RESEARCH AND ACTIVITY REPORT 2019-20

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Bhubaneswar

Research and Activity Report 2019-20

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MESSAGE

Shri Bikram Keshari Arukha
Minister
Forest & Environment Department
Govt. of Odisha

I am happy to learn that Regional Plant Resource Centre (RPRC), Bhubaneswar is bringing out its Research and Activity Report (2019-20).

Regional Plant Resource Centre (RPRC), a reputed centre for fundamental and applied scientific research, has been implementing various research projects while achieving its goal in the field of plant taxonomy and conservation, biotechnology, biochemistry, microbiology, horticulture, medicinal and aromatic plants. Apart from intensive research on plant biodiversity assessment, microbial applications, wild edible fruits, mushrooms, mangroves, orchids and phyto-chemicals of selected medicinal plants, few other aspects like bio-fuel production, viral indexing of economically important crops including tissue culture of banana are noteworthy as important thrust and frontier areas of plant sciences. It has consistently maintained and added to its rich collections of rare and endangered plants, orchids, bamboos, palms, cacti and succulents, mangroves, medicinal & aromatic plants, for developing a repository of bio-resources for research and improvement.

I appreciate the effort of all concerned for publishing the Research and Activity Report (2019-20) and hope that it would be immensely useful for students, teachers, researchers and conservationists.

(Bikram Keshari Arukha)

MESSAGE

Dr. Mona Sharma, IAS
Additional Chief Secretary
Forest & Environment Department
Govt. of Odisha

The Regional Plant Resource Centre, a Research & Development organization, under the Forest and Environment Department in Odisha has been implementing several innovative research projects for bio prospecting indigenous macro and microflora for wider use through fundamental and applied research. The centre has strengthened and augmented its research and development programmes for inventorising, conserving, propagating, and documenting the rich biological wealth of the region. Production of quality planting materials on commercial scale, germplasm conservation and re-introduction of rare and endangered plants including mangroves & orchids are some of the significant activities of the organization.

I am happy to learn that RPRC has also successfully completed several research projects funded by different Departments of Government of India and Odisha. I hope the Institute would continue to endeavor in finding solutions to meet recent challenges in conserving the biological diversity of the State.

I express my appreciation for bringing out this research & activity report (2019-20) which would be a positive source of information to and disseminate the findings of various research activities being undertaken by the Institute.

Best wishes.

(Dr. Mona Sharma)

FROM CHIEF EXECUTIVE'S DESK

Smt. Pusazhule Mekro, IFS
Chief Executive

It is my pleasure to bring out this research & activity report for the period 2019-20 to address various research and development activities undertaken at RPRC. The report presents implementation of our research programmes which are prioritized to address issues pertaining to conservation and bio-resource utilisation relevant to the eastern ghats in general and Odisha state in particular. In continuation to our effort to establish germplasm banks of various plant groups for conservation and scientific enquiry, the center initiated and maintained advance line of research focusing the prioritized areas such as germplasm conservation and re-introduction of RET and other important special group of plants including mangroves and orchids,biodiversity mapping of eastern ghats, screening of wild edible fruits, mushroom and medicinal plants for active bio-molecules, nutraceauticals, antioxidants, microbial interactions on mangroves and orchids, viral indexing of crop plants, biofuel and micro-propagation of plantation crops and endangered plants.

Scientists were allotted various research projects with financial support from state Forest & Environment Department under state plan budget after rigorous evaluation by the recently formed Research Advisory Committee (RAC) headed by the PCCF in the Government. The centre has implemented several such research projects covering various thrust areas of research relevant to the state as per recommendation of RAC.

The center has been nurturing academic intellect by guiding Ph.D. and M.Sc. students. A six month Project training progarmme for M.Sc. (Biotech) students from various organizations is being organized to provide hands on training to fulfill the requirement of their M.Sc. degree. Several research papers in national and international journals have been brought out by the Centre and many new processes and technologies have been developed.

The scientists, research fellow and supporting staff of RPRC made sustained effort and contributed to growth of the institute, and I extend my sincere thanks to them for their endeavour. Financial support received from various agencies of Govt. of India and Odisha is gratefully acknowledged. We are grateful to Additional Chief Seceretary, Forest and Environment Department, Government of Odisha for providing the research grant under state plan budget and support provided by Director, (Environment) is thankfully acknowledged.

(Pusazhule Mekro)

INTRODUCTION

Regional Plant Resource Centre (RPRC), popularly called 'Ekamrakanan', was established in 1985 at Bhubaneswar, the capital city of Odisha over an area of 487 acre and can be approached from the land mark CRPF square on NH-5 and is about 1.5 Km. towards northern side of CRPF square. RPRC is an autonomous R & D organization under the Forest and Environment Department, Govt. of Odisha. The centre undertakes scientific research and applied activities to conserve, propagate and document plant resources of the region. Commercial production of horticultural plants, germplasm conservation of rare and endangered, wild edible fruits, medicinal plants and mangroves are some significant activities of the organization. The center is recognised as a Research and Development organisation by the State Government and Government of India to undertake research leading to Ph.D. degree by Several Universities. During the last 35 years of its existence, the Regional Plant Resource Centre has established itself as a leading research institute of the country and a major centre for conservation of biological diversity of plants. The Botanic Garden raised and maintained by the institute with its panoramic views attract visitors from far and wide. The RPRC Campus has a beautiful lake of about 40 acres and a very rich and diverse flora of more than 2000 species both natural and introduced. Due to conducive conditions, RPRC campus also supports rich faunal diversity (36 species of mammals, 148 species of birds, 38 reptile species, 15 species of amphibians, 15 fish species, 104 species of butterflies, 58 species of arachnids and 41 odonate species have been recorded so far). During recent years, the centre has gained a distinctive position among the leading research institutions in plant sciences and a major centre for conservation of plant diversity of Eastern India.

The Centre has implemented 27 research projects funded by different Departments of Government of India and Odisha during the year 2019-20. The research activities of RPRC are guided by the Scientific Advisory Committee (SAC) and Research Advisory Committee (RAC).

MANDATE

The center has a mandate of promoting plant conservation, research and to augment plant resources for sustainable development in the following areas

- Germplasm collection of selected plant groups (living collections) for long term conservation and research.
- Survey, evaluation, propagation and conservation of medicinal, aromatic, oil-yielding and other economic plants including rare/endangered species.
- Genetic manipulation of plants through cell, tissue and organ culture, somatic embryogenesis, transformation techniques and other biotechnological approaches.
- Studies on production, conversion and utilization of biomass especially of fuel-wood species.
- Provide necessary expertise and assistance in landscaping, garden lay out, green belt development, plant identification and impart training on plant propagation and nursery technologies.
- Dissemination of information through publication of scientific and popular articles.
- Co-operate and collaborate with other national and international institutions to promote the cause of conservation of biological diversity of plants and exchange of seed and plant materials.

EXECUTIVE SUMMARY

Research activities carried out during 2019-2020 in RPRC under various field of Research and Development projects are summarized below:

Plant Diversity Assessment, Conservation and Monitoring

Quantitative assessment of tree diversity in Chandaka Wildlife Sanctuary has been undertaken. In the present study, the trees, shrubs, climbers and herbs occurring in the entire area were collected, identified and listed. Emphasis was laid on plant biodiversity documentation in special habitats and recording data on special groups of plants like weeds, epiphytes, lithophytes, grasses, wetland plants etc.

Studies on structure and composition of tree vegetation in different forest types in Similipal Biosphere Reserve have been described. The quantitative assessment of plant resources of Similipal Biosphere Reserve has been made by Regional Plant Resource Centre. While *Shorea robusta, Terminalia alata, Anogeissus latifolia, Xylia xylocarpa* and *Protium serratum* were the five most dominant species for the buffer zone, *Shorea robusta, Syzigium cumini, Dillenia pentagyna, Kydia calycina* and *Protium serratum* were the five major species of quantitative importance in the sample plots of core area of Similipal Biosphere Reserve. Sal was more dominant in core than buffer zone as could be observed from high values of stem density, frequency and dominance.

Structure and composition of tree vegetation in tropical moist deciduous forests of Nayagarh Forest Division as influenced by different intensities of disturbance have also been studied. This study was performed in representative sample plots harbouring moist deciduous forest patches located in three Reserve forests (Central RF, Radadimaua RF and Sapua RF) of Nayagarh Forest Division, Odisha, India.

Systematic studies of the family Solanaceae in Eastern Ghats, India has been undertaken to present the taxonomic review of the species of Solanaceae, based on comparative chemotaxonomic, morphological studies. Solanaceae Juss. is one of the largest and most important families of flowering plants, and major crop plant species such as *Solanum tuberosum* L., *Solanum lycopersicum* L., *Solanum melongena* L., *and Capsicum annum* L. belong to these taxa. Special emphasis was given to the primary sculpture, periclinical walls and secondary sculpture with special reference to Scanning Electron Microscope study of the rest of the genus.

Microbial Diversity and Applications

The work on optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide (EPS) by some selected Fungi. Polysaccharides from fungal source has been proved to be effective in different plethora of biomedical fields. Present study has been carried out on EPS production by Fusarium proliferatum, novel fungus as no reports on its EPS production capability has been reported yet. The present work include morphological , physiological and molecular characterization and identification of the fungus. The main objective of this study was to optimize fermentation conditions for better EPS production extracellularly under submerged culture conditions where we have screened different culture conditions, media and incubation period and temperature by following the OFAT (one factor at a time) method.

Standardization of nursery technology by application of PGPF (Plant growth promoting fungi) under different soil compositions and its impact on quality of *Piper longum*: A RET medicinal plant of Odisha, has been undertaken. Plant growth promoting properties of microbial resources are becoming important as they help in improving plant growth under abiotic and biotic stress conditions. An experiment under pot culture condition using submerged culture of native microflora of *Piper longum* having potential of phosphate solubilisation and plant growth promotion properties has been carried out.

Studies on harnessing the potential of endophytes against root knot nematodes *Meloidogyne icognita* in banana. This is a Net-working project under BPCL-NER –Banana programe funded by DBT, Govt. of India. Banana is important staple food and agricultural products in most tropical countries and consumed widely all over the world. The root-knot nematodes (*Meloidogyne icognita* and other *Meloidogyne* spp.) can cause the banana yield reduction for 20–30% commonly. Endophytic microbes play a vital role in plant protection and growth promotion The use of these microorganisms is preferred compared to chemical fertilizers and pesticides because of their lower cost and their contribution to sustainable agriculture. Recent reports have observed that endophytes efficiently promoted plant growth and that the endophytes may be biocontrol candidates against plant parasitic nematode .

A study has been undertaken on evaluation of fungal bioinoculants on growth and development of some forest plantation tree species under field conditions. Plant growth, development and production of their useful products may be improved through nutrient management which can be achieved by application of either chemical and/or biological fertilizers. Plant growth promoting microorganisms are useful tool for growth and development of plants of different sectors viz., agriculture, forestry, horticulture and ornamentals . The process of inoculating microbes to the soil in a forest nursery could be an effective method to achieve higher growth and establishment of tree species on native sites. The development of quality planting material through bioinoculation practices not only endow with economic benefit to the end users but improve soil fertility and ultimately sustainability in native sites. Hence, a present study was carried out on bioinoculation of two fungi (phosphate solubilising) on *Pongamia pinnata* and *Dalbergia sissoo* in pot culture condition

Extraction, purification and characterization of bioactive secondary metabolites and enzymes from endophytic fungi dealt with the laboratory experiments carried out to enhance the potential of L-asparaginase production by *Fusarium* sp. and secondary metabolite "piperine" by fungal endophyte of Piper longum . L- asparaginase is an anticancer enzyme and sourced from many bacteria and fungi. Though, bacterial enzyme causes more allergic reactions, search for fungal origin of this enzyme is vital. In view, a fungal endophyte *Fusarium* sp. was used for the Mass scale culture preparation under submerged condition in SD medium. The data obtained on its media optimization , extraction and purification may be compiled and published as ready reference for the scientific community.

Propagation, conservation and re-introduction of RET & other important plants

Research work on phytochemical evaluation, nutritional analysis, propagation and reintroduction of selected threatened plants of Odisha has been initiated. Vegetative parts of *Hypericum gaitti* has been evaluated as possible source of anti-depressant drugs hypericin, pseudohypericin and hyperforin. It has been observed that the aerial parts of *H. gaitii* accumulated the chlorogenic acid and the several flavonoids, namely, hyperoside, isoquercetine, quercitrine, quercetine, and hyperforin. However, the quantification of the above secondary metabolites is yet to be done with corresponds to the peak area of respective standards.

Comparative assessment of the nutritional, anti-nutritional properties and neurotoxicity of *Cycas sphaerica* endosperms and leaves has also been undertaken. The endosperms and leaves of *Cycas sphaerica* was collected from Rajini Reserve Forest, Khurda, Odisha. Both were rinsed with distilled water to remove any dust particles. After that they were air dried at 37˚C temperature, grinded into fine powder and kept in air tight containers for further analysis. Proximate analysis of the various parts of *Cycas sphaerica* was determined using the protocol prescribed by Association of Official Methods of Analysis. Proximate analysis of *Cycas sphaerica* endosperms and leaves were carried out to examine the nutritional value of the plants.

For scaling-up of propagation methods for *Hypericum gaitii* and propagation of Cycas sphaerica from bulbils, an experiment on vegetative propagation using bulbils as explants was laid and successful method of root induction in bulbils has been achieved. Successful sprouting, formation of young leaves and inductions of healthy roots were observed after four months of the treatment .

Re-introduction of *Lasiococca comberi, Hypericum gaitii, Cycas sphaerica* in few new habitats and their performance evaluation has been undertaken in three different reintroduction sites of Odisha (Chandaka-Damapada W/L sanctuary, Rajin Reserve Forest, Khurda and Mandasaru gorge, Kandhamal). This year the growth performance of reintroduced *C. sphaerica* plants in all three sites have been assessed and found to be quite encouraging .

Studies on restoration of wild orchid population in Chandaka & RPRC through reintroduction of in vitro raised seedlings has been initiated. In this project, in vitro propagation methods used for *Vanda tessellata, Cymbidium aloifolium, Acampe praemorsa* and *Aerides odorata* for mass production of seedlings. These seedlings were used for the population restoration programs.

Mass propagation and breeding facility for orchids has been established in RPRC. For the cultivation of orchid in a commercial scale, the major factor is the availability of quality planting materials. Currently, the all the planting materials are being produced through tissue culture. Among the different ornamental orchids, *Dendrobium* hybrids in general are being used for the cut flower production because of their long vase life. Among the many varieties *Dendrobium* Sonia, *Dendrobium* Rynco Green, *Dendrobium* White Fairy, *Dendrobium* Burana White, *Dendrobium* Bina Zumbo Paul and *Dendrobium* Pink Fragrance are of highly important for the commercial scale cultivation. In this project, mass production of planting materials are produced using tissue culture techniques for these varieties.

Standardization of micro-propagation methods for *Anogeissus latifolia, Santalum album* and *Desmodium oogeinense* an important and endangered forest trees has also been carried out.The micro-propagation has proven to be the method of choice for rapid multiplication of selected forest tree species, where the seed and

vegetative propagation is a problem. In the present study, three important and endangered forest tree species were selected for micro-propagation i.e. *Anogeissus latifolia, Santalum album* and *Desmodium oogeinense*

Research on conservation of salt-sensitive back-mangroves *Heritiera fomes* and *H. littoralis* through re-introduction in protected area has been implemented through application of vegetative propagation technique. In this project, attempt has been made to initiate re-introduction of *Heritiera littoralis*, locally called dhala sundari, a special group of mangrove plant using vegetatively propagated saplings inorder to create and enhance public awareness on biodiversity conservation, promote social, ecological & economic benefits of the coastal people of the country in general and the State of Odisha in particular.

Studies on Medicinal plants and Wild edible fruits

A study on Phytotherapeutic investigation of *Piper trioicum* as neurological disorder: An insight into therapeutic avenues towards Alzheimer's disease has been undertaken. Due to its high medicinal value and based on the ethno medicinal uses of Indian System of Medicine (ISM), *P. trioicum* is used as a memory enhancer but till date there is no claim to treat neurodegenerative disorder like Alzheimer's disease. So, the project has been undertaken to validate neuroprotective and memory enhancing properties.The research investigation was carried out to screen the presence of phytochemicals in hydroalcohol extract of *P. trioicum* (HPT). The HPT contains high amount of alkaloid, steroid and flavonoids. Biological activities were carried out by performing in vitro, ex-vivo and in vivo antioxidant activities.

Work has been done on pharmacological profiling of *Geophila repens* and *Bacopa floribunda* and evaluation of their therapeutic potential against Alzheimer's disease. Alzheimer's disease (AD) is one of the most recognised neurodegenerative diseases that impairs memory, cognitive functions and may lead to dementia in late stage of life. The study highlights the isolation, structural elucidation and quantification of Pentylcurcumene (PC), a terpene from hydroalcohol extract of *G. repens* (GRHA). Based on results of our current findings, it is concluded that Pentylcurcumene, a terpene is an important bioactive molecule in *G. repens* has potential anticholinesterase activities and it could be a potential source of drug in Alzheimer's disease (AD). Bioautography method will be extremely helpful in the identification of other novel anticholinesterase compounds in *G. repens*. Further investigations are to be needed in in vivo model to establish the key mechanism and establish the proper pathway of Pentylcurcumene to combat neurological diseases like Alzheimer's disease.

A study has been carried out on *Hydrolea zeylanica* pertaining to alteration of the expression of glucose transporter protein in type-2 diabetic rats. In this project, the role of bioactive principles in the most active hydroalcohol fraction of *H. zeylanica* (HAHZ) in bringing glycaemic control, GC-MS and LC-MS analysis were investigated. To better understand the mode of inhibition of HAHZ, molecular mechanism of action of HAHZ in oxidative stress induced diabetes, streptozotocin-induced oxidative stress and metabolic changes in diabetic rats were studied.

Ameliorative effects of *Homalium zeylanicum* on diabetes-induced oxidative stress and inflammation in Wistar rats has also been studied to establish scientifically the efficacy of this plant for combating diabetes-induced oxidative stress and inflammation in Wistar

Unexplored *Ardisia solanacea* and *Aegiceras corniculatum* plants of Myrsinaceae family were evaluated as alternate source of embelin and other related compound as substitutes for over exploited RET medicinal species *Embelia ribes* and *E. tsjeriam-cottam*. In this research work, apart from embelin, other related compounds like,

Epigallocatechin gallate (EGCG) , Epigallocatechin (EGC) and Quercetin have been evaluated. Epigallocatechin gallate (EGCG) , Epigallocatechin (EGC) and Quercetin compounds were evaluated from the fractions of four compounds using different solvent (methanol, chloroform, petroleum ether and hexane) extracts of leave and fruits of *Ardisia solanacea* and *Aegiceras corniculatum*. The EGCG and EGC compound were analyzed (HPLC) using mobile phase water:acetonitrile:methanol:ethyl acetate:glacial acetic acid.

Bixa orellena and *Nyctanthes arbortristis* have been evaluated for antifungal activity using *Aspergillus flavus* and *Aspergillus niger* as target experimental model. Antifungal activity against *Aspergillus flavus* and *Aspergillus niger* was tested using three methods, radical growth method, Agar diffusion method and biomass reduction method. Four solvent extracts of *Bixa oreallana* and *Nyctanthes arbortristis* leaves were prepared using soxlet extraction method.

Effect of temperature on withanolide contents of *Withania somnifera* plants grown by seeds stored at different temperatures has been studied in addition to phytochemical analysis. It was observed that no major differences were found in the withanolide content of *Withania somnifera* with respect to different temperatures in which the seeds were stored.

Qualitative and quantitative analysis of essential amino acids and pectin in lesser known wild edible fruits of Odisha have been performed. Pectin is a natural product which is found in the cell walls of all higher plants. It is the methylated ester of polygalacturinic acid. In terms of nutrition and health, pectin has several biological and physiological functions which have been shown to lower blood cholesterol level, helps in prostate cancer treatment and acts as a potential carrier for drug delivery. Keeping in mind the growing need for alternative bionutrition resources, these wild edible fruits need to be popularized for their edibility and medicinal properties. This study dealt with estimation & evaluation of composition of various essential amino acids and pectin in selected wild edible fruits of Odisha to promote bioprospecting, conservation and domestication of promising species. In this research work, 4 wild edible fruits of Odisha viz. *Grewia tiliifolia, Limonia acidissima, Streblus asper* and *Olax scandens* were subjected to evaluation of all Essential amino acids.

Underutilized and under exploited wild edible mangrove fruits of Odisha coast were evaluated for their nutritional and antioxidant properties. In this research work, fruits of the mangrove species viz. *Bruguiera sexangula* (Odia name-Bandari), *Rhizophora mucronata* (Odia name- Rai), and *Xylocarpus granatum* (Odia name- Sisumara), belonging to Bhitarkanika National Park in Kendrapada District of Odisha, were subjected to comparative evaluation of nutritional properties and further analysis of antioxidant potential of the nutritionally superior fruit species.

Bioenergy and Biofuel

A study on elucidation of genetic network with response to salt and drought stress in *Saccharum bengalense* Retz. was undertaken. The major source of bioethanol production is from the biomass; thus availability of the raw material is the major hurdle. In recent years, dedicated energy crops have been developed those are able to grow on waste of abundant agriculture lands. *Saccharum bengalense* is growing wildly in different parts of the state of Odisha belonging to the sugarcane family. The species has shown resistance to drought in field condition. In pot experiments, plants also have shown resistance to salts. It is possible that, the plant *Saccharum bengalense* do have unique genetic network with regard to managing different types of stress. In this study, novel genetic networks have been identified with regard to the salt and water stress through transcriptome sequencing.

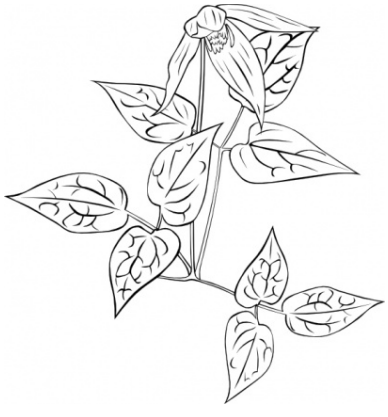
Horticulture and Floriculture

To regulate ripening and enhance fruit shelf-life in banana, an important fruit crop for food security, work has been initiated to identify novel and key candidate proteins/genes responsible for ripening during different developmental and ripening stages of banana through Omics-based approaches. Work has also been focused on tissue-specific proteome expression and mass spectrometry identification of banana during fully developed and ripening stages to identify differentially expressed proteins.

Studies have been carried out on Mass Propagation of Local *Musa* Varieties of Odisha for commercialization Using Tissue Culture Techniques. In this work, effect of cobalt toxicity on rooting culture of Gaja Bantala was investigated.

Studies on production of quality planting material of ripe banana and Analysis of genetic fidelity through molecular marker have been undertaken. In this project, effect of IBA and Activated charcoal in Yangambi and Champa Varieties during rooting culture was investigated. This apart, virus indexing test had been initiated with the help of National Research Centre For Banana (NRCB), Tiruchirapalli, Tamil Nadu through Polymerase Chain Reaction (PCR) for the detection of Banana Bunch Top Virus (BBTV), Cucumber Mosaic Virus (CMV),Banana Streak Mosaic Virus (BSMV) and Banana Bract Mosaic Virus (BBrMV).

Work on Standardization of protocol for micro-propagation of potato varieties has been carried out . Due to the high susceptibility of potato to diseases, especially to viruses, several studies have started micropropagation by using meristems and shoot tips as explants in order to produce virus free seed potato. In addition to low quality of the crop output, productivity changes from year to year and hence the deficit in the supply of the crop leads to its high price. The choice of suitable variety (Kufri Pukhraj) is of paramount importance for successful commercial cultivation of potato. This current work aimed at developing a suitable protocol for large scale commercial production of virus-free potato tuber seeds applicable for Odisha state.



RESEARCH ACHIEVEMENTS

Plant Biodiversity Assessment, Conservation and Monitoring

Study of the quantitative ecology, phenology and phyto-sociology of dominant forest trees of Odisha.

(State Plan Funded)

Principal Investigator: Dr. Pratap Chandra Panda, Principal Scientist

Research Fellow: Prabhat Dash, Subrat Kar (SRF)

(a) Quantitative assessment of tree diversity in Chandaka Wildlife Sanctuary

In the present study, the trees, shrubs, climbers and herbs occurring in the entire area were collected, identified and listed. For quantitative ecological study, the area was divided into smaller sampling grids and transects of 1000 m X 5 m (0.5 ha) were laid in representative vegetation types to capture maximum diversity. For each transect, data on geographical coordinates, soil types, forest types and general information about the sites were collected. All the tree species occurring in each transect were enumerated and field level data on density, abundance, frequency, regeneration potential, use value of each of them were recorded. Each species was photographed and GPS data of place of occurrence were recorded in case of rare and economically important species so that the species can be relocated. Emphasis was laid on plant biodiversity documentation in special habitats and recording data on special groups of plants like weeds, epiphytes, lithophytes, grasses, wetland plants etc

A total number of 2108 individuals of tree species with ≥ 30 cm GBH were recorded from 79 sample quadrates (39.5 ha) of Chandaka- Domapada Sanctuary. They represent 113 species belonging to 83 genera under 36 families (Table-1, Fig.1) The family Fabaceae (including Papilionaceae, Mimosaceae and Caesalpiniaceae) with 28 species was the most dominant taxon in terms of species content followed by Euphorbiaceae (10 species), Rubiaceae (10 species), Verbenaceae (8 species) and Moraceae (8 species). Eighteen (18) families were represented by single species only. The top 10 species and their contribution to density, basal area and IVI in studied area were *Xylia xylocarpa* (IVI=30.979), *Diospyros sylvatica* (IVI=14.164), *Aegle marmelos* (IVI=13.842), *Careya arborea* (IVI=11.606), *Strychnos potatorum* (IVI=11.351), *Tectona grandis* (IVI=11.033), *Strychnos nux-vomica* (IVI=11.000), *Lagestroemia parviflora* (IVI=10.681), *Holarrhena pubescens* (IVI=8.908) and *Cassia fistula* (IVI=7.556). The values of diversity indices such as Shannon-Weiner Index, Simpson Index and Evenness Index were calculated as 3.962, 0.03 and 0.83 respectively.

Fig.1. Floristic survey and quantitative assessment

Table-1: Biodiversity parameters of tree species enumerated from Chandaka Wildlife Sanctuary

No. of tree species	113
No. of genera	83
No. of families	36
Number of individuals	2108
Stand Density (No. of stems ha-1)	222.211
Total Basal Area (m2)	34.517
Stand Basal Area (m2 ha-1)	11.2
Maximum tree GBH (cm)	120
Mean tree GBH (cm)	43.7
Shannon-Weiner Index	3.962
Simpson Index	0.03
Evenness Index	0.83

A total of 2108 trees were enumerated from on the study sites of Chandaka-Dampara sanctuary and the total basal area (BA) was found to be 34.517 sq. m. The stand density was calculated as 222.211 stems/ ha. The stand density and basal area showed decreasing trend with increasing girth class in Chandaka. The highest stem density of 618.83 stems/ ha and basal area of 8.613 sq. m/ ha was recorded for lowest girth class of 30-60 cm GBH. The lowest stem density of 1.94 stems/ ha and basal area of 0.149 sq. m/ ha was calculated for trees of lowest girth class (91-120 cm.

Table-2: Girth class distribution according to density and basal area of tree species enumerated from Chandaka Wildlife Sanctuary

Girth Class	Density (Stem ha-1)	Basal Area (m2 ha-1)
30-60 cm	618.83	8.613
61-90 cm	63.63	2.445
91-120 cm	1.94	0.149

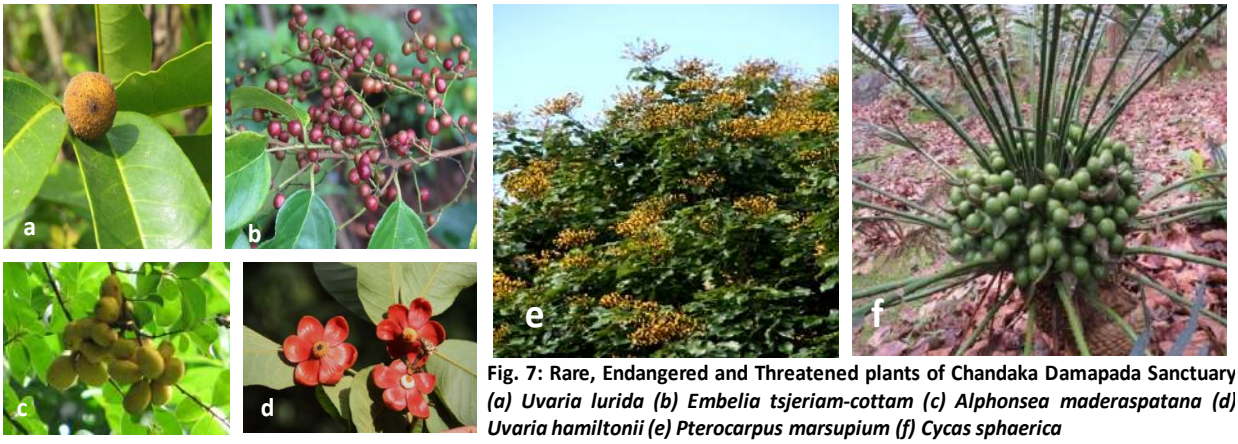


Fig. 7: Rare, Endangered and Threatened plants of Chandaka Damapada Sanctuary ***(a) Uvaria lurida (b) Embelia tsjeriam-cottam (c) Alphonsea maderaspatana (d) Uvaria hamiltonii (e) Pterocarpus marsupium (f) Cycas sphaerica***

(b) Structure and composition of tree vegetation in different forest types in Similipal Biosphere Reserve

The vegetation of Similipal has been classified under three broad forest types e.g. Semi Evergreen Forest, Moist Deciduous Forest and Dry Deciduous Forest with several seral types and degradation stages. A recent study made by RPRC on quantitative assessment of forest biodiversity in Tropical Semi Evergreen Forests and Dry Deciduous Hill Forests of Similipal taking 4 sample transects of 1000m X 5 m each (2.0 ha) from each forest type, it was found that Semi Evergreen Forest is quite rich in terms of species richness, stand density, basal area and with higher diversity indices (Table- 1, 2 and 3).

Table-3: Comparison of diversity, density and dominance of tree species in Semi-evergreen and Dry Deciduous Hill forests of Similipal

Diversity Index	Semi Evergreen	Basal Area (m2 ha-1)
Forest	Dry Deciduous Hill Forest	8.613
Species Richness	78	49
No. of Individuals	1241	972
Stand Density (No. of stem ha-1)	620.5	486
Stand Basal Area (m2 ha-1)	37.7135	19.7803
Shannon Index	2.3109	3.3685
Simpson Index	0.0113	0.0561
Evenness index	0.5304	0.8655

The quantitative assessment of plant resources of Similipal Biosphere Reserve has been made by Regional Plant Resource Centre taking 10 nos. of 1000 X 5 m transects (5 ha) in the buffer area and 16 transects of the same size in the core zone (8 ha). A total number of 106 tree species from buffer area and 126 species from buffer area have been recorded. Shorea robusta (Sal) was the most dominant species in terms of frequency, density and dominance in both core and buffer zones of Similipal (Table – 4 & 5). While *Shorea robusta, Terminalia alata, Anogeissus latifolia, Xylia xylocarpa* and *Protium serratum* were the five most dominant species for the buffer zone, *Shorea robusta, Syzigium cumini, Dillenia pentagyna, Kydia calycina and Protium serratum* were the five major species of quantitative importance in the sample plots of core area of Similipal Biosphere Reserve. Sal was more dominant in core than buffer zone as could be observed from high values of stem density, frequency and dominance.

Table-4: Stand density and Basal Area in Core and Buffer zones under different girth class

	30-60 cm		61-90 cm		91-120 cm		121-150 cm		≥150 cm	
	Density (Stem ha-1)	Basal Area (m2 ha-1)	Density (Stem ha-1)	Basal Area (m2 ha-1)	Density (Stem ha-1)	Basal Area (m2 ha-1)	Density (Stem ha-1)	Basal Area (m2 ha-1)	Density (Stem ha-1)	Basal Area (m2 ha-1)
Buffer Zone (10)	306.00	4.28	120.00	5.32	64.40	5.56	46.00	6.77	58.00	16.12
Core zone (16)	406.55	5.92	183.09	8.11	112.73	9.81	55.82	8.00	36.91	9.80

Table-5: Diversity parameters for tree species in Similipal

Diversity Measures	
Area sampled	13 ha
Species Richness (No. of spp.)	158
Stand Density (No. of stem ha-1)	860.76
Stand Basal Area (m2 ha-1)	40.38
Shannon Index (H')	3.83
Simpson Index (Cd)	0.08
Evenness index	0.76

(C) Structure and composition of tree vegetation in tropical moist deciduous forests of Nayagarh Forest Division as influenced by different intensities of disturbance

In view of growing threat to biodiversity, it is important to assess and quantify the loss in terms of alterations of natural communities and their structural attributes as influenced by anthropogenic disturbances. Understanding the response of forest vegetation to different intensities of human disturbance would help in prioritizing conservation efforts to be taken up. The present study was undertaken to assess the impact of different intensities of disturbance on tree species diversity and community composition in three forest blocks of Nayagarh Forest Division of Eastern Ghat region in Odisha experiencing varying degrees of human interference. This study was performed in representative sample plots harbouring moist deciduous forest patches located in three Reserve forests (Central RF, Radadimaua RF and Sapua RF) of Nayagarh Forest Division, Odisha, India.

In all three Reserved Forests (Central RF, Radadimaua RF and Sapua RF) of Nayagarh Forest Division, Odisha selected for the study, the villages are located in the forest fringes and the villagers travel a distance of 1-8 km for collection of forest produce and grazing of animals, the nearby forests being the most frequently visited. Based on the distance of sample plots from the nearby village-clusters and intensity of anthropogenic disturbances described above, the study area (sample plots) has been classified into highly disturbed (HD), moderately disturbed (MD) and undisturbed (UD) categories.

In each stand, density, frequency and basal area of the tree species (GBH>30 cm) were estimated in randomly placed belt transects of 1000m X 5 m (0.5 ha). A total of 25 transects - eight transects in undisturbed sites (UD), eight transects in moderately disturbed (MD) sites and nine in highly disturbed (HD) sites were laid for tree enumeration. The number of individuals of each standing tree with ≥ 30 cm GBH were counted physically and girth at breast height (GBH) was taken at a height of 1.37 m above the ground level. The seedlings/ saplings, shrubs and climbers were enumerated from two 5mX5m quadrates and herbs from two 1m X 1m quadrates located within each transect.

