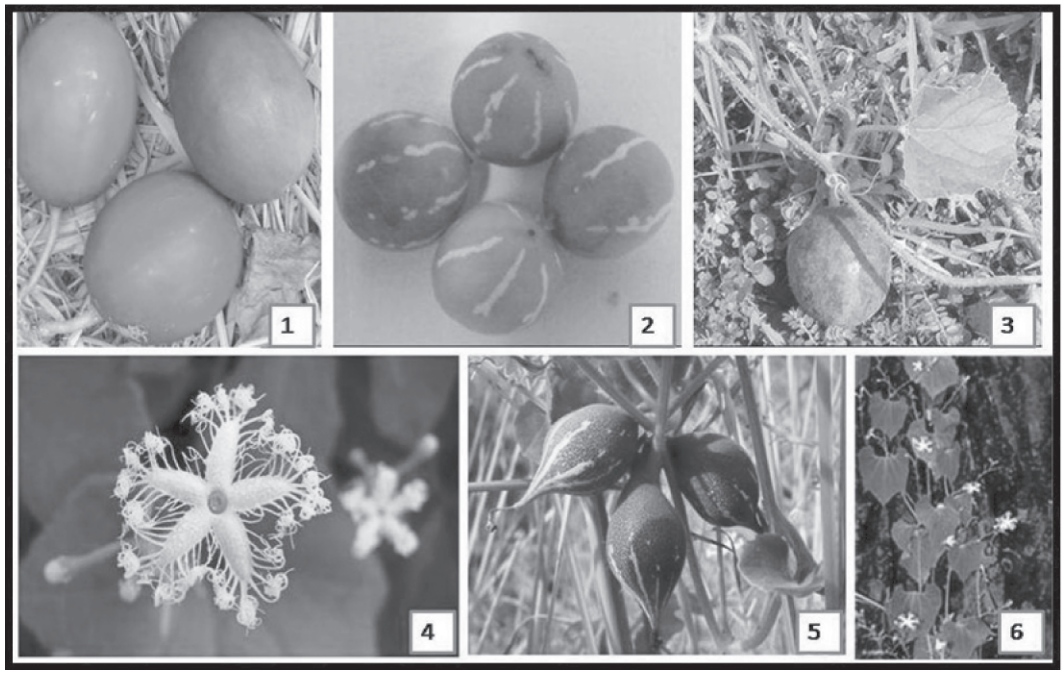

Fig.1. Morphological details of exprimented wild cucurbits, 1: Fruits of *T. tricuspidata*, 2: Fruits of *D. palmatus*, 3: Fruit of *C. melo*, 4: Flowers of *T. cucumerina*, 5: Fruits of *T. cucumerina*, 5: Vegetative parts of *T. cucumerina*

bacterial strains were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was assessed by estimation of Minimum Inhibitory Concentration (MIC) (Rai *et al.,* 2010) by broth dilution assay with standard Kanamycin. For the estimation of MIC, 5.0 mg of each extract was dissolved in 10 ml of trypticase soya broth to get 500 µg/ml and for the standard 0.5 mg was taken to get 50 µg/ml.

2.4. Data Interpretation

After the incubation, the tubes showing no visible growth after 8 h till 24 h were considered to be inhibition of bacteria which represent MIC (minimum inhibitory concentration) values of a respective concentration. Inoculums control showed visible growth due to the absence of antimicrobial agents, where as the broth control showed no growth due to absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each replicates. For the bactericidal and bacteriostatic studies, the sample tubes were kept under observation until 72 h after readings for MIC were taken.

3. Results and Discussion

All the four extracts (n-butanol, methanol, acetone and aqueous) of selected wild cucurbits were screened for their antibacterial activity. The extracts of selected wild cucurbits (plant parts) showed significant MIC (minimum inhibitory concentration) values against all used tested microbial strains. It was observed that non-edible cucurbits (*T. tricuspidata* and *D. palmatus*) were more effective as compared to the other two edible cucurbits (*C. melo* and *T. cucumerina*). The comparative results of the used wild cucurbits on selected microbial strains were analysed. It was noticed that, the *T. tricuspidata* showed lowest MIC values followed by *D. palmatus, C. melo* and *T. cucumerina* (Table 1-4). It was further observed that the fruit extracts of experimental plants exhibited higher antibacterial activity followed by leaves and root extracts. Among the used solvent extracts, methanol extract of experimental plant parts showed lowest MIC values followed by acetone, aqueous and n-butanol (Table 1-4). The growth of the experimental bacterial strains was inhibited significantly by the used extracts of selected wild cucurbits (Table 1-4). Among the tested strains, it was noticed that the growth inhibition of *S. pyogens* and *S. mutans* was more with the acetone and methanol extracts of experimental plant parts (fruits and leaves) (Fig. 2-3). The methanol extract of *T. tricuspidata* fruits showed highest inhibitory effect in low concentration (lowest MIC = 200 µg/ml) against *S. pyogens* (Fig. 2) while the acetone and methanol extracts of *C. melo* fruits showed lowest MIC (200 µg/ml) against *S. mutans* (Fig. 3). It was observed that the extracts of *T. tricuspidata* had significant inhibitory effect against all the tested microbial strains. It was further noticed that the methanol extract of fruits of this vine showed lowest MIC values against *S. pyogens* followed by leaf and root extract (Table 1).

When MIC values of *D. palmatus* (fruit, leaves and root) extracts were analysed, it was observed that leaves and fruits extracts showed significant activities. The methanol extract of fruit showed lowest MIC values (300 µg/ml) against all strains followed by acetone extract of fruits (Table 2). Experiment with the solvent extracts of *C. melo* plant parts exhibited that the acetone and methanol extracts of fruits had lowest MIC against *S. mutans* followed by *S. pyogenes*, *V. cholerae*, *S. typhi* and *S. flexneri* (Table 3). In a comparison experiment on the antibacterial activities of *T. cucumerina*, it was seen that only n-butanol, acetone and methanol extracts of leaves showed antibacterial activities (500 µg/ml) but the MIC was higher than the other vines indicating its lower efficiency in comparison to other (Table 4).

Literature survey revealed that there is no reports of comparative antibacterial activities in terms of MIC are available on selected wild cucurbits but researchers earlier have also documented the preliminary antibacterial activity of *T. tricuspidata, T. cucumerina, D. palmatus* and *C. melo* plant parts by disc diffusion (DD) and agar well diffusion methods (AWD) such as Kage *et al.* (2009) reported the antibacterial activity of *T. cucumerina* AWD assay against *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Reddy *et al.* (2010) reported the antibacterial activity of *T. cucumerina* against gram-negative and gram-positive bacteria such as *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella paratyphi*, *S. aureus, E. coli, Streptococcus faecalis, Proteus vulgaris*, *Klebsiella pneumoniae, P. aeruginosa* and *Serratia marcescens*. Kavita *et al.* (2012) reported the antibacterial activity of *D. palmatus*. Vadnere *et al.* (2013) reported the antimicrobial activity of *D. palmatus* using AWD assay against *Salmonella typhimurium* and *B. cereus.* Saboo *et al.* (2013) reported the antibacterial activity of *T. tricuspidata* using AWD assay against *E. coli, S. aureus, B. subtilis and P. aeruginosa*. Patel and Kishnamurthy, (2013) reported the antibacterial activity of *D. palmatus*. Gupta and Wagh, (2014) reported the antibacterial activity of *D. palmatus* plant pars using AWD assay against *S. aureus, Micrococcus luteus, B. cereus* and *P. aeruginosa*. Gavrakar *et al.* (2014) reported the antibacterial activity of *C. melo* fruits against gram-negative bacteria using AWD assay against *E. coli, P. aeruginosa, B. cereus* and *S. aureus.* Siddeeg *et al.* (2014) reported the antibacterial activity of *C. melo* seed oil using AWD assay against Gram-negative bacteria.

Table 1
Estimation of MIC values of *Tricosanthes tricuspudata* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
TTLNB	GC	GC	GC	GC	GC
TTLM	400 µg/ml	500 µg/ml	500 µg/ml	300 µg/ml	300 µg/ml
TTLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTLAQ	GC	GC	GC	GC	GC
TTFNB	GC	GC	GC	GC	GC
TTFM	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml	200 µg/ml
TTFA	400 µg/ml	500 µg/ml	500 µg/ml	400 µg/ml	400 µg/ml
TTFAQ	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTRNB	GC	GC	GC	GC	GC
TTRM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TTRA	GC	GC	GC	GC	GC
TTRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252; Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 2
Estimation of MIC values of *Diplocyclos palmatus* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
DPLNB	GC	GC	GC	GC	GC
DPLM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPLAQ	500 µg/ml	GC	GC	500 µg/ml	500 µg/ml
DPFNB	500 µg/ml	GC	GC	GC	GC
DPFM	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml	300 µg/ml
DPFA	300 µg/ml	400 µg/ml	400 µg/ml	300 µg/ml	300 µg/ml
DPFAQ	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
DPRNB	GC	GC	GC	GC	GC
DPRM	GC	GC	500 µg/ml	500 µg/ml	500 µg/ml
DPRA	GC	GC	GC	GC	GC
DPRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 3
Estimation of MIC values of *Cucumis melo* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
CMLNB	GC	GC	GC	GC	GC
CMLM	GC	GC	GC	GC	GC
CMLA	GC	GC	GC	GC	GC
CMLAQ	GC	GC	GC	GC	GC
CMFNB	GC	GC	GC	GC	GC
CMFM	500 µg/ml	500 µg/ml	500 µg/ml	200 µg/ml	500 µg/ml
CMFA	500 µg/ml	500 µg/ml	500 µg/ml	200 µg/ml	500 µg/ml
CMFAQ	GC	GC	GC	500 µg/ml	500 µg/ml
CMRNB	GC	GC	GC	GC	GC
CMRM	GC	GC	GC	GC	GC
CMRA	GC	GC	GC	GC	GC
CMRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D.palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

Table 4
Estimation of MIC values of *Trichosanthes cucumerina* extracts (µg/ml, n=3)

Plant Extract	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
TCLNB	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLM	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLA	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
TCLAQ	GC	GC	GC	GC	GC
TCFNB	GC	GC	GC	GC	GC
TCFM	GC	GC	GC	GC	GC
TCFA	GC	GC	GC	GC	GC
TCFAQ	GC	GC	GC	GC	GC
TCRNB	GC	GC	GC	GC	GC
TCRM	GC	GC	GC	GC	GC
TCRA	GC	GC	GC	GC	GC
TCRAQ	GC	GC	GC	GC	GC
Standard	25 µg/ml	12.5 µg/ml	25 µg/ml	12.5 µg/ml	12.5 µg/ml
IC	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol, AQ: aqueous, A: acetone, NB: n-butanol, GC: Growth in all concentrations, IC: Inoculums control; *Streptococcus mutans*: MTCC 497, *Streptococcus pyogenes*: MTCC 1926, *Vibrio cholerae* : MTCC 3906, *Shigella flexneri*: MTCC 1457, *Salmonella typhi:* MTCC 1252, Concentration: for Standard- 6.25 – 50 µg/ml; for extracts- 100 – 500 µg/ml)

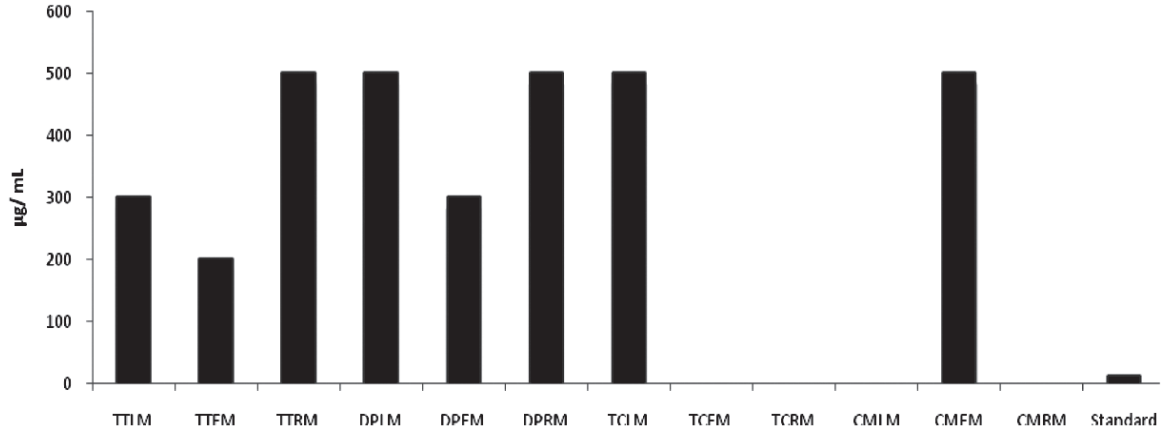

Fig. 2. Comparative antibacterial potential of methanol extract of selected plant parts against *S. pyogenes* (TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol)

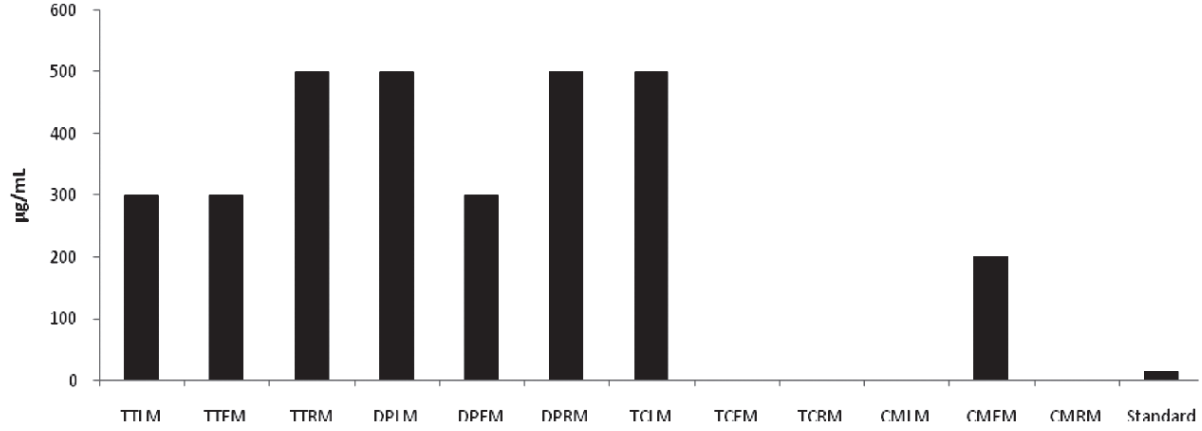

Fig. 3. Comparative antibacterial potential of methanol extract of selected plant parts against *S. mutans* (TT: *T. tricuspidata*, TC: *T. cucumerina*, DP: *D. palmatus*, CM: *C. melo*, L: Leaf. F: Fruits, R: Root, M: Methanol)

4. Conclusion

The present study highlights comprative antimicrobial potential of wild cucurbits available in Similipal Biosphere Reserve. The results obtained from the exprimental wild cucurbits like *T. tricuspidata*, *D. palmatus*, *T. cucumerina* and *C. melo* showed significant antibacterial activity against selected microbial pathogens. Among the said cucurbits, the methanol extract of *T. tricuspidata* fruits showed lowest MIC values against *S. pyogenes* (MTCC 1926) and methanol and acetone extract of *C. melo* root against *S. mutans* (MTCC 497). The bacteriostatic effects of the extracts were also indicated. The exprimented wild cucurbits posses significant antimicrobial activity against some selected bacterial strains. Further research can explore the bioactive compounds present in these plants responsible for such activities and successful utilisation of these compounds for formulation of new antimicrobial drugs which not only could check microbial infections but also might fight against AMR.

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Combination effects of dimethoate and atrazine on pigment fluorescence of *Anabaena doliolum* Bhar: Prediction of toxicity

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ABSTRACT

Effects of an organophosphorus insecticide dimethoate (O,O-dimethyl-S-(N-methyl carbamoylmethyl) phosphorodithioate) and herbicide triazine (2-chloro-4-ethylamino-6-isopropylamino -S-triazine) were measured separately and also in combination on growth and pigment fluorescence of the cyanobacterium *Anabaena doliolum* with 4 days exposure. Both the pesticides reduced the growth and chl-*a* content of the cultures but enhanced the fluorescence from phycobilisomes (PBS), PS II and PS I. PS II fluorescence at 580 nm excitation was increased more than that of PBS and PS I and atrazine more effectively accelerated PS II fluorescence than that of dimethoate. The EC_{50}s were 33.17±1.85 µM and 11.94±0.83 µM for dimethoate and atrazine, respectively, determined from a significant first order polynomial relation between PS II fluorescence and pesticide concentrations. The predicted EC_{50}s, calculated from concentration addition equation, ranged between 12.76 and 28.16 µM and the observed EC_{50} in combination of the pesticides ranged between 12.62±0.93 µM and 28.74±1.39 µM. It was found to have additive effects in the combination.

1. Introduction

Pesticides and herbicides have become the integral parts of modern agricultural systems. The registration of pesticides for agricultural use worldwide is based on the ecological risk assessment of chemicals applied alone, even though the protection of crops against pests is known to be achieved by the administration of many pesticides together or in succession. On the other hand, it is impossible to test every possible combination of pesticides at all possible concentration levels for prediction of their environmental toxicity. Information on the possible interactive effects of pesticide mixtures have been gained from several single-species work in the laboratory (Mohapatra and Mohanty, 1992; Mohapatra and Schiewer, 1996; 2000; Panda *et al.*, 1998; Backhaus *et al.*, 2004; Dong *et al.,* 2009; Jena *et al*., 2012).

Atrazine is a triazine herbicide widely used for the control of weeds and grasses in crops (Mehlera *et al.*, 2008; Dong *et al.*, 2009). It also causes damage to the gill epithelium and kidney, and increases the renal excretion of sodium, chloride and proteins in the rainbow trout (Fisher-Scherl *et al.*, 1991) and carp (Neskovic *et al.*, 1993). The herbicide is known to decrease the photosynthetic efficiency and accelerate pigment fluorescence of *Anabaena doliolum* and has many other interactive phytotoxicity (Van den Brink, *et al.,* 2009; Nayak and Mohapatra, 2011; Moore and Locke, 2012). Dimethoate is a conventional organophosphorus insecticide widely used to control a variety of pests on agricultural and animal farms. Researchers have demonstrated that organophosphorus insecticides significantly affect the health and safety of animals (Farag *et al.*, 2003; Tian *et al.*, 2005; Ali *et al.*, 2009) and plants (Mohapatra and Mohanty, 1992; Mohapatra *et al.*, 1997;

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2010; Pandey and Gopal, 2012). The residues of the pesticides have also been reported in surface and ground water (Miller *et al.*, 1999; Du Preez *et al.*, 2005; Banks *et al.*, 2005; Murphy *et al.*, 2006; Van den Brink *et al.,* 2009).

Effects of combined application of atrazine and other chemicals have shown additivity, synergism as well as antagonism (Anderson and Zhu, 2004; Fu *et al.,* 2013; Xing *et al.,* 2013). Atrazine and lindane combination increased abundance of the phytoplankton taxa *Cyclotella* sp. at the highest treatment level, which was due to synergistic effects on the microinvertebrate population. Dimethoate, malathion, parathion and chlorpyrifos has imposed synergistic effects on the toxicity of atrazine to many micro-invertebrates and animal species via the inhibition of acetylcholinesterase (AChE) (Cometa *et al.*, 2007; Choung *et al.,* 2011; Fu *et al.,* 2013). However, there is no report of the combination effect of atrazine and dimethoate to cyanobacteria. The present paper attempts to reveal if at all there is synergism of atrazine and dimethoate as reported in case of microinvertebrates and animals, in the cyanobacterium *Anabaena doliolum* Bhar.

2. Materials and methods

The filamentous and heterocystous cyanobacterium *Anabaena doliolum* Bhar. was maintained in non-absorbent cotton stoppered 500 ml borosilicate conical flasks containing 250 ml BG-11 medium (Rippka *et al.*, 1979) spiked with micronutrients. Actively growing cells were harvested and diluted with fresh medium 2 hours before inoculation to an initial inoculum density of $0.5x10^6$ cells/ ml. All stock and experiment cultures were grown in a culture room at 25 ± 2^0 C under continuous illumination with white light fluorescent tubes providing an irradiance of 40 $\mu E/m^2$ s and were hand shaken twice daily to keep the cultures under active phase of growth.

