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# Ecotype diversity of numerical and structural chromosomes of *Drimia indica* (Roxb.) Jessop (Hyacinthaceae) as revealed from karyotype analysis

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ABSTRACT

*Drimia indica* (Roxb) Jessop commonly known as Indian squill, a perennial medicinal bulbous plant, is used in traditional medicine. Cytological studied for four ecotypes were analyzed from Odisha viz. ODi-16 (Khandapada), ODi-22 (Nayagarh), ODi-24 (Daspalla), ODi-26 (Odagaon) and found that diploid chromosome number 2n=20 with a anuploid number from Nayagrah (2n=16). Detailed karyotype analysis showed structural chromosome variations among the ecotypes and high number of secondary constricted chromosomes are available in comparatively higher altitude (178m) from Nayagarh area. Chromosome length varied from 196.36 µm in ODi-22 (Nayagrah) to 211.39 µm in ODi-26. Karyotypes showed more variations in Type A and Type B chromosomes as compared to Type C (Median constricted chromosomes) and Type D (Sub median constricted chromosomes). TF% varied from 31.37% to 34.28%. The occurrence of natural cytological abnormalities with sticky bridge formation, early separation, lagerds, DNA fragmentation in the ecotypes might be due to microevolution and stabilization of chromosomes for adaptation. Chromosome polymorphism could be an important parameter along with morphology and other DNA markers for phylogenetic analysis and species evolution.



## 1. Introduction

Indian squill, *Drimia indica* (Roxb) Jessop (*Syn. Urgenia indica* Kunth.) of the family Hyacinthaceae, is a perennial medicinal bulbous plant. In traditional medicine, bulbs of *D. indica* are having important therapeutic uses. This genus is extremely polytypic genus comprising of hundreds of species occurring in India, Africa and Mediterranean region (Ahmed *et al*., 2006). Nine species are commonly seen in India (Hemadri and Swahari, 1982) which contradicts with taxonomic revision by Deb and Dasgupta (1987), who recognized five species in India. It is reported to be used in chronic bronchitis and asthma, anthelmintic, cardio-tonic in heart insufficiency, deobstruent, digestive, expectorant, stomachic, diuretic (Blumenthal *et al*., 1998). It is also used in rheumatism, leprosy, skin diseases, internal pain and scabies (Kirtikar and Basu, 1988). Out of the phytochemicals, the glycosides, scillarin-A and scillarin-B have been reported in fresh squill (Prajapati *et al*., 2003). Other constituents found in squill include flavonoids, carbohydrates, antifungal glycoproteins, steroids, alkaloids, tannins, coumarins and saponins (Abbas *et al*., 2012; Siva Kameshwari *et al*., 2012, 2013; Bashir *et al*., 2013). Pharmacological evaluations revealed the presence of antibacterial, antifungal (Shenoy *et al*., 2006), laxative and spasmodic (Abbas *et al*., 2012), antioxidant, antiangiogenic and pro-apoptotic activities in *U. indica* (Deepak and Salimath, 2006). Since Indian squill bulbs have long been used as a source of natural product with pharmaceutical and biocidal applications, numerical and structural alteration of chromosomes play a major role in polyploidization and thus variation in phytochemical constituents.

The genus *Drimia* (*Syn. Urginea*), wild onion, has been studied for biosystematics (Shiva Kameshwari *et al*., 2012); cytogenetics (Raghavan, 1935; Sen, 1974; Jha and

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Sen, 1983a, b; Subramanian, 1987; Dixit and Yadav, 1989; Yadav and Dixit, 1990) and DNA markers (Harini *et al*., 2008, Kawalkar 2010, Desai *et al*., 2012; Alluri *et al*., 2015). Ethno-medicinally bulbs of *D. indica* are reported to be antiulcerous, antinematodal, antitumorous, antiarthrities properties and also used to warts, abscesses, boils of skins, cardiac diseases, antidote to scorpion sting (Chittoor *et al*., 2012). The bulbs contain literally hundreds of phytocompounds that defend cells against free radical damage by blocking the development of heart diseases, cancer, rheumatism, edema, gout, asthma, dog bites, cut wound, infertility in man. Due to these medicinal properties of *D. indica* bulbs have been exploited commercially from natural habitats. As per IUCN criteria, threat status of *D. indica* is vulnerable for states like Chhattisgarh and Madhya Pradesh. Phylogenetic problems are being reported using morphology and cytology, DNA and protein, mitochondrial and nuclear genomes (Nylander *et al.,* 2004; Shiva Kameshwari, 2013).

Hence, it is necessary to initiate awareness, conservation and cultivation of Indian squill. Anthropogenic pressures such as habitat degradation are largely responsible for genetic depletion and loss of genetic diversity (Shiva Kameshwari *et al*., 2012). Therefore, genetic analysis of ecotypes is quite important for its active principle variation and there is urgent need for conservation and sustainable utilization of this economically important medicinal plant. The potential ecotype variation is very high in Odisha which is unexplored and that create interest for cytological study from various ecological area for karyotype and chromosomal analysis to ascertain the ploidy changes, if any, and possible role of genomic plasticity in adaptation of the plant and its implication in active principle accumulation.

## 2. Materials and methods

### *2.1. Cytological analysis*

Four ecotypes of *Dremia indica* were collected from different parts of Odisha and maintained in green house of Department of Botany, Utkal University, Bhubaneswar (Table 1). Actively growing root tips (1.5-2 mm) were pre-treated in half saturated para dichlorobenzene (pDB) and aesculin mixture (1:1) for 4 h at 18$^{o}$C in refrigerator and then fixed in 1:3 acetic acid:ethanol overnight at room temperature. Fixed roots were treated in 45% glacial acetic acid for 15 min. Root tips were stained in 2% aceto-orcein followed by cold hydrolysis with 5N HCl at 4$^{o}$C for 5 min. Chromosome squash were made using 45% glacial acetic acid and were observed under Olympus BX-53 microscope. Digital microphotographs were captured in Micro Publisher 5.0 RTV camera using QCapture Pro 7 (Canada) software for karyotype analysis.

Total chromosome length was estimated by adding the length of all chromosomes in the karyotype by applying formula ðr$^2$h, where 'r' is the radius and 'h' is the length of the chromosome respectively. Analysis of the chromosome type was conducted according to Levan *et al*. (1964), and that of the karyotype in accordance with the classification standard of Stebbins (1971) modified by Das and Mallick (1993). Form percentage (F %) of individual chromosome was calculated.

## 3. Results and discussion

Chromosome numbers of all the four cultivars showed 2n = *2x* = 20 except ODi-22 collected from Nayagarh with 2n=16 chromosomes. The size variation of chromosomes within the karyotype was obtained in all the ecotypes ranged from small to large size. All the somatic chromosomes are classified as Type A with comparatively large chromosomes having nearly median (nm) primary and nearly median or sub-median (nsm) secondary constrictions; Type B with medium sized chromosomes having nearly sub-median (NSM) primary constriction and nearly sub terminal (nst) secondary constriction; Type C with medium size chromosome having nearly median primary constriction (nm) and Type D with small to medium size chromosomes having nearly sub-median (nsm) primary constriction (Table 1). Although all the ecotypes showed numerical variation in diploid somatic chromosome number with four Types of chromosome, the numerical differences of different Types of chromosomes were recorded which was revealed by karyotype formulae of all the genotypes (Figs. 1 – 4, 1a-4a) showing definite differences in their chromosome structure recorded as follows;

Ecotype ODi-16 (Khandapara)

The karyotype formula of ODi-16 was assigned to be $1A_{nm,nsm}+1A_{nm,nst}+2B'_{nsm,nst}+1C_{nm}+5D_{nsm}$ with 2n = 20 chromosomes (Figs. 1,1a). The total chromosome length was found to be 176.72 µm with a total form percentage of 33.35%.

Ecotype ODi-22 (Nayagarh)

The karyotype formula of ODi-22 was assigned to be $2A_{nm,\ nsm}+2B_{nsm,nsm}+1B'_{nsm,nst}+1C_{nm}+2D_{sm}$ with 2n = 16 chromosomes (Figs. 2, 2a). The total chromosome length was found to be 163.64 µm with a total form percentage of 34.28%.

Ecotype ODi-24 (Daspalla)

The karyotype formula of ODi-24 was assigned to be $2A_{nm,\ nsm}+1B_{nsm,\ nsm}+1C_{nm}+6D_{nsm}$ with 2n = 20 chromosomes (Figs. 3, 3a,). The total chromosome length, volume, TF%

Table 1. Detailed karyotype analysis of the four ecotypes of *D. indica* with different chromosomal parameters.

| Ecotype | Place of collection | Latitude/longitude/ altitude | Somatic chromosome number (2n=2x) | Karyotype formula | NSC+ | Total chromosome length (µm±SE) | Total F% |
|---|---|---|---|---|---|---|---|
| **ODi-16** | Khandapada | 20.26°N, 85.17°E, 65m | 20 | $1A_{nm,nsm}+1A'_{nm,nst}+2B'_{nsm,nst}+1C_{nm}+5D_{nsm}$ | 4 | 176.72±2.80 | 33.35 |
| **ODi-22** | Nayagarh | 20.12°N, 85.10°E, 178m | 16 | $2A_{nm,nsm}+2B_{nsm,nsm}+1B'_{nsm,nst}+1C_{nm}+2D_{nsm}$ | 5 | 163.64±3.43 | 34.28 |
| **ODi-24** | Daspalla | 20.33°N, 84.85°E, 122m | 20 | $2A_{nm,nsm}+1B_{nsm,nsm}+1C_{nm}+6D_{nsm}$ | 3 | 196.36±3.55 | 32.74 |
| **ODi-26** | Odagaon | 20.02°N, 84.98°E, 32m | 20 | $3A_{nm,nsm}+1B_{nsm,nsm}+1C_{nm}+5D_{nsm}$ | 4 | 211.39±4.58 | 31.37 |

+ NSC = Number of secondary constricted chromosome

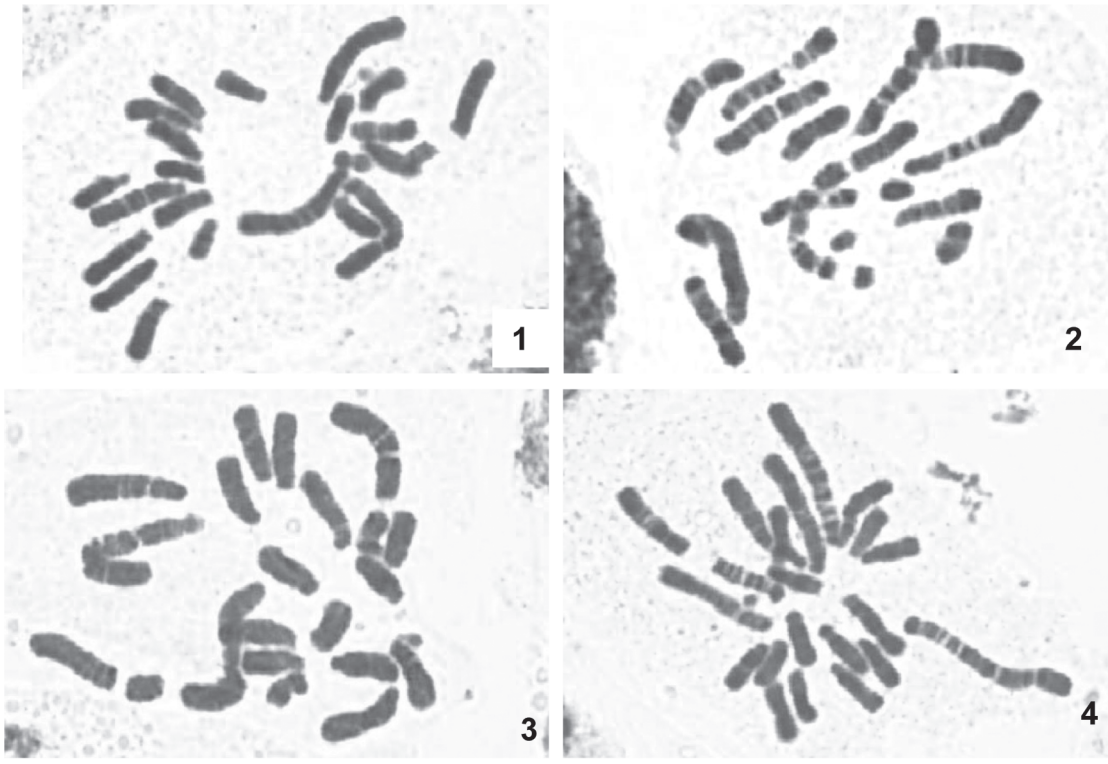
and INV were found to be 196.36µm and 32.74 % respectively.

Ecotype ODi-26 (Odagaon)

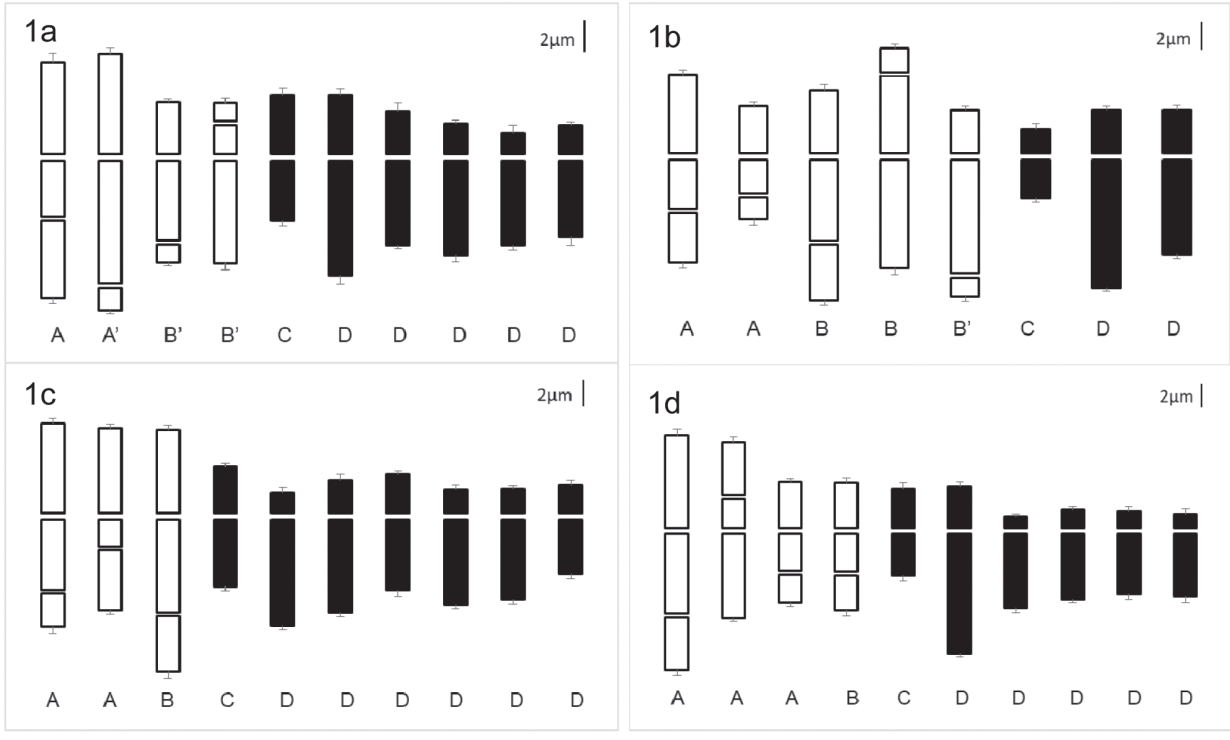
The karyotype formula of ODi-26 was assigned to be $3A_{nm,nsm}+10_{nsm,\ nsm}+1C_{nm}+5D_{nsm}$ with 2n = 20 chromosomes (Figs. 4, 4a). The total chromosome length was found to be 211.39 µm with a total form percentage of 31.37 %. The ideogram of the genomic constitutes varied significantly as depicted in Figs. (1a-4a).

The chromosome structure and genomic length variation as evident from karyotype and idiogram (1a-4a) confirms the structural alteration of chromosomes and stabilization of cytotypes in ecotypic level. However, heteromorphicity in respect of centromeric position indicated the occurrence of a deletion at the short arm of the respective chromosome was noticed in *Allium* species (Mahbub *et al.,* 2014) having distinct CMA- and DAPI-banding patterns to localize intensity and percentage of GC- and AT-rich repeats. Differences in chromosome length might be due to differential condensation and spiralization of the chromosome arms and species-specific compaction of DNA threads along with nucleosomes (Das and Mallick, 1989) with altered non-histone proteins (Chattopadhyay and Sharma, 1990). The alteration in the TF% might be due to chromosomal alteration due to break and reunion of the chromosome arms in early stages of evolution in the genome. Duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution might be the reason there is a structural alteration of the chromosome morphology as well as the variation of secondary constricted chromosomes in the above ecotypes (Das and Das, 1994; Rai *et a*., 1997; Ghosh *et al.,* 2013; Das *et al.,* 2015). High TF% in all the ecotypes indicates the alteration of chromosome structure in the genome. These factors indicate greater genome stability conferring resistance to the cultivars against biotic or abiotic environmental stresses which is a characteristic feature of ecotypes that need to confirm in future by fluorescent *in situ* hybridization (FISH) or genomic *in situ* hybridization (GISH) as shown (Doležel *et al.,* 2004, Jeridi *et al.,* 2011, Nath *et al*., 2015).

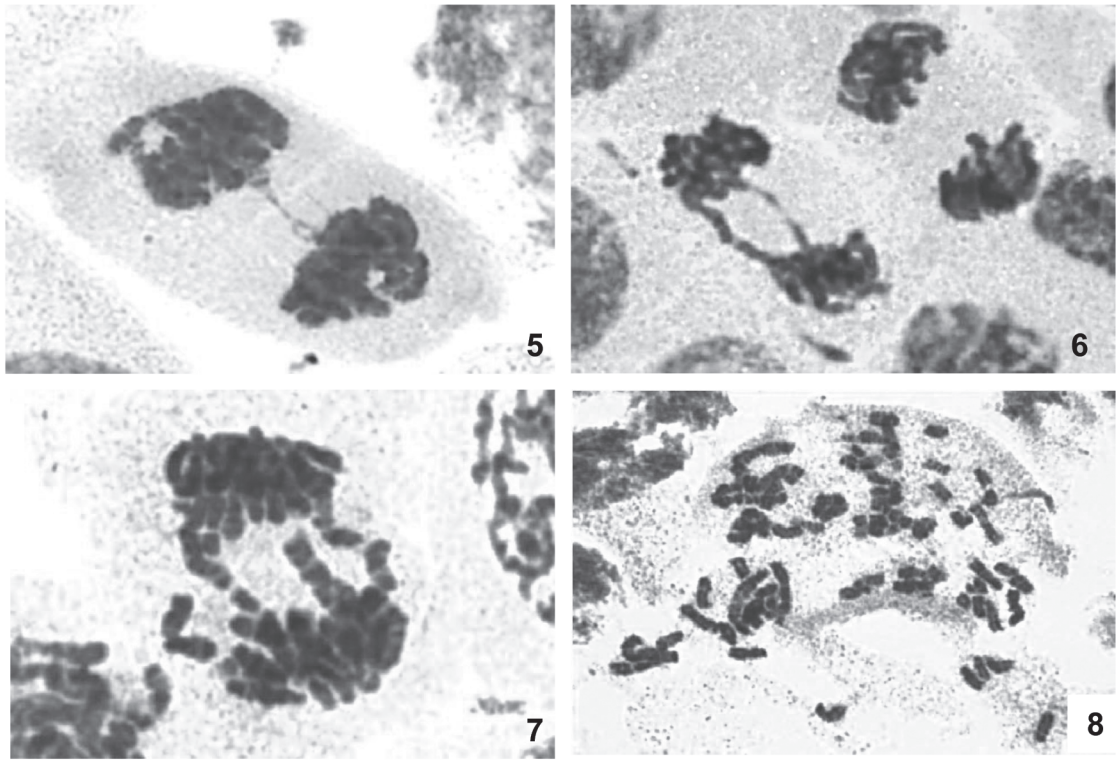
Chromosome polymorphism within species is often ignored by systematic botanist and comparative evolutionary biologists. However, the cytocypic variation are found in this study might be due to macroevolution within the species. The occurrence natural cytological abnormalities with sticky bridge formation (Fig. 5), early separation (Fig. 6), lagerds (Fig. 7), DNA fragmentation (Fig. 8) in some of the ecotypes might be due to microevolution and stabilization of chromosomes of the species in a specific condition for adaptation. Polymorphism may have profound impact on phylogeny reconstruction, species delimitation, and studies of character evolution as suggested by John (1999). The interpopulation variation pattern found in *D. indica* is very complicated and difficult to conclude. The morphological complexity is accompanied by a high degree of cytological variation has been reported earlier (Jha and Sen, 1983a, b, Shiva Kameshwari *et al*., 2013, Nath, 2015). *D. indica* seems to have high genomic and phenotypic plasticity and phylogeny. Of the four studied ecotypes the vegetative character could not show great variations but anuploids number 2n=16 was obtained in ecotypes from Nayagrah (ODi-22). On the other hand, the reproductive characters have shown less variation and are almost uniform. Cytological studies recorded the presence of diploid and aneuploid populations (Figs. 1-4) 2n = 16 was new records




Figs. 1-4. Metaphase plates of four ecotypes of *D. indica* of Odisha; (1) Khandapada, (2) Nayagarh (3) Daspalla (4) Odagaon. Magnification bar = 5 µm.




Figs. 1a-4a. Idiogram of four ecotypes of *D. indica* of the corresponding metaphase plates.




Figs. 5-8. Chromosome aberration are found along with root tips cells (5) Sticky bridge (6) Early separation of chromosome (7) Lagard chromosomes (8) Chromosome fragmentation.

for the species. These morphological variations along with cytological variations and similarities are considered responsible for designating them as cytotypes. But there may be a chance of variation in secondary metabolites which need to be studied. Such type of investigations on variations would not only indicate the principal feature of evolution within species but may also lead to exploitation of certain distinct genotypes for commercial purposes. Several populations are recorded with ploidy and anuploidy with connecting link from tetraploid 2n = 40 to diploid 2n = 20 (Shiva Kameshwari *et al*., 2010, 2013).

