

## 2.7 Forest Protection

### Overview

Insect pests and disease problems of forest plant species are the major deterrents of productivity on the one hand and on the other, affect the long term programme of afforestation and environment amelioration. Research in ICFRE institutes is mainly centred on biology of insect pests and pathogens, integrated pest management, host plant resistance against insect pests and pathogens, biological control and use of beneficial micro-organisms for enhancement of productivity.

### Summary of the Achievements Under the Theme

#### Insect/Marine Pests

- Life table and behavioural mechanisms involved in oviposition by *L. invasa* was studied for 3 generations in Coimbatore. The total life cycle period from egg to adult was recorded as 125-130 days.
- Biology and parasitisation efficiency of parasitoids of the gall insect *Leptocybe invasa* viz. *Megastigmus* sp. and *Quadrasitichus mendelli* were studied.
- Infestation of *Indarbela quadrinotata*, a pest of *Casuarina equisetifolia*, was the highest in the North-eastern agro-climatic zone of Tamil Nadu. There was no significant reduction in growth of the trees due to the pest attack during the period of one year. Pungam (*Pongamia pinnata*) oil and Jatropha (*Jatropha curcas*) oil were promising.



A Native Parasitoid *Megastigmus* sp. Recorded on Eucalyptus Gall Wasp *Leptocybe invasa*

- The potential biopesticidal fractions were extracted, characterized and identified from *Aegle marmelos* seed oil and tested against teak insect pest *Hyblaea puera* at Nilambur and Kulathupuzha nurseries in Kerala.
- A total 197 clones of *Eucalyptus* were assessed for their susceptibility and resistance/tolerance against gall insect in a field trial at Satyavedu.
- *Pongamia pinnata* seeds during post harvest storage were attacked by unidentified lepidopteran species which caused higher damage than that of an unidentified bruchid beetle. Methyl parathion protected seeds for a longer period and pest mortality was higher than the plant based extract treated seed lots.
- Bio-efficacy of chemical pesticides (Quinalphos, Imidacloprid and Dimethoate) and botanicals (neem oil, jatropha oil and pongamia oil) and a commercial neem product (Neemazal 1% ) were tested on the targetted pests of *Ailanthus excelsa* and *Gmelina arborea* and their effective doses for control of the pests were determined.
- A new sandal seed borer *Araecerus fasciculatus* Linn.(Anthribidae: Coleoptera) was found to cause serious damage to sandal seeds. The overall percentage of damage stood at 20%. Two new weevils viz, *Peltotrachelus cognatus* Faust and *Myloccerus delicatulus* Boheman were found as defoliators of sandal.
- Mangrove plants *Excoecaria agallocha*, *Avicennia officianalis* and *A. marina* in Godavari, Krishna and Sarada-Varaha deltas were found to be infested by several marine wood boring pests including certain rare pholadids, namely, *Lignopholas chengi*, *L. rivicola* and *L. fluminalis*.
- Test timbers of *Bombax ceiba* and *Paraserianthes falcataria* treated with



TBTM-MMA compound were found to have curtailed fouling growth and wood borer attack to a great extent even after 30 months of marine exposure trials at Vishakhapatnam and Kochi harbours but for sphaeromatid borer invasion of a few panels at Kochi.

- Bio-deterioration studies at Machilipatnam and Nizampatnam ports in Andhra Pradesh on marine materials, including wood showed that the two environs are relatively safe from biofouling point of view, but are potentially hazardous from the view point of marine wood borer activity that exercises a great bearing on fishing operations in these regions.
- Integrated management package for white grubs in teak nursery at Kanchangaon, Mohagaon Project Division, Mandla (Madhya Pradesh Forest Development Corporation Ltd.) was developed.
- Insecticidal properties of some plant extracts (*Azadirachta indica*, *Melia azedarach*, *Acorus calamus*, *Adhatoda vesica* and *Clerodendron viscosum*) were evaluated against *Hertia vitessoides* Moore (Lep: Pyralidae), a major pest of *Aquilaria malaccensis* Lamk.
- Potential insect pests of selected Bamboo species in Assam were recorded.
- Sixteen species of insects and 2 species of mites have been documented on *Acacia nilotica*.
- Major biotic factors responsible for khejri mortality were found to be *Ganoderma lucidum* and *Acanthophorus serraticornis*. The khejri mortality percentage varied from 18 to 23%. Maximum mortality was noted in Nagaur and minimum in Jhunjhunu district.
- Pheromone traps were used to manage *Ips longifolia* Steb. in Chir pine Forests in Himachal Pradesh. Doses of pheromone were standardized.
- Time-mortality bioassay using 7 doses of Baculovirus were applied on 3<sup>rd</sup> instar larvae of *Lymantria obfuscata*. LT<sub>50</sub> for the doses, were calculated.
- Ten spots colony of the spider were recorded and geo referenced in Himachal Pradesh. Ten complete mature colony of the spider were collected and reared in the laboratory. Seventeen prey species belonging to 6 families under 4 orders were recorded.
- *Cateremna tuberculosa* has been reported to be as a pest of stored seeds of Chilgoza pine.
- Biology of *Phloeobius crassicollis* was studied. Insect was reported as new pest of ten bamboo species on which average attack percentage was recorded from 2 to 51% with maximum attack of 51% in *Bambusa wamin*.
- Biology of *Aphrodisium hardwickianum* white was studied. *Quercus dilatata* was also found to be attacked by this beetle. Para-dichlorobenzene in kerosene oil @ 10 ml / emergence hole was found to be most effective measure. Mechanical control by hammering stone into the exit hole for adult was also effective.
- Bioassay of various extracts of aerial plant parts of *Calotropis procera* and *Plumbago zeylanica* were tested against larvae of shisham defoliator, *Plecoptera reflexa* and poplar defoliator, *Clostera cupreata*. 2% methanol and acetone extracts gave about 60-70% mortality after 72 hrs.
- A new biopesticidal product “Vilvekam-Aegle marmelos seed oil based biopesticide” was developed, and released.

#### Diseases, Pathogens and Beneficial Microbes

- A total of 222 fungal isolates of 40 genera were isolated from the fruits and seeds of *Elaeocarpus munronii*, *Dysoxylum malabaricum*, *Dipterocarpus indicus*, *Vateria*



*indica*, *Garcinia gummigatta*, *Poeciloneuron indicum*, *Syzygium malabaricum*, *Myristica malabarica*, *Knema attenuata*, *Madhuca longifolia*, *Hopea ponga*, *Kingiodendron pinnatum* and *Cinnamomum sulphuratum*.

