Role of Soil Moisture in Ectomycorrhizae Formation and Growth of Eucalyptus [*Corymbia citriodora* (Hook.) K. D. Hill and L. A. S. Johnson] Seedlings.

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Abstract

Mycorrhizal fungi are obligate bio-trophs of plant roots and are a major factor in improving plant growth. Eucalyptus seedlings were planted in the field and inoculated with two native ectomycorrhizae *Scleroderma citrinum* and *Russula rosea.* The seedlings roots were observed at regular interval of ectomycorrhizal colonization and also growth parameters were measured. Some seedlings were maintained as control without inoculation. Rhizospheric soil moisture level of the seedlings were analyzed at different seasons. Ectomycorrhizal fungi inoculated seedlings showed better growth than that of uninoculated seedlings. Rhizospheric soil moisture of *Scleroderma citrinum* inoculated was maximum at the spring season which shown in relation with the growth of *S. citrinum* inoculated seedlings at the 4th month. Similarly, at the summer, autumn and winter season rhizospheric soil moisture of *Russula rosea* inoculated seedlings were found to be higher which is inconsistentwith the growth of *R. rosea* inoculated seedlings. Increase in soil moisture during the rainy season was also found to be important factor controlling in ectomycorrhizal activity and colonization. Decrease in soil moisture level during spring and autumn season was found to decrease in percent of ectomycorrhizal colonization to the roots length. However, growth of individual etcomycorrhizal fungi differs with seasonal variation, soil chemistry and host interaction.

Keywords: Ectomycorrhzae, growth, soil moisture.

1 Introduction

Most of the eucalypt plantations in India started during decades 1960 – 1980. During this period it became one of the main species for planting by foresters, scientist and workers because of their ability to resist drought, fast growth and also to meet the needs for pulp and paper. Eucalypt plantations were raised under social forestry projects in many states because of its ability to regenerate degraded forest and waste lands.

Mycorrhizal development in association with plant roots are affected by different factors (Harley, 1969). Extremes of soil temperature, pH, moisture etc., and the presence of antagonistic soil microorganism can affect and regulate the fungal symbiont (Marx, 1980). Several studies has documented that inoculation of ectomycorrhiza on eucalypt increased growth, increased biomass production in nursery seedlings (Grove *et al.*, 1991), improve survival and increased tolerance of seedling to environmental stress (Burgess and Malajczuk, 1989). Seasonal variation in the periodicity of growth and mycorrhizal activity occurs in the ecosystems and may be substantial enough to change the mycorrhizal status (Allen, 1983). Harvey *et al.* (1978) found that most of the ectomycorrhizal tips are active between May to October. Ectomycorrhizal active root tips reduce with increase in temperature and decrease in moisture content in horizons. Many studies reveal that fungal association do not benefit the host plant when water becomes limiting (Dosskey *et al.*, 1991). This effect could be due to the fact that fungal symbionts are directly affected by water availability, with each having decreased performance when water is limited (Theodorou, 1978). Difference between ectomycorrhizal fungi to colonize plant roots (Heinrich and Patrick, 1986) shows poor relation between plant growth and root colonization (Jones *et al.*, 1990). Successful establishment and inoculation of isolated mycorrhizal fungi in field condition have been carried out with different plant species in past few years. Growth and survivality of seedlings depends on their ability to adapt under environmental stress. When the soil moisture becomes maximum in all soil layers during rainy season, the ectomycorrhizal activity was recorded to be maximum (Singh, 1998). A comparison of pH of soil layers between different seasons has been observed pH in the acidic range supports maximum mycorrhizal activity.

The objective of the study was to investigate the ectomycorrhizal colonization to the roots of the seedlings at different soil moisture level of different season inoculated with two dominantly found native ectomycorrhizal fungi and its effect in growth of *Corymbia citriodora* (Hook.) K. D. Hill and L. A. S. Johnson seedlings.

2 Materials and Method

2.1 Experimental design

Seedlings of *Corymbia citriodora* were planted in the field, Nagaland University campus, Lumami (26°13.29?N and 94°28.430?E). Seedlings were then allowed to establish on the field with proper irrigation. Two species of ECM forming fungi viz. *Scleroderma citrinum* and *Russula rosea* were isolated and inoculated near the root of the seedlings as mycelia suspension. The isolates were maintained in the modified Melin Norkans agar (MMN, Marx, 1969). Twenty seedlings were maintained for each fungus and 20 seedlings maintained as control. In the control seedlings no fungal inoculums was added.