## 4. Conclusion

Cytological studied for four ecotypes of *Drimia indica*, commonly known as Indian squill, a perennial medicinal bulbous plant, ODi-16 (Khandapada), ODi-22 (Nayagarh), ODi-24 (Daspalla), ODi-26 (Odagaon) were under taken. Diploid chromosome number 2n=20 was found in all ecotypes except a anuploid 2n=16 from Nayagrah. Detailed karyotype analysis showed structural chromosome variations among the ecotypes and high number of secondary constricted chromosomes. TF% also varied from from sub-median to nearly median type. The occurrence of natural cytological abnormalities might lead to cytotypes during microevolution.

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# Substitution of BAP with meta-Topolin (m-T) in multiplication culture of *Musa* species

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ABSTRACT

Meta-Topolin (mT), a benzyladenine analog [N 6-(3-hydroxybenzylamino) purine], is a highly active cytokinin. Benzyladenine (BA) is the most widely used cytokinin in the micropropagation industry due to its effectiveness and affordability. The effect of the cytokinin (meta-Topolin) on micropropagation of banana cultivar Patakpura was studied and compared to BA (6-benzylaminopurine). *In vitro* cultures were sub-cultured on MS media containing BAP and m-T. Results obtained after six weeks of growth showed that there were statistically significant differences among the parameters analyzed for different treatments. Higher multiplication rates were recorded with cultures treated with m-T compared to BAP.



## 1. Introduction

Bananas and plantains (*Musa* spp.) are among the most important fruit crops in the world and are staple food for millions across the globe (FAO, 2010). Presently banana is grown in around 150 countries across the world on an area of 50.34 million hectare producing 106.84 million tonnes (FAO, 2015). World total banana and plantain production ranks at the 5th place after cereals, and there is still much scope for yield improvement (Jain *et al.*, 2004). It represents an essential source of nutrients for millions of people, particularly in tropical and subtropical regions, as well as a cash crop in many developing countries. In general, banana cultivars are considered as good sources of carbohydrates, proteins, vitamins and minerals (Anbazhagan *et al.,* 2014). Banana is generally propagated vegetatively through suckers, which grow from lateral buds originating from corms and suckers. Thus, banana propagation through conventional method using young shoots or part of the tuber is not an ideal method. This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material (Hussein, 2012).Tissue culture plants have been reported to produce 39% higher yield than plants from sword suckers (Pradeep *et al*., 1992) likely performed better than in banana (Faisal *et al*., 1998). Shoot apex, nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al*., 2001) and cytokinin helps in shoot multiplication (Cronauer and Krikorian, 1984a). Growth regulators namely auxin, cytokinin, gibberellin and abscisic acid like kinetin, indole-3-acetic acid, benzyl-aminopurine etc were used for the *in vitro* regeneration of various plants (Ali *et al*., 2014 and 2015; Momena *et al*., 2014). Cytokinins such as benzyl-aminopurine (BAP) are known to reduce apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana (Madhulatha *et al*., 2004). Effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different banana cultivars (Buah *et al*., 2010; Farahani *et al*., 2008; Rahman *et al*., 2006; Resmi and Nair, 2007).Meta-

Topolin is first isolated from poplar leaves. Meta-Topolin is a natural constituent of plant tissues, together with its 9-â-D-ribofuranosyl and 9-â-v-glucopyranosyl derivatives. Meta-Topolin is more active than zeatin and benzyladenine in the promotion of shoot formation in plant tissue cultures (Werbrouck *et al*., 2008). The aim of this work was to assess the potential of meta-Topolins as alternatives to BA in banana tissue culture.

## 2. Material and methods

The present investigation was carried out to study the effect of meta-Topolin during multiplication culture of *Musa* species through tissue culture in the Banana Tissue culture Laboratory, Regional Plant Resource Centre, Bhubaneswar.

Patakpura variety is a locally available cultivar of banana found in coastal region of Odisha. It is very popular for its sweet taste and pleasant flavour. Tissue cultured plants of banana variety Patakpura were taken as source of explants. Aseptically established *in vitro* plantlets were cut transversely to separate leaves and produce a section of pseudostem approximately 1 cm in length, including an intact vegetative bud. The lower part of the pseudostem was trimmed to remove darkened or necrotic tissues and the sheath removed carefully by peeling. The explants were then cut into half longitudinally.These explants were cultured in bottles containing modified Murashige and Skoog (1962) including macro- and micro-elements (Vuylsteke, 1998) with growth regulators such as auxin (IAA), cytokinin (BA) and (meta-Topolins) m-T. After adjusting the pH to 5.8, the media was autoclaved at 121°C and 103 kPa for 20 min. An air conditioned culture room with controlled temperature of 25 ± 2ºC and light intensity of 2000-3000 lux for a photo-period of 16 h of light by cool white fluorescent tubes was used to incubate the tissue cultured plantlets. The photo-period was controlled manually by turning on / off the light switches. Artificial lighting was provided by providing cool-white, fluorescent tubes to carry out most of the micropropagation work. Sub-cultures were made at intervals. After inoculation, observationsof the growth responses, multiplication efficiency of BAP and m-T were compared.

## 3. Results and discussion

In this experiment, banana var. Patakpura was used to study the effect of naturally occurring PGR (Plant Growth Regulator) and synthetic PGR on multiplication culture. During this 4$^{th}$ multiplication culture of the Patakpura variety, eight treatments (T1 to T8) of Basal MS Medium was used with different concentrations of BAP+ IAA and BAP +m-T. During 7-14 days of multiplication culture, less contamination of cultures was observed. During this period explants turned green in colour and clusters of 3-4 proliferating buds with 1-3 auxiliary buds were regenerated from the basal parts of explants around the meristematic region, which is shown in Table 1.

After 15 days of inoculation, a combination of BAP + IAA or BAP + m-T) in 5$^{th}$ multiplication culture of Patakpura exhibited differential effects on shoot length, number of shoots and fresh weight. Interactive effect of BAP and synthetic hormone m-T was observed among different parameters in multiplication culture. The MS basal media containing BAP+ mT at a concentration of 3mg + 0.1mg showed significant increase in high fresh weight (6.75 gm) compared to treatment of BAP + IAA (2.96gm). In terms of shoot number, medium with 3 mg BAP + 0.1 mg m-T resulted in production of 3.79 shoots in comparison with the combination BAP + IAA (3.1).

Table 1

Effects of different concentration of BAP, m-T and IAA on multiplication culture of banana var. Patakpura

| Code | Treatment | Concentration | Mean Fresh Weight (gm) | Mean Shoot Length (cm) | Mean no. of shoots/explant |
|---|---|---|---|---|---|
| T1 | BAP + m-T | 1mg + 0.1mg | 3.90 ± 0.54 | 2.14 ± 0.54 | 6.10 ± 1.19 |
| T2 | BAP + m-T | 2mg + 0.1mg | 4.25 ± 0.47 | 2.75 ± 0.18 | 6.78 ± 1.15 |
| T3 | BAP + m-T | 3mg + 0.1mg | 6.75 ± 0.28 | 3.79 ± 0.60 | 7.80 ± 1.13 |
| T4 | BAP + m-T | 4mg + 0.1mg | 3.65 ± 0.64 | 2.79 ± 0.69 | 6.4 ± 1.30 |
| T5 | BAP + m-T | 1mg + 0.1mg | 2.96 ± 0.79 | 2.89 ± 0.15 | 5.10 ± 0.69 |
| T6 | BAP + m-T | 2mg + 0.1mg | 3.16 ± 0.69 | 2.23 ± 0.45 | 5.40 ± 1.71 |
| T7 | BAP + m-T | 3mg + 0.1mg | 3.80 ± 0.53 | 3.14 ± 0.78 | 5.95 ± 1.05 |
| T8 | BAP + m-T | 4 mg + 0.1mg | 3.76 ± 0.46 | 3.18 ± 0.57 | 5.20 ± 0.69 |

Figure 1: Effect of meta-Topolin with BAP in 5th subculture of multiplication stage of Patakpura variety of banana

Figure 2: Effect of BAP with IAA in 5th subculture of multiplication stage of Banana var. Patakpura

After 21 days of culture, it was observed that the Patakpura explants produced large numbers of shoot buds during multiplication culture. The culture showed higher regeneration capacity in medium containing m-T compared to IAA supplementation with BAP. From the study it was found that MS medium supplemented with BAP at the concentration of 3mg, showed highest average fresh weight (6.75 gm), highest shoot length (4.23 cm) and more number of shoot per explants (7.8) as compared to the other combinations of BAP supplemented with m-T and IAA. The combination of BAP (3 mg) and mT (0.1 mg) showed maximum proliferation compared to the treatment combination (BAP+ IAA) in 5th subculture of multiplication culture (**Figure 1 & 2**).

Meta-topolin is reported to promote *in vitro* shoot proliferation and improve quality of shoots in many plant species (Aremu *et al*., 2012). With application of such cytokinins, Mongomake *et al*. (2015) and Taylor *et al*. (2012) have found enhanced germination of somatic embryos and induction of shoot organogenesis from cotyledon explants in Cassav (*Manihot esculenta*) by Li *et al*. (1998). The effect of *meta-Topoline* on micro-propagation of banana cv. Patakpura as assessed during the present study is comparable to the results obtained by Mongomake *et al*. (2015 and Raemakers *et al*. (1993) in Cassava.

**Conclusion**

From the above result and observation during multiplication culture of banana var. Patakpura, it was observed that the presence of BAP along with IAA in the culture medium induced efficient shoot multiplication. The use of combination BAP along with IAA and m-T in MS medium was found to enhance fresh weight, average number of shoots and shoot length at a concentration of 3mg/l BAP + 0.1 mg m-T. Though the cultural processes described above have to be fully optimized, it is observed that use of m-T brings important and open up new opportunities for development of rapid plant regeneration systems with potential applications. From this experiment, it was concluded that meta-Topolin was quite effective in inducing multiple shoots, which needs further study.

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# Effect of AM *Rhizophagus irregularis* inoculation on growth and physiology of *Eleusine coracana* (L.) Gaertn. grown under Zn stress

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ABSTRACT

Heavy metals at more than threshold concentration interfere with usual physiological processes of plants. Arbuscular mycorrhizal (AM) fungi which form symbiotic association with plant root is reported to ameliorate abiotic and biotic stress. The present study was conducted to evaluate the effects of AM fungi *Rhizophagus irregularis* inoculation to *Eleusine coracana* growing under different concentration of Zinc in pot culture experiment. Plant growth parameters such as root length, shoot length and bio-mass, physiological parameters such as total chlorophyll, carbohydrate, protein, reducing sugar, free amino acid and proline content, antioxidative enzyme CAT and GPX activity were analyzed. Zn concentration of 100 ppm enhanced plant growth and physiology, where as high concentration of Zn (300 & 500 ppm) caused stress to the plant resulting in reduction in AM root colonization and spore density. However, the AM inoculation alleviated the Zn stress in all the treatments by enhancing anti-oxidation enzyme activities. The AM inoculation has, therefore, the potential to alleviate Zn stress in *E. coracana*.



## 1. Introduction

Zinc (Zn) is a heavy metal and essential micronutrient for plants (Reeves and Baker, 2000). It acts as cofactor for several enzymes such as RNA polymerases, anhydrases, dehydrogenases, oxidases, peroxidases (Cakmak, 2000) and regulates synthesis of protein, nucleic acid metabolism, Photosynthesis, carbohydrate metabolism and auxin synthesis (Palmer and Guerinot, 2009). The acute deficiency of Zn results in physiological stress with several visible symptoms like stunted growth, small leaves, chlorosis of leaves, necrotic leaf tips, sterility of spikelets etc. (Sharma *et al*., 2013). Besides, at high concentrations Zn is reported to be potentially toxic (Ozdener and Aydin, 2010). Anthropogenic releases of zinc and its compounds to the environment are from dust and fumes from mining, zinc production and processing facilities, brass works, coal and fuel combustion, refuse incineration, iron and steel production etc. (EPA 1980d; Raymond *et al*., 2011).

Mycorrhiza is the symbiotic relationship between a group of fungi and roots of higher plants (Smith and Read, 2008). Arbuscular Mycorrhiza (AM) is the most widespread mycorrhizal symbiosis in which the fungus develops hyphae, arbuscules and vesicles by entering the cortical cells of the plant roots. AM fungi provide a direct physical linkage between the soil and plant roots by their extrametrical mycelia. The AM association is reported to enhance plant tolerance to biotic and abiotic stresses (Augé, 2001; Beltrano *et al.,* 2008). The interaction between AM colonization and accumulation of toxic elements is an area of considerable interest relating to both production of safe food and bioremediation programs (Smith *et al.,* 2009).

*Eleusine coracana* (L.) Gaertn. commonly known as finger millet is one of the nutritious staple crops of India cultivated since ancient times. Its grains are rich source of calcium, magnesium, potassium, methionine and tryptophan, which is lacking in the diets of poor people living on starchy

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foods like cassava, polished rice and maize meal (Fernandez *et al*., 2003; Devi *et al*, 2014). Wheat and rice provide food security, but crops like finger millet promise nutritional security for the world (Singh and Raghuvanshi, 2012). It is a climate resilient crop and considered as a future crop in the context of climate change (Gupta *et al*., 2017). The present study was designed to assess the effect of Zn stress and AM association on growth and physiology of *E. coracana*.

## 2. Materials and methods

### *2.1 Seed collection and surface sterilization*

Seeds of *Eleusine coracana* were collected from Regional Pulse Research Centre, Odisha University of Agriculture and Technology (OUAT), Berhampur, Odisha**.** Healthy seeds were selected and surface sterilized in 0.1% mercuric chloride for 5 minutes followed by washing with sterile distilled water several times to remove the traces mercuric chloride from seed. The surface sterilized seeds were then processed for seed germination.

### *2.2 AM inocula*

*Rhizophagus irregularis* (Blaszk., Wuet, Renker & Buscot) C. Walker & A. Schüßler 2010 (Formerly *Glomus intraradices*) was procured from Ambika Biotech and Agro Services, Madhya Pradesh by the generic name Root Care containing 100000IP (IP: Infective Propagule) containing spores and hyphae per kg of the carrier material.

### *2.3 Zn treatment*

Zinc Sulphate ($ZnSO_4$) was used as the source of Zn. To prepare 1000ppm stock solution, 4.39g of Zinc sulphate was added to 1000ml water. Appropriate dilutions were made to the stock solution to get different concentration of treatment solutions.

### *2.4 Seed germination study*

For seed germination study 10 no. of surface sterilized seeds were placed in sterilized petriplates over different Zn treatment solution (0, 100, 200, 300, 400, 500 and 600ppm) saturated cotton pads with for germination. The seeds were allowed germinate at 25ÚC under darkness for 3 days.

### *2.5 Pot culture and experimental design*

Pot culture experiment was carried out in polybags containing sterilized potting mix composed of dry soil and sand (2:1, v/v) sieved through 2mm diameter sieve with organic manure (3:1, v/v). Potting mixture was sterilized by autoclaving for 1hr at 121$^{o}$C and 15psi on alternate days 3 times to make the substrate free from any mycorrhizal contamination. The basic physico-chemical properties of the potting substrate were pH: 6.3, EC: 0.052 $dSm^{-1}$, organic carbon: 16g $kg^{-1}$, Avl. N: 148 mg $kg^{-1}$, Avl. P: 93 mg $kg^{-1}$, Avl. K: 298 mg $kg^{-1}$, Zn: 1.83 mg/kg. Each poly bag was filled with 2kg of substrate mixed with 20g of AM inocula containing approx. 2000IP and 12 no. of *E. coracana* seeds were sown. After 7 days of seed germination appropriate level of Zn dissolve in water was added. Experiment was randomized with 4x2 factorial designs consisting of four Zn addition level (0, 100, 300 and 500ppm) and two inoculation treatments (non-mycorrhizal and mycorrhizal). The treatments were (1) T0 (NM): Non-Mycorrhizal + 0ppm Zn, (2) T0(M): Mycorrhizal + 0ppm Zn, (3) T100 (NM): Non-mycorrhizal + 100ppm Zn, (4) T100(M): Mycorrhizal + 100ppm Zn, (5): T300 (NM): Non-mycorrhizal + 300ppm Zn, (6) T300(M): Mycorrhizal+300ppm Zn, (7) T500(NM): Non- mycorrhizal + 500ppm Zn, (8) T500(M): Mycorrhizal + 500ppm Zn stress. Each treatment had 3 replicates and different parameters were analyzed after 45days growth.

### *2.6 Growth and morphological parameters*

For study of root and shoot length, the root and shoot were detached and individual length of root and shoot length were measured and expressed in centimeter. Fresh weight of the root and shoot were measured in electric balance. The plant materials were then kept in a hot air oven at 80ÚC for 3 hr and the dry weight was measured. Both fresh weight and dry weight expressed in g.

### *2.7 Biochemical parameters*

Biochemical parameters such as chlorophyll content (Arnon *et al.,* 1949), total carbohydrate by the Anthrone reagent method (Hedge and Hofreiter, 1962), total protein content by Lowry Method (Lowry *et al.,* 1951), reducing sugar (Nelson, 1994), Free amino acid (Moore and Stein, 1963) and Proline content (Bates *et.al*., 1973) were estimated.

### *2.8 Anti-oxidative enzyme activity*

Leaf tissues were ground to a fine powder in liquid $N_2$ and then homogenized in 2ml of 50 Mm/l potassium phosphate buffer (pH 7.0), 1mM EDTA, 2mM D-iso ascorbic acid, 2% (w/v) PVP & 0.05% (w/v) Triton X-100 using a chilled mortar and pestle. The homogenate was centrifuged at 10,000rpm for 10 min at 4$^{0}$C and the supernatant were collected and used for the enzyme assay. Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of $H_2O_2$ disappearance at 240nm (Aebi, 1984) and GPX activity was measured spectrophotometrically at 470 nm as increase in absorbance due to guaiacol oxidation

(Hemeda & Klein, 1990). The enzyme activity was expressed as U $g^{-1}$ protein.

*2.9 Estimation of mycorrhizal root colonization*

The roots were washed thoroughly and were cut (1cm), cleared in 10%KOH, bleached in $H_2O_2$ for 5 min, acidified with 2% HCl and stained in trypan blue (0.05%) as per Phillips and Hayman (1970). The level of colonization in each root segment was measured by the method of Giovannetti & Mosse (1980) which involved gentle squashing of stained root segment on a microscope slide after covering with a cover slip. The percentage of mycorrhizal colonization was estimated by following formula:

$$\text{Mycorrhizal colonization (\%)} = \frac{\text{No. of root colonized with AM}}{\text{Total no. of roots inspected}} \text{ X } 100$$

$$\text{Occurance (\%) of a particular AM structure} = \frac{\text{No. of root with a particular AM structure}}{\text{Total no. of roots colonised with AM}} \text{ X } 100$$

*2.10 Estimation of AM spore density*

AM fungal spores were extracted from potting substrate by wet sieving and decanting method of Gerdemann & Nicholson (1963) followed by sucrose density gradient centrifugation technique as described by Daniel and Skipper (1982). The AM spores were washed into a filter paper using a stream from wash bottle and the filter paper containing spores were kept in a petriplates. Isolated spores were counted over a gridded filter paper under stereo zoom microscope at 40× magnification.

*2.11 Statistical analysis*

The significant difference between parameters by the level of Zn addition and AM inoculation is statistically analyzed by two way analysis of variance (ANOVA) at $P< 0.05$ using MS excel.

## 3. Results & discussion

*3.1. Germination study*

The percentage of seed germination of *E. coracana* in different conc. of Zn was presented in Figure 1. In control condition (0ppm) there was 100% seed germination and as conc. of Zn increased there was decrease in the rate of germination. At 600ppm of Zn there was 50% seed germination, hence it was considered as LC50. The findings indicated that higher than 200ppm Zn is showing toxicity and inhibiting the germination rate. Ionic toxicity and osmotic stress caused of drastic effects of heavy metal salts on seed germination (Shaukat *et al.,* 1999).

*3.2 Mycorrhizal root colonization and AM spore density*

The mycorrhizal root colonization was not detected in non-inoculated plants. Among the mycorrhiza inoculated treatments AM structures hyphae, arbuscules and vesicles were observed (Table 1). The AM colonization increased with increasing Zn conc. at 100ppm than control but decreased at 300 and 500ppm. The formation of vesicle is drastically reduced (32%) in response to 500ppm Zn than hyphae and arbuscules. The spore density was also highest at 100ppm and again decreased with increasing conc. of Zn.

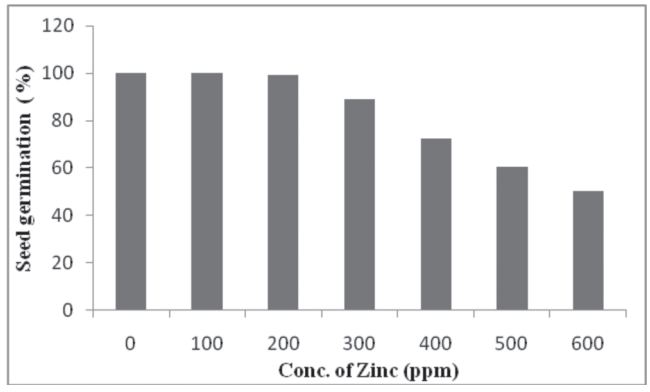


Figure 1. Seed germination under different concentration of Zn

Regarding the effects of heavy metal on mycorrhizal colonization positive, negative or neutral reports are available. AM root colonization of maize and mycorrhizal spore density in the heavy metal metal Cd, Zn, Pb, and Cu polluted field were higher than that in the uncontaminated field (Weissenhorn *et al*., 1995b) and higher mycorrhizal colonization in *Viola calaminari*a was observed in highly contaminated sites with Zn and Pb (Hildebrandt *et al*.,1999). Weissenhorn *et al*. (1995a) and Diaz *et al*. (1996) reported no correlation between AM association and the degree of heavy metal pollution in a field soil. Chao and Wang (1990) reported mycorrhizal infection rate of maize was reduced by

Table 1

Mycorrhizal root colonization (%) in *Eleusine coracana* under different treatments.

| Zn Conc. | Total AM (%) | Arbuscules (%) | Vesicle (%) | Spore Density (No. of spore /100g soil) |
|---|---|---|---|---|
| 0ppm | 95 | 88 | 84 | 116 |
| 100ppm | 100 | 89 | 68 | 138 |
| 300ppm | 90 | 77 | 61 | 105 |
| 500ppm | 86 | 73 | 52 | 94 |

the addition of heavy metals (Zn, Cu, Ni, Cr, Pb and Cd). Pb contamination inhibited mycohrrizal colonization of leguminous tress *Robinia pseudoacacia* (Yang *et al.,* 2015) and tomato plant (Chen *et al*., 2005). All these reports suggested impact of heavy metal on mycorrhizal colonization differ among host plant species. Thus, the observation of present study revealed that the *E. coracana* is sensitive to high level of Zn stress and reducing the AM colonization.

