

## Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of *Pogostemon patchouli* Pellet

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Received: 19 July 2008 / Accepted : 12 April 2009 / Published : 21 April 2009

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**Abstract:** Patchouli (*Pogostemon patchouli* Pellet) is an important aromatic crop cultivated for its essential oil used in cosmetics. arbuscular mycorrhizal (AM) fungi is known to modify several aspects of plant physiology and phytochemical constituents. Hence, a study was conducted on the efficacy of certain AM fungi in the improvement of some growth parameters and content of some phytochemical constituents in the leaves of *P. patchouli*. Patchouli seedlings were raised in soil inoculated with isolates of seven indigenous AM fungi, viz. *Acaulospora scrobiculata*, *Gigaspora margarita*, *Glomus aggregatum*, *G. geosporum*, *G. mosseae*, *Sclerocystis pakistanika* and *Scutellospora heterogama*. Seedlings raised in the presence of AM fungi generally showed an increase in growth, nutritional ingredients (sugars, N, P, K, Zn, Ca and Mn), total chlorophyll, and secondary metabolites in the leaves of patchouli compared to those from seedlings grown in the absence of AM fungi, the extent of increase, however, being varied with the AM fungi species. Furthermore, it was found that the phosphorus concentration was positively correlated with all growth parameters and content of phytochemical constituents (except essential oil), and that *G. aggregatum* seemed to be the best AM symbiont for the patchouli plant used in this experiment.

**Keywords:** AM fungi, *Pogostemon patchouli*, patchouli, growth parameters, phytochemical constituents

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## Introduction

Patchouli (*Pogostemon patchouli* Pellet. Family: Lamiaceae) is an important aromatic crop native to Phillipines. It is cultivated for its essential oil used in cosmetics. Production of patchouli oil in India is negligible (about 100-150 kg/year), as against the global production of around 700-800 tonnes/year. Presently, India is importing over 200 tonnes of the oil from Indonesia, Malaysia and Singapore [1]. Hence, there is a good scope for growing patchouli as a main crop or intercrop with other plantation crops. The use of microbial inoculants is playing an important role in sustainable agriculture. Utilisation of mycorrhizal biofertilisers in the cultivation of medicinal and aromatic plants is of recent interest. Arbuscular mycorrhizal (AM) fungi have been used to enhance the plant growth and yield of medicinal crops and to help maintain good soil health and fertility that contributes to a greater extent to a sustainable yield and good quality of the products [2]. AM fungi can also alter plant-water relation and response to drought [3]. Due to their ability to increase nutrient uptake and water transport, AM fungi are being frequently used in sustainable agriculture [4]. Though these fungi are not host specific, recent studies [5,6] have clearly brought out host preference in AM fungi, thus emphasising the need for selecting efficient AM fungi for inoculating a particular host [7-9]. The productivity of many plants is dependent on the formation of AM fungi. However, there have been only few attempts to study the impact of AM inoculation on patchouli plant [1,10].

As a result of this symbiotic association between AM fungi and host plants, phosphorus content also has an effect on phytochemical constituents and growth parameters in plants [11-13]. Phosphorus plays an important role as an energy carrier during photosynthesis [14]. Therefore, AM fungi may function as a metabolic sink causing basipetal mobilisation of photosynthesis to roots, thus providing a stimulus for greater photosynthetic activity [13]. AM fungi, as obligate symbionts, also depend for their growth and activity on the supply of carbon compounds from the photosynthetic partner [15,16]. AM symbiosis can cause an important carbohydrate gain in the host plant and up to 20% of total photoassimilate substances can be transferred to the fungal partner [17]. Photosynthetic activity and carbohydrates, which are the photoassimilate substances, are very important in terms of parameters that explain the physiological activity of the plants mentioned above. For this reason, this study aims to screen for efficient AM fungi for patchouli plant and also to study the effect of the association of different indigenous AM fungi on some growth parameters, viz. reducing and total sugars, chlorophyll level, and content of secondary metabolites, viz. total phenols, ortho-dihydroxyphenols, alkaloids, flavonoids, tannins, saponins and essential oil in the leaves of *P. patchouli*. The relationship between the phosphate content and these parameters in the mycorrhizal patchouli plant is also investigated.

