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IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI, *TRICHODERMA VIRIDE* AND *PSEUDOMONAS FLUORESCENS* ON THE GROWTH ENHANCEMENT OF *VITEX NEGUNDO*

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In the present investigation a pot experiment with sixteen different combinations of *Glomus mosseae* (G), *Acaulospora laevis* (A), *Trichoderma viride* (T) and *Pseudomonas fluorescens* (P) was carried out to assess the interaction between bioinoculants and their consequent effect on the growth and nutrition of *Vitex negundo* a medicinal plant. The result obtained indicated the dependence of *Vitex negundo*, on mycorrhizal symbiosis. The different growth parameters increased significantly after 120 days of inoculation in comparison to control. Among all the growth parameters plant height (85.1±2.50), fresh shoot weight (21.6±0.57), dry shoot weight (16.0±0.51), fresh root weight (24.2±0.69), dry root weight (15.1±0.54), root length (40.2±1.67), leaf area (29.0±0.35), spore count number (78.4±5.55), percent root colonization (92.6±1.48), total chlorophyll content (2.055±0.003), phosphorus contents in roots (0.406±0.003) and shoots (0.243±0.005) were highest in combination of *G. mosseae + A. laevis + T. viride + P. fluorescens*. These results indicate that combined inoculation with two native AMF isolates (*G. mosseae* and *A. laevis*), *T. viride* and *P. fluorescens* significantly increased growth and other parameters. Thus, the improvement in plant growth could be attributed to the enhancement of nutrients via an increase in the absorbing root surface area due to mycorrhizal symbiosis.

Key words- *Vitex negundo, G. mosseae, A. laevis , T. viride , P. fluorescens.*

The demand for the medicinal plants has been increasing rapidly with the consumption of crude drugs. This led to an increase in the cultivation of medicinal plants to maintain a steady supply to support the increasing demand due to a decline in their natural populations. Recently, medicinal plants are being propagated through tissue culture (Shahzad *et al*. 1999) to meet the demand for planting stocks. Arbuscular mycorrhizal (AM) fungi are associated with more than 80% of terrestrial plant families (Trappe 1987). Arbuscular mycorrhiza is the mutualistic symbiotic association between most vascular land plant species and fungi of the phylum Glomeromycota (Smith and Read 2008). These fungi form an important component of the soil microbial mass and regulates several essential biological processes at the plant soil interface. The role of AM fungi in enhancing plant growth as well as improving host resistance to certain plant diseases is well documented (Wright and Milliner 1994). Further, the main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, Zn, Cu, S, Fe, Mg, Ca and Mn and the supply of these nutrients to the host roots (Sundar *et al*. 2010, Javot *et al*. 2007). Mycorrhiza (AM) is present in most medicinal plants, agronomic and vegetable crops. This type is characterized by the presence of arbuscules in the region of the root cortex, vesicles may or may not be present, and they function as reserve organs and also for fungal multiplication. Arbuscular mycorrhiza are recognized as most common type of mycorrhizae with diverse host range (Mathur and Vyas 2007). The AM hyphae growing through soil pore spaces can affect phosphate absorption beyond the depleted zone. The fungal hyphae transport phosphate over large distances into the root cortical cells. The phosphate absorbed by AM fungi from soil solution is accumulated in the vacuoles of the fungus as polyphosphate (Poly-P) granules. The (Poly P) granules in fine branches of arbuscules are broken down by enzymatic activities and releasing phosphorus (inorganic) in the cytoplasm.

A synergistic interaction of AM fungi with

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saprobic fungus *Trichoderma* also resulted in enhanced growth responses in host plants (Calvet *et al.* 1993, Camprubi *et al.* 1995, Rani *et al.* 1999). Similarly, *Pseudomonas fluorescens* enhances biomass yield in *Catharanthus roseus* under water deficit stress (Jaleel *et al.* 2007). Keeping the above in view the present study was to examined the role of AM fungi *G. mosseae, A. laevis* , *T. viride* and *P. fluorescens* in improving the growth of *Vitex negundo.*

MATERIALS AND METHODS

Soil sample collection -To isolate the dominant AM fungi, composite rhizospheric soil samples of *Vitex negundo* were collected. This was done by digging out a small amount of soil close to the plant roots upto 15 to 30 cm depth, which were kept in sterilized polythene bags at 10^0C for further processing.

