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# Variability in growth, nutrition and phytochemical constituents of *Sphaeranthes amaranthoides* (L.) Burm. as influenced by indigenous arbuscular mycorrhizal fungi

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## Abstract

A study was conducted under green house nursery condition of the efficacy of seven indigenous arbuscular mycorrhizal fungi (AMF) in the improvement of growth, biomass, nutrition and phytochemical constituents, namely total phenols, Ortho di-hydroxy phenols, flavonoids, alkaloids, tannins and saponins in the leaves of *Sphaeranthes amaranthoides* (L.) Burm. Seedlings were raised in polythene bags containing soil inoculated with isolates of seven diffeent indigenous AMF viz., *Acaulospora marrowae*, *Archaeospora trappei*, *Gigaspora margarita*, *Glomus aggregatum*, *G. pakistanika*, *G. walkeri* and *Scutellospora heterogama*. *S. amaranthoides* seedlings raised in the presence of AM fungi generally showed an increase in plant growth, nutritional status, and phytochemical constituents over those grown in the absence of AM fungi. The extent of growth, biomass, nutritional status and phytochemicals constituents enhanced by AM fungi varied with the species of AM fungi inhabiting the leaves of *A. amaranthoides* seedlings. Considering the various plant growth parameters, nutritional status of the plant, total phenols, ortho di-hydroxy phenols, alkaloids, flavonoids, tannins and saponins in the leaves, it was observed that *Glomus walkeri* is a the best AM symbiont for *S. amaranthoides* used in this experiment.

**Keywords:** biomass, native AM Fungi, nutrition, phytochemical constituents, *Sphaeranthes amaranthoides*

## INTRODUCTION

The introduction of beneficial organisms into soil is a present crux of applied mycorrhizal research. 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 (Rajan *et al*., 2004). The activity has gained momentum in recent years due to the higher cost and hazardous effects of heavy doses of chemical fertilizers. These fungi are a ubiquitous group of soil fungi colonizing the roots of plants belonging to more than 90 per cent of the plant families (Brundreff, 1991). These zygomycetous fungi represent an important component in the soil microbial biomass due to their ubiquity and their direct involvement in essential processes at the plant-soil interface (Honley and Smith, 1983). Interest in this association is mainly because of the manifold benefits conferred on the host by the fungi. They are known to improve the nutritional status of plants as well as their growth and development and to protect plants against root pathogens and confer resistance to drought and soil saline condition (Bagyaraj and Varma, 1995). Two hundred and ten species of AM fungi are recognized and classified (Giovannetti and Gianinnazzipearson, 1994) . Due to the wide host range they inhabit, there exists a wide variation in the ways they benefit the host, which in turn are related to the extent of the colonization of host roots by the fungus. The extent of the root colonization varies with several soil and climatic factors apart from the host involved. However, these fungi show a preferential colonization to hosts and thus the extent to which the host benefits depends of the fungal species involved in the symbiosis (Miller *et al*., 1987). The existence of inter- and intra-specific variations among the plant species involved in relation to their phosphorus requirement and the ability of the host to translocate the native soil phosphorus further determine the efficacy of these fungi (Koide, 1991). Thus it is essential to screen for an efficient AM fungus for a particular host in order to harness the maximum benefit from the fungus. Furthermore, since AM fungi cannot be grown on laboratory media, production of a large quantity of the inoculum for inoculation of he soil under field conditions is difficult. Nevertheless, since most of the commercially important medicinal crops are raised under nursery conditions before being transplanted to the main field, the inoculation of soil in the nursery would not only result in the saving of the inoculum needed but also help in better establishment of the transplanted seedlings.

The are few published reports on the influence of AM fungi on the growth, nutrition and phytochemical

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constituents of medicinal plants (Gupta and Janardhanan, 1991; Earanna *et al*., 2002). *Sphaeranthes amaranthoides* is an important medicinal plant and commonly called 'Kesavarthini.' The leaves are bitter, acrid, sweet, thermogenic, diuretic, expectorant and are used for the treatment of epilepsy, chronic asthma, cough, bronchitis, leprosy, hermia, haemorrhids, helminthiasis and dyspepsia. The powdered leaf is good for skin diseases and is considered as a nervine toxic (Kiritkar and Basu, 1995). Hence the present investigation was done to screen an efficient AM fungus for *S. amaranthoides* and also to study the effects of the association on the growth, biomass, nutritional status and phytochemicals, *viz.*, total phenols, ortho di-hydroxy phenols, alkaloids, flavonoids, tannins and saponins in the leaves of *S. amaranthoides*.