## Materials and Methods

### *Cultivation of plants*

This investigation was carried out under nursery condition in a glasshouse. Seedlings of *Pogostemon patchouli* were obtained from the nursery unit of Department of Horticulture at Tamil

University, India. The identity of the plant was ascertained [18] and seedlings through stem cuttings (uniform 5-cm length) were raised on sterile sand-soil (1:1) mix. Three-week-old seedlings were transplanted to pots (24-cm dia.) containing 5 kg of sand-soil (1:3) mix which was classified as fine entisol, isohyperthermic kanhaplustalfs. The soil pH was 7.4 (1:10 soil-to-water ratio) and it contained 3.2 µg available phosphorus (extractable with NH<sub>4</sub>F + HCl) and an indigenous AM fungal population at 60 spores/50g of soil. The AM fungal species (*Acaulospora scrobiculata*, *Gigaspora margarita*, *Glomus aggregatum*, *G. geosporum*, *G. mosseae*, *Sclerocystis pakistanica* and *Scutellospora heterogama*) used in this study were isolated from rhizosphere soil of patchouli plants. These AM fungal species were isolated by wet-sieving and decanting technique [19]. The species-level identification of different AM fungal species was done following the keys provided by Schenck and Perez [20]. These fungi were multiplied using sterilised sand and soil mix(1:1v/v) as substrate and guinea grass (*Panicum maximum* Jacq.) as host. After 90 days of growth, shoots of guinea grass were severed and the substrate containing hyphae, spores and root bits was air-dried and used as inoculum. The inoculum potential (IP) of each culture was estimated by adopting the most probable number (MPN) method as outlined by Porter [21]. The soil in each pot was mixed with this inoculum so as to maintain an initial IP of 12,500 per pot. One set of plants without inoculation was used as control. Each treatment with 5 replications was performed in the glasshouse and watered regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution without phosphate was prepared [22] and then added to the pots at 50 ml per pot once every 20 days.

#### *Harvesting and analysis of plants*

Seventy-five days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, nutritional status, and some physiological and phytochemical constituents. Plant height was measured from soil surface to the growing tip of the plant. Dry biomass was determined after drying the plant sample at 60°C to constant weight in a hot-air oven. The root system was removed and assessed for AM fungal infection by grid-line intersect method [23] after clearing the roots with 10% KOH and staining with trypan blue (0.02%) as described by Phillips and Hayman [24]. Soil sample (100 g) was collected from each pot and subjected to wet-sieving and decantation method as outlined by Gerdemann and Nicolson [19] to estimate the population of spores. The essential oil content in the leaves of mycorrhizal and non-mycorrhizal plants were determined by hydro-distillation method by using Clevenger-type apparatus. Phosphorus, nitrogen, potassium and calcium content of the plant tissue were determined by employing the vanadomolybdophosphoric acid [25], micro-Kjeldahl [26], flame photometric and versenate titration method [25] respectively. Atomic absorption spectrophotometry was employed to estimate zinc and manganese content in the plant samples using respective hollow cathode lamps.

The content of chlorophylls in the leaves were determined spectrophotometrically [27]. The total and reducing sugar content in the leaves was estimated by employing Nelson-Somogyi reaction using glucose as standard [28]. The determination of total phenols and ortho-dihydroxyphenols was

done using Folin-Ciocalteu reagent as outlined by Farkas and Kiraly [29] and Arnov's reagent with catechol as standard [30] respectively. Aluminium chloride colorimetric method was used for determination of flavonoids with quercetin as standard [31]. Alkaloid content was estimated by extraction and precipitation by ammonium hydroxide [32]. Tannin was determined by absorbance measurement of its complex with ferric chloride and potassium ferrocyanide using tannic acid as standard [33]. Saponin was estimated by extraction with 20% ethanol with subsequent solvent fractionations as outlined by Zakaria [33].

#### *Statistical analysis*

The generated data were subjected to statistical analysis by completely randomised block design and the means were separated by Duncan's Multiple Range Test (DMRT). The data were also analysed by linear regression and analysis of variance using SAS, and the means were compared between treatments by the t-test.

## **Results and Discussion**

### *Growth response, nutritional status and physiological parameters*

The responses of patchouli plants to inoculation with different AM fungi were found to vary. Mycorrhizal inoculation resulted in a significant increase in height, biomass, and nutrient content of the plants (Tables 1-2). Those inoculated with *Glomus aggregatum* showed highest increase in all growth parameters and nutritional components, followed by *Glomus mosseae*. However, *S. pakistanika* and *A. scrobiculata* did not show any significant differences from control (Tables 1-2). All mycorrhiza-treated plants were heavily colonised at the rate of between 48.5%-98.5% (Table 1). However, there was no positive correlation between plant growth parameters and mycorrhizal colonisation.

