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Response of *Alpinia galanga* Willd to inoculation with different arbuscular mycorrhizal fungi in Ambo, Ethiopia.

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The efficacy of eight arbuscular mycorrhizal (AM) fungi on 'Pera-rattai' or greater galangal (*Alpinia galanga* Willd.) was studied in glasshouse condition. Seedlings were raised in poly bags containing soil inoculated with isolates of different AM fungi, viz., *Acaulospora scrobiculata*, *Gigaspora margarita*, *Glomas aggregatum*, *G. intraradices, G. fasiculatum, G. macrocarpum, G. mosseae* and *Scutellospora heterogama*. Pera-rattai seedlings raised in the presence of AM fungi generally showed an increase in plant growth, nutrients and content of secondary metabolites over those grown in the absence of the inoculation with AM fungi. The extent of improvement by AM fungi varied with the species of AM fungi inhabiting the roots and rhizomes of Pera-rattai seedlings. Plants inoculated with *Glomas aggregatum* showed significantly greater plant height, dry plant biomass, P, K, Zn, Cu and Fe and content of secondary metabolites compared to other treatments. Considering the various plant growth parameters, nutrients and content of secondary metabolites of the plants, it was observed that *Glomas aggregatum* and *Glomas intraradices* would become the best AM symbionts for Pera-rattai compared to others.

Key words: *Alpinia galanga,* Arbuscular mycorrhiza, growth, biomass, nutrition, secondary metabolites.

Introduction

Medicinal plants are the most important source of medicines and play a key role in world health (Kala, 2005). These plants may be considered as famous chemical factory for biosynthesis of a huge array of secondary metabolites (Dhyani and Kala, 2005). The most important of these bio-active constituents of plants are alkaloids, flavonoids, tannins, saponins, and polyphenolic compounds (Kiritkar and Basu, 1975). Many of these chemicals are utilized as medicine, dyes, and pesticides and or of commercial importance. In order to maintain a sustained supply of raw materials to the drug industries,

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these plants are encouraged to be cultivated in cropped field soils in recent years (Alsabahi *et al*., 1999). The introduction of beneficial organisms into soil is present cru of applied mycorrhizal research. Utilization of mycorrhizal bioinoculants in the cultivation of medicinal and aromatic plants is of resent interest. A scientifically managed system of soil-mycorrhiza-bacteria-plant association is useful in conserving energy by reducing fertilizer requirement of medicinal crops and meeting production targets in nutritionally deficient soils. The arbuscular mycorrhizal (AM) fungi used to enhance the plant growth and yield of medicinal crops has gained momentum in recent years because of the higher cost and hazardous effects of heavy doses of chemical fertilizers (Srivastava *et al.,* 1996).

They are known to improve the plant growth, biomass, nutritional status and development, protect plants against root pathogens and confer resistance to drought and soil salinity conditions and to help maintain good soil health and fertility that contributes a greater extent to a sustainable yield and good quality of the products (Jeffries, 1987; Smith and Reed, 1997). There has been considerable interest in the potential use of AM fungi in agricultural systems (Chen *et al*., 2001; Kahneh *et al.,* 2006). Though these fungi are not host-specific, recent studies have clearly brought out host preference in AM fungi, thus emphasizing the need for selecting efficient AM fungi for inoculating a particular host (Dhillion, 1992; Mathur *et al.,* 2006). Host preference has been reported in many forest tree species like *Calliandra calothyrsus* (Reena and Bagyaraj, 1990), *Casuarina equisetifolia* (Vasanthakrishna *et al.,* 1995), *Tectona grandis* (Rajan *et al.,* 2000), *Garcinia indica* (Lakshmipathy *et al.,* 2003) and a few medicinal species like *Phyllanthus amarus, Withania somnifera* (Earanna, 2001), *Coleus forskohlii* (Gracy and Bagyaraj, 2005) and *Andrographis paniculata* (Chiramel *et al.,* 2006). Also there are few published reports on the influence of AM fungi on the growth, nutrition and phytochemical constituents of medicinal plants (Earanna, 2001; Gracy and Bagyaraj, 2005; Chiramel *et al.,* 2006). Pera-rattai (*Alpinia galanga* Willd. Family- Zingiberaceae) is one of the important perennial rhizomatous medicinal plants with numerous medicinal properties. The juice extracted from dried rhizomes and roots, used as an official drug of Indian and Ethiopian Pharmacopoeia. The rhizome contains flavonoids viz., quercetin, kaempferol, isorhamertin, kaempferide, gelangin and essential oil such as methyl cinnamate, cineole, camphene, ∞-pinene and borneol (Anonymous, 1978). Leaves also yield volatile oil. The rhizomes are used for the treatment of rheumatism and cathedral infections especially in bronchial catarrh, carminative and stomachic (Anonymous, 1997). Hence, the present investigation was undertaken to screen for an efficient AM fungus for *A. galanga* and also to study the effects of

