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Full Paper

Effect of *Glomus mosseae* and plant growth promoting rhizomicroorganisms (PGPR's) on growth, nutrients and content of secondary metabolites in *Begonia malabarica* Lam.

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Abstract: *Begonia malabarica* Lam. (Begoniaceae) is one of the important medicinal plants whose main secondary metabolites are luteolin, quercetin and β-sitosterol. The leaves are used for the treatment of respiratory tract infections, diarrhoea, blood cancer and skin diseases. A study was undertaken to determine the effect of arbuscular mycorrhizal (AM) fungus, Glomus mosseae, and some plant growth promoting rhizomicro-organisms (PGPR's) on the growth, biomass, nutrients, and content of secondary metabolites of B. malabarica plant under green house conditions. Various plant growth parameters (total plant biomass, mycorrhizal parameter, shoot and root phosphorus), mineral content (potassium, iron, zinc, and copper), and secondary metabolites (total phenols, ortho-dihydroxy 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 mosseae + Bacillus coagulans + Trichoderma viride performed better than with other treatments or uninoculated control plants. The results of this experiment clearly indicated that inoculation of B. malabarica with G. mosseae along with PGPR's enhanced its growth, biomass yield, nutrients and secondary metabolites.

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Keywords: *Begonia malabarica, Glomus mosseae,* PGPR's, *Bacillus coagulans*, *Trichoderma viride*, secondary metabolites

Introduction

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 [1]. Utilisation of biofertilisers in the cultivation of medicinal and aromatic plants is of recent interest. 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 phytochemical constituents, tolerance to drought, salinity, transplant shock, resistance to plant pathogens, and synergistic interaction with other beneficial soil microorganisms such as N2-fixers and P-solubilisers [2-4]. 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 of soil beyond root hairs [5,6].

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 (PGPRs) have been reported to enhance the activity of mycorrhizal fungi and consequently plant growth [7-13]. Therefore microbial inoculants can help maintain good soil health and fertility that contribute to a greater extent to a sustainable yield and quality of products [1]. However, the information available on the use of these beneficial microorganisms in medicinal plants is meagre.

Begonia malabarica Lam. is one of the important medicinal plants, belonging to the family *Begoniaceae* and commonly called as 'rathasoori' or 'senthandu'. The main secondary metabolites of *B. malabarica* are luteolin, quercetin and β-sitosterol [14]. The leaves are used for the treatment of respiratory tract infections, diarrhoea, blood cancer, and skin diseases [14]. It is found to grow in lateritic and acidic soil. Lateritic soil is, in general, poor in nutritional status and is especially deficient in phosphorus. The present study was undertaken to study the effect of AM fungus, *Glomus mosseae* and the PGPRs, *Bacillus coagulans* and *Trichoderma viride*, singly and in combination on the growth, biomass, nutrients, and content of secondary metabolites of *B. malabarica* raised under glasshouse condition.

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Materials and Methods

B. malabarica seedlings were raised in seed pans containing a sand:soil mix (1:1 v/v). The seedlings after germination were maintained for four weeks. *G. mosseae* maintained as a pot culture using sterilised sand:soil mix (1:1 v/v) as the substrate and guinea grass (*Panicum maximum* Jacq.) as the host was used in the present study. The substrate along with the roots of guinea grass was air-dried. The hyphae, spores and root segments in the dried substrate served as the mycorrhizal inoculum. *Bacillus coagulans*, 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 800 ml medium. After 3 days of growth for *B. coagulans* and 7 days for *T. viride*, the cultures were used for inoculation along with *G. mosseae* at the time of sowing and the plants were maintained in a glasshouse for 90 days. The microbial cultures were separately mixed with sterile lignite powder and their populations were determined by serial dilution plate method.

Pots of 4.5-kg capacity were filled with a sandy loam soil:sand (1:1 by volume) potting mix. The soil used was of an alfisol-type kaolinitic, isohyperthemic typic kanhaplustafs. The potting mixture had a pH of 6.2 and contained 2.7 ppm available phosphate (NH_4F + HCl extractable). A planting hole was made at the centre of the pot. Ten grams each of of *G. mosseae* (1400 IP g^{-1}), *B. coagulans* (2.8 X 10^8 cfu g^{-1}), and *T. viride* (3.4 X 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 glasshouse 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 samples at 60ºC to constant weight in a hot air oven. The phosphorus and potassium content of the plants were estimated by vanadomolybdate phosphoric acid and flame photometric method respectively [15]. An atomic absorption spectrophotometre 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 [16]. The contents of secondary metabolites, i.e. total phenols [17], ortho dihydroxy phenols [18], flavonoids [19], alkaloids [20] and tannins [21] were assayed in the plant leaf samples.

