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Ectomycorrhizal influences on selected tree species from Central Himalayan Region of India

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Abstract

Mycorrhizae mediated processes are know to influence the growth performance of host species in plant communities, but not much is know about their role in competitive outcome of host species. In view of the great potential of suitable mycorrhizal fungi, pure culture technique developed and commercialized in western countries to be applied for harnessing this natural source of biofertilizer for growth, improvement and for the greening of wasteland in India. The comparative symbiotic efficiencies of two ectomycorrhizal fungi, *viz.*, *Amanita hemibapha* and *Rusulla vesca* were assessed in association with the seedlings of ban oak (*Quercus leucotrichophora*) and chir pine, (*Pinus roxburghii*) major tree species of the Indian Himalayan region. The over all grwoth performance was found to be positively influenced, depending on the species of ectomycorrhizal fungi used. While the oak performed better in comparision to pine, when grown in a mixed culture in the presence of *Russula vesca*, pine was found to perfom better in the presence of *Amanita hemibapha*.

Keywords: Ectomycorrhizae, *Quercus leucotrchophora*, *Pinus roxburghii*, seedlings, growth improvement

1. Introduction

Mycorrhiza-mediated processes generally influence nutrition and competition in plants, and soil nutrient cycling (Smith and Read, 1997). More than 90% plant species have association with mycorrhizal fungi (Trappe, 1987) but little is known about the effects of mycorrhizal symbiosis on plant species composition and competition (Ozinga et al., 1997). Mycorrhizae are known to influence plant performance through the positive influence they confer on their hosts. The benefits, for example, lead to improved growth of host plants and increased tolerance to drought and diseases (Molina et al., 1992). The importance of mycorrhizal fungi in determining plant diversity relative to other mechanisms such as species competition and species coexistence has remained largely unattended. The role of mycorrhizal fungi in nutrient uptake by host plant may vary from one group of fungi to another and with changing environmental condition. Through a conceptual model, Aerts (2002) schematically showed that the type of mycorrhizal association, such as ericoid mycorrhizal fungi and arbuscular mycorrhizal fungi determine the plant species which dominate in heathland ecosystem. It is likely that the effect of mycorrhizal fungi on the nutrient uptake of the host plant also varies from one species to another within the same group of mycorrhizal association. Evidence is accumulating to indicate that different species of ectomycorrhizal fungi evoke differential responses on host plants (Cairney, 1999; Saikkonen et al., 1999).

The main objective of this study was to examine whether competitive outcome of the tree species occurring together in forests varies with the change in associated ectomycorrhizal species. The basic assumption is that in the course of evolution the most beneficial fungal associate of the host is selected.

The tree species studied were *Quercus leucotrichophora* (ban oak) and *Pinus roxburghii* (chir pine). Having

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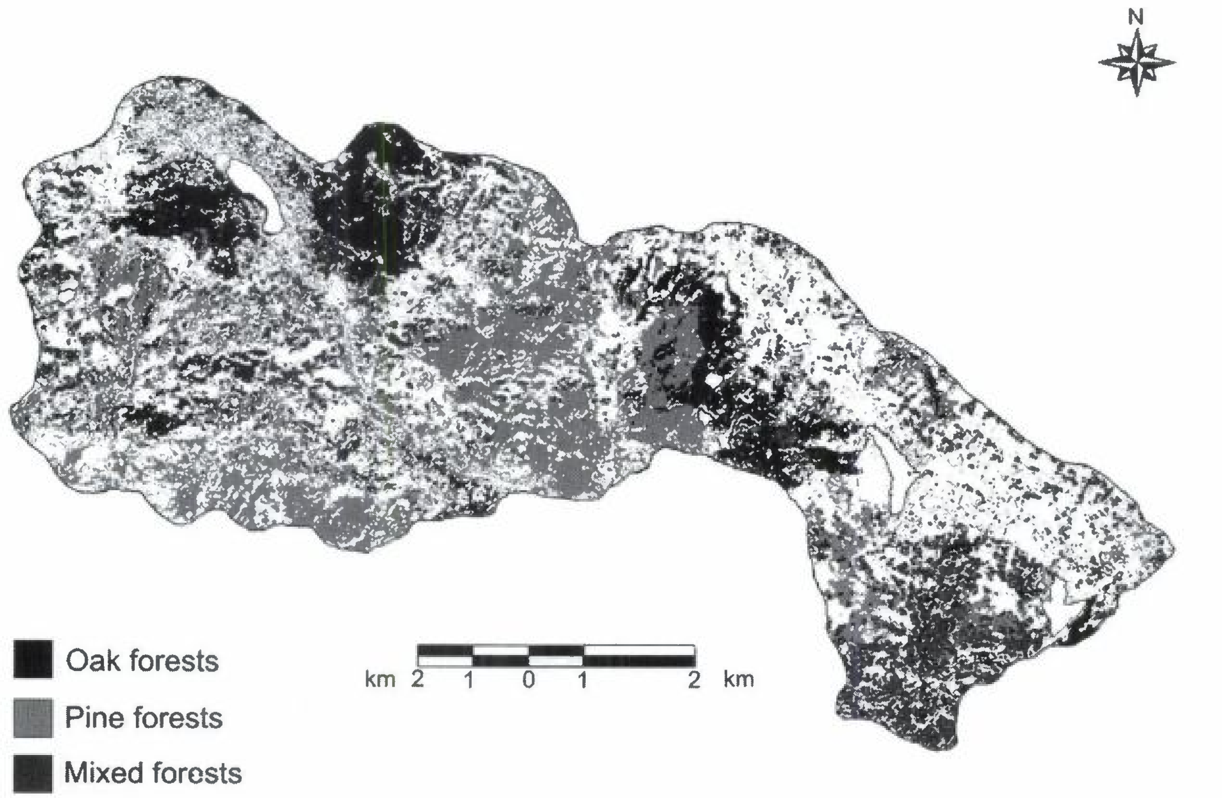


