

Differential effect of some arbuscular mycorrhizal fungi on growth of *Piper longum* L. (Piperaceae)

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Abstract

The potential for arbuscular mycorrhizal fungi (AMF) to influence the host species depends on their affinities and effects. Twenty three different AMF strains from nearby localities were evaluated for their symbiotic response with *Piper longum* (long pepper). Saplings were raised in 10 cm thick sand-soil mix inoculated with isolates of different AMF fungi. Almost all the studied AMF strains showed increase in plant growth, biomass and nutrient content (N&P) over the control, while retarded growth response was observed with the inoculation of 6 different AMF species. Considering the shoot length, total biomass, nutrient content, chlorophyll content and root infection, pre-inoculation with 6 AMF species viz; *Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp. 2, *G. mosseae* and *G. etunicatum* appeared to be the most promising AM fungi for inoculating this medicinal plant.

Keywords: Arbuscular mycorrhizal fungi, *Piper longum*, Biomass, *Glomus* spp., Root infection,

Introduction

Piper longum L (Hindi: Pipli, Pipal, Piper; Assamese: Pipoli, Family: Piperaceae) is the most commonly known species of long pepper. It forms an important component in ayurvedic principles/preparations such as 'Trikadu' (dry ginger, black pepper & long pepper) and 'Panchakolam'. *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 & 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, concurrently entangled in prioritized list by national medicinal plant board of India. It is cultivated in Arunachal Pradesh as well as it grows in wild condition in different parts of the State in India (Misra & Dutta, 2003). It is being collected from forest and sold to local and outside agencies. The rising demands have otherwise dramatically declined the stock in wild thereby necessitates the provocation of large-scale cultivation. Arunachal Pradesh renders wide scope for large scale cultivation of *P. longum* due to its congenial climate and vast land resource.

AMF are soil fungi colonizing most of the plant roots and forming an association called endomycorrhiza. More than 90% of plants and 80% of plant families in all terrestrial environments form the association (Harley & Harley, 1987) with these obligate fungi belonging to the group Glomeromycota (Schubler *et al.*, 2001). These fungi are known to improve the nutritional status of the host, particularly that of phosphorous and thereby enhance their growth, development and yield (Bagyaraj & Varma, 1995; Bagyaraj, 2007). Different AMF species induce differential growth responses in terms of biomass production and clonal growth patterns of co-existing plant species (Heijden *et al.*, 1998). AMF are not host specific and show extremely wide host range but their affinity to the host is always preferential (Rogers *et al.*, 1994), thus

suggesting the need for selecting efficient AM fungi for a particular host (Abbott & Robson, 1982; Gracy & Bagyaraj, 2005). Hence in the present investigation it was envisaged to screen and select efficient AM strains for this particular host in order to harness the maximum benefit from the fungus in the nursery.

Materials and methods

A green house experiment was conducted to observe the different effect of the indigenous AM fungi on growth, nutrient and chlorophyll content of medicinal plant *Piper longum*. The soil used in this study was collected from the botanical garden, Rajiv Gandhi University from a depth of 0-15 cm and has been classified as sandy loam. The soil had a pH of 5.5 ± 0.02 and it contained 3.98 ± 0.65 ($\mu\text{g g}^{-1}$) available phosphorous. The experimental design was completely randomized block design with 10 replicates for each treatment. Treatments consisted of saplings without AMF (control) and pre-infected saplings with any one of the 23 AMF species. For each AMF species, inoculation beds were prepared separately in green house. The beds were filled up with sand-soil mix and the rooting medium was inoculated into the bed with culture of individual AMF species by uniformly distributing its propagules i.e. large inoculum of endogone sporocarps and mycelium. Soil-based AM inoculum at the rate of 40 g kg^{-1} soil was taken out from a pure culture pot of AMF for preparing a bed. Beds were 10 cm thick where inoculum were placed by preparing a layer ($1/4^{\text{th}}$ of sand-soil mixture) so that growing root of cuttings can pass through this inoculum layer, then the remaining soil was topped over the inoculum layer, levelled and pressed down. Large numbers of piper cuttings of uniform size were planted temporarily in each bed.

