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Influence of *Glomus walkeri* Blaszk and Renker and plant growth promoting rhizomicroorganisms on growth, nutrition and content of secondary metabolites in *Sphaeranthes amaranthoides* (L.) Burm.

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The study was undertaken to determine the effect of arbuscular mycorrhizal (AM) fungi, *Glomus walkeri* Blaszk. & Renker and some plant growth promoting rhizomomicroorganisms (PGPR'S) on growth, biomass, nutrition and content of secondary metabolites of *Sphaeranthes amaranthoides* (L.) Burm.under glass house conditions. Various plant growth parameters (total plant biomass, mycorrhizal parameters, shoot and root phosphorus), mineral content (potassium zinc, iron and copper) and secondary metabolites (total phenols, ortho di-hydroxy phenols, tannins, flavonoids and alkaloids) were determined and found to vary with different treatments. Among all the treatments plants inoculated with microbial consortium consisting of *Glomus walkeri* + *Bacillus subtilis* + *Trichoderma viride* performed better than with other treatments and uninoculated control plants. The results of this experiment clearly indicated that inoculation of *S. amaranthoides* with *G. walkeri* along with PGPR'S enhanced its growth, biomass, nutrition and secondary metabolities.

Key words: *Sphaeranthes amaranthoides, Glomus walkeri, Bacillus subtilis, Trichoderma viride,* growth, nutrition, secondary metabolites

Introduction

Medicinal plants are nature's best gift to cure a number of diseases of men and animals. India has 16 agro climatic zones and medicinal plants are distributed across diverse habitats and landscapes. In India, Tamilnadu State is under strategic geographical location and possess an invaluable treasure of

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medicinal plants holding a major share in cultivation and export of medicinal plant species including *Sphaeranthes amaranthoides* (*L.*)*Burm. S. amaranthoides* (*Asteraceae*) is one of the important medicinal plant, commonly known as "Kesavarthini". The juice expressed from the plant is used in vitiated conditions of vata, epilepsy, hemicranias jaundice, hepatopathy and gastropathy. The roots are bitter, acrid, sweet, thermogenic, diuretic, expectorant, febrifuge and stomachic. They are useful in strangury, diabetes, leprosy, fever, pectoralgia, cough, hermia, haemorrhids, helminthiasis and dyspepsia. The powdered leaf is good for skin diseases and is considered as a nervine tonic. The flowers are highly esteemed as an alternant, depurative refrigent and tonic. (Kiritkar and Basu, 1975).

Ecosystems are composed of many organisms interacting in a multiple complex relationships with their environment and with each other. Biological relationships may be antagonistic, neutral or beneficial (Wright, 2005). Modern agriculture emphasizes eco-friendly technologies such as organic farming and application of bio-fertilizers in crops. Current research in drug discovery from medicinal plants involves a multifaceted approach with the advent of innovative technologies and the importance being given to sustainable agriculture. An introduction of Arbuscular Mycorrhizal (AM) fungi is known to increase the growth of many plant species including medicinal plants. This is attributed to an increased uptake of nutrients, production of growth promoting substances and phyto-chemical constituents, tolerance to drought, salinity, transplant shock, resistance to plant pathogens and synergistic interaction with other beneficial soil micro organisms (Bagyaraj and Varma,1995; Jeffries *et al.,* 2003) It has been established that mycorrhizal plants grow better in infertile soils because of improved mineral nutrients through hyphae, which help in exploring a greater volume soil beyond root hairs (George *et al.,* 1992 ; Rajan *et al.,* 2002).

With the advent of innovative technologies and the importance being given to sustainable agriculture, AM fungal association is of great economic significance on the growth of agricultural and medicinal crops. Certain plant growth promoting rhizomicroorganisms (PGPR'S) have been reported to enhance the activity of mycorrhizal fungi and consequently plant growth (Fitter and Garbaye, 1994; Gurumurthy, 1997; Lakshmipathy *et al.,* 2001; Eranna *et al.,* 2002; Selvaraj *et al.,* 2008). Therefore microbial inoculants can help to maintain good soil health and fertility that contribute to a grater extent to a sustainable yield and quality of products (Wright, 2005). However, the information available on the use of these beneficial microorganisms in medicinal plants particularly in *S.amaranthoides* is meager. Hence, the present study was undertaken to determine the effect of AM fungus, *Glomus walkeri*

(isolated from rhizophere soils of S. amaranthoides, a wagaiyur isolate. Thanjavur,Tamilnadu,India) and the PGPR's, *Bacillus subtilis* and *Trichoderma viride,* singly and in combination on growth, biomass, nutrients and content of secondary metabolites of *S. amaranthoides* raised under glass house condition.

