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# Nitrogen Fixation and Seed Yield of Chickpea Cultivars as Affected by Microbial Inoculation, Crop Residue and Inorganic N Fertilizer

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> THE CONCEPT of isotope dilution was applied for estimation of N<sub>2</sub> fixation by two chickpea cultivars (local varieties) under pot experiment conditions. Chickpeas, Giza 2 and Giza 195, were examined for their response to inoculation with either Rhizobium (Rh) or arbuscular mycorrhiza fungi (AM) and their combination. <sup>15</sup>N-ammonium sulfate and <sup>15</sup>N-labelled wheat straw were applied at a rate of 36 and 72 kg N ha<sup>+1</sup>, respectively as N fertilization sources. Rhizobium inoculation or N fertilization significantly increased the total nodule number and nodule dry weight per pot. Dual inoculation with Rh and AM gave the best results of nodulation and colonization percentage comparable to those recorded with the individual inoculants. Results of dry matter accumulation, seed yield and NPK uptake followed the same trend. Nitrogen derived from mineral fertilizer (Ndff) and organic residues (Ndfr) were highly affected by AM colonization as compared with other treatments. Chickpeas utilized similar amounts of N either derived from inorganic fertilizer or crop residue. On the other hand, chickpeas inoculated with Rhizobium were more dependent on nitrogen derived from air (Ndfa). Dual inoculation was superior over individual inoculation whereas the best values of Ndfa were obtained. Generally, the obtained results showed no significant difference between chickpea cultivars or N sources, but suggest that the combined use of Rhizobium and AM fungi is a promising technology to improve fertility of poor sandy soils and to enhance grain yield.

> Keywords: Arbuscular mycorrhiza, Biological nitrogen fixation, Chickpea cultivars, N sources, <sup>15</sup>N isotope dilution, *Rhizobium*, Yield.

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Plant residues are known to affect soil physical properties, availability of soil nutrients and soil biology. So, it can be used as an alternative nutrient source, especially for N and P in nutrient deficient soil, where it is used as vital resources for replenishing soil organic matter and supplying the major nutrients (Ajwa and Tabatabai, 1994), depending on their rate of degradation caused by biological decomposition. The application of plant residues can play an important role in enhancing and sustaining crop production (Tian *et al.*, 1993).

Raju *et al.* (1991) reported that green manuring (FYM) was more effective than inoculation with *Rhizobium* in increasing N, P and K uptake. Organic amendments together with microbial inoculation could enhance the microbial activities in the root zone, including  $N_2$  fixation and mycorrhizal colonization (Ishac *et al.*, 1986).

Arbuscular mycorrhizal fungi (AMF) are known to increase nutrient uptake, particularly P to the host plant (Daft and Nicolson, 1969; El Ghandour *et al*, 1996a,b). A synergistic effect of AMF was found with *Rhizobium*-peanut symbiosis (Ishac *et al.*, 1988; El-Ghandour *et al.*, 1997) and chickpea (Subba Rao *et al.*, 1986; El Ghandour *et al.*, 1996) in either sandy or alluvial soils. Inoculation with selected rhizobial strains significantly increased the dry matter of aerial parts and seed yield over the uninoculated control in field trial (Beck, 1992). Also, AMF improved biological nitrogen fixation via enhancement of competitive ability of introduced rhizobia strains (Thiagarajan and Ahmad, 1993).

The objectives of this study were therefore: (1) To determine the effect of wheat straw and mineral nitrogen as nutritional sources on N<sub>2</sub> fixation and grain yield of chickpea in the presence of AMF and /or *Rhizobium* inoculants, (2) To compare the recoveries of N from wheat straw and inorganic forms under different inoculants, using <sup>15</sup>N labeled materials, (3) To assess the interaction between inoculants and two N sources on dinitrogen fixation.

### **Material and Methods**

#### Pot experiment

Earthenware pots (28 cm diameter and 26 cm height) were filled with 6 kg of sandy soil from the farm area of Soil and Water Department, Atomic Energy Authority, Egypt. The soil chemical properties were pH (H<sub>2</sub>O 1:1) 7.9; total N 0.07%; organic matter 0.05%; P-Olsen 0.04% and CaCO<sub>3</sub> 0.41%. Mechanical analysis indicated the presence of 90.5% sand; 4.7% silt and 4.8 % clay.