Earlier studies also showed the same trend for medicinal plants subjected to AM inoculation [5, 34-36] and these studies also indicated the host preference for the AM fungi. Bagyaraj and Varma [37], Chiramel et al.[6] and Rajeshkumar et al. [36] stressed the need for selecting efficient native AM fungi for plant species. The present study conducted with an objective of screening for efficient indigenous AM fungi for patchouli plant has also resulted in varied plant growth responses to different AM fungi. The extent of increase in phosphorus and zinc content in the plant varied among the fungi studied, with plants grown in the presence of *G. aggregatum* containing significantly highest content of these nutrients, followed by those grown in the presence of *G. mosseae*. Such a variation in the plant nutrient content in relation to fungal species for other medicinal plant species is well documented [6,35]. The enhancement in growth and nutritional status is also related to the per cent root colonisation apart from several soil and environmental factors.

**Table 1.** Different native AM fungi and their influence on growth and oil content in leaf of *Pogostemon patchouli*

Treatment	Plant height (cm)		Plant dry weight (g/plant)			AM fungal colonisation in root (%)	Number of AM fungal spores / 100 g of soil
	Shoot	Root	Shoot	Root	Total		
Control (without AM fungi)	58.5 <sup>a</sup>	24.2 <sup>a</sup>	18.5 <sup>a</sup>	12.6 <sup>a</sup>	31.1 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
<i>Acaulospora scrobiculata</i>	62.4 <sup>a</sup>	26.2 <sup>a</sup>	20.4 <sup>a</sup>	13.2 <sup>a</sup>	33.6 <sup>a</sup>	50.0 <sup>a</sup>	310 <sup>a</sup>
<i>Gigaspora margarita</i>	65.8 <sup>b</sup>	28.4 <sup>b</sup>	21.2 <sup>b</sup>	14.6 <sup>b</sup>	35.8 <sup>b</sup>	75.0 <sup>b</sup>	420 <sup>b</sup>
<i>Glomus aggregatum</i>	72.5 <sup>c</sup>	29.5 <sup>c</sup>	25.5 <sup>c</sup>	16.4 <sup>c</sup>	41.9 <sup>c</sup>	98.5 <sup>c</sup>	765 <sup>c</sup>
<i>Glomus geosporum</i>	63.4 <sup>b</sup>	26.5 <sup>b</sup>	21.2 <sup>b</sup>	14.4 <sup>b</sup>	35.6 <sup>b</sup>	72.5 <sup>b</sup>	422 <sup>b</sup>
<i>Glomus mosseae</i>	69.5 <sup>c</sup>	27.2 <sup>c</sup>	23.5 <sup>c</sup>	16.1 <sup>c</sup>	39.6 <sup>c</sup>	80.0 <sup>b</sup>	685 <sup>c</sup>
<i>Sclerocystis pakistanika</i>	62.2 <sup>a</sup>	25.8 <sup>a</sup>	20.4 <sup>a</sup>	13.0 <sup>a</sup>	33.4 <sup>a</sup>	48.5 <sup>a</sup>	214 <sup>a</sup>
<i>Scutellospora heterogama</i>	64.2 <sup>b</sup>	26.4 <sup>a</sup>	20.6 <sup>a</sup>	13.2 <sup>a</sup>	33.8 <sup>a</sup>	52.5 <sup>a</sup>	265 <sup>a</sup>

Note: Means (n=5) in each column followed by the same letter are not significantly different ( $P < 0.05$ ) from each other according to DMR test.

The major concern in mycorrhizal technology for crop production is the existence of a great difference in the functional compatibility of AM fungi with medicinal crops [13,38,39]. In this study, inoculation with *G. aggregatum* and *G. mosseae* was found to be most effective in increasing the biomass (Table 1). AM fungi are known to improve plant growth and physiological parameters, mainly through uptake of phosphorus and other nutrients [13,40]. Bagyaraj et al.[41] and Lakshmiopathy et al.[42] reported that different strains of AM fungi differ in the extent they increase nutrient uptake, physiological parameters and plant growth. Hence, some workers suggested the need for selecting efficient AM fungi that can be used for inoculating different plants [38]. In the present study, mycorrhizal inoculation enhanced the P, Zn, Ca and Mn content in the root and leaf of patchouli plant, which contributed to the enhanced growth of the plant (Table 2). Mycorrhizal treatment resulted in an increase in the number of spores in the rhizosphere soil and this was maximum in *G. aggregatum* (Table 1), followed by that in *Glomus mosseae*. It is well known that enhanced nutritional status of a plant is manifested in its improved growth [40].

