ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5):1137-1141

ORIGINAL RESEARCH ARTICLE

Co-inoculation of AM fungi (*Glomus macrocarpum* and *Acaulospora laevis*) and *Bacillus megaterium* var. *phosphaticum* on the growth and nutrient contents of *Solanum xanthocarpum* Schrad. and Wendl

Nandini R.T.S* and P. Tholkappian

Department of Microbiology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

Received 27 May 2012; Revised 29 Sep 2012; Accepted 09 Oct 2012

ABSTRACT

Arbuscular mycorrhizal (AM) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomass. Phosphorous is essential for growth and productivity of plants and plays an important role in plants in many physiological activities. *Solanum xanthocarpum* is an important medicinal herb in Ayurvedic medicine. It is used to cure diseases bronchial, asthma, misperistalsis, piles and dyysuria and for rejuvenation. The present study was carried out to investigate the effect of AM fungi and *Bacillus megaterium* var. *phosphaticum* inoculations as individual, dual and consortium for the growth and nutrients content of *S. xanthocarpum*. A significant increased over control in percent root colonization (61.92%), spore number (65.80 /25 g of soil) of AM fungi. Plant height (61.54 cm), dry weight (7.92 g) and nutrient content N (36.70 g/plant), P (21.15 g/plant) and K (25.83 g/plant) were recorded.

Key words: Arbuscular mycorrhizal, *Solanum xanthocarpum.*

1. INTRODUCTION

The AM fungi abundant and ecologically very important for the plants in tropical countries and have been recognized as a promising alternative technology for reducing fertilizer requirement for the major crop species [1,2]. Soils in the tropics are either poor in phosphorus (P) and other essential nutrients have an immobile form of P [3]. Hence, AM fungal inoculum could be added to the soil for better uptake of P to enhance crop production in tropical countries. The soluble P released PSM (Phosphate Solubilizing Microorganism) is activity taken up by mycorrhizal roots [4]. Dual inoculation of PSM and AMF may enhance the plant acquisition of P from insoluble P sources [5].

The history of plants to be utilized as medicine as medicine in thousands of years old. Medicinal plants are considered one of the major sources of drug in modern as well as traditional medicinal systems throughout the world. India with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine (Ayurveda, Siddha Unani, Amchi and local health traditions) provide a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people [6].

S. xanthocarpum is commonly known as the Indian night shade (English) or Kantankattiri (Tamil). It occurs throughout India, in dry situations as a weed along the roadsides and wastelands. It is also distributed in Ceylon, Asia, Malaya, Tropical, Austrana and Polynessia [7]. Roots of *S. xanthocarpum* possess the ability to cure asthma, flatulence, sore throat, toothache, bronchinal spasm and constipation. Stem, flowers and fruits are bitter and carminative and are prescribed for relief in burning sensation in the feet. Whole plant is used for skin infections and paste of the plant is orally used for chest congestion. The present work was focused to evaluate the efficiency of the interaction between AM fungi (*G. macrocarpum* and *A. laevis*) and *B. megaterium* var. *phosphaticum* on the growth and nutrient contents of *S. xanthocarpum*.

2. MATERIALS AND METHODS

The present study was conducted at Department of Microbiology, Annamalai University, Annamalai Nagar, India. The seeds of *S. xanthocarpum* were collected from Marunthuvalmalai hills of Kanyakumari District, Tamil Nadu. Seeds were soaked in 5% sodium chloride and sterilized with

***Corresponding Author:** Nandini R.T.S**, Email:** nandinirts@yahoo.com

0.1% $HgCl_2$ for 1 min followed by thorough washings with sterile distilled water atleast for 7-10 times. The sterile seeds were used for sowing in cement pots of size (45 x 30 x 45 cm) filled with sand and garden soil (1:3).

The efficient strains of AM fungi (*G. macrocarpum* and *A. laevis*) were previously isolated and *B. megaterium* var. *phosphaticum* were also isolated from soil of *S. xanthocarpum* and maintained at Department of Microbiology, Annamalai University. Phosphobacterial slurry prepared with Carboxy Methyl Cellulose (CMC) was pelleted with seeds of *S. xanthocarpum* followed by shade drying. The AM fungi were applied 3cm below the soil surface as thin layer @ of 5.0 g/pot before sowing. The pots culture experiment was conducted with individual, dual and consortium inoculation of *G. macrocarpum*, *A. laevis* and *B. megaterium* var. *phosphaticum* with the following treatment in triplicates.

