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

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Abstract: The aim of the agricultural production is to best supply the human requirements. This study aimed to investigate the effects of *Glomus intraradices* Schenk and Smith (Arbuscular Mycorrhiza Fungus = AMF) inoculation and Phosphorous (P) and Nitrogen (N) fertilizations on the some plant characteristics and nutrient content of soybean (*Glycine max* L. Amsoy-71). 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, soybean

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.

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 (Mosse *et al*., 1976; Redente and Reeves, 1981; Abd-Alla *et al*., 2000). 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 (Marschner, 1995). AMF takes the nutrients (especially phosphorous) which are in the forms that plants cannot uptake and transfer to plants (Papastylianou, 1993; George *et al*., 1995; Smith and Hayman, 1997).

Kumar *et al*. (1998) investigated the effects of AMF and *Rhizobium* inoculations on the nodulation, root colonization, nitrogen fixation and the yield of chickpea. They stated that inoculation increased the nodulation, root colonization, seed amount, dry matter and nitrogen and phosphorous contents of both seeds and hays.

It has been also reported that phosphorous contents of seeds inoculated with Arbuscular Mycorrhizae increased and development and growth of following generation ameliorated (Koide and Lu, 1995; Merryweather and Fitter, 1996; Zhu and Smith, 2001).

AM researches the tolerance levels of plants against the drought, salinity and high heavy metal contents (Jones and Hutchinson, 1986; Shetty *et al*., 1994, 1995; Sharples *et al*., 2000).

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 (Tüfenkçi *et al*., 2000; Shockley *et al*., 2004). Tüfenkçi *et al*. (2000) reported that AMF inoculation increased the phosphorous content and positively affected the nitrogen uptake in chickpea.

An important characteristic of soybean is that it utilizes more nitrogen and phosphorous from the environment by the help of nodulation bacteria and Mycorrhizal fungi living in its roots. Besides nitrogen and phosphorous fixation, the uptakes of zinc, copper, manganese and iron also increase (Hayman, 1982; Smith and Read, 1997). Thanks to *Mycorrhiza*, researchers have long known that microorganisms play important role in soil productivity and plant nutrition. It has also known that plants supply nutrients not only with their roots but also with the help of mycorrhizal fungus (Bolan, 1991).

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Smith and Read (1997) 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.

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 investigate the effects of AMF inoculation and phosphorous and nitrogen applications on the yield and nutrient content of soybean.

MATERIALS AND METHODS

Material: This study was carried out at the glasshouse of the Department of Horticulture, Yuzuncu Yil University, Van, Turkey, in 2004.

Soybean cv. Amsoy-71 was used as plant material and OM/95 isolate of *Glomus intraradices* (*Gi*), a mixture of root, soil mycelium and spores, was used as AMF material (Demir and Onoğur, 1999). 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.

Methods: In the analysis of soil, the texture was determined by Bouyoucous (1951) hydrometric method pH in 1:2.5 soil: water suspension (Jackson, 1958), lime by calcimetric methods, organic matter by the modified Walkley (1947) Black method salt content by Richards (1954), total nitrogen by Kjeldahl method (Kacar, 1994), available phosphorous by the method Olsen *et al*. (1954), potassium, calcium and magnesium by an extraction with 1 N neutral ammonium acetate (Thomas, 1982), available iron, manganese, zinc and copper by mixing with dipropylenetriamine (DTPA) (Kacar, 1994).

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 (Kacar, 1984), phosphorous was determined by spectrophotometrically by the indo-phenol-blue method (Kacar, 1984). Potassium, calcium, magnesium, iron, manganese, zinc and copper contents in the extracts were determined using atomic absorption spectrophotometry (Kacar, 1984).

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

The analysis of growing medium: As 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 (Aydeniz, 1985).

