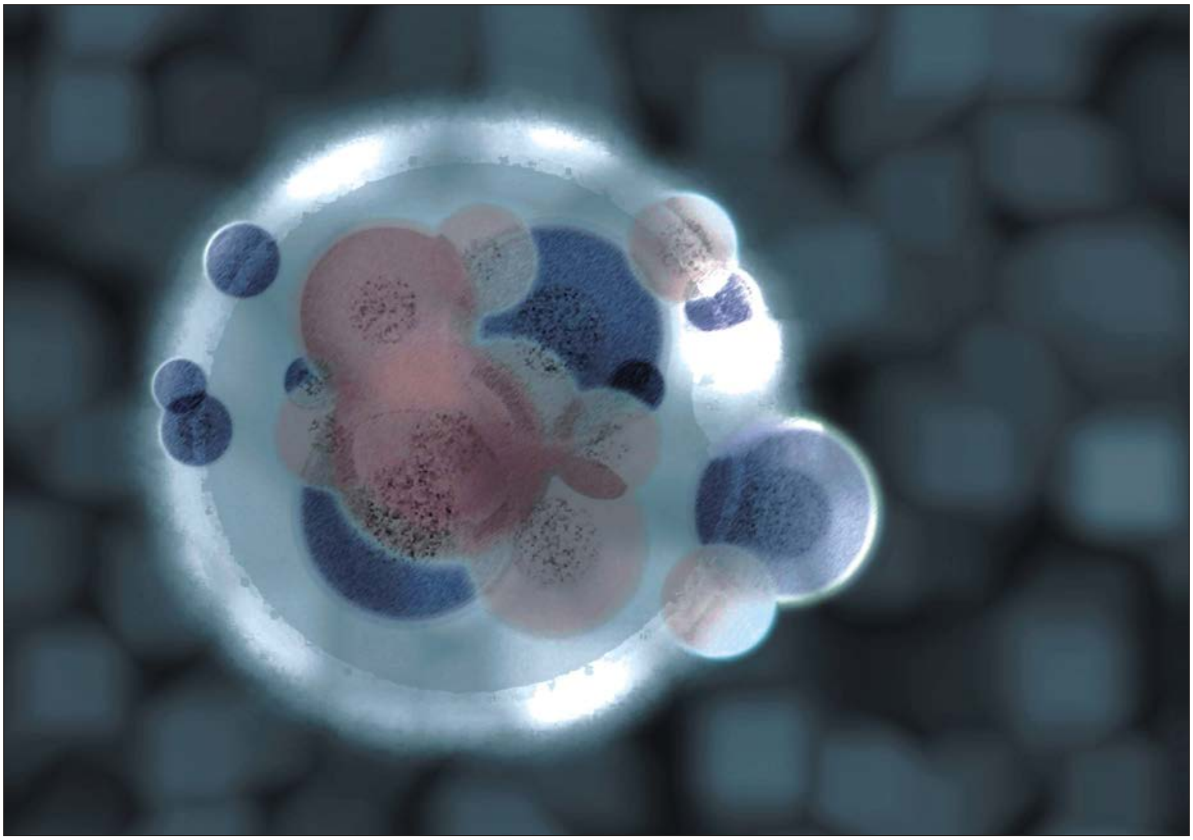

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Effects of Arbuscular Mycorrhiza Fungus Inoculation and Phosphorous and Nitrogen Fertilizations on Some Plant Growth Parameters and Nutrient Content of Chickpea

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Abstract: The study aimed to investigate the effects of *Glomus intraradices* Schenk and Smith (Arbuscular Mycorrhiza Fungus = AMF) inoculation beside phosphorous (P) and nitrogen (N) fertilizations on the some plant characteristics and nutrient content of chickpea (*Cicer arietinum* cv. Aziziye-94). At the end of the study, while there were significant effects of inoculation on the plant height, the fresh and dry weights of shoots and phosphorous, potassium, calcium, zinc and copper contents in shoots, there was no significant effect on the nitrogen, magnesium, iron and manganese contents in shoots. Inoculation decreased the contents of magnesium and copper. Increasing doses of phosphorous and nitrogen applications significantly increased the plant height, the fresh and dry weight in shoots and the contents of nitrogen, phosphorous, iron, manganese and copper.