Treatments

T_1 - Control

T_2 - *G. macrocarpum*

T_3 - *A. laevis*

T_4 - *B. megaterium* var. *phosphaticum*

T_5 - *G. macrocarpum* + *A. laevis*

T_6 - *G. macrocarpum* + *B. megaterium* var. *phosphaticum*

T_7 - *A. laevis* + *B. megaterium* var. *phosphaticum*

T_8 - *G. macrocarpum* + *A. laevis* + *B. megaterium* var. *phosphaticum*

Samples were collected from each treatment on 30, 60 and 90 DAS and analyzed for different parameters. The plant height was measured from ground level to apical meristem and expressed in cm. The dry matter content was estimated by drying the plants in Hot air oven at 60° C till a constant weight was obtained and expressed in grams. The N, P, K contents of the plant was estimated by Microkjeldahl, Vandomolybdate and Flame photometer [8.9,10] respectively. AM fungal spores per 25 g of rhizosphere soil and AM fungal root colonization percentage were estimated by following the standard methods s[11, 12]. The data were statistically analyzed by Randomized Block Design (RBD).

3. RESULTS AND DISCUSSION

Pot culture experiment was carried out to study the effect of AM fungi (*G. macrocarpum* and *A. laevis*) and *B. megaterium* var. *phosphaticum* as individual, dual and consortium in *S. xanthocarpum*. The effect of AM fungi and *B. megaterium* var. *phosphaticum* on growth parameters (plant height and dry matter content) on 30, 60 and 90 Days were presented in (**Table 1**).

Among the eight treatments, the maximum plant height was recorded in T_8 (inoculation of AM fungi and *B. megaterium* var. *phosphaticum*) as 61.54 cm followed by dual inoculation treatments T_5, T_6 and T_7 (59.64, 58.43 and 57.92 cm). In individual inoculation treatments (T_2, T_3 and T_4) recorded the plant height 56.06, 55.40 and 53.50 respectively, whereas the minimum plant height was observed in control (without inoculation) T_1 as 51.10 cm.

The least dry matter production observed in control (without inoculation of AM fungi and *B. megaterium* var. *phosphaticum*) as 3.98 g/plant. In individual inoculation treatments T_2, T_3 and T_4 recorded the dry weight of 6.25, 5.72 and 5.10 g/plant. The next best response observed in dual and consortium treatments *viz*., T_5, T_6, T_7 and T_8 (7.34, 7.12, 6.84 and 7.92 g/plant).

Simultaneous application of PSM and AM fungus has been shown to stimulate plant growth more than inoculation of either organism alone in certain situations when the soil is P deficient [13]. The maximum plant height and dry matter production in alfalfa with dual inoculation of *G. fasciculatum* and *B. megaterium* [14]. The fact that plant growth and nutrient uptake increased in the presence of AM fungi suggested a strong synergistic relationship between root colonization, P uptake and growth promotion. Many studies showed the synergistic effect of co inoculation of PSM and AM fungi on growth and nutrition on plants.

The increase of N contents in plant parts could be to increase in translocation of soil N to the plants that was possibly mediated by AM fungus [15]. *G. mossae* and *A. laevis* (AMF) also showed better result for increasing the P content of *L. usitatissimum*. The higher P uptake in plants inoculated with PSM and AM fungus in presence of MRP may be attributed to greater absorption of P by AM [16].

In the present study the maximum N, P and K contents was observed in inoculation of T_8 (AM fungi and *B. megaterium* var. *phosphaticum*) as 36.70, 21.15, 25.83 g/plant at 30 DAS. The nest best response recorded in dual inoculation T_5 (35.36, 19.65 and 24.45 g/plant) T_6 (34.70, 19.02 and 23.95 g/plant) and T_7 (34.10, 18.26 and 23.16 g/plant) followed by single inoculation T_2 (32.76, 16.73 and 21.75 g/plant), T_3 (32.10, 16.20 and 20.98 g/plant) and T_4 (30.76, 14.69 and 19.60

g/plant). The least amount of plant N, P and K contents was recorded in control (without inoculation) T_1 as 28.20, 11.73, 15.98 g/plant (**Table 2**).