Figure 1. Major forest types of study area (Nainital).

considerable overlaps in the altitudinal distribution (generally between 500 to 1800 m and 1200 to 2000 m, respectively, Fig. 1., Tiwari and Joshi, 2006), these tree species tend to dominate their respective forest, though mixed stands of these are not uncommon. The ectomycorrhizal fungi selected for the study are *Amanita hemibapha* and *Russula vesca.* It has been reported earlier that in the Northwest Himalaya, species of most ectomycorrhizal genera show host specificity. Majority of species are associated either with oak or conifers (Lakhanpal, 1997), and in this respect they resemble the pattern described in the global summary of ectomycorrhizae (Molina et al., 1992). In Himalaya, though both *Amanita* and *Russula* species are found in pine as well as in oak forests, the later is much more abundant in oak forest in comparision to pine and the riverse is true in case of *Amanita* sp. (Pande et al., 2004). We hypothesize that because of greater root colonization, oak seedlings would have competitive advantage over pine seedlings in the presence of *Russula vesca* and pine over oak in the presence of *Amanita hemibapha.* An interesting feature of this study is to examine if cahnge in competitive outcome takes place due to change in the mycorrhizal partener. To the best of our knowledge there are no reports indicating the significance of the associated ectomycorrizal species on the outcome of competition between mycorrhizal host plants.

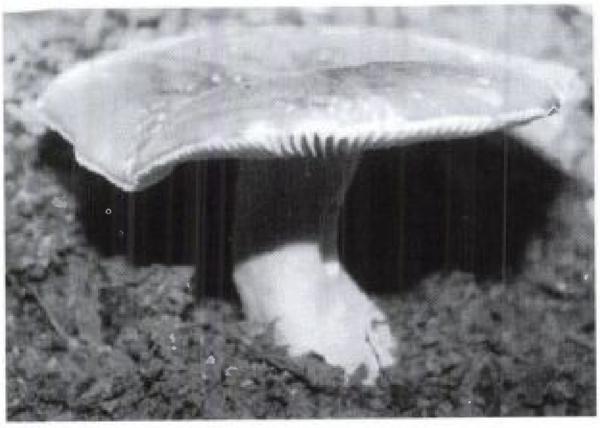
2. Material and Methods

A glasshouse experiment was conducted to assess the improvement in growth and fitness of oak and pine seedlings artificially inoculated with selected ectomycorrhizal fungi. For this, sporocarps of two species, *Amanita hemibapha* and *Russula vesca* (Fig. 2) were collected from the study sites located in mixed forests of District Nainital (Uttarakhand state) in the Central Himalayan region of India. The sporocarps were brought to the laboratory, surface sterilised with 30% H_2O_2 and transferred to modified Melin Norkran's (MMN) agar medium plates (previously autoclaved) under aseptic conditions (Marks, 1969). The plates were incubated at 25°C and the pure cultures were obtained after 15–20 days by repetitive subculture for 2 to 3 times.

Bulk inocula were produced as mycelia on paddy husk substrate supplemented with MMN broth medium. For this, two-litre conical flasks, each containing 200 g paddy husk mixed with 1 litre MMN broth medium were sterilized. The pure cultures of the two fungi were prepared and inoculated separately in different conical flasks, under aseptic conditions. The cultures were allowed to grow for 60 days to get dense mycelial growth on the paddy husk.

