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Original Research Paper

Growth Response of Wild Variety of *Phyllanthus emblica* L. Plants with Inoculation of Arbuscular Mycorrhizal Fungi (*Glomus fasciculatum*) and Different Sources of Carrier Materials

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ABSTRACT

Greenhouse studies were conducted under nursery conditions to understand the effect of arbuscular mycorrhizal (AM) fungi and different carrier materials on *Phyllanthus emblica* L. The seedlings raised with the inoculation of AM fungi and vermicompost showed a significant increase in dry weight of shoot and root, and phosphorus nutrition in shoots compared to uninoculated/control plants, However, the inoculation of AM fungi with peat and perlite, and AM fungi alone does not show much favourable results. AM fungi with vermicompost showed increased per cent root colonization and spore number/ 50 g of soil. It can be concluded that AM fungi (*Glomus fasciculatum*) with carrier material of vermicompost is a good inoculant for *Phyllanthus emblica* at nursery level.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are key components for plant survival and to create an intimate link between plant roots and soil (Ezawa et al. 2000). They play major role in acquisition of mineral nutrients by acquiring both mobile and immobile elements (Marschner & Dell 1994). AM fungal extrametrical hyphae release polysaccharides, which help in aggregation of soil particles (Srivastava et al. 1996), and enhance the ability of plants to cope with environmental stresses generally prevalent in degraded wasteland ecosystems (Sylvia & Williams 1992). They improve survival of planting stock, and seedling growth, which are essential for the survival of plants in degraded wastelands (Fagbola et al. 2001), and contribute to a long term stability of soil and plant ecosystems by efficient recycling of nutrients.

It is well established fact that AM fungi form symbiotic associations with diverse groups of plant species, but many studies have revealed that AM fungi do show host preference (Bagyaraj et al. 1980, Gaur et al. 1998, Ramana et al. 1999). For that reason to get maximum benefits in terms of crop production efficient mycorrhizal fungus may be inoculated. According to Bagyaraj (1992) activity of effective indigenous AM fungi may be promoted to get maximum benefits in terms of AM fungal association by proper cultural practices.

The cultural practices like crop rotation, cover crops, and phosphorus management can be used for sustainable crop production and to mass multiply the effective indigenous AM fungi (Douds & Johnson 2003). The is great importance of microorganisms to AM fungal spore germination and development and use of carrier materials like, vermicompost, peat and perlite or any other similar

material favouring the growth of microorganisms during onfarm production of healthy AM inoculum (Lakshman 1997). But, literature on effect of these carrier materials on crop production and onfarm production of AM fungi is very meagre. Therefore, this study has been undertaken to evaluate the growth response of *Phyllanthus emblica* L. (wild variety) plants to AM fungi. The carrier materials used are vermicompost, peat and perlite along with AM fungi (*Glomus fasciculatum*) during inoculation.

MATERIALS AND METHODS

Soil and plant materials: The physico-chemical characteristics of soil were determined as per Jackson (1973). Organic matter was determined according to Piper (1950). Electric conductivity was measured using bridge meter, and pH was determined in 1:1 (w/v) soil to water ratio. The soil used for the experiments was sandy loam with pH 7.0, organic carbon; 0.84%, nitrogen 1.41 mg/kg, potassium 2.41 mg/kg, phosphorus 0.18 mg/kg, zinc 2.02 mg/kg, copper; 1.04 mg/kg, magnesium 1.42 mg/kg and E.C. 10.17 mmho/cm. The soil was steam sterilized for one hour on two consecutive days. *Phyllanthus emblica* L. (wild variety) seeds were collected from Range Forest Office Seed Development Unit, Dharwad. Seeds were germinated in small plastic cups containing sterilized soil. Before sowing, the seeds were surface sterilized in 2% sodium hypochlorite and washed with distilled water for 2-3 times.

