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Effect of ectomycorrhizae on growth and establishment of sal (*Shorea robusta*) seedlings in central India

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Abstract. *Pyasi A, Soni KK, Verma RK. 2013. Effect of ectomycorrhizae on growth and establishment of sal (*Shorea robusta*) seedlings in central India. Nusantara Bioscience 5: 44-49.* The aim of the present study was to develop ectomycorrhiza in sal sapling outside the sal growing areas. For this purpose sal seedling were raised at Jabalpur which is around 80 km away from natural sal forest (Motinala, Madhya Pradesh, India). Seed sowing was done with inoculation of ectomycorrhizal inocula prepared by isolating the fungi from surface sterilized young basidiocarp of *Lycoperdon compactum* and *Russula michiganensis*. The inocula of ectomycorrhizal fungus were prepared in wheat grains treated with gypsum. The synthesis of ectomycorrhiza was observed in the sapling planted in the experimental field at Jabalpur with production of basidiocarp of *Lycoperdon compactum* near saplings. The mycorrhized saplings also showed higher growth indices.

Key words: ectomycorrhizal inoculum, ectomycorrhiza synthesis, nutrient uptake, sal forest

Abstrak. *Pyasi A, Soni KK, Verma RK. 2013. Pengaruh ektomikoriza terhadap pertumbuhan dan pembentukan bibit sal (*Shorea robusta*) di India tengah. Nusantara Bioscience 5: 44-49.* Tujuan penelitian ini adalah untuk mengembangkan ektomikoriza pada anakan pohon sal yang ditanam di luar wilayah pertumbuhan aslinya. Untuk itu bibit sal ditanam di Jabalpur yaitu sekitar 80 km jauhnya dari hutan alam sal (Motinala, Madhya Pradesh, India). Penaburan benih dilakukan dengan inokulasi inokulum ektomikoriza yang disiapkan dengan mengisolasi basidiocarp muda jamur *Lycoperdon compactum* dan *Russula michiganensis* yang permukaannya disterilkan. Inokulum jamur ektomikoriza dipersiapkan dalam biji gandum yang diperlakukan dengan gipsum. Sintesis ektomikoriza teramati pada anakan pohon yang ditanam di kebun percobaan di Jabalpur dengan produksi basidiocarp *Lycoperdon compactum* di dekat anakan pohon. Anakan yang bermikoriza juga menunjukkan indeks pertumbuhan yang lebih tinggi.

Kata kunci: inokulum ektomikoriza, sintesis ektomikoriza, serapan hara, hutan sal

INTRODUCTION

Sal (*Shorea robusta* C.F. Gaertn.; Figure 1) is one of the most important sources of hardwood timber in India. Density of sal forest has significantly reduced from sal dense 65.6% in 1976 to 11.1% in the year 1999 followed by sal open 11.2% and sal medium 18.2%. The overall change has been estimated to be 42.1% of the total forested area (Chauhan et al. 2003). Decreasing sal forest cover is one of the top-ranked problems of forest department. The primary reason for decreasing it so rapidly is poor regeneration. The seed of sal is recalcitrant, and start to germinate just before it detaches from the tree. It immediately needs appropriate moisture, nutrient, and mycorrhiza for its establishment. A mycorrhiza, in general, is a symbiotic relation between a fungus and the roots of a vascular plant. Ectomycorrhization refers to the infestation of cortical tissues of root by hyphae of mycorrhizal fungi (Harley 1959). Mycorrhizae form a mutualistic

Figure 1. Flowers of sal (*Shorea robusta* C.F. Gaertn.)

relationship with the roots of most plant species. This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose supplied by the plant. The carbohydrates are translocated from their source to root tissue and on to fungal partners. In return, the plant gains the benefits from the mycobionts in terms of water and mineral nutrients thus improving the plant's mineral absorption capabilities. Plant roots alone may be incapable of taking up phosphate ions that are demineralized, for example, in soils with a basic pH. The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources, and make them available to the plants they colonize (Bowen et al. 1974). The absence of mycorrhizal fungi can also slow plant growth in early succession or on degraded landscapes. Fungi have been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic contaminated soils and coal mine restoration (Bauman et al. 2013).