different AM fungi on growth, biomass, nutrients and content of secondary metabolites viz., total phenols, orthodihydroxy phenols, alkaloids, flavonoids, tannins and saponins in the roots and rhizomes of *A. galanga*.

Materials and methods

An Experiment was carried out under glasshouse conditions. The soil used in this study was collected from an uncultivated field at a depth of 0-30cm and classified as fine, entisol, isohyperthermic, kanhaplustalfs. The soil pH was 6.8 (1:10 soil to water extract ratio), and it contained 2.2 ppm available phosphorus (extractable with NH_4F+HCL) and an indigenous AM fungal population of 85 spores/50 g of soil. The rhizome bits (approximately uniform 5cm length of *A. galanga*) was sterilized in 5% chloramine T solution for 30 min., washed and sown in poly bags (10x15cm) containing sterilized soil: vermiculite mix (1:1 v/v). Ruakura nutrient solution (10% concentration) without phosphate was prepared (Smith *et al*., 1983) and then applied to the poly bags of 50ml per bag once in every 10 days. After 24 days seedlings were transplanted to poly bags of size 25x15cm containing 2.5 kg of unsterilized soil: sand in the ratio of 3:1 (v/v). The AM fungal species used in the study were either isolated or obtained from different places of India and Ethiopia as mentioned in Table 1. These fungi were multiplied using sterilized sand: soil mix (1:1 v/v) as the substrate and onion as the host. After 90 days of growth, the shoots of onion was removed, the substrate containing hyphae, spores and root bits was air dried and used as inoculum. The inoculum potential (IP) of each culture was estimated adopting the most probable number (MPN) method as outlined by Porter (1979). The soil in each poly bags was mixed with these inoculum at different rates so as to maintain an initial IP of 12,500 per poly bag. Each poly bag containing the potting mixture, with or without AM inoculums, as the treatment may be, was planted with one seedling of Pera-rattai. One set of plants without inoculation was treated as the control. Each treatment with 5 replicates was maintained in a glass house and watered regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution (Smith *et al*., 1983) without phosphate was added to the poly bags at the rate of 50 ml per poly bag once in 15 days. One hundred and twenty days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, nutritional status and content of secondary metabolites. Plant height was measured from soil surface to the growing tip of the plant. Rhizome length and diameter was recorded after harvest. Dry biomass was determined after drying the plant sample at 60ºC to a constant

weight in a hot air oven. Soil samples (100 g) were collected from each poly bag and subjected to wet sieving and decantation method as outlined by Gerdemann and Nicolson (1963) to estimate the population of spores. Fine terminal feeder roots were stained using 0.02% trypan blue as described by Philips and Hayman (1970) and the percent root colonization was estimated by adopting gridline intersection method (Giovannetti and Mosse, 1980). Estimation of soil aggregates (<50µm size), which indirectly denotes the extent of external hyphae in soil, was done as described by Van Bavel (1980).

Phosphorus and potassium contents of the plant tissues were determined by employing the vanadomolybdate phosphoric yellow colour and flame photometric methods (Jackson, 1973) respectively. Atomic adsorption spectrophotometry was employed to estimate zinc, copper and iron contents of the plant samples, using respective hollow cathode lamps. The content of secondary metabolites viz., total phenols (Farkas and kiraly, 1962), ortho dihydroxy phenols (Mahadevan and Sridhar, 1996), flavonoids, tannins, saponins and alkaloids of the plant samples were assayed (Sadasivam and Manickam, 1996; Zakaria, 1991). Data thus generated were subjected to statistical analysis of completely randomized design (CRD) and the means were separated by Duncan's Multiple Range Test (DMRT) (Little and Hill, 1978).