AM inoculum production: AM fungal spores were isolated from rhizosphere soil of *Phyllanthus emblica* L. collected from Forest Department, Dharwad. AM fungal spores were isolated following the wet sieving and decanting method (Gerdmann & Nicolson 1963). The spores were identified as per Schenk & Perez (1990). Species of *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Enterospora* were isolated from 100g of rhizosphere soil samples. *Glomus fasciculatum* was dominant in the soil, therefore, selected for use in this experiment. Isolated spores were multiplied using Jowar (*Sorghum vulgare* L.) as host plant for three months in culture pots filled with autoclaved soil (121°C for one hour at 15 psi) from the same site. After 60 days Jowar plants were cut at ground level, the roots were chopped into 1 cm pieces and mixed with the soil from rhizosphere of host plants. This soil based inoculum was used for inoculation. 15 g of air dried AM fungal inoculum was given to each pot as thin layer, 2 cm below the soil surface. The inoculum consisting of 3 g root bits plus 12 g rhizospheric soil of host plants with hyphae and sporocarps (105 clamydospores per 50 g soil approximately). Hogaland plant nutrient solution without phosphorus was given to the seedlings at the interval of 15 days.

Treatments: Five treatments are set in triplicate with *Phyllanthus emblica* plants in nursery condition as follows.

1. Control (CN)
2. Mycorrhiza (*Glomus fasciculatum*) inoculated
3. Mycorrhiza (*Glomus fasciculatum*) inoculated + Vermicompost
4. Mycorrhiza (*Glomus fasciculatum*) inoculated + Peat
5. Mycorrhiza (*Glomus fasciculatum*) inoculated + Perlite

Experimental pots were kept free of weeds, pest and irrigated every alternate day in order to maintain moisture. The plants were harvested after 60, 120 and 180 days interval after sowing. Observations such as dry weight of shoot (DWS) and dry weight of root (DWR) were recorded. After the harvest, experimental plant shoot and root were oven dried at 70°C until a constant weight was obtained to determine the dry weight.