## MATERIALS AND METHODS

This investigation was carried out under nursery condition in a glass house. The soil used in this study was collected from an uncultivated field at a depth of 0-30 cm and was classified as fine, entisol, isohyperthermic kanhaplustalfs. The soil pH was 7.2 (1:10 soil to water extract ratio), and it contained 2.7 ppm available phosphorus (extractable with $NH_4F$ + HCl) and an indigenous AM fungal population of 60 spores/50 g of soil. Nursery was raised by sterilizing the seeds of *S. amaranthoides* with 5 per cent chloramines T solution for 30 minutes, then washing and sowing in polythene bags (10 x 15cm) containing sterilized soil: vermiculite mix (1:1 v/v). Ruakura nutrient solution at 50 ml per bag was applied once in 10 days. After 30 days seedlings were transplanted to polythene bags of size 25x15cm containing 2kg of unstrilised soil: sand: compost in the ratio of 2:1:0.5 (v/v/v).

The AM fungal species used in this study (Table 1) were isolated from the rhizosphere soil of Kesavarthini plants cultivated at the herbal garden of Tamil University, Tamilnadu, India. These AM fungal species were isolated by using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The species level identification of different AM fungal species was done following the keys provided by Trappe (1982) and Schenck and Perez (1990). These fungi wee multiplied using sterilized sand:soil mix (1:1 v/v) as the substrate and onion as the host. After 90 days of growth, shoots of onion was 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 adopting the Most Probable Number (MPN) method as outlined by Porter (1979). The soil in each polythene bag was mixed with this inoculum at different rates so as to maintain an initial IP of 12,500 per polythene bag. Each bag containing the potting mixture, with or without AM inoculum as the treatment may be, was planted with one seedling of *S. amaranthoides*. One set of plants without inoculation was the control. Each treatment with 5 replications was maintained in a glass house and watered regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution (Harley and Smith, 1983), without phosphate was added to the polythene bags at the rate of 50 ml per polythene bag once in 15 days.

Ninety days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, nutritional status 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 a constant weight in a hot air oven. Soil sample (50 g) was collected from each polythene bag and subjected to wet sieving and decantation method as outlined by Gerdemann and Nicolson (1963), to estimate the Population of spores. The root system was removed and assessed for AM fungal infection by the grid-line intersect method (Giovanetti and Mosse, 1980) after clearing the roots with 10 per cent KOH and staining with trypan blue (0.02%) as described by Phllipsand Hayman (1970). Estimation of soil aggregates (<50 mm size), which indirectly denote the extent of external hyphae in soil, was done as described by Van Bavel (1980).

Phosphorus and potassium content of the plant tissue were determined by employing the vanadomolybdate phosphoric yellow colour and flame photometric method (Jackson, 1973), respectively. Atomic absorption spectrophotometry was employed to estimate zinc, copper and iron content of the plant samples, using respective hollow cathode lamps. Sturdiness quotient, biovolume index (a measure of the total volume of a seedling), and quality index, which reflects the quality of a seedling, were determined using the formulae given by Hatchell (1985). The content of secondary metabolites, *viz.*, total phenols (Farkas and Kiraly, 1962), ortho dihydroxy phenols (Mahadevan and Sridhar, 1996), flavonoids, tannins, saponins and alkaloids in the leaves of the tested plants, were assayed according to the methods described by Sadasivam and Manickam, (1996) and Zakaria (1991). The data thus generated were subjected to statistical analysis of completely randomized block design and the means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

## RESULTS AND DISCUSSION

In the field survey, '*Kesavarthini*' (*S. amaranthoides*) plants growing in uncultivated P-deficient sandy loam soils were almost same as in cultivated soils. Microscopic examination of their roots revealed extensive colonization by AM fungi with 94.5 per cent level of infection. A large number of inter and intra-matrical vesicles were noticed between 120mm and