Key words: Arbuscular Mycorrhiza, *Glomus intraradices*, nitrogen, phosphorous, inoculation, chikpea

INTRODUCTION

Adequate nourishment of people depends on not only animal food products which are rich for animal protein but also the legumes which are rich for vegetal protein. Nowadays, legumes are getting more important in human nutrition in Turkey due to the high price of animal products.

Chickpea, with 21-24% protein content, is one of the highly nutritive legumes. Chickpea's protein content is higher than other edible legumes such as bean, broad bean and lentil. Moreover histidin, an aminoacid, content of chickpea is even higher than human milk[1].

Turkey is an important chickpea producer and exporter and it is proposed that its chickpea production will reach to 1.7 million tonnes with the help of high-yielding and Ascochyta resistant cultivars in 2015[2]. There are two kinds of symbiotic relationships between legumes and microorganisms. The first is related to *Rhizobium* bacteria fixing atmospheric nitrogen and the second is related to Arbuscular Mycorrhiza Fungus (AMF) ameliorating the phosphorous uptake. Dual application of AMF and *Rhizobium* acts as biological fertilization in legumes. Moreover, there are ameliorative synergetic effects of this dual application[3-5]. In the symbiotic relation of AMF, fungi supply nutrient and water to plants. Therefore, a kind of coexistence occurs to be helpful to the both organisms[6]. AMF takes the nutrients (especially phosphorous) which are in the forms that plants cannot uptake and transfer to plants[7-9]

The effects of AMF and *Rhizobium* inoculations on the nodulation, root colonization, nitrogen fixation and the yield of chickpea were investigated. These researchers stated that inoculation increased the nodulation, root colonization, seed amount, dry matter and nitrogen and phosphorous contents of both seeds and hays[10].

It has been also reported that phosphorous contents of seeds inoculated with Arbuscular Mycorrhizae increased and the development and growth of following generation ameloriated[11-13].

AM also increases the tolerance levels of plants against the drought, salinity and high heavy metal contents[14-17].

Many researchers are interested in AMF due to its contribution to phosphorous uptake. In many studies, it has been stated that AMF which live together symbiotically with 90% of plant communities in the nature play an important and decisive role in the phosphorous uptake of plants[18-20]. It was reported that AMF inoculation increased the phosphorous content and positively affected the nitrogen uptake in chickpea[19].

An important characteristic of soybean is that it utilizes more nitrogen and phosphorous from the environment by the help of nodulation bacteria and

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Mycorrhizal fungi living in its roots. Besides nitrogen and phosphorous fixation, the uptakes of zinc, copper, manganese and iron also increase[21,22]. Thanks to *Mycorrhiza*, researchers have long known that microorgamisms play important role in soil productivity and plant nutrition. It has also known that plants supply nutrients with not only with their roots but also with the help of mycorrhizal fungus[23].

It was reported that besides increase in nutrient uptake, mycorrhizal fungi increased the resistance of plants to a-biotic stress conditions such as salinity, excessive heat, drought, heavy metal toxicity and secreted growth promoting substance for plants[22].

Available phosphorous amount is not sufficient in soil of the Lake Van Basin and supplemental phosphorous fertilizer cannot be dissolved easily because of soil pH. Therefore, this study aimed to investigated the effects of AMF inoculation and phosphorous and nitrogen applications on the yield and nutrient content of chickpea.

MATERIALS AND METHODS

The experiment was carried out in greenhouse conditions from March to May in 2004.

Chickpea cv. Aziziye-94 was used as plant material and OM/95 isolate of *Glomus intraradices* (Gi), a mixture of root, soil, mycellium and spores was used as AMF material[24]. A mixture of soil, sand and pumice was used as plant growth medium. Three doses of ammonium sulphate (0 mg N kg^{-1}, 100 mg N kg^{-1} and 200 mg N kg^{-1}) and triple super phosphate (0 mg P_2O_5 kg^{-1}, 50 mg P_2O_5 kg^{-1} and 100 mg P_2O_5 kg^{-1}) were separately applied into the growing media of each application.

In the analysis of soil, the texture was determined by Bouyoucous' hydrometric method[25], pH in 1:2.5 soil: water suspension[26], lime by calcimetric methods[27], organic matter by the modified Walkley Black method[28], salt content by Richards[29], total nitrogen by Kjeldahl method[30], available phosphorous by the method Olsen *et al.*[31], potassium, calcium and magnesium by an extraction with 1 N neutral ammonium acetate[32], available iron, manganese, zinc and copper by mixing with dipropylenetriamine (DTPA)[30].