### *3.2 Growth parameters*

Growth parameters like shoot length, root length, fresh weight and dry weight showed enhancement at 100ppm Zn, but significant reduction at 300 and 500ppm Zn addition in both mycorrhizal and non mycorrhizal plants (Table 2). However, all the growth parameters were higher in mycorrhizal plants than non mycorrhizal plants. Similar results were reported in tomato (López-Millán *et al*., 2009) and barley (Wu *et al*., 2008). Enhancement of growth at 100ppm of Zn indicated that it might have compensated the Zn deficiency in the soil. The reduction in plant growth under high levels of Zn was water potential, hampered nutrient uptake and secondary stress such as oxidative stress (John *et al.,* 2009). Shetty *et al.* 1994 reported that the plant growth inhibition in zinc contaminated sites was due to interference of zinc with phosphorous uptake by plants and the application of arbuscular mycorrhizae (AM formerly VAM) fungi increased plant biomass even at elevated levels of Zn in the soil.

### *3.3 Biochemical parameters*

The total chlorophyll, total carbohydrate, reducing sugar and protein content (Table 3) in the leaves of *E. coracana* showed increase at 100ppm Zn, but there was significant decrease at higher conc. like 300 and 500ppm of Zn in both mycorrhizal and non mycorrhizal plants. Interesting to note that mentioned parameters were higher in mycorrhizal plants than non mycorrhizal plants in all the treatments. Further, total free amino acid and proline content (Figure 2 a & b) was recorded to increase in with Zn stress which was again reduced by AM association.

The reduction in chlorophyll content might be due to Zn induced oxidative stress (Gallego *et al.,* 1996). The heavy metal stress affect the synthesis of chlorophyll enzymes (Padmaja *et al.,* 1990), thereby reducing the photosynthesis of the plants and reduce the growth of plants under abiotic stress (Wu and Xia, 2006). Like other heavy metals, excess Zn reported to show marked alterations in electron transport, membrane permeability and uptake and transloca-tion of nutrient elements (Wang *et al*., 2009b). Chavan and Banerjee (1980) reported that Zn toxicity appear to be due to Fe deficiency. The absorption and translocation of plant nutrients like Fe, Mg, K, P and Ca depended on Zn concentration in soil (Cayton *et al*., 1985) and high level of Zn might have caused mineral imbalance. The findings indicated that 100ppm of Zn might have compensated the Zn deficiency of soil and resulted increased in growth and biochemical parameters, but at high conc. (300 & 500ppm) Zn have become toxic to the plant and inhibit their physiology. The improvement in plant growth and physiology with AM association at high Zn Conc. as observed in the present study was supported by Lingua *et al*., (2008)

Increase in free amino acid content with increasing Zn conc. can be correlated hydrolysis of protein to amino acid leading to decrease in protein content. Assessment of proline content is an important parameter to evaluate the effect of stress on plants (Mohanty and Patra, 2011). Proline acts as a non-enzymatic free radical scavenger, an osmo-protection (Khan *et al.* 2002) and a redox potential buffer (Molinari *et al*. 2007), thereby protecting the cells from damage. The reduction in the amount of free amino acid with AM association might be due to reduction in protein degradation. The lesser accumulation proline in mycorrhizal than non mycorrhizal plant can be related to stress amelioration effect of AM association under abiotic stress (Borde *et al*., 2011; Damaiyanti *et al*., 2015)

### *3.4 Antioxidative enzyme activity*

Findings of the present study revealed that anti-oxidative enzyme CAT and GPX activity increased in

Table 2

Growth parameters of *E. coracana* under different treatments

| Different treatments | Shoot length (cm) | Root length (cm) | Fresh Weight (g) | Dry Weight (g) |
|---|---|---|---|---|
| T0 (NM) | 34.5±1.1 | 14.8±0.37 | 2.06±0.08 | 0.831±0.03 |
| T0(M) | 36.3±1.4 | 16.1±0.40 | 2.37±0.06 | 0.852±0.01 |
| T100 (NM) | 34.8±1.2 | 15.9±0.41 | 2.15±0.04 | 0.861±0.09 |
| T100(M) | 37.1±1.7 | 17.6±0.47 | 2.31±0.06 | 0.881±0.009 |
| T300 (NM) | 32.4±1.1 | 13.1±0.31 | 1.51±0.03 | 0.799±0.01 |
| T300(M) | 35.8±1.3 | 14.7±0.44 | 1.88±0.05 | 0.82±0.07 |
| T500(NM) | 30.1±1.0 | 10.3±0.27 | 0.93±0.08 | 0.733±0.06 |
| T500(M) | 33.9±1.1 | 12.4±0.34 | 1.23±0.06 | 0.772±0.06 |

Table 3

Biochemical parameters of *Eleusine coracana* under different treatments

| Different treatments | Total Chlorophyll (mg/g FW) | Carbohydrate content (mg/g FW) | Reducing sugar content (mg/g FW) | Protein content (mg/g FW) |
|---|---|---|---|---|
| T0 (NM) | 0.969±0.023 | 36.37±0.83 | 7.13±0.36 | 9.63±0.43 |
| T0(M) | 0.983±0.027 | 37.82±0.88 | 8.79±0.49 | 10.87±0.28 |
| T100 (NM) | 0.997±0.026 | 36.89±0.76 | 9.53±0.11 | 11.23±0.33 |
| T100(M) | 1.378±0.029 | 40.11±0.9 | 10.21±0.13 | 12.54±0.39 |
| T300 (NM) | 0.672±0.026 | 25.9±0.95 | 6.43±0.23 | 6.97±0.21 |
| T300(M) | 0.796±0.031 | 30.19±0.81 | 7.76±0.16 | 8.37±0.39 |
| T500(NM) | 0.362±0.021 | 21.04±0.93 | 4.8±0.21 | 4.05±0.25 |
| T500(M) | 0.562±0.03 | 24.24±0.89 | 5.35±0.1 | 5.01±0.19 |

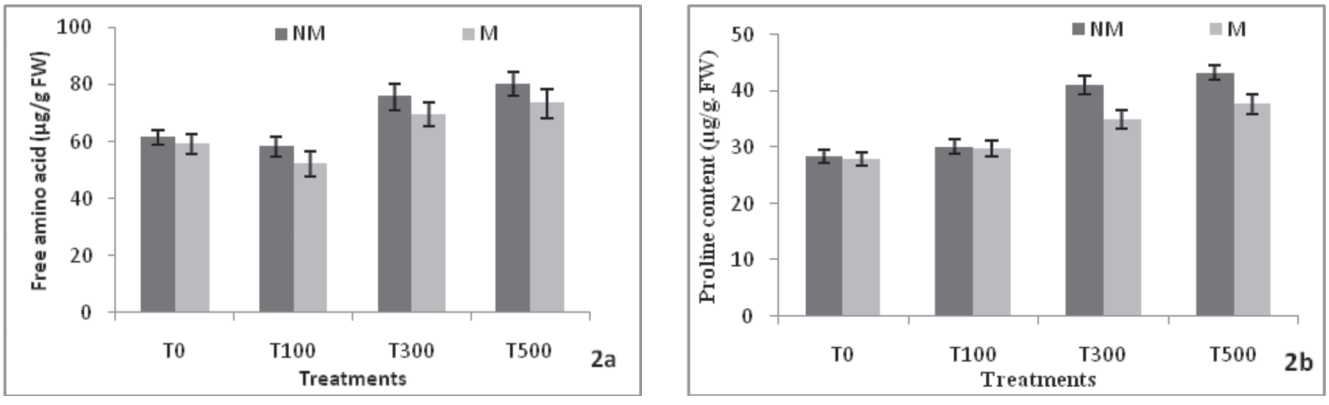


Figure 2: Free amino acid (a) and proline content (b) of *E. coracana* under different treatments

mycorrhizal plants than non mycorrhizal plant in all the treatments (Figure 3 a &b). Plants scavenge ROS generated in response to stress by stimulating antioxidant enzymes (Rout *et al*., 2017). Though Zn is essential for biological system, excess of Zn can promote generation of Fenton-type ROS (Emamverdian *et al*. 2015). AM association is reported to enhance antioxidant enzyme activity in mycorrhizal plant than non mycorrhizal plant in salinity stress (Latef, 2011), and heavy metal lead (Yang *et al*., 2015) and Zn stress (Rout *et al*., 2019). The induction of antioxidant enzymes during appressoria formation attributed to a defense response of plants during the early stage of symbiosis development (Hajiboland *et al*., 2010). The general stimulation SOD, CAT, POD and APX of plant roots associated with AM compared to non-AM roots (He *et al.*, 2007) could be related to enhanced activity of CAT and

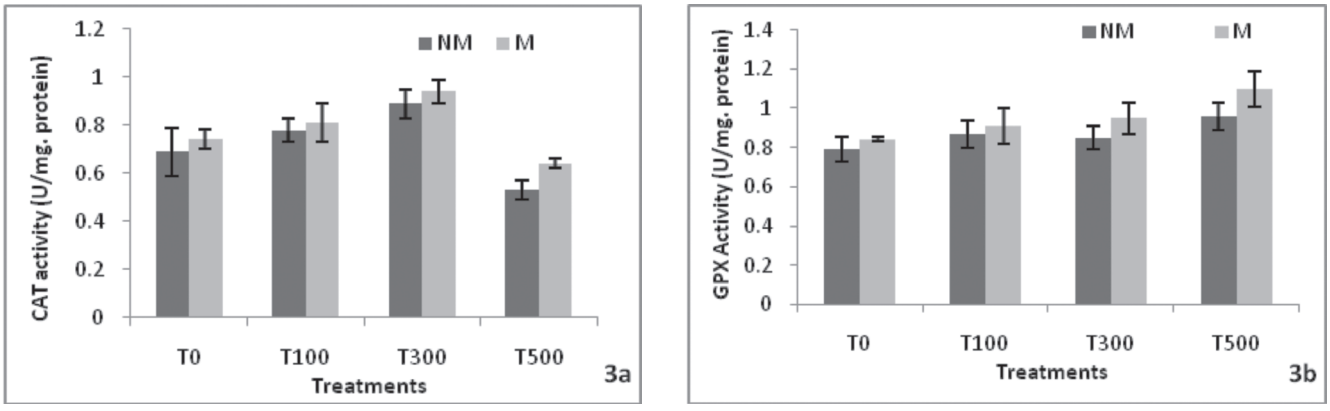


Figure 3(a-b): Antioxidant enzyme CAT (a) and GPX (b) activity in *E. coracana* under different treatments.

GPX of mycorrhizal plant in Zn stress. The CAT activity was highly sensitive to Zn stress than GPX as its activity was drastically reduced at 500ppm Zn.

## 4. Conclusion

The AM inoculation under Zn stress improves the plant growth in terms of better biomass accumulation, physiology in terms of cholorophyll, carbohydrate, protein and reducing sugar content of *E. coracana.* Enhance antioxidative enzyme activities and proline accumulation may contribute for ROS scavenging activity in mycorrhizal plants. The stress ameliorative effect of AM association is higher at low conc. (100ppm) than high conc. (500ppm) which is positively correlated to percentage of root association. The present study suggested that AM association enhanced the plant tolerance to Zn stress but very high level of stress is inhibitory for AM colonization. Further study is required to understand the AM-heavy metal interaction and mechanism of stress alleviation.

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# Contribution of wild edible plants to the food security, dietary diversity and livelihood of tribal people of Keonjhar district, Odisha

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ABSTRACT

Edible wild plants (EWPs) refer to both indigenous and naturalized exotic plants occurring in the natural environment. These plants form an important source of supplementary food in the times of food stress for native communities. They provide sustainable livelihood, food security and also play an important role in nutritional requirement of the poor tribal people of Odisha. Keonjhar district of Odisha, dominated by tribal population, occupies an important place in the mineral resource map of Eastern India and excessive mining activities have been responsible for considerable depletion of biological resources, which affect the livelihood of the poor local people. The field survey conducted during the year 2016-18 in Keonjhar district of Odisha recorded the use value of 160 species of wild edible plants belonging to 119 genera and 63 families. This includes 80 species yielding edible fruits, 75 species used as leafy vegetables, 22 species bearing edible flowers, 8 species producing edible tubers and 12 species with edible seeds. Some plants are found to have multiple use values. The study will help in generating information on diversity, distribution, utilitarian values, socio-economic potential and conservation implications of wild edible plants.



## 1. Introduction

Forest plays an important role in maintaining environmental stability and to meet the essential requirements of almost all the living organisms on earth. The wild plants provide food, fodder, medicine, construction materials and many other items since time immemorial. Majority of the tribal communities of India live close to or within forests and depend on wild products and biomass for their food and energy needs (Mohapatra & Sahoo, 2010; Mahapatra & Mitchell, 1997). Even today, wild plants constitute an important part of the staple food of many tribal communities. Besides, wild edible plants play a significant role to meet the supplement or substitute food in times of scarcity like drought, flood and famine. According to an estimate, 80% of forest dwellers in Bihar, Odisha, Madhya Pradesh, West Bengal and Himachal Pradesh depend on forest for 25 to 50 % of their annual food requirements (Tiwari, 1994). Knowledge about the wild edible plants is being transmitted from the tribal medicine-man/ elderly persons living in the area for longer time to young persons of their community. Indigenous rural and tribal people living in the vicinity of the area collect, process and use a good number of plants for their own consumption and to earn their livelihood through sale of these items in local markets. The diversity in wild plant species offers variety of family diet and contributes to household food security, but the lack of awareness, limited nutritional data and the poor perception that most wild plants are of poor nutritional value have led many wild food plants to be neglected by local people and researchers (Padulosi *et al*., 2002; Mishra *et al*., 2008). The nutritional and medicinal values of these species have never been assessed. Considering their importance and non-availability of published account on the subject, an effort has been made to compile all available information on wild edible plants of Keonjhar district, Odisha, India. Keonjhar district occupies a distinct place in the tribal map of Odisha as well as India. According to 'Food Security Atlas of Rural Odisha" (WFP, 2008), the status of Keonjhar

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district is under 'severely unsecured' category though the district is bestowed with sound forest cover with abundance of wild edible plants. In view of this, the authors conducted a survey to document the little and less known uses of plants consumed or marketed by the tribals. The use values of non-domesticated crops known in local communities require proper study and documentation in order to validate, quantify and disseminate useful information (Edison *et al*., 2006). A harmonious blend to indigenous knowledge with modern science is essential to promote sustained utilization of these wild and potential source of nutrition (Horo & Topno, 2015).

## 2. Materials and methods

### *2.1 Study area*

Keonjhar district has an area of 8240 km$^2$, and lies between 21$^0$ 1' N to 22$^0$ 10' N latitude and 85$^0$ 11' E to 86$^0$ 22' E longitude (Fig 1). It is one of the predominant tribal districts of Odisha. It is surrounded by Singhbhum district of Jharkhand in the North, Jajpur in the South, Dhenkanal and Sundargarh in the West and Mayurbhanj and Bhadrak in the East. It lies at an altitude of 480 meter. To the West is a range of lofty hills which contains some of the highest peaks of Odisha namely Gandhamardan (3477ft), Mankadnacha (3639ft), Gonasika (3219ft) and Thakurani hills (3003ft). About half of the area of this district spreading over 4043 km$^2$ is covered with forests of northern tropical moist deciduous type. The river Baitarani comes out of Gonasika hills and flows to the north touching the border of Singhbhum district of Jharkhand. The soil is mostly red throughout the district and in the south there is a small patch of black cotton soil. The important minerals available in huge quantity in the district are iron-ore, manganese and chromites.

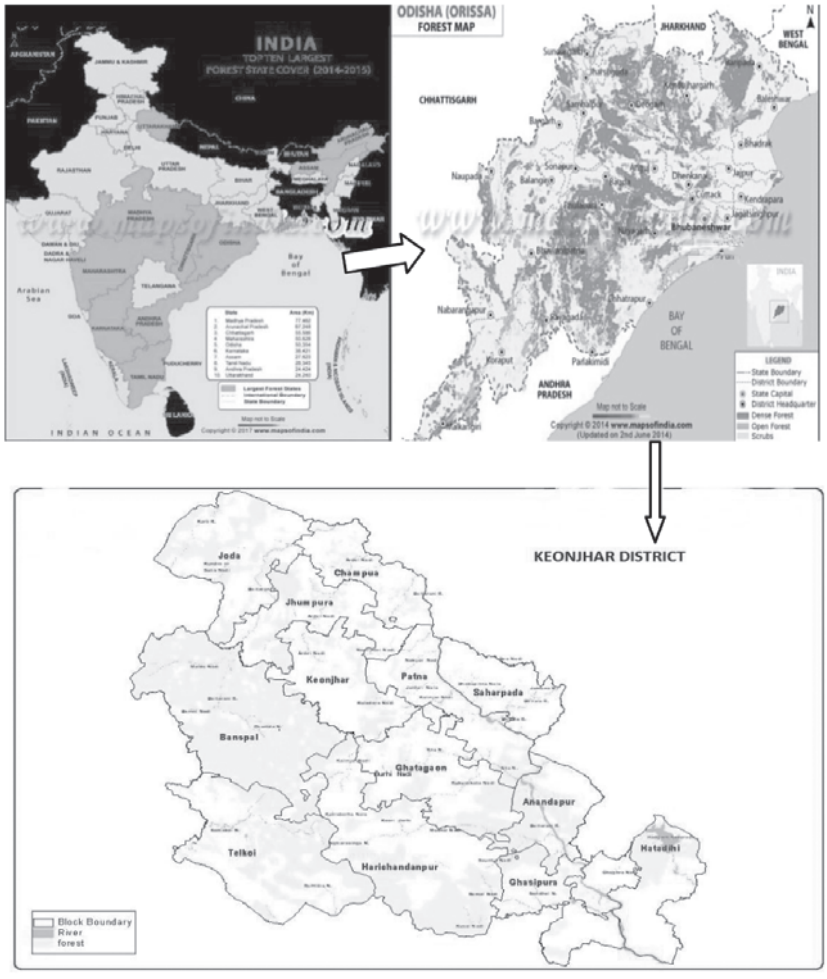


Fig.1. Map showing the location of Keonjhar district, Odisha (Study site)

*2.2. Climate, soil and population*

The climate of the district is characterized by an oppressively hot summer with high humidity; May being the hottest month. The lowest temperature in December is as low as 11.7$^0$C. The average annual rainfall is 1,534.5 mm. The varied soil, topography and climate are conducive for rich plant diversity.

The scheduled tribe population of Keonjhar district is 8,18,878 as per 2011 census belonging to 46 tribes. Out of these the principal tribes are Bathudi, Bhuyan, Bhumji, Gond, Ho, Juang, Kharwar, Kisan, Kolha, Kora, Munda, Oraon, Santal, Saora, Sabar and Sounti. The concentration of scheduled tribes is the highest in Keonjhar Sub-Division and lowest in the Anandpur Sub-Division.

*2.3 Data collection*

Several field trips were undertaken in different tribal dominated areas of Keonjhar district, Odisha during 2016-2018 to collect information about wild edible plants in different seasons. Some elderly persons of the tribe were the key informants to share their inherited knowledge. They shared knowledge on the flowering and fruiting time, vernacular names, uses of the edible parts and their mode of consumption. Tribal inhabitants helped in the collection of plant samples. The forest range officials not only supported the survey but also assisted in the forest walk as authors' guide. The rural markets have been surveyed to access the sale and marketing of the species. Data collection was done through interviews and discussions with the tribal and rural sellers in the rural market.

The plant specimens were collected from the field and the voucher specimens were processed and preserved using standard herbarium techniques. Photographs of the plants or plant parts were also taken in the field for record. The species were identified in consultation with 'The Botany of Bihar and Orissa' (Haines, 1921-1925) and 'Flora of Orissa' (Saxena and Brahamam, 1994-1996). The specimens have been deposited in the Herbarium of the P. G. Dept of Botany, North Orissa University, Baripada, Odisha. The plants are enumerated alphabetically with their botanical names, families, local names, plant parts used and mode of consumption.

## 3. Results and discussion

During the present survey, 160 wild edible plants have been recorded which are consumed by the tribes and local inhabitants of Keonjhar district as food and/or medicine. Out of the documented edible wild taxa, 75 species are reported to be used as leafy vegetables, 80 species as fruits, 22 species as edible flowers, 12 species as seeds and 8 species as tubers (Fig 2). Some of the noted species having multiple edible parts are *Amorphophallus paeoniifolius, Artocarpus heterophyllus, Azadirachta indica, Brassica campestris, Buchanania lanzan,Cucurbita maxima, Madhuca indica, Mangifera indica, Moringa oleifera, Semecarpus anacardium, Shorea robusta, Tamarindus indica* etc. These species are listed in Table 1 with botanical description and the methods of consumption.