The commercial formulation of atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) and dimethoate (O, O-dimethyl-S- (N-methyl carbamoylmethyl) phosphorodithioate) were used for treatment in liquid medium. Stock solution (10 mM) of each pesticide was always freshly prepared in aqueous medium by using sterile BG11 medium and was used to achieve the desired treatment concentrations (0-100 µM for dimethoate and 0-50 µM for atrazine) for toxicity assessment of individual toxicant. The treatments were made in 50 ml cotton stoppered borosilicate culture tubes, each containing 20 ml of the culture and were incubated for 4 days. The combination effect was also measured after 4 days treatment by taking the pesticides in ratios determined by the concentration addition equation of Berenbaum (1985). The ratio of the components in the binary mixture as well as the predicted EC50 were determined by the equation

$$EC_{50z} = (EC_{50x} \times EC_{50y} \times 100)/(P_1EC_{50y} + P_2EC_{50x})$$

Where

x, y and z are dimethoate, atrazine and the combination, respectively. P_1 and P_2 are the percentage of dimethoate and atrazine in the mixture, respectively.

The EC_{50} values of dimethoate and atrazine were determined from the PS II fluorescence response of the cyanobacterium to graded concentrations of the pesticides

The absorbance of the homogeneous suspension was measured at 678 nm by a uv-vis spectrophotometer (systronics, India) against freshly prepared sterile BG11 medium as blank. The measurement of chlorophhyll *a* was made following the standard extraction protocol for cyanobacteria and the absorbance values of the extracts were converted to biomass following the equations of Hirschberg and Chamovitz (1994). Photosynthetic pigment fluorescence was measured with the help of a varian spectrofluorimeter (Varian, Australia) after standardizing the cultures into equal cell density (10^7 cells/ ml). Two millilitre of the homogenious suspension was taken in a spectrofluorimeter cuvette and was dark adapted for five minutes. Fluorescence emission from PS II and phycobilisomes (PBS) was measured at 685 nm and 660 nm, respectively on excitation of culture with a 580 nm monochromatic beam. Similarly chlorophyll *a* specific fluorescence emission from PS II and PS I was also measured at 685 nm and 725 nm, respectively on excitation of culture with a 440 nm monochromatic beam (Mohapatra *et al.*,1997). The excitation and emission band passes were 5 nm in each case.

Three replicates were taken for each treatment. The mean values of the replicates are presented in tables. The EC_{50} of individual pesticide was calculated from the PS II fluorescence response of the cyanobacterium. The combination treatments were made by taking the pesticides in different ratios and the predicted and observed EC_{50}s were compared by independent t test.

3. Results and discussion

Dimethoate, at all concentrations, caused reduction in the growth of the cyanobacterium, which was found significant at ≥ 10 µM. Consequently significant reduction in chl-*a* content was observed in this concentration range (Table 1). On the other hand, there was a concentration dependent increase in fluorescence yield from PS I, PS II and PBS, measured with excitation of phycobiliproteins and chl *a*. Comparison of PBS and PS II fluorescence at 580 nm

excitation showed that PS II fluorescence yield was enhanced more than of PBS. As a result there was a continuous reduction of the PBS/PS II fluorescence ratio which was, however, not significantly different from each other. At concentrations ≥ 50 µM, severe reduction in pigment content resulted in lower fluorescence yield. With 440 nm excitation there was enhancement of PS I and PS II fluorescence yield, which was found concentration dependent. PS II fluorescence was enhanced more than of PS I resulting in significant decrease of PS I/PS II fluorescence ratio.

In higher plants and cyanobacteria, dimethoate is known to increase fluorescence due to limited PQ function (Mohapatra *et al.,* 1997, 2010; Mohapatra and Schiewer, 2000). The insecticide enhances fluorescence emission from both the photosystems as well as from PBS primarily by membrane perturbations (Mohapatra *et al.*, 1996, 1997; 2010; Pandey and Gopal, 2012). Such fluorescence enhancement is attributed to the acceptor limitation of photosystems caused by reduced PQ cycle and delinked PBS-PS II electron flow. In *Solanum melongena* and *Chlorella vulgaris* it has been observed that dimethoate severely impaired photosynthesis more like a herbicide affecting PS II-PS I electron flow (Mohapatra *et al.*, 2010; Jena *et al.*, 2012).

Atrazine was found more effective than dimethoate to reduce the growth and chl *a* content of *A. doliolum* and at all selected concentrations the reduction was found significant (Table 1). The herbicide also caused enhancement of PS II and PBS fluorescence emission with 580 nm excitation, which was significantly higher than the enhancement effected by the corresponding concentration of dimethoate. PS II fluorescence was strongly induced compared to PBS resulting in continuous concentration dependent decrease of PBS/PS II fluorescence ratio. With chl-*a* excitation also there was significant induction of PS II and PS I fluorescence. As expected the PS II fluorescence enhancement was significantly higher than that of PS I resulting in decrease of the PS I/PS II fluorescence ratio with increase of atrazine concentration.

Atrazine is known to enhance PS II fluorescence yield like other herbicides (DCMU, simazine) by blocking the PS II-PS I electron flow at the level of Q_A (Roberts *et al*., 1990; Lazar, 2003; Nayak and Mohapatra, 2011). Interaction of the herbicide with D1 protein of PS II blocks Q_A-Q_B electron flow resulting higher single turn over events at the level of Q_A. The increase in PBS fluorescence in the present case with atrazine treatment is an indication of impaired PBS-PS II excitation transfer, presumably due to membrane perturbations as observed with other hydrophobic chemicals (Mohapatra *et al*., 1997; Lazar, 2003; Jena *et al*., 2012; Pandey and Gopal, 2012).

PS II fluorescence yield per mg chl-*a* was taken as the parameter for determination of the effective concentrations (EC) of pesticides. The parameter could be well correlated with pesticide concentrations through first order polynomial function (Fig. 1). In case of both the pesticides there was significant correlation between the fluorescence rise and the pesticide concentrations. The EC_{50} (at 95 % CI) of dimethoate and atrazine were found to be 33.17 ± 1.85 µM and 11.94 ± 0.83 µM, respectively.

Assessment of fluorescence of individual photosynthetic components with specific pigment excitation is the most efficient way to evaluate adverse impact of insecticides on photosynthetic activity. It represents the energy transfer from chl-*a* pigments to PS reaction centers (Lutz *et al*., 1998). In the present study fluorescence emissions from PS II was taken as the parameter to evaluate the combination effects of dimethoate and atrazine on *A. doliolum*. Nine different combinations, applying the concentration addition model, were taken and the predicted EC_{50}s were calculated. The predicted EC_{50} ranged from 12.76 – 28.16 µM and increased with increase in the concentration of dimethoate in the combination. From the PS II fluorescence yield the observed EC_{50}s were in the range from 12.62 ±0.93 µM to 28.74± 1.39 µM (Table 2). Like the predicted ones the observed EC_{50}s also increased with increase in the concentration of dimethoate in the combination. With higher dimethoate content in the mixture

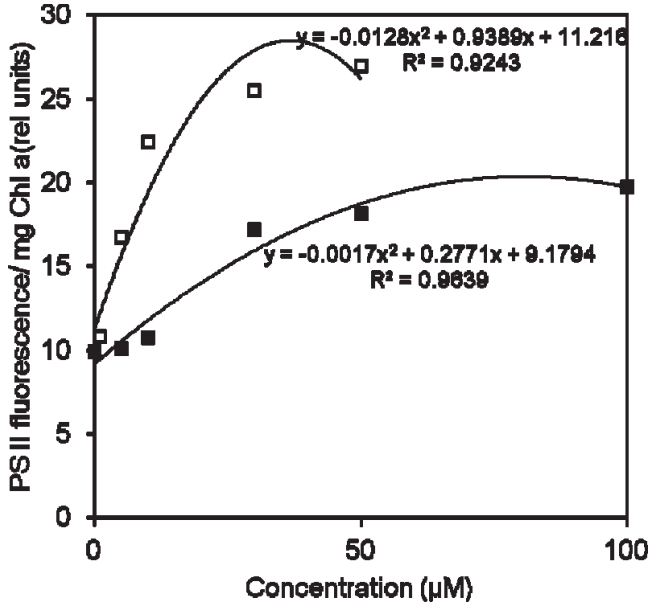

Fig. 1. Effect of dimethoate (■) and atrazine (□) on PS II fluorescence of *Anabaena doliolum*. The regression lines are the result of first order polynomial relation between the fluorescence yield and the concentrations.

Table 1
Culture absorbance (Abs; 678 nm), Chl-*a* content (µg/10^7 cells) and pigment fluorescence (rel. units) of *Anabaena doliolum* treated separately with dimethoate and atrazine

Conc (µM)	Abs	Chl *a*	PBS	PS II	PBS/PS II	PS II/mg Chl	PS I	PS II	PS I/PS II
Dimethoate									
0	0.316	2.384	47.39	23.72	1.998	9.950	1.726	3.168	0.545
5	0.311	2.375	47.64	23.98	1.987	10.097	1.765	3.394	0.520
10	0.293	2.296	48.97	24.65	1.987	10.736	1.932	4.216	0.458
30	0.159	1.897	50.42	32.58	1.548	17.174	2.024	4.799	0.422
50	0.083	1.284	50.26	23.33	2.154	18.170	1.687	4.018	0.420
100	0.009	0.095	3.402	1.879	1.811	19.779	0.085	0.212	0.401
Atrazine									
0	0.326	2.379	47.86	23.65	2.024	9.941	1.732	3.179	0.545
1	0.307	2.368	49.77	25.62	1.943	10.819	1.839	4.176	0.440
5	0.257	2.286	53.65	38.34	1.399	16.772	2.287	6.719	0.340
10	0.185	1.995	54.82	44.71	1.226	22.411	2.465	7.129	0.346
30	0.043	1.748	51.63	44.58	1.158	25.503	2.315	6.932	0.334
50	0.006	0.165	6.64	4.45	1.492	26.970	0.217	0.642	0.338

Table 2
Effect of combination of dimethoate and atrazine on PS II fluorescence of *Anabaena doliolum* measured after 4 days. The values of the pesticides given in parentheses are the % of the chemical in the combination. Observed EC50 have been calculated from the PS II fluorescence yield. The t values given in the last column are the results of independent comparison

Dimethoate (µM)(%)	Atrazine (µM)(%)	Predicted (P) EC50 (µM)	Obereved PS II /mg Chl a	Observed (O) EC50 (µM)	O/P ratio	t value
3.317(10)	10.746(90)	12.76	23.74	12.62± 0.93	0.989	1.62
6.634(20)	9.552(80)	13.69	22.88	13.29± 0.89	0.971	1.19
9.950(30)	8.358(70)	14.78	22.13	15.11±0.82	1.022	1.83
13.267(40)	7.164(60)	16.05	21.66	16.14±0.76	1.006	1.08
16.584(50)	5.970(50)	17.56	20.72	17.74±1.04	1.010	0.94
19.901(60)	4.776(40)	19.38	20.04	19.56±1.12	1.009	0.83
23.218(70)	3.582(30)	21.63	19.55	21.42±1.38	0.990	1.22
26.534(80)	2.388(20)	24.47	18.27	24.84±1.76	1.015	1.36
29.851(90)	1.194(10)	28.16	17.98	28.74±1.39	1.021	1.21

the observed EC_{50}s were insignificantly higher than that of the predicted ones whereas the reverse was noted when the concentration of atrazine was high in the combination. The t-values showed that in none of the combinations there was significant difference between the predicted and observed EC_{50}s, thus confirming the fact that the combination effect of dimethoate and atrazine in the cyanobacterium is purely additive.

Researches have shown that OP insecticides primarily cause neurotoxicity via the inhibition of acetylcholinesterase (Cometa *et al.,* 2007). Atrazine treatment significantly increased the toxicity of dimethoate, chlorpyrifos malathion and parathion when applied in combination or the target organisms were preexposed to atrazine before OP treatments (Choung *et al.,* 2011; Fu *et al.,* 2013; Xing *et al.,* 2013). Fu *et al.,* (2013) reported that atrazine treatment significantly enhanced toxicity of chlorpyrifos but inclusion of a recovery period after atrazine exposure eliminated the synergism. In *Chironomus tentans* atrazine alone up to 1000 µg/l did not show significant toxicity to the midges in a 48-h bioassay.

However, atrazine concentrations as low as 1 µg/l in combination with dimethoate, 10 µg/l in combination with, demeton-S-methyl and 100 µg/l in combination with disulfoton (all at EC_{25}) significantly enhanced the toxicity of each organophosphate insecticide (Anderson and Zhu, 2004; Choung *et al.,* 2011). Similarly addition of atrazine (10 µg/l) significantly increased the toxicity of terbufos to *Ceriodaphnia cf dubia* (Choung *et al.,* 2011). This indicated that synergism of atrazine with OP insecticides is common in invertebrates and fish though antagonism is observed with some pesticides (Cometa *et al.*, 2007; Choung *et al.,* 2011, Fu *et al.,* 2013). However, in the present observation in *Anabaena doliolum* such synergism was not seen when photosynthesis was taken as a parameter. It was instead additive effects which may be attributed to the same site and similar mechanism of action of both the pesticides.

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Comparative assessment of structure, composition and diversity of tree species of tropical moist deciduous forests in three forest ranges of Nayagarh Forest Division, Odisha, India

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ABSTRACT

Quantitative assessment of tree species diversity from representative sample plots of three forest ranges of Nayagarh Forest Division of Odisha, India was made. Sixty six (66) transects (1000 m X 5 m) were laid in Nayagarh (20 nos), Dasapalla (20 nos.) and Mahipur (26 nos.) forest ranges for enumeration of the tree species having ≥ 30 cm GBH. A total of 162 tree species belonging to 115 genera under 44 families were recorded. Maximum of 128 tree species from Nayagarh, 119 species from Mahipur and minimum of 89 species from Daspalla range were enumerated. *Shorea robusta, Buchanania lanzan, Lannea coromandelica, Terminalia alata* and *Cleistanthus collinus* were the predominant tree species of the study area. The families Rubiaceae, Euphorbiaceae, Fabaceae, Moraceae and Mimosaceae were found to contribute to maximum species richness, stand density and basal area. The stem density varied in the range of 466.54 stem ha^{-1} in Dasapalla range to 530.30 stem ha^{-1} in Nayagarh range. The stand basal area was maximum (31.62 m2 ha^{-1}) in Dasapalla range followed by Mahipur (17.13 m2 ha^{-1}) and Nayagarh (13.16 m2 ha^{-1}). However, highest value of Shannon-Weiner Index (3.61) was recorded for Nayagarh range and lowest (3.51) for Manipur forest range. The tree density and species richness decreased with increasing girth class. Highest number of species and maximum density was recorded for 30-60 cm girth class in all the three forest ranges.

1. Introduction

Though tropical forests occupy only 7% of the earth's land surface (Laurance, 1999; Dirzo and Raven, 2003), they harbour 60% of all known terrestrial species and provide significant local, regional and global human benefits by providing a range economic goods and ecosystem services (Gardner *et al*., 2009). Over the past century tropical forests have been suffering from exceptional rates of change as they are degraded or destroyed by human activities. The combined influence of persistently high rates of deforestation and forest degradation (FAO, 2006), over-harvesting, invasive species and global environmental change threatens to make tropical forests the epicentre of current and future extinctions (Bradshaw *et al*., 2009).

Trees form the major structural and functional basis of tropical forest ecosystems can serve as robust indicators of changes and stresses at the landscape level (Sahu *et al*., 2012). Plant diversity inventories in tropical forests have mostly been concentrated on tree species rather than other life forms, because they constitute an important aspect of forest ecosystems and fundamental to total tropical forest biodiversity (Rennolls and Laumonier, 2000). They provide resources and habitat structure for almost all other species and form the major biotic component in the forest ecosystem (Cannon *et al*., 1998).

In India, most of the quantitative plant biodiversity inventories of tropical forests made so far are from the

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forests of the Western Ghats (Sukumar *et al*., 1992; Ganesh *et al*., 1996; Pascal and Pelissier, 1996; Ghate *et al*., 1998; Parthasarathy, 1999; Parthasarathy and Karthikeyan, 1997; Ayyappan and Parthasarathy, 1999; Chittibabu & Parthasarathy, 2000; Jayakumar and Nair, 2013; Reddy *et al*., 2008a) and that of the Coromandal coast (Parthasarathy and Karthikeyan, 1997; Parthasarathy and Sethi, 1997). However, Eastern Ghat region, largely covering the states of Odisha, Andhra Pradesh and Tamilnadu is poorly studied except those of Kadavul and Parthasarathy (1999a), Kadavul and Parthasarathy (1999b) and Arul Pragasan & Parthasarathy (2010) in Tamilnadu; Naidu & Aniel Kumar (2015) and Reddy *et al*. (2008b) in Andhra Pradesh; Sahu *et al.,* (2007) and Panda *et al.,* (2013) in Odisha.

In order to fulfill the above gap and to generate baseline data on forest trees occurring in tropical moist deciduous forests of Odisha, a comparative assessment of tree species diversity from representative sample plots of three forest ranges of Nayagarh Forest Division of Odisha was made.

2. Materials and methods

2.1. Study sites

Nayagarh Forest Division of Odisha, India is a part of Eastern Ghats region and occupies an area of 3067.28 sq. kms (Fig.1.). The forests comprising of Reserved Forests, Proposed Reserved Forests, Degraded Protected Forests and Village Forests (RFs, PRFs, DPFs and VFs) cover an area of 1063.16 sq. kms, which is 34.66 % of the geographical area of the division. The forests of the district is predominated by mixed Sal Forest, Dry Peninsular Sal forest, Miscellaneous Forest, Northern tropical dry deciduous forest and South Indian Moist Mixed Deciduous forest. In the lower hills and plains the forest is dominated by Sal but as the altitude increases in upper hill slopes the miscellaneous species is found abundantly. The altitude varies in the range of 47m to 932 m above MSL.

The forests are well drained by a large number of rivers, rivulets, streams and nallahs. The most important river of this district is Mahanadi and major portion of drainage water from the forests flows into this river. A small portion of forests of the Southern part, South-West part, Western portion and South-West portion drain in to the Rushikulya River of Ganjam District.

Three prominent seasons are observed in this area (hot and dry summer, hot and humid rainy season and moderate winter season). The highest temperature in summer is around 39^0 to 44^0 C but at some places it is around 35^0C. The monsoon temperature is around 30^0 C with relative humidity varying in the range of 70% to 90%. The winter temperature

Fig. 1. Map showing location of study area

from December to February goes down to nearly 8^0 to 10^0 C. The mean annual rainfall is 1500 mm approximately out of which 80% is received during June-September. Most of the rainy days are distributed in monsoon season i.e. from 3rd week of June to 2nd week of September.

The rocks occupying the greater part of the study area include Khondalite, acid charnockite, garnetiferous granite gneiss, granulites, quartz and pegmatite veins, sandstones. Sub-recent and recent deposits of laterite, alluvium and soil overlie these successions. The soils occurring in the division are dominantly very deep, well drained, fine loamy in texture and are acidic in reaction. They are slightly eroded and have medium available water capacity. The soil types of such characteristics have been classified as Typic Haplustalfs with deep well drained and moderately eroded soil.

For the present study, a total of 66 transects (1000 m X 5 m) were laid in Nayagarh (20 nos), Dasapalla (20 nos.) and Mahipur (26 nos.) forest ranges of Nayagarh Forest Division for quantitative assessment of tree diversity. The geographical location of the sampling sites in these forest ranges are given in Table 1.

2.2. Field methods

The moist deciduous forest patches in three forest ranges of Nayagarh Forest Division viz. Nayagarh, Dasapalla and Mahipur were identified based on information available in recent Working Plan for Nayagarh Forest Division, interaction with forest officials, ground truthing and analysis of dominant species and their common associates as reported by Champion & Seth (1968) and ICFRE (2013). A total of 66 transects each measuring 0.5 ha (1000 m X 5 m) were laid in moist deciduous forest patches of Nayagarh (20 nos), Dasapalla (20 nos.) and Mahipur (26 nos.) forest ranges and all standing trees of ≥ 30 cm GBH (girth at breast

Table-1:
Geographical location of the sampling sites in Nayagarh Forest Division

Forest Range	Latitude	Longitude	Altitude
Dasapalla	20° 11.460' N-20° 25.076' N	84° 33.676' E- 85° 39.859' E	44-248 m
Mahipur	20° 07.158' N -20° 16.142' N	84° 49.450' E-85° 13.884'E	76-168 m
Nayagarh	19° 58.328'N-20° 14.431' N	84° 28.175'E-85° 14.355'E	58-279 m

height, *i.e.* 1.37 m above ground level) were enumerated. Data on number of individuals of each species, their GBH and height were measured. Associated shrubs, climbers and herbs were listed and field information on regeneration potential of trees was also noted. Plants were identified using regional floras (Haines, 1921-25, Mooney, 1950, Gamble and Fischer, 1915-1935 and Saxena and Brahmam, 1994-96) and by matching them with authentic herbarium specimens housed in different Indian Herbaria. The voucher specimens were housed in the Herbarium of Regional Plant Resource Centre (RPRC), Bhubaneswar, India.