For mineral content analysis, plant samples were oven-dried at 68°C for 72 h and then were ground. Nitrogen was determined by Kjeldahl method[33], phosphorous was determined by spectrophotometrically by the indo-phenol-blue method[33]. Potassium, calcium, magnesium, iron, manganese, zinc and copper contents in the extracts were determined using atomic absorption spectrophotometry[33].

Experimental pots were disinfected with 10% of formaldehyde. Then autoclaved growing medium (1500 g), inoculum (250 g) and soybean seeds mixed with growing medium (250 g) and sand (500 g) were put into each pot, respectively.

The experiment was designed as Completely Randomized Block Design with three replications, each having three plants per pot. Pots were placed in a glasshouse and plants were watered with distilled water during the experiment. Plants were properly protected against the plant disease. Experiment was ended 2.5 months after seed sowing when the plants flowered. Some plant growth parameters such as plant height and fresh and dry matter contents of plants were determined.

Results were analysed with COSTAT statistical package programme and the means were grouped with Duncan Multiple Comperation method[34].

RESULTS

Analysis of growing medium: It is seen from the Table 1, growth medium was slightly alkaline; had low organic matter, medium lime, no saline, inadequate for nitrogen and phosphorous, adequate for potassium[35] and sufficient for available Fe, Mn, Zn and Cu[36].

Effects of applications on some plant growth criteria: Gi inoculation, phosphorous and nitrogen applications significantly affected some of the plant growth criteria of chickpea at different levels. Gi inoculation significantly ($p<0.01$) affected the plant height and shoot fresh weight. The nitrogen and phosphorous applications also significantly ($p<0.001$) affected these mentioned traits. While the NxP interaction and the interaction of Gi inoculation and nitrogen application significantly ($p<0.01$) affected only the plant height, the interaction of Gi inoculation and phosphorous application significantly ($p<0.001$) affected only the shoot dry weight (Table 2). However, the interaction of all there factors on these three traits was not significant .

The plant height and fresh and dry weights of shoots significantly increased by the increasing doses of phosphorous and the highest values for these traits obtained from the 100 mg P_2O_5 kg^{-1} application as 37.56 cm $plant^{-1}$, 6.81 and 2.87 g pot^{-1}, respectively (Table 2). The 100 mg N kg^{-1} application gave the highest plant height and shoot fresh weight values as 37.94 cm $plant^{-1}$ and 6.71 g pot^{-1}, respectively. The 200 mg N kg^{-1} application gave the highest shoot dry weight values as 2.71 g pot^{-1} and it had also higher shoot fresh weight as 6.67 g pot^{-1} (Table 2). Gi inoculation significantly increased

Table 1: Some physical and chemical properties of experimental soil

Texture class	pH (1:2.5)	Salt (%)	Lime (%)	Organic matter (%)	Total N (%)	Available P (ppm)	Exchangeable K (ppm)	Ca (%)	Mg (ppm)	Fe (ppm)	Available Mn (ppm)	Zn (ppm)	Cu (ppm)
Sandy-clay-loam	8.03	0.057	14.4	0.92	0.07	6.98	4800	0.58	221	10.3	11.2	4.4	2.6

Table 2: Means values of the Gi inoculation, nitrogen and phosphorous applications on some of the plant growth parameters in chickpea

		Plant height (cm)	Fresh weight (g/pot)	Dry weight(g/pot)
Phosphorous (mg kg^{-1})				
0		35.33b	6.15c	2.08c
50		36.78a	6.54b	2.59b
100		37.56a	6.81a	2.87a
Nitrogen (mg/kg)				
0		35.61b	6.12b	2.44b
100		37.94a	6.71a	2.40b
200		36.11b	6.67a	2.71a
Gi Inoculation				
With		37.78a	6.93a	2.81a
Without		35.33b	6.03b	2.22b
Applications	DF	F-values	F-values	F-values
Inoculation	1	484.0**	372.80**	402.63**
Nitrogen	2	28.8***	72.80***	21.64**
Nitrogen x inoculation	2	14.5**	03.09ns	02.23ns
Phosphorous	2	17.7***	16.41**	85.62***
Phosphorous x inoculation	2	02.7ns	00.04ns	28.44**
Phosphorous x nitrogen	4	06.3**	01.11ns	02.74ns
Phosphorous x nitrogen x inoculation	4	01.8ns	00.09ns	01.39ns