Effects of the applications on some of the plant growth criteria: *Gi* inoculation, phosphorous and nitrogen applications significantly affected some of the plant growth criteria of soybean at different levels. *Gi* inoculation significantly ($p<0.01$) affected the plant height and shoot fresh weight. Nitrogen application significantly ($p<0.001$) affected the fresh and dry weight of shoot. The interaction of *Gi* inoculation and nitrogen application significantly ($p<0.05$) affected the plant height. There were significant effects of phosphorous on plant height ($p<0.01$), shoot fresh weight ($p<0.001$) and shoot dry weight ($p<0.05$) (Table 2).

The shoot fresh weight was also significantly ($p<0.001$) affected by the interactions of phosphorous and *Gi* inoculation. There were significant effects of phosphorous and the interaction of phosphorous and nitrogenous on the plant height ($p<0.001$), shoot fresh weight ($p<0.001$) and shoot dry weight ($p<0.05$). Moreover, the interaction of all there factors on shoot dry weight was significant ($p<0.001$).

By increasing doses of phosphorous and especially with the dose of 100 mg P_2O_5 kg^{-1}, 7.5, 36.3 and 21.0% increases compared to the control dose were obtained in plant height, fresh plant weight and plant dry weight, respectively. The dose of 50 mg P_2O_5 kg^{-1} also increased the plant fresh weight by 27.8%. There was 6.6% difference between the fresh weight values of the doses of 50 and 100 mg P_2O_5 kg^{-1}. The dose of 50 mg P_2O_5 kg^{-1} had no significant increase in the plant dry weight, but the dose of 100 mg P_2O_5 kg^{-1} increased the plant dry weight by 20.8% (Table 2).

Plant criteria increased with increasing doses of nitrogenous. Although there were 3.9 and 3.7% increases in plant height with 100 and 200 mg N kg^{-1}, respectively,

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)	Exchangeable Ca (%)	Exchangeable Mg (ppm)	Available Fe (ppm)	Available Mn (ppm)	Available Zn (ppm)	Available Cu (ppm)
Sandy-clay-loam	8.03	0.057	14.4	0.92	0.07	6.98	4800	0.48	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 yield criteria

		Plant height (cm)	Fresh weight (g pot)	Dry weight (g pot)
Phosphorous (mg P_2O_5 kg)				
0		33.89b#	2.48c	0.28b
50		35.28ab	3.17b	0.29b
100		36.44a	3.38a	0.34a
Nitrogen (mg kg)				
0		34.33	2.54c	0.20c
100		35.67	3.11b	0.31b
200		35.61	3.37a	0.41a
Gi Inoculation				
With		37.04a	3.31a	0.34
Without		33.37b	2.71b	0.27
Applications	df	F-values	F-values	F-values
Inoculation	1	130.68**	13.26**	15.43ns
Nitrogen	2	2.16ns	52.59***	98.54***
Nitrogen × inoculation	2	4.73*	3.84ns	4.36ns
Phosphorous	2	7.12**	106.53***	3.84*
Phosphorous × inoculation	2	0.09ns	9.86***	0.16ns
Phosphorous × nitrogen	4	6.75***	12.09***	6.05**
Phosphorous × nitrogen × inoculation	4	1.11ns	8.64***	0.63ns

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(s) are significantly different

these were statistically insignificant. However, there were significant increases in plant fresh weight at these two doses as 22.4 and 32.7%, respectively. There was 8.4% difference between the fresh weight values of the doses of 100 and 200 mg N kg^{-1}. The dry weight was significantly increased compared the control dose by the doses of 100 and 200 mg N kg^{-1} as 55 and 105%, respectively. There was 32.3% difference between the plant dry weight values of the doses of 100 and 200 mg N kg^{-1} (Table 2).

Plant height, fresh plant weight and plant dry weight increased with the application of mycorrhiza as 11.0, 22.1 and 25.9%, respectively.

Effects of the application on the plant macro element content: *Gi* inoculation significantly ($p<0.05$) affected the only P and Ca contents of shoot. Nitrogen application significantly affected the N and P contents of shoot at $p<0.001$ level; the K and Mg contents of shoots at $p<0.01$ level, the Ca content of shoot at $p<0.05$ level (Table 3).

