Open Access
CODEN: IJBNHY
ISSN: 2278-778X
Original Research Article
International Journal of Bioassays

Effect of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi with and without rock phosphate on four forest tree seedlings

Sharanappa Jangandi[1], Chaitra B. Negalur[3], Narayan[2] and H.C. Lakshman[3]
[1]Zonal Agricultural and Horticultural, Research Station, Hiriyur, Chitradurga-577598, Karnataka, India.
[2]Department of Environmental Science, Kuvempu University. Shankarghatta- 577451, Shimoga, Karnataka, India.
[3]P.G. Department and Studies in Botany, Microbiology Laboratory, Karnatak University, Dharwad-580 003, India.

Received: November 27, 2016; **Revised:** December 12, 2016; **Accepted**: December 23, 2016
Available online: 1st January 2017

Abstract: The effect of phosphorus solubilizing bacteria *Bacillus polymyxa* and AM-mycorrhizal fungi *Rhizophagus fasciculatus* with and without rock phosphate treatments on growth of *Terminalia paniculata* and *T. tomentosa* were studied in nursery. The results showed that the combined inoculation of both PSB, AM fungi and rock phosphate produced vigorous plant growth of tree seedlings for quick planting. The experiments clearly demonstrated that the combined inoculation of PSB and AM fungi brought marked increase in plant growth, dry matter, and P uptake when, compared to individual inoculants or non-inoculated plants. The increase in growth was attributed to the increase in P uptake in shoots of the seedlings. The results indicated that both organisms have synergistic effect with additional 250 mg RP/kg rock phosphate treatment for *T.paniculata* Roth. and 150 mgRP/kg for *T.tomentosa* W.& A. in green house conditions.

Key words: *Terminalia paniculata; T. tomentosa;* Rock phosphate; *Rhizophagus fasciculatus; Bacillus polymyxa.*

Introduction

Forest trees are short-term renewable resource available to the mankind which not only are necessary for ecology and aesthetics but also as a source for obtaining basic necessities for people. Though timber production as of now does not suffice the needs of the ever-growing human population, there is definitely a scope to increase the production of timber. Keeping these needs in view the production of timber seedlings at nursery level are most essential. This could be possible, if the production can be increased with the application of biofertilizers like PSB and AM mycorrhiza, the mycobiont undergoes pronounced alterations of root system besides ensuring ecological sustainability (Mukerji and Sharma, 1996). AM fungi offer a great potential for sustainable plant growth (Lakshman, 2010).

In recent days, the potential of phosphate solubilising bacteria (PSB) and vesicular-arbuscular mycorrhiza (AM) fungal association on different plants has been well documented (Barea *et al.,* 1975; Barea and Azcon, 1983; Poonguzhali *et al.,* 2008; Lakshman, 2009). The simultaneous dual inoculation of phosphate solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi has been shown to stimulate plant growth in phosphorus deficient soil (Piccini and Azcon, 1987). However, therefore only a few reports, on forest seedlings inoculation by using PSB and AM at nursery level. The data on the above parameters were analyzed following randomized complete block design with 4 triplicates and the treatment means by Ducan's multiple rage test.

The main objective of this study was to evaluate the efficiency of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi individually and in combination in making P in rock phosphate available to four forest 'plants grown in P deficient soil.

Materials and Methods

One week old seedlings of two forest crops viz., *Terminalia paniculata* and *T. tomentosa* were subjected to the following microbial inoculations as follows:

1. Non-inoculated (control) without using PSB, AMF or rock phosphate,
2. Rock phosphate alone,
3. Phosphate solubilizing bacteria alone
4. Vesicular -arbuscular mycorrhiza fungi alone
5. PSB + Rock phosphate,
6. AMF + Rock phosphate,
7. PSB + AMF + Rock phosphate.

Before the inoculation of bioinoculants and phosphate of *Terminalia paniculata* and *T.tomentosa* seeds of both the tree species were soaked in tap water for 3-4 days and their surface were sterilized in 2% Streptomycin. These seeds were placed in large seed germinating pots containing sterilized three parts of pure soil and one part of garden soil. One week old seedlings were transplanted in to earthen experimental pots. Each pot

***Corresponding Author**:
Dr. Chaitra B Negalur,
Microbiology laboratory,
P.G. Department of Botany,
Karnatak university, Dharwad,
Karnataka, India.
E-mail: chainegalur@gmail.com

measuring 25X35cm diameter containing 6kgs of sterilized garden soil and pure sand in (V:V) 1:1 ratio.

