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IMPACT OF DUAL INOCULATION WITH *Rhizobium sesbania* AND VESICULAR-ARBUSCULAR MYCORRHIZA ON GROWTH AND NUTRITION OF *Sesbania aegyptiaca* IN A CALCAREOUS SOIL

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ABSTRACT

A pot experiment was carried out to study the effect of inoculation with single and dual inoculants of symbiotic N_2-fixer *R. sesbania* and the vesicular-arbuscular (VA) mycorrhizal fungus *Glomus macrocarpus* on growth and nutrition of *S. aegyptiaca* in a calcareous soil amended with rock phosphate (0.5%) or fertilized with different levels of superphosphate. A significant increase in fresh and dry weights of *S. aegyptiaca* was obtained after inoculation of plants particularly with dual inoculant, in the presence of ½ dose of superphosphate. Incorporation of dual inoculants in the presence of ½ dose of P significantly increased the accumulation of N, P and K in *S. aegyptiaca* plants. *R. sesbania* inoculant significantly increased the N content in *S aegyptiaca* plants in comparison with VA mycorhiza inoculated plants, while VA mycorrhiza inoculant significantly increased the P and K contents in plants compared with *R. sesbania* inoculated treatments. Number and dry weights of nodules were increased in inoculated plants. The maximum N_2-ase activity of *S. aegyptiaca* plants roots was recorded in 45 days old plants inoculated with dual inoculum particularly in the presence of low dose of superphosphate. The introduced biopreparations were able to colonize actively the rhizosphere of *S. aegyptiaca* plants in the presence of chemical fertilizer. The maximum counts of total bacterial count in rhizosphere of 60 days old plants were found in the presence of dual inoculant and ½ dose of superphosphate. High percentage of VA mycorrhiza colonization in *S. aegyptiaca* roots was recorded, indicating good ecological adaptation of the mycorrhizal fungus to the calcareous soil. The high percentage of VA mycorrhiza colonization was found in inoculated and fertilized treatments. The colonization was increased by elapsing of growth time. The remarkable increase in VA mycorrhiza colonization was recorded in treatments inoculated with dual inoculum and fertilized with superphosphate.
Key words: N_2-fixing bacteria, VA mycorrhiza, Biofertilizers, *Sesbania*, *Rhizobium,* Calcareous soil, Superphosphate, Rock phosphate

INTRODUCTION

Sesbania is considered to be one of the most important forage legumes for many tropical and subtropical regions. Several investigators indicated that many of *Sesbania* species can provide a wide range of products, *e.g*., forage, green manure, firewood, gum, pulp, paper and edible leaves and flowers, (Allen and Allen, 1981 and Legocki *et al.*, 1984). In addition, due to their nodulation *Sesbania* species are potentially suitable for improving fertility and consequently the utilization of salt-affected soils (Dommergues, 1981). In Egypt, *Sesbania aegyptiaca* grows widely as a wild legume plant. Interest in *S. aegyptiaca* cultivation as fodder and green manure in Egypt has considerably increased over the past decade in order to face the great need in animal feeding. Meanwhile Egyptian agricultural authorities were obligate to move towards desert and new reclaimed soils for increasing cultivated lands to face the great increment of populations. The genus *Sesbania* as legumes requires large amounts of phosphorus for nodulation, N_2-fixation and plant growth (Israel, 1987 and O'Hara *et al.*, 1988). Although the considerable addition of phosphorus to alkaline soils in Egypt, the amount of available phosphorus for plant is usually low, where the added P fertilizer is rapidly transformed to unavailable form. Therefore, supply of adequate P is an essential factor for plant growth particularly in newly reclaimed soils such as calcareous soil. However, the use of high rates of soluble P-fertilizers increases the costs of agricultural production. Rock phosphate may be desirable alternative to high cost soluble P fertilizer. It may be cheap source of P nutrient, but its agronomic effectiveness is highly variable and generally lower than that of soluble P fertilizers. Hence, the microbial solubilization of this alternative is necessary to increase the amounts of biologically mobilized P. The effect of VA mycorrhiza inoculation on the availability of P was reviewed by many investigators (Siqueira, 1987; Tarafdar and Marschner, 1995; Armanios *et al.*, 1996; El-Sawah, 2000 and Hauka, 2000). They reported that VA mycorrhiza plays an important role in improving the uptake of mineral nutrients of low mobility in soil by plant. Legume inoculation with *Rhizobium* spp. had positive effect on plant growth, nodulation and N_2-fixation (Moawad *et al.*, 1988; Zahara *et al.*, 1990; Badr El-Din and Moawad, 1991; Gohar *et al.*, 1991 and Monib *et al.*, 1994). Dual inoculation with *Rhizobium* spp. and VA mycorrhiza induced pronounced increases in plant dry weight, nodulation and nutrient content of different legume plants (Punj and Gupta, 1988; Ishac *et al.*, 1994 and Armanios *et al*., 1996). The synergistic effects of inoculation of legumes with VA mycorrhiza and *Rhizobium* in low P soils are well documented (Subba Rao *et al.*, 1986 and Brown *et al.,* 1988).