The interaction of nitrogen and AMF applications significantly affected the K, Ca and Mg contents of shoots (at $p<0.001$, $p<0.01$ and $p<0.05$ level, respectively). Phosphorous application significantly affected the N, P and K contents of shoot at $p<0.001$ level, the Ca content at $p<0.01$ level, the Mg content at $p<0.05$ level. The interaction of phosphorous and AMF applications only significantly affected the P content at $p<0.05$ level of shoots. The interaction of P and N applications significantly affected the N, P and Mg contents of shoots at $p<0.001$ level, the Ca content at $p<0.01$ level; the K content at $p<0.05$ level. The interactions of all true applications significantly affected the N and Mg contents of shoots at $p<0.05$ level and the Ca content at $p<0.01$ level.

As seen from Table 3, by increasing doses of phosphorous and especially with the dose of 100 mg $P_2O_5 kg^{-1}$, 9.5, 20.9 and 14.6% increases compared to the control dose were obtained in the contents of N, P and Mg, respectively. However, the dose of 100 mg P_2O_5 kg^{-1} decreased the plant K content by 41.1%. There was 18.4% decrease between the plant K content values of the doses of 50 and 100 mg $P_2O_5 kg^{-1}$. There was 7.2% increase in the plant Ca content compared to control at the dose of 50 mg P_2O_5 kg^{-1}. The dose of 100 mg $P_2O_5 kg^{-1}$ had insignificant decrease (2.6%) in the plant Ca content compared to the dose of 50 mg $P_2O_5 kg^{-1}$.

The plant N and P contents significantly increased (33.1 and 19.2% increases, respectively when compared the control dose with the dose of 200 mg N kg^{-1}) with the increasing doses of nitrogenous doses. There were 13.3 and 8.6% differences between the N and P contents of the doses of 100 and 200 mg N kg^{-1}, respectively (Table 3).

Increasing doses of nitrogenous irregularly affected the plant K content. While there was 10.0% decrease in this trait with the dose of 100 mg N kg^{-1}, there was 6.3%

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

		Nitrogen (%)	Phosphorous (%)	Potassium (%)	Calcium (%)	Magnesium (%)
Phosphorous (mg P_2O_5/kg)						
0		6.545b#	0.707c	1.576a	4.496b	0.792b
50		7.110a	0.770b	1.322b	4.821a	0.832ab
100		7.169a	0.855a	1.117c	4.699a	0.908a
Nitrogen (mg kg)						
0		5.941c	0.709c	1.385a	4.498b	0.863a
100		6.978b	0.778b	1.259b	4.708a	0.789b
200		7.906a	0.845a	1.372a	4.780a	0.879a
Inoculation						
With		6.975	0.794a	1.499	4.851a	0.864
Without		6.907	0.741b	1.177	4.472b	0.824
Applications	df	F-values	F-values	F-values		F-values
Inoculation	1	0.46ns	47.83*	12.37ns	19.66*	4.72ns
Nitrogen	2	121.17***	89.99***	13.36**	8.42*	11.17**
Nitrogen × inoculation	2	1.20ns	4.24ns	25.72***	12.46**	7.01*
Phosphorous	2	47.49***	241.50***	12.64***	7.37**	3.41*
Phosphorous×inoculation	2	1.72 ns	4.06*	1.64ns	2.81ns	2.82ns
Phosphorous × nitrogen	4	18.49***	44.67***	3.88*	5.53**	8.98***
Phosphorous × nitrogen × inoculation	4	2.92*	0.96ns	1.18ns	4.52**	3.75*

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(s) are significantly different

