

## 2.7 Forest Protection

### Overview

Research projects on insect pests and disease problems of forest plant species are being carried at ICFRE institutes. Integrated methods of management with special emphasis on host plant resistance against insect pests and pathogens have been worked out. Application of beneficial microorganisms for improvement of biomass production of the tree species and management of key insect pests and pathogens has been carried out.

### Insect Pests

- Database was developed in MS-ACCESS with several tables, forms and queries for computerization of National Forest Insect Collection. Data entry of all the 18,000 species was done in the database along with their physical verification and photos. For putting this database on net, it was converted into MySQL, PHP form.
- One new genus and 13 new species of encyrtids were described as new to science and two new records from India.
- Acetone, methanol and petroleum ether extracts of *Tagetes minuta* at 2% concentration caused 30-50% mortality of larvae of major insect pests of shisham and poplar.
- Biology of the bamboo borer, *Phloeobius crassicollis* was studied in detail in the laboratory.
- Relative resistance in selected clones of *Casuarina equisetifolia* against the bark feeding borer, *Indarbela quadrinotata* was identified through analysis of biochemical and physical nature of the clones.
- One hundred clones of eucalypts at three different agro-climatic zones of Tamil Nadu,
- Andhra Pradesh and Kerala were categorized for resistance/tolerance and susceptibility against the gall insect, *Leptocybe invasa*.
- In all, 19 fouling and 11 wood boring species were found to be new introductions to Visakhapatnam port and, of these, the annelid *Hydroides operculatus* (Treadwell) and the mollusc *Siphonaria cf. kurracheenensis* Reeve were recorded for the first time from India.
- Test panels of *Bombax ceiba* and *Paraserianthes falcataria* treated with TBTM-MMA preservative undergoing marine trials at Visakhapatnam and Kochi harbours showed that a few panels at the latter harbour were attacked by sphaeromatid wood borers in 23 months.
- A wood boring gastropod, *Thais blanfordi* (Melvill) was recorded for the first time from the fouling assemblages along the east coast.
- Attack of stem borer, *Botocera rufomaculata*, which girdles the main tree bole was found to be controlled by injecting 10 ml water emulsion of 0.5% dichlorvos.
- Petroleum ether extract of seeds of *Annona squamosa* was recorded to exhibit 72.99 to 75.43% antifeedant activity against the larvae of *Eutectona machaeralis* and *Hyblaea puera*. Foliar spraying of 0.5% neem commercial formulation was recorded to inhibit 80% egg laying, besides 90% antifeedant effect on young larvae.
- Soil treatment with phorate or methyl folidal @ 300gm/bed (size 12m x 1.25m) in combination and alternatively with EPN juveniles @ 250-300/ bed proved effective



in reducing the seedling mortality by root feeding white grubs.

- Seedlings treated with monocrotophos (0.01%) and kept under canopy in exclusion trials, laid down at Rajasthan and Gujarat, exhibited the best average growth performance in almost all the parameters after a period of 48 weeks.
- Thirty seven insect species belonging to 5 insect orders viz. Lepidoptera, Coleoptera, Hemiptera, Orthoptera, Hymenoptera and 24 families, Class Arachnida, were found to be associated with 5 temperate medicinal plants in the nurseries.
- *Polygraphus longifolia* appears in the chir pine forest earlier and attack the weak tree first, thereafter other insect stem borers made the tree their destiny for food, shelter and egg laying. Fire incidence and excessive resin tapping increased the susceptibility of the trees to the beetle incidence. Neem oil @ 5% acts as repellent and is effective in preventing the beetles attack.
- *Metarhizium* based mycoinsecticide product named PESTSTAT (in powder and liquid forms), is developed and is ready for release and application in the field.

### Diseases, Pathogens and Beneficial Microbes

- Resistant and susceptible clones of *Dalbergia sissoo* have been identified against *Fusarium solani* causing vascular wilt.
- On the basis of DNA sequencing, two species of *Ganoderma*, *G. resinaceum* and *G. weberianum* have been separated from the collection. The latter is a new record for India. Fifty one ITS sequences have been submitted to Gen Bank, (National Centre for

Biotechnology Information), USA and have been released at NCBI web site.

- Volatile effect of citronella oil, garlic oil, lemongrass oil and ajwain oil to inhibit growth and germination of spoilage fungi in stored medicinal plant produce was successfully established.
- Diagnostic kit was developed after standardizing protocols for DNA extraction of *Cylindrocladium quinqueseptatum* from soil, diseased plant parts and post-inoculation pre-symptomatic Eucalyptus plant samples infected with leaf, twig and seedling blight which will be helpful in identification of the pathogen and for disease forecasting.
- Fifteen 18S rDNA gene and twenty eight internal transcribed spacer region of nuclear rDNA gene of different isolates of fungus *Cordyceps sinensis* of Himalayan meadows were amplified, sequenced and submitted to NCBI and accession numbers were allotted.
- *Aspergillus flavus*, *Curvularia* sp. and *Fusarium* sp. were found infesting CCA treated timber wafers of *Anogeissus acuminata* (Yon) and *Mangifera indica* (Mango) even at a preservative retention of  $27.6 \text{ kg.m}^{-3}$ .
- Four genera and seven species of wood-decaying fungi have been recorded for the first time on sal and bijasal stored wood.
- Severe infestation of insect pest (*Achaea janata*) and charcoal root rot disease, caused by *Rhizoctonia bataticola*, have been recorded on mehndi (*Lawsonia inermis*) in Pali and Sojat in Rajasthan.
- Microbial inoculants, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Azotobacter*



*chroococum* and *Azospirillum brasiliense* inoculated to the stem cuttings of Eucalypts clones enhanced the rooting 40 % more than the IBA treated cuttings.

- Artificial inoculation of the six isolates of *Frankia* on *Casuarina* cuttings raised in inert media exhibited that the strain CjCbe1 produced higher nodules (7/cuttings) and nodule weight (55 mg/nodule).
- The microbes *Aspergillus* sp. and *Trichoderma* spp. were found to promote plant growth with soil amendments as they enhanced 10-20% survival and 43% height of sandal seedlings.

#### Projects under the theme

Project	Project Completed During the Year	Ongoing Projects	New projects Initiated During the Year
Plan	17	25	11
Externally Aided	10	10	04
<b>Total</b>	<b>27</b>	<b>35</b>	<b>15</b>

#### 2.7.2 Insects Pests, Diseases and their Control

##### Butterfly Diversity in Garhwal Himalayas

Data on species richness, abundance, food plants/sources, mating, egg laying period and other baseline stand parameters of over 200 species have been collected from Ban Oak forests from 6 sites in Garhwal.

##### Biodiversity of Parasitic Chalcidoidea of Uttarakhand

Parasitic chalcidoidea were sorted out into 18 families of this group in the laboratory under stereozoom microscope. Family Eulophidae was the most abundant and species rich family followed by Pteromalidae, Encyrtidae, Eucharitidae, Mymaridae, Eupelmidae, Aphelinidae and Trichogrammatidae. Several new species have been identified and work on their description is in progress.

#### Database of National Forest Insect Collection (NFIC)

Database has been developed in MS-Access and also in My Sql and PHP formats. Data on taxonomic classification, collection records, host information, etc. of about 18,000 insect species of forestry importance has been incorporated. High resolution pictures of about 16,000 species of insects have also been incorporated to give identification about the species.

#### Database of Forest Insects of Some Important Tree Species and their Management

Data have been collected for nine forestry tree species viz. *Shorea robusta*, *Dalbergia sissoo*, *D. latifolia*, *Acacia catechu*, *A. nilotica*, *Albizia lebbek*, *Ailanthus excelsa*, bamboo and *Tectona grandis*, for the insect pests associated, distribution, host range, nature of damage, period of occurrence and management techniques. Different user interface forms have been designed and listed successfully. These forms are integrated to design complete user interactive system. Presently data of four species viz. sal, shisham, bamboo and teak related information has been entered in the software.

##### Taxonomy

One new species belonging to family Tanaostigmatidae (Hymenoptera: Chalcidoidea), *Cynipencyrtus indicus* Singh has been described. This is the first record of the Genus *Cynipencyrtus* from India.

*Proleurocerus litoralis* Hayat and Kazmi (Encyrtidae) parasitizing egg masses of *Eurybrachys tomentosa* (Fulgoridae) from Doon Valley, Uttarakhand, were recorded. This is also a new host record for the species. Earlier this species was known from females only. Therefore, its males were also described.

First host Record of *Cotesia koebelei*, (Riley 1889) (Hymenoptera: Braconidae: Microgastrinae) on *Hyposidra talaca* Walker



(Lepidoptera: Geometridae) was recorded from Doon Valley.

### **Taxonomy of Braconid Parasitoids (Hymenoptera) from Central India**

Survey of important forestry and adjoining agroforestry areas of Maharashtra, covering localities of districts: Akola, Washim, Hingoli, Parbhani, Wardha, Yavatmal, Nanded, Latur, Beed, Osmanabad, Solapur, Jalgaon, Nashik, Thane and Mumbai were carried out for collection of braconids. Over all, 176 samples of insect fauna were collected by sweeping method out of which 876 braconids were sorted out and preserved. Two hundred thirteen samples of leaf miners, defoliators, gall forming insects and larvae / pupae of insect pests were collected for emergence of braconids from various forest tree species. In all, 8 species of braconids were recovered. *Apanteles antipoda*, *A. machaeralis* from teak skeletonizer, *Eutectona machaeralis* and *A. hyblaeae* from the larvae of teak defoliator, *Hyblaea puera*; *Dolichogenidea* sp. and *A. tachardiae* from defoliator of *Acacia nilotica*; *Apanteles* sp. from an unidentified semilooper on *Bridelia retusa*; *A. caniae* from *Papilio demoleus* and *Apanteles tiracholae* from *Agrotera basinotata*, defoliator of *Lagerstroemia parviflora*. Four species viz. *Bracon jalgaonensis* sp. nov., *Chelonus wardhaensis* sp. nov., *Chelonus hingoliensis* sp. nov., and *Doryctes indicus* sp. nov. collected by sweeping method were recorded as new species to science.