Results and discussions

Growth response, nutritional status, mycorrhizal development and content of secondary metabolites of plants raised in soils were assessed for the impact of inoculation with different AM fungi. The response of the Pera-rattai plants to inoculation with different AM fungi were found to be varied. Mycorrhizal inoculation resulted in a significant increase in plant height, biomass, nutrient content and content of secondary metabolites of *A. galanga* seedlings. However, there was no positive correlation between plant growth parameters and mycorrhizal colonization. Plants inoculated with *Glomus aggragatum, Glomus intraradices* and *Glomus mosseae* showed significantly greater plant height, shoot, root and rhizome dry biomass compared to other treatments (Table 1). Earlier studies also showed the same trend in medicinal plants due to AM inoculation (Gracy and Bagyaraj, 2005; Chiramel *et al.,* 2006; Chandrika *et al.,* 2002) and these studies also indicated the host preferences among the AM fungi. Bagyaraj and Varma (1995) and Jeffries (1987) stressed the need for selecting efficient AM fungi for plant species. The present study was conducted with an objective of screening for an efficient AM fungus for Pera-rattai plants has also resulted in varied plant growth responses to different AM fungi. *Glomus aggregatum* significantly enhanced the plant height compared to all other treatments expect for *G. intraradices*. Plant biomass was increased in

plants treated with *G. aggregatum* followed by *G. intraradices*, compared to uninoculated control. In general, mycorrhizal inoculation increased the percent mycorrhizal root colonization and spore numbers in soil. Considering the percent root colonization observed in this study, *G. aggregatum* and *G. intraradices* inhabited in significantly higher percentage of roots compared to other AM fungi (Table 2). In uninoculated control plants also the percentage of root colonization was observed as 30.62% due to the seedlings were grown in unsterilized soils after transplanted to the poly bags. Similarly spore number was also higher in soil samples inoculated with *G. aggreatum* followed by the soil samples inoculated with *G. intraradices* indicating, the better proliferating ability of this fungus with *A. galanga* as the host. It is well known that enhanced nutritional status of a plant manifests in its improved growth (Jeffries, 1987). Nutritional status of Pera-rattai plants like phosphorus, potassium, zinc, copper and iron contents was significantly higher in plants raised in soil inoculated with AM fungi (Table 3). Highest dry matter production and P, K, Zn, Cu, and Fe concentration in roots and rhizomes were observed in plants treated with *G. aggregatum* and *G. intraradices* (Table 3). The extent of increase in plant P, K, Cu, Fe and Zn contents varied among the fungi studied with seedlings grown in the presence of *G. aggregatum* containing a significantly higher content of these nutrients, followed by those grown in the presence of *G. intraradices*. Such a variation in the plant nutrient status in relation to the fungal species and other medicinal plant species is well documented (Gracy and Bagyaraj, 2005; Chiramel *et al.,* 2006; Chandrika *et al.,* 2002). The enhancement in growth and nutritional status is also related to the percent root colonization apart from several soil and environmental factors. The main effect of mycorrhizal fungi in improving plant growth was improved uptake of nutrients, especially phosphorus, potassium, copper, iron and zinc due to the exploration of the external hyphae of the soil beyond root hair zone when phosphorus is depleated (Chiramel *et al.,* 2006). Increased phosphorus uptake has been attributed not only to increased surface area of absorption but also to enhanced hyphae translocation (Chiramel *et al.,* 2006).

The content of secondary metabolites viz., total phenols, orthodihydroxy phenols, flavonoids, alkaloids, tannins and saponins of Pera-rattai seedlings were found to be significantly higher in plants raised in soil inoculated with AM fungi (Table 4), with seedlings raised in the presence of *G. aggregatum* showing the most increase of all phytochemical constituents in the plant tissues. Such a variation in the phytochemical constituents in relation to the fungal species for other medicinal plant species is also well documented (Chiramel *et al.,* 2006; Rajeshkumar *et al*., 2008). Recently Ponce *et al.,* (2004) reported that

the analysis of extracts obtained from roots and shoots of *Trifolium repens* revealed that the composition of the flavonoid mixtures varied with growing conditions. Quercetin, acacetin and rhamnetin accumulated in roots of inoculated plants, whereas they were not detected in non-inoculated plants. The different composition of the flavonoids extracted from shoots and roots of white clover grown with or without the AM fungus clearly shows that metabolism of these molecules is strongly affected when the plant is AM colonized (Ponce *et al.,* 2004).