Mycorrhizal root colonisation was determined by grid-line intersect method [22] after staining the root samples with acid fuchsin (0.2%) [23]. Extrametrical chlamydospore numbers in the root-zone soil were enumerated by wet-sieving and decantation method [24]. The data thus generated were

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subjected to statistical analysis of completely randomised block design and the means were separated by Duncan's Multiple Range Test [25].

Results and Discussion

In general, inoculants appreciably enhanced plant height especially for *B. coagulans* treatment (29.2 cm) (Table1), which was significantly superior over other treatments. This was followed by *Glomus mosseae* + *Bacillus coagulans* + *Trichoderma viride* (28.0 cm). There was no significant difference in the number of leaves and branches of PGPR-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. mosseae* + *B. coagulans* + *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 the investigation of Earanna et al. for Periwinkle [11] and of Sivakumar et al. for *Pelargonium graveolens* inoculated with *Glomus fasciculatum* and some PGPR's [12].

Single inoculation with *G. mosseae* or dual inoculation with *G. mosseae* + *B. coagulans* also significantly enhanced the total dry weight of *B. malabarica* plants. Those similarly inoculated with *G. mosseae* + *B. coagulans* + *T. viride* showed maximum shoot and root dry weight (9.7 g/plant), the lowest biomass being recorded in control (Table 1). This may be due to synergistic interaction of the AM fungi and PGPRs in the rhizosphere of the plants [3,8,12].

Table 1. Effect of AM fungus and PGPRs on growth and biomass of *B. malabarica*

Treatment	90 DAT			Plant biomass (g/plant)		
	Plant height(cm)	No. of leaves	No. of branches	Shoot	Root	Total
Uninoculated control	16.5^{e}	16.2^{e}	3.2^{e}	1.3^{d}	1.4^{d}	2.7^{e}
Glomus mosseae (G.m)	26.8^{c}	25.6^{d}	4.4^{c}	5.4^{c}	4.2^{b}	9.6^{a}
Bacillus coagulans (B.c)	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.m + B.c	28.5^{b}	30.1^{b}	4.8^{b}	5.4^{b}	4.2^{b}	9.6^{a}
G.m + T.v	26.5^{c}	29.6^{c}	4.6^{c}	4.8^{c}	4.2^{b}	9.0^{b}
B.c + T.v	20.5^{d}	28.9^{c}	4.8^{b}	4.6^{c}	4.0^{c}	8.6^{b}
G.m + B.c + T.v	28.0^{a}	32.4^{a}	5.2^{a}	5.6^{a}	4.9^{a}	9.7^{a}

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

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Maximum per cent root colonisation were recorded in the plants inoculated with *G. mosseae* + *B. coagulans* + *T. viride* (95.2 %) (Table 2). Similarly, spore number was maximum when the plants were inoculated with *G. mosseae* + *B. coagulans* (682.4/100g soil) and *G. mosseae* + *B. coagulans* + *T. viride* (585.2/100 g soil), the lowest number being recorded in uninoculated control plants (Table 2). Synergistic interactions have been reported between the free-living rhizosphere bacteria, N_2 fixing organisms and mycorrhizal fungi [26,11] with respect to the per cent root colonisation and spore number.

The leaf phosphorus, potassium, zinc, copper and iron content were maximum in the plants treated with *G. mosseae* + *B. coagulans* + *T. viride* (27.14 mg/plant, 15.2 mg/plant, 507.2µg/plant, 89.2 µg/g, and 94.2 µg/g respectively), in contrast with the plants inoculated with *G. mosseae* alone (15.20 mg/plant; 10.5 mg/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 colonisation. The phosphorus, potassium, zinc, copper and iron content were lowest in the uninoculated control plant. Such an increased P, K, Zn, Cu and Fe uptake due to mycorrhizal inoculation with PGPRs was also reported by earlier workers [3,27].

