Original Article

Bulletin of Environment, Pharmacology and Life Sciences

Online ISSN 2277 – 1808
Bull. Environ. Pharmacol. Life Sci.; Volume 1 [6] May 2012: 60 - 65

Website: www.bepls.com

The Influence of Bioinoculants on Growth and Mycorrhizal Occurrence in the Rhizosphere of M*entha spicata* linn

Aditya Kumar
School of Biological Sciences,
Shoolini University, Solan, Himachal Pradesh-173229, INDIA
Ph. 08894893438
Email: adityagohar@*yahoo.com*

ABSTRACT

Arbuscular mycorrhizae (AM) are the important beneficial micro- organisms of the soil edaphon in most agro-ecosystems. In the present investigation, attempt was made in order to evaluate the potential of two arbuscular mycorrhizal fungi (Glomus mosseae and Acaulospora laevis) along with Trichoderma viride on growth attributes of Mentha spicata. All the inoculated seedlings showed significant results over control after 45 and 90 days of inoculation in polyhouse pot experiment. Dual inoculation of G.mosseae and A.laevis showed the maximum mycorrhizal inoculation effect. The possibility of arbuscular mycorrhizal fungi (AMF) along with other microflora has been ensured for primary establishment of seedlings under nursery conditions.

KEY WORDS: Arbuscular Mycorrhizal Fungi, *Glomus mosseae, Acaulospora laevis,* medicinal plant

INTRODUCTION

The anxiety of man to explore nature and discover new forms and aspects of life has led to a vast heritage of knowledge and ideas. The diversity of microbial flora and their role in the ecology has always raised a number of quarries in human mind. The micro organisms have posed influence in the social and economic structure of human civilization from time immortal. The microbial communities are responsible for development of soil structure through various biogeochemical cycles in order to maintain the soil health and quality [1].

The mycorrhizal symbiosis represents a series of complex feedbacks between host and fungus that is governed by their physiology and nutrition [2]. Arbuscular mycorrhizal fungi (AMF) are considered as the major component of the soil edaphon, which received an increased attention globally, as that the adverse soil conditions of different agroclimatic region of the world provide a micro ecosystem that can be manipulated and managed by these fungi for increased plant growth and productivity. 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]. The existence of inter and intra- specific variations among the plant species involved in relation to their phosphorus requirement and the ability of the host to translocate the native soil phosphorus further determine the efficacy of these fungi [4].

Natural products including medicinal plants have a great significance due to their wide range of therapeutic potential to treat a large number of ailments, it becomes necessary to enhance their biomass production and their quality in order to fulfill the need of society. Therefore, it requires formulation of planning and strategies for their conservation and enhancement of their products. Hence, keeping the above facts in consideration, in the present study, analysis has been made to see the effect of AM fungi (*Glomus mosseae* and *Acaulospora laevis*) and *T. viride,* alone and in dual combination on different growth as well as physiological parameters of *Mentha spicata* after 45 and 90 days of inoculation.

MATERIAL AND METHODS

Study Site:

The study was undertaken in poly house of Botany Department where all the conditions (27-35ºC temperature, 80% humidity, 15000-19000 lux. light intensity) were controlled during experiment.

Selection of Bioinoculants:

Two dominant strains of AM fungi i.e. *G.mosseae* and *A.laevis* were isolated from rhizospheric soil of selected medicinal plant and mass multiplied by using different hosts and substrates. *T.viride* was isolated from soil by Warcup's soil plate method and mass cultured by using wheat bran: saw dust medium. Two months old seedlings of preferred medicinal plants were procured from Dr.Y.S. Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh.

Sample Collection and Processing:

The soil samples were collected from the root zone of each plant after 45 and 90 days. The soil samples were wet sieved for AM spore population using the 'wet sieving and decanting technique' and quantification of AM spores was done by 'grid line intersect method' [5,6]. The fine terminal roots were removed from the root, rinsed with tap water and stained with trypan blue according to 'rapid clearing and staining method' [7]. The percent AM root colonization was calculated by using following equation;

%AM root colonization = (Total number of root segments colonized / Total number of root segments examined) ×100

Record of Data:

The effect of different treatments was recorded at 45 and 90 days after planting (DAP). The plant growth parameters like plant height (measured in cm. from soil surface to the growing tip of plant), % root colonization, AM spore count, root length (cm.) were recorded. Moreover, roots and shoots were harvested and weighted separately for their fresh weight (cm.) and the oven dried to 70ºC for dry weight (cm.). Leaf area ($cm.^2$) was studied using leaf area meter (Systronics 211).