Ectomycorrhiza plays an important role in sal forest ecosystem as it forms mutualistic association with a variety of basidiomyceteous and gasteromyceteous fungi. The main ectomycorrhiza forming fungi in central India have already been studied and reported of which the common fungi are: *Astraeus hygrometricus*, *Boletus edulis*, *Boletus fallax*, *Geastrum triplex*, *Lycoperdon compactum*, *Scleroderma bovista*, *Scleroderma geaster* and *Scleroderma verrucosum*, *Russula adusta*, *Russula cinerella*, *Russula delicula*, *Russula leelavathyi*, *Russula michiganensis* (Bakshi 1974; Soni et al. 2011; Pyasi et al. 2011, 2012). The basidiocarp of ectomycorrhizal fungi are produced on soil surface inside sal forest. The actively growing basidiocarp helps young seedlings in absorbing nutrients mainly the phosphorous and other trace elements. The life of basidiocarp is very short usually 2-3 days but it production can persist for more than 3 consecutive rainy months.

In present study isolation of two ectomycorrhizal fungi in pure culture were made and used them to synthesize ectomycorrhiza in sal sapling planted in non-sal forest area.

MATERIALS AND METHODS

Location of experiment

The experiment was conducted at research experimental area of Forest Pathology Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India (Figure 2) which is located on N $23^0$06’074” E $79^0$59'386", elevation above sea level 415 m. It is around 80 km away from natural sal forest. The place experiences warm weather during April-June with average temperature ranges between 41ºC-21ºC during these months and 27ºC-8ºC during the winters (November-February). The annual rainfall is 1386 mm, and monsoon arrives at this place at the beginning of July and prolongs up to September.

Isolation of ectomycorrhizal fungi and preparation of inocula

Fresh and tender fruit bodies of *Lycoperdon compactum* G.H. Cunn. and *Russula michiganensis* Shaffer was collected from sal forest and washed under running tap water for five minutes. After that it was placed in aqueous solution of sodium hypochlorite, 0.1% (w/v available chlorine) for ten minutes and then the basidiocarps were cut into small and thin slices using a sterile razor blade. These sections were again floated into double distilled autoclaved water to remove disinfectants. These sections were inoculated onto Norkrans and PDA media containing a trace of Bavistin (R) and Streptomycin sulfate in Petri dishes (Figure 3D) and incubated at 25-27^{0}C. After growth, the fungi were transferred to culture tubes (Figure 3E).

