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Growth responses in *Acacia nilotica* inoculated with VAM fungus (*Glomus fasciculatum*), *Rhizobium* sp. and *Trichoderma harzianum*

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Experiments were conducted to determine the influence of VAM fungi, *Rhizobium* sp. and *Trichoderma harzianum* individually as well as in combinations on the biomass yield of *A. nilotica*. The biomass yield of different treated and untreated plants were determined by observing various parameters. Dual inoculation with VAM and *Rhizobium* sp. resulted in maximum plant growth and nodulation. It was concluded that the VAM and *Rhizobium* was the best biofertilizer for the better biomass production of multipurpose plant of *A. nilotica*.

Key words : Biomass, *Acacia nilotica*, *Rhizobium*, VAM and *Trichoderma harzianum*

INTRODUCTION

Restoration and maintenance of soil fertility is a basic but critical ecological problem especially in the region where the soils are nutrient deficient, particularly in phosphorus and nitrogen. Lack of microbial nutrient cycling is the problem of wastelands. Hence there is a need to restore the soil fertility for the establishment of green cover. The degraded soils are reclaimed through afforestation programmes with multiutility forest legume tree species along with beneficial microbes. Leguminous plants can form two types of symbiotic associations with microorganisms. One with *Rhizobium* sp. involved in N_2 fixation, the other with VAM fungi, concerned with the better uptake of P and other nutrients (Crush, 1974). It is now established that the enhanced growth of plant is due to absorption of ions especially P by the fungus from the soil and subsequent transfer to plant (Hayman and Mosse, 1972). Inoculation of legumes with VA mycorrhizal fungi can stimulate nodulation and N_2 fixation (Mosse, 1981). The purpose of the present investigation was to assess the role of VAM fungi, *Rhizobium* sp. and *Trichoderma harziamum* alone and in combinations in improving the seedling quality.

MATERIALS AND METHODS

Native VAMF endophyte, *Glomus fasciculatum* isolated from the rhizosphere of *A. nilotica* was multiplied and maintained on *Zea mays* for three months. *Rhizobium* sp. isolated from the nodules of the plants was cultured and maintained in yeast extract mannitol broth. *Trichoderma harzianum* isolated from the rhizoplane of *A. nilotica* was multiplied on wheat bran:sawdust:water (3:1:4) medium. The medium

was sterilzied in autoclave for 1 h at 121°C. The autoclaved medium inoculated with *T. harzianum* and incubated in BOD incubator for 10 days at 28±1°C for mass production of the inoculum for present investigation.

Seeds were treated with concentrated H_2SO_4 and thoroughly washed with running tap water and soaked in water overnight. Germinated seeds were rolled with *Rhizobium* inoculum. Ten per cent mycorrhizal inoculum of *G. mosseae* by weight consisting of spores and root pieces were inoculated in each pot (30x25 cm). A thin layer of mycorrhizal inoculum was placed below the surface before sowing seeds. Ten per cent (by wt) of fungal inoculum of *T. harzianum* was added in each pot. Soil without any microbial addition served as control. The experiment was designed with the following treatments with *A. nilotica.*

Soil (control)
Soil + *G. fasciculatum*
Soil + *Rhizobium* sp.
Soil + *Rhizobium* sp + *G. fasciculatum*
Soil + *T. harzianum*
Soil + *T. harzianum* + *G. fasciculatum*

Five replicates of each treatment were taken. Interaction between various treatments was studied after 45 and 90 days and following parameters were observed.

1. Shoot lenght in cm
2. Shoot dry wt in g
3. Root length in cm
4. Root dry wt in g
5. Nodule Number
6. Nodule wt in g
7. Percentage VAM infection

For dry wt, root and shoot pieces were dried in hot air oven at 80°C for 12 h.

RESULTS AND DISCUSSION

It is evident from Table 1.1 and 1.2 that seedling inoculated with either *G. fasciculatum* or *Rhizobium* sp. or *Trichoderma harzianum* showed increased growth over control. Seedlings inoculated with *Rhizobium* sp. and *G. fasciculatum* showed increased growth parameters over the individual inoculations or over other combined inoculations. The height of the plants, root and shoot dry weights, nodule number, nodule dry wt. and percentage VAM infection was significantly more in combination of *Rhizobbium* sp. and *G. fasciculatum* as compared to other treatments. Results of the present study show that in low phosphate soils, the plant growth and nodulation are dependent on the mycorrhizal symbiosis as in the case of earlier studies (Crush 1974; Mosse *et al.* 1976). Inoculation of seedlings with native *Rhizoium* sp. or VAM fungi either individually or in combinations increased the seedling growth and biomass over uninoculated controls. The plants require relatively larger amount of P for optimum growth, nodulation and nitrogen fixation (Carling *et al.* 1978).

Table 1.1 : The response of *A. nilotica* to inoculation of *Glomus fasciculatum*, *Rhizobium* sp. and *Trichoderma harzianum* after 45 days

Treatment	Shoot Lenght (cm)	Shoot Dry weight(g)	Root Length (cm)	Root dry Weight (g)	Nodule Number	Nodule Weight (g)	VAM infection (%)
Control	32 ± 0.093	0.8 ± 0.093	18 ± 0.127	0.1 ± 0.000	16 ± 0.935	0.074 ± 0.0007	2 ± 0.707
G. fasciculatum	40 ± 0.093	1.3 ± 0.093	23 ± 0.093	0.2 ± 0.035	22 ± 0.353	0.124 ± 0.0009	20 ± 0.707
Rhizobium sp.	3 4± 0.093	0.9 ± 0.093	28 ± 0.127	0.2 ± 0.035	29 ± 0.935	0.069 ± 0.001	-
G. mosseae							
+ *Rhizobium* sp.	47 ± 0.127	1.4 ± 0.093	31 ± 0.135	0.3 ± 0.035	44 ± 0.935	0.126 ± 0.0007	29 ± 0.935
T. harzianum	39 ± 0.127	0.8 ± 0.093	29 ± 0.093	0.2 ± 0.035	20 ± 0.935	0.036 ± 0.001	-
G. fasciculatum +							
T. harzianum	40 ± 0.353	1 ± 0.061	30 ± 0.692	0.3 ± 0.093	6 ± 0.935	0.005 ± 0.0003	25 ± 0.353

± = Standard deviation

Table 1.2 : The response of *A. nilotica* to inoculation of *Glomus fasciculatum*, *Rhizobium* sp. and *Trichoderma harzianum* after 90 days

Treatment	Shoot Lenght (cm)	Shoot Dry weight(g)	Root Length (cm)	Root dry Weight (g)	Nodule Number	Nodule Weight (g)	VAM Infection (%)
Control	35 ± 0.093	0.93 ± 0.009	30 ± 0.127	0.61 ± 0.009	29 ± 0.353	1.16 ± 0.003	1 2± 0.707
G. fasciculatum	49 ± 0.154	2.90 ± 0.003	32 ± 0.127	1.33 ± 0.03	34 ± 0.353	0.20 ± 0.012	49 ± 0.612
Rhizobium sp.	46 ± 0.093	2.63 ± 0.009	35 ± 0.093	1.33 ± 0.003	36 ± 0.707	0.22 ± 0.003	-
G. mosseae							
+ *Rhizobium* sp.	55 ± 0.154	2.94 ± 0.009	36.5 ± 0.093	1.51 ± 0.009	58 ± 0.935	0.36 ± 0.009	60 ± 0.707
T. harzianum	47 ± 0.127	2.70 ± 0.009	34 ± 0.093	0.94 ± 0.009	28 ± 0.353	0.18 ± 0.007	-
G. fasciculatum +							
T. harzianum	50 ± 0.154	2.72 ± 0.007	35.2 ± 0.093	1.36 ± 0.009	30 ± 0.707	0.19 ± 0.003	36 ± 0.612

± = Standard deviation

It has been clearly shown that VAM can improve plant growth through increased uptake of P especially in lowfertile soils (Safir *et al.*, 1972; Mosse *et al.*, 1973; Gerdemann, 1975). Inoculations with *Rhizobium* sp. and VAM fungi had a significantly positive effect on growth and nodulation in *A. nilotica*. Similar results were reported by Bagyaraj *et al.* (1979); Varma (1979); Bala *et al.* (1989); Dixon *et al.* (1993); Mandal and Kaushik (1995) and Mehrotra (1995). Dual inoculation increased the efficiency of the shoot and root system in providing the plant with essential levels of P and N, for grwoth in *A. nilotica*. Data clearly indicate that dual inoculation with VAM and *Rhizobium* show a significant increase in biomass

productivity. Hence in the seedling production of *A. nilotica* due consideration should be given to symbiotic organisms such as mycorrhizal fungi and nodulating bacteria. Improvement of seedling quality is essential for the survival of seedlings in degraded areas of low soil fertility and biological activity. However, tests for VAM, plants and soil responses under field conditions are needed for specific VAM effects.

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REFERENCES

Bagyaraj, D. J., Manjunath, A. and Patil, I.R.B. (1979). Interaction between VA mycorrhiza and *Rhizobium* and their effect on soyabean in field. *New Phytol.* **82 :** 141-145.

Bala, K., Rao, A. V. and Tarafdar, J.C. (1989). Occurence of VAM associations in different plant species of the Indian desert. *Arid Soil Research and Rehabilitation* **3 :** 391-396.

Carling, D. R., Riehle, W. G., Brown, M. F. and Johnson, D. R. (1978). Effects of a vesicular - arbuscular mycorrhizal fungus on nitrate - reductase and nitrogenase activities in nodulating and non-nodulating legumes. *Phytopathol.* **58 :** 1590-1596.

Crush, J. R. (1974). Plant growth responses to vesicular - arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legume. *New Phytol.* **73 :** 743-749.

Dixon, R. K., Garg, V. K. and Rao, M. V. (1973). Inoculation of *Leucaena* and *Prosopis* seedlings with *Glomus* and *Rhizobium. Arid soil Research and Research and Rehabilitation* **7(3) :** 133-144.

Gerdemann, J.W. (1975). Vesicular arbuscular mycorrhizae. In : *The Development and Function of Roots,* (eds) J. G. Torrey and D. T. Clarkson, Academic Press, New York, pp. 575-595.

Hayman, D. S. and Mosse, B. (1972). Plant growth response to vesicular - arbuscular Mycorrhiza. III. Increased uptake of labile P from soil. *New Phytol.* **71 :** 41-47.

Mehrotra, M. D. (1995). A practical approach to mycorrhizal contrainerised seedlings in forest nurseries. *Indian Forester* **121(2) :** 670-672.

Mosse, B. (1981). Vesicular-arbuscular Mycorrhizal research for tropical agriculture. *Research Bulletin* **194:** 5-81.

Moosse, B., Hayman, D.S. and Arnold, D.J. (1973). Plant growth responses to vesicular arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P deficient soils labelled with ^{32}P. *New Phytol.* **72:** 809-875.

Mosse, B., Powell, C.L. and Hayman, D. S. (1976). Plant growth responses to vesicular - arbuscular mycorrhiza. IX. Interactions between VA mycorrhizal, rock phosphate and symbiotic nitrogen fixation. *New Phytol.* **76:** 331-342.

Safir, G.R., Boyer, J.S. and Gerdemann, J.W. (1972) Nutrient status and mycorrhizal enhancement of water transport in soyabean. *Plant Physiol.* **49:** 700-703.

Varma, A. 1979. Vesicular-arbuscular mycorrhiza and nodulation in soyabean. *Folia Microbiologica.* **24:** 666-671.

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