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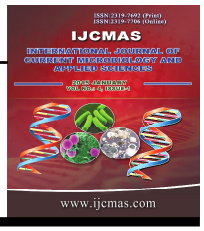

Original Research Article

Effect of Arbuscular Mycorrhizal fungi on growth and biomass enhancement in *Piper longum* L. (Piperaceae)

H. S. Seema and Rajkumar H. Garampalli*

Department of Studies in Botany, University of Mysore, Manasagangotri,
Mysore – 570006, Karnataka, India
**Corresponding author*

A B S T R A C T

Piper longum is a species of long pepper known for treating various kinds of ailments in ayurveda. The effect of inoculation of AM fungi on growth performance of *Piper longum* was evaluated for their symbiotic response in greenhouse condition. Three AM fungi, *Glomus fasiculatum*, *Acaulospora fovata* and *Gigaspora margirata* were used as inoculums that were selected from trap culture. The results show, number of leaves in A. *fovata* inoculated pots increased when compared to other inoculated pots and control. Considering the root, shoot length and whole weight of fresh plant, control pots showed better performance when compared to inoculated pots. Dry weight of total biomass, mycorrhizal dependency and mycorrhizal inoculation effect of *Gi. margirata* show increased effect when compared to *G. fasiculatum*, *A. fovata* and control. The results of present study indicated that *Gi. margirata* can be considered as good growth promoter for better biomass yield in *Piper longum* L.

K e y w o r d s

Arbuscular mycorrhizal fungi, *Piper longum*, *Gigaspora margirata*, *Glomus fascilatum*, *Acaulospora fovata*, Biomass enchancement.

Introduction

Piper longum L. (Piperaceace) commonly known as pippali is the dried ripe fruits; pippalimulam is the roots of this plant (Sivarajan and Balachandran, 1996) which is the accepted source of the drugs in ayurveda. The fruits of the plant are very well- known medicine for diseases of the respiratory tract, viz. cough, bronchitis, asthma, etc; as counter irritant, analgesic when applied locally for muscular pains and inflammation and general tonic. P. *longum* is described in the ayurvedic and unani systems of medicine as a valuable drug used for treatment of various kinds of ailments (Viswanathan, 1995; Sivarajan and Balachandran, 1996). Due to increasing demand at national and global level with the trend of 16.3% increase per annum have added value to this plant species, in prioritized list by national medicinal plant board of India. P. *longum* is a native of North East India. It occurs in the hotter parts of India, from Central Himalayas to Assam, Khasi and the Mikir hills, the lower hills of west Bengal and the evergreen forests of the Western Ghats from Konkan to Travancore

(Sumy *et al.,* 2000; Sivarajan and Balachandran, 1996). The rising demand for this species has exhausted its wild stock and necessitated large-scale cultivation. Therefore, a scientific approach towards its cultivation with the help of AM technology could increase productivity and provide an additional source of income to local people.

Arbuscular mycorrhizal fungi are ecologically important component in soil communities (Borowicz, 2001) and form mutalistic with roots of most land plants (Smith and Read, 2008). For this two- way interaction, host plants provide carbohydrates to AMF in return for several benefits of nutrients and signals. AMF extensive hyphal networks could explore more soil volume where roots could not arrive (Borowicz, 2001). Through hyphal network, by vividly described as "super-highways" (Barto *et al.,* 2012), AMF could transport phosphorus, nitrogen, sulphurous, water, and microelements (Borowicz, 2001, Sieh *et al.,* 2013, Govindarajulu *et al.,* 2005). Mycorrhization is beneficial to the host plant as it stimulates the growth of the seedlings (Machineski *et al.,* 2009) and accumulates nutrients in the aerial parts (Ngwene *et al.,* 2010), besides protecting the plant from pathogens (Elsen *et al.,* 2008). Recent studies have shown that mycorrhizal symbiosis, with its medicinal potential which can be an alternative for maximizing the production of chemical compounds (Ratti *et al.,* 2010; Oliveira *et al.,* 2013), with the produced phyto mass having a higher concentration of active principles (Toussaint *et al.,* 2007; Chaudary *et al.,* 2008; Ratti *et al.,* 2010; Ceccarelli *et al.,* 2010; Dave and Tarafdar, 2011; Karagiannidis *et al.,* 2011). It also have been found to enhance plant growth, photosynthetic activity, nutrient content, act antagonistically towards soil borne fungal pathogens, and modify plant metabolites (Smith and Read, 1997). AMF may be useful in the development of effective methods of plant cultivation and may improve the quality and quantity of obtained material (Khaosaad *et al.,* 2006; Muthukumar *et al.,* 2006). The main aim of the present study was to examine the biomass enhancement in *Piper longum* by inoculation of different AM fungal species and bioprospecting the possibility of AM fungal application in order to improve the cultivation practices of medicinal plants.