Table 1

List of wild edible leafy vegetables with their vernacular names, parts used and method of consumption

| Sl. | Botanical name | Family name | Vernacular/ Common name | Parts used | Habit | Method of consumption |
|---|---|---|---|---|---|---|
| 1 | *Achyranthes aspera* L. | Amaranthaceae | Chirchithi | Leaves | Herb | Young leaves & shoots together with other greens are cooked & eaten. |
| 2 | *Alternanthera sessilis* (L.) R.Br.ex DC | Amaranthaceae | Madaranga | Leaves | Herb | Leafy shoots are cooked with spices. |
| 3 | *Aegle marmelos* (L.) Corr. | Rutaceae | Bela | Fruit | Shrub | Eaten raw, toasted. Ripe fruits are eaten as drinks, snacks, sweet taste. |
| 4 | *Alangium salvifolium* (L.f.)Wang. | Alangiaceae | Ankula | Fruits | Tree | Fleshy ripe fruits are cherished by children & tribes though they emit fishy smell. It has a lot of medicinal value. |
| 5 | *Allmania nodiflora* (L.) R.Br. ex Wight | Amaranthaceae | Marakata | Leaves | Herb | Leaves are used as common greens. |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | *Allophylus serratus* (Roxb.) Kurz | Sapindaceae | Khandakoli | Fruits | Shrub | Ripe fruits are relished by rural children |
| 7 | *Alocasia fornicata* (Roxb.) Schott | Araceae | Saree ala | Leaves | Herb | Tender leaves & petioles are cooked with spices & tamarind pulp to make curry. |
| 8 | *Alocasia macrorrhiza* (L.) G.Don | Araceae | Manasaree ala | Leaves | Herb | Tender leaves & petioles are cooked with spices & tamarind pulp to make curry. |
| 9 | *Alternanthera sessilis* (L.) R.Br. ex DC | Amaranthaceae | Gurundi saga, Mudurunga | Leaves | Herb | Leaves are fried/roasted and taken. Very commonly used. |
| 10 | *Amaranthus spinosus* L. | Amaranthaceae | Kanta maris, Kanta khara | Leaves | Herb | Leaves & shoots are cooked with salt & chilli |
| 11 | *Amaranthus tricolor* L. | Amaranthaceae | Lal bhaji | Leaves | Herb | Whole plant is cooked with spices & eaten as vegetables. |
| 12 | *Amaranthus viridis* L. | Amaranthaceae | Bhaji saga | Leaves | Herb | Leaves & shoots are chopped & cooked with oil, salt & garlic. |
| 13 | *Amorphophallus campanulatus* Bl. | Araceae | Ola | Leaves Tuber | Herb | Young tender leaves are cooked with tamarind, tomato & spices & eaten with rice. |
| 14 | *Amorphophallus paeonifolius* (Dennst.) Nicolson | Araceae | Olua | Tuber | Herb | Curry made with boiled tuber with tamarind pulp & fried with oil and garlic. |
| 15 | *Annona reticulata* L. | Annonaceae | Repdei Daru/ Chini balar | Fruits | Shrub | Ripe fruits are sweet & tasty with sweet aroma. |
| 16 | *Annona squamosa* L. | Annonaceae | Repdei Daru/ Chini balar | Fruits | Shrub | Ripe fruits are sweet & tasty with sweet aroma. |
| 17 | *Antidesma acidum* Retz. | Euphorbiaceae | Nununia | Leaves | Shrub | Leaves are cooked & eaten. |
| 18 | *Ardisia solanacea* Roxb. | Myrsinaceae | Laidak | Leaves, flowers | Shrub | Leaves are boiled with dal & cooked to eat with rice. Flowers are also edible. |
| 19 | *Artocarpus heterophyllus* L | Moraceae | Panasa | Fruits | Tree | Ripened fruit is eaten as such, used in ice-cream, candies, desserts, homemade cakes. Unripe fruits are cooked as curry, fried as snacks. |
| 20 | *Artocarpus lacucha* Roxb. ex Buch.- Ham. | Moraceae | Dheucha | Fruits | Tree | Ripe fruits are edible & eaten as snacks. Seeds are toasted & eaten. |
| 21 | *Averrhoa carambola* L. | Averrhoaceae | Karmanga | Fruits | Tree | Fruit is eaten fresh, cooked or pickled. |
| 22 | *Azadirachta indica* A. Juss | Meliaceae | Neem | Leaves Flowers Fruits | Tree | Young leaves are cooked or fried with potato, brinjal & tomato. Sometimes leaves made into curries. Rice cake is made by mixing the leaves, gram & rice. Children eat the fruits while wandering. |
| 23 | *Bacopa monnieri* (L.) Pennell | Scrophulariaceae | Panikundi | Leaves | Herb | Used as greens |

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| 24 | *Bambusa bambos* L. | Poaceae | Kantabaunsa | Leaves | Herb | Young shoots called 'Karadi' are cut into small pieces, cooked with spices & used as delicacy. These are dried & preserved for future. |
| 25 | *Basella alba* L. | Basellaceae | Poi saga | Leaves, stem, flowers | Herb | Stems, floral buds & leaves are used in curry making. |
| 26 | *Bauhinia purpurea* L. | Caesalpiniaceae | Kuliari | Leaves, flowers | Shrub | Young shoots along with tender leaves are fried as greens & also cooked as curry. Flowers are also edible. |
| 27 | *Bauhinia retusa* Roxb. | Caesalpiniaceae | Kanchana | Leaves, flowers | Shrub | Tender leaves & flowers are cooked & fried. |
| 28 | *Bauhinia vahlii* Wight & Arn. | Caesalpiniaceae | Sialilata | Leaves, seed | Climber | Seed kernel roasted. |
| 29 | *Bauhinia variegata* L. | Caesalpiniaceae | Kanchana | Leaves, flowers | Shrub | Tender leaves are fried. Edible flowers are deep fried to make snacks. |
| 30 | *Begonia picta* Sm. | Begoniaceae | Lundi ara | Leaves | Shrub | Leaves are boiled, then cooked as curry. |
| 31 | *Boerhavia chinensis* (L.) Asch. & Schweinf. | Nyctaginaceae | Puruni, khapra saga | Leaves | Herb | Leaves with tender shoots are fried with oil, salt and mustard. |
| 32 | *Boerhavia diffusa* L. | Nyctaginaceae | Puruni khapra saga | Leaves | Herb | Leaves with tender shoots are fried with oil, salt & mustard. |
| 33 | *Borassus flabellifer* L. | Arecaceae | Tala | Fruits | Tree | Ripe fruits are juicy and tasty. Pulp is used in making cakes, jaggery, sweets etc. |
| 34 | *Brassica campestris* L. | Brassicaceae | Sorisa | Leaves, flowers | Herb | Inflorescence along with tender leaves are cut into small pieces and fried with onion or garlic. |
| 35 | *Brassica napus* L. var *glauca* (Roxb.) Schulz | Brassicaceae | Sorisa saga | Leaves, flowers | Herb | Leaves and flowers are cooked & consumed by all tribes. |
| 36 | *Bridelia retusa* (L.) Spreng. | Euphorbiaceae | Kasi phala | Fruits | Tree | Fruits are eaten by forest dwellers. |
| 37 | *Buchanania lanzan* Spreng. | Anacardiaceae | Chara | Fruits, seeds | Tree | Ripe fruits are palatable and relished by all. Seed kernels are used in making sweets, ladoos and desserts. |
| 38 | *Canthium dicoccum* (Gaertn.) Teijsm & Binnend. | Rubiaceae | Kukurhada/ Karuna | Fruits | Shrub | Both ripe & raw foods are eaten. |
| 39 | *Capparis zeylanica* L. | Capparaceae | Asadhua | Fruits | Shrub | Raw fruits are cooked and ripe fruits are edible as such. |
| 40 | *Carissa spinarum* L. | Apocyanaceae | Dudkoli | Fruits | Shrub | Ripened fruits are edible |
| 41 | *Casearia graveolens* Dalz. | Flacourtiaceae | Benchi | Fruits | Shrub | Ripened fruits are edible. |
| 42 | *Cassia occidentalis* L. | Caesalpiniaceae | Chako ara | Leaves | Herb | Tender leaves are cooked. |
| 43 | *Cassia tora* L. | Caesalpiniaceae | Chako ara | Leaves | Herb | Tender leaves are cooked. |

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| 44 | *Catunaregam spinosa* (Thunb.) Tirveng. | Rubiaceae | Salag | Fruits | Shrub | Raw fruits are boiled & sieved to remove tannin content, then cooked to make curry & eaten with rice. Ripe fruits are edible as such. |
| 45 | *Celastrus paniculata* Willd. | Celastraceae | Kujuri saga | Flowers | Climber | Flowers cooked as vegetable. |
| 46 | *Celosia argentea* L. | Amaranthaceae | Laenga, Khukari Sirgiti ara | Leaves | Herb | Leaves & young shoots are collected fried/roasted with chilly & onion |
| 47 | *Centella asiatica* (L.) Urb. | Apiaceae (Umbelliferae) | Chauka ara Thalkudi (Odia) | Leaves | Herb | Leaves are fried as greens. Sometimes leaves are made into chutney. |
| 48 | *Chenopodium album* L. | Chenopodiaceae | Bathua ara/ Betua ara | Leaves | Herb | Tender leafy shoots are cooked to make a delicious bhaji eaten by all. |
| 49 | *Cicer arietinum* L. | Fabaceae | Buta saga | Leaves, seeds | Herb | Young leaves & shoots are collected, roasted & eaten. Seeds are eaten commonly. |
| 50 | *Citrus medica* L. | Rutaceae | Jambira/ bada nimbu | Fruits | Shrub | Fruits are squeezed & eaten with garlic & chilly and also used in making sarbat, pickles, jellies, etc. |
| 51 | *Clausena excavata* Burm. f. | Rutaceae | Agnijala/ Agnijalini | Fruits | Shrub | Ripe fruits are sweet & eaten by tribes. |
| 52 | *Cleome viscosa* L. | Cleomaceae (Capparaceae) | Hurhuria saga | Leaves | Herb | Young plants are consumed. |
| 53 | *Cleome monophylla* L. | Cleomaceae | Hurhuria saga | Leaves | Herb | Young plants are consumed. |
| 54 | *Coccinia grandis* (L.) Voigt. | Cucurbitaceae | Banakunduri/ Kainchikakudi | Leaves Fruits | Herb | Young leaves are cooked as vegetables. Fruits are used as vegetables & eaten raw. |
| 55 | *Colocasia esculenta* (L.) Schott | Araceae | Saru ara, Pechki | Leaves | Herb | Young shoots and tender leaves are chopped and cooked with spices and tamarind pulp. |
| 56 | *Commelina benghalensis* L. | Commelinaceae | Kansiri, Kena saga | Leaves | Herb | Leafy young shoots are fried with other greens. |
| 57 | *Corchorus capsularis* L. | Tiliaceae | Nalita | Leaves | Herb | Tender leaves are boiled sieved then fried with mustard seeds & mustard oil. |
| 58 | *Cordia obliqua* Willd. | Ehretiaceae | Bahala saga | Leaves Floral buds | Shrub | Leaves along with floral buds are cooked with tomatoes & spices. |
| 59 | *Cucurbita maxima* Duchesne | Cucurbitaceae | Kakharu saga | Leaves, stem, fruits, flowers, seeds | Climber | Leaves & young stems are fried or cooked as curry along with potato, tomato, pumpkin & mustard paste. |
| 60 | *Dendrocalamus strictus* Roxb. | Poaceae | Baunsa | Leaves | Herb | Young shoots are cooked into delicious dish. These are dried & stored for future. |
| 61 | *Digera muricata* (L.) Mart. | Amaranthaceae | Kari Gandhari | Leaves | Herb | Young leafy shoots are boiled & consumed. |
| 62 | *Dillenia aurea* Sm. | Dilleniaceae | Rai/ Karmata | Fruits, flower | Tree | Fruits are cooked when raw or ripe. Flowers are made into chutney. |

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| 63 | *Dillenia indica* L. | Dilleniaceae | Ou | Fruits | Tree | Made into jams, jellies, pickles, chutneys & cooked into curry. |
| 64 | *Dillenia pentagyna* Roxb. | Dilleniaceae | Rai | Fruits | Tree | Buds & fruits are eaten cooked or raw. |
| 65 | *Dioscorea alata* L. | Dioscoreaceae | Khamba alu | Tubers | Climber | Boiled tubers are fried, cooked with vegetables or made into chips for snacks. |
| 66 | *Dioscorea belophylla* Voigt ex Haines | Dioscoreaceae | Bhatkanda/ Mandei alu | Tubers | Climber | Consumed as vegetables. |
| 67 | *Dioscorea bulbifera* L. | Dioscoreaceae | Pita alu | Tubers | Climber | Sliced & washed repeatedly to remove bitterness, then they are boiled/fried & eaten. |
| 68 | *Dioscorea glabra* Roxb. | Dioscoreaceae | Kanta alu/ Pindalu | Tubers | Climber | Cooked as vegetables. |
| 69 | *Dioscorea hispida* Dennst. | Dioscoreaceae | Kanda | Tubers | Climber | Cooked as an ingredient of curry. |
| 70 | *Dioscorea wallichii* Hook. f. | Dioscoreaceae | Pitalu | Tubers | Climber | Boiled & cooked with vegetables, also used in dry form for use in food shortage. |
| 71 | *Diospyros malabarica* (Desr.) Kostel. | Ebenaceae | Mankada kendu | Fruits | Climbing herb | Ripe fruits are sweet, smelly & edible. |
| 72 | *Diospyros melanoxylon* Roxb. | Ebenaceae | Kendu/ Tiril | Fruits | Climbing herb | Ripe fruits are sweet & pulpy. |
| 73 | *Diospyros sylvatica* Roxb. | Ebenaceae | Kalicha/ Sara tiril | Fruits | Tree | Ripe fruits are edible. |
| 74 | *Emilia sonchifolia* (L.) DC. | Asteraceae | Uli aa (Kl) | Leaves | Herb | Leaves can be used raw or cooked. |
| 75 | *Enhydra fluctuans* Lour. | Asteraceae | Muchri ara (Mu) | Leaves | Herb | Whole leafy plants are boiled & cooked with oil & spices. |
| 76 | *Erycibe paniculata* Roxb. | Convulvulaceae | Joraikuli | Fruits | Shrub | Ripe berries are pulpy, sweet & edible. |
| 77 | *Eryngium foetidum* L. | Apiaceae | Chutni/Jangli dhania(Mu) | Leaves | Herb | Leaf paste is made into chutney with chilly and garlic/onion. Plant is used as condiment. |
| 78 | *Euphorbia granulata* Forssk. | Euphorbiaceae | Kantha arak (San) | Leaves | Herb | Young leafy shoots are boiled & consumed. |
| 79 | *Euphorbia hirta* L. | Euphorbiaceae | Dudhia, Marang dudhai | Leaves | Herb | Tender leaves are cooked & eaten. |
| 80 | *Ficus auriculata* Lour. | Moraceae | Dumri | Fruits | Tree | Ripe figs are eaten raw and immature figs are cooked as vegetables. |
| 81 | *Ficus hispida* L. f. | Moraceae | Dimiri | Fruits | Tree | Raw as vegetable and ripe are eaten as such. |
| 82 | *Ficus racemosa* Linn. | Moraceae | Dumri | Fruits | Tree | Raw fruits are cooked with spices and eaten as curry, tasty like mushroom. Fully ripe fruits have pleasant odour and are eaten are such. |

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| 83 | *Ficus religiosa* L. | Moraceae | Aswastha | Leaves | Tree | Tender reddish leaves are boiled, sieved and then fried with oil. |
| 84 | *Ficus semicordata* Buch. -Ham. ex J. E. Sm. | Moraceae | Pudhei | Fruits | Tree | Raw fruits are cooked into curry. Ripe figs are eaten raw and made into jams. |
| 85 | *Flacourtia indica* (Burm. f.) Merr. | Flacourfiacaeae | Bhaincha | Fruits | Shrub | Fruits are edible, have pleasant flavor and sweet taste. Sufficiently acidic, palatable, used for jam and jelly. |
| 86 | *Garcinia xanthochymus* Hook.f.ex. | Clusiaceae | Amba | Fruits | Tree | Raw and ripe fruits are eaten as mangoes. They are cooked and pickled. |
| 87 | *Gardenia gummifera* Linn. f. | Rubiaceae | Bhurudu | Fruits | Tree | Ripe fruits with pinkish pulp, tasty and eaten as snacks. |
| 88 | *Glinus oppositifolius* A. DC. | Aizoaceae | Pita saga | Leaves | Herb | Whole plant is eaten cooked with potato & brinjal. |
| 89 | *Glycosmis pentaphylla* (Retz.) DC. | Rutaceae | Dubduba | Fruit | Shrub | Ripe berries are sweet and tasty, eaten by children. |
| 90 | *Gmelina arborea* Roxb. | Verbenaceae | Gambhari | Fruit | Tree | Sweet ripe fruits are sometimes eaten by children. |
| 91 | *Grewia asiatica* L. | Tilliaceae | Pharsa koli | Fruit | Tree | Ripe fruits are eaten. |
| 92 | *Grewia hirsute* Vahl. | Tiliaceae | Sunaragada or kukurpelia | Fruit | Shrub | Ripe fruits are sweet, acidic and fragrant. |
| 93 | *Grewia tilifolia* Vahl. | Tiliaceae | Dhamana | Fruit | Tree | Fruit is edible which is seedy with scanty pulp having a good acidic flavor. |
| 94 | *Indigofera cassioides* Rottl. ex DC. | Fabaceae | Gileri | Leaves, Flowers | Shrub | Leaves are cooked (rare). Pink flowers are boiled and sieved out. Dry bhaji is made with onion, tomato, garlic etc. to make a delicious recipe. |
| 95 | *Ipomoea aquatica* Forssk. | Convulvulaceae | Kalama saga | Leaves | Herb | Leaves & tender shoots are fried into a delicious recipe. |
| 96 | *Justicia adhatoda* L. | Acanthaceae | Juani | Leaves | Herb | Leaves are cooked & eaten. |
| 97 | *Lagenaria siceraria* Standley | Cucurbitaceae | Lau | Leaves Fruits | Climber | Tender leafy shoots are cooked with other vegetables. |
| 98 | *Lantana camara* L. | Verbenaceae | Putus | Fruits | Shrub | Ripe fruits are relished by the children. |
| 99 | *Lepidium sativum* L. | Brassicaceae | Himba saga | Leaves | Herb | Leaves and young shoots are roasted and eaten. |
| 100 | *Leucas aspera* (Willd.) Link | Lamiaceae | Gayasa Tupu saga, Pitta saga | Leaves | Herb | Young shoots & leaves (alone) are cooked & eaten with rice. |
| 101 | *Leucas cephalotes* (Roth) Spreng. | Lamiaceae | Tupu saga Kointha | Leaves | Herb | Leaves are boiled/fried & cooked by all tribes. |
| 102 | *Limonia acidissima* L. | Rutaceae | Kaitha | Fruits | Tree | Pulp of ripe and raw fruits is made into chutney with sugar, salt and chilly. Pulp may be eaten as such. |

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| 103 | *Limnophila heterophylla* (Roxb.) Benth. | Scrophulareaceae | Hidimichi | Leaves | Herb | All tribes consume tender leafy shoots cooked with other greens. |
| 104 | *Litsea glutinosa* (Lour.) Robins. | Lauraceae | Bagha airee | Fruits | Tree | Ripe fruits are eaten sometimes. |
| 105 | *Luffa cylindrica* (L.) M. Roem. | Cucurbitaceae | Tadri | Fruits | Climber | Young fruits are cooked as vegetables. |
| 106 | *Madhuca indica* Gmel. | Sapotaceae | Mahua | Flowers, seeds, fruits | Tree | Fleshy corolla is sun dried made into paste with rice & baked to cakes. Country liquor is distilled from the flower. Fruits are cooked as vegetable. Edible oil is extracted from seeds. |
| 107 | *Mangifera indica* L. | Anacardiaceae | Amba/oli | Fruits, seeds | Tree | Raw fruits are cooked. Used in making pickles, chutney, amchur & serbet. Delicious ripe fruits are eaten as such. The ripe fruits can be used in the preparation of juice, squash, jam, jelly and aam sadha. Seed kernel is boiled & ground to paste along with rice to make cakes in the time of food scarcity. |
| 108 | *Manilkara hexandra* (Roxb.) Dubard | Sapotaceae | Khirokoli | Fruits, seed | Tree | Ripe fruits are sweet & eaten fresh or dries. Edible oil is extracted from seed. |
| 109 | *Marsilea minuta* L. | Marsileaceae | Sunsuni ara | Leaves | Herb | Leaves and shoots are cooked & eaten. |
| 110 | *Medicago sativa* L. | Fabaceae | Ghipari | Leaves | Herb | Cooked & eaten with rice. |
| 111 | *Mentha spicata* L. | Lamiaceae | Pudina | Leaves | Herb | Leaves are used in making chutney. Used in salads . |
| 112 | *Merremia quinquefolia* (L.) Hall. f. | Convulvulaceae | Chadhei saga | Leaves | Climber | Leaves & tender shoots are cooked & eaten with rice. |
| 113 | *Meyna spinosa* Roxb.ex. Link. var. *pubescens* Robyns. | Rubiaceae | Salara | Fruits | Shrub | Ripe fruits are edible. |
| 114 | *Momordica dioica* Roxb. ex Willd. | Cucurbitaceae | Kankada | Leaves Fruits | Climber | Young leaves are cooked like fruits. |
| 115 | *Moringa oleifera* Lam. | Moringaceae | Sajana saga | Leaves, Flowers, fruits, seeds | Tree | Leaves are fried with moong dal & coconut. Leaves are cooked mixed with other vegetables & spices (curry). Flowers are made into pakoda with besan. |
| 116 | *Molluga pentaphylla* L. | Molluginaceae | Pitagima | Leaves, Floral buds | Herb | Leaves are eaten cooked with brinjal & potatoes. |
| 117 | *Morus alba L.* | Moraceae | Tutkoli | Fruit | Shrub | Ripe fruits are edible. |
| 118 | *Mukia maderaspatana* (L.) Roem. | Cucurbitaceae | —— | Fruits | Climber | Fleshy ripe fruits are edible. |
| 119 | *Murraya koenigii* (L.) Spreng. | Rutaceae | Bhrusunga | Leaves | Shrub | Used in tadka to increase flavor of the food item and as snacks and chutneys. |