2.3. Data analysis

The phytosociological characteristics of individual species and their communities like (a) Frequency (percent of all transects in which a species was present), (b) density (ratio of total number of trees to total number of transects) and (c) abundance (ratio of total number of trees to total number of transects of occurrence) were recorded. The relative importance of any species in the community was assessed by determining the Importance Value Index (IVI) following Curtis and Mcintosh (1950); where the relative values of frequency, density and basal cover for a species was derived as the value expressed in terms of percentage of the sum of the values for all the species in the plant community (Mueller-Dombois and Ellenberg, 1974). Family Importance Value (FIV) was taken as the sum of relative density, relative diversity, and relative basal cover of all species belonging to a botanical family. The relative diversity of a family was evaluated as the number of species within the family expressed as percentage of total number of species within all the families represented in the community (Mori *et al.,* 1983). The dominance was determined by Simpson's index ($Cd = \Sigma(n_i/N)^2$, and diversity as Shannon's Index ($H = -\Sigma(n_i/N) \log (n_i/N)$, where $n_{i\,=}$ importance value index of species i, N= sum of importance value index for the community. Evenness was calculated by Pielou's index ($D = -\Sigma p_i^2 / \text{In } S$), where *S* is the species richness of the community (Magurran, 1988). Species similarity among different regions was computed using Jaccard's Coefficient of Similarity (Jaccard, 1908). The density and basal area in respect of species were calculated on the basis of data recorded from all the transects (0.5 ha each) of the particular Forest Range and the values were expressed per hectare basis for comparison. The girth (GBH) was converted into basal area (BA) as $BA = GBH^2/4\pi$.

3. Results

3.1. Floristic composition and species richness

A total number of 16063 individuals of tree species with ≥ 30 cm GBH were recorded from 33 ha of area sampled in Daspalla, Mahipur and Nayagarh forest ranges. They represent 162 species belonging to 115 genera under 44 families. The family Rubiaceae with 13 species was the most dominant taxon in terms of species content followed by Euphorbiaceae (11 species), Fabaceae and Moraceae (9 species each) and Mimosaceae (8 species) and 12 families were represented by single species only (Tab. 5). Of these, 89 species (4695 individuals) occur in Daspalla range, 119 species in Mahipur range (6065 individuals) and 128 species (5,303 individuals) were reported from Nayagarh range. Of the three, Nayagarh range was observed to be more species-rich. In terms of similarity in species occurrence, all the three ranges had an average similarity of 0.55 (55%), which implies that 55% of species are common to all the three studied ranges. Mahipur and Nayagarh forest ranges shared maximum similarity of 0.63 in terms of species presence and least (0.55) between Dasapalla and Nayagarh forest ranges. In general, though there is no correlation between species richness and stand density, highest number of species (128 species) and highest stand density (530.30 stems/ha) were observed in Nayagarh range. However, in 10 ha of sampled area in Daspalla forest range, lowest number (89 species) of tree species were recorded but the stand density (469.50 stems/ha) was higher than that of Mahipur range, where as many as 119 species were found to occur.

The values of diversity indices like Shannon-Weiner Index and Simpson Index varied greatly among the three forest ranges studied. Shannon's Index varied from 3.51-3.61, the highest value being for Nayagarh range and lowest for Mahipur range. Simpson Index ranged between 0.06 and 0.11 across the three study sites. The species accumulation curves for the three study sites were initially steep as the area of sample plots increased up to 4 ha but then the rise with increasing number of sampling plots was

much slower. However, the species area curve didn't reach an asymptote (Fig. 2).

3.2. Importance value index (IVI)

The IVI depicts the sociological structure of a species in its totality in the community. Tropical deciduous forests of Odisha state are dominated by the single species, *Shorea robusta* (Sal). This species scored highest IVI of 63.85 in Daspalla range, 93.84 in Mahipur range and 91.60 in Nayagarh range showing the dominance of the species in terms of density, basal area and frequency of occurrence. The top 10 species and their contribution to density, basal area and IVI in the three forest ranges of Nayagarh Forest Division are shown in (Table 3). (The variation in stand density, basal area and frequency of five dominant species namely, *Shorea robusta, Lannea coromandelica, Madhuca indica, Buchanania lanzan* and *Terminalia alata* occurring in all the three ranges are presented in (Table 3) for the purpose of comparison.)

3.3. Family composition

The total of 16,063 numbers of trees belonging to 162 species under 115 genera and 44 families were enumerated from representative sample plots located in Daspalla, Mahipur and Nayagarh forest ranges. In terms of tree density, Dipterocarpaceae with the lone species *Shorea robusta* and with 5,627 individuals dominated the tropical moist deciduous forests of Nayaharh district, followed by Anacardiaceae (1,687 stems) and Combretaceae (1,329 stems). Dipterocarpaceae, alone contributed to 35.03% of the tree population having Family Importance Value (FIV) of 80.16. The top 10 families comprised of 13,106 individuals contributed to 70.03% of total FIV. Twelve families were represented by single species with 5989 individuals and they accounted for 30.28% of total FIV, which includes the predominant species *Shorea robusta* (Dipterocarpaceae). Dipterocarpaceae scored the maximum FIV of 80.16, followed by Anacardiaceae (FIV=21.83), Combretaceae (FIV=20.21) and Euphorbiaceae (FIV=19.53). The FIV of Dipterocarpaceae although represented by single species. (FIV=80.16) is greater than the FIV of all speciose families such as Rubiaceae, Euphorbiaceae and Fabaceae (Table 5).

3.4 Stand Density, Basal Area and Girth Class distribution

A total of 4695, 6065 and 5303 trees were enumerated from the sample sites of Dasapalla (10 ha), Mahipur (13 ha) and Nayagarh (10 ha) forest ranges respectively. The stand density varied in the range of 530.30 stems ha^{-1} in Nayagarh forest range to 466.54 stems ha^{-1} in Mahipur forest range. In Daspalla range, maximum stand density of 219.700 stems/ha was observed under the lowest girth class of 30-60 cm but maximum stand basal area (10.529 m^2/ha) was recorded under the highest girth class of >150 cm. In case of Mahipur range, stand density and stand basal area went on decreasing with increasing girth classes. However, stand density showed a decreasing trend with increasing girth class in Nayagarh range but stand basal area went on decreasing till it attained a girth class of 120 cm and again increased with higher girth class of 121-150 cm and >150 cm. The total basal area showed variation in the range of 316 m^2 in Dasapalla range to 131.59 m^2 in Nayagarh range. Similarly, the stand basal area was recorded the highest (31.62 m^2/ha) for Dasapalla range followed by Mahipur (17.13 m^2/ha) and the lowest in Nayagah range (13.16 m^2/ha) (Table 4).

The terms of height class distribution of trees in all the three forest ranges, maximum percentage of standing trees were in the height range of 6.0 m-20.0 m. Individuals with less than 5 m height or more than 25 m height were quite few in number (Table 6).

Table 2
Key diversity attributes of forests in Nayagarh Forest Division, Odisha.

Description	Daspalla (10ha)	Mahipur (13ha)	Nayagarh (10ha)	Total for all sites (33ha)
Number of tree species	89	119	128	163
Number of individuals	4695	6065	5303	16063
Stand Density (Stems ha^{-1})	469.5	466.54	530.3	486.76
Total Basal area (m^2)	316.23	222.66	131.59	670.47
Stand Basal Area (m^2 ha-1)	31.62	17.13	13.16	20.32
Shannon-Weiner Index	3.54	3.51	3.61	3.68
Simpson Index	0.06	0.11	0.1	0.08
Evenness Index	0.79	0.73	0.74	0.72

Table 3
Density, basal area and IVI of the ten dominant species in three forest ranges of Nayagarh Division, Odisha

Daspalla Range				Mahipur Range				Nayagarh Range			
Species	Stand Basal area (m^2)	Stand Density	IVI	Species	Stand Basal area (m^2)	Stand Density	IVI	Species	Stand Basal area (m^2)	Stand Density	IVI
Shorea robusta	11.83	104	63.85	*Shorea robusta*	9.36	172.62	93.84	*Shorea robusta*	5.85	234.30	91.60
Cleistanthus collinus	1.07	38.2	15.36	*Terminalia alata*	0.78	26.46	12.83	*Terminalia alata*	0.49	25.80	11.38
Lannea coromandelica	1.74	24.7	14.59	*Buchanania lanzan*	0.39	21.77	9.11	*Buchanania lanzan*	0.41	26.20	10.73
Schleichera oleosa	1.59	16.5	12.37	*Diospyros melanoxylon*	0.41	15.38	8.12	*Diospyros melanoxylon*	0.55	9.70	8.64
Madhuca indica	1.45	20.7	12.15	*Cleistanthus collinus*	0.28	11.62	6.60	*Madhuca indica*	0.30	12.00	7.35
Protium serratum	1.40	20.8	12.02	*Dalbergia paniculata*	0.35	8.08	6.34	*Lannea coromandelica*	0.26	12.40	6.94
Buchanania lanzan	0.68	25.7	11.00	*Madhuca indica*	0.26	9.15	6.19	*Pterocarpus marsupium*	0.35	13.30	6.38
Desmodium oojeinensis	1.10	17.5	10.36	*Pterospermum xylocarpum*	0.33	10.38	6.09	*Semecarpus anacardium*	0.22	8.90	5.66
Terminalia alata	1.18	15.2	10.34	*Syzigium cumini*	0.32	9.62	6.00	*Terminalia chebula*	0.19	9.60	5.28
Anogeissus latifolia	1.05	11.3	8.21	*Lannea coromandelica*	0.23	8.46	5.98	*Careya arborea*	0.13	6.30	4.47
Total of above 10 species	23.07	294.6	170.24	Total of above 10 species	12.71	293.54	161.08	Total of above 10 species	8.74	358.50	158.43
Remaining 79 species	8.55	174.90	129.76	Remaining 109 species	4.42	173.00	138.92	Remaining 118 species	4.42	171.80	141.57
All species (89 species)	31.62	469.5	300.00	All species (119 species)	17.13	466.54	300.00	All species (128 species)	13.16	530.30	300.00

Table 4
Distribution of girth classes and their contribution to basal area and stand density in three forest ranges of Nayagarh Forest Division, Odisha.

	Girth Class									
Sites	30-60 cm		61-90 cm		91-120 cm		121-150 cm		>150	
	Stand Density (stems/ ha)	Stand Basal Area (m^2 /ha)	Stand Density (stems/ ha)	Stand Basal Area (m^2 /ha)	Stand Density (stems/ ha)	Stand Basal Area (m^2 /ha)	Stand Density (stems/ ha)	Stand Basal Area (m^2 /ha)	Stand Density (stems/ ha)	Stand Basal Area (m^2 /ha)
Dasapalla	219.700	3.331	92.5	4.220	6.490	6.247	5.460	7.816	37.800	10.529
Mahipur	300.000	0.437	97.000	0.421	47.077	0.355	20.077	0.284	8.462	2.154
Nayagargh	412.300	0.627	100.300	0.407	8.100	0.069	5.500	0.083	4.000	1.301

Table 5
Species content, number of individuals, contribution to basal area and FIV of the plant families in Nayagarh Forest Division, Odisha.

Sl No.	Family	No of Species	No of individuals	Basal area (m^2)	FIV
1	Rubiaceae	13	672	24.23	15.77
2	Euphorbiaceae	11	1303	31.30	19.53
3	Fabaceae	9	849	39.38	16.68
4	Moraceae	9	134	8.92	7.69
5	Mimosaceae	8	198	8.10	7.35
6	Combretaceae	7	1329	51.26	20.21
7	Meliaceae	7	168	3.86	5.92
8	Verbenaceae	7	62	1.54	4.91
9	Anacardiaceae	6	1687	51.27	21.83
10	Caesalpiniaceae	6	160	4.23	5.31
11	Ebenaceae	6	826	30.90	13.43
12	Rutaceae	6	150	3.02	5.06
13	Annonaceae	5	24	0.42	3.28
14	Bignoniaceae	5	126	4.62	4.54
15	Flacourtiaceae	5	159	3.38	4.56
16	Sterculiaceae	4	257	8.34	5.91
17	Apocynaceae	3	109	1.91	2.80
18	Burseraceae	3	317	17.94	6.49
19	Lythraceae	3	126	2.55	3.00
20	Oleaceae	3	47	0.71	2.24
21	Boraginaceae	2	8	0.18	1.30
22	Celastraceae	2	29	0.97	1.55
23	Dilleniaceae	2	93	5.79	2.67
24	Lauraceae	2	37	1.21	1.64
25	Loganiaceae	2	107	4.00	2.49
26	Malvaceae	2	34	1.04	1.59
27	Myrsinaceae	2	9	0.20	1.31
28	Myrtaceae	2	284	8.09	4.20
29	Sapindaceae	2	240	18.84	5.53
30	Sapotaceae	2	481	21.61	7.44
31	Tiliaceae	2	39	0.61	1.56
32	Ulmaceae	2	10	0.26	1.33
33	Acanthaceae	1	1	0.07	0.63
34	Alangiaceae	1	46	0.71	1.01
35	Arecaceae	1	18	0.60	0.81
36	Bombacaceae	1	49	2.73	1.32
37	Cochlospermaceae	1	1	0.01	0.62
38	Dipterocarpaceae	1	5627	298.49	80.16
39	Lecythidaceae	1	207	6.46	2.87
40	Leeaceae	1	4	0.08	0.65
41	Melastomataceae	1	2	0.04	0.63
42	Ochnaceae	1	29	0.55	0.88
43	Opilliceae	1	3	0.05	0.64
44	Rhamnaceae	1	2	0.03	0.63

Table 6
Height class-wise proportion of tree individuals in Nayagarh Forest Division, Odisha.

Height	Study sites			Individuals	% of Individuals
	Daspalla	Mahipur	Nayagarh		
≤ 5m	51	357	569	977	6.08
6-10m	640	1183	1353	3176	19.77
11-15m	1315	2118	1184	4617	28.74
16-20m	1119	1147	1320	3586	22.32
21-25m	675	735	788	2198	13.68
>25m	895	525	89	1509	9.39
Total	4695	6065	5303	16063	100

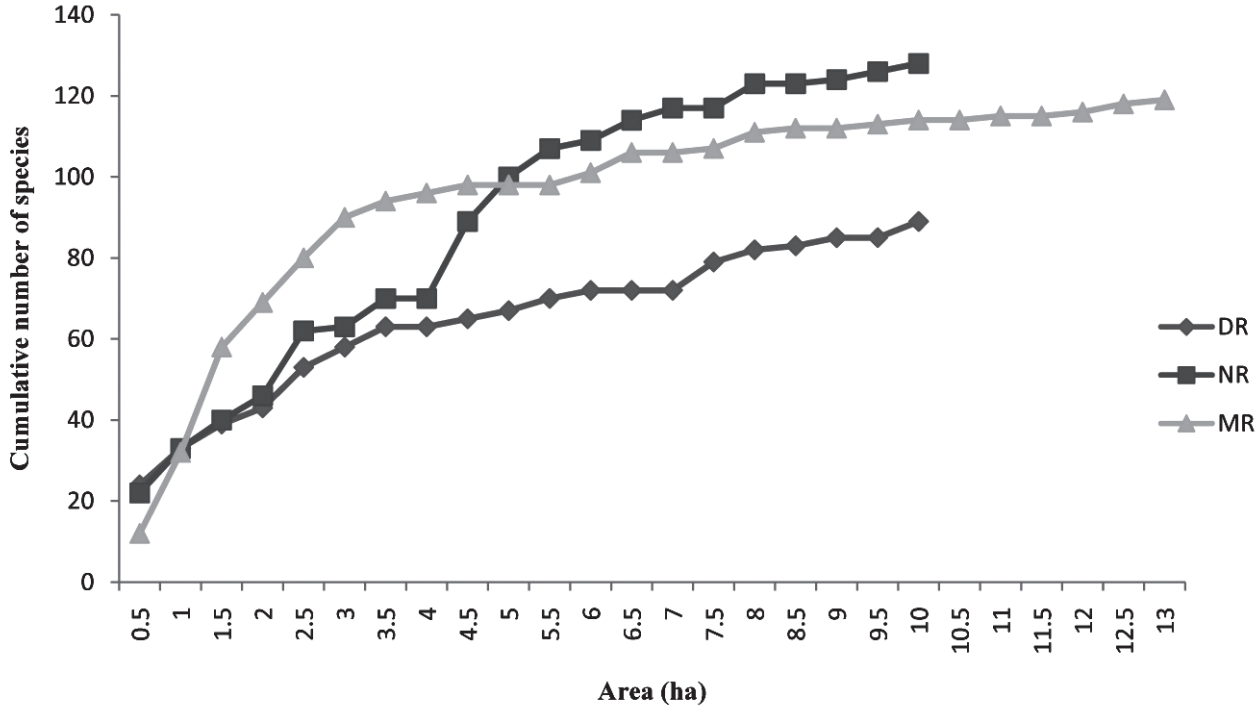

Fig. 2. Species accumulation curve for three different study sites in Nayagarh Forest Division, Odisha

4. Discussion

The predominant forest types of the Eastern Ghats of Odisha are tropical dry deciduous, tropical moist deciduous and semi-evergreen types (Champion and Seth, 1968) and are highly diversified and species rich. The density, abundance and distribution of individual species are measurable indicators of plant diversity (Wattenberg and Breckle, 1995). The species richness of 163 species over 33 ha sampled area in three forest ranges of Nayagarh Division reflects a moderate level of diversity in forests of northern Eastern Ghats. The result of the study compared well with other large-scale inventories conducted in tropical forests both in India and elsewhere. For example, 63 species were recorded for 50 ha plot at Mudumalai Forest Reserve, India, to 996 species in 52 ha at Lambir, Malaysia (Condit *et al.,* 2000). In a recent assessment of species richness in southern Eastern Ghats, Arul Pragasan and Parthasarathy (2010) recorded 272 species in the 60 ha area sampled using similar sampling and data collection procedures.

The stand density of 469.50 stems/ ha now reported for Daspalla range, 466.54 for Mahipur range and 530.30 stems/ha remains within the range reported for other tropical forests of Indian subcontinent; 352 stems ha^{-1} in northern Eastern Ghats (Panda *et al*., 2013); 443 stems ha-1 in Malyagiri hills of Odisha (Sahu *et al*., 2012); 298 stems ha”1 at Mudumalai Forest Reserve, India and 689 stems

ha"1 at Sinharaja, Sri Lanka (Condit *et al*., 2000). Chittibabu and Parthasarathy (2000) reported tree density in the range of 266 trees ha"1 to 632 trees ha"1 from tropical evergreen forests of Koli Hills of Western Ghats of India and between 270 to 673 trees ha-1 in the Anamalais (Ayyappan and Parthasarathy, 1999). Density of trees (30 cm GBH and above) in tropical forests ranges between 245 and 859 (Ashton 1964; Campbell *et al*., 1992; Richards, 1996) with intermediate values of 448 to 617 stems ha-1 in Costa Rica (Heaney and Proctor, 1990) and 639 to 713 stems ha-1 in Central Amazonia (Ferreira and Prance, 1998). The mean stand density of trees now reported for Nayagarh Forest Division (486.76 stems ha-1) is well within the reported range for tropical forests of India.

The species diversity depends on the adaptation of species which increases with the stability of community and Shannon's Index (H') is generally higher for tropical forests (Knight, 1975). In Indian forests, the value is reported to vary in the range of 0.83 to 4.0 (Singh *et al*., 1984). In the present study, Shannon's Index of diversity of tree species in all three sites varied between 3.54 to 3.61 which are within the reported range for the forests of Indian sub-continent (Gandhi and Sundarapandian, 2014; Ayyapan and Parthasarathy, 1999; Pandey, 2003; Panda *et al*., 2013). Comparison of diversity indices is very difficult because of the difference in the area sampled and lack of uniform plot dimensions. However, the index now determined is lower than the value reported for Northern Andhra Pradesh (Reddy *et al*., 2011), Niyamgiri hills, Odisha (Dash *et al*., 2009). The higher dominance index could be attributed to single-species dominance in the forest ecosystem.

