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Full Length Research Paper

Arbuscular Mycorrhizal Fungi on Growth of Two Multipurpose Tree Species of Garhwal Region, Uttarakhand, India

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Abstract
All trees are said to be multipurpose; some however, are more multipurpose than others. Multipurpose trees species (MPTS) are understood as 'those trees and shrubs which are deliberately kept and managed for more than one preferred use, product and/or service; the retention or cultivation of these trees is usually economically but also sometimes ecologically motivated in a multiple-output land-use system. Some MPTS have received much research attention and are therefore more widely known than others. Fodder trees and fuelwood species and sometimes fruit trees represent important groups of MPTS. The Indian subcontinent, with its peculiar central position in the old world and diverse edapho-climatic conditions, offers a unique opportunity for biological diversity in its flora and fauna. Once covered by dense forests, this region has now been deforested by the timber harvesting, grazing and conversion towards agricultural land uses. Increasing demands for fuel, fodder, timber and other forest products continue the process. Plantation of MPTS can play an important role in restoring productivity, ecosystem stability and biological diversity to degraded lands. MPTS are those deliberately grown and managed for more than one purpose. Usually the goals are economic and ecological including major products and services in a multiple-use system, especially agroforestry. These trees are intended for purposes other than the monopurpose timber production characteristic of natural forests and plantations. Thus they include such species that may serve various purposes in different contexts. Keeping this in view the present study was taken up with an objective to study the response of some medicinal tree species to AM fungi for germination and growth parameters.

Keywords: *AM Fungi, Colonization, Fodder, Inoculum, Mycorrhizae*

Introduction

Arbuscular mycorrhizal fungi (VAM/AM) are geographically ubiquitous and occur over a broad ecological range. They are commonly associated with plants in agriculture, horticulture, pastures,etc. About 90% of vascular plants form arbuscular micorrhiza (Kendrick and Birch, 1985) .There are over 300000 receptive hosts in the world flora of 160 species of VAM/AM fungi (Schenk and Perez, 1987; Mukerji., 1996). The advantage of AM fungi in crop productivity raises concern for their survival and preservation in soils. The conservation in soils covers many aspects of agricultural crop production and management programme (Hayman and Mosse, 1972; Tilak *et al*., 1985). Beneficial function of AM fungi are concerned with the uptake, translocation and transfer of nutrients, mainly phosphorous from soils to host cells therby improving nutrition and growth of host plant particularly the plant nutrients which are available at low concentration in soil. Arbuscular mycorrhizal (AM) fungi are known for improving plant growth through their influence on root geometry with enhanced production of roots aided by an increase in an uptake of major nutrients, leading to increased photosynthetic activity.

Mycorrizae are the structures formed by the association of root in plant with fungi. Such fungus association of root in plant with fungi forms symbiotic association with many plants under natural conditions. Fungi receive carbohydrates from host plant while plant receives mobilized phosphorous and other nutrient through mycorrizal roots. The mycorrizal habit has perhaps evolved as a survival mechanism for both partners in association allowing each to survive in adverse environment of low soil fertility, water stress, temperature extreme and salinity where they could not. They increased nutrient uptake from soil by increasing the absorptive surface area of the root system. The extrametrical hyphae proliferates in bulk soil and available the poorly mobile element such as P, Zn, and Cu. Mycorrizal plant are well nourished as compared to non- mycorrizal plant in nutrient deficient soils. The VAM/AM confers drought tolerance to plant by increasing the water absorption capacity of the root system or by altering the host physiology. The external hyphal network contributes to the process of creating a stable soil aggregate structure, improving soil structure for better aeration and water percolation. The present investigations were carried out in the nursery of Department of Forestry and Natural Resources, HNB Garhwal University to study the germination and other growth parameters of multipurpose tree species as influenced by AM fungi.

Materials and methods

Study area

The present study was conducted in the Chauras campus of HNB Garhwal University and experiments were carried out in the nursery and pathology laboratory of Department of Forestry and Natural Resources, HNB Garhwal University.

Collection of Soil sample

Soil samples were collected from different locations of Chauras campus, H.N.B. Garhwal University. For collection of soil we removed the litters from the ground, and then collected soil from the rizosphere of plants /weed at depth upto 5-10cm.with the help of spade (Khurpi) and collected in uv sterilized polybags.The collected soil samples were dried at room temperature for 3-5 days.