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| 120 | *Nelumbo nucifera.* Gaertn. | Nymphaeaceae | Padma | Pedicels and floral buds | Herb | Tribal people cut the petioles into small pieces and use them as vegetables. Children eat the floral buds. |
| 121 | *Nymphaea nouchali* Burm. f. | Nymphaeaceae | Kain | Pedicels | Herb | Pedicels are cooked as vegetables. |
| 122 | *Oxalis corniculata* L. | Oxalidaceae | Ambiliti, Netho sag | Leaves | Herb | Fresh leaves are fried with boiled dal. As the leaves are sour, they are chewed as mouth freshner. |
| 123 | *Paederia foetida* L. | Rubiaceae | Psaruni | Leaves | Climber | Leaves are made into paste & used to make curry with potato & tomato. After cooking there is no bad smell. Leaves are fried with besan/ gram flour also. |
| 124 | *Passiflora foetida* L. | Passifloraceae | Bisiripi | Fruits | Herb | Ripe fruits are edible. |
| 125 | *Phoenix acaulis* Buch.-Ham. ex Roxb. | Arecaceae | Bhuin khajuri | Fruits | Shrub | Ripe fruits are edible though have scanty pulps. |
| 126 | *Phoenix sylvestris* (L.) Roxb. | Arecaceae | Khajuri | Fruits | Shrub | Ripe fruits are sweet. Fruits are used to make juice, jelly and jam. |
| 127 | *Phyllanthus acidus* (L.) Skeels | Euphorbiaceae | Naara koli | Fruits | Shrub | Mature fruits are too tart and used in making pickles. |
| 128 | *Phyllanthus emblica* L. | Euphorbiaceae | Aanla | Fruits | Shrub | Fruits are eaten raw and also made into pickled. Salted dried fruits are mouth freshener. Fruits are dried, powdered and preservers. |
| 129 | *Physalis minima* L. | Solanaceae | Tomatilo | Fruits | Herb | Raw fruits are sometimes used as vegetable during fruit scarcity. |
| 130 | *Pithecellobium dulce* (Roxb.) Benth. | Mimosaceae | Sima kaian | Fruits | Tree | Pulpy aril of ripe fruit is sweet and edible. |
| 131 | *Polyalthia cerasoides* (Roxb.) Bedd. | Annonaceae | Budhi chamadi | Fruits | Shrub | Ripe fruits have sweet pulp which is edible. |
| 132 | *Polygonum barbatum* L. | Polygonaceae | Madara | Leaves | Herb | Tender leafy shoots are cooked with tomato & spices. |
| 133 | *Polygonum glabrum* Willd. | Polygonaceae | Sauri ara | Leaves | Herb | Tender leafy shoots are cooked with tomato & spices. |
| 134 | *Polygonum plebeium* R. Br. | Polygonaceae | Muthi sag, Pimpdi sag | Leaves | Herb | Whole leafy plant is ground along with chilly, turmeric & spices. The paste is wrapped in 'Sal' leaves & baked in fire (earthen oven). It is called 'patrapoda' & is taken with rice. |
| 135 | *Portulaca oleracea* L. | Portulacaceae | Balbalia, Nunia sag | Leaves | Herb | Leaves are roasted & eaten. |
| 136 | *Protium serratum* (Wall. ex Colebr.) Engl. | Burseraceae | Rimuli | Fruits | Tree | Ripe fruits are eaten raw. Sometimes these are made into chutneys/ pickles. |
| 137 | *Rungia parviflora* Nees | Acanthaceae | Kawoa sag Hasa arak | Leaves | Herb | Green leaves are consumed as vegetables. |

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| 138 | *Rungia pectinata* (L.) Nees. | Acanthaceae | Pimpidi saga | Leaves | Herb | Green leaves are consumed. |
| 139 | *Schleichera oleosa* (Lour) Oken | Sapindaceae | Kusum/ kasam daru | Fruits, seeds | Tree | Ripe fruits are edible with sweet taste. Seeds are eaten roasted. Oil extracted from the seed in cooking and massaging. |
| 140 | *Semecarpus anacardium* Linn. | Anacardiaceae | Bhalia | Fruits, seed | Tree | Fleshy orange receptacle is eaten when ripe. Kernel of seed is roasted and eaten. |
| 141 | *Sesbania grandiflora* (L.) Poir. | Fabaceae | Agasthi | Leaves Flowers | Shrub | Flowers are cooked as curry with spices and also fried into delicious pakoda. |
| 142 | *Shorea robusta* Gaertn.f. | Dipterocarpaceae | Sal/sargi | Fruits, seeds | Tree | Raw fruits are cooked as vegetables and eaten with rice. Seeds are roasted and taken as snacks. Oil is used in cooking and massaging. |
| 143 | *Solanum nigrum* Sw. | Solanaceae | Katha koli | Fruits | Herb | Raw fruits are cooked as vegetable (rare). |
| 144 | *Solanum torvum* Sw. | Solanaceae | Dengabheji | Fruits | Herb | Raw fruits are used as vegetables. |
| 145 | *Solanum viarum* Dunal | Solanaceae | Bheji | Fruits | Herb | Raw fruits are used as vegetables. |
| 146 | *Solanum virginianum* Orleg. | Solanaceae | Kantaregi | Fruits | Herb | Raw fruits are used as vegetables. |
| 147 | *Spondias pinnata* (L. f.) Kurz | Anacardiaceae | Ambada | Fruits | Tree | Raw fruits are eaten raw, pickle or cooked. Ripe fruits are used as such. |
| 148 | *Streblus asper* Lour. | Sterculiaceae | Sahada | Fruits, flowers | Shrub | Ripe fruits are edible and flowers are cooked. |
| 149 | *Streblus taxoides* (Heyne ex Roth) Kurz | Sterculiaceae | Jhumpura | Fruits | Shrub | Raw fruits are cooked as vegetables. |
| 150 | *Syzygium cerasoides* (Roxb.) Chatt. & Kanjilal f. | Myrtaceae | Kudedaru | Fruits | Tree | Edible ripe fruits mildly acidic with scanty pulp. |
| 151 | *Syzygium cumini* (Linn.) Skeels | Myrtaceae | Jamu | Fruits | Tree | Ripe fleshy fruits are commonly eaten. |
| 152 | *Tamarindus indica* L. | Caesalpiniaceae | Tentuli | Fruits, flowers, leaves, seeds | Tree | Ripe and unripe fruits are eaten. Pulp is used for flavoring curry and used in making sauces, chutneys, pickles, beverages etc. Tender leaves and flowers are edible as well as mouth freshener. Seed kernels are eaten burnt. |
| 153 | *Tamilnadia uliginosa* (Retz) Tirveng. & Sastre | Rubiaceae | Tolaka | Fruits | Tree | Ripe fruits are eaten as such or after being boiled or made into curries. |
| 154 | *Toxocarpus kleinii* Wt. & Arn. | Asclepiadaceae | - | Flowers | Herb | Flowers are cooked to make delicious dish like mushroom. |
| 155 | *Trianthema portulacastrum* L. | Aizoaceae | Purni | Leaves | Herb | Young plants are eaten by all tribes. |

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| 156 | *Woodfordia fruiticosa* (L.) Kurz | Lythraceae | Icha/ dhatki | Flowers | Shrub | Children suck the petals to remove nectar while wandering in the jungle. Flowers are highly medicinal. |
| 157 | *Ziziphus mauritiana* Lam. | Rhamnaceae | Barakoli | Fruits | Tree | Mature raw fruits and ripe fruits are eaten raw, pickled, candied and also preserved for future. |
| 158 | *Ziziphus nummularia* (Burm.f.)Wt & Arn. | Rhamnaceae | Jangli barakoli | Fruits | Tree | Ripe fruits are edible. |
| 159 | *Ziziphus oenoplia* (L.) Mill. | Rhamnaceae | Kanteikoli | Fruits | Shrub | Sweet acidic ripe fruits are edible. |
| 160 | *Ziziphus rugosa* Lam. | Rhamnaceae | Chunkoli | Fruits | Shrub | Ripe fruits being sweet in taste are eaten as such. |

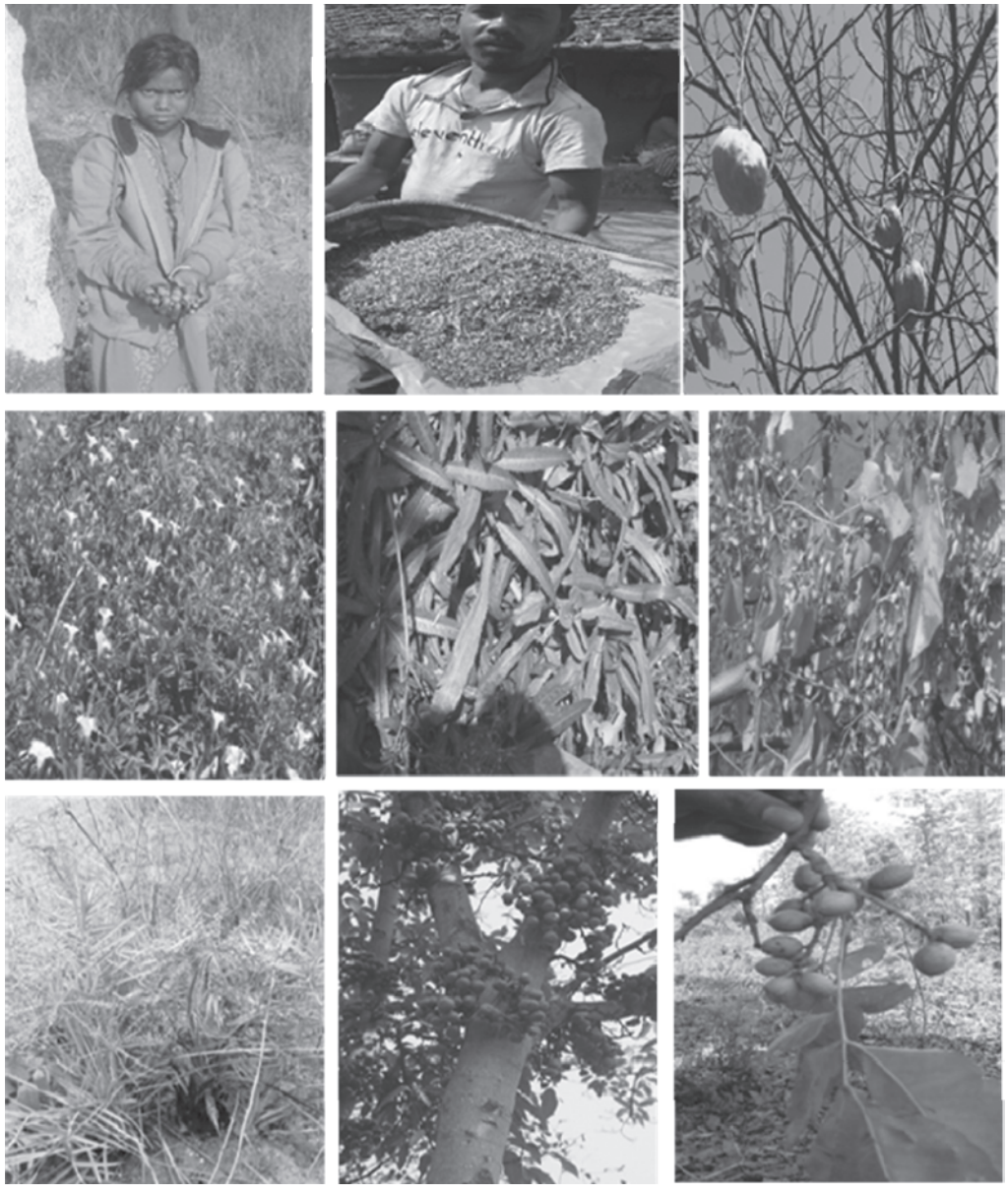

Fig. 2 (a) A tribal girl collecting edible fruits; (b) Collection of flowers of *Indigofera cassioides;* (c) *Annona reticulata* (d) *Merremia quinquefolia;* (e) *Eryngium foetidum;* (f) *Dioscorea wallichii;* (g) *Phoenix acaulis;* (h) *Ficus racemosa* and (i) *Schleichera oleosa*

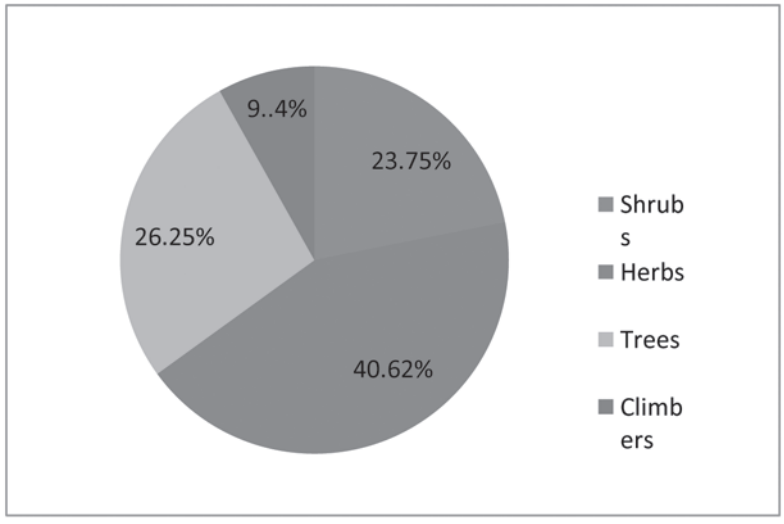


Fig.3. Wild edible food plants in terms of habit

The edible taxa listed in the above table belong to 160 species under 119 genera and 63 families (Fig. 3). A good number of leafy vegetables like *Alternanthera sessilis, Bauhinia purpurea, Brassica campestris, Chenopodium album, Cicer arietinum, Cordia obliqua, Eryngium foetidum, Ipomoea aquatica, Leucas aspera, Marsilea minuta* and *Moringa oleifera* are generally cooked by all communities. These species are marketable and provide opportunity to supplement household income. Edible flowers of *Indigofera cassioides, Brassica campestris, Moringa oleifera, Bauhinia variegata, Cordia obliqua, Madhuca indica, Sesbania grandiflora* and *Toxocarpus* species are sold in the market depending on their season of availability. Varieties of edible wild foods also include fruits, seeds and tubers which are safe for consumption. Tubers of *Dioscorea* species and fruits of *Mangifera indica, Diospyros melanoxylon, Buchanania lanzan, Tamarindus indica, Schleichera oleosa, Artocarpus heterophylla, Emblica officinalis, Ficus racemosa* and many others are consumed in large scale and also traded outside. Though commercial use of Sal (*Shorea robusta)* seeds has picked up during last two to three decades, the extraction of oil from it for domestic consumption is in practice since long. Now it is being use for making vegetable ghee (Vanaspati), sweets and chocolates. Kusum and Mahua seeds also yield edible oils. Seeds of *Buchanania lanzan* are highly nutritive, tasty and costly. The tubers are the most important food crops for the tribes in lean seasons.

## 4. Conclusion

Wild edible plants are of high social and economic value to the tribal people and those living in forest fringe villages, which ensures food security and household income. However, habitat loss and over-exploitation of NTFPs has been the causes of depletion of the forest resources. Many edible plants are rapidly shrinking due to increasing population and anthropogenic pressure along with the traditional knowledge base. Present research on wild edibles may help the forest planners on linking livelihood and socio-economic development with biodiversity conservation. Sustainable harvesting, value addition and cultivation are essential for judicious utilization of the wild edible plants.

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# Cyanobacterial diversity of Bhitarkanika mangrove forests, Odisha

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ABSTRACT

Bhitarkanika National Park in the district of Kendrapara is represented by the mangrove forests of Brahmani and Baitarani Delta of the Odisha coast. Cyanobacteria diversity was assessed in this mangrove forest in five major areas viz. Bhitarkanika, Dangamal, Kalibhanjdian, Gupti and Ekakula. A total of 29 cyanobacteria were isolated and identified from these sites and grouped in to eleven genera. The genus *Lyngbya* was found to be most abundant in this mangrove forest. Among these five areas, Kalibhanjadian showed maximun species richness (11) and dominance (0.44) whereas, minimum richness was recorded for Ekakula (4) and minimum dominance at Bhitarkanika (0.22). Both Shannon index and Simpson's diversity index indicated that Bhitarkanika is having the highest cyanobacteria diversity and Kalibhanjadian, the lowest. The diversity and distribution of cyanobacteria in the study sites will throw light on the health and functioning of the mangrove ecosystem.



## 1. Introduction

Mangrove ecosystems are the most unique environment and are considered as one of the highly productive natural ecosystems of the world. They serve as the base of an elaborate and productive food web in the tropical and subtropical coastal marine environments. The exceptional diversity and distribution of the flora and fauna in an estuary is mainly controlled by the fluctuations in the physico-chemical characteristics of water and active participation of the microorganisms which perform various bio-geochemical and nutrient cycles by degradation of foliage litters. Dynamic mangrove ecosystem supports the growth of many microbes such as phosphate solubilizing, nitrogen fixing, sulphate reducing and methanogenic bacteria, wood degrading fungi as well as photosynthetic microbes like cyanobacteria and microalgae, which perform complex interactions for maintaining nutrient cycle and ecological balances in this ecosystem (Rao and Rao, 2015). The knowledge on microbial diversity and distribution in a mangrove forests would improve the understanding of their functionality, interaction and the role they play in such an ecosystem. These microorganisms, due to their characteristic adaptation and active participation for sustainable development of mangrove ecosystem, could be utilized as industrially important microbes for different value-added products. Several studies on microbial diversity and physico-chemical constituents has been reported for various mangroves ecosystems of Pichavaram (Tamilnadu) and Sundarban (West Bengal), which explains the physiology and stability of this ecosystem (Silambarasan *et al*., 2011; Ramanathan *et al*., 2008; Satpati *et al*., 2013).

Cyanobacteria and microalgae are microscopic photosynthetic organisms, which constitute the world's phytoplankton. These are available in diverse kind of aquatic environments ranging from fresh water to very saline water, very cold arctic region to hot springs. Most cyanobacteria are cosmopolitan species which can grow and sustain in adverse environmental conditions. These cyanobacteria play

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a major role as primary producers and constitute the integral component of the microbiota and their food web in mangrove ecosystem along the tropical coasts (Kathiresan and Bingham, 2001; Sakthivel, 2004). Further, the delta being the slowest stretch of river flow accumulates the major portion of effluents and nutrient carried over by the river system and thus creates a typical ecological environment suitable for growth of cyanobacteria. These organisms can produce various complex compounds such as lipid, carbohydrates and proteins using simple inorganic substances from the ecosystem. Few of them even synthesize bioactive compounds such as polyhydroxyalkanoates, pigments, oils, proteins, polysaccharides, vitamins, antioxidants, UV-protectants, health products etc. (Blackburn and Volkman, 2012). Therefore, exploration of diversity of these bioresources from different ecosystems will not only be helpful for further bioprospecting and biotechnological applications but also to understand the ecosystem functioning as a whole.

Geographically, Bhitarkanika is located between 20°4'-20°82 N Latitudes and 86°45'-87°50' E longitudes in the Kendrapara district of Odisha. It is the second largest mangrove ecosystem of India which consists of mangrove forests, rivers, river deltas, creeks, estuaries, backwater, accreted land and mud flats. These areas exhibit bidirectional tidal fluxes and thus form extensive, low gradient inter-tidal zones available for mangrove colonization (Chadha and Kar, 1999). Bhitarkanika is one of the richest and diverse mangrove ecosystems in terms of mangrove plants as well as microbial community. Most of the literatures of this region are focused on the flora and the fauna. But in Bhitarkanika mangrove region, due to vast expanse of water and inaccessible forests, the microbial diversity remains unexplored. Only a few reports about the microbial diversity with special emphasis to soil bacteria are available (Mishra *et al*., 1995; Gupta *et al*., 2007; Thatoi *et al*., 2012). Preliminary surveys on microalgae and cyanobacteria were reported by Rath and Adhikary (2006) and Thatoi *et al.* (2012) from estuaries and mangrove soils of Bhitarkanika. Hence, this study was attempted to assess the detailed cyanobacteria diversity of Bhitarkanika to understand their ecological importance in particular and for further biotechnological applications, in general.


**Fig. 1.** Sample collection sites of Bhitarkanika mangrove forest

## 2. Material and methods

### *2.1. Sample collection*

The study area was divided in to five major areas of Bhitarkanika as shown in the Fig 1. Samples were collected during 2017-19 from different niches of mangrove sediments, estuarine water, pneumatophores and from the surfaces of woods and shells of the study area by random sampling method. Approximately fifty samples were collected from different sampling sites in autoclaved plastic bags, glass vessels and water storage bottles for further analysis in laboratory.

### *2.2. Isolation and identification of cyanobacteria*

Isolation of cyanobacteria was done by successive serial dilution and repeated spread plate and streak plate method on BG-11 agar plates. Cyanobacteria filaments that grew in the culture plates were isolated and subsequently transferred to BG-11 media in cotton stopped conical flask.

The purified cyanobacteria were observed under microscope for morphological characterization and identified following manual by Desikechary (1959).

*2.3. Cyanobacterial culture and maintenance*

Each cyanobacteria isolate was cultured in Erlenmeyer flasks with complete BG-11 medium or $BG_0$-11 (without nitrate) as per their nitrogen requirement (Ripka *et al*., 1979). The cultures were maintained in a temperature-controlled incubator at 27 ± 2ÚC under illumination with white fluorescent light (photon) with photoperiod of 14:10 hours. The isolates were sub-cultured at regular intervals to maintain their growth.

*2.4. Cyanobacteria diversity index calculation:*

The cyanobacteria isolated under each genus were counted for estimating diversity, richness and evenness of cyanobacteria in study area. Generic diversity was estimated by Shannon-Weiner Index (H) (Shannon and Weaver, 1949) and Simpson's diversity and Simpson's dominance (Simpson 1949) using the following formulae.

Shannon-Weiner Index, $H = \Sigma pilnpi$

Species evenness = $\frac{H}{Hmax}$

Simpson's Dominance $D = \Sigma(pi)^2$

Simpson's Diversity Index, $1 - D = 1 - \Sigma(pi)^2$

Species Richness (S)= Total no of isolates present in the arae

where pi = Total number of isolates of genus i /total number of all isolates

$H_{max}$= ln(S) i.e maximum diversity possible where

D= Simpson's Dominance

## 3. Results

*3.1. Isolation and identification of cyanobacteria strains*

About ten to fifteen samples were collected from different micro-habitats of each five major areas of Bhitarkanika forest (Fig. 1) with GPS locations for possible isolation of cyanobacteria strains. After the initial growth of cyanobacteria in BG-11 media and agar plates, they were separated by repeated streaking on fresh plates with microscopic observation till the pure cultures were obtained. Then each pure culture was grown again with $BG_0$-11 (without nitrate) to check the presence of heterocyst in them. Those isolates who can sustain their growth in $BG_0$-11 medium were further cultured in that medium and those which could not sustain their growth without nitrate for more than 15 days were cultured in complete BG-11 medium. A total of 29 cyanobacteria strains were isolated from five different areas having six isolates from Dangamal and Bhitarkanika area, eight isolates from Gupti, eleven isolates from Kalibhanjadian and four isolates from Ekakula area. The cyanobacterial samples isolated from soil and water samples and from other sources from different areas were mentioned in Table 1. The isolates belonged to both unicellular and filamentous forms and filaments with or without heterocysts. Morphologically branched and unbranched filaments were also observed. On the basis of microscopic observations and morphological characterization, the isolates were identified and grouped under the genera *Lyngbya, Aphanocapsa, Gloeocapsa, Anabaena, Oscillatoria, Phormidium, Scytonema, Calothirix, Trichodesmium, Leptolyngbya* and *Fristchiella* (Plate 1).

