Arbuscular Mycorrhizal Colonization in Ghora Neem (*Melia azedarach* L.) Seedlings Grown from Pre-sowing Treated Seeds

M. S. Rahman[1], B. M. Khan[1], M. A. U. Mridha[2]
M. K. Hossain[1] and P. P. Dhar[2]
[1]Institute of Forestry and Environmental Sciences
University of Chittagong, Chittagong 4331, Bangladesh
[2]Department of Botany, University of Chittagong
Chittagong 4331, Bangladesh

Abstract

The colonization status of arbuscular mycorrhizal (AM) fungi of *Melia azedarach* L. seedlings grown from seven different types of pre-sowing treated seeds and the spore population in the rhizosphere soils were studied. The percent root colonization varied significantly and ranged between 14.81-95.0 in different treatments. The superior seedlings showed heavy root colonization as compared to other seedlings. The intensity of colonization and spore population also varied significantly in different treatments. Spore population was not correlated with the percent root colonization and seedling growth parameters. Four AM genera such *Glomus*, *Acaulospora*, *Entrophospora* and *Gigaspora* were identified from the rhizosphere soils of the seedlings. The importance of AM fungi has been ensured from this study for the primary establishment of *M. azedarach* seedlings in the nursery.

সারসংক্ষেপ

বীজের সাত প্রকার ভিন্ন ভিন্ন প্রাক-অঙ্কুরোদগম ট্রিটমেন্ট থেকে উৎপন্ন ঘোড়া নিম (*Melia azedarach* L.) চারাসমূহের আরবাসকুলার মাইকোরাইজাল ছত্রাকের উপনিবেশন (colonization) এবং মূল-সংযুক্ত মাটিতে স্পোর পপুলেশনের অবস্থা পরীক্ষা করা হয়। মূল উপনিবেশনের শতকরা হার তাৎপর্যপূর্ণভাবে ভিন্ন, যা বিভিন্ন ট্রিটমেন্টে ১৪.৮১% থেকে ৯৫% হয়। সবচেয়ে ভাল চারাসমূহ তুলনামূলকভাবে অনেক বেশি মূল উপনিবেশন প্রদর্শন করে। উপনিবেশনের তীব্রতা এবং স্পোর পপুলেশন বিভিন্ন ট্রিটমেন্টে তাৎপর্যপূর্ণভাবে ভিন্ন হয়। মূল উপনিবেশন এবং চারা বৃদ্ধির সাথে স্পোর পপুলেশনের মিল পাওয়া যায়নি। চারটি আরবাসকুলার মাইকোরাইজাল ছত্রাকের গণ, যথা *Glomus*, *Acaulospora*, *Entrophospora* এবং *Gigaspora* চারার মূল-সংযুক্ত মাটি থেকে শনাক্ত করা হয়। এ পরীক্ষা থেকে নার্সারীতে ঘোড়া নিম চারা এবং প্রাথমিক প্রতিষ্ঠার জন্য আরবাসকুলার মাইকোরাইজাল ছত্রাকের গুরুত্ব নিশ্চিত হয়েছে।

Key words : Arbuscular mycorrhiza, colonization, *Melia azedarach*, pre-sowing treatment, spore population

Introduction

Melia azedarach L. is a deciduous tree commonly known as ghora neem under Meliaceae family. It is a fast growing tree species, usually planted along roadsides, which differs from neem tree (*Azadirachta indica*) in having longer leaflets and white flowers (Zabala 1990). This species can grow well in drained fertile slightly acidic soils of pH 5.5 to 6.5 (Davidson 1985). The species is grown in the Himalayas up to 1800 metres above sea level (Banerjee 1998) and extends to most tropical and subtropical countries (Zabala 1990). The wood is generally used for tool handles, cabinets, furniture, face veneer for plywood, etc.

Arbuscular mycorrhizal (AM) fungi belong to lower group of fungi and are widely distributed throughout the world. AM fungi infect fine feeder roots and protect the host plants from root pathogens, enhance drought resistance (Verma and Jamaluddin 1994) and help nutrient uptake, mainly phosphorous (Smith and Read 1997). The importance of mycorrhizae in improving the quality and survival of forest tree seedlings and their growth after planting has been well recognized (Bakshi 1974).