### **Studies on Termites**

Vials of termite species belonging to the family Termitidae were studied. In all, 33 species belonging to the family Termitidae have been studied. Apart from it, 7 species belonging to the families Kalotermitidae (5 species), Rhinotermitidae (1 species) and Stylotermitidae (1 species) have also been studied. Correct geographical distribution of all the 40 species has also been given.

### **Microlepidoptera**

A seed borer identified as *Cateremna tuberculosa* Meyrick is reported infesting the seeds of the Chilgozapine for the first time. Taxonomy of the same is updated by studying the wing venation and genitalia.

### **Insect Pest Surveys, Incidence and Biology**

#### **Bamboo Pests**

In central India surveys on insect pests of bamboos in nurseries revealed 4 types of insects namely leaf rollers, grasshoppers, aphids and whitegrubs. The leaf rollers were identified as *Cryptisia coclesalis*, *Pyrausta bambucivora*; grasshoppers as *Hieroglyphus banian*, *Poecilecerus pictus* and *Schistocerea gregaria*; aphids as *Oregma bambusae*; and white grub as *Holotrichia consanguinea*. Similarly in plantations, 4 types of insects viz., leaf rollers, grasshoppers, aphids, culm borers and hare were recorded to damage the different species of bamboos.

#### **Biology of Bamboo Borer, *Phloeobius crassicollis***

*Phloeobius crassicollis* is an important pest of bamboos and was observed to attack six different species of green standing bamboos namely; *Bambusa bambos*, *B. tulda*, *B. vulgaris*, *Gigantochloa aproviolacea*, *Dendrocalamus giganteus* and *D. strictus*. The incidence of attack of the borer in *Bambusa bambos* ranged from 7.18% to 11.2%. The beetles emerge in the month of May-June and lay 40-45 eggs on the node of bamboo culm. Incubation period was recorded as 10-12 days. Longevity of the female and male adults were 20-25 and 17-20 days, respectively. Larvae enter into the node and feed into the inner surface of bamboo. Larval period was observed 9-10 months. Mature larvae make an oval pupal chamber on the edge of the larval gallery at internodes and pupated in a crowded manner in a group of 2-11. The pupal period lasts for 15-20 days. Life cycle is completed in one year.



### **Insect Pests of Fast Growing Tree Species in Tamil Nadu and Kerala - *Ailanthus excelsa*, *Melia dubia*, *Gmelina arborea*, *Thespesia populnea*, *Morus alba*, *Bombax ceiba* and *Dalbergia sissoo***

About 55 species of insects were recorded damaging seedlings, saplings and young trees of these species in surveyed areas(six nurseries, eleven plantations and three natural forest locations in Tamilnadu and four nurseries, seven plantations and three natural forest locations in Kerala). They were categorized into major and minor based on their intensity of attack and nature of damage caused. Of the 55 species of insects recorded, 10 were new records. The natural enemy complex involving three larval and pupal parasitoids, seven predators and two microbes associated with the key pests were also documented. A pest calendar was prepared for benefit of end users.

### **Insects Associated with Fruits and Seeds of Selected Endemic Trees of Western Ghats**

The incidence of occurrence in percentage in respect of coleopterans was 37.78, lepidopterans was 26.50 and dipterans were 36.22. Diversity of fruits and seeds insects was more in Subramanya ( $H= 1.8099$ ) as compared to Makuta ( $H=0.808$ ).

### ***Myristica dactyloides* (Ramapathre) Mortality at Elimane, Karnataka**

Surveys revealed incidence of no minor or major insect pests associated with the tree. Dead branches from the upper canopy of *M. dactyloides* trees were chopped down for further investigation and were kept under cage condition for insect emergence. Two unidentified species of Coleoptera (Family Cerambycidae) were found to emerge from the pieces of dead branch. Unidentified Hymenoptera parasites were also collected.

### **Insect Pests of Cone and Seeds of *Pinus gerardiana* (Chilgoza)**

Eight sites of Kinnaur and Chamba districts i.e. Pangi 2750m, Labrang 2915m, Jhangi (Akpa) 2742m, Kilba 1894m, Akpa FRH 2503m, Korathi 2753m, Bharmour 2143m and Rispa 2406m were surveyed for the insect pest infestation on cones and seeds. Cones were infested by cone borer *Dioryctria abietella* throughout the range of natural occurrence of Chilgoza pine but at Kilba 69% of cones were found to be damaged. A seed borer identified as *Cateremna tuberculosa* Meyrick is reported infesting the seeds of the Chilgoza pine for the first time. Symptoms of the seed borer attack start appearing during month of July and by August almost 50% of seeds were found damaged. Damage continued till December by which 94% seeds were found damaged.

### ***Indarbela quadrinotata* Pest of Different varieties of *Emblica officinalis***

Survey was conducted in Clonal Seed Orchard (CSO) of *Emblica officinalis* at Sonaghati (Research & Extension Circle, Betul) for monitoring the status of insect pests and diseases. Incidence of bark eating caterpillar *Indarbela quadrinotata* was recorded in seven different varieties viz. Krishna, Francis, NA-6, Chakaiya, Kanchan, NA-7 and local variety of *E. officinalis*.

### **Control**

### **Chemical**

### **Insect Pest Management in Selected Forestry Species Nurseries**

Diseases and insect pests in *Dysoxylum malabaricum*, *Garcinia gummigutta*, *Myristica malabarica*, *Vateria indica*, *Azadirachta indica*, *Pongamia pinnata*, *Emblica officinalis* and *Sapindus emarginatus* in different nurseries were studied. Incidence of leaf blight and leaf spot disease was observed in all selected species.



About 35% Gall infestation and 100% defoliator infestation was observed along with scale insect in *Pongamia pinnata* seedlings. Infestation of leaves (100%) by *Meconellicoccus hirsutus* was observed in Sulikere nursery for *Emblica officinalis* seedlings.

#### **IPM Model for White Grubs in Teak Nursery**

Monitoring, observations and experimentations on *Holotrichia rustica*, *H. mucida* and *Schizonycha ruficollis* revealed relationship of beetles emergence and rising relative humidity. Data indicate that rainfall 2- 3 weeks prior to the date of emergence did not induce beetle emergence, due possibly to the lower atmospheric relative humidity (< 50%). After the increase in RH, even moderate amount of rains induced the emergence of beetles. Spraying on *Ziziphus jujuba* and *Z. mauritiana* host-traps with monocrotophos or dimethoate 0.05% twice at the interval of 15 days proved effective in reducing the egg laying by the adults. Laboratory experiments with the EPNs against laboratory produced white grubs proved effective. Treatment of the teak beds within 1 week after the adult emergence with phorate/methyl folidol @ 300g/ bed (size 12 m X 1.25m) in combination and alternately with the cadavars of EPN *H. indica* and *S. carpocapsae* @ 250 – 300 juveniles(ijs)/ bed in good watering conditions proved effective in reducing the incidence of seedling mortality. The ijs were also recovered after 1 month of the release proving their survival in the released soil. Based on the observations, a model guideline for the management of white grubs developed.

#### **Control of Bamboo Borer, *Phloeobius crassicollis***

Dursban 0.04% was found most effective followed by endosulphan, whereas, cypermethrin and Deltamethrin were found least effective. Among systemic insecticides, imadacloprid

0.04% was found effective followed by monocrotophos and dimethoate.

#### ***Pongamia pinnata* Galls and their Management**

Detailed surveys indicated the infestation of three gall inducers on *Pongamia pinnata* is attacked by three gall inducers viz., i) Leaf gall inducer- *Eriophyes cheriani* Massee (Eriophyidae: Acarina: Arachnida), ii). Ovary gall inducer - *Asphondylia pongamiae* Mani (Cecidomyiidae: Diptera: Insecta) and iii). Stem gall inducer - Agromyzid fly (Agromyzidae : Diptera: Insecta). Among these, the ovary gall inducer is most important as they directly affect the seed production upto the extent of 100% seed loss. Lopping of ovary gall infested branches and burning/burying them is ideal to manage the ovary gall inducer. In case of severe infestation, Chlorpyriphos or Imidacloprid may be sprayed at the time of bud formation.

#### **Pests of *Ailanthus excelsa*, *Gmelina arborea* and *Dalbergia sissoo***

Chemical pesticides viz, Thiodicarb, Flubendiamide, Monocrotophos, Chloripyriphos at 0.025 to 0.075% were effective in controlling the pests, *Atteva fabricilla*, and *Eligma narcissus* (*Ailanthus excelsa*) *Myllocarbus discolor*, *M. viridanus* (*Dalbergia sissoo*) and *Eupterote geminata* (*Gmelina arborea*) in nurseries and in young plantation.

