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Influence of mycorrhizal developmental stages and plant age on rhizosphere mycoflora of Pinus kesiya (Royle)

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Quantitatively the population was recorded to be high around the mycorrhizal roots. Some fungi were specific to different stages of mycorrhizal development. *Rhizopus nigricans* and *Cunninghamella elegans* were recorded at 5% mycorrhizal association stage. *Fusarium* sp. was found at 20% mycorrhizal association, while *Mucor* spp. were obtained at 60% stage. *Verticillium* sp. had the highest frequency of occurrence in the beginning of mycorrhizal association but later on *Penicilium* spp. were found to be the most common. Sugar content of mycorrhizal and nonmycorrhizal roots were determined to assess their effect on the mycorrhizospheric micropopulation. The mannitol and trehalose were present only in mycorrhizal roots.

INTRODUCTION

Since the introduction of the term rhizosphere in 1904, it is well understood, that there is greater microbial activity in this region than in soil away from the roots. Numerous researchers have tried to investigate the various aspects like the influence of higher plants on rhizosphere microflora (Mishra, Kanaujia 1973; Bowen 1969), the effects of root exudates (Bovira 1956; Mishra 1970), chemical fertilizers (Mishra, 1972), defoliation (Srivastava, Mishra 1970), other chemicals (Mishra, Singh, Kanaujia 1973) and light (Harley, Waid 1955) on the rhizosphere microflora.

The knowledge on the mycorrhizosphere i.e. rhizosphere of mycorrhiza is meagre. Tribunerkaya (1955), Katzenlson (1960), Rambelli (1973) have compared the micropopulation of mycorrhizosphere and nonmycorrhizosphere regions. In normal root the exudates pass directly to soil and rhizosphere microorganisms thrive on such nutrients. In the case of mycorrhizosphere, however, the root exudates are available to the microorganisms only after being subjected to a sort of screening by the fungal symbiont and the nutrients to be absorbed by the plants from the soil have to move through the fungal sheath. During this process many changes may occur in the nature of leachets which may or may not be favourable for the growth of microbial population. Studies on such aspects are not available to understand the role of the fungal symbiont in regulating the flow of root exudates.

The main aim of this study was to investigate the distribution pattern of mycorrhizospheric mycopopulation at different stages of the fungal association.

MATERIALS AND METHODS

A site for present study was selected at the altitude of 1500 M Shillong (Meghalaya) near a natural old pine stand. The soil was sandy loam with a pH 5.

For the study of mycorrhizosphere micropopulation, soil was assesseptically collected in the month of July and August 1977 from the vicinity of roots of *Pinus kesiya* (Royle) raised in nursery beds near to this old pine stand. Sampling was done at the different stages of mycorrhizal development. Samples were collected when the plants were 12, 15, 20, 28, 36 and 45 days old. Sampling was done from five plants selected randomly. A composite sample was made from five samples collected for each stage of mycorrhizal development. For the isolation of fungi, Peptone Dextrose Rose Bengal Agar and Malt extract media (Johnson, Curl 1972) were used. Warcup's (1950) method was adopted for isolation of fungal species and 0.003 g of soil was used as an inoculum in each plate. Fungal plates were incubated at $25 \pm 1^{\circ}C$ for five days. After incubation the colonies were counted by a digital colony counter and examined for identification.

Roots of seedlings collected at the different stages indicated above were washed several times in running water and kept in a mixture of formaldehyde, acetic acid and ethyl alcohol (5/5/90 v. v.) for one day to remove the fine particles of soil and ultimately washed in sterile water. The roots were examined for mycorrhizal development cutting sections by hand transversely. The sections were stained in cotton blue and mounted in lactophenol. The root sections were examined under

the microscope for mantle development and to determine the percentage of mycorrhizal association in each stage. The sugar contents of root at different stages of mycorrhizal development was determined by chromatography (S m i t h 1960).

RESULTS

The results are presented in the Tables 1-4 and Figures 1-2. A remarkable effect of mycorrhizal development on soil fungi was noticed. Table 1 reveals that some fungi. viz: *Rhizopus nigricans, Cunninghamella elegans* and *Verticillium terrestre* were restricted to five per cent level of mycorrhizal association (IV stage). Different species of

Table 1
Distribution of fungi in the mycorrhizosphere of *Pinus kesiya*

Species	IVth stage	Vth stage	VIth stage
Mucor haemlis	—	—	+
Mucor sp.	—	—	+
Rhizospus nigricans	+	—	—
Cunninghamella elegans	+	—	—
Verticillium terrestre	+	—	—
Verticillium spp.	+	+	+
Verticillastrum sp.	+	+	—
Gliocladium spp.	+	+	+
Penicillium spp.	—	+	+
Fusarium oxysporum	—	+	—
Humicola sp.	+	+	—
Total number of fungal species	7	6	5

Fusarium were present at twenty percentage of mycorrhizal association and *Mucor hiemalis* and *Mucor* sp. were confined at the sixty per cent stage. At a low level of mycorrhizal association pathogenic fungi like *Fusarium* spp. were dominant. At a high degree of mycorrhizal association saprophytic forms i.e. *Penicillium* spp. were number predominant (Table 4).

Quanititatively the fungal population increased in proportion to the increasing level of mycorrhizal development (Fig. 1).