Materials and methods

S. amaranthoides seedlings were raised in seed pans containing sand: soil mix (1:1 v/v). The seedlings after germination were maintained for four weeks. *G. walkeri* maintained as a pot culture using sterilized sand: soil mix (1:1 v/v) as the substrate and onion (Allium cepa L.) as the host was used in the present study. The substrate along with the roots of onion was air-dried. The hyphae, spores and root segments in the dried substrate served as the mycorrhizal inoculum. *Bacillus subtilis* which is not only a PGPR but also a mycorrhiza helper bacterium (MHB) was grown in nutrient broth and *Trichoderma viride* in potato dextrose broth each in a 2 L flask containing 800ml medium. After 3days of growth for B.subtilis and 7days for T.viride, the cultures were used for inoculation along with *G .walkeri* at the time of sowing and the plants were maintained in a glass house for 90 days. The microbial cultures were separately mixed with sterile lignite powder and their populations were determined by serial dilution plate method.

PVC Pots of 4.5 kg capacity were filled with a sandy loam soil: sand (1:1 v/v) potting mix. The soil used was of an alfisol-type Kaolinitic, isohyperthermic typic kanhaplustafs. The potting mixture had a pH of 6.4 and contained 2.8 ppm available phosphate(NH_4 + HCl extractable A planting hole was made at the centre of the pot. Ten grams each of G.walkeri (1400 IP g^{-1}) B.subtilis ($2.8x10^8$ cfu g^{-1}) and T. viride (3.4x 10 8 cfu g^{-1}) inocula were added as per the treatment allocation shown in Table 1. One seedling was maintained per pot with 5 replications for each treatment. The plants were kept in a glass house and watered regularly.

The plants were harvested 90 days after planting. Growth parameters, viz., plant height, number of leaves and branches were recorded at harvest. Dry weight of shoot and root was recorded after drying the sample at 60 ^{0}C to constant weight in a hot air oven. The phosphorus and potassium content were estimated by vanadomolyb-date phosphoric acid and flame photometric method, respectively (Jackson, 1973). An atomic absorption spectro-photometric was employed to estimate zinc, copper and iron content of the plant leaf samples, using respective hollow cathode lamps. Acid phosphatase activity was estimated in the root-zone soil as per the procedure given by Tabatabai (1982). The content of secondary metabolites, i.e total phenols (Mc Donald *et al.,* 2001) ortho di-

hydroxy phenols (Mahadevan and Sridhar,1996) flavonoids (Chang *et al.,* 2002) alkaloids (Harborne, 1973), and tannins (Zakaria, 1991) were assayed in the plant leaf samples.

Mycorrhizal root colonization was determined by grid-line intersect method (Giovannetti and Mosse, 1980) after staining the root samples with acid fuchsin (0.2%) (Phillips and Hayman, 1970). Extrametrical chlamydospore numbers in the root-zone soil were enumerated by wet- sieving and decantation method (Gerdemann and Nicolson, 1963). The data has generated were subjected to statistical analysis of completely randomized block design and the means were separated by Duncan's Multiple Range Test (DMRT) (Little and Hills, 1978).

Results and discussion

In general, inoculants appreciably enhanced plant height for *B. subtilis* treatment 29.2 cm (Table 1) which was significantly superior over than treatments. This was followed by *G.walkeri* + *B subtilis* + *T.viride* (28.0 cm). There was no significance in the number of leaves and branches of PGPR's inoculated and uninoculated control plants. The maximum number of leaves and branches on 90 days after transplanting (DAT) were recorded in plants inoculated with *G. walkeri* + *B.subtilis* +*T. viride* (32.4/plant and 5.2 / plant respectively), which was significant over all other treatments, the lowest number of leaves and branches being recorded in control plants (Table 1). Such a response of improved plant growth was also obtained in Periwinkle (Earanna *et al.,* 2002) and in *Pelargonium graveolens* (Sivakumar *et al.,* 2002) inoculated with *Glomus fasciculatum* and some PGPR's.