- Natural fungal flora of seeds of *D. malabaricum*, *E. munronii*, *M. malabarica*, *P. indicum* and *V. indica*, being pathogenic on seeds and seedlings was proved.
- A new fungal species viz., *Penicilloipsis indcus* was identified from the seeds of *D. malabaricum*. New host records of fungi i.e., *Beltrania rhombica* on seeds of *P. indicum* and *Bartalinia lateripes* on seeds of *G. gummigatta* were also reported.
- Control of forest seed pathogenic fungi has been achieved by using the leaf and bark extracts of *Prosopis juliflora* and *Cleistanthus*.
- The cause of culm rot and bamboo blight disease in Assam was identified as *Fusarium udum*. The most effective fungicides found *in-vitro* are being tried for its management.
- Seven species of fungi belonging to different genera were isolated from canker disease of rohida (*Tecomella undulate*) and established cultures in laboratory for pathogenicity test.
- Major biotic factor responsible for khejri mortality was found to be *Ganoderma lucidum* and *Acanthophorus serraticornis*. The khejri mortality percentage varied from 18.08 to 22.67 %. Maximum mortality was noted in Nagaur and minimum in Jhunjhunu district.
- *Cylindrocladium quinqueseptatum* was isolated from 28 samples out of 68 samples collected from Uttar Pradesh. The artificial inoculation and disease development protocols have also been developed.
- Cultures of fungal pathogens of poplar are regularly maintained, viz., *Alternaria*, *Botryodiplodia*, *Curvularia*, *Drechslera*, *Phoma/Phyllosticta* and *Sclerotium*.
- Diversity of nine isolates of *Curvularia* sp., causing leaf spot disease in poplars and their management using four different media such as PDA, CDA, MEA and SA was studied.
- Clones of *Casuarina equisetifolia* (250 no.) screened for infection of *Subramanianospora vesiculosa* (blister bark disease) under controlled condition revealed that 36 clones were resistant and having higher phenolic content.
- Morphological variations were studied for the four virulent isolates of *Fusarium solani* f. sp. *dalbergiae*, the causal organism of wilt disease of *Dalbergia sissoo*. Their mass cultures were prepared in broth for artificial inoculation on promising germplasm.
- Seventeen clones procured from G&TP Division, FRI were artificially injected with conidial suspension of the 4 strains and observations for disease expression and extent are being made.
- Effective antagonistic species of *Trichoderma* against the four pathogen strains of *Fusarium solani* f. sp. *dalbergiae* were tested and found to cause mycoparasitism by hyphal lysis and coiling.
- *Trichoderma viride* formulation was found significantly superior to all the treatments and control in increasing the number and biomass of leaves of *Stevia rebaudiana*. In *Asparagus racemosus*, *Trichoderma piluliferum* and *T. viride* were significantly superior to other treatments and control in increasing the root biomass.
- Natural decay resistance of imported woods was tested against *Trametes versicolor*, a white rot fungus suggested that teak wood from Ghana was resistant to decay.
- DNA finger printing using RFLP-PCR was done for twenty promising clones of *D. sissoo*.



- RAPD-PCR was conducted with isolates of *Cordyceps sinensis*, collected from different locations in Uttarakhand for studying the variability among the isolates. Nine population lines were identified.
- Internal transcribed region of nrDNA (ITS regions) were amplified and sequenced for the authentic identification of the isolates of *Cordyceps sinensis*. Blast search results affirmed their identity as *Cordyceps sinensis* (the new name is *Ophiocordyceps sinensis*).
- For the first time, beta tubulin gene region of *Cordyceps sinensis* has been sequenced, studied and deposited in gene bank.
- Cordycepin, ergosterol and adenosine was detected in some of the *Cordyceps sinensis* isolates, collected from different geographical locations in Uttarakhand. Some of the bioactive principles were found to be in a higher quantity in the cultivated *Cordyceps sinensis* in comparison to the wild.
- A new protocol for artificial cultivation of fruiting bodies of *G. lucidum* was developed using used tea leaves-wheat straw spawn, poplar branch billets and by creating a low-cost mist chamber. The fruiting bodies were harvested within 90 days which is the shortest reported time in its artificial production.
- For extending the results to the user groups, a one-day training programme on cultivation of *Ganoderma lucidum* was conducted on 11<sup>th</sup> March 2011. Local mushroom growers and farmers (21 No.) were given demonstration and hands-on training in glasshouse of Forest Pathology Division, FRI, Dehradun.
- Ten strains of *Frankia* were isolated and identified from the rhizospheres of casuarinas in Tamil Nadu.
- Suitable and effective forms of ectomycorrhizal (ECM) fungal inoculum for growth improvement of seedlings of commercially important plantation tree species like *Acacia auriculiformis*, *Acacia mangium*, *Casuarina equisetifolia*, *C. junghuhniana*, *Eucalyptus camaldulensis* and *E. tereticornis* were identified.
- Standardized suitable culture medium and ideal pH and temperature conditions for mass production of different isolates of ECM fungi (*Laccaria fraterna* and *Pisolithus albus*) under *in-vitro* conditions.
- Diversity of mycorrhizal associations with *Dipterocarpus* and *Shorea* species in Assam were assessed.
- The effect of Arbuscular Mycorrhizal was studied on the growth performance of *Mesua ferrea* L. and *Aquilaria agallocha* Roxb.
- Antifungal properties against *Rhizoctonia bataticola* and *Fusarium solani* was found in *Citrullus colocynthis* and against *Alternaria alternata* in *Datura stramonium*.
- Qualitative and Quantitative phosphate solubilizing, siderophore and HCN production characteristics of 68 isolates of fluorescent pseudomonads from rhizosphere soil of G-48 clone of *Populus deltoides* were studied.
- As an alternate to the application of chemical hormones (IBA), for induction of rooting and nutrient enhancement in *Eucalyptus* clones beneficial microbial inoculants like *Azospirillum*, *Pseudomonas*, *Bacillus* and *Azotobacter* were applied and effective rooting and nutrient enhancement achieved.
- Isolates of 216 Plant Growth Promoting Rhizobacteria (PGPR) and 26 different AM fungi were isolated and identified from the rhizosphere samples of 6 different fast growing native tree species of Tamil Nadu and Kerala.



- Nucleotide Sequences were constructed for different isolates of PGPR isolated from the rhizosphere of fast growing native tree species and the same have been submitted to European Molecular Biology Laboratory (EMBL) and NCBI Database.
- Efficacy of PGPRs for production of IAA and Phosphate solubilization was determined.
- Molecular characterization of Phosphate Solubilizing Bacteria (PSB), isolated from the *Ailanthus excelsa* rhizosphere samples was done and identified as *Bacillus megaterium*.
- One-day interactive meeting for developing a network on shisham mortality was organized on Sep. 2011, which was attended by scientists from HAU, Hissar; Dr. Y S Parmar University of For. & Hort., Nauni, Solan; G. B. Pant Univ. of Agr. & Tech., Pantnagar; ICFRE Institutes (Jodhpur, Jorhat, Jabalpur, Ranchi and Allahabad); Forest Officers from Haryana, Punjab, Uttarakhand and Uttar Pradesh forest departments and Scientists of concerned Divisions of FRI.

### Projects under the Theme

Projects	Completed Projects	Ongoing Projects	New Projects Initiated During the Year
Plan	12	22	23
Externally Aided	7	5	1
<b>Total</b>	<b>19</b>	<b>27</b>	<b>24</b>

## 2.7.2 Insect Pests, Diseases and Control

### Insect Pests

#### Insect Pest Surveys, Incidence and Biology

##### Insect Pests of Bamboo

*Phloeobius crassicollis* was reported as new pest on the following ten bamboo species in and around Dehradun: *Bambusa bamboos*, *B. nutans*, *B. polymorpha*, *B. tulda*, *B. vulgaris*,

*B. wamin*, *Dendrocalamus giganteus*, *D. strictus*, *D. calostachyus* and *Gigantochloa atroviolacea*. Average attack percentage on these species was recorded from 2 to 51% with maximum attack of 51% in *Bambusa wamin*.

From Assam, 23 insect pests belonging to the various orders of Lepidoptera, Orthoptera, Coleoptera were recorded infesting following bamboo species: *Bambusa tulda*, *B. balcooa*, *B. pallida* and *B. nutans*. Based on the incidence and intensity of insect pest attack, *Antonina* sp., *Psara licarsisalis*, *Crocidophora* sp., *Hexacentrus unicolor* (Tettigoniidae) and *Oxya nitidula* (Walk) (Acrididae) were categorized as major pests, causing serious damage. During the survey, the natural enemies like entomopathogenic fungus, *Beauveria bassiana*, was collected from *Crocidophora* sp.; two different predatory spiders (*Oxyopes* sp.) on the larvae of *Crocidophora* sp., and *Psara licarsisalis* were also recorded. Paint brush swift *Baoris farri* (Moore) was also recorded as new host on *Bambusa tulda*.

##### Field Manual of Insect Pests

Insect pests of *Dalbergia sissoo* and *Populus deltoides* were collected and photographed for preparation of the field manuals.