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

		Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
Phosphorous (mg P_2O_5 kg)					
0		410c	8.75b	81.04b	34.69a
50		443b	11.25a	100.30a	31.80b
100		460a	11.52a	71.45b	34.34a
Nitrogen (mg kg^{-1})					
0		407a	10.97a	80.53	30.89b
100		452a	10.85a	80.25	35.21a
200		456a	9.70b	91.74	34.73a
Inoculation					
With		447	11.08	86.34	32.64b
Without		429	9.93	82.01	34.57a
Applications	df	F-values	F-values	F-values	F-values
Inoculation	1	8.67ns	15.99ns	1.66ns	55.91*
Nitrogen	2	46.22***	11.61**	1.88ns	5.76*
Nitrogen × inoculation	2	3.26ns	10.02**	6.50*	4.73**
Phosphorous	2	22.48***	53.66***	13.49***	6.23**
Phosphorous × inoculation	2	0.86ns	3.24ns	14.24***	7.59**
Phosphorous × nitrogen	4	0.94ns	20.43***	2.39ns	77.12***
Phosphorous × nitrogen × inoculation	4	1.99ns	18.72***	2.28ns	10.56***

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(s) are significantly different

increase in this trait with the dose of 200 mg N kg^{-1}. Similarly, there was 9.4% decrease in the plant Mg content with the dose of 100 mg N kg^{-1} and then there was 1.9% increase in this trait with the dose of 200 mg N kg^{-1}.

The application of mycorrhiza only and significantly increased the plant P and Ca contents as 7.2 and 8.5%, respectively. However, there were insignificant decreases in the N, P and Mg contents.

Effects of applications on the plant micro element content: *Gi* inoculation significantly (p<0.01) affected the Cu content. Nitrogen application significantly affected Fe, Mn and Cu contents at p<0.001, p<0.01 and p<0.05 level, respectively. The interactions of nitrogen and inoculation applications significantly affected the Mn, Zn and Cu contents at p<0.01, p<0.05 and p<0.05 levels, respectively (Table 4).

Phosphorous application significantly affected Fe, Mn and Zn contents at p<0.001 level and the Cu content at p<0.01 level. Interaction of phosphorous and AMF significantly affected the Zn and Cu contents at p<0.001 and p<0.01 levels, respectively. Interaction of P and N significantly affected the Mn and Cu contents at p<0.001 and p<0.01 levels, respectively. Interactions of there factors (P, N and AMF) significantly (p<0.001) affected the contents of Mn and Cu.

As seen from Table 4, by increasing doses of phosphorous and especially with the dose of

100 mg P_2O_5 kg^{-1}, 12 and 31.7% increases compared to the control dose were obtained in the contents of Fe and Mn, respectively. The dose of 50 mg P_2O_5 kg^{-1} increased the same traits as 8 and 28.6%, respectively. There were 5.2 and 4.6% differences between the Fe and Mn contents of the doses of 100 and 200 mg P_2O_5 kg^{-1}, respectively.

While there was 23.8% increase in the plant Zn content with the dose of 50 mg P_2O_5 kg^{-1}, there was 13.0% decrease in this trait with the dose of 100 mg P_2O_5 kg^{-1}. There were decreases in the plan Cu content as 9.3 and 1.0% the doses of 100 and 200 mg P_2O_5 kg^{-1}, respectively. The plant Fe content significantly increased (11.0 and 12.0% increases, respectively) when compared the control dose with the doses of 100 and 200 mg N kg^{-1}. The plant Mn content significantly increased (13.1%) when compared the control dose with especially the dose 200 mg N kg^{-1}. Moreover, there was 13.9%, but insignificant increase in the Plant Zn content. The plant Cu content significantly increased (14.0%) when compared the control dose with especially the dose 100 mg N kg^{-1}.

The mycorrhiza inoculum only decreased the plant Cu content as 5.9%. The application of mycorrhiza also insignificantly increased the plant Fe, Mn and Zn contents as 4.2, 11.6 and 5.3%, respectively.

DISCUSSION

At the end of the study, the dose of 100 mg P_2O_5 kg^{-1} gave 2.55 cm, 0.9 g, 0.06 g, 0.715%, 0.148%, 0.116% better responses compared to the control dose in plant height, fresh plant weight, plant dry weight and the contents of N, P and Mg, respectively. However, the plant K content decreased with the increasing doses of phosphorous. The lowest K content was obtained from, the dose of 100 mg P_2O_5 kg^{-1}.