Single inoculation with G. walkeri or dual inoculation with *G.walkeri* and *B.subtilis* also significantly enhanced the total dry weight inoculated with *G.walkeri* + *B.subtilis* + *T.viride* showed maximum shoot and root dry weight (9.7 g/plant), the lowest biomass being recorded in control (Table1). This may be due to synergistic interaction of the AM fungi and PGPR's in the rhizosphere of the plants (Lakshmipathy *et al.,* 2002; Sivakumar *et al.,* 2002)
Maximum percent root colonization was recorded in the plants inoculated with *G.walkeri* + *B.subtilis* + *T.viride* (95.2%) (Table 2). Similarly, spore number was maximum when the plants were inoculated with *G.walkeri* + *B. subtlis* (682.4/100 g soil) and *G.walkeri* + *B. subtilis* + *T. viride* (585.2/100 soil), the lowest number being recorded in un inoculated control plants (Table 2). Synergistic interactions have been reported between the free- living rhizosphere bacteria, N^2 fixing organisms and mycorrhizal fungi (Mayer and Lindermann, 1986; Eranna *et al.,* 2002) with respect to the percent root colonization and spore number.

Table 1. Influence of AM fungus, *Glomus walkeri* and PGPR's on growth and biomass of *S.amaranthoides*

Treatment	90 days			Plant biomass g/ plant		
	Plant height (cm)	No of leaves	No of branches	Shoot	Root	Total
Uninoculated Control	16.5 [e1]	16.2[e]	3.2[e]	1.3[d]	1.4[d]	2.7[e]
Glomus walkeri (Gw))	26.8[C]	25.6[d]	4.4[c]	5.4[c]	4.2[b]	9.6[a]
Bacillus subtilis (Bs))	29.2[a]	20.6[e]	3.5[d]	5.5[a]	2.2[d]	7.7[c]
Trichoderma viride (T.v.)	16.5[e]	20.8[e]	3.6[d]	1.4[d]	1.8[e]	3.2[d]
G.w + B.s	28.5[b]	30.1[b]	4.8[b]	5.4[b]	4.2[b]	9.6[a]
G.w + T.v	26.5[c]	29.6[c]	4.6[c]	4.8[c]	4.2[b]	9.0[b]
B.s + T.v	20.5[d]	28.9[c]	4.8[b]	4.6[c]	4.0[c]	8.6[b]
G.w + B.s +T.v	28.0[a]	32.4[a]	5.6[a]	5.6[a]	4.9[a]	9.7[a]

[1]Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test($P<0.05$)

Table 2. Influence of *Glomus walkeri* and PGPR's on % root colonization, spore number in the root zone soil and nutrient status in the leaves and acid phosphatase activity in the soil of *S. amaranthoides*

Treatment	Percent Root colonization	Spore number/ 100g of soil	Leaf P (mg/plant)	Leaf K (mg/plant)	Leaf Zn (µg/g)	Leaf Cu (µg/g)	Leaf Fe (µg/g)	Acid phosphatase activity (µg/g/soil/hr
Uninoculated Control	28.9[e]	124.0[e1]	1.58e	2.2[f]	38.6[e]	18.6e	22.4e	5.06e
Glomus walkeri (G.w)	87.2[b]	482.6[b]	15.20[c]	10.5[c]	160.5[d]	53.6c	60.5c	14.40[c]
Bacillus subtilis (B.s.)	30.5[d]	160.5[d]	3.50[d]	2.5[e]	56.2[e]	42.5d	48.2d	6.02[d]
Trichoderma viride (T.v.)	31.2[d]	140.6[d]	3.05[d]	2.9[e]	62.0[e]	40.2d	41.5d	6.08[d]
G.w + B.s	83.5[d]	682.4[a]	20.22[b]	12.5[b]	394.5[b]	60.8b	92.5b	23.03[c]
G.w + T.v	62.8[c]	320.5[c]	16.56[c]	11.4[b]	251.8[c]	56.8c	90.5b	13.03[c]
B.s + T.v	45.2[d]	285.0[c]	13.45[bc]	8.2[d]	120.2[d]	38.4d	85.6b	18.05[b]
G.w +B.s +T.v	95.2[a]	585.2[a]	27.14[a]	15.2[a]	207.2[a]	89.2a	94.0a	33.5[a]