The chlorophyll content in every inoculated plant was seen to be higher than that in uninoculated control (Table 3). In particular, the amount of chlorophylls a + b increased significantly ( $P < 0.01$ )(Table 3). The content of chlorophylls a, b and a + b increased in *Glomus aggregatum* treated plants by 14%, 12% and 18% respectively compared with those in uninoculated control plants. The

**Table 2.** Influence of native AM fungi on N, P, K, Zn, Mn and Ca content in shoot and root of *Pogostemon patchouli*

Treatment	Nitrogen (g/plant)		Phosphorus (g/plant)		Potassium (g/plant)		Zinc (µg/plant)		Manganese (µg/plant)		Calcium (µg/plant)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Control (without AM fungi)	0.285 <sup>a</sup>	0.052 <sup>a</sup>	0.204 <sup>a</sup>	0.065 <sup>a</sup>	0.282 <sup>a</sup>	0.048 <sup>a</sup>	0.216 <sup>a</sup>	0.192 <sup>a</sup>	0.016 <sup>a</sup>	0.008 <sup>a</sup>	29.0 <sup>a</sup>	18.5 <sup>a</sup>
<i>Acaulospora scrobiculata</i>	0.310 <sup>a</sup>	0.058 <sup>a</sup>	0.216 <sup>a</sup>	0.072 <sup>a</sup>	0.284 <sup>a</sup>	0.052 <sup>a</sup>	0.312 <sup>a</sup>	0.210 <sup>a</sup>	0.026 <sup>a</sup>	0.009 <sup>a</sup>	30.0 <sup>a</sup>	19.2 <sup>b</sup>
<i>Gigaspora margarita</i>	0.312 <sup>b</sup>	0.062 <sup>b</sup>	0.244 <sup>b</sup>	0.085 <sup>b</sup>	0.302 <sup>b</sup>	0.054 <sup>b</sup>	0.610 <sup>b</sup>	0.312 <sup>b</sup>	0.036 <sup>b</sup>	0.010 <sup>b</sup>	36.0 <sup>c</sup>	19.8 <sup>c</sup>
<i>Glomus aggregatum</i>	0.340 <sup>c</sup>	0.064 <sup>c</sup>	0.262 <sup>c</sup>	0.098 <sup>c</sup>	0.312 <sup>b</sup>	0.054 <sup>b</sup>	0.710 <sup>c</sup>	0.352 <sup>c</sup>	0.039 <sup>c</sup>	0.012 <sup>c</sup>	38.5 <sup>c</sup>	20.0 <sup>c</sup>
<i>Glomus geosporum</i>	0.308 <sup>b</sup>	0.060 <sup>b</sup>	0.248 <sup>b</sup>	0.082 <sup>b</sup>	0.286 <sup>a</sup>	0.052 <sup>a</sup>	0.610 <sup>b</sup>	0.285 <sup>b</sup>	0.028 <sup>b</sup>	0.011 <sup>b</sup>	29.5 <sup>a</sup>	20.2 <sup>c</sup>
<i>Glomus mosseae</i>	0.316 <sup>b</sup>	0.062 <sup>b</sup>	0.258 <sup>c</sup>	0.092 <sup>c</sup>	0.292 <sup>a</sup>	0.049 <sup>a</sup>	0.642 <sup>c</sup>	0.314 <sup>b</sup>	0.029 <sup>b</sup>	0.011 <sup>b</sup>	30.2 <sup>b</sup>	19.2 <sup>b</sup>
<i>Sclerocystis pakistanika</i>	0.305 <sup>a</sup>	0.052 <sup>a</sup>	0.218 <sup>a</sup>	0.072 <sup>a</sup>	0.284 <sup>a</sup>	0.048 <sup>a</sup>	0.314 <sup>a</sup>	0.210 <sup>a</sup>	0.024 <sup>a</sup>	0.009 <sup>a</sup>	29.5 <sup>a</sup>	19.0 <sup>a</sup>
<i>Scutellospora heterogama</i>	0.306 <sup>a</sup>	0.052 <sup>a</sup>	0.224 <sup>1</sup>	0.074 <sup>a</sup>	0.282 <sup>a</sup>	0.049 <sup>a</sup>	0.316 <sup>a</sup>	0.214 <sup>a</sup>	0.025 <sup>a</sup>	0.009 <sup>a</sup>	29.5 <sup>a</sup>	18.8 <sup>a</sup>

