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Effect of arbuscular mycorrhizal (AM) fungus and plant growth promoting rhizomicroorganisms (PGPR's) on medicinal plant *Solanum viarum seedlings*

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Abstract

A green house nursery study was conducted to assess the interaction between arbuscular mycorrhizal (AM) fungus, *Glomus aggregatum* and some plant growth promoting rhizomicrooganisms (PGPR's), *Bacillus coagulans* and *Trichoderma harzianum,* in soil and their consequent effect on growth, nutrition and content of secondary metabolities of *Solanum viarum* seedlings. Triple inoculation of *G. aggregatum+B. coagulans+T. harzainum* with *Solanum viarum* in a green house nursery study resulted in maximum plant biomass (plant height 105 cm and plant dry weight 12.17 g), P, Fe, Zn, Cu and Mn and secondary metabolities [total phenols (129.6 μg g^{-1} f.wt.), orthodihydroxy phenols (90.6 μg g^{-1} f.wt.), flavonoids (3.94 μg g^{-1} f.wt.), alkaloids (5.05 μg g^{-1} f.wt.), saponins (5.05 μg g^{-1} f.wt.) and tannins (0.324 μg g^{-1} f.wt.)] of *S. viarum* seedlings. The mycorrhizal root colonization and spore numbers in the root zone soil of the inoculated plants increased. The enzyme activity namely acid phosphatase (53.44 μg PNP g^{-1} soil), alkaline phosphatase (40.95 μg PNP g^{-1} soil) and dehydrogenase (475.5 μg PNP g^{-1} soil) and total population of *B. coagulans* (12.5×10^4 g^{-1}) and *T. harzianum* (12.4×10^4 g^{-1}), in the root zone soil was found high in the triple inoculation with *G. aggregatum+B. coagulans+T. harzianum* that proved to be the best microbial consortium.

Key words

Solanum viarum, *Glomus aggregatum*, *Bacillus coagulans, Trichoderma harzianum,* Secondary metabolites

Introduction

Medicinal plants are nature's best gift to cure a number of diseases of men and animals. *Solanum viarum* Dunal is an important medicinal plant belonging to the family Solanaceae and commonly called as Medicinal Solanum or Marunthu Kathiri or Soda apple (Nee, 1999). This plant concerns its cultivation as the richest source of solasodine, a nitrogenous analogue of diosgenin. The gelatinous layer surrounding the seeds and leaves contains this glycoalkaloid (Saini, 1966). Solasodine is used as the important starting material for the synthesis of cortisone and other steroid drugs. These drugs have been shown to be effective in cancer treatment, domestic contraceptive formulations, treatment of patients with Addison's disease, rheumatic arthritis, and as anabolic agents. The extracts also possess some nematicidal and bactericidal properties (Chandra and Srivastava, 1978). Arbuscular mycorrhizal fungi are a unique group of soil fungi that form symbiotic association with the higher plants and facilitate the uptake of diffusion limited plant nutrition, such as P, Zn, Cu, Fe and Mn (Tinker, 1984). The role of AM fungi and PGPR's in improving crop plants growth is well documented (Bhowmik and Singh, 2004). These fungi show a preferential colonization to the hosts, and thereby, the extent to which a host is benefited depends on the fungal species involved in the symbiosis (Smith and Read, 1997). Mycorrhizal fungi interact with a wide range of other soil organisms in the root or in the rhizosphere of the soil. Some form a symbiotic association and in turn modify the host physiology (Fitter and Garbaye, 1994). Certain plant growth promoting rhizomicroorganisms (PGPR's) have been reported to enhance the activity of mycorrhizal fungi and consequently plant growth (Jayanthi *et al.*, 2003; Sumana *et al.*, 2003). 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. However, the information available on the use of these beneficial microorganisms in medicinal plants is meagre. Hence the present investigation was aimed to study the effect of AM fungus, *Glomus aggregatum* and PGPR's, *B. coagulans* and *T. harzianum*, singly and in combination on the growth, biomass, nutrient and secondary metabolities of *S.viarum* raised under green house condition.