The purpose of the present study is to evaluate the influence of seed inoculation with *R. sesbania* and/or VA mycorrhiza on growth, nodulation and nutrient content of *S. aegyptiaca* plants, in pots receiving superphosphate (at different levels) or rock phosphate fertilizers in calcareous soil. The effect of inoculation on N_2-ase activity of plant root system, VA mycorrhiza colonization and total bacterial count was also investigated.

MATERIALS AND METHODS

A pot experiment was conducted during summer 1999 under greenhouse conditions at Agric. Res. Exper. Sta., Fac. of Agric., Mansoura Univ. The experiment was carried out to investigate the effect of inoculation 0with *R. sesbania* and *G. macrocarpus*, separately or mixed, on growth, nutrient uptake by *S. aegyptiaca* plants, nodulation, nitrogenase activity, total bacterial counts and VA mycorrhiza colonization in rhizosphere soil.

Soil:

A calcareous soil (35.0% $CaCO_3$ and pH 7.9) was obtained from a surface layer (0-20 cm) in Bourg El-Arab region, Egypt. The soil was air dried, ground to pass through a 2.0 mm sieve. The main physical and chemical properties of the soil are recorded in Table (1).

Table (1): Physical and chemical analysis of the used soil.

Physical properties		Chemical properties	
Particle size distribution		$CaCO_3$ %	35.0
Sand %	53.9	pH	7.9
Silt %	2.9	Total N %	0.6
Clay %	7.7	Available P (ppm)	2.1
Texture	Sandy	Available K meq/100 g	0.45
		E. C. m mhos/m	3.1

Organisms:

Effective isolate of *R. sesbania* was obtained from Microbiol. Dept., Fac. of Agric., Mansoura Univ., Egypt. Local strain of *G. macrocarpus* was provided from Fac. of Sci., Mansoura Univ., Egypt.

Cultivar:

Seeds of *Sesbania* (*S. aegyptiaca*) were used in this study. They were provided from Forage Crops Department, El-Serw Agricultural Research Station, Agric. Res. Center (ARC), Giza Egypt.

Compost preparation:

2 kg finely powdered wheat straw and 1 kg shredded small pieces of wheat straw were mixed in a pile. Wheat straw pile was amended with 120 g $(NH_4)_2 SO_4$ dissolved in 7.5 L H_2O. The pile was kept at 22°C. Two doses of $CaCO_3$, 20 g each, were added after 2 and 3 days. Moisture was kept constant a round at 40% for 5 weeks. The pile was remixed every two days. The composted wheat straw was mixed with soil at the rate of 0.5% (w/w).

Seeds inoculation:

Rhizobial inocula:

250 ml Erlenmeyer flasks containing 100 ml YMB medium portions were inoculated with *R. sesbania* and incubated on rotary incubating shaker (200 rpm/min) for 48 hours at 30 ± 1°C. 1.3 x 10^{10} cell were used as inoculum for each pot

VA Mycorrhiza inoculant:

Seeds planted over the thin layer from segments of highly infected onion roots and 5 ml of spores suspension (~ 180 spores/ml) were added on the seeds and then covered with some of the same soil.

Plant growth conditions:

The soil was mixed with the composted wheat straw. Each plastic pot (20 x 25 cm) was filled with 5 kg of soil. Seeds were distributed at the rate of 5 seeds/pot. 10 experimental treatments were included, which were divided to three groups according to inoculation treatments. The first group comprised four experimental treatments (control) didn't receive bio-fertilization (one treatment had no P fertilization, the 2nd had full P dose (31 kg P/fed), the 3rd had ½ dose of superphoaphate (15.5 kg P/fed) and the 4th had 0.5% (w/w) rock phosphate). The second group comprised three experimental treatments recieved ½ dose of superphosphate and microbial inoculation (*R. sesbania*, *G. macrocarpus* and *R. sesbania* + *G. macrocarpus*). The third group had three experimental treatments that received 0.5% (w/w) rock phosphate (26.4% P_2O_5, Abou Zaabal Phosphate Fertilizers Co.) and microbial inoculation (*R. sesbania*, *G. macrocarpus* and *R. sesbania* + *G. macrocarpus*). 0.5% rock phosphate and ½ dose of superphosphate were added before sowing. An activated dose (15 Kg N/Fed) of NH_4NO_3 (33.3% N) and 12 Kg K_2O/Fed (K_2SO_4 48%) were added after 10 days from sowing. The experiment was laid out at Randomized Complete Block Design with three replicates. The plants were thinned to 3 seedling/pot after two weeks of sowing. Irrigation stood at 60% of the water holding capacity (WHC) with tap water. The pots were incubated under natural greenhouse conditions. All pots were irrigated with equal volume of tap water whenever, the available water content decreased till 40% of WHC.

Microbiological analysis:

After 20, 40 and 60 days from sowing, rhizospheric soil was microbiologically analyzed for determining the densities of total bacteria on Allen (1969) medium. Mycorrhizal infection level was estimated microscopically on a representative sample of fresh root as described by Trouvelot *et al.* (1986), after clearing and staining (Phillips and Hayman, 1970).

Nitrogenase activity:

The N_2-ase activity of roots at 45 and 60 days after sowing was measured using the acetylene reduction assay (Hardy *et al.*, 1973). Values of N_2-ase activity were recorded as n moles C_2H_4/g root/h.

Chemical analysis:

After 30, 60 and 90 days from planting, inoculated and uninoculated representative plants were carefully uprooted. The root was dipped in water to remove soil particles, washed with distilled water. Total fresh and dry (after drying at 70°C for 48 hr) weights of plant were determined. The oven dried plants were powdered and mineralized by sulfuric-perchloric acids (Piper, 1950). Content of total nitrogen, P and K was determined by the method of Jackson (1973).

Statistical analysis:

Differences between treatments were determined using the statistical procedures for agricultural research (Gomez and Gomez, 1984) and the significance of differences among treatments was tested at the 5% and 1% probability level. The data were tabulated to reveal the effect of the main studied factors and significance of the different ways of interaction.

RESULTS AND DISCUSSION

Weight yields:

The effect of microbial inoculation on both fresh and dry weights of plants is presented in Tables (2 and 3). The results obtained show that, in general, inoculation with the used biopreparations, in the presence of ½ dose of super phosphate recorded highest weights of *Sesbania* plants in comparison with full dose in the absence of inoculation. However, inoculated plants, which received 0.5% rock phosphate also recorded highest weights than those of the corresponding uninoculated treatments. The highest weights were recorded in treatments of dual inoculation. The maxima increases in fresh weight of plants fertilized with full dose of super phosphate or 0.0 phosphate, after 90 days, were 11.68% and 85.43%, respectively, in soil inoculated with dual inoculant and fertilized with ½ dose of super phosphate fertilizer. In the same trend, the maxima increases, after 90 days, in dry weights of *Sesbania* plants inoculated with dual inoculant and fertilized with ½ dose of super phosphate were 45.99% and 262.2% over those of uninoculated but fertilized with full dose or unfertilized plants, respectively. Contrary, the non fertilized-non inoculated treatments showed the lowest yields. On the other hand, it was found that in uninoculated treatments, the addition of mineral phosphate had a beneficial effect on plant growth. The beneficial effect of the used organisms on plant growth can be attributed not only to N_2-fixation (Fayez, 1989) and nutrient availability (Hauka *et al.*, 1990 & 1996), but also to the production of growth promoting substances excreted in rhizosphere soil (Sundaro-Rao, 1988 and Hauka *et al.*, 1990). These results are in agreement with those reported by Kothari *et al.* (1990), Heggo & Barakah (1993) and El-Sawah (2000) who reported that in calcareous soil composed inoculation improved the dry matter of maize plants.