The occurrence of 311 species of trees, shrubs, climbers and herbs has been reported from all 25 transects (12.5 ha) laid in Nayagarh Forest Division, Odisha, India under Highly Disturbed (HD), Moderately Disturbed (MD) and Undisturbed (UD) categories (Table-6). This includes 104 species of trees, 49 species of shrubs, 51 liana/ climber

species and 187 species of herbs. Undisturbed (UD) stand was the richest in terms of species diversity, where 195 plant species (62.70%) were found to be present. In the present study lowest species richness (62 species) and stand density (206.22 trees/ha) was noted in Highly disturbed (HD) sample plots and highest species diversity (77 species) and stand density (744 trees/ ha) in case of Undisturbed forest stands (Table-7). The values of the two diversity indices viz; Shannon, Simpson, Fisher's alpha and Margaleff's indices varied greatly across the three study stands. While Shannon's Index varied from 3.30-3.42, Simpson Index ranged between 0.74 and 0.80, Fisher's alpha from 14.45 to 15.18 and Margaleff's index for species richness varied between 29.65 and 34.71.

Highest stand density of 744 stems ha−1 was recorded for undisturbed forests and lowest (206.22 stems ha−1) in highly disturbed forests. The stand density was found to differ considerably amongst three disturbance gradients. The stand basal area varied from 12.74 m2 ha−1 in highly disturbed (HD) forests to 23.27m2 ha−1 in Moderately Disturbed (MD) and 36.95m2 ha−1 in undisturbed (UD) forests.

Table-6: Taxonomic composition of highly disturbed (HD), moderately disturbed (MD) and undisturbed (UD) forest stands.

	No. of species				No. of genera	No. of families
	HD	MD	UD	Total		
Trees	62	72	77	101	78	37
Shrubs	17	35	30	49	39	23
Climbers/Lianas	22	43	27	51	43	21
Herbs	138	122	70	187	121	31
Total	239	272	204	388	281	75

Table-7: Key Diversity attributes of trees in highly disturbed (HD), moderately disturbed (MD) and undisturbed (UD) forest stands.

Variable	HD (4ha)	MD (4ha)	UD (4ha)	Total (12ha)
No. of tree Species	62	72	77	101
No. of genera	54	59	63	79
No. of family	28	31	35	37
Number of individuals	742	1733	2976	5451
Stand density (No. of Stems ha-1)	185.50	433.25	744.00	454.25
Total basal area (m2)	50.18	93.10	147.80	291.07
Stand basal area (m2 ha-1)	12.54	23.27	36.95	24.26
Maximum tree gbh (cm)	269	300	360	360
Mean tree gbh (cm)	77.11	69.38	69.29	70.38
Shannon-Weiner Index	3.24	3.20	3.42	3.44
Simpson Index	0.11	0.12	0.09	0.10
Evenness Index	0.78	0.75	0.79	0.74
Fisher's alpha Index	16.10	15.17	14.45	17.61
Margaleff (M Base 10.) Index	34.83	31.80	29.65	26.76

Fig. 3: Important trees species of Nayagarh Forest Division-(a) *Pterospermum* xylocarpum (b) *Terminalia bellirica (c) Oroxylum indicum (d) Litsea glutinosa (e) Bauhinia purpurea (f) Aegle marmelos (g) Erythrina suberosa (h) Butea monosperma (i) Xylia xylocarpa (j) Mimusops hexandra and (k) Memecylon umbellatum.*

Fig. 4: Field work in Nayagarh Forest Division and Similipal

Systematic studies of the family Solanaceae in Eastern Ghats, India

(State Plan Funded)
Principal Investigator: Dr. C. Kalidass, Scientist
Research Fellow: Ms. Madhusmita Mallia, JRF

Linnaeus (1753) recognized two groups from the family Solanaceae in his book Species Plantarum i.e. Pentandria monogynia and Didynamia monogynia. Within the Pentandria monogynia, *Atropa, Capsicum, Datura, Hyoscyamus, Mandragora, Nicotiana, Physalis*, and *Solanum* were grouped and slightly away but still within the group where *Brunfelsia, Cestrum* and *Lycium* were placed. The second group Didynamia monogynia included *Browallia* and *Schwenckia*. In this grouping, *Lycopersicon* and *Melongena* were grouped under *Solanum*.

Solanaceae Juss. is one of the largest and most important families of flowering plants, and major crop plant species such as *Solanum tuberosum* L., *Solanum lycopersicum* L., *Solanum melongena* L., and *Capsicum annum* L. belong to these taxa. Several species of pharmaceutical interest due to their secondary metabolites, species of economic relevance and toxic species are also classified in this cosmopolitan family. The greatest species diversity of Solanaceae is observed in the Eastern Ghats, India. The first, proposed by Hunziker (2001) and based on morphological and chemical criteria, comprises approximately 2300 species in 92 genera distributed across the subfamilies Solanoideae, Cestroideae, Juanulloideae, Salpiglossoideae, Schizanthoideae, and Anthocercidoideae. The second, a more recent proposal, was presented by Olmstead et al. (2008) in a molecular study conducted on a sample of 89 genera and 190 species. The authors proposed seven subfamilies: Solanoideae, Cestroideae, Nicotianoideae, Petunioideae, Schizanthoideae, Goetzeoideae, and Schwenckioideae. Both proposals agree that Solanoideae is the most derived subfamily in relation to the Cestroideae. Though a number of studies have been done in India in the past to understand the systematic, morphology and taxonomy of flowering plants in various families and genera, the family still pose problems for taxonomists. However, no study on taxonomy, nomenclature. So, the current study aimed to present the taxonomic review of the species of Solanaceae, based on comparative chemotaxonomic, morphological studies of Solanaceae.

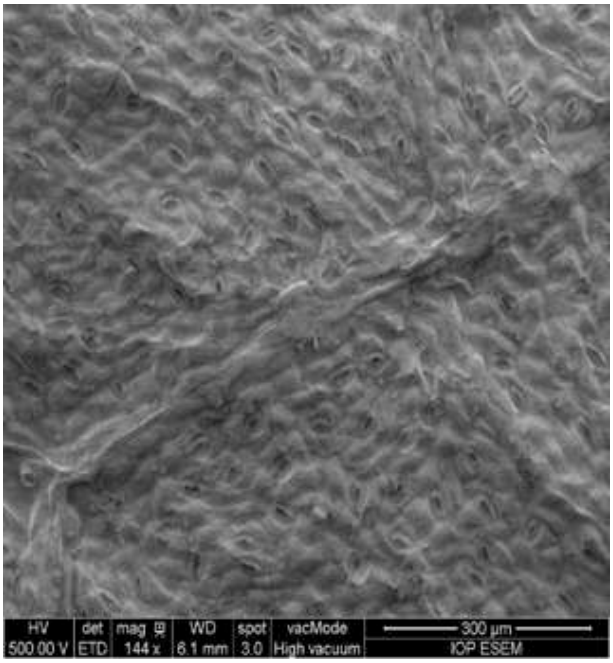
Currently, we are carried out the study on the basis of fresh samples collected from the field trips and analysis of existing herbarium specimens and also more number specimens from various Herbarium of Eastern Ghats, India. We conducted field trips (20 days) for surveyed and exploration of the genus *Solanum L., Physalis, Datura., Cestrum, Capsicum* and *Withania* in different regions of the Eastern Ghats (Fig. 1). In addition, we have been consulted National herbaria of BSI and Central National Herbarium, Howrah (CAL) and observed 164 herbarium sheets for *Physalis* species, 179 sheets for *Datura* species, 150 sheets for *Withania somnifera*, 55 sheets for *Lycianthes*, 115 sheets for *Solanum lycopersicon*, 230 sheets for *Cestrum* species. Additional to that we also consulted electronic materials of International herbaria of Royal Botanic Gardens, Kew (K) and NYBG through the website. We were the first to report species of *Physalis angulata* var. pendula and description of a new species of *Cestrum pandanii* sp. nov. from the Eastern Ghats (Fig.2).

The seeds of one genus belonging *Physalis* were studied by Scanning Electron Microscope. Micro and macromorphological characters including the seed shape, colour, size, seed shape pattern was studied and their data are presented here (Fig. 3). The seeds were distinguished on the basis of seed topography, presence of pores and hirsutous nature. Special emphasis was given to the primary sculpture, periclinical walls and secondary sculpture. SEM morphology of seed and systematics of Solanaceae of Eastern Ghats of India has not been done so far. Moreover, we will be completing the Scanning Electron Microscope study of the rest of the genus.

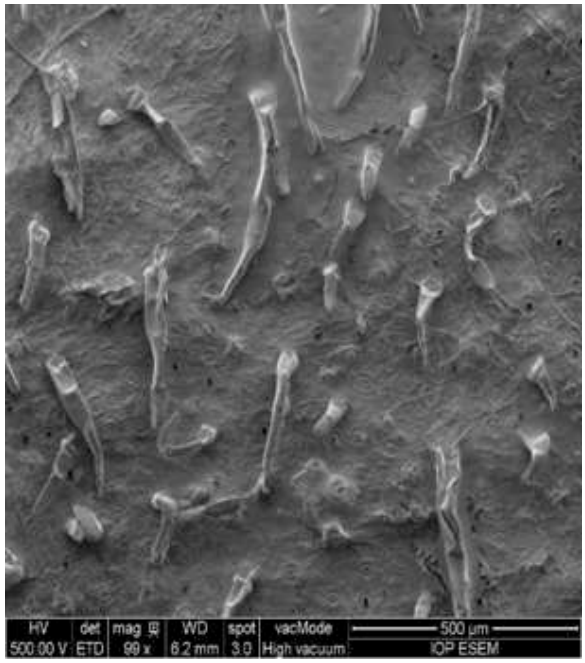
Key to the genus of Solanaceae in Eastern Ghats, India

Calyx neither inflated nor closing the fruit				
	Fruit a capsule			
		Capsule spinous, Flowers more than 15cm…..		*Datura*
		Capsule non spinous, Flower less than 5 cm….		*Nicotiana*
	Fruit a berry			
		Corolla tubular ……..		*Cestrum*
		Corolla campanulate or rotate		
			Filaments longer than the anther…..	*Capsicum*
			Filaments shorter than the anther…..	*Solanum*
Calyx inflated closing the fruit				
	Fruiting with calyx solitary in number…….			*Physalis*
	Fruiting with calyx more than 3 in number...……..			*Withania*

Fig. 1. Patterns of the Solanaceae family



C. diurnum



C. pandanii sp. nov.

C. pandanii sp. nov.

Fig. 2. A new species for Cestrum through SEM study by taking the leaf section

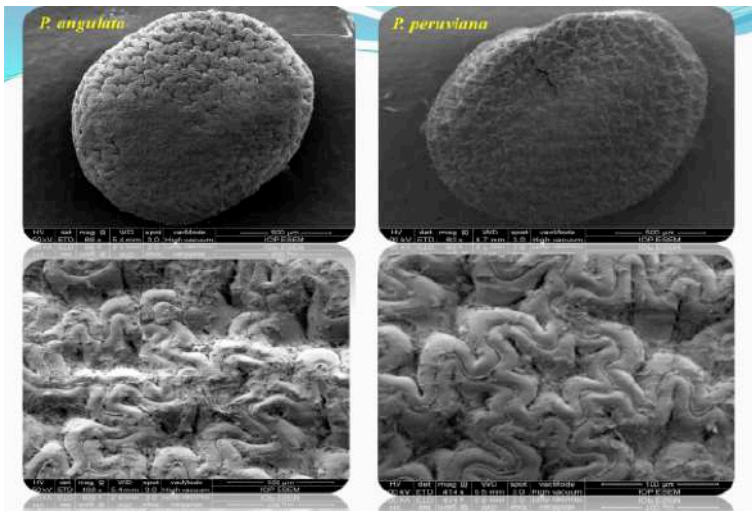


Fig. 3. Scanning electron microscope micrographs of *Physalis* seeds

Microbial Diversity and Applications

Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by some selected Fungi

(S&T Dept., Govt. of Odisha funded)

Principal Investigator: Dr. Nibha Gupta, Principal Scientist
Research Fellow : Smita Behera , JRF

Polysaccharides from fungal source has been proved to be effective in different plethora of biomedical fields. Present study has been carried out on EPS production by *Fusarium proliferatum*, novel fungus as no reports on its EPS production capability has been reported yet. The present work include morphological , physiological and molecular characterization and identification of the fungus. The main objective of this study was to optimize fermentation conditions for better EPS production extracellularly under submerged culture conditions where we have screened different culture conditions, media and incubation period and temperature by following the OFAT (one factor at a time) method . Among all malt extract broth was selected as favorable medium for EPS yielding 81.4±0.48 mg/l. Output confirms 9 days of incubation period, pH 6.0 and temperature 25°C best for the growth and exopolysaccharide yield.

For more specific conclusion Plackett Burman and screening designs were opted for selection of more suitable media components in different combinations. Malt extract broth medium used as basal medium and supplementation of xylose, glucose, tryptophan, olive oil, Tween 80, vitamin C, K_2HPO_4 and $CaCl_2$ was done at different ratio. A total of sixteen media were formulated using these supplements for the study. Finally medium 12 was selected as best for the EPS yield. Furthermore permutation and combination in medium 12 was performed to ensure proper result. Hence the optimized medium formulated includes: malt extract medium (basal medium), xylose 4%, glucose 4%, tryptophan 0.1%, olive oil 3%, Tween 80 0.2%, vitamin C 0.2%, K_2HPO_4 0.2%, $CaCl_2$ 0.5%, pH 6.0, incubation period of 9 days at temperature 25 °C yielding 4067 ± 153.08 mg/l of EPS. The biochemical study of the crude sample indicated the presence of protein, DPPH activity, phenolics and reducing sugar. The crude EPS was purified through Sepharose 6B column and characterized for sugar moiety , functional groups and structural organization. HPTLC, FTIR and LCMS analysis exhibited the monomeric composition of maltose, fructose, xylose, galactose, glucose, raffinose and sorbose with functional group of –OH, C=O, -C-O-C with glycosidic likanges and suggested the production of glycan by this fungi .

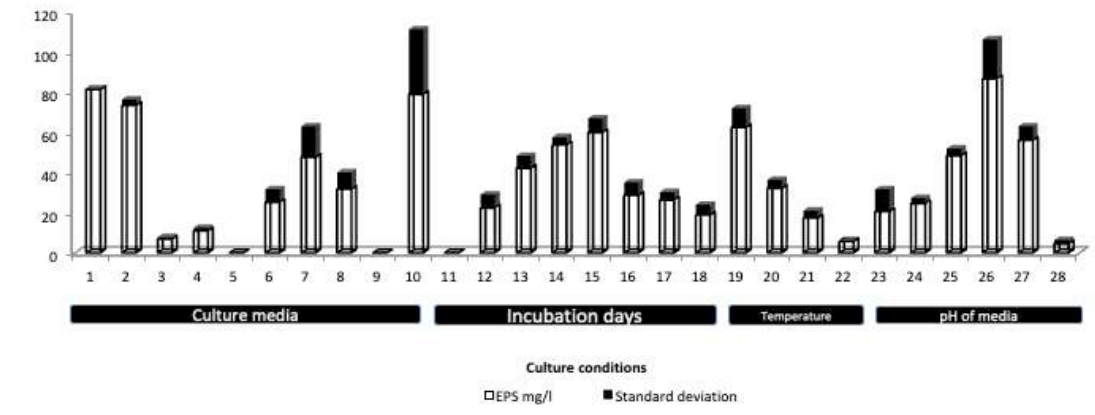


Fig. 1 EPS production by *F. proliferatum* under submerged culture conditions

Culture media : Malt extract, Sabouraud dextrose, Potato dextrose, Czapek dox , Mushroom complete medium, Yeast malt extract, Tien and Kirk, Glucose yeast extract peptone, Yeast and mould , liquid medium ; Incubation period : 3, 5, 7,8,9,12,15,and 20 days ; Temperature 25, 30,35, 40° C; pH of medium : 3,4, 5, 6, 7 , 8

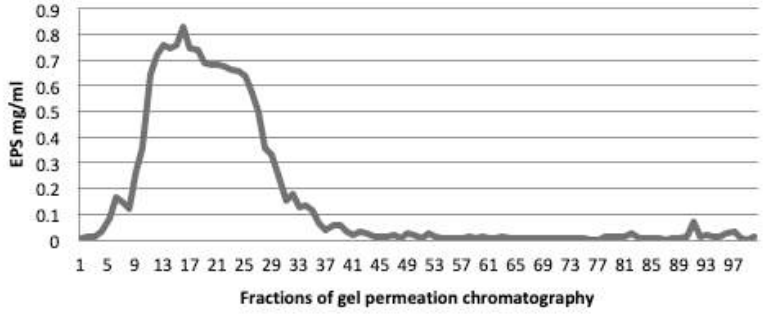


Fig.2 Purification of EPS through Sepharose 6 B gel permeation column chromatography

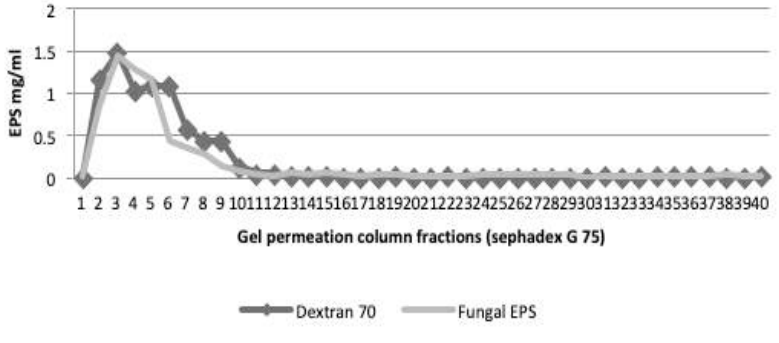


Fig. 3 Estimated value of EPS and Dextran 70 through gel permeation sephadex G 75 column

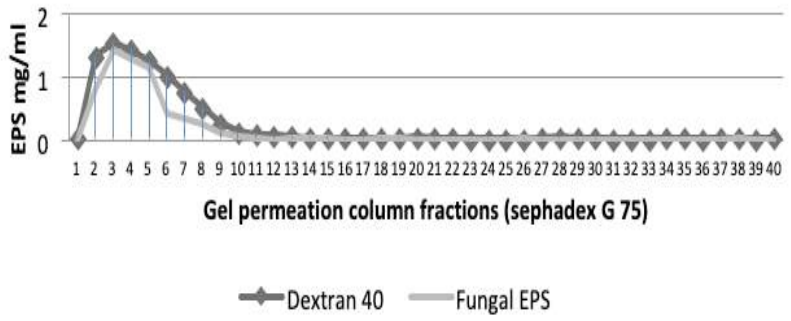


Fig.4 Estimated value of EPS and Dextran 40 through gel permeation sephadex G 75 column

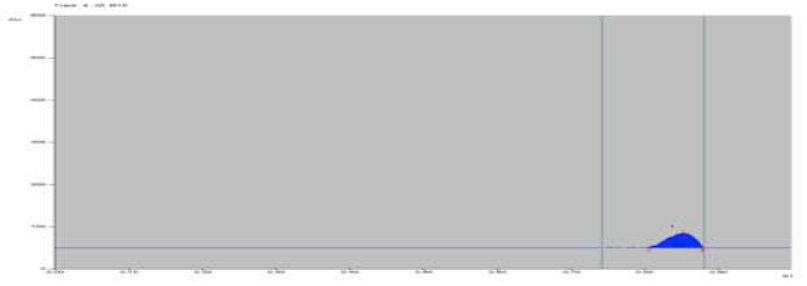


Fig. 5 HPTLC profile of sample on silica plates developed in solvent system Butanol: Pyridine (5:1 v/v ratio) shows absorbance at 285nm (A.U=Arbitory units Y-axis) plotted against Rf value (X-axis).

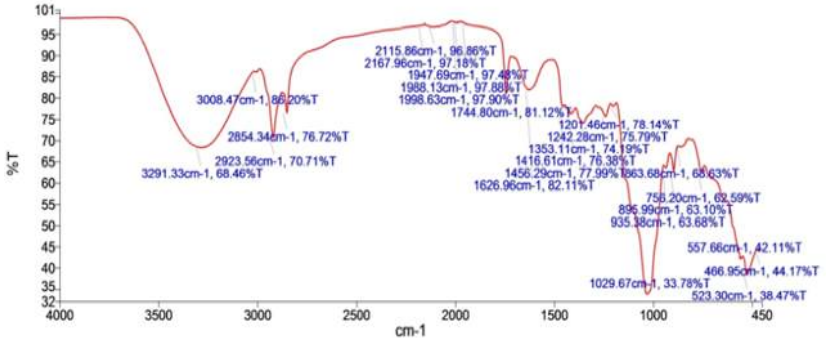


Fig.6 FTIR analysis profile of partially purified EPS sample of *Fusarium* sp. with percentage of Transmittance at Y-axis and wave no. at X-axis.

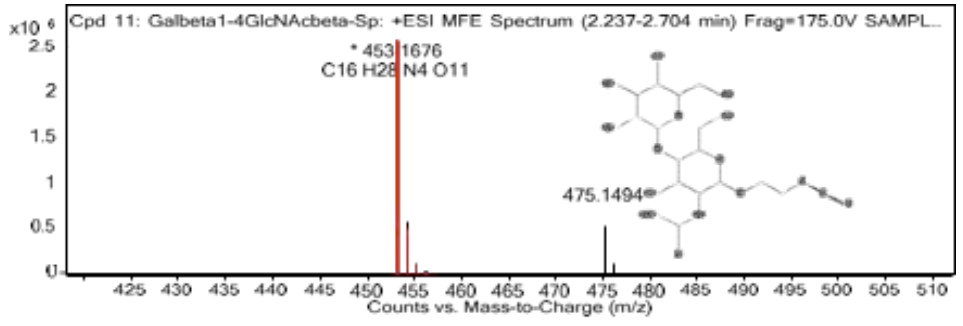


Fig. 7 LCMS analysis of probable compound (glycan) present in EPS

Standardization of nursery technology by application of PGPF (Plant Growth promoting fungi) under different soil compositions and its impact on quality of *Piper longum*: A RET medicinal plant of Odisha

(NMPB, GOI Funded)

Principal Investigator: Dr. Nibha Gupta, Principal Scientist

Plant growth promoting properties of microbial resources are becoming important as they help in improving plant growth under abiotic and biotic stress conditions. Medicinal plants are associated with native endophytes. Microbes endowed such potential of plant growth promotion h could be exploited for the development and enhancement of productivity under agriculture, horticulture and forestry sector. In view, an experiment under pot culture condition using submerged culture of native microflora of *Piper longum* having potential of phosphate solubilisation and plant growth promotion properties was carried out. The present study was carried out with completely randomized design with 12 treatments and 20 replications along with control. The experiments were conducted on Piper longum seedlings grown by vegetative propagation method in two different season along with control and different fungal inoculation either individual and /or dual sets. The morphological parameters recorded on plant height, root length, biomass, leaf no., area as well as physiological growth parameters like relative growth rate, leaf area ratio, net assimilation rate, quality index and chlorophyll content of the plants of 160 days of growth. All data subjected to analysis of variance as one factor at a time in different experimental sets. Data recorded on growth performance of plants revealed better effect on enhancement of growth of plants in rainy and winter season as compared to control. The plants developed good growth under inoculated condition in rainy season and growth promotion effect of fungi exhibited the higher shoot height, leaf no and fruit no. where as winter season promoted more leaf area of the plants. Overall, piperine content of the fruits was more or less similar in both the season. However, winter season supported richer physiological state of plant in terms of total carbohydrate , reducing and non reducing sugar. However, physiological parameters of fungi inoculated plants during rainy season exhibited higher variation than the winter season. Over all potential for growth promotion and development of *Piper longum* is clearly visible due to inoculation of *P. admetzi* in both seasons. This fungus also perform better in dual inoculation with *F. Moniliforme* , *A. acculeatus* and *Paecilomyces lilacinus*. *A. niger* also performed good in combination with *F. moniliforme* and *Paecilomyces liacinus*. Though *A. acculeatus* supports good for plant promotion during winter season individually , plants of rainy season under inoculated conditions performed better for the implementation as conservation and production strategies is concerned. However, inoculation of *P. admetzi* during winter season gives more leaf area useful for more appropriate strategies for commercial promotion of *Piper longum* for vegetative propagation and commercialization in large scale.

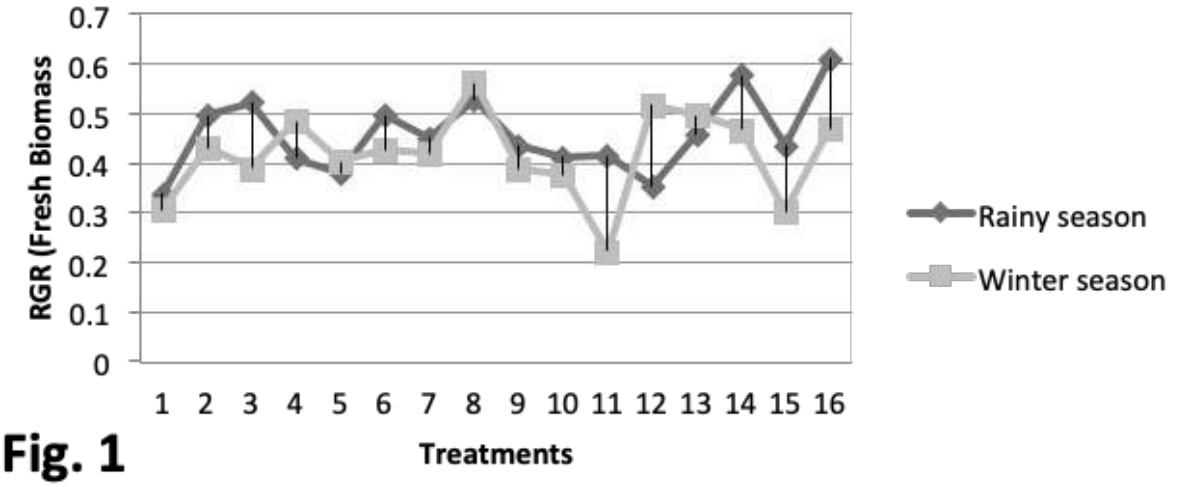


Fig. 1

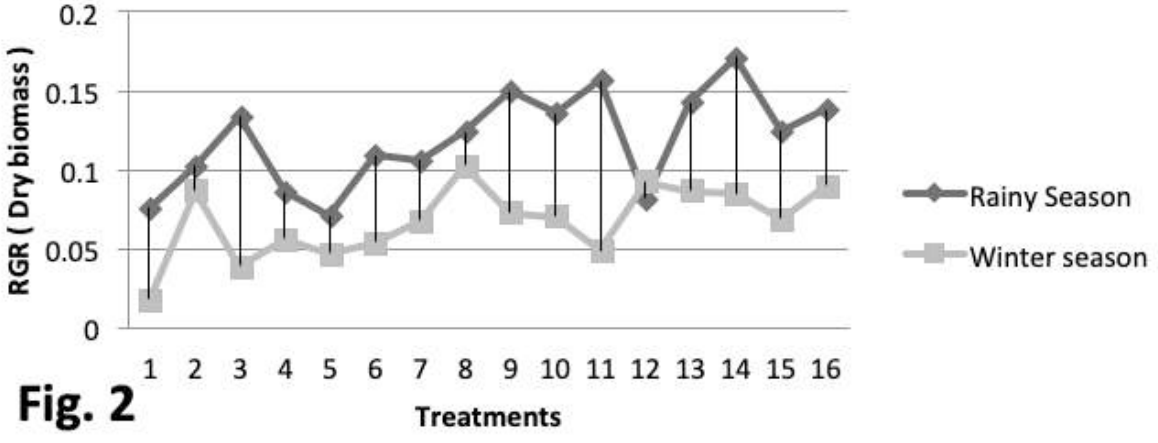


Fig. 2

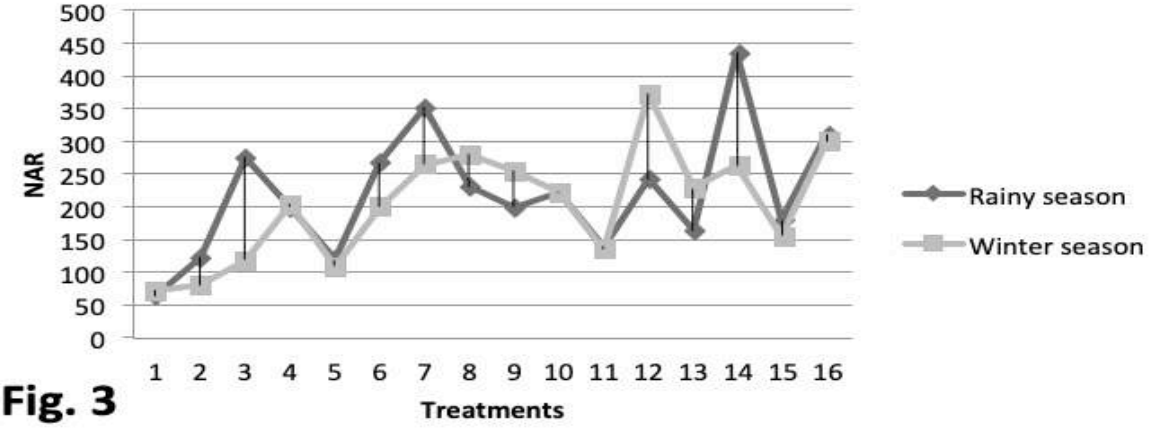


Fig. 3

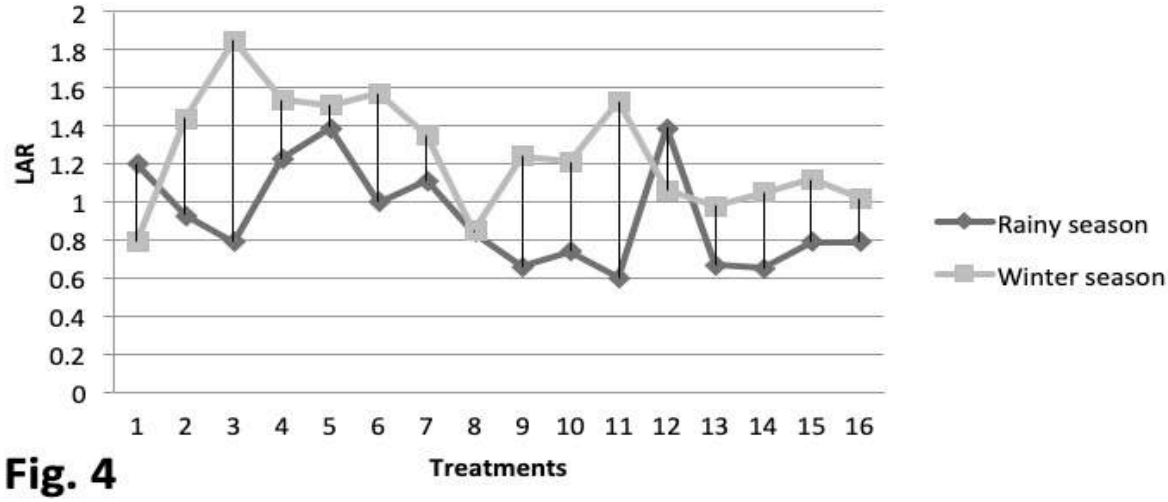


Fig. 4

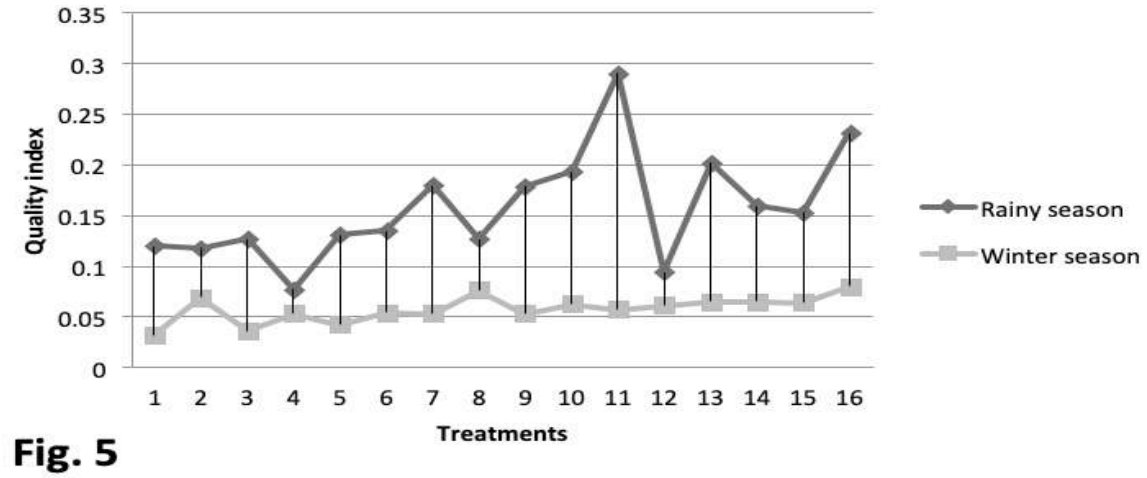


Fig. 5

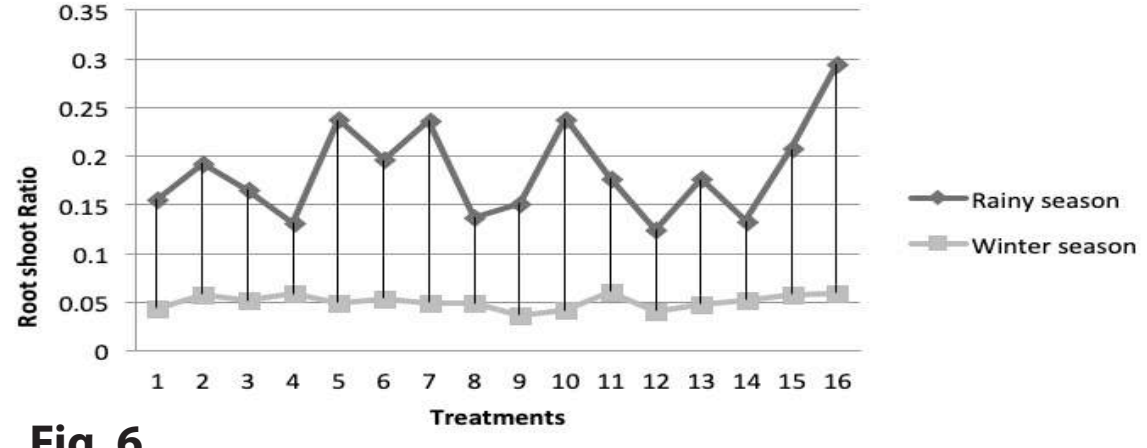


Fig. 6

Harnessing the potential of endophytes against root knot nematodes *Meloidogyne icognita* in banana.