Materials and Methods

The potting mixture used in the study was unsterilized sand: soil: FYM (1:1:0.25 by volume). The potting mixture had a pH of 7.4 and contained 2.7 ppm available phosphates (NH_4 F+HCl extractable). Polyvinyl chloride pots of 18 cm diameter were filled with sterile brown sandy loam soil (3 kg pot^{-1}). AM fungi were maintained in onion (*Allium cepa* L.) as the host plant was inoculated to the raised nursery beds measuring 1 x 2 m at the rate of 1.5 kg m^{-2}. Thirty day old *S. viarum* seedlings raised in sterilized nursery soil beds were transplanted to the pots. One seedling was maintained per pot. Sixty gram of dry soil inoculum containing 400-500 spores /50g soil was mixed in the top of 6 cm of the soil of each treatment pot. *B .coagulans* was multiplied on nutrient broth for 3 days at 30°C under a stationary condition. *T. harzianum* was grown on potato dextrose broth for 7 days. The microbial cultures were separately mixed in a sterile lignite powder and their populations were determined by serial dilution plate method. *B. coagulans* inoculum containing 2.8 $x10^8$ cfu g^{-1} and *T. harzianum* inoculum containing 3.4$x10^8$ cfu g^{-1} were added as per the treatment allocation. Control plants received 6g of soil containing non mycorrhizal root pieces of onion. The amount of AM inoculum for each treatment was so adjusted that equal quantity of soil inoculum could be added to each pot. Pots were maintained under green house condition and watered regularly. The experiment was laid out in randomized complete block design with four replications. The treatments were T_1 Control (without AM fungus and PGPR's) T_2 *Glomus aggregatum (Ga)* alone T_3 *Bacillus coagulans (Bc)* alone T_4 *Trichoderma harzianum (Th)* alone T_5 Ga + Bc T_6 Ga + Th T_7 Bc + Th T_8 Ga + Bc + Th

Plant growth parameters, *viz*. plant height (cm) and plant dry weight (g) were recorded at 90 days after transplanting (DAT). Shoot and root biomass was determined after drying the plant samples to constant weight at 60°C in a hot air oven. Nitrogen (N), phosphorous (P) and potassium (K) content of shoot and root was determined by micro-Kjeldhal, vanadomolybdate phosphoric yellow colour and flame photometric methods respectively (Jackson, 1973). An atomic absorption spectrophotometer was employed to estimate Zn, Cu, Mn and Fe content of the plant leaf and root samples, using respective hollow cathode lamps. Acid phosphatase and alkaline phosphatase and dehydrogenase activities were estimated from the root zone soil, as per the procedure given by Tabatabai (1982). The population of *B. coagulans and T. harzianum* in the root zone soil was estimated by the dilution plate method using nutrient agar and potato dextrose agar medium respectively. Soil samples (100 g) were collected from each pot and subjected to wet-sieving and decantation as outlined by Gerdemann and Nicolson (1963) to estimate the population of AM fungal spores. Fine terminal feeder roots were stained using 0.05% trypan blue as described by Phillips and Hayman (1970) and the percent root colonization was estimated by adopting the gridline intersect method (Giovannetti and Mosse, 1980). The content of secondary metabolites, *i.e.* total phenols (Donald *et al*., 2001), ortho dihydroxy phenols (Mahadevan and Sridhar, 1996), flavonoids (Chang *et al*., 2002), alkaloids (Harborne, 1973), saponins and tannins (Zakaria, 1991) were assayed in the plant leaf samples. 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 (Little and Hills, 1978).