Statistical Analysis:

Statistically interpretation of data was done by using analysis of variance (ANOVA) followed by post hoc test through computer software SPSS 16.0 (SPSS Inc. Chicago, IL). Means were then ranked at P=0.05 level of significance using Duncan's Multiple Range Test for comparison.

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi are well known to enhance the nutritional status of several plants and thereby aid in increased growth and yield. The present investigation was carried out in order to evaluate the potential of AM fungi and *T.viride* on growth and physiological parameters of *M.spicata*. Results elucidated that the seedlings of all plants under investigation varied in their response to inoculation with AM fungi and *T.viride* in different combinations or treatments of inoculation and also showed the dependence of these plants on such types of inoculations. Data represented in Tables 1.1 and 1.2 indicates that different bioinoculants used in the experiment triggered the growth performance of *M.spicata* seedlings.

Shoot Length:

AM symbiosis significantly increased the shoot length of *M.spicata* after 45 and 90 days of inoculation. Results indicate that maximum increase in shoot length was registered in dual combination of *A.laevis* plus *G.mosseae* (30.43±4.66, 40.80±2.46). In single inoculation, the plants nourished with *A.laevis* (26.26±0.32, 30.86±1.88) showed maximum increment in plant height. The increased growth of vegetative parameters is the consequence of increased root proliferation via. increased nutrient and water uptake due to mycorrhizal inoculation. In the present study, inoculating *M.spicata* with AM fungi could have played an important role in altering the rhizosphere environment by shortening the distance, the nutrients had to diffuse through the soil to the roots and therefore, contribute increase in height by increasing the nutrients uptake. Parkash *et al.* [8] studied the effect of soil sterilization on bioinoculant's activity in establishment of *Acacia catechu* and reported a significant increase in shoot length due to co-inoculation of AM fungi.

Shoot Biomass:

Results depicted in Tables (1.1 and 1.2) indicate that forty five and ninety days after inoculation (DAI), maximum shoot weight (fresh and dry) was recorded in the treatment of *A.laevis* plus *G.mosseae* (4.010±0.10, 0.798±0.05; 7.985±0.05, 1.507±0.05). The higher biomass production in inoculated *M.spicata* could be due to higher moisture absorption by the inoculated plants. The shoot production depends not only on the vigour of the tree, but also on the environmental factors such as site conditions, soil moisture, season and pH [9,10]. Soil based AM inoculum in the present investigation enhanced growth of plants which might be due to the higher reproduction of AM fungi in the soil based inoculum, which sprouted rapidly from extracellular and intracellular hyphae

present in the soil and root inoculum. AM inoculation usually enhanced growth and shoot biomass of plants [11,12]. Similarly, Prasad *et al.* [13] also reported that *Gladiolus* plants inculated with AM fungi (*G.fasciculatum*) showed enhanced growth as compared to control.

Root Biomass:

Mycorrhizal infection significantly stimulated the *M.spicata* root growth. At the end of experimental period, a significant effect of mycorrhizal symbiosis was observed in the root weight (fresh and dry) of inoculated plants than non inoculated plants. Forty five and ninety DAI, the maximum fresh root weight was observed in the plants treated with *A.laevis* plus *G.mosseae* (2.168±0.09, 5.361±0.41). Similarly, higher dry root weight was registered in the plants inoculated with *A.laevis* plus *G.mosseae* (0.633±0.04) and *A.laevis* plus *T.viride* (1.174±0.09) respectively. The reason may be that there was no indigenous microbe to compete with the inoculated strain in pure culture so that the effect of inoculation was more obvious. The contribution of AM fungi to plant growth is not limited to improved nutrition but they also change the morphogenetic characters of roots. Mycorrhizal fungi decrease the meristematic activity of root apices and thus lead to an increase in the number of adventitious roots [14]. Mycorrhizal plants had a greater number of lateral roots in the present investigation. Singh and Subba Rao [15] who worked on wheat crop reported a significant increase in the yield of root and shoot. Similar was the observation made by Turjaman [16] who reported a significantly increased shoot and root dry weight in plants of *Dyera polyphylla* and *Aquilaria filaria*, when inoculated with AM fungi. In the present investigation, the effect of AM fungi and *T.viride* was more pronounced in aerial biomass than in root biomass (Table 1.1, 1.2) which may be because of arbuscular mycorrhizal colonization, caused a proportionally greater allocation of carbohydrates to the shoot than to the root tissue [17].