As AM fungi increased the uptake of phosphorus and other nutrients, they may also increase the synthesis of secondary metabolites. The increase in total phenols, and OD phenols in inoculated plants could be attributed to triggering of pathway of aromatic biosynthesis (Mahadevan, 1991). Krishna and Bagyaraj (1984) reported an increase in phenols in the roots of *Arachis hypogeae* colonized by *G. fasciculatum*. Hemalatha (2002) also reported an increase in total phenols, OD-phenols, flavonoids, alkaloids and tannins in the roots and leaves of *Ocimum basilicum* and *Coleus amboinicus* in mycorrhizal inoculated plants. Codignola *et al.,* (1989) found that *Glomus versiforme* inoculated with *Allium porum* showed higher level of phenols in the leaves and roots. Increased accumulations of phenols are important in the stress resistance mechanism. In the present study, the content of secondary metabolites, viz., phenols, OD-phenols, flavonoids, alkaloids, tannins and saponins were significantly higher due to inoculation of AM fungi *G. aggregatum* and *G. intraradices* compared to other treatments.

Mycorrhizal fungi are also implicated in improving the soil structure by increasing soil aggregation by their hyphae (Miller and Jastrow, 1992). Soil aggregation is a measure of the amount of extrametrical hyphae, which is in turn related to the efficiency of the fungus (Reena and Bagyaraj, 1990). This observation was further strengthened by the present study as AM fungi used in this study significantly improved the aggregation of soil compared to the uninoculated treatments (Table 2). Soil aggregation was highest in soil inoculated with *G. aggregatum* followed by *G. intraradices* compared to uninoculated control.

Species and strains of AM fungi have differed to the extent by which they increase nutrient uptake, secondary metabolites and plant growth (Gracy and Bagyaraj, 2005; Chiramel *et al.,* 2006; Krishna and Bagyaraj, 1984). Hence, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants has been stressed (Jeffries, 1987; Bagyaraj and Varma, 1995). The efficiency refers to ability of the fungus to increase plant growth in a phosphorus deficient soil (Smith and Reed, 1997). This 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 and other nutrients from soil. In the present study, mycorrhizal parameters, such as percent root colonization and extrametrical spores, were considerably higher in *G. aggreatum* and *G. intraradices* inoculated treatments compared to the uninoculated control. The extent of colonization and the spore count varied with different AM fungi. Higher root colonization and the sporulation allow more fungal-host contact and exchange of nutrients, hence better plant growth. AM fungi differ greatly in their symbiotic effectiveness which depends on their preference for particular soils or host plant specificity (Rajeshkumar *et al*., 2008), direct ability to stimulate plant growth, rate of infection, competitive ability and tolerance to applied chemicals. Giving weightage to the growth, biomass, nutritional status and content of secondary metabolites, but not neglecting the other parameters, *G. aggregatum* and *G. intraradices* (Ethiopian strains) were found to be the best and the next best fungus respectively for inoculating Pera-rattai in the nursery in order to get healthy, vigorously growing seedlings in the nursery that could establish and perform better when planted in Ethiopian soils. It can be concluded that Pera-rattai seedlings show varied responses to different AM fungi and *Glomus aggregatum* confers higher benefits compared to all other fungi used in this study. Further considering the ability for higher root colonization, plant biomass, nutrient status and secondary metabolites when compared to the plants inoculated with other fungi, suggested clear and specific relationship exists between a particular species of fungus and the plant. The importance of proper selection of efficient AM fungi for the right medicinal plants and environment may be the key for successful use in agriculture.