Fungal symbiont colonization started in the seedlings after twenty eight days of sowing (IV stage). In forty five days old (VIth stage) seedlings sixty per cent of roots possessed fungal symbiont (Table 2 and

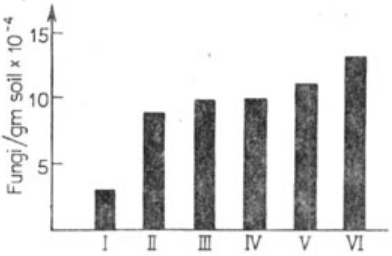

Fig. 1. Stages of mycorrhiza development

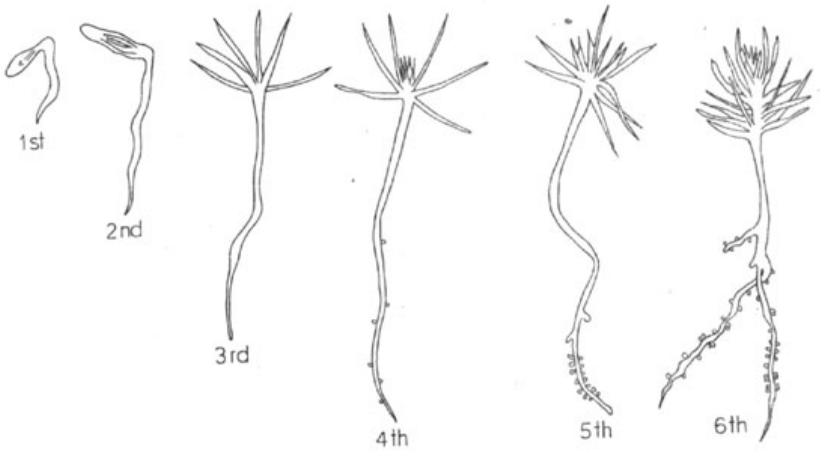

Fig. 2. Developmental stages of mycorrhiza in *Pinus kesiya*

Table 2

Different developmental stages of mycorrhizal association of *Pinus kesiya* (Royle)

Different stages of *Pinus kesiya*	Percentage of mycorrhizal development	Fungal sheath thickness, μm	Type of association	Mean shoot/ /root ratio mm
IVth	5	7.5	ectomycorrhiza	30/45
Vth	20	11.25	ectomycorrhiza	38/56
VIth	60	27.25	ectomycorrhiza	40/62

Fig. 2). The thickness of the mantle was recorded nearly four times greater than at the initial stage of mycorrhizal development (Table 2).

Only on ectomycorrhizal type of association was recorded in *Pinus kesiya*.

In the sugar content from initial stages of seedlings to highest mycorrhizal development stages varied qualitatively and quantitatively. At highest mycorrhizal association, trehalose, rhamnose and manintol were the main sugars while at a low level of mycorrhizal development the important sugars were fructose, trehalose and glucose (Table 3).

Table 3
Distribution of sugars at different developmental stages of *Pinus kesiya* seedlings

Sugars	Ist	IInd	IIIrd	IVth	Vth	VIth
Fructose	+	+	+	+	—	—
Trehalose	—	—	—	+	+	+
Fucose	+	+	—	—	—	—
Rhamnose	—	—	—	—	—	+
Glucose	+	+	+	+	+	+
Manintol	—	—	—	—	+	—

\+ Present
— Absent

Table 4
Percentage occurrence of fungal species at different mycorrhizal root rhizosphere

Species	IVth stage	Vth stage	VIth stage
Mucor hiemalis Wehm.	—	—	6.8
Mucor sp.	—	—	3.4
Rhizospus nigricans Ehr.	10	—	—
Cunninghamella elegans Lendn.	8	—	—
Verticillium terrestre (Link) Lind.	12	—	—
Verticillium sp.	44	27.7	22.03
Gliocladium sp.	8	9.09	6.8
Humicola sp.	6	7.27	—
Penicillium spp.	—	45.4	61.2
Fusarium oxysporum Schlecht.	9.09	0.00	—
Verticillastrum sp.	12	10.9	—

DISCUSSION

The microbial population in the rhizophere region is affected mainly by the age of plants (Rovira 1956; Foster, Rovira 1976; Mishra

1968). However, certain other factors, viz: defoliation (Srivastava, Mishra 1970), chemical fertilizers (Mishra 1972) and cover vegetation (Mishra 1972) and cover vegetation (Mishra 1968; Sharma 1977) also influence the rhizospheric micropopulation. In the case of mycorrhizal roots the fungal symbiont plays the primary role in the determination of rhizosphere microbial population (Rambelli 1973; Neal, Bolhen, Zak 1941). Table 1 and fig. 1 clearly revealed that microbial population is influenced strongly both in quality and quantity withean increase in degree of mycorrhizal association. Short root initials covered with a thick fungal mantle were resistant to infection but an incomplete fungal sheath probably could not prevent the penetration of pathogens (Marx, Devey 1969), (Table 4). Pathogenic forms were dominant at a low degree of mycorrhizal development but at the high degree of mycorrhizal association saprophytic forms were more common. This clearly suggests that ectomycorrhizal fungi and fungal mantle protect the plant root pathogens (Marx, Devey 1969) by forming a mechanical barrier. The development of mycorrhizae and the mantle thickness influenced the rhizosphere population. This change in population is influenced by many factors but one of them may be the distribution and presence of sugars in roots. The mycorrhizal roots store more simple sugars as the mycorrhizal association increases. The ectomycorrhizae in our study functioned as a mechanical barrier to certain pathogenic forms like *Fusarium* species and provided a conducive environment for the saprophytic fungi. This type of selection may help better plant growth causing a lower incidence of diseases in well developed ectomycorrhizal plants. The exact role of fungal symbionts on the mycorrhizosphere is, however, still a debatable topic and requires further investigations.

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