(DBT, GOI Funded)

Principal Investigator: Dr. Nibha Gupta , Principal Scientist

Research Fellow : Jiban Jyoti Panda, JRF

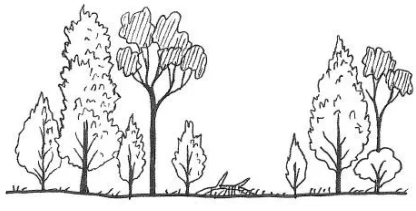
This is a Net-working project under BPCL-NER –Banana programe funded by DBT, Govt. of India. Banana is important staple food and agricultural products in most tropical countries and consumed widely all over the world. However, large scale plantations is being declined due to many diseases and pests, including various species of plant-parasitic nematodes. To date, numerous nematode species recognized to cause the most serious damage to banana. The root-knot nematodes (*Meloidogyne icognita* and other *Meloidogyne spp*.) can cause the banana yield reduction for 20–30% commonly. Endophytic microbes play a vital role in plant protection and growth promotion The use of these microorganisms is preferred compared to chemical fertilizers and pesticides because of their lower cost and their contribution to sustainable agriculture .Recent reports have observed that endophytes efficiently promoted plant growth and that the endophytes may be biocontrol candidates against plant parasitic nematode. There are different community structures of endophytes for the different disease levels of banana roots. Investigating the distribution of culturable endophytes in roots and effectively screen for endophytes could improve our knowledge of the antagonistic ability against root-knot nematodes at different disease degrees of banana roots. As a major objective of the group project, we have surveyed and collected various samples from banana cultivars of Assam, isolated endophytic microbes and prepared pure culture extracts and evaluated for nematicidal properties in collaboration with AAU, Assam and TNU, Coimbtore. Total phenolic content was estimated using the culture filtrate for all fungal and bacterial endophytes isolates and expressed in term of mg/ml. In case of all 51 fungal endophytes , phenolic content ranges from 0.11 to 1.49, found in B 9 and B 26 respectively. All bacterial evaluated for the production of phenolic content and a range was observed from 0.13 to 0.29 (mg/ml). Fungal endophytes were tested for ascorbic acid equivalent antioxidant capacity (table 6) and one organism B 17 and B 23 was found with high producing capacity of 0.027 (mg/ml) and lowest observed in case of B 31 with a value of 0.004 (mg/ml). Among microbial isolates from our centre, 4 fungi and three bacteria were found with inhibitory properties against nematode tested. These seven cultures haves been characterized for their extracellular useful activity (enzymes and secondary metabolite production). Fungal culture B10 exhibited good production of exopolysaccharides in culture medium. most of the fungal and bacterial isolates have been endowed with P and Zn solubilization capacity. Bacterial isolates did not exhibit any extracellular enzymatic activity except BC 18 which was amylase positive during screening. Two selected bacterial strain (BC 1 RPRC & EB 4 TNU to be used for the field experiment) were screened for antimicrobial activity against *Aspergillus, Penicillium, Fusarium, Cladosporium, Curvularia, Humicola , Mucor* and *myceloid* fungal strains. Bacterial strain BC 1 exhibited inhibitory activity against all fungi tested. However, EB 4 was found to be inhibitory towards growth of *Aspergillus, Fusarium* and *Cladosporium* sp. Evaluation of these bacterial strains under pot culture conditions and subsequently field trial is under process at TNU , Coimbatore and AAU , Assam.

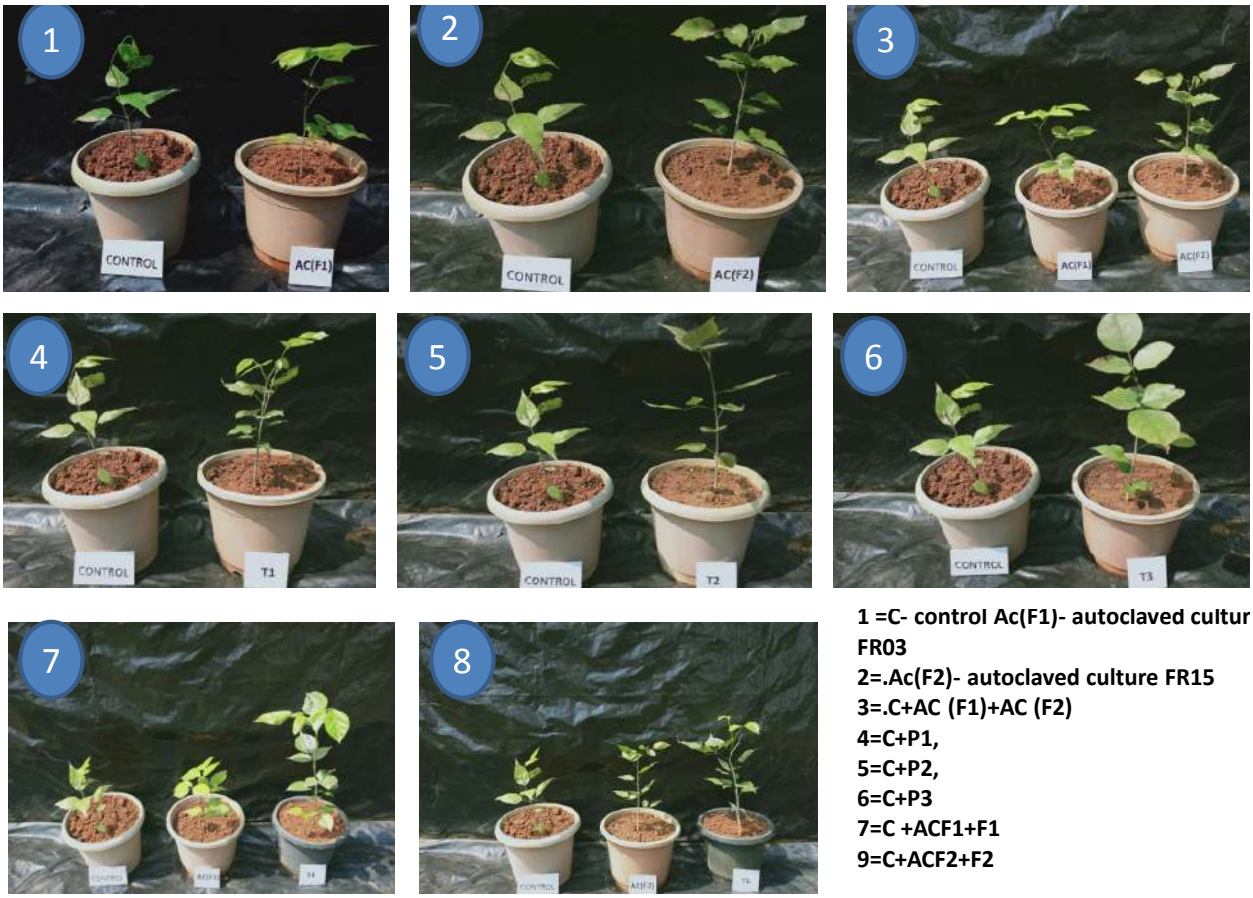
Evaluation of fungal bioinoculants on growth and development of some forest plantation tree species under field

(State Plan Funded)

Principal Investigator: Dr. Nibha Gupta , Principal Scientist
Research Fellow : Rahul Behera, JRF , Madhuchanda Sahoo, JRF

Plant growth, development and production of their useful products may be improved through nutrient management which can be achieved by application of either chemical and/or biological fertilizers . Plant growth promoting microorganisms are useful tool for growth and development of plants of different sectors viz., agriculture, forestry, horticulture and ornamentals . Use of beneficial microbes as bioinoculants /biofertilizers would reduce the cost of chemical fertilizers involved in different agriculture and plantation programe. Most of the tropical soils are phosphate fixing, hence free form of phosphate is not readily available to the plants. Application of Mineral solubilizers to the seedlings helps in their establishment in such type of problematic soils. The process of inoculating microbes to the soil in a forest nursery could be an effective method to achieve higher growth and establishment of tree species on native sites. The development of quality planting material through bioinoculation practices not only endow with economic benefit to the end users but improve soil fertility and ultimately sustainability in native sites. Hence, a present study was carried out on bioinoculation of two fungi (phosphate solubilising) on Pongamia pinnata and *Dalbergia sissoo* in pot culture condition. Periodical analysis of growth of plants indicated the positive effect of fungal inoculation on growth of both type of seedlings over uninoculated control. Supplementation of phosphatic fertilizer alongwith fungal inoculation has also enhanced the growth promoting effect of these fungi. Field Application of these fungal inoculants on *Pongamia pinnata* has indicated their helpful potential towards growth and establishment of seedlings in native field as all transplanted seedlings are established and growing well as compared to uninoculated control plants. Supplementation of these fungal cultures into the rhizosphere of *Dalbergia sissoo* in plantation sites did not exhibit promising results. However, this was an initial and preliminary trail. More experiments may be carried out with reference to the edaphic factors, ecotypes, specialized plant environment which make presence of microbes and their activity essential for growth and establishment of plants in general and/or specific environment. However, work done on fungal inoculation on *Pongamia pinnata* in nursery condition as well as field environment is promising and establish the role of microbes in growth and development of plantation forest trees. This technology may be useful for the development of quality planting materials required for the plantation programe in future.





1 =C- control Ac(F1)- autoclaved cultur FR03
2=.Ac(F2)- autoclaved culture FR15
3=.C+AC (F1)+AC (F2)
4=C+P1,
5=C+P2,
6=C+P3
7=C +ACF1+F1
9=C+ACF2+F2

Fig. 1 Pot experiment on *Pongamia pinnata*

Fig-2. Pot experiment on *Pongamia pinnata* in nursery condition

Fig-3. Field application of bioinoculants on *Pongamia pinnata*

Extraction, purification and characterization of bioactive secondary metabolites and enzymes from endophytic fungi

(State Plan Funded)

Principal Investigator: Dr. Nibha Gupta , Principal Scientist

Research Fellow : Swagatika Mishra, SRF, Sangram Samal , JRF, Ms. Rupa Acharya, JRF

The research project entitled "Extraction, purification and characterization of bioactive secondary metabolites and enzymes from endophytic fungi" dealt with the laboratory experiments carried out to enhance the potential of L-asparaginase production by *Fusarium* sp. and secondary metabolite "piperine" by fungal endophyte of *Piper* longum. L- asparaginase is an anticancer enzyme and sourced from many bacteria and fungi. Though, bacterial enzyme causes more allergic reactions, search for fungal origin of this enzyme is vital. In view, a fungal endophyte Fusarium sp. was used for the Mass scale culture preparation under submerged condition in SD medium. Ammonium sulphate precipitation at 80% level was confirmed best for protein purification. Subsequently, the Sephadex G-50 gel filtration through coloumn chromatography was followed for partial purification of enzyme. Finally, ion exchange chromatography by using DEAE cellulose was followed for the further purification of enzyme. Different fractions collected during purification processes were analysed for enzyme activity. The partially purified enzyme was subjected to characterization for its optimum requirement of pH, temperature, incubation period and substrate. The enzyme was activity at wide range of pH, 15-30 min of incubation. It preferred 37^0 C for good activity. As natural phenomenon, the enzyme utilizes L –aspargine, L –arginine and phenyl alanine were also exhibited as good substrate for this enzyme. It is important to note that this enzyme did not show any activity in presence of Glutamine means its is Glutaminase free L-asparaginase which has good clinical value. The crude enzyme was evaluated for its anticancer properties (outsourcing) which was reported negative against 10 cancer cell lines tested. The non evaluable data may be due to purity and concentration of samples used. This has to be repeated more times to reach any constructive conclusion. However, very less reports are available in literature as far as L-asparaginase from *Fusarium* is concern. The data obtained on its media optimization , extraction and purification may be compiled and published as ready reference for the scientific community.

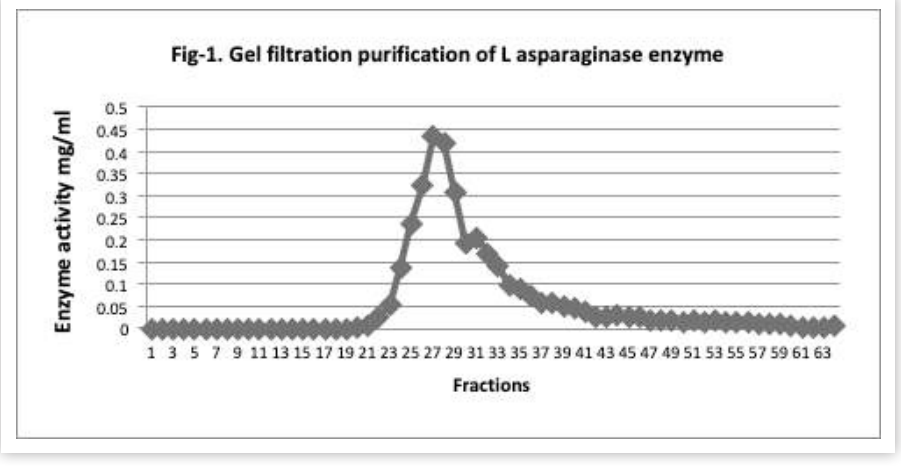


Fig-2. Gel filtration purification of protein

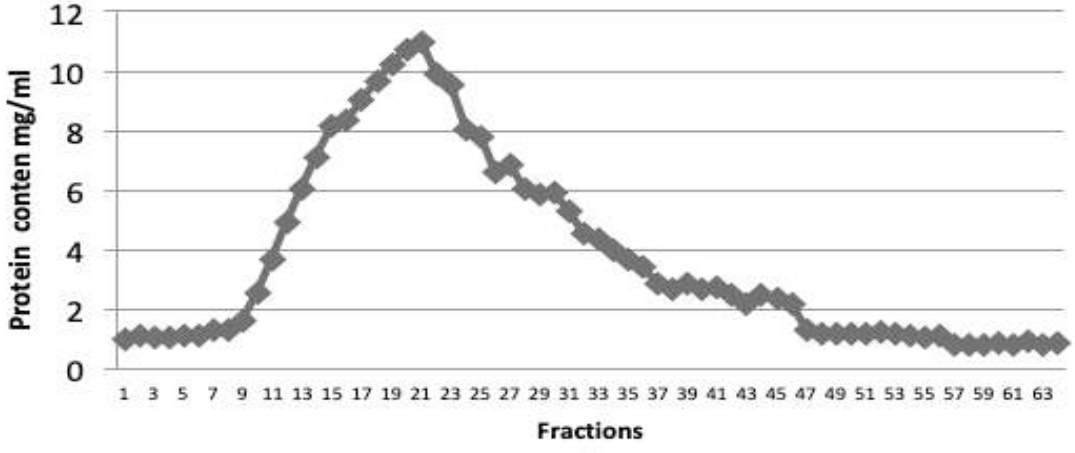
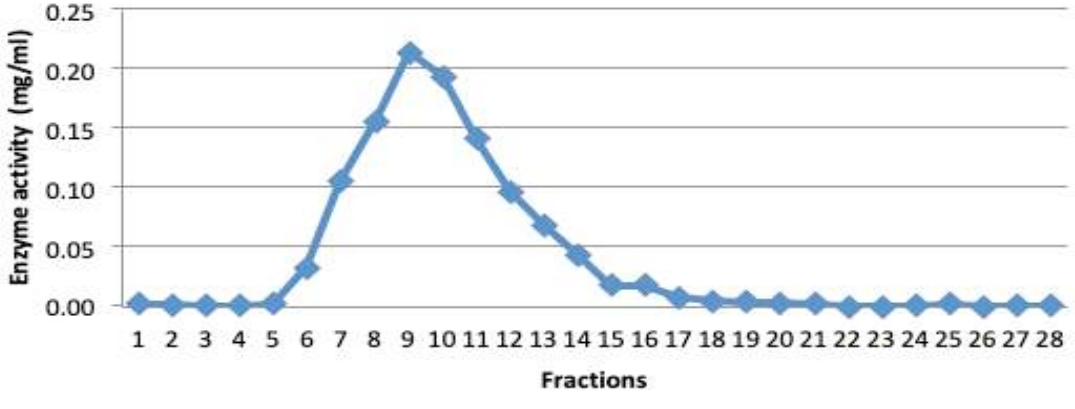


Fig-3. Ion exchange purification of L asparaginase



Two fungal strains have been selected for the production and of secondary metabolites . Standardization for the suitable media , pH and incubation period and temperature was carried out. Fungal strain A preferred CZ media, 7 days of incubation, 4.5 pH for the better growth . Repeated mass culture followed by solvent extraction was done. Second fungal culture was also mass multiplies and treated for ethyl acetate extraction , followed by purification with two stage solvent combination through column chromatography. Repetitive experiments on mass culture, extraction purification and biological evaluation is prerequisite to assign credit to fungal metabolite for its bioactivity.

Propagation, conservation and re-introduction of RET & other important plants

Phytochemical evaluation, nutritional analysis, propagation and reintroduction of selected threatened plants of Odisha

(State Plan Funded)

Principal Investigator: Dr. Pratap Chandra Panda, Principal Scientist

Research Fellow:Pradeep Kamila, SRF

(a) Phytochemical evaluation of vegetative parts of *Hypericum* species as possible source of anti-depressant drugs hypericin, pseudohypericin and hyperforin

The aerial parts of *Hypericum gaitii* collected from Nawana forest range of Similipal Biosphere Reserve (SBR) was used for extraction of different phyto-constituents. After collection, the plant samples were thoroughly washed, dried in room temperature and crushed in to powder. 0.5gm of powder was extracted in 50mL of 100% methanol by ultrasonicator. Then the prepared extract was filtered and kept in refrigerator for further analysis. HPLC analysis was done using a Shimadzu Prominence LC-20A (Shimadzu Europa GmbH, Duisburg, Germany) chromatographic system equipped with two LC-20AD model pumps, a thermostat CTO-10AC with injector and a SPD-M20A detector. We observed that the aerial parts of *H. gaitii* accumulated the chlorogenic acid and the several flavonoids, namely, hyperoside, isoquercetine, quercitrine, quercetine, and hyperforin. However, the quantification of the above secondary metabolites is yet to be done with corresponds to the peak area of respective standards.

Fig-1. Flower of *H.gaiti*

(b) Comparative assessment of the nutritional, anti-nutritional properties and neurotoxicity of *Cycas sphaerica* endosperms and leaves

The endosperms and leaves of *Cycas sphaerica* was collected from Rajini Reserve Forest, Khurda, Odisha. Both were rinsed with distilled water to remove any dust particles. After that they were air dried at 37 ˚C temperature, grinded into fine powder and kept in air tight containers for further analysis.

Proximate analysis of the various parts of *Cycas sphaerica* was determined using the protocol prescribed by Association of Official Methods of Analysis (AOAC, 2012) methods. Carbohydrates were determined by difference of the sum of percentage of all the proximate composition from 100. The anti-nutrients like phytate, oxalate, and tannin of endosperms and leaves of *Cycas sphaerica* were analyzed. Phytate was measured following the procedure of Wheeler and Ferrel (1971), whereas oxalate content was determined using the protocol of Holloway et al. (1989). Tannin content was ascertained by the method of Maxson and Rooney (1972). Amino acid analysis was done with the help of HPLC. Vitamins content was analyzed by using the reverse-phase high-performance liquid chromatography (RP-HPLC). For phyto-constituent analysis, the concentrated methanol extract was analyzed on a Shimadzu QP2010 GC–MS system with 2010 GC.

Table 1. Proximate composition of *Cycas sphaerica* plant parts

Sl. No.	Parameters	Endosperm (g/100g)	Leaf (g/100g)
1	Protein	16.80	30.81
2	Fat	3.61	1.9
3	Carbohydrate	66.48	28.68
4	Ash	2.32	5.76
5	Fiber	0.3	22.85
6	Moisture	10.49	9.99

Proximate analysis of *Cycas sphaerica* endosperms and leaves were carried out to examine the nutritional value of the plants and the results are represented in Table 1. The crude protein of the endosperms was found to be (16.80 %), fat content (3.61 %), carbohydrate contents (66.48 %). The amino acid composition of *C. sphaerica* endosperms and leaves showed the occurrence of 17 amino acids presented in a tabular form (Table 2.) Among the essential amino acids, leucine is the predominant amino acid in both endosperms and leaves of *C. sphaerica.* The analyses of the anti-nutrient composition of the endosperms and leaves of *C. sphaerica* are carried out for the first time and presented in Table 3. Among the plant parts of *C. sphaerica*, leaves contained rich quantity of phytate. The tannin content of *C. sphaerica* endosperms was 0.06 mg/100 and is far below the toxic levels. The GC-MS analysis of methanol extract of *C. sphaerica* endosperms and leaves disclosed the presence of various bioactive constituents which were represented in Table 4. A total of 18 and 20 numbers of compounds were detected in endosperms and leaves of *C. sphaerica*, respectively. 3-O-Methyl-D-glucose was found to be a major constituent in the endosperms (33.23%) and in pith (31.79%). The *C. sphaerica* endosperms and pith revealed the presence of carbohydrates, fatty acids, sterols, vitamins and hydrocarbons.

Table 2. Amino acid composition of *Cycas sphaerica* plant parts (g/100 g protein)

Essential amino acids	Endosperm	Leaf
Histidine	0.07	0.15
Threonine	0.50	0.35
Valine	0.05	0.08
Methionine	0.37	0.35
Phenylalanine	0.35	0.48
Isoleucine	0.30	0.26
Leucine	0.92	0.75
Lysine	0.46	0.51

Table 3. Anti-nutritional factors in different plant parts of *Cycas sphaerica*

	Endosperm	Leaf
Anti-nutrients		
Oxalate (mg/gm) dry wt.	1.90	0.9
Phytate (mg/gm) dry wt.	0.07	0.58
Tannin (TAE mg/gm) dry wt.	6.0	0 .14

Table 4. Chemical constituents identified in ethanol extract of *Cycas sphaerica* plant parts by GC-qMS

Sl. No	RT	Compounds	Molecular formula	Endosperm	Leaf
1	5.88	(2E,4E)-7-hydroxyocta-2,4-dienoic acid methyl ester	$C_9H_{14}O_3$	nd	nd
2	9.96	Mannose	$C_6H_{12}O_6$	nd	nd
3	12.35	Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$	26.30	nd
4	13.28	3-O-Methyl-D-glucose	$C_7H_{14}O_6$	33.23	31.79
5	13.77	5-Undecyne	$C_{11}H_{20}$	nd	12.07
6	14.07	1-Tetradecyne	$C_{14}H_{26}$	nd	0.76
7	14.30	1-Pentadecyne	$C_{15}H_{28}$	nd	0.91
8	14.87	10-Undecenoic acid methyl ester	$C_{13}H_{26}O_2$	0.32	nd
9	15.10	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	0.41	nd
10	15.44	Palmitic acid	$C_{16}H_{32}O_2$	7.23	13.80

11	15.76	Tetradecanoic acid, 13-methyl-, ethyl ester	$C_{17}H_{34}O_2$	nd	0.27
12	16.43	Palmitoleic acid	$C_{16}H_{30}O_2$	nd	nd
13	17.14	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{30}O_2$	0.20	nd
14	17.24	Oleic acid methyl ester	$C_{19}H_{36}O_2$	1.15	nd
15	17.41	1-Octadecyne	$C_{18}H_{34}$	nd	0.78
16	17.93	cis-Vaccenic acid	$C_{18}H_{34}O_2$	18.66	15.62
17	18.19	Stearic acid	$C_{18}H_{36}O_2$	1.32	3.48
18	19.37	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	0.05	nd
19	19.72	Arachidonic acid methyl ester	$C_{21}H_{34}O_2$	0.08	nd
20	20.06	Paullinic acid	$C_{20}H_{38}O_2$	0.22	0.27
21	20.53	Icosapentaenoic acid	$C_{20}H_{30}O2$	nd	0.04
22	21.98	9-Octadecenoic acid-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	0.16	nd
23	22.59	Glyceryl linolenate	$C_{21}H_{36}O_4$	0.24	0.08
24	25.95	Octadecanoic acid, 4-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	nd	0.11
25	30.60	β-Tocopherol	$C_{28}H_{48}O_2$	0.69	nd
26	30.88	γ-Tocopherol	$C_{28}H_{48}O_2$	nd	0.26
27	31.15	Stigmasterol acetate	$C_{31}H_{50}O_2$	nd	0.63
28	31.45	Brassicasterol acetate	$C_{30}H_{48}O_2$	nd	nd
29	32.04	9-Hexacosene	$C_{26}H_{52}$	nd	14.18
30	32.31	α-Tocopherol	$C_{29}H_{50}O_2$	nd	0.09
31	34.35	Campesterol	$C_{28}H_{48}O$	1.67	0.44
32	34.98	Stigmasterol	$C_{29}H_{48}O$	0.84	0.99
33	36.59	β-Sitosterol	$C_{29}H_{50}O$	4.35	3.20

RT-Retention time (min); nd- Not detected

(c) Scaling-up of seed propagation methods for *Hypericum gaitii* and propagation of *Cycas sphaerica* from bulbils

For macro-propagation of *Hypericum gaitii*, seeds were stored at room temperature for 2-3 days for embryo maturation and then sown in porous medium containing leaves mould and sand (1:1) in plastic pots for germination test. In case of *Cycas sphaerica*, bulbils were collected from mature plants by scraping, treated with bavistine and planted in sterile rooting medium containing sand and soilrite (1:1) mixture with regulated watering.

The seeds of *H. gaitii* are very minute in size and seed germination was achieved after 5-10 days but the percentage of germination was quite low (Figure 2). However, subsequent seedling establishment was found to be very difficult.

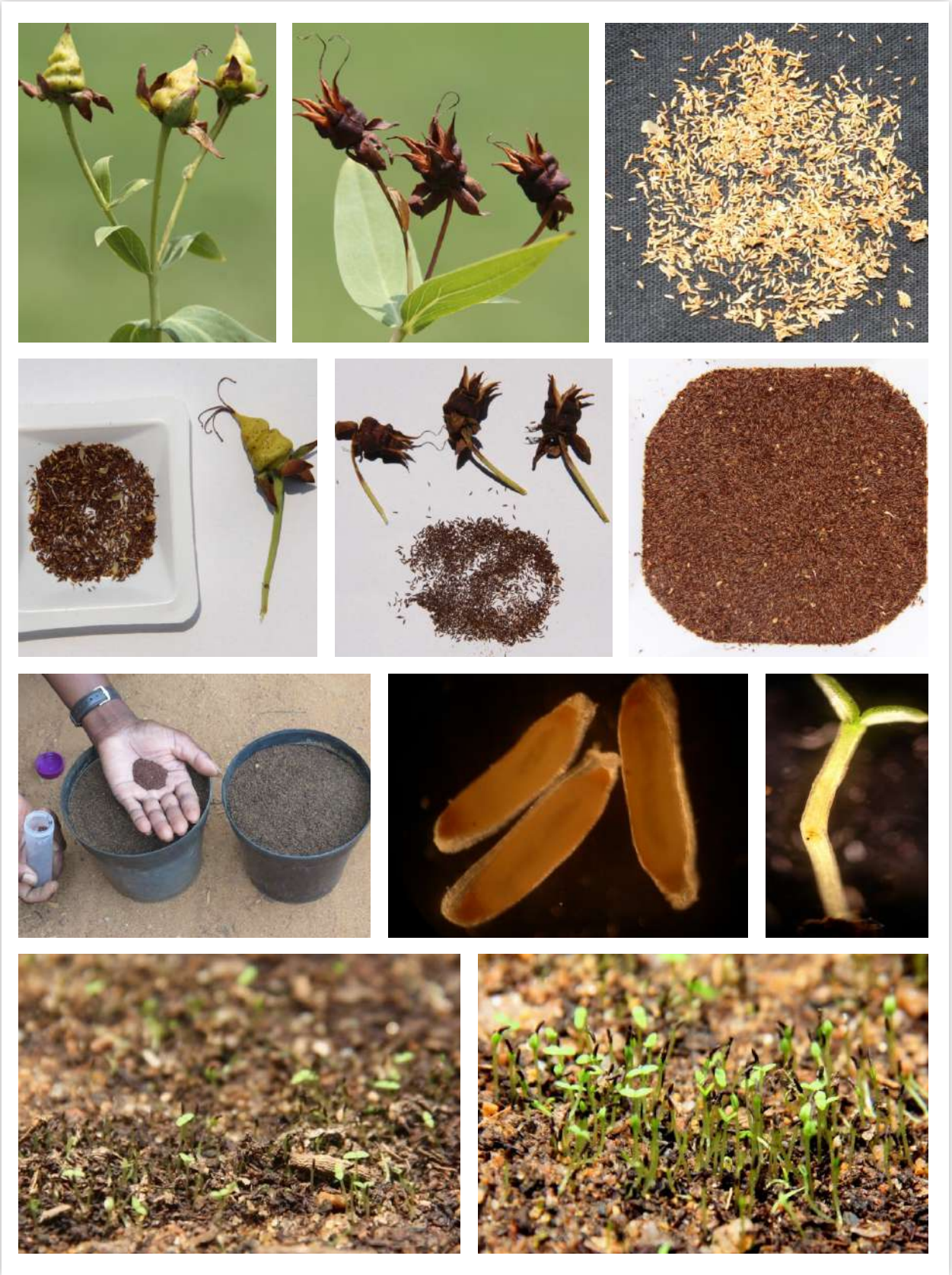


Fig-2. Propagation of *Hypericum gaitii* from seeds

Experiment on vegetative propagation using bulbils as explants was laid and successful method of root induction in bulbils has been achieved. Successful sprouting, formation of young leaves and inductions of healthy roots were observed after four months of the treatment (Figure 3).

Fig-3. Propagation of *Cycas sphaerica* from seeds

(d) Re-introduction of *Lasiococca comberi*, *Hypericum gaitii*, *Cycas sphaerica* in few new habitats and their performance evaluation

The growth performances of reintroduced *Lasiococca comberi* were recorded at regular intervals and data height and stem diameter of those plant were recorded. Similarly, a total of 1350 well established seedlings of *Cycas sphaerica* were reintroduced in three different reintroduction sites of Odisha (Chandaka-Damapada W/L sanctuary, Rajin Reserve Forest, Khurda and Mandasaru gorge, Kandhamal). This year the growth performance of reintroduced *C. sphaerica* plants in all three sites have been assessed and found to be quite encouraging (Figure 5).

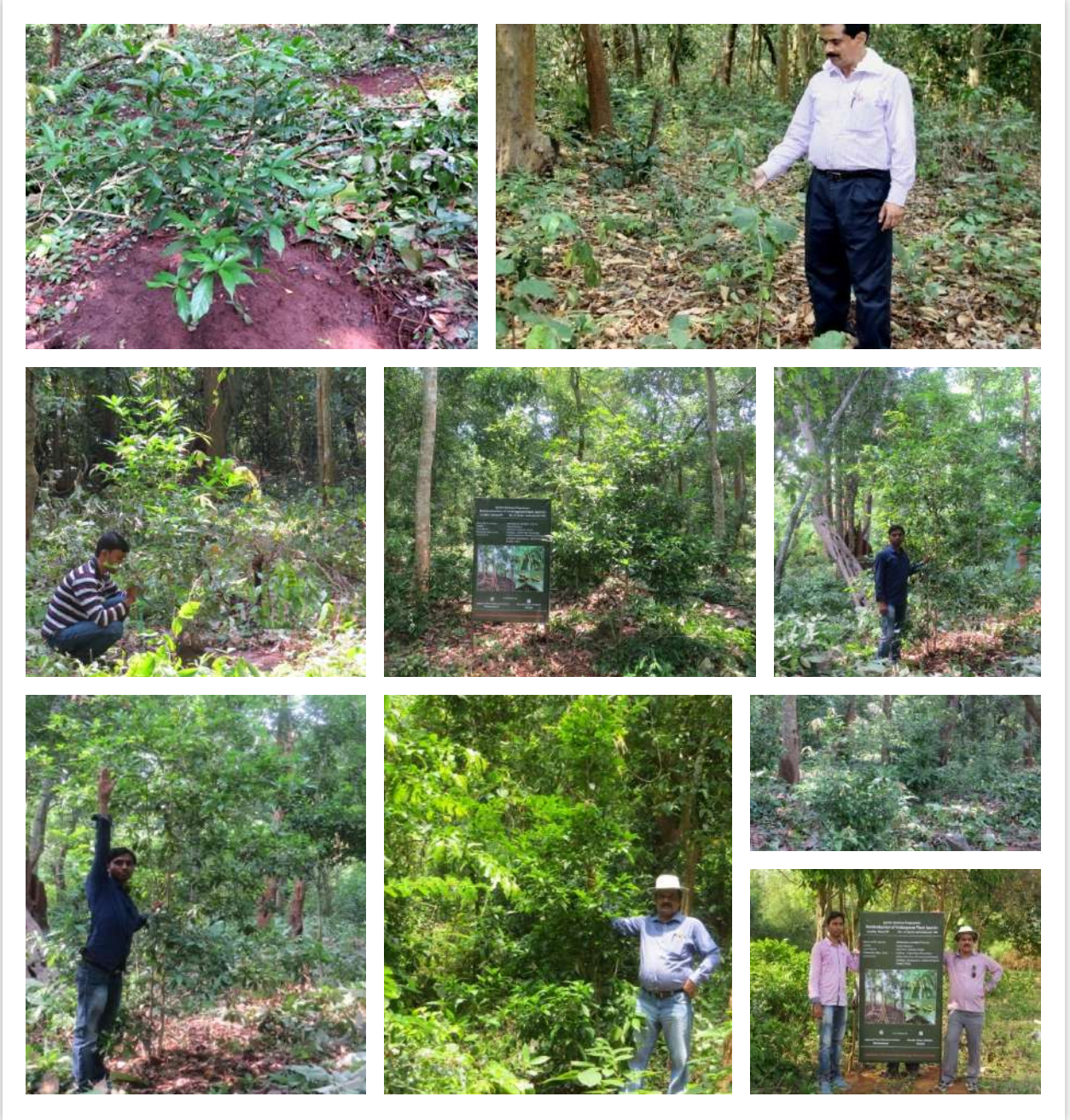


Fig-4. Growth performance of reintroduced plants *Lasiococca comberi* at different reintroduction sites

Fig-5. Reintroduction of *Cycas sphaerica* at different reintroduction sites of Odisha

Restoration of wild orchid population in Chandaka & RPRC through reintroduction of *in vitro* raised seedlings

(State Plan Funded)
Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist
Reseach Fellow: Nishi Padma Samal, JRF, Rudra Narayan Dehuri,FA

In India, about 1331 species have been reported and majority of them are confined to the north eastern regions. In the state of Odisha, 150 species have been reported. Similipal Biosphere Reserve is the best climate for orchid growth and development and thus has housed 97 orchids with terrestrial and epiphytic habits. Of the 400 endemic orchids of India, 23 species are growing in the state; importantly, species like *Eria meghasaniensis*, *Zeuxine mooneyi, Cirrhopetalum panigrahianum, Liparis udaii, L. espeeviji, Odisha cleistantha, Habenaria panigrahiana* are exclusive to the state. Among the different genera, maximum diversity of Habenaria has been reported with 17 numbers of species, followed by Dendrobium represented by 12 numbers of species. It is more important to note that in the recent surveys or reports it has been indicated that the population of the orchids are disappearing from the nature in much faster rate. In the state, few of the endemic orchids of India are growing, the best example is the *Dendrobium regium*; populations are under severe threat Though reported from many parts of the country, from the recent observations, it is reported that the major viable populations are only growing at the Similipal Biosphere Reserve. Eria meghasaniensisis exclusively to our state, the populations are under severe threat. Conservation approaches need to be in place to restore the populations (Yam et al., 2010). One of the best immediate approaches would be the mass propagation of the orchid using biotechnological tools and introduce the seedlings/plantlets in to the nature (Cribb et al., 2003).