The micro element contents (especially Fe, Zn and Cu) changed with the increasing doses of phosphorous. The dose of 100 mg P_2O_5 kg^{-1} gave 50 and 2.75 ppm better responses compared to the control dose in the contents of Fe and Mn, respectively. The highest increase in the plant Cu content (18.99 ppm) was obtained from the dose of 50 mg P_2O_5 kg^{-1}. All these results were in parallel with the findings of Tufenkçi *et al.* (2005). Kahiluoto *et al.* (2001) reported that phosphorous application increased the plant dry matter.

The fresh and dry weights were significantly affected by the increasing doses of nitrogenous applications. The dose of 200 mg N kg^{-1} gave 0.83 g and 0.21 g better responses compared to the control dose in the fresh dry plant weight, respectively. The 1.28 cm increase was obtained from the nitrogenous application, but it was statistically insignificant. There were 1.965, 0.136, 0.281 and 0.016% increases in the plant N, P, Ca and Mg contents, respectively with the dose of 200 mg N kg^{-1}. The Fe and Cu contents increased with the dose of 200 mg N kg^{-1} as 49 and 3.84 ppm, respectively. The 11.21 ppm increase in the Zn content was insignificant, though. The Mn content decreased as 1.27 ppm with the dose of 200 mg N kg^{-1}.

The plant heigh and fresh plant weight were significantly affected by mycorrhiza application. Mycorrhiza application gave 3.67 cm and 0.6 g better responses compared to the control dose in these traits. The 0.07 g increase in the plant dry by mycorrhiza application weight was insignificant. While mycorrhiza application increased the plant N, P, K, Mg, Ca, Fe, Mn and Zn contents, it decreased the plant Cu content. The changes only in P, Ca and Cu were significant. Kung'u (2004) reported that mycorrhiza application significantly increased the plant N, P and K contents in *Senna spectabilis*. Chulan and Martin (1992) stated that mycorrhiza application significantly increased the plant dry matter content in *Theobroma cacao*. Aggangan and Dela Cruz (1991) also reported that mycorrhiza application significantly increased the plant dry matter content (631%) in *L. leucocephala*. Yoshitaka and Yamamoto (1986) determined that mycorrhiza application significantly increased the plant P, Ca and Mg contents in soybean. Abdel-Fattah (2001) reported that mycorrhiza application significantly increased phosphates enzymes of soybean plants as well as the plant N and P contents. Kung'u (2004) found 213% increase in plant dry matter contents in mycorrhiza application.

Researchers have demonstrated that vesicular-arbuscular mycorrhiza fungi, not only increases phosphorus uptake, but also plays an important role in the uptake of other plant nutrients and water (Huang *et al*., 1985; Ellis *et al*., 1985). Sander and Sheikh, (1983), reported that the inflows of phosphorus to mycorrhiza roots can be greater than inflows to comparable non-mycorrhiza roots by up to 2-5 times. Li *et al.* (1991), demonstrated that about 10% of the total potassium uptake in mycorrhizal coach grass was due to hyphal uptake and transport.

Investigate the effects of *Gi* inoculation besides P and N applications on the plant growth and nutrient content in soybean. *Gi* inoculation significantly increased some of the plant growth criteria and P, K and Zn contents. Moreover, N and P applications had also significant effects on same of the plant growth criteria and the N, P, Fe, Mn and Cu contents.

At the end of the study, it was concluded that *Gi* inoculation significantly improved the plant growth and some nutrient contents such as P, K and Zn in soybean. Several researches have been reported that soil

microorganisms, mainly AMF, have important role in the uptake of phosphorous (Koide, 1991; Kothari *et al*., 1991; Sylvia and William, 1992; Smith and Read, 1997; Achakzai *et al*., 2002). On the other hand, *Gi* inoculation in significant increase in the Fe content and decreases in the Mg, Mn and Cu contents and AMF increased the root area of plant by producing abundant amounts of hypes and by up taking plant nutrient in relatively remote areas (Gür, 1975; Abbott and Robson, 1981; Bolan, 1991).

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.

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