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Effect of Arbuscular Mycorrhizal Fungus And Plant Growth Promoting Rhizomicro-Organisms On Productivity Of Strobilanthes Ciliatus Nees. , An Endemic To Western Ghats, South India.

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ABSTRACT: *A pot culture investigation was conducted to know the influence of inoculation with the Arbuscular Mycorrhizal Fungus Glomus aggregatum and the Plant Growth Promoting Rhizomicroorganisms (PGPR) Trichoderma harzianum and Bacillus coagulans singly and in combination on productivity of Strobilanthes ciliatus. The plant height, number of leaves per plant and plant dry matter were significantly higher in plants inoculated with Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum. The maximum root colonization and spore number were also observed in plants inoculated with Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum.*

KEY WORDS: *Strobilanthes ciliatus, Arbuscular Mycorrhiza, Glomus aggregatum, Bacillus coagulans, Trichoderma harzianum.*

I. INTRODUCTION

The genus Strobilanthes belongs to Acanthaceae is known for its diversified habits, gregarious nature and infrequent but elegant flowering. *Strobilanthes ciliatus* Nees. is one of the endemic species that has got several therapeutic properties. It has a strong aroma and is widely used in Ayurveda as a source of the drug 'Sahacharya'. The entire plant is used as a source of medicine. Roots are useful in the treatment of rheumatalgia, lumbago, sciatica, limping, chest congension, strangury, fever, leucoderma, skin diseases, inflammations, cough, bronchitis, odontalgia and general debility. Leaves and bark are also used oil which is of good medicinal value.
Currently, cultivation of medicinal plants has increased due to their wide range of therapeutic potential to treat a large number of ailments. Here comes the importance of sustainable agriculture, which includes the exclusion of most synthetic pesticides and fertilizers, causing adverse effect on health and fertility of soil. Arbuscular Mycorrhizal 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 (1). Mycorrhizae are found in a wide range of habitats, usually in the roots of angiosperms, gymnosperms and pteridophytes (2). As 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 root colonization of the host roots by the fungus(3). In mutualistic association, the plant gains the benefit s of the mycelium's higher absorptive capacity for water and mineral nutrients, especially phosphorous (P) and other low mobile mineral nutrients (4). Mycorrhizal pants are often more resistant to the effects of drought (5, 6). A M fungi enhancing the activity of beneficial soil organisms, like nitrogen fixers and phosphate solubilizers with consequential beneficial effect on plant growth. So the present study was undertaken to understand the response of *Strobilanthes ciliatus* to the AM fungus *Glomus aggregatum* and the plant growth Promoting Rhizomicroorganisms (PGPR), *Bacillus coagulans* and *Trichoderma harzianum* singly and in combination.

II. MATERIALS AND METHODS

The plants studied were grown in pots in polyhouse conditions. The soil used in this study was collected from an uncultivated field at a depth of 0-30cm and was classified as fine, entisol, isohyperthermic kanhaplusstalfs. The soil p^H was 6.9 and it contained 2.7 ppm available phosphorous (extractable with NH_4F+HCl) and an indigenous AM fungal population of 60 spores/ 50g of soil. Nursery was raised by planting stem cuttings of *S. ciliatus* in polybags (10x15 cm) containing sterilized soil: farm yard manure (1: 1 v/v). Ruakura nutrient solution at 50ml per polybag was applied once in 10 days. After 30 days seedlings were transplanted to polythene bags of size 25x15 cm containing 2kg of unsterilized soil: sand: compost in the ratio of 2: 1: 0.5 (v/v/v).

The AM fungal species used in this study were isolated from the rhizosphere soil of *S. ciliatus* collected from Neriamangalam, the foot hills of Western Ghats, Kerala, India. These AM fungal were isolated by using wet sieving and decanting technique (7). These fungi were 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 (8). *Bacillus coagulans* was grown in nutrient agar medium and *Trichoderma harzianum* in potato dextrose broth. After 3 days of growth of *B. coagulans* and 8 days of growth of *T. harzianum* cultures were used for inoculum along with *Glomus aggregatum* at the time of transplanting. The microbial cultures were separately mixed in sterile lignite powder and their populations were determined by serial dilution plate method. Thirty days old seedlings were transplanted to pots. 60g of dry soil inoculum containing 400-500 spores were mixed in the top (6cm) of the soil in each treatment pots. *B. coagulans* ($2.8x10^8$cfu g^{-1}) and *T. harzianum* ($3.4x10^8$cfu g^{-1}) inocula were added as per the following treatments:

T1: Uninoculated control
T2: Inoculated with *Glomus aggregatum* (Ga)
T3: Inoculated with *B. coagulans* (Ba)
T4: Inoculated with *T. harzianum* (Th)
T5: Inoculated with Ga + Bc
T6: Inoculated with Ga + Th
T7: Inoculated with Bc + Th
T8: Inoculated with Ga + Bc+ Th.

Pots were irrigated twice a week for the first 4 weeks and subsequently at weekly intervals to maintain enough moisture and the plants were raised for 90 days after transplanting. Growth parameters like plant height and number of leaves were recorded. Shoot and root biomass of the test plant were determined after drying the samples at 60^0c to attain a constant weight in a hot air oven. Fresh root samples were stained using 0.05g /100g solution trypan blue (9) and the per cent root colonization was estimated by adapting the gridline intersect method (10) and extrametrical spores in the root zone soil were enumerated by wet sieving and decantation method (7). All the data were subjected to analysis of variance for a completely random design (CRD) with five replicates. The mean values were further separated by DMRT (Duncan's Multiple Range Test) for significant difference $p \leq 0.05$ (11).

