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RESEARCH PAPER

Effect of Arbuscular Mycorrhizal (AM) fungi on growth enhancement of black pepper (*Piper nigrum* L.) at nursery stage

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Abstract : An experiment was conducted to study the effect of different Arbuscular Mycorrhizal (AM) fungi on rooting and growth of black pepper and also to assess the symbiotic efficacy and the rhizosphere population of beneficial microflora in nursery stage at College of Horticulture, Sirsi for three years (2013-14 to 2015-16). The results of the investigation had clearly showed significant improvement in growth of black pepper when black pepper cuttings were inoculated with *Glomus fasciculatum* similar to that of cuttings treated with IBA (1000ppm) as compared to rest of the AM fungi and un-inoculated control. A matching trend was recorded with respect to per cent root colonization and spore counts, population of beneficial rhizosphere microflora *viz*., free living nitrogen fixers and phosphate solubilizers. The inoculation of AM fungi also enhanced the shoot phosphorus concentration in black pepper.

Key Words : AM fungi, *Glomus fasciculatum,* P concentration, Rhizosphere microflora

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INTRODUCTION

Arbuscular Mycorrhizal (AM) fungi are used to inoculate many crop plants to improve the nutrition and development of host plants (Jeffries, 1987 and Sreenivasa *et al.*, 1993). Some scientists have observed wide variations among and within different species of AM fungi in their ability to promote plant growth (Rao *et al.*, 1983). Though AM fungi are not host specific, recent studies have indicated host preferences for AM endophytes (Hetrick, 1984 and Sreenivasa and Rajashekhara, 1989), thus, suggesting the need to select suitable AM fungi for a particular host plant. Besides host preference, the mycorrhizal fungi associated with plant roots are also known to increase the efficiency of absorption of nutrients particularly phosphorus, zinc, copper, manganese, iron etc. and thus enhance growth of plant species (Gerdemann, 1968). This improved efficiency has been shown to be dependent on plant species (Gerdemann, 1968), the fungal species (Mosse,

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1972a) and the soil type used (Mosse, 1972b). Although VAM fungi have extremely wide host range (Mosse, 1973) specific host preference has been suggested for better interactions (Bagyaraj *et al.*, 1989). This preferential association between certain plant and fungal species can be evaluated with respect to combinations which provide the greatest plant growth stimulations and highest root colonization or sporulation. Arbuscular mycorrhizal (AM) fungi are ubiquitous and are present in various environments such as mangrove vegetation, desert area, different soils, and are known to enhance plant biomass through better uptake of nutrients and water, resistant to drought and increased tolerance to invading plant pathogens. Therefore, the present investigation was undertaken to study the performance of different AM endophytes on growth of black pepper cuttings at nursery stage.

MATERIAL AND METHODS

Effect of different Arbuscular Mycorrhizal (AM) fungi on rooting and growth of black pepper and their symbiotic efficacy in terms of per cent root colonization, spore counts and the population of beneficial rhizosphere microflora *viz.*, free living nitrogen fixers and phosphate solubilizers were studied under nursery conditions at College of Horticulture, Sirsi for three consecutive years between 2013-14 and 2015-16. There were altogether eight treatments each replicated nine times. Pure cultures of AM fungi were maintained in Rhodes grass using sterile potting mixture in earthen pots (Sreenivasa and Bagyaraj, 1988). The potting mixture consisted of red soil: sand: FYM (2:1:1) and 5 kg potting mixture filled in polybags (30 cm x 15 cm size, 250 gauge). Inoculums of different AM fungi were placed at 2 cm below the potting mixture as a thin layer. A single node cutting of black pepper with a leaf was planted in each bag based on the number of infective propagules. The experiment was laid out in a Complete Randomized Design. Per cent root colonization was determined by staining the roots with trypan blue (Phillips and Hayman, 1970). The mycorrhizal spore counts were determined by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The population of free living nitrogen fixers and phosphate solubilizers in the rhizosphere of black pepper was estimated by serial dilution and standard plate count method using Waksmann No. 77 and Pikovskaya's medium, respectively. Shoot phosphorus (P) concentration was determined by vanado-molybdate yellow color method (Jackson, 1967). Observations were recorded at 90 days after planting and the data was statistically analyzed using excel programme.

RESULTS AND DISCUSSION

The results of the investigation on influence of different AM fungi on growth of black pepper had clearly showed significant variations. The growth of black pepper had significantly improved when the black pepper cuttings were either inoculated with *Glomus fasciculatum* or treated with IBA (1000ppm) when compared to other AM fungi and un-inoculated control (Table 1). A matching trend was recorded with respect to per cent root colonization and spore counts of AM fungi (Table 2), as well as population of beneficial rhizosphere microflora *viz*., free living nitrogen fixers and phosphate solubilizers and it was also observed that the phosphorus concentration had increased in the shoots inoculated with *Glomus fasciculatum.* Better growth and establishment of pepper cuttings was due to infection and colonization

Table 1: Influence of AM fungi on rooting and growth of black pepper (Three years pooled data between 2013-14 and 2015-16)