# Fertilization

The soil was enriched with rockphosphate (RP) Sibaiya 11, El-Nasr Company {P<sub>2</sub>O<sub>5</sub>, 29.2 %; reactivity 46 % (in percent of total P)}, at rate of 50 kg ha<sup>-1</sup> before planting. Labeled ammonium sulfate with 4.3 % <sup>15</sup>N atom excess (a.e.) was applied at rate of 15 mg N kg<sup>-1</sup> soil (equivalent to 36 kg N ha<sup>-1</sup>) 15 days before planting to ensure that <sup>15</sup>N-fertilizer pool is stabilized into the soil. Wheat straw labeled with <sup>15</sup>N (2% atom excess, and collected from previous experiments) had the following chemical composition, 88.1 % organic matter; 50.9% organic carbon; 0.36% N; 0.07 % P; C/N ratio 141 were applied at rate of 2% w/w (equivalent to 72 kg N ha<sup>-1</sup>). Ground plant residues were incorporated into the soil 30 days before planting.

#### Sowing and inoculation treatments

A mixture of arbuscular mycorrhizal fungi (AM) spores to be used as inoculum was isolated from faba bean field in Inshas area, by wet sieving method as described by Gerdemann and Nicolson (1963). A thin layer of inoculum (200m1) was placed 2cm below the soil surface in the pots before sowing to produce the mycorrhizal plants.

Seeds of *Cicer arietinum* (chickpea) Giza 2 and Giza 195, (obtained from Institute of Field Crops Research, Agriculture Research Centre, Giza, Egypt) were treated with inoculant of *Rhizobium leguminosarum* (Rh) bv. *cicer*, strain ARC 200c having a population of 7.5  $\times 10^9$  cells/g air-dry peat-moss carrier at the rate of 20 g/100 seeds. This peat-based inoculum was obtained from Agricultural Microbiology Department, Institute of Soil, Water and Environment, Agriculture Research Centre, Giza, Egypt. The seeds were sown immediately. Seeds of the control treatment received neither AMF nor *Rhizobium* inocula.

#### Experimental layout

The experiment was laid out in a simple complete randomized block design consisting of 4 inoculation treatments; two sources of nitrogen,  $(^{15}NH_4)_2$  SO<sub>4</sub> and labelled wheat crop residues, and one source of phosphorus (Rock P). Each treatment was replicated three times. The plants were harvested after 120 days, separated into shoot and seeds, dried at 70 °C for constant weight, then ground and kept for analyses. Mycorrhizal colonization percentage was determined by the slide techniques of Daft and Nicolson, (1969). The data of nodulation (numbers and dry weights of nodules) were recorded. Total N, P and K contents in plants (shoot and seeds) were determined according to Page *et al.* (1982). Dinitrogen fixation and %Ndfa in shoot and seeds were estimated using

the combined equation after Hardarson *et al.* (1991). In this respect, wheat (non-fixing plant) was used as a reference crop for application of isotope dilution technique.

#### Calculations

Amounts of nitrogen that derived from fertilizer (NdfF) organic residues (Ndfr) and air (NdfA) were calculated using the following equations:

$$\%$$
NdfF = <sup>15</sup>N% a.e. in plant / <sup>15</sup>N% a. e. in fertilizer x 100 (1)

%Ndfr =  ${}^{15}$ N% a.e. in plant /  ${}^{15}$ N% a. e. in crop residues x 100 (2) The  ${}^{15}$ N direct method was followed as described by Hood *et al.* (1999)

$$%$$
NdfA =100 [1 - ( $%$ Ndff<sub>F</sub> / n x  $%$ Ndff<sub>NF</sub>] +  $%$ Ndff<sub>F</sub> (1/n) -1 (3)

where *n* is the amount of fertilizer N applied to the fixing crop divided by the amount applied to the non-fixing crop.  $%Ndff_F$ ,  $%Ndff_{NF}$  is the percentages of N derived from fertilizer by fixing and non-fixing crop, respectively.

#### Statistical analysis

The data obtained in the current study were subjected to ANOVA analysis followed by Duncan's multiple range test (DMRT) at 0.05 probability.