Results and Discussion

In general, inoculants appreciably enhanced plant height especially for *G .aggregatum* alone (54.5 cm), *G. aggregatum* + *B. coagulans* (58.2 cm) and *G. aggregatum* +*B. coagulans* + *T. harzianum* treatments (60.5 cm), which was significantly superior over other treatments (Table 1). Single inoculation with *G. aggregatum* or dual inoculation with *G. aggregatum* + *B. coagulans* also significantly enhanced the total dry weight of *S.viarum* plants. Those similarly inoculated with *G. aggregatum* +*B. coagulans* + *T.*

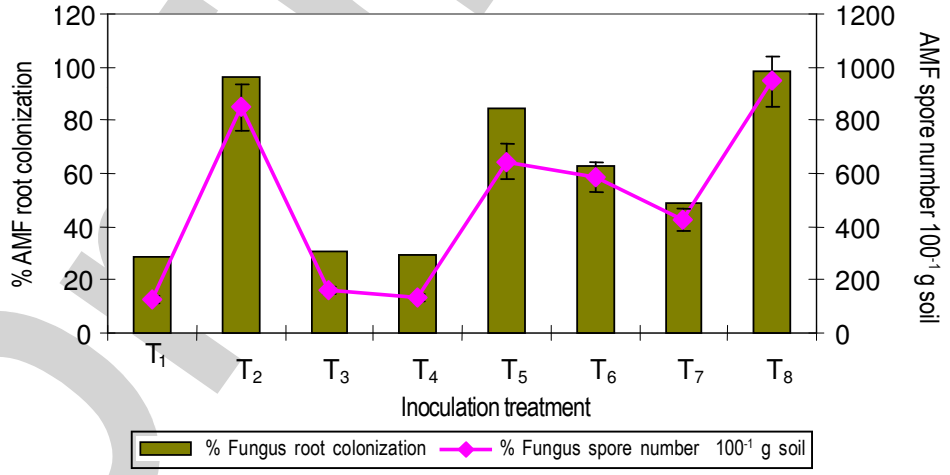

Fig. 1: Percent root colonization in the roots and AMF spore numbers in the root zone of plant *Solanum viarum* T_1 Control ; T_2 *Glomus aggregatum* alone (Ga); T_3 *Bacillus coagulans* alone (Bc); T_4 *Trichoderma harzianum* alone (Th); T_5 Ga + Bc T_6 Ga + Th T_7 Bc + Th T_8 Ga + Bc + Th

Table - 1: Effect of AM fungus and PGPR's on growth and biomass of plant *S. viarum*

Inoculation treatment		Plant height (cm)			Plant dry weight (g plant^{-1})		
		Shoot	Root	Total	Shoot	Root	Total
T_1	Control (without fungus and PGPR's)	38.5±1.8^{d}	24.5±1.4^{d}	63.0±3.2^{d}	4.85±0.6^{d}	3.62±0.6^{d}	8.47 ±1.6^{d}
T_2	*Glomus aggregatum* (Ga) alone	54.5±2.4^{b}	38.2±1.6^{b}	92.7±4.8^{b}	6.42±1.2^{b}	4.82±0.8^{b}	11.24±2.2^{b}
T_3	*Bacillus coagulans* (Bc) alone	42.2±1.8^{c}	25.6±1.4^{d}	67.8±3.4^{d}	4.93±0.8^{d}	3.65±0.6^{d}	8.58±1.6^{d}
T_4	*Trichoderma harzianum* (Th) alone	42.4±1.8^{c}	30.4±1.6^{c}	72.8±4.2^{c}	5.05±1.0^{c}	4.13±0.8^{c}	9.18±1.8^{c}
T_5	Ga + Bc	58.2±2.8^{b}	39.4±1.8^{b}	97.6±4.8^{b}	6.65±1.4^{b}	4.85±0.8^{b}	11.50±2.2^{b}
T_6	Ga + Th	54.4±2.4^{b}	38.2±1.6^{b}	92.6±4.8^{b}	6.43±1.2^{b}	4.82±0.8^{b}	11.25±2.2^{b}
T_7	Bc + Th	48.2±1.8^{c}	38.4±1.6^{b}	86.6±3.8^{c}	5.15±1.0^{c}	4.15±0.8^{c}	9.30±1.8^{c}
T_8	Ga + Bc + Th	60.5±3.2^{a}	44.5±2.2^{a}	105.0±5.2^{a}	7.12±1.8^{a}	5.05±1.0^{a}	12.17±2.4^{a}