Table 3: Means values of nitrogen, phosphorous, potassium, calcium and magnesium contents of chickpea

		Nitrogen (%)	Phosphorous(%)	Potassium (%)	Calcium (%)	Magnesium (%)
Phosphorous (mg/kg)						
0		6.60b	0.654c	0.815b	3.892a	0.836
50		6.67b	0.681b	0.891a	3.723b	0.849
100		7.03a	0.750a	0.915a	3.763b	0.82
Nitrogen (mg/kg)						
0		6.16b	0.681b	0.878	3.935a	0.864a
100		6.97a	0.656c	0.888	3.960a	0.865a
200		7.18a	0.749a	0.855	3.493b	0.775b
Inoculation						
With		6.92	0.714a	0.894a	3.966a	0.825
Without		6.92	0.676b	0.854b	3.627b	0.844
Applications	DF	F-Values	F-Values	F-Values	F-Values	F-Values
Inoculation	1	09.43ns	119.40**	43.33*	481.81**	0.19ns
Nitrogen	2	31.15***	76.93***	02.42ns	150.80***	9.12**
Nitrogen x inoculation	2	01.90ns	11.01**	01.40ns	63.10***	0.17ns
Phosphorous	2	04.23*	204.61***	17.35***	03.39ns	1.58ns
Phosphorousxinoculation	2	02.60ns	09.58***	01.32ns	01.04ns	3.10*
Phosphorous x nitrogen	4	02.16ns	17.43***	17.22***	06.66***	3.26*
Phosphorous x nitrogen x inoculation	4	00.91ns	20.87***	02.22ns	10.68***	6.49**

ns= not significant; *: Significant at $p<0.05$ level; **: Significant at $p<0.01$ level; ***: Significant at $p<0.001$ level, Values followed by the different letter are significantly different

the plant weight and the fresh and dry shoot weights compared to the noninoculated ones as relatively 6.9, 14.9 and 2.7%, respectively (Table 2).

Effects of the application on the plant macro element content: The effects of Gi inoculation, nitrogen and phosphorous applications some of on the macro element content of chickpea plants are seen at Table 3. Gi inoculation significantly affected the P ($p<0.01$), K ($p<0.05$) and Ca ($p<0.01$) contents of shoot. Nitrogen application significantly affected the N, P and Ca contents of shoot at $p<0.001$ level; the Mg content of shoots at $p<0.01$ level. The interaction of nitrogen and AMF applications significantly affected the P and Ca contents of shoots (at $p<0.01$ and $p<0.001$ level, respectively). Phosphorous application significantly affected the P and K contents of shoot at $p<0.001$ level and the N content at $p<0.05$ level. The interaction of phosphorous and AMF applications only significantly affected the P and Mg contents of shoots at $p<0.001$ and $p<0.05$ levels, respectively. The interaction of P and N applications significantly affected the P, K and Ca contents of shoots

Table 4: Means values of iron, manganese, zinc, and copper contents of chikpea

		Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
Phosphorous (mg/kg)					
0		376.09b	16.91c	83.6	24.45c
50		389.86ab	18.61b	84.33	26.29b
100		406.99a	19.56a	85.53	29.14a
Nitrogen (mg/kg)					
0		328.34c	16.55b	78.90c	18.74c
100		395.32b	18.99a	83.36b	29.39b
200		449.28a	19.54a	91.20a	31.75a
Inoculation					
With		397.28	18.3	85.87	21.11b
Without z		384.68	18.42	83.1	32.15a
Applications	DF	F-Values	F-Values	F-Values	F-Values
Inoculation	1	05.12ns	00.23ns	193.44**	326.39**
Nitrogen	2	98.45***	42.17***	34.66***	518.42***
Nitrogen x inoculation	2	01.64ns	04.00ns	08.15*	396.75***
Phosphorous	2	06.02**	20.22***	01.10ns	014.81**
Phosphorous x inoculation	2	00.77ns	08.41**	00.37ns	04.79*
Phosphorous x nitrogen	4	01.29ns	13.76***	01.39ns	06.18**
Phosphorous x nitrogen x inoculation	4	00.61ns	01.66ns	01.92ns	04.52**

ns= not significant; *: Significant at $p<0.05$ level; **: Significant at $p<0.01$ level; ***: Significant at $p<0.001$ level, Values followed by the different letters are significantly different

at $p<0.001$ level, the Mg content at $p<0.05$ level. The interactions of all tree applications significantly affected the P and Ca contents of shoots at $P<0.001$ level and the Mg content at $p<0.05$ level.