The treatments were set up in a completely randomized design replicated thrice. The PSB used in the experiment was *Bacillus polymyxa* having 6.2 × 10^8 CFUs/g of carrier based culture inoculated @ 1 g/per each plant. Inoculum of *Rhizophagus fasciculatus* maintained in green house with Maize (*Zea mays*) as host (containing 270-300 spores /100 g soil) was applied @ 25 g per each plant. Each experimental pot contained 6 kg of sterilized garden soil. The soil had the pH of 6.8, E.C. of 0.32 mmhs cm^{-1}, 237 kg N ha^{-1}, 14.2 kg P_2O_5 ha^{-1} and 156 kg K_2O_5 ha^{-1}. Seedlings were watered on alternate days. Twice in a week 10ml of Hoagland solution was provided to each pot. After 180 days of sowing, plants samples were drawn and observations on plant height, dry weight of plants were measured. The plant material was oven dried for 72hrs at 70^0c, per cent mycorrhizal colonization of plants roots were cleared following of the procedure (Phillips and Hayman 1970). AM fungal spores were isolated according to Wet-Sieving and decanting procedure of Gerdemann and Nicolson, 1963. P uptake in the plants was determined by following the standard method (Vanadomolybdate phosphoric yellow colour the procedure proposed by (Jackson, 1973).

Results and Discussion

The mean height of plants increased by six to five and half times over the control plants at 180 days. The bacterial population remained significantly higher in the rhizosphere of mycorrhizal plants. The inoculation of PSB or AM Fungi alone did not appear to influence the plant dry matter and per cent of mycorrhizal colonization (Tables 1-2). However, a slight increase in plant height, dry matter and P uptake was observed in the plants treated with PSB plus rock phosphate and AM fungus plus rock phosphate. But the inoculation of PSB plus AM fungi with additional rock phosphate brought significantly increased plant growth, dry matter, percent AM fungi colonization and P uptake of *T. paniculata* (Tables 1-2). The combined action of bacteria solubilizing rock phosphate and mycorrhiza which enable the plant roots to rapidly absorb solubilizing P might have enhanced the ability of plants to utilize the phosphorus in case of *T. tomentosa.*

Table 1: The Effect of combined inoculation of AMF and PSB with different dosages of rock phosphate to *Terminalia paniculata* on plant growth, root length, plant biomass, percent root colonization, spore number and % P content in shoot for 180 days.

Treatments	Plant height (cm)	Root length (cm)	Plant dry weight (g)	Root/ shoot ratio (%)	% AMF colonization	AMF spore number/ 25g.soil	P-uptake in shoot (mg/plant)
Non-inoculated (control)	21.2a	15.2a	7.3b	0.72a	--	--	11.2a
50mgRp	34.5b	15.4b	9.5d	0.45c	34.3b	74	13.2b
100mgRp	34.7a	15.5b	9.6e	0.45g	43.2b	74	16.2b
150mgRp	35.7b	15.7c	9.6d	0.44e	47.2c	76	16.8c
200mgRp	37.2c	16.2b	11.1e	0.44b	51.0g	79	18.3a
50+AMF	42.0a	17.1a	14.3b	0.41c	49.1a	105	24.1ab
50+PSB	38.3c	16.5e	12.2b	0.43d	43.2c	43	25.2e
50+AMF+PSB	49.1d	18.4e	14.3b	0.37g	58.3a	111	27.5g
100+AMF	49.5a	23.5e	14.1c	0.47d	61.0c	117	27.0b
100+PSB	47.3b	21.4a	13.2a	0.45b	48.4b	46	27.1c
100+AMF+PSB	52.1c	32.1b	16.6a	0.61c	73.2a	127	31.0e
150+AMF	58.0g	33.2a	18.3c	0.57b	64.1b	119	31.1a
150+PSB	56.5e	32.3b	17.2c	0.57d	54.1e	47	32.2b
150+AMF+PSB	69.2b	37.5c	23.1b	0.54e	76.3g	131	32.2c
200+AMF	73.7b	41.1a	24.2c	0.56d	72.2b	123	31.1d
200+PSB	68.4e	41.3d	22.0b	0.60c	53.0a	48	32.0e
200+AMF+PSB	84.1b	43.2c	27.4a	0.51c	83.2d	146	34.3g

*Means in each column followed by the same letter are more significantly different (P<0.05) from each other according to DMR test.

Table 2: The Effect of combined inoculation of AMF and PSB with different dosages of rock phosphate to *Terminalia tomentosa* on plant growth, root length, plant biomass, percent root colonization, spore number and % P content in shoot for 180 days.