The seeds of ban oak (*Quercus leucotrichophora*) and chir pine (*Pinus roxburghii*) were germinated on moist

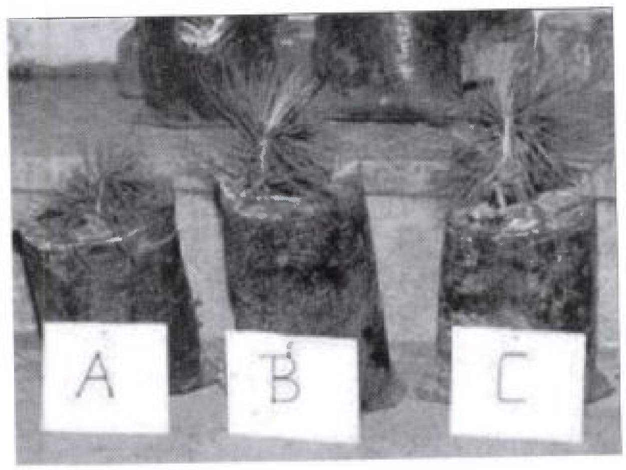
(a)



(b)

Figure 2. Sporocarps of the ectomycorrhizal fungal species used (a) *Amanita hemibapha*, (b) *Russula vesca*.

(a)



(b)

Figure 3. Seedlings of oak (a) and pine (b) showing the effect of two inoculated ectomycorrhizal species. A: Control; B: Inoculated with *Russula vesca*; C: Inoculated with *Amanita hemibapha*.

sterilized sand in a glass house after surface sterilization. Potting mixture was prepared by mixing sand and soil in the ratio 1:2. Characteristics of the soil are depicted in Table 1.

For each fungus, 10 g paddy husk inoculum was thoroughly mixed with 3 kg of sterilized sand-soil mixture that was filled in the nursery bags sterilized with alcohol. For control, no fungal inoculum was added in the sand-soil mixture. Two seedlings were transferred in each bag using the following combinations.

Control	Inoculated with *Amanita hemibapha*	Inoculated with *Russula vesca*
Oak+Oak	Oak+Oak	Oak+Oak
Oak+Pine	Oak+Pine	Oak+Pine
Pine+Pine	Pine+Pine	Pine+Pine

Each treatments was replicated five times. After six months, the seedlings were carefully removed by brushing the polybags and washed under gently flowing tap water. Different growth parameters, *viz*. root length, shoot length, collar diameter, dry mass of shoot, root and the number of mycorrhizal and non-mycorrhizal fine roots were recorded.

3. Results

In both oak and pine, the seedlings inoculated with ectomycorrhizal fungi showed significantly ($P<0.001$) more growth in respect of all parameters (shoot length, root length, collar diameter) over the uninoculated controle (Fig. 3). The ectomycorrhizal fungi, *Russula* and *Amanita*, significantly increased the number of fine roots (Fig. 4). The *Russula* inoculated oak seedlings showed more mycorrhizal roots compared to those inoculated with *Amanita,* whereas *Amanita* favoured pine seedlings in general for the production of mycorrhizal roots. The dry

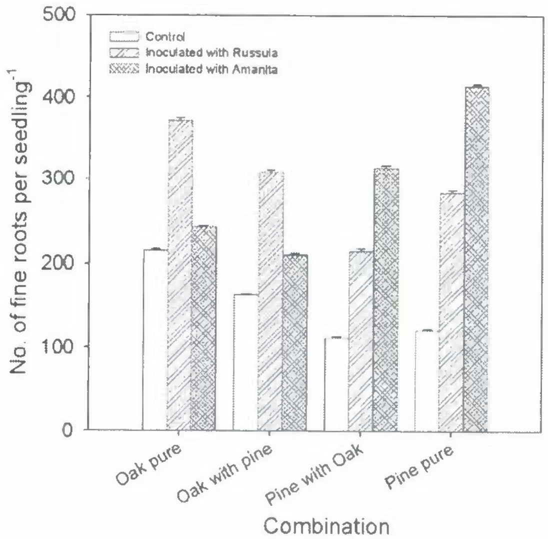


Figure 4. Effect of mycorrhizal fungi on competitive outcome in total fine roots production of 6 months old oak and pine seedlings. 'Oak with pine' indicates oak seedlings grown with pine and compared with pure oak seedlings, whereas 'pine with oak' indicate their comparison with pure pine.