Table (2): Fresh weights (g/plant) of biologically inoculated *Sesbania* grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	30 day			60 day			90 day		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
1: Uninoculated treatments:									
Without P fertilization	0.20	0.70	0.90	0.25	2.10	2.35	3.70	21.15	24.85
Full dose of superphosphate	0.27	0.84	1.11	0.60	4.30	4.90	12.86	28.40	41.26
½ dose of superphosphate	0.22	0.78	1.00	0.33	2.91	3.24	7.25	23.27	30.52
0.5% rock phosphate	0.21	0.72	0.93	0.27	2.66	2.93	7.35	22.46	29.81
2: Inoculated treatments:									
A. Superphosphate (SP)									
Rhizobium + ½ dose SP	0.33	1.22	1.55	0.58	4.65	5.23	11.98	28.57	40.55
VAM + ½ dose SP	0.28	0.87	1.15	0.56	4.34	4.90	11.27	27.17	38.44
Rhizobium + VAM + ½ dose SP	0.32	1.27	1.59	0.63	4.67	5.30	15.77	30.31	46.08
B. Rock phosphate (RP)									
Rhizobium + 0.5% RP	0.23	0.83	1.06	0.37	3.27	3.64	10.26	24.31	34.57
VAM + 0.5% RP	0.20	0.81	1.01	0.33	3.15	3.48	6.75	26.28	33.03
Rhizobium + VAM + 0.5% RP	0.30	1.22	1.52	0.45	3.84	4.29	9.55	29.13	38.68
F-test	**	**	**	**	**	**	**	**	**
N-LSD 5%	0.034	0.032	0.041	0.048	0.520	0.520	1.470	4.660	4.900
N-LSD 1%	0.045	0.043	0.055	0.064	0.700	0.700	1.970	6.480	6.620

VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

Table (3): Dry weights (g/plant) of biologically inoculated *Sesbania* grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	30 day			60 day			90 day		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
1: Uninoculated treatments:									
Without P fertilization	0.04	0.11	0.15	0.10	0.35	0.45	0.71	3.47	4.18
Full dose of superphosphate	0.11	0.17	0.28	0.29	0.41	0.70	2.48	7.89	10.37
½ dose of superphosphate	0.09	0.15	0.24	0.17	0.37	0.54	2.01	7.77	9.78
0.5% rock phosphate	0.08	0.15	0.23	0.12	0.37	0.49	1.43	6.88	8.31
2: Inoculated treatments:									
A. Superphosphate (SP)									
Rhizobium + ½ dose SP	0.18	0.28	0.46	0.34	0.80	1.14	3.34	11.49	14.83
VAM + ½ dose SP	0.13	0.27	0.40	0.25	0.77	1.02	2.27	8.37	10.64
Rhizobium + VAM + ½ dose SP	0.18	0.30	0.48	0.39	0.79	1.18	3.78	11.36	15.14
B. Rock phosphate (RP)									
Rhizobium + 0.5% RP	0.13	0.24	0.37	0.19	0.39	0.58	1.99	7.17	9.16
VAM + 0.5% RP	0.12	0.22	0.34	0.17	0.40	0.57	1.92	6.83	8.75
Rhizobium + VAM + 0.5% RP	0.16	0.22	0.38	0.15	0.50	0.65	1.97	7.33	9.30
F-test	**	**	**	**	**	**	**	**	**
N-LSD 5%	0.030	0.022	0.045	0.035	0.025	0.041	0.029	0.720	0.730
N-LSD 1%	0.038	0.029	0.060	0.047	0.034	0.055	0.039	0.970	0.980

VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

N, P and K content in *Sesbania*:

Data presented in Table (4) show that N, P and K in *Sesbania* plants were found to be affected positively by inoculation treatments with *R. sesbania* and VAM. The inoculated plants contained more nutrients levels than non inoculated plants. Generally, the increase in nutrient content of *Sesbania* plants was much higher in inoculated plants that received ½ dose of P mineral fertilizer than plants fertilized with the recommended dose of fertilizer or with non inoculated non fertilized treatments. Both the content and uptake of nutrients increased with age of plants. The increases may be due to more dry matter accumulation with plant age progress and its metabolic processes. The highest accumulation in nutrients was scored by plants inoculated with dual inoculants. It could be concluded that N, P and K content in *Sesbania* plants were increased significantly by the synergistic effect mainly between both symbiotic N_2-fixer and VA mycorrhiza. The present study also revealed that *R. sesbania* is capable of enhancing the absorption of N and P fertilizers by the plants. VAM also had beneficial effect on P and N nutrition of plants. The extra P was taken up from the soluble P fraction associated with such phosphate sources or released from them in the soil. Mycorrhiza, therefore, ensure a better utilization of the available phosphate rather than mobilization of the insoluble fraction. It is well known that phosphate is stored and probably transported within the fungal system as polyphosphate granules. Mycorrhizal roots may thus act as storage organs of applied phosphate absorbed before it becomes immobilized in soil. The intensive assimilation of N and P as well as the enhancement of root uptake capacity may be responsible for the increment of K accumulation by the *Sesbania* plants after inoculation. This may be attributed to the functional link between N, P and K within the plant (Kumar *et al.*, 1979). The ability of associative N_2-fixer and VA mycorrhiza to increase the mineral N- and P-uptake by plants has been demonstrated previously (Hauka *et al.*, 1990 and Belimov *et al.*, 1995). The effect of increasing nutrient content in plant can be explained by an enhancement of the biological N_2-fixation or by activities of nitrogen fixing bacteria and VA mycorrhiza on N & P cycling in soil as well as the possibility of interaction between the *R. sesbania* and mycorrhizal fungus in helping the plant to use sparingly N_2 and soluble phosphate (Azcon *et al.*, 1978 and Azcon-aguilar *et al.*, 1986). Ba and Guissou (1996) suggested that VAM plants take up more P from soil and rock phosphate than non-VAM plants. Likewise, VAM are able to increase N-concentration in plants by an indirect P-supply, a direct uptake of N-compounds from soil by hyphae and an indirect effect on nitrate reductase activity in plants (Barea *et al.*, 1986).