Treatments	Plant height (cm)	Root length (cm)	Plant dry weight (g)	Root/ shoot ratio (%)	% AMF colonization	AMF spore number/25g.soil	P-uptake in shoot (mg/plant)
Non-inoculated (control)	19.7a	13.8a	6.8b	0.70a	--	--	12.0a
50mgRp	23.2c	14.1b	8.3d	0.44ab	33.4b	69	12.4c
100mgRp	23.5b	14.5g	8.4e	0.62d	34.7c	71	13.1b
150mgRp	23.1bc	14.7b	8.5b	0.62c	39.3d	82	13.2ab
200mgRp	23.7b	14.9d	8.6c	0.63b	42.1e	87	14.5d
50+AMF	29.2a	21.2c	11.1d	0.73b	53.0a	103	17.1g
50+PSB	27.4c	18.1b	10.0e	0.66a	44.4ab	89	17.3e
50+AMF+PSB	38.7a	23.3e	13.6g	0.66b	58.1a	105	21.2b
100+AMF	46.0ab	28.29d	15.2e	0.61a	49.2c	107	19.5a
100+PSB	43.5b	25.1b	14.2a	0.57e	47.4a	91	17.5ab
100+AMF+PSB	52.4g	27.3c	16.8c	0.54b	63.2d	102	24.0b
150+AMF	67.3e	32.2a	18.5b	0.49g	71.0e	109	29.2c
150+PSB	64.2c	41.0b	19.1a	0.64d	54.9c	92	27.3de
150+AMF+PSB	73.1b	41.3a	24.7g	0.56b	74.3b	214	33.4b
200+AMF	61.2a	33.2c	17.1b	0.54e	61.4a	113	32.1a
200+PSB	63.4ab	35.0e	18.3c	0.55b	52.1d	97	31.2c
200+AMF+PSB	66.bd	35.1c	19.2b	0.53a	68.3c	112	30.0d

*Means in each column followed by the same letter are more significantly different (P<0.05) from each other according to DMR test.

Inoculation with AM fungus *Rhizophagus fasiculatus* with different levels 50mg, 100mg, 150mg and 200mg/kg soil, showed positive impact on Terminalia *paniculata* and *Terminalia tomentosa*. There was an increased plant height (84cm), root length(43.2cm), percent root colonization 83.2%, spore number 146/50gm. Soil with higher P content in Shoots of *Terminalia paniculata* after the combined inoculation of AM fungus phosphate solubilizer at 200mg rock phosphate treatment at 180 days. However, 150 mg rock phosphate treatment with AM fungus and *Bacillus polymyxa* brought significantly increased plant growth, biomass yield and P uptake in shoots of *Terminalia tomentosa* at 180 days. In both the seedlings PSB do not influence on AM fungal, spore population or percent of root colonization. Similarly, there was increased root/shoot ratio in control or non-inoculated plants over the inoculated plants at 180days. Though, all the inoculants increased plant height, dry weight, P uptake over the control plants, the combined inoculation of *Rhizophagus fasciculatus* and *B. polymyxa* registered the maximum plant height at 180 days after sowing. Combined inoculation with additional rock phosphate recorded significant AMF colonization and P uptake. *B. polymyxa* and arbuscular mycorrhizal fungi mutually help each other's development and this can result in synergistic interactions. Similar findings have been reported in other tree species (Young, 1990; Saxena and Tilak, 1996; Kalavathi *et al.,* 2000; Lakshman, 2010). In the present investigation, results revealed that the combined inoculation of *B. polymyxa* and *R. fasciculatus* with additional rock phosphate significantly increased plant height, P uptake and AM colonization over PSB alone or AM fungi alone inoculated plants. These results are consistent with earlier reports of (Young, 1990; Kalavathi *et al,* 2000; Haut *et al.,* 2002). It is relevant to mention that the possible synergistic effect would be the uptake by AM fungal hyphae and translocation into the plant of P released by PSB in soil (Garbage, 1991, Lakshman, 1994,1999, 2009,2010). Certain plant hormones like IAA and GA produced by the bacterial culture might induce the growth of other associated organisms like VA-mycorrhizal fungi (Azcon *et al.,* 1978). Thus, for maximizing the better growth and vigour of *T. paniculata* and *T. tomentosa* seedlings at nursery level, combined application of PSB, AM fungal and rock phosphate is recommended to obtain healthy timber seedlings stock at nursery level. It can be concluding that AM Fungus (*Rhizophagus fasiculatus*) Plus *Bacillus polymyxa* with addition rock phosphate 200 mg/kg soil is most stage. Similarly, AM fungus (*Rhizophagus fasiculatus*) plus *Bacillus polymyxa* with additional 150mg rock phosphate per kg soil can be recommended for *Terminalia tomentosa* seedlings at nursery stage.