Table 1. Effect of soil inoculation with different arbuscular mycorrhizal fungi on plant growth of *Alpinia galanga*

Treatments*	Plant height (cm/plant)				Plant dry biomass (g/plant)			Total biomass (g/plant)
	Root length	Shoot length	Rhizome length	Rhizome diameter	Shoot	Root	Rhizome	
Uninoculated	15.8^{e}	30.5^{d}	13.2^{d}	2.6^{d}	3.550^{e}	1.030^{d}	4.350^{d}	8.930^{d}
Acaulospora scrobiculata (TN)	16.6^{d}	32.2^{d}	14.3^{c}	2.8^{c}	3.865^{d}	1.214^{c}	4.415^{c}	9.494^{c}
Gigaspora margarita (TN)	17.4^{c}	36.4^{c}	14.2^{c}	3.0^{b}	3.924^{c}	1.265^{b}	4.516^{b}	9.705^{b}
Glomus aggregatum(Ambo)	18.5^{a}	38.2^{a}	14.9^{a}	3.2^{a}	4.362^{a}	1.652^{a}	4.615^{a}	10.629^{a}
Glomus intraradices (Addis)	18.2^{a}	38.0^{a}	14.7^{a}	3.1^{a}	4.345^{a}	1.614^{a}	4.598^{a}	10.557^{a}
Glomus fasciculatum (Ban)	17.8^{c}	36.2^{c}	14.2^{c}	2.9^{b}	3.912^{c}	1.260^{b}	4.514^{b}	9.686^{b}
Glomus macrocarpum (Guder)	15.9^{e}	31.4^{d}	13.4^{d}	2.6^{d}	3.622^{e}	1.114^{d}	4.365^{d}	9.105^{d}
Glomus mosseae (Ambo)	18.0^{b}	37.8^{b}	14.4^{b}	3.0^{b}	4.328^{b}	1.605^{a}	4.516^{b}	10.449^{a}
Scutellospora heterogama (TN)	17.2^{c}	35.2^{c}	14.1^{c}	2.8^{c}	3.866^{d}	1.295^{b}	4.506^{c}	9.667^{b}

Means of five replications with same superscript in each column do not differ significantly at P=0.05 level by Duncan's Multiple Range Test. *TN - Isolate from Bharathidasan University, Tamil Nadu, India, Ambo - Isolate from Ambo University College, Ethiopia,Guder - Isolate from Guder farm, Ambo University College, Ethiopia, Addis - Isolate from Addis Ababa University, Ethiopia,Ban - Isolate from University of Agricultural Sciences, Bangalore, India.

Table 2. Effect of soil inoculation with different AM fungi on mycorrhizal root colonization, spore number in root-zone soil and percent aggregation of rhizosphere soil.

Treatments*	AMF colonization in roots (%)	Spore number/100 g soil	Percent aggregation of rhizosphere soil
Uninoculated	30.62[d]	85.0[e]	18.0[d]
Acaulospora scrobiculata (TN)	48.05[c]	270.0[d]	36.0[c]
Gigaspora margarita (TN)	75.68[b]	315.0[c]	39.0c
Glomus aggregatum(Ambo)	92.45[a]	560.0[a]	52.0[a]
Glomus intraradices (Addis)	90.10[a]	545.0[a]	48.0[a]
Glomus fasciculatum (Ban)	72.65[b]	482.0[b]	42.0[b]
Glomus macrocarpum (Guder)	42.12[d]	120.0[e]	20.0[d]
Glomus mosseae (Ambo)	81.06[b]	516.0[a]	44.0[b]
Scutellospora heterogama (TN)	52.18[c]	245.0[d]	38.0[c]

Means with same superscript in each column do not differ significantly at P=0.05 level by Duncan's Multiple Range Test,*TN - Isolate from Bharathidasan University, Tamil Nadu, India, Ambo - Isolate from Ambo University College, Ethiopia., Guder - Isolate from Guder farm, Ambo University College, Ethiopia, Addis - Isolate from Addis Ababa University, Ethiopia, Ban - Isolate from University of Agricultural Sciences, Bangalore, India.