From Chandaka-Damapara Sanctuary, three species such as *Vanda tessellate* and *Acampe praemorsa* has been reported from different studies (Misra, 2004). These orchids are distributed in many districts of the state as well as other parts of the country. Flowering is not a problem, so also the production of the seeds. However, it has been seen that the seed germination rate in many cases are very limited. Population restoration programs have been initiated in many parts of the world by introducing the in vitro raised seedlings. For orchid propagation tissue culture technology is essential, that provides the nutrients for the seed germination and development of seedlings. In this project, in vitro propagation methods used for *Vanda tessellata, Cymbidium aloifolium, Acampe praemorsa* and *Aerides odorata* for mass production of seedlings. These seedlings were used for the population restoration programs.

Development of protocols for in vitro production of seedlings of Vanda tessellata, Acampe praemorsa, Aerides odorata and Cymbidium aloifolium.

Vanda tessellata

The capsules bearing the seeds were collected and then inoculated on Murashige and Skoog's 1962 nutrient medium for production of seedlings.After inoculation, the cultures were observed regularly and observations were recorded in the Table 1. It was found that the seed took 120 days for completion of the germination. Of the three media tested, germination responses were recorded from the MS medium containing growth regulators.

Again, from the two medium containing growth regulators, the medium containing NAA and BAP germinated faster as compared to the medium containing BAP (Table. 1). The seeds on the medium containing NAA and BAP completed germination at 120 days whereas on only BAP supplemented medium took 150 days for the completion of germination (Fig. 1). On both the mediums, protocorms were formed, however, the protocorms on NAA containing medium did not grew further, subsequently died. The PLBs developed on the BAP containing medium will be sub-cultured on to the new mediums for further production of leaf and roots.

Table 1. Effects of growth regulators on the seed germination of *Vanda tessellata*

Sl. No.	Nutrient Medium			Remark
	MS	MS containing NAA 2.0 mg/l + BAP 0.5 mg/l	MS containing BAP 0.5 mg/l	
1	0.0	30% germination	30 % germination	Protocorm formed, later died

Fig. 1. *In vitro* propagation of *Vanda tessellata*

a) Closer view of the flower; b) Capsule bearing seeds; c) Seed germination on MS medium containing NAA 2.0 mg/l and BAP 0.5 mg/l; d) Seed germination on MS medium containing BAP 0.5 mg/l.

Acampe praemorsa

Capsules were collected from the natural habitat of Keonjhar and brought to the laboratory. All of these were found to be matured, thus were used for the production of seedlings under in vitro conditions. After washing, the capsules were surface sterilized with the commercial bleach and alcoholand inoculated on the nutrient medium as mentioned in the Table 2. The two-medium tested for seed germination, the medium containing IBA 2.0 mg/l showed higher germination rate as well as for the production of leaf and root. The seeds on IBA medium initiated the germination process at 15 days of culture showing swelling of the embryo and chlorophyll synthesis started at 21 days of culture (Fig-2). Subsequently, the seeds found to be completed the germination process at 30 days of culture. Protocorms were formed at 45 days of culture, leaves initiated at 90 days and finally root production initiated at 150 days of culture. In the IBA containing medium, 72 % of the seeds germinated; at 160 days of culture each shoot had 2.66 leaves and 2.00 roots. It was observed that the protocorms form on the IBA medium produced new protocorms.

Table 2. Effects of growth regulators on the seed germination of Acampe praemorsa

Sl, No.	Growth regulators (mg/l)	% of germination	No. of leaves / shoot	Number of roots/ shoots	Remarks
1	MS medium	31.33	1.33	0.0	No shoot multiplication
2	MS+ IBA 2.0 mg/l	72.00	2.66	2.0	Shoot multiplication

Fig-2. In vitro propagation of *Vanda tessellata*

a) Closer view of the flower; b) Capsule bearing seeds; c&d) Seed germination on MS medium containing e) Seed germination on MS medium containing IBA 2.0 mg/l.

Aerides odorata

Young protocorms were used as the explant and effect of three growth regulators were evaluated as mentioned in the Table 3. While NAA and BAP combinedly used mostly for the production of new shoots, IBA was used for the induction of roots. Protocorms on MS medium without any growth regulators produced 2.66 numbers of new shoots at 120 days of culture. Each new shoot at 180 days of culture produced 2.0 number of leaves and 1.66 number of roots. Inclusion of BAP and NAA in the nutrient medium produced more new shoots; MS medium containing BAP 0.5 mg/l and NAA 1.0 mg/l produced a maximum of 5.0 number of new shoots. The shoots also enhanced the production of more leaves and roots as compared to MS medium (Fig.4). Presence of IBA in the nutrient medium also produced new shoots, however, effects were not significantly higher. The growth regulators produced 2.66 no. of leaves and 3.33 no. of roots from each shoot.

Table 3. Effects of growth regulators on the protocorms cultured *in vitro* of Aerides odorata

Sl, No.	Concentration (mg/l)			No. of shoot/ex-plant	No. of leaves /shoot	Number of roots/shoots
	BAP	NAA	IBA			
1	0.0	0.0	0.0	2.66	2.0	1.66
2	0.50	1.0	0.0	5.00	2.3	2.66
3	0.50	2.0	0.0	3.33	3.3	3.00
4	0.0	0.0	2.0	2.66	2.30	3.33

Fig-3. *In vitro* propagation of *Aerides odorata*

a). Closer view of the flower; b) protocorms in the culture medium; c) Protocorm multiplication on MS medium containing BAP 0.5 mg/l and NAA 1.0 mg/l; d) Production of leaf and root on MS medium containing IBA 2.0 mg/l; e) Shoot elongation on MS medium containing IBA 2.0 mg/l. f) Acclimated plants growing at the poly house.

Cymbidium aloifolium

Young seedlings developed in vitro were used as explants for the production of multiple shoots. Three different combinations of BAP and NAA were added in the nutrient medium for the production of new shoots. The shoots on MS medium alone did not produce any new shoot even after 130 days of culture. On this medium,the shoot had 2.3 no. of leaves and 1.66 no of roots (Table 4). Supplementation of BAP and NAA in the medium not only produced new shoots but also produced a greater number of leaves and roots. Among the three combination of growth regulators, BAP 1.0 mg/l and NAA 1.0 mg/l produced highest number of new shoots. On this medium also each shoot had produced 2.33 leaves and 1.3 number of roots (Fig. 4). The best hormone combination for induction of more leaves and roots found to be in the medium containing BAP 0.5 mg/l and NAA 1.0 mg/l.

Table 4. Effect of growth regulators on the shoot explants grown *in vitro* of *Cymbidium aloifolium*

Sl. No.	Concentration (mg/l)			No. of shoot/ex-plant	No. of Leaves / shoot	Number of roots/shoots
	BAP	NAA				
1	0.0	0.0	1.33	2.2	2.66	1.66
2	0.25	1.0	2.33	3.66	3.00	2.66
3	0.50	1.0	4.00	4.33	3.00	3.00
4	1.00	1.0	5.00	2.66	1.00	3.33

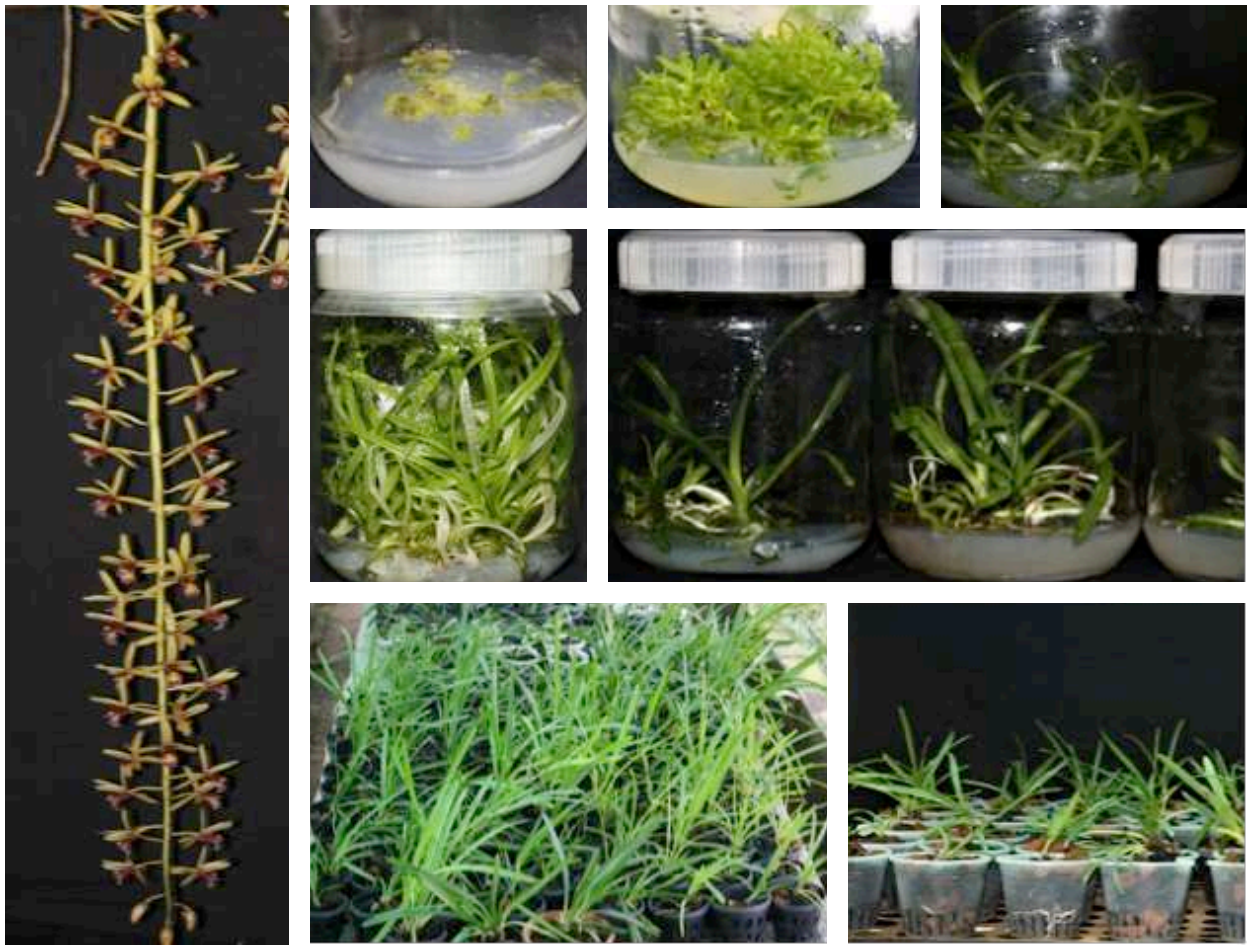


Fig. 4. In vitro propagation of *Cymbidium aloifolium*

a). Closer view of the flower; b) protocorms in the culture medium; c) Protocorm multiplication on MS medium containing BAP 0.5 mg/l and NAA 1.0 mg/l; d) Production of leaf and root on MS medium containing IBA 2.0 mg/l; e-g) Shoot elongation on MS medium containing IBA 2.0 mg/l. h&i) Acclimated plants growing at the poly house.

Introduction of the seedlings at different natural habitats

Seedlings of the orchids mentioned at the Table5 were introduced at the Chandaka Reserve Forest and their performance were monitored after 10 months of introduction. Survival of the orchids found to be low, in all the species recorded to be closure to 50% (Fig. 5). With the application of fertilizer new shoot and roots were produced. Maximum number of new roots were noticed in the *Aerides odorata*, whereas in *Vanda tessellata* lesser number of new roots were recorded. Similarly, in *Cymbidium aloifolium* maximum new leaves were developed during 10 months of planting. Though mature plants of *Cymbidium aloifolium* were planted, flower production was not noticed. However, the plants of the Vanda tessellata produced leaves after six months of planting on the host plant.

Table 5. Orchid introduction at the Chandaka Reserve Forest

Sl. No.	Name of the species	Number of plants introduced	Survived	Comments
1.	*Cymbidium aloifolium*	40	25	Survival rate is low, new leaf and roots developed.
2.	*Cymbidium bicolor*	10	5	Survival rate is low, new leaf and roots developed.
3.	*Aerides odorata*	20	10	Survival rate is low, new leaf and roots developed.
4.	*Aerides multiflora*	20	10	Survival rate is low, new leaf and roots developed. Flowers also produced.
5.	*Rhyncostylis retusa*	10	5	Survival rate is low, new leaf and roots developed. Flowers also produced.
6.	*Vanda tessellata*	10	4	Survival rate is low, new leaf and roots developed. Flowers also produced.

Fig-5. Introduction of orchid plants at Chandaka Reserve Forest a,b,c). *Cymbidium aloifolium, d) Aeridesodorata & f) Rhyncostylis retusa*

Establishment of mass propagation and breeding facility for orchids

(RKVY, GOI Funded)

Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist

Reseach Fellow: Sulagna Subhasmita Jena, SRF

Mass production of planting materials through tissue culture

Orchids are known for their high ornamental value, being used extensively for the cut flower production. Growing orchids is becoming an excellent source of income in many parts of the world. For the cultivation of orchid in a commercial scale, the major factor is the availability of quality planting materials. Currently, the all the planting materials are being produced through tissue culture. Among the different ornamental orchids, Dendrobium hybrids in general are being used for the cut flower production because of their long vase life. Among the many varieties *Dendrobium* Sonia, *Dendrobium* Rynco Green, *Dendrobium* White Fairy, *Dendrobium* Burana White, Dendrobium Bina Zumbo Paul and Dendrobium Pink Fragrance are of highly important for the commercial scale cultivation. In this project, mass production of planting materials are produced using tissue culture techniques for these varieties.

Young shoot buds from the in vitro plants were used as the explants for the mass production of plants. The explants were cultured onMurashige and Skoog's medium (MS) containing 5.0 mg/l 6-Benzylaminopurine for production of multiple shoots. From each shoot, about 15 new shoot buds were produced within the 120 days of culture. These shoots were further multiplied on MS containing BAP 0.5 mg/l. The young shoot buds were transferred to MS medium containing 2.0 mg/l Indole-3-butyric acid (IBA) for shoot elongation and root production. On this medium, each shoot reached to the height of 3-4 cm and produced three to four roots at 120 days of culture (Fig. 7).

a). Closer view of the flower; b) protocorms in the culture medium; c) Protocorm multiplication on MS medium containing BAP 0.5 mg/l and NAA 1.0 mg/l; d) Production of leaf and root on MS medium containing IBA 2.0 mg/l; e-g) Shoot elongation on MS medium containing IBA 2.0 mg/l. h&i) Acclimated plants growing at the poly house.

Fig.1 *In vitro* propagation of *Dendrobium* orchids

Development of molecular marker

Molecular markers have been developed for *Dendrobium regium* and *Dendrobium formosum*. Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) were used for the for the development molecular marker profiles. The primers already identified for *Dendrobium* orchids were used and profiles were developed using polymerase chain reaction (PCR) (Fig. 2).

a) SSR profiles of *Dendrobium regium*, b) ISSR profiles of *Dendrobium formosum* M: DNA ladder; the lanes 10-12 in top panel and lanes 13-15 in bottom panel are the negative controls.

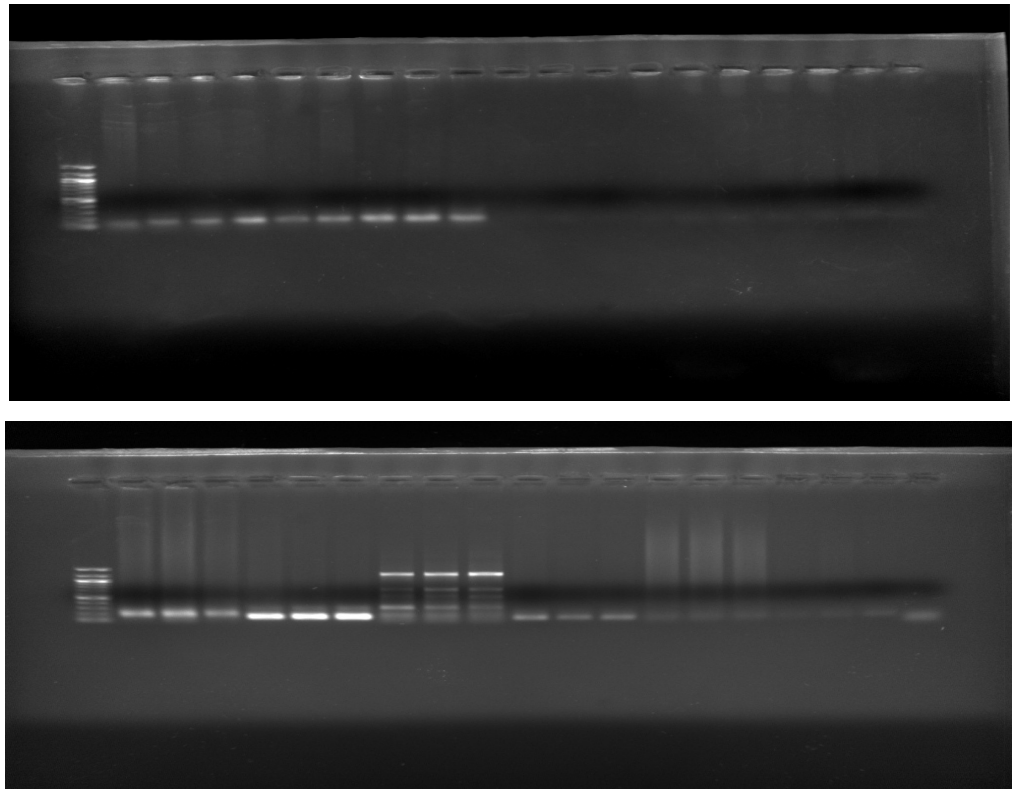


Fig. 2 Molecular marker profiles of *Dendrobium* orchids

Standardization of micro-propagation methods for *Anogeissus latifolia*, *Santalum album* and *Desmodium oogeinense*, an important and endangered forest trees

State Plan Funded)

Principal Investigator: Dr. Giridara-Kumar Surabhi, Senior Scientist

Research Fellow: Bhagyalaxmi Bhuyan, JRF

The micro-propagation has proven to be the method of choice for rapid multiplication of selected forest tree species, where the seed and vegetative propagation is a problem. In the present study, three important and endangered forest tree species were selected for micro-propagation i.e. *Anogeissus latifolia, Santalum album and Desmodium oogeinense*.

Santalum album: The visible shoot bud induction was seen after 10-days of inoculation in nodal segments on basal medium, 1.0 and 1.5mg/l BAP supplemented medium. But no bud induction in 2.0 and 3.0 mg/l BAP supplemented medium even after 10-days. For shoot proliferation different concentrations of NAA with optimum BAP concentration was tested.

Fig.1: Multiple soot initiation from nodal explants of *Santalum album* on same basal medium after 70-days of initial culture.

Fig.2: Shoot growth induction from nodal explants of *Desmodium oojeinense* after 30-days of culture, on 1mg/L BAP supplemented medium.

Desmodium oojeinense: The shoot induction were observed on nodal explants with basal medium, 1.0 and 1.5mg/L BAP supplemented medium. However, the growth is very slow on 1.5mg/l BAP supplemented medium. The shoot proliferation was tested on medium supplemented with kinetin and BAP at different concentrations and 10 mg /L BAP given optimum shoot growth. Further, different combinations of NAA with BAP tested for shoot multiplication in the study.

Conservation of salt-sensitive back-mangroves *Heritiera fomes* and *H. littoralis* through re-introduction in protected area: application of vegetative propagation technique

(DBT, GOI funded)

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Research Fellow: Satyajit Mahatab, JRF

In this project, attempt has been made to initiate re-introduction of *Heritiera littoralis*, locally called dhala sundari, a special group of mangrove plant using vegetatively propagated saplings inorder to create and enhance public awareness on biodiversity conservation, promote social, ecological & economic benefits of the coastal people of the country in general and the State of Odisha in particular. This species re-regenerates naturally through seeds with very poor rate of germination along with other adverse & irregular flowering and fruiting habits, less seed viability which make it difficult to produce adequate natural recruits as well as planting materials to facilitate artificial regeneration through seeds. In order to arrest gradual depletion, the reduced, scattered and sporadic populations need to be augmented with the re-introduction of the species propagated through vegetative means on priority basis.

Firstly, artificial/adventitious rooting was induced in micro stem cuttings using nursery-grown germplasm of *H. littoralis* as a source of explants for the rooting experiment. Cost-effective black-taping method (alternate to conventional air-layering with polythene-moss-ball) was employed to induce rooting through pre-girdling of the hard-wood micro-stem cuttings of this difficult-to-root species with exogenously applied rooting hormones like IBA+NAA. In so doing, around 80% rooting success was achieved followed by hardening of the rooted micro-cuttings with around 50% of survival rate. Secondly, the saplings raised from micro-cuttings were further subjected to various level of secondary hardening process against salinity stress (NaCl) under captive (mist house) nursery conditions. Presently, Biochemical and anatomical analysis of quick rooting in micro-stem cuttings and salt tolerance during hardening, are being conducted. Besides, analysis of growth & development of the hardened saplings under nursery (shade-net house) conditions is going on using Plant Efficiency Analyzer (PEA) procured under this project. The OJIP Analysis pertaining to Chlorophyll fluorescence analysis for evaluation of plant health/ productivity is under progress.

Fig-1. Measurement of Chlorophyll fluorescence values of propagated & hardened saplings of *Heritiera littoralis*

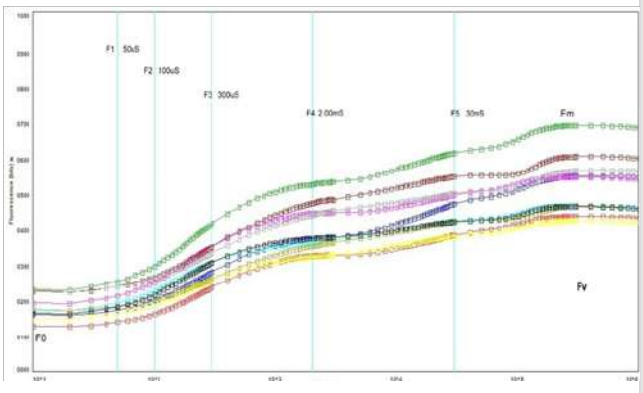


Fig-2. Chlorophyll fluorescence spectrum of propagated & hardened saplings of *Heritiera littoralis*

Studies on Medicinal Plants and Wild Edible Fruits

Phytotherapeutic investigation of *Piper trioicum* as neurological disorder: An insight into therapeutic avenues towards Alzheimer's disease

(State Plan Funded)

Principal Investigator : Dr. Atish Kumar Sahoo, Senior Scientist

Research Fellow: Satish Kumar Kanhar, SRF

Piper is an economically and ecologically important genus in the family Piperaceae. This family comprises more than 2,000 species of shrubs, herbs, and lianas. *Piper trioicum* (Piparaceae) is distributed in South Asian countries and the whole plant is used as rubefacient, diuretic, hepatoprotective and used for treatment of diabetes, muscular pains, headache, toothache, internal remedy for cholera in folk medicine and the root is used as diuretic. Due to its highly medicinal value and based on the ethno medicinal uses of Indian System of Medicine (ISM), *P. trioicum* is used as a memory enhancer but till date there is no claim to treat neurodegenerative disorder like Alzheimer's disease. So, the project has been undertaken to validate neuroprotective and memory enhancing properties.

- Phytochemical extraction process by hydro alcohol (70%)
- Phytochemistry analysis and biological activities of hydroalcohol extract of P. trioicum
- Study of key markers (acetylcholinesterase, AChE and butyrylcholinesterase, BChE)
- Enzyme kinetics study and mode of inhibitions
- HPTLC bioautography test for enzyme inhibition
- Neurodegenerative properties analysis by in vivo model.

The research investigation was carried out to screen the presence of phytochemicals in hydroalcohol extract of *P. trioicum* (HPT). The HPT contains high amount of alkaloid, steroid and flavonoids. Biological activities were carried out by performing in vitro, ex-vivo and in vivo antioxidant activities. Further, HPT demonstrated potential neuroprotective activities as evidenced from enzymatic assays (acetylcholinesterase and butyrylcholinesterase). In DPPH free radical scavenging activity, IC50 was found to be 46.65 µg/ml (Fig. 1A). The antioxidant potential was attributed to the presence of phenolic and flavonoids of HPT that may have the ability to donate hydrogen radicals to neutralize the DPPH radicals. Ex-vivo antioxidant CAP-e assay was performed by using RBC to evaluate antioxidant potential of HPT. In CAP-e assay, HPT demonstrated antioxidant potential with IC50 of 61.04 µg/ml (Fig. 1B). Phenols and flavonoids of HPT were responsible for neutralizing reactive oxygen species by preventing further damage and impairing function of RBCs. The antioxidant potential of HPT was evaluated by ORAC assay that relies on fluorescence quenching effect of antioxidants. It is based on principle of inhibition of peroxy free radicals by antioxidants. The antioxidant potential was expressed in terms of net AUC. Net AUC of HPT was found to be 56.86 (Fig. 1C-D).

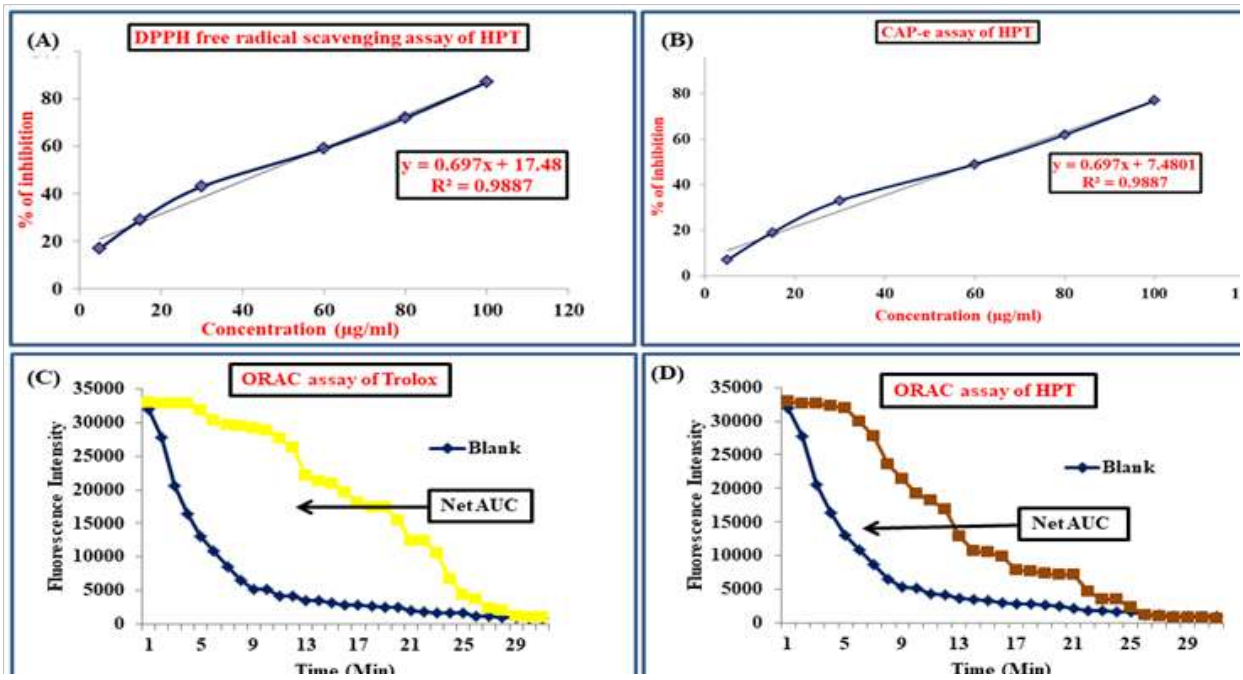


Fig. 1. A. DPPH free radical scavenging assay of hydroalcohol extract of *Piper trioicum* (HPT) B. CAP-e assay of HPT C. ORAC assay of trolox D. ORAC assay of HPT

The current investigation demonstrated acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of HPT. In AChE and BChE inhibitory activities, IC50 of HPT were found to be 73.06 ± 0.19 and 92.37 ± 0.29 µg/ml, respectively. The anticholinesterase potential may attribute to the presence of phenolics and flavonoids in HPT. Enzyme kinetics of HPT for AChE and BChE inhibitor was performed in the form of double reciprocal (Lineweaver-Burk) plot against 1/[s] versus 1/[v]. HPT showed reversible competitive inhibition towards AChE and BChE. The cholinesterase property was attributed to the high alkaloid content in HPT. HPTLC bioautographic assay was performed and bioactive compound was identified as white spot on HPTLC plate (Rf 0.58) (Fig. 3). The current findings suggested the modulatory effect of HPT on the activities of AChE, BChE and β-secretase (BACE1) in animal model (Table 1, Fig. 2).

***Table 1**. Effect of HPT on AChE and BChE activity on rats*

Groups	AChE (µM/min/gm tissue)	BChE (µM/min/gm tissue)
Normal control	4..37 ± 0.18	3.17 ± 0.76
Toxic control	9.22 ± 0.39	7.38 ± 0.14
Positive control	5.53 ± 0.41	4.29 ± 0.22
HPT (low dose)	7.62 ± 0.56	6.11 ± 0.98
HPT (high dose)	6.19 ± 0.74	5.80 ± 0.57

The present experimental results showed significant decrease in the level of total protein (TP), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) in aluminium chloride induced AD rats in toxic control group whereas malondialdehyde (MDA) level was significantly increased in toxic control group (aluminum chloride induced) as compared to the normal control group. However, administration of HPT (high dose, 400 mg/kg b.w.) caused significant elevation in the level of TP, SOD, CAT, GSH and GPx and decrease in the level of MDA as compared to toxic control group and compared to positive control group (galantamine treated) experimental animals (Fig. 4).

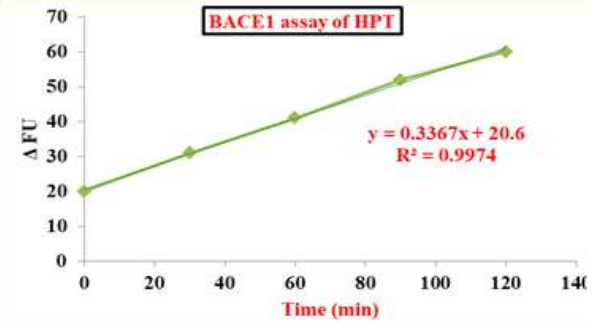


Fig. 2. BACE1 assay of hydroalcohol extract of *Piper trioicum* (HPT)

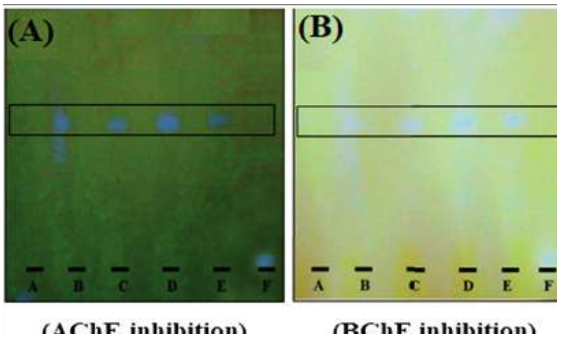


Fig. 3. Fingerprint of hydroalcohol extract of *P. trioicum* (HPT)

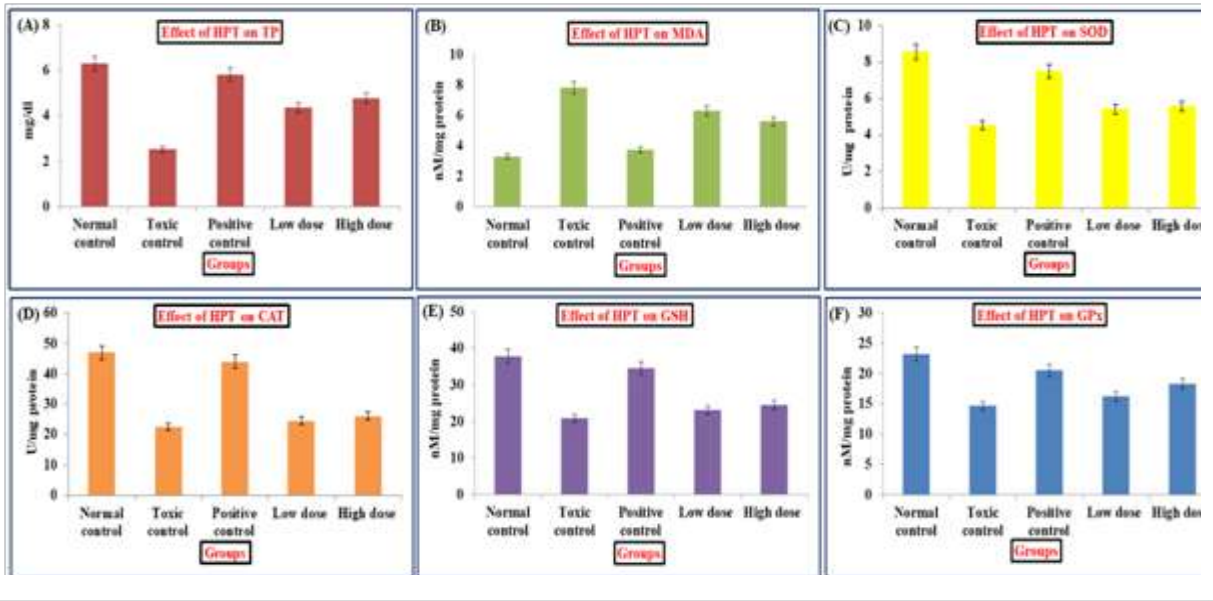


Fig. 4. Effects of HPT on total protein (TP), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) in experimental rats. (A): TP, (B): MDA, (C): SOD, (D): CAT, (E): GSH and (F): GPx

Pharmacological profiling of *Geophila repens* and *Bacopa floribunda* and evaluation of their therapeutic potential against Alzheimer's disease

(Fellowship Project , S & T Dept., Govt. of Odisha Sponsored)
Principal Investigator : Dr. Atish Kumar Sahoo, Senior Scientist
Research Fellow: Umesh Chandra Dash, BPRFS Fellow

Alzheimer's disease (AD) is one of the most recognised neurodegenerative diseases that impairs memory, cognitive functions and may lead to dementia in late stage of life. The pathogenic cause of AD remains incompletely understood and FDA approved most of the synthetic or natural drugs are partial inhibitors rather than curative such as galanthamine is obtained from Galanthus spp. Huperzine A found in Huperzia spp., gingkolides a diterpenoids from Gingko biloba. *Geophila repens* (L.) I.M. Johnst (Rubiaceae) is commonly known as Snake Pennywort, Krishnamanduki (Sanskrit) and locally called as "Karimuthil". It is a small creeping perennial herb with long stems, commonly found in China, Africa and India. As per the ethnobotanical claim, the aerial parts of this plant are used in coughs, diarrhoea, oedema, leprosy, piles, fever, inflammatory swellings, antimicrobial, antifungal, and memory enhancing properties. As clinical trial is time consuming and expensive, so ex- vivo CAP-e assay and in vitro DPPH and ORAC assay were performed to evaluate the antioxidant capacity of pentylcurcumene (PC) to protect cells from oxidative damage. In current research, we investigated the anticholinesterase activities of PC and this observation was further extended to the bioautography detection of PC towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities. In addition, enzyme kinetics and computational molecular docking analysis were carried out between receptor (protein) and ligand (PC) to find out inhibitory potential of AChE and BChE in human and mouse homology models in AD.