#### **Management of Teak Heartwood Borer, *Alcterogystia cadambae* Moore**

The pest incidences were recorded in the identified areas of Doginal and Kirwati in Yellapur division. Three light traps were installed (one is solar power) for the monitoring of the pests in the infested plantations. Adult activity was monitored from light trap collections from the infested plantations. Mechanical control by larval traps and soil taps were tested and role of bird predators documented. Biocontrol by nematode injections were tested at



Doginal plantations. Field observations and field trials on trunk treatments with nematodes and fumigants were conducted. A package of practices for management of the borer is developed.

### **Insect Pest Problem of Sandal under Cultivation and their Management**

Five silvi-horticultural models of Sandal situated in Bevananahalli, Muddennahalli, Gottipura, Yelwala (Mysore) and Jarackbandae were continuously surveyed for insect pests and diseases. The common insect pests found in these models were sap-suckers (*Oxyrachis tarandus*, *Saisettia hemispherica*, *Parasaissetia nigrum*, *Ceroplastis actiniformis*, *Aonidiella orientalis* and *Paratachardina silvestrii*). The defoliators recorded on sandal, were *Crotogonus* sp. and *Dasychira mendosa*). Surveys on young sandal plantations grown with *Acacia auriculiformis* revealed the incidence of a notorious stem borer, *Purpuricenus sanguinolentus* (Cerambycidae: Coleoptera). This is the first report of this cerambycid beetle on sandal. The bark eating caterpillar (*Indarbela quadrinota*) was observed in almost all models. The Red coffee borer (*Zeuzera coffeae*) was observed in one of the sandal models. The blight symptoms were very few.

### ***Indarbela quadrinotata* Management in *Casuarina equisetifolia* Plantations**

Assessment of the bark eating caterpillar incidence in the *C. equisetifolia* plantations raised in different Agro-climatic Zones of Tamil Nadu was made and, altogether, 18 plantations have been studied so far. Impact of the bark eating caterpillars infestation on the growth of Casuarina trees is in progress and collection of periodical growth data and maintenance of plots being carried out. A few entomopathogens have been collected from the bark eating caterpillar. Pure cultures were developed from these specimens for studying their efficacy on the pest.

### **Insect Pests of *Buchanania lanza***

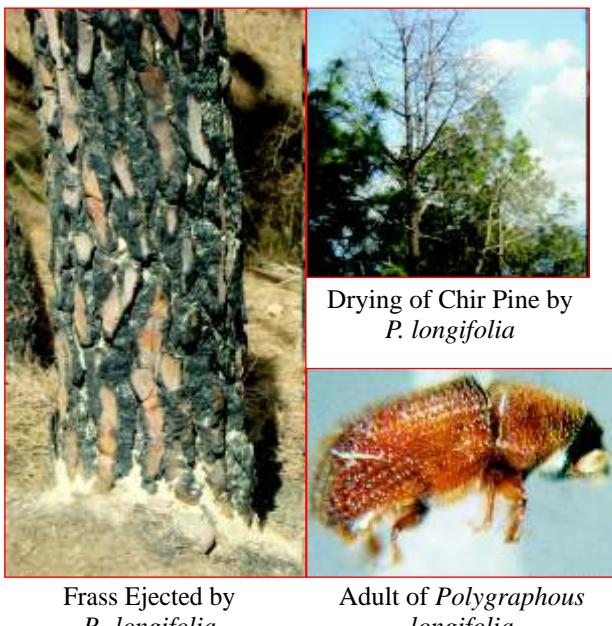
Out of 7 chemicals viz. dichlorvos 0.5%, monocrotophos 0.07%, endosulfan 0.05%, dimethoate 0.05%, petrol, kerosene and para-dichlorobenzene + kerosene- 10 ml each; water emulsion of dichlorvos 0.5% (10 ml) showed cent per cent killing of stem borer *Batocera rufomaculata* within 60 days in natural stand of *B. lanza*. Out of 4 chemicals viz monocrotophos 0.05%, endosulfan 0.05%, bavistin 0.2% & alpha NAA (40 ppm); foliar spraying of combination of endosulfan 0.05% + bavistin 0.2% + alpha NAA 40 ppm proved best for maximum number and weight of fruit production of *B. lanza*. Foliar spraying of monocrotophos 0.05%, endosulfan 0.05%, cypermethrin 0.03%, fenvalerate 0.03%, deltamethrin 0.002%, alphamethrin 0.003%, bipro super- *B. bassiana* 7 ml/lit, neemraj supreme- neem oil 0.5%, found significantly superior over control in killing of larvae within 72 hrs.

### **Management of Insect Borer Complex in Chir Pine Forests**

Chir pine stem has been reported to be attacked by 12 species of insects. On the basis of population and nature of damage, 4 species - *Polygraphus longifolia*, *Platypus biformis*, *Cryptorhynchus rufescens* and *Sphaenoptera aterrima* were graded as serious. *Polygraphus longifolia* was observed as most destructive insect bark borer. Three plots of size 1000 sq m were marked at D-113, Sairighat Forest (Solan Forest Division) and Platoo Forest (Hamirpur Forest Division) and insect fauna of Chir pine and their natural enemies were recorded from randomly selected trees. To evaluate the effectiveness of tree trap for *Polygraphus longifolia* and other beetles, billets of two sizes (80 cm L X 70 cm GBH and 100 cm L X 90 cm GBH) were kept at 5 experimental sites and the data on insect activity and population abundance of *P. longifolia*, *Cryptorhynchus rufescens* and



*Sphaenoptera aterrima* were recorded along with moisture content of the logs. Trees with girth range of 90 to 180 cms were found to be highly susceptible to infestation in comparison to young (below 90 cms) and mature (above 180 cms) stands. Fire incidence and excessive resin tapping increased the susceptibility of the trees to the beetle incidence.



Frass Ejected by  
*P. longifolia*

Adult of *Polygraphous*  
*longifolia*



Hole Formation below the Bark



Hole Formation on the Bark



Polygamous Gallery Formation on  
Bast Layer

### Biocontrol

#### ***Metarhizium* Based Mycoinsecticide for the Insect Management in Forest Plantations and Nurseries**

Twenty five *Metarhizium* isolates were maintained in the laboratory for studying the virulence and biocontrol potential against major pests on important forest tree species. The Teak defoliators, *Hyblaea puera*, *Paliga machoeralis* and insect pests of *Ailanthus excelsa* were found susceptible to all the collected *Metarhizium* isolates but pathogenicity varied among the isolates. Bioassay with Mahagony borer, *Hypsipyla robusta* reared using artificial diet revealed that 7 isolates were pathogenic to them. Pathogenicity of different isolates against arboreal termites, *Odontotermes* spp. was tested in the laboratory with different dosages/time and the LD<sub>50</sub> and LT 50 were calculated. Field evaluation of selected isolates against *Ailanthus* pests showed mortality of 30-34%. Mass multiplication of fungus in grains, solid media and agro wastes was tested. A mycoinsecticide product named as PESTSTAT in two forms, as powder and liquid is ready for release and application in the field.

#### **Microsporidia as Biocontrol Agents Against Lepidopteran Pests**

A total of 94 lepidopterans were tested and microsporidian parasites were isolated from 29 species. Bio assay study was carried out on



*Hyblaea puera*, *Catopsilia*, *Papilio demoleus* and *Papilio polytes* larvae by inoculating different concentrations of spores isolated from their respective hosts. Morphometry of 29 species of microsporidia were studied. Studies on morphology, Pathogenicity, rate of multiplication and life cycle of microsporidian spores in *Hyblaea puera* are completed in major test species. TEM studies were carried out for selected species. Cross-infectivity studies were carried out using *H. puera* spores to other forest pests to examine the infection potential.

### Natural Enemies of Teak Defoliators in Madhya Pradesh

Periodical surveys were conducted in teak forests of Madhya Pradesh for collection of natural enemies (parasitoids, predators and pathogens) of major insect pests of teak, *Hyblaea puera* and *Eutectona machaeralis*. The larvae of *H. puera* were noticed to be attacked by five species of parasitoids namely, *Echthromorpha notularia*, *Trophocampa indubia*, an unidentified species of *Brachymeria* and three unidentified species of *Sturmia*, three species of insect predators namely, *Calleida splendidula*, *Canthecona furcellata* and *Chrysoperla carnea*, one species of bird predator namely, *Corvus macrohynchos* and unidentified spiders, and a species of fungal pathogen namely, *Aspergillus flavus*. The larvae of *E. machaeralis* was observed to be attacked by eight species of parasitoids namely, *Apanteles machaeralis*, an unidentified species of *Apanteles*, *Brachymeria* and *Sturmia*, *Cremastus hepaliae*, *Trophocampa indubia* and *Xanthopimpla cera* and an unidentified species of nematode, two species of insect predators namely, *Canthecona furcellata* and *Chrysoperla carnea*, and unidentified spiders, and three species of fungal pathogens namely, *Aspergillus niger*, *Beauveria bassiana* and *Fusarium oxysporum*. Carried out laboratory culture of *A. flavus*,

*A. niger* and *B. bassiana* and conducted pathogenicity tests against target pests. Rearing technique of a potential polyphagous insect predator, *C. furcellata* and its alternative hosts have been developed.