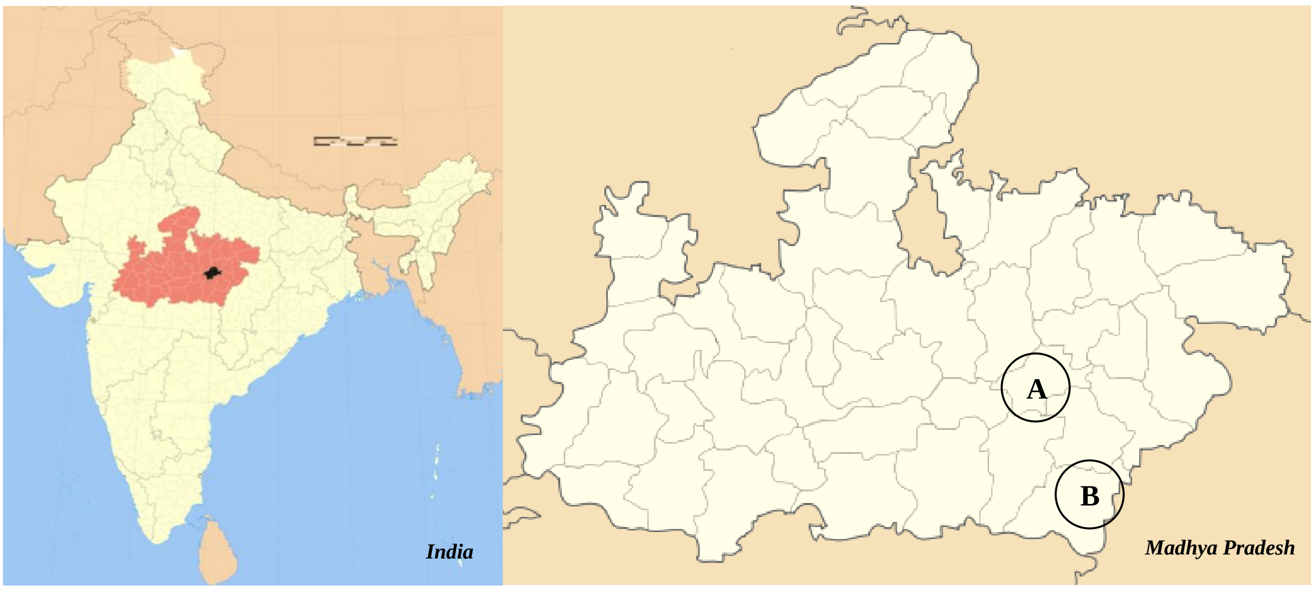

Figure 2. Map showing location of experiment at Jabalpur (A) and source of ectomycorrhizal fungi at Motinala (B); both in Madhya Pradesh, India.

For preparation of Ectomycorrhizal inocula, the culture isolated as above were multiplied in water soaked boiled wheat grains treated with gypsum salt and a pinch of Bavistin in a narrow mouth glass bottles (Figure 3F). Six to eight mycelial agar discs of mycorrhizal fungi were inoculated into the wheat grains. After 10-12 weeks of incubation at 25-27oC the inoculum was ready for use (Kannan and Natarajan 1988).

Inoculation and planting of seedlings

The experimental seedlings received the following treatment and are arranged in CRD on a cemented platform, i.e. (i) Control, (ii) Sal litter, (iii) ECM1 (*Russula michiganensis*), (iv) Sal litter+ECM1, (v) Vermicompost+ RCM1, (vi) ECM2 (*Lycoperdon compactum*), (vii) Sal litter+ECM2, (viii) Vermicompost+ECM2, and (ix) Vermicompost.

Sal litter collected from sal forest of Motinala, Balaghat District, Madhya Pradesh, India (Figure 2) and added to the local soil in the ratio of 1:4 (v/v). The vermicompost was also mixed in the same ratio. The potting mix was filled in black polyethylene bags of 7x9". Ten g inocula of ectomycorrhizal fungi were given to each seedling of treatment numbers 3-8. The inoculum was placed just below seeds during seed sowing in pre monsoonal period. After five months the seedlings were planted in the micro-plots with 1x0.75m spacing at the Institute campus in RCBD. Four blocks each with all the above-mentioned treatments were made. The seedlings were watered with tube well water as and when required.

Observation and recording of data

The basidiocarp of ectomycorrhizal fungi is produced on soil surface near inoculated seedlings. The life of basidiocarp was recorded 2-3 days but it production persist for more than 3 consecutive months of rainy season. Basidiocarp of *Lycoperdon compactum* is produced in close vicinity of roots in artificially inoculated sal saplings during July-August.

Heights of saplings, leaf breath and length were measured using standard centimeter scale. Diameter of saplings at collar region was measured using Varner's callipers. Leaf area index was calculated by multiplying maximum length of leaf with breadth (cm^2). Growth indices of seedlings were calculated after 9 months of planting using the following formula:

$$\text{Growth Indices} = \frac{H \times CD \times LAI}{H + CD + LAI}$$

H = Height; CD = Collar diameter; LAI = Leaf area index

Statistical analysis

Data obtained on growth indices were analyzed by one-way analysis of variance (ANOVA) using Sx Statistics software.