##### Biology of *Leptocybe invasa*

Life table and behavioural mechanisms involved in oviposition by *L. invasa* was studied for 3 generations in Coimbatore. The total life cycle period from egg to adult was recorded as 125-130 days. Trial with 2 coloured sticky traps deployed in 3 nurseries for 2 seasons showed that adult wasps preferred yellow colour.

##### Insect Pests of Sandal

Five silvi-horticultural models of sandal situated in Bevanahalli, Muddannahalli, Gottipura, Yelwala (Mysore) and Jarackbandae were continuously surveyed for insect pests. It was observed that insect pests problems were



more and diseases were less on sandal. Coccids (sap-suckers) were economically more important pest as was than any other insect pest. Three new stem borers were recorded during the study viz *Purpuricenus sanguinolentus* Olivier and *Exocentus* sp. and *Derolus volvulus*. The stem borer *Purpuricenus sanguinolentus* was found to cause extensive damage to sandal plants. A new sandal seed borer *Araecerus fasciculatus* Linn.(Anthribidae: Coleoptera) was found to cause serious damage to sandal seeds whose overall damage stood at 20 %. Two new weevils defoliators were also recorded viz. *Peltotrachelus cognatus* Faust and *Myllocerus delicatulus* Boheman. Three new lepidopterous defoliators recorded were *Amata passalis* (Artiidae), *Micronia aculeate* (Uraniidae) and *Parallelia* sp. (Noctuidae). One grasshopper species, *Crotogonus* sp., was observed on all the silvi- horti models of sandal. Pollen feeders collected from sandal plants were identified as *Oxycetonia versicolor* and *O. juncunda*. Nine new parasites collected on coccids of sandal have been identified and documented. Check-list on the entomofauna of sandal was also updated.

#### **Insect pests of *Emblica officinalis*, *Pongamia pinnata*, *Garcinia* sp. and *Dysoxylum* sp.**

Nearly 35% gall infestation and 100% defoliation was observed in *Pongamia pinnata* seedlings along with scale insect. *Emblica officinalis* seedlings were found to be completely defoliated by *Meconellicoccus hirsutus* in Sulikere nursery and Lakkunda nursery. At the latter site 20% infestation was seen in *Garcinia*, and 15% in *Dysoxylum* seedlings.

#### **Fouling Organisms**

*Thais blanfordi* (Melvill), a gastropod, was found to be associated with fouling assemblages for the first time in the country at Machilipatnam port. Recruitment of fouling organisms on rubber, asbestos, cement, asphalt, FRP, PVC and brass

showed that different forms preferred different substrata for settlement.

#### **Marine Borers in the Mangrove Habitats of Northern Andhra Pradesh**

Rare pholadids, namely, *Lignopholas chengi*, *L. rivicola* and *L. fluminalis* were recorded from the mangroves in the Godavari delta. Mangrove habitats in Srikakulam, Visakhapatnam, East Godavari and Krishna districts were surveyed and damage caused to vegetation was assessed. Mangrove plants such as *Excoecaria agallocha*, *Avicennia officianalis*, *A. marina* were found to be infested by several species of marine wood boring organisms. Important pest species were recognized to be *Sphaeroma terebrans* (Pillbugs), *Martesia striata* (Piddocks), *Lyrodus pedicellatus*, *Nototeredo knoxi*, *Nausitora hedleyi*, *Bankia campanellata*, *B. destructa* and *B. philippinensis*.

#### **Control**

#### **Chemical**

#### **Eucalyptus Gall Wasp, *Leptocybe invasa***

Screening of 2 plant based extracts (Citronella oil, Neem oil), and some pesticides (Acetamiprid, Imidacloprid and Methyl parathion) through foliar and soil application showed that incidence of galls was reduced with the application of pesticides. In order to standardize preventive methods of management of gall wasps, comparison of gall resistance with and without susceptible clones were attempted and found that some resistant clones showed gall incidence from 10- 30%.

#### ***Phloeobius crassicornis***

*Phloeobius crassicornis* is a major pest of bamboos. Newly emerged beetle feed on the outer surface of the bamboo culm, preferably at the nodes, during May-June and damages the new culm. Chlorpyrifos at 0.04% and 0.05% concentration gave 78 and 81% control, respectively.



### **Oak Stem Borer, *Aphrodisium hardwickianum* White (Coleoptera: Cerambycidae)**

Monocrotophos (36 EC) and dimethoate (30 EC) at concentrations of 0.4% each and 5-10 ml of fumigant (saturated solution of para-dichlorobenzene in kerosene oil per emergence hole) were found to be the most effective measure. Mechanical control by hammering stone into the exit hole for adult was also effective.

### **Insect Pests of *Ailanthus excelsa* (*Atteva fabriciella* and *Eligma narcissus*) and *Gmelina arborea* (*Tingis beesoni*)**

Quinalphos, Imidacloprid, Dimethoate at 0.025 to 0.05% were effective in controlling these pests.

### **Integrated Management Package**

Package on white grubs in teak nursery was developed for Madhya Pradesh Forest Development Corporation Ltd. In this package different activities like installation of light trap unit, different dates of sowing of teak seeds, pruning of host trees around the nurseries, application of biopesticides cakes (neem and jatropa) and prophylactic / curative treatment of phorate 100 gm per bed (size 10x1m), synthetic pyrethroids i.e. deltamethrin 0.005% followed by alphamethrin 0.01% was recommended against these key insect pests.

### **Efficacy of TBTM-MMA Preservative Against Fouling Organisms**

In continuously exposure trials of TBTM-MMA treated test panels of *Bombax ceiba* and *Paraserianthes falcataria* for the last 30 months at Visakhapatnam and Kochi harbours, it was found that monthly recruitment of fouling organisms was negligible in treated panels, whereas, untreated panels (controls) showed higher biomass loads. All treated panels at Visakhapatnam remained free from any wood borer attack throughout the period but a few treated panels at Kochi were attacked by sphaeromatid wood borers.

### **Biocontrol**

#### **Eucalyptus Gall Wasp, *Leptocybe invasa***

Culture of Eucalyptus gall wasp population and the parasites *Megastigmus* sp. and *Quadrastichus mendelli* in nursery plant were raised on Eucalyptus clones in breeding chamber. Studies on the biology and parasitisation efficiency of *Megastigmus* sp. and *Quadrastichus mendelli* showed that *Megastigmus* sp. took approximately 40-45 days and *Quadrastichus mendelli* took 28-32 days to complete the life cycle. The parasitisation efficiency of *Quadrastichus mendelli* was found higher than that of *Megastigmus* sp.

#### **Oak Stem Borer, *Aphrodisium hardwickianum* White (Coleoptera: Cerambycidae)**

Following natural enemies of the borer were recorded (a) Insects: an undetermined Elatrid beetle (b) Birds (i) Himalayan Woodpecker, *Dendrocopos himalayensis*, (ii) Brown-fronted Woodpecker, *Dendrocopos auriceps*, (iii) Scaly-bellied Woodpecker, *Picus squamatus* and (iv) Greater Yellownape and *Picus flavinucha*.

#### **New Biocontrol Opportunities for Prickly Acacia and *Acacia nilotica***

*Anamolococcus indicus* (sap.-sucker) *Physcita* sp. (leaf weber) and *Deoderus* sp. (beetle) were found to be the potential candidates for biological control of *A. nilotica*. Based on the elaborate host specificity studies under lab and nursery conditions, involving 9 species of Acacias, these insects were screened.

#### **Microsporidia as Biocontrol Agents**

A total of 94 lepidopterans were tested and microsporidian parasites were isolated from 29 species. Morphometry of 29 species of microsporidia were studied. Bio-assay study using microsporidian parasites was carried out on larvae of *Hyblaea puera*, *Catopsilia* sp., *Papilio demoleus* and *P. polytes* by inoculating different concentrations of spores isolated from their respective hosts.