Nodulation status:

The effect of microbial inoculation on number and dry mass of nodules was investigated. Mean values for number and dry weight of nodules per plant in all treatments are given in Table (5). The obtained data showed that nodulation was significantly affected by rhizobial inoculation. Much number and high dry weights percentages of root nodules were recorded in the inoculated treatments fertilized with superphosphate than

those fertilized with rock phosphate. The highest number and dry weights of root nodules were found in treatments received dual inoculant + ½ dose superphosphate followed by treatments of *R. sesbania* + ½ dose of superphosphate, VAM + ½ dose of superphosphate, *R. sesbania* + VAM + 0.5% rock phosphate, *R. sesbania* + 0.5% rock phosphate and VAM + 0.5% rock phosphate. The number of nodules from all treatments increased with the advancing age of test plants. On the other hand, it was found that in uninoculated treatments, the addition of mineral phosphate had a beneficial effect on nodule numbers, volume and dry weights. The results agreed with those obtained by Mahmoud (2000). He reported that inoculation of *Sesbania* with VAM and phosphate dissolver bacterium increased both number and weight of root nodules.

Table (5): Number of nodules and dry their weights of biologically inoculated *Sesbania* plants grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	45 days		75 days	
	No./ plant	Dry Wt/g plant	No./ plant	Dry Wt/g plant
1: Uninoculated treatments:				
Without P fertilization	13	0.04	22	0.05
Full dose of superphosphate	29	0.10	38	0.13
½ dose of superphosphate	33	0.09	41	0.12
0.5% rock phosphate	21	0.04	28	0.08
2: Inoculated treatments:				
A. Superphosphate (SP)				
Rhizobium + ½ dose SP	47	0.14	52	0.19
VAM + ½ dose SP	40	0.11	48	0.15
Rhizobium + VAM + ½ dose SP	47	0.15	60	0.21
B. Rock phosphate (RP)				
Rhizobium + 0.5% RP	36	0.13	51	0.14
VAM + 0.5% RP	33	0.12	43	0.13
Rhizobium + VAM + 0.5% RP	41	0.14	56	0.17
F-test	**	**	**	**
N-LSD 5%	3.81	0.033	3.89	0.039
N-LSD 1%	5.10	0.044	5.21	0.052

VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus)*, N-LSD, new least significant difference.

Nitrogenase activity:

The effect of microbial inoculation on acetylene reduction activity is shown in Table (6). Examination of the data presented in this table showed that the uninoculated controls had the lowest nitrogenase activities. On the other hand, the inoculated treatments, generally, showed high nitrogenase activities. The maximum activity of nitrogenase was found in 45 days old plants inoculated with dual inoculum of both *Rhizobium* and VA mycorrhiza in the presence of half dose of superphosphate (113.74 nmol C_2H_4/h/g root) or 0.5% rock phosphate (109.56 nmol C_2H_4/h/g root). The vital role played by

ATP in nitrogenase activity also explains these results. These findings are in harmony with data of Hegazi (1983), Abdel Magid *et al.* (1988). The results also showed that the nitrogenase activity was decreased with increasing plant age in all treatments. Monib *et al*. (1994) found that acetylene reducing activity was decreased by increasing the plant age.