Table 3. Effect of different AM fungi on root and rhizome P, K, Zn, Cu and Fe content of *Alpinia galanga*

Treatments*	Total Phosphorus (mg/g dry weight)		Total Potassium (mg/g dry weight)		Zinc content (µg/g dry weight)		Copper content (µg/g dry weight)		Iron content (µg/g dry weight)	
	Root	Rhi**	Root	Rhi**	Root	Rhi**	Root	Rhi**	Root	Rhi**
Uninoculated	0.18[d]	0.24[c]	1.16[d]	1.28[d]	4.30[d]	4.36[d]	2.80[d]	2.95[d]	4.16[d]	4.24[d]
Acaulospora scrobiculata (TN)	0.30[c]	0.33[d]	1.68[c]	1.72[c]	4.75[c]	4.82[c]	3.10c	3.24[c]	4.60[c]	4.78[c]
Gigaspora margarita (TN)	0.38[c]	0.41[c]	1.92[b]	1.99[b]	5.32[b]	5.38[b]	3.50[b]	3.62[b]	5.26[b]	5.32[b]
Glomus aggregatum(Ambo)	0.48[a]	0.53[a]	2.12[a]	2.24[a]	6.74[a]	6.84[a]	4.85[a]	4.99[a]	6.22[a]	6.30a
Glomus intraradices (Addis)	0.44[a]	0.48[a]	2.08[a]	2.12[a]	5.87[a]	5.92[a]	4.66[a]	4.78[a]	6.10[a]	6.18[a]
Glomus fasciculatum (Ban)	0.41[b]	0.43[b]	1.96[b]	1.98[b]	5.16[b]	5.22[b]	4.65[a]	4.72[a]	5.12b	5.24[b]
Glomus macrocarpum (Guder)	0.22[d]	0.24[c]	1.24[d]	1.30[d]	4.12[d]	4.24[d]	3.15[d]	3.24[d]	4.24[d]	4.32[d]
Glomus mosseae (Ambo)	0.42[b]	0.43[b]	1.98[b]	2.08[b]	5.64[a]	5.72[a]	4.56[a]	4.62[a]	5.36[b]	5.48[b]
Scutellospora heterogama (TN)	0.32[c]	0.35[d]	1.72[c]	1.85[c]	4.82[c]	4.92[c]	3.62[b]	3.68[b]	4.62[c]	4.74[c]

Means of five replications with same superscript in each column do not differ significantly at P=0.05 level by Duncan's Multiple Range Test., **Rhi – Rhizome, *TN - Isolate from Bharathidasan University, Tamil Nadu, India., Ambo - Isolate from Ambo University College, Ethiopia., Guder - Isolate from Guder farm, Ambo University College, Ethiopia., Addis - Isolate from Addis Ababa University, Ethiopia., Ban - Isolate from University of Agricultural Sciences, Bangalore, India.

Table 4. Effect of different native AMF on the content of secondary metabolites in the roots of *Alpinia galanga*

Treatments*	Total phenol (µg/g)		OD phenol (µg/g)		Flavonoids (µg/g)		Alkaloids (µg/g)		Tannins (µg/g)		Saponins (µg/g)	
	Root	Rhi**	Root	Rhi**	Root	Rhi**	Root	Rhi**	Root	Rhi**	Root	Rhi**
Uninoculated	82.0[d]	120.4[d]	42.5[d]	65.2[d]	2.1[d]	3.14[d]	1.6[c]	3.16[d]	0.180[b]	0.260[c]	0.114[c]	0.160[c]
Acaulospora scrobiculata (TN)	90.5[d]	128.4[c]	52.5[c]	75.8[b]	2.6[c]	3.25[c]	1.9[b]	3.28[d]	0.182[b]	0.264[c]	0.117[b]	0.168[b]
Gigaspora margarita (TN)	114.2[c]	132.5[c]	56.4[b]	76.2[b]	2.9[c]	3.28[c]	2.1[a]	3.32[c]	0.192[a]	0.286[a]	0.118[b]	0.168[b]
Glomus aggregatum(Ambo)	140.5[a]	165.8[a]	66.2[a]	85.4[a]	3.6[a]	4.12[a]	2.2[a]	3.96[a]	0.198[a]	0.254[a]	0.120[a]	0.172[a]
Glomus intraradices (Addis)	140.2[a]	162.4[a]	64.5[a]	82.3[a]	3.5[a]	4.06[a]	2.1[a]	3.84[a]	0.192[a]	0.288[a]	0.121[a]	0.171[a]
Glomus fasciculatum (Ban)	132.4[b]	145.6[b]	58.2[b]	80.0[b]	3.1[b]	3.42[b]	2.1[a]	3.64[b]	0.184[b]	0.264[b]	0.119[b]	0.170[a]
Glomus macrocarpum (Guder)	96.4[d]	120.5[d]	44.5[d]	66.2[d]	2.6[d]	3.24[c]	1.7[c]	3.18[d]	0.182[b]	0.262[c]	0.115[c]	0.164[c]
Glomus mosseae (Ambo)	138.2[b]	158.5[b]	59.5[b]	82.4[a]	3.4[a]	3.92[a]	2.0[a]	3.68[b]	0.190[a]	0.272[b]	0.116[c]	0.172[a]
Scutellospora heterogama (TN)	120.4[c]	122.6[d]	48.2[c]	72.7[c]	2.9[c]	3.62[b]	1.8[b]	3.42[c]	0.182[b]	0.268[b]	0.116[c]	0.168[b]