### **Braconid Parasitoids (Hymenoptera: Braconidae) from Central India**

Extensive survey for the collection of Braconid parasitoids and their host insects was carried out in Chhattisgarh (185 localities of 16 districts) and Maharashtra (385 localities of 34 districts). Twelve new species (*Apanteles lakhaensis*; *Apanteles neocajani*; *Apanteles neohyblaeae*; *Apanteles neotaeniaticornis* and *Rogas jalnaensis*, *Anisocyrta gilvicorpa* sp. nov.; *Bracon jalgaonensis* sp. nov.; *Chelonus (Chelonus) wardhaensis* sp. nov.; *Chelonus (Microchelonus) hingoliensis* sp. nov.; *Doryctes indicus* sp. nov.; *Parahormius longicorpus* sp. nov. and *Parahormius longiflagellatus* sp. nov.) have been described as new to the science. Five genera and 14 species have been recorded for the first time from India. Twelve genera and 39 species have been recorded for the first time from Chhattisgarh and Maharashtra. Consolidated host-record of Indian Braconid species has also been prepared.

### **Studies on Larval Parasitoids of Major Defoliators of Teak and Sal Forests of Orissa**

Surveys in ten districts of Orissa were carried out and 202 samples of larvae and pupae of insect pests of teak and sal defoliators collected. Rearing of field collected samples yielded 180 specimens of *Apanteles* out of which following fifteen species were identified: *A. antipoda*, *A. belippa*, *A. bambusae*, *A. caniae*, *A. creatonoti*, *A. detrectans*, *A. effrenus*, *A. expulsus*, *A. lamprosemae*, *A. leptothecus*, *A. machaeralis*, *A. neocajani*, *A. neotaeniaticornis*, *A. tachardiae* and *A. fuseinervis*. Natural field parasitisation of all these 15 species was calculated. All these *Apanteles* spp. are indigenous and were recorded for the first time from Orissa.

### **Entomopathogenic Nematode for the Management of Termites and White Grubs**

The laboratory culture of one exotic and six unidentified (native) populations of EPNs

native to central India, was maintained *in vivo*. One of the EPN isolate (*Steinernema dharnaii* sp. nov.) has been identified (at molecular level) as new-to-science by CABI, Kew, UK. Process for the identification of other unidentified native EPN populations is in progress in collaboration with the Zoological Survey of India, Dehradun.

### **Insect Pests of Medicinal Plants-*Abelmoschus moschatus*, *Gloriosa superba* and *Withania somnifera***

On muskdana, *Abelmoschus moschatus*, three insect pests viz. *Anomis flava* and *Sylepta derogata* (defoliators) and red bug *Dysdercus cingulatus* (sap sucker); on kalihari, *Gloriosa superba*, two defoliators viz. *Polytela gloriosa* and *Amsacta lacieneus* and on Ashwagandha, *Withania somnifera*, two insect pests viz. sap suckers, *Plautia crossota* and aphids were recorded. *Ichneumon* sp. and *Stermia* sp. were recorded as parasites on the insect pests of *A. moschatus* and *G. superba*. Seasonal history of the key insect pests *A. flava*, *S. derogata*, *P. gloriosae* and *D. cingulatus* was also studied.

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

The final Time-Mortality Bioassay using 7 doses of Baculovirus, viz. 1.70E+09, 8.50E+08, 4.25E+08, 2.13E+08, 1.06E+08, 5.31E+07 and 2.66E+07 were applied on fifty 3<sup>rd</sup> instar larvae for each dose. Time-Mortality-Bioassay data were subjected to Probit Analysis using SPSS software. The results indicated that  $LT_{50}$  for the doses, were 0.372, 20.582, 8.223, 12.492, 12.403, 10.556 and 10.810 days, respectively, meant the days required to kill the larvae after administering the viral dose.

As the laboratory trials were successful for sex-pheromone, a experimental field trial of sex-pheromone was conducted in some selected spots of Shamshi. Fifty pheromone-traps were mounted on the ground and pheromone ampule was filled with 1 microliter of the extracted pheromone. The site was revisited after 15 days





Male IGM Killed in Pheromone

and the catch of male moths from all traps were counted. Altogether 650 adult male were killed.

Assessment of impact of first field trial of baculoviral experiment was done at the selected sites of Oak forest in Charwag village of Sarahan District. The effect of baculovirus was assessed by counting the number of egg-mass recorded during July-August and comparing the numeric strength with the record of egg-mass during last two years. It was found that there was a reduction of production in egg-mass measuring 92%.

#### **Predatory Efficiency of *Stegodyphus sarasinorum* Karsch (Arachnida: Araneae: Eresidae) against Insect Pests**

Five field surveys were conducted in areas like Nogli, Gesipul, (Rampur), Sunni, Basantpur, Arki, Gablog, Bangora, Dadhau, Renuka and Kunj-Kayer falling in the lower hill and mid-hill regions of Himachal Pradesh. Twenty three different spots



Social Spider Nest Reared in Lab

were screened in search of social spider nest and only in 10 spots, colony of the spider was recorded and geo-referenced. Ten mature colonies of the spider were collected and reared in the laboratory. Following 15 trees and shrubs were recorded as the host tree where the spider constructed the web viz. *Mallotus philippensis*, *Artemesia* sp., *Lagerstroemia* sp., *Curessus* sp., *Platyclusus orientalis*, *Kigelia pinnata*, *Prunus dulcis*, *P. domestica*, *Dalbergia sissoo*, *Eucalyptus* sp., *Zyzyphus jujuba*, *Phyllostachys* sp., *Callistemon viminalis*, *Acacia catechu*, *Punica granatum* and one unidentified. Following 17 prey insect species belonging to 6 families under 4 orders were recorded: *Granida albosporsa*, *Holotrichia longipennis*, *Anomala* sp., *Apogonia* sp., *Melolontha* sp., *Brachytrypes portontosus*, *Anlanches milaris*, *Chrotogonus* sp., *Apriona cinerea*, *Deudoris epijarbus*, *Closstera cupreata*, *Plectora reflexa*, *Agrotis epsilon*, *Heterotermes indicola*, *Pieris brassicae* and *Pieris rapae*.

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

It was observed that *Polygraphus longifolia* Steb. (Coleoptera: Scolytidae) passes through three to four generations during a year. For management, pheromone traps were used. Pheromone, Ipsdienol (C<sub>10</sub>H<sub>16</sub>O) at 1 mg, 2 mg, 4 mg and 8 mg loading/ concentration in four types of pheromones traps viz. Fero-T<sup>TM</sup>, Del-Ta<sup>TM</sup>, Wot-T<sup>TM</sup> and Fligh-T<sup>TM</sup> by following factorial RBD design were evaluated at Plateau chir pine forest in Barsar Forest Range under Hamirpur Forest Division.

#### **Botanicals**

#### **Bioassay Against Major Defoliators of Poplar and Shisham**

Laboratory reared 3<sup>rd</sup> instar larvae of Shisham defoliator, *Plecoptera reflexa* and poplar defoliator, *Clostera cupreata* were tested using Acetone (A) and methanol (M) extraction of aerial parts of *Calotropis procera* (CP) and



*Plumbago zeylanica* (PZ). It was found that 2% concentrations of PZA and PZM gave 63 and 73%, whereas, CPM and CPA gave 67 and 63% larval mortality after 72 hrs respectively.

*Atteva fabriciella*, *Eligma narcissus* (*Ailanthus excelsa*) and *Tingis beesoni* (*Gmelina arborea*)

Pongamia, Jatropha and Neem oil at 5 different concentrations (2-3%) were found effective in controlling these insect pests. The commercial product, Neem azal (1%) tested at 10 different concentrations exhibited that the product at 10 and 20 ppm was effective for managing *E. narcissus* and *T. beesoni*, respectively.