Isolation of dominant AM spores from soil samples

Isolation of dominant AM spores *G. mosseae* and *A. laevis* was done by 'Wet sieving and decanting technique' by (Gerdemann and Nicolson 1963). With this technique, 50g of soil was soaked in 250 ml water for 24 h. The supernatant was then passed through a gradient of sieves with pore sizes ranging from 150 µm to 45 µm arranged one above the other in an ascending order. Each sieve was then washed in water which was filtered through Whatman No.1 filter paper. This filter paper was then observed under a stereobinocular microscope to observe various kinds of spores, it was mounted on Polyvinyl lactic acid (PVLA) for further studies.

Quantification of AM spores and identification of AM fungi

This was done by the Grid line intersect method (Adholeya and Gaur 1994). Spores were counted under a stereobionocular microscope with a counter. The AM spores (*G. mosseae* and *A. laevis*) were identified with the identification manuals by Walker (1983), Schenck and Perez (1990) and Mukerji (1996),

Mycorrhizal root colonization

Roots were washed from the soil blotted dry to determine mycorrhizal root colonization. Mycorrhizal root colonization was done by the Rapid clearing and staining method (Phillips and Hayman 1970). The percentage of AM root colonization was determined as

Percent root colonization =

$$\frac{\text{Number of root segments colonized}}{\text{Number of root segments studied}} \times 100$$

Mass production of AM spores and mass culture of *Pseudomonas fluorescens*

Dominant AM spores of *Glomus mosseae* and *Acaulospora laevis* were isolated from rhizospheric soil of *Vitex negundo* and mass multiplied with maize as host plant.

Pseudomonas fluorescens (MTCC No. 103) was procured from IMTECH (Institute of Microbial Technology, Chandigarh, India) and multiplied in nutrient broth medium (1.25g peptone, 0.75g beef extract, 1.25g NaCl, 250 ml distilled water) for 24 h for suitable bacteria growth.

Mass production of *Trichoderma viride*

T. viride was isolated from the soil and then further mass produced by using wheat bran, saw dust and distilled water in the ratio of 3:1:4.

Experimental site

The experiment was set up in green house condition, D.A.V. College for Girls Yamunanagar, Haryana, India.

Pot mixture preparation

The soil was sterilized for two consecutive days at 15 lb pressure with 121^0C temperatue, and for two hours. Earthen pots (25×25 cm) were selected. To each pot 10 percent inoculum of AM fungi, *T. viride* and *P. fluorescens* alone and in combinbation were added.

Different treatments used during the present investigation were as follows

1) Control (Without any inoculum)
2) *Trichoderma viride*
3) *Glomus mosseae*

4) *Acaulospora laevis*
5) *Pseudomonas fluorescens*
6) *G. mosseae* + *A.laevis*
7) *G. mosseae* + *T. viride*
8) *Glomus mosseae* + *P. fluorescens*
9) *A.laevis* + *T. viride*
10) *A.laevis* + *P. fluorescens*
11) *Trichoderma viride* + *P. fluorescens*
12) *G. mosseae* + *A.laevis* + *T. viride*
13) *G. mosseae* + *T. viride* + *P. fluorescens*
14) *G. mosseae* + *P. fluorescens* + *A.laevis*
15) *A.laevis* + *T. viride* + *P. fluorescens*
16) *G. mosseae* + *A.laevis* + *T. viride* + *P. fluorescens*

The effect of these bioinoculants were recorded on different parameters of *Vitex negundo* after 120 days of inoculation. In control set no inoculum was added. Single seedling of *Vitex negundo* was planted in each pot and placed in green house conditions. Plants were watered regularly. Hoagland's nutrient solution without phosphorus (100ml/pot) was added to each plant after regular intervals of 15 days. Each treatment was replicated five times.

Analysis of growth parameters

For root and shoot fresh and dry weight, roots and shoots were harvested after 120 days, weighed for their fresh weight and then oven dried for 2 hours at 70^0 C for dry weight. Amount of chlorophyll a, chlorophyll b and total chlorophyll was calculated by method of Arnon (1949). Leaf area by leaf area meter (Systronics 211). Estimation of phosphorus was done by 'Vanadomolybdo phosphoric yellow colour method' (Jackson 1973).