#### **Results and Discussion**

#### Nodulation and root colonization as affected by Rhizobium and AMF

Root colonization percentage was higher with combined inoculation (Rh+AMF) than with single AMF inoculation (Table 1). Root colonization was significantly higher with cultivar Giza 2 than with Giza 195 when combined inoculant (Rh + AMF) was applied. These results are closed to those reported by Subba Rao *et al.* (1986). The results also show that the native soil was lacking indigenous AM spores. Nodulation performance was induced with *Rhizobium* inoculant either alone or combined with AM. The absence of nodules in both control and AM treatments indicates the lack of rhizobia in experimental sandy soil. This finding was inconsistent with those of Subba Rao *et al.* (1986) who found that inoculation with *Glomus fasciculatum* alone has improved nodulation. On the other hand, present data are in accordance with the previous findings regarding the improvement of nodules (number and dry weight) with dual inoculation of *Rhizobium* and AM. In this respect, nodulation was higher to some extent in soil fertilized with labelled ammonium sulfate

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than with labelled wheat residue, but there is no significant difference between chickpea cultivars. Hafeez et al. (1998), Rupela et al. (1998) and Sattar et al. (1998) showed a significant effect of genotypes and *Rhizobium* inoculation on nodule number and mass, but Hafeez et al. (1998) stated that nodule number was not a useful parameter. Accordingly, Salih (1984) found a significant difference between *Rhizobium* strains and cultivars for nodule dry weight, but differences in nodulation of chickpea cultivars were not paralleled by differences in seed yield.

 TABLE 1. Nodule number, nodule dry weight and AM root colonization of chickpea cultivars as affected by inoculation with Rh and AMF and nitrogen sources .

Treatment	<sup>15</sup> N-	ammonium	sulfate	<sup>15</sup> N-Wheat straw			
	Nodule no pot <sup>-t</sup>	Nodule DW g pot <sup>1</sup>	AM root colonization (%)	Nodule no pot <sup>-1</sup>	Nodule DW g pot <sup>-1</sup>	AM root colonization (%)	
Giza 2				······································			
Uninoculated	0	0	0	0	0	0	
Rhizobium (Rh)	60 c	0. <b>39</b> b	0	45 c	0.51 b	Ō	
AM Fungi	0	0	495	0	0	43 c	
Rh + AMF	86 a	0.56 a	65 a	62 a	0.66 a	58 a	
Giza 195	-						
Uninoculated	0	0	0	0	0	0	
Rhizobium (Rh)	62 c	0.38 b	0	50 b	0.32 d	0	
AM Fungi	0	0	41 d	0	0	38 d	
Rh + AMF	72 Ь	0.53 a	45 c	61 a	0.46 c	51 b	
_c.v. (%)	7.52	12.21	3.49	6.40	5.52	5.32	

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

Rh = Rhizobium

AMF = Arbuscular mycorrhiza fungi

#### Dry matter, seed yield and nutrient uptake by chickpea cultivars

Inoculation with *Rhizobium* and AMF individually or combined improved dry matter accumulation in shoot and seed yield of both cultivars (Table 2). A similar trend was noticed for N, P and K uptake by shoot and seed under <sup>15</sup>N-labelled fertilizer addition. Generally, dual inoculation was superior over individual inoculation. Statistically, there was no significant difference between chickpeas in shoot and seed yields. Shoot N was higher in Giza 195 than in Giza 2. A reversible trend was recorded with shoot P. Nitrogen and phosphorus content of seeds was similar in both cultivars under different inoculation treatments. Similarly, potassium content of either shoot or seed was the same in both cultivars. Alagawadi and Gaur (1988) found that single inoculation of *Rhizobium* increased the nodulation, whereas combined inocula of *Rhizobium* and phosphate-solubilizing bacteria (*Pseudomonas striata* or

Bacillus polymyxa) increased nodulation, soil P and also the dry matter content and seed yield. Also, they found that straw and seed yields as well as N and P uptake were higher in dual inoculation combined with 10 kg N+ 60 kg  $P_2O_5$ ha<sup>-1</sup> as rockphosphate comparable to control. In a pot culture experiment using a silty loam soil, Sattar *et al.* (1995) stated that inoculation of five chickpea cultivars gave significant increase in nodule parameters, N accumulation and seed yield with significant strain x cultivar interactions. Data released in the present work proved the enhancing effect of AM fungi on nutrient uptake especially phosphorus that released from the most cheap rockphosphate form.