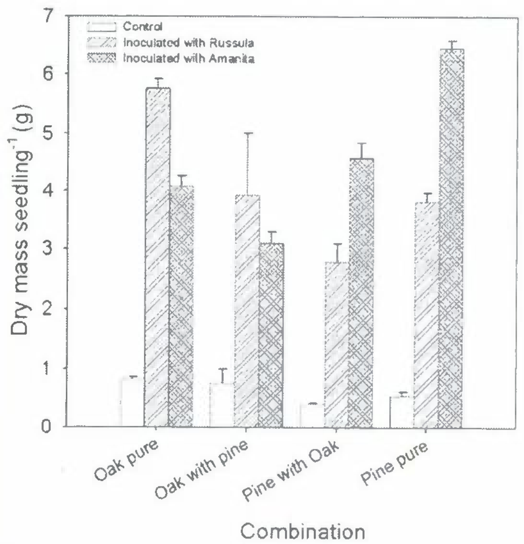


Figure 5. Effect of mycorrhizal fungi on competitive outcome in total dry mass of 6 months old oak and pine seedlings. 'Oak with pine' indicates oak seedlings grown with pine and compared with pure oak seedlings, whereas 'pine with oak' indicate their comparison with pure pine.

mass of oak seedlings inoculated with *Russula* and *Amanita* was seven- and five-fold greater than that of uninoculated seedlings, respectively. A six and eleven-fold increase in the total pine seedling mass was observed when inoculated with *Russula* and *Amanita,* respectively.

In both the species, seedling mass was greater when the seedlings were grown separately than when grown in mixture (Fig. 5). Evidently, competition reduced the growth

Table 1. Important characteristics of soil used in the pot experiments.

Moisture	38.3%
Soil temperature	25°C
Sand	60%
Silt	30%
Clay	10%
pH	6
Carbon	1.6%
Nitrogen	0.246%
Available P	62.35 kg/h
Potassium	436.8 kg/h
Litter cover	90%

of seedlings of both species with or without inoculation. However, the competitive outcome between oak and pine seedlings changed with the change in the fungal associate, i.e., *Russula vesca* favoured oak while *Amanita hemibapha* favoured pine. It should however be kept in view that these experiments dealt with a very early stage of seedlings and the results are indicative of the competitive outcome. In addition, the distance between seedlings in the pot experiments, as in the present study is relatively smaller than what happens in nature. The study nevertheless warrents a more elaborate and long-term experimentation. From the results it can also be concluded that favourable effect of *Russula vesca* was more on oak seedlings and that of *Amanita hemibapha* on pine seedlings (Fig. 3).

4. Discussion

Evidence suggest that mycorrhizal fungi may be involved in the regulation of competition between plant species particularly when neighbouring individuals differ in their response to mycorrhizal association (Allen and Allen, 1990). The mycorrhizal fungi may affect competition between plants in more ways than one, often by triggering a chain of reactions. Oak and pine have many common ectomycorrhizal fungi and this sharing might play a significant role in deciding the competitive success of one host species over the other. For example, the mycorrhizal fungi may increase soil nutrient and water availability, which in turn may cause a greater leaf expansion and ability to compete for light (Hetrick et al., 1989). They may also modify the outcome of competition through inter-plant mycelial connections and transfer of material (Hartnett et al., 1993; Simard et al., 2002) and changes in the rhizosphere chemistry (Chang and Li, 1998). A fungal species that modifies the competitive outcome between neighbouring species must facilitate supply of nutrients and water at different relative rates (Allen and Allen, 1984, 1986). In the present experiment two-ectomycorrhizal fungi differed in their symbiotic effect on the hosts, thereby bringing about change in their competitive outcomes. The fungal species, the association of which favoured the host species in competition seemed to give a relative advantage to it through a better access to resources, such as water, over other forest species. Modifications such as these can have significant implications for community dynamics.

There are data to suggest that host plants also bring about difference in the life history traits such as sporulation or infection of different mycorrhizal fungi (Sanders, 1993; Sanders et al., 1992; Bever et al., 1996). Streitwolf-Engel et al. (1997) have shown strong differential effects of the fungal species on morphology and pattern of clonal growth of plants, thus affecting their spatial arrangement in communities. Observations such as these and of the present study suggest that mycorrhizae play a significant role in determining the organisation of plant communities. Thus, the differential interaction of plants and fungi is reciprocal, and also an important factor in overall community organisation. The present experiment dealt with a very early phase of seedling growth, and the results of competitive outcome between the host species may change as time progresses, nevertheless it throws light on the importance of fungal flora involved in mycorrhizal association to plant community dynamics. To conclude, this preliminary study on ectomycorrizal components of the Central Himalayan oak indicates that the fungal species seems to have a marked influence on competitive outcome of the seedlings, their growth and health status. These species can, therefore be used as efficient bioinnoculants for a particular tree species and act as biofertilizer in nursery management of these tree species.

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