1. Hydroalcohol fraction of Geophila repens (GRHA) and isolation of bioactive compound by standard column chromatography method.

- Spectroscopic analysis of isolated compound (PC) to determine its structure
- High performance thin layer chromatography (HPTLC).

2. Biological activities in terms of evaluating the drug activities

- Cell Based antioxidant protection in erythrocytes (CAP-e) assay of PC.
- Oxygen radical absorbance capacity (ORAC) assay of PC.
- Cholinesterase inhibition assay of PC and mode of cholinesterase inhibition assay of PC and bio autography test of PC.
- Molecular modelling and automated setup of PC.

The study highlights the isolation, structural elucidation and quantification of Pentylcurcumene (PC), a terpene from hydroalcohol extract of *G. repens* (GRHA). Extensive literature survey revealed that there are no official analytical methods available for estimation of PC. High performance thin layer chromatography (HPTLC) method has been developed and validated for quantitative determination of PC in *G. repens* because of its reliability in

quantitation of analytes at nanogram level, cost effectiveness, simple, and sensitive. Regarding the bioactivity of PC, cellular antioxidant studies e.g. DPPH radical scavenging activities, oxygen radical absorbance capacity (ORAC) and cell-based antioxidant protection in erythrocytes (CAP-e) studies were performed. The advantage of the ORAC assay is the wide range of applications as it can be used for both lipophilic and hydrophilic samples to quantify the antioxidant capacity toward different oxidants e.g. hydroxyl radicals, peroxyl radicals and peroxynitrite.

Chromatographic separation was performed and the compound (PC) was isolated (17.14 mg; 0.0114% with respect to GRHA) which passed the terpene chemical test. Melting point was recorded at 56-57 °C and further, the purity of PC was cross checked by TLC as appeared a single prominent fluorescence spot at Rf 0.51 (benzene:methanol, 7.5:2.5, v/v) at 254 and 366 nm, respectively. UV spectrum with absorbance maxima was recorded ($CHCl_3$), λmax at 266 nm indicated the presence of CH_3-group attached to benzene ring (Fig. 1). Further to validate the purity of PC, HPTLC is an efficient and routine analytical technique was undertaken to analyse the drug sample in nanogram (ng) level. This technique is widely accepted by researchers due to its sensitivity, less time consuming and cost effectiveness (Fig. 1 & 2)

Cellular antioxidant protection assay was performed in erythrocytes (RBC model from rat) to evaluate the antioxidant capacity of PC in living system. RBC model was chosen because it is simple and RBC does not produce ROS or undergo apoptosis. The decrease in fluorescent intensity of DCF-DA (fluorescent probe) in the presence of peroxy radical generator AAPH marked the cellular protection ability of GRHA and PC in RBC. The antioxidant potential of GRHA and PC in terms of IC50 values reported at 35.03 ± 0.69 and 43.92 ± 0.87 µg/mL, respectively and both results were comparable to the reference drug gallic acid (23.69 ± 0.49 µg/mL) (Fig. 3).

Likewise, the antioxidant potential of PC was evaluated by ORAC assay that relies on fluorescence quenching effect of antioxidants. ORAC reaction is highly sensitive and allows limit of detection (LOD) lower than nM. It is based on in situ production of peroxy free radicals by AAPH that are able to react with substrate resulting in change in fluorescent intensity (FI) and increase in the rate of fluorescent decay. Quantification of scavenging potential of GRHA and PC towards peroxyl radicals were evaluated in terms of net area under curve (AUC). Antioxidant protections were calculated by subtracting resultant AUC. AUC of GRHA and PC were 13.45 ± 1.05 and 15.19 ± 0.89, respectively. Both results were found comparable to trolox 24.80 ± 0.94 (Fig. 3C). PC showed good peroxyl radical scavenging activity (Fig. 3).

In AChE inhibitory activities, IC50 of GRHA and PC were 65.96 ± 0.43 and 73.12 ± 0.56 µg/mL whereas, in BChE inhibitory activities, IC50 of GRHA and PC were recorded 86.03 ± 0.47 and 97.65 ± 0.46 µg/mL, respectively. In both assays, IC50 of galantamine was recorded 26.34 ± 0.45 (AChE inhibition) and 28.35 ± 0.43 µg/mL (BChE inhibition) (Fig. 4A and D). Results suggest that Pentylcurcumene in G. repens is a first-line cholinesterase-inhibitor may be relevant in slowing AD progression due to its effective radical scavenging activities. In enzyme kinetics studies of PC as AChE and BChE inhibitor showed in the form of double reciprocal (Lineweaver-Burk) plot against 1/[s] versus 1/[v] (where 's' is substrate concentration and 'v' is reaction velocity). After analysing mode of inhibition from the plot with graphical analytic tool, the nature of AChE and BChE inhibition caused by both GRHA and PC showed reversible competitive inhibition as evidenced by intersection at Y-axis with Vmax 0.8 and 0.6, respectively (Fig. 4B and E). Our findings and the mechanism of inhibition specifies that GRHA and PC have competed at the active sites on AChE and BChE surface by blocking the substrates (ATCI and SBTC) binding on both enzymes, respectively which are in full agreement with present achieved results (Fig. 4).

Additionally, HPTLC bioautography tests were performed to identify the cholinesterase inhibitory effect of bioactive principles present in GRHA. It is a simple and less time-consuming process to detect the anticholinesterase activity of PC at nanogram level. Experimental evidences of our earlier work on HPTLC bioautographic test shows the localization of bioactivity molecules on Rf range 0.42-0.58 were responsible for anticholinesterase activities. In order to correlate with in vitro experimental results, computational tool of molecular docking studies was performed to explore the possible binding interaction of Pentylcurcumene (PC) with active sites of AChE (human/

mouse) and BChE (human/mouse). Pentylcurcumene (PC) was docked by using Glide module of Schrodinger molecular modelling with docking scores -5.6, -6.1, -6.9, and -4.18 for hAChE, mAChE, hBChE and mBChE, respectively (Table 1B). The docking results showed that the 2D plot of protein-ligand interaction was within 5Å and ligand was docked pretty well for both BChE (human) and AChE (human and mouse) (Fig. 5; Table 1).

Table 1: Melting point, spectroscopic analysis and molecular docking study of Pentylcurcumene (PC)

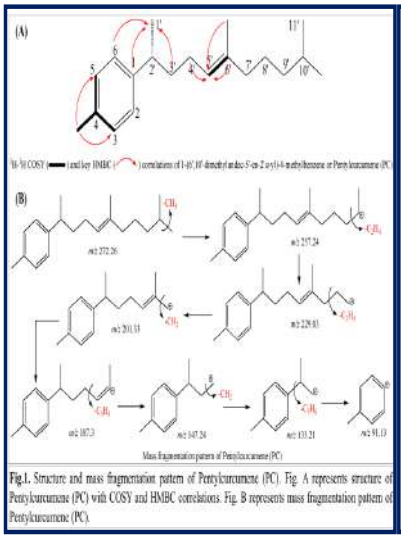
	(A) Spectroscopic analysis data of Pentylcurcumene (PC)
	Experimental data
Parameter	
Melting point	56-57 ˚C
UV (λmax)	
(CHCl3)	214, 241, 266 (C6H5-CH3) nm
IR (KBr, vmax cm-1)	2916.08 (aromatic C-Hstretch), 2848.73 (alkenyl C-Hstretch), 1698.84 (C=Cstretch), 1463.76 (aromatic C=Cstretch), 1271.33 (alkyl C-Hstretch).
ESI-HRMS	m/z 272.26 [M+H]+, 257.24 [C19H29]+, 229.03 [C17H25]+, 201.33 [C15H21]+, 187.3 [C14H19]+, 147.24 [C11H15]+, 133.21 [C10H13]+, 91.13 [C7H7]+
1H NMR	1H NMR (CDCl3, 300 MHz): δH 7.26 (H-2), 7.11 (H-3), 7.13 (H-5), 7.22 (H-6), 2.34 (Ar-CH3), 1.25 (H-1´, s), 2.32 (H-2´, t), 1.65 (H-3´, m), 1.68 (H-4´, m), 2.37 (H-5´, t), 1.63 (6´-CH3, m), 1.60 (H-7´, m), 1.58 (H-8´, m), 1.30 (H-9´, m), 1.25 (H-10´, d), 0.90 (10´-CH3, s), 0.92 (H-11´, s)
13C NMR	13C NMR (CDCl3, 75 MHz): δC 129.41 (C-1), 126.14 (C-2), 124.32 (C-3), 130.18 (C-4), 128.12 (C-5), 126.14 (C-6), 24.93 (Ar-CH3), 22.91 (C-1´), 77.65 (C-2´), 24.93 (C-3´), 29.29 (C-4´), 77.44 (C-5´), 77.23 (C-6´), 32.81 (6´-CH3), 29.46 (C-7´), 29.58 (C-8´), 29.66 (C-9´), 76.81 (C-10´), 29.81 (10´-CH3), 29.90 (C-11´)

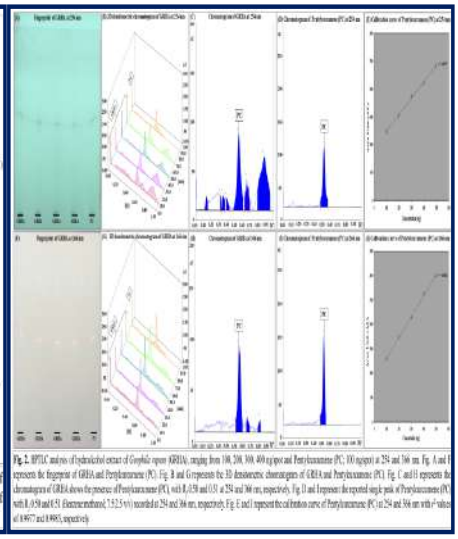
(B) Molecular docking of Pentylcurcumene (PC)

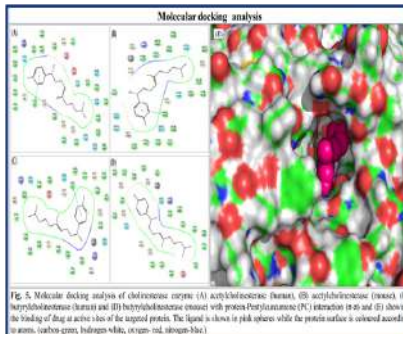
Species	Receptor	PDB-ID	Docking Score
Human	AChE(H)	4M0E	-5.6
Mouse	AChE(M)	5DTI	-6.1
Human	BChE(H)	1P0I	-6.9
Mouse	BChE(M)	Homology model	-4.18

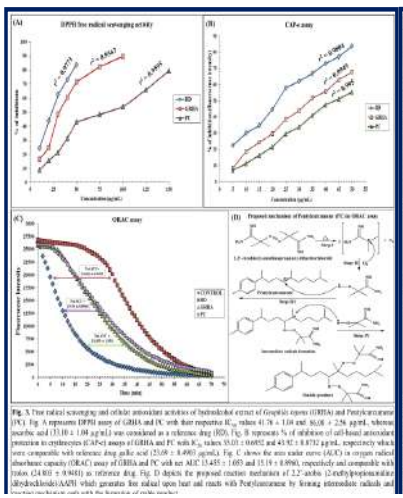
UV-Visible, IR, 1HNMR, 13CNMR and Mass spectroscopy analysis of isolated molecule Pentylcurcumene (PC). Molecular docking scores of Pentylcurcumene (PC) as ligand towards acetylcholinesterase (AChE; human/rat) and butyrylcholinesterase (BChE; human/rat) were analysed and protein structures were obtained from protein data bank (http://www.rcsb.org).

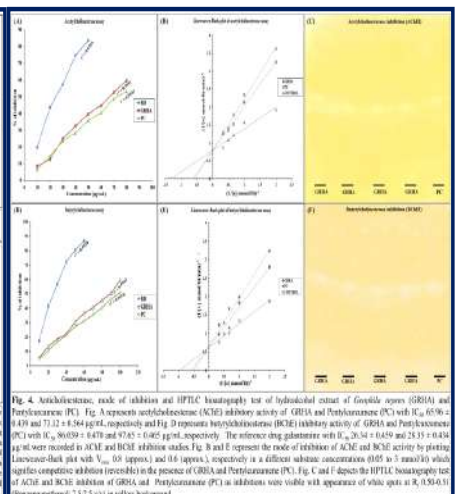
Based on results of our current findings, it is concluded that Pentylcurcumene, a terpene is an important bioactive molecule in G. repens has potential anticholinesterase activities and it could be a potential source of drug in Alzheimer's disease (AD). The molecular docking results support anticholinesterase activities of Pentylcurcumene with good agreement towards Alzheimer's disease. An improved and advanced HPTLC tool resulted in a successful implementation of bioautography detection method of Pentylcurcumene in *G. repens* towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities. Bioautography method will be extremely helpful in the identification of other novel anticholinesterase compounds in *G. repens*. Further investigations are to be needed in in vivo model to establish the key mechanism and establish the proper pathway of Pentylcurcumene to combat neurological diseases like Alzheimer's disease.











Hydrolea zeylanica alters the expression of glucose transporter protein in type-2 diabetic rats

(State Plan Funded)
Principal Investigator : Dr. Atish Kumar Sahoo, Senior Scientist
Research Fellow: Sandeep Kumar Swain, SRF

Hydrolea zeylanica (L.) Vahlis (Hydroleaceae), is a perennial, creeping herb native to India. It is commonly known as "Blue water leaf" or "Water olive". It is widely distributed in tropical regions of Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Taiwan, Chinese Provinces, Thailand, Viet Nam and Australia. The plant grows in rice paddies, pond margins, stream sides, swampy or inundated soils. In India, the leaves and young shoots are traditionally consumed which are rich in nutrition, dietary fibers and plays a pivotal role in reducing the malnutrition. *H. zeylanica* is an aquatic plant widely used as leafy vegetable in some parts of India. In south Odisha and Hazaribag district of Jharkhand, India, decoction of leaves is used as household remedy for diabetes. In India, the leaves of this plant are used ethnomedicinally for various diseases e.g. diabetes, wound healing, antiseptic and ulcer.

As far as we know, no previous research has investigated antidiabetic properties of *H. zeylanica*. We therefore examined various bioactivities of successive extracted fractions of *H. zeylanica* and investigated the role of bioactive principles in the most active hydroalcohol fraction of *H. zeylanica* (HAHZ) in bringing glycaemic control, GC-MS and LC-MS analysis were performed. To better understand the mode of inhibition of HAHZ, molecular mechanism of action of HAHZ in oxidative stress induced diabetes, streptozotocin-induced oxidative stress and metabolic changes in diabetic rats were studied.

- Phytochemical investigation of H. zeylanica by GC-MS/MS and LC-MS/MS.
- Experimental design of single dose one day study and multi dose 28 days study.
- Evaluates the ameliorates effect of H. zeylanica in type-II diabetic induced oxidative stress disorders.
- In vivo studies of the serum marker status of H. zeylanica in streptozotocin induced diabetic rat.
- In vivo studies of the liver and pancreas oxidative markers in streptozotocin induced diabetes to evaluate the effectiveness of HAHZ in diabetic rat.
- Evaluate the expression of glucose transporter protein in STZ-induced type-II diabetic rat by western blotting technique.
- Histopathological observation of liver and pancreas.

The present findings confirm that *H. zeylanica* has remarkable antidiabetic activities as evidenced from in vivo studies by modulating the liver and pancreas function with the improvement the cellular morphology of both organs in streptozotocin-induced diabetic rats. A total of 32 compounds from H. zeylanica were first reported by GC-MS/MS and LC-MS/MS analysis (Fig. 1). However, among 32 compounds R-limonene, perillartine, N-formyl-L-lysine, limonen-6-ol, pivalate, lidocaine and gamolenic acid in *H. zeylanica* were responsible for antioxidant and antidiabetic activities.

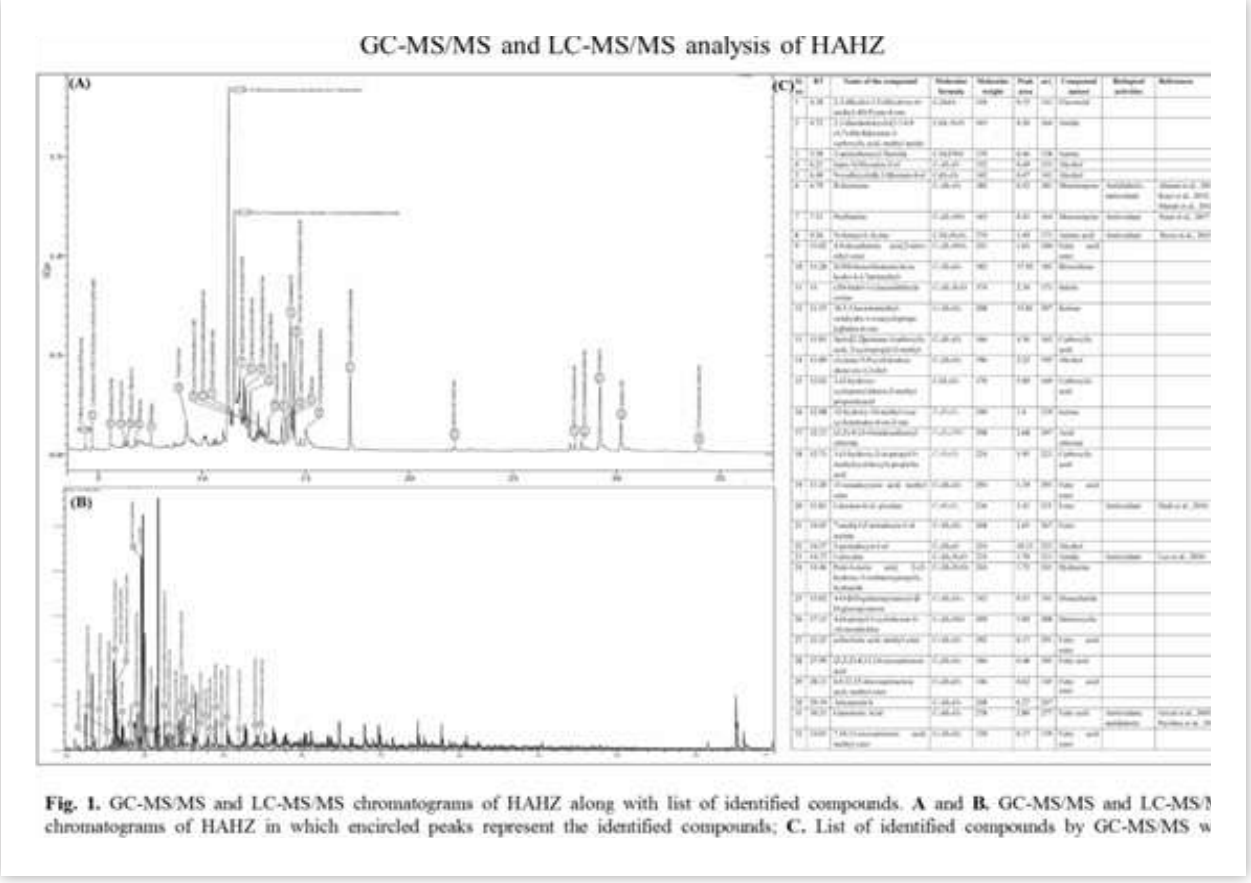


Fig. 1. GC-MS/MS and LC-MS/MS chromatograms of HAHZ along with list of identified compounds. **A** and **B**. GC-MS/MS and LC-MS/MS chromatograms of HAHZ in which encircled peaks represent the identified compounds; **C.** List of identified compounds by GC-MS/MS w

In single-dose one day study, the serum glucose (SG) level was investigated in STZ-induced diabetic rats. After 6 h of single dose administration of HAHZ-300 and 400 mg/kg caused significant reduction ($p < 0.001$) 15.89 ± 0.03% and 20.08 ± 0.12%, respectively in SG level as compared to positive control (Fig. 2A).

OGTT was performed on the 15th day of the experiment. We observed no significant change in SG level in normal group rats at 2 h post oral glucose administration and this was due to the enhanced release of insulin. But there was a marked increase in fasting glucose level and AUC in negative control which indicates significant ($p < 0.001$) impairment in glucose tolerance to exogenous glucose administration as compared to normal whereas, HAHZ-400 mg/kg significantly ($p < 0.001$) reduced the SG level (131.1 ± 0.59 mg/dL) and AUC (12539) and were comparable to the positive control (Fig. 2B-C).

ITT was performed on the 21st day of treatment. After 1.5 h of insulin (2 UI/kg, i.p.) administration, blood glucose level was significantly ($p < 0.001$) decreased in negative control in comparison to the normal and positive control. However, after 5.5 h of insulin administration, rats in negative control group showed significant increase in blood glucose level in comparison to positive control and HAHZ-400 mg/kg (108.33 ± 0.666 mg/dL). Also, the plasma glucose disappearance rate (KITT) was significantly ($p < 0.001$) lowered in negative control, but HAHZ-400 mg/kg significantly ($p < 0.001$) recovered the KITT (19.10 ± 0.08/min) which was comparable to positive control (Fig. 2D).

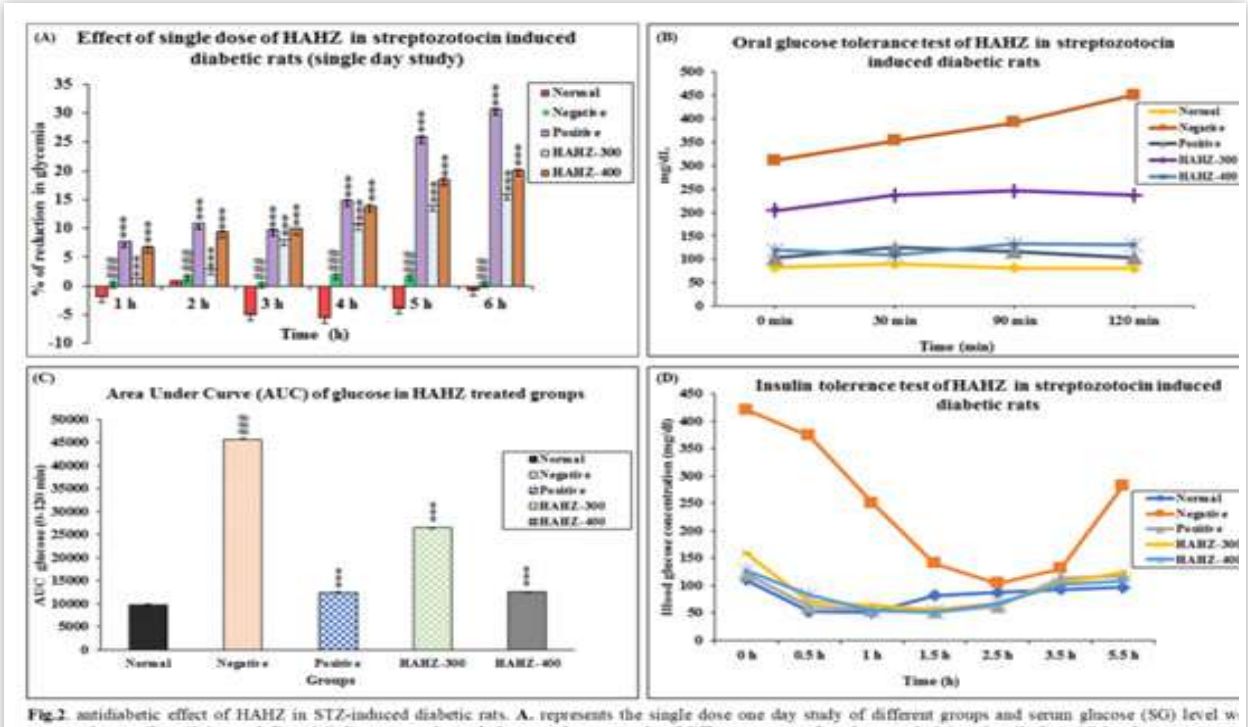


Fig.2. antidiabetic effect of HAHZ in STZ-induced diabetic rats. **A.** represents the single dose one day study of different groups and serum glucose (SG) level w measured up to 6h at 1h interval. **B and C**. Represents the oral glucose tolerance study of different groups after glucose administration (2g/kg). SG levels were measu at 0, 30, 60, 90 and 120 mins and AUC after post glucose load were represented. **D.** Represents the effect of insulin on different group and SG level were measured for

SGOT, SGPT and ALP are associated with the occurrence of T2DM, on completion of 28 days study period, the level of SGOT, SGPT and ALP (significantly ($p < 0.001$) elevated in negative control as compared to normal. By comparing the results from this study, HAHZ-400 mg/kg showed significant ($p < 0.001$) reduction in SGOT (65.33 ± 1.52 U/L), SGPT (22.30 ± 0.15 U/L) and ALP level (208.38 ± 2.22 U/L) and were found comparable to positive control (Fig. 3A).

After 28 days of diabetic induction of experimental study, negative control significantly ($p < 0.001$) elevated the TG, TC, LDL-C in comparison to normal. However, pretreatment of HAHZ-400 significantly ($p < 0.001$) reduced the elevated level of TG (138.06 ± 1.12 mg/dL), TC (99.82 ± 2.22 mg/dL), and LDL-C (39.29 ± 3.10 mg/dL) which were found similar to positive control. Even HDL-C level was significantly ($p < 0.001$) decreased in negative control in comparison to the normal whereas HAHZ-400 treated group significantly elevated the HDL-C level to 32.78 ± 0.42 mg/dL and found similar to the positive control (Fig. 3B).

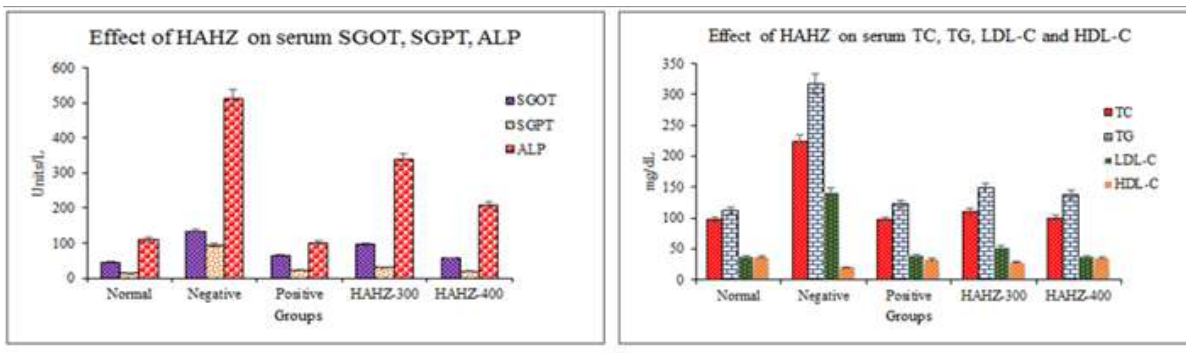


Fig.3. Biochemical estimation of serum. A. represents the Effect of HAHZ on serum SGOT, SGPT, ALP, TP and TB in STZ induced diabetic rats. B. represe the Effect of HAHZ on serum TG, TC, LDL-C and HDL-C in STZ-induced diabetic rats

Our results demonstrated that negative control showed significant ($p < 0.001$) increase in MDA level in serum, liver and pancreas as compared to the normal. But, the pretreatment of HAHZ-400 showed significant decrease in MDA level in serum (4.24 ± 0.08 nM/mg protein), liver (1.47 ± 0.13 nM/mg protein) and pancreas (3.11 ± 0.56 nM/mg protein) and were comparable to positive control (Fig. 4A). The present study also analyzed the SOD level significant ($p < 0.001$) decreased in serum, liver and pancreas in negative control as compared to the normal. But, pretreatment with HAHZ-400 significantly ($p < 0.001$) increased the SOD level (serum 8.11 ± 0.11; liver 6.43 ± 0.22; pancreas 14.44 ± 0.29 U/mg protein) and these results were found comparable to positive control (Fig.4B). While in T2DM, another oxidative stress parameter CAT level significantly ($p < 0.001$) decreased in negative control as compared to normal. But, HAHZ-400 showed significant ($p < 0.001$) increase in the CAT level (serum; 40.44 ± 0.27 U/mg protein, liver; 24.12 ± 0.18 U/mg protein, pancreas; 26.42 ± 0.73 U/mg protein) and found comparable to positive control (Fig. 4C).

Similarly, the decrease in glutathione reductase (GSH) activities was observed in serum, liver and pancreas in negative control diabetic rats as compared to the normal. Pre-treatment with HAHZ-400 showed significant ($p < 0.001$) increase in GSH (serum; 0.70 ± 0.05 U/mg protein, liver; 19.86 ± 0.20 U/mg protein, pancreas; 19.33 ± 0.20 U/mg protein) similar to positive control (Fig. 4B).

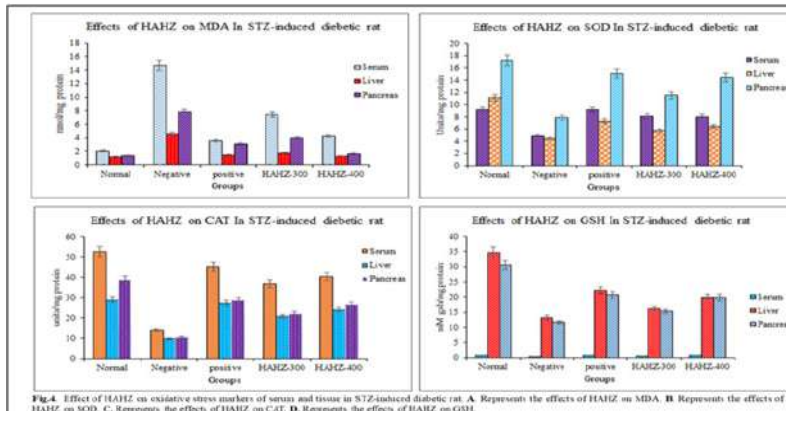


Fig.4. Effect of HAHZ on oxidative stress markers of serum and tissue in STZ-induced diabetic rat. **A.** Represents the effects of HAHZ on MDA. **B.** Represents the effects of HAHZ on SOD. **C.** Represents the effects of HAHZ on CAT. **D.** Represents the effects of HAHZ on GSH.

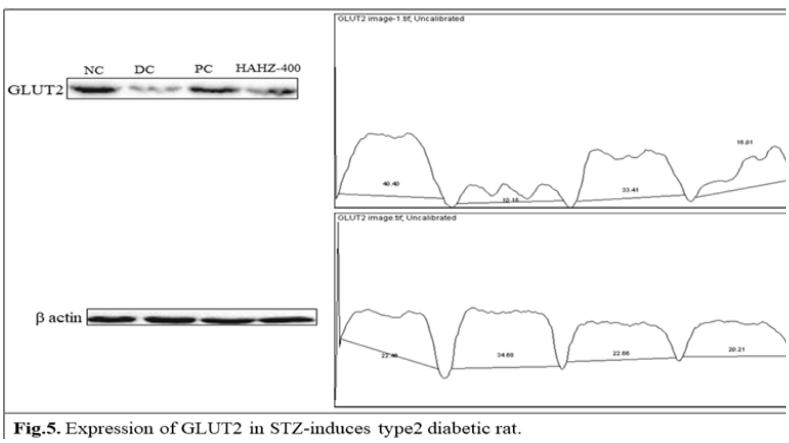


Fig.5. Expression of GLUT2 in STZ-induces type2 diabetic rat.

As shown in Figure 5, GLUT2 from the liver of normal control, diabetic control, positive control (GLB 5mg/kg; b.w) and HAHZ-400 treated, rats were detected using western blotting between the marker protein 19-250 kDa in size. GLUT2 appeared to decrease more than 20% in the STZ-induced, diabetic model rats, compared to the normal rats. However, in the case of the GLB-5mg/kg treated diabetic rats, and HAHZ treated diabetic rats GLUT2 expression increased significantly in the liver tissues, compared to the diabetic control rats (Fig. 4). Therefore, it may be explained that the decreased hyperglycaemia in HAHZ-400 treated type 2 diabetic model rats could be due to the increased GLUT2 in liver of rats in this group.

Ameliorative effects of *Homalium zeylanicum* on diabetes-induced oxidative stress and inflammation in Wistar rats.