AMF used for inoculation were *Acaulospora delicata*, *A. rugosa*, *Gigaspora candida*, *Glomus aggregatum*, *G. albidum*, *G. aurantium*, *G. claroideum*, *G. clarum*, *G. coronatum*, *G. etunicatum*, *G. fasciculatum*,

G. geosporum, *G. glomerulatum*, *G. hoi*, *G. intraradices*, *G. macrocarpum*, *G. mosseae*, *G. occultum*, *G. versiforme*, *G. xanthium*, *Glomus* sp. 2, *Glomus* sp. 4, and *Glomus* sp. 5. An inoculated or uninoculated sapling along with rhizospheric soil, without disturbing the rooting zone was taken from respective nursery bed and planted by making a core in the centre of pots containing 200 g autoclaved sand-soil mixture. The AMF spores were isolated using wet sieving and decanting method (Gerdemann & Nicolson, 1963) from the trap culture of soil samples collected from seven different field sites of Arunachal Pradesh, viz, Open forest (Rajiv Gandhi university), Jhum field (Midpu village), Forest area (Karsingsa), Settled agriculture (Doimukh village), Home garden (Rajiv Gandhi University), Pine forest (Ziro town), and Tea garden (Harmutty), an adjoining area of Assam.

The experimental pots were maintained in the green house at a temperature of $22 \pm 1^\circ\text{C}$, with 12 h fluorescent illumination with 8000 lx light intensity and water was supplied daily to maintain the soil moisture level close to field capacity. Inorganic nutrients were thoroughly incorporated to the soil at the rate (mgkg^{-1}) at potting stage. N and P in the form of urea and single super phosphate and K as murate of potash was used in the ratio of 66:110:30 respectively.

Harvesting was carried out for 90 d after planting (DAP) and further destructive and non-destructive growth measurements were taken. The plant parameters like plant height (measured from soil surface to the growing tip of the plant), were recorded at 90 d interval after planting (DAP). Shoot and root dry weights were determined after drying the plant samples at 60°C to a constant weight in a hot air oven. The phosphorus content of the shoot and root was determined by the method of Hanson (1950). For determination of total nitrogen, microkjeldhal method of Bremner & Mulvaney (1982) was followed. Chlorophyll content of leaf was measured by using the method of Arnon (1949).

Statistical analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's multiple range test (DMRT) ($P < 0.05$) using computer statistics programme SPSS (2000) Version 10.1.0.

Results

The effect of inoculation of different AM fungi on growth performance of *P. longum* plants was evaluated in

Table 1. Effect of AM fungal inoculation on shoot length, shoot fresh weight & shoot dry weight of *P. longum* saplings.