### Termites and White Grub Management Using Entomopathogenic Nematode

Laboratory culture of waxmoth, *Galleria mellonella* was maintained round the year. The mature larvae of the same were periodically separated out of the culture and utilized as fictitious host. The culture of 2 exotic and 4 native populations of entomopathogenic nematode was maintained round the year on fictitious host, i.e., waxmoth larvae in laboratory. Experiments were laid out relating to improved parameters for economical and successful mass-multiplication of the native populations/ isolates from central Indian states. Experiments were carried out for bioassaying potentiality of native populations/ isolates of EPNs against termite and white grub pests. For isolating more populations from central India, surveys were carried out and 40 samples were collected from two sites comprising of teak plantation areas of Kundam Project (M.P. Forest Development Corporation, Jabalpur (Slimnabad, Jirri, Dhimarkheda) and Dindori Forest Division, Dindori, (Karanjia, Jagatpur, Amarkantak) in Madhya Pradesh. Investigation on insecticidal tolerance/ compatibility of EPNs to commonly used insecticides/biopesticides has been initiated.

### Management of Indian Gypsy Moth (*Lymantria obfuscata*) in Himachal Pradesh

Two aspects of ecofriendly biological control measures, viz. using baculovirus (LONPV) as microbial insecticides and utilizing female sex pheromone to trap & kill male moth, were adopted in this project. Bioassay experiment using 5 dilutions from stock solutions of baculovirus has been conducted on 75 larvae



in each test. Ninety Percent mortality was observed. Pheromone was extracted and purified from the abdominal glands of 100 adult females using acetone solvent and stored. This would be applied during next breeding season.

### **Evaluation of Biopesticidal Products for the Management of Teak Defoliator and Skeletonizer in Forest Nursery**

Planned and time bound use of a neem based biopesticide (0.5%) was found to cause 90% antifeedant effect to the larvae of teak skeletonizer. It was also proved to inhibit over 80% egg laying of teak defoliator. Spraying with biopesticides like, 0.05% of spinosad (Actinomycete) 45 % EC Actinomycete Biological Product; botanical combination agropest *bt* @ 0.05%; cigna 5.40% w/w (Insect Growth Regulator) for direct mortality with 3–7 days residual effects against teak defoliator and skeletonizer in forest nursery. EPNs *H. indica* and *S. carposcapsae* were reared and their bioefficacies evaluated for the first time against forest insect pests. EPN, *H. indica* in laboratory bioassay (dose-range 3 to 30 ijs larva<sup>-1</sup>) in 72 hrs post-exposure caused mortality up to 76.47 % at 10 ijs larva<sup>-1</sup> 100% at 30 ijs larva<sup>-1</sup>. Leaf treatment method (dose-range 32 to 280 ijs larva<sup>-1</sup> or 3.88 ijs to 32.00 ijs cm<sup>-2</sup>) resulted in mortality range of 78.94% at 130 ijs larva<sup>-1</sup> or 15.53 cm<sup>-2</sup> and 100% at 265 ijs larva<sup>-1</sup> or 32.00 cm<sup>-2</sup>. Field spraying experiment indicated requirement of 5000 to 10000 infective juveniles/ litre for the mortality. First time 3 native EPN populations (1 *Steinernema* spp. and 2 *Heterorhabditis* spp.) were isolated and being maintained successfully, as no previous reports from the central Indian forest floor is available. PDBC strains of *H. indica* was found compatible with chemical insecticides, viz., imidacloprid, monocrotophos, endosulfan, chlorpyriphos and Thiamethaxam (actara), and biopesticides like neem product,

agropest *bt* and derisome (botanical products), bioprahah (*Photorhabdus* bacteria), conserve (Spinosad actinomycete product) and signa (Insect Growth Regulator). Taking clues from tolerance of EPNs with chemical insecticides and biopesticides, combination with EPN @ 5000 and 10,000/ litres proved effective in presence of good amount of atmospheric relative humidity.

### **Biocontrol for Prickly Acacia: Exploration in India**

The study sites were selected for survey and laying out of exclusion trials in Rajasthan (Jodhpur, Hanumangarh, Bharatpur and Pali) and in Gujarat (Bhuj, Nadiad, Junagarh and Gandhinagar). Regular and random samplings were carried out in different survey sites. Data on temperature, humidity, light intensity, longitude & altitude were recorded in each site. The samples collected during survey were identified. The rust fungus sample collected during survey from Verawal, Tarapur, and Nadiad in Gujarat was identified as *Revenelia evansii*. Two species of mites viz., *Amblyseius alstoniae* and *Pronematus* sp. were collected from Gujarat, while three species of mites pests viz., *Amblyseius alstoniae*, *Tenuipalpus* sp. and *Oligonychus* sp. were collected from Rajasthan. Rust and mites have been prioritised for host-specificity tests for *acacia* seedlings. Data of exclusion trials on plant height, number of shoots, number of leaves per plant, basal stem diameter, root length and total wet weight of seedlings (above ground and below ground) of 12 weeks, 24, 36 and 48 weeks old seedlings of *A. nilotica*, kept under observation for exclusion trials at Jodhpur, Pali, Hanumangarh and Bharatpur (Rajasthan) and Gandhinagar, Nadiyad, Junagarh and Bhuj (Gujarat), has been analyzed. Plant parasitic nematode species isolated from dead and dried trees of *A. nilotica* from Ratipar Forest Reserve in Gujarat were identified as



*Hoplolaimus indicus*, *Aphelenchus avenae* and *Helicotylenchus* sp. These nematodes were isolated and separated out from the soil samples collected from dried and diseased trees of *A. nilotica* at Ratipar and Bhuj (Gujarat). All the three species were found ecto- semiendo and endoparasitic in nature, which feed mainly on cortical tissues. In seedlings, neither necrosis nor stunting of root has been observed at Bhuj (Gujarat).

### Botanicals

#### Pests of *Ailanthus excelsa*, *Gmelina arborea* and *Dalbergia sissoo*

Crude extracts of *Ailanthus excelsa*, *Lantana camara*, *Aegle marmelos* and *Pongamia*, *Jatropha* and *Neem* oil were tested for mortality, feeding deterrent and antifeedant effects on the targeted pests. None of the crude extracts of the said plant species was effective for any of the pests tested- *Atteva fabricilla*, and *Eligma narcissus* (*Ailanthus excelsa*) *Mylllocerus discolor*, *M. viridanus* (*Dalbergia sissoo*) and *Eupterote geminata* (*Gmelina arborea* ). However, the oils of *Jatropha*, *Pongamia* and *Neem* at 2-3% were found effective in controlling the *Ailanthus* and *Gmelina* defoliators in nurseries and in young plantation.

#### Biopesticidal Property of *Aegle marmelos* Seed Oil

Three compounds out of 13 extracted from fruit, pulp and seeds of *A. marmelos* showed biopesticidal effect and were found to be effective against teak defoliator *H. puera* with 70 % larval mortality. Field level biopesticidal evaluation of individual compounds and crude extracts against teak insects in State Forest Nurseries at Nilambur and Kulathupuzha, Kerala revealed that they are found to be very effective against *H. puera* in Nilambur teak nursery when compared to Kulathupuzha and also it was observed as a growth promoter.



Application of *A. marmelos* Seed Oil Pre Formulations against *H. puera* on Teak at Nilambur, Kerala

#### Biopesticides from *Annona squamosa* Against Teak Defoliators

Out of five solvents extract and one water extract of 0.05% *Annona squamosa* were evaluated for antifeedant activity against teak skeletonizer larvae. Petroleum ether extract exhibited 72.99% antifeedant activity ( $P<0.005$ ), followed by 69.99% by ethyl acetate ( $P<0.05$ ), as compared to other extracts. Further, confirmatory bioassays with concentrations/doses ranging from 25ppm to 3000ppm of each promising extract for the determination of optimum doses exhibited over 70% antifeedancy above 800ppm of crude ethyl acetate extract ( $P<0.001$ ). These two extracts also inhibited feeding in teak defoliator larvae with antifeedancy being 75.43 and 78.84% over control ( $P<0.001$ , df. = 24, F=14.00, SE(d) $\pm$  =10.85, LSD( $P<0.05$ )=22.40). Effective concentration for 50% antifeedant effect for ethyl acetate was calculated to be 524.80 ppm with upper and lower fiducial limits, respectively being 769.80 and 435.78. EC<sub>50</sub> for petroleum extract was 220.80 with upper and lower fiducial limits being 357.78 and 114.52. Column Chromatography allowed isolation of three major



fractions from petroleum ether extract, as confirmed by TLC, time to time, codes 6a, 6b and 6c and four chromatographic fractions from ethyl acetate extract; codes 3a, 3b1, 3b2 and 3b, which exhibited antifeedant and growth regulatory effect. Results indicated 3b1 fraction at higher concentration of 0.08 to be effective with significantly superior ( $P<0.001$ ,  $df=30$ ,  $F=8.34$ ,  $SE(d)\pm=10.64$ ,  $LSD(P<0.05)=21.95$ ) antifeedant activity over other treatments against teak skeletonizer, *Eutectona machaeralis*, with over 74.49% leaf protection. Nevertheless, concentration of 400 ppm was statistically at par with 800 ppm with 47.83% leaf protection ( $P<0.038$ ,  $df=29$ ,  $F=2.94$ ,  $SE(d)\pm=17.92$ ,  $LSD(P<0.05)=37.38$ ). Lowest concentration of fraction 3b2 was at par with control with no significant antifeedant activity ( $P>0.05$ ). Fraction 3b of ethyl acetate proved most promising with significantly superior antifeedant activity ( $P<0.001$ ,  $df=30$ ,  $F=174.01$ ,  $SE(d)\pm=3.97$ ,  $LSD(P<0.05)=8.10$ ) at and above 0.05% (500 ppm) concentration (89.79% leaf protection). Increase in activity was noticed after increasing the concentration of the extract. The full grown larval weight after being, pre-pupal fed continuously on treated leaves and pupal weights were significantly affected with mortality at larval and pre-pupal stages, as compared to control ( $P<0.05$ ). While there was statistically non-significant ( $P>0.05$ ) difference in initial larval weights, concentrations above 400 ppm were significantly superior ( $P<0.033$ ) in affecting the final larval weights. Similarly, pre-pupal weights were also affected. There was no development of pupae in treatments above 100 ppm. Similar results were obtained with ethyl acetate extract

against teak skeletonizer, *Eutectona machaeralis* at and above 400 ppm. In this case, there was no pupal development, even at the lowest concentration tested.