Dry matter accumulation in shoot and seed yield of both cultivars treated with  $^{15}$ N-labelled wheat straw (Table 3) did not significantly vary from those recorded with labelled chemical fertilizer (Table 2) except for the shoot dry matter and shoot N of Giza 195. A similar effect of inoculation treatments was noticed. Against the statement that cereal straw and carbonaceous materials with low N and wide C/N ratio may reduce soil mineral N and decrease N uptake (Evans *et al.*, 1997; Ladd, 1987; Smika *et al.*, 1969; Wagner and Zapata, 1982), wheat straw application in the present study, has improved N content of shoot and seeds especially under inoculation. This was most pronounced in shoot N of Giza 195 (169 mg N pot<sup>-1</sup>) inoculated with *Rhizobium* plus AM fungi. Shoot P of Giza 2 was less than with chemical fertilizer. There was no significant difference either between cultivars or fertilizers form when seed P was considered. A similar trend was noticed with K uptake with the exception of a high accumulation in shoots of Giza 2 treated with labelled straw.

#### Biological nitrogen fixation and fertilizer or crop residue N uptake

Contribution of biological nitrogen fixation was recorded only with *Rhizobium* alone or in combination with AM fungi under chemical N fertilization (Table 4). Total N<sub>2</sub> fixed (shoot + seed) was higher in dual inoculation than in Rh solely thereby providing evidence for the enhancement effect of AMF on nodulation and consequently N<sub>2</sub> fixation (Subba Rao *et al.*, 1986). A similar effect of *Bradyrhizobium japonicum* combined with *Azospirillum brasilense* on N<sub>2</sub> fixation by soybean was recorded earlier (Galal, 1997). *Rhizobium* - cultivar interaction was noticed, since Giza 195 accumulated more N from air than Giza 2. Such significant effects of chickpea genotypes and inoculation with rhizobia were also observed for %Ndfa, %Ndff where the %Ndfa values ranged from 70 to 81% (Sattar *et al.*, 1998) and these percentages are close to that recorded in the present study (72-82%). Similarly,

El Hadi and Elsheikh (1999) using protein content of chickpea seeds as indicator for  $N_2$  fixation found that Rhizobium strains were infective and effective in N fixation of six genotypes. In a pot experiment, the acetylene reduction assay revealed that chickpea cultivars had accumulated in their shoots about the same amount of N from fixation (Silsbury, 1989).

 TABLE 2. Dry matter accumulation, seed yield and nutrient uptake by chickpea cultivars as affected by inoculation treatments in soil amended with <sup>15</sup>N- ammonium sulfate.

Treatment	Dry matt	Dry matter g pot		N uptake mg pot		P uptake mg pot <sup>-1</sup>		K uptake mg pot <sup>-1</sup>	
	Shoot	Seed	Shoot	Seed	Shoot	Seed	Shoot	Seed	
Giza 2								<u>.</u>	
Uninoculated	6.0 b	2.4 b	10.2 d	21.5 c	5.0 d	0.2 d	0.9 Ъ	0.12 b	
Rhizobium (Rh)	7.8 a	3.7 ab	45.5 c	44.7 bc	6.5 bc	0.3 c	0.9 Ъ	0.2 ab	
AM fungi	7.2 a	5.1 a	57.0 bc	59.0 Ь	6.9 b	0.4 bc	0.8 b	0.3 ab	
Rh + AMF	7. <b>4</b> a	6.3 a	80.0 Ь	95.0 a	7.9 a	0.6 a	1.0 ab	0.33 a	
Giza 195									
Uninoculated	5.2 Ь	2.7 b	43.0 c	20.0 c	0.7 e	0.14 e	0.9 Ь	0.14 b	
Rhizobium (Rh)	6.7 a	4.8 ab	81.0 b	51.0 bc	0.9 d	0.35 bc	1.5 a	0.2 ab	
AM fungi	5.8 a	4.4 ab	52.0 c	36.0 bc	1.2 c	0.4 Ь	0.8 b	0.3ab	
Rh + AMF	7.4 a	6.1 a	119.0 a	92.0 a	1.3 bc	0.6 a	1.3 ab	0.2 ab	
c.v. (%)	25.6	36.3	23.4	29.5	4.85	10.21	26.7	41.2	

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

TABLE 3. Dry matter accumulation, seed yield and nutrient uptake by chickpea cultivars as affected by inoculation treatments in soil amended with  $^{15}$ N- wheat straw.