AMF species	SL (cm)	SFW (g)	SDW (g)
Control	07.3 ± 0.519^a	0.26 ± 0.023^a	0.07 ± 0.008^a
<i>Acaulospora delicata</i>	12.2 ± 0.771^b	0.66 ± 0.019^{ad}	0.15 ± 0.003^{cdef}
<i>A. rugosa</i>	08.5 ± 0.645^a	0.34 ± 0.027^{ab}	0.08 ± 0.002^a
<i>Gigaspora candida</i>	17.5 ± 0.925^{efg}	0.78 ± 0.113^{bcd}	0.17 ± 0.005^{cdef}
<i>Glomus aggregatum</i>	08.3 ± 0.829^a	0.34 ± 0.041^{ab}	0.07 ± 0.009^a
<i>G. albidum</i>	17.8 ± 0.984^{efg}	0.84 ± 0.040^{cd}	0.24 ± 0.005^g
<i>G. aurantium</i>	08.3 ± 0.667^a	0.34 ± 0.034^{ab}	0.07 ± 0.007^a
<i>G. claroideum</i>	12.2 ± 0.359^b	0.88 ± 0.038^{cd}	0.21 ± 0.035^{fg}
<i>G. clarum</i>	16.0 ± 0.651^{cdef}	2.09 ± 0.098^{hi}	0.47 ± 0.020^k
<i>G. coronatum</i>	14.0 ± 0.463^c	0.59 ± 0.028^{abcd}	0.13 ± 0.004^{bcd}
<i>G. etunicatum</i>	16.5 ± 0.838^{def}	1.72 ± 0.257^{gh}	0.36 ± 0.029^j
<i>G. fasciculatum</i>	31.0 ± 1.083^j	2.77 ± 0.307^j	0.54 ± 0.024^l
<i>G. geosporum</i>	19.3 ± 0.718^{gh}	1.34 ± 0.205^{ef}	0.264 ± 0.018^g
<i>G. glomerulatum</i>	11.5 ± 0.696^b	0.63 ± 0.039^{abc}	0.19 ± 0.003^e
<i>G. hoi</i>	05.0 ± 0.493^a	0.23 ± 0.015^a	0.06 ± 0.003^a
<i>G. intraradices</i>	10.2 ± 0.704^b	0.35 ± 0.020^{ab}	0.09 ± 0.001^{ab}
<i>G. macrocarpum</i>	12.5 ± 0.907^b	0.41 ± 0.030^{ab}	0.11 ± 0.013^{abc}
<i>G. mosseae</i>	20.7 ± 0.707^{hi}	1.82 ± 0.308^g	0.37 ± 0.038^j
<i>G. occultum</i>	10.5 ± 0.538^b	0.54 ± 0.033^{abc}	0.14 ± 0.007^{abcde}
<i>G. versiforme</i>	22.8 ± 0.704^i	1.90 ± 0.282^j	0.38 ± 0.053^j
<i>G. xanthium</i>	14.2 ± 0.772^c	1.02 ± 0.112^{de}	0.29 ± 0.007^h
<i>Glomus</i> sp. 2	21.0 ± 0.820^h	1.78 ± 0.318^j	0.35 ± 0.025^i
<i>Glomus</i> sp. 4	15.2 ± 0.773^{cde}	1.41 ± 0.110^{efg}	0.30 ± 0.072^{hi}
<i>Glomus</i> sp. 5	15.0 ± 0.454^{cd}	0.89 ± 0.037^c	0.24 ± 0.007^g

SL = Shoot length; SFW = Shoot fresh weight; SDW = Shoot dry weight. Values are means of 10 replicates \pm standard error. Values with the same letter within same column for each parameter are not significantly different at $p \leq 0.05$ with respect to species main effect. Decrease values underlined with same letter within same column for each parameter are not significantly different at $p \leq 0.05$ levels by Duncan's multiple range test with respect to species main effect.

pot conditions. The results obtained after completion of the experiment showed a positive effect of AM inoculation on different growth parameters viz. root and shoot length, fresh and dry weight of root and shoot, total biomass, chlorophyll content and percent root colonization of *P. longum* plants.

Effects on shoot length

With the exception of 4 AM species (i.e. *Acaulospora rugosa*, *Glomus aggregatum*, *G. aurantium* & *G. hoi*), inoculation with all other 19 AMF species showed a significant increase in shoot length in comparison to uninoculated control plants. The highest shoot length was observed with the inoculation of *G. fasciculatum*, which was followed by other 5 inoculants viz. *Glomus versiforme*, *Glomus* sp. 2, *G. mosseae*, *G. geosporum* and *G. etunicatum* while the lowest being observed in *G. intraradices* inoculated plants. Among the 23 tested AMF species, inoculation with 3 species viz. *Acaulospora rugosa*, *G. aggregatum* and *G. aurantium* had more shoot length than the non-mycorrhizal plants but the increase was non-significant. On the other hand, plants inoculated with *G. hoi* showed less shoot length than the control plants (Table 1).

Effects on shoot fresh weight & shoot dry weight

Out of 23 AM species tested for efficiency, 9 species failed to show a significant effect in increasing both the shoot fresh weight and shoot dry weight over the un-inoculated control plants. Again *G. hoi* inoculated plants showed decrease in both shoot fresh weight and dry weight in comparison to the un-inoculated control plants. The maximum percent increment of shoot weight and shoot biomass production was about 10 fold & 7 fold, respectively in plants inoculated with *G. fasciculatum*. This AMF species was followed by other 5 mycorrhizal species, viz. (*G. clarum*, *G. versiforme*, *G. mosseae*, *Glomus* sp. 2 & *G. etunicatum*) in respect to their shoot fresh weight and shoot biomass increment capacity (Table 1).