In most of the studies relating to vegetation composition and site quality of forests, basal area acts as an important attribute (Mani and Parthasarathy, 2005; Parthasarathy and Karthikeyan, 1997; Srinivas and Parthasarathy, 2000). The basal area recorded in the present study ranged from 13.16 m^2 ha^{-1} in Nayagarh range to 31.62 m^2ha^{-1} in Daspalla range. These values are within the reported range for tropical deciduous forests in other parts of Eastern Ghats (Jha and Singh, 1990, Reddy and Prachi, 2008, Sahu *et al*., 2012 and Panda *et al*., 2013). However, the values of basal area determined in the present study are lower as compared to some other tropical forests of India (Kadavul and Parthasarathy 1999b; Reddy *et al*., 2008; Reddy *et al*., 2011; Parthasarathy and Karthikeyan, 1997; Parthasarathy *et al*., 1992; Prakasha *et al*., 2008).

The diameter distribution reflects the disturbance effect within the forests (Denslow, 1995; Hett and Loucks, 1976) and helpful in detecting trends in regeneration patterns (Poorter *et al*., 1996). The low basal area values in almost all the three ranges of the present study revealed the extent of forest disturbance with poor representation of trees in higher girth class. However, higher basal area under 121-150 cm and >150 cm girth class in Daspalla range is indicative of better forest regeneration following reduction in human interference. Tree density decreased with increasing size class of trees indicates how well the growing forest is utilizing site resources. A few small-to-medium sized trees per hectare may imply that land is not being fully utilized by the tree crop (Hitimana et al., 2004). Distribution curves that drop exponentially with increasing GBH are characteristic for many sites in India (Khamyong *et al.,* 2004) and the present findings are in conformity with the above observation.

Quantitative floristic data from the present study will provide base-line information on distribution, richness and relative abundance of taxa for formulating management and conservation actions.

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Improvement of seedling and clone establishment capability of *Pongamia pinnata* (L.) Pierre grown in fly ash using mixed microbial inoculums

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ABSTRACT

Among various energy plants, *Pongamia pinnata* is one of the most important species because of its multipurpose uses, high oil content and high medicinal values and need to be cultivated in large-scale for commercial use. In order to facilitate survival and growth of suitable species in marginal and wastelands and for meaningful utilization of fly ash, fly ash with different concentrations and combinations of growing media were used as substrates supplemented with microbial inoculums for better establishment of vegetatively propagated plants of *Pongamia pinnata*. Maximum growth, biomass production and better nodulation in the plant was achieved using fly ash and coir pith in the proportion of 4:1 and garden soil and coir pith in the proportion of 3:2 as compared to dilution with sand and pure fly ash.

1. Introduction

Increased demand and the progressive worldwide shortage of fossil fuels warrants for search of alternate source of energy. Biofuels in the form of bioethanol from microbial fermentation and biodiesel from vegetable oil or any non-edible oil provide good hope as alternate source of energy. Now a days many plants with high oil content are being tried as source of biodiesel. In this context it has been observed that many plants like *Pongamia pinnata (Millettia pinnata)*, *Jatropha* spp., *Azadirachta indica*, *Simarouba glauca* etc. yield oil that can be used as biodiesel and are known as biodiesel plants. Among various energy plants, *Pongamia pinnata* is one of the valuable and important plants because of its multipurpose commercial uses (Naik *et al.*, 2008) including its high medicinal value. *Pongamia pinnata* seeds contain about 40j oil, which can be converted to biodiesel by trans-esterification method (Meher *et al*., 2006). However, the unavailability of good arable land stands a great hurdle in this direction and only hope is to develop technology for growing *P. pinnata* in marginal and waste lands with stress soil conditions.

The state of Odisha is now facing the problem of rapid wasteland formation due to contamination by flyash generated from thermal power plants using excessive coal for power generation. Every year thermal power plants in India produce more than 100 million tonnes of fly ash, which is expected to reach 175 million tonnes in the near future (Jamwal Nidhi, 2003). Flyash is a highly insoluble particulate substance generating heavy toxic effects in environment. Disposal of this huge quantity of fly ash is posing a great problem due to its limited use in the manufacturing of bricks, cements, ceiling and other civil construction materials. This would further bring changes in land-use patterns and contribute to land, water and atmospheric degradation, if proper management plans for handling ash are not undertaken (Kalra *et al*., 1996). The countries like Germany, Denmark, France, U.K., USA, and the Netherlands utilize fly ash (up to 70 %) for construction purpose, but in India it's utilization is less than 15 % (Sinha and Basu, 1998). Use of fly ash in agriculture provides a feasible alternative for its safe disposal to improve the soil environment and enhance the crop productivity. However, a

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judicious management strategy has to be developed to abate the land pollution from the heavy metals present in the fly ash. Flyash being a fossil byproduct contains many micro and macro nutrients for plants but being insoluble, are not readily available to plants. There is a great scope of utilization of flyash as plant growth substrate through microbial amelioration (Misra *et. al*., 2000).

The present research work aims at finding out a suitable dilution factor for utilization of flyash as plant growth substrate through microbial amelioration using consortia of microbes of suitable N_2 fixer, phosphate solubilizer and AM fungi using decomposed coir pith as Mixed Microbial Inoculums for Reclamation (MMIR) to minimize the toxicity and promote proper utilization of flyash for successful growth and establishment of seedlings and clonally propagated *Pongamia pinnata* plantlets. Plantlets obtained from rooting of stem cuttings in different media containing fly ash were studied for their survival, growth, biomass yield and other parameters. Methods of vegetative propagation of *P. pinnata* has been standardized by many authors (Palanisamy and Kumar 1997; Thatoi *et al.*, 2002, Thirunavoukkarasu *et al.*, 2002, but there has been no study on mass propagation and subsequent establishment of *P. pinnata* using fly ash as growing media. In view of this, the present study was undertaken to make a comparative assessment and growth of *P. pinnata* in pure fly ash and fly ash amended with sand, garden soil and decomposed coir pith in different concentrations and combinations.

2. Materials and methods

2.1. Collection of samples

Fly ash samples were collected from ash pond of NALCO, Angul and brought to the laboratory. Coir pith was collected from coir industries located at Satasankha, Puri district, Odisha. The psysico-chemical properties of fly ash were analyzed following standard method of Jackson (1973). Further the study of microbial population of flyash was studied using standard plate technique of (Alexander, 1962).

Three ingredients such as garden soil, sand and decomposed coir pith were mixed with fly ash samples in different proportions to prepare the growing media.

2.2. Microbial decomposition of coir pith

Coir pith is a highly insoluble lignocellulosic material produced in huge quantities by the coir industries and is a rich source of organic carbon. This is not easily biodegradable and dumped along roads and wastelands causing pollution. Attempt was made to facilitate early decomposition of coir pith to provide nutrients for plant growth by mixing it with a lignocellulolytic fungi *Pleurotus* sp., a phosphate solubilizer (*Bacillus* sp.), rock phosphate and sodium chloride in a cement-concrete tank and then by providing regulated watering. The process of decomposition was carried out under anaerobic condition by covering the tank with polythene sheets for a period of one month.

2.3. Pot culture experiment

Flyash was diluted with different ingredients like garden soil, sand and decomposed coir pith in the proportions of 4:1 (fly ash: sand/ soil/ coir pith:: 4:1) and 3:2 (fly ash: sand/ soil/ coir pith:: 3:2). Thirty polypots were prepared for each category of growing medium and other thirty containing pure fly ash**.** The experiment was conducted during the month of January-April, 2009. Stem cuttings with three nodes were taken from healthy tree of *P. pinnata* during the early morning hour when the leaves are turgid. The leaves from the basal node were annexed by sharp sterile knife and basal straight cut was made just below the node. The upper end of the cutting was sealed with paraffin wax to prevent infection and to minimize the loss of water from the cut ends. The lower ends of stem cuttings were dipped in .01% $HgCl_2$ solution for 2 minutes and washed several times with sterile distilled water. The cuttings were then planted in polypot containing different proportions of fly ash and then transferred to a mist chamber and arranged in different groups in randomized block design. Intermittent mist of fresh water is provided two times for a period of thirty minutes during day time with the help of sprinklers. The rooted cuttings were uprooted after 90 days of planting and data on growth parameters were recorded. Morphological features such as number of leaves, shoot length, root length, plant biomass, and number of nodules formed and biochemical parameters such as total chlorophyll and total sugar contents were estimated (Arnon, 1942).

3. Results and discussion

Analysis of physic-chemical properties revealed that the pH of fly ash is high (8.0), water holding capacity (37.2%) is very low and percentage of carbon is negligible. Due to these adverse physico-chemical properties, the fly ash is not suitable for plant growth. The findings of the study is in conformity with Sarkar and Rano (2007), who reported alkaline nature of fly ash with poor water holding capacity affecting plant growth. In view of this, vast stretches of land close to thermal power stations get contaminated due to fly ash deposits and remain hostile to plant growth.

As could be seen from the data presented in Table-1, with addition of increasing doses of sand, garden soil and decomposed coir pith to raw fly ash, pH gradually goes on decreasing making it acidic and suitable for plant growth.

Table1
Physico-chemical properties of fly ash amended with different doses of sand, garden soil and coir pith

Types of growing medium	pH	Water-holding capacity (%)	Organic carbon content (%)
GS	6.4	31.4	1.84
FA	8.0	37.2	.85
FA:CP:: 4:1	7.1	86.2	1.63
FA:GS:: 4:1	6.8	40.22	1.37
FA:S:: 4:1	6.7	39.0	0.81
FA:CP:: 3:2	6.0	168.0	1.89
FA:GS:: 3:2	6.0	46.9	1.66
FA:S:: 3:2	6.7	41.6	0.78

FA-Fly ash, CP-Coir pith, GS-Garden soil, S-Sand, C-Carbon

On the other hand, the water holding capacity and organic carbon content increases remarkably when fly ash is amended with decomposed coir pith. The flyash when mixed with coir pith in a proportion of 4:1, the water holding capacity increases from 37.2% to 86% and to 168% in the proportion of 3:2. Similarly, the organic carbon content increases from 0.85% to 1.63% when fly ash is mixed with coir pith in the proportion of 4:1 and to 1.89% in the proportion of 3:2. Assessment of microbial diversity in raw fly ash revealed that the micro-organisms like *Rhizobium*, *Nitrosomonas*, *Nitrobacter* and *Ammonifier* responsible for N_2 cycle were very low in comparison to garden soil. Therefore it can be concluded that the flyash has very poor N_2-fixation activity which is not conducive for plant growth. However, amendment of fly ash with coir pith and garden soil increases the microbial population in it as compared to its combination with sand. Mitra *et al.* (2003) recommended the application of organic amendments to the stress soil prior to microbial inoculation.

With regard to growth performance of *Pongamia pinnata* clones, it was observed that the media containing fly ash and coir pith in the proportion of 4:1 and both garden soil and coir pith mixed with fly ash in the proportion of 3:2 were responsible for significant improvement in growth parameters like number of leaves, number of nodules, plant height and biomass yield in comparison to dilution with sand and those grown on pure flyash (Table-3). The

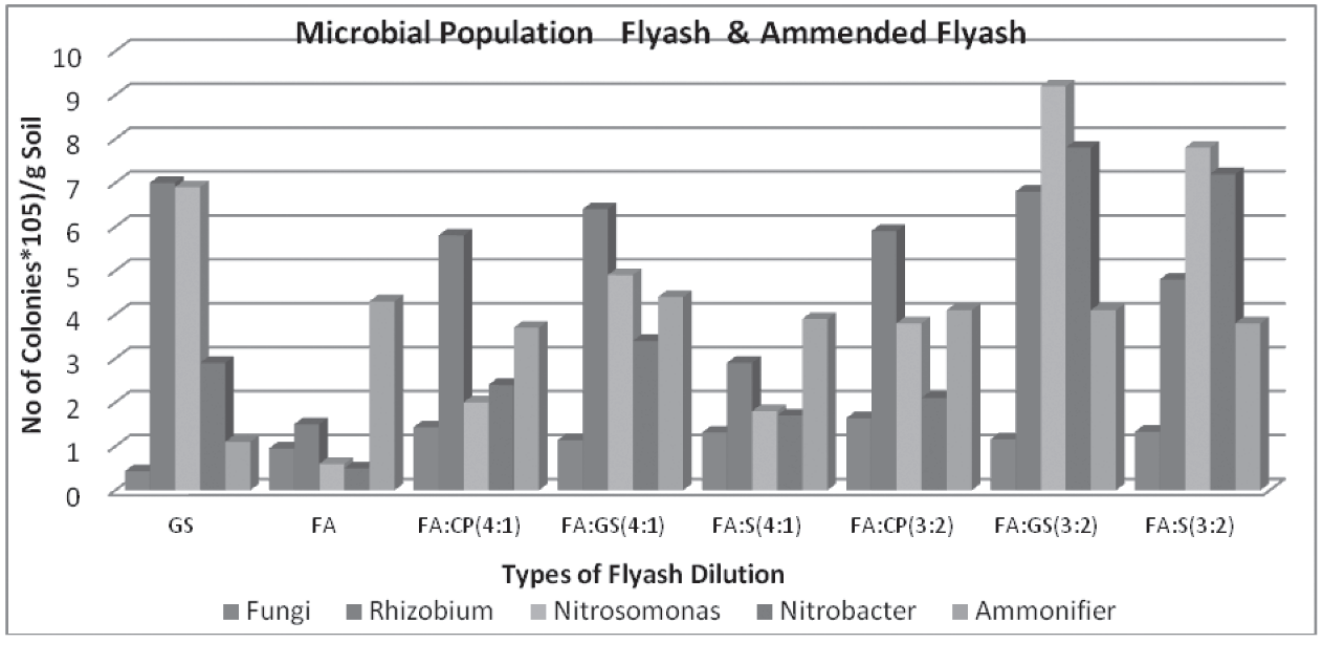

Fig 1: Microbial population in fly ash and amended fly ash (CFU isolated by Pour-plate method using Czapek's Dox media for fungi, Rhizobium by YMA media, *Nitrosomonas* by Winogradsky's medium, *Nitrobacter* by Nitrite agar and *Ammonifier* by yeast extract Mannitol agar media)

Table 3
Growth and nodulation response of *Pongamia pinnata* clones in differently diluted flyash

Parameters	Treatments							
	GS	FA	FA:CP::4:1	FA:GS::4:1	FA:S::4:1	FA:CP::3:2	FA:GS::3:2	FA:S::3:2
Leaf No.	41±16	15±8.5	38±9.5	25±5.0	26±5.0	31.8±9.0	39.4±13.0	17±3.73
Nodule No.	60±3.5	0±0	97±13	50±9.0	41±3	97±20.39	36±16	10±9.59
Shoot Length (cm)	43.3±3.8	27.9±3.0	38.2±5.06	30.6±2.93	20.14±5.58	43.3±6.64	53.62±12.37	41.56±6.36
Root Length (cm)	33.0±6.8	18.2±10.84	39.0±6.82	34.8±3.69	24.14±15.58	37.18±11.36	53.88±15.0	35.48±12.0
SDW (gm)	4.46±6.8	1.35±1.1	3.96±1.42	3.63±.16	1.59±.32	5.12±1.23	6.02±2.67	3.6±2.39
RDW (gm)	1.86±.81	0.70±0 .10	.75±.46	1.25±.45	1.25±.08	89±.09	1.89±.40	0.59±.05

SDW: Shoot Dry Weight, RDW: Root Dry Weight

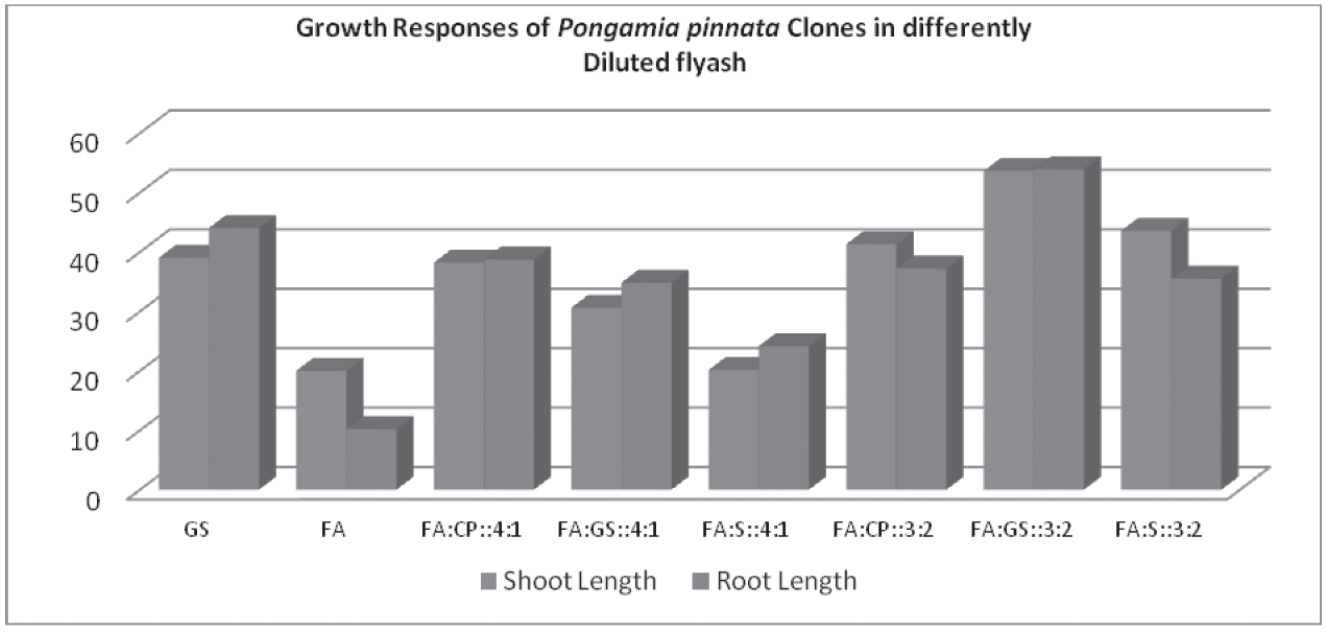

Fig 2: Growth response of *Pongamia pinnata* in differently diluted fly ash

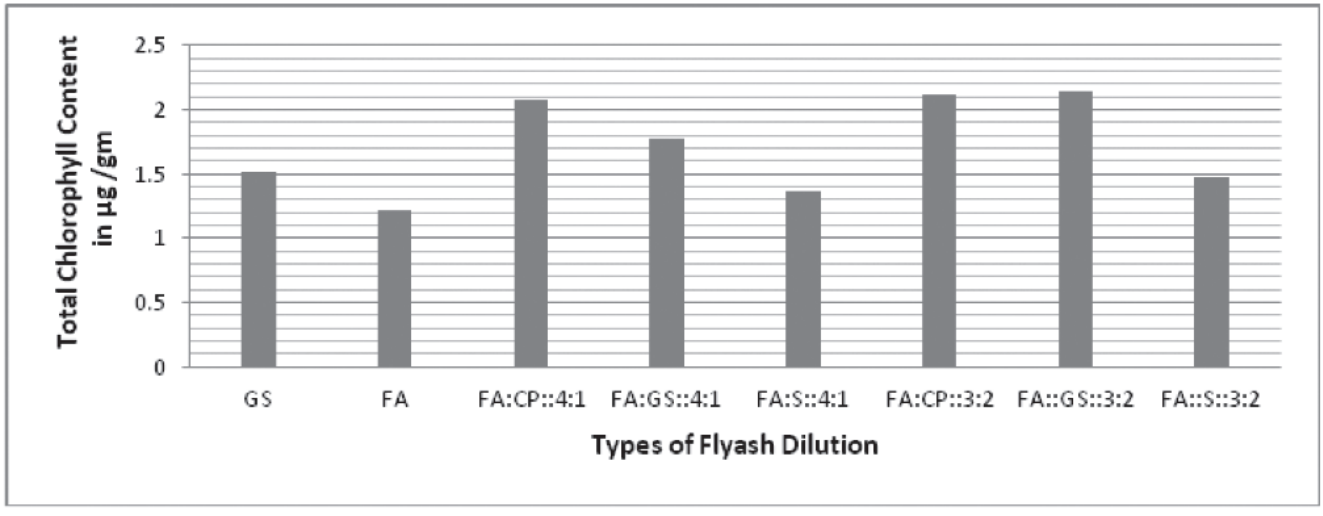

Fig 3: Changes in total chlorophyll content of *P. pinnata* grown in fly ash amended with varying doses of sand, soil and coir pith after 90 days of planting