2.2 Plant and fungal parameters

After the inoculation fungal colonization was monitored at regular interval under the stereo microscope. Representing root tips were characterized on the basis of colour, branching shape and presence of emanating hyphae (Agerer, 1998). Development of ectomycorrhiza was counted by counting ultimate dichotomy as one mycorrhiza. Percentage of mycorrhizal colonization/ cm (Sharma, 1981) was calculated as follows:

$$\text{Ectomycorrhizal colonization}(\%) = \frac{\text{Total number of dichotomous branched rootlets}}{\text{Total number of lateral rootlets}} \times 100$$

Seedlings growth parameters were measured every 4th month such as shoot height, leaf length, root length and root collar diameter. Rhizospheric soil moisture was also analyzed of different season by gravimetric method (Allen *et al.*, 1974).

3. Results

At the spring season rhizospheric soil moisture of *Scleroderma citrinum* inoculated seedlings was found higher, but at the summer, autumn and winter season rhizospheric soil moisture of *Russula rosea* inoculated seedlings was found higher (Table-1). Seedlings growth has also shown better of *Scleroderma citrinum* inoculated seedlings at the 4th month (Table-2). However, the growth of *Russula rosea* inoculated seedlings has shown better growth gradually in all growth parameters at the 8th, 12th and 16th month old seedlings (Table-2).

Ectomycorhizal colonization was observed after 6th month of inoculation to the roots of the seedlings. Colonization of *Russula rosea* to the root of the inoculated seedlings was found higher at the 6th, 8th and 12th month of seedlings (Fig. 1), but *Scleroderma citrinum* colonization was found higher at the 16th month. Control seedlings also found to be colonized by the ectomycorrhiza (Fig.-1). It is expected due to using of unsterile substrate, which are probably colonized by some air borne fungal spores.

Table 1: Rhizospheric soil moisture levels of four different seasons

Fungal treatment	Spring	Summer	Autumn	Winter
M1	16%	27%	21.21%	22.21%
M2	17%	24%	20.25%	20.35%
M0	13%	20%	20%	18%

Each value is mean of three replicates

M1= *Russula rosea*, M2= *Scleroderma citrinum*, M0= control

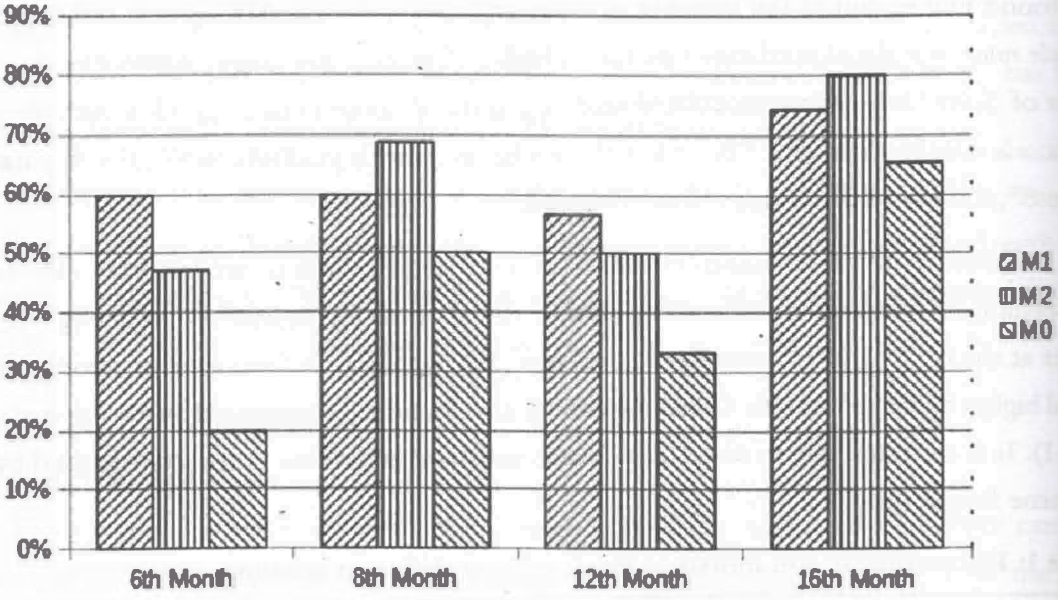
Table 2: Growth parameters of the *Corymbia citriodora* seedlings