Note: Means (n = 5) in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

concentration of phosphorus was positively correlated with that of the chlorophylls in mycorrhizal plants, although it was not significant (Table 5). The correlation coefficients were determined as  $r = 0.962$ ,  $0.753$ , and  $0.867$  for chlorophylls a, b and a + b respectively (Table 5). The partial correlations for all chlorophyll concentrations were also found to be non-significant ( $P > 0.05$ ).

One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. In this study, phosphorus and the chlorophyll content in all mycorrhizal plants were found to be higher than those in the uninoculated control, which indicated that the photosynthetic rate was improved by the AM fungi. Amelioration of the rate of photosynthesis and higher phosphorus level in leaves as a result of AM inoculation were also reported in other studies [13,43]. Since the photosynthetic process is known to be positively influenced by phosphorus, a positive correlation found in this study for phosphorus concentration in all mycorrhizal plants compared with that of uninoculated control of similar size also indicated its effect on photosynthesis. Furthermore, the effect of AM fungi on leaf morphology leading to an increase in the leaf area and leaf hydration is also probably partly caused by enhanced phosphorus level [44].

**Table 3.** Influence of native AM fungi on chlorophyll content and amount of sugars in the leaves of *P. patchouli*

Treatment	Chlorophyll a (mg g <sup>-1</sup> )	Chlorophyll b (mg g <sup>-1</sup> )	Chlorophylls a + b (mg g <sup>-1</sup> )	Carbohydrate content	
				Reducing sugars (%)	Total sugars (%)
Control (without AM fungi)	0.156 <sup>a</sup> ± 0.014	0.332 <sup>a</sup> ± 0.004	0.488 <sup>a</sup> ± 0.012	0.28 <sup>a</sup> ± 0.12	0.75 <sup>a</sup> ± 0.015
<i>Acaulospora scrobiculata</i>	0.198 <sup>b</sup> ± 0.012	0.398 <sup>b</sup> ± 0.004	0.596 <sup>b</sup> ± 0.014	0.48 <sup>a</sup> ± 0.24	0.82 <sup>b</sup> ± 0.025
<i>Gigaspora margarita</i>	0.202 <sup>b</sup> ± 0.002	0.401 <sup>b</sup> ± 0.004	0.603 <sup>b</sup> ± 0.014	0.62 <sup>b</sup> ± 0.42	0.85 <sup>b</sup> ± 0.024
<i>Glomus aggregatum</i>	0.214 <sup>c</sup> ± 0.002	0.406 <sup>c</sup> ± 0.006	0.620 <sup>c</sup> ± 0.014	0.96 <sup>c</sup> ± 0.62	1.36 <sup>c</sup> ± 0.045
<i>Glomus geosporum</i>	0.199 <sup>b</sup> ± 0.002	0.402 <sup>b</sup> ± 0.004	0.601 <sup>b</sup> ± 0.012	0.84 <sup>bc</sup> ± 0.54	0.92 <sup>b</sup> ± 0.032
<i>Glomus mosseae</i>	0.212 <sup>c</sup> ± 0.002	0.404 <sup>c</sup> ± 0.002	0.616 <sup>c</sup> ± 0.012	0.92 <sup>c</sup> ± 0.62	1.24 <sup>c</sup> ± 0.044
<i>Sclerocystis pakistanika</i>	0.196 <sup>b</sup> ± 0.002	0.399 <sup>b</sup> ± 0.002	0.595 <sup>b</sup> ± 0.014	0.62 <sup>b</sup> ± 0.42	0.86 <sup>b</sup> ± 0.025
<i>Scutellospora heterogama</i>	0.198 <sup>b</sup> ± 0.004	0.401 <sup>b</sup> ± 0.002	0.599 <sup>b</sup> ± 0.012	0.64 <sup>b</sup> ± 0.42	0.88 <sup>b</sup> ± 0.024

Note: Means (n=5) in each column followed by the same letter are not significantly different ( $P < 0.05$ ) from each other according to DMR test.