Treatment	Dry matter g pot		N uptake mg pot <sup>-1</sup>		P uptake mg pot		K uptake mg pot <sup>-1</sup>	
	Shoot	Seed	Shoot	Seed	Shoot	Seed	Shoot	Seed
Giza 2								•
Uninoculated	6.0 ab	2.0 b	31.8 d	18.0 c	0.8 f	0.2 a	1.1 Ե	0.1 c
Rhizobium (Rh)	6.4 ab	4.8 a	69.0 cd	64.0 ab	0.8 e	0.3 a	1.5 ab	0.2 ab
AM fungi	7.5 ab	3.7 ab	39.0 ef	83.6 b	1.3 c	0.6 a	1.6 ab	0.2 ab
Rh + AMF	7.4 ab	6.8 a	89.0 bc	90.0 a	1.6 b	0.5 a	1.7 ab	0.3 a
Giza 195								
Uninoculated	5.2 b	2.5 b	26.0 b	14.0 c	0.9 d	0.2 a	1.0 b	0.1 c
Rhizobium (Rh)	8.0 ab	4.6 a	140 ab	59.0 ab	1.3 c	0.3 a	1.0 Ь	0.2 bc
AM fungi	9.0 a	4.8 a	152.0 a	75.0 a	1.8 a	0.4 a	2.0 a	0.2 ab
Rh + AMF	9.0 a	6.1 a	169.0 a	82.0 a	1.7 a	0.3 a	1.4 ab	0.2 ab
c.v. (%)	24.6	28.4	22.9	29.8	3.24	6.6	29.9	28.8

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

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Nitrogen derived from fertilizer (Ndff) was significantly affected by inoculation. The highest value of fertilizer N uptake by total plant (shoot + seeds) was induced by AMF inoculation indicating the promotion effect of fungi on plant growth and nutrient uptake. The %Ndff is still lower than %Ndfa as affected by inoculation. A similar trend was observed by Sattar *et al.* (1998).

Nitrogen derived from air and organic residues (Table 5) were to some extent, identical to those resulted with  $^{15}$ N labelled labelled chemical fertilizer (Table 4). This indicates the importance of crop residues as well as chemical fertilizer in development of soil N and plant nutrition. The application of  $^{15}$ N direct approach showed no significant differences between N derived from labelled barley or maize and that of labelled ammonium sulfate (Hood *et al.*, 1999). However, it has been widely accepted that organic inputs play a significant role in the long - term build up of soil organic matter and associated soil stabilization.

#### Nitrogen recovery

Residue (%Ndfr) or fertilizer (%Ndff) N recovery shown in Table 6, indicated the positive influence of inoculation on N recovered by chickpeas as compared with the uninoculated control. It is clear that AMF colonization improved the %N fertilizer recovery (28.3%, 21.5%) and % residue recovery (3.7%, 7.1%) against the other inocula treatments for Giza 2 and Giza 195, respectively. Percentage of residue N values were lower than those of% fertilizer N recovery indicating the slow degradation of these residues and slow release of mineralized-N to be available for plant utilization. These results are in agreement with previous findings (Hood *et al.*, 1999). Also, the present results of residue N recovery (1.4%-7.1%) were on line with those of Haggar *et al.*, (1993) and Vanlauwe *et al.*, (1996) who found that crop N recovery from organic inputs such as plant residues or manures is often less than 20%.

In conclusion, inoculation with rhizobia and AMF is an appropriate technique for enhancing chickpea yield and at the same time reducing the N fertilizer requirement. Crop N recovery from organic residues could substitute a part of fertilizer-N demand. In this respect, the application of direct <sup>15</sup>N method to measure N release from organic residues is an appropriate and valuable technique. However, the results of the present work should be continued under field conditions.

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 TABLE 4. Nitrogen derived from fertilizer (NdfF) and air (NdfA) to shoot and seeds of chickpea cultivars as affected by inoculation treatments in soil
 amended with <sup>15</sup>N- ammonium sulfate.

Treatment	Nitrogen d	erived from fer	tilizer mg pot	Nitrogen derived from air mg pot <sup>-1</sup>		
	Shoot	Seed	Total	Shoot	Seed	Total
Giza 2					· · · · ·	
Uninoculated	2.2 d	4.5 Ъ	6.7 b	-	-	_
Rhizobium (Rh)	2.7 d	2.2 c	4.9 c	32 3 h	33 O h	65 3 h
AM fungi	12.5 a	13.0 a	25.5 a	-	-	-
Rh + AMF	4.8 c	3.8 Ъ	8.6 b	60.0 a	78.0 a	138 0 a
Giza 195						190.04
Uninoculated	9.0 ab	4.4 b	13.4 b	-	~	-
Rhizobium (Rh)	4.9 c	1.8 c	6.7 c	60.0 b	38.0 h	98 O b
AM fungi	11.4 a	7.9 a	19.3 a	-	-	-
<u>Rh + AM</u> F	4.8 c	2.8 с	7.6 c	95.0 a	77.3 a	172.3 a

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

# TABLE 5. Nitrogen derived from organic residues (NdfF) and air (NdfA) to shoot and seeds of chickpea cultivars as affected by inoculation treatments in soil amended with <sup>15</sup>N- wheat straw.

