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SYNERGISTIC EFFECT OF DUAL INOCULATION (VESICULAR-ARBUSCULAR MYCORRHIZAE) ON THE GROWTH AND NUTRIENTS UPTAKE OF *MEDICAGO SATIVA*

I.A. KHAN[1*], N. AYUB[2], S.N. MIRZA[1], S.M. NIZAMI[1] AND M. AZAM[3]

[1]Department of Forestry & Range Management, University of Arid Agriculture,Rawalpindi, Pakistan
[2]Department of Microbiology, Quaid-e-Azam University. Islamabad, Pakistan
[3]Department of Mathematics and Statistics, University of Arid Agriculture, Rawalpindi,Pakistan.
**Corresponding author: Telephone# 051-9062269*

Abstract

Inoculation effect of single and dual vesicular arbuscular mycorrhizal (VAM) with *Gigaspora rosea, Glomus intraradices* + *Gigaspora rosea* and *Glomus etunicatum* + *Glomus intraradices* on the growth and nutrients uptake (NPK) on *Medicago sativa* were carried out. Yield in the response of shoot and root dry weight was significantly increased due to dual inoculation than single inoculation. The dry weight of dual inoculation in shoot was 10.61g and 12.64 g and in root was 3.11 and 3.68 g, respectively while the nitrogen, phosphorus and potassium uptake % was also more due to dual inoculations.

Introduction

The rangeland productivity in Pakistan is 10 to 50% of its potential due to inadequate and erratic rainfall, poor soil conditions, over grazing etc., (Qurashi *et al.*, 1993). Soils of rangelands in Pakistan are calcarious, alkaline and dominated by mica mineralogy and also difficient in phosphorus upto 90% (Rashid & Qayyum, 1990, Memon *et al*., 1992). Grasses are common but shrubs are dominant in Rangelands of Pakistan. Legume forage are also being gradually introduced in our rangelands to increase and improve the forage productivity.

Vesicular arbuscular mycorrhizae occur widely under various environmental conditions and are found in association with a number of leguminous crop (Islam & Ayanaba, 1981). Arbuscular mycorrhizal fungi are known to occur wildely under various environmental condition and are found in association with forage crops (Carrenho *et al.*, 2001., Chen *et al;* 2005., Souchie *et al;* 2006). Growth enhancement efforts of *Aspergillus fumigatus* in mung bean and cluster bean an well documented (Trafdar *et al.*, 1992, 1995). The beneficial effects of mycorrhizae on alfalfa production are associated with a better nutrient balance (Hamel *et al.*, 1992).

The beneficial effects of inoculation with vesicular arbuscular mycorrhizae fungi (VAM fungi) have been shown by several workers (Guehl & Garbaye, 1990; George *et al.*, 1995). The mycorrhizae has the beneficial effect on the phosphate nutrition of crop plants in soil low in phosphorous has been qorated (Chen *et al*., 2005; Duponnois, 2006). In the present study, a pot experiment was carried out to assess the effect of inoculating *Medicago sativa* with *Gigaspora rosea, Glomus intraradices* + *Gigaspora rosea* and *Glomus etunicatum* + *Glomus intraradices* to understand the interaction effects in term of biomass and nutrient uptake.

In legumes, VAM fungi increase nodulation and N2 fixation as a consequence of improved phosphorus nutrition. (Athar *et al*., 2005). Colonization with AM fungi improves nitogenase activity in soybean crop (Fonseca, *et al.*, 1993). Sundaresan *et al;*

(1987) studied uptake of N by VAM fungus *Glomus faciculatum* in cowpeas at 4 different moisture levels. It was found that total N content increased only in roots but not in the shoots. Abdelgadir (1998) studied 455 soybeans (*Glycine max* (L.) Merr.) varieties from different parts of the world to determine their ability to grow in P deficient soils and to determine the effects of vesicular arbuscular (VA) mycorrhizal fungi in the humid tropics. The result indicated that VA mycorrhizal fungi differed in their effects on plant growth, suggested the importace of evaluating the compatibility of VA mycorrhizal fungus and plant host.

Materials and Methods

Pots experiments were conducted during 1996-1998 at Quaid-I-Azam University, Islamabad and University of Arid Agriculture, Rawalpindi, Pakistan. Inoculation of *Gigaspora rosea*, *Glomus intraradices* + *Gigaspora rosea*, *Glomus etunicatum* + *Glomus intraradices* and one control were used to conduct experiment in order to study the effects of Mycorrhizal inoculation on growth and nutrients uptake.

Autoclaved soil with the following composition (Table 1) was used in 16 cm diameter earthen pots. The seeds of *Medicago sativa* were obtained from the Fodder Section, National Agricultural Research Centre, Islamabad. All experiments were arranged in open air under natural field conditions using Completely Randomized Design.

First experiment: *Medicago sativa* fodder seeds, three VAM species and one control were used with three replications. Twelve pots were filled with autoclaved soil. Inoculation with VAM was done by laying method (Jackson, 1972). Pots were kept in open air under natural field conditions. Four plants were grown in each pot. Plants were harvested just after seed formation.

Second experiment: Mycorrhizae inoculated and uninoculated plants were tested by the method (Phillips & Hayman, 1970) in order to study and culture for nutrient uptake from unfertilized soil. Oven dried shoot portion from each pot was ground to determine the total nitrogen by Cottenie (1980) method, phosphorus and potassium percentage by Anon., (1990) method, phosphorus and potassium reading were taken by spectrophotometer and Flame photometer, respectively. The data regarding different plant characters under study were subjected to analysis of variance technique to determine significance of mean among the treatments by Steel & Torrie (1980) and comparisons of treatment means accomplished by least significant difference (L.S.D.) test at 0.05% level of significance.

Results and Discussion

Shoot and root dry weight increased significantly due to inoculation of mycorrhizae (Table 2 and Fig. 1). The dry weight of shoot and root without inoculation was 9.12 and 2.67 g whereas the shoot dry weight was 9.81, 10.61 and 12.64 g, while root weight was 2.88, 3.11 and 3.68 g, respectively when inoculated with *Gigaspora rosea*, *Glomus intraradices* + *Gigaspora rosea* and *Glomus etunicatum* + *Glomus intraradices*. The dual inoculations with *Glomus inraradices* + *Gigaspora rosea* and *Glomus etunicatum* + *Glomus intraradices* were significantly more effective than single inoculation (*Gigaspora rosea*).

Table 1. Chemical characteristics of soil used in the experiments.

pH	7.4
Moisture	32%
Total organic carbon	0.6%
Total nitrogen	16 mg kg^{-1}
Phosphorus	5.3 mg kg^{-1}
Potassium	140 mg kg^{-1}

Table 2. Mycorrhizae inoculation effects on shoot and root dry weights of *Medicago sativa.*

Treatments	**Shoot dry weight (g)**	**Root dry weight (g)**
Control	9.12 d	2.67 d
Gigaspora rosea	9.81 c	2.88 c
Glomus intraradices + *Gigaspora rosea*	10.61 b	3.11 b
Glomus etunicatum + *Glomus intraradices*	12.64 a	3.68 a

Any two means not sharing a letter differ significantly at 0.05 probability level.
LSD (0.05) for shoot wt = 0.6043, LSD (0.05) for root wt = 0.1786

Table 3. Mycorrhizae inoculation effects on nitrogen, phosphorous and potassium uptake (%) of *Madicago sativa*.

Treatments	**Nitrogen uptake %**	**Phosphorous uptake %**	**Potassium uptake %**
Control	3.48 d	0.39 c	2.14 c
Gigaspora rosea	3.69 c	0.43 c	2.27 b
Glomus intraradices + *Gigaspora rosea*	4.11 b	0.64 b	2.34 ab
Glomus etunicatum + *Glomus intraradices*	4.93 a	0.77 a	2.41 a

Any two means not sharing a letter differ significantly at 0.05 probability level.
LSD (0.05) for N = 0.1575**,** LSD (0.05) for P = 0.0842**,** LSD (0.05) for K = 0.1191

The inoculum mixture of two species was significantly more effective than single species inoculum. These results are supported by Mamta & Tilak (1987) who studied the effect of *Rhizobium* spp. and mycorrhizal fungus (*Glomus versiforme*) on the growth of mungbean.

The findings of Nakatsubo (1997) who conducted a culture experiment to examine the effect of arbuscular mycorrhizal (AM) fungi on the growth and reproduction of *Kummerowia striata*, a common annual legume of river flood plains of Japan, are also relevant. The plants were grown from seeds in pots with nutrient poor sandy soil collected from a fluvial bar. Arbuscular mycorrhizal infection increased the above ground biomass and seed production. These results suggested that AM fungi may influence the establishment of *Kummerowia striata* in nutrient poor disturbed habitats. Tarafdar *et al*., (1992) reported that many fungi and *Aspergillus fumingtus* in Particular enhanced the growth of mung bean and cluster bean.

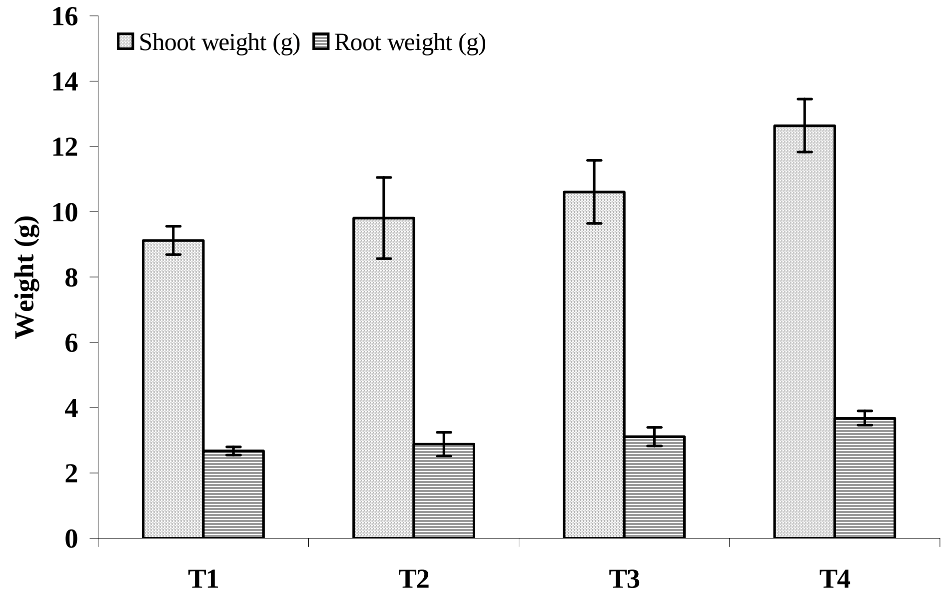

Fig. 1. Shoot and root dry weight (g) of *Medicago sativa* as influenced by arbuscular mycorrhizae inoculated and uninoculated

T_1 = Control, T_2 = *Gigaspora rosea*, T_3 = *G. intraradices* + *Gigaspora rosea*, T_4 = *G. etunicatum* + *G. intraradices*

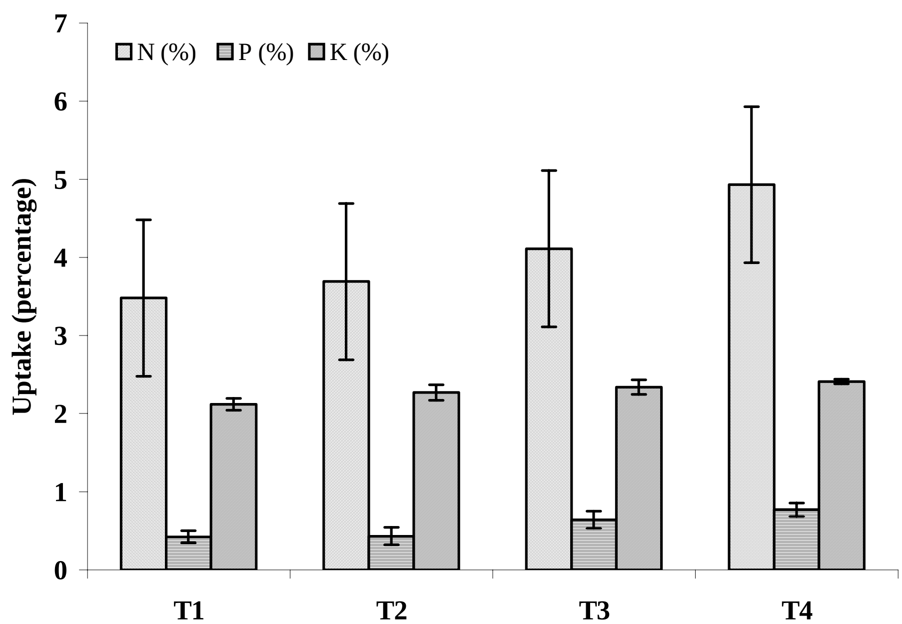

Fig. 2. Nitrogen, phosphorus and potassium (%) in arbuscular mycorrhizae inoculated and uninoculated *Medicago sativa*.

T_1 = Control, T_2 = *Gigaspora rosea*, T_3 = *G. intraradices* + *Gigaspora rosea*, T_4 = *G. etunicatum* + *G. intraradices*

There was a statistically significant effect of mycorrhizae on nitrogen uptake percentage of grass species (Table 3 and Fig. 2). The nitrogen, phosphorus and potassium uptake percentage without inoculation was 3.48, 0.39 and 2.14%, respectively. The inoculation with *Gigaspora rosea*, *Glomus intraradices* + *Gigaspora rosea* and *Glomus etunicatum* + *Glomus intraradices* increased the nitrogen uptake to 3.69, 4.11 and 4.93%, respectively, Phosphorus uptake was 0.43, 0.64 and 0.77%, respectively, while the potassium increased to 2.27, 3.34 and 2.41%, respectively. The results were in accordance with the findings of Alvey *et al*., (2001) provided strong evidence that cereal/legume rotations can enhance P nutrient uptake of cereal microbiologically through arbuscular mycorrhizae.

Tarafdar *et al.*, (1992) reported many soil fungi produced phosphates as extracellular enzyme and *Aspergillus fumigatus* had a particular capacity to produce phosphates which lead to increased phosphate uptake in mung-bean and cluster-bean. The colonization of arbuscular mycorrhizae improved P utilization, distribution in soybean crop. Ruiz-Lozano (2006) reported that VAM improved the uptake of nutrients by extra radiacal mycorrhizal hyphae.

Similar effects of mycorrhizae were also reported by Mamta & Tilak (1987). They studied the effect of *Rhizobium* species and mycorrhizal fungus (*Glomus versiforme*) on nutrient of mungbean. Single inoculation with *G. versiforme* had non significant effect over the corresponding controls, while dual inoculation significantly Improved/increased nutrent status of mungbean.

These result are supported by Johnny (1999) who reported that the legumes formed tripartite symbioses with arbuscular mycorrhizal fungi (AMF) and rhizobia which variably influenced plant productivity. The result suggested that specific AMF + rhizobia combinations enhanced plant growth and yield significantly in some strains. This estimate compared favorably with similar data reported by George *et al*., (1995) and Chen *et al.*, (2005). They reported that colonization of plants roots by arbuscular mycorrhizal fungi greatly increased the plant uptake of phosphorus and nitrogen. The most prominent contribution of arbuscular mycorrhizal fungi was increased uptake of nutrients by extra-radical mycorrhizal hyphae. Many tested fungal isolates increased phosphorus and nitrogen uptake of the plant by absorbing phosphate, ammonium and nitrate from soil. The contribution of arbuscular mycorrhizal fungi to plant phosphorus uptake however, was usually much larger than the contribution to plant nitrogen uptake.

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