Effects on root fresh weight, root dry weight & total biomass

Only 3 out of 23 species (*Glomus* sp. 2, *G. fasciculatum* & *G. clarum*) showed a significant increase over un-inoculated control in respect to root fresh weight and root dry weight as well as total biomass. Significantly highest total biomass (0.84 g) production was observed in *G. fasciculatum* inoculated plants when compared to un-inoculated control plants. Ten AMF species (*Gigaspora candida*, *Glomus albidum*, *G. etunicatum*, *G. geosporum*, *G. glomerulatum*, *G. mosseae*, *G. xanthium*, *G. versiforme*, *Glomus* sp. 4 & *Glomus* sp. 5) could not significantly increase the root fresh weight and root dry weight of the *P. longum* over un-inoculated control plant but they showed a significant increase in total biomass in comparison to control plants due to significantly high increase in shoot biomass. Among these above mentioned species, Inoculation with 4 AMF species (*A. delicata*, *G. claroideum*, *G. coronatum* & *G. occultum*) showed a non-significant effect on biomass production whereas 6 AMF species (*A. rugosa*, *G. aggregatum*, *G. aurantium*, *G. hoi*, *G. macrocarpum* & *G. intraradices*) caused a depression to the biomass in comparison to control plants (Table 2).

Effects on chlorophyll content of *P. longum*

A significant increase in chlorophyll content over the control was observed in plants inoculated with *G. fasciculatum*, *G. clarum*, *G. versiforme* and *G. etunicatum* only. Nine AMF inoculants showed non-significant increase whereas inoculation with remaining 10 AMF species showed rather a decrease in chlorophyll content in comparison to control plants.

Table 2. Effect of AM fungal inoculation on root fresh weight, root dry weight & total biomass.

AMF species	RFW (g)	RDW (g)	Total biomass (g)
Control	0.62 ± 0.033 ^a	0.17 ± 0.018 ^a	0.23 ± 0.019
<i>Acaulospora delicata</i>	0.46 ± 0.035 ^a	0.12 ± 0.010 ^{def}	0.27 ± 0.010 ^a
<i>A. rugosa</i>	0.26 ± 0.027 ^b	0.08 ± 0.004 ^f	0.16 ± 0.005 ^b
<i>Gigaspora candida</i>	0.56 ± 0.031 ^a	0.15 ± 0.031 ^{abc}	0.32 ± 0.030 ^{cdef}
<i>Glomus aggregatum</i>	0.36 ± 0.028 ^b	0.08 ± 0.005 ^f	0.15 ± 0.011 ^b
<i>G. albidum</i>	0.59 ± 0.036 ^a	0.14 ± 0.028 ^{abcd}	0.37 ± 0.033 ^{cdefg}
<i>G. aurantium</i>	0.36 ± 0.042 ^b	0.09 ± 0.007 ^{ef}	0.16 ± 0.004 ^b
<i>G. claroideum</i>	0.31 ± 0.046 ^b	0.09 ± 0.001 ^{ef}	0.30 ± 0.034 ^{abcd}
<i>G. clarum</i>	1.03 ± 0.048 ^c	0.24 ± 0.018 ^c	0.71 ± 0.031 ^j
<i>G. coronatum</i>	0.38 ± 0.028 ^b	0.10 ± 0.002 ^{def}	0.23 ± 0.004 ^a
<i>G. etunicatum</i>	0.76 ± 0.041 ^{ab}	0.17 ± 0.013 ^a	0.53 ± 0.033 ^{hi}
<i>G. fasciculatum</i>	1.46 ± 0.130 ^c	0.30 ± 0.019 ^d	0.84 ± 0.041 ^k
<i>G. geosporum</i>	0.80 ± 0.033 ^b	0.11 ± 0.010 ^{cdef}	0.38 ± 0.020 ^{defg}
<i>G. glomerulatum</i>	0.48 ± 0.046 ^{ab}	0.13 ± 0.013 ^{abcde}	0.31 ± 0.015 ^{abcde}
<i>G. hoi</i>	0.33 ± 0.036 ^b	0.08 ± 0.002 ^f	0.14 ± 0.005 ^b
<i>G. intraradices</i>	0.45 ± 0.030 ^{ab}	0.12 ± 0.003 ^{bcdf}	0.21 ± 0.002 ^b
<i>G. macrocarpum</i>	0.34 ± 0.040 ^{ab}	0.09 ± 0.007 ^f	0.21 ± 0.014 ^b
<i>G. mosseae</i>	0.66 ± 0.033 ^{ab}	0.20 ± 0.130 ^{abc}	0.57 ± 0.039 ⁱ
<i>G. occultum</i>	0.57 ± 0.043 ^a	0.15 ± 0.015 ^{ab}	0.29 ± 0.021 ^{abc}
<i>G. versiformae</i>	0.77 ± 0.031 ^{ab}	0.18 ± 0.017 ^{ab}	0.55 ± 0.044 ⁱ
<i>G. xanthium</i>	0.57 ± 0.029 ^a	0.15 ± 0.027 ^{abc}	0.45 ± 0.025 ^{gh}
<i>Glomus</i> sp. 2	1.12 ± 0.197 ^c	0.29 ± 0.014 ^d	0.64 ± 0.023 ^j
<i>Glomus</i> sp. 4	0.55 ± 0.030 ^a	0.15 ± 0.017 ^{abc}	0.44 ± 0.082 ^g
<i>Glomus</i> sp. 5	0.57 ± 0.029 ^a	0.16 ± 0.016 ^{ab}	0.40 ± 0.017 ^{fg}