References

1. Azcon, R., Azcon Aguilar, C. and Barea, J.M. "Effects of plant hormones present in bacterial cultures on the formation and response to VA-mycorrniza". *New Phytol* 80 (1978):359-364.
2. Barea, J.M., Azcon, R. and Hayman, D.S. "Possible synergestic interactions between Endogbne and phosphate solubilizing bacteria in low phosphate soils". In: *Enomycorrnizjas* (Eds.) F. B. Sanders, B. Mosse and P. B. Tinker. (1975): 409- 418.
3. Barea, J.M., Azcon, R. and Zcon-Agular, C. "Interaction between phosphate-solubilizing bacteria and VA-mycorrniza to improve the nutrition of rock-phosphate by plants in non-acidic soils". Third International Congress on Phosphorus compounds U.S.A. Nov. 15-19 (1983): 127-152.
4. Garbage, J. "Biological interactions in the mycorrhizosphere". *Experientia* 47(1991):370-375.
5. Huat, O. K.M., Awang, K., Hashim, A and Majd, N. M. "Effects of fertilizers and vesicular-arbuscular mycorrhizas on the growth and photosynthesis of *Azadirachta exelsa* (Jack) Jacob seedlings". *Forest Ecol. Mang* 158.1-3 (2002): 51-58.
6. Jackson, M.L. *"Soil Chemical Analysis".* Prentice of India (P) Ltd., New Delhi (1973).
7. Kalavathi, B.P., Santhakrishnan, P. and Divya, M.P. "Effect of VA mycorrnizal fungi and phosphorus solubilizing bacterium in Neem". *Indian Forester,* 72(2000): 67-70.
8. Lakshman, H.C. "VA-mycorrhizal studies on some important timber tree species". Ph.D. Thesis, Karnataka University, Dharwad, India (1994): 348pp.
9. Lakshman, H.C. "Dual inoculation of VA-mycorriza and *Rhizobium* is beneficial to *Pterocarpus marsupium Rox.* timber tree species". *Ecol. Env. Con.* 5.2(1999): 133-135.
10. Lakshman, H.C. 2009. "Growth Response of and Nitrogen fixation of *Phaseolus lunatus* (Lima Bean) with the inoculation of AM Fungi and *Rhizobium". Asian. Sci.* 4.1-2: 37-41.
11. Lakshman, H.C. (eds.) "Bioinoculants for integrated plant growth". M.D. Publications (Pvt.) Ltd. New Delhi. India. (2010):554.
12. Mukerji, K.G. and Dixon, R.K. "Mycorrhizae in forestation". In: *Proc. Internal Symp. Rehbi. Trop. Rain Forest Ecology* (eds.) Majid, N. M., Malek, I.A.A., Hamzah, M.Z. and Jusoff, K. University Pert. Malaysia. (1992): 66-82.
13. Mukerji, K.G. and Sharma, M. "Mycorrhizal relationships in forest eco-system". In: Forests -A *Global Perspective.* Eds. S. K. Majumdar, E. W. Miller and F. J. Brenner. *The Pennsylvania Academy.of Science,* U.S.A. (1996): 95-125.
14. Phillips, J.M. and Hayman, D.S. "Improved procedure of clearing and staining of vesicular-arbuscular mycorrnizal fungi for rapid assessment of infection". *Brit. Mycol. Soc,* 55 (1970): 158-161.

15. Piccini, D. and Azcon, R. "Effect of phosphate solubilizing bacteria and vesicular-arbuscular mycorrnizal fungi on the utilization of Bayovar rock Phosphate by alfafa plants using a sand-vermiculite medium". *Plant. Soil.* 101(1987): 45-50.

16. Poonguzhali, S. Madhiyan, M and Sa TM. "Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorous utilization of plants". J. Micro. Biol. Biotechnol. 18(2008):773-777.

17. Saxena, A.K. and Tilak, K.V.B.R. "Interaction of soil microorganisms with vesicular-arbuscular mycorrhiza". In: *New Approaches in Microbial Ecology.* (Eds: J. P. Tiwari, G. Saxena, N. Mittal, I. Tewari and B. P. Chamola). Aditya book publishers, New Delhi. (1996):187-204.

18. Young, C. "Effect of phosphorus solubilizing bacteria and VAM fungi on the growth of tree species in subtropical and tropical soils". *Soil Set PI. Nutr.* 36(1990): 225-231.

Cite this article as:

Sharanappa Jangandi, Chaitra B. Negalur, Narayan and H.C. Lakshman. "Effect of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi with and without rock phosphate on four forest tree seedlings". *International Journal of Bioassays* 6. 01 (2017): 5204-5207.

DOI: http://dx.doi.org/10.21746/ijbio.2017.01.003

Source of support: Nil

Conflict of interest: None Declared