Means in each column followed by the same letter are not significantly different (P<0.05) from each other according to DMRT

Table - 2: Effect of AM fungus and PGPR's on enzyme activities and the population of *Bacillus coagulans* and *T. harzianum* from root zone soil of plant *Solanum viarum*

	Treatments	Acid phosphatase (µg PNP g^{-1} soil)	Alkaline phosphatase (µg PNP g^{-1} soil)	Dehydrogenase activity (µg PNP g^{-1} soil)	*Bacillus coagulans* x 10^4 g^{-1} soil	*Trichoderma harzianum* x 10^4 g^{-1} soil
T_1	Control (without AM fungus and PGPR's)	36.80 ± 2.8^{d}	36.50 ± 2.8^{d}	318.5 ± 6.4^{d}	0.3 ± 0.02	0.2 ± 0.01
T_2	*Glomus aggregatum* (Ga) alone	44.71 ± 3.4^{ab}	38.15 ± 3.2^{b}	418.5 ± 7.2^{b}	2.1 ± 0.06	1.8 ± 0.02
T_3	*Bacillus coagulans* (Bc) alone	39.58 ± 3.2^{c}	37.45 ± 3.2^{c}	330.5 ± 6.6^{c}	5.5 ± 0.08	0.5 ± 0.02
T_4	*Trichoderma harzianum* (Th) alone	38.44 ± 2.8^{c}	37.21 ± 3.2^{c}	328.4 ± 6.4^{c}	0.9 ± 0.02	5.4 ± 0.2
T_5	Ga + Bc	46.54 ± 3.6^{ab}	39.46 ± 3.4^{b}	460.5 ± 7.8^{b}	10.5 ± 0.2	1.9 ± 0.02
T_6	Ga + Th	43.14 ± 3.4^{b}	38.75 ± 3.2^{b}	452.8 ± 7.8^{b}	10.2 ± 0.2	8.5 ± 0.2
T_7	Ba + Th	39.67 ± 3.2^{c}	37.14 ± 3.2^{c}	396.2 ± 6.8^{c}	8.5 ± 0.2	9.2 ± 0.4
T_8	Ga + Bc + Th	53.44 ± 3.8^{a}	40.95 ± 3.4^{a}	475.5 ± 8.8^{a}	12.5 ± 0.8	12.5 ± 0.8

Values superscripted with identical letters within each column do not differ significantly different (P<0.05) from each other according to DMRT

Table - 3: Effect of AM fungus and PGPR's on the content of secondary metabolites in the leaves of plant *S.viarum*