III. RESULTS AND DISCUSSION

Arbuscular mycorrhiza plays a key role in increasing nutrient uptake and thereby increases the growth and yield (12). The present study was carried out in order to evaluate the role of an AM fungus and Plant Growth Promoting Rhizomicroorganisms (PGPRs) on productivity through microbial inoculation. All the inoculated treatments significantly increased the plant height as compared to uninoculated plants. Similar results were observed in the medicinal plant Kalmegh(13). Inoculation with *Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum* resulted in higher plant height compared to all other inoculations and control (Table I) is followed by inoculated with *Glomus aggregatum + Bacillus coagulans* and *Glomus aggregatum + Trichoderma harzianum* respectively. The inoculation of *Strobilanthes ciliatus* with *Glomus aggregatum* alone significantly increased the plant height as compared the plants inoculated with *Bacillus coagulans* alone and *Trichoderma harzianum* alone and in combinations (Bc + Th). Number of leaves per plant was also significantly larger in inoculated treatments as compared to the control, highest numbers of leaves was observed in plants inoculated with Ga + Bc + Th. Similar effect was observed in total plant dry matter (Table I). This may be due to increased plant height as well as increased biomass and leaf yield of inoculated test plants; which may be related to the action of native inoculants. This is in conformity with earlier observations in *Coleus aromaticus* [14], in *Phyllanthus amarus* [15] and in *Plantago ovata* [16], such enhanced dry biomass due to inoculations with *Glomus mossae* was well documented in *Andrographis paniculata* [17]. Plant growth promotion by rhizomicroorganisms may be due to the synergistic interaction of AM fungi and PGPR's in the rhizosphere of the plants (18, 19).

Mycorrhizal fungi enhancing the number and activity of beneficial soil organisms like nitrogen fixers and phosphate solubilizers with consequential beneficial effect on plant growth has been reported [20]. In the present study, mycorrhizal root colonization and spore numbers in the root zone soils were significantly more in *Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum* inoculated plants as compared to the control (Table 2). This indicates the efficacy of inoculated AM fungi against native AM fungi present in soil for better colonization.

This supports the well documented fact that inoculation with effective AM fungi enhances mycorrhizal root colonization [21, 22]. The root zone soil of *S. ciliatus* plants inoculated with *Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum* had higher *B. coagulans* number followed by plants treated with *Glomus aggregatum + Bacillus coagulans* and *B. coagulans* alone when compared with control plants (Table 2). This suggests a synergistic activity were in mycorrhizal helper bacteria 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 [23]. The present study clearly highlights the beneficial effect of inoculation with *Glomus aggregatum + Bacillus coagulans + Trichoderma harzianum* on the growth parameters like plant height, number of leaves, plant dry biomass etc. of an important medicinal plant 'Karimkurinji'.

TABLE I*:*
Influence of Glomus aggregatum (Ga) and PGPR's (Bacillus coagulans + Trichoderma harzianum) on the plant growth parameters of Strobilanthes ciliatus at 90 DAT.

Inoculation treatment		*Plant height*		*Number of leaves*	*Plant Dry biomass (g/plant)*		
		Shoot	*Root*		*Shoot*	*Root*	*total*
1	Control (uninoculated)	62.5^{d}	24.2^{d}	34^{d}	16.6^{d}	12.6^{d}	39.2^{d}
2	Glomus aggregatum alone (Ga)	91.2^{b}	52.6^{b}	44^{b}	24.8^{b}	23.2^{b}	48.0^{b}
3	Bacillus coagulans alone (Bc)	68.4^{c}	26.4^{c}	36^{c}	18.4^{c}	13.2^{c}	41.6^{c}
4	Trichoderma harzianum alone (Th)	67.5^{c}	25.6^{c}	34^{d}	16.8^{c}	13.0^{c}	39.8^{d}
5	Ga + Bc	94.2^{b}	56.2^{b}	48^{b}	26.4^{a}	23.8^{a}	50.2^{a}
6	Ga + Th	93.8^{b}	54.4^{b}	44^{b}	25.8^{b}	23.4^{b}	49.2^{b}
7	Bc + Th	68.5^{c}	26.8^{c}	38^{c}	18.5^{c}	13.4^{c}	42.9^{c}
8	Ga+Bc+Th	98.6^{a}	58.2^{a}	52^{a}	27.2^{a}	24.2^{a}	51.4^{a}

Means followed by the same letter with in a column do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test, DAT = Days After Transplanting.

TABLE II:
Influence of Glomus aggregatum (Ga) and PGPR's (Bacillus coagulans + Trichoderma harzianum) on mycorrhizal root colonization, spore numbers and population of B. coagulans and T. harzianum in the root zone soil of Strobilanthes ciliatus at 90 DAT.

Inoculation Treatment		*Mycorrhizal root colonization (%)*	*Total No. of spores/100g of soil*	*Population of B.coagulans (Xx 10^4 c.f.u /g soil)*	*Population of T.harzianum (Xx 10^5 c.f.u /g soil)*
1	Control (uninoculated)	-	-	-	-
2	*Glomus aggregatum* alone (Ga)	92.4^{b}	985^{b}	-	-
3	*Bacillus coagulans alone* (Bc)	-	-	5.0^{c}	-
4	*Trichoderma harzianum* alone (Th)	-	-	-	4.7^{c}
5	Ga+ Bc	93.5^{b}	990^{b}	8.5^{b}	-
6	Ga +Th	86.4^{c}	972^{c}	-	6.8^{b}
7	Bc + Th	-	-	9.2^{b}	7.2^{b}
8	Ga +Bc + Th	98.5^{a}	1085^{a}	10.5^{a}	8.4^{a}

Means followed by the same letter with in a column do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test; c.f.u. = colony forming units; DAT = Days After Transplanting.

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