Isolation of AM fungi from collected soil

For isolation of spores of AM fungi from soil samples, Gardeman and Nicolson's (1963) wet sieving and decanting technique was used for the present study.

Mycorrizal colonization in roots

For mycorrizal colonization in roots Phillips and Haymen (1971) technique was used. The stained roots were cut into a segments of 1 cm length.100 segment were mounted in lactophenol and press lightly with thumb to prepare to squash. These squash preparation were observed under in compound microscope to study the characteristics of AM Fungi in roots. A root segment was considered to be mycorrizal if it shows the presence of hyphae, vesicles or arbuscles.

Identification of mycorrhizal spores

For identification, mycorrhizal spores were mounted on glass slides in lacto phenol and were observed under compound microscope using the standard monographs given by Schenck and Perez (1990).

Inoculum production of AM Mycorrhizal spores

Earthen pots of 6 diameters were filled with fumigated soils: (1:1) mixture and covered with aluminum foil. This ensures most of the natural fungal spores of putting mixtures are killed. Seeds of *Sorghum halepense* (sudan grass) were surface sterilized with 0.1% sodium hypo chloride for 5 minutes and then wash repeatedly with distilled water. These seeds were sown in pots with AM fungal spores of *Glomus macrocarpum* isolated from the soils of Chaurus Campus. Germinating seeds in contact with the AM fungal spore, which germinate and hyphae penetrates the roots of the seedling and multiply. At harvest the roots of seedling were shredded into small pieces and mixed with the rhizosphere soil to obtain homogenous inoculums.

Nursery preparation

The soil was collected from the nursery of Forestry Department and fumigated with 1% formaldehyde for 24 hrs. This fumigated soil was filled in the polybags. Following four treatments were taken in planting polybags for the comparative study and determination of the potential efficacy of the AM fungi for two MPTS test plants.
A: Control (Fumigated soil C)
B: Fumigated soil with compost (FS+C)
C: Fumigated soil with Mycorrhiza (FS+ M)
D. Fumigated soil with mycorrhiza and compost (FS+M+C)
Following two test plants were taken for the present study:

Bauhinia variegata L Caesalpiniaceae

It is a medium sized tree with an elongated spreading crown and bluish-green foliage. Bark is dark brown. Leaves 6-12X 6.5-14cm, cleft 0.8-2.5cm, sub curvaceous; cordate at base with 11-15 nerves; petioles 2-4 cm long. Flower white or purplish variegated, 4-5 cm long, appearing on leafless branches, in few flowered racemes. Calyx cylindrical or turbinate, 2.5-3 cm long, with 5 teeth. Petals 4-5 cm long, obovate, with red purple veins. Fertile stamens 3-5. Pods 15-30X 1.5-2.5 cm, 10-15 seeded, glabrous.Wood is used for agricultural implements and fuelwood. Ash of dried leaves is taken in cough. Young flowers are eaten as vegetable. Seed contain 16.5% of fatty oil. Leaves provide good quality fodder. Fibre from bark variously used. The tree yield a gum.

Querus leucotricophora A. Camus (Synon. *Q. incana* Roxb.) (Fagaceae)

It is evergreen tree attains a height up to 40 m. Bark pale grey to blackish and rough. Leaves ovate –lanceolate 5-11.5X 2.5-5 , cm acuminate, cuspidate, serrate, coriaceous glossy dark green above densely white pubescent beneath, base cuneate, lateral nerves 12-20 pairs. Male catkin 5-10 cm long, pale white, slender, pubescent, interrupted. Female flowers solitary or clustered on short spikes, axillary. Acron ovoid, 1-1.6 cm long, smooth cup covering half or more than the half of the nut.