RESULTS AND DISCUSSION

Results

Basidiocarps of *Lycoperdon compactum* were observed in groups of 1-3 near ECM2 treated saplings in two blocks in close vicinity forming ectomycorrhizal association with feeder roots of sal at Jabalpur, Madhya Pradesh, India (Figure 3A-B). Mycorrhiza developed by the winter season (Figure 4A-B). No basidiocarp was observed in control and other treatments. Basidiocarps produced are short-lived usually 2-3 days which upon maturation releases basidiospores. At Jabalpur basidiocarp was produced 3 times during July-August 2012 when rainy season was at its peak. The basidiocarp is rough, tough, globose, yellowish pinkish brown 2-5 cm in diameter, a typical gasteromyceteous shape, with a small rhizoidal stalk at base. Young sporophore is hard, depressed above, exoperidium glabrous, smooth 1-1.5 mm thick leathery, endoperidium smooth very thin. Gleba olivaceous, amber when young, becomes powdery chocolate brown at maturity. Spores abundant, round, hyaline to olivaceous brown, verrucose 4.5-5.0 µm (Figure 3C). The fungus grows on PDA agar medium with milky white, irregular colonies, rhizoidal, radiate, slow growing 35 mm. in diam. after 7 days at 25±2^{0}C.

The maximum growth indices were observed in vermicompost+ECM2 treatment, which was 3.4 times more than control followed by ECM2, 3.0 times more, vermicompost+ECM1, 2.1 times more, sal litter+ECM2, 2.1 times more and sal litter+ECM1, 1.8 times more. Other treatments have no significant effect on growth indices of sal saplings (Table 1).

Table 1. Growth indices of sal saplings treated with different organic amendments and cultures of ectomycorrhizal fungi after 9 months of planting in micro-plots.

Control	ECM1	Sal Litter	Sal litter+ECM1	Vermicompost+ECM1	ECM2	Sal Litter+ECM2	Vermicompost+ ECM2	Vermicompost
Growth indices								
2.35	3.64	3.44	4.20	5.03	6.99	4.90	7.98	3.03
$CD_{0.05}$ = 1.55								

Note: ECM1 = Ectomycorrhizal fungus, *Russula michiganensis*; ECM2 = Ectomycorrhizal fungus, *Lycoperdon compactum.*

Discussion

Mycorrhiza is a beneficial association between fungi and root of higher plants. About 85 percent higher plants (gymnosperms and angiosperms) form this type of relationship in forests (Bakshi 1974). In fact, this sustainable relationship is a deal between mycobionts and phycobionts for their survival, and to some extent, it may prove to be obligatory for both the partners. This