Treatments	Root length (cm)	Root weight (g)	Root dry weight (g)	No. of leaves
Control	8.33	0.09	2.16	1.66
Glomus fasciculatum	24.60	1.60	6.53	4.66
Glomus macrocarpum	17.16	1.36	4.83	3.33
Glomus mosse	14.2	1.15	3.86	2.60
Glomus intraradices	11.66	0.43	2.20	2.00
Gigaspora margarita	15.16	1.17	3.96	3.00
Sclerocystis dussii	6.50	0.43	2.10	2.00
IBA (1000 ppm)	23.30	1.43	6.03	5.00
S.E.±	1.62	0.06	0.53	0.31
C.D. (P=0.05)	4.81	0.17	1.57	0.92

Note: IBA: Indole butyric acid

of *Glomus fasciculatum* as the AM inoculum contained three major propagules (components) *viz.*, chlamydospores, infected root bits with arbuscles and hyphae that would have resulted in better colonization synergy between plant and fungi. Few workers have indicated that the different rates of growth of fungi through root cortex may result in different levels of colonization (Smith and Walker, 1981). Daft and Nicolson (1966) also correlated increased growth and yield of crops to the extent of mycorrhizal colonization. Similar observations were recorded by Sreenivasa (1992) and Sreenivasa *et al.* (1993) in chilli. Similarly, the population of free living nitrogen fixers and phosphate solubilizers were also found to be significantly highest in the rhizosphere of black pepper inoculated with *Glomus fasciculatum* as compared to other AM fungi and un-inoculated control (Table 3). Increased populations of beneficial microflora in mycorrhizal plants were earlier ascribed to increase in root biomass and root exudates (Graham and Menge, 1982). It is a well known fact that higher microbial activity is because of availability of various nutrients in higher concentrations in the rhizosphere region. Any change in this region might affect the rhizosphere microflora and in turn plant growth.

In the present study, root length, root weight, shoot weight and number of leaves were significantly highest in cuttings inoculated with either *G. fasciculatum* and or cuttings treated with IBA (1000ppm) when compared to other AM fungi and un-inoculated control plants (Table 2). AM fungi are known to be associated with increased growth of many plant species as they increase the uptake and translocation of not only P but also other nutrients (Abbott and Robson, 1982 and Sreenivasa *et al.*, 1993). Many workers have reported increased uptake of P, Zn, Cu, Mn, and Fe due to mycorrhizal inoculation in crop plants (Rao *et al.*, 1983 and Sreenivasa, 1992). In the present study, per cent root colonization, spore counts and shoot phosphorus concentration were found to be significantly highest in cuttings inoculated with *G. fasciculatum* followed by cuttings treated with IBA

Table 2: Influence of AM fungi on per cent root colonization and spore counts in the rhizosphere of black pepper (Three years pooled data between 2013-14 and 2015-16)

Treatments	% root colonization	Spore count / 50 g soil
Control	42.60	47.60
Glomus fasciculatum	90.00	228.30
Glomus macrocarpum	85.30	222.00
Glomus mosse	78.00	190.30
Glomus intraradices	51.00	132.00
Gigaspora margarita	79.60	181.30
Sclerocystis dussii	54.33	118.60
IBA (1000 ppm)	45.60	60.00
S.E.±	2.57	5.30
C.D. (P=0.05)	7.63	15.74

Note: IBA: Indole butyric acid

Table 3: Influence of AM fungi on the population of free living nitrogen fixers and P-solubilizers in the rhizosphere of black pepper (Three years pooled data between 2013-14 and 2015-16)

Treatments	Free living N_2 fixers ($x10^4$/g soil)	PSB ($x10^4$/g soil)	Shoot P content (%)
Control	14.00	5.40	0.06
Glomus fasciculatum	29.60	10.73	0.18
Glomus macrocarpum	29.30	11.06	0.19
Glomus mosse	19.60	8.60	0.14
Glomus intraradices	21.30	6.40	0.09
Gigaspora margarita	18.30	8.80	0.10
Sclerocystis dussii	14.30	5.00	0.05
IBA (1000 ppm)	14.33	6.26	0.17
S.E.±	2.05	0.53	0.04
C.D. (P=0.05)	6.08	1.57	0.11

Note: PSB: Phosphate solubilising bacteria, IBA: Indole butyric acid

(1000ppm) (Table 3). In a field response study on chilli to AM inoculation in black clayey soil, Sreeramulu and Bagyaraj (1986) observed higher values for growth, yield, P and Zn contents in plants inoculated with *Glomus fasciculatum* as compared to un-inoculated plants. The results of the investigation clearly indicated the superiority of *G. fasciculatum* and IBA (1000ppm) in increasing P status of plants and in turn plant growth and yield of black pepper. Similar increase in growth, yield and nutrient content in plants inoculated with *Glomus etunicatum* and *G. mosseae* was reported by Sreenivasan *et al*. (2012) and Hemalatha *et al*. (2012) in amla (*Emblica officianalis Gaerten*.) and French bean, respectively.

The present investigation had clearly brought out that the AM fungus, *Glomus fasciculatum* was highly efficient in increasing the growth and establishment of black pepper (shoot cuttings) followed by was treatment of cuttings with IBA (1000ppm) at nursery stage.

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