JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 5021-5027

Composite Treatment of Ectomycorrhizal Fungus *Scleroderma bovista* with Two Mycorrhiza Helper Bacteria Augmented Banj Oak (*Quercus leucotrichophora* A. Camus) Growth

Anurag Yadav[1]*, R.C. Dubey[2] and Kusum Yadav[3]

[1]Department of Microbiology, College of Basic Science & Humanities, S.D. Agricultural University, S.K. Nagar, Gujarat -385 506, India.
[2]Department of Botany and Microbiology, Gurukula Kangri University, Haridwar, Uttarakhand- 249 409, India.
[3]Department of Biochemistry, University of Lucknow, Lucknow - 2260 07, India.

(Received: 20 August 2014; accepted: 14 September 2014)

Effect of composite inoculation of ectomycorrhizal fungi *Scleroderma bovista* along with two mycorrhizosphere bacterial inoculants was studied on banj oak (*Quercus leucotrichophora* A. Camus) plant growth in nursery glasshouse experiment. Seven treatments comprising of uninoculated control, two bacterial controls, one ectomycorrhizal (ECM) fungal control, two combinations of mycorrhizosphere bacteria and ECM fungus along with one composite inoculation of two bacteria with ECM fungus were used in the study. Dual treatment of *S. bovita* with *Pseudomonas fluorescens* MB9 yielded maximum shoot length (15.26 cm) and collar diameter (0.320 cm) of the plant. Triple inoculation of *S. bovista* with the two bacteria yielded maximum root length (36.86 cm), lateral roots (24.8), short roots (351.6), dry weight (6.746 g) and ectomycorrhizal colonization (39.2%). The study suggests the use of composite inoculation of native mycorrhizosphere bacteria like *Bacillus subtilis, P. fluorescens* and others with *Scleroderma bovista* for the better growth of *Q. leucotrichophora*, which is a slow growing plant.

Key words: Growth enhancement, *Scleroderma bovista*, mycorrhiza helper bacteria, ectomycorrhiza.

It is well known fact that some microorganisms from ectomycorrhizal mantle have the ability to enhance ECM fungal growth (Garbaye and Bowen, 1989) and are called 'helpers' (Garbaye and Bowen, 1989). Initially the enhancement in ECM formation due to co-inoculation with bacteria was reported on *Eucalyptus diversicolor* seedlings (Dunstan et al., 1998), which was followed by other supporting reports. Such type of bacteria can therefore be used along with ECM fungus for plant growth enhancement. These stimulatory bacterial isolates can also be of special interest in improving ECM occurrence in nurseries and forests. Mycorrhiza helper bacteria (MHB) when co-inoculated with ECM fungi help plant growth by affecting the plant root receptivity to mycorrhizal fungi, modulating root-fungus recognition and attachment, changing physico-chemical properties of soil, enhancing ECM fugal survival in soil (Frey-Klett and Garbaye, 2005) or by inhibiting plant pathogens (Schelkle and Peterson, 1997). Due to massive association of ECM fungus with plant, the associated fungus holds strong competitive advantaged against other fungi (Villeneuve et al., 1991). Common root pathogens may also get suppressed due to ECM co-inoculation with helper bacteria (Schelkle and Peterson, 1997).

* To whom all correspondence should be addressed.
Tel : +91 2748 278097;
E-mail: anuragyadav123@gmail.com

Although composite inoculation of LECM fungi with mycorrhiza helper bacteria or plant growth promoting bacteria has already been successfully tried on plants including pine and spruce (Shishido et al., 1996), Douglas fir (Frey-Klett et al., 1999), *E. diversicolor* (Dunstan *et al.*, 1998) and others but the same has not yet been tried on *Q. leucotrichophora*, the chief oak species of Western Himalaya which constitutes dense forest patches in the region. Even though the ECM fungi like *Amanita hemibapha* and *Rusulla vesca* have been reported to be associated with *Q. leucotrichophora* (Veena et al., 2007), the effect of *S. bovista*, along with or without mycorrhizosphere bacteria, on *Q. leucotrichophora* growth is needed to be clarified. The present study tries to establish the helper effect of two bacterial isolates on *Scleroderma bovista* to augment *Q. leucotrichophora* plant growth. *Q. leucotrichophora* (ban oak or banj oak) is chief oak species of Western Himalaya and constitute dense forest patches in the region.