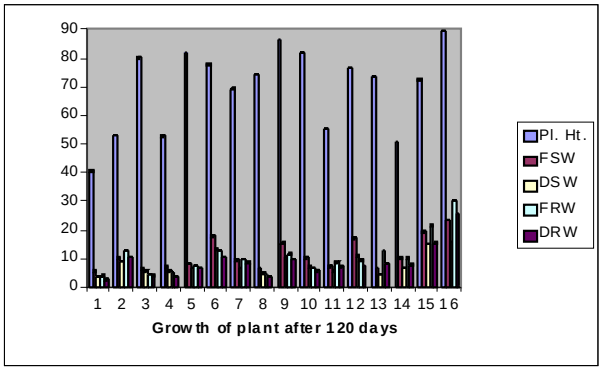
Statistical analysis

All results were analysed using analysis of variance, (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were ranked at P=0.005 level of significance using Duncan's Multiple Range Test of comparison.

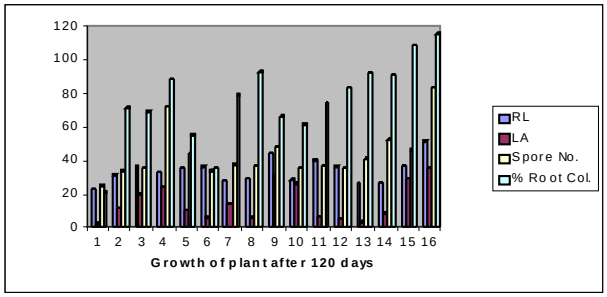
RESULTS

The effect of inoculation with *P. fluorencens, T. viride* and two AM fungi (*G.mosseae* and *A. laevis*) on growth of *Vitex negundo* differed significantly (Table 1.1-1.2), (Plate-1-2, Graph1.1, 1.2, 1.3).

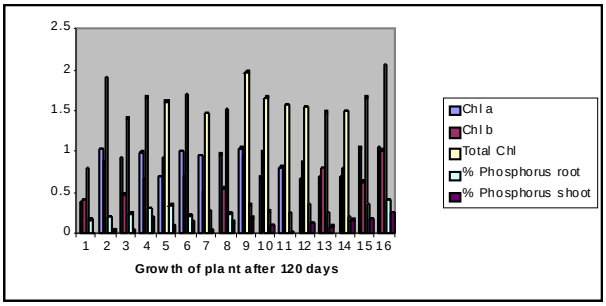
Graph 1.1 *Vitex negundo* after 120 day showing Plant height, Fresh shoot weight, Dry shoot wt., fresh root wt., Dry root wt



Graph1.2. Root length, Leaf area, spore no., % Root colonization.



Graph 1.3. Chlorophyll a, b, Total Chlorophyll, % Phosphorous root, % Phosphorus shoot..



Change in plant height was significant in all the plants and maximum plant height was observed in mixed consortium of *G . mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* treated plants (85.1±2.50) which was four times higher than the uninoculated plants (Table-1). Fresh shoot weight (21.6±0.57), dry shoot weight (16.0±0.51), fresh root weight (24.2±0.69), dry root weight (15.1±0.54) and root length