(Fellowship Project , S & T Dept., Govt. of Odisha Sponsored)

Principal Investigator : Dr. Atish Kumar Sahoo, Senior Scientist

Research Fellow: Diptimayee Rout, BPRFS Fellow

Homalium zeylanicum (Gardner) Benth. (Salicaceae), commonly known as 'Kalladamba', is distributed in Western Ghats, Andhra Pradesh, Tamil Nadu, and Kerala of India. Ethnobotanically, the bark and leaf of this plant are traditionally used in diabetes, rheumatism, malaria and wound healing by the isolated section of the society in Rayalaseema region of Andhra Pradesh, India. So, further investigation on this plant for the presence of active constituents needs to be validated. Hence this project has been undertaken to establish scientifically the efficacy of this plant for combating diabetes-induced oxidative stress and inflammation in Wistar rats.

1. Phytochemical investigation and chemical profiling of the hydroalcohol extract of bark and leaf of H. zeylanicum.
2. In vitro free radical scavenging assays as DPPH, hydroxyl, nitric oxide and superoxide dismutase, metal chelating assays of hydroalcohol extract of bark and leaf of *H. zeylanicum*.
3. In vitro antidiabetic studies as α-amylase inhibition, α-glucosidase inhibition assays of the hydroalcohol extract of bark and leaf of *H. zeylanicum*.
4. In vitro anti-inflammatory studies as protein denaturation assays of the hydroalcohol

   extract of bark and leaf of *H. zeylanicum*.
5. Ex vivo ORAC and Cap e assay for the determination of cellular antioxidant protection of the hydroalcohol extract of bark of *H. zeylanicum*.

The bark and leaf of the plant *H. zeylanicum* (Gardner) Benth., (Salicaceae) were collected from Tirumala Hills, Chittoor District, Andhra Pradesh, India. The plants were botanically identified by Dr. P.C Panda, Regional Plant Resource Centre, Bhubaneswar, Odisha and Voucher specimen was deposited in the herbarium of RPRC for future references.

Percentage yield of plant extracts

Percentage of yield of hydroalcohol extract of bark and leaf of *H. zeylanicum* (HAHZB; HAHZL) was found to be 8.4% and 7.6% respectively.

Phytochemical analysis of extracts:

The quantitative phytochemical analysis of the HAHZB and HAHZL reported the presence of major group of chemicals e.g. Flavonoids, Tannins, Saponins, Alkaloids, Steroids, Terpenoids. These phytochemicals are the major contributors for the antioxidant activity of H. zeylanicum, and exhibited a correlation in the radical scavenging activities.

GC-MS/MS analysis of HAHZB

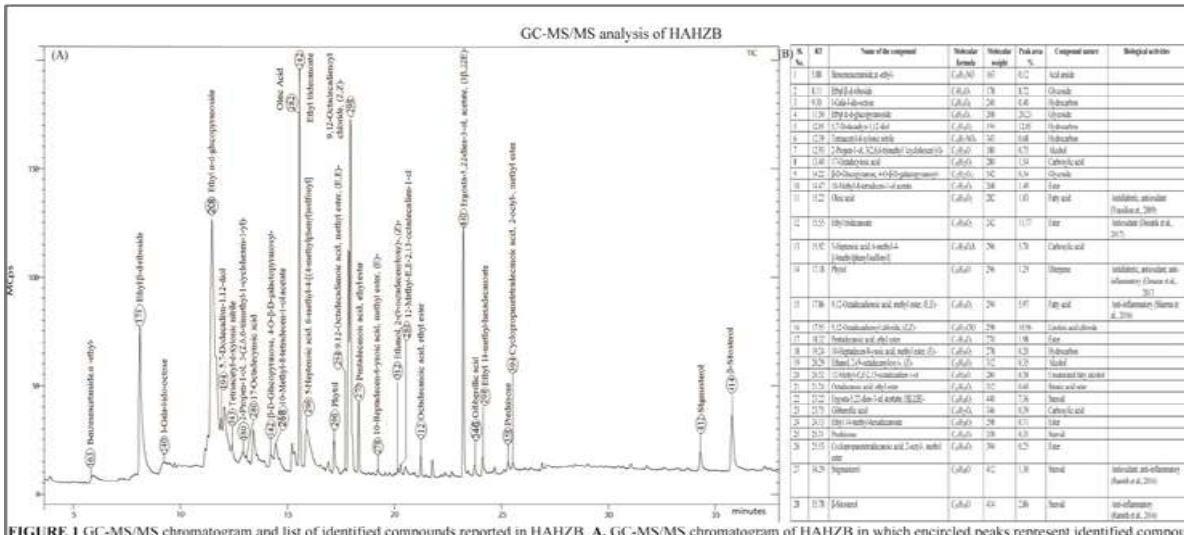


FIGURE 1 GC-MS/MS chromatogram and list of identified compounds reported in HAHZB. **A.** GC-MS/MS chromatogram of HAHZB in which encircled peaks represent identified compou
B. List of identified antioxidants, antidiabetic and anti-inflammatory compounds that are reported in HAHZB by GC-MS/MS study. HAHZB-hydroalcohol fraction of bark of *H. zeylanicum*.

GC-MS/MS analysed the secondary metabolites of HAHZB and validated by comparing the mass spectra of compounds with the standard mass spectra of NIST library (Version-11). A total of 28 no. of compounds belongs to glycoside, carboxylic acid, hydrocarbon, fatty acid, diterpene, ester, acid chloride and steroid groups were represented/described with peak number, retention time (RT), compound name, molecular formula, molecular wt. and peak area (Figure. 1)

GC-MS/MS analysis of HAHZB detected 15 dominant compounds e.g. ethyl β-d-riboside (8.72%), ethyl α-d-glucopyranoside (20.23%), 5,7-dodecadiyn-1,12-diol (12.05%), 17-octadecynoic acid (1.54%), 10-methyl-8-tetradecen-1-ol acetate (1.49%), oleic acid (1.03%), ethyl tridecanoate (11.77%), 5-heptenoic acid, 6-methyl-4-[(4-methylphenyl)sulfonyl] (5.70%), phytol (1.29%), 9,12-octadecadienoic acid, methyl ester, (E,E)- (5.97%), 9,12-octadecadienoyl chloride, (Z,Z)- (10.96%), pentadecanoic acid, ethyl ester (1.98), ergosta-5,22-dien-3-ol, acetate, (3β,22E)- (7.36%), stigmasterol (1.30%), β-sitosterol (2.86%) (Figure.1). Out of which, 6 compounds have antioxidant, anti-inflammatory and anti-diabetic activities.

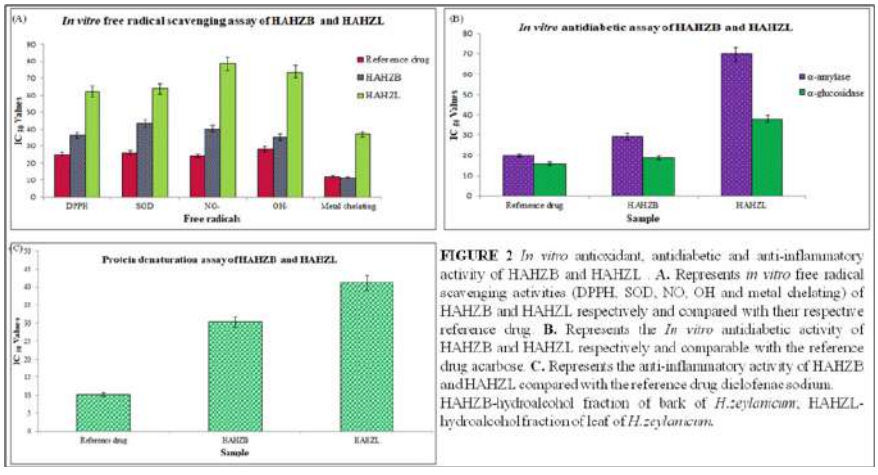


FIGURE 2 *In vitro* antioxidant, antidiabetic and anti-inflammatory activity of HAHZB and HAHZL . **A.** Represents *in vitro* free radical scavenging activities (DPPH, SOD, NO, OH and metal chelating) of HAHZB and HAHZL respectively and compared with their respective reference drug. **B.** Represents the *In vitro* antidiabetic activity of HAHZB and HAHZL respectively and comparable with the reference drug acarbose. **C.** Represents the anti-inflammatory activity of HAHZB and HAHZL compared with the reference drug diclofenac sodium. HAHZB-hydroalcohol fraction of bark of *H.zeylanicum*, HAHZL-hydroalcoholfraction of leaf of *H.zeylanicum*.

In vitro antioxidant study of HAHZB and HAHZL has been conducted and compared with their respective standard drugs. The IC50 values were recorded for DPPH (HAHZB 36.23 ± 0.27 µg/mL; HAHZL 62.12 ± 0.43 µg/mL, and ascorbic acid 25.12 ± 0.41 µg/mL). In SOD assay (HAHZB 43.34 ± 0.87 µg/mL; HAHZL 63.9 ± 0.32 µg/mL and, quercetin 26.21 ± 0.38 µg/mL). The in vitro test results of NO assays were (HAHZB 40.11 ± 0.82 µg/mL; HAHZL 78.53 ± 0.74 µg/mL; ascorbic acid 24.13 ± 0.52 µg/mL). The test results were reported for OH assays (HAHZB 35.23 ± 0.89 µg/mL; HAHZL 73.69 ± 0.76 µg/mL, and gallic acid 28.24 ± 0.61 µg/mL). In the metal chelating assays, the IC50 values were (HAHZB 11.54 ± 0.97 µg/mL; HAHZL 36.96 ± 0.76 µg/mL; and, EDTA 12.27 ± 0.83 µg/mL) and the values for each of the parameters studied has been depicted in figure 2. For the anti-diabetic studies of H. zeylanicum bark and leaf fractions were conducted by performing the in-vitro α-amylase and α-glucosidase studies. The IC50 values of each fraction was recorded for α-amylase (HAHZB 29.12 ± 0.62 µg/mL; HAHZL 69.88 ± 0.91 µg/mL; and, acarbose 19.89 ± 0.72 µg/mL). The test results obtained for α-glucosidase were (HAHZB 18.55 ± 0.27 µg/mL; HAHZL 37.92 ± 0.73 µg/mL; and, acarbose 16.02 ± 0.67 µg/mL), respectively. Protein agglutination of anti-inflammatory study was performed and IC50 values of each of the fractions were recorded (HAHZB 30.34 ± 0.67 µg/mL; HAHZL 41.21 ± 0.73 µg/mL and, diclofenac sodium 10.16 ± 0.22 µg/mL) as shown in figure 2. All the results indicated its sound pharmacological potential. Hence, it is concluded that the plant parts might be effective and can be optional herbal drugs to cure diabetic condition by scavenging free radicals and combating inflammation.

Ex vivo ORAC and CAP-e assay of HAHZB

ORAC assay of HAHZB was performed by measuring the net area under the curve (AUC) of HAHZB. The net AUC of HAHZB and standard drug trolox was found to be 13.38 and 22.25 respectively (Figure 3). The cellular antioxidant potential of HAHZB was measured by conducting the CAP-e assay on RBCs obtained from Wistar rats.

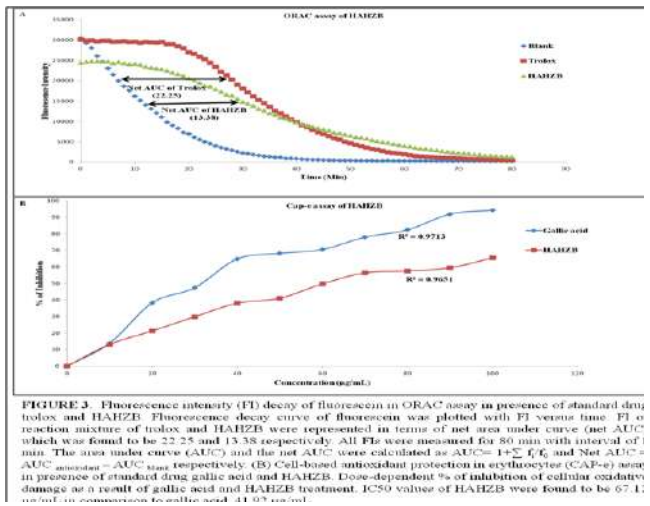


FIGURE 3. Fluorescence intensity (FI) decay of fluorescein in ORAC assay in presence of standard drug trolox and HAHZB. Fluorescence decay curve of fluorescein was plotted with FI versus time. FI of reaction mixture of trolox and HAHZB were represented in terms of net area under curve (net AUC) which was found to be 22.25 and 13.38 respectively. All FIs were measured for 80 min with interval of 1 min. The area under curve (AUC) and the net AUC were calculated as AUC= $1+\sum f_i/f_0$ and Net AUC = $AUC_{antioxidant} - AUC_{blank}$ respectively. (B) Cell-based antioxidant protection in erythrocytes (CAP-e) assay in presence of standard drug gallic acid and HAHZB. Dose-dependent % of inhibition of cellular oxidative damage as a result of gallic acid and HAHZB treatment. IC50 values of HAHZB were found to be 67.1 µg/mL in comparison to gallic acid 41.92 µg/mL.

Percentage of Inhibition of oxidative damage by HAHZB was calculated by comparing the cells treated with AAPH (oxidizing agent). HAHZB demonstrated cellular oxidative defence with IC50 67.12 µg/mL, whereas, the IC50 of standard drug gallic acid was 41.92 µg/mL (Figure 3). The present study shows that HAHZB and HAHZL both having antioxidant, antidiabetic and anti-inflammatory potential which further can validate through an animal model and can proceed for the pipeline of drug development.

Evaluation of unexplored *Ardisia solanacea* and *Aegiceras corniculatum* plants of Myrsinaceae family as embelin and other related compound producing substitutes for over exploited RET medicinal species *Embelia ribes* and *E. tsjeriam-cottam*

(NMPB, GOI Funded)

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Research Fellow: Swadha Baral, SRF

Re-emergence of interest in herbal plant-based health care globally on the one hand and unsustainable collection from the wild without adequate efforts at conservation and sustainable harvesting results in a large number of species coming under serious threat of extinction leading to use of substitutes. To overcome the above mentioned constraint, finding out suitable cultivable substitutes for these RET medicinal plants (whole or plant parts) possessing identical/common medicinal properties is the need of the hour. Since, the active principle embelin & other related compounds such as Phenol, Flavonoid are reported to present largely in the plants under Myrsinaceae family, other Non-RET species (of the same family) viz. *Ardisia solanacea* Roxb. (tinkoli) and *Aegiceras corniculatum* (L.) Blanco (khalsi) are proposed for isolation and quantification of embelin possibly as a new source of embelin that can act as a suitable substitutes for *Embelia ribes & E. tsjeriam-cottam*. Both the proposed plants are quite available in the wild in Odisha but detailed study on them regarding this approach has not yet been initiated. Furthermore, in this study, only the fruit and the leaf parts were considered for isolation of embelin to least exploit the plant species and hence protecting them. Though some data are available regarding the presence of embelin in roots, barks, but choosing these parts of plant can be detrimental for the survival of the plant. Reports on the availability of embelin in leaves *E. ribes* (Swamy et al., 2007; Raghu et al., 2011) encouraged us to analyse embelin content in the leaves (in addition to fruit part) of the proposed species. Apart from its natural occurrence, both the species can be propagated and cultivated following conventional seed and vegetative propagations (Basak et al., 2003; Basak and Mahapatra, 2010).

During this period of research work, apart from embelin, other related compounds like, Epigallocatechin gallate (EGCG) , Epigallocatechin (EGC) and Quercetin have been evaluated in *Ardisia solanacea* and *Aegiceras corniculatum*, as alternate sources in place of RET plants *Embelia ribes* and *E.tsjeriam-cottam* belonging to the same family Myrsinaceae. Epigallocatechin gallate (EGCG) , Epigallocatechin (EGC) and Quercetin compounds were evaluated from the fractions of four compounds using different solvent (methanol, chloroform, petroleum ether and hexane) extracts of leave and fruits of Ardisia solanacea and *Aegiceras corniculatum*. The EGCG and EGC compound were analyzed (HPLC) using mobile phase water:acetonitrile:methanol:ethyl acetate:glacial acetic acid. The retention time of EGCG compound was found to be around 11.00 minute. Besides, the effective mobile phase for quercetin was optimized (Methanol: ortho phosphoric acid (65:35 v/v)). The detection was carried out using variable wavelength UV–VIS detector set at 369 nm. (Fig. 1 & Fig. 2)

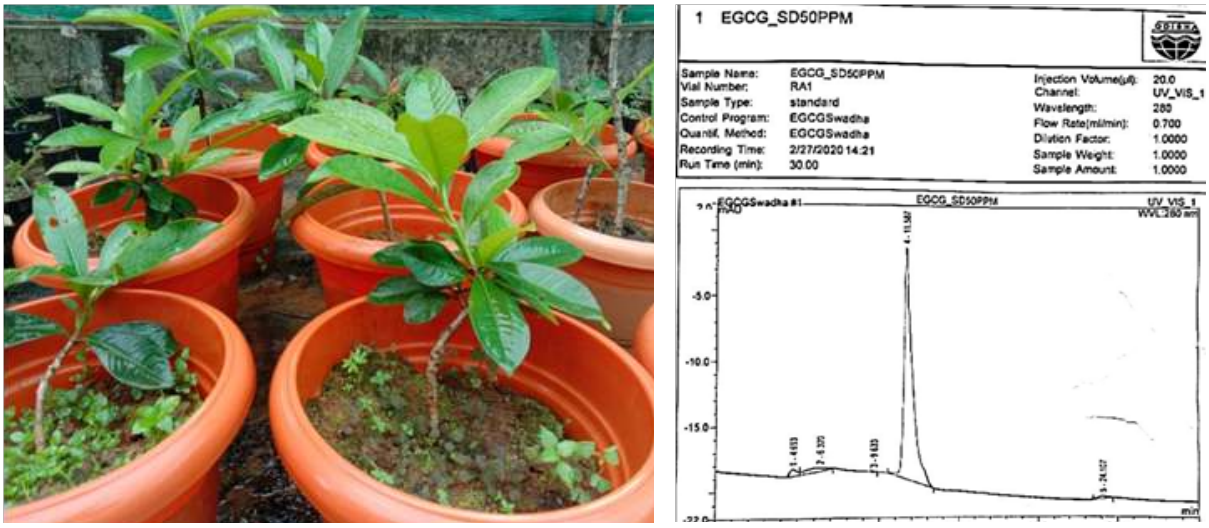


Fig.1. Nursery grown Saplings of Ardisia solanacea and EGCG standard recorded through HPLC

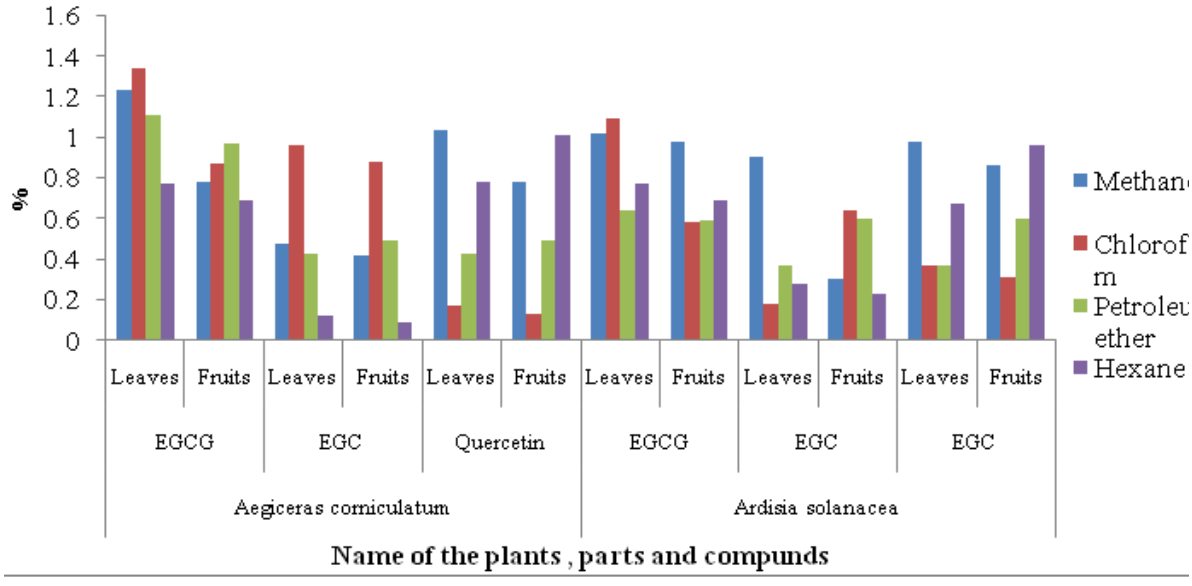


Fig.2. Quantitative analysis of EGCG, EGC & Quercetin in various plant parts of *A. corniculatum* and *A. solanacea*

Evaluation of *Bixa orellena* and *Nyctanthes arbortristis* for antifungal activity using *Aspergillus flavus* and *Aspergillus niger* as target experimental model.

(State Plan Funded)

Principal Investigator: Dr. Sunita Bhatnagar, Senior Scientist

Research Fellow: Rashmi Das, JRF

Antifungal activity against *Aspergillus flavus* and *Aspergillus niger* was tested using three methods, radical growth method, Agar diffusion method and biomass reduction method. Four solvent extracts of *Bixa oreallana* and *Nyctanthes arbortristis* leaves were prepared using soxlet extraction method. Same were used for all the activities to fulfill the following objectives

1. Evaluation of antifungal activity of solvent extracts of *Nyctanthes arbortristis* and *Bixa orellana* leaves against *Aspergillus flavus* and *Aspergillus niger.*
2. Assessment of effect of solvent extracts of both the plants on aflatoxin content produced by the *Aspergillus flavus*.

Antifungal activity of *Nyctanthes arbortristis* using Radical growth method on *Aspergillus flavus*

The radical growth is expressed in terms of percentage of growth inhibition. Growth inhibition was observed highest on 2nd day observation i.e.60% in ethyl acetate leaf extracts of *Nyctanthes arbortristis* at 1mg/ml doses, while other extracts showed mild growth inhibition as compared to the *A.flavus* (control) (Fig 1). Whereas in *A.niger* (control) treated plates highest inhibition was also observed in ethyl acetate with 41.4% at 1mg/ml doses on 2nd day observation (Fig 2).

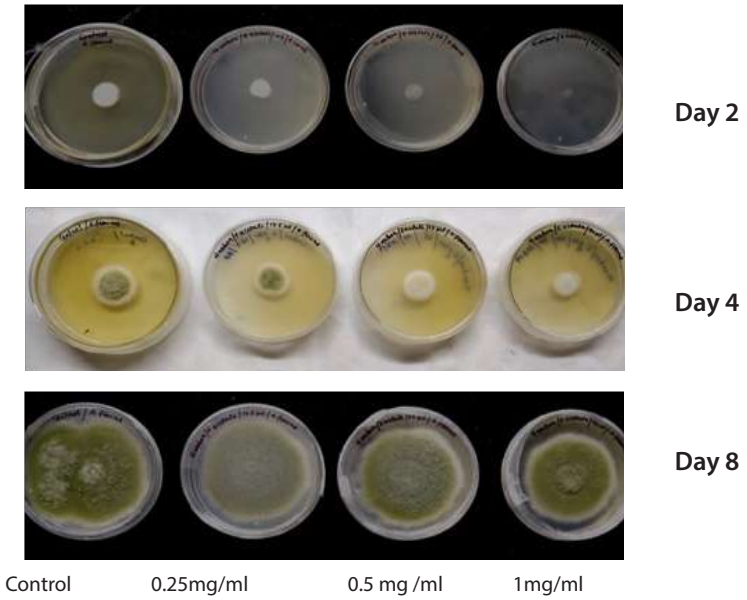


Fig 1- Effect of *N. arbortristis* (ethyl acetate extract) on radical growth of *A.flavus*

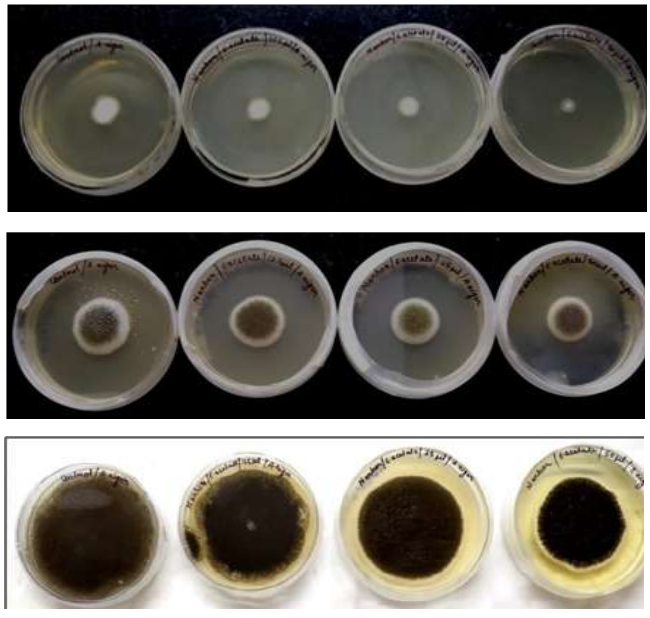


Fig 2- Effect of *N. arbortristis* (ethyl acetate extract) on radical growth of A. niger

Ethyl acetate extract of Nyctanthes arbortristis was most active against all the methods, In both the models higher dose of 1mg/ml was able to reduce the biomass more than 50 percent. None of the extracts of *Bixa oreallana* exerted any significant antifilarial activity.

Effect of temperature on withanolide contents of *Withania somnifera* plants grown by seeds stored at different temperatures.

(State Plan Funded)

Principal Investigator: Dr. Sunita Bhatnagar, Senior Scientist

Research Fellow: Smiti Jena, JRF

The project work was under taken with the following objectives.

1. Effect of temperature on the shelf life of seeds of *Withania somnifera*.
2. Effect of temperature on withanolides in plants raised from seeds stored at three temperatures.

Germination of seeds stored at variable temperatures.

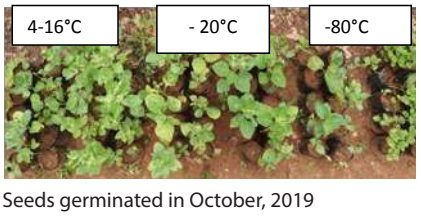
Quality planting material was procured in the form of Seeds in the month of July, 2018 from Central Institute of medicinal and aromatic plants, Lucknow. Batch testing of the seeds was done and it was found that they are 100 percent viable. Seeds were stored at three different temperatures, i.e.,4-16 degree Celsius, -20 and -80 degree Celsius. Every month seeds were taken out and germinated from August, 2018 onwards. Data related to previous year was reported earlier. Same experiment continued till January, 2020. It was observed that even after 18 months of storage, there was no effect on the seed viability, whereas when stored at room temperature they lose viability within 3 months. Further medicinal plants hold their activity because of the active principles in them, so in the present year State plan project phytochemical analysis along with the crude withanoloide content was studied in the plants raised from the seeds stored at the three temperatures as mentioned in the experimental protocol. (Figs 1-2)

Effect on Withanoloide content

It was observed that no major differences were found in the withanolide content of *Withania somnifera* with respect to different temperatures in which the seeds were stored. It was also observed that the intensity of bands reduced with progress in months. The observation indicates the decline of withanolide contents with the increase in months. TLC results showed better bands in almost all leaf samples than root samples, which indicate that leaves of Ashwagandha might have more withanolide content as compared to the roots. (Fig. 2)

Seeds germinated in April, 2019

Seeds germinated in July, 2019



Seeds germinated in October, 2019

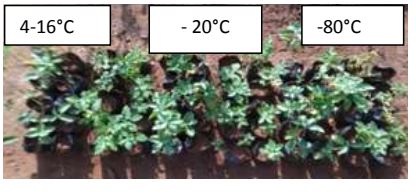
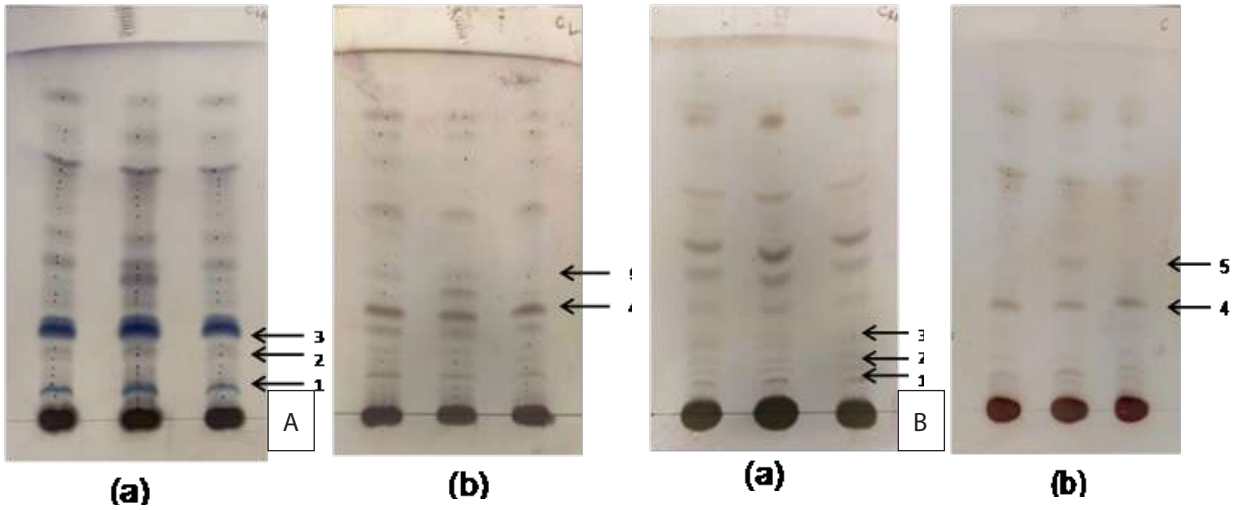


Fig.1Seed germination of *W.somnifera* at diferent time intervals.



1. Withaferin A: 0.10±0.01
2. Withanone: 0.18±0.02
3. Withanolide A: 0.23±0.02
4. Withaferin A: 0.34±0.01
5. Withanolide D: 0.51±0.02

Fig. 2. TLC of A- Leaf extracts and B- Root extracts of Withania somnifera for analysis of withanolides during fourth and fifth month. TLC of plant samples in (a) solvent 1 (b) solvent 2

Qualitative and quantitative analysis of essential amino acids and pectin in lesser known wild edible fruits of Odisha

(State Plan Funded)

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Research Fellow: Lizaranee Behuria, JRF, Jyoti Ranjan Barik, JRF

Essential amino acids are generally considered as essential for humans. The use of underutilized wild edible plants helps in the fight to malnutrition associated problem and increasing the health status of the rural population. Fruits have emerged as the most important source because of high nutritive value as well as the protecting ability from many diseases. When compared proteins between fruits and the other plant crops, it is clear that proteins of fruits are of high quality as to their content of essential amino acids relative to human body's requirement. Several authors had reported that these wild edible fruits provide functional as well as nutraceutical properties. According to the recent reports, wild fruits were found as an important source of protein (Mahapatra et al., 2012; Nayak and Basak, 2015; Rout et al., 2015). Purified essential amino acid compounds have been identified, isolated and analyzed through HPLC (Ayessou et al., 2014; Muhammad et al., 2015) and GC-MS analysis (Golden et al., 2001; Ozcan and Baycu, 2008; Avinash et al., 2010) from various wild edible fruits.

Pectin is a natural product which is found in the cell walls of all higher plants. It is the methylated ester of polygalacturinic acid. In terms of nutrition and health, pectin has several biological and physiological functions which have been shown to lower blood cholesterol level, helps in prostate cancer treatment and acts as a potential carrier for drug delivery. Keeping in mind the growing need for alternative bionutrition resources, these wild edible fruits need to be popularized for their edibility and medicinal properties producing value added product, boosting indigenous production of high grade pectin.

Present study dealt with estimation & evaluation of composition of various essential amino acids and pectin in selected wild edible fruits of Odisha to promote bioprospecting, conservation and domestication of promising species. In this research work, 4 wild edible fruits of Odisha viz. *Grewia tiliifolia, Limonia acidissima, Streblus asper* and *Olax scandens* were subjected to evaluation of all 9 Essential amino acids (metheonine , phenylalanine, lysine, leucine, isoleucine, tryptophan, threonine & valine) using various methods like Spectrophotometer analysis, TLC and HPLC. According to the results obtained from this study, all the studied fruits possessed EAA. As per spectrophotometric analysis, *Streblus asper* appeared to have high EAA content followed by *Limonia acidissima*, *Olax scandens* and *Grewia tiliifolia*. However, as per HPLC analysis, wild fruits viz. *Grewia tiliifolia* and *Olax scandens* showed promising essential amino acid content (especially Leucine and Valine) and deserved to be popularized and domesticated. In order to find out promising pectin producing wild fruit species, a total 9 parameters (yield, degree of esterification, titrable acidity, ascorbic acid, moisture, methoxyl content, equivalent wt., anhydrouronic acid and galacturonic acid) have been analysed to evaluate and characterize pectin of good quality amongst 4 selected wild fruits viz. *Syzigium cumini, Diospyros malabarica, Pithecellobium dulce* and *Spondias pinnata*. Based on high yield, degree of esterification, methoxyl content and anhydrouronic acid content (which are major indicators of good quality pectin), it can be opined that *Syzigium cumini* and *Diospyros malabarica* fruits have good quality Pectin and deserved to be popularized and domesticated (Figs. 1-4).