Means of five replications with same superscript in each column do not differ significantly at P=0.05 level by Duncan's Multiple Range Test., **Rhi – Rhizome, *TN - Isolate from Bharathidasan University, Tamil Nadu, India, Ambo - Isolate from Ambo University College, Ethiopia., Guder - Isolate from Guder farm, Ambo University College, Ethiopia., Addis - Isolate from Addis Ababa University, Ethiopia., Ban - Isolate from University of Agricultural Sciences, Bangalore, India.

References

Alsabahi, R.H., Safiyeva, S. and Craker, L.E. (1999). Antimicrobial activity of some Yemeni medicinal plants. Journal of herbs, spices and medicinal plants 6: 75-83.

Anonymous (1978). The Ayurvedic formulary of India, part I, 1st edition, Government of India press, Fridabad, India pp-386.

Anonymous (1997). Indian medicinal plants: A sector study. Occasional paper No.54, Export-Import Bank of India, Quest publication, Bombay, India pp-140.

Bagyaraj, D.J. and Varma, A. (1995). Interactions between arbuscular mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid tropics. Advanced Microbial Ecology 14: 119-142.

Chandrika, K., Lakshmipathy, R. and Balakrishna, G. (2002). Response of *Centella asiatica* (L.) urban. to mycorrhizal inoculation. Journal of Soil Biology and Ecology 22(1&2): 35-39.

Chen, B., Christie, P. and Li, X. (2001). A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. Chemosphere 42: 185-192.

Chrimal, T., Bagyaraj, D.J. and Patil, C.S.P. (2006). Response of *Andrographis paniculata* to different arbuscular mycorrhizal fungi. Journal of Agricultural Technology 2(2): 221-228.

Codignole, A., Verotta, L., Spanu, P., Maffei, M., Scannerini, S. and Bonfate-Fasole, P. (1989). Cell wall bound phenols in roots of vesicular-arbuscular mycorrhizal plants. New Phytologist 112: 221-228.

Dhillion, S. (1992). Evidence for host-mycorrhizal preference in native grassland species. Mycological Research 96: 359-362.

Dhyani, P.P. and Kala, C.P. (2005). Current research on medicinal plants: five lesser known but valuable aspects. Current science 88: 335.

Earanna, N. (2001). VA mycorrhizal association in medicinal plants of South eastern dry zone of Karnataka and response of *Phyllanthus amarus* and *Withania somnifera* to inoculation with VAM fungi and plant growth promoting rhizomicroorganisms. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India pp 225.

Farkas, G.L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant disease and disease resistance. Phytopathology 2: 105-150.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spore of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting. Transactions of British Mycological Society 46: 235-244.

Giovannetti, M. and Mossea, B. (1980). An evaluation of technologies for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.

Gracy, L. S. and Bagyaraj, D.J. (2005). Influence of different AM fungi on growth, nutrition and forskholin content of *Coleus froskohlii*. Mycological Research 109:795-798.

Hemalatha, M. (2002). Synergistic effect of VA- mycorrhizae and *Azospirillum* on the growth and productivity of some medicinal plants. Ph.D. Thesis, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India pp 108.

Jackson, M.L. (1973). Soil chemical analysis. Prentice Hall (India) Pvt. Ltd, New Delhi pp-140.

Jeffries, P. (1987). Use of mycorrhizae in agriculture. CRC Critical Review 5: 319-357.

Kahneh, E., Ramezanpour, H., Haghparast Tanha, M.R. and Shirinferk, A. (2006). Effects of arbuscular mycorrhizal fungi and phosphorous supplement on leaf P, Zn, Cu and Fe concentrations of tea seedlings. Caspian Journal of Environmental Sciences 4(1): 53-58.