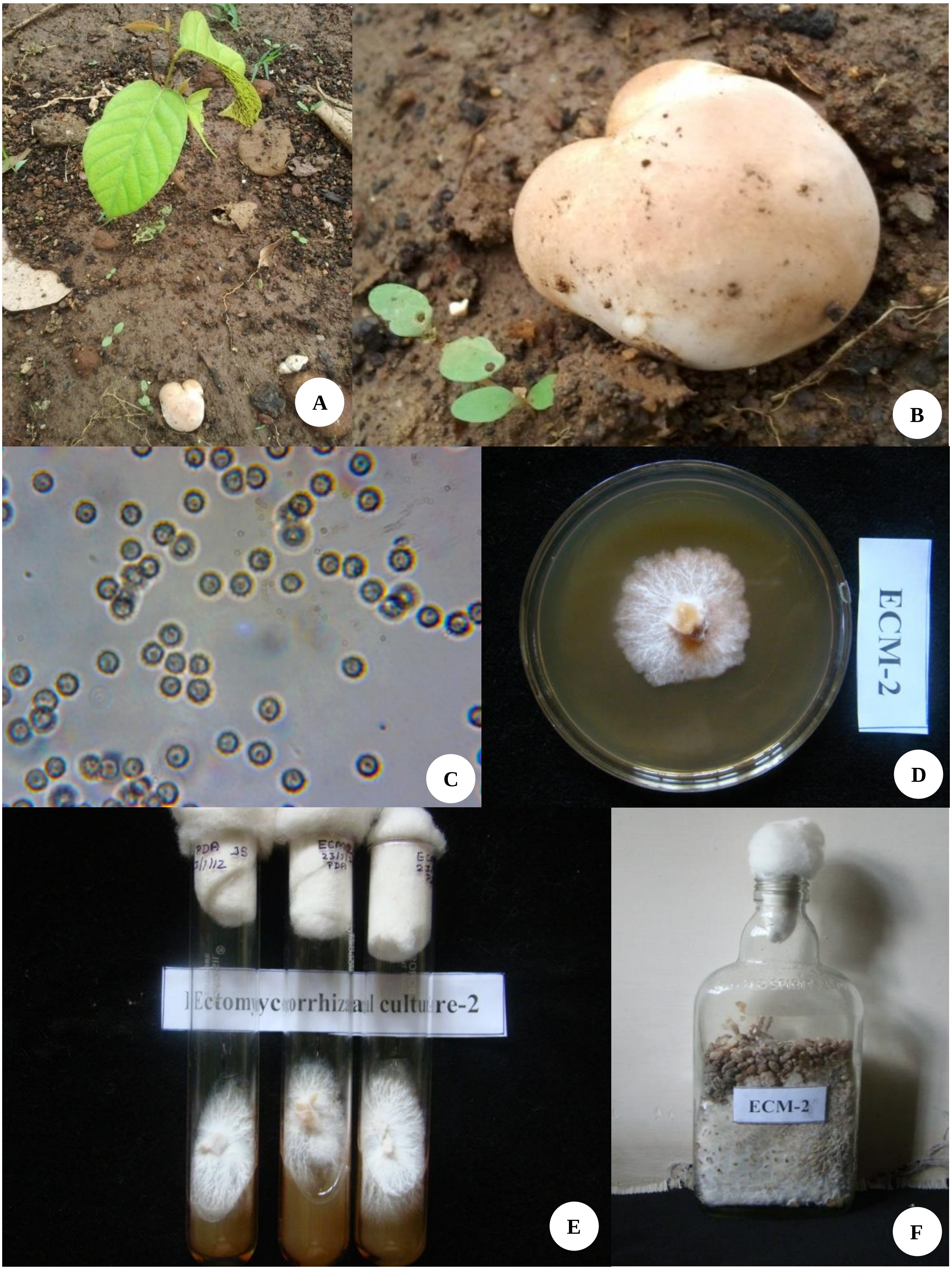

Figure 3. *Lycoperdon compactum*, an ectomycorrhiza forming fungus associated with sal saplings. A. Fruit body near the sapling of Shorea robusta, B. Single basidiocarp, C. Basidiospores (40x), D. Seven days old pure culture on PDA plate. E. Seven days old pure culture on PDA slants, F. 45 days old spawn in wheat grains.