Materials and Methods:

Selection of AM fungus for inoculation

Individual AMF spores showing hyphal connection were isolated by the wet sieving and decanting method (Gerdemann and Nicolson, 1963) from the air-dried rhizosphere soil samples collected from Western Ghats of Karnataka region. Selected AM fungal species were cultured and mass multiplied using *Sorghum bicolor* L. as host plant. The soil used in this study was collected from the botanical garden, Department of Studies in Botany, University of Mysore, Mysore, Karnataka from a depth of 0-30 cm and has been classified as sandy loam. The soil has a pH of 7.79 and it contained 47.8 ($\mu g g^{-1}$) available phosphorous. The soil and sand was kept in a hot air oven for 2 days at 150^0 C for dry sterilization. The soil and sand (1:1 V/V) was used for mass multiplication of AM fungi inoculum by trap culture method (Walker and Vestberg, 1994). To culture single or individual AM fungal species, single spore isolation method was followed, which later served as inoculum for experimental medicinal plant. For this, the host plant is allowed to grow up to 80 days of period under greenhouse conditions by trap culture method and after removing the host plant the biomass obtained along with

the soil adhered were treated as inoculums and stored at 4^0 C till further use.

AM fungal inoculation in pot experiment:

The selected species of monospecific AMF inoculums isolated by trap culture method was used to inoculate the *Piper longum* plants. A green house experiment was conducted to analyze the efficacy of individual species of AM fungi on the growth parameters enhancement of medicinal plants. The soil used in this study was collected from the botanical garden, Department of Studies in Botany, University of Mysore, Mysore, and Karnataka. The soil and sand sterilization was carried out as mentioned above. The soil and sand (1:1 V/V) was prepared and the experimental design was design in pots with 3 replicates for each treatment. The plantlets of *Piper longum* were collected from Biotechnology Center, Hulimavu, Bangalore. Sterile soil + Sand + Medicinal plant served as control pot. Sterile soil + Sand + Medicinal plant + each of the AMF were considered as treated pots. 50g of AM fungi inoculum was added to each pots containing 200g autoclaved sand: soil mixture. Three AM fungal species (Fig. 1) were used as inoculum (*Glomus fasiculatum*, *Acaulospora fovata* and *Gigaspora margirata*). The experimental pots were maintained in the green house at a temperature of 25 ± 1^oC, and watered regularly to maintain the soil moisture level close to field conditions. In this experiment no inorganic nutrients were added to the plants. Harvesting was carried out after 80 days of planting and further destructive and non-destructive growth measurements were taken. The plant parameters, like number of leaves, root length, shoot length; whole plant weight, root weight and shoot weight were recorded at 20 days interval after planting (DAP).