Many researchers studied the association, distribution and activity of AM fungi in some tropical trees (Raman and Gopinathans 1992, Mohankumar and Mahadevan 1988). The selection of the most appropriate plant fungus association for each specific environmental and ecological situation is one of the main challenges in current research on AM. Very little work has been done on AM occurrence both in the soil and trees of Bangladesh (Mridha *et al.* 1995). Ghora neem is increasingly planted in both public and private plantation programmes in Bangladesh. The present study is an attempt to find out the colonization status of AM fungi in roots and spore populations of the fungi in the rhizosphere soils of ghora neem seedlings grown from pre-sowing treated seeds.

Materials and methods

Study site and fruit collection

The experiment was carried out in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh. The seeds were sown and grown in the soils used in the nursery. The soil was sieved well (< 3 mm) and mixed with decomposed cow dung in a ratio of 3:1 uniformly. Initially the soil contained root segments and 150 AM spores in 100 g dry soil on an average. Fruits of *Melia azedarach* were collected from the plus trees of the University Campus. Some fruits were stored, whereas others were soaked in cold water for 12 hours, and seedstones were extracted by hand. The seedstones were dried under sunlight for three days. Fruits and seedstones of uniform size were selected from the lot.

Experimental design and treatments

A randomized complete block design with three replications was used in the study. A total of 150 seeds were subjected to seven different pre-sowing treatments. Three replications of each treatment consisted of 150 seeds. These were sown in the nursery bed at equal depth and 10 cm apart from each other in random plots. The treatments used in the experiment were as follows :

T0 : Whole fruit with pulp

T1 : Extracted seedstones without pulp

T2 : Soaking the extracted seedstones in cold water for 24 hours

T3 : Soaking the extracted seedstones in liquid farmyard manure for 24 hours

T4 : Soaking the extracted seedstones in conc. H_2SO_4 for 1 minute followed by cold water washing

T5 : Soaking the extracted seedstones in conc. H_2SO_4 for 2 minutes followed by cold water washing

T6 : Cracking the extracted seedstones with hammer.

Determination of growth parameters

The seedlings were allowed to grow for two months. Ten seedlings from each replication were randomly selected and uprooted very carefully. Five seedlings along with rhizosphere soils were used to assess AM colonization and spore population and other five to estimate the growth parameters. After taking the shoot height and root length they were oven-dried at 70°C for 48 hours until a constant weight is obtained.

Assessment of mycorrhizal colonization and spore population

The collected root samples were washed carefully to make free from adhering soil. Fine roots were cut into small segment of approximately 1 cm for determination of percent AM colonization and intensity of colonization. From these, 100 segments were randomly selected for staining. Some segments were preserved in 10% formalin solution in plastic container for future use. The root segments were washed thoroughly in water and stained in aniline blue following the methods of Phillips and Hayman (1970) and with some modifications followed at Mycorrhizae Laboratory in Chittagong University (Mridha *et al.* 1999). The stained root segments were mounted in slides and studied under a compound microscope. The percent of root colonization, mycelium (only), vesicle (with mycelium) and arbuscule (with mycelium) were calculated as follows :

$$\frac{\text{Total number of positive segments}}{\text{Total number of segments studied}} \times 100$$

Rhizosphere soils of all the species were collected from the polythene bag separately where the seedlings were grown. The collected soils were analyzed for AM spore populations by following the wet sieving and decanting method (Gerdemann and Nicolson 1963). The identification of different genera was made by following Schenck and Perez (1990).

Results and discussion

The percent root colonization and the growth parameters (like height, root length, collar diameter, and dry weight) of the ghora neem seedlings significantly varied with the pre-sowing treated seeds grown in the nursery (Table 1). The mean percent root colonization of AM fungi was significantly highest in T5 (95%), T4 (94.11%) and T2 (94.73%) treatments and lowest in T1 (14.81%). It was positively correlated with the mean height of the seedlings in treatments T5 and T4. Similarly, percent root colonization was positively correlated with root length, shoot and root dry weight in the seedlings of T5, T4, T3, T0 and T6 treatments and was negatively correlated with collar diameter of T1 and root-shoot dry weight of T1 and T2. Considering the total spore population of AM fungi, the average population was found highest in T3 (245) followed by T5, T2 and lowest in T6 (161). Spore population was not correlated with the percent root colonization and the growth parameters of the seedlings.

