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EFFECT OF NATIVE DOMINANT AM FUNGUS AND PGPRs ON GROWTH AND BIOCHEMICAL CHARACTERISTICS OF MEDICINALLY IMPORTANT *INDIGOFERA ASPALATHOIDES* VAHL. ex. DC

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ABSTRACT

The effect of native dominant AM fungus *Glomus aggregatum* isolated from the rhizosphere soil of *Indigofera aspalathoides Vahl. Ex DC* and plant growth promoting rhizo-microorganisms (PGPRs) viz., *Bacillus coagulans* and *Trichoderma viride* on the growth and biochemical parameters of the medicinally important *I. aspalathoides* was studied in pot culture. Infection percentage and number of spores in the rhizosphere soil were higher in plants treated with triple inoculation (*Glomus aggregatum + Bacillus coagulans + Trichoderma viride*). Dry matter content of *I. aspalathoides* was high in dual inoculation (*G. aggregatum + B. coagulans*) whereas plant height, protein and amino acid contents were higher in triple inoculation. The macro- and micronutrients of the test plant except manganese were also higher in *G. aggregatum* and PGPRs treated plants than the uninoculated control. Total chlorophyll, carotenoids, lipid and phenol contents were higher in triple inoculation of *I. aspalathoides* than the other treatments. The inoculation of efficient AM fungus in combination with the two PGPRs had positively influenced the growth and biochemical constituents of *I. aspalathoides* whereas *B. coagulans* and *T. viride* as individual inoculations did not show significant effect on the test plants.

Keywords: *Indigofera aspalathoides, Glomus aggregatum*, PGPRs, Biomass, Biochemical constituents

INTRODUCTION

Pharmaceutical companies depend largely on whole plants or their specific parts for the extraction of secondary metabolites, particularly alkaloids, flavonoids, steroids and terpenoids (Agarwal and Paridhavi, 2007). This has motivated farmers to take up cultivation of medicinal herbs in a more systemic manner. With the increasing demand for phytochemicals, the commercial medicinal plant growers started using chemical fertilizers to boost the yield of the plants. Of late, the traditional medical practitioners and growers realized the rising toxicity in plant based medicines due to manuring with chemical fertilizers (Anuradha *et al.*, 2001). In recent years, use of artificially produced inoculums of mycorrhizal fungi and other PGPRs for field, horticulture and medicinal plants have increased due to their multifarious role in plant growth and yield and resistance to climatic and edaphic stresses, pathogens and pests (Raman and Mahadevan, 1996). AM fungi improve plant growth in different ways like increased phosphorus uptake (Schweiger *et al.*, 2007), increase in biomass of plants (Javot *et al.*, 2007), synergistic interaction with beneficial microbes such as phosphate solubilizers (Glick, 1995) etc.

I. aspalathoides Vahl ex. DC. belongs to the family Leguminosae and subfamily Papilionaceae (Fabaceae) and is exploited extensively in traditional medicinal preparations. The plant is said to be indigenous to the southern India, especially southern Tamil Nadu and Sri Lanka. The decoction of leaves, flowers and tender shoots are used to treat leprosy and malignant tumors (Mathew, 1995). The whole plant, rubbed with butter, is applied to reduce oedematous tumors. The leaves are applied to abscesses; and oil obtained from the root is used to anoint the head in erysipelas. The plant exhibits antimycobacterial properties.

In this context, the present investigation was carried out to assess the efficiency of native dominant AM fungus *Glomus aggregatum* isolated from the rhizosphere soil of *I. aspalathoides* and PGPRs such as *Bacillu. coagulans* and *Trichoderma viride* on the growth and various biochemical parameters of *I. aspalathoides* plants raised in pot culture.

MATERIALS AND METHODS

Selection of planting material

Seeds of *I. aspalathoides* were immersed in 5 % sodium chloride, the floating seeds were discarded and viable seeds were used for further sowing in pots of size 22 cm x 20 cm filled with sand and soil in the ratio 1:3.