RL = Root length; RFW = Root fresh weight; RDW = Root dry weight.

Values are means of 10 replicates ± standard error. Values with the same letter within same column for each parameter are not significantly different at $p \leq 0.05$ with respect to species main effect. Decrease values underlined with same letter within same column for each parameter are not significantly different at $p \leq 0.05$ levels by Duncan's multiple range test with respect to species main effect.

Effect on nutrient uptake

Out of 23 species, merely 15 species showed significant increase of nitrogen over the un-inoculated plants (Table 3). The maximum nitrogen concentration was recorded in plants inoculated with *G. clarum* which was followed by other 4 AMF species viz. *G. occultum*, *G. versiforme*, *G. etunicatum* and *G. mosseae* respectively. The shoot nitrogen content of plant was not affected by inoculation with the remaining species. Similarly, the shoot phosphorous content was significantly higher in plants inoculated with 5 AMF species only viz. *G. clarum*, *G. etunicatum*, *G. mosseae*, *G. versiforme*, *Glomus* sp. 2 compared to that of controls, whereas the inoculation of plant with the remaining species showed non-significant increase of phosphorous over the un-inoculated control.

Percent infection of different AMF in *P. longum* plant

Out of 23 AMF species, 11 species produced ≥40% root infection in the root of *P. longum* (Fig.1). Inoculation with *G. fasciculatum* showed significantly highest infection (56%) which was followed by *Glomus* sp. 4 (52%), *Glomus* sp. 2 (50%) and *G. claroideum* (50%) (Fig.1). The *Gigaspora* species resulted into >40% infection. It was also recorded that both *Acaulospora* species had <35% infection whereas all *Glomus* species

Table 3. Chlorophyll content, phosphorous & nitrogen content of *P. longum* plant due to AMF inoculation.