The intensity of root colonization by AM fungi also varied in different treatments (Table 2). The average mycelium colonization was noticed significantly highest (95%) in T5, T2 and T4 (85%) which was followed by T3 (70%) and the lowest in T0, T1 and T6. Considering the vesicle formation, the highest colonization was found in T2 (32%) and lowest in T1 (0%). Similarly, highest arbuscule colonization was obtained in T4 (91%) followed by T2, T5, and no arbuscule formation was observed in T1. Different fungi genera in the rhizosphere soils were identified by taking 20 spores randomly from each treatment. *Glomus* and *Acaulospora* were found in all the treatments except in T1. In addition, *Gigaspora* was observed in T1 and *Entrophospora* was in T3 and T5.

Findings of the present study show that mycorrhizal colonization increased significantly with the increase of seedlings growth. The intensity of colonization i.e. mycelium, vesicle

Table 1. Colonization of arbuscular mycorrhizal fungi in roots and spore populations of the fungi in the rhizosphere soils in relation to growth parameters of *M. azedarach* seedlings grown from pre-sowing treated seeds.

Treatements	Mean height (cm)	Mean root length (cm)	Mean collar diameter (mm)	Mean dry weight (g)		Mean % root colonization	Total spore population
				Shoot	Root		
T0	13.76e	7.53d	2.031c	0.41d	0.043d	25.0d	186e
T1	16.76d	10.56c	2.562bc	0.74c	0.11c	14.81e	192d
T2	19.13c	11.56bc	2.96abc	0.84.c	0.16b	94.73a	229b
T3	21.76b	12.1ab	3.33ab	1.08b	0.20b	81.66b	245a
T4	24.6a	12.76ab	3.5ab	1.35a	0.25a	94.11a	212c
T5	25.3a	13.36a	3.7a	1.52a	0.27a	95.0a	231b
T6	19.56c	11.43bc	2.73abc	0.83c	0.17b	70.0a	161f

Means followed by the same letter (s) in a column are not significantly different at $P<0.05$, Duncan's Multiple Range Test (DMRT).

Table 2. Intensity of colonization in roots and total spore population in the rhizosphere soils in different treatment of *M. azedarach* seedlings.

Treatments	Mean intensity of colonization		
	Mycelium (%)	Vesicle (%)	Arbuscule (%)
T0	10.0c	10.0d	20.0e
T1	14.81c	0.0e	0.0f
T2	95.0a	32.0a	84.0b
T3	70.0b	17.0cd	67.0cd
T4	85.0a	15.0cd	91.0a
T5	95.0a	25.0b	71.0c
T6	20.0c	20.0bc	61.0d

Means followed by the same letter (s) in a column are not significantly different at $P<0.05$, Duncan's Multiple Range Test (DMRT).

and arbuscule also varied with the other parameters of the seedlings and percent root colonization. The higher percent root colonization by AM fungi could fairly be correlated with the growth parameters of the seedlings. It showed similarity with teak seedling's study of Verma and Jamaluddin (1995). It is noticeable in the present study that *Glomus* and *Acaulospora* were present with the seedlings of ghora neem grown in the nursery soil. These endomycorrhizal fungi may be utilized for mass multiplication as well as seedling inoculation for better establishment of seedlings under field condition. In this study, the total spore density in general could not be correlated with the mycorrhizal colonization, possibly because of the presence of a diverse population of AM fungal species (Giovannetti *et al.* 1988 and Mehrotra 1998). Mycorrhization of forest plant draws considerable attention over the last few years because of their role as bio-fertilizers for improving host growth (Sampangiramaiah 1993). More studies are needed to select suitable indigenous AM fungal strains for the production of quality seedlings at the nursery stage and to promote the forester's awareness on the role of mycorrhiza.

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