TABLE- 1: GROWTH RESPONSE OF *VITEX NEGUNDO* AFTER 120 DAYS

S.no.	Treatments	Change in plant height (cm)	Fresh Shoot Weight (gm)	Dry Shoot Weight (gm)	Fresh Root Weight (gm)	Dry Root Weight (gm)	Root Length (cm.)	Leaf Area (sq. cm)	Spore Count	%Root Colonization
1.	Control	19.3±1.55^{ij}	5.5±0.39^{i}	3.1±0.10^{i}	2.9±0.22^{i}	1.2±0.17^{i}	12.3±0.89^{i}	3.14±0.50^{f}	26.4±5.32^{h}	30.3±3.41^{i}
2.	T	31.4±1.93^{hi}	7.1±0.70^{hi}	4.7±0.31^{h}	3.9±0.22^{hi}	2.1±0.17^{h}	14.4±0.56^{h}	4.62±0.29^{h}	33.6±3.21^{g}	67.9± 4.89^{de}
3.	G	53.3±1.48^{gh}	10.0±0.54^{gh}	5.4±0.36^{gh}	5.3±0.26^{h}	2.1±0.25^{h}	18.1±0.51^{g}	6.74±0.59^{fg}	35.4±3.58^{g}	68.7±1.83^{de}
4.	A	49.3±1.61^{h}	9.1±0.44^{h}	5.5±0.44^{gh}	5.1±0.40^{h}	3.2±0.15^{gh}	23.3±0.80^{f}	6.14±0.29^{g}	73.0±3.94^{b}	86.6±3.73^{b}
5.	P	57.7±1.55^{gh}	11.4±1.00^{g}	5.3±0.34^{gh}	6.7±0.52^{h}	3.9±0.80^{gh}	18.4±0.52^{g}	6.16±0.47^{g}	44.6±2.41^{de}	52.9±4.76^{g}
6.	G+A	59.6± 0.80^{gh}	11.7±0.34^{g}	6.5±0.43^{g}	7.2±0.51^{g}	4.4±0.44^{g}	22.3±0.72^{f}	6.98±0.31^{fg}	33.6±2.70^{g}	35.0±2.29^{h}
7.	G+T	72.3±1.27^{d}	14.3±0.80^{f}	6.6±0.38^{fg}	9.0±0.45^{g}	5.6±0.52^{f}	23.0±0.38^{de}	10.4±0.35^{ef}	37.2±2.39^{fg}	79.1±1.22^{c}
8.	G+P	62.6±1.40^{f}	10.1±0.40^{gh}	6.8±0.65^{fg}	8.4±0.34^{g}	4.6±0.44^{g}	31.2±1.30^{c}	8.36±0.46^{f}	36.4±2.61^{fg}	67.6±4.60^{de}
9.	A+T	55.5±1.31^{gh}	9.9±0.48^{gh}	4.4±0.34^{h}	12.2±0.29^{e}	5.9±0.26^{f}	25.8±2.19^{e}	11.6±0.25^{ef}	48.2±5.76^{cd}	66.0±3.65^{e}
10.	A+P	68.1±1.20^{e}	12.4±0.88^{g}	6.8±0.40^{fg}	11.5±0.44^{f}	7.2±0.36^{de}	28.4±0.76^{d}	14.6±0.49^{e}	35.8±1.92^{g}	61.3± 2.98^{f}
11.	T+P	74.7±1.43^{c}	17.4± 0.45^{d}	7.1±0.53^{f}	12.5±0.40^{e}	6.6±0.41^{e}	27.1±0.49^{de}	12.5±0.27^{f}	36.4±3.21^{fg}	71.9±3.57^{d}
12.	G+A+T	73.7±1.74^{cd}	15.2±0.60^{e}	9.0±0.61^{e}	12.5±0.38^{e}	6.8±0.47^{e}	28.8±0.61^{d}	14.2±0.31^{e}	34.6±2.97^{fg}	80.8±3.99^{c}
13.	G+T+P	72.1± 0.85^{d}	17.0±0.68^{d}	10.4±0.43^{d}	14.2±0.76^{d}	7.4±0.45^{d}	36.2±2.77^{b}	19.8±0.31^{d}	41.0±2.24^{ef}	77.6±4.33^{c}
14.	G+P+A	79.3± 0.72^{b}	20.4±0.35^{b}	14.8±0.46^{b}	18.6±0.44^{c}	9.3±0.47^{b}	36.8±2.17^{b}	22.2±0.36^{c}	52.0±4.18^{c}	86.8±2.96^{b}
15.	A+T+P	83.3±1.20^{b}	19.1± 0.54^{c}	12.8±0.52^{c}	21.4± 0.51^{b}	8.1±0.53^{c}	32.8±3.11^{b}	25.8±0.48^{b}	45.0±3.16^{de}	88.9±2.41^{ab}
16.	G+A+T+P	85.1±2.50^{a}	21.6±0.57^{a}	16.0±0.51^{a}	24.2±0.69^{a}	15.1±0.54^{a}	40.2±1.67^{a}	29.0±0.35^{a}	78.4±5.55^{a}	92.6±1.48^{a}
	L.S.D. (P=0.05)	1.8411	0.7614	0.5604	0.5687	0.5425	1.882	0.4967	4.5969	4.3425
	ANOVA (F 15,32)	749.435	315.858	362.042	958.132	312.978	148.451	1983.360	76.151	137.192

Treatments

1) Control (Without any inoculum) 2) *Trichoderma viride* (*T*) 3) *Glomus mosseae* (*G*) 4) *Acaulospora laevis* (*A*) 5) *Pseudomonas fluorescens* (*P*) 6) *G* + *A* 7) *G* + *T* 8) *G* + *P* 9) *A* + *T* 10) *A* + *P* 11) *T* + *P* 12) *G* + *A* + *T* 13) *G* + *T* + *P* 14) *G* + *P* + *A* 15) *A* + *T* + *P* 16) *G*+*A*+*T*+*P*

* Mean of fine replicates, ± = standard deviation mean values followed by deferent alphabet (s) within a column do not differ significantly over one another at p≤ 0.05 led by duncan's Multiple range test.