Root Length:

It is evident from Tables (1.1 and 1.2), AM inoculation significantly increased the root length of all inoculated plants over control. After 45 days of inoculation, maximum increase in root length was found in the plants treated with *A.laevis* plus *T.viride* (17.50±0.35) followed by *G.mosseae* (15.5±0.20) and *A.laevis* plus *G.mosseae* (14.6±0.20). Similarly, maximum root growth was observed in *A.laevis* plus *G.mosseae* (26.63±0.61) followed by *A.laevis* (24.53±0.30) and *T.viride* (23.8±0.20) after 90 days of inoculation. These improvements were likely achieved via. mycorrizal contribution to phosphorus uptake and the ability of AM fungi to stimulate plant synthesis of certain phytohormones such as ABA and cytokinins. AMF colonization enhances soil aggregation through extraradical hyhae that are external to plant root and exuding the glycoprotein, glomalin, from extraradical hyphae that cements soil microaggregates in to large soil aggregate structure [17]. The improved soil tilth that occurs enhances air and water percolation and improves root system assess to soil water and nutrients.

Leaf Area:

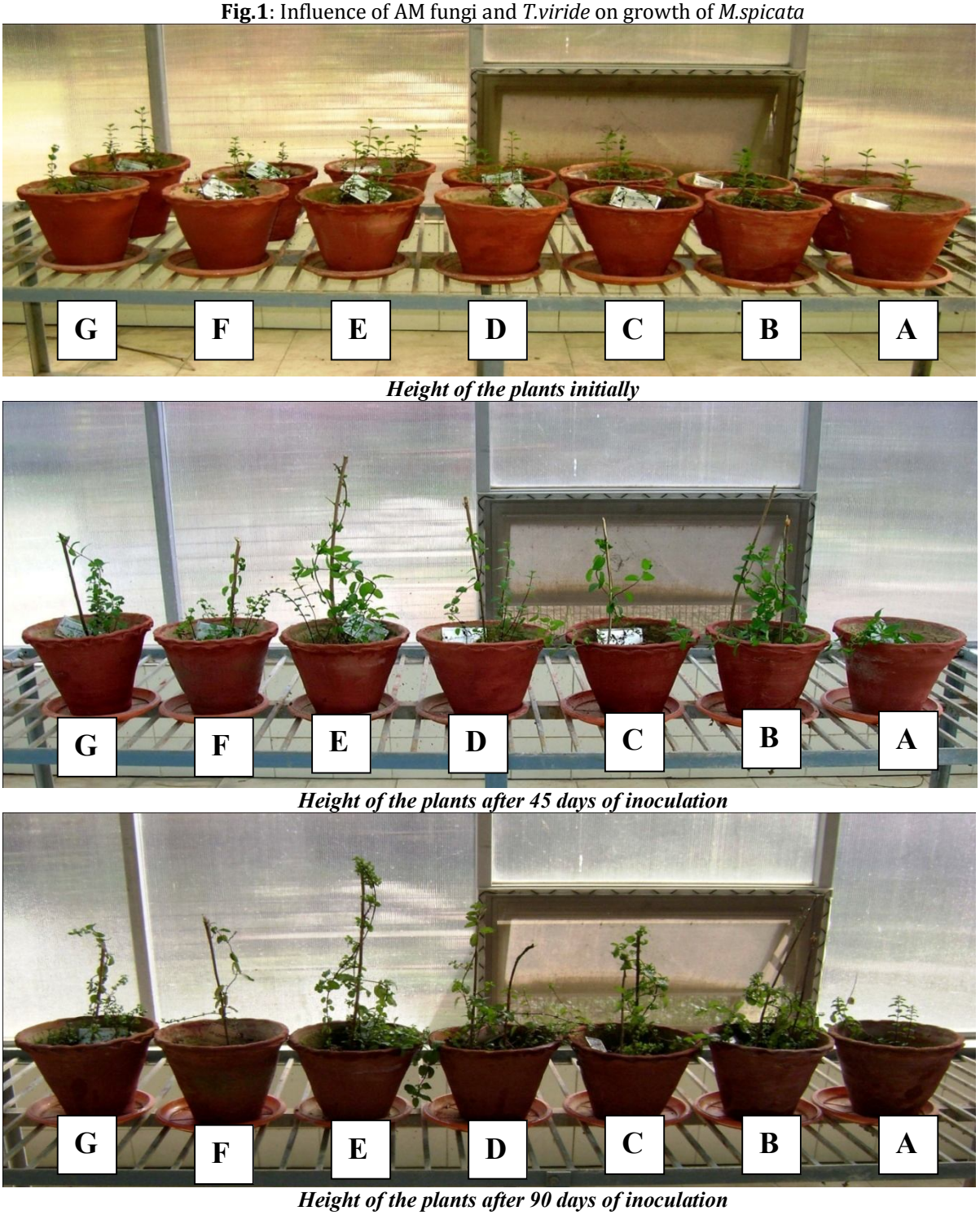
Tables 1.1 and 1.2 envisaged that mycorrhizal inoculation significantly increased leaf area after 45 and 90 days of inoculation. After 45 days of inoculation, maximum increased leaf area was reported in the plants treated with *G.mosseae* (4.36±0.20) followed by *A.laevis* plus *G.mosseae* (3.90±0.10) and *T.viride* (3.66±0.15). Similarly, after ninety days of inoculation, highest leaf area was registered in *A.laevis* plus *G.mosseae* (5.86±0.20) followed by *G.mosseae* (4.83±0.20) and *A.laevis* (4.66±0.25). Increased leaf area in the present investigation are in accordance with the study of Amico *et al.