AM spore isolation and mass multiplication

The spores of native dominant *G. aggregatum* were collected from the rhizosphere soil of *I. aspalathoides* plants from three localities of Kanyakumari district, Tamil Nadu viz., Marunduvalmalai hills, Munchirai and Veli hills by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The spores were mass multiplied using funnel culture technique and the root based inoculum was used for further treatment (Selvaraj and Subramanian, 1990).

PGPRs

Authenticated cultures of PGPRs namely *Bacillus coagulans* (MTCC 2499) and *Trichoderma viride* (MTCC 793), used in the present study were procured from Agriculture College and Research Institute, TNAU, Tiruchirapalli. The bacterial and fungal cultures were maintained in nutrient agar and potato dextrose agar medium respectively for further use (Mahadevan and Sridhar, 1996).

AM fungus and PGPRs inoculation

Seed pelleting with the PGPRs, *B. coagulans* and *T. viride*, was done before sowing (Subba Rao, 1999). The seeds were spread on a polythene sheet and the liquid based inoculums of both the PGPRs were sprinkled over the seeds. The inoculum coated seeds were air dried before sowing. The mass multiplied soil and root based inoculum of AM fungus was applied 3 cm below the pot culture soil as a thin layer at the rate of 5.0 g/pot (Kumutha *et al.*, 2006).

Experimental set up

The experiment was carried out in random block design with five replicates. Eight treatments consisting of various combinations of AM fungus and PGPRs and uninoculated control were maintained in pot culture. Soil in control pots were sterilised (T_0) and they did not receive any biofertiliser inoculation. Plants in T_1 experimental pots were inoculated with *G. aggregatum*, T_2 inoculated with *T. viride* and T_3 inoculated with *B. coagulans*. T_4 was subjected to dual inoculation with *B. coagulans* and *T. viride*, T_5 received *G. aggregatum* and *B. coagulans* inoculation and T_6 received *G. aggregatum* and *T. viride* inoculation. T_7 was treated with all the three bioinoculants, *G. aggregatum, B. coagulans* and *T. viride.*

Estimation of AM fungal colonization and enumeration of AM spores:

Roots collected from *G. aggregatum* and PGPRS inoculated *I. aspalathoides* were stained with 0.05% Tryphan blue in lactophenol according to Phillips and Hayman (1970). The percentage colonization of cleared and stained root segments were estimated (Krishna and Dart, 1984 and McGonigle *et al.*, 1990).

The AM fungal spores were isolated and counted from 100 g soil using wet sieving and decanting method of Gerdemann and Nicolson (1963). Taxonomic identification of spores to species level was based on spore size, color, ornamentation and wall characteristics (Schenck and Perez, 1990).

Estimation of plant biomass and plant pigments

The dry matter content and height of the plants were determined after 90 days of treatment. The plant pigments, chlorophyll and carotenoids were extracted from leaves using acetone. The total chlorophyll was estimated according to Arnon (1949) and Witham *et al.* (1971) and the total carotenoids were estimated using the method proposed by Goodwin (1954).

Estimation of plant nutrients

The plant macronutrients namely nitrogen, phosphorus and potassium were estimated according to Piper (1950), Jackson (1973) and Jones and Isaac (1969) respectively. The micronutrients present in plant samples were estimated using atomic absorption spectrometry (model-Shmiadzu) in Ayya Nadar Janaki Ammal College according to the method described by Clarson (2002).

Estimation of biochemical constituents

The protein content was estimated according to Lowry *et al.* (1951) and the total free amino acids by Troll and Canon (1953). The estimation of total phenols in plants was carried out by the protocol put forth by Farkas and Kiraly (1962). The total lipid was estimated with dry plant sample according to Sato and Murata (1988).

The estimation of alkaloids, flavonoids and saponins were performed according to Harborne (1999), Bohm and Kocipai Abyazan (1994) and Obadoni and Ochuko (2001) respectively.