AMF species	Chlorophyll content (mg/g)	N (%)	P (%)
Control	0.39 ± 0.074 ^a	0.36 ± 0.056 ^a	0.08 ± 0.019 ^a
<i>Acaulospora delicata</i>	0.37 ± 0.069 ^a	0.46 ± 0.034 ^{abcd}	0.09 ± 0.011 ^{ab}
<i>A. rugosa</i>	0.06 ± 0.008 ^b	0.48 ± 0.035 ^{abcd}	0.09 ± 0.021 ^a
<i>Gigaspora candida</i>	0.23 ± 0.032 ^a	0.48 ± 0.062 ^{abcd}	0.11 ± 0.012 ^c
<i>Glomus aggregatum</i>	0.35 ± 0.085 ^a	0.50 ± 0.034 ^{cde}	0.11 ± 0.006 ^c
<i>G. albidum</i>	0.30 ± 0.070 ^a	0.50 ± 0.040 ^{cde}	0.10 ± 0.017 ^{bc}
<i>G. aurantium</i>	0.05 ± 0.015 ^b	0.44 ± 0.047 ^{abcd}	0.11 ± 0.031 ^c
<i>G. claroideum</i>	0.41 ± 0.080 ^a	0.50 ± 0.042 ^{cde}	0.11 ± 0.006 ^c
<i>G. clarum</i>	1.16 ± 0.262 ^b	0.63 ± 0.042 ^g	0.13 ± 0.012 ^d
<i>G. coronatum</i>	0.52 ± 0.035 ^a	0.45 ± 0.044 ^{abcd}	0.09 ± 0.009 ^{ab}
<i>G. etunicatum</i>	1.40 ± 0.315 ^c	0.61 ± 0.049 ^{efg}	0.21 ± 0.020 ^e
<i>G. fasciculatum</i>	0.98 ± 0.095 ^b	0.52 ± 0.043 ^{def}	0.09 ± 0.020 ^{ab}
<i>G. geosporum</i>	0.39 ± 0.083 ^a	0.49 ± 0.033 ^{bcde}	0.09 ± 0.017 ^{ab}
<i>G. glomerulatum</i>	0.21 ± 0.015 ^a	0.38 ± 0.035 ^{abc}	0.09 ± 0.012 ^{ab}
<i>G. hoi</i>	0.52 ± 0.082 ^a	0.50 ± 0.051 ^{cde}	0.08 ± 0.019 ^a
<i>G. intraradices</i>	0.24 ± 0.039 ^a	0.51 ± 0.045 ^{def}	0.11 ± 0.020 ^c
<i>G. macrocarpum</i>	0.15 ± 0.021 ^a	0.39 ± 0.046 ^{abcd}	0.10 ± 0.020 ^b
<i>G. mosseae</i>	0.53 ± 0.066 ^a	0.53 ± 0.053 ^{def}	0.13 ± 0.027 ^d
<i>G. occultum</i>	0.29 ± 0.039 ^a	0.63 ± 0.045 ^f	0.09 ± 0.013 ^{ab}
<i>G. versiforme</i>	0.89 ± 0.155 ^b	0.59 ± 0.054 ^{eg}	0.20 ± 0.012 ^e
<i>G. xanthium</i>	0.55 ± 0.088 ^a	0.50 ± 0.048 ^{cde}	0.09 ± 0.016 ^{ab}
<i>Glomus</i> sp. 2	0.55 ± 0.032 ^a	0.52 ± 0.062 ^{def}	0.11 ± 0.005 ^c
<i>Glomus</i> sp. 4	0.44 ± 0.053 ^a	0.51 ± 0.041 ^{def}	0.08 ± 0.022 ^a
<i>Glomus</i> sp. 5	0.49 ± 0.081 ^a	0.37 ± 0.032 ^{ab}	0.09 ± 0.015 ^{ab}