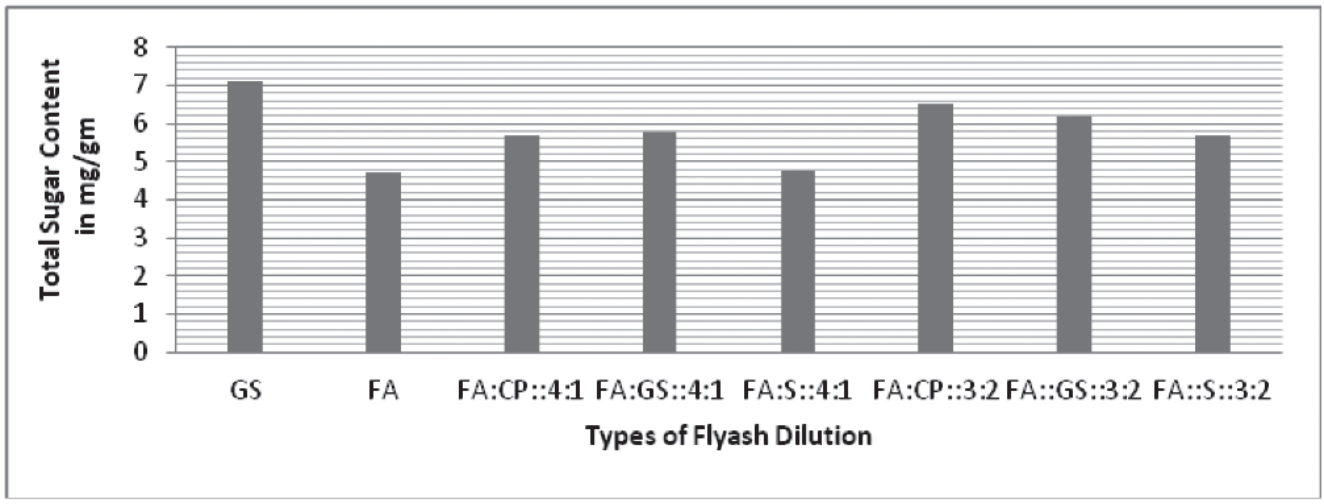

Fig. 4. Changes in total sugar content of *P. pinnata* grown in flyash and amended fly ash after 90 days of planting

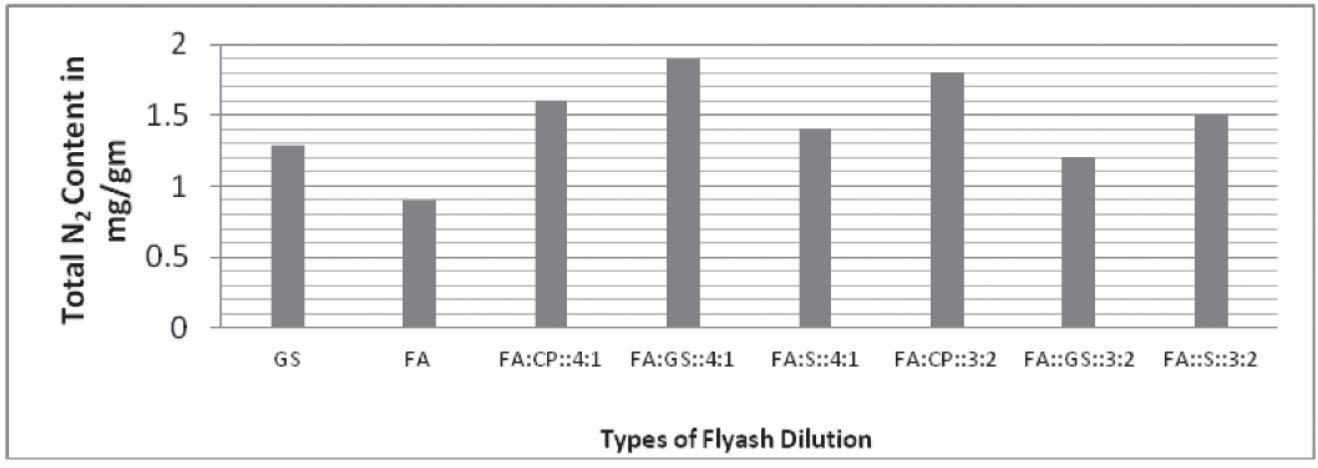

Fig. 5. Changes in N_2 content of *P. pinnata* clones grown in fly ash and amended fly ash (90 Days after planting)

increased number of nodule formation in the plant grown in a medium containing fly ash and coir pith in the proportion of 4:1 indicates improvement in *Rhizobium* establishment in the rhizosphere and enhancement of nitrogen fixing ability. Similar trend with regard to increase in chlorophyll, total sugar and protein contents was also noticed. The result is in agreement with the findings that enhancement in growth of seedlings in stress soil condition under the Mixed Microbial Inoculums (MMIR) treatment may be attributed to the release of nutrients (carbon and energy source) from decomposed coir pith to the soil favouring growth of the beneficial microorganisms and subsequent enhancement of soil enzyme activities and increase in the availability of nutrients to plants (Yunus *et al.,* 2010).

4. Conclusion

The present paper aims at standardizing suitable media and mixtures containing fly ash as a major component and methods of microbial amelioration for enhanced growth and establishment of clonally propagated plants of *Pongamia pinnata* in marginal soils and wastelands. Decomposed coir pith- a lignocellulosic waste, proved to be a very good material for amendment of flyash to promote growth and establishment of the plants under field conditions. Along with proper utilization of fly ash and coir pith, both of which are wastes and pose environmental problems, the findings of the study will help in raising large-scale plantation of *Pongamia pinnata*- a biodiesel plant species in marginal and wastelands.

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Genotypic response of high-yielding lowland rice varieties of Odisha to cadmium

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ABSTRACT

Cadmium contamination in agricultural soils is of global concern because its concentration in soils is enhanced now-a-day due to application of pesticides and phosphatic fertilizers. Three high yielding low land rice varieties of Odisha, var. Hiranmayee, var. Mrunalini and var. Jagannath, were investigated for their genotypic responses under low doses (0 to 500 µM) of cadmium in hydroponic cultures. Rice varieties were grown in YS nutrient solution with different concentrations of Cd and photosynthetic pigment analysis, heavy metal tolerance indices, phytotoxic symptoms and overall plant growth parameters were evaluated every 5 day interval for 20 days. Chlorophyll (*Chl*) and carotenoids pigment levels significantly decreased over time with increasing Cd concentration. Increased *Chl* a/b ratio indicated a marked fluctuations in the *Chl* b level. Total *Chl* content reduced with a prolongation of treatments, proportionately with the test concentrations. Chlorosis was primarily observed from 14th day of the treatment. Root growth was inhibited with a conspicuous blackening and loss of adventitious rootlets at 200 and 500 µM of Cd^{2+} in the solution, while 10 and 50 µM Cd^{2+} in nutrient solution stimulated the plant growth. Tolerance index values reported in var. Mrunalini to be of higher Cd tolerance while var. Jagannath to be lower Cd tolerance at Cd level below 500 µM.

1. Introduction

Plants are continuously being subjected to biotic and abiotic stress due to diverse anthropogenic activities from various sources like industrial effluents and wastes, urban run-off, sewage treatment plants, agricultural fungicides and mining operations. Such environmental pollutants are progressively affecting the agricultural land and other different ecosystems which largely contribute to soil pollution (Macfarlane and Burchett, 2001) that is not only contaminating the agricultural land, but also incorporating fast into the plant tissue (Berrow and Webber, 1972; Williams and David, 1976). Various abiotic stresses caused due to heavy metals, e.g., aluminium (Al), lead (Pb), and cadmium (Cd), are associated with the growing problems of agricultural soil contamination on a global scale (Azevedo *et al*., 2012). Cadmium contamination in agricultural soils is of particular concern as its supplementation in agricultural soils is enhanced, mainly due to application of pesticides and phosphatic fertilizers. Cadmium is a highly water-soluble heavy metal, easily available in the soil. It is fast uptaken by plants owing to its high mobility in the soil-plant system and easily accumulates in the edible plant parts, causing yield reduction. Consumption of heavy metal contaminated plant products severely affects mammalian tissues including kidneys, liver and lungs (Nakadaira and Nishi, 2003).

Cadmium is a stable element due to its long biological half-life and therefore stress effects of cadmium in the plant system is severe and prolonged. Common visual symptoms include leaf chlorosis, leaf and root necrosis and a prominent growth inhibition and tissue-size (Hernandez and Cooke, 1997). Phytotoxicity of the metal is mainly due to the binding of the metal to the SH-containing proteins, membrane phospholipids and oxidative phosphorylation (Wagner, 1993), leading to an impairment of cell respiration, inhibition of enzyme activities and protein denaturation (Das *et al.*, 1997; Hernandez and Cooke, 1997; Mittra *et al.*, 2009). Several

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methods like chemical remediation and phytoextraction have been proposed to reduce Cd concentration in paddy soil (Ishikawa *et al*., 2006; Makino *et al*., 2008). However, growing concentration of Cd in the soil in contaminated areas is a serious concern for rice yield. Present study aims to investigate the low doses of Cd in three low-land high-yielding rice varieties with regard to their efficacy in cope with the Cd stress and other physiomorphological effects.

2. Materials and methods

2.1. Plant materials

Rice seeds (*Oryzae sativa* L.) of var. Hiranmayee, var. Mrunalini and var. Jagannath were collected from Rice Research Station, Orissa University of Agriculture and Technology, Odisha. The agronomic characteristics of the three varieties showed that var. Hiranmayee had the lowest 135 days crop duration time where as maximum was 150 days in var. Jagannath following by var. Mrunalini (146 days). The average maximum yield was 5654 ka/ha in var. Mrunalini followed by 5453 kg/ha in var. Hiranmayee and 5000 kg/ha in var. Jaganath.

2.2. Chemical treatment

Cadmium available as the hydrated form of cadmium chloride (E-Merck) was prepared as a stock of 1 mM in deionized, double distilled water. Appropriate amounts of the stock solution were diluted in MilliQ water to attain working concentrations (10µM-500µM).

2.3 Seed germination

Seeds were surface sterilized with a 0.5% Bevistin (fungiside) for 15 min and a brief treatment of 70% ethyl alcohol for 3 min followed by an intermittent thorough washing with deionised water. Twenty-five seeds were imbibed in the petri dishes for 12h and incubated in dark for 72h at 37^0C. Germination was considered when the coleoptiles were longer than 2 mm.

2.4 Hydroponic culture

Uniformly germinated seeds were selected and transferred to the plastic cups containing Yoshida's Nutrient solution (Yoshida, 1975) and spiked with different concentrations of Cd^{2+}. Seeds immersed in the nutrient solution without the test solution served as control. Three replicates were carried out for each treatment and the required analysis was performed from the 5^{th} day till 20^{th} day.

2.4 Morphological parameters

Phytotoxic symptoms such as the leaf color and variations in the plant health were analyzed by direct visualization of the samples. Growth parameters such as the length of roots and shoots were measured directly using a ruler at every 5 day interval. Metal Tolerance Index was calculated based on the root length study as per the formula: Metal Tolerance index = [(Maximum root length in Cadmium solution- Maximum root length in Control condition)/ Maximum root length in Control condition] ×100.

2.5. Estimation of photosynthetic pigments

Photosynthetic pigments were extracted from 0.5g of the fresh leaf using 80% acetone as the extracting solution and the absorbance of the extract was read at 646.8 nm, 663.2 nm for chlorophyll and 470 nm for carotenoids. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations (mg/ g) were calculated using the equations given by Arnon (1949).

3. Results and discussion

3.1. Plant growth and morphology

Phytotoxic symptoms were noted at ≥ 100µM of Cd^{2+} in all the three varieties (Figs. 1a & 1b). The concentration of Cd^{2+} at 10 µM rather promoted length of root and shoot growth in all the three test varieties throughout the experiment. In var. Jaganath the shoot length increased at 10 µM. Leaf chlorosis and root length inhibition was observed in all the three varieties at ≥ 100 µM Cd^{2+}, while the severity of the root inhibition was accordingly to increasing Cd concentration (Figs. 2a-c). Root length inhibition was the most rapidly observable parameter under Cd toxicity (Guo and Marschner,1995), already reported in other crops (Cheng and Zhou, 2002; Song *et al.,* 2002; Zhou, 2003). The graphical representation for root length elongation (Figs. 2a-c) was in accordance with the morphological data. Shoot length was affected too as the length of shoot was found decreased with increased concentration of Cd^{2+} in all the varieties. However, shoot growth was drastically decreased in 500 µM of Cd^{2+} in var. Hiranmayee (Fig. 1a) followed by var. Mrunalini (Fig.1b) whereas var. Jagannath showed not such significant changes. Other visual symptoms to Cd stress were development of spiny shoots, root curling and discolouration of root-hairs. The effect of Cd treatment on the variety Jagannath did not show any significant variation in the morphological traits. on the contrary, 10µM Cd treatment in all the three varieties did not show any phytotoxic symptoms. Root and shoot length in all the three varieties progressively reduced with the number of days at 100µM, 200 µM and 500 µM of Cd^{2+} ion supplementation (Figs. 2a-c & 3a-c). Differences in plant growth was the most easily detectable parameter for plants under stress (Bindhu and Bera, 2001). Plant height was significantly reduced at 100 µM and 200µM Cd stress while

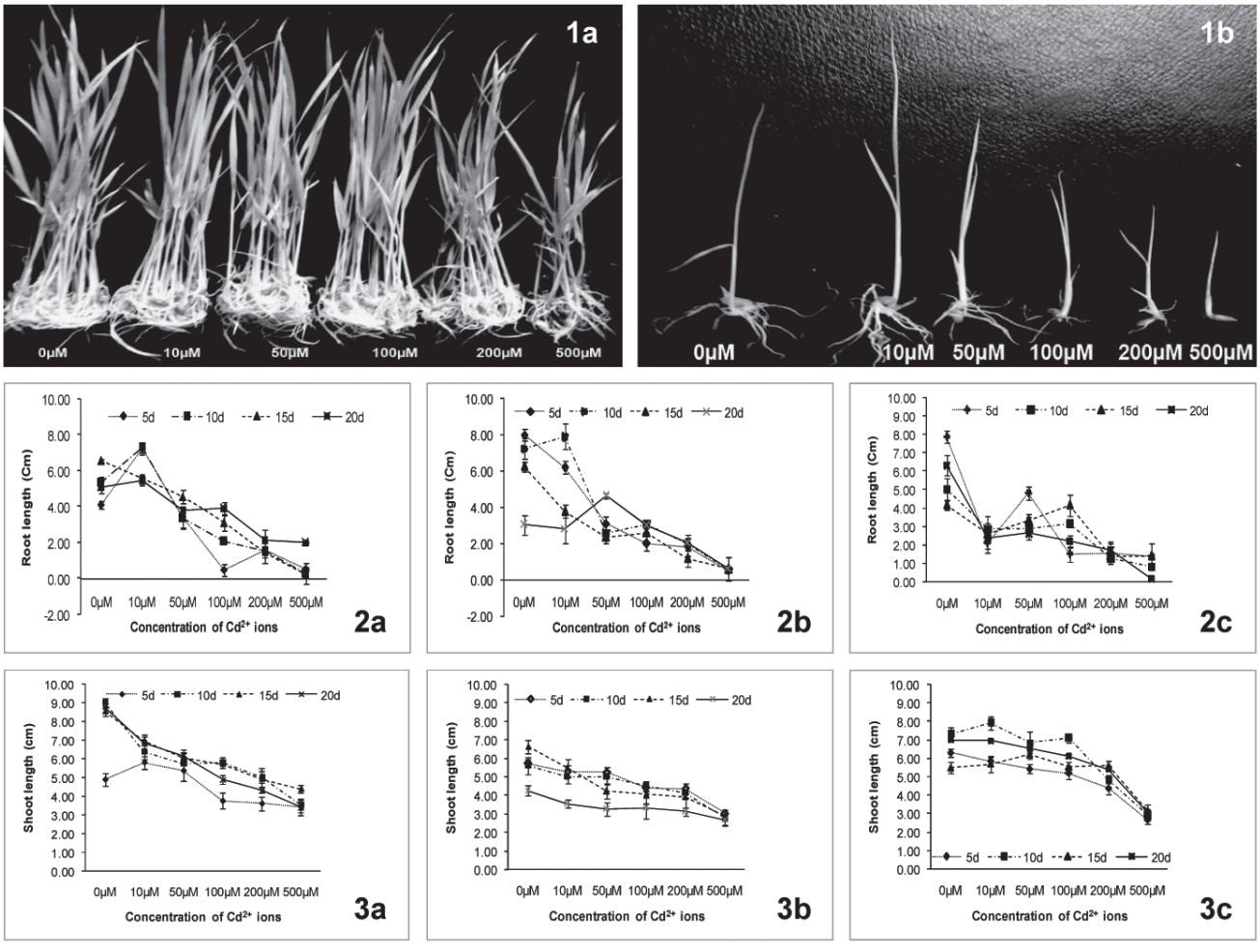
50 µM Cd treatment did not significantly affect the seedlings. Among the varieties it was found that the root of var. Mrulani found significantly susceptible to Cd stress than that of var. Jagannath followed by var. Hiranmayee. In contrast the shoot length was decreased significantly in var. Mrulani with increased Cd concentration (Fig. 1b). Root and shoot length significantly decreased with increase in concentration of Cd. The shoot system was less affected as compared to root at 500µM of Cd.

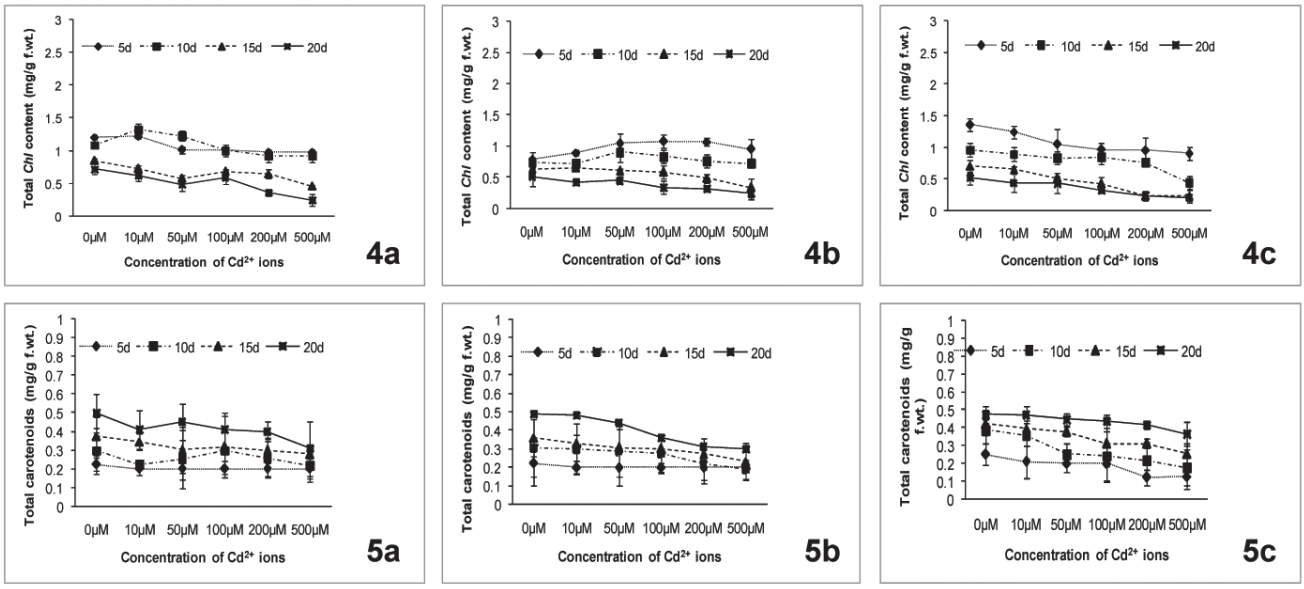
3.2. Metal tolerance indices

All the three rice varieties varied in their metal tolerance indices (MTI) values (data not shown). Var. Mrunalini was noted to have the highest tolerance index while var. Jagannath showed lower tolerance values consistent at all selected concentrations. MTI for roots could be a measured for the root-to-shoot translocation of the heavy metal ions. Higher values for root MTI might be a defense strategy adopted by the plant species to protect the system from the deleterious effects of elevated Cd levels. Tolerance values in var. Hiranmayee was noted to be higher at 500 µM Cd level, which might be a mechanism to adopt at high abiotic stress. Such tolerance indices may vary with plant species and genotypes based on the rate of Cd intake and its subsequent translocation (Metwally *et. al.,* 2005; Salt *et. al.,* 1995).