* [18] who observed increment in AMF infection, root weight, leaf area, photosynthetic activity, stomatal conductance and root hydraulic conductivity in tomato, when inoculated with *G.clarum*. A significant variation in the proportion and composition of leaf volatiles in *Citrus jambhiri* inoculated with *G.intraradices* has well documented [19]. Larger leaf area of mycorrhizal plants than non-mycorrhizal plants which may be due to more CO_2 assimilation and more transpiration rate in mycorrhizal plants has also been well documented [20,21]. All these findings support the results of present investigation.

Spore Count and Percent Root Colonization:

Result indicates that a varied degree of spore population and mycorrhizal root colonization has been reported in all inoculated plants. Maximum spore population was observed in the plants treated with *A.laevis* plus *G.mosseae* (27.0±2.0, 35.66±2.51) after 45 and 90 days of inoculation. Similarly, the intensity of mycorrhizal root colonization was found highest in the plants inoculated with *G.mosseae* plus *T.viride* (61.28±1.18, 100.00±0.0) followed by *A.laevis* plus *G.mosseae* (38.8±0.42, 91.54±1.41).

Among the single inoculated treatments, highest mycorrhizal colonization was observed in the plants inoculated with the AM fungi i.e. *A.laevis* (36.19±0.33) and *G.mosseae* (67.99±1.16) after 45 and 90 days respectively, thus supporting the well documented fact that inoculation with effective AM fungi enhances mycorrhizal root colonization [22]. Highest mycorrhizal root colonization in *M.spicata* was observed when *G.mosseae* was co-inoculated with *T.viride*. This may be because of synergestic interaction between AM fungi and *T.viride*. The stimulation was attributed to volatile compounds produced by *Trichoderma* spp. [23]. Increase in rhizosphere spore density as a consequence of mycorrhizal inoculation has been reported in Cowpea and rice [24,25]. Another important feature observed in the study was that the rhizospheric spore density decreased with increase in root colonization in some cases during course of experimentation.