Statistical analysis:

Pot culture was set up in randomized block design and the data obtained were subjected to one way Analysis of Variance as per the procedure described by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Indigofera aspalathoides plants were removed on 90 days after sowing to find out the effect of native efficient AM fungus, *G. aggregatum* and PGPRs namely *B. coagulans* and *T. viride* on its growth and biochemical parameters. The dominance of *G. aggregatum* in the rhizosphere soil of *I. aspalathoides* over other native AM fungi has been proved in the earlier screening and diversity studies (Sundar, 2009).

The roots of *G. aggregatum* and PGPRs inoculated *I. aspalathoides* showed more than 85% colonization in all the treatments (Table 1). The higher AM spore number was recorded in rhizosphere soils of T_7 (94.08%) followed by T_5 (93.22%). The population of AM spores identified in the root zone soil of *I. aspalathoides* was also higher in all the treatments. The highest AM spore number was recorded in soils of T_7 (604/100 g soil) plants. Our results coincide with the findings of Anusuya and Senthilkumar (2003) in *G. mosseae* and *T. harzianum* inoculated *Dianthus caryophyllus*. This higher colonization indicates the efficacy of inoculated native dominant AM fungi over the AM fungi present in the rhizosphere soil. The hormones and vitamins synthesized by the PGPRs may contribute significantly to mycorrhizal development and subsequent plant growth (Bagyaraj, 2005).

The biomass of *I. aspalathoides* was found to be high in T_7 plants (3.81 g/plant) followed by T_5 (3.72 g/plant). Minimum dry matter content was noted in control plants (Table 2). *I. aspalathoides* in T_1 showed higher plant height than control, T_2 and T_3 plants. Maximum growth (30.19 cm/plant) was recorded in T_7 plants. This increase in plant biomass and height may be due to the synergistic relation between the bioinoculants. Dual inoculation with *G. mosseae* plus *T. harzianum* was found to increase the growth, biomass and alkaloid content of *Andrographis paniculata* (Arpana, 2000). Earanna *et al.* (2003) also recorded maximum plant height and dry matter production in *Phyllanthus amarus* with the inoculation of *G. mosseae* with either *B. coagulans* or *T. harzianum*. Dual inoculation of *G. geosporum* with either *B. coagulans* or *T. harzianum* had significantly increased the growth parameters in *Gloriosa superba* (Elango, 2004). Gupta *et al.* (2006) who studied the growth and development of maize with reference to AM fungi found positive correlation between increase in height and AM inoculation.

The total chlorophyll and carotenoid contents were significantly higher ($P < 0.05$) in AM fungal treatments alone and in combination with PGPRs than the uninoculated plants (Table 2). They were high in *G. aggregatum* + *B. coagulans* inoculated plants followed by the other AM fungal treatments. This increase might be due to increase in the cytokinin content, which prevents chlorophyll degradation and increases ion transport (Allen, 1981). Similar results also have been obtained in *Phyllanthus amarus* by Earanna *et al.* (2003), in *Gloriosa superba* by Elango (2004) and in *Bombyx mori* by Kumutha *et al.* (2006).

G. aggregatum inoculation of *I. aspalathoides* had shown increased levels of plant macronutrients (Table 3). Maximum nitrogen content was observed in T_7 (101.54 mg/plant) followed by T_5 (99.68 mg/plant). Significant difference ($P < 0.05$) over control was observed in all the *G. aggregatum* treatments and also in T_3 and T_4. This upholds the observation made earlier in *Coleus aromaticus* (Earanna *et al.*, 2001) and in *Phyllanthus amarus* (Earanna *et al.*, 2003).

The phosphorus and potassium contents were high in *G. aggregatum* treatment co-inoculated with *B. coagulans* and *T. viride*. All the *G. aggregatum* treatments were determined to be better than the control and the PGPRs alone treated *I. aspalathoides* plants (Table 3). The P and K contents were found to be low in T_0 plants (Table 3). The cause for the increased uptake of nutrients may be, under appropriate conditions mycorrhizal plants are credited with 3-5 times higher rate of phosphorus uptake per unit root length than non-mycorrhizal plants of the same dependent species (Bolan, 1991; Tinker *et al.*, 1992 and Schachtman *et al.*, 1998). Further, *B. coagulans* is known to solubilize unavailable forms of phosphorus and convert them to available form thus resulting in better phosphorus uptake by AM fungi.