140mm in size. The vesicles were globose to subglobose and the subtending hyphae were simple. Based on the morphological characters, the AM fungal isolate was identified as *Glomus* species. Altogether seven AM fungi were isolated from root-zone soils and identified (Table 1). Among them *Glomus aggregatum* and *Glomus walkeri* were predominant. However, *Acaulospora*, *Archaeospora*, *Gigaspora* and *Scutellospora* rarely occurred. Soil-borne auxiliary cells of *Gigaspora* and *Scutellospora* were also isolated and identified.

**Table 1.** Different native AM fungi and their influence on the growth of *S. amaranthoides*

| Treatment | Plant height (cm) | | Plant dry weight (g/plant) | |
|---|---|---|---|---|
| | Shoot | Root | Shoot | Root |
| Control (without AM fungi) | 62.0[a] | 23.5[a] | 16.5[a] | 12.5[a] |
| *Acaulospora marrowae* | 68.6[b] | 28.6[b] | 19.8[b] | 18.2[b] |
| *Archaeospora trappei* | 66.4[b] | 28.2[b] | 18.8[b] | 17.5[c] |
| *Glomus walkeri* | 90.6[d] | 52.4[d] | 24.5[d] | 23.2[d] |
| *Glomus aggregatum* | 84.5c[d] | 42.0[cd] | 23.8[c] | 19.9[b] |
| *Glomus pakistanika* | 72.4[c] | 34.5[c] | 20.2b[c] | 19.8[b] |
| *Gigaspora margarita* | 86.5[d] | 46.2[cd] | 24.0[c] | 20.4[cd] |
| *Scutellospora heterogama* | 68.5[b] | 29.2[b] | 19.2[b] | 17.6[c] |

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 growth response, nutritional status and mycorrhizal development of plants raised in sandy loam soils were assessed for the impact of inoculation with different native AM fungi. The responses of the Kesavarthini plants to inoculation with different AM fungi were found to be varied. Mycorrhizal inoculation resulted in a significant increase in height, biomass, nutrient content and phytochemical constituents of *S. amaranthoides* seedlings. However, there was no positive correlation between plant growth parameters and mycorhizal colonization. Earlier studies also showed the same trend for medicinal plants subjected to AM inoculation (Rajan *et al*., 2004; Earanna *et al*., 2002; Chandrika *et al*., 2002) and these studies also indicated the host preference to the AM fungi. Jeffries (1987) and Bagyaraj and Varma (1995) stressed the need for selecting efficient native AM fungi for plant species. The present study conducted with an objective of screening an efficient indigenous AM fungi for *S. amaranthoides* seedlings has also found varied plant growth responses to different AM fungi.

Mycorrhizal treatments resulted in an increase in the number of spores in the rhizosphere soils and this was maximum with *Glomus walkeri* followed by *G. aggregatum*. It is well known that enhanced nutritional status of a plant is manifested in its improved growth.

*S. amaranthoides* plants grown in the presence of AM fungi showed a general increase in such growth parameters as plant height and total dry weight as against those grown in soils uninoculated with AM fungi (Table 1).

The nutritional status of *S. amaranthoides* seedlings, *viz*., phosphorus, potassium, zinc, copper and iron content, was also significantly higher in plants raised in soil inoculated with AM fungi (Table 2). Seedlings raised in the presence of *Glomus walkeri* showed an increase of 108%, 81%, 82%, 80.5% and 82.5% in the tissue P, K, Zn, Cu and Fe content, respectively, as compared to the seedlings raised as uninoculated control. The extent of increase in plant P, K, Zn, Cu and Fe content varied among the fungi studied with seedlings grown in the presence of *G. walkeri* containing significantly highest content of these nutrients, followed by those grown in the presence of *Gigaspora margarita* and *Glomus aggregatum*. Such a variation in the plant nutrient status in relation to the fungal species for other medicinal plant species is well documented earlier (Reena and Bagyaraj, 1990; Rajan *et al*., 2004). The enhancement in growth and nutritional status is also related to the per cent root colonization apart from several soil and environmental factors.