Its leaves provide valuable fodder for livestock in hill tracts and leaves can be harvested throughout the year when there is scarcity of other foliage. Its wood is used for construction, plough and bed sticks, as well as fuel. It is also considered as an ideal firewood species. Gum of tree medicinally used for gonorrheal and digestive disorders. Decomposed leaves used as organic manure. It is an important tree of social forestry. Planting polybags with different soil mix (treatments) was filled to the level of about 1-2cm below the upper margin of the bags. The polybags were watered, so that soil is moist but not sodden.5 seeds of test plant were sown in each polybags and seeds were covered with soil mix taking care that thickness of the cover was not more than the diameter of the seeds. Nursery observations were taken at 15 days intervals starting from one month after sowing. Five seedlings from each treatment were selected randomly and brought to laboratory for further study. The parameters recorded are:

a) Shoot height
b) No. of leaves
c) Shoot fresh weight
d) Shoot dry weight
e) Root fresh weight
f) Root dry weight
g) Leaves fresh weight
h) Leaves dry weight

Results and Discussion

During the present study spores of following species of arbuscular mycorrizal (AM) fungi were isolated from the rhizosphere of different plant species of which the *Glomus fasciculatum* was found most dominant.

1. *Glomus fasciculatum*
2. *Glomus macrocarpum*
3. *Glomus microcarpum*

Bauhinia variegate

After 15 days of seed sowing the maximum (6.1` cm) growth was recorded in treatment C (FS+M) and maximum number of leaves (3.0) was recorded in treatment C (FS+M).The minimum (5.2 cm) growth was recorded in B (FS+C); After 30 days of seed sowing the maximum (10.5cm) growth was recorded in treatment C (FS+M).At this stage height and number of leaves were observed more (5.0) in treatment C (FS+M) than the other treatment. The minimum (7.7cm) growth was recorded in treatment B (FS+C) ;After 45 days the maximum (13.3cm) growth was recorded in treatment C (FS+M). The height and leaves were observed more in treatment C (FS+M) and minimum (8.5cm) growth recorded in treatment B (FS+C); After 60 days the maximum (14.9 cm) growth was recorded in treatment C (FS+M) and maximum (11) number of leaves was observed in treatment C (FS+M) and minimum (9.0 cm) growth was recorded in treatment B (FS+C) (Table-1; Fig. 1).

Table- 1. Growth of *Bauhinia variegata* seedling in polybags.

Time (Days)	Treatment	Shoot Height (cm)	No. of leaves	Root fresh weight (gm)	Root dry weight (gm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Leave fresh weight (gm)	Leave dry weight (gm)	Root length
15	Control	5.7	2.0	-	-	-	-	-	-	-
	FS+C	5.2	2.0	-	-	-	-	-	-	-
	FS+M	6.1	3.0	-	-	-	-	-	-	-
	FS+C+M	5.3	2.0	-	-	-	-	-		-
30	Control	8.9	4.0	-	-	-	-	-	-	-
	FS+C	7.7	4.0	-	-	-	-	-	-	-
	FS+M	10.5	5.0	-	-	-	-	-	-	-
	FS+C+M	9.5	3.0	-	-	-	-	-	-	-
45	Control	9.5	4.0	0.299	0.100	0.159	0.054	0.490	0.156	5.1
	FS+C	8.5	4.0	0.291	0.095	0.245	0.099	0.109	0.059	8.1
	FS+M	13.3	8.0	0.163	0.061	0.132	0.046	0.490	0.159	5.8
	FS+C+M	12.9	5.0	0.159	0.050	0.088	0.027	0.279	0.169	4.8
60	Control	10.1	5.0	0.429	0.149	0.242	0.058	0.744	0.249	11.5
	FS+C	9	6.0	0.264	0.098	0.204	0.060	0.391	0.116	10.5
	FS+M	14.9	11.0	0.390	0.110	0.327	0.058	1.200	0.375	9.5
	FS+C+M	13.8	7.0	0.389	0.114	0.227	0.079	0.847	0.309	7.5

Growth After 15 days

Growth After 30 days

Growth After 45 days

Growth After 60 days

Fig 1: Growth of *Quercus leucotricophora* seedlings in polybags

After 15 days of seed sowing there was no germination in test plants. Therefore the data were recorded after 30 days. In *Quercus leucotricophora* seedling the maximum (5.1 cm) shoot growth was recorded in treatment D (FS+C+M).The numbers of leaves were recorded maximum (3.0) in A (Control) . The minimum (3.5 cm) shoot growth the observation at treatment C (FS+M)and number of leaves were recorded minimum in FS+C after 30 days. After 45 days the maximum growth (9.1cm) was recorded in treatment D (FS+C+M) and minimum (7.3 cm) shoot growth was recorded in treatment B (FS+C).After 60 days the maximum (9.7 cm) shoot growth was recorded in treatment D (FS+C+M) and minimum (5.1 cm) shoot growth was recorded in treatment A (Control). The numbers of leaves were recorded maximum (6) in treatment D (FS+M+C) and minimum (4) were recorded in treatment A (Control) (Table-2; Fig. 2).