MATERIALS AND METHODS

The study was conducted in nursery glasshouse in the Department of Forestry, Kumaun University, Nainital. The fallen healthy acorns of oak were collected from healthy *Q. leucotrichophora* tree in early March. Collection was done from the same tree to overcome genetic variations. Seeds were washed with plenty of tap water, soaked in mild detergent and re-washed three or four times. Seeds were surface sterilized by soaking in 30% H_2O_2 for 15 minutes (Hauer and Dawson, 1996) and then immediately washed with 2 L sterilized distilled water to remove H_2O_2 traces. Seeds were then placed in plates containing sterilized, acid-washed and neutralized sand in germination chambers for germination and kept at 25°C for one month.

Two mycorrhizosphere bacterial strains, *viz. B. subtilis* MB14 and *P. fluorescens* MB9, isolated from *Q. leucotrichophora* mycorrhizosphere and found to enhance ECM fungal growth *in vitro* (data unpublished), were used in the study. Mass bacterial culture was done in 1 L flasks containing nutrient agar medium. These flasks were inoculated and incubated at 30°C for four days. Mass inoculum of *S. bovista* was prepared by gently floating the agar discs containing inoculum on Modified Melin Norkrans (MMN) (Marx, 1970) broth in 1 L flasks. The flasks were kept at 25°C for one month in rotary shaker at 60 rpm. After desired incubation, separation of mycelia from medium was done by pouring the medium in a flask containing funnel with Whatman filter paper number 42. The separated mycelia were mixed in an electric blender for breaking and thorough mixing of hyphae.

Potting mixture was prepared by mixing sand and soil in 3:1 ratio (Table 1). Mixture was autoclaved at 20 lbs $inch^{-2}$ for 20 minutes (Rizvi and Khan, 2009) for two subsequent days. The soil mixture was cooled to room temperature.The test bacteria and ECM fungal mycelium were mixed at the dose of 10^8 bacteria and mycelium g^{-1} soil and sprayed to the soil. Four controls used in experiment were; i) the uninoculated control, ii) inoculated fungal control (EMF) and iii) two inoculated bacterial controls (B1 and B2). The three treatment combinations used in the study were i) *S. bovista* with *B. subtilis* MB14 (EMF+B1), ii) *S. bovista* with *P. fluorescens* MB9 (EMF+B2) and iii) *S. bovista* with *B. subtilis* MB14 and *P. fluorescens* MB9 (EMF+B1+B2). All the treatments had five replica.

About 1.5 kg of seeded soil mixture was filled in polythene bags (15 x 23 cm) and one-month-old *Q. leucotrichophora* plants were transplanted in it. Polybags were transported to green house in sleepy hollow nursery in Nainital. Watering of nursery seedlings was done after every 15 day interval or whenever required with 50 mL distilled water to each pot.

Plant growth parameters

Plant growth parameters *viz.* plant height, dry wt. and ectomycorrhizal occurrence were tri-monthly checked for nine months. Pots from seven treatments were arranged in five replicates in randomized block design. For sampling, plants contained in pots were randomly selected and their polythene bags were removed and soil was gently brushed off from roots. Root system of seedlings was thoroughly washed with running tap water to remove adhering debris and blot dried. Plant root and shoot lengths were measured. Plant dry weights were estimated by keeping them in oven at 85°C overnight and then weighing them immediately with electronic balance. Short roots

and mycorrhizal tips were counted with the help of magnifying glass. White or cream colored swollen root tips were considered mycorrhizal. Ectomycorrhizal roots were also examined microscopically by staining the T.S. of roots with cotton blue and lactophenol.

RESULTS

The results clearly indicate that dual inoculation of one fungal partner with any of the two bacterial partners or triple inoculation of two bacterial partners (*B. subtilis* MB14 and *P. fluorescens* MB9) with one fungal partner (*S. bovista*) resulted in increased plant root length, shoot length, collar diameter, lateral roots, short roots and ectomycorrhizal occurrence, significantly higher than uninoculated and inoculated controls (Table 2, 3 and 4). Overall dry weight root-shoot ratio of oak seedlings decreased with increase in inoculation treatment level in the order of *B. subtilis*/*P. fluorescens* > *S. bovista* > *S. bovista* with *B. subtilis* /*P. fluorescens* > *S. bovista* with *B. subtilis* and *P. fluorescens*. No ectomycorrhizal formation was observed in uninoculated control and bacterial treatment controls. *S. bovista* colonized *Q. leucotrichophora* seedlings (Table 3) with 20.6 % or more colonization rate in all inoculated seedlings.