The maximum percentage of root colonization and spore number recorded in consortium treatment (T_8) as 61.92% and 65.80/25 g of soil respectively. In dual inoculation treatment the higher per cent root colonization and spore number was observed in T_5 (60.38% and 65.80/25 g of soil), T_6 (59.75% and 63.15/25 g of soil) and T_7 (58.93% and 62.14/25 g of soil) followed by individual inoculation of *G. macrocarpum* T_2 (57.44% and 60.29/25 g of soil), *A. laevis* T_3 (56.72% and 59.72/25 g of soil) and *B. megaterium* var. *phosphaticum* T_4 (55.20% and 57.84/25 g of soil). The minimum per cent root colonization and spore number was observed in (T_1) control 52.00% and 53.80/25 g of soil (**Table 3**).

Root colonization percentage in fennel in in**o**culated treatments with two species of mycorrhizal fungi (*G. fasciculatum* and *G. macrocarpum*) was substantially more than non-inoculated treatments[17]. PSB like *B. megaterium* var. *phosphaticum*, *B. brevis* and *B. subtilis* behaved as mycorrhizae helper bacteria because they promoted root colonization and spore number when associated with mycorrhizal fungi and phosphate solubilizing bacteria combinations [16,17].

Thus the present study reveals that the combined inoculation of AM fungi (*G. fasciculatum* and *G. macrocarpum*) and *Bacillus megaterium var. phosphaticum* more effective in *S. xanthocarpum* plant. These bioinoculants have also proved to improve the growth and nutrient content of medicially important plants. *S. xanthocarpum* are while the plant has used successfully in Ayurvedic medicine for centuries more clinical trials should be conducted to support its and therapeautical uses.

Table 1: Effect of AM fungi and *B. megaterium* var. *phosphaticum* on the growth parameters on *S. xanthocarpum*

T. No	Treatments	Plant height (cm)			Dry weight (g)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	Control	7.58	29.86	51.10	0.30	3.45	3.98
T2	*Glomus macrocarpum*	12.44	35.82	56.06	0.84	4.60	6.25
T3	*Acaulospora laevis*	11.92	34.67	55.40	0.78	4.45	5.72
T4	*B. megaterium* var. *phosphaticum*	10.78	33.10	53.50	0.60	4.15	5.10
T5	*Glomus macrocarpum* + *Acaulospora laevis*	14.62	38.85	59.64	1.06	5.15	7.34
T6	*Glomus macrocarpum* + *B. megaterium* var. *phosphaticum*	14.02	37.92	58.43	1.02	5.02	7.12
T7	*Acaulospora laevis* + *B. megaterium* var. *phosphaticum*	13.58	37.38	57.92	0.98	4.90	6.84
T8	*Glomus macrocarpum* + *Acaulospora laevis* + *B. megaterium* var. *phosphaticum*	15.78	40.40	61.54	1.20	5.47	7.92
	SE	0.54	0.75	0.90	0.05	0.13	0.27
	CD	1.10	1.52	1.82	0.12	0.28	0.56

Table 2: Effect of AM fungi and *B. megaterium* var. *phosphaticum* on the NPK content on *S. xanthocarpum*

T. No	Treatments	N (g/plant)			P (g/plant)			K (g/plant)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	Control	27.60	54.45	97.90	11.13	32.00	66.34	15.98	29.74	58.66
T2	*Glomus macrocarpum*	32.76	60.70	104.76	16.73	37.70	73.50	21.75	35.30	65.25
T3	*Acaulospora laevis*	32.10	60.28	103.58	16.20	37.25	72.62	20.98	34.65	64.44
T4	*B. megaterium* var. *phosphaticum*	30.76	58.55	101.66	14.69	35.60	70.84	19.60	33.00	62.70
T5	*Glomus macrocarpum* + *Acaulospora laevis*	35.36	64.10	108.56	19.65	40.90	76.97	24.45	38.55	68.69
T6	*Glomus macrocarpum* + *B. megaterium* var. *phosphaticum*	34.70	63.50	107.60	19.02	40.20	76.10	23.95	37.88	67.92
T7	*Acaulospora laevis* + *B. megaterium* var. *phosphaticum*	34.10	62.46	106.70	18.26	39.34	75.30	23.16	36.97	67.00
T8	*Glomus macrocarpum* + *Acaulospora laevis* + *B. megaterium* var. *phosphaticum*	36.70	65.84	110.58	21.15	42.54	78.76	25.83	40.20	70.44
	SE	0.65	0.85	0.94	0.74	0.79	0.87	0.67	0.81	0.86
	CD	1.30	1.70	1.88	1.48	1.59	1.75	1.34	1.62	1.72

Table 3: Effect of AM fungi and *B. megaterium* var. *phosphaticum* on percent root colonization and spore number on *S. xanthocarpum*

T. No	Treatments	Per cent root colonization			Spore number/25 g of soil		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T1	Control	25.40	35.10	52.00	30.34	40.68	53.80
T2	*Glomus macrocarpum*	29.29	40.35	57.44	36.02	46.87	60.29
T3	*Acaulospora laevis*	28.75	39.75	56.72	35.26	46.02	59.72
T4	*B. megaterium* var. *phosphaticum*	27.60	38.38	55.20	33.60	44.27	57.84
T5	*Glomus macrocarpum + Acaulospora laevis*	31.50	42.96	60.38	39.28	48.89	63.92
T6	*Glomus macrocarpum + B. megaterium* var. *phosphaticum*	30.94	42.12	59.75	38.75	48.10	63.15
T7	*Acaulospora laevis + B. megaterium* var. *phosphaticum*	30.45	41.70	58.93	37.70	47.25	62.14
T8	*Glomus macrocarpum + Acaulospora laevis + B. megaterium* var. *phosphaticum*	32.64	44.32	61.92	40.92	50.64	65.80
	SE	0.54	0.65	0.73	0.80	0.84	0.90
	CD	1.10	1.32	1.48	1.62	1.70	1.82

REFERRENCE

1. Mosse B. 1981. Vesicular-arbuscular mycorrhizal research in tropical agriculture. Research Bulletin 194, p 82. Hawaii Institute of Tropical Agriculture and Human Resource, University of Hawaii, Honolulu, Hawaii.
2. Yost R.S and R.L Fox. 1979. Contribution of mycorrhizae to P nutrition of crops growing on an oxisol. Agron J. 71: 903-908.
3. Menge J.A. 1983. Utilization of vesicular arbuscular mycorrhizal fungi in agriculture. Can J Bot. 61: 1015-1024.
4. Barea J.M., R. Azcon and C. Azcon-Aguilar. 1983. Interactions between phosphate-solubilizing bacteria and VA mycorrhiza to improve the utilization of rockphosphate by plants in non acidic soils. In: 3rd International Congress on Phosphorus Compounds, Brussels. Institut Mondial du Phosphate, Casablanca, pp 127–144.
5. Piccini D and Azcon R. 1987. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the utilization of Bayovar rockphosphate by alfalfa plants using a sand vermiculite medium. Plant Soil 50 :45–50
6. Pandey M.M., S. Rastogi and A.K Rawat. Indian drug for general wealth care: An overview Internet. J. Altern Med. 6:1.
7. Shenth A.K. 2005. The herbs of Ayurveda. A.K. Sheth Publishers, Vol. IV. 1044.
8. Piper C.S. 1950. Soil and plant analysis, Inter. Sci. Pub. Inc., New York, U.S.A., p. 368.
9. Jackson M.L. 1973. Soil chemical analyses, Prentice Hall, New Delhi, pp.111-120.
10. Jones, J.B and R.A. Isaac. 1969. Comparative elemental analyses of plant tissue by spark emission and atomic absorption spectroscopy. Agronomy J., 61:393-394.
11. Daniels, B.A. and Skipper, H.D. 1982. Methods recovery and quantitative estimation of propagules from soil. In: Schenck NC, (eds) Methods and Principles of Mycorrhizal Research, American phytopathological society, St. Paul, Minnesota. pp. 224.
12. Philip, J.F. and D.S. Hayman. 1970. Improved procedures for clearing root parasitic and staining vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-160.
13. Mohammad Sahir Khan and Almas Zaidi. 2006. Influence of composite inoculation of phosphate solubilizing organisms and an arbuscular mycorrhizal fungus on yield, grain protein and phosphorus and nitrogen uptake by green gram. Archives of agronomy and soil science.

14. Piccini D and R. Azcon 1987 Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the utilization of Bayovar rockphosphate by alfalfa plants using a sand vermiculite medium. Plant Soil 50 :45–50
15. Kapoor, R., B. Giri and K.G. Mukerji. 2004. Improved growth and essential oil yield quality in (Foeniculum vulgare mill) on mycorrhizal inoculation supplemented with P-fertilizer. Bioresource Technol., 93: 307-311.
16. Frey-Klett, P., J.C. Pierrat and J. Garbaye. 1997. Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria biocolor* and Douglas fir. Appl. Environ. Microbiol, 63: 139-144.