	Inoculation treatment	Total phenols (µg g^{-1} f.wt.)	Orthodihydroxy phenols (µg g^{-1} f.wt.)	Flavonoids (µg g^{-1} f.wt.)	Alkaloids (µg g^{-1} f.wt.)	Saponins (µg g^{-1} f.wt.)	Tannins (µg g^{-1} f.wt.)
T_1	Uninoculated control (without AM fungus and PGPR's)	95.0±4.8^{e}	78.5 ±3.2^{d}	2.84 ±0.4^{d}	4.85 ±0.6^{d}	0.180±0.02^{d}	0.295 ±0.02^{d}
T_2	*Glomus aggregatum* (Ga)alone	125.5±2.4^{b}	88.5 ±3.8^{b}	3.45 ±0.6^{b}	4.96 ±0.8^{b}	0.184±0.04^{b}	0.315 ±0.04^{b}
T_3	*Bacillus coagulans* (BC)alone	118.2 ±2.8^{b}	82.4 ±3.6^{c}	2.95 ±0.4^{c}	4.88 ±0.8^{c}	4.88 ±0.8^{c}	0.306 ±0.02^{c}
T_4	*Trichoderma harzianum* (Th) alone	112.4 ±2.2^{d}	80.2 ±3.2^{c}	2.86 ±0.4^{d}	4.86 ±0.6^{d}	4.86 ±0.6^{d}	0.302 ±0.02^{d}
T_5	Ga+Bc	126.8 ±2.4^{b}	89.2 ±3.8^{a}	3.86 ±0.6^{a}	4.98 ±1.8^{a}	4.98 ±1.8^{a}	0.319 ±0.02^{a}
T_6	Ga+Th	124.3±5.2^{c}	87.4 ±3.8^{b}	3.24 ±0.8^{b}	4.95 ±0.8^{b}	4.95 ±0.8^{b}	0.318 ±0.02^{b}
T_7	Ba+Th	120.2 ±5.2^{e}	86.2 ±3.8^{b}	2.90 ±0.6^{c}	4.90 ±1.0^{c}	4.90 ±1.0^{c}	0.304 ±0.02^{d}
T_8	Ga+Bc+Th	129.6 ±5.2^{a}	90.6 ±4.8^{a}	3.94 ±1.0^{a}	5.05 ±1.0^{a}	5.05 ±1.0^{a}	0.324 ±0.01^{a}

Means in the same column followed by the save superscript do not differ significantly according to duncan's multiple range test (P<0.05)

Table - 4: Influence of AM fungus and PGPR's on nutrient status in the roots and leaves of plant *S. viarum*

	Treatment	P (g plant^{-1})		K (g plant^{-1})		Zn (g plant^{-1})		Cu (g plant^{-1})		Mn (g plant^{-1})		Fe (g plant^{-1})	
		Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves
T_1	Control (without AMF and PGPR's)	0.65	0.204	0.048	0.282	0.192	0.216	18.5	29.0	0.008	0.016	0.156	0.252
T_2	*Glomus aggregatum* (Ga) alone	0.072	0.244	0.054	0.302	0.210	0.312	19.2	33.4	0.010	0.026	0.162	0.258
T_3	*Bacillus coagulans* (Bc) alone	0.069	0.214	0.052	0.288	0.208	0.248	18.8	32.2	0.009	0.021	0.158	0.254
T_4	*Trichoderma harzianum* alone (Th)	0.068	0.212	0.049	0.284	0.206	0.236	18.6	32.0	0.009	0.020	0.154	0.252
T_5	Ga + Bc	0.074	0.256	0.054	0.306	0.214	0.316	19.6	33.8	0.012	0.028	0.164	0.260
T_6	Ga + Th	0.073	0.248	0.053	0.304	0.210	0.312	19.4	33.2	0.011	0.026	0.162	0.258
T_7	Bc + Th	0.071	0.239	0.051	0.301	0.208	0.311	19.2	33.4	0.010	0.024	0.160	0.256
T_8	Ga + Bc + Th	0.098	0.262	0.056	0.312	0.216	0.324	19.8	34.2	0.013	0.029	0.164	0.264

Values are mean of three replicates

harzianum showed maximum shoot and root dry weight (12.17g plant^{-1}), the lowest biomass being recorded in control (Table 1). Maximum percent root colonization were recorded in the plants inoculated with *G. aggregatum +B. coagulans + T .harzianum* (Fig. 1), similarly, spore number was maximum when the plants were inoculated with *G. aggregatum + B. coagulans and G. aggregatum +B. coagulans + T .harzianum* , and the least being recorded in uninoculated control plants (Fig. 1). Phosphorus, potassium, zinc, copper, manganese and iron content of leaf samples were maximum in the plants treated with *G. aggregatum B. coagulans + T. harzianum* in contrast with the plants inoculated with *G.aggregatum* alone (Table 4). The acid phosphatase alkaline phosphatase and dehydrogenase activities in the root zone soil of all the inoculated seedlings was significantly higher than uninoculated control (Table 2). Secondary metabolites (total phenols, ortho dihydroxy phenols, flavanoids, alkaloids, saponins and tannins) in the leaf and fruit were maximum in plants treated with *G. aggregatum +B. coagulans + T. harzianum* (129.8, 81.5, 3.62, 5.08, 0.285 and 0.454 µg g^{-1} respectively), followed by the plants dually inoculated with *G . aggregatum +B . coagulans* (Table 3).