**Table 2.** Influence of native AM fungi on P, K, Zn, Cu and Fe contents in the shoot and root of *P. amboinicus*

| Treatment | Phosphorus content (mg/plant) | Potassium content (mg/plant) | Zinc content ( g/plant) | Copper content ( g/plant) | Iron content ( g/plant) |
|---|---|---|---|---|---|
| Control (without AM fungi) | 3.29[a] | 3.2[a] | 162.8[a] | 61.7[a] | 59.5[a] |
| *Acaulospora marrowae* | 5.50[b] | 4.1[b] | 195.0[bc] | 74.9[b] | 64.2[b] |
| *Archaeospora trappi* | 5.38[ab] | 4.2[c] | 198.5[b] | 105.8[c] | 72.8[c] |
| *Glomus walkeri* | 6.56[d] | 5.4[d] | 296.9[d] | 112.3[d] | 95.6[d] |
| *Glomus aggregatum* | 6.23[d] | 4.2[c] | 280.9[c] | 108.9[d] | 92.2[d] |
| *Glomus pakistanika* | 5.95[c] | 4.4[c] | 241.3[bc] | 98.5[c] | 88.5[c] |
| *Gigaspora margarita* | 6.35[d] | 4.6[c] | 286.2[d] | 109.2[d] | 94.2[d] |
| *Scutellospora heterogama* | 5.45[b] | 3.4[a] | 223.2[bc] | 104.4[c] | 88.2[c] |

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 phytochemical constituent *viz*., total phenols (Ortho di-hydroxy phenols, flavonoids, alkaloids, tannins and saponins) of *S. amaranthoides* seedlings were found to be significantly higher in plants raised in soil inoculated with AM fungi (Table 3), with seedlings raised in the presence of *G. walkeri* showing the highest increase for all the phytochemical constituents studied 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 earlier (Rajan *et al*., 2004; Rao *et al*., 2004).

Other parameters studied in relation to effects of soil inoculation with AM fungi are shown in Table 4.

*G. walkeri* and *Glomus aggregatum* inhabited a significantly higher percentage of roots compared to other AM fungi. Similarly, spore number was also highest in the soil samples inoculated with *G. walkeri* followed by soil inoculated with *Glomus aggregatum*, indicating a better proliferating ability of these fungi with *S. amaranthoides* as the host.

**Table 3.** Influence of different native AM fungi on phytochemical constituents in the leaves of *S. amaranthoides*

| Treatment | Total ( g/g fresh wt.) | O-Dihydroxy-phenols ( g/g fresh wt.) | Flavonoids ( g/g dry wt.) | Alkaloids ( g/g dry wt.) | Tannins ( g/g dry wt.) | Saponins ( g/g dry wt.) |
|---|---|---|---|---|---|---|
| Control (without AM fungi) | 95.0$^{a}$ | 65.2$^{a}$ | 3.12$^{a}$ | 4.32$^{a}$ | 0.280$^{a}$ | 0.160$^{a}$ |
| *Acaulospora marrowae* | 120.5$^{c}$ | 75.2$^{d}$ | 3.25$^{b}$ | 4.38$^{b}$ | 0.290$^{b}$ | 0.172$^{b}$ |
| *Archaeospora trappi* | 114.2$^{b}$ | 70.2$^{b}$ | 3.16$^{b}$ | 4.36$^{b}$ | 0.298$^{b}$ | 0.176$^{b}$ |
| *Glomus walkeri* | 130.5$^{d}$ | 85.4$^{d}$ | 3.76$^{d}$ | 5.12$^{d}$ | 0.435$^{d}$ | 0.210$^{d}$ |
| *Glomus aggregatum* | 128.2$^{d}$ | 82.3$^{c}$ | 3.62$^{c}$ | 4.86$^{c}$ | 0.382$^{c}$ | 0.195$^{c}$ |
| *Glomus pakistanika* | 118.5$^{b}$ | 80.5$^{c}$ | 3.58$^{c}$ | 4.82$^{c}$ | 0.365$^{c}$ | 0.192$^{b}$ |
| *Gigaspora margarita* | 129.2$^{d}$ | 83.4$^{d}$ | 3.64$^{cd}$ | 5.01$^{d}$ | 0.395$^{d}$ | 0.199$^{c}$ |
| *Scutellospora heterogama* | 115.5$^{b}$ | 72.72$^{b}$ | 3.18$^{c}$ | 4.38$^{b}$ | 0.340$^{ab}$ | 0.185$^{b}$ |