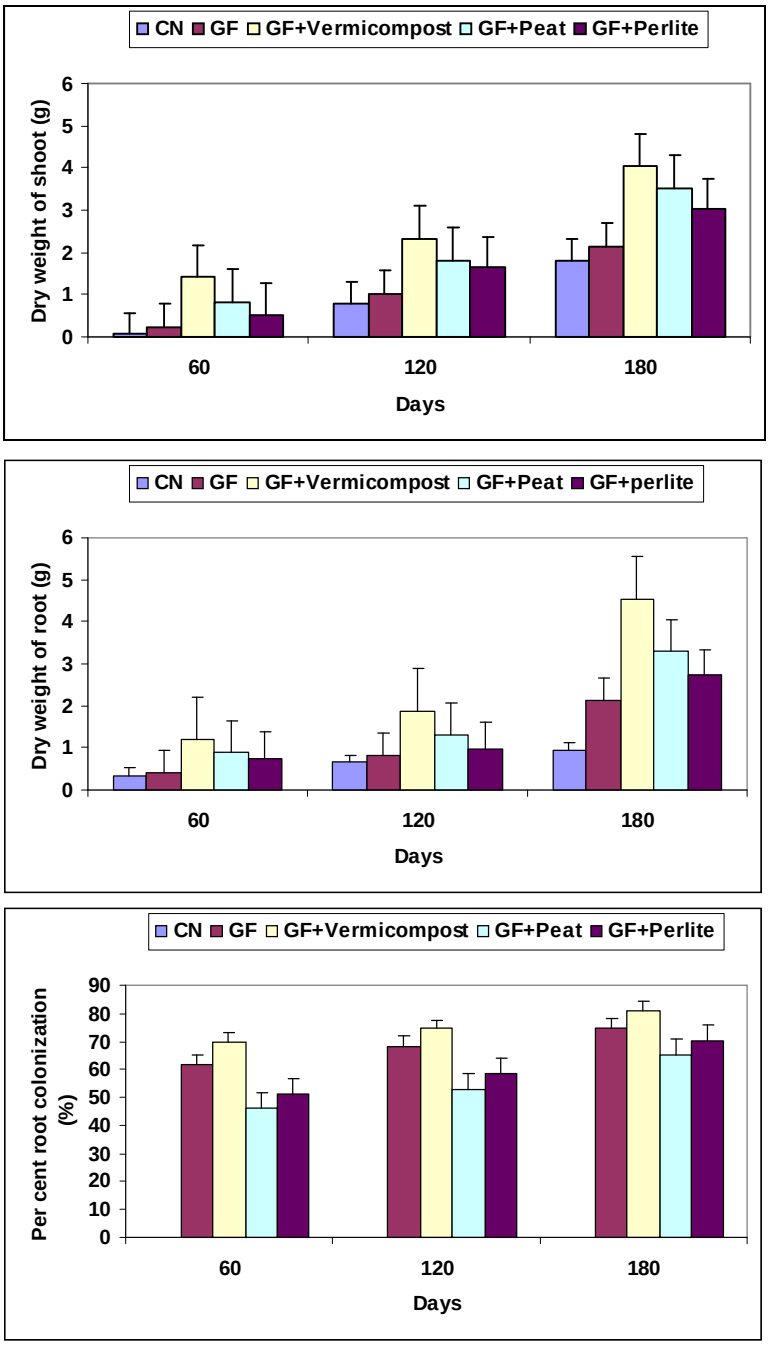

Fig. 1: Effect of AM fungal inoculation on dry weight of shoot, root and percent root colonization in *Phyllanthus emblica* plants (wild variety) at different intervals. CN-control; GF-*Glomus fasciculatum.*

Root colonization: The percent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by Phillips & Hayman (1970). The following formula was used to calculate the root colonization (Giovannetti & Mosse 1980).

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

Mycorrhizosphere spores determination: Spores were separated from the soil by wet sieving and decanting technique (Gerdemann & Nicolson 1963). 50 g of soil was collected and mixed with water. The mixture was pour through different sieve sizes (250, 106, 45µm). After several times of sieve washing, the supernatant was collected in petridish and spores counted under binocular-microscope.

Phosphorus content: The phosphorus content in the shoots was determined by the vanado-molybdate phosphoric acid yellow color method outlined by Jackson (1973).

RESULTS AND DISCUSSION

The soil used was sandy loamy, slightly acidic with low percentage of available phosphorus. The AM fungal (*Glomus fasciculatum*) inoculation along with carrier material (*Glomus fasciculatum* + vermicompost) resulted in increased dry weight of shoot and root, spore number/50 g of soil, percent root colonization, and percent of phosphorus uptake in shoot when compared with control (Table 1 and Figs. 1 and 2).

In general, significantly increased growth was observed in plants treated with mycorrhiza (*Glomus fasciculatum*) + vermicompost (Fig. 1), where after 60, 120 and 180 days of sowing, plants showed increased dry weight of shoot and root over other treatments like *Glomus fasciculatum* + peat, *Glomus fasciculatum* + perlite and *Glomus fasciculatum* alone and control.

The response of the *Phyllanthus emblica* plants to inoculation with *Glomus fasciculatum* and carrier materials varied. Percent mycorrhizal root colonization in experimental plants was low in early days inoculation of 60 days, but steadily increased after 120 days. However, it was observed that after 180 days of inoculation there was increase in percent root colonization and spore number in *Phyllanthus emblica* plants (Fig. 1). The hyphae, arbuscules and vesicles were predominant sign of infection in wild variety of *Phyllanthus emblica* plants.

Phyllanthus emblica plants treated with mycorrhiza (*Glomus fasciculatum*) + vermicompost performed well and showed increased growth and phosphorus uptake over other treatments. Percent root colonization and spore number per 50 g of soil (Fig. 2) also increased in experimental plants when treated with mycorrhiza (*Glomus fasciculatum*) + vermicompost followed by *Glomus fasciculatum* alone, *Glomus fasciculatum* + perlite and GF + peat. Mycorrhizal colonization increased when vermicompost was added (Gutierrez-Miceli et al. 2008). Few studies have tested the effect of vermicomposts on AM fungi. Cavender et al. (2003) have reported an increase in AM fungal colonization of roots of *Sorghum bicolor* after vermicompost application. Peat lacks beneficial symbiotic arbuscular mycorrhizal fungi, which are important to crops in sustainable systems. However, several studies have shown a negative impact of peat on AM fungi (Mauritz Vestberg et al. 2008). Peat has been found to inhibit AM fungal colonization. From the present study it can be concluded that AM fungi (*Glomus fasciculatum*) with vermicompost mixture is best suitable for inoculating *Phyllanthus emblica* plants in nursery.