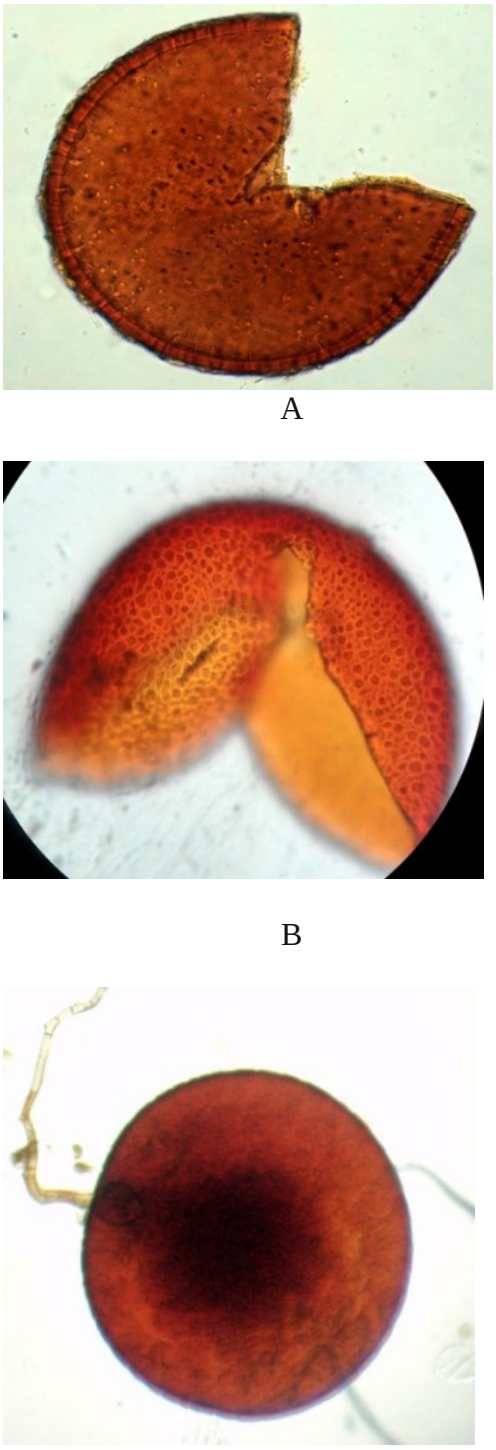

Fig.1 *Glomus fasiculatum* (a), *Acaulospora fovata* (b) and *Gigaspora margirata* (c) selected for the inoculation of *Piper longum L.*

Chemical properties of soil

Chemical properties of soil of inoculums, sterilized and unsterilized soils were collected in sterile polyethylene bags using soil auger, at a depth of 0–30 cm and the soil samples was analyzed for soil pH, Electrical conductivity and available phosphorus at Central Sericulture Research and Training Institute, Mysore, Karnataka.

Estimation of fresh and dry weight

The root and shoot portions of the plants were separated. The root portion was washed gently to remove all the adhering soil particles in running tap water. Both the portions were gently pressed in folds of filter paper to remove excess moisture. The fresh weight was determined and the samples were wrapped in a paper and kept in a hot air oven at 72^0 C for 48 hours, removed, cooled in desiccators and reweighed to record dry weight.

Mycorrhizal dependency

Dry weight of roots and shoots, dry matter data and degree of response to mycorrhizal dependency were calculated as being the difference between the biomass of the shoot of inoculated and non-inoculated plants and was expressed as a percentage of the dry biomass of inoculated plants. A mycorrhizal dependency value was calculated according to the formula of Plenchette *et al.,* (1983).

$$MD = \frac{\text{Dry weight of inoculated plants}}{\text{Dry weight of non-inoculated plants}} \text{ X } 100$$

Mycorrhizal inoculation Effect (MIE)

Dry weight of roots and shoots, dry matter data was recorded. The mycorrhizal inoculation effect (MIE) to assess the growth improvement brought about by inoculation with a mycorrhizal fungus in sterilized soil with indigenous AM fungi. MIE was calculated by the following formula (Bagyaraj *et al.,* 1988).

MIE = Dry weight of inoculated plants - Dry weight of non-inoculated plants / Dry weight of inoculated plants X 100

Statistical analysis

All the data were statistically analysed using one-way ANOVA (Analysis of variance) by SPSS. Differences among treatments were determined using Tukey's multiple range tests (TMRT) at a significant level of $p \leq 0.05$. Data are presented as Mean ± Standard deviation (SD).

Result and Discussion

The effect of inoculation of three AM fungi on growth and performance of *P. longum* plants was evaluated and results obtained showed a positive effect of AM inoculation on different growth parameters viz. number of leaves, root length, shoot length, whole plant weight, root weight and shoot weight of *P. longum* plants (Table 1).

Chemical properties of soil

Form this result sterilized soil shows high phosphorous content when compared to unsterilized soil, whereas the pH of sterilized soil was basic in condition whereas in the unsterile it showed acidic condition. The electrical conductivity also shows high in sterilized as compared to unsterilized soil. Among the inoculums, *A. fovata* showed maximum variation in pH, EC, Avail P of soil physio-chemical parameters.

Effects on AM fungi inoculation in Field experiment

Among the 3 AM fungi, *A. fovata* proved to be excellent inoculum for *P. longum*, whereas Gi. *margirata* was inferior. Root and shoot length with the AM species shows highest length in control sterilized soil and *A. fovata*, whereas in G. *fasiculatum* shows least. The root and shoot portions of the plants were separated. The fresh weight of root showed was high with *Gi. margirata* when compared to the control. Whereas, control showed high shoot weight when compared to *Gi. margirata*. The total biomass weight of fresh weight was significantly increased in *A. fovata* when compared to G. *fasiculatum* (Table 1 and Fig. 2).