Table (6): Nitrogenase activity (nmol C_2H_4/g root/h) of biologicallyss inoculated *Sesbania* plants grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	N₂-ase activity (nmol C_2H_2/g root/h)	
	45 day	75 day
1: Uninoculated treatments:		
Without P fertilization	12.89	8.22
Full dose of superphosphate	28.99	14.12
½ dose of superphosphate	21.78	10.33
0.5% rock phosphate	15.77	10.21
2: Inoculated treatments:		
A. Superphosphate (SP)		
Rhizobium + ½ dose SP	80.85	39.38
VAM + ½ dose SP	46.78	20.21
Rhizobium + VAM + ½ dose SP	113.74	53.83
B. Rock phosphate (RP)		
Rhizobium + 0.5% RP	101.77	39.38
VAM + 0.5% RP	35.78	15.77
Rhizobium + VAM + 0.5% RP	109.56	51.87
F-test	**	**
N-LSD 5%	7.39	5.59
N-LSD 1%	9.90	7.48

VAM, vesicular - arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

Populations of total bacteria:

The effect of microbial inoculation on total bacterial counts was investigated. Data presented in Table (7) show that the introduced bacteria were able to actively colonize the rhizosphere of *Sesbania* plants compared with uninoculated plants. Dual inoculation had positive effect on counts in comparison with single inoculation. *Rhizobium* inoculated treatments contained more total bacterial counts than VAM containing treatments. The improvement in the viability of tested bacteria may be due to the inoculation with different biopreparations, *i.e*., *Rhizobium* + VA mycorrhiza, (Belimov *et al*., 1995) as well as to the compost and mineral fertilizers addition (Eweda and Vlassak, 1988 and El-Sawah *et al.*, 1995). The population size of tested bacteria indicated an active rhizosphere of plant. The highest numbers of total bacteria was found in most combined inoculation treatments. The treatments fertilized with recommended dose of P was the most active followed by treatments fertilized with half dose of superphosphate than treatments fertilized with 0.5% rock phosphate. The density of total bacteria in the rhizosphere of *Sesbania* plants increased with the increase in plant development.

Table (7): Population of total bacteria* in rhizosphere of biologically inoculated *Sesbania* plants grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	Counts of total bacteria (x 10^6 cell/g soil)		
	20 day	40 day	60 day
1: Uninoculated treatments:			
Without P fertilization	1.66	3.13	8.31
Full dose of superphosphate	3.01	5.07	14.17
½ dose of superphosphate	2.88	4.81	13.31
0.5% rock phosphate	2.88	3.74	13.25
2: Inoculated treatments:			
A. Superphosphate (SP)			
Rhizobium + ½ dose SP	3.39	5.11	14.99
VAM + ½ dose SP	3.18	4.91	14.47
Rhizobium + VAM + ½ dose SP	3.79	6.77	16.34
B. Rock phosphate (RP)			
Rhizobium + 0.5% RP	2.98	4.88	14.44
VAM + 0.5% RP	3.08	4.89	13.67
Rhizobium + VAM + 0.5% RP	3.47	6.63	15.91
F-test			**
N-LSD 5%	NS	NS	3.14
N-LSD 1%	NS	2.33	4.37

VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus)*, N-LSD, new least significant difference. *Counts in zero time before cultivation1.08 x 10^6 cfu/g dry soil.

Mycorrhizal infection:

The effect of microbial inoculation on VAM colonization in calcareous soil is presented in Table (8). The obtained results showed that considerable VAM infection occurred in the plants inoculated by *Rhizobium* or VAM in comparison with uninoculated control samples. However, higher VAM infection occurred in plants inoculated with dual inoculum than single form. The high VAM colonization was observed with the increase in plant age. The inoculation with *G. macrocarpus* successfully developed a high level of infection in *Sesbania* plants, which indicated good probability of ecological adaptation of mycorrhizal fungus to the calcareous soil. Very low infection was observed in the uninoculated control plants. The rate of fungal colonization was higher after 90 days as compared to plants with either 30 or 60 days. Colonization in soils fertilized with 0.5% rock phosphate was higher than that in soils fertilized with ½ dose of superphosphate in the absence of biofertlization. Azcon-aguilar *et al.* (1986) found that the essential synergistic effect of bacterial inoculation increased the degree of mycorrhizal infection. The high colonization of VA mycorrhiza may be also due to compost addition which can stimulate the growth of hyphae, that would achieve further infection (St-Jhon *et al*., 1983).