(a) *Grewia tiliifolia* (Dhamura)

(b) *Olax scandens* (Bhadabhadi)

(c) *Diospyros malabarica* (Mankad kendu)

(d) *Syzigium cumini* (Bada jamu)

Fig.1. Studied Wild edible fruits for EAA (a & b) and Studied Wild edible fruits for Pectin (c & d)

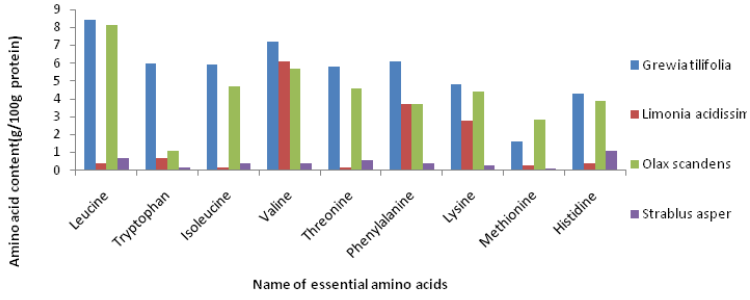


Fig.2. Essential Amino Acids (g/100g protein) in 4 Wild Edible Fruits of Odisha

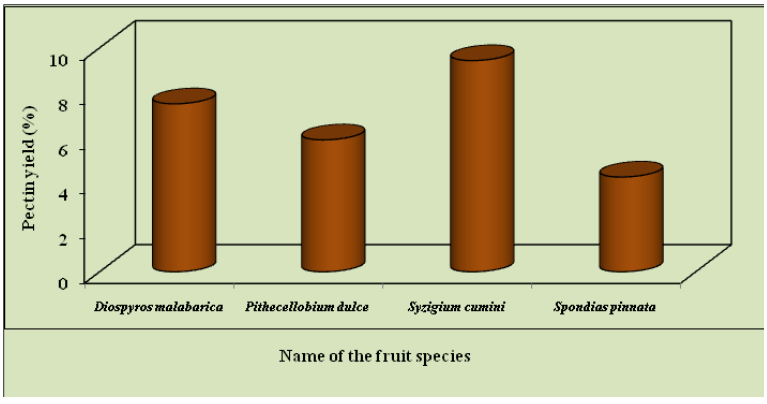
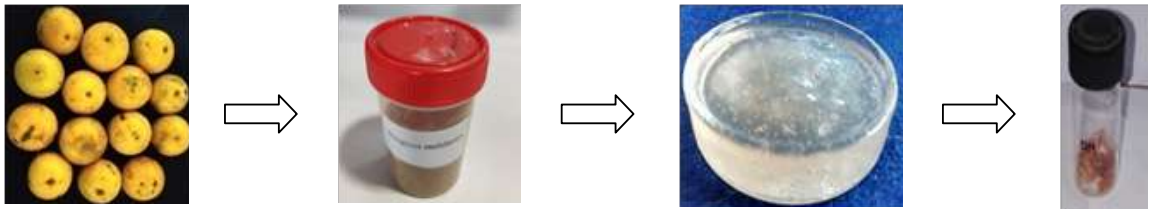


Fig.3. Pectin yield in 4 selected wild edible fruits



Diospyros malabarica (Mankad kendu)

Powder sample

Wet Pectin

Dry Pectin

Fig.4. Chronology of Pectin production.

Evaluation of underutilized and under exploited wild edible mangrove fruits of Odisha coast for their nutritional and antioxidant properties

(State Plan Funded)

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Research Fellow: Bastabik Mohan Das, JRF

Mangrove species grow at the edge between the coastal and land area in subtropical and tropical regions of the world and are highly adapted to various temperatures, coastal winds, extreme tidal waves, salinity fluctuations, coastal water turbulence, river run-off and anaerobic soil. The unique ecology and ecosystem services, plant morphological characteristics, and traditional uses of mangrove plants have already drawn the attention to researchers over the years. Mangroves possess unique biochemical functions in their ecosystem and are considered as a novel / natural products. Mangroves are rich resources of compounds like polyphenols and tannins. Leaves and fruits also possess phenolic compounds, alkaloids and flavonoids which serve as novel bioactive compound. Several studies have already been carried out on the nutritive values and presence of potent micronutrient in the fruits of different plant species, but less study have been documented with fruits of mangrove species (Halder et al., 2013, 2015). During recent years, there has been a growing interest to evaluate various mangrove fruits for their nutritional value (Rout et al., 2015; Sudirman et al., 2014; Patil and Chavan 2013; Jacoeb et al., 2013).

In this research work, fruits of the mangrove species of Odisha viz. *Bruguiera sexangula* (Odia name-Bandari), *Rhizophora mucronata* (Odia name- Rai), and *Xylocarpus granatum* (Odia name- Sisumara), belonging to Bhitarkanika National Park in Kendrapada District of Odisha, were subjected to comparative evaluation of nutritional properties and further analysis of antioxidant potential of the nutritionally superior fruit species. A total 8 nutritional parameters viz. moisture,carbohydrate, protein, sugar,reducing sugar, nonreducing sugar and acidity content were estimated in all the studied fruits and *Xylocarpus granatum* was found superior fruits. Subsequently this superior fruit was subjected to evaluation of antioxidant activity based on 6 parameters of non-enzymatic activity (carotenoid, flavonoid, phenol, reducing power, DPPH & FRAP) and 3 parameters of enzymatic activity (Peroxidase, Catalase & superoxide dismutase). Though several mangroves are reported to be used in traditional medicine, only some of them were tested for biological activities and a very few were studied for antioxidant activity. *Xylocarpus granatum*, a RET mangrove species, thus deserved to be conserved for more other related studies.

Fig.1. Fresh & Dried powdered mangrove fruit samples used for nutritional analysis

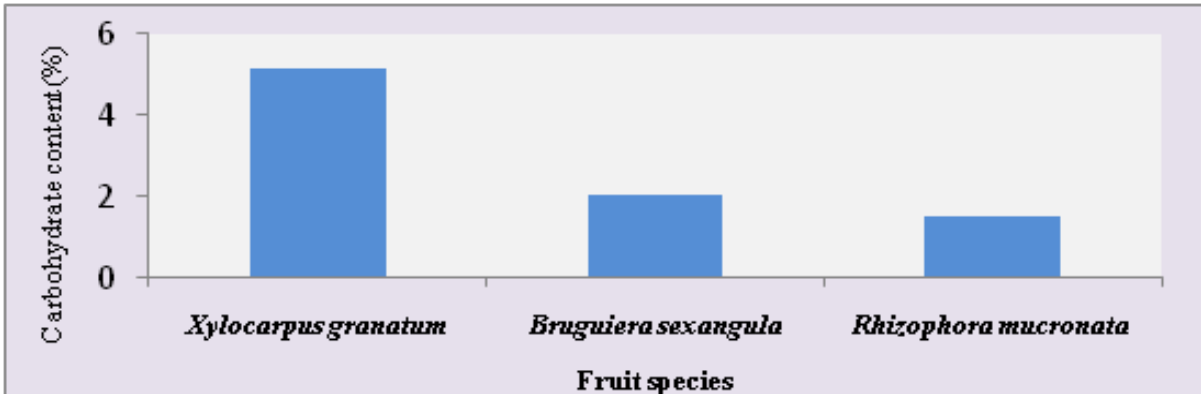


Fig.2. Carbohydrate content in wild edible mangrove fruits of Odisha

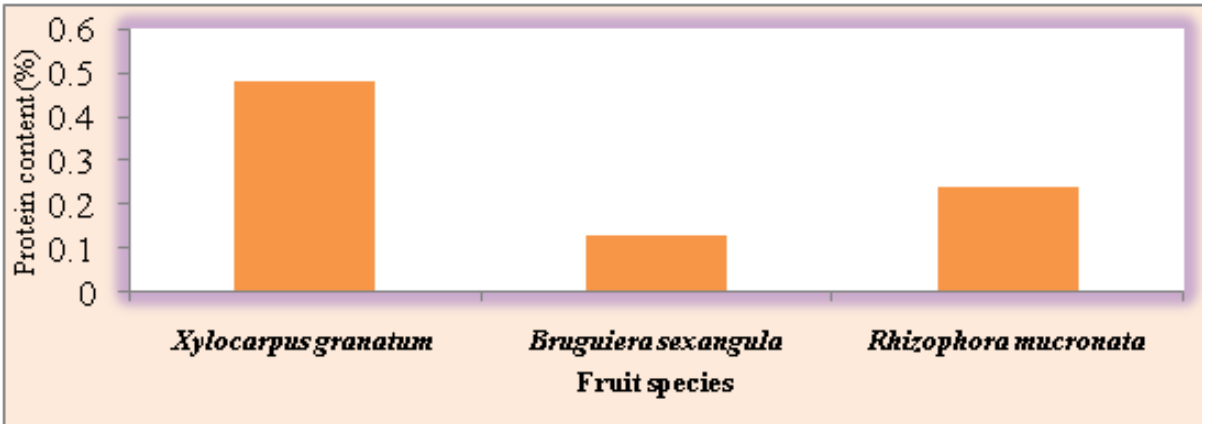


Fig.8. Protein content in wild edible mangrove fruits of Odisha

Studies on Bioenergy and Biofuel

Elucidation of genetic network with response to salt and drought stress in *Saccharum bengalense* Retz.

(State Plan Funded)

Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist

Research Fellow: Debabrata Dash, SRF

Availability of energy for meeting different activities is the key factor for the development of a country. Fossils fuels, currently used as the major source of energy are limited and polluting the environment; thus it is required to use renewable source of energy. Among the different types of the bioenergy options, bioethanol is being used in many countries as the technology of the production is matured. The major source of bioethanol production is from the biomass; thus availability of the raw material is the major hurdle. In recent years, dedicated energy crops have been developed those are able to grow on waste of abundant agriculture lands.

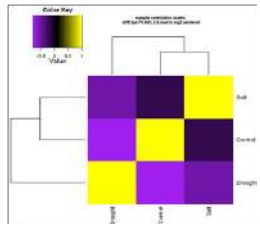
Saccharum bengalense is growing wildly in different parts of the state of Odisha belonging to the sugarcane family. Three years cultivation practice conducted at our institute has shown excellent biomass production potential. The grass is able reach 16 feet height. The species has shown resistance to drought in field condition. In pot experiments, plants also have shown resistance to salts. It is possible that, the plant *Saccharum bengalense* do have unique genetic network with regard to managing different types of stress. In this study, novel genetic networks have been identified with regard to the salt and water stress through transcriptome sequencing.

Genetic network with response to salt

The plants were grown on 300mM and 400 mM NaCl and found that though there was significant difference in the performance of the plants as compared to control, were able to grow on 300 mM and 400 mM of NaCl. RNA from the treated plants were isolated and used for sequencing using Illumina platforms. The gene transcription data generated were used for the identification of the genes those are differentially expressed either are up-regulated or are down regulated with the treatment of salt Trinity package and the transcript abundance were estimated using kallistosoftware.Differentially expressed unigenes/isoforms were calculated using p-value cutoff 0.05. Significantly differentially expressed genes/isoforms were identified with p-value cutoff 0.05 and log2FoldChange (>=2 and <=-2). From the analysis it was found that 239 numbers of unigenes were upregulated and 89 no. downregulated with the treatment of salt. With the analysis of the isoforms of the genes, it was fund the 452 numbers of the genes were upregulated whereas 341 genes were downregulated.

Genetic network with response to drought

The plants were allowed to grow on pots for different periods and found that the plants could survive for 30 days without any application of irrigation.The biomass accumulation found to be lower than that of the control. From the RNA sequencing analysis, it was found that a greater number of genes were upregulated (864) and downregulated (298) as compared to the salt stress. Similar were the cases with comparisons of expression with the gene isoforms. With the 30days of drought treatment 1548 numbers of genes were upregulated whereas 744 gene isoforms were downregulated.



The expression of all the genes present in the control as well as in the salt and drought treated samples were measured and used for correlation analysis. The correlation plot is mentioned in the Fig.1. From the analysis, it was found that there is no correlation of gene expression between the three sets of experiments and the values are shown to be less than zero. From the cluster analysis it is shown that the gene expression pattern in control is closer to salt as compared to drought. This might be due to the higher levels of differential expression of genes in the drought treated samples.

Horticulture and Floriculture

Omics-approach to regulate ripening and enhance fruit shelf-life in banana: an important fruit crop for food security

(RKVY, GOI Funded)

Principal Investigator : Dr. Giridara-Kumar Surabhi, Senior Scientist

Research Fellow: Dinesh Pradhan, JRF

The fruit harvested from bananas and plantains are important components of food security in the tropical world and provide income to the farming community through local and international trade. In developing countries, post-harvest losses of fruits and vegetables account for almost 50% of the produce. Therefore, ripening associated softening is the obvious target to extend fruit shelf-life and to control the post-harvest losses. To date, no in-depth work has been focused on identifying key ripening proteins/genes and utilizing them for controlling ripening process to promote fruit shelf-life in banana. This project aimed to identify novel and key candidate proteins/genes responsible for ripening during different developmental and ripening stages of banana through omics-based approaches.

The climacteric fruit such as banana, ripening is preceded by a characteristic burst in ethylene levels, which triggers metabolic/structural changes associated with the maturation process, including loss of mesocarp firmness. The pulp softening depends on the alteration of the cell wall structural properties, with massive depolymerisation/solubilisation of cell wall components. Although some processes appear to be common to most species, many others seem to be unique, involving the activation of different sets of cell wall-modifying enzymes. Cell wall modification during fruit ripening is a highly organized process that involves complex interplay among various cell wall hydrolases.

In the present study, proteins were isolated adopting phenol extraction method from banana fruit tissues (pulp and peel) and quality checked by SDS-PAGE. The protein samples were subjected to orbitrap fusion mass spectrometry. The identified proteins were categorized based on their functional role such as hormone regulation, sugar metabolism, RNA processing, cell wall modification etc. The pectinesterase (PE) is a ubiquitous cell-wall-associated enzyme that presents several isoforms that regulates plant cell wall modification and subsequent breakdown. Pectinesterase functions primarily by altering the localized pH of the cell wall resulting in alterations in cell wall integrity. This enzyme is known to extensively decrease the rigidity of cell wall structure and solubilisation of pectins during fruit softening. It catalyses the de-esterification of pectin into pectate and methanol. Pectin is one of the main components of the plant cell wall. Interestingly, in our study one number of pectinesterase (accession no-M0RPM3) in pre-climacteric and four numbers of pectinesterase (accession no-M0SC42, M0RPM3, M0TGN6 and M0SC43) in climacteric stage were identified. Previously, pectinesterase enzymes were identified in peach fruit tissues of different stages such as pulp tissues at two-ripening stages, mesocarp tissues of ripe and unripe stages and pulp tissues of four ripening stages. This enzyme was also identified in pulp tissues of jujupe fruit at two-matured stages and pulp tissues of papaya at ripe and unripe stages. Based on the identification of pectinesterase enzyme in previous studies from different fruits, it clearly indicates that this enzyme plays a key role in banana fruit ripening process.

Pectate lyase is involved in the maceration and soft rotting of fruit tissue. It is responsible for the eliminative cleavage of pectate, yielding oligosaccharides with 4-deoxy-α-D-mann-4-enuronosyl groups at their non-reducing ends (Wang et al., 2019). This protein is maximally expressed late in pollen development. It has been suggested that the pollen expression of pectate lyase genes might relate to a requirement for pectin degradation during pollen tube growth (Wing et al., 1990). Interestingly, in our study two pectate lyase enzymes (accession no- M0U687 and M0TAX1) were identified in climacteric stage.

β-hexoaminidase is a member of glycosyl hydrolase families which are known to break the glycosidic bonds between carbohydrates and non-carbohydrates molecules and it cleaves the terminal N-acetyl-D-hexoamine residues and generates the paucimannosidic N-glycans present in most plant glycoproteins (Gutternig et al., 2007). It was identified in pulp and pericarp tissues of capsicum at four developmental stages and three ripening stages (Jagadeesh et al., 2004; Ghosh et al., 2011). This enzyme was also identified in pericarp tissues of tomato at four ripening stages (Meli et al., 2010). In our study, we have identified one number of β-hexoaminidase protein (accession no-M0T2F6) which was present in climacteric stage. The present study reveals that the β-hexoaminidase involvement during banana fruit ripening.

Xyloglucan is the most abundant hemicellulose in the primary cell walls of non-graminaceous plants, where it coats and cross-links adjacent cellulose microfibrils through non-covalent associations (Saladie et al., 2006). This enzyme is associated with ripening and softening of different fruits, where they can determines the maintenance of cell wall integrity or weakening. It acts as a storage reservoir of seeds in some plant species such as nasturtium, where it accumulates as large deposits on the inside of the cotyledon on cell wall during seed development and is subsequently hydrolysed during germination process (Buckeridge et al., 2000).

The α-mannosidase enzyme are known to hydrolyse the glycosidic bonds between carbohydrates, as well as between carbohydrate and non-carbohydrate. It is a main member of glycosyl hydrolase (GH) family 38 which cleaves the terminal α-mannosidic linkages from both the high mannose type and plant complex type N-glycans which are present in glycoproteins (Hossain et al., 2009; Ghosh et al., 2011). Three numbers of α-mannosidase were identified from pericarp tissues of late ripening stages in blueberries (Chea et al., 2019). Previously, α-mannosidase enzyme was identified from pericarp tissues of tomato (Meli et al., 2010) and capsicum (Ghosh et al., 2011) during ripening stages. Interestingly, in our study one number of α-mannosidase (accession no-M0TE13) and four numbers of α-mannosidase (accession no-M0TWG0, M0TLF4, M0U935, M0TWG0) were identified in pre-climacteric and climacteric stage, respectively. More abundant number of this enzyme present in climacteric stages suggested that α-mannosidase plays a key role in regulating the ripening process of banana fruit.

β-galactosidase (β-gal) plays an important role in the fruit ripening process (Lawson et al., 2018). It participates in cell wall metabolism via its ability to catalyse galactosyl metabolism from the large and complex side chains of cell wall (Ban et al., 2018; Lawson et al., 2018). β-galactan is mainly present on side chains of the polysaccharide rhamnogalacturonan-I (Schols et al., 1995). These chains are combined with glucan chains of cellulose (Zykwinska et al., 2007), forming a dense network that contributes to the extensibility, strength, and porosity of the cell wall (Ulvskov et al., 2005; Larsen et al., 2011). β-gal, a glycosidase, contains a consensus sequence of the putative active site, G–G– P–[LIVM]–x–Q–x–E–N–E–[FY], of glycosyl hydrolase family 35 (GH35) proteins (Henrissat, 1998). The role of β-gal is to remove terminal, non-reducing β-D-galactosyl residues of hemicellulose and pectin from the cell wall (Smith and Gross, 2000). The β-gal enzyme plays a key role to accelerate fruit softening by increasing

the porosity of the cell wall and enhancing the access of other cell wall-degrading enzymes (Pena and Carpita, 2004; Ng et al., 2013, 2015). In our study, two β-galactosidase enzymes (Accession no-M0SQP6, M0RVL3) and four β-galactosidase enzymes (Accession no-M0SQP6, M0SX47, M0TFY5, M0RVL3) were identified in pre-climacteric and climacteric stage, respectively.

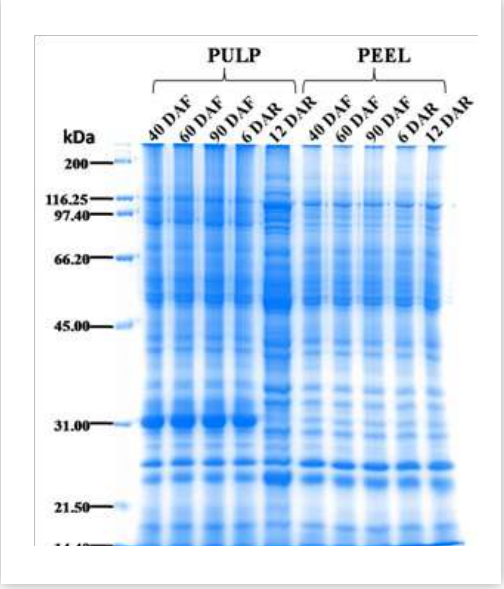


Fig.1: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile showing separation of proteins from banana pulp and peel tissues of different developmental and ripening stages by using phenol extraction method. Known amount of proteins (100µg) was loaded in each lane and proteins were resolved on 12% SDS-PAGE followed by colloidal coomassie blue staining.

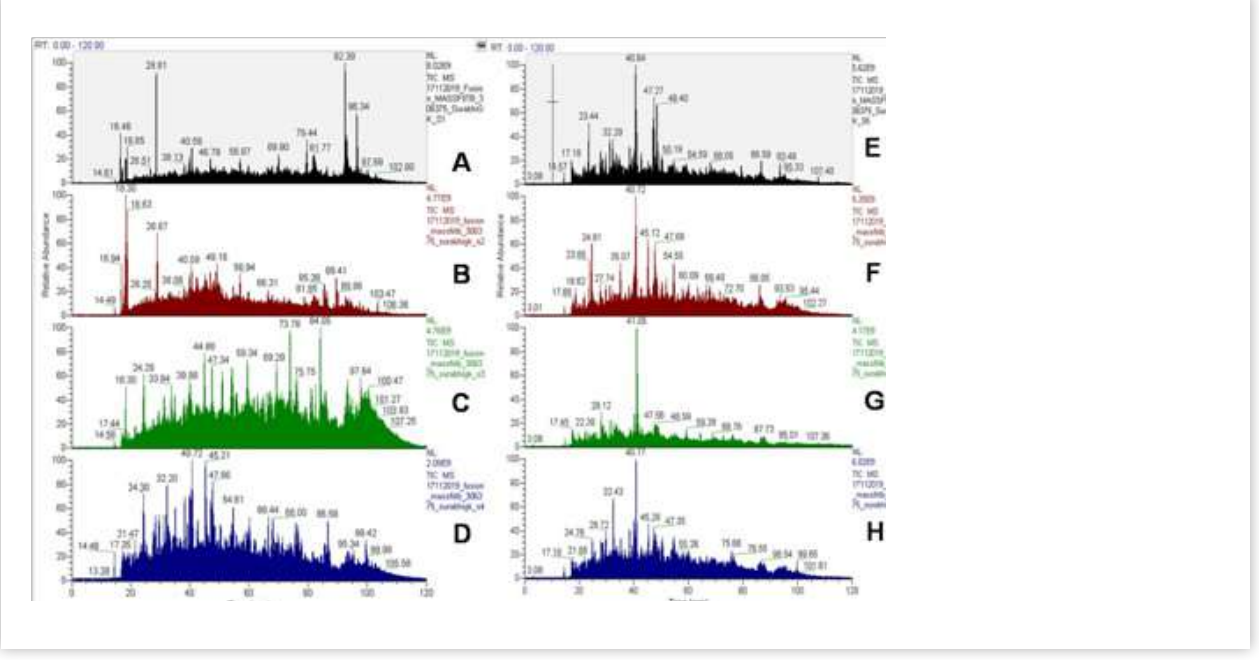


Fig.2: The chromatogram maps of the protein samples which were obtained after running 120 min. The chromatograms obtained from (A).40-DAF-pulp, (B).60-DAF pulp,(C).6-DAR pulp, (D).40-DAF peel, (E). 60-DAF peel, (F).90-DAF peel, (G). 6-DAR peel, (H).12-DAR peel protein, respectively. DAF: day after flowering; DAR: day after ripening.

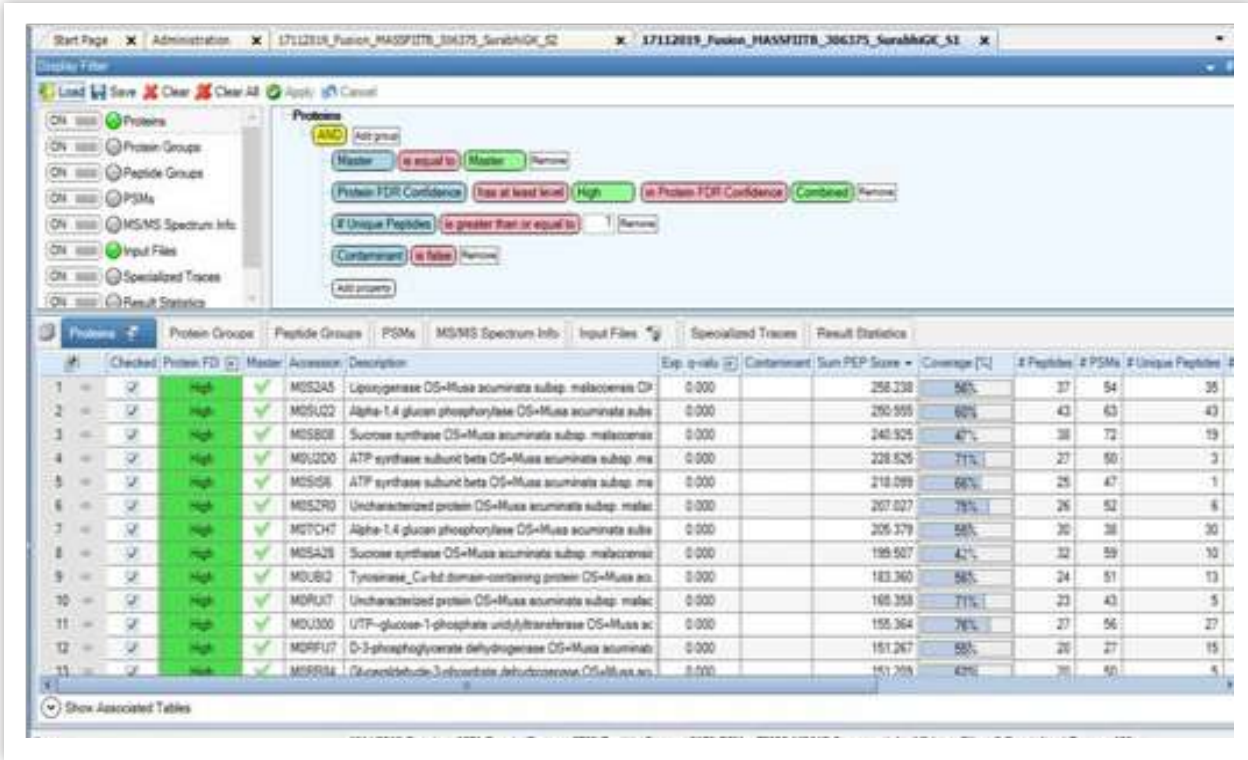


Fig.3: Image depicting the results of banana fruit proteins identified using high resolution orbitrap fusion mass spectrometry. The protein search was performed against Musa acuminata database from Uniprot using Proteome Discoverer 2.2.version.

Table-1: Table depicting cell wall modifying proteins which were identified through orbitrap fusion mass spectrometry from banana fruit tissue (pulp) at climacteric stage.

Accession No./Protein I.D.	Origin/ Taxonomy	Protein description	Sum peptide score	Coverage peptide (%)	A.A.	Protein molecular weight (kDa)	Unique peptide	Peptide sequence
M0TGN6	*Musa acuminata* subsp.malaccensis	Pectinesterase	8.193	4	507	54.4	2	DDPNEPTGIVIHI
M0RPM3	*Musa acuminata* subsp. malaccensis	Pectinesterase	8.182	4	566	62.1	2	AGAYLENVEVG
M0T2F6	*Musa acuminata* subsp. malaccensis	Beta-hexosaminidase	8.503	4	550	62.1	2	YTVEDAYEVVD AK
M0S3Q7	*Musa acuminata* subsp. malaccensis	Beta-hexosaminidase	8.544	16	115	13.2	1	SNQYENTSLVQI GVNTK
M0TTV0	*Musa acuminata* subsp. malaccensis	Xyloglucan endotransglucosylase/ hydrolase	11.621	14	286	32.6	3	SGQPYTVQTNV AHGK
M0RMW1	*Musa acuminata* subsp. malaccensis	Xyloglucan endotransglucosylase/ hydrolase	11.614	10	326	34.6	3	LDPSSGCGFASN
M0TLF4	*Musa acuminata* subsp. malaccensis	Alpha-mannosidase	32.441	5	1077	120.9	5	INITEMNLSANQ R
M0T6Z4	*Musa acuminata* subsp. malaccensis	Alpha-mannosidase	5.388	3	731	82.3	2	QDLAEANANVR
M0SQP6	*Musa acuminata* subsp.	Beta-galactosidase	5.45	2	87	95.4	1	GYFDAPEGNDP AIDFTGMGK

Tissue-specific proteome expression and mass spectrometry identification of banana during fully developed and ripening stages to identify differentially expressed proteins

(State Plan Funded)

Principal Investigator : Dr. Giridara-Kumar Surabhi, Senior Scientist

Research Fellow: Subhankar Mohanty, SRF

The comparative proteomic approaches can enable the identification of protein species with changes in abundance levels during the process of fruit ripening. Because, the proteins are the effectors of gene expression and the gene transcript level is not always well correlated with the protein level (i.e. due to post translational modifications), and larger-scale efforts that involve protein profiling have received more attention. In this study, proteomes of banana fruit at 90-DAF (peel and pulp) and 12-DAR (peel and pulp) were subjected to mass spec identification using orbitrap fusion mass spectrometry. The protein-protein interaction studies were performed for annotated proteins from four different samples, considered in this study. Based on the results, several proteins such as regulatory, signaling, sugar, hormonal regulation and cell wall metabolism related proteins were tightly interacting in a network mode during the ripening process in banana (Fig.1). The knowledge obtained through this study would be highly useful for selecting the right candidates and utilize them in controlling ripening and to enhance fruit shelf-life in banana.

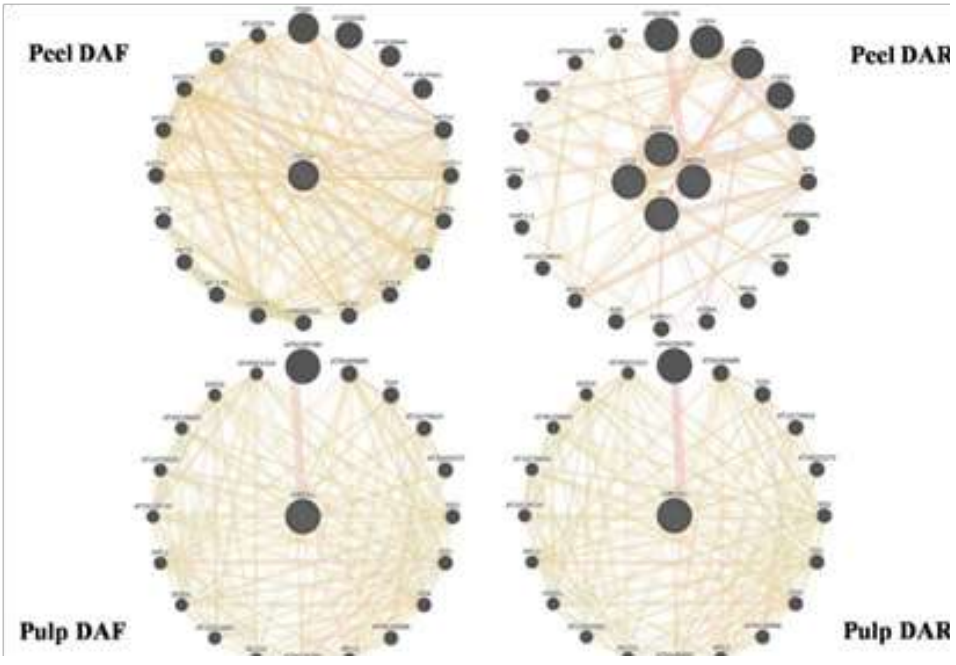


Fig.1: The depicted image showing annotated list of all proteins interacting from co-expression based on domains in a network mode for four different tissues studied in the experiment.

Mass Propagation of Local Musa Varieties of Odisha, Commercialization Using Tissue Culture Techniques

(RKVY, Govt. of India Funded)

Principal Investigator: Dr. Bandita Deo, Senior Scientist

Research Fellow: Nimisha Mohapatra, JRF

Effect of cobalt toxicity on Rooting culture of Gaja Bantala

Cobalt is an essential element for both plant and animal but at higher concentration cobalt is reported to be toxic. At lower concentration, cobalt has been reported to have a positive effect on plants. In our study root length, the other morphological parameters such as shoot length,number of leaves also decreased with the increase in cobalt concentration (Fig.1 and Fig 2).So it is concluded that cobalt at high levels may inhibit the root and shoot growth directly by inhibition of cell division or cell elongation or combination of both.

Fig 1 :Micropropagated Gaja Bantala shoots inoculated in rooting medium containing IAA along with different concentration of CoCl2

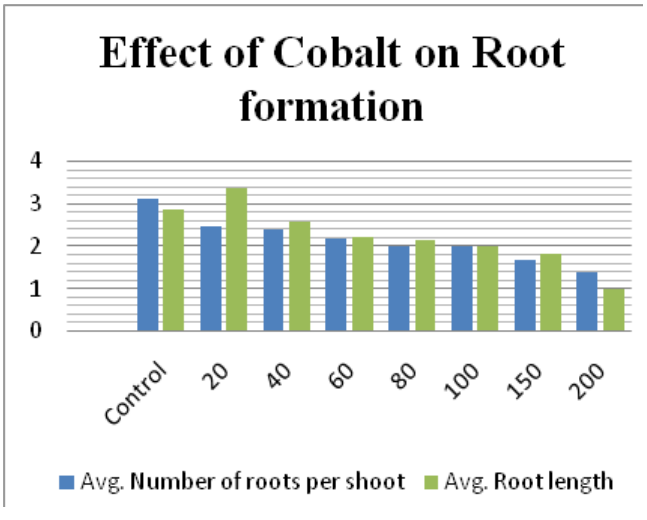


Fig 2 :Graphical representation of Cobalt on Shoot and Root formation of variety Gaja Bantal

Production of quality planting material of ripe banana. Analysis of genetic fidelity through molecular marker.

(State Plan Funded)

Principal Investigator: Dr Bandita Deo, Senior Scientist

Research Fellow: Bikram Keshari,Field Assistant: TinkuTinu Prasad Routray

Study on the effect of IBA and Activated charcoal in Yangambi and Champa Varieties during rooting culture Basal MS Media enriched with IBA and Activated Charcoal (AC)having different concentration shown the effective result in the term of root initiation period(Fig.1,2).But in term of number of roots per plant, length of the root of explant as compared to other combination was slightly inreased (Fig.3).

Fig.1 MS medium supplemented with IBA and AC at 21 days culture of Yangambi

Fig.2 MS medium supplemented with IBA and AC at 21 days culture of Champa

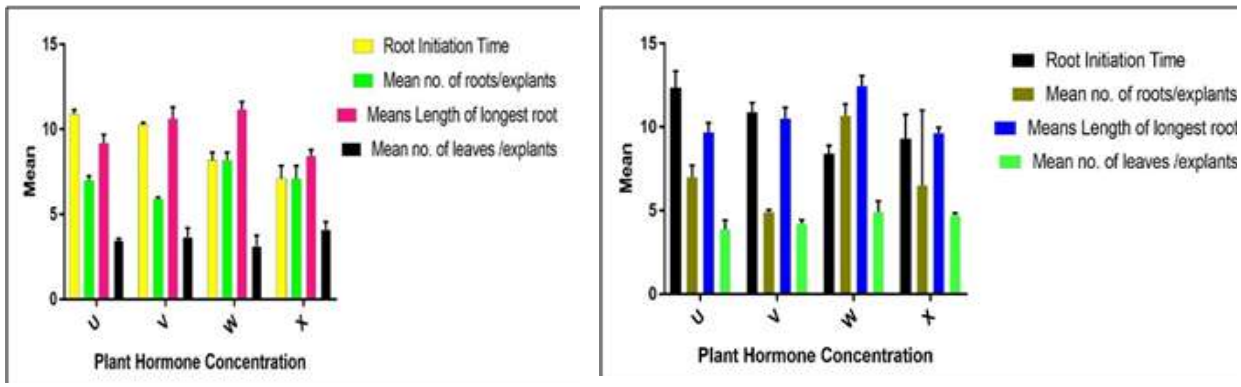


Fig.3. Tissue cultured rooted plantlets of Yangambi and Champa transferred to primary hardening chamber

After the rooting culture explants undergo for acclimatization. Once the plantlets are ready for shifting outside the laboratory, they are carefully acclimatized to adapt to the greenhouse and later to least protected field conditions. Before transfer to primary hardening, plantlets were gently washed in water to remove agar medium from roots to avoid contamination. Tissue cultured plantlets Yangambi (Fig.4) and Champa (Fig.5) were transferred to primary hardening.