The micronutrients namely copper, iron and zinc analysed in the *I. aspalathoides* were also found to be positively influenced by mycorrhizal inoculation. Copper and zinc contents were high (19.42 ppm/plant and 24.56 ppm/plant) in triple inoculation of *I. aspalathoides* with *G. aggregatum*, *B. coagulans* and *T. viride.* All the treatments registered significantly higher ($P < 0.05$) copper and zinc contents except the uninoculated (T_0), T_2 and T_3 plants (Table 3). The iron content was high in T_5 (155.28 ppm/plant). Enhanced uptake of Zn, Cu and Fe nutrients by inoculation with AM fungi has also been reported by several workers (Murugan, 2002; Hemalatha, 2002 and Rajeshkumar, 2002).

There was only a marginal difference in manganese content between the various treatments, and the difference was insignificant ($P > 0.05$) between the various treatments with *G. aggregatum* and PGPRs and also with T_0 plants (Table 3). Manganese uptake and its concentration in plants are sometimes not affected and more often are lower in mycorrhizal plants (Lambert and Weidensaul, 1991). The reason for decrease in Mn concentration is most likely an indirect effect caused by AM induced changes in rhizosphere microorganisms particularly Mn reducers (Kothari *et al.*, 1991).

Table 1. Effect of *G. aggregatum* and PGPRs on root colonization and spore number in the roots and rhizosphere soil of *I. aspalathoides.*

Treatments	Percentage colonization	AM fungal spore number/100 g soil
Glomus aggregatum	89.16	520
G. aggregatum + *B. coagulans*	93.22	592
G. aggregatum + *T. viride*	90.35	558
G. aggregatum + *B. coagulans* + *T. viride*	94.08	604
SEM	1.36	14.82
CD at 5% level	2.79	29.86

Each value is a mean of five replicates

Table 2. Effect of *G. aggregatum* and PGPRs on the plant biomass, plant height, total chlorophyll and carotenoid contents of *I. aspalathoides*

Treatments	Dry weight (g)	Plant height (cm)	Total chlorophyll (mg / g)	Carotene (mg / g)
Control	2.68	19.70	0.501	0.501
Glomus aggregatum	3.25*	24.18*	0.678*	0.8*
Trichoderma viride	2.83	21.22	0.591	0.672
Bacillus coagulans	2.88*	21.90*	0.624	0.691*
B. coagulans + *T. viride*	2.91*	22.30*	0.644*	0.744*
G. aggregatum + *B. coagulans*	3.72*	29.06*	0.771*	0.86*
G. aggregatum + *T. viride*	3.36*	25.21*	0.715*	0.81*
G. aggregatum + *B. coagulans* + *T. viride*	3.81*	30.19*	0.752*	0.857*
SE	0.09	0.87	0.061	0.096
CD at 5% level	0.17	1.61	0.128	0.184

Each value is a mean of 5 replicates; *, indicates significance over control.

Table 3. Macro- and micronutrient contents of *I. aspalathoides* influenced by *G. aggregatum* and PGPRs.

Treatments	Plant macronutrients (mg/plant)			Plant micronutrients (ppm/plant)			
	N	P	K	Cu	Fe	Mn	Zn
Control	70.32	40.34	51.3	10.04	120.26	34.34	15.24
Glomus aggregatum	93.12*	61.24*	59.78*	16.78*	143.46*	36.92	21.36*
Trichoderma viride	79.48	47.2	55.67	10.95	130.14	35.36	17.01
Bacillus coagulans	85.06*	59.06*	58.01	12.56	138.23	36.89	19.18
B. coagulans + *T. viride*	86.14*	60.5*	58.96	13.78*	140.10	37.03	20.6*
G. aggregatum + *B. coagulans*	99.68*	70.1*	63.2*	18.63*	155.28*	38.94	23.89*
G. aggregatum + *T. viride*	93.77*	62.86*	60.84*	17.47*	147.32*	38.23	22.08*
G. aggregatum + *B. coagulans* + *T. viride*	101.54*	68.78*	64.56*	19.42*	153.16*	39.07	24.56*
SEM	5.82	3.02	4.01	1.50	9.94	2.48	2.41
CD at 5% level	11.64	6.04	8.06	2.98	19.89	4.96	4.7

Each value is a mean of 5 replicates, *Values indicate significance over control.