Effects on dry weight of total biomass

Gi. *margirata* showed 10.64g total biomass when compared to the control plant which had less biomass. Mycorrhizal dependency values were recorded using roots and shoots dry matter data. The degree of response to mycorrhizal dependency were showed that *Gi. margirata* 118.61% was highest percentage followed by *A. fovata* at 116.27%, whereas *G. fasiculatum* it showed 109.14% which was least among the inoculated plants. In mycorrhizal inoculation effect also showed a slight difference in *Gi. margirata* which had 15.69% followed by *A. fovata* 13.99% and G. *fasiculatum* 8.37% which was the least among the inoculated plants.

Table.1 Effects on AM fungi inoculation in field experiment

	no of leaves	root length	shoot length	whole weight	root weight	shoot weight
CONTROL	30.33 ± 10.69^{b}	33.7 ± 6.71^{a}	72.8 ± 19.38^{a}	41.06 ± 8.84^{a}	10.10 ± 3.11^{c}	30.93 ± 6.10^{a}
GLM	33 ± 12.16^{a}	25.4 ± 6.35^{b}	38.33 ± 2.48^{c}	32.48 ± 16.45^{b}	16.53 ± 7.31^{b}	13.82 ± 7.64^{c}
AC	38.33 ± 19.85^{a}	32.06 ± 5.17^{a}	58.8 ± 35.87^{b}	40.54 ± 15.21^{a}	12.85 ± 3.62^{c}	27.85 ± 12.00^{b}
GIG	27.66 ± 17.61^{b}	31.43 ± 7.63^{a}	52 ± 31.45^{b}	39.1 ± 33.00^{a}	26.58 ± 21.95^{a}	12.28 ± 10.92^{c}

Values are means of 3 replicates ± standard Deviation. Values with the same letter within same column for each parameter are not significantly different at $p \leq 0.05$ levels by Tukey's multiple range tests with respect to species main effect.

Fig.2 30 days, 60 days, 80 days plants of *piper longum* in a green house condition

Piper longum Linn. is a well known plant being used in home remedies as well as in Ayurveda. Several papers have revealed the potential of AM fungi to enhance plant growth and alter secondary metabolite production (Nemec and Lund, 1990; Abu-Zeyad *et al.*, 1999; Fester *et al.*, 1999; Kapoor *et al.*, 2002a, b; Rojas-Andrade *et al.*, 2003; Copetta *et al.*, 2006). Results of the present experiments confirm various reports on enhanced plant growth due to AM inoculation to medicinal plants (Earanna, 2001; Bobby and Bagyaraj, 2003; Nisha and Rajeshkumar, 2010; Vasanthakrishna *et al.*, 1995; Rajan *et al.*, 2000). In our result, all AM fungi proved to increase significantly the number of leaves, root and shoot length, total biomass fresh and dry weight and mycorrhizal dependency and mycorrhizal inoculation effect on *P. longum*. Specially, biomass accumulation of the plantlets was significantly promoted by 3 AMF species i.e., *Gigaspora margirata, Acaulospora fovata* and *Glomus fasiculatum.* In most cases, we found increase in Control and *A. fovata* of number of leaves, root and shoot length, whole weight. In effect of dry weight, mycorrhizal dependency and mycorrhizal inoculation effect, *Gi. margirata* showed highest percentage when compared to the other inoculums.

The results also showed a negative growth response induced in *Glomus fasiculatum*. It may possibly be due to the host preference of AM species as reported by many workers in some medicinal plant species like *Phyllanthus amarus* and *Withania somnifera* (Earanna, 2001) and *Coleus forskohlii* (Gracy and Bagyaraj, 2005). Singh and Gogoi (2011) examined the augmented growth of long pepper in response to arbuscular mycorrhizal inoculation. They evaluated six AM inoculms with *Piper longum* and found that mycorrhizal inoculation with four AMF species, viz., *Glomus fasciculatum*, *G. clarum*, *G. etunicatum* and *G. versiforme* greatly enhanced long pepper growth both in the nursery and field conditions. In the present study *Gigaspora margirata* significantly increased the biomass of *P. longum* was found to be the most excellent for endorsement of favourable growth, nutritional responses and economic yield in *P. longum* production under protected conditions.

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