Table-2. Growth of *Quercus leucotricophora* seedling in polybags (Fig. 2)

Time (Days)	Treatment	Height (cm)	No. of leaves	Root fresh weight (gm)	Root dry weight (gm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Leave fresh weight (gm)	Leave dry weight (gm)	Root length
30	Control	4.5	3.0	-	-	-	-	-	-	-
	FS+C	4.5	1.0	-	-	-	-	-	-	-
	FS+M	3.5	2.0	-	-	-	-	-	-	-
	FS+C+M	5.1	2.0	-	-	-	-	-	-	-
45	Control	5.1	4.0	-	-	-	-	-	-	-
	FS+C	7..8	6.0	-	-	-	-	-	-	-
	FS+M	6.5	4.0	-	-	-	-	-	-	
	FS+C+M	9.7	6.0	-	-	-	-	-	-	-
60	Control	6.6	4.0	0.634	0.114	0.554	0.368	1.047	0.539	10.2
	FS+C	9.2	6.0	0.536	0.160	0.379	0.205	1.70	0.238	7.9
	FS+M	7.1	5.0	0.544	0.179	0.315	0.132	1.25	0.283	5.59
	FS+C+M	10.1	7.0	0.575	0.119	0.254	0.184	1.65	0.293	7.5


Growth After 15 days


Growth After 30 days


Growth After 45 days

Growth After 60 days

Fig 2: Growth of *Quercus leucotricophora* seedlings in polybags

Conclusion

AM fungal spores multiply only in association with plant roots which act as suitable ecological niche for germination of spores. The present study has confirmed the effect of arbuscular mycorrhizal fungi in nursery of two MPTS test plants for 60 days. The mycorrhizal fungi significantly increase the growth of seedlings of three test plants i.e. *Bauhinia variegate and Quercus leucotricophora* as reported by earlier workers (Mukerji et al., 1996; Dixon et al.,1997; Sarwar and Mukerji, 1995). Thus arbuscular mycorrhizal symbiosis results in improved primary growth of seedlings.

Although test plant *Quercus leucotricophora* is ectomycorrhizal but efforts have been made to inoculate oak seeds with *Glomus macrocarpum.* The greenhouse studies on container-grown oak seedlings have shown benefits due to mycorrhiza. Some species of oak shows better shoot and root mass after 20 weeks in greenhouse condition (Dixon et al. 1984; Mitchell et al. 1984). Our results shows some good results (Table-2; Fig. 2) of AM fungi on oak seedlings.
Addition of soils from under mature *Q. garryana* led to formation of mycorrhizas and improved growth in container-grown *Q. garryana* seedlings (Devine et al. 2009). Addition of soils from under an oak canopy increased mycorrhizal colonization, leaf mass, and foliar N in *Quercus ellipsoidalis* (Dickie et al. 2007). The effects of mycorrhizal fungi on seedling growth, either as single fungal species or in assemblages, are difficult to assess in oaks, such as *Q. garryana,* that are adapted to Mediterranean climates with seasonal drought. The Arbuscular mycorrhizal fungal spores isolated from the rhizosphere soil around the oak trees and seedling shows more number of spores per 10 grams of soil in comparison to other sites.

The seedlings of the test plants were raised in nursery successfully. The mycorrhizal spore *Glomus fasciculatum* was found most dominant in the Chauras campus soil. The two test plants showed better growth in the nursery. Mycorrhizal fungi can play a significant role in production of these MPTS species. The *Quercus leucotricophora* showed some better growth in comparison to our earlier studies in the nursery (Chamola et al. 2014). Therefore arbuscular mycorrizal fungi can play a significant role in the establishment of tree seedlings in the nursery.

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