Improved response in plant growth with AM and PGPR's obtained in the present investigation supports Earanna *et al.* (2002) for periwinkle, Selvaraj *et al*., (2008) for *Begonia melabonica* and of Sivakumar *et al.* (2002) for *Pelargonium graveloleus* inoculated with *Glomus spp*. and some PGPR's . Single inoculation with *G. aggregatum* or dual inoculation with *G. aggregatum + B. coagulans* also significantly enhanced the total dry weight of *S. viarum* plants. Those similarly inoculated with *G. aggregatum +B. coagulans + T. harzianum* showed maximum shoot and root dry weight. This may be due to synergistic interaction of the AM fungi and PGPR's in the rhizosphere of the plants (Lakshmipathy *et al.*, 2002; Muthuraju *et al*., 2002; Sivakumar *et al*., 2002).

Maximum percent root colonization were recorded in the plants inoculated with *G. aggregatum +B. coagulans + T. harzianum* synergistic interactions have been reported between the free – living rhizosphere bacteria, P-solubilizing bacterial organisms and mycorrhizal fungi (Earanna *et al*., 2002; Khan *et al*., 2007) with respect to the percent root colonization and spore number.

The phosphorus, potassium, zinu, copper, manganese and iron content were maximum in the plants treated with *G. aggregatum +B. coagulans+T. harzianum* probably due to the enhanced mycorrhizal colonization resulting in efficient uptake (Lakshmipathy *et al*., 2002; Selvaraj *et al*., 2008).

The acid phosphatase alkaline phosphatase and dehydrogenase activities 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 (Table 2). The highest value was recorded in the root zone soil of the plants inoculated with *G. aggregatum +B. coagulans+T. harzianum* (53.44 , 40.95 and 475.5 µg PNP g^{-1} soil respectively), followed by that of the *G. aggregatum +B. coagulans* inoculated plants (46.54 , 39.46 and 460.5 µg PNP g^{-1} soil respectively), enhanced enzyme activities are recorded in the present study similar to that of neem inoculated with AM fungi and PGPR's. The root zone soil of plants inoculated with *G. aggregatum+B. coagulans+T. harzianum* had higher *B. cogulans* population suggesting the stimulatory effect and synergistic activity in MHB, which enhances the activity of *G. aggregatum* by producing organic acids, which serve as a carbon source to the fungus or by

producing hydrolytic enzymes thus enabling the AM fungus to penetrate and ramify in the root system of the host (Sumana *et al*., 2003; Duponnois *et al*., 1991).The root zone soils of plants inoculated with *G. aggregatum +B. coagulans + T. harzianum* had higher *Trichoderma harzianum* population suggesting the antagonistic effect of soil borne disease and stimulating effect of Am symbiont on *T. harzianum* supports earlier works of Selvaraj *et al.* (2008).

The higher secondary metabolites (total phenols, ortho dihydroxy phenols, flavanoids, alkaloids, saponins and tannins) in plants treated with *G.aggregatum+B. coagulans+T. harzianum* or in *G. aggregatum+B. coagulans* is 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 (Selvaraj *et al*., 2008).

From this study, it can be concluded that the "Microbial consortium "consisting of *G. aggregatum+B. coagulans+T. harzianum* seems to be best suited for medicinal plant *S. viarum* growth, biomass, nutrients and content of secondary metabolites.

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