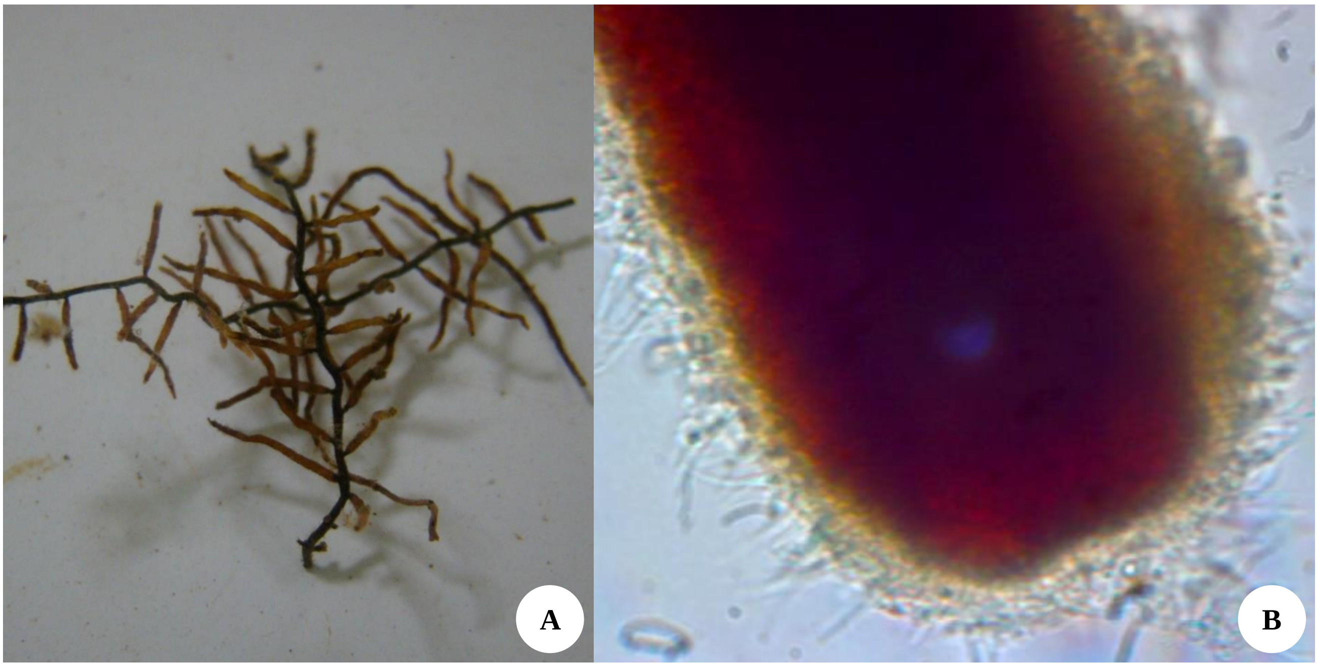

Figure 4. Development of ectomycorrhiza in sal. A. Ectomycorrhizal roots collected during winter season. B. Mantle formation in sal root

beneficial relationship can be exploited in establishing new plantations at deteriorating sites and in degenerating forest covers. In the present study, we established an ectomycorrhizal synthesis *in vivo* by inoculating two ectomycorrhizal fungi during seed sowing along with other treatment like sal litter and vermicompost which provides sal seeds the natural consortium of microbes which support its growth. But these natural consortiums of microbes when used alone are not very much effective in growth of sal saplings. These are more effective when used along with the cultures of ectomycorrhizal fungi. Among the two ectomycorrhizal fungi used only *Lycoperdon compactum* produces basidiocarps in short duration. Another species *Lycoperdon perlatum and Russula parasurea are reported to form mycorrhiza with P. patula* in Nilgiri Biosphere Reserve Tamil Nadu, India (Mohan 1991). In Malaysia two other dipterocarps also developed ectomycorrhiza with locally isolated ectomycorrhizal fungi (Lee et al. 2008). Many scientists from India and abroad have artificially established mycorrhization in *Pinus* and *Eucalyptus* spp. and have got fruitful results (Alexander 1981; Marx and Ross 1970). In central India, Sharma et al. (2009) have synthesized ectomycorrhiza in a monocot host, *Dendrocalamus strictus* with *Cantharellus tropicalis.* In our study we used wheat grains for production of inocula of ectomycorrhizal fungi similarly Kannan and Natarajan (1988) also produced spawn of *Laccaria laccata* and *Amanita muscaria* but in sorghum grains. In the present study, we have tried sal litter collected from natural sal forest to inoculate the seedling in non-sal area similarly Lakhanpal (1987) used natural roots of mycorrhizal trees for artificial inoculation of *Pinus gerardiana* and *Picea smithiana* seedlings to develop mycorrhiza.

CONCLUSION

Artificial application of ectomycorrhizal fungus synthesize mycorrhiza in feeder roots at non sal growing area and helpful in initial growth and establishment of sal seedling in central India.

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