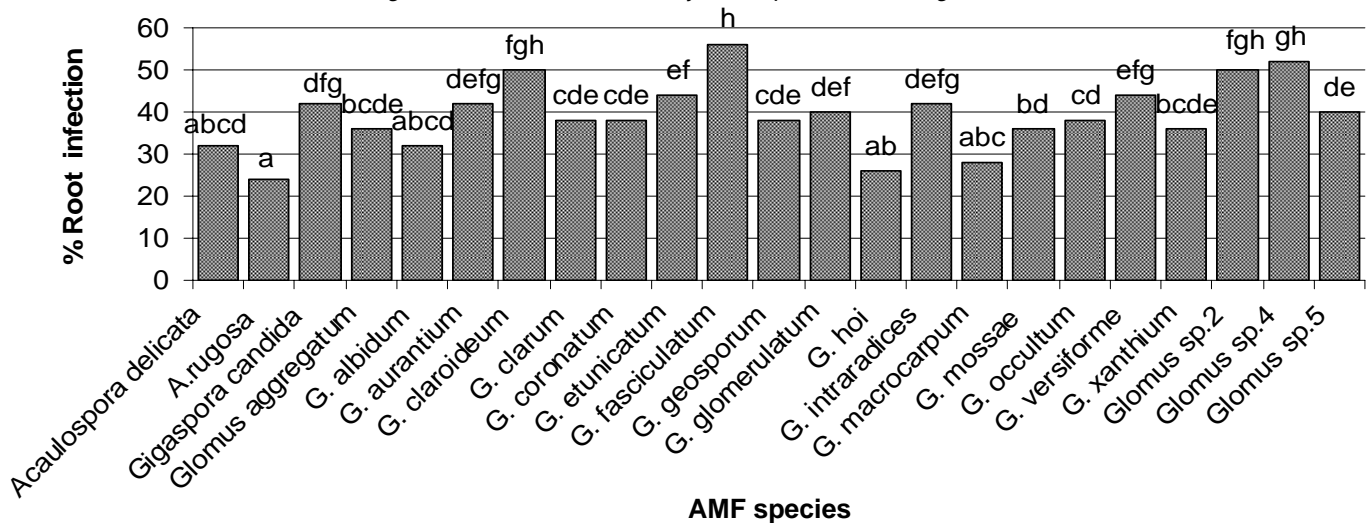
Values are means of 10 replicates ± standard error. Values with the same letter within same column for each parameter are not significantly different at $p \leq 0.05$ with respect to species main effect. Decrease values underlined with same letter within same column for each parameter are not significantly different at $p \leq 0.05$ levels by Duncan's multiple range test with respect to species main effect.

excluding 3 viz. *G. albidum*, *G. hoi* and *G. macrocarpum* showed >35% infection. *A. rugosa* treated plants had lowest (24%) infection in the roots.

Discussion

Results of the experiments confirm various reports on enhanced plant growth due to AM inoculation to medicinal plants (Earanna, 2001; Bobby & Bagyaraj 2003; Nisha & Rajeshkumar, 2010) and forest trees species (Vasanthakrishna *et al.*, 1995; Rajan *et al.*, 2000). Most of the AM fungi resulted in significant increase in plant height, root and shoot dry weight, plant biomass, chlorophyll and nutrient contents (N&P) of *P. longum*. Specially, biomass accumulation by the seedlings was significantly promoted by 13 AMF species. In most cases, increase in shoot length and biomass was significant due to the inoculation of *G. fasciculatum* followed by other 5 inoculants viz, *G. versiforme*, *Glomus* sp. 2, *G. mosseae*, *G. geosporum* and *G. etunicatum*, respectively. 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 & Bagyaraj, 2005). It has been reported that species of AM fungi differ significantly in their ability to improve plant growth and other aspect of plant performance also (Liu & Luo 1988; Liu, 1989). The concentrations of N and P in shoots were improved by all 20 three AMF species, though significant increase was observed with the inoculation of 4 AMF only viz, *G. etunicatum*, *G. clarum*, *G. mosseae* and *G. versiforme*.

Fig. 1. Percent root infection by AMF species in *P. longum* roots.



Bar with the same letter are not significantly different at $p \leq 0.05$ levels by Duncan's multiple range test with respect to species main effect.

These results are consistent with the findings of Azcon *et al.* (1991) that the differences in functional compatibility, the ability of AMF to improve plant nutrition and growth existed among AMF even when the root colonization was found to be similar for *G. clarum* and *G. coronatum* and others. Mycorrhizal inoculation also increased phosphorous content of plants. Phosphorous being a constituent of phosphonucleotides which tend to increase cell division (Black, 1965) might increase the plant growth. The increased 'P' content may be attributed to increase in uptake of P facilitated due to AM colonization through various mechanisms. Hattingh *et al.* (1973) suggested that faster movement of P into mycorrhizal hyphae and solubilization of soil P could be some of the means for increasing P content in mycorrhizal plants. Faster movement of P into the mycorrhizal hyphae is achieved due to increased affinity of P ions and thereby decreasing the threshold concentration required for absorption of P. Further solubilization of soil P is achieved by the release of organic acids and phosphates (Bolan, 1991). Increase in the N content in AM inoculated plants has been reported by many workers (Kessel *et al.*, 1985). They described the increase in N content to improved plant nutrition and not to the fungal activity. Thus, mycorrhiza may improve the N nutrient not as a result of extensive absorbing surfaces, but by some mechanism that accelerates other parts of N uptake processes (Megan *et al.*, 1978). Increased chlorophyll accumulation was observed in all AM inoculated plants. Higher P levels in tissues as a result of root colonization by the AM can be expected to increase the chlorophyll content, as P is one of the important components of chlorophyll. Increase in chlorophyll content due to AM symbiosis has been also reported by Adivappar (2001), Richmond & Lang (1975) and Shivaputra *et al.* (2004).