Dual treatment of *S. bovita* with *P. fluorescens* yielded maximum shoot length (15.26 cm) and collar diameter (0.320 cm) of the plant. Triple inoculation of *S. bovista* with the two bacteria yielded maximum root length (36.86 cm), lateral roots (24.8), short roots (351.6), dry weight (6.746 g) and ectomycorrhizal colonization (39.2 %). Stimulation of lateral root formation seems to be a common feature of MHB (Duponnois, 1992; Poole et al., 2001; Schrey et al., 2005) which leads to enhancement in number of active sites for plant-fungus interaction. In a similar kind of study on *E. diversicolor,* treatment of an unidentified bacterium (Slf14) and *Bacillus* sp. (Elf28) yielded higher shoot dry weight than uninoculated control or ECM inoculated control (*L. bicolor* S238) (Dunstan et al., 1998).

Table 1. Characteristics of soil used in experiment

Parameter	Value
pH	6.42
Moisture	22.08%
Organic carbon	2.70%
Total nitrogen	0.26%
Available phosphorus	0.0084%
Carbon/Nitrogen ratio (C:N)	10.38
Water holding capacity	7.94%
Sand	72.80%
Silt	21.0%
Clay	6.2%

Table 2. Growth (cm) of *Q. leucotrichophora* plants with various inoculation treatments[#]

Treatments	Three months			Six months			Nine months		
	Shoot length	Root length	Collar diameter	Shoot length	Root length	Collar diameter	Shoot length	Root length	Collar diameter
Control	6.26	9.56	0.156	8.04	15.72	0.168	11.42	16.50	0.246
EMF	8.12	12.50	0.138	11.16	24.82	0.234	13.92	27.66	0.278
B1	6.60	11.94	0.150	8.68	16.38	0.174	10.22	13.80	0.228
B2	6.38	10.88	0.146	8.50	15.08	0.166	12.18	17.38	0.206
EMF+B1	8.76	14.20	0.146	12.50	25.86	0.248	13.12	29.64	0.296
EMF+B2	8.58	14.68	0.148	12.10	24.08	0.262	15.26	32.94	0.320
EMF+B1+B2	8.28	15.24	0.148	12.02	23.10	0.250	14.74	36.86	0.304
SE	0.83	1.45	0.04	1.32	2.15	0.044	1.78	2.60	0.027
CD at 5%	1.09	1.90	0.003	1.73	2.81	0.057	2.33	3.39	0.035
F	*8.70	*10.43	ns0.327	*10.78	*24.41	*4.879	*5.21	*60.34	*12.46

EMF — *S. bovista*, B1 — *B. subtilis* MB14, B2 — *P. fluorescens* MB9
[#]Values are mean of five replicates, * Significant at 5% ANOVA

DISCUSSION

Our results demonstrate that *S. bovista* has positive effects on *Q. leucotrichophora* growth under glasshouse nursery. Plant growth augmentation was further enhanced when fungus was co-inoculated with *P. fluorescens* MB9 and *B. subtilis* MB14. Similar kind of observations have been reported in previous co-inoculation studies of *Laccaria laccata with B. amyloliquefaciens* on Douglas fir with 60-80% mycorrhization increase (Duponnois and Garbaye, 1991), *L. laccata* with *Agrobacterium* sp. on *Pinus sylvestris* (Leyval and Berthelin, 1991), *L. laccata* with *B. subtilis*, *Bacillus* sp, *P. fluorescens* and *Pseudomonas* sp. on Douglas fir (Duponnois and Garbaye, 1992), *L. laccata* with *P. fluorescence on Q. robur* with 30% to 53% mycorrhization increase (Garbaye et al., 1992), *Laccaria bicolor* with *P. fluorescens* BBc6 on Douglar fir with 70% mycorrhization increase (Frey-Klett et al., 1997), *Pisolithus alba with* fluorescent pseudomonad strains HR13 and HR26 on *Acacia hilosericea* (Founoune et al., 2002). Considerable helper effect was noticed on the

Table 3. *S. bovista* colonization on *Q. leucotrichophora* roots with various inoculation treatments#