3.3 Total photosynthetic pigments

Lowering in the total chlorophyll levels was noted in all the three varieties while carotenoid content tend to increase as a means to counter act the cadmium stress effects on the total chlorophyll content (Fig 3a-c). Chlorophyll *b* was largely affected than chlorophyll *a* at 200µM and 500 µM Cd doses. Previous research studies explained the lowering in the chlorophyll content at higher Cd doses (Figs. 4a-c) might be due to degradation of chlorophyll or inhibition of its biosynthetic enzymes (Somashekaraiah *et al.*, 1992; Vajpayee *et al.*, 2000). Often, cadmium treatment in rice affects the donor side of the PSII *in vitro* was first evident by Bazzaz *et al.,* 1974. Hence, such damages in the chloroplasts severely hampers with the process of photosynthesis (Bazzaz *et al.*, 1974). There are not much of significant changes of carotinoids in alteration of Cd concentration in all the tested three varieties of rice (Figs. 5a-c).





Figs. 1a&b. Differential root and shoot growth responses of var. Hiranmayee (Fig. 2a) and var. Mrunalini (Fig. 2b) at different dosed of Cd treatment. Figs. 2a-c. Effect of different low doses of Cd on root growth of three varieties of rice. Figs. 3a-c. Effect of different low doses of Cd on shoot growth of three varieties of rice. Figs. 4a-c. Effect of different low doses of Cd on *Chl* content of three varieties of rice. Figs. 5a-c. Effect of different low doses of Cd on carotenoid content of three varieties of rice.

4. Conclusion

Increasing heavy metal threat in the field of agriculture makes it essential to investigate the three important paddy crops for their efficacy to cope with the cadmium effects. All the varieties were more or less affected in their plant height and photosynthetic effects. The common phytotoxic effects like chlorosis and root stunting and discolouration was common in all the varieties. Photosynthetic pigment system is also affected, mostly the chlorophyll b. The level of carotenoid content, however, varied according to the chlorophyll content as a means to compensate the pigment loss. It was evident that, var. Hiranmayee was comparatively tolerant while var. Jagannath had the least phytotoxic effect due to the Cd stress.

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Qualitative estimation of bioactive compounds and evaluation of antimicrobial activity of *Strychnos nux-vomica* L. leaf extracts

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ABSTRACT

Strychnos nux-vomica, an important wild medicinal plant of Odisha, was evaluated for the possible bioactive compounds through qualitative screening and thin layer chromatography (TLC). The Minimum Inhibitory Concentration (MIC) was determination for the anti-microbial activity of its leaf extracts (n-hexane, acetone, methanol and aqueous) against five bacterial strains (*Streptococcus pyogenes, Streptococcus mutans*, *Shigella flexnerii*, *Salmonella enteric-typhii* and *Vibrio cholerae*) and two fungal strains (*Candida parapsilosis* and *Aspergillus tubingensis*). The results of phytochemical screening revealed the presence of saponin, tannin, alakaloids, flavonoids, phenolic compounds, steroids and terpenoids in the leaf extracts. With n-hexane and aqueous extracts, the MIC of 400 µg/ml was found effective against *Streptococcus pyogenes*, *Shigella flexnerii* and *Candida parapsilosis*. TLC showed visible bands with aqueous and n-hexane extracts. The results of the present study corroborates the claims of tribals and traditional healers about the use of *S. nuxvomica* for the treatment of bacterial and fungal infections.

1. Introduction

An impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Abraham and Thomas, 2012). Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system . The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants in their primary health care needs (Kamaraj *et al.,* 2012). In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal.

Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates and invertebrates. Numerous investigations have proved that medicininal plants as well as microorganisms contain diverse classes of bioactive compounds such as tannins, alkaloids, flavonoids, terpenoids, phenols, etc (Chitemerere and Mukanganyama, 2011). Plants have been a major focus of investigations for novel biologically active compounds and the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Kamaraj *et al.,* 2012).

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Strychnos nux-vomica (Poison nut) belongs to family Loganiaceae and is locally known as Kochila. It is a medium-sized, deciduous tree with a straight trunk, leathery leaves, funnel-shaped, greenish-white flowers and orange coloured (when matured), globose fruits. Leaves are used for treating chronic wounds and ulcers (Arivoli and Tennyso, 2012). Fruit are used as appetizer, tonic and useful in the treatment of leucoderma, blood disorders, piles, ulcers, pneumonia, haemoptysis, occipital headache, anemia, jaundice, itching, and urinary infection (Gamble and Fischer, 1957; Han *et al.,* 2008; Ghosh, 1935). Bark is used as tonic to cure epilepsy (Pattanaik, 2006; Gruenwald, 2000; Singh *et al.,* 2009).

Thus the main aim of this work was to detect the various bioactive components present in leaf extracts of *Strychnos nux-vomica* and to determine the antibacterial activity of the species to prove its use as a safe and potent antibacterial agent.

2. Materials and methods

2.1. Collection of plant samples and preparation of extracts

Leaves of Strychnos nux-vomica plants were collected from Chandaka forest, Khordha, Odisha, India. The collected plant parts were dried at room temperature under shade and powdered using mechanical devices after drying. The leaf powder was kept in thimble and extraction was carried out using the Soxhlet apparatus (Tiwari *et al.,* 2011). The residues were collected and left for air drying later and the dried crude extracts were stored in refrigerator for further experimental work.

2.2. Phytochemical screening

Five grams of powered leaf samples were soaked in 55 ml test tube (Borosil, India) containing 30 ml each of aqueous (distilled water), acetone, chloroform, n-hexane, methanol and toluene. All these were kept at room temperature for overnight. Then the solvent extracts were filtered through Whatman No. 1 filter paper (Himedia, India) and were used for the preliminary qualitative phytochemical analysis following standard procedures (Harborne, 1973; Sofowara, 1993; Trease and Evans, 1989).

2.3. Antimicrobial study

Four different solvents (n-hexane, acetone, methanol and aqueous) as per their polarity index were used for antibacterial activity testing. The extracts of experimental plant were screened for antibacterial activity against five bacterial strains [*Streptococcus pyogenes* (MTCC-1926), *Streptococcus mutans* (MTCC-497), *Shigella flexnerii* (MTCC-1457), *Salmonella enteric-typhii* (MTCC-1252) and *Vibrio cholera* (MTCC-3906)] and two fungal strains [*Candida parapsilosis* (MTCC-2513) and *Aspergillus tubingensis* (MTCC-4285)] collected from the IMTECH, Chandigarh, India. Nutrient broth (Hi-Media, India) was used to maintain broth cultures. An additional 1.8 gm of agar (Hi-Media, India) per 100 ml made up the nutrient agar medium. The medium was autoclaved at 15 psi pressure in a temperature of 121^0 C for 20 min to ensure sterilization. The media used for fungal culture was Sabouraud's dextrose agar/ broth of (Hi media, India).

Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) using two fold serial dilution methods (CLSI, 2002; CLSI, 2009). Selected colonies of aforesaid microbes were picked off from a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of nutrient broth (Hi-media, India) and Sabouraud's dextrose broth. The broth was incubated for 6±1 hours at 35±2 ^{0}C for bacteria and 24-48 hours at 28±2 ^{0}C for fungal strain or until there was visible growth appeared. McFarland 0.5 standard was used to adjust the turbidity to get 10^5 colony forming units (CFU)/ml. McFarland standard was prepared by standard methods (Chapin, 2003) using barium chloride and sulphuric acid (1.17 % of $BaCl_2$. $2H_2O$ with 1 % of H_2SO_4) and visual comparison was carried out (Carlberg, 1985; CLSI, 2009; Versalovic *et al.,* 2011) using Wickerham Card (B005R43DK8, Carolina Biological Supp. Comp.) or white card with black lines (Jiang, 2011). Each crude leaf extracts of 16 mg extract dissolved in 10 ml of DMSO to get desired drug concentrations.

MIC was calculated by two fold serial broth dilution method for leaf extracts/solvents with standard Ampicilin (Hi-media, Mumbai, India) for bacterial strains and Amphotericine B (Hi-media, India) for fungal strains. The method includes 24 tubes of 5 ml capacity were arranged in 3 rows/replications with each row containing 8 tubes. Nutrient broth of 1.9 ml for bacteria and Sabouraud's dextrose of 1.9 ml for fungal strain was taken to first tube and 1ml to other 7 tubes was added in each row or to replication. Crude extract (16 mg in 10 ml of DMSO) of 100 µl was added to the first tube in each row and after mixing the content; 1 ml was serially transferred from first tube to the second tube, then 1ml from second to third, third to four, four to five, five to six then six to seven in each of the rows. This provide extract concentrations of 1600, 800, 400, 200, 100, 50, 25 and 12.5 µg/ml in the first to seventh tube respectively in each row. Finally, 1 ml (10^5 CFU/ml) of bacterial suspension and fungal suspension were added to first, second and third rows of tubes respectively. All the test samples and control/standard tubes were then incubated

for 12-18 hours at 35±2 °C for bacteria and 48 hours at 30 ± 1 ^{0}C for fungal strain (Bayati and Mola, 2008). After the incubation, the tubes of lowest concentration showing no visible growth after 8 hours till 12 hours were considered to be inhibition of bacteria and 24 hours till 48 hours for fungus (Liete *et al.,* 2014**)** which represent MIC values. Inoculums control showed visible growth due to no antimicrobial agents whereas, the negative control DMSO showed no growth due to absence of microbes. Triplicates were maintained and the experiment was repeated thrice, for each replicates the average readings were taken for all the experiments designed. Data mentioned for MIC values is mean ± SD for all readings.

2.4. Thin Layer Chromatography(TLC)

For TLC, the readymade aluminum sheets (20×20 cm) of TLC silica gel 60 F254 (Merck, Germany) were used. The samples were applied on the silica gel by capillary made up of glass. Thin layer chromatography of different solvent systems was prepared for leaf extracts. Out of which Toluene/Glacial acetic acid (3:1) showed higher band separation in both the extract (hexane, acetone, methanol and aqueous) of *S. nux-vomica*. All the solvents used are of laboratory grade (Merck, India). The R_f was calculated for different compounds by dividing the distance of the compound travelled from the original position by the solvent travelled from the original position (the solvent position). All the experiments were repeated three times and the mean data recorded for all the observations.

3. Results and discussion

Plants are known as the "chemical factories" of nature as they provide the richest source of organic chemicals on earth (Prabha *et al.,* 2014). The results of qualitative phytochemical screening of experimental plants (leaf extracts) showed the presence of seven different phytochemical like saponin, tannin, alkaloids, flavonoids, phenolic compounds, steroids and terpenoid. It was observed that methanol and acetone extracts showed highest number of bioactive compounds followed by aqueous and n-hexane extracts (Table 1). No bioactive compounds were detected in toluene extract of *S. nux-vomica* leaf. Similar results have been reported in *S. nux-vomica* extracts by Magdalin Joy and Reginald Appavoo (2014).

Table 1
Qualitative estimation of bioactive compounds of *Strychnos nux-vomica* leaf extracts

Phytochemicals	Extracts					
	Aqueous	n-hexane	Toluene	Acetone	Chloroform	Methanol
Saponin	+	-	-	-	-	+
Tannin	+	+	-	+	-	+
Alkaloids	+	+	+	+	-	+
Flavonoids	-	-	-	+	-	+
Phenolic compounds	+	-	-	+	-	+
Steroids	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-

(+ = presence, - = absence)

Since no information was available in published literature with respect to phenolic content of the leaves of *S. nux-vomica* (Eldahshan and Abdel-Daim, 2015), an attempt was made to find out potential bioactive compounds in the leaves. Only few workers studied the effect of different solvent based leaf extracts on different microbial strains like *Staphylococcus aureus, Salmonella, Klebsiella pneumonia, Aspergillus niger*, *Aspergillus terreus, Aspergillus flavus* (Gnanavel *et al.,* 2012); *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Aeromonas hydrophyla, Pseudomonas aeruginosa* (Senthilkumar *et al.,* 2005). In our work, leaf extracts of four different solvent systems tested showed anti-microbial activity (MIC values) against all pathogenic bacterial and fungal strains at different levels. Among the four solvents screened, n-hexane and aqueous leaf extract showed excellent (MIC = 400µg/ml) antimicrobial activity against 3 pathogenic strains and MIC value of 800µg/ml) for other 4 strains. While highest antimicrobial activity (MIC = 800µg/ml) was observed in acetone and methanol solvent extracts of leaf against 3 pathogenic strains, the lowest antibacterial activity (MIC = 1600 µg/ml) was recorded against the other 4 strains. Similar results have been also obtained earlier in medicinal plants like *S. nux-vomica* (Prabha *et al.,* 2014; Magdalin Joy and Reginald Appavoo, 2014; Thambi and Cherian, 2015), *Lawsonia inermis* (Moharana *et al.,* 2014), *Tinospora*

cordifolia (Kumari, 2012) and *Tylophora indica* (Jahan *et al.,* 2013). Of the seven microbial strains evaluated, the best MIC value (400 µg/ml) was observed in n-hexane and aqueous leaf extract against *Streptococcus pyogenes*, *Shigella flexnerii* and *Candida parapsilosis* but the least MIC value (1600 µg/ml) was noticed in acetone and methanol leaf extract against *Streptococcus mutans*, *Salmonella enteric-typhii*, *Vibrio cholera* and *Aspergillus tubingensis* (Fig. 1). Ampicilin was used as standard for bacterial strains with highest MIC at 25µg/ml and a lowest MIC 12.5µg/ml while Amphotericine B was used as standard for fungal strains with a MIC of 25µg/ml (Fig. 1). Similar experiment was conducted by Kalaivanan *et al.,* (2014), in which they prepared leaf extracts of different solvents like hexane, chloroform, ethyl acetate and methanol and treated them against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli, Salmonella typhimurium, Shigella flexneri, Proteus mirabilis, P. vulgaris* and *Vibrio cholera*. Among these extracts, they found methanol extract having higher efficacy against *S. flexnerii* and *S. aureus*.

As the results of Table 1 revealed the presence of bioactive compounds, TLC screening was carried out. The TLC of *S. nux-vomica* showed the visible spot in aqueous extract at mean R_f 0.50 (lemon yellow), R_f 0.55 (lemon green) and visible spot of n-hexane extract at mean R_f 0.40 (light bottle green), R_f 0.44 (light bottle green), R_f 0.48 (light bottle green) using mobile phase Toluene/acetic acid (3:1 v/v) (Fig. 2). Rathi *et al.* (2008) optimized TLC procedure of methyl extract of fruit pulp of Kochila (*S. nux-vomica*) in the mobile phase chloroform / ethyl acetate / diethyl amine (0.5:8.5:1, v/v/v) and found bands at R_f 0.42 and 0.55, of the two R_f values one was coinciding with our R_f value i.e. 0.55 in aqueous extract. In such an experiment, Mathivanan *et al.* (2014) optimised TLC procedure of methanol leaf extract of *S. nux-vomica* in the mobile phase methanol/chloroform (1:9, v/v) and found R_f 0.48, 0.60,

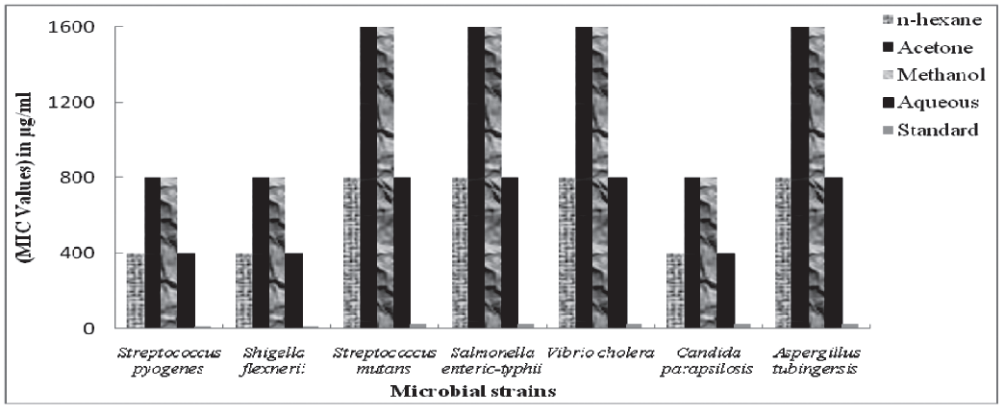

Fig. 1 Antimicrobial activity of *S. nux-vomica* leaf extracts against pathogenic strains

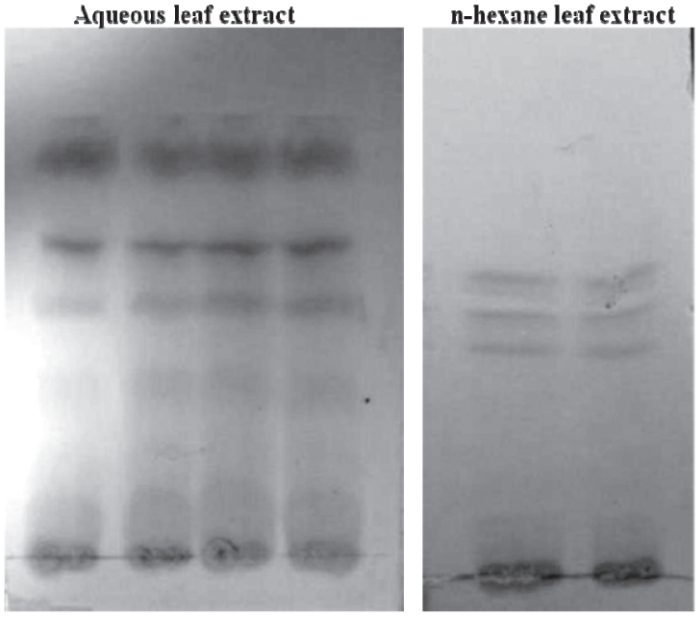

Fig. 2. TLC Fingerprinting of *S. nux-vomica*

0.71 and 0.83. From these four values, one was coinciding with our R_f value i.e. 0.48 in n-hexane extract.

Recent research indicated that the antibiotics are becoming ineffective against common pathogens such as *S. pyogenes* (Feng *et al.,* 2010; Gracia *et al.,* 2009). In addition, currently fungal infections are ranked fourth of nosocomial infections. The yeast candida is implicated in more than 75% of invasive or systemic fungal infections (Richardson, 2005). About 25% of all medicine available in the market have been derived directly or indirectly from plants (De Smet, 1997; WHO 2005). Herbal medicines are generally believed to be safe, but it is important to evaluate their biological safety aspect before use so that harmful consequences could be avoided (Kunle *et al.,* 2012).The present study validates medicinal uses of *S. nux-vomica* against bacterial infections. The antimicrobial activity of the plant may be attributed to the various phytochemical constituents present in the crude extract of leaf. The work reported here is of preliminary nature and aimed at finding out the antimicrobial activity of this medicinal plant and the result of the study established good antibacterial and antifungal activity of *S. nux-vomica* leaf extracts. The study indicates the plants could be a potential source of newer antimicrobial agents. Further work on the types of phytoconstituents and purification of individual groups of bioactive compounds can reveal the potential of the plant extract to control microbial infections and as an effective application for control of a broad spectrum microbes causing severe skin problems, upper respiratory tract infections, eye infections, onychomycosis, nosocomial infection etc.

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Ethno-medicinal uses of some threatened medicinal plant species of Mahanadi Wildlife Division, Boudh-Nayagarh district, Odisha, India

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ABSTRACT

Mahanadi Wildlife Division in Odisha harbours diversified vegetation mostly belonging to tropical moist deciduous forests and rich flora including a number of important and threatened medicinal plants. Due to habitat destruction and overexploitation, the wild populations of several medicinal plant species have declined considerably in recent years. In the present communication, first-hand information collected from local inhabitants on ethno-medicinal uses of 18 prioritized threatened medicinal plants of Odisha like *Rauvolfia serpentina*, *Mesua ferrea, Oroxylum indicum, Gardenia gummifera, Gloriosa superba* etc. have been provided. Needs for periodic threat assessment and strategy for conservation of these important species including reintroduction in natural habitats have been emphasized.

1. Introduction

Plants are universally recognized as a vital part of the world's biological diversity and an essential resource for the planet. Many thousands of wild plants have great economic and cultural importance, providing food, medicine, fuel, clothing and shelter for humans around the world. Plants also play a key role in maintaining the Earth's environmental balance and ecosystem stability. They also provide habitats for the world's animal and insect life. Many plant species are threatened by habitat transformation, over-exploitation, invasive alien species, pollution and climate change and are now in danger of extinction. The disappearance of such vital and large amounts of biodiversity presents one of the greatest challenges for the world community: to halt the destruction of plant diversity that is essential to meet the present and future needs of humankind (Anonymous, 2009).