Kala, C.P. (2005). Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Conservation Biology 19: 368-378.

Kiritkar, K.R. and Basu, B.D. (1975). Indian medicinal plants, Indological and oriental publishers, New Delhi, India pp-425.

Krishna, K.R. and Bagyaraj, D.J. (1984). Growth and nutrient uptake of peanut inoculated with the mycorrhizal fungus *Glomus fasciculatum* compared with non-inoculated plants, Plant and Soil 77:405-408.

Lakshmipathy, R., Balakrishna, G. and Chandrika, K. (2003). Symbiotic response of *Garcinia indica* to VA mycorrhizal inoculation. Indian Journal of Forestry 26: 143-146.

Little, T.M. and Hill, F.J. (1978). Agricultural Experimentation: Design and Analysis. John Wiley and Sons. Inc., USA.

Mahadevan, A. (1991). Post infectional defense mechanisms. Today and Tomorrow Publishers, New Delhi pp-871.

Mahadevan, A. and Sridher, S. (1996). Methods in physiological plant pathology. Sivagami Publications, Madras, India pp-325.

Mathur, N., Joginder, S., Sachendra, B., Avinash, B. and Vyas, A. (2006). Increased nutrient uptake and productivity of *Plantago ovate* Forsk. By AM fungi under field conditions. American – Europian Journal of Scientific Research 1(1): 38-41.

Miller, R.M. and Jastrow, J.D. (1992). The role of mycorrhizal fungi in soil conservation. In: Mycorrhizae in sustainable Agriculture (Eds. G.J. Bethlenfalvay and R.C. Linderman). ASA Special Publications, WI, USA pp 29-44.

Phillips, J.H. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of British Mycological Society 55: 158-161.

Ponce, M.A., Scervino, J.M., Erra-Balsells, R., Ocampo, J.A. and Godeas, A.M. (2004). Flavonoids from shoots and roots of *Trifolium repens* (white clover) grown in presence or absence of arbuscular mycorrhizal fungus, *Glomus intraradices*. Phytochemistry 65: 1925-1930.

Porter, W.M. (1979). The most probable number method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Australian Journal of Soil Research 17: 515-519.

Rajan, S.K., Reddy, B.J.D. and Bagyaraj, D.J. (2000). Screening of mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecology and Management 126: 91-95.

Rajeshkumar, S., Nisha, M.C. and Selvaraj, T. (2008). Variability in growth, nutrition and phytochemical constituents of *Plectranthus ambonicus* (Lour) Spreng. as influenced by indigenous arbuscular mycorrhizal fungi. Maejo International Journal of Science and Technology 2(2): 38-45.

Reena, J. and Bagyaraj, D.J. (1990). Responses of *Acacia nilotica* and *Calliandra calothrysus* to different VA mycorrhizal fungi. Arid soil Research and Rehabilitation 4: 261-268.

Sadasivam, S. and Manicam, A. (1996). Biochemical methods. New Age International (P) Ltd. New Delhi, India pp-325.

Smith, G.S., Johnson, C.M. and Conforth, I.S. (1983). Comparison of nutrient solutions for growth of plants in sand culture. New Phytology 94: 537-548.

Smith, S. E. and Read, D.J. (1997). Mycorrhizal symbiosis. Academic Press, Cambridge pp 9-61.

Srivastava, D., Kapoor, R., Srivastava, A.k. and Kukerji, K.G. (1996). Vesicular arbuscular mycorrhiza. In: Concepts in mycorrhizal research (ed. K.G. Mukerji). Kluwe, Dordrecht pp 1-34.

Van Bavel, C.H.M. (1980). Mean weight diameter of soil aggregates as a statistical index of aggregation. Soil Science Society of American Proceedings 14: 20-23.

Vasanthakrishna, M., Bagyaraj, D.J. and Nirmal nath, J.P. (1995). Selection of efficient VA mycorrhizal fungi for *Casuarina equisetifolia* - Second screening. New Forests 9: 157-162.

Zakaria, M. (1991). Isolation and characterization of active compounds from medicinal plants. Asia Pacific Journal Pharmacology 6(1): 15-20.

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