Table (8): Level of root mycorrhizal infection in biological inoculated *Sesbania* plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	30 days			60 days			90 days		
	Infection parameters (%)								
	F	M	A	F	M	A	F	M	A
1: Uninoculated treatments:									
Without P fertilization	4.4	4.2	0.0	6.1	5.8	5.7	9.9	8.7	7.2
Full dose of superphosphate	6.6	6.2	4.2	10.0	9.8	7.9	19.9	17.0	15.5
½ dose of superphosphate	7.1	6.0	4.7	10.6	10.7	8.0	24.0	22.5	18.1
0.5% rock phosphate	7.5	7.3	4.8	12.0	11.5	8.5	25.5	22.0	20.1
2: Inoculated treatments:									
A. Superphosphate (SP)									
Rhizobium + ½ dose SP	12.1	9.5	9.0	15.4	13.3	9.9	41.1	33.0	26.6
VAM + ½ dose SP	22.9	20.3	12.0	41.7	38.4	29.9	83.0	66.7	46.2
Rhizobium + VAM + ½ dose SP	30.2	27.1	18.3	44.0	40.3	34.2	92.7	74.3	84.7
B. Rock phosphate (RP)									
Rhizobium + 0.5% RP	9.3	7.7	5.2	12.5	11.1	9.7	27.7	24.5	19.9
VAM + 0.5% RP	19.0	15.7	11.0	34.1	32.2	28.0	71.3	68.1	46.4
Rhizobium + VAM + 0.5% RP	27.3	22.0	14.2	47.5	43.7	32.0	89.2	73.8	55.0
F-test	**	**	**	**	**	**	**	**	**
N-LSD 5%	3.33	3.23	1.93	4.01	4.43	2.77	3.30	4.44	4.41
N-LSD 1%	4.46	4.33	2.58	5.37	5.93	3.71	4.41	5.94	5.91

F, frequency of root infection; M, intensity of cortical infection; A, arbuscule frequency in roots; VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

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تـأثير التلقيح المـزدوج بالرايزوبيـا والميكـوريزا علـى نمـو وتغذيـة السيسـبان فـى الأراضى الجيرية

فتحى إسماعيل على حوقة
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تتجه الجهود فى مصر حالياً نحو إستغلال الأراضى المستصلحة حديثاً مثل الأراضى الجيرية فى زيادة إنتاجية المحاصيل بصفة عامة والبقولية منها بصفة خاصة سواء كانت محاصيل بذرية أو محاصيل علف ، إلا أنه يعيب هذه الأراضى الجديدة غالباً غياب أو ندرة أعداد مجموعات بكتيريا الرايزوبيا وكذلك فطريات الميكوريزا مقارنة بأراضى الدلتا القديمة - والتى تحتوى على هذه الميكروبات الهامة طبيعياً – مما يستلزم معه إثراء هذه الأراضى الجديدة بمثل هذه الأنواع الميكروبية الهامة لرفع خصوبتها وزيادة إنتاجيتها.

أجريت تجربة أصص تحت ظروف الصوبة بمحطة التجارب والبحوث الزراعية بمزرعة كلية الزراعة جامعة المنصورة بغرض دراسة تأثير التلقيح المفرد والمزدوج بكل من الرايزوبيا وفطريات الميكوريزا على نمو نباتات السيسبان ومحتواها من العناصر ، وكذلك الأعداد الكلية للبكتيريا فى ريزوسفير هذه النباتات ونسبة إصابة الجذور بالميكوريزا بالإضافة إلى أعداد العقد الجذرية وأوزانها الجافة وكذلك نشاط إنزيم النيتروجينيز فى جذور هذه النباتات ، وذلك تحت ظروف الأراضى الجيرية ، ولقد إستخدم فى هذه الدراسة نوعين من الأسمدة الفوسفاتية هى صخر الفوسفات بنسبة ٠,٥% وكذلك سماد السوبرفوسفات بمستويات مختلفة (صفر ، ٥٠% ، ١٠٠% من الجرعة الموصى بها) ، ولقد أوضحت النتائج مايلى :

أدت اللقاحات بصفة عامة إلى زيادة معنوية فى كل من الأوزان الغضة والجافة للنباتات ، وكذلك محتوى هذه النباتات من عناصر النيتروجين والفوسفور والبوتاسيوم وذلك على طول فترات التجربة .