*3.2. Genetic diversity and abundance of cyanobacteria samples*

Kalibhanjadian recorded highest abundance of cyanobacteria genera followed by Gupti, Bhitarkanika & Dangamal and lowest was recorded from Ekakula as presented in Table 2. Percent abundance of cyanobacterial genera is presented in Fig. 2. The most abundant genera among all isolates were found to be *Lyngbya* (45%) followed by *Aphanocapsa* (10%), *Anabaena* (10%) and *Oscillatoria* (7%). The occurrence of other genera was sporadic. Species richness was found to be maximum in Kalibhanjadia. However, evenness index was highest in Ekakula (0.99) followed by Bhitarkanika (0.87) and lowest in Kalibhanjadian (0.48). All the diversity indices for various areas are graphically represented in Fig 3. Shannon index and Simpson's diversity index revealed the sequence of Bhitarakanika as Dangamala>Ekakula>Gupti>Kalibhanjadian. Simpson's dominance index was maximum in Kalibhanjadian and minimum in Bhitarkanika and Dangamal area.

## 4. Discussion

Availability of fresh water from Brahmani, Baitarani, Dhamara and Bhitarkanika (Maipura river) rivers and saline water from sea in core area of Bhitarkanika, provide a wide range of niches for different cyanobacteria species to grow. In the present study, 29 cyanobacteria species have been isolated from the mangrove soil, water and from surface of other hard substrates like tree bark, pneumatophores and submerged woods (Table 1, Plate 1). Most of the cyanobacteria isolated were filamentous forms which were found attached to any substratum or soil surface than that

Table1

Cyanobacteria isolates from Bhitarkanika forest region.

| Area | Sampling site | Isolated from | Code of isolate | Name |
|---|---|---|---|---|
| Bhitarakanika and Dangamal | BD-1 | Boat surface and water | BD-1a | *Lyngbya* sp. |
| | BD-2 | Water | BD-2a | *Fristchiella* sp. |
| | | | BD-2b | *Apahnocapsa* sp. |
| | BD-3 | Soil and stem | BD-3a | *Trichodesmium* sp. |
| | BD-4 | soil | BD-4 | *Oscillatoria pseudogeminata v. unigranulata* |
| | BD-7 | Bark | BD-7 | *Lyngbya* sp. |
| Gupti | G-1 | Soil | G-1 | *Lyngbya keutzingii* |
| | G-2 | Water | G-2 | *Apahnocapsa koordersi* |
| | G-3 | Water | G-3 | *Gloeocapsa* sp. |
| | G-4 | Soil | G-4 | *Anabaena sphaerica* |
| | G-5 | Soil | G-5 | *Lyngbya mesotricha* |
| | G-7 | Soil | G-7 | *Lyngbya* sp. |
| | G-13 | Water | G-13 | *Anabaena fertilissima* |
| | G-18 | Pneumatophore of *Rhizophora apiculata* | G-18 | *Lyngbya*sp. |
| Kalibhanjadian | K-3 | Bark of *Tamarix ericoides* | K-3 | *Lyngbya* sp. |
| | K-5 | Soil | K-5 | *Lyngbya* sp. |
| | K-8 | Water | K-8 | *Lyngbya* sp. |
| | K-9 | water | K-9a | *Lyngbya* sp. |
| | | | K-9b | *Phormidium tenue* |
| | K-10 | Water | K-10a | *Chlorococcus* sp. |
| | | | K-10b | *Leptolyngbya lignicola* |
| | K-12 | Pneumatophore of *Heritiera littoralis* | K-12 | *Aphanocapsa* sp. |
| | K-13 | Bark of *Excoecaria agallocha* | K-13 | *Microcoleus* sp. |
| | K-14 | Pneumatophore of *Avicennia officinalis* | K-14 | *Lyngbya* sp. |
| | K-15 | Root of *Phoenix* sp. | K-15 | *Lyngbya*sp. |
| Ekakula | EK-6 | water | EK-6 | *Anabaena vaginicola* |
| | EK-8 | Dried Stem | EK-8 | *Scytonema* sp. |
| | EK-9 | Dried Stem | EK-9 | *Oscillatoria pseudogeminata* |
| | EK-12 | Soil | EK-12 | *Calothirix* sp. |

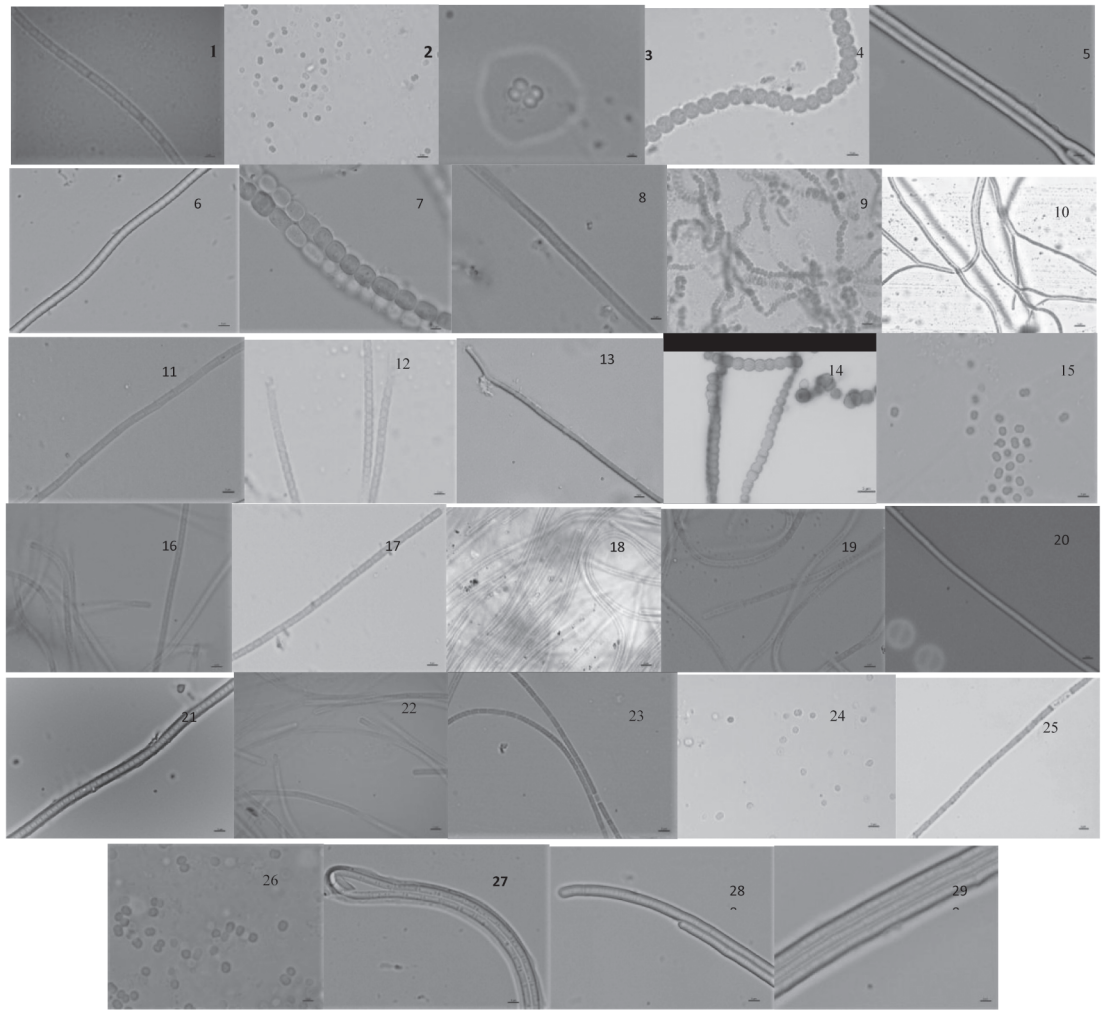

Plate:1 Photographs of cyanobacterial isolates:1) *Lyngbya keutzingii*(G-1) 2) *Apahnocapsa koordersi*.(G-2) 3) *Gloeocapsa* sp.(G-3) 4) *Anabaena sphaerico* (G-4) 5) *Lyngbya mesotricha* (G-5) 6) *Lyngbya* sp. (G-7) 7) *Anabaena fertilissima* (G-13) 8)*Lyngbya* sp. (G-18) 9) *Anabaena vaginicola* (EK-6) 10) *Scytonema* sp. (EK-8) 11) *Oscillatoria pseudogeminate* (EK-9) 12)*Calothirix* sp. (EK-12) 13)*Lyngbya* sp. (BD-1a) 14) *Fristchiella* sp. (BD-2a) 15)*Apahnocapsa* sp. (BD-2b) 16)*Trichodesmium* sp. (BD-3a) 17) *Oscillatoria pseudogeminata v. unigranulata.* (BD-4b) 18)*Lyngbya* sp. (BD-7) 19)*Lyngbya* sp. (K-3) 20) *Lyngbya* sp. (K-5) 21)*Lyngbya* sp. (K-8) 22) *Lyngbya* sp. (K-9a) 23) *Phormidium tenue* (K-9b) 24) *Chlorococcus* sp. (K-10a) 25) *Leptolyngbya lignicola* (K-10b) 26) *Apahnocapsa* sp. (K-12) 27)*Microcoleus* sp. (K-13) 28) *Lyngbya* sp. (K-14) 29)*Lyngbya* sp. (K-15)

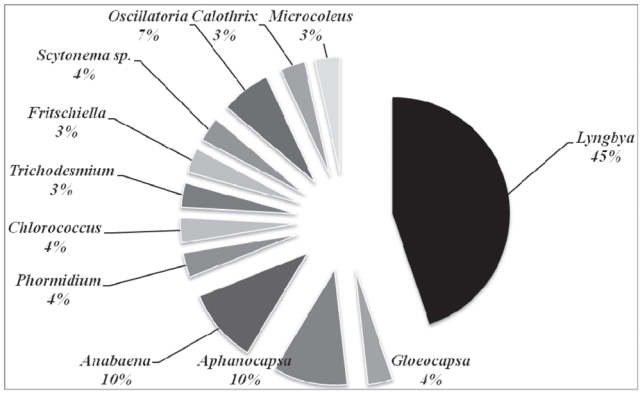


Fig.2. Percent abundance of cyanobacterial genera in Bhitarkanika forest.

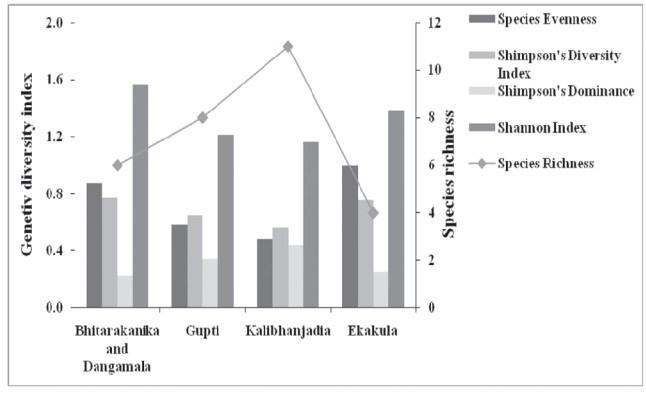


Fig. 3. Genetic diversity of cyanobacterial population from Bhitarkanika forest.

Table 2

Abundance of cyanobacteria sample collected from different areas of Bhitarkanika.

| Genus | Total no. of isolates | Bhitarkanika and Dangamal | Gupti | Kalibhanjadian | Ekakula |
|---|---|---|---|---|---|
| *Lyngbya* | 13 | 2 | 4 | 7 | - |
| *Gloeocapsa* | 1 | | 1 | - | - |
| *Aphanocapsa* | 3 | 1 | 1 | 1 | - |
| *Anabaena* | 3 | | 2 | - | 1 |
| *Phormidium* | 1 | - | - | 1 | - |
| *Chlorococcus* | 1 | - | - | 1 | - |
| *Trichdesmium* | 1 | 1 | - | - | - |
| *Fritscheilla* | 1 | 1 | - | - | - |
| *Scytonema sp.* | 1 | - | - | - | 1 |
| *Oscilatoria* | 2 | 1 | - | - | 1 |
| *Calothrix* | 1 | - | - | - | 1 |
| *Microcoleus* | 1 | - | - | 1 | - |
| Total | 29 | 6 | 8 | 11 | 4 |

of planktonic forms. The species richness, dominance and diversity of the cyanobacteria were analyzed to predict how well the species are distributed within an area. Kalibhanjadian area showed maximum abundance (Table 2, Fig.2) of cyanobacteria which might be due to the geographical position and physico-chemical properties of soil and water sample of the area (Ahad *et al*., 2015). It is a dense mangrove forest surrounded by two rivers in both the sides and other side is opening to Dhamra port. Therefore, it might have a wide range of variation in pH, salinity, chloride content and effluents from the rivers which influence the cyanobacterial growth and species richness. Moreover, the mangrove forest with diverse type of plant roots and pneumatophores give much surfaces to the filamentous cyanobacteria to grow. However, Bhitarkanika and Dangamal area showed maximum diversity of cyanobacteria with minimum dominance (Fig. 3), which might be due to the existence of a number of cricks in this area that divides the total area in to various micro-niches thus allowing diverse cyanobacteria to grow. Further analysis of physico-chemical parameters of these areas will explain more about the possible cause of such diversity distribution of cyanobacteria.

## 5. Conclusion

Biotic and abiotic factors influence the distribution of cyanobacteria in any environment. Basic knowledge of ecological factors is important for understanding the ecology and biodiversity of cyanobacteria. Total 29 species belonging to 11 genera were isolated from the mangrove forest samples from which *Lyngbya* spp. is showing maximum abundance and the maximum cyanobacterial diversity was found in Bhitarkanika and Dangamal region. This shows the rich diversity of cyanobacteria in the ecosystem which may possibly be explored further for biotechnological applications.

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# Rice stubble as a potential bio-energy substrate

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ABSTRACT

Stubble burning has greatly contributed to the rising air pollution and consecutively the health issues in India. The objective of this research was to conduct the bio-methane potential (BMP) assay of rice straw and convert the agricultural crop waste-to-energy (WtE). Batch experiments under anaerobic conditions were performed at 38 °C with rice straw and the inoculum was sourced from an industrial biogas plant. The total biogas production was continuously measured for 15 days with an ANKOM wireless gas production system. The experimental results demonstrated a biogas production of nearly 140 mL/g FM. Considering the thumb rule calculations, approximately 0.5 KWh electricity can be expected from 1 kg of rice straw. Post-digestion, the slurry from the anaerobic reactors where biogas is produced could be further utilized as organic fertilizers, further improving the organic content and quality of soil. The experiments demonstrated the possibility to generate energy from the otherwise discarded and wasted rice stubble as a sustainable alternative. Employing such residue-based biogas plants could not only generate a green energy, but can also provide farmers an incentive from the electricity and organic fertilizers produced and ultimately prevent the practice of stubble burning.



## 1. Introduction

Rice is the staple food for more than half of the total human population globally. India is the second largest rice producer in the world where an annual production of nearly 13,915 Gg (Giga gram) is reported from Punjab and Haryana alone (Grover and Chaudhry, 2019). Waste is an obvious repercussion of such a huge activity. Rice crop residues rank as the world's third largest agricultural wastes and in India, the rise of mechanical harvesters has significantly contributed in generating increasing crop residues. Mechanical harvesters are undeniably effective and save time and labor. However, a consequence remains that a remarkable portion of the crop residue is left on the fields and numerous studies report that as much as 1.5 kg of stubble is generated per kg or rice harvested (Kumar *et al*., 2015).

When it comes to managing these crop residues after harvest in India, the most widely practiced method till date remains to set the fields on fire. Open-burning remains the cheapest, less labor intensive and fastest method to easily discard and prepare for the next crops. The 'on-farm' burning practice employed to manage these huge volumes of crop residues emits greenhouse gases and adds particulate matter to the ambient air along with destroying the nutrients otherwise present in the crop residues. The soil quality is also damaged due to the heat generated during the burning which affects the soil ecosystem and the microbes (Chakma *et al*., 2016).

The fate of rice crop residues could be altered from being treated as waste to a resource instead. Rice crops with its high organic and carbohydrate content bears a

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potential for biogas production. Biogas is a mixture of majorly methane ($CH_4$) and carbon dioxide ($CO_2$) produced with the help of microorganisms under anaerobic conditions from several types of organic wastes from agricultural, animal, domestic as well as industrial sources (Manyi *et al*., 2013).Generating biogas could be a cost-effective, carbon neutral process where rice growing farmers could utilize the gas either for cooking or heating purposes or could further convert the calorific-rich methane to generate electricity. The aim of this research remains in determining the bio-methane potential (BMP) of rice straw and evaluate its competence as a source of energy than being discarded or burnt as waste.

## 2. Materials and methods

### *2.1. Substrate*

Rice straw was collected from a farmer nearby in Bhubaneswar, Odisha, India. The air-dried rice straw was chopped with scissors into about 2 cm lengths.

### *2.2. Dry Matter (DM) and Volatile Solids (VS) of rice straw and the inoculum*

Known quantities of sample were taken and dried in an oven at 105$^0$C. The volatile solids were quantified by combusting dried samples at 550$^0$C for 4 h (Verein Deutscher Ingenieure 2006). Samples were taken in triplicates and the average values are presented.

### *2.3. Experimental Set-up for determination of biogas production from rice straw*

Anaerobic batch reactors were prepared following the guidelines of the German standard VDI 4630 (Verein Deutscher Ingenieure 2006). Borosil glass bottles with 1100 mL volume were fed with 495 mL of inoculum and were mixed with 5 g of rice straw. Reference samples were prepared by considering 5g of distilled water instead of the substrate to determine the biogas produced from the inoculum only. Wireless gas measurement system ANKOM (N1v0, 4RF2; RFS#194) was employed where the readings are transmitted to the computer every minute and the experiments were performed for 15 days. The biogas yield was calculated from the pressure values measured by the ANKOM gas systems based on Avogadro's principle and the experiments were performed in duplicates on a water bath maintained at 38$^0$C.

Table 1

Dry mass (DM) and Organic dry mass (oDM) of rice straw and the inoculum bearing active microbial consortia from an industrial biogas plant considered for batch experiments.

| | Dry mass (%) | Organic Dry Mass (%DM) |
|---|---|---|
| Rice straw | 92.5 | 85.8 |
| Inoculum | 7.2 | 66.8 |

## 3. Results and discussion

Rice straw demonstrated its competence as a suitable substrate for biogas production and as expected, the organic matter was high in the agricultural residue (Table 1).

While the references samples (i.e. pure inoculum without any given substrate) showed a cumulative biogas potential of approximately 210 mL on an average, the batch reactors with rice straw displayed an average production of nearly 894 mL (Fig. 1). A lag phase was evident in the biogas production with the rice straw. This is expected to

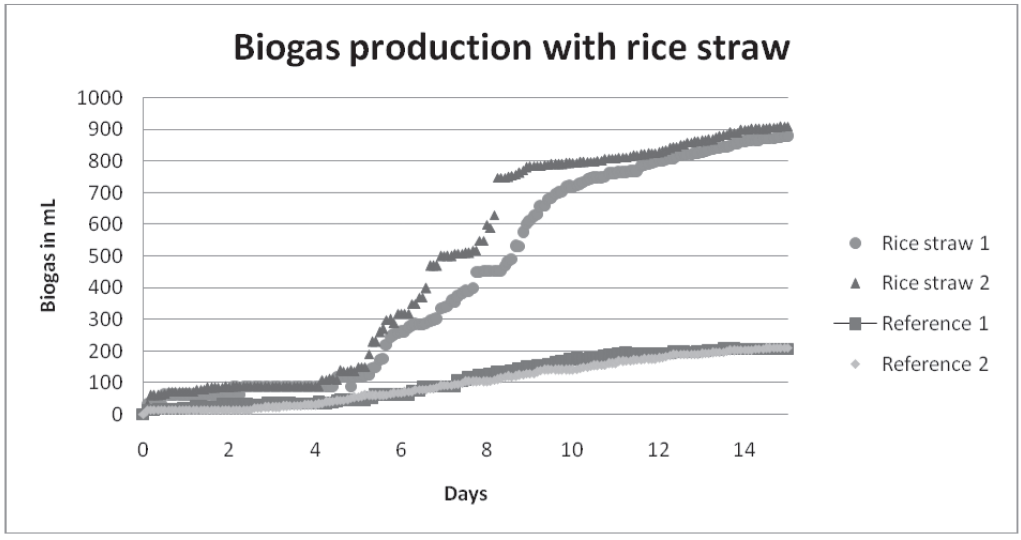


Fig.1. Biogas production with rice straw mixed with inoculum from a biogas plant (in duplicates) in batch reactors. Pure inoculum without substrates was considered as reference (in duplicates).

be due to the high lignin content (ranging between 6.4-19.4%) in rice straw and its complex and recalcitrant lignocellulosic structure containing hemicellulose (32.3-37.1%) and cellulose (25.4-35.5%) which inhibit the first phase during anaerobic digestion i.e. hydrolysis stage after which the anaerobic microorganisms sequentially convert the organic matter to methane and carbon dioxide (Jin and Chen, 2006; Chen *et al.* 2008).

The results attained in the experiments indicate a biogas potential of rice straw to generate roughly 140 mL biogas per gram fresh mass of rice straw. Considering the standard calculations that 1 $m^3$ biogas could generate nearly 3.2 KWh electricity (Agency for Renewable Resources, 2019), this amount of biogas could roughly correspond to a production of nearly 0.5 KWh of electricity per kg of rice straw. In other words, 1 kg of rice straw could provide electricity to power 1 bulb of 60W for nearly 8 hrs.