The amounts of reducing and total sugars significantly differed in control and inoculated plants ( $P < 0.05$ ) (Table 3). Moreover, the concentration of total phosphorus positively correlated with reducing and total sugar content in the treated plants (Table 5). The correlation co-efficient of reducing and total sugars were  $r = 0.982$  and  $0.304$  respectively and found to be non-significant. Symbiotic interactions in AM association are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus [45]. It has been demonstrated, using mycorrhizal and non-mycorrhizal clover plants of comparable plant size and growth rate and with similar N and P content,

that AM fungal colonisation stimulates the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and for growth reduction of the autotroph [46]. In this study, the content of carbohydrate compounds (reducing and total sugars) of mycorrhizal plants was also generally higher than that in the uninoculated control, and a positive correlation was determined between phosphorus concentration and all sugar content. Phosphorus plays the most important role during the breakdown of carbohydrates and synthesis of polysaccharides. In particular, phosphorus is very effective in the synthesis of starch from glucose [13]. As AM fungi increase the uptake of phosphorus, they may also increase the synthesis of carbon compounds [17]. Thus, it is seen that photosynthesis activity, increasing as a result of this symbiotic association, has a close relationship with the increase in the function of AM fungi.

#### *Phytochemical constituents*

The content of secondary metabolites (total phenols, ortho-dihydroxy phenols, alkaloids, flavonoids, tannins and saponins) in the leaves of patchouli plants were found to be significantly higher in those raised in soil inoculated with AM fungi (Table 4), with plants raised in the presence of *G. aggregatum* showing the most increase. (However, for all fungi tested there was no significant increase in essential oil content.) Such a variation in the phytochemical constituents in relation to fungal species for other medicinal plant species is also well documented [6,47]. The concentration of total phosphorus was also positively correlated with that of all secondary metabolites in the inoculated plants (Table 5). The correlation coefficients ( $r$ ) of total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids, tannins and saponins were 0.69, 0.62, 0.63, 0.92, 0.65 and 0.64 respectively (Table 5).

As AM fungi increase the uptake of phosphorus and other nutrients, they may also increase the synthesis of secondary metabolites. The increase in total phenols and ortho-dihydroxyphenols in inoculated plants could be attributed to the triggering of pathway of aromatic biosynthesis [48]. Krishna and Bagyaraj [49] reported an increase in phenols in the root of *Arachis hypogaea* colonised by *G. fasciculatum*. Hemalatha [50] also reported an increase in total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids and tannins in the root and leaf of inoculated *Ocimum basilicum* and *Coleus amboinicus*. Codignola et al. [51] found that *Glomus versiforme* inoculated with *Allium porum* showed a higher level of phenols in both leaves and roots. Dhillion [52] confirmed host-mycorrhizal preference in some grassland species.



**Table 4.** Influence of different native AM fungi on phytochemical constituents in the leaves of *P. patchouli*

Treatment	Total phenols (µg/g fresh wt.)	Ortho-di-hydroxy phenols (µg/g fresh wt.)	Alkaloids (µg/g dry weight)	Flavonoids (µg/g dry weight)	Tannins (µg/g dry weight)	Saponins (%)	Essential oil content in the leaves (%)
Control (uninoculated)	95.0 <sup>a</sup>	64.2 <sup>a</sup>	4.31 <sup>a</sup>	3.12 <sup>a</sup>	0.280 <sup>a</sup>	0.160 <sup>a</sup>	0.56 <sup>a</sup>
<i>Acaulospora scrobiculata</i>	120.5 <sup>b</sup>	72.3 <sup>b</sup>	4.32 <sup>a</sup>	3.22 <sup>a</sup>	0.291 <sup>b</sup>	0.172 <sup>b</sup>	0.59 <sup>b</sup>
<i>Gigaspora margarita</i>	124.2 <sup>b</sup>	70.2 <sup>b</sup>	4.38 <sup>b</sup>	3.24 <sup>a</sup>	0.294 <sup>b</sup>	0.172 <sup>b</sup>	0.62 <sup>c</sup>
<i>Glomus aggregatum</i>	130.5 <sup>c</sup>	85.4 <sup>c</sup>	5.12 <sup>c</sup>	3.76 <sup>c</sup>	0.335 <sup>c</sup>	0.192 <sup>c</sup>	0.65 <sup>c</sup>
<i>Glomus geosporum</i>	124.2 <sup>b</sup>	74.2 <sup>b</sup>	4.76 <sup>b</sup>	3.62 <sup>b</sup>	0.320 <sup>bc</sup>	0.190 <sup>c</sup>	0.59 <sup>b</sup>
<i>Glomus mosseae</i>	128.5 <sup>c</sup>	83.4 <sup>c</sup>	4.92 <sup>c</sup>	3.64 <sup>b</sup>	0.332 <sup>c</sup>	0.191 <sup>c</sup>	0.62 <sup>c</sup>
<i>Sclerocystis pakistanika</i>	121.4 <sup>b</sup>	70.5 <sup>b</sup>	4.38 <sup>b</sup>	3.26 <sup>a</sup>	0.295 <sup>b</sup>	0.178 <sup>b</sup>	0.58 <sup>a</sup>
<i>Scutellospora heterogama</i>	122.5 <sup>b</sup>	71.2 <sup>b</sup>	4.32 <sup>a</sup>	3.27 <sup>a</sup>	0.294 <sup>b</sup>	0.172 <sup>b</sup>	0.57 <sup>a</sup>