### Vilvekam-Aegle marmelos Seed Oil Based Biopesticide

Eight suitable preformulations from aqueous and organic extracts from the processed tissues (half fruit, pulp and seeds) of *A. marmelos* were developed and tested at the doses of 2000, 5000 and 10,000 ppm in comparison with neem formulation and synthetic pesticide against the target insect pests, *Hyblaea puera* and *Spodoptera litura*. Based on the promising results obtained from the laboratory and field trials a new product “Vilvekam-Aegle marmelos seed oil based biopesticide” was developed, and released.

### Field Level Biopesticidal Applications



### Insect Resistant Germplasm

#### Screening for Eucalyptus Gall Resistant Clones

A trial with 179 high yielding clones of *Eucalyptus* located at Satyavedu, Andhra Pradesh was screened for the attack of the gall insect, *Leptocybe invasa*. Data so far collected showed that about 19 clones were showing resistance/

tolerance to the gall insect. Consistency of the resistant nature of these clones needs to be watched further.

#### Others

#### Integrated management of Khejri mortality

Field surveys were made in 4 districts viz., Nagaur, Sikar, Churu and Jhunjhunu to assess the



extent of mortality. The percentage khejri mortality ranged between 18.08 to 22.67 % with an average mortality of 20.93 % in all the surveyed districts. Eggs and larvae of different instars of *Acanthophorus serraticornis* have been collected for rearing in the lab condition. The treatments were given as recommended by CAZRI, AFRI & ARS. Before treatments, observations were recorded on DBH, root infection with borer/fungus defoliation percentage, and weight of loong production by visual and actual record. The bio-ecology of *Acanthophorus serraticornis* was studied in the laboratory as well as in the insectary conditions. The eggs have been laid by female beetles in the month of September-October in the moist soil around the collar region of trees. The eggs are oval in the shape, white and measured 4.76 mm in length and 2.38 mm in width. Incubation period varied from 9-11 days. The maximum length of newly hatched 1<sup>st</sup> instar larvae was 25 mm, creamish-white with

blackish brown head. The 1<sup>st</sup> instar larvae life was of 45-50 days. Life generations of *Acanthophorus serraticornis* overlap considerably and different instars larvae have been collected from fields during January to March. A workshop was organized to review the work done by AFRI and suggestions and future line of action have been finalized.

### **Influence of Climate on Bionomics of *Pityogenes scitus* Blanchard (Coleoptera: Scolytidae) in Himachal Pradesh**

Five Forests viz. D-91 Bhawan Ki Dhar under Solan Forest Range, D-73 Mashobra under Mashobra Forest Range, Jamunda Forest under Kotgarh Forest Range, Jangi Forest under Akpa Forest Range and Brundhar Forest under Manali Forest Range were selected. Emergence of beetle and climatological data for five localities were collected.

### **Different Pheromones Traps Evaluated in Chir Pine Forests**





## Diseases and their Control

### Seed Borne Diseases

A total of 222 fungal isolates of 40 genera were isolated from the fruits and seeds of *Elaeocarpus munronii*, *Dysoxylum malabaricum*, *Dipterocarpus indicus*, *Vateria indica*, *Garcinia gummi-gatta*, *Poeciloneuron indicum*, *Syzygium malabaricum*, *Myristica malabarica*, *Knema attenuata*, *Madhuca longifolia*, *Hopea ponga*, *Kingiodendron pinnatum* and *Cinnamomum sulphuratum*. Natural fungal flora of seeds of *D. malabaricum*, *E. munronii*, *M. malabarica*, *P. indicum* and *V. indica* were found to be pathogenic on seeds and seedlings. A new fungal species viz., *Penicillopsis indcus* was identified from the seeds of *D. malabaricum*. New host records of fungi i.e., *Beltrania rhombica* on seeds of *P. indicum* and *Bartalinia lateripes* on seeds of *G. gummigatta* were reported. Control of forest seed pathogenic fungi has been achieved by using the leaf and bark extracts of *Prosopis juliflora* and *Cleistanthus* sp.

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 isolated and identified from *Pinus gerardiana* (chilgoza) seeds. *Penicillium citrinum* was the most predominant fungus affecting Chilgoza seeds in storage. It was observed that 40.07% of seed rot was found at 25°C and even 8.27% seed rot was observed at 0°C due to *Penicillium citrinum*.

### Diseases of Sandal Wood

Five silvi-horticultural models of sandal situated in Bevananahalli, Muddannahalli, Gottipura, Yelwala (Mysore) and Jarackbandae were continuously surveyed for insect pests and diseases. The diseases of sandal plants were very predominant during the seedling stages only.

Some of the common diseases observed in all the five models are damping-off (*Fusarium* sp.), root disease (*Phytophthora* sp. and *Pythium* sp.), foliar blight (*Botrytis* sp.), root decay (*Cylindrocarpon* sp.) and tip blight (*Phoma* sp.). The symptoms of root decay and tip blight were found in the 2-3 years old plants also. The management strategies were adopted to reduce the inoculum level at the field by following cultural and biological methods.

### Bacterial Wilt in Teak

The impact of bacterial wilt diseases in hi-tech teak plantations on length of lamina and intermodal length of apical region of the Teak stem was found to be less in Ridomil (0.3 % and 0.2%) followed by Streptocyclin (0.1%) as compared to control. Powdery mildew occurs in moist conditions and is usually seen in the wetter spring and fall seasons. Spraying of sulphur (0.2%) is recommended but it may injure tender foliage, especially in hot weather so it must be used carefully under strict scientific supervision. Biological agents include *Cladosporium cladosporoides*, *C. oxysporum* and *Trichoderma virens* which are eco-friendly and give quite fruitful results.

### Management of Diseases of Aonla, Neem and Khamer

Varieties viz. Kanchan, Anand-2 and NA-7 showed more susceptible reaction to *Fusarium oxysporum*. On the basis of laboratory evaluation, Ridomil 0.2% was found the most effective against the growth of *F. oxysporum* followed by Ridomil 0.1%, Bavistin 0.2% and Dithane M 45 0.2%. Comparative efficacy of six fungicides recorded at 3 intervals (15, 25 & 35 days) confirmed that Bavistin, Dithane M 45 and Ridomil at both concentrations and Fytolan at 0.2% were effective to control the disease in nurseries while Thiram and Foltaf were less effective.



Sites prone to wilt infection have been selected from Madhya Pradesh and Maharashtra. Three pathogens viz. *Fusarium solani*, *Verticillium nigrescens* and *Rhizoctonia solani* causing wilt in Aonla, Neem, and Khamer have been identified. Susceptibility of the pathogen to different fungicides and biological agents has been determined under controlled laboratory condition.

**Culm Rot and Bamboo Blight Disease in Assam**

Pathogenicity tests carried out in the lab as well as potted seedlings revealed *Fusarium udum* as the causal organism of the disease. Two biocontrol agents (viz. *Trichoderma viride* and *T. harzianum*), 8 fungicides and 1 antibiotic were tested *in vitro* against the fungal pathogen. The most effective fungicides as well as biocontrol agents were selected for *in-vitro* tests. Four

fungicides viz., Mass, Bavistin, Moximate and Indophil M 45 were found effective under *in-vitro* condition were tried in the potted bamboo seedlings against culm rot and bamboo blight disease.



Pathogenicity Test in the Lab



Pathogenicity Test in Potted Seedlings



*In-vitro* Evaluation of Fungicides Against *Fusarium udum*



### Host Specificity of Rust Fungus in *Acacia* Species

Host specificity test on 14 species of *Acacia* was conducted for rust fungus, *Ravenelia evansii* in polyhouse at Jodhpur, only eight species performed well in the climatic conditions of temperature and relative humidity at Jodhpur. The cross-infectivity and host specificity test were designed using the seven species of *Acacias* including *A. nilotica indica* against rust infection.