According to estimates by IUCN, 10% of all plant taxa are under some sort of threat globally. The over exploitation of resources and deforestation in the tropical forests has resulted in depletion of biodiversity. The main threat to tropical biodiversity is habitat loss (Bowels *et al,* 1998). The Red Data Book of Indian Plants (Nayar & Sastry, 1987, 1988, 1990) listed only 814 species of threatened plants (4.7%) under different categories out of the known 17000-18000 species of flowering plants in the country. It is essential to develop species-specific conservation strategies for the RET species with emphasis on both *in situ* and *ex situ* conservation approaches. The conservation, domestication and sustainable utilization of threatened medicinal plants assume great importance in view of their utility as a healthcare need for the vast majority of tribal and rural population in a country like India.

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The use of a large variety of plants in Odisha to cure diseases is an age-old practice for people living in forest and rural areas, where they lack access to modern medical facilities, and do not have the capacity to afford for modern allopathic drugs. The forest has been the source of medicinal plants since man became aware of the preventive and curative properties of plants and started using them for human health care. Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual, and health care of rural and tribal people in the state.

The Conservation Assessment and Management Prioritization (CAMP) workshop for medicinal plants of Odisha was held in October 2007 at Bhubaneswar came out with a prioritized list of 41 species of medicinal plants those require conservation intervention (Ved *et al*., 2008) . The objective of the workshop was to assess the threat on medicinal plants of Odisha, based on the criteria developed by the International Union for Conservation of Nature and Natural Resources (IUCN). During a CAMP process, the wild and captive status for each taxon under consideration was reviewed with emphasis on distribution and habitat, population characteristics, level of present and perceptible threat, biology of the species etc. and based on these criteria each species was assigned a IUCN Red list category.

In the present study, the ethno-medicinal uses of 18 of the 41 prioritized medicinal plant species used by the tribal and local people in and around Mahanadi Wildlife Division, Boudh and Nayagarh districts of Odisha have been collected and presented in this paper.

2. Study area

The Mahanadi Wildlife Division was created by inclusion of a part of Satkosia (WL) sanctuary situated south of the river Mahanadi in Boudh and Nayagarh district and Baisipalli (WL) sanctuary in Nayagarh district in the year 1999. The Division is located between 20° 23.8' to 20° 36.8' N Latitude and 84° 35.4' to 84° 58.5' E Longitude (Fig.1). The total geographical area of Mahanadi Wildlife Division is 437.29 Sq. Kms which includes the whole area of Baisipalli Wildlife Sanctuary (168.35 Sq. Km.) and part of Satakosia Gorge Sanctuary (268.94 Sq. Km.). It consists of four Reserve Forests viz. Padmatola RF and Arakhpadar RF in Boudh District, Mahanadi RF and Baisipalli RF in Nayagarh District. The Division consists of three Ranges, viz Chamundia, Kusanga and Banigochha.

As per Champion and Seth (1968) classification and Forest Survey of India (FSI, 2011), the forests found in this division broadly fall under four forest type groups: 1. 2B/C3- Orissa semi evergreen Forest, 2. C3/C2- Moist Peninsular sal Forest, 3. 3C/C3- Moist mixed deciduous

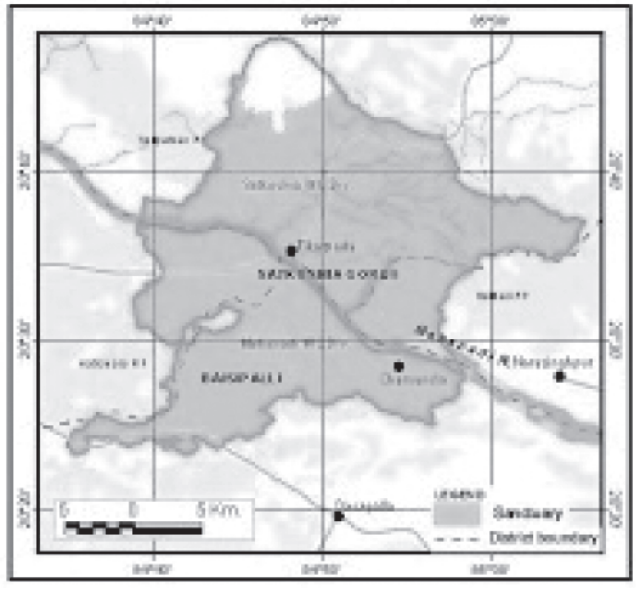

Fig.1. Location map of Mahanadi Wildlife Division

forests and 4. 4RS1- Riparian Fringing Forest. Within these main types intermediate edaphic and seral sub types like bamboo brakes are also found. Flora of these two sanctuaries is dominated by sal (*Shorea robusta*) and its common associates.

This region is dominated by tribes such as Kondhs, Gonds, Mundas and Saoras. The tribals occupy a putative role in the Jagannath cult of Orissa, the Saoras are the early worshipers of Lord Jagannath and till today they have been performing major role in the religious rights of Jagannath temple.

3. Methodology

The field study was carried out during 2014-15 in different tribal villages and forest area at regular intervals in all the seasons and the information on the use of medicinal plants listed in the proceedings of the CAMP workshop for Odisha state (Ved *et al*., 2008) was obtained through structured questionnaires, complemented by free interviews and informal conversations (Huntington, 2000). The interviews were individually carried out during the first contacts with the local population, local specialists considered by the community as having exceptional knowledge about the use of plants were identified. More than 100 knowledgeable persons or medicine men, Vaidyas, experienced and aged persons and local healers of the villages were consulted for recording local name; parts of plants used methods of drug preparation and recommended doses. Personal interviews and group discussions with local inhabitants revealed some very valuable and specific information about the plants, which were further authenticated by crosschecking with published literature. In

addition, voucher specimens were collected and preserved as herbarium specimens to ascertain correct botanical identity of each species. The species were identified in consultation with Haines (1921-1925) and Saxena and Brahmam (1994-1996) and by matching with authentic specimens available in different Herbaria. The medicinal plants collected are listed here with their botanical names followed by family name, their local names and the parts used for medicinal purpose. The threat category to which a species is assigned as per CAMP workshop (Ved *et al*., 2007) has also been indicated.

4. Results and discussion

It was observed that the local people and tribals use more than 350 different wild and cultivated plant species to cure various ailments and diseases. Out of these, the ethno-medicinal/ ethno-botanical uses of only 18 threatened medicinal plants prioritized during the CAMP workshop for medicinal plants of Odisha (Ved et al., 2008) have been enumerated here (Table-1). The species are arranged alphabetically with their botanical names, family, local names, threat assessment category and local uses. Among these species, 2 species were under "Critically Endangered (CR)" category, 7 species under "Endangered (E)" and 9 species under "Vulnerable (VU)" category. Of these, *Gardenia gummifera, Gloriosa superba, Hedychium coronarium, Mesua ferrea, Oroxylum indicum, Pterocarpus marsupium, Rauvolfia serpentina, Saraca asoca, Symplocos racemosa* etc. were the species frequently used by the locals for various ailments and therefore, need conservation actions.

Table 1
Ethno-medicinal uses of the Endemic and RET plants of Mahanadi Wildlife Division of Boudh-Nayagarh district, Odisha

Plant name	Family	Local name	Habit	IUCN status (Ved *et al*., 2008)	Uses
Celastrus paniculata Willd.	Celastraceae	Laibeheda	Climber	VU	The seed oil is massaged against rheumatic joints and paralysis.(K,S) The seed oil is applied externally on the affected part for 15 days to cure all kinds of skin diseases.(K,M)
Gardenia gummifera L.f.	Rubiaceae	Gurudu	Shrub	VU	10 gm of root along with 2 black pepper made into paste & taken twice daily in empty stomach for 15 days with cold water cures metrorrhagia (S, M, K) The gum of the plant is applied on rheumatic swelling to get relief.(K)
Gloriosa superba L.	Liliaceae	Agnisikha	Climber	EN	The paste of rhizome is applied externally for 21 days to check bleeding from piles.(S) Tuber extract 1tsp taken twice a day for 5 days is administered upto 3 months pregnancy for abortion.(M)
Hedychium coronarium Koenig.	Zingiberaceae	Dulala Champa	Herb	VU	Decoction of the rhizome (10 ml) is taken once daily for 1 month against rheumatism.(K,M)
Litsea glutinosa (Lour.) Robins.	Lauraceae	Ledhachali	Tree	VU	Paste of bark is applied externally to relieve pain due to internal injury and in sprain. (K,S) Crushed stem bark is applied on boils.(K)
Mesua ferrea L.	Clusiaceae	Nageswar	Tree	VU	Leaf paste is massaged on head against hemicrania. (K) The flower paste is applied locally twice daily for 7 days against piles.(S,K)
Operculina turpethum (L.) Silva-Manso	Convolvulaceae	Dudhalomo	Climber	VU	Powdered roots (10gm) mixed with fruits juice (20gm) of *Phyllanthus emblica* is given twice a day for 10 days to check by diabetes.(K,G)

Oroxylum indicum (L.) Vent.	Bignoniaceae	Phemphana	Tree	EN	Bark paste is massaged on joints to relieve of pains and swellings and the juice of bark is given in empty stomach in backache. (K,S)
Paederia foetida L.	Rubiaceae	Prasaruni	Climber	VU	The leaf juice along with black pepper taken once daily for 7 days against swelling and pain of body.(K,S) 50 gm of leaves along with 100 gm of raw rice made into paste & is taken daily for 7 days cure lower back pain.(K) Crushed root mixed with cattle feed, is given for stomach disorder of cow.(K)
Piper longum L.	Piperaceae	Pipali	Creeper	EN	Fruit powder (10 gm) mixed with dry ginger powder (10 gm) is taken every day at bed time for free bowel movement.(S)
Pterocarpus marsupium Roxb.	Fabaceae	Piasal	Tree	EN	The wood is used for making wooden articles & furnitures.(ALL)
Pueraria tuberosa (Willd.) DC.	Fabaceae	Bhuin kakharu	Climber	VU	The tuber paste is applied thrice daily for 3 days against neck swelling of the cattle. (S)
Rauvolfia serpentina (L.) Benth. ex Kurz	Apocynaceae	Sarpagandha	Undershrub	EN	Root paste (5gm) applied on the affected part and a piece of root given to patient to chew for 3 days against snake bite. (S,M,K) Bark juice (5ml) given twice a day for two days against stomachache.(S) Five gm. of the root paste is given at bed time for one month against high blood pressure & hypertension. (K G,S)
Saraca asoca (Roxb.) de Wilde	Caesalpiniaceae	Ashok	Tree	CR	The decoction of bark (40 gm) taken once in the morning in empty stomach for 15 days to cure gynaecological disorders.(K,M,S) The bark paste applied externally and bandaged on the affected part for 5-7 times cure bone fracture. (S,K)
Scindapsus officinalis (Roxb.) Schott.	Araceae	Gaja pipali	Climber	VU	Water extract of the leaves is given in the morning and the fruit is cooked and eaten for 6 months to cure rheumatism .(K)
Stereospermum chelonoides (L. f.) DC.	Bignoniaceae	Patuli	Tree	EN	Seeds are made into a necklace to wear around the neck to prevent malaria fever. (K)
Symplocos racemosa Roxb.	Symplocaceae	Lodha	Tree	CR	Stem bark decoction with honey (3:2) is given to children below 10 years against liver complaints (K) 20ml of bark decoction taken twice daily in empty stomach for 7 days cure leucorrhoea. (K,S)
Uraria picta (Jacq.) Desv.	Fabaceae	Iswarjata	Undershrub	E	The root is prescribed for coughs & fever (M,G)

(VU- Vulnerable, CR-Critically endangered, EN- Endangered, K- Kondhs, G- Gonds, S- Saoras, M- Mundas)

The tribal folk have a wide range of herbal remedies which are most popular and effective according to their faith and understanding. Plants are real benefactors, whereas the aboriginals are real researchers, who in their struggle to have healthy living, confront with nature and explore new medicinal herbs for self help which in turn are exploited or over exploited. In the present investigation, 18 RET medicinal plant species used by different tribes inhabiting Mahanadi Wildlife Division for their healthcare needs have been identified. In the process wild collection and marketing of herbal drugs, several endangered species of medicinal plants are over-exploited and wild populations of them get substantially reduced. There appears a need to create awareness among tribals about the proper identification, sustainable harvesting and on-farm cultivation of rare species. During the present investigation, species like *Gardenia gummifera, Gloriosa superba*, *Pterocarpus marsupium*, *Mesua ferrea*, *Paederia foetida*, *Piper longum*, *Rauvolfia serpentina*, *Saraca asoca* etc. were found to be exploited heavily and need conservation interventions urgently.

Systematic approaches to conservation of medicinal plants and adoption of scientific practices of sustainable resource utilization have been emphasized by Uniyal *et al*. (2006). Conservation of the species in natural habitat may be the best option to save the species from near extinction. Alternatively, reintroduction of rare and endangered species into their natural habitat could be the last resort for recovery and maintaining viable populations of these threatened plant species (Maunder, 1992).

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Ethno-botanical uses of coastal sand dune plants of West Bengal

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ABSTRACT

The ethnobotanical uses of 52 plant species occurring on sand dune of coastal West Bengal from East Medinipur district to Talsari of Odisha have been described in this paper. Botanical name, family and medicinal uses have provided for each species.

Coastal sand dunes are the natural structures which protect the coastal environment by absorbing energy from wind, tide and wave action (Corre, 1991) and play a vital role in protecting the coast from erosion and flooding (Desai, 2000). Dune vegetation has immense ecological and economic significance (Banerjee, 1994). It provides a range of ecological services including stabilization of shoreline, protection of hinterland from natural hazards, eco-tourism and contribute to the livelihood of local people by providing a wide varieties of useful plants having medicinal and food values.

The coastal belt of West Bengal is rich in plant resources, which harbour many economic and medicinal plant species. The local communities along the coastal belt depend upon these resources for their livelihood and utilize many plants for food, timber, fibre, fuel and medicine. Over-exploitation of costal plant species for fuel, fodder, house construction, medicine etc. has resulted in loss of plant diversity including those of rare, endangered and threatened plants. The traditional knowledge possessed by the local inhabitants on medicinal values of plants need to be documented to make people aware of their economic importance to facilitate conservation and sustainable utilization of these valuable resources. The present paper reports the ethnobotanical uses of 52 such plant species occurring in the sand dunes along the coastal belt of Purba Medinipur, West Bengal.

The study was carried out along 76 km costal belt of Bay of Bengal from the East (Purba) Medinipore district of West Bengal to Talsari of Odisha coast. The study area lies between 21°362 502 2 N and 19°482 002 2 N latitudes and 85°522 402 2 E to 87° 372 002 2 E longitudes. Plant samples were collected and enumerated by laying random quadrates of 10m X 10m sizes at different distances from the high tide mark. Information on medicinal uses of plant species were collected during August, 2008 to December 2012 from different ethnic groups, traditional healers, Ayurvedic practitioners and others through interaction and discussions. The botanical names, family and ethno-botanical uses of 52 species of plants belonging to 50 genera and 31 families collected from coastal sand dunes of West Bengal coast are presented in Table-1.

Arun *et al*. (1999) enumerated 154 species of plants occurring in Indian coastal sand dunes belonging to 108 genera and 41 families, of which medicinal uses of 52 species

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Table 1
Enumeration of medicinal plants and their traditional uses along the coast of West Bengal

Botanical name	Family	Uses
Acanthus ilicifolius L.	Acanthaceae	Plant parts like leaves are used in rheumatism and asthma.
Achyranthes aspera L.	Amaranthaceae	Plant decognotion is used as an emmengogue, in piles and skin eruptions.
Ageratum conyzoides L.	Asteraceae	Herb infusion is given in stomach ailments such as diarrhoea, dysentery and intestinal colic with flatulence.
Alternanthera sessilis R. Br.	Amaranthaceae	It is used for indigestion, burning sensation, diarrhoea and fever and also used as leafy vegetables.
Anacardium occidentale L.	Anacardiaceae	Bark and leaves infusion is used to relief from toothache and sore gums. Roasted and raw kernels are eaten as a desert, employed in confectionery and are highly nutritious.
Argemone maxicana L.	Papaveraceae	Leaves are useful in cough and skin diseases. Roots are useful in guinea worm infection, skin disease and leprosy.
Azadirachta indica A.Juss	Meliaceae	Flowers are fried and eaten. The oil extracted from flowers, fruits, seeds keeps skin clean and protect from infection also acts as mosquito repellent.
Barringtonia acutangula Gaertn.	Barringtoniaceae	Fruit is bitter, anthelmintic, astringent. Leaf juice is given in diarrhoea.
Borassus flabellifer L.	Arecaceae	Root is diuretic and anthelmintic. Fruits are used in dyspepsia, flatulence, colic and skin diseases.
Caesalpinia bonduc (L.) Roxb.	Caesalpiniaceae	Leaf paste is applied on swollen testicles; useful against jaundice and rheumatism.
Calophyllum inophyllum L.	Clusiaceae	Seed oil is used as a stimulant embrocating in rheumatism and gout; Oil cures scabies and other cutaneous disease. Stem bark is astringent.
Calotropis gigantea (Linn.) R. Br. ex Ait.	Ascleapiadaceae	Root bark is diaphoretic and expectorant; acts as a might stimulant. Powdered root bark gives release diarrhoea and dysentery.
Canavalia maritima (Aubl.) Thou.	Fabaceae	Young pods and seeds are used as vegetables.
Cassia occidentalis L.	Caesalpiniaceae	Whole plant has purgative, febrifuge and diuretic properties; plant decoction is used in sores, dysentery and stomach troubles.
Casuarina equisetifolia L.	Casuarinaceae	Bark is a tonic and astringent, useful in diarrhoea and dysentery.
Catharanthus roseus L.	Apocynaceae	Whole plant body has important medicinal property including tie treatment of cancer, fever etc.
Cissus quadrangularis L.	Vitaceae	Stem and root paste is used in bone fractures.
Citrullus colocynthis L.	Cucurbitaceae	Commonly known as bitter cucumber to the local people, fruits and roots are useful in kidney infection, jaundice etc.
Clerodendrum inerme (L.) Gaertn.	Verbenaceae	Fresh and dry leaves possess alternative and febrifugal properties. Root boiled in coconut oil is useful in rheumatism.
Cocos nucifera L.	Arecaceae	Roots are astringent and diuretic. Juice of young fresh spadix is intoxicating; useful in dyspepsia and diarrhoeas and leprosy. Fresh unripe fruit pulp is diuretic.

Crotalaria retusa L.	Fabaceae	Root powder mixed with spices used as a remedy for colic. leaves is used in fevers
Croton bonplandianum Baill.	Euphorbiaceae	Leaves are useful in skin diseases and wounds.
Cynodon dactylon (L.) Pers.	Poaceae	Plant decoction is diuretic; useful in dropsy and anasarca.
Cyperus rotundus L.	Cyperaceae	The root extract oil instilled into eyes in conjunctivitis reduces the pain, redness and ocular discharges.
Eragrostis viscosa Retz.	Poaceae	Used as livestock fodder.
Evolvulus alsinoides (L.) L.	Convolvulaceae	Herb is used to cure dysentery, chronic bronchitis, fever, hiccups and jaundice and as antiseptic.
Gisekia pharnaceoides L.	Aizoaceae	Leaves and roots are useful for skin infection and stomach ache.
Hemidesmus indicas (L.) R. Br.	Asclepiadaceae	Root and black pepper paste is used in stomach pain and diarrhoea.
Ipomoea aquatica L.	Convolvulaceae	Leaves are eaten as vegetables with high food value.
Ipomoea pes-caprae (L.) R. Br.	Convolvulaceae	It is a sand binder; leaves and roots are useful for gonorrhoea, rheumatism, skin infection and stomach ache.
Jatropha gossypifolia L.	Euphorbiaceae	Roots are used for leprosy; bark decoction as emmemagogue; leaves to cure stomach ache, venereal diseases and as blood purifier.
Kyllinga triceps Roth.	Cyperaceae	The juice of the leaves is used in the skin injury by the ethnic races.
Lantana camara L.	Verbenaceae	Invasive species of this particular zone.
Launaea sarmentosa (Willd.) Schult-Bip. ex O. Kuntze	Asteraceae	Good sand binder and plant juice is applied for the treatment of rheumatism.
Leucas aspera (Willd.) Link	Lamiaceae	Leaf juice is used for chronic skin eruptions and painful swellings.
Mimosa pudica L.	Mimosaceae	Root paste in the water collected after washing the raw rice is given orally for the snake bite. Leaf paste is applied to glandular swellings.
Opuntia stricta (Haw.) Haw.	Cactaceae	Baked fruit is given for whooping cough.
Pandanus fascicularis Lam.	Pandanaceae	Flowers are used in perfumes. Leaves are useful in making mats and baskets.
Pedalium murex L.	Pedaliaceae	The mucilaginous infusion formed from leaves, fruits or seeds in water or milk is used in the treatment of urino-genital diseases such as Gonorrhoea, dysuria etc.
Phoenix paludosa (L.) Roxb.	Arecaceae	Fruits are edible. Popularly used as thatching material and in fencing.
Phoenix sylvestris (L.) Roxb.	Arecaceae	Dried leaves are used as brooms. Fruits are eaten after ripening. It is also used in fencing.
Phyla nudiflora (L.) Greene.	Verbenaceae	Fresh plant paste or poultice is applied as sappurent for boils, swollen cervical glands and chronic indolent ulcers.
Pongamia pinnata (L.) Pierre	Fabaceae	Dried flowers decoction is given to diabetics,seed oil in scabies, leucoderma.
Prosopis juliflora (Swartz.) DC.	Mimosaceae	The leaves having insecticidal effect.