Note: Means (n = 5) in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

**Table 5.** Relationships between total phosphorus content and other organic constituents in *Glomus aggregatum* treated plants

Content	Y =	a +	b.x +	c.x <sup>2</sup> +	d.x <sup>3</sup>	R <sup>2</sup>	r
Chlorophyll a <sup>P3</sup>		26.37 (-0.96 ns)	- 243.42 (0.96 ns)	748.24 (-0.96 ns)	- 763.02	0.92	0.962 NS
Chlorophyll b <sup>P3</sup>		- 5.47	56.73	- 180.94	190.67	0.54	0.753 NS
Total chlorophyll <sup>P3</sup>		- 0.87 (0.85 ns)	11.32 (- 0.84 ns)	- 160.98 (- 0.85 ns)	-	0.76	0.867 NS
Reducing sugars <sup>P3</sup>		10.72 (- 0.94 ns)	- 98.97 (0.95 ns)	324.42 (- 0.95 ns)	- 348.36	0.97	0.982 NS
Total sugars <sup>P3</sup>		36.12 (- 0.18 ns)	- 313.64 (0.17 ns)	796.52 (- 0.17 ns)	- 725.94	0.95	0.304 NS
Total phenols <sup>P3</sup>		- 7.21 (0.27 ns)	- 76.75 (- 0.28 ns)	- 256.28 (0.30 ns)	281.42	0.58	0.69 NS
Ortho di-hydroxy phenols <sup>P3</sup>		- 5.42 (0.24 ns)	64.75 (- 0.24 ns)	- 242.24 (0.24 ns)	- 264.42	0.44	0.62 NS
Flavonoids <sup>P3</sup>		- 19.90 (0.13 ns)	- 166.12 (- 0.10 ns)	- 421.54 (0.08 ns)	338.08	0.40	0.63 NS
Alkaloids <sup>P3</sup>		- 24.61 (0.8 ns)	238.52 (- 0.8 ns)	- 737.96 (0.8 ns)	750.35	0.84	0.92 NS
Tannins <sup>P3</sup>		14.12 (0.12 ns)	135.64 (- 0.12 ns)	- 418.56 (0.12 ns)	- 248.42	0.52	0.65 NS
Saponins <sup>P3</sup>		12.12 (0.10 ns)	124.42 (- 0.10 ns)	- 406.24 (0.10 ns)	- 236.46	0.48	0.64 NS

Note: P3 = Polynomial fit (degree 3)

NS = Correlation coefficient is not significant (P > 0.05).

ns = Partial correlation is not significant (P > 0.05).

## Conclusions

*P. patchouli* has been shown to exhibit varied responses to different AM fungi. Consideration of such factors as the growth parameters, nutritional status, and content of phytochemical constituents suggests that a specific relationship exists between a particular species of fungus and the plant. Giving weight to plant physiological growth parameters and phytochemical constituents but not neglecting the other parameters, *Glomus aggregatum* and *Glomus mosseae* seem to be the best and the next best fungus respectively for inoculating *P. patchouli* in the nursery in order to obtain healthy, vigorously growing seedlings that should perform better when planted in sandy loam soils, hence demonstrating and confirming that proper selection of efficient AM fungi for the right medicinal plant and environment is the key for their successful use in agriculture.

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