Fungal treatment	4th month			8th month			12th month			16th month		
	M1	M2	M0	M1	M2	M0	M1	M2	M0	M1	M2	M0
Shoot Height (cm)	120	138	89	236	195	144.4	339.2	284.5	262.4	534.4	532.2	314.6
Root length (cm)	30.4	38	24	66	62	55	251	240	160	360	345	170
Leaf length (cm)	13.1	13.9	12	16	14.2	13.4	14.8	14	12	17	16.4	15.8
Root collar diameter (cm)	0.6	0.62	0.62	0.8	0.8	0.6	0.84	0.84	0.68	1.04	0.96	0.8

Each value represents the mean data of five seedlings

M1= *Russula rosea*, M2= *Scleroderma citrinum*, M0= control

Fig 1: Mycorrhizal colonization to the roots of the seedlings



Each value represents the mean data of five seedlings

M1= *Russula rosea*, M2= *Scleroderma citrinum*, M0= control

4 Discussion

Enhancement of plant growth is a major effect of mycorrhizal infection. Studies have revealed that soil moisture influence in plant growth and ectomycorrhizal formation. At low soil moisture loss of cell turgidity may occur (Mexal and Read, 1973) while in very wet soils, oxygen deficiency limits fungal activity (Slankis, 1974). Consequently, mycorrhizal infection, developments are restricted to excessive soil moisture. The present experiment shows that increase in soil moisture of different season *i.e.* at the interval of every 4th month increased in seedlings growth. Seedlings growth of *Scleroderma citrinum* inoculated has shown better growth at the 4th month of seedlings which are in consistent with the data of higher rhizospheric soil moisture at the spring season of *Scleroderma citrinum* inoculated seedlings. Similarly, rhizospheric soil moisture of *Russula rosea* inoculated seedlings was found higher at the summer, spring and winter season which found in relation with growth of the seedlings at the 8th, 12th and 16th month.

Russula rosea colonization was found higher at the 6th, 8th and 12th month, the data are consistent with the higher rhizospheric soil moisture of the seedlings during the summer and the autumnseason.But at the 16th month colonization of *Scleroderma citrinum* colonization was higher than the percentage of colonization of *Russula rosea* to the root length. Mycorrhizal species have different tolerance to various soil moisture levels (Bougher and Malajczuk, 1990). Ectomycorrhizal activity and growth also vary with seasonal variation. There are different other edaphic factors and soil chemistry which influence in mycorrhizal colonization such as the organic matter, soil temperatures. Increase in soil moisture found to effect in seedlings growth viz. root length, leaf length, shoot length and root collar diameter and increase in ectomycorrhizal colonization of both the fungal inoculated seedlings as compared to control treatment. Thomson *et al.* (1994) hypothesized that growth response of *Eucalyptus globulus* to inoculation with ectomycorrhizal fungi was positively correlated with the colonize root length. Similarly, several studies has been observed the relationship between plant growth and ectomycorrhizal colonization, which implies that ectomycorrhizal fungi can be screened on their ability to colonized plant root and growth of the host plant. Although potentially beneficial, nursery inoculation is not always straight forward and requires selection of compatible and efficient ECM fungal isolates tuned for specific target plant and growth conditions (Oleveira *et al.*, 2011)

In conclusion, mycorrhizal technology has a major relevance in forest production of exotic plants and survivability of the seedlings. Field performance of loblolly pine seedlings with specific ectomycorrhizae on a reforestation site in South Carolina has showed significantly higher height survival and root collar diameter than the controls and volume index approximately three times greater (Marx, 1977). Control seedlings have shown ectomycorrhizal colonization, however inoculation of ectomycorrhiza increase the percent of colonization as well as the biomass of the ectomycorrhizae than the uninoculated treatment. It is necessary to understand more on the symbiosis of individual ectomycorrhizal species to the host plant, its effects and the factors controlling in colonization of ectomycorrhizal fungi.

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