The N, P and K contents of shoots significantly increased by the increasing doses of phosphorous and the highest values for these traits were obtained from the 100 mg P_2O_5 kg^{-1} application as 7.03, 0.75 and 0.92%, respectively. However, the Ca content of shoots significantly decreased by the increasing doses of phosphorous and the highest value was obtained from the 0 mg P_2O_5 kg^{-1} application as 3.89% (Table 3).

The N and P contents of shoots significantly increased by the increasing doses of phosphorous and the highest values for these traits were obtained from the 200 mg N kg^{-1} application as 7.18 and 0.75%, respectively (Table 3). However, the Ca and Mg contents of shoots did not change by the 100 mg N kg^{-1} application, but then significantly dropped by the 200 mg N kg^{-1} application as 3.49 and 0.78%, respectively. Gi inoculation significantly increased the P, K and Ca contents of shoots compared to the noninoculated ones as relatively 5.6, 4.6 and 9.3%, respectively (Table 3).

Effects of applications on the plant micro element content: Gi inoculation significantly ($p<0.01$) affected the Cu and Zn contents. Nitrogen application significantly ($P<0.001$) affected Fe, Mn, Zn and Cu contents. The interactions of nitrogen and inoculation applications significantly affected the Zn and Cu contents at $P<0.05$ and $P<0.001$ level, respectively. Phosphorous application significantly affected Mn and Cu contents at $P<0.001$ level and the Fe content at $p<0.01$ level. Interaction of phosphorous and AMF and interaction of P and N significantly affected the Mn and Cu contents at $p<0.001$ and $p<0.01$ levels, respectively. Interactions of all these three factors (P, N and AMF) significantly ($p<0.001$) affected only the content of Cu (Table 4).

Increasing doses of phosphorous significantly increased the Fe, Mn and Cu contents in shoot. The application of 100 mg P_2O_5 kg^{-1} caused the highest values of these mineral matters as 406.99, 19.56 and 29.14 ppm, respectively (Table 4).

Increasing doses of nitrogen also significantly increased the Fe, Mn, Zn and Cu contents. For example, the application of 200 mg N kg^{-1} caused the highest values for there minerals as 449.28, 19.54, 91.20 ppm and 31.75 ppm, respectively. AMF inoculation significantly decreased the Cupper content, but increased the Zn content (Table 4) as 3.3 and 52.3%, respectively.

DISCUSSION

This study aimed to investigate the effects of Gi inoculation besides P and N applications on the plant growth and nutrient content in chickpea. Gi inoculation significantly increased all of the plant growth parameters investigated and P, K, Ca and Zn contents. Several studies reported that the most important contribution to P uptake has been employed by soil microorganisms, mainly by AMF[37,38]. Present fundings are in agreement with the literature. Moreover N and P applications had also significant effects on all of the plant growth criteria and most of the plant nutrient contents. Several researches have been reported that soil microorganisms, mainly AMF, have important role in the uptake of phosphorous[21,37-40]. On the other hand, Gi inoculation insignificant increase in the Fe content and decreases in the Cu content.

The ameliorative effect of AMF could be explained by changes in root system of chcikpea. AMF have increased the root area of plant by producing abundant amounts of hypes and by up taking plant nutrient in relatively remote areas[23,41,42]. N_2 fixation and the plant development have been also ameliorated in legumes because of its positive effects on P uptake; legumes need high amount of phosphorous for nodulation; therefore, require Gi inoculation in P deficient soil. It has been also reported that for maximum crop yield only 50% of the required fertilizer might be supplied with bioinoculants[43]. As we consider our results, Gi inoculation along with moderate levels of fertilizer (50 mg P_2O_5 kg^{-1} and 100 mg N kg^{-1}) could be recommended for chickpea production.

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