Ricinus communis L.	Euphorbiaceae	Seed oil gel is useful in dermatitis; protective in occupational eczemas and dermatitis.
Saccharum spontaneum L.	Poaceae	Grass is used as fodder; also used for thatching and for making ropes.
Salicornia brachiata Roxb.	Chenopodiaceae	Leaves and young shoots are eaten.
Salvadora persica L.	Salvadoraceae	Plants are used for making salads and are often taken as fried snacks with rice.
Tamarix troupii Hole	Tamaricaceae	Used as remedy of ulcer.
Tephrosia purpurea (L) Pers.	Fabaceae	Excellent medicine for eczema when applied with turmeric.
Tephrosia villosa (L.) Pers.	Fabaceae	Root paste and powder is effective for brushing the teeth and also applied for the relief of pain of Scrotum.
Thespesia populnea (L.) Soland ex. Corr.	Malvaceae	Roots are used for relif from Cholera and dysentery.

have been dealt in this paper. The economic value of sand dune plants as potential source of food, fodder and pharmaceuticals have been highlighted by Sridhar and Bhagya (2007). The sand dune species of costal West Bengal are extremely important resources, which play a vital role in the economic and social life of local inhabitants. Conservation and judicious utilization of the costal plant wealth, which have been degraded considerably due to over-exploitation, clearing of forest for industrialization, rapid urbanization, pisciculture and human settlements have been suggested. Besides, it is essential to undertake detailed phytochemical investigation to validate ethno- botanical knowledge possessed by the local people.

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Eragrostis pilosa (L.) P. Beauv (Poaceae): An addition to the grass flora of Andaman and Nicobar Islands, India

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ABSTRACT

Eragrostis pilosa (L.) P. Beauv (*Poaceae*) collected as new addition to the grass flora of Andaman and Nicobar Islands. Eight species of *Eragrostis* have been enlisted in earlier reports from this region. The paper embodies vivid description, photographic plate, illustration along with distribution and taxonomic status.

During a botanical exploration to South Andaman, numerous species of Poaceae were collected and documented. After critical dissection, herbarium consultation and thorough study of relevant literature (Pandey & Diwakar, 2008; Bor, 1960, Kabeer & Nair, 2009) it was found that *E. pilosa* was neither previously reported nor collected from this region. Thus, the current collection forms the first record of the species for the grass flora of Andaman and Nicobar Islands.

Members of *Eragrostis* Wolf. occur mainly in the tropical and temperate regions worldwide. The genus is represented by 423 species (Giraldo-Canas et al., 2012, among which 44 species are found in India (Kabeer & Nair, 2009, Vivek, 2014) and 8 species in Andaman and Nicobar Islands.

Eragrostis pilosa (L.) P. Beauv, Esc. Agrostogr. 71, 162, 175. 1812; Stapf in Hook.f. Fl. Brit. India 7: 323. 1896; Rang & Tadul, Handb. S. India Grasses 225. 1921; Gamble Fl. Madras 3: 1827. 1934; Bor, Grass. Burma Ceylon India Pakistan 512. 1960; Sharma *et al.,* in Biol. Mem. 2 (1 & 2): 164. 1977; Britto & Matthew, Fl. Tamil Nadu Carnatic 3(2): 1856. 1983; Matthew, Fur. III. Fl. Tamil Nadu Carnatic 4: 778. 1988; Mayur., Flow. Pl. Madras City. 296. 1994 (rev.ed.); Moulik, Grass. Bamb. India 2: 606. t. 134. 1997; Daniel & Umamahesh, Fl. Gulf Mannar 550. 2001; Pallith; Pock. Fl. Sirumalai Hills 286. 2001. *Poa pilosa* L., Sp. Pl. 1: 68. 1753.

Annual. Culm 10 – 65 cm; erect to geniculate; node glabrous, blackish purple; internode 2 – 20 cm, cylindrical, glabrous, olive green. Leaf blade 1 – 18 cm × 0.2 – 0.3 mm, lanceolate, apex acute, veins at lower surface serrulate; ligule 0.1 – 0.2 mm, membranous, apex fimbriate; leaf sheath 2 – 5 cm × 2 – 3 mm, mouth bearded with glandular hairs. Panicle 3 – 30 × 0.3 – 7 cm. Spikelets 0.2 – 1.5 cm × c. 0.4 cm, pedicelled; florets 3 – 29; lanceolate, apex acute; rachis 1 – 5 cm, angular, denticulate; rachilla 2 – 12 cm; pedicel 1 – 8 cm, denticulate. Lower glume 0.3 – 1 mm × 0.1 – 0.5 mm, membranous, glabrous, margin entire, mid-nerve prominent, slightly raised, dentate. Upper glume 0.4 – 1.4 × 0.2 – 0. 6 mm, linear to lanceolate, apex acute, membranous, margin entire, mid-nerve prominent, denticulate. Lemma 0.8 – 2 × 0.4 – 1.2 mm, ovate to

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lanceolate, apex acute to attenuate, margin entire, 3 nerved, mid-nerve prominent, denticulate. Palea 0.4 – 1.8 × 0.1 – 0.3 mm, elliptic, apex acute, membranous, hyaline, margin entire, infolded, 2 nerved, 2 keeled, keels denticulate. Lodicules 2, 0.1 – 0.2 mm, membranous. Stamens 3, 0.4 – 0.6 mm long, brownish white. Ovary 0.1 – 0.2 mm × c. 0.1 mm, yellowish; stigma 0.3 – 0.5 mm, plumose, white. Caryopsis c. 0.8 × 0.4 – 0.5 mm, elliptic to oblong, brownish. (Fig.1).

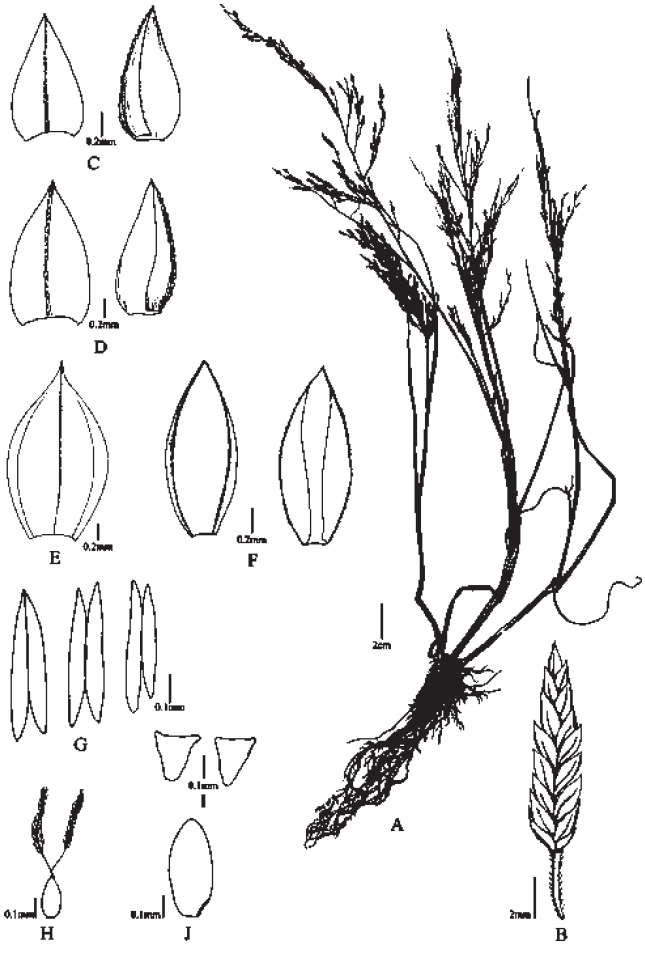

Fig.1 *Eragrostis pilosa* (L.) P. Beauv. : A. habit; B. Spikelet; C. lower glume; D. upper glume; E. lemma; F. palea; G. anthers; H. pistil; I. lodicules; J. caryopsis.

Flowering & Fruiting: May – March

Habitat & Ecology: The species was observed growing on road sides, in open grasslands near Sippighat, Port Blair, South Andaman. The associated species were *Eragrostis uniloides, Echinochloa colonum* and other seasonal grasses.

Distribution: INDIA: Andhra Pradesh, Arunachal Pradesh, Bihar, Goa, Gujarat, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh (Kabeer & Nair, 2009) and Andaman & Nicobar Islands (present study).

Species examined: INDIA: Andaman and Nicobar Islands, Port Blair, Sippighat, South Andaman, 16.07.2015, Longitude 92^{0}73’33" Latitude 11^{0}66’67", Reshma Lakra 32298 (PBL, CNH).

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Notes on diversity and distribution of genera *Pteris* Linn. and *Pteridium* Gleditsch ex Scopoli in Odisha

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ABSTRACT

Three species of *Pteris* and one species of *Pteridium* Gleditsch ex Scopoli are reported as new distributional records for Odisha state from Similipal Biosphere Reserve. Correct botanical name, diagnostic features, phenology and ecology of each species has been provided.

The taxonomic study of pteridophytes of Odisha has not been given due attention and no comprehensive account is yet available. However, Haines (1924), Mooney (1950), Datta *et al.* (1985), Saxena and Brahmam (1994-96) and Panigrahi (1998) have reported occurrence of some ferns and fern allies of different parts of the state. A comprehensive account of the ferns of Koraput district was provided by Das *et al.* (1989). Biswal *et al.* (2011) and Sahu *et al.* (2013) reported the occurrence of two additional species from within the geographical boundary of Odisha.

The authors conducted intensive field work for about five years in the Similpal Biosphere Reserve and reported the occurrence of as many as 99 taxa belonging to 31 fern families. Out of which, three species of *Pteris* (Pteridaceae) and one taxon namely *Pteridium revolutum* (Bl.) Nakai (Dennstaedtiaceae) turned out to be new distributional records for the state of Odisha.

Genus *Pteris* is a large and complex genus containing many apomictic and allopolyploidy taxon, but the identification has never been a problem as the diagnostic characters are constant and not too difficult to recognize. The usual features like stipe-base scale-characters are not very diagnostic in this case. Though many infra-generic categories have been described under the genus, Fraser-Jenkins (2015) considered such separations are meaningless based on the latest findings. The genus *Pteris* is represented by about 250 species, mainly distributed in tropical and sub-tropical regions of the world. In Odisha, Saxena and Brahmam (1994-96) reported seven species of Pteridaceae from the state, while, Panigrahi (1998) mentioned about the occurrence of eight species of *Pteris* from the state. During the present study, eight species of *Pteris* have been collected from Similipal alone. Three taxa namely *Pteris arisanensis* Tagawa, *P. ensiformis* Burm. f. and *P. longipes* D. Don turned out to be new distributional records for Odisha.

Since these taxa are new to the state flora, correct botanical name with author citation, brief descriptions etc. are provided below. A taxonomic account of genus *Pteris* as reported by various workers from Odisha is given in Table 1.

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1. *Pteris arisanensis* Tagawa, Acta Phytotax. Geobot. 5: 102. 1936.

Plants 1-1.5 m tall. Rhizome erect, short, 1.5-2 cm in diam., apex with black-brown scales. Fronds clustered (6-8 per plant); stipe basally brown, upper part straw-colored, slightly lustrous, as long as fronds, 3-4 mm in diam., glabrous; lamina 2- or 3-pinnatipartite, oblong-ovate in outline, 50-70 × 20-30 cm; lateral pinnae 5-15 pairs, opposite, slightly decumbent, sessile or basal pairs shortly stalked, lanceolate, 15-25 (-33) × 3-4 (-5.5) cm, base rounded-cuneate and slightly oblique, deeply pectinately divided to winged costa, apex long caudate; basal pair of pinnae often each with basiscopic pinnule similar to main part of pinnae but smaller; segments 25-35 pairs, alternate, subspreading or oblique, falcate-oblong, 20-30 × 5-8 mm, base slightly enlarged, margins entire, apex obtuse or mucronate; terminal pinna similar to lateral pinnae; costae with 6-10 mm wide wings, prominent abaxially, straw-colored, glabrous, grooved adaxially, with spines; veins conspicuous and convex on both sides, decumbent, 2-forked at base, basiscopic vein of segment base arising from rachis, and acroscopic vein from base of costa, 2 opposite veins of pinna base arriving at margin of incision and forming a fork or triangle, or sometimes interlinked into a continuous triangular mesh, and other veins outward from mesh separate and extending to base of incision; lamina green, yellowish green, or brown-green, sub-leathery when dried, glabrous.

Specimens examined: RRLB-12641, NCR & AKB, 30.04.2011. Bhanjabasa, Similipal

Ecology: In dense forests, among rock boulders in accumulated humus rich soil near streams; 100-1800 m.

Fertile: March- October.

Note: This species is very close to diploid apomictic species of *P. confusa* described from South India and Sri Lanka (misreported from Arunachal Pradesh by Singh & Panigrahi, 2005), but the range of variations of *P. confusa* includes large deltate fronds with long pinna-lobes, which do not occur in large plants of *P. arisanensis*, hence both the taxa are not conspecific.

2. *Pteris ensiformis* Burm.f., Fl. Ind. 230. 1768.

Plants 30-50 cm tall. Rhizome ascending or prostrate, slender, 4-5 mm in diam., apex with black-brown scales. Fronds dense, dimorphic; sterile fronds 1.5-2(-3) cm apart, shorter than fertile fronds; stipe and rachis straw-colored, slightly lustrous, stipe 10-30 cm (stipes of sterile fronds shorter), 1.5-2 mm in diam., glabrescent; lamina oblong-ovate, 10-25 × 5-15 cm, pinnate to bipinnate; pinnae 2-6 pairs, opposite, slightly decumbent, upper ones sessile, lower pairs shortly stalked; sterile fronds often pinnate, triangular in outline, 2.5-3.5(-8) × 1.5-2.5(-8) cm, acuminate; pinnules (1 or)2 or 3 pairs, opposite, contiguous, sessile, decumbent, oblong-oblanceolate to broadly lanceolate, basally decurrent and entire, upward and apices with acute teeth, apex obtuse; pinnae of fertile fronds distant (basal pairs 5-7 cm apart), 1-3-forked, middle fork longest, apical pinnae not decurrent at base, basal two pairs sometimes pinnate; pinnules 2 or 3(or 4) pairs, decumbent, narrowly linear, basally decurrent, margins entire except at apices, sterile parts with dense teeth, apically acuminate; midvein straw-colored, adaxially prominent; veins dense, often forked; lamina gray-green to brown-green, sometimes with nearly white bands along each side of mid-vein, herbaceous when dried, glabrous.

Specimens examined: NOU-475, AKB, 16.06.2007, Upper Barakamda; RRLB-12618. NCR & AKB.

Ecology: Wet acidic soils beneath forests, stream sides; 100-1000 m.

Fertile: May –Feb.

Note: The varieties described under the species show range of variation and subsequently nested in one species i.e. *P. ensiformis.* A white variegated species found in the wild are cultivated in many countries and should not create any taxonomic confusion.

3. *Pteris longipes* D. Don, Prodr. Fl. Nepal. 15. 1825.

Plants 1.2-1.5 m tall. Rhizome erect, short, 1.5-2 cm in diam., woody, apex with dark brown scales. Fronds clustered; stipe dark straw-colored to light brown, slightly lustrous, 70-80 cm, 6-8 mm in diam., firm, glabrous; lamina 3-pinnatipartite, triangular-ovate in outline, 60-70 × 35-45 cm; lateral pinnae 12-20 pairs, subopposite or alternate, oblique, sessile, lanceolate, 10-12 × 1.5-2 cm, base truncate, pectinately divided nearly to costae, apex caudate (2-3 cm), with linear lobes; segments 25-28 pairs, alternate, interlinked and slightly decumbent, oblong, ca. 10 × 3-4 mm, base slightly enlarged, apex obtuse and crenate; apical pinnule same as middle lateral ones, stalked (ca. 1 cm); costae prominent abaxially, straw-colored, glabrous, slightly grooved adaxially, with needle like spines on both sides; veins conspicuous on both surfaces, oblique, 2-forked at base, and opposite two veins oblique up to margin of incision; lamina pale green to green-brown, herbaceous when dried, glabrous.

Specimens examined: RRLB- 12666, NCR& AKB, 30.04.2011. Upper Barakamda.

Table 1
Reports on occurrence of *Pteris* species in Odisha by various workers

Odisha (Saxena & Brahmam,1996)	Odisha (Panigrahi,1998)	Similipal (Present study)
Pteris biaurita	*P. biaurita*	*P. arisanensis**
P. cretica	*P. cretica*	*P. biaurita*
P. heteromorpha	*P. heteromorpha*	*P. cretica*
P. nemoralis	*P. linearis*	*P. ensiformis**
P. pellucida	*P. nemoralis*	*P. longipes**
P. quadriaurita	*P. quadriaurita*	*P. pellucida*
P. vittata	*P. venusta*	*P. quadriaurita*
	P. vittata	*P. vittata*

* New records for Odisha

Ecology: Occasional in moist-deciduous forests as undergrowth.

Fertile: March-October.

Genus *Pteridium* has long been treated as a dubious one. All taxa reported earlier were placed under single species prior to the study by Tryon (1941), who attempted to solve the taxonomy of the genus. Subsequently, the taxonomy of the genus as occurring in South Asia and Europe was worked out by Fraser-Jenkins (1992, 1997 and 2008). By and large, the genus requires a great deal of taxonomic research. *P.aquilinum* was the sole representative of the genus in the state till date but the present work adds one more species namely, *P. revolutum* to it.

Pteridium revolutum (Bl.) Nakai, Bot. Mag.(Tokyo) 39: 109.1925; Chandra, Ferns of India (Enumeration, Synonyms & Distribution). 1-459. 2008. *Pteris revoluta* Bl. Enum. Pl. Java 214. 1828.

Plants up to 1 m tall. Rhizome long creeping. Fronds subleathery when dried, margins often revolute; stipe straw-colored or brown, 35-50 cm, 5-8 mm in diam. at base, adaxially grooved, densely clothed with pallid hairs when young, glabrous when old; lamina 3-pinnate-pinnatifid, broadly triangular or ovate-triangular in outline, 30-80 × 30-50 cm, apex acuminate; pinnae 4-6 pairs, opposite, decumbent, oblong, base subtruncate, stalked (2-3 cm), apex acuminate; basal pinnae 2-pinnate-pinnatifid, slightly triangular, 20-30 × 10-15 cm, stalked (2-3 cm); pinnules to 12-18 pairs per pinna, opposite or alternate, spreading, sessile, lanceolate, 6-8 × 1-1.5 cm, base truncate, not adnate to costule, deeply pinnate, apex shortly caudate-acuminate; ultimate pinnules ca. 20 pairs per pinna, opposite or alternate, slightly obliquely spreading, lanceolate-falcate, ca. 8 × 3 mm, base connate to costule, often entire, apex obtuse or acute; apical pinnae 2-pinnate-pinnatifid, lanceolate; segments approximate, with pallid or light brown hairs abaxially; veins prominent abaxially, grooved adaxially; rachises, costae, and costules approximate, with pallid or light brown hairs or verrucose, glabrescent.

Specimens examined: RRL-B-12686, NCR& AKB, 31.05.11, Jenabil, Similipal

Ecology: Sunny slopes, open grassland on forest fringe.

Fertile: May –Sept.

Note: *P. aquilinum* as reported from various parts of India, so also from Similipal is considered to be an European species (Fraser Jenkins, 2015), but further study is necessary to conclude its taxonomic and ecological stance. Both *P. revolutum* and *P. aquilinum* are highly toxic and carcinogenic due to the presence of cyanide Thiaminase and the carninogen Ptaquiloside, which is often passed in to the milk by cattle eating this bracken fern. It may be fatal to livestock grazing on them and the human beings as well.

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