Treatments	Three months			Six months			Nine months		
	lateral roots	short roots	EMF colonz.(%)	lateral roots	short roots	EMF colonz.(%)	lateral roots	short roots	EMF colonz. (%)
Control	7.4	82.6	—	8.0	184.4	—	8.8	218.8	—
EMF	12.8	145.2	20.6	9.4	210.6	24.2	14.6	289.4	31.0
B1	11.0	103.6	—	14.2	150.4	—	16.8	255.0	—
B2	14.0	92.4	—	15.4	141.2	—	16.6	270.6	—
EMF+B1	13.2	163.6	23.2	11.2	271.2	27.2	18.4	311.2	32.0
EMF+B2	15.6	198.0	25.6	18.2	256.4	31.4	20.6	325.0	34.8
EMF+B1+B2	16.4	211.4	28.0	28.0	265.4	30.6	24.8	351.6	39.2
SE	2.1	13.1	2.4	2.8	12.4	2.5	2.7	12.9	3.3
CD at 5%	2.8	17.1	3.1	5.0	16.2	3.2	3.5	16.8	4.3
F	*10.0	*77.4	*149.6	*28.3	*96.9	*191.5	*17.1	*60.6	*159.8

EMF — *S. bovista*, B1 — *B. subtilis* MB14, B2 — *P. fluorescens* MB9colonz. —colonization#Values are mean of five replicates, * Significant at 5% ANOVA

Table 4. Dry weight of *Q. leucotrichophora* plants under different inoculation treatments#

Treatment	Three months				Six months				Nine months			
	Shoot (g)	Root (g)	Total (g)	Root Shoot Ratio	Shoot (g)	Root (g)	Total (g)	Root Shoot Ratio	Shoot (g)	Root (g)	Total (g)	Root Shoot Ratio
Control	0.202	0.326	0.528	1.618	0.812	1.250	2.062	1.570	1.144	1.868	3.012	1.646
EMF	0.244	0.376	0.620	1.548	1.732	2.614	4.346	1.516	2.134	2.960	5.094	1.394
B1	0.246	0.354	0.600	1.440	0.890	1.220	2.110	1.386	1.456	2.184	3.640	1.504
B2	0.206	0.364	0.570	1.782	1.006	1.468	2.474	1.470	1.580	2.326	3.906	1.474
EMF+B1	0.274	0.378	0.652	1.382	1.710	2.724	4.434	1.594	2.408	3.654	6.062	1.522
EMF+B2	0.268	0.382	0.650	1.434	1.830	2.458	4.288	1.344	2.736	3.196	5.932	1.168
EMF+B1+B2	0.242	0.390	0.632	1.622	1.890	2.906	4.796	1.538	2.812	3.934	6.746	1.402
SE	0.062	0.021	0.027	0.151	0.115	0.126	0.191	0.163	0.133	0.150	0.208	0.125
CD at 5%	0.024	0.027	0.035	0.197	0.150	0.164	0.249	0.212	0.173	0.196	0.272	0.163
F	*11.67	*5.20	*14.47	*4.27	*87.77	*174.84	*204.13	*4.67	*121.87	*133.86	*230.26	*7.00

EMF — *S. bovista*, B1 — *B. subtilis* MB14, B2 — *P. fluorescens* MB9#Values are mean of five replicates, * Significant at 5% ANOVA

formation of *L. fraterna* due to treatment with MHB isolates from France (*P. fluorescens* BBc6 and *B. subtilis* MB3) and indigenous isolates from Australia (*Bacillus* sp. Elf28 and pseudomonad Elf29) on *Eucalyptus diversicolor* seedlings (Dunstan et al., 1998).

Mycorrhizosphere bacteria isolated from *Q. leucotrichophora* and identified as *B. subtilis* MB14 and *P. fluorescens* MB9 can positively influence the *S. bovista* for modulating *Q. leucotrichophora* growth. However these bacteria strains moderately enhanced plant root and shoot growth when inoculated alone. The ability of these bacteria to increase ECM colonization suggests a direct bacterial effect on the metabolic status of ECM fungi. It is also suggested that these bacteria survive well in the trehalose rich environment of vegetative fungal hyphae (Danell et al., 1993). Many bacteria have been reported to release iron from a mineral, biotite, in oak-*S. citrinum* ectomycorrhiza (Uroz et al., 2007). It is also possible that dual inoculation enhances the photosynthetic rate, which enhances the mobilization of soluble sugars to host roots, thus increasing fungal growth and activity in the roots (Amijee et al., 1989; Hetrick, 1989).