### **Insecticidal Properties of Some Plant Extract Against *Heortia vitessoides***

Evaluation of insecticidal properties of some plant extract was carried out against *Heortia vitessoides* Moore (Lep: Pyralidae), a major pest of *Aquilaria malaccensis* Lamk, a world renowned Agar oil yielding tree. Bio-efficacy of locally available botanicals for the management of *Heortia vitessoides*- a major defoliator in nurseries as well as young plantations of *Aquilaria malaccensis* was studied. Results of bioassay test with botanicals viz., *Azadirachta indica*, *Melia azedirach*, *Acorus calamus*, *Adhatoda vesica* and *Clerodendron viscosum* (tested at 2.5%, 5.0% and 10.0% concentration) showed the maximum of 95.0% antifeedant activity in case of *A. indica* followed by 90.0% in case of *A. calamus*.

### ***Tagetes minuta* Extract Against Major Pests of Shisham and Poplar**

It was observed that after 72 hours of exposure of major pests of shisham and poplar at 1% concentration of different chemical extracts JGPE (Jungli Gainda in Petroleum Ether), JGA (Jungli Gainda in Acetone) and JGM (Jungli Gainda in Methanol) were found effective, whereas, J.G.W. (Jungli Gainda in Water) was observed not effective. The bioassay of effective chemical extracts (JGM, JGA and JGPE) was done and it was observed that at 2% concentration the mortality of larvae was observed 33.33-53 per cent.

### **Panchagavya and Dasagavya**

The organic preparations Panchagavya and Dasagavya tested against key insect pests of *Casuarina* revealed that the organic formulations



not only controlled the insect pests but also enhanced the growth under nursery conditions. Application of 12 rounds of Panchagavya and Dasagavya at 15 days intervals at 13% controlled the infestation of mealy bug on casuarinas and increased the growth 20% compared to the control. Similarly the concentration of 5% reduced the gall formation to 40% on Eucalyptus and increased plant growth 14% while the concentration 3% exhibited 30% reduction in teak defoliator incidence and 12% increase in plant growth.

### Toxins of Soil Actinomycetes Against Major Forest Insect Pests

Out of 340 soil samples collected from forests of Madhya Pradesh, Maharashtra and Chhattisgarh, 9 actinomycetes/ bacteria were isolated on potato dextrose agar plates following serial dilution technique and pour plate method. Among the 3 actinomycetes isolated, *Streptomyces* sp. was identified and confirmed at Institute of Microbial Technology, Chandigarh. Culture technique of soil actinomycetes was developed for production of toxins, protocol for extraction of antibiotics and its fractions for chemical characterization of toxins of isolated actinomycete, *Streptomyces* sp. Toxicity tests of its culture filtrate, isolated antibiotics and different fractions was conducted against major insect pests of teak, siris and mahaneem. Commercially available bioproducts (ivermectin and spinosad) of soil actinomycetes was evaluated against major insect pests of teak, siris, bamboo and mahaneem and worked out their relative efficacy and toxicity ( $LC_{50}$ ) through Probit analysis. Field-cum-laboratory tests of ivermectin and spinosad were conducted against the early last instar larvae of major insect pest of above noted species. Field trial in nursery stage at insectary of this institute was carried out to test the toxicity of ivermectin and spinosad. The results revealed that management of these pests may be achieved by foliar application of bioproducts (ivermectin and spinosad).

### Insect Pests of Medicinal Plants

#### Management of Potential Insect Pests of Important Medicinal Plants Grown in Arid and Semi-arid Regions

A check-list of five species of insect pests and three species of diseases has been prepared. Severe infestation of *Achaea janata* (Noctuidae), a semilooper has been noticed on all mehndi (*Lawsonia inermis*) growing areas at Sojat road (Pali). Bio-ecology of the key pests viz. *Achaea janata* and *Aphid gossypii* have been studied. Soil treatment (10g *Trichoderma* + 5-0 kg Vermicompost + 15 g Phorate) and foliar Spray (0.02% Pratirodh) was found to be the best against mehndi defoliator (*Achaea janata*) and aphid (*Aphid gossypii*) attack. The mean dry weight of the treated plot was recorded 2.47 kgs with a net return of ` 185 per metre<sup>2</sup> plot as compared to untreated control with mean dry weight 2.00 kg and net return of ` 150/metre<sup>2</sup> plot. Seed treatment using *Trichoderma harzianum* @ 10g/kg seed + soil treatment with *Beauvaria bassiana*-1 + Phorate granules was found to be the best against downy mildew and termites in Isabgol. The treated (5m x 5m) plot yielded 104.88 kgs stover/plot as compared to untreated 76.56 kgs stover/plot with net return of ` 3670.80/- and ` 2679.60/- per plot, respectively.

#### Control of Insect Pests of Important Medicinal Plants in Himachal Pradesh

In total, 37 insect species belonging to 5 insect orders viz. Lepidoptera, Coleoptera, Hemiptera, Orthoptera, Hymenoptera and 24 families, Class Arachnida, were collected from selected 5 medicinal plants raised in the nurseries. The study on the biology of *Plusia orichalcea* Fab. on *Saussurea costus* infesting *Picrorhiza kurrooa* Royle ex Benth., *Acrtium lappa* Linn., *Heracleum candicans* Wall. ex DC, *Angelica glauca* Edgew., *Saussurea costus* Falc. and *Valeriana jatamansi* Jones revealed that the



insect was most active from second week of April to last week of June. Four overlapping generations were studied from March to June. There were 5-6 larval instars,  $16.25 \pm 5.4$  days larval period,  $10.75 \pm 2.6$  days pupal period and total lifecycle has been completed in 35 days in the laboratory. Three generations of the pest had been studied in the laboratory. The pest starts appearing in the field during March and its maximum activities recorded in May and June. In the laboratory, heavy larval (3<sup>rd</sup> and 4<sup>th</sup> instars) mortality has been recorded. The fecundity of a female varied from 113 to 228 eggs and the total life cycle was completed in 27 to 38 days during different months. Two species of larval parasitoids viz., *Apanteles glomeratus* and *Apanteles ruficrus* (Haliday) were reported and the extent of parasitisation by these species was 13.3, 21.2 and 25.0 per cent in April, May and June, respectively. Entomopathogen, which resulted in large scale mortality of larval and pupal population in field as well as in laboratory was identified as *Bacillus cereus* Var. *Mycoides* (Flugge) Smith, Gorden and Clark. The study indicated that these biological control agents can play an important role in eco-friendly management of pest. Different insecticides and biopesticides viz. Grownim @ 5.0 %, Monocrotophos @ 0.03%, Endosulphan @ 0.1%, Furadan3G @ 30 gm/m sq., Dursban @ 0.02% Rogar @ 0.05%, Neem cake @ 500 gm/m sq and Summer oil @ 5.0 % in *Valeriana jatamansi* were evaluated in the nursery and it was found that endosulphan @ 0.1% and Chloropyriphos @ 0.02 % was effective in controlling pest in nursery. Insecticidal residual analysis of treated plants were got done from UH&F Nauni, IHBT Palampur and IIM, Jammu where the results revealed that Endosulfan @ 0.1%, Monocrotophos @ 0.03%, Carbofuran @, Chloropyriphos @ 0.02 % have got the residual effect in the used part of plant. Hence, these insecticides are not a good option to control the insect pest in nursery.

### Insect Resistant Germplasms

#### Screening Resistance in *Eucalypts* spp. for Gall Insect Pest

About 210 clones of *Eucalyptus* identified for high yielding were collected from 9 different organizations and assembled at nursery and Vegetative Multiplication Gardens (VMGs) of the Institute of Forest Genetics and Tree Breeding, Coimbatore. For screening these clones at a hot spot area at Satyavedu, Andhra Pradesh a trial was established and about 179 clones have been planted at the trial. Screening of these high yielding clones for the attack of gall insect, *Leptocybe invasa* at the trial at Satyavedu (Andhra Pradesh) and at VMGs at Panampally (Kerala) and Bharathiar University (Tamil Nadu) are in progress.

A total 100 *Eucalyptus* clones raised in clonal trials at three different locations of Tamil Nadu, Kerala and Andhra Pradesh were evaluated for pests and diseases problems importantly for the gall insect, *Leptocybe invasa*. Field data collected over a period of two years showed that about 25 clones were highly susceptible to the attack of the gall insect. Low to Moderate level of attack was observed on 71 clones. About 4 clones consistently expressed only ovipositional damage and no gall development was seen. Studies on life cycle of the gall insect, *Leptocybe invasa* varied between 82-95 days.

#### *Casuarina equisetifolia* Against the Serious Bark Feeding Pest, *Indarbela quadrinotata*

Relative resistance in 10 selected clones of *Casuarina equisetifolia* was identified against the serious bark feeding pest, *Indarbela quadrinotata* in terms of biochemical and physical compounds like phenols, tannins, free fatty acids and phenolic acids of clones imparting resistance. Behavioural response of *I. quadrinotata* on different *Casuarina* clones



further revealed the influence of phenols, tannins, free fatty acids and phenolic acids on the feeding and reproduction behaviour of the bark feeder.