Fig.1: Influence of AM fungi and *T.viride* on growth of *M.spicata*



Height of the plants initially

Height of the plants after 45 days of inoculation

Height of the plants after 90 days of inoculation

A-Control, B-*Trichoderma viride*, C-*Glomus mosseae*, D-*Acaulospora laevis*, E-*A.laevis* + *G.mosseae*, F-*G.mosseae* + *T.viride*, G-*A.laevis* + *T.viride*

Table: 1.1- Bioinoculation effect of Arbuscular Mycorrhizal Fungi and *T.viride* on growth parameters of *M.spicata* after 45days

Treatments	Change in height (cm.)	Leaf area (sq.cm.)	Root length (cm.)	Fresh shoot weight (gm.)	Dry shoot weight (gm.)	Fresh root weight (gm.)	Dry root weight (gm.)	AM Spore number/10 gm. of soil	AM Root colonization (%)
Control	*15.23±0.68^{e}	1.63±0.20^{e}	5.63±0.25^{g}	1.012±0.01^{f}	0.134±0.04^{e}	0.624±0.03^{e}	0.142±0.03^{e}	9.0±1.0^{e}	11.68±0.68^{f}
Trichoderma viride	26.0±0.65^{b}	3.66±0.15^{b}	7.53±0.30^{f}	1.555±0.04^{e}	0.238±0.04^{d}	0.977±0.03^{c}	0.229±0.02^{d}	11.66±1.52^{d}	18.91±0.82^{e}
Glomus mosseae	22.63±0.20^{cd}	4.36±0.20a	15.5±0.20^{b}	2.104±0.05^{c}	0.350±0.04^{c}	2.089±0.06^{a}	0.547±0.04^{b}	23.33±1.52^{b}	28.7±1.36^{d}
Acaulospora laevis	26.26±0.32^{b}	2.7±0.10^{d}	12.53±0.20^{d}	1.930±0.33^{d}	0.375±0.06^{c}	0.856±0.04^{d}	0.237±0.04^{d}	19.0±1.0^{c}	36.19±0.33^{c}
A.laevis + G.mosseae	30.43±4.66^{a}	3.9±0.10^{b}	14.6±0.20^{c}	4.010±0.10^{a}	0.798±0.05^{a}	2.168±0.09^{a}	0.633±0.04^{a}	27.0±2.0^{a}	38.8±0.42^{b}
G.mosseae + T.viride	19.5±0.43^{d}	3.36±0.15^{c}	11.53±0.15^{e}	2.835±0.06^{b}	0.544±0.04^{b}	0.915±0.04^{cd}	0.243±0.039^{d}	19.33±1.52^{c}	61.28±1.18^{a}
A.laevis + T.viride	24.0±0.65^{bc}	2.5±0.10^{d}	17.50±0.35^{a}	2.758±0.04^{b}	0.542±0.04^{b}	1.146±0.01^{b}	0.352±0.04^{c}	25.66±1.52^{ab}	35.82±2.83^{c}

* Each value is an average of three replicates
Means values followed by different alphabet/s are significant over one another by Duncan's Multiple Range Test at P= 0.05.
± Standard Deviation

Table: 1.2- Bioinoculation effect of Arbuscular Mycorrhizal Fungi and *T. viride* on growth parameters of *M.spicata* after 90 days