### Stem canker disease in rohida

Survey was carried out to select experimental site and collection of diseased samples, isolation and identification of pathogens responsible for stem canker of rohida (*Tecomella undulata*) in Barmer. Seven fungi have been isolated from different sites from infected rohida trees. The fungi have been identified as *Lasioidiploidia theobrome*, *Stemphyllum* sp., *Alternaria* sp. and rest of the fungi were unidentified. Individual fungus species and in combination were inoculated on healthy branches of Rohida for pathogenicity testing.

### Khejri Mortality

Field surveys were made in 4 districts of Rajasthan viz., Nagaur, Sikar, Churu and Jhunjhunu to assess the extent of mortality. The mortality of khejri ranged between 18 to 23 %. Based on the actual data of tree mortality in the randomly selected pockets at different localities, in addition to *Ganoderma lucidum* and *Macrophomina phaseolina* was isolated from infected khejri roots. Eggs and larvae of different instars of *Acanthophorus serraticornis* have been collected for rearing in the lab condition. Six sites were selected in five districts viz; Surani (Balesar Road, Jodhpur), Raghunatpura (Didwana, Nagaur), Jhareli (Jayal, Nagaur), Goshala (Fatehpur, Sikar), Churu (Churu) and Sultana (Jhunjhnu) for the laying out of experiment. The treatments were given as recommended by CAZRI, AFRI and ARS. Before treatments, observations were recorded on DBH,

root infection with borer/fungus defoliation percentage, and weight of loong production by visual and actual record.

Fruiting bodies of *Ganoderma lucidum* were collected from infected khejri trees. The fungus was isolated and multiplied on sorghum seeds. Freshly prepared culture of *Rhizoctonia bataticola* causing charcoal root rot in khejri trees was inoculated on the young seedling for pathogenicity test. Till now, three different strains of *Trichoderma* spp. have been isolated from the soil collected from different sites.

### Diseases of Medicinal Plants and Their Bio-control

Diseases of *Rauvolfia serpentina*, *Withania somnifera* and *Chlorophytum borivillianum* were recorded from Madhya Pradesh and Chhattisgarh. A new damping-off disease of *W. somnifera* caused by *Sclerotium rolfsii* was reported for the first time from India. Antagonistic organisms viz. *Streptomyces* sp. and *Bacillus amylolequifaciens* were isolated from the soil. The effect of antagonistic organisms and biopesticides (1 litre cow urine + 100 g each leaves of *Azadirachta indica*, *Ailanthus excelsa* and *Calotropis procera*) was tested on the major pathogens of *R. serpentina* and *C. borivillianum* in the laboratory. The experiment on the effect of *Trichoderma harzianum*, *Bacillus firmus*, *B. amyloliquefacien* and, *Streptomyces* sp, Bavistin and biopesticides were conducted on the leaf spot and inflorescence disease of *R. serpentina* in the field. Among the above treatments bavistin 0.5% + *Streptomyces* sp. showed best results for disease control as well as seed production. In another experiment, the effect of systemic fungicide, biopesticide and bio-controlling agents on the foliar diseases of *C. borivillianum* were noticed. In this experiment, two fortnightly spray of the culture of *Streptomyces* sp. shows significant disease control as well as better rhizome production. One experiment on the selection of



potting mixture for the root development of *W. somnifera* was conducted by using different combination of potting mixtures. Neem cake 50%+ soil mix 50% proved best potting mixture for the over all growth of the *W. somnifera* as well as reduction in root-knot caused by *Meloidogyne incognita*.

### Screening for Resistance Against Diseases

The studies on blister bark disease resistance in 250 clones of *Casuarina equisetifolia* were carried out in a nursery experiment by artificially inoculating the pathogen, *Subramanianospora vesiculosa* which exhibited that 36 clone were resistant to the pathogen. The rest of the clones were found expressing the resistance and susceptibility to the pathogen at varying degrees. Analysis of total phenols was also carried out and correlated with the level of resistance expressed by different clones.

Resistance against *Fusarium solani* wilt disease of *Dalbergia sissoo* was tested in seventeen clones by using direct inoculation (injection) with four virulent strains. Only one clone No. 103 showed resistance while clone no. 49 and 1003 were the most susceptible ones.

### Antifungal Properties of Plants

Antifungal properties of seven selected plant parts (leaves, roots and seeds of *Datura stramonium*, fruits of *Balanites aegyptiaca*, roots and fruits of *Citrulus colocynthis* and flowers of *Tephrosia purpurea*) were evaluated against fungal pathogens. For antifungal assay, pure cultures of fungi were procured from National Type Culture Collection, Forest Pathology Division of FRI, Dehradun. Total fourteen extracts were tested against five fungi for their antifungal properties: (1) Aqueous extract of *Citrulus colocynthis* roots showed good antifungal activity against *Rizoctonia bataticola*, (2) Alcoholic extract of *Citrulus colocynthis* roots showed antifungal activity against *Fusarium solani*,

(3) Aqueous and ethanolic extract of *Citrulus colocynthis* leaves showed mild to moderate antifungal activity against selected fungi, (4) Aqueous extract of *Datura stramonium* seeds showed good antifungal activity against *Alternaria alternata* and (5) Alcoholic extract of *Datura stramonium* seeds showed moderate to mild antifungal activity against all five fungi.



Inhibition zone by Aqueous Extract of *Citrulus colocynthis* Roots Against *F. solani*



Inhibition zone by Aqueous Extract of *Citrulus colocynthis* Fruit Against *F. solani*

### Dynamics of Litter Decomposition in Sal Forest

Natural sal sites were selected from Madhya Pradesh, Chhattishgarh, and Orissa. Litter samples have been collected and analyzed for carbon flux and NPK. Microbial flora and fauna associated with litter decomposition have been



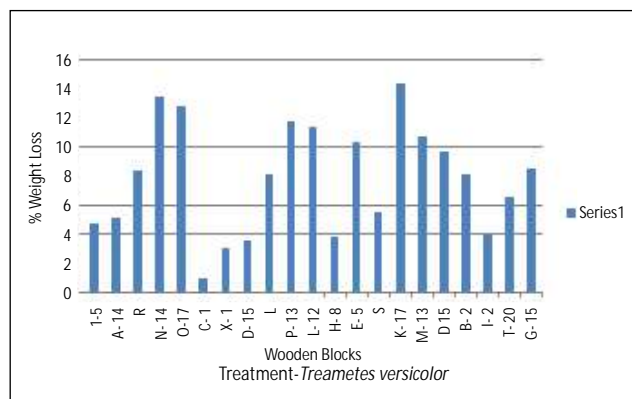
studied. Fungi forming ectotrophic mycorrhizae (*Russula* and *Pulveroboletus* among basidiomycetes and *Geastrum*, *Astraeus* and *Scleroderma* among gasteromycetes) with sal trees have been collected and identified. Other beneficial supporting microbes including *Trichoderma*, *Cladosporium* and PSB have been cultured and preserved for further use under field condition.

### Fungal Degradation in Catamarans

Fungal infested timber from catamarans from the third field station, Pudimadaka was collected. The fungal samples were cultured and maintained. Timber test coupons were treated to a gradient of CCA absorptions. CCA treated coupons were subjected to experimentation with pure cultures of individual fungi. In general, lower loadings of CCA along with control were infested, whereas, higher loadings resisted infestation.

### Natural Decay Resistance in Imported Woods

Natural decay resistance tested through accelerated laboratory tests with white rot fungus *Trametes versicolor*, revealed that beech wood (*Fagus grandifolia*) from France gave highest percentage weight loss (14.36), followed by ash wood (*Fraxinus americana*) (13.48) and beech wood from Belgium (12.84), whereas, minimum weight loss was observed in teak wood from Ghana (1.06) blocks.



### Molecular Variability

RAPD-PCR was conducted with isolates of *Cordyceps sinensis* collected from different locations in Uttarakhand for studying the variability among the isolates which identified nine population lines. Internal transcribed region of nrDNA (ITS regions) were amplified and sequenced for the authentic identification of the isolates of *Cordyceps sinensis*. BLAST search results affirmed their identity as *Cordyceps sinensis* (the new name is *Ophiocordyceps sinensis*). For the first time beta tubulin gene region of *Cordyceps sinensis* has been sequenced, studied and deposited in gene bank.