The present study also supports the findings of Zhu *et al.* (2000) that certain AM fungi may be preferentially associated with particular plant species and helping the host plant to get a better nutrient status. It has been reported that even if AM fungi are considered to have a wide host range, there is some degree of ecological specificity between AM fungi and plants (Rosendahl *et al.*, 1992). The efficiency of the fungus to increase plant growth in a phosphate-deficient soil depends on the ability to form extensive and well-distributed hyphae in soil to form extensive colonization in the root system and to absorb P from soil. Hence, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants has been stressed by different workers (Abbott & Robson 1982; Bagyaraj & Varma 1995; Jeffries, 1987). The results also showed a negative growth response induced by 6 AM species (*A. rugosa*, *G. aggregatum*, *G. aurantium*, *G. hoi*, *G. intraradices* & *G. macrocarpum*) cogitating that the development of this mutual association relies on recognition between AMF species and the host plants. Wang *et al.* (2008) observed the similar response with the

inoculation of *G. versiforme* in cucumber seedlings indicating that this species requires enough time for the proper establishment that results in the imbalance of the carbohydrate distribution between the shoots and the roots of the plants. It has been also found that AMF species are different in their carbon demands from the host plant (Saikkonen *et al.*, 1999). They drain carbohydrates from host plants in demand for life at the cost of root biomass loss of host plants (Thomson *et al.*, 1986; Fredeen & Terry 1988). This finding of the present study is also consistent with the reports of Gaur & Adholeya (2005) and Bethlenfalvai *et al.* (1982) that a negative growth response by the host or parasitism of the fungus might result from extensive intra-radical fungal growth in want of sufficient extra radical mycelium. A direct relationship between degree of plant growth stimulation or depression and degree of colonization has been reported by Clapperton & Reid (1992) suggesting that the root-colonizing organisms are most likely to effect the changes to assimilate partitioning. Further, several workers have also suggested that lipids in hyphae and spores of AM fungi might cause a significant drain in the host carbohydrate supply thus resulting in negative growth response (Ho & Trappe, 1973; Cox *et al.*, 1975).

Growth depression in plants when colonized by AM fungi have been reported in many studies (Mosse, 1972; Sanders *et al.*, 1977; Menge *et al.*, 1978; O'Bannon *et al.*, 1980; Azcon & Ocampo, 1981; Buwalda & Goh 1982; Schenck & Smith 1982; Nuffelen & Schenck, 1984). This negative effect has been attributed to the root system architecture (Baylis, 1970), high concentration of available inorganic nutrients (Buwalda & Goh, 1982), or high inoculum density of AM fungi (Clapperton & Reid, 1992). It must also be noted that AM fungi harbour some endo-symbiotic bacteria (Bianciotto *et al.*, 1996) which may play a role causing these apparent growth depressions in plant. Schenck & Smith (1982) found *Gigaspora* species as plant growth depressive however; in the present study *G. candida* significantly increased the biomass of *P. longum*. Growth depression in acid soils might also result from lack of activity of microorganisms that form mutualistic associations with the plants that form arbuscular mycorrhiza (Yost & Fox, 1979; Abbott & Robson, 1985). The AM fungal species causing growth depressions in the experiments with piper plants may represent fungal cheaters as they did not enhance the growth and mineral nutrition of the host plants. In the present study, only 6 AMF namely *Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp. 2, *G. mosseae* and *G. etunicatum* species tested were 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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