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Effects of Bacterial Inoculation and Molybdenum Application on Nutrient Element Content of Beans

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ABSTRACT

This study was carried out to determine the effect of different doses of molybdenum with bacterial inoculation on macro and micro- elements content of bean grains. In this study, Akman 98 dry bean variety was used as plant material, Molybdenum dosages were Mo0 = Molybdenum free, Mo1 = 0.05 mg/kg, Mo2 = 0.10 mg/kg and *Rhizobium tropici* (CIAT899), bacterium of *Phaseolus* plant, was used as inoculation material. The study was planned according to the randomized complete blocks in factorial design with three replicates. Following the performed field trial, nutrient elements (N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn) of bean grains were determined. According to the results obtained from the study, the effect of molybdenum application and inoculation on nutrient elements of bean grains was different and statistically significant. This effect on nutrients showed that the nutrients in the bean grains generally increased due to the increase in molybdenum doses. Mineral content of bean grains were determined as nitrogen (N) 3.59-4.39, protein content 22.44-27.44, phosphorus (P) 0.36-0.46 potassium (K) 1.83-2.33 magnesium (Mg) 0.17-0.20 calcium (Ca) (%), as mg/kg (Fe) 37.59-65.25 copper (Cu) 5.27-7.69 manganese (Mn) 14.98-16.96 zinc (Zn) 14.77-27.28. The nutrient contents of inoculated bean grains were found to be higher than the non-inoculated ones. Nutrient contents were different depending on the inoculation and dose applications, and this difference was found to be statistically significant.

1. Introduction

The beans enrich the soil with nutrients by removing the nutrients found in the lower layers of the soil to the soil surface through the advanced root systems. Beans meet most nitrogen needs from the soil air by utilizing the free nitrogen of the air through the bacterium *Rhizobium phaseoli* with a common life feature in its roots, and it enriches the soil in which they grow with nitrogen. In this way, an average of 5 kg /da of nitrogen per year are able to fixed. The loss of nitrogen in this way is less than that of nitrogenous fertilizers, it does not lead to pollution of drinking water and does not cause the quality deficiency resulting from chemical fertilization (Akçin, 1976).

When the microorganisms, which bind the free nitrogen of the air to the soil by establishing symbiotic life with leguminous plants and generally known as *Rhizobium* spp. are not given to the soil, they are generally found in the soil in limited number or they are not effective. For this reason, the amount of nitrogen bound to the soil by biological means is low in the situations without inoculation (Gök and Onaç, 1995). If the seed is cultivated by inoculation with effective bacterial strains, nodosites will occur early in the development of the plant roots and the plant may complete its development without being affected by lack of nitrogen in the soil. The nitrogen supplied to the plant through nodosites enters the plant metabolism as organic compounds and the plant can easily benefit from these compounds (Haktanır and Arcak, 1997).

Bergersen (1971) reported that molybdenum plays a role in the basic mechanism of nitrogen fixation by activating nitrogenase enzyme and it is an important element for bacteria that co-live with high plants and fix nitrogen. Many investigators have shown that bacterial inoculation in legumes affects nitrogen content in vegetative growth, dry matter formation, grain yield, nodulation, vegetative growth, nodule and granule (Gök and Onaç, 1995).

Molybdenum deficiency is observed in plants grown in soil with molybdenum below 0.025 kg in decadence (Aydemir, 1985). Some plants have higher requirements for molybdenum. For example, in terms of nitrogen determination, *Leguminous* plants and *Cruciferae* family plants, especially cauliflower and cabbage, have higher need for molybdenum. This research was carried out to determine bacterial inoculation,

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which is important in protein rich grain yield, and to determine the effect of different molybdenum doses on nutrient content of bean grains due to increased nodulation and nitrogen detection in plants.

2. Materials and Methods

In this study, Akman 98 bean (*Phaseolus vulgaris L.*), which is the registered product of Anatolia Agricultural Research Institute, was used as bean variety and, as bacteria, *Rhizobium tropici* (CIAT899) was used, which was provided from the biological laboratories of Soil, Fertilizer and Water Resources Central Research Institute Directorate, Ankara. The trial was set up according to randomized block experimental designs with three replications and it was carried out in field conditions as a two-year study. In the experiment, each plot size was 2.5 m x 2 m, row spacing was 50 cm, and row top was 20 cm. This is a complete factorial treatments design, with factor A (2 levels: inoculated and non-inoculated) and factor B (3 levels: Mo_0= without molybdenum, Mo_1=0.05 mg/kg, Mo_2=0.10 mg/kg).