In this study, co-inoculation treatment resulted in enhancement in number of lateral and short roots, similar to that reported with strains of *P. fluorescens* and *Laccaria bicolor* for increase in number of short roots in Norway spruce (*Picea abices*), supposedly due to IAA secretion (Karabaghli et al., 1998). Stimulatory infection of *Pisolithus alba* and increased root and shoot biomass of *Acacia hilosericea*, co-inoculated with fluorescent pseudomonad strains (HR13 and HR26) has also been reported from previous study (Founoune et al., 2002). ECM plants inoculated only with bacterial treatments were not different in root and shoot height, collar diameter and dry weight compared to control. No ECM occurrence was reported in these treatments.

The survival of nursery plantations in forest requires proper measures and is crucial for the successful reforestation program. Reports prove that plants inoculated with ECM fungi thrive better in degraded nutrient-poor and arid soils (Barea, 1991; Barea, 2000). Our study shows that *S. bovista* can increase *Q. leucotrichophora* growth under glasshouse conditions because this fungus is much adapted to the Himalayan environmental conditions (Yadav and Yadav, 2012). The fungus *S. bovista* is much abundant in Himalayan region due to puffball nature of its mushrooms.

Thus it can be conclude that ECM fungal effects plant growth can be further improved by its co-inoculation with MHB, which could prove useful in environmentally stressed climates (Barea, 1997; Requena et al., 1997) like Himalayan forests. Our results also show that co-inoculation of selected free-living bacteria isolated from adverse environments and ECM fungi can improve the formation and function of the ECM symbiosis, particularly when the conditions for plants growth are also adverse. Hence, to restore the *Q. leucotrichophora* forest cover in Himalaya and to prevent soil erosion, dual inoculation treatment with *S. bovista* and mycorrhizosphere bacteria (*B. subtilis* MB14 and *P. fluorescens* MB9) is advocated. Such practices would help nurserymen in plantations since co-inoculation increases plant biomass without affecting its root-shoot ratio. Moreover, same mycorrhizal rate can be obtained with lower doses of fungal inoculum due to MHB co-inoculation.

REFERENCES

1. Amijee, F., Tinker, P.B., Stribley, D.P. The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytologist*, 1989; **111** (3): 435-446.
2. Barea, J. Rhizosphere and mycorrhiza of field crops. In. *Biological Resource Management Connecting Science and Policy* (Balázs E, Galante E, Lynch J, Schepers J, Toutant J-P, Werner D, et al., eds). Springer Berlin Heidelberg, 2000; pp 81-92.
3. Barea, J.M. Mycorrhiza-bacteria interactions on plant growth promotion. In. *Plant growth promoting rhizobacteria Present status and future prospects* (Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S, eds). Paris: OECD, 1997; pp 50-158.
4. Barea, J.M. Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. In. *Advances in soil science* (Stewart BA, editor.). 15. New York Berlin Heidelberg: Springer, 1991; pp 1-39.
5. Danell, E., Alström, S., Ternström, A. *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom