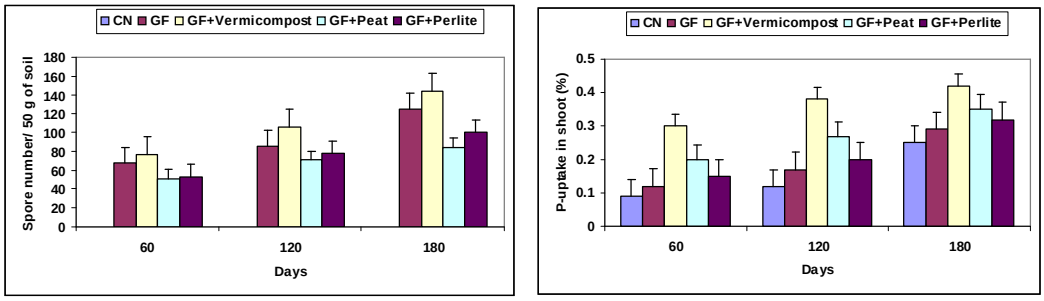

Fig. 2. Effect of AM fungal inoculation on spore number/50 g of soil and P-uptake of shoot in *Phyllanthus emblica* plants (wild variety) at different intervals. CN-control; GF-*Glomus fasciculatum.*

Table 1: Effect of soil inoculation with AM fungi (*Glomus fasciculatum*) and carrier materials on dry weight of shoot (DWS) and root (DWR), per cent colonization (PC), spore number (SN) and phosphorus (P) uptake in shoot of *Phyllanthus emblica* (wild variety). CN-Control; GF-*Glomus fasciculatum.* Means sharing a letter in columns are not significantly different according to Duncan's test $P < 0.05$. All the values are mean of three replicated ± standard error.

	DWS (g)	DWR (g)	PC (%)	SN	P (%)
60 Days					
CN	0.073e±0.006	0.346e±0.027	0.000e±0.000	0.000e±0.000	0.090e±0.000
GF	0.214d±0.009	0.399d±0.002	61.667b±0.882	68.000b±0.577	0.120d±0.000
GF+Vermicompost	1.410a±0.018	1.208a±0.004	70.000a±0.577	76.667a±0.882	0.300a±0.000
GF+Peat	0.819b±0.012	0.897b±0.004	46.000d±0.577	50.667d±0.333	0.200b±0.000
GF+Perlite	0.540c±0.012	0.762c±0.017	51.000c±0.577	53.333c±0.333	0.150c±0.000
120 Days					
CN	0.790e±0.004	0.657e±0.017	0.000e±0.000	0.000d±0.000	0.120e±0.000
GF	1.009d±0.003	0.819d±0.011	68.000b±0.577	85.667b±0.333	0.170d±0.000
GF+Vermicompost	2.329a±0.016	1.883a±0.006	74.667a±0.882	105.667a±7.172	0.380a±0.000
GF+Peat	1.811b±0.036	1.327b±0.014	53.000d±1.000	70.333c±1.333	0.270b±0.000
GF+Perlite	1.650c±0.043	0.980c±0.007	58.667c±0.333	77.333bc±3.528	0.200c±0.000
180 Days					
CN	1.812e±0.007	0.937e±0.012	0.000e±0.000	0.000e±0.000	0.250e±0.000
GF	2.138d±0.006	2.139d±0.008	74.667b±0.333	125.000b±2.887	0.290d±0.000
GF+Vermicompost	4.046a±0.032	4.528a±0.016	81.000a±0.577	144.000a±3.215	0.420a±0.000
GF+Peat	3.535b±0.035	3.317b±0.012	65.333d±0.577	84.667d±0.882	0.350b±0.000
GF+Perlite	3.040c±0.031	2.723c±0.030	70.333c±0.333	100.333c±0.882	0.320c±0.000

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