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. mosseae* + *B. coagulans* + *T. viride* (33.5 µ/g soil/hr), followed by that of the *G. mosseae* + *B. coagulans*-inoculated plants (23.03 µ/g soil/hr) (Table 2). Enhanced acid phosphatase activity in the root-zone soil of Neem due to inoculation with AM fungi was also reported earlier [28].

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Table 2. Influence of AM fungus and PGPRs on % root colonisation, spore number in the root zone soil, and nutrient status in the leaves of *B.malabarica*

Treatment	Percent root colonisation	Spore number/ 100 g 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^{e}	1.58^{e}	2.2^{f}	38.6^{f}	18.6^{e}	22.4^{e}	5.06^{e}
Glomus mosseae (G.m)	87.2^{b}	482.6^{b}	15.20^{c}	10.5^{c}	160.5^{d}	53.6^{c}	60.5^{c}	14.40^{c}
Bacillus coagulans (B.c)	30.5^{d}	160.5^{d}	3.40^{d}	2.8^{e}	56.2^{e}	42.5^{d}	48.2^{d}	6.02^{d}
Trichoderma viride (T.v)	31.2^{d}	140.6^{d}	3.08^{d}	2.9^{e}	62.0^{e}	40.2^{d}	41.5^{d}	6.08^{d}
G.m + B.c	83.5^{b}	682.4^{a}	20.22^{b}	12.5^{b}	394.5^{b}	60.8^{b}	92.5^{b}	23.03^{b}
G.m + T.v	62.8^{c}	320.5^{c}	16.56^{c}	11.4^{b}	251.8^{c}	56.8^{c}	90.5^{b}	13.03^{c}
B.c + T.v	45.2^{d}	285.0^{cd}	13.45^{bc}	8.2^{d}	120.2^{d}	38.4^{d}	85.6^{b}	18.05^{c}
G.m + B.c + T.v	95.2^{a}	585.2^{a}	27.14^{a}	15.2^{a}	507.2^{a}	89.2^{a}	94.0^{a}	33.5^{a}

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

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The leaf secondary metabolites (total phenols, ortho dihydroxy phenols, flavonoids, alkaloids and tannins) were maximum in the plants treated with *G. mosseae* + *B. coagulans* + *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. mosseae* + *B. coagulans* (124.2 µg/g, 75.6 µg/g, 3.28 µg/g, 4.36 µg/g, and 0.382 µg/g respectively) (Table 3). This is also apparently due to the enhanced mycorrhizal colonisation and nutrient status of the plants. Such an increased content of secondary metabolites due to mycorrhizal inoculation with PGPRs was reported by earlier workers [29,30].

Table 3. Influence of AM fungus and PGPR's on the content of secondary metabolites in the leaves of *B. malabarica*

Treatment	Total phenols (µg/g fresh wt.)	O-dihydroxy-phenols (µg/g fresh wt.)	Flavonoids (µg/g fresh wt.)	Alkaloids (µg/g dry wt)	Tannins (µg/g dry wt)
Uninoculated control	94.0^{e}	63.5^{e}	3.12^{e}	4.25^{e}	0.285^{e}
Glomus mosseae (G.m)	123.8^{b}	75.2^{b}	3.26^{b}	4.28^{b}	0.380^{b}
Bacillus coagulans (B.c)	118.2^{c}	70.4^{d}	3.21^{c}	4.26^{d}	0.286^{d}
Trichoderma viride (T.v)	110.6^{d}	69.2^{d}	3.16^{d}	4.32^{c}	0.285^{d}
G.m + B.c	124.2^{b}	75.6^{b}	3.28^{b}	4.36^{b}	0.382^{b}
G.m + T.v	112.4^{d}	73.2^{c}	3.24^{c}	4.21^{d}	0.365^{c}
B.c + T.v	110.5^{d}	70.6^{d}	3.18^{d}	4.23^{d}	0.314^{d}
G.m + B.c + T.v	129.8^{a}	81.5^{a}	3.62^{a}	5.08^{a}	0.454^{a}

Note: 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

From this study, it can be concluded that the "microbial consortium" consisting of *G. mosseae*, *B. coagulans* and *T. viride* seems to be best suited for *B. malabarica*. The results of these experiments clearly indicate that inoculating *G. mosseae* along with plant growth promoting rhizosphere microorganisms encourages the ability of *G. mosseae* and enhances the growth, biomass, nutrients, and content of secondary metabolites of *B. malabarica*.

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