Screening of 132 families of *Acacia auriculiformis*, 80 families of *A. mangium* and 64 germplasm collections of *Ailanthus excelsa* populations for insect pest resistance and selection of pest resistant candidates revealed varying levels of pest resistance nature of different germ plasm of these species for their key pests.

Characterization of the allelochemical profiles like phenols tannins, lipids, five fatty acids, phenolic acids and volatiles of selected germ plasm of teak, casuarina and eucalypts enabled to identify the influence of these chemicals with the attraction/deterrence of insect pests. Evaluation of behavioural responses of insect pests further confirmed the role of these allelochemicals in food and oviposition preference of the pests on their host species. A data bank constitutes 120 volatile chemicals responsible for insect pest resistance and susceptible clones/provenances of teak, casuarinas, and eucalyptus was also developed.

### DISEASES AND THEIR CONTROL

#### Screening for Resistance Against Diseases

Twenty clones of *Dalbergia sissoo* were tested against four virulent strains of *Fusarium solani* by direct inoculation. Clone No. 14 and 6 exhibited stabilized resistant reaction. Experiment on flooding of different shisham clones followed by inoculation with *F. solani* showed that all clones exhibited wilt disease establishing that shisham plants become susceptible to fungal infection after water logging.

Blister bark disease resistance was tested in 150 clones of *Casuarina equisetifolia* against 3 isolates of *Trichosporium vesiculosum* and clone numbers TNIPT -7 and TNIPT -11 showed resistance against the disease under nursery conditions.

#### *Acacia nilotica* Mortality

The effect of biotic stresses, climatic change and physico- chemical attributes of soil in four agro-climatic zones each in Punjab and Haryana was studied to understand the mortality of kikar. Many factors attribute for the mortality major being injuries due to developmental activities like road widening, construction, laying of pipelines and electricity lines, drainage, etc.

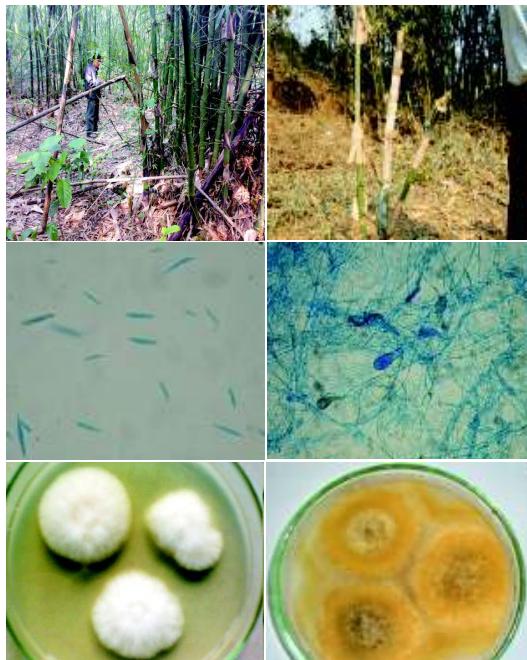
#### Blister Bark Disease in *Casuarina equisetifolia*

Etiology and histopathological aspects of blister bark disease of *Casuarina equisetifolia* were studied and found that temperature between 25°C to 35°C was ideal for the growth of *Trichosporium vesiculosum* under laboratory conditions. In pathogenicity test conducted with artificial inoculation of *T. vesiculosum* at nursery revealed that 5 months old seedlings were susceptible for blister bark disease. Histopathology studies showed that the pathogen initially infect the root cortex zone and, thereafter, spread into cambium of shoot zone. It was also found that vertisol type of soil are more favourable for spread of disease than alfisol type soil.

#### Diseases of Bamboo

The samples of leaves infected were collected from seedlings, cultured and identified in laboratory as leaf rust, *Dasturella divina* and leaf tip blight, *Helminthosporium solani* in central India. Different species of bamboos in plantations were surveyed periodically throughout the year at 15 days interval. Leaf rust caused by *Dasturella divina* was recorded on leaves of different bamboo species from December 2009 to January 2010.

Studies were done on the incidence and management of culm rot and bamboo blight disease in Assam and the field tours conducted in Sibsagar, Dibrugarh, Tinsukia, Dhemaji, Goalpara, Tezpur and Bongaigaon districts of Assam exhibited the presence of bamboo blight



*Fusarium* sp. Isolated from the Diseased Sample

Unidentified Fungal Species

disease in all the surveyed sites. The per cent of blight incidence in new culms ranges from 21 to 61% in the surveyed districts and the highest disease per cent infection of culms was observed in Golaghat district (61.29%) followed by Tinsukia (29.18%) and the least was observed in Sibsagar district (20.93%). Studies on fungal culture from the collected diseased samples revealed the association of *Fusarium* sp. and one unidentified species with blight disease of bamboos in Assam. Pure cultures of these fungi are being maintained in laboratory.

#### Diseases and Pests in *Gmelina arborea*

To identify major diseases and insect pests associated with *G. arborea* and to assess the occurrence of disease and pest infestation in relation to soil and plant nutrient status with special reference to potassium, field surveys were conducted in 36 plantation sites and 5 nurseries within 8 districts of Jharkhand. Plant specimen of affected plant parts were collected for identification of the causal organisms. The severity index due to the pathogens and insect

pests was developed. Soil and plant samples collected from all the sites were analysed so as to relate plant and soil K status with severity index.

#### Diseases of Important Medicinal Plants

Charcoal root rot disease caused by *Rhizoctonia bataticola* was found to cause severe damage to mehndi plants with an ultimate loss of mehndi yield at Sojat. Soil treatment (10g *Trichoderma* + 5-0 kg Vermicompost + 15g Phorate) and foliar Spray (0.02% Pratirodh) was found to be the best against mehndi defoliator (*Achaea janata*), aphid (*Aphid gossypii*) attack and leaf blight disease. The mean dry weight of the treated plot was recorded 2.47 kg with a net return of ` 185 per metre<sup>2</sup> plot as compared to untreated control with mean dry weight 2.00 kgs and net return of ` 150/metre<sup>2</sup> plot. Seed treatment using *Trichoderma harzianum* @ 10g/kg seed + soil treatment with *Beauvaria bassiana*-1 + Phorate granules was found to be the best against downy mildew and termites in Isabgol. The treated (5m x 5m) plot yielded 104.88 kg stover/ plot as compared to untreated 76.56 kg stover/ plot with net return of ` 3670.80/- and ` 2679.60/- per plot, respectively.



Mehndi Brown Leaf Spot Disease Caused by *Alternaria* species



Pupal Density of White Fly on Mehndi



Full Grown Larva of *Achaea janata* on Mehndi Plant



### Natural Decay Resistance of Imported Woods

Among the imported woods tested for natural decay resistance teak wood from Tanzania and Australia were found highly resistant against all test decay fungi, whereas beach wood from France and Belgium and Ash wood from France were not resistant.

### Fungi Associated with Fruits and Seeds

A total 150 fungi isolated from the different fruit/seed samples were identified. Among these 40 were potential plant pathogens, reported for their pathological effects in several other hosts. Isolates included storage fungi like *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Rhizomucor variabilis*, *Mucor mucedo*, *Trichoderma viride*, *T. koningii*, *T. pseudokoningii* and *T. harzianum*. These fungi play a major role in causing the seed health problems like seed rotting, shrinkage, discolouration, and abortive and poor germination. *Beltrania rhombica* on *Poeciloneuron indicum* seeds is reported as new host record. 75-90% *Fusarium verticilloides* infection was found in *D. malabaricum* fruits and seeds. In *C. sulpharatum* and *H. ponga* seeds though there was fungal infection, viability was not affected. In *S. malabaricum* and *M. longifolia*, fungal infection was less.

Ten fungal species i.e. *Alternaria alternata*, *Aspergillus niger*, *Cephalosporium* sp., *Chaetomium globosum*, *Cladosporium* sp., *Fusarium equiseti*, *Fusarium oxysporum*, *Penicillium citrinum*, *Rhizopus stolonifer* and *Trichothecium roseum* were identified and stored seeds of *Pinus gerardiana*. *Penicillium citrinum* was the most predominant fungus affecting Chilgoza seed in storage. It was observed that 40% of seed rot was found at 25°C and even 8% seed rot was observed at 0°C due to *Penicillium citrinum*.

### Molecular Variability in Fungi

Morphological and cultural variation in 63 fruiting bodies of *Ganoderma lucidum*

collected from Delhi (NCR), Haryana, Punjab, Uttar Pradesh, and Uttarakhand studied. Vegetative compatibility tests were performed between different isolates of *G. lucidum*. OPA3 primer has differentiated isolates of *G. lucidum* on the basis of host species. On the basis of DNA sequencing two species of *Ganoderma*, *G. resinaceum* and *G. weberianum* have been separated from the collection, the latter is a new record for India. Fifty one ITS sequences have been submitted to GenBank, (National Center for Biotechnology Information), USA and have been released at NCBI web site. The sequences were also incorporated and released by the other gene banks viz. European Molecular Biology Laboratory Nucleotide Sequence Database, UK (<http://www.ebi.ac.uk/embl/>) and DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp/>).