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 4.** Effects of soil inoculation with AM fungi on percent root colonisation, spore numbers in root zone soil, percent aggregation of rhizosphere soil, sturdiness quotient, biovolume and quality index of *S. amaranthoides*

| Treatment | AM fungi colonisation in root (%) | Number of AM fungi spore / 100 g of soil | Aggregation of soil | Sturdiness quotient | Biovolume index | Quality index |
|---|---|---|---|---|---|---|
| Control (without AM fungi) | 0$^{a}$ | 0$^{a}$ | 18$^{a}$ | 16.4$^{a}$ | 2442.2$^{a}$ | 0.45$^{a}$ |
| *Acaulospora marrowae* | 53.5$^{b}$ | 320$^{b}$ | 36$^{b}$ | 16.8$^{b}$ | 2526.4$^{b}$ | 0.46$^{b}$ |
| *Archaeospora trappi* | 45.5$^{b}$ | 310$^{b}$ | 39$^{b}$ | 16.9$^{b}$ | 2527.2$^{b}$ | 0.47$^{b}$ |
| *Glomus walkeri* | 92.5$^{d}$ | 760$^{d}$ | 52$^{d}$ | 17.4$^{d}$ | 3245.4$^{d}$ | 0.58$^{d}$ |
| *Glomus aggregatum* | 85.5$^{cd}$ | 680$^{cd}$ | 48$^{c}$ | 17.1$^{c}$ | 3162.8$^{c}$ | 0.55$^{c}$ |
| *Glomus pakistanika* | 82.0$^{cd}$ | 620$^{cd}$ | 42$^{c}$ | 16.9$^{c}$ | 2526.4$^{b}$ | 0.47$^{b}$ |
| *Gigaspora margarita* | 82.5$^{cd}$ | 620$^{cd}$ | 45$^{c}$ | 17.0$^{c}$ | 2975.9$^{b}$ | 0.54$^{c}$ |
| *Scutellospora heterogama* | 63.0$^{c}$ | 460$^{c}$ | 32$^{b}$ | 16.6$^{b}$ | 2469.4$^{b}$ | 0.46$^{b}$ |

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.

Mycorrhizal fungi are also implicated in soil structure improvement 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 is further strengthened by the present study as mycorrhizal fungi used in this study significantly improved the aggregation of soil compared to that of the control (Table 4). Soil aggregation was highest in soil inoculated with *G. walkeri* and *Glomus aggregatum* in that order. Other seedling parameters (sturdiness quotient, biovolume index and quality index) were all found be higher than those of the control, the increase being to the extent of 6.08%, 33.20% and 29.4%, respectively (Table 4). Such values indicate a sturdier stem and a greater dry weight of the plant, qualities which are desirable among nursery seedlings (Hatchell, 1985).

AM fungi differ greatly in their symbiotic effectiveness which depends on their preference for particular soils or host plant specificity (Dhillion, 1992), direct ability to stimulate plant growth, rate of infection, competitive ability and tolerance to applied chemicals. Giving weighting to quality index, but not neglecting the other parameters, *Glomus walkeri* and *Glomus aggregatum* were found to be the best and the next best fungus, respectively, for inoculating *S. amaranthoides* in the nursery in order to obtain healthy, vigorously growing seedlings that could establish and perform better when planted in sandy loam soils.

## CONCLUSIONS

*S. amaranthoides* seedlings show varied responses to different AM fungi, with *Glomus walkeri* conferring greater benefits compared to all other fungi used in this study. Further considerations *viz.*, the ability for higher root colonization, plant biomass, biovolume index and mineral and phytochemical constituents suggested that a clear and specific relationship exists between a particular species of fungus and the plant.

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