Climate change, pollution, energy crisis and food security remain the pressing challenges worldwide for a growing population. Agricultural activities including biomass burning, animal rearing and manure management, fermentation etc. contribute to nearly 50% of the total anthropogenic methane emissions globally.Removing the crop residues from the field and capturing and utilizing the methane instead remain an effective mitigation strategy. Utilization of organic fertilizers from the biogas reactors could improve soil fertility further contributing in maintaining food security (Mussoline, 2013). By considering the eco-friendly, zero-waste generating biogas technology from the rice stubble, farmers in India could end the practice of stubble burning and prevent the aforesaid alarming effects of such practices concerning the health of the population and environment as-well.

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# Updated checklist of the genus *Eragrostis* Wolf (Poaceae) in Eastern Ghats, India

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ABSTRACT

An updated checklist of the genus *Eragrostis* Wolf (Poaceae) occurring in in Eastern Ghats of India based on field collections, study of herbarium specimens and literature survey is presented in this paper. A total of 23 species are recognized under the genus, of which *Eragrostis deccanensis* Bor is endemic to Peninsular India and distribution of *Eragrostis nairii* Kalidass is restricted to Odisha state.



## 1. Introduction

*Eragrostis* Wolf, a large genus of Poaceae tribe Eragrostideae, is comprised of 406 species (POWO, 2019) and distributed mainly in tropical and subtropical regions of the world. (Chaisongkram *et al.,* 2013). It is commonly known as 'love grass' and characterized by its paleas with unwinged keels, lemmas with obtuse or acute apex, rachilla internodes not bearing tufts of hairs near the base of each floret and stamens 2 or 3. Karthikeyan *et al*. (1989) recognized 36 species from India, but thereafter, many new taxa viz., *Eragrostis amabilis* var. *peramangalamensis* (Umamaheswari & Daniel, 1998), *E. brownie* (Thoiba & Pradeep, 2018), *E. burmanica* (Sridevi & Binojkumar, 2001), *E. collinensis* (Vivek et al., 2013), *E. dayanandanii* (Ravichandran *et al*., 1996) *E. henryi* (Vivek *et al*., 2013), *Eragrostis jainii* (Vivek *et al*., 2013) *E. kiwuensis* (Matthew, 1999), *E. minor* var. *rajasthanensis* (Vivek *et al*., 2016), *E. nairii* (Kalidass, 2015), *E. nilgiriensis* (Vivek *et al*., 2013), *E. paniciformis* (Veldkamp *et al*., 2017), *E. schweinurthii* (Matthew, 1999) *E. subsecunda* (Sreekumar & Nair, 1991) and *E. tremula* var. *gajanandii* (Singh *et al*., 2011) have been described from India.

As part of ongoing studies on the grasses of Eastern Ghats, the authors have collected many species of *Eragrostis* from Odisha and Tamil Nadu parts of Eastern Ghats. The specimens were identified in consultation with pertinent literature (Bor, 1960; Rao *et al*., 2017; Vivek *et al*., 2013d, 2015). Besides the field collections by the authors, specimens deposited in CAL, MH and RRPC were also examined. In addition, the published papers on grasses such as Panda & Choudhury (1984), Jha (1995), Mondal & Mukherjee (1991), Panda & Das (1995), Panda *et al*.(1994), Subbiah *et al*.(2012) were referred for preparation of the checklist. A total of 23 species of *Eragrostis* are known to occur in Eastern Ghats and are enumerated below.

## 2. Systematic Enumeration

1. ***Eragrostis amabilis*** (L.) Wight & Arn. in R.Wight, Cat. Indian Pl. 2: 105. 1834.

Distribution: INDIA (Andaman, Andhra Pradesh, Bihar, Daman & Diu, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh & West Bengal) and OLD-WORLD TROPICS.

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**2.** ***Eragrostis aspera*** (Jacq.) Nees, Fl. Afr. Austral. III: 408.1841.

Distribution: INDIA (Andhra Pradesh, Bihar, Karnataka, Maharashtra, Odisha, Rajasthan & Tamil Nadu) and AFRICA.

**3.** ***Eragrostis atrovirens*** (Desf.) Trin. ex Steud., Nom. Bot., ed. 2, 1: 562. 1840.

Distribution: INDIA (Andaman, Andhra Pradesh, Bihar, Daman & Diu, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh & West Bengal), AFRICA, JAPAN, MARIANAS, MALESIA, SRI LANKA and TAIWAN.

Note: The species is characterized by its persistent and stout rhachilla with winged, ribbed internodes, florets falling entire upwards and paleas with relatively short entirely scaberulous keels.

**4.** ***Eragrostis cilianensis*** (All.) Vignolo ex Janch., Mitt. Naturwiss. Vereins Univ. Wien 5(9): 110. 1907.

Distribution: INDIA (Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu & Uttar Pradesh) and OLD WORLD.

**5.** ***Eragrostis ciliaris*** (L.) R.Br. in Tuckey, Narr. Exped. Zaire: 478. 1818.

Distribution: INDIA (Andaman, Andhra Pradesh, Bihar, Daman & Diu, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh & West Bengal) and TROPICS & SUBTROPICS.

**6.** ***Eragrostis ciliata*** (Roxb.) Nees in C.F.P.von Martius, Fl. Bras. Enum. Pl. 2: 512. 1829.

Distribution: INDIA (Andhra Pradesh, Bihar, Gujarat, Odisha & Tamil Nadu), MYANMAR to INDONESIA.

**7.** ***Eragrostis coarctata*** Stapf in Hook.f., Fl. Brit. India 7(22): 313. 1896.

Distribution: INDIA (Andhra Pradesh, Bihar, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh & West Bengal), BANGLADESH, NEPAL and MYANMAR.

**8.** ***Eragrostis deccanensis*** Bor, Grasses Burma, Ceylon, India & Pakistan: 507. 1960. *Eragrostis phleoides* Stapf in Hook.f., Fl. Brit. India 7(pt. 22): 313. 1896, *nom. illeg.*, non Stapf 1896. *Steirachne deccanensis* (Bor) Kalidass in J. Biol. Rec. 3(1): 190. 2018.

Distribution: INDIA (Andhra Pradesh, Karnataka, Kerala, Odisha and Tamil Nadu), Endemic.

**9.** ***Eragrostis gangetica*** Steud., Syn. Pl. Glumac. 1:266. 1854. *E. dayanandanii* Ravich., Krishnan & N. P. Samson in Kew Bull. 51(1): 155. 1996.

Distribution: INDIA (Andaman, Andhra Pradesh, Bihar, Daman & Diu, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh & West Bengal), AFRICA and ASIA.

Note: *Eragrostis. dayanandanii* was treated as an endemic grass of Tamil Nadu by Clayton *et al*. (2006) and Kabeer & Nair (2009). Later Vivek *et al*. (2015) merged this species under *E. gangetica*.

**10.** **Eragrostis japonica** Trin. in Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math. 1: 405. 1831. *Eragrostis diarrhena* Steud., Syn. Pl. Glumac. 1: 266. 1854. *Eragrostis namaquensis* Nees ex Schrad. Linnaea 12(4): 452. 1838.

Distribution: INDIA (Andhra Pradesh, Assam, Bihar, Gujarat, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Nagaland, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh & West Bengal), AFRICA, ASIA and AUSTRALIA.

Note: It is a highly variable species. Earlier authors distinguished *E. diarrhena* from *E. japonica* by the dense panicles and oblong to lanceolate spikelets.

**11.** ***Eragrostis macilenta*** (A.Rich.) Steud., Syn. Pl. Glumac. 1: 268. 1854.

Distribution: INDIA (Andhra Pradesh & Tamil Nadu), AFRICA and MADAGASCAR.

**12.** ***Eragrostis minor*** Host, Fl. Austriaca 1: 135. 1827. *Eragrostis poaeoides* P. Beauv., Ess. Agrost. 162. 1812. *Eragrostis pappiana* (Chiov.) Chiov. Ann. Bot. (Rome) 8 (3): 371. 1908

Distribution: INDIA (Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Odisha, & Tamil Nadu) and OLD WORLD TROPICS.

Note: This species can be easily recognized in the field by its greenish-yellow spikelets, loosely imbricate florets and 3 stamens.

**13.** ***Eragrostis nigra*** Steud., Syn. Pl. Glumac. 1: 267.1854.

Distribution: INDIA (Andhra Pradesh, Assam, Bihar, Himachal Pradesh, JammuKashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh & West Bengal), CHINA, INDO-CHINA, INDONESIA and SRI LANKA.

Note: It is very closely resembles *E. atrovirens* but can be easily distinguished from by its spikelets olive green

to blackish (vs. spikelets laden gray in *E. atrovirens*) and panicle branches divided near base (vs. panicle branches not divided near base in *E. atrovirens*).

**14.** ***Eragrostis nairii*** Kalidass in J. Econ. Taxon. Bot. 39(1):126. 2015.

Distribution: INDIA (Odisha), Endemic.

Note: Reported occurrence of this neo-endemic species from Nilgiri by Pavithra *et al*. (2017) need confirmation.

**15.** ***Eragrostis nutans*** (Retz.) Nees ex Steud., Nom. Bot., ed. 2, 1: 563.1840.

Distribution: INDIA (Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh & West Bengal), CHINA, MALESIA, SE ASIA and SRI LANKA.

Note: *E. nutans* is allied to *E. atrovirens* but differs by its globose (not ellipsoid) grain, narrower and denser panicles, persistent paleas and smaller spikelets.

**16.** ***Eragrostis papposa*** (Roem. & Schult.) Steud., Nom. Bot., ed. 2, 1: 564.1840.

Distribution: INDIA (Andhra Pradesh & Punjab), AFRICA, PAKISTAN and SPAIN.

**17.** ***Eragrostis pilosa*** (L.) P.Beauv., Ess. Agrost. 71. 1812.

Distribution: INDIA (Andhra Pradesh, Arunachal Pradesh, Bihar, Goa, Gujarat, Jammu-Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu & Uttar Pradesh) and OLD WORLD TROPICS.

**18.** ***Eragrostis riparia*** (Willd.) Nees in C.F.P.von Martius, Fl. Bras. Enum. Pl. 2: 512. 1829.

Distribution: INDIA (Andhra Pradesh, Bihar, Karnataka, Kerala, Maharashtra, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh & West Bengal), MYANMAR, NEW GUINEA, PHILIPPINES and SRI LANKA.

**19.** ***Eragrostis tenella*** (L.) P.Beauv. ex Roem. & Schult. Syst. Veg., ed. 15 bis 2: 576. 1817. *Eragrostis amabilis* var. *insularis* (C.E.Hubb.) P. Umam. & P. Daniel in J. Econ. Taxon. Bot. 22(1): 216. 1998. *Eragrostis amabilis* var. *peramangalamensis* P.Umam. & P. Daniel in J. Econ. Taxon. Bot. 22(1): 216. 1998.

Distribution: INDIA (Andhra Pradesh, Karnataka, Kerala, Maharashtra & Tamil Nadu), MADAGASCAR, MASCARENES and SRI LANKA.

Note: Veldkamp (2002) while revising the genus *Eragrostis* for Malesia, merged *E. tenella* and *E. viscosa* under *E. amabilis*.

**20.** ***Eragrostis tenuifolia*** (A. Rich.) Hochst. ex Steud., Syn. Pl. Glumac. 1: 268. 1854.

Distribution: INDIA (Andhra Pradesh, Bihar, Daman & Diu, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu & Uttar Pradesh), AFRICA, AUSTRALIA and NEW GUINEA.

Note: It is often confused with *E. pilosa* due to the presence of open panicle and long pilose hairs in the axils of its branches but it is easily distinguished from the former by the nerveless glume and jagged outline of the spikelets.

**21.** ***Eragrostis tremula*** Hochst. ex Steud., Syn. Pl. Glumac. 1: 269. 1854. *E. tremula* var. *gajanandii* Singh, Bala Purohit, Indian For. 137(6): 797. 2011.

Distribution: INDIA (Andhra Pradesh, Bihar, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh & West Bengal), AFRICA, AFGHANISTAN and MYANMAR.

**22.** ***Eragrostis unioloides*** (Retz.) Nees ex Steud., Syn. Pl. Glumac. 1: 264. 1854.

Distribution: INDIA (Andaman, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh & West Bengal), AFRICA, ASIA, MYANMAR, NEPAL and SRI LANKA.

Note: Pinkish spikelets and the granular lemmas are the notable feature to identify the species in the field.

**23.** ***Eragrostis viscosa*** (Retz.) Trin. in Men., Acad. Sci. Petersb. Ser. 6.1:397. 1830.

Distribution: INDIA(Andhra Pradesh, Bihar, Daman & Diu, Gujarat, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh & West Bengal), AFRICA, MALESIA, MYANMAR, PHILIPPINES and SRI LANKA.

Note: Except the viscid nature of inflorescence, culm nodes and leaf sheath axils, it is practically impossible to segregate the species from *E. amabilis*.

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# *Habenaria plantaginea* Lindl. (Orchidaceae): A new record for Eastern part of the Chhotonagpur Plateau, West Bengal, India

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ABSTRACT

The taxonomy and micro-morphology of the terrestrial orchid species *Habenaria plantaginea* Lindl. (Orchidaceae) is reported here as a new distributional record for the state of West Bengal based on field collection, literature survey and laboratory work. Botanical description, line drawings, colour photographs of different plant parts, notes on ecology, distribution pattern, stereo-microscopic and SEM studies of the taxon has been provided for authentication of identity.



## 1. Introduction

Orchidaceae is regarded as second largest family of the Liliopsida and contains 25,000-35,000 species under 800-1,000 genera in the world (Dressler, 2006). About 90% of the orchid species are epiphytes and rest grow in terrestrial habitats. In India, about 1300 species of orchids belonging to 140 genera are reported to occur and maximum concentration of species can be found in eastern Himalayas, the Western ghats, Eastern ghats and the South Indian hills. The genus *Habenaria* is represented by about 876 species (Batista *et al*., 2013) and these are widely distributed in all continents except Antarctica. India is well represented with 72 species of *Habenaria*, of which 30 are endemic (Misra, 2007; Prasad & Venu, 2015). The presence of 12 species of *Habenaria* from West Bengal state has so far been reported (Choudhury *et al*., 2011).

*Habenaria plantaginea* Lindl., a terrestrial orchid having prominent underground bulbs was observed in the slope of Ajodhya hills (Chamtaburu) in Purulia District, West Bengal at an altitude of about 2336 ft under the canopy of *Terminalia chebula*. Ajodhya hills are the eastern most part of the Chhotonagpur plateau and also considered by some as the extended part of Eastern Ghats ranges.

Review of literature revealed that very little work has been done on this group. Stern (1997) worked on vegetative anatomy of Habenariinae and Bhaurav and Rajaram (2016) analysed the distribution, density and characteristics of 18 species of *Habenaria* occurring in Western Ghats of India. Some sporadic work on orchids, in general, has been done in different parts of India such as from Andhra Pradesh (Miria *et al*., 2012), Madhya Pradesh (Mujaffar *et al*., 2013), Tamil Nadu (Kottaimuthu *et al*., 2008; Christudhas and Mary, 2015) and Jharkhand (Kumar *et al*., 2007). The present paper deals with the taxonomy, micro-morphology and SEM studies of *Habenaria plantaginea*.

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## 2. Materials and methods

Field survey was conducted at Ajodhya hills of Purulia district, the Eastern part of the Chhotonagpur Plateu, West Bengal. The floral part were dissected, measured and photographs of both the vegetative and reproductive parts of the flower were taken under the Lica Stereo zoom Microscope (Model-S8APO). The SEM study of pollinia was done at Central Research Facilities, IIT, Kharagpur, West Bengal, India.

## 3. Enumeration of the species

**Habenaria plantaginea** Lindl. Gen. Sp. 323.1835; Prain, Bengal Pl.1032. 1903. *Plantaginorchis plantaginea* (Lindl.) Szlach. Richardiana 4 (2): 65. 2004.

### Botanical description

***Habitat:*** Terrestrial (Fig. No.2. a, c). ***Habit:*** Annual herb; erect; 40-45 cm tall (Fig No.1. a). ***Root:*** Roots adventitious, some modified to form ovoid or globose bulb (Fig N.1.a. & 2.h). ***Stem:*** Erect, cylindrical, glabrascent, herbaceous. ***Leaf:*** Exstipulate, simple, alternate, short-petioled or sessile, 10×3 cm long, leaf base sheathing; lamina elliptic to oblong, base obtuse, apex acute, succulent, soft in texture, glabrous, venation parallal (Fig No.2. f). ***Inflorescence:*** Racemose (Fig No.2.b, d, e & 3. a). ***Flower:*** Resupinate, bractate, pedicilate, bisexual, zygomorphic, hypogynous, white in colour, up to 15mm long (fig No. 4.b & c). ***Calyx:*** Sepals 3, anterior sepal (near to mother axis) fused, lateral 2 petals free, spreading and reflexed; anterior sepals cover the petals forming hood which further cover the column (Fig No.1.f & 3.d). ***Corolla:*** Petals 5, zygomorphic, bilabiate, upper 3 with long lips, deeply lobed, labelum to about 13 mm long, two side lobes much larger than middle lobe; the base of the upper petals spurred, spurs about 35-37 mm long; lower lip 2-lobed, free, 1.5-2 mm long, elliptic, apex acute, 1-2 nerved, hyaline, both the lips adnate at the base of the column (Fig No.3. c). ***Androecium:*** Formed to gynostemium, column short, anther cells adnate to the front of the column, discrete or rarely touching, their bases often prolonged in to tubes or a sack like structure containing translator (Fig No. 1. g. & 3. e, f), caudicles short, glands exposed, flat and discoid or elliptic or elongate with truncate end, pollinia 2, clavate or pyriform (Fig No. 1. h & 5. i, j, k). ***Gynoecium:*** Carpel 3; stigma 2; placenta parietal (Fig No. 3. h). ***Fruit:*** Dry dehiscent, bract persistent, 1.5 cm long, green in colour (Fig No. 1. i & 3. g).

**Flowering and fruiting:** August to October.

### SEM study of the pollinia

The examination of the pollinia taken under Scanning Electron Microscope (SEM) generated some important information. The whole pollinia 2-3mm long, consisting 4 different parts viz. pollen sac, caudicles, translator and corpusculum (Fig No.4. i). Corpusculum looks like ligules at one side and short at the other side (Fig No.4. j). Translator very long, about 1.5-2 mm in size, translator narrow on one side and wider on the other (Fig No.4. i). Translator is dorsi-ventrally flattened. Caudicles very short, covering translator. Pollen sac in cluster, cell surface of pollinium rectangular or reticulate in shape; every cell consists of series of rectangular or triangular massula; retipilate thickening present on the masulla (Fig No. 4. n); retipilate thickening consists of muri with distinct columella and sometime columella are fused with each other and forms bridge like tagetum (Fig No. 4. o).

### Distribution

This orchid species is native to tropical and sub-tropical parts of the world, like India (Fig No. 1), Nepal, Sri Lanka, Bangladesh, and Southeast Asia. The plant prefers well drained shady localities. In India it usually grows in moist and dry deciduous forests with rich humus and leaf litters.

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**Fig. 1.** Illustration of the plant, **a.** Habit sketch, **b-c.** Flower with spur, **d.** Lateral view of flower, **e.** Front view of flower, **f.** Column cover with hood, **g.** Column with pollinium, **h.** Single pollinia, **i.** Fruit with bract, **j.** T.S. of the ovary.


**Fig.2. a-c,** Habitat of the plant, **b.** Inflorescence, **d-e.** Flower, **f.** Leaf, **g.** Fruit, **h.** Plant with bulb.

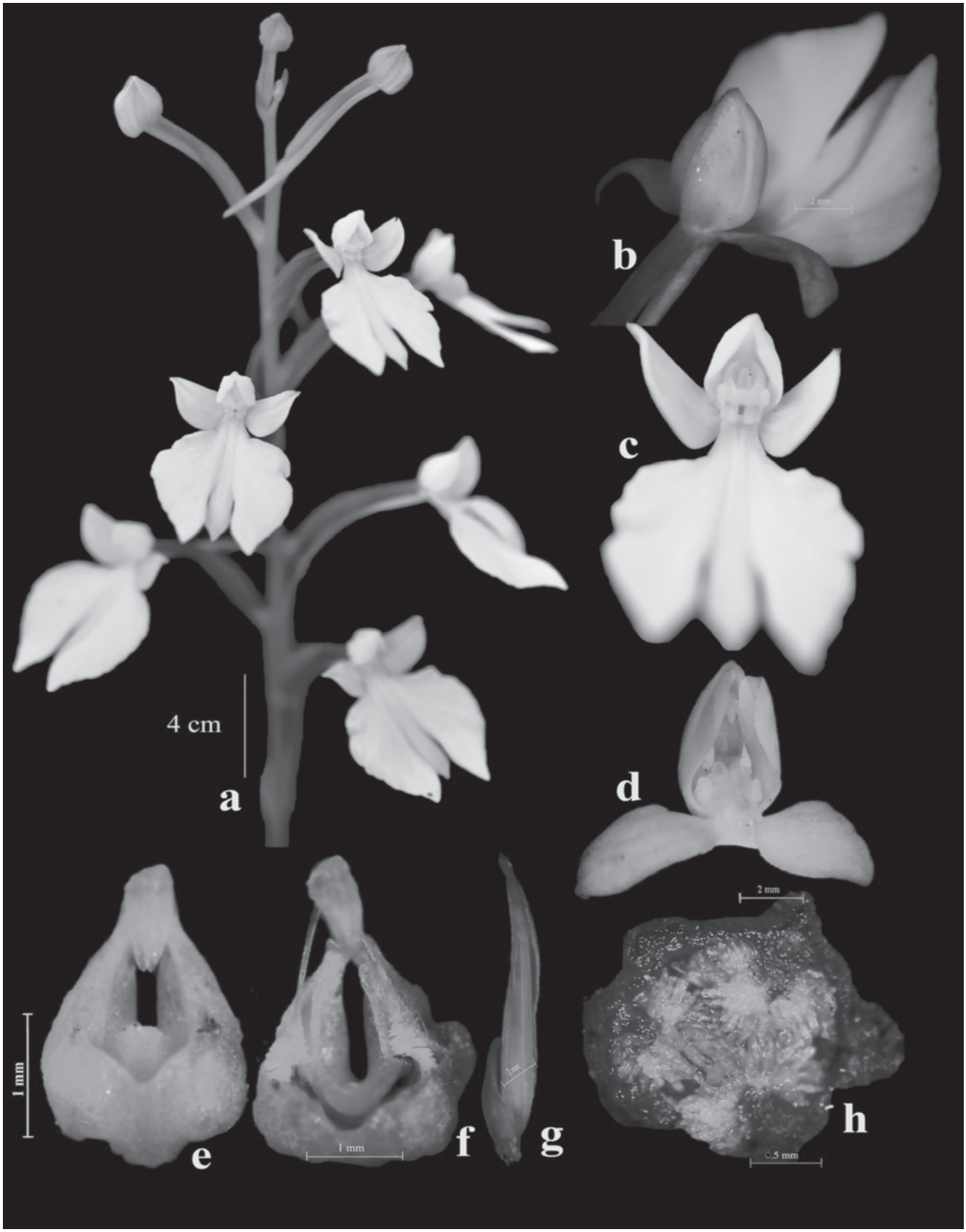


**Fig No. 3**. **a.** Inflorescence of the plant, **b.** lateral view of flower, **c.** Front view of the flower, **d.** Flower with pollinia, **e-f.** Column, **g.** Fruit with bract, **h.** T.S. of the Ovary.