Cantharellus cibarius. *Mycological Research*, 1993; **97** (9): 1148-1152.
6. Dunstan, W.A., Malajczuk, N., Dell, B. Effects of bacteria on mycorrhizal development and growth of container grown *Eucalyptus diversicolor* F. Muell. seedlings. *Plant Soil*, 1998; **201** (2): 241-249.
7. Duponnois, R. Les bactéris auxiliaires de la mycorrhization du Douglas (*Psudotsuga menziesii* (Mirb.) Franco) par *Laccaria laccata* souche S238. France: Thése de ì Université de Nancy I; 1992.
8. Duponnois, R., Garbaye, J. Aplication of AMB (auxillary mycorrhization bacteria) during inoculation of douglas fir by *Laccaria laccata* S238 in a forest nursery. *Revue Forestiere Francaise*, 1992; **44** (6): 491-500.
9. Duponnois, R., Garbaye, J. Effect of dual inoculation of Douglas fir with the ectomycorrhizal fungus *Laccaria laccata* and mycorrhization helper bacteria (MHB) in two bare-root forest nurseries. *Plant Soil*, 1991; **138** (2): 169-176.
10. Founoune, H., Duponnois, R., Ba, A., Sall, S., Branget, I., Lorquin, J., et al. Mycorrhiza helper bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. *New Phytologist*, 2002; **153** (1): 81-89.
11. Frey-Klett, P., Churin, J.-L., Pierrat, J.-C., Garbaye, J. Dose effect in the dual inoculation of an ectomycorrhizal fungus and a mycorrhiza helper bacterium in two forest nurseries. *Soil Biology and Biochemistry*, 1999; **31** (11): 1555-1562.
12. Frey-Klett, P., Garbaye, J. Mycorrhiza helper bacteria: A promising model for the genomic analysis of fungal-bacterial interactions. *New Phytologist*, 2005; **168** (1): 4-8.
13. Frey-Klett, P., Pierrat, J.C., Garbaye, J. Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas Fir. *Applied and Environmental Microbiology*, 1997; **63** (1): 139-144.
14. Garbaye, J., Bowen, G.D. Ectomycorrhizal infection of *Pinus radiata* by some microorganisms associates with the mantle of ectomycorrhizas. *New Phytology*, 1989; **112**: 383-388.
15. Garbaye, J., Churin, J.-L., Duponnois, R. Effects of substrate sterilization, fungicide treatment, and mycorrhization helper bacteria on ectomycorrhizal formation of pedunculate oak (*Quercus robur*) inoculated with *Laccaria laccata* in two peat bare-root nurseries. *Biology and Fertility of Soils*, 1992; **13** (1): 55-57.
16. Hauer, R.J., Dawson, J.O. Growth and iron sequestering of pine oak (*Quercus palustris*) seedlings inoculated with soil containing ectomycorrhizal fungi. *Journal of Arboriculture*, 1996; **22**: 122-130.
17. Hetrick, B.A.D. Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. In. *Nitrogen, phosphorus and sulphur utilization by fungi* (Boddy L, Marchand RM, Read DJ, eds). Cambridge: Cambridge University Press, 1989; pp 205-226.
18. Karabaghli, C., Frey-Klett, P., Sotta, B., Bonnet, M., Le Tacon, F. *In vitro* effects of *Laccaria bicolor* S238N and *Pseudomonas fluorescens* strain BBc6 on rooting of de-rooted shoot hypocotyls of Norway spruce. *Tree Physiology*, 1998; **18**: 103-111.
19. Leyval, C., Berthelin, J. Weathering of a mica by roots and rhizospheric microorganisms of pine. *Soil Science Society of America Journal*, 1991; **55** (4): 1009-1016.
20. Marx, D. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. V. Resistance of mycorrhizae to infection by vegetative mycelium of *Phytophthora cinnamomi*. *Phytopathology*, 1970; **60** (10): 1472-3.
21. Poole, E.J., Bending, G.D., Whipps, J.M., Read, D.J. Bacteria associated with *Pinus sylvestris–Lactarius rufus* ectomycorrhizas and their effects on mycorrhiza formation *in vitro*. *New Phytologist*, 2001; **151** (3): 743-751.
22. Requena, N., Jimenez, I., Toro, M., Barea, J.M. Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in mediterranean semi-arid ecosystems. *New Phytologist*, 1997; **136** (4): 667-677.
23. Rizvi, R., Khan, A. Response of Eggplant (*Solanum melongena* L.) to fly ash and brick kiln dust amended soil. *Biology and Medicine*, 2009; **1** (2): 20-24.
24. Schelkle, M., Peterson, R. Suppression of common root pathogens by helper bacteria and ectomycorrhizal fungi *in vitro*. *Mycorrhiza*, 1997; **6** (6): 481-485.
25. Schrey, S., Schellhammer, M., Ecke, M., Hampp, R., Tarkka, M. Mycorrhiza helper bacterium *Streptomyces* AcH 505 induces differential gene expression in the ectomycorrhizal fungus *Amanita muscaria*. *New Phytologist*, 2005; **168**: 205 - 216.
26. Shishido, M., Massicotte, H., Chanway, C.

Effect of plant growth promoting *Bacillus* strains on pine and spruce seedling growth and mycorrhizal infection. *Annals of Botany*, 1996; **77** (5): 433-442.

27. Uroz, S., Calvaruso, C., Turpault, M., Pierrat, J., Mustin, C., Frey-Klett, P. Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology*, 2007; **73** (9): 3019-3027.

28. Veena, P., Palni, U.T., Singh, S.P. Ectomycorrhizal influences on selected tree species from Central Himalayan region of India. *Symbiosis* (*Rehovot*), 2007; **44** (1/3): 115-119.

29. Villeneuve, N., Le Tacon, F., Bouchard, D. Survival of inoculated *Laccaria bicolor* in competition with native ectomycorrhizal fungi and effects on the growth of outplanted Douglas fir seedlings. *Plant Soil*, 1991; **135** (1): 95-107.

30. Yadav, A., Yadav, K. Physiological study of two ectomycorrhizal fungi isolated from Kumaun Himalaya. *Indian Forester*, 2012; **138** (1): 17-21.