Table 4. Effect of *G. aggregatum* and PGPRs on the total protein, total free amino acids, total lipid and total phenol content of *I. aspalathoides.*

Treatments	Total protein (mg/g) fresh weight	Total free amino acids (mg/g) fresh weight	Total lipid (µg/g) dry plant	Total phenols (µg / g) fresh plant
Control	0.70	0.49	91.50	107.45
Glomus aggregatum	0.94*	0.60*	108.20*	130.60*
Trichoderma viride	0.81	0.51	96.60	119.20*
Bacillus coagulans	0.89	0.54	98.40	112.30
B. coagulans + *T. viride*	0.90*	0.55*	99.30	121.40*
G. aggregatum + *B. coagulans*	1.05*	0.70*	120.60*	123.10*
G. aggregatum + *T. viride*	0.99*	0.62*	110.70*	140.70*
G. aggregatum + *B. coagulans* + *T. viride*	1.02*	0.67*	125.50*	145.00*
SE	0.096	0.027	4.30	4.54
CD at 5% level	0.191	0.054	8.60	9.11

Each value is a mean of 5 replicates, *Values indicate significance over control.

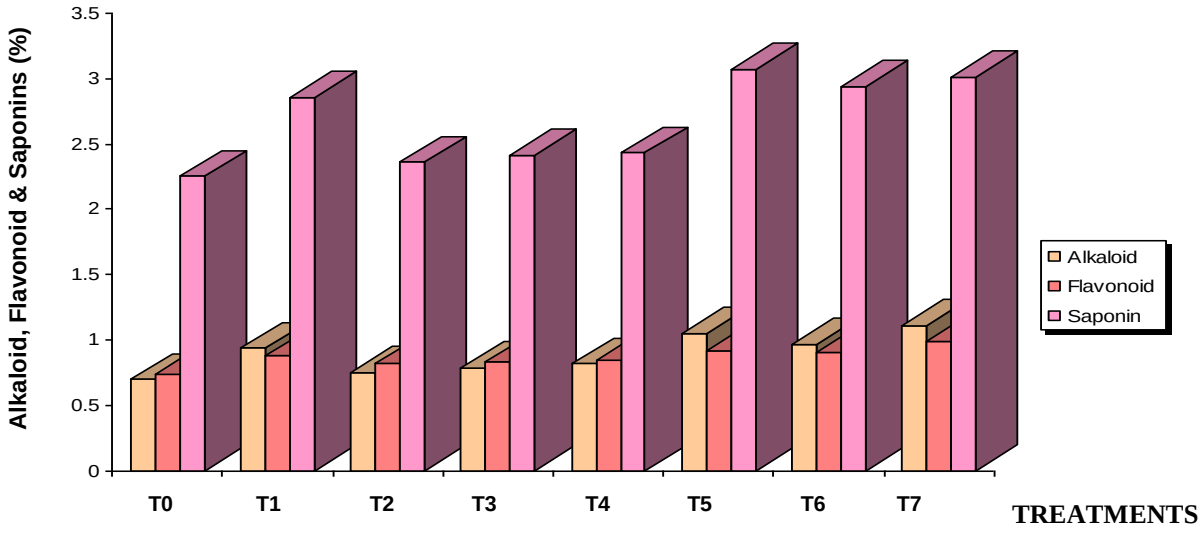

Fig.1. Quantitative analysis of phytochemical constituents of *G. aggregatum* and PGPRs treated *I. aspalathoides*

Maximum protein (1.47 mg/g plant) and amino acid (0.7 mg/g fresh plant) were observed in T_5 inoculated plants (Table 4). The control plants showed minimum protein and amino acid contents. Krishna and Bagyaraj (1983) found that protein and amino acid levels increased in *G. fasciculatum* inoculated *Arachis hypogeae* roots. A three fold higher protein content was found in mycorrhizal than in non-mycorrhizal red clover roots and in tobacco and onion (Dumas *et al.*, 1989).