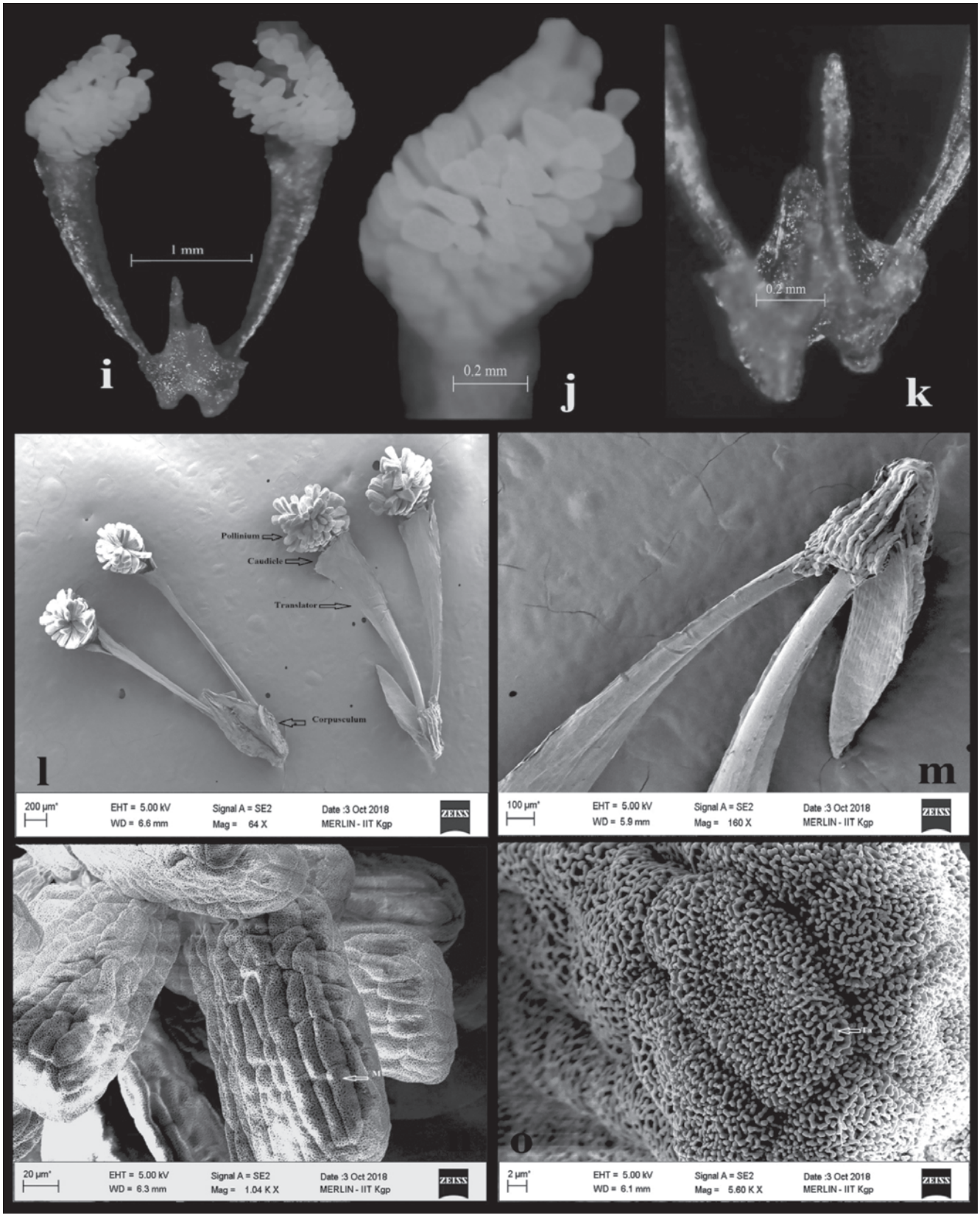


**Fig No. 4. i.** Pollinia, **j.** Single pollinium showing cluster of cell, **k.** Corpusculum, **l.** SEM structure of pollinia, **m.** SEM structure of Corpusculum with legule like appendage, **n.** Series of Masulla, **o.** Surface of pollen showing Muri and Tagellum. M- Masulla, Ta- Tagellum

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# *Riccia cavernosa* Hoffm. (Ricciaceae, Marchantiales, Hepaticopsida), an addition to Bryoflora of South India

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ABSTRACT

*Riccia cavernosa*is collected from Sri Krishnadevaraya University Botanic Garden and Siddarampuram forest Nursery, Ananthapuramu district, Andhra Pradesh, is reported here as a new distributional record for South India.



The genus *Riccia* (Mich.) L. (Ricciaceae; Marchantiales, Hepaticopsida), comprised of 150 species worldwide (Daniel *et al*., 2014), are the most common thalloid terrestrial liverworts distributed throughout the world. By habit they distinctly form rosettes and show very simple morphological characters. The genus *Riccia* is represented by 36 species in India, of which 18 are reported from different parts of South India (Singh, 2014).

As a part of our exploration of bryophytes in Andhra Pradesh, during 2016 and 2019 we could collect curious specimens belonging to the genus *Riccia* from Sri Krishnadevaraya University Botanical Garden and Siddarampuram forest nursery located in Ananthapuramu district, Andhra Pradesh. After critical microscopic examination, they found belonging to *Riccia cavernosa,* which till date has not been recorded in any locality of South India (Daniels 2010; Dandotiya *et al*., 2011; Uwe Schwarzi, 2013; Sandhyarani *et al*., 2014 and ENVIS: Bryophytes of Kerala, 2019) and hence form a new distributional record for the region.

Bryophyte explorations were conducted in all districts of Andhra Pradesh during 2016 to 2019 including Ananthapuramu district. Specimens from the soil were scraped manually with the help of bent and sharpened flat spoon and placed in zip lock polythene cover with labeled field number. Field observations were recorded in the field notes and live photographs were taken by using DSLR-Camera (Nikon D-3300). Collected materials were brought to the laboratory, made air dried at room temperature and preserved in brown paper packets (12 × 18 cm) with detailed labels on them. Critical examination of the specimens was done by using temporary slides and plant parts were separated by using micro forceps (Varin) VR-15 curved, VR-11 straight with fine sharp edges. Slides were observed under light microscope (Olympus CH20i), light stereo microscope (Olympus SZ61) and micro measurements were taken using ocular micrometer (ERMA) 19 mm, 100 segments in 1 cm. Photographs were taken by using Moto g3 turbo equipped with 13 MP camera, 4 x wide digital zoom, different dimensions were measured and identified belonging to *Riccia*

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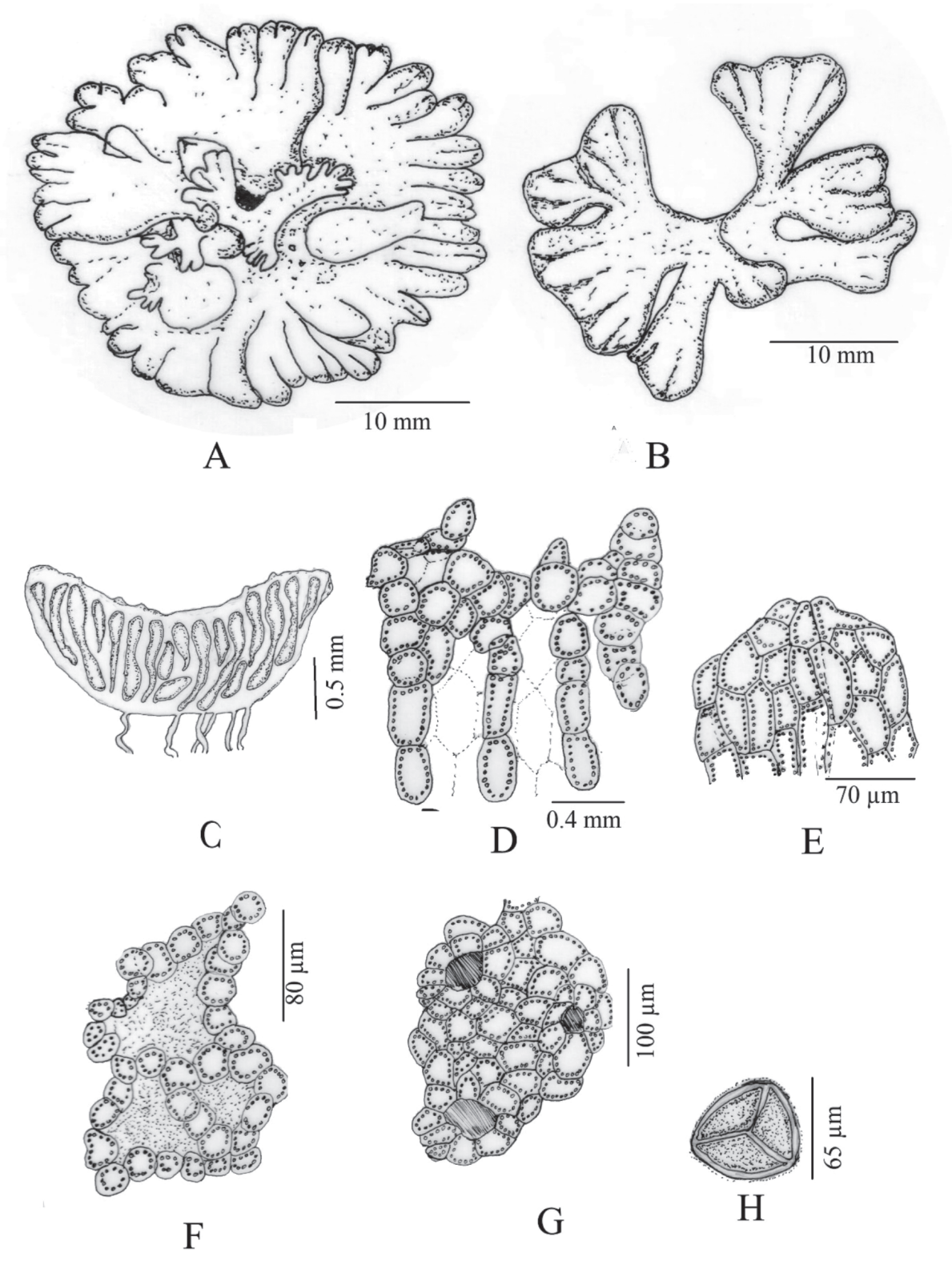


Figure 1. ***Riccia cavernosa*** Hoffm. *Deutschl. A. Complete thallus, B. Partial Thallus, C. T.S. of Thallus, D. Epidermal cells and assimilation tissue, E. Epidermal dome, F. Air chambers, G. Epidermal cells with pores, H. Spore.*

*cavernosa* by using standard floras. Technical description, voucher specimens, illustration are provided for the species and specimens were deposited in Sri Krishnadevaraya University Herbarium (SKU), Ananthapuramu. Abbreviated names used for the collectors are: AS (AnanthaneniSreenath) and BR (Boyina Ravi Prasad Rao).

***Botanical description***

**Riccia cavernosa** Hoffm. Deutschl. Fl. 2: 95 1796. (Figure 1).

Monoecious thallus,medium-sized to large in complete regular rosettes up to 30 mm across; brightgreen to yellowish green; often becoming tinged with light red along the margins, older parts lacunose (cavernose) when dry, margins yellowish, spongy. Branches repeatedly furcate, shortly to deeply divided, and overlapping, oblong to obovate, apex obtusely rounded, shortly emarginate, thallus margins rounded, obtuse; ventral surface rounded, green. Scales absent.Dorsal surface porous, appears spongy with large air cavities, each air chamber cells 4 to 6sided, walls slightly bulging, up to 80-105 x 50-55 µm. Assimilatory tissue up to 0.8 mm thick, air chambers generally in a single storey, appearing to be several storeys in section, due toobliquely sloping cavities, bounded by unistratose walls of chlorophyllous cells; storage tissue occupying ventral part of thallus, rhizoids present both tuberculate and smooth. Gametophytic stages are not visualized; in cross section capsules numerous in 1-3 rows slightlyprojecting ventrally. Spores light brown to dark brown, reticulate, globosely triradiate to sub globose, mark on proximal view, 68-85 µm in diameter.

*Habitat:* The species naturally grows on moist sandy soil in nursery areas and few thalli appear in rainy season.

*Specimens examined:* India, Andhra Pradesh, Ananthapuramu District, Sri Krishnadevaraya University Botanical Garden, 02 September 2019, 51629 SKU, BR & AS & Siddarampuram forest nursery, 04 October 2019, 57006, SKU, BR & AS.

*Distributions: World* - Africa, Australia, Bangladesh, Europe, Iran, Macronesia, Nepal, North America, Pakistan, Russia, South America and *India* - Himachal Pradesh, Madhya Pradesh, Nagaland and Rajasthan.

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# *Turnera subulata* Sm. (Passifloraceae): A species new to the flora of Odisha, India

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ABSTRACT

*Turnera subulata* Sm. (Passifloraceae) is reported here as a new distributional record for the state of Odisha, India. A detailed note on the nomenclature, botanical description, phenology, ecology and distributional of this species is provided in this paper.



The genus *Turnera* L. with about 140 species belongs to the family Passifloraceae (APG III, 2003) and members of the genus are distributed mainly in the Tropical America and Africa (Thulin *et al.* 2012). Among these, *Turnera ulmifolia* and *T. subulata* have been reported from Peninsular India (Gamble 1935; Manilal and Sivarajan, 1982; Srinivasan 1983; Tripathi, 1993) and only one species such as *Turnera ulmifolia* L. is known to occur in Odisha (Saxena and Brahmam, 1995). During the botanical exploration of Khurda District, Odisha, we have collected some interesting specimens of the genus *Turnera* L. from Bhubaneswar. On critical examination of plant specimens and consultation of pertinent literature (Srinivasan, 1983; Kumar, *et al.*, 2000), it was identified as *Turnera subulata* Sm. Perusal of literature revealed that the species has so far not been reported from Odisha (Saxena and Brahmam, 1995). Thus, occurrence of *Turnera subulata* in Bhubaneswar is a new distributional record for the flora of Odisha state. The species is enumerated herewith with brief description, notes on distribution, phenology, taxonomical notes. Besides, field photographs are provided here to facilitate easy identification. The voucher specimens have been deposited in the Herbarium of Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India.

**Turnera subulata** Sm. in Rees, Cycl. 36: no. 2. 1817; Baker in Steenis, Fl. Males. 4 236 1951; Srinivasan, in Nair and Henry, Fl. Tamil Nadu, India 1 169 1983; Mohan and Henry, Fl. Thiruvananthapuram 206 1994. *Turnera elegans* Otto in Nees, Hort. Phys. Berol 36 1820. *Turnera ulmifolia* L. var. *elegans* (Otto) Urban, Monogr. Turn. 139 1883; Gamble, Fl. Pres. Madras 1 523 1935; Matthew and Britto in Matthew, Fl. Tamil Nadu Carnatic 1 627 1983. **Fig.1**

Annual, erect large herbs, up to 60 cm in height; branchlets slender, densely clothed white hairs, ascending highly branched. Leaves simple, alternate; elliptic-obovate, 4-7× 2-3 cm long, base cuneate or decurrent; margin serrate, apex obtuse or acute, pubescent, base one pair of gland-dotted; lateral nerves 7 pairs. Petiole 1.2 cm long, slender, pubescent. Inflorescences solitary in leaf base, axillary.

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Flowers bisexual, 5-merous; bracteolate, one pair, linear, 1 cm long. Pedicel 8mm long, adnate partially or totally to the petiole. Calyx valvate, companulate, 1.5 cm in diameter, pubescent, base united; calyx-lobes lanceolate, each lobe, 1 cm long, pubescent, margin entire, apex obtuse with a pointed mucro, 2-4 mm long. Corolla 5 free, twisted to left, 1.5-3 cm in diameter, pale yellowish white, basal bluish black spot, 5 lobed; corolla lobes, widely obovate, each lobe, 2.2 cm long, glabrous. Stamens 5, connate at base calyx- tube; filament 7 mm long, glabrous; anther yellow, 3-6 mm long, basifixed. Ovary tricarpel, ellipsoid, glabrous, 2-5 mm long; style 3, free, 6 mm long, glabrous; stigma plumose, bright yellow.

*Flowering*: June – August *Fruiting*: Not observed.

*Distribution*: Native of Tropical America and naturalized all over the country. INDIA (Almost all states).

*Specimens examined*: INDIA, Odisha State, Khurda District, Bhubaneswar, VIP colony, N20Ú18'789'' E085Ú40'366", ±102 MSL, 06. 08. 2016, *Kalidass & Murugan* 9498 (RPRC).

It is commonly found in moist and the road side,

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Fig. 1 *Turnera subulata* Sm. (Passifloraceae): A. habit, B. flowering twig

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# Does *Mimosa hamata* Willd. (Mimosoideae: Fabaceae) occur in Tamil Nadu?

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ABSTRACT

The occurrence of *Mimosa hamata* Willd. (Mimosoideae: Fabaceae) in Tamil Nadu was previously considered doubtful. Now, the existence of this armed straggler is confirmed based on the present fresh collections from Dindigul, Madurai and Tirunelveli districts. The confusion with regard to its distribution and taxonomy is discussed in this paper.



*Mimosa* L. is the second largest genus of the subfamily Mimosoideae of Fabaceae (Savassi-Coutinho *et. al*., 2012) and is comprised of 540 species (Lewis *et al*., 2005; Bessega *et al*., 2008). The genus is principally Neotropics in distribution, with centers of diversity in central Brazil, subtropical South America and Mexico (Simon & Proenca, 2000; Simon *et al*., 2011). In India, it is represented by 11 species (Sanjappa, 1992), of which three are distributed in Tamil Nadu (Vajravelu, 1983). The genus differs from other similar mimosoids by the absence of glands on the anthers and it is easily recognized in the field by its bipinnate and paripinnate leaves, 3–6-merous and iso- or diplostemonous flowers and articulated or non-articulated craspedium fruit (Barneby, 1991).

During the present systematic studies on legumes of Tamil Nadu, the authors have collected some interesting specimens of *Mimosa* from Dindigul, Madurai and Tirunelveli districts of Tamil Nadu. A critical examination of the specimens coupled with study of relevant literature confirmed it as *Mimosa hamata*. Scrutiny of literature on distribution of this species in Tamil Nadu revealed that it is remain doubtful. Mayurnathan (1929) reported this species from Madras, whilst Livingstone & Henry (1994) omitted the species in flowering plants of Madras city and its immediate neighborhood owing to the lack of authentic specimens. Similarly, Vajravelu (1983) included it in the Flora of Tamil Nadu under the addenda based solely on the authority of Matthew (1981). However, this species was not reported either in Flora of Tamil Nadu Carnatic (1983) or Flora of Tiruchirapalli District (1998). Moreover, this species was not reported in any of the works pertaining to the flora of Tamil Nadu (Kottaimuthu, 2014; Manickam *et al*., 2008; Matthew 1999; Nair & Nayar 1982; Natarajan *et al*., 2002; Pallithanam, 2001; Sankar *et al*., 2012; Senthilkumar & Krishnamurthy, 1993; Vajravelu *et al*., 1987). Therefore, the present collections form the first authentic report of occurrence of this species from Tamil Nadu. A brief description along with relevant notes are provided here for easy identification.

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***Mimosa hamata*** Willd. Sp. Pl. 4: 1033. 1806; Baker in Hook.f., Fl. Brit. India 2: 291. 1878; Gamble, Fl. Pres Madras 1: 421. 1919; Sanjappa, Leg. India: 68. 1992; Pull. & Ramamurthy, Fl. Eastern Ghats 2: 98. 2001. **(Fig.1)**

A medium sized much branched shrub up to 2 m. high, branches pubescent, armed with hooked prickles. Leaves stipulate; stipules setaceous c. 3 mm long, hairy; bi-pinnate, rachis 1.2-5.0 cm long, sometimes prickly. Pinnae 3-6 pairs, 7-25 mm long, leaflets 6-10 pairs, more or less sessile, 2-3 x 1-2 mm, linear or ovate-oblong, acute, mucronate, glabrous above, pilose below. Flowers in globose heads, solitary or paired in upper axils; peduncles longer than the leaves, 1.5-3.5 cm long, puberulous; heads c. 10-13 mm in diameter. Flowers pink, tetramerous, sessile; bracts linear, spathulate, pilose. Calyx ca. 1 mm. Corolla tube ca. 3 mm long, lobes c. 1.5 mm long. Stamens 8. Ovary stalked, hairy. Pod 5-7.5 cm long, c. 1.0 cm broad flat, falcate, velvety, sutures emarginated between the joints and armed with hooked prickles. Seed ovoid, flattened, reddish brown.

*Flowering & Fruiting:* April and August.

*Distribution:* INDIA (Andhra Pradesh, Karnataka, Madhya Pradesh, Punjab & Tamil Nadu) and PAKISTAN (Baluchistan, Punjab & Sind).

*Specimens examined:* Tamil Nadu: Dindigul District; Nilakottai-Dam site, 180 m, 10.8.2013, R. Kottaimuthu 151076; Madurai District, Ayalanallur grave yard, 160 m, 20.7.2013, R. Kottaimuthu 52393; Tirunelveli District, Kadayam-Courtallum, 180 m, 10.6.2009, R. Kottaimuthu 35279 (Saraswathi Narayanan College Herbarium).

Fig. 1: Fruiting twig of *Mimosa hamata* Willd.

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