Initial growth of *Vitex negundo*

Initial growth of *Vitex negundo*

Growth of *Vitex negundo* after 120 days

Growth of *Vitex negundo* after 120 days

Plate-1 :

1) Control (Without any inoculum) 2) *Trichoderma viride* 3) *Glomus mosseae* 4) *Acaulospora laevis* 5) *Pseudomonas fluorescens* 6) *G* + *A* 7) *G* + *T. viride* 8) *G* + *P. fluorescens*

Plate-2:

9) *A* + *T. viride* 10) *A* + *P. fluorescens* 11) *Trichoderma viride* + *P. fluorescens* 12) *G* + *A* + *T. viride* 13) *G* + *T. viride* + *P. fluorescens* 14) *G* + *P. fluorescens* + *A* 15) *A* + *T. viride* + *P. fluorescens* 16) *G* + *A* + *T. viride* + *P. fluorescens*

TABLE 2 : CHLOROPHYLL CONTENT AND PHOSPHORUS CONTENT OF *VITEX NEGUNDO* AFTER 120 DAYS

S.no.	Treatments	Chlorophyll Content (mg/ ml)			Percent Phosphorus	
		Chl a	Chl b	Total Chl	Root	Shoot
1.	Control	0.383±0.002^{i}*	0.412±0.002^{i}	0.792±0.002^{i}	0.163±0.002^{i}	0.006±0.001^{i}
2.	T	1.027±0.002^{c}	0.854±0.004^{d}	1.888±0.002^{c}	0.194±0.003^{h}	0.035±0.004^{h}
3.	G	0.918±0.002^{f}	0.477±0.003^{h}	1.395±0.002^{h}	0.234±0.001^{g}	0.045±0.005^{gh}
4.	A	0.981± 0.007^{de}	0.666±0.003^{ef}	1.654±0.003^{de}	0.320±0.002^{de}	0.199±0.003^{b}
5.	P	0.684±0.004^{g}	0.917±0.006^{c}	1.606±0.004^{e}	0.347±0.003^{d}	0.107±0.002^{f}
6.	G+A	0.998±0.001^{d}	0.690± 0.003^{e}	1.686±0.005^{d}	0.216±0.003^{gh}	0.150±0.003^{e}
7.	G+T	0.944±0.006^{ef}	0.511±0.005^{g}	1.457±0.004^{f}	0.293±0.005^{e}	0.044±0.004^{gh}
8.	G+P	0.952±0.005^{e}	0.543±0.009^{fg}	1.497±0.002^{ef}	0.240±0.005^{fg}	0.134±0.004^{ef}
9.	A+T	1.033±0.004^{b}	0.918±0.002^{c}	1.951±0.003^{b}	0.371±0.002^{b}	0.185±0.004^{c}
10.	A+P	0.665±0.002^{h}	0.983±0.004^{b}	1.650±0.002^{de}	0.273±0.004^{ef}	0.096±0.003^{fg}
11.	T+P	0.805±0.003^{fg}	0.752± 0.005^{e}	1.559±0.004^{e}	0.253±0.003^{f}	0.023±0.004^{hi}
12.	G+A+T	0.662±0.002^{h}	0.862±0.007^{d}	1.531±0.005^{e}	0.356±0.004^{c}	0.126±0.004^{ef}
13.	G+T+P	0.678±0.005^{g}	0.790±0.006^{e}	1.467±0.005^{g}	0.253±0.005^{f}	0.085±0.004^{g}
14.	G+P+A	0.681±0.007^{g}	0.790±0.003^{e}	1.475±0.003^{g}	0.197±0.003^{h}	0.162±0.004^{de}
15.	A+T+P	1.035±0.002^{b}	0.624±0.003^{i}	1.655±0.003^{de}	0.354±0.004^{c}	0.168±0.001^{d}
16.	G+A+T+P	1.046±0.002^{a}	1.004±0.002^{a}	2.055±0.003^{a}	0.406±0.003^{a}	0.243±0.005^{a}
	L.S.D. ($P \leq 0.05$)	0.0051	0.0059	0.0044	0.004	0.045
	ANOVA (F15,32)	11626.575	7899.573	33029.686	2640.360	1904.461

Treatments

1) Control (Without any inoculum) 2)*Trichoderma viride* (*T*) 3)*Glomus mosseae* (*G*) 4) *Acaulospora laevis* (*A*) 5) *Pseudomonas fluorescens* (*P*) 6) *G* + *A* 7) G + T 8) G + P 9) A + T 10)A + P 11)T + P 12) G + A + T 13) G + T + P 14) G + P + A 15) A + T + P16) G+A+T+P

* Mean of fine replicates, ± = standard deviation mean values followed by deferent alphabet (s) within a column do not differ significantly over one another at p< 0.05 led by duncan's Multiple range test.

(40.2±1.67) was found in consortium of *G . mosseae + A. laevis + T. viride + P. fluorescens* and least in uninoculated control. Table-1 showed that biomass of all the inoculated plants of *V. negundo* increased significantly in terms of fresh and dry shoot and root weight after 120 days of inoculation. The increase in shoot and root weight may be due to more absorption of nutrients via an increase in root surface area. *Vitex negundo* inoculated plants with AMF grew markedly better than non inoculated control plants. Similarly, the highest value of leaf area was registered in those plants which were treated with *G.mosseae + A.laevis + T.viride + P.fluorescens* (29.0±0.35) which showed significant difference from control (3.14±0.50) but there was no significant difference among few inoculated plants. Percentage mycorrhizal root colonization and AM spore number also increased in all AM treated plants over control (Table-1). Inoculated treatments showed higher percentage of colonized roots as compared to control.

After 120 days of inoculation synergistic effects was observed and percent mycorrhizal root colonization (92.6±1.48) and spore count (78.4±5.55) was highest in plants treated with *G. mosseae + A. laevis + T. viride + P. fluorescens.* Chlorophyll content was found to increase in all treated plants than control. The highest increase in chlorophyll a (1.046± 0.002), chlorophyll b (1.004± 0.002) and total chlorophyll (2.055±0.003) were maximum in plants treated with *G . mosseae + A. laevis + T. viride + P. fluorescens* and minimum chl a (0.383±0.002), chl b (0.412±0.002) and total chl (0.792±0.002) in control plants after 120 days of inoculation (Table-2), (Plate 1-2). Similar trend was observed for phosphorus content of root and shoot. The P content of root (0.406±0.003) was higher over shoot (0.243±0.005) and was significantly higher in mixed consortium(G+A+T+P).

DISCUSSION

In general, the response of *Vitex negundo* to different treatments for different parameters was better as compared to control. In this experiment, none of the single, double and

triple inoculation treatments had significant effects in showing the consistent plant growth promoting effects on all experimental parameters as observed in mixed inoculation. All the observed parameters were found maximum in four combination treatment i.e., *G. mosseae + A. laevis + T. viride + P. fluorescens* for inoculating *Vitex negundo* plants. The enhanced growth is because AM fungi are known to possess the ability to increase nutrient uptake of plants by developing association with roots (Schreiner *et al*. 1997) and sometimes also promotes the growth of other rhizospheric microorganisms and thus enhances plant growth (Johansson *et al*. 2004). The other reason can be once host roots are colonized by the AM fungi, it changes the root exudates released and produces phosphatase enzyme in the rhizosphere. These phosphatases produced by extraradical hyphae of AM fungi could hydrolyze extracellular phosphate ester bonds and ultimately made P available to the plants (Joner *et al.* 2000). Inoculation with AM fungi (*G . mosseae + A laevis*) significantly increased plant height as compared to uninoculated plants (Yadav *et al.* 2012, Yadav *et al*. 2011). Similarly, the growth of plant *Amorpha fruticosa* by inoculation of AM fungi has been observed by Song *et al*. (2012). Similarly Kumar (2012) showed that AM fungi could have played an important role in altering the rhizosphere environment. Among both the AM fungi studied *Acaulospora laevis* was found to be much compatible strain for *Vitex negundo* than *G. mosseae* by increasing the capacity of the root systems to absorb and translocate nutrients extensive mycelia.

The possible outcome of improved plant growth indicates the improvement in fresh shoot and root weight and hence better biomass accumulation and this in turn improves P uptake. As phosphorus is essential for the process of nitrogen fixation, in this experiment also, mixed inoculation might have influenced the plants with both P and N uptake. This may be the cause for enhanced leaf area, root length and chlorophyll content of *Vitex negundo* seedlings inoculated with *G . mosseae + A. laevis + T. viride + P. fluorescens.* The hyphae of AMF have the tendency to extract nitrogen and transport it from the soil to plants. They contain enzymes that breakdown organic nitrogen and contain nitrogen reductase which alters the forms of nitrogen in the soil. Higher values of growth and other physiological parameters indicate higher quality of the seedlings and hence better establishment in the soil. It was found that root colonization and AM spore number were greatly influenced by synergistic effect of AM fungi, *P. fluorescens* and *T. viride*. *Pseudomonas* species are also effective in root colonization as they produce secondary metabolites (O' Sullivan and O' Gara 1992) that enhances AMF growth and thus mycorrhizal spore number and colonization.

The P and N contents of the plants showed the same trend which resembled the earlier works performed on other crops (Akhtar and Siddiqui 2010). Content of P was observed more in roots than shoots. Present findings also indicated that AM inoculated plants along with *T. viride* and *P. fluorescens* had a higher phosphorus content than control. Mutualistic association thus improve the phosphorus mineralization and its acquisition by plants. This may be due to synergistic interaction between both the AM fungi and other plant growth promoting bioinoculants. It has also been found that PGPR possess a wide variety of other direct mechanisms to support mycorrhizal symbiosis. They solubilize the bound phosphorus from the soil and release P into the soil, which is then taken up by AM colonized roots, their interaction with AM fungi therefore occasionally produces positive effects by enhancing plant growth and protection (Xavier and Germida 2003). In general, with all treatments, the contents of Chlorophyll a and b in mycorrhizal plants were significantly greater than those of non- mycorrhizal ones at all stages of plant growth. There results are in accordance with the results obtained by (Sharma *et al*. 2008). AM treated plants allow the root system

to exploit a greater volume of soil by extending among the root zone and by exploring smaller soil pores not reached by the root hairs. The total photosynthesis pigments increased due to mycorrhizal colonization which was more than double the concentration found in uninoculated control. Similar results were shown by Tanwar *et al.* (2011) with enhanced growth and yield of *Capsicum annum*.

The improved soil texture increases air and water percolation and facilitates the root system access to soil water and nutrients. Phosphorus (P) has an important role as energy carrier during photosynthesis. AM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots thus providing a stimulus for greater photosynthetic activity (Bevege *et al*. 1975). AM symbiosis needs carbon source from symbiotic partner synthesized by the process of photosynthesis and it was found that upto 20% of the total photoassimilates can be transferred to the fungal partner (Graham 2000). Synergistic effect of inoculation of AM fungi, *T.viride* and *P. fluorescens* was found beneficial for plant growth by other workers (Tanwar *et al.* 2011, Neetu *et al*. 2011) and the effect of AM fungi with *T. viride* was found beneficial for plant growth by many other workers (Kumar 2012, Bhromsiri and Bhromsiri 2010) There are several studies which have focused on mycoparasitic nature of *Trichoderma* species and hence its contribution to plant health (Allay and Chakraborty 2010).

CONCLUSION

The current study shows that inoculation with plant growth promoting microorganisms (G+A+T+P) enhances the overall growth performance of *Vitex negundo* plants grown under green house conditions. Based on the response of different characters like plant growth, mycorrhizal spore number and root colonization , P content and other parameters it can be concluded that the quadruple inocuation (*G. mosseae + A. laevis + T. viride + P. fluorescens)* and triple inoculation (*A. laevis + T. viride + P. fluorescens)* are the best consortia of microorganisms for inoculating *V. negundo* plants. Inoculation with such a microbial consortium may result in healthy, vigorously growing *V. negundo* seedlings. This technology, being simple and ecofriendly can be adopted easily by any nursery men for inoculating *V. negundo* seedlings in the nursery. This combination can be tested further in the field condition and can be recommended to farmers after proper confirmation.

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