### Active Principal in Medicinally Important Fungus

Cordycepin, ergosterol and adenosine were detected in some of the *Cordyceps sinensis* isolates collected from different geographical locations in Uttarakhand. Some of the bioactive principals have been found to be in a higher quantity in the cultivated *Cordyceps sinensis* in comparison to the wild. Comparison of the cordycepin content, determined in the sporophores of *Cordyceps sinensis* and literature reported with those determined in the cultured samples, revealed that the cordycepin yield was high in eleven cultured samples of which seven isolates ISO 3 (0.024%), ISO 6 (0.029%), ISO 10 (0.013%), ISO 21 (0.026%), ISO 22 (0.013%), ISO26 (0.016%) and ISO 33 (0.02%) showed significantly high cordycepin content. The results of study demonstrated that *Cordyceps sinensis* could be cultivated on Jhangora grains where the cordycepin production was found to be much higher than that of naturally occurring sporophores. This study is the first report regarding the cordycepin production in the cultured Indian isolates of *Cordyceps sinensis*.



Cultivated Fruiting Body of *G. lucidum*

### Cultivation of *Ganoderma lucidum*

An economical cultivation protocol of a medicinally important fungus *Ganoderma lucidum* was successfully developed using poplar billets for the first time and has been passed on successfully to the Mushroom growers in Dehradun and nearby areas.

### Wood Decay Fungi and their Management

Specimens of 102 wood decay fungi were collected from 22 hosts. One new species, *Nitschkia tectonae* and three new records of fungi were reported from India viz. *Australohydnum dregeanum*, *Hjorstamia fresii* and *Schizopora flavipora*.

Nine hyper parasitic fungi colonizing decaying wood have been isolated. Out of them, *Trichoderma viride* and *Aspergillus flavus* were selected and tested *in-vitro* for biological control of 10 wood decaying fungi (*Trametes cingulata*, *Stereum hirsutum*, *Pycnoporus sanguineus*, *Trametes feei*, *Phellinus badius*, *Lenzites elegans*, *Phellinus pachyphloeus*, *Lenzites acuta*, *Earliella scabrosa*, *Gleophyllum striatum* and *Flavodon flavus*). Different concentration of Ammonium tetraborate, Potassium tetraborate, Manganese sulphate, Magnesium sulphate, Zinc sulphate, Copper sulphate, Borax boric acid and Urea, were tested to evaluate the efficacy of these chemicals against *Flavodon flavus* and *Trametes cingulata*.

### Root Rot and Stem Decay in *Acacia catechu* and their Control

Fungi causing root rot and stem decay diseases in *Acacia catechu* were collected from Khandwa (6 samples) and Yavatmal (27 samples). Wood decay and disease causing fungi (*Auricularia*, *Daldinia concentrica*, *Flavodon flavus*, *Ganoderma lucidum*, *Lenzites palisoti* Monodictys, *Phellinus badius*, *P. gilvus*, *Stachylidium*, *Schizophyllum commune* and *Torula*) were isolated from fruit bodies and diseased samples. During survey, over 60% mortality in *Acacia catechu* plantation (compartment 282 & 310, 22 ha) at Hivari Range in Yavatmal was recorded due to root rot disease caused by *Ganoderma lucidum*. The site was poor in murrum soil. Young plantation (one year old) at Mukutbandh and Ghosa range of Pandharkawda division was surveyed and up to 5% seedlings were found dead due to root rot disease. Ten years old plantation (mixed with sissou and neem) was surveyed at Isapur (Digras), Pusad Division and found almost healthy (only 4% plants were found dead due to *Ganoderma lucidum*).

### 2.7.3 Mycorrhizae, Rhizobia and other useful Microbes

#### Mycorrhizae

Different types of ectomycorrhizal (ECM) fungal inocula (Basidiospores, Vermiculite based vegetative mycelial and alginate bead inocula) of *Laccaria fraterna* (1 Isolate) and *Pisolithus albus* (4 Isolates) inoculated singly to *Acacia auriculiformis*, *A. mangium*, *Casuarina equisetifolia*, *C. junghuhniana*, *Eucalyptus camaldulensis* and *E. tereticornis* seedlings grown in sterilized and unsterilized potting media under nursery condition. It was found that the vegetative mycelial inoculum of *P. albus* (Isolate 3) for *A. auriculiformis*, *C. equisetifolia* and *C. junghuhniana*; Isolates 2 and 4 for *A. mangium*



and basidiospore inoculum (Isolate 3) for *E. camaldulensis* and *E. tereticornis* were suitable for growth improvement of the seedlings in nursery. Nodule population was found higher in seedlings grown in sterilized potting medium inoculated with vegetative mycelial inoculum of *P. albus* isolate 3, followed by isolates 1 and 2 in Casuarinas. All the ECM inoculated plants had more number of ECM colonized roots (myco-tips) especially the basidiospore inoculum of *P. albus* (Isolate 3) showed significantly more myco-tips in all the tree species.

### Plant Growth Promoting Rhizobacteria (PGPR)

In all, 216 PGPR isolates and 26 different AM fungi were isolated and identified from the rhizosphere samples of 6 different fast growing native tree species in Tamil Nadu and Kerala. Germplasm of all these PGPR isolates are maintained in the laboratory. Population density of PGPRs and AM fungi was determined and the maximum population was recorded in *Melia dubia* rhizosphere followed by *Gmelina arborea*. Maximum percent of root colonization and soil spore population of AM fungi was observed in *G. arborea* followed by *M. dubia* and *Dalbergia latifolia*. Season and soil type influenced the population density of PGPRs and AM fungi.



Bacterium, *Azospirillum amazonense* Isolated from Rhizosphere of *Neolamrckia cadamba*



Bacterium, *Bacillus megaterium* Isolated from Rhizosphere of *Ailanthus excelsa*

PGPR isolates were screened for production of IAA and phosphate solubilization efficiency and the best isolates were selected for nursery experiments. Salt tolerance capacity of PGPR isolates to various concentrations of sodium was also studied under *in-vitro* conditions and some of the isolates were found tolerant.

Nucleotide Sequences of different isolates of PGPR (Plant Growth Promoting Rhizobacteria) isolated from the rhizosphere of six different fast growing native tree species in Tamil Nadu and Kerala have been submitted to European Molecular Biology laboratory (EMBL) and NCBI Database.

Molecular characterization of Phosphate Solubilizing Bacteria (PSB) isolated from the *A. excelsa* rhizosphere samples was done and identified as *Bacillus megaterium*. Nursery experiments revealed significant growth increments in bio-inoculants applied seedlings of *A. excelsa*, *A. triphysa* and *N. cadamba* over control in 90 and 180 DAI.

A nursery experiment organized to study the effect of selected PGPR isolates on seedlings of shola species such as *Mappia foetida*, *Symplocos cochinsinensis*, *Syzygium cuminii*, *Syzygium arnottianum*, *Michelia nilagirica* and *Michelia champaca* revealed that bio-inoculants inoculated seedlings had better seedling health and growth parameters over uninoculated control seedlings.



Microbial inoculants (*Azospirillum*, *Pseudomonas*, *Bacillus* and *Azotobacter*) tested on 30 *Eucalyptus* clones for effective rooting and nutrient enhancement in comparison with IBA revealed that PGPRs inoculated *Eucalyptus* stem cuttings responded better for rooting than IBA treated cuttings under controlled conditions (37° C & 65 % RH). The PGPR, *B. megaterium* inoculated clone no. 14 showed vigorous rooting after 14 days and found better than IBA treated cuttings with percentage of rooting was 81.00. Similarly, *P. fluorescens* showed better root initiation in clone No. 111 after 14 to 15 days of inoculation. Clone No. 17 showed 70% of root initiation after 15 days of inoculation. However, the clones 1, 196, 19, 31, 16, 7, 9, 188, 186, 16 and 63 showed late response to PGPRs inoculation collected from Sathyavedu and Karunya. These clones produced rooting only after 20 days of inoculation with percentage of rooting was 45.00–58.00.