Total lipid and total phenol contents (Table 4) showed maximum levels in *I. aspalathoides* inoculated with *G. aggregatum*, *B. coagulans* and *T. viride* (125.50 µg/g dry plant and 145.00 µg/g fresh plant respectively). The plants treated with *G. aggregatum* (either alone or in combination with *B. coagulans* and *T. viride*) showed significantly higher ($P < 0.05$) lipid and phenol contents than the other two PGPR treatments and control plants (Table 4). The increase in total phenols in AM inoculated plants could be attributed to the triggering of pathways of aromatic biosynthesis (Mahadevan, 1991). Hemalatha (2002) also reported an increase in total phenols in the leaves and roots of *Ocimum basilicum* and *Plectranthus amboinicus* in dually inoculated plants. The increase of total lipids in mycorrhizal plants might be due to the increase in neutral lipids, principally triacylglycerols that are reportedly supplied to the plant roots by the fungal hyphae (Gasper *et al.*, 1997). Elango (2004) reported significant increase in total lipid content in mycorrhizal Glory lily plants.

The alkaloid and flavonoid contents of *I. aspalathoides* were high in triple inoculation of *G. aggregatum* with *B. coagulans* and *T. viride* (1.11% and 0.99% respectively). Saponin content was higher in T_5 inoculation (3.07%). The single or dual inoculation of *G. aggregatum* with *B. coagulans* or *T. viride* had showed effective improvement of alkaloid, flavonoid and saponin contents of the test plants (Fig. 1). The increase might be attributed to the production of hormones (Allen *et al.*, 1980 and Azcon *et al.*, 1978) and the uptake of phosphorus and other nutrients by AM fungus (Sreenivasa and Bagyaraj, 1988 and Krishna *et al.*, 1998). Significant difference in alkaloid, flavonoid and saponin contents were not observed between *B. coagulans* and *T. viride* treated plants ($P > 0.05$). However, all the AM fungal treatments were superior to the other PGPR treatments, with significant response being observed in all AM fungal treatments over the control plants (Fig. 1). Similar results were obtained by Krishna *et al.* (1998) on *G. fasciculatum* and *B. megaterium* treated essential oil bearing grass, *Citronella java.* Elango (2004) also reported higher levels of flavonoids in the roots and rhizomes of *Gloriosa superba* plants treated with *G. geosporum*, *B. coagulans* and *T. harzianum.*

In the present investigation, *I. aspalathoides* inoculated with the native dominant AM fungi *G. aggregatum* along with *B. coagulans* and *T. viride* showed significantly higher plant growth, dry matter content, pigments like chlorophyll and carotenoids, biochemical constituents such as plant macro- and micronutrients, protein, amino acids, phenols, lipids and the content of secondary metabolites than the uninoculated plants and the PGPRs *B. coagulans* and *T. viride* alone inoculated plants. AM fungi along with other plant protecting fungi such as *T. viride* are important for the establishment of medicinally important plants *Stevia rebaudiana* (Sharma *et al.*, 2007). In our

study, dual inoculation of *G. aggregatum* and *B. coagulans* and the triple inoculation recorded maximum dry matter and biochemical constituents with no significant difference between the two treatments.

Productive and sustainable agriculture insists on agronomic practices such as manuring at regular intervals, removal of weeds and management of pests and diseases. Root colonizing microbes are known to exert beneficial effects on plant growth and resistance to drought and pathogens via direct or indirect mechanisms (Antoun and Prevost, 2005). As *I. aspalathoides* plants which are indigenous to this region, have extensive medicinal properties, application of chemical fertilizers is normally not recommended, since it may have some implications on the secondary metabolite contents of the plants. In this context, application of these effective microorganisms namely *G. aggregatum, B. coagulans* and *T. viride* will be safer and will also boost the growth and productivity of the plants which in turn will have better realization for the growers in terms of sale of plant biomass to the drug manufacturers.

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