Treatment	Nitrogen d	erived from res	idue mg pot <sup>1</sup>	Nitrogen de	rived from air mg pot <sup>-1</sup>		
	Shoot	Seed	Total	Shoot	Seed	Total	
Giza 2	<u>_</u>					· · · · · · ·	
Uninoculated	4.1 b	2.2 b	6.3 b	-	-	-	
Rhizobium (Rh)	3.7 b	2.6 b	6.3 b	39.3 b	33.3 Ь	72.6 b	
AM fungi	5.1 a	10.9 a	16.0 a	-	-	-	
Rh + AMF	4.5 a	3.6 b	8.1 b	54.3 a	48.6 a	102.9 a	
Giza 195							
Uninoculated	3.4 d	1.8 d	5,2 d	-	- ·	-	
Rhizobium (Rh)	7.0 c	2.4 d	9.4 c	88.2 b	33.0 b	121.2 b	
AM fungi	21.3 a	9.8 a	31.1 a	-	-	-	
Rh + AMF	6.8 c	2.5 d	9.3 c	113.2 a	50.0 a	163.2 a	

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

TABLE 6. N recovery (%) in shoot and seeds of chickpea cultivars as affected by inoculation treatments in soil amended with <sup>15</sup>N-ammonium sulfate or wheat straw.

Treatment	<sup>15</sup> N-ammonium sulfate			<sup>15</sup> N-wheat straw			
	Shoot	Seed	Total	Shoot	Seed	Total	
Giza 2		· · · · · ·					
Uninoculated	2.4	5.0	7.4 b	0.9	0.5	1.4 c	
Rhizobium (Rh)	3.0	2.4	5.4 b	0.8	0.6	1.4 c	
AM fungi	13.9	14.4	28.3 a	1.2	2.5	3.7 a	
Rh + AMF	5.3	4.2	9.5 b	1.0	0.8	1.8 b	
Giza 195							
Uninoculated	10.0	4.9	14.9 b	0.8	0.4	1.2 c	
Rhizobium (Rh)	5.4	2.0	7.4 c	1.6	0.5	2.1 b	
AM fungi	12.7	8.8	21.5 a	4.9	2.2	7.1 a	
Rh + AMF	5.3	3.1	8.4 c	1.6	0.6	2.2 b	

Means in the same column followed by the same letter are not significantly different at  $P \le 0.05$ . Egypt. J. Microbiol. 37, No. 3 (2002)

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م دراسة مدى استجابة الصنفين جيزة 2 جيزة ، 195 للتلقيح البكتري بواسطة الريزوبيا أو فطر الميكورييزا منفردين أو فى مخلوط منهما أضيف سماد سلفات الأمونيوم المؤشر وكذلك بقايا حطب القمح المؤشر كمصدرين للتسميد الآزوتى . الجذريه وكانت أفضل النتائج مع اللقاح المزدوج . نفس الاتجاه تم رصده مع نسبة الإصابة بفطر الميكوريزا ، تراكم المادة الجافة ، محصول الحبوب وكذلك إمتصاص الآزوت والفوسفور والبوتاسيوم . الآزوت المنفرد من السماد المعدني وكذلك البقايا العضوية تأثر إيجابيا بفطر الميكورييزا مقارنة بالمعاملات العضوية تأثر إيجابيا بفطر الميكورييزا مقارنة بالمعاملات كانت كمية الآزوت المنفرد منهما تكاد أن تكون متماثلة .

النباتات الملقحة بواسطة الريزوبيا اعتمدت أساسيا على الآروت المثبت حيويا وفى هذا الصدد كان اللقاح المزدوج أكثر كفاءه مقارنة بالمعاملات الآخرى . لم تعطى النتائج المتحصل عليها فروق واضحة وقاطعة بين صنفى الحمص أو مصدرى الآروت بينما أعطت دلالة على اعتماد التلقيح البكتيرى أو فطر الميكوريز اكتقانة واعدة من شأنها تحسين خصوبة التربة الفقيرة خصوبيا مثل التربة الرملية وبالتالى العمل على زيادة الإنتاجية الحصولية .