Treatments	Change in height (cm.)	Leaf area (sq.cm.)	Root length (cm.)	Fresh shoot weight (gm.)	Dry shoot weight (gm.)	Fresh root weight (gm.)	Dry root weight (gm.)	AM Spore number/10 gm. of soil	AM Root colonization (%)
Control	*19.86±0.58^{g}	2.2±0.1^{e}	10.5±0.2g	1.856±0.03^{f}	0.386±0.06^{e}	1.479±0.06^{f}	0.415±0.05^{d}	13.33±1.52^{f}	50.5±1.40^{g}
Trichoderma viride	30.33±0.75^{b}	4.26±0.05^{c}	23.8±0.2^{c}	7.159±0.09^{b}	1.214±0.04^{c}	3.255±0.03^{d}	0.785±0.10^{b}	18.33±1.52^{e}	63.92±0.30^{e}
Glomus mosseae	28.93±1.35^{b}	4.83±0.20^{b}	15.86±0.37^{f}	5.185±0.14^{d}	1.126±0.10^{c}	2.153±0.03^{e}	0.525±0.09^{cd}	31.0±2.0^{b}	67.99±1.16^{d}
Acaulospora laevis	30.86±1.88^{b}	4.66±0.25^{b}	24.53±0.30^{b}	6.644±0.04^{c}	1.364±0.05^{b}	4.732±0.09^{b}	1.148±0.02^{a}	22.66±1.52^{d}	55.08±0.40^{f}
A.laevis + G.mosseae	40.8±2.46^{a}	5.86±0.20^{a}	26.63±0.61^{a}	7.985±0.05^{a}	1.507±0.05^{a}	5.361±0.41^{a}	1.167±0.10^{a}	35.66±2.51^{a}	91.54±1.41^{b}
G.mosseae + T.viride	24.56±1.05^{c}	3.9±0.36^{c}	16.8±0.10^{e}	3.760±0.03^{e}	0.912±0.06^{d}	2.315±0.10^{e}	0.643±0.08^{bc}	31.0±2.64^{b}	100.0±0.00^{a}
A.laevis + T.viride	30.7±2.84^{b}	3.36±0.20^{d}	21.26±0.15^{d}	5.116±0.01^{d}	1.163±0.11^{c}	3.838±0.09^{c}	1.174±0.09^{a}	27.33±1.52^{c}	75.17±2.60^{c}

* Each value is an average of three replicates
Means values followed by different alphabet/s are significant over one another by Duncan's Multiple Range Test at P= 0.05.
± Standard Deviation

This could be due to reason that more spore germination might have taken place after infection to the newly emerged roots as the days advanced [26,27].
The tremendous advances in research on mycorrhizal physiology and ecology over the past 40 years have led to a greater understanding of the multiple roles of AMF in the ecosystem. Beneficial AMF are one of the important cornerstones of sustainable agricultural systems. The results of the present study clearly brought out the beneficial effect of inoculation with *G.mosseae*, *A.laevis* and *T.viride*, alone and in different combinations on various growth parameters of *M.spicata*. As mycorrhizal fungi are beneficial for plant establishment, this study provides a good scope for commercially utilizing the efficient strains of AM fungi to exploit them for their beneficial effects with other beneficial

rhizospheric microflora in establishment of seedlings, increase in productivity and reduce the fertilizer application required for obtaining economic production of this plant under field conditions.