[1]Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P<0.05$)

The leaf phosphorus, potassium, zinc, copper and iron content were maximum in the plants treated with *G.walkeri* + *B.subtilis* + *T.viride* (27.14 mg/ plant,15.2mg/ plant, 507.2 µg/g, and 94.2 µg/g, respectively) in contrast with the plants inoculated with *G.walkeri* alone (15.20 mg/ plant, 10.5mg /plant, 160.5 µg/g, 53.6 µg/g and 60.5 µg/g respectively) (Table 2). This is probably due to the enhanced mycorrhizal colonization. The phosphorus, potassium, zinc, copper and iron content were lowest in the un inoculated control plant. Such an increased P, K, Zn, Cu and Fe uptake due to mycorrhizal

inoculation with PGPR's was also reported by earlier workers. (Lakhmipathy *et al.,* 2002; Thanuja, 2000).

The acid phosphatase activity in the root-zone soil of all the inoculated seedlings was significantly higher compared to that in the root-zone soil of uninoculated control plants. The highest value was recorded in the root-zone of the plants inoculated with *G. walkeri* + *B.subtilis* + *T.viride* G. (33.5 µg/g soil/hr) followed by that of the *G.walkeri* + *B.subtilis* inoculated plants (23.03 µg/g soil/hr). Enhanced soil phosphatase activity in the root-zone soil of neem due to inoculation with AM fungi was also reported earlier (Sumana, 1998). The leaf secondary metabolites (total phenols, ortho di-hydroxy phenols, flavonoids, alkaloids and tannins) were maximum in the plants treated with *G. walkeri*+ *B.subtilis* + *T.viride* (129.8 µg/g, 81.5 µg/g, 3.62 µg/g ,5.08 µg/g and 0.454 µg/g respectively), followed by the plants dually inoculated with *G. walkeri* + *B. subtilis* (124.2 µg/g, 75.6 µg/g, 3.28 µg/g, 4.36 µg/g and 0.382µg/g, respectively (Table 3). This was also apparently due to the enhanced mycorrhizal colonization and nutrient status of the plants. Such an increased content of secondary metabolites due to mycorrhizal inoculation with PGPR's was reported by earlier workers (Elango, 2004 and Selvaraj *et al.,* 2009).

Table 3. Influence of *Glomus walkeri* and PGPR's on the Content of secondary metabolites in the leaves of *S. amaranthoides*

Treatment	Total phenols (µg/g fresh weight)	Ortho di-hydroxy µg/g fresh weight	Flavonoids µg/g fresh weight	Alkaloids µg/g fresh weight	Tannins µg/g fresh weight
Uninoculated Control	94.0[e][1]	63.5e	3.12e	4.25e	0.285e
Glomus walkeri (G.w.)	123.8[b]	75.2b	3.26b	4.25b	0.380b
Bacillus subtilis (B.s.)	118.2[c]	70.4d	3.21c	4.26d	0.286d
Trichoderma viride (T.v.)	110.6[d]	69.2d	3.16d	4.32c	.285d
G.w + B.s	124.2[b]	75.6b	3.28b	4.36b	0.382b
G.w + T.v	112.4[d]	73.2c	3.24c	4.21d	0.365c
B.s + T.v	110.5[d]	70.6d	3.18d	4.23d	0.314d
G.w + B.s +T.v	129.8[a]	81.5a	3.62a	5.08a	0.454a

[1]Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P<0.05$)

Conclusions

It is concluded that the "microbial consortium" consisting of *G.walkeri* + *B. subtilis* + *T.viride* seems to be best suited for *S.amaranthoides*. The results clearly indicated that inoculation of *G.walkeri* along with PGPR's encourages the ability of *G.walkeri* and enhances the growth, biomass, nutrients and content of secondary metabolites of S. *amaranthoides*.

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