The AM fungi and PGPRs inoculated clones (C111, C14, C19, C53) also showed higher content of P than control clones. Out of 30 clones, clone nos 111, 191, 116, 76, 101, 154, 186, 14, 69 and 123 showed better performance in root initiation, number of lateral roots, root length and shoot length. The nutrient status (P) was also studied in the microbial inoculants, inoculated *Eucalyptus* clones and it was found that content of P was higher in the microbial inoculants applied *Eucalyptus* clones.

#### **Diversity of Mycorrhizal Associations with *Dipterocarpus* and *Shorea* Species in Assam**

The survey and collection work was carried out in two study sites: Site-I includes Amsoi, Kulsi, Dhupdhara for *S. robusta* and site -II includes Jeypore, Digboi and Margerita for *D. retusus* and Jeypore for *S. assamica*. Composite samples (24 nos.) of rhizosphere soil, composite samples of ectomycorrhizal roots (24 nos.) and ECM fruit bodies (12 nos.) were collected from the selected sites. Morphological and anatomical details of ECM roots and ECM

fruit bodies were worked out for their proper identification and taxonomic details. *Russula* species was reported to be associated with all selected tree species, therefore, it was selected for nursery trials. Mass inoculum of *Russula* species was raised on wheat grains in polypropylene bags.

Rhizosphere soil was analyzed for the isolation, quantification and colonisation of AM with roots of selected plant species. *Glomus* sp. was most dominant strain reported but percent colonisation was fewer (2-4%). Mother inoculum of AM fungi was raised with living host (wheat) in small earthen pots (10 Nos., 650 g capacity). Mass inoculum of AM fungi for nursery trials was raised in bigger earthen pots (4 Kg capacity, 25 nos.) with living host (wheat). Seeds (500 nos.) of *Dipterocarpus retusus* and *Shorea robusta* were collected and sown in nursery bags (20x21 cm) filled with sterilized soil. To observe the efficacy of mycorrhizae with selected host species, four sets of experiments were laid: (i) Control (not inoculated), (ii) Inoculated with Ectomycorrhizal fungi, (iii) Inoculated with Endomycorrhizal fungi and (iv) Inoculated with Ecto + Endomycorrhizal fungi.



Mass Inoculum of *Russula* sp.



Mass Inoculum of *Glomus* sp.



## Nursery Trials

Seed of *S. robusta*

Soil Sterilization

Seeds of *D. retusus*

## Arbuscular Mycorrhizal Diversity in Meghalaya

Two dominant and efficient strains (*Glomus* sp. and *Gigaspora* sp.) which were prevalent in the forest soil were isolated and

inoculum production and mass multiplication through trap culture and pot/plot culture completed. The seedlings of two selected plant species (*Mesua ferrea* L. and *Aquilaria agallocha* Roxb.) are being raised by inoculation experiment with VAM fungi.



Collection of Rhizospheric Soil Samples

## AM Fungi and Rhizobium for *Acacia nilotica* and *Ailanthus excelsa* in Western Rajasthan

Rhizosphere soil and root samples of *Acacia nilotica* and *Ailanthus excelsa* were collected from various forest nurseries viz; AFRI model nursery, Bhuteshwer nursery, Jodhpur; Navalgarh forest nursery, Jhunjhunu and high tech nursery of Forest Department, Sojat Road (Pali). In plantations, rhizospheric soil samples of *Acacia nilotica* var. *indica* were collected from various sites viz., Nagaur (4), Bikaner (2), Barmer (3), Pali (5) and Sirohi (5 sites) districts. For *Acacia nilotica* var. *cupressiformis*, samples were collected from Nagaur (1), Pali (5 sites) and Sirohi district (5) and samples of *Ailanthus excelsa* from

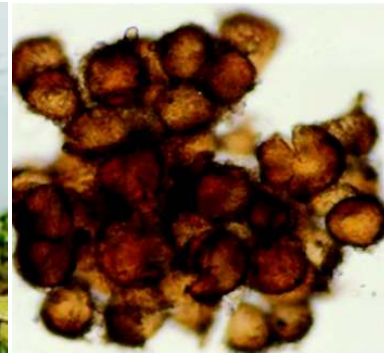
Nagaur (1), Bikaner (1) and Barmer (3) district. Soil samples were analyzed for pH, EC, (%) organic carbon (% OC), phosphorous (P) and isolation of AM fungi carried out. The important genera identified were *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis*. Among these four genera, *Glomus* occurred most frequently. The different species of *Glomus* were recorded as *G. aggregatum*, *G. fasciculatum*, *G. mosseae*, *G. macrocarpum*, *G. microcarpum*, *G. constrictum* and *Glomus* sp. Out of which, *G. fasciculatum* was dominant species in all the sites of nurseries as well as in plantations. The spore population varied from site to site and ranged between 163 to 480 propagules per 100 g soil.



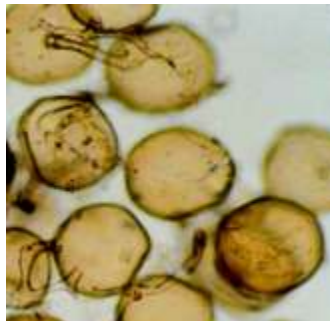
*Acacia nilotica* var. *indica*  
at Sirohi



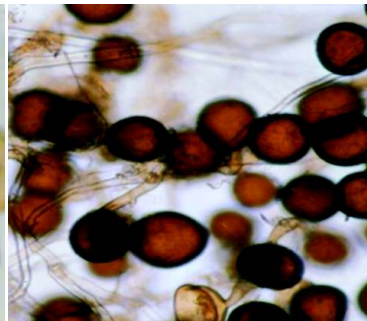
*Acacia nilotica* var. *cupressiformis*  
at Pali



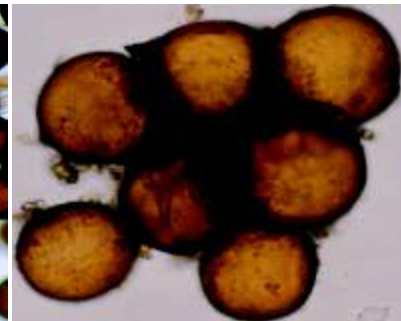
*Sclerocystis* sp.



*Glomus aggregatum*



*Glomus microcarpum*



*Glomus* sp.

### Frankia

The technique developed to inoculate and establish the association of *Frankia* successfully with the rooted cuttings of casuarina was tested in a nursery trial using 10 strains of *Frankia* isolated from *C. equisetifolia* and *C. junghuhniana*. In the trial, 93 clones of *C. equisetifolia* and seedlings of *C. junghuhniana* were used. Nodulation occurred both in the rooted stem cuttings of *C. equisetifolia* and seedlings of *C. junghuhniana* after 30 days. Further, the data collected on growth parameters showed increased shoot length, root length, collar

diameter and biomass with the *Frankia* inoculated seedlings. Analysis of nitrogenase activity of 10 strains of *Frankia* showed that the strains collected from the coastal and stressed sites had higher nitrogenase activity (137.57 to 256.18 nmol of C<sub>2</sub> H<sub>4</sub> mg protein/hr). Similarly, estimation of nitrogen content of the *Frankia* inoculated seedlings showed an average of 0.159 mg/g of Nitrogen content. A field trial has been established at Karaikal with *Frankia* inoculated seedlings of *C. equisetifolia* and *C. junghuhniana*.



*Frankia* Nodule Formation in  
Rooted Stem Cutting of Casuarina



*Frankia* Structure