A combined dendrogram of 82 isolates of *Cylindrocladium quinqueseptatum* was generated after RAPD-PCR by primer OPE-2, OPE-3, OPE-5. Eight internal transcribed spacer region sequence of nuclear rDNA and six beta tubulin gene sequences of different isolates of fungus *Cylindrocladium quinqueseptatum* were submitted to GenBank NCBI (<http://www.ncbi.nlm.nih.gov>) and accession numbers were allotted. Diagnostic kit was developed after standardizing protocols for DNA extraction of this pathogen from soil, diseased plant parts and post-inoculation pre-symptomatic Eucalyptus plant samples infected with *Cylindrocladium quinqueseptatum* leaf, twig and seedling blight. It is helpful in identification of *Cylindrocladium quinqueseptatum* and can be used for disease forecasting.

Screening of 60 primers was done for RAPD (Random amplified polymorphic DNA) out of which, 32 primers showed consistent polymorphism. Out of 32 primers 18 primers were analyzed for polymorphism. A combined



dendrogram for all the isolates was constructed using UPGMA method. PCR-RFLP of 15 isolates was done. Fifteen 18S rDNA gene and twenty eight internal transcribed spacer region of nuclear rDNA gene of different isolates of fungus *Cordyceps sinensis* of Himalayan meadows were amplified, sequenced and submitted to NCBI and accession numbers were allotted.

Colony character, pigmentation, growth rate, spore type, size and germination in *Drechslera* sp. isolates causing leaf blight disease in poplars were studied on four growth media (PDA, CDA, MEA and SPA). Diversity of the isolates of *Phyllosticta/Phoma* sp. causing foliar diseases in poplars was studied through various cultural and morphological parameters namely colony type, colony colour, pigmentation, growth rate, spore type and size and germination.

#### **Bioactive Principles in *Cordyceps sinensis***

Different isolates of *Cordyceps sinensis* were grown in Jhingora as per the protocol developed by FRI Dehradun. Twenty five isolates were powdered in liquid nitrogen after the growth of 6 months and were analyzed for their bioactive principles by HPTLC for the presence of cordycepin, adenosine and ergosterol contents. Presence of these bioactive principles indicates the medicinal value of the isolates.

#### **Wood Decay in Stored Tropical Timber**

Seven hundred forty five specimens of wood decaying fungi were collected on wood logs of 34 host species from 12 wood depots of Maharashtra and 25 wood depots of Orissa. Twenty genera and 44 species of wood decaying fungi were identified. Of these, 4 genera viz. *Hapalopilus*, *Ceriporiopsis*, *Schizophora*, and *Postia*; and 7 species: i.e. *Hapalopilus nidulans*, *Ceriporiopsis merulinus*, *Trametes ochraceae*, *Postia placenta*, *Schizophora paradoxa*, *Pycnoporus coccineus* and *Pycnoporus cinnabarinus* were recorded for the first time from

Orissa and Maharashtra on sal and bijasal. Twenty five cultures were also maintained for further studies. An experiment was conducted to test toxicity (*in-vitro*) at different concentration of urea and zinc sulphate against decay resistance of *Flavodon flavus*.

#### **Marine Lignicolous Fungi in Traditional Wooden Craft**

Fungal infested timber from catamarans made of *Anogeissus acuminata* was collected from the fishing villages, namely, Pedajalaripeta and Bhimunipatnam in Visakhapatnam district. Fungi in the samples collected were cultured and 13 isolates separated. Timber wafers of *Anogeissus acuminata* and *Mangifera indica* were treated with Copper Chrome Arsenic wood preservative to different gradients of absorptions and exposed to individual fungal isolates for their infesting activity. Among various fungi, *Aspergillus flavus*, *Curvularia* sp. and *Fusarium* sp. were found to be infesting the wafers even at a preservative retention of  $27.6 \text{ kg m}^{-3}$ .

#### **Control**

#### **Chemical Control of Diseases of *Buchanania lanza***

Out of 6 treatments viz. dithane 0.1%, 0.2%, bavistin 0.1%, 0.2%, redomil 0.1% & 0.2%, foliar spraying of redomil 0.2% followed by redomil 0.1% proved highly effective against wilt diseases caused by *Fusarium oxysporum* in nursery stage.

#### **Biological Control and Botanical Fungitoxicants**

Bagasse formulation of *Trichoderma piluliferum* and *T. viride* significantly increased the root biomass in treated *Asparagus racemosus* plants and controlled the root diseases.

Isolation of 15 antagonistic bacteria, a fungus and an actinomycete from the soil samples were collected from different localities of



Madhya Pradesh and Chhattisgarh. Identification and pathogenicity tests were conducted on plants of *Withania somnifera*, *Rauvolfia serpentina* and *Chlorophytum borivillianum*. Bioassay test against four fungal pathogens were confirmed with antagonistic microorganisms isolated from soils of different nurseries and with biopesticide (cow urine+*Azadirachta indica* leaves+*Ailanthus excelsa* leaves+*Calotropis procera* leaves). Four antagonistic bacteria/actinomycetes showed their effectiveness in controlling growth of pathogen *in vitro*.

Samples of *Balanites aegyptica* (root, leaves and bark), *Tephrosia purpurea* (leaves, root, seed), *Citrulus colocynthis* (leaves) were collected from the vicinity of Jodhpur (Rajasthan) and shade dried for preparation of ethanol and water extract. The extracts were used @ 50mg/ml/solvent for further studies. The pure culture of the fungal pathogens viz. *Rhizoctonia solani*, *R. bataticola*, *Fusarium solani* and *Alternaria alternata* were procured from Forest Pathology Division, FRI, Dehradun and maintained. The ethanolic extracts were tested against the four test fungi. Ethanolic extract of *Citulus colocynthis* (leaves) was found effective in inhibiting the growth of *Fusarium solani*.

Methanol extract of *Sapindus mukorossi* (Gyarahdevi) exhibited  $IC_{50}$  at all the concentrations against all the test fungi barring *Alternaria* sp., *Fusarium oxysporum* and *Colletotrichum gloeosporioides* ( $IC_{50}$  2.0%). Butanol extract of Gyarahdevi exhibited  $IC_{50}$  at all the concentrations against all the test fungi barring *Alternaria* sp. and *F. oxysporum*.

Volatile effect of citronella oil, garlic oil, lemongrass oil and ajwain oil to inhibit growth and germination of spoilage fungi in stored medicinal plant produce was successfully established. Volatile effect of combination of oils was also tested against spoilage fungi during

storage of medicinal plant produce. All combinations of oils were found effective against all spoilage fungi for *Withania somnifera* stored roots. Combination of lemon grass and eucalyptus oils and citronella and eucalyptus oils were not effective in checking fungal infestation in *Stevia rebaudiana* stored leaves.

### 2.7.3 Mycorrhizae, Rhizobia and other Useful Microbes

#### Ectomycorrhizal (ECM) Fungi

Standardized suitable culture medium, ideal pH and temperature conditions for mass production of different isolates of two ECM fungi, *L. fraterna* and *P. albus* under *in-vitro* condition. Further the study on identification of suitable type of inoculum for growth improvement of the targeted tree species in nursery revealed that vegetative mycelial inoculum of *P. albus* isolate 3 for *A. auriculiformis*, *C. equisetifolia* and *C. junghuhniana* and *P. albus* isolates 2 and 4 for *A. mangium* was found suitable. Whereas the basidiospore inoculum of *P. albus* isolate 3 was found suitable for *E. camaldulensis* and *E. tereticornis*. Nodule population was found higher in seedlings grown in sterilized potting medium inoculated with vegetative mycelial inoculum of *P. albus* (Isolate 3), followed by same type of inoculum of *P. albus* (Isolates 1 and 2) in Casuarinas. While auscularlly the types of ECM inoculum showed good number of ECM colonized roots (myco tips), interestingly the basidiospore inoculum of *P. albus* (Isolate 3) exhibited significantly very high myco tips in all the tree species.

Diversity of mycorrhizal associations with *Dipterocarpus* and *Shorea* species in Assam. Ectomycorrhizal fungi and rhizosphere soil along with mycorrhizal roots of *Dipterocarpus retusus*, *Shorea robusta* and *S. assamica* were collected and photographed in nature. Morphological



characters of fruit bodies like colour, shape, size, odour and number of gills per centimeter were recorded in field. Five different ectomycorrhizal fungi were recorded and out of which three were identified as *Russula*, *Lactarius* and *Amanita* sp. *Russula* was dominant species and reported to be associated with all selected species.

### Arbuscular Mycorrhizal (AM) Fungi

Rhizosphere soils were analyzed and *Glomus* species was found to be most dominant strain among the endomycorrhizal association with selected plant species. However, percent infection of *Glomus* species was very less. All species were preserved as wet and dried for future study and reference. Pure cultures of associated fungi were raised on potato dextrose agar and are being maintained in laboratory.

Surveys were carried out in Nongkhylliem Reserve Forest, Nongpoh, Meghalaya (Umtasor and Nongpoh) for collecting rhizospheric soil samples. A total of 80 rhizospheric soil samples from different plant species were collected and screened for endomycorrhizal qualitative and quantitative analysis. The two dominant and efficient endo-mycorrhizal strains (*Glomus* sp. and *Gigaspora* sp.) which were prevalent in these forest soils were isolated and are under further process for inoculum production and mass multiplication trial in lab.

Roots and rhizosphere soil samples collected from the root zone of native tree species such as *Ailanthus excelsa*, *A. triphysa*, *Neolamarckia cadamba*, *Gmelina arborea*, *Melia dubia* and *Dalbergia latifolia* in different parts of Tamil Nadu and Kerala were processed and estimated percent root colonization and soil spore population of Arbuscular Mycorrhizal (AM) fungi. Twenty seven different AM fungi belonging to three genera such as *Acaulospora*, *Gigaspora* and *Glomus* were recorded from the

rhizosphere of different native tree species. Among them, the genus *Glomus* was found dominant and followed by *Acaulospora*.