Fig.4. Yangambi Plantlets in primary Hardening

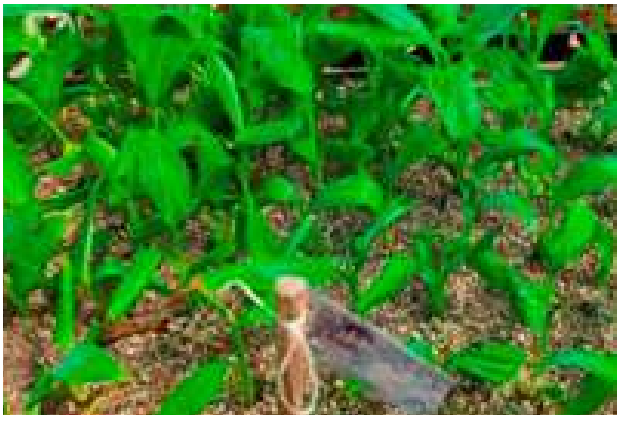


Fig.5. Champa Plantlets in primary hardening

Virus indexing Test:

The banana leaves of tissue culture Yangambi and Champa plantswere collected from the secondary hardening chamber and sentfor virus indexing testto National Research Centre For Banana (NRCB), Tiruchirapalli, Tamil Nadu through Polymerase Chain Reaction (PCR) for the detection of Banana Bunch Top Virus (BBTV), Cucumber Mosaic Virus (CMV),Banana Streak Mosaic Virus (BSMV) and Banana Bract Mosaic Virus (BBrMV).

Detail Report –BBTV & BSMYV by PCR, CMV and BBrMV testing by RT-PCR

S. No	Plant Species	Name of the Variety	Sample Code	Test For			
				BBTV	BSMYV	CMV	BBrMV
1	Banana	Yangambi	YAN1	Negative	Negative	Negative	Negative
2	Banana	Yangambi	YAN2	Negative	Negative	Negative	Negative
3	Banana	Yangambi	YAN3	Negative	Negative	Negative	Negative
4	Banana	Yangambi	YAN4	Negative	Negative	Negative	Negative
5	Banana	Yangambi	YAN5	Negative	Negative	Negative	Negative
6	Banana	Champa	C1	Negative	Negative	Negative	Negative
7	Banana	Champa	C2	Negative	Negative	Negative	Negative
8	Banana	Champa	C3	Negative	Negative	Negative	Negative
9	Banana	Champa	C4	Negative	Negative	Negative	Negative
10	Banana	Champa	C5	Negative	Negative	Negative	Negative

Signature of the scientist

Dr. R. SELVARAJAN, Ph.D.,
Principal Scientist (Plant Pathology)
National Research Centre for Banana (ICAR),
Thogamalai Road, Thayanur (Po),

From the result it was indicated that all the tissue culture plants of variety Yangambi and Champa produced from the banana laboratory of RPRC are free from all four major virus [Banana Bunch Top Virus (BBTV) ,Cucumber Mosaic Virus (CMV),Banana Streak Mosaic Virus (BSMV) and Banana Bract Mosaic Virus (BBrMV)].

Standardization of protocol for micro-propagation of potato varieties

(State Plan Funded)

Principal Investigator: Dr. C. Kalidass, Scientist

Research Fellow: Sanjukta Das, JRF

Potato is one of the most important food crops after wheat, maize and rice; historically contributing to food and nutrition security in the world. The major constraint in potato cultivation in India is insufficient availability of quality seed. Use of healthy seed in any vegetative propagated crops is very important because of the prevalence of a number of viral, fungal and bacterial diseases which considerably reduce the yield. The seed alone accounts for 40-50% of total cost of cultivation. Therefore, the availability of healthy uniform planting material comprises the most important aspect for potato production and recent biotechnological approaches have been successfully integrated with diseases free seed production scheme. Tissue culture technique is most extensive method to produce disease free quality plantlets and microtuber in potato. The application of tissue culture techniques for rapid propagation of potato became more widely used in many countries.

Traditionally the potato is propagated by planting healthy, high-quality seed tubers. Potato seed is, therefore, as assemblage of small tubers used for planting the crop (Simmonds, 1997). However repeated clonal multiplication of tubers for seed production is characterized by low multiplication rates, physiological decline, progressive accumulation of pathogens, especially degenerative viral diseases and high cost of storage and transport. As a result, the availability of quality potato seed is a major constraint for potato production. Tissue culture is used to increase propagation rates and to modify the germplasm of plants. Tissue culture has been applied to improve production of potato by germplasm conservation, pathogen free potato plants and micropropagation (Khalafalla, 2001). In vitro propagation of potato using single nodal cutting is the best method of rapid multiplication rates with maximum genetic stability (Chandra and Naik, 1993). Rapid production of pathogen free potato plants

through meristem culture, micropropagation and elimination of virus free plants are likely using meristem culture (Jha and Ghosh, 2005). Due to the high susceptibility of potato to diseases, especially to viruses, several studies have started micropropagation by using meristems and shoot tips as explants in order to produce virus-free seed potato. In addition to low quality of the crop output, productivity changes from year to year and hence the deficit in the supply of the crop leads to its high price. The choice of suitable variety (Kufri Pukhraj) (Fig.1) is of paramount importance for successful commercial cultivation of potato. This current proposal aims at developing a suitable protocol for large scale commercial production of virus-free potato tuber seeds applicable for Odisha state.

During our experimental study, surface sterilization pre-treatments of explants with combination of Mercuric chloride with the fungicide Bavistin, was found effective. The effects of basal nutrients were found to be significant for the plant growth and it was decided that full strength medium was good for sprout length and maximum number of nodes and leaves. Effect of different media comprising cytokinin, auxin and vitamins were used to evaluate the effect of growth regulators on in vitro propagation of potato variety Kufri Pukhraj. We have been screening the PGR, Media, sterility of explants, duration of sterilant, half & full strength of media and different stages of micropropagation of potato plant var. Kufri Pukraj. Nodal explants of potato variety Kufri Pukhraj was cultured on MS medium supplemented with different 6-BAP combinations. The nodes from germinated germplasms were used to develop in vitro shoots cultures. Elongated shoots were further multiplied and kept in maintained culture room. For micro-propagation of this variety, MS + 6-BAP (1mg/l, 1.5mg/l, 2mg/l, 2.5mg/l) was used in initiation and elongation stage (Fig.2). The initiation media was manipulated through sucrose concentration, and cytokinin type to improve the expressive response of explant and its survival capacity after inoculation. The effects of such media on shoot, root and micro-tubers induction were observed (Fig.3). In this experiment, optimum concentration of sterilant with suitable time duration were also recorded. Highest frequency of shoots was achieved IAA (1.0mg/l) and BAP (0.6 mg/l). The *in vitro* propagation protocol should be developed and can be successfully adopted for mass propagation of this economically important crop. Microtuber production and collection and storage should be continued.

Fig.1. Kufri Pukhraj variety of potato; a). Foliage: Dark grey-green. Leaves closed with large sized follicles, rachis green. Leaflets ovate to lanceolate, smooth glossy surface with entire margin; b). Flowers: White, moderate flowering. Anthers orange-yellow, well developed, high pollen stain ability. Stigma round; c). Sprouts: Blue-purple; d). Tubers: White, large, oval, slightly tapered, smooth skin, fleet eyes, yellow flesh.

Fig.2. Effect of shoot elongation of various PGR for the *in vitro* shoot growth

Fig.3. Sucrose effect on microtuber formation during different development stage

RESEARCH PROJECTS

EXTERNAL FUNDED PROJECTS				
Sl.No	**Title**	**PI**	**Funding**	**Period**
1	Harnessing the potential of endopytes against root knot nematode Meloidogyne icognita in banana	Dr. N.Gupta Principal Scientist	DBT,MS&T, GOI	2018-2021
2	Standardization of nursery technology by application of PGPF (Plant growth promoting fungi) under different soil conditions and its impact on quality of *Piper longum* : A RET medicinal plant of Odisha	Dr. N.Gupta Principal Scientist	NMPB, Dept of AYUSH, GOI	2016-2020
3	Optimization of submerged culture requirements for the production of mycelia growth and exopolysaccharide by some selected Fungi	Dr. N.Gupta Principal Scientist	S&T Dept., GoO	2018-2020
4	Establishment of mass propagation and breeding facility for orchids	Dr. N. R. Nayak Senior Scientist	RKVY, GOI	2017-2020
5	Establishment of tissue culture based mass propagation facility of banana and plantains	Dr. N. R. Nayak Senior Scientist	RKVY, GOI	
6	Omics'- approach to regulate ripening and enhance fruit shelf life in banana: an important fruit crop for food security.	Dr. G.K.Surabhi	RKVY, GOI	2017-2020
7	Mass Propagation of Local *Musa* Varieties of Odisha, Commercialization using Tissue Culture Techniques.	Dr. B. Deo	RKVY, GOI	2017-2020
8	Evaluation of unexplored *Ardisia solanacea* and *Aegiceras corniculatum* plants of Myrsinaceae family as embelin and other related compounds producing substitutes for overexploited RET medicinal species *Embelia ribes & E. tsjeriam-cottam*.	Dr. U.C. Basak	NMPB, Dept of	2016-2020
9	Conservation of salt-sensitive back mangroves *Heritiera fomes* and *Heritiera littoralis* through re- introduction in protected area: application of vegetative prpogation technique	Dr. U.C. Basak	DBT, MS&T GOI	2018-2021

STATE PLAN FUNDED PROJECTS				
Sl. No.	Title	PI	Funding	Period
1	Study of quantitative ecology, phenology & phytosociology of dominant forest trees of Odisha: Collection of additional field data for compilation of the pictorial guide.	Dr.P.C. Panda	F & E Dept., GoO	2019-2020
2	Phytochemical evaluation, nutritional analysis, propagation & reintroduction of some selected threatened plants of Odisha	Dr.P.C. Panda	F & E Dept., GoO	2019-2020
3	Quantitative assessment of plant biodiversity in some selected districts of Odisha	Dr.P.C. Panda & Dr.C. Kalidass	F & E Dept., GoO	2019-2020
4	Extraction, purification and characterization of bioactive secondary metabolites and enzymes from endophytic fungi	Dr. N. Gupta	F & E Dept., GoO	2019-2020
5	Evaluation of fungal bioinoculants on growth and development of some forest plantation tree species under field conditions.	Dr. N. Gupta	F & E Dept., GoO	2019-2020
6	Production of quality planting materials of ripe banana: Analysis of genetic fidelity through molecular marker	Dr. B. Deo	F & E Dept., GoO	2019-2020
7	Restroration of wild Orchid population in Chandaka & RPRC through reintroduction of in vitro raised seedlings	Dr. N. R. Nayak	F & E Dept., GoO	2019-2020
8	Elucidation of genetic network with response to salt and drought stress in *Saccharum bengalense* Retz.	Dr. N.R. Nayak	F & E Dept., GoO	2019-2020
9	Phytotherapeutic investigation of *Piper trioicum* as neurological disorder: An insight into therapeutic avenues towards Alzheimer's disease.	Dr. A. K. Sahoo	F & E Dept., GoO	2019-2020

10	*Hydrolea zeylanica* alters the expression of glucose transporter protein in type-2 diabetic rats.	Dr. A. K. Sahoo	F & E Dept., GoO	2019-2020
11	Evaluation of some underutilized and underexploited wild edible mangrove fruits of Odisha coast for their nutritional and antioxidant properties	Dr. U. C. Basak	F & E Dept., GoO	2019-2020
12	Qualitative and quantitative analysis of essential amino acids and pectin in lesser known wild edible terrestrial fruits of Odisha.	Dr.U. C. Basak	F & E Dept., GoO	2019-2020
13	Standardization of micro-propagation methods for *Anogeissus latifolia* and *Santalum album, Desmodium oogeinense* endangered forest trees.	Dr. G. K. Surabhi	F & E Dept., GoO	2019-2020
14	Tissue-specific proteome expression and mass spectrometry identification of banana during fully developed and ripening stages to identify differentially expressed proteins.	Dr. G. K. Surabhi	F & E Dept., GoO	2019-2020
15	Evaluation of *Bixa oreallana* and *Nyctanthes arbortristis* for antifungal activity using *Aspergillus flavus* and *Aspergillus niger* as target experimental models.	Dr. S. Bhatnagar	F & E Dept., GoO	2019-2020
16	Effect of temperature on withanolide contents of the plants grown by seeds stored at different temperatures.	Dr. S. Bhatnagar	F & E Dept., GoO	2019-2020
17	Standardization of protocol for micro- propagation of potato varieties.	Dr. C. Kalidass	F & E Dept., GoO	2019-2020
18	Systematic studies of the family Solanaceae in Eastern Ghats in India	Dr. C. Kalidass	F & E Dept., GoO	2019-2020

PUBLICATIONS

Research Paper (2019-20)

Behera, S., Kar, S. K., Rout, K. K., Barik, D. P., Panda, P. C. & S. K. Naik (2019). Assessment of genetic and biochemical fidelity of field-established *Hedychium coronarium* J. Koenig regenerated from axenic cotyledonary node on meta-topolin supplemented medium. Industrial Crops and Products 134: 206-215.

Das, B., Ray, A. Sahoo, A., Jena, S., Singh, S., Kar, B., Patnaik, J., Panda, P. C., Mohanty, S. and S. Nayak (2020). Quantitative and chemical fingerprint analysis for quality control of *Zingiber zerumbet* based on HPTLC combined with chemometric methods. Plant Biosystems, 1-13.

Das, P. K., Kamila, P. K. & P. C. Panda (2020). Nothapodytes nimmoniana (J. Graham) Mabb. (Icacinaceae)- An addition to the forest trees of Odisha, India. NeBio 11 (2): 63-66.

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Kamila, P. K., Das, P. K. Mallia, M. Kalidass, C. & P. C. Panda (2020). *Gynochthodes cochinchinensis* (DC.) Razafim. & B. Bremer (Morindeae: Rubioideae: Rubiaceae): an addition to the woody climbers of India. Journal of Threatened Taxa 12(3):15395–15399.

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Kanhar, S., Roy P.P., A.K. Sahoo (2020). Computational and experimental validation of free radical scavenging properties of high-performance thin-layer chromatography quantified Phenyl myristate in *Homalium nepalense*. Journal of Separation Science, 1–10. https://doi.org/10.1002/jssc.201901178.

Maharana, P.K. and U. C. Basak (2020). Studies of Non-Enzymatic and Growth Changes in Two Vegetatively Propagated Mangrove Species i.e. *Excoecaria agallocha* and *Cerbera manghas* at NaCl Stress During Hardening. Intl. J. Sci. & Res.9(3):1343-48.

Maity, P., A.K. Nandi , M Pattanayak, D. K. Anna, I K. Sen, I Chakraborty, S K Bhanja , A. K. Sahoo , Nibha Gupta and S. S Islam (2020). Structural characterization of heteroglycan from an edible mushroom *Termitomyces heimii*. International Journal of Biological Macromolecules 151 :305-311.

Mallick, S. N., Sahoo, T., Naik, S. K. & P. C. Panda (2020). Ethnobotanical study of wild edible food plants used by the tribals and rural populations of Odisha, India for food and livelihood security. Plant Archives 20 (1): 661-669.

Mohapatra, N. and B. Deo (2019). Substitution of BAP with meta-Topolin (m-T) in multiplication culture of *Musa* Species. Plant Science Research 41(172):8-11.

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Pradhan,B. and B. Deo (2019). Detection of phytochemicals and in vitro propagation of Banana (*Musa* variety Gaja Bantal).journal of Medicinal Plant Studies.7(1): 46-49.

Ray, A., Jena, S., Dash, B., Sahoo, A., Kar, B., Patnaik, J. Panda, P. C., Nayak, S. &Sahoo, T., Acharya, L. K. & P. C. Panda (2020). Plant diversity along disturbance gradients in tropical moist deciduous forests of Eastern Ghats of India. Plant Archives 20(1):207-217.

Rout, P. and U. C. Basak (2020). Effects of salinity and exogenous proline application on protein profiling and antioxidant enzyme activities during ex vitro shoot multiplication in hypocotyls of two *Bruguiera* species. Plant Cell Biotechnology and Molecular Biology. 21(3-4), 37-48.

Sahoo, T., Acharya, L. K. & P. C. Panda (2020). Structure and composition of tree species in tropical moist deciduous forests of Eastern Ghats of Odisha, India in response to human induced disturbances. Environmental Sustainability. https://doi.org/10.1007/ s42398-020-00095-0.

Swain, S.K., Dash, U.C., Kanhar, S., A.K. Sahoo (2020). Ameliorative effects of *Hydrolea zeylanica* in streptozotocin-induced oxidative stress and metabolic changes in diabetic rats, Journal of Ethnopharmacology, 247, 1-13.

Books/ Booklets/Book Chapters/Research Reports

Book Chapter

Gupta, N. (2020). *Trichoderma* as biostimulant: Factors responsible for plant growh promotion. In *Trichoder*ma : Agricultural applications and beyond .Eds C. Manoharachary, H.B. Singh and Ajit Varma. Book series of Soil biology, Springer. Cham http:/doi.org/10.1007/978-3-030-54785-5_13

Surabhi G.K., Rout A. (2020) Glycinebetaine and crop abiotic stress tolerance: an update, In: Roychoudhury A., Tripathi D.K. (eds) Protective chemical agents in the amelioration of plant abiotic stress, John-Wiley, pp.24-44; ISBN:978-1-119- 551638.

Surabhi G.K., Seth J.K. (2020) Exploring in-built defense mechanism in plants under heat stress, In: Wani S., Kumar V. (eds) Heat stress tolerance in plants: physiological, molecular and genetic perspectives, John-Wiley, pp.239-282; ISBN:978-1-119-43236-4.

Surabhi G.K., Badajena B. (2020) Recent advances in plant heat stress transcription factors, In: Wani S. (eds) Transcription factors for abiotic stress tolerance in plants, Elsevier; pp.153-200; ISBN:978-0-128-19334-1.

Research Report

Basak, U.C and P.C. Panda (2020). Research Report 2018-19. Pub. by Chief Executive, Regional Plant Resource Centre, Bhubaneswar.100pp.

TRAINING & EDUCATION

Short term Training to the students of M.Sc. / B.Tech. / M/Tech. and other courses are provided every year from January to June for a duration of 6 months. Training is imparted on the "Advance Plant Biotechnology". The students have to submit their CV along with the forwarding letter from their Institutes head or the Project head. The applications are to be received by end of November every year. Students enroll themselves by end of December and the training starts from January (6 months). Students are allotted to various Scientists and have to work under them for completing their Thesis. At the end of the course they are issued a course completion certificate.

Sl.No.	Name of the candidate	Title of the dissertation	Guide	Year
1	Liza Sahoo SOA, University, BBSR	Development of Molecular Markers for *Dendrobium* Fairy White Orchid	Dr. N.R. Nayak, Senior Scientist	2020
2	Gopasila Mallick, AMITY, Mumbai	Development of Molecular Markers for *Dendrobium* Sonia Orchid	Dr. N.R. Nayak, Sr. Scientist	2020
3	D. P. Ipsita Swain, SOA University.,BBSR	Control of lethal browning of tissue culture plantelets of banana CV CHAMPA with antioxidants.	Dr. B Deo, Sr. Scientist	2020
4.	T. Shivani N Mohanty, SOA	Quantitative analysis of essential amino acids in some wild edible fruits of Odisha.	Dr. U.C Basak Sr. Scienist	2020
5	Hena Khodiyar, C P S Berhampur	Phytochemical and antioxidant activity of medicinal plant- *Melia azedarach*.	Dr. S. Bhatnagar, Sr. Scientist	2020
6	Ankita Pradhan, SOA University, BBSR	An effective protocol for in vitro propagation of cultivated banana *Musa accuminata* colla: effects of plant growth regulators.	Dr. C. Kalidass	2020

Ph.D. Awarded/Pursuing

Sl. No.	Name of the Supervisor/ candidate	Title of the Doctoral program	University registered/Year	Status
1. Dr. Pratap Chandra Panda, Pr. Scientist				
	Subrat Kumar Kar	Assessment of genetic diversity, nutritional value and productivity of seagrasses of Chilika lagoon, Odisha, India	Ravenshaw University	Pursuing
	Samarendra Narayan Mallick	Study of the diversity, distribution, collection and use of wild edible food plants by the tribals of Odisha	Ravenshaw University	Pursuing
	Tirthabrata Sahoo	Quantitative assessment of plant biodiversity in tropical dry deciduous forests of Eastern Ghats: A case study of Nayagarh District, Odisha	Siksha O Anusandhan University	Pursuing

2	**Dr. Nibha Gupta, Pr. Scientist**			
	Hruda Ranjan Sahoo	Bioprospecting of phosphate solubilising fungi and their application for improving the growth of some RET medicinal palnts. (Reg. No.: 03- Life Science 2012-13)	Dept. of Life Science, Utkal University, Bhubaneswar	Pursuing
	Smita Behera	Optimization of cultural and nutritional conditions for enhanced production of exopolysaccharide by some fungi	Dept. of Life Science, Utkal University, Bhubaneswar	Pursuing
3	**Dr. Nihar Ranjan Nayak, Sr. Scientist**			
	Sulagna Subhasmita Jena	Production of Quality Planting Materials and elucidation of diversification of flowering time (FT) gene in Orchids.(R.No.03/ Biotech/2015-16)	Dept. of Biotechnology Utkal University, Bhubaneswar	Pursuing
	Johnnita Tirkey	Development of molecular tools for *Dendrobium* species and their hybrids (Orchidaceae) for application in horticulture industry. (Reg. No 06-Biotechnology, 2017-18)	Dept. of Botany Utkal University, Bhubaneswar	Pursuing
	Debabrata Dash	Optimization of Various Parameters for the Production of Second Generation Bioethanol from the Efficient Biomass Producing Plants of Odisha. (Reg. No 07-Biotechnology, 2017-18)	Dept. of Biotechnology Utkal University, Bhubaneswar	Pursuing
4	**Dr. Giridara Kumar Surabhi, Sr. Scientist**			
	Subhankar Mohanty	A proteome approach to investigate fruit ripening and identification of key ripening proteins/genes in banana.	Utkal University	Pursuing
	Dinesh Pradhan	Transcrption profiling and molecular characterization of candidate fruit ripening associated genes in banana.	Utkal University	Pursuing
	Kousik Mukherjee	Studies on genetic diversity of sal (*Shorea robusta* Gaertn.) tree population in Odisha using molecular markers and development of mass prpogation protocoil through *in vitro* culture	Utkal University	Pursuing
	Mr. Subhankar Mohanty	A proteome approach to investigate fruit ripening and identification of key ripening proteins/genes in banana.	Utkal University	Pursuing

5	**Dr. Atish Kumar Sahoo, Sr. Scientist**			
	Satish Kanhar	Phytochemical and Biological Evaluation of three Indian *Homalium* species with special reference to Hepatoprotective activity in CCl4-Inuced oxidative stress in Wistar rats. (Regd. No. 02-Pharmacy-2016-17).	Utkal University	Pursuing
	Umesh Chandra Dash	Pharmacological profiling of *Geophila repens* and *Bacopa floribunda* and evaluation of their therapeuticpotential against Alzheimer disease. (Regd. No. 10-Biotechnology-2016-17).	Utkal University	Pursuing
	Sandeep Kumar Swain	Ethnopharmacological significance and therapeuticevaluations of *Hydrolea zeylanica* in experimentallyinduced type2 diabetes in rats. (Regn.no. 01-Biotechnology-2017-18	Utkal University	Pursuing
	Deeptimayee Rout	Ameliorative effects of *Homalium zeylanicum* ondiabetes induced oxidative stress and inflammation inWistar rats. (Regd. No. 11-Biotech-2016-2017).	Utkal University	Pursuing
6	**Dr. Uday Chand Basak, Sr. Scientist**			
	Jyotimayee Nayak	Screening of some medicinally important wild edible fruits of Odisha for nutrient content, antioxidant and antibacterial activities. (Reg. No.: 08-Botany-2012-2013)	Utkal University	Pursuing
	Pragyan Aparichita Patra	Evaluation of some lesser known wild edible fruits for their nutritional, antinutritional and antioxidant properties.(Reg. No. 06-Biotech-2016-2017)	Utkal University	Pursuing
	Swadha Baral	Essential amino acid profiling of some wild edible fruits of Odisha. (Reg. No. 04-Biotechnology-2017-18)	Utkal University	Pursuing
7	**Dr. Sunita Bhatnagar, Sr. Scientist**			
	Dipti Ranjan Behera	Bioassay guided isolation of antifilarial agents from selected medicinal plants using Setaria cervi as target parasite.	Utkal University, Bhubaneswar.	Pursuing

LIBRARY

At present the library of the centre has a collection of 2670 books on the thrust areas of Taxonomy, Biotechnology, Medicinal and Aromatic Plants, Tissue Culture, Microbiology, Physiology and Biochemistry, Forestry and Ecology, Molecular Biology, Horticulture and Floriculture, Ornamental Plants, Orchids and many other areas. Number of periodicals and journals of leading institutions and firms on related areas of importance are subscribed by the library. About 22 Indian Journals of repute are included as annual subscription. Library holdings also include non-book materials such as transparencies, cassettes, floppies, audio-visuals, CD's, DVD's and number of Topo sheets of Odisha. Internet facility is provided to its users. Reprography service at nominal payment is available to the research personnel. The Library's collection is referred by the Research Fellow of various institutions such as OUAT, IMMT, CRRI, CTCRI, Utkal University, Berhampur University, Sambalpur University, North Orissa University, IGIPS, Andhra University, Kalyani University, Jadavpur University and many other research institutions

HERBARIUM

The Centre has a modern Herbarium with a collection of 14,000 accessions belonging to 1600 species. The herbarium specimens have been digitized and made available to researchers as well as scientific communities through a web-based application.

EX-SITU CONSERVATION & GERMPLASM COLLECTION

RPRC has rich living collections of different plant groups. Till date, cacti and other succulents, wild and exotic orchids, species with fragrant flowers, endangered and threatened plants, medicinal plants, mangroves, palms, bamboos, wild edible fruit plants, cultivars of Hibiscus and Rose have been introduced to the living collection division and are being studied. Following are some of the ex-situ collections available in RPRC.

RET Corner: Conserved more than 30 RET species like *Cycas sphaerica, Cordia macleodii, Gnetum ula, Homalium tomentosum, Hypericum gaitii, Pomatocalpa decipiens* etc.

Rose Garden: The Centre has collection of around 1000 varieties of roses. The available varieties include Alec's Red, Black Lady, Double delight, French perfume, Nurjahan, Surkhab, Tiara etc,

Fragrance Flower Garden: "Garden of Species with Fragrant Flowers" of around 70 species like *Cananga odorata, Gardenia jasminoides, Magnolia coco, Murraya paniculata* etc.

Jagannath Vatica: A special garden housing 125 species of plants used in different rituals of Lord Jagannath.

Palmetum: Representing nearly 60 species of palms including *Archontophoenix alexandrae, Calamus spp, Corypha umbraculifera, Dypsis lutescens, Livistonia chinensis, Ravenea rivularis* etc.

Bambusetum : Having collection of around 30 species of bamboos like *Arundinaria chino, Arundo donax, Bambusa balcooa, Dinochloa maclellandii, Melocanna baccifera, Phyllostachys nigra, Pseudosasa japonica* etc.

Hibiscus Garden : The garden has a collection of 52 varieties of *Hibiscus* species.

Medicinal Plant Garden: This germplasm garden houses 250 species of medicinal plants collected from all over the country.

Cacti and other Succulents: The Centre houses more than 1000 Varieties and cultivars of cacti & succuents, both for sale and display for visitors.

Wild Edible Fruits Garden : Housed more than 100 native species with an aim of *ex-situ* conservation and evaluation for nutritional values & bioprospecting.

Orchids: Having germplasm collection of nearly 100 species of Orchids. Many hybrids are also available for sale.

FLOWER SHOW

In order to promote interest and elicit public awareness towards floriculture & other ornamental crops/flowers, the centre has been organizing annual flower show in the month of January in its excellent recreational botanical garden spreading over 110 acres with sprawling lawns, trees and flower beds, fountains, children park and boating facility in the lake. The special attractions of the flower show are being competition, display and sales of roses, cacti, orchids, succulents, herbal plants and the agri-horticultural tools and products etc. enjoyed by huge gatherings of plant lovers and visitors from local, far & wide.

STATE LEVEL ANNUAL FLOWER SHOW, 2020

11th-12th January, 2020

The Regional Plant Resource Centre (RPRC) and Plant Lovers' Association (PLA), Bhubaneswar organise the State Level Annual Flower Show in the premises of the Botanic Garden of RPRC (Ekamrakanan) on second Saturday and Sunday of January every year since 2006. This year, the Annual Flower Show has been organised on 11th and 12th January, 2020 with the support of Odisha Mining Corporation Ltd. and the Directorate of Horticulture, Odisha.

On this occasion, Dr. Mona Sharma, IAS, Additional Chief Secretary, Forest & Environment Department, Government of Odisha inaugurated the State Level Annual Flower Show, 2020 and visited the cut flower, Bonsai, Orchid sections, flower arrangements, floral displays, Rangoli, Vegetable carving, Plant Bazaar, Nurseries and exhibition stalls etc. She visited the Exhibition Stall and Floral Display made by Regional Plant Resource Centre, Bhubaneswar and released the Research Report, 2018-19 brought out by Institute. Dr. Sandeep Tripathy, IFS, Principal Chief Conservator of Forests and HoFF, Odisha attended the function as Guest of Honour. Sri Rajiv Kumar, IFS, Chief Executive, RPRC & MD, Odisha Forest Development Corporation Ltd.; Sri Khirod Pattnaik, President, Plant Lovers' Association and other dignitaries attended the function. The scientists of RPRC and office bearers of Plant Lovers Association were also present. An attractive "Floral Gate" has been erected at the entry point, which has become a major attraction for the visitors.

A "Plant Bazaar" has been organised at the venue of the show, where ornamental plants, flowering plants, seeds, seedlings, garden tools and implements, fertilisers, pesticides are being made available to public at reasonable cost. Besides nurseries and seed firms, exhibition stalls have been opened by the Directorate of Horticulture, Odisha, Ekamra Vana, Central Horticultural Experiment Station etc. A total of 46 organizations/ firms/ nurseries have participated and exhibited ornamental plants/ seeds/ fertilizers etc. for display and sale. This year, a "Orchid" section has been introduced and more than 100 attractive species/ varieties of hybrid and wild orchids are on display.

The Plant Lovers' Association also organised "Garden Competition" in the Capital city of Bhubaneswar to encourage the residents for raising Gardens and growing plants to add to the beauty of the City. This year over more than 95 entries for Garden competitions in different categories were received. The Association also organised a Painting and Poster Competition among school children and more than 500 children from different schools of Bhubaneswar participated. The winners of Garden and Painting competitions will be awarded with prizes and trophies in a function was held at 4 PM on 11th January, 2020. Dr. Hari Shankar Upadhyay, IFS, PCCF (Wildlife) & Chief Wildlife Warden, Odisha was the Chief Guest of the function and gave away prizes to the winners of Garden and Painting competitions. Sri Maloth Mohan, IFS, PCCF & Chairman, Odisha Biodiversity Board and Sri P. K. Jha, IFS, Special Secretary to Government, Housing and Urban Development Department, Government of Odisha was the Guests of Honour.

This year, 500 numbers of entries have been received in Potted Flowering, Foliage, Cut Flower, Flower Arrangement, Group Display, Bonsai, Orchids, Rangoli, vegetable carving and other categories. The winners under different categories was awarded with prizes and trophies in a function to be held at 4 PM on 12th January, 2020. Sri R. Vineel Krishna, IAS, Managing Director, Odisha Mining Corporation Ltd. shall grace the occasion as Chief Guest and give away the prizes. Dr. K. Murugesan, IFS, Director (Environment)-cum-Special Secretary to Government, Forest & Environment Department, Government of Odisha will be the Guests of Honour. Sri Rajiv Kumar, IFS, Chief Executive, RPRC and Sri Khirod Pattnaik, President, PLA was also on the dais.

As in previous years, about 1,00,000-1,50,000 visitors are expected to visit the Flower Show during these 2-days. Entry to the Botanic Garden was free for these two days and free parking inside the garden was provided for the visitors. The visitors certainly liked the ambience with beautiful landscape, sprawling lawns, greenery, picturesque lake with migratory birds and enjoy the children's corner and boating in Ekamrakanan Lake.

The scientists, staff, students and workers of RPRC; the office bearers and members of PLA had put their best efforts to make this State Level Flower Show, 2020 a success. The financial assistance from OMC and Directorate of Horticulture, Odisha and help and assistance from different participating institutions and individuals have been thankfully acknowledged.

REGIONAL PLANT RESOURCE CENTRE

NAYAPALLI, BHUBANESWAR-751015

BALANCESHEET AS ON 31.03.2020

LIABILITIES	SCHEDULE	AMOUNT(RS.)	ASSETS	SCHEDULE	AMOUNT(RS.)
General Fund	1	9,51,12,135	Fixed Assets	5	9,96,50,987
Grant for Non-recurring Expenses	2	15,25,75,414	Work-in-Progress	6	3,69,86,813
Advance Received for Contract Work	3	72,20,370	Fund Transfer to opening new scheme account		45,65,034
Current Liabilities	4	69,61,757	Current Assets		
			Loans & Advances	7	8,84,41,788
			Cash in Hand		7,723
			Cash at Bank		3,22,17,331
Total		26,18,69,676	Total		26,18,69,676

17/11/2020
Chief Executive
Regional Plant Resource Centre
Bhubaneswar

For PARTHA S. MISHRA & CO.
Chartered Accountants
Sanjaya Kumar Patra
CA S.K. Patra (FCA, DISA)
Partner, M. No-301529

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