After these plots were made ready for planting and after the surface sterilization of the seeds was made with 0.5% sodium hypochlorite, they were sown by inoculation of *Rhizobium tropici* bacteria developed in YMB (Yeast mannitol broth). The study was carried out on a total of 24 plots including 3 molybdenum dozes, 2 inoculation factors and 4 replications. The amount of seed was calculated for each plot, and placed in a polyethylene bag. Seed surfaces were soaked by adding 10% sucrose solution in 1% ratio to the bean seeds in the polyethylene bags which were going to be used in the parcels to be inoculated. 1% inoculant was added to the bag, and the bags were swayed gently, allowing the inoculant to stick to the seeds. The seeds were dried in the shade and then planted immediately. So as to prevent contamination, first the control was done and the parcels with bacterial inoculation were planted. The planting was made in a plant population of 50 cm between rows and 20 cm row top. It was made in the evening to avoid the negative effects of sun rays on bacteria. In the field trial macro element base fertilizer was applied to all parcels during sowing as 4 kg N/da $(NH_4)_2SO_4$ (%21 N), 6 kg P_2O_5/da TSP (% 45 P_2O_5), 5 kg K_2O/da K_2SO_4 (% 50 K_2O) and micro element base fertilizer was given as 5 mg kg^{-1} Fe, 12 mg kg^{-1} Mn, 2 mg kg^{-1} Zn, 1 mg kg^{-1} Cu.

The analysis of the soil sample used in the test was performed according to texture: Bouyocous (1951), pH: Richards (1954), EC: U.S. Salinity Lab. Staff (1954), organic matter: Smith and Weldon (1941), $CaCO_3$: Hızalan and Ünal (1966), total nitrogen: Bremner (1965), phosphorus: Olsen et al., (1954), changeable cations: Knudsen et al. (1982), trace elements Soltanpour and Workman (1981). Some physical and chemical properties of the research soil are given in Table 1. After 90 days of vegetation, plants were harvested by hand. Following the harvest, nutrient element (N, P, K, Ca, Mg, Fe, Cu, Mn and Zn) analysis of plant seeds was performed using method of wet decomposition (Bayraklı 1987) with sulphuric acid (Lindsay and Norwell 1978).

Table 1
Some physical and chemical characteristics of soil used at experiment.

Properties	Value	Properties		Value
pH	7.67	Fe	mg kg^{-1}	3.42
EC (dSm^{-1})	0.313	Cu		5.88
% $CaCO_3$	22.42	Mn		36.18
% Organic Matter	1.20	Zn		2.46
% Clay	24.2	B		3.92
% Silt	32.6	Mo		0.015
% Sand	43.2	Ca	me /100g	1.8
Class	Loam	Mg		1.6
N (mg kg^{-1})	111.23	K		0.99
P (mg kg^{-1})	13.60			

Statistical Analysis

Data were analyzed as a factorial experiment in a completely randomized manner with three replications using the JMP statistical software version 5.1 (SAS Institute INc., Cary, NC, USA). Sources of variation were treatments, incubation day and their interaction. Means were compared by Student's t-test at a significance level of 0.05.

3. Results and Discussion

In order to determine the effect of bacterial inoculation and molybdenum application of Akman 98 bean variety on the nutrients of the beans, nutrients were determined as (N, P, K, Ca, Mg, Fe, Cu, Mn, Zn) in the bean grains in this study. According to the results obtained from the study, the effect of molybdenum application and inoculation on nutrient elements of the bean grains was different and statistically significant (Table 2). It was found that with the increase of the molybdenum doses of this effect to the nutrients, the nutrients in the bean grains were generally increased. Mineral content in bean seeds were as following; (Fe) 37.59-65.25 (mg / kg) in the form of calcium (Ca) 0.17-0.20 (%), magnesium (Mg) 0.17-0.20 (%), copper (Cu) 5.27-7.69 (mg/kg) manganese (Mn) 14.98-16.96 (mg/kg) zinc (Zn) 14.77-27.28 (mg/kg). The nutrients in the bean grains of the plants that were inoculated were found to be higher than those that were not inoculated. In addition, the effect of molybdenum doses on the nutrients of the plant was also found to be statistically significant. Mo is an important micronutrient element for biological N-fixation in soil and acts as a central metal ion for both NA (Neuraminidase) and NR (Nitrat redüktaz) enzymes in cofactors (Mendel and Hänsch 2002).

Table 2
The results of variance analysis of rhizobium inoculation and Mo application on the macro and micro nutrient element content