Survey was carried out for seeing the association of AM fungi for *Acacia auriculiformis*, *Tectona grandis*, *Casuarina equisetifolia* and *Eucalyptus camaldulensis* plants. Results showed high level of mycotrophy with AM fungi in all selected species Altogether 10 species of fungi belonging to 5 genera were recorded. Predominant species were *Gigaspora*, *Glomus* and *Sclerocystis* in Shimoga area and only *Gigaspora* and *Glomus* species were predominant in Sravanabelogola and Doddaballapura area. Efficacy of treatments with AM inoculum in nursery experiments revealed that if inoculum is provided at seedling stage, the growth of plants can be boosted tremendously. Overall, fortification of the root zone with AM fungi has yielded improved growth, biomass and survival of seedlings, and seedling quality index. The treatment is very economical and eco-friendly.

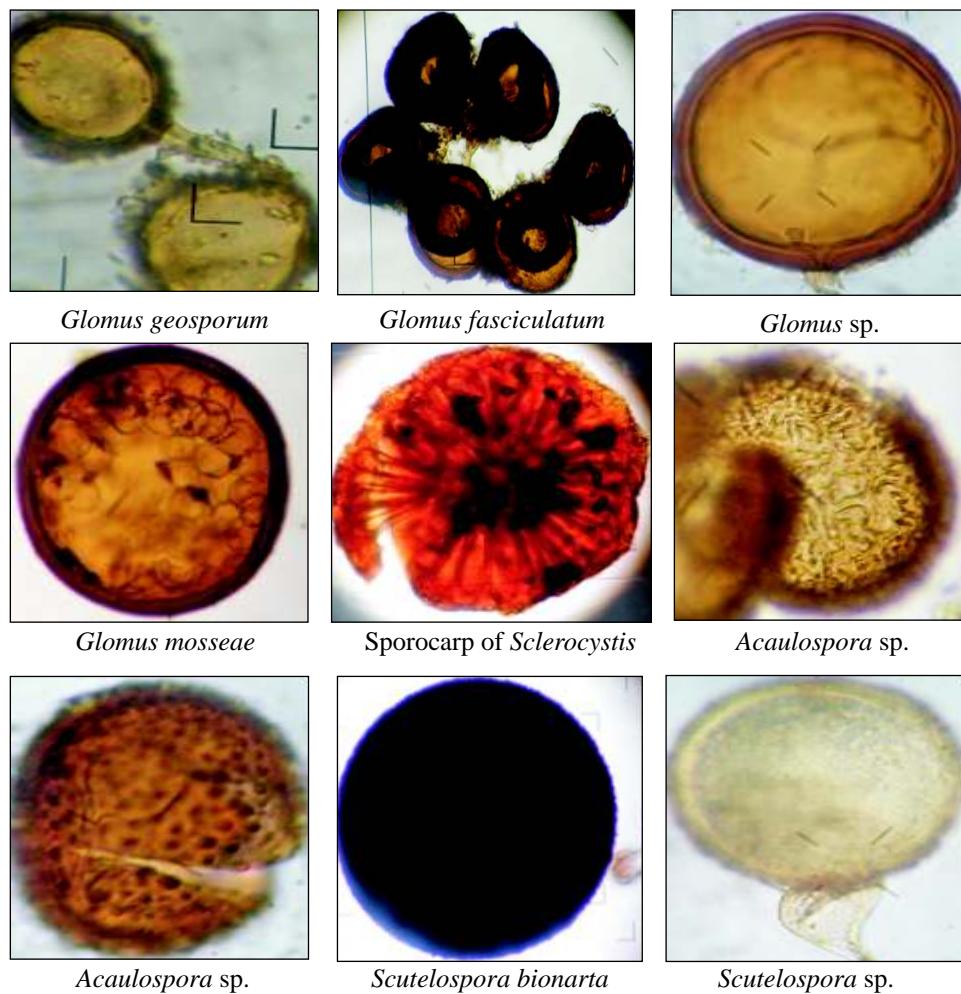
Five AMF of mehndi genera such as *Glomus*, *Gigaspora*, *Scutellospora*, *Sclerocystis* and *Acaulospora* and among the species of *Glomus* viz., *G. fasciculatum*, *G. aggregatum*, *G. mosseae*, *G. macrocarpum*, *G. microcarpum*, *G. intraradices*, *G. reticulatum*, *G. constrictum*, *G. multicaulae*, *G. geosporum* and *G. convolvulus* were recorded. *Glomus multicaulae*, first time reported on mehndi from Pushkar and Ajmer. *Sclerocystis indica* was recorded on mehndi and ashwagandha from Nagaur and Pushkar (Ajmer), respectively. The distribution of different AM species viz., *G. aggregatum* (35%), *G. mosseae* (15%), *G. fasciculatum* (20%), *G. macrocarpum* (10%), *Glomus* sp. (15%), *Scutellospora* (3%) and *Acaulospora* (2%) were recorded in ashwagandha, whereas, in mehndi, distribution of AM fungi recorded as *G. fasciculatum* (55%),



*G. aggregatum* (20%), *G. macrocarpum* (12%), *Glomus* sp. (8%), *Acaulospora* (5%), *Scutellospora* (3%) and *Sclerocystis* (2%) with the dominancy of *G. fasciculatum*. The maximum root infection was recorded (85%) from Sojat (Pali) and minimum (46%) from Pushkar, Ajmer. While, in ashwagandha, maximum root colonization (78%) was recorded from Harima Krihifarm (Nagaur) and minimum (34%) from Ajmer. Root infection was recorded 54 per cent, while, AM spores were found 210/100 gm soil. Both the species viz mehndi and ashwagandha were found highly mycorrhizal in nature. The root infection was found in the form of intercellular, intracellular hyphae, vesicles and arbuscular structures in the

roots. In VAM inoculation experiments, the maximum (53.6cms) shoot length was recorded in seedlings inoculated with pure culture of *G. fasciculatum*. However, all the treatments were found effective in all parameters i.e., enhancing shoot height, basal stem diameter, number of spores/10gm of soil and percentage of infection in mehndi, whereas, in case of ashwagandha, the maximum shoot height, basal stem diameter was 70.5 cms and 10.5 mm, respectively in seedlings inoculated with consortium inoculums with dominancy of *G. aggregatum*. No. of spores/10gm of soil and percentage of infection were also recorded high as compared to other treatments.

### AM Spores Collected from Mehndi & Ashwagandha





### Microbial Inoculants

The Plant Growth Promoting Rhizobacterias (PGPRs) such as *Bacillus megaterium*, *Pseudomonas fluorescens*, *Azotobacter chroococum* and *Azospirillum brasiliense* were tested on rootings of 30 Eucalyptus clones under nursery conditions. The PGPRs increased the rooting and growth of Eucalyptus cuttings equivalent to IBA treated cuttings. The clones C-14 and C-111 responded better for rooting when the cuttings were inoculated with *Pseudomonas fluorescens* or *Azotabacter chroococum* at the rate of 5 ml/cutting as compared to IBA (4000 ppm) treated cuttings.

Plant Growth Promoting Rhizobacterial (PGPRs) isolates were isolated from the soil samples collected from native tree species viz., *Ailanthus excelsa* (69 isolates), *A. malabaricum* (12 isolates.), *Anthocephalus cadamba* (33 isolates), *Gmelina arborea* (45 isolates), *Melia dubia* (28 isolates) and *Dalbergia latifolia* (27 isolates) and maintained in laboratory for further biochemical tests.

Population density of the PGPRs isolated from various soil samples of the selected native tree species was also estimated. Among them, the samples collected under *N. cadamba* trees had maximum colonies than other tree species.

Species level identification of the genera such as *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* isolated from the samples of *Ailanthus. excelsa*, *A. triphysa* and *Neolamarckia cadamba* were done. Identification of the PGPRs isolated from the samples of *G. arborea*, *M. dubia* and *D. latifolia* is under progress.

*Frankia* is the Nitrogen fixing actinomycete and particularly associated with *Casuarina* species. Six strains (Cj Cbe1, Cjkl, CjN1, Ce Py1, CePy2, CeSc1) of *Frankia* were isolated from the nodules of *Casuarina equisetifolia* and *C. junghuhniana* and were grown in artificial medium. The strain CjCbE1 exhibited better performance with the seedlings and cuttings of *Casuarinas* spp. as it produced higher nodule numbers (7/cutting) and nodule weight (55mg/nodule). Normally the cuttings of Casuarinas raised in inert media do not show root nodules. However, in the present study the nodulation was achieved in the cuttings of *Casuarina* spp by artificial inoculation of *Frankia* in the inert media.

Germplasm of growth promoting organisms were collected from 11 different localities of M.P. Experiments on *Gmelina arborea*, *D.sissoo* and *J.curcas* were conducted in root trainers and growth promoting organisms were selected for these species. Twenty different fungi isolated from rhizosphere soil were screened for plant growth promoting properties (germination% and height). Of them, *Aspergillus* sp. and four *Trichoderma* spp., were found growth promoting and selected for use in nursery. Application of plant growth promoting microbes (AM fungi, *Aspergillus* sp. and *Azospirillum*) and soil amendments (mix of *Lucaena*, teak leaves and FYM in equal ratio, v/v) increased 10-20% survival of sandal seedlings. When out planted in the field, it also increased plant height up to 43%.