REFERENCES

1. Jeffries, P. & Barea, J.M. (2001). Arbuscular Mycorrhiza- A key component of sustainable plant and soil ecosystems. In: The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. Springer-Verlag, Heidelberg, p. 95-108
2. Rapparini, F., Llusia, J. & Penuelas, J. (2008). Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of *Artemisia annua* L. *Plant Biol*., 10: 108-122.
3. Rajeshkumar, S., Nisha, M.C. & Selvaraj, T. (2008). Variability in growth, nutrition and phytochemical constituents of *Plectranthus amboinicus* (Lour) Spreng. as influenced by indigenous arbuscular mycorrhizal fungi. Mj. *Int. J. Sci. Tech*., 2(02): 431-439.
4. Koide, R.T. (1991). Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol*., 117: 365-386.
5. Gerdemann, J.W. & Nicolson, Y.H. (1963). Spores of mycorrhizae *Endogone* species extracted from soil by wet seiving and decanting. Trans. *Brit. Mycol. Soc*., 46: 235-244.
6. Adholeya, A. & Gaur, A. (1994). Estimation of VAMF spore in soil. Myco. News, 6(1): 10-11.
7. Philips, J.M. & Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc*., 55: 158-161.
8. Parkash, V., Sharma, S., Kaushish, S. & Aggarwal, A. (2009). Effect of soil sterilization on bio-inoculants activity in establishment of *Acacia catechu* Willd. *Phytomorphology*, 59(1-2): 57-63.
9. Kijkar, S. (1991). Producing rooting cuttings of *Eucalyptus camaldulensis*. AASEAN- Canada forest tree seed centre project handbook, pp 25.
10. Sylvia, D.M. & Williams, S.E. (1992). Vesicular arbuscular mycorrhizae and environment stress (Eds. Bethlenfalvay, G.J. and Linderman, R.G.) Mycorrhizae in Sustainable Agriculture, Am. Soc. Agron. Spec. Publ., 54: 101-124.
11. Duponnois, R., Plenchette, C. & Ba, A.M. (2001). Growth stimulation of seventeen fallow leguminous plants inoculated with *Glomus aggregatum* in Senegal. Eur. J. Soil Biol., 37: 181-186.
12. Diop, T.A., Wade, T.K., Diallo, A., Diouf, M. & Gueye, M. (2003). *Solanum* cultivar responses to arbuscular mycorrhizal fungi: growth and mineral status. Afr. J. Biotechnol., 11: 429-443.
13. Prasad, V., Manjunath, G.T.S. & Reddy, C.N. (2000). Influence of *Glomus fasciculatum* inoculation on growth and phosphorus uptake in *Gladiolus* sp. Myco. News, 11(4): 17-18.
14. Berta, G., Fusconi, A., Trotta, A. & Scannerini, S. (1990). Morphogenetic modifications induced by mycorrhizal fungus *Glomus* strain E_3 in the root system of *Allium porrum* L. New Phytol., 114: 207-215.
15. Singh, C.S. & Subba Rao, N.S. (1987). Yield and phosphorus content of wheat (*Triticum aestivum*) influenced by co-inoculation with *Azopitrillura brasilense* and *Glomus fasciculatum.* (Eds. Verma, A.K., Oka, A.K., Mukerji, K.G., Tilak, K.V.B.R. and Raj, J.) Mycorrhiza Round Table, Proceedings of a National Workshop. I.D.R.C., New Delhi, India, p. 335-346.
16. Turjaman, M., Tamai, Y., Santoso, E., Osaki, M. & Tawarya, K. (2006). Arbuscular mycorrhizal fungi increased early growth of non- timber forest product species *Dyera polyphylla* and *Aquillaria filaria* under greenhouse conditions. *Mycorrhiza*, 16: 459-464.
17. Wright, S.F. & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil, 198: 97-107.
18. Amico, J.D., Torrecillas, A., Rodriguez, P., Morte, A. & Sanchez- Blanco, M.J. (2002). Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. J. Agric. Sci., 138: 387-393.
19. Nemec, S. & Lund, E. (1990). Leaf volatiles of mycorrhizal and non- mycorrhzal *Citrus jambhiri* Lush. J. Essent Oil. Res., 2:287-297.
20. Amerian, M.R., Stewart, W.S. & Griffiths, H. (2001). Effect of two species of AM fungi on growth assimilation and leaf water relations in maize (*Zea mays*). Asp. Appl. Biol., 63: 73-76.
21. Borde, M., Kedar, H. & Jite, P.K. (2009). Growth performance of *Vitis vinifera* with *Glomus fasciculatum*. Myco. News, 21(2): 12-15.
22. Rajan, S.K., Reddy, B.J.D. & Bagyaraj, D.J. (2000). Screening of mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecol. Manag., 126: 91-95.
23. Graham, J.H. & Sylvertsen, J.P. (1984). Influence of vesicular arbuscular mycorrhizae on the hydraulic conductivity of roots of two citrus rootstocks. *New Phytol*., 7: 277-284.
24. Muthukumar, T. & Udaiyan, K. (2002). Growth and yield of cowpea as influenced by changes in arbuscular mycorrhizal fungi in response to organic manuring. J. Agro. Crop Sci., 188 (2): 123-132.
25. Secilia, J. & Bagyaraj, D.J. (1994). Evaluation and first- year field testing of efficient vesicular arbuscular mycorrhizal fungi for inoculation of wetland rice seedlings. *World J. Microb. Biot.,* 10: 381-384.
26. Harikumar, V. & Potty, V.P. (2002). Technology for mass multiplication of arbuscular mycorrhizal (AM) fungi for field inoculation of sweet potato. *Myco. News*, 14(**1**): 11-12.
27. Gaur, A. & Adholeya, A. (2002). Arbuscular mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol. Fert. Soils*, 35(**3**): 214-218.