وجدت زيادة معنوية فى تركيز النيتروجين فى النباتات الملقحة بالرايزوبيا عنه فى حالة النباتات الملقحة بالميكوريزا ، بينما وجدت زيادة فى تركيز الفوسفور والبوتاسيوم فى النباتات الملقحة بالميكوريزا مقارنة بمثيلتها الملقحة بالرايزوبيا .

سجلت النباتات الملقحة المسمدة تزايداً فى أعداد العقد وأوزانها الجافة عن مثيلتها الغير ملقحة .

تزايد نشاط إنزيم النيتروجنيز فى جذور نباتات السيسبان ووصل إلى أعلى قيمة له بعد ٤٥ يوم من الزراعة مع التلقيح المزدوج وفى وجود مستوى منخفض من السوبرفوسفات وقد لوحظ تناقص نشاط هذا الإنزيم بمرور الوقت .

وقد أدت اللقاحات المستخدمة إلى تأثير إيجابى بزيادة ملحوظة فى كثافة أعداد المجتمع البكتيريى فى ريزوسفير النباتات الملقحة .

وجدت زيادة فى مستوى الإصابة لجذور نباتات السيسبان بالميكوريزا فى المعاملات الملقحة والمسمدة كيماوياً ، وقد تزايدت نسبة الإصابة مع الوقت فى نهاية التجربة ، كما سجلت معاملات التلقيح المزدوج مع التسميد بالسوبرفوسفات زيادة ملحوظة عن بقية المعاملات .

Table (4): Nutrient contents (%) in plants and nutrients-uptake (mg/plant) by biologically inoculated *Sesbania* grown in calcareous soil amended with composted wheat straw (0.5%).

Treatments	N%			N-uptake			P%			P-uptake			K%			K-uptake		
	30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day	30 day	60 day	90 day
1: Uninoculated treatments:																		
Without P fertilization	0.59	0.66	0.85	0.89	2.97	35.53	0.15	0.20	0.19	0.23	0.90	7.94	1.00	0.85	1.54	1.50	3.83	64.37
Full dose of superphosphate	0.75	0.78	1.02	2.10	5.46	105.8	0.21	0.34	0.35	0.59	2.38	36.3	1.09	1.57	1.93	3.05	10.99	200.1
½ dose of superphosphate	0.73	0.73	1.02	1.75	3.83	99.76	0.20	0.29	0.29	0.48	1.57	28.36	1.07	1.47	1.88	2.57	7.94	183.9
0.5% rock phosphate	0.66	0.66	1.00	1.52	3.38	83.10	0.19	0.27	0.27	0.44	1.32	22.44	1.04	1.36	1.86	2.39	6.66	154.6
2: Inoculated treatments:																		
A. Superphosphate (SP)																		
Rhizobium + ½ dose SP	1.09	1.11	1.25	5.01	12.65	185.4	0.20	0.28	0.31	0.92	3.19	45.97	1.07	1.55	2.66	4.92	17.67	394.5
VAM + ½ dose SP	1.05	1.06	1.18	4.20	10.81	122.6	0.22	0.41	0.39	0.88	4.18	41.50	1.15	1.66	2.68	4.60	16.93	285.2
Rhizobium + VAM + ½ dose SP	1.18	1.22	1.35	5.66	14.40	204.4	0.23	0.44	0.42	1.10	5.19	63.59	1.17	1.82	2.73	5.62	21.48	413.3
B. Rock phosphate (RP)																		
Rhizobium + 0.5% RP	1.02	1.11	1.21	3.77	6.44	110.8	0.20	0.28	0.28	0.74	1.62	25.65	1.06	1.46	1.88	3.92	8.47	172.2
VAM + 0.5% RP	0.78	0.99	1.05	2.65	5.64	91.88	0.20	0.36	0.36	0.68	2.05	31.50	1.09	1.71	2.42	3.71	9.75	211.8
Rhizobium + VAM + 0.5% RP	1.11	1.20	1.22	4.22	7.80	113.5	0.23	0.40	0.42	0.87	2.60	39.06	1.10	1.79	2.48	4.18	11.64	230.6
F-test	**	**	**	**	**	**	**	**	**	**	**	**		**	**	**	**	**
N-LSD 5%	0.031	0.028	0.026	0.041	0.67	14.84	0.034	0.032	0.029	0.054	0.121	4.21	NS	0.092	0.100	0.127	0.93	9.06
N-LSD 1%	0.042	0.039	0.035	0.055	0.89	19.86	0.048	0.043	0.039	0.073	0.162	5.64	NS	0.123	0.132	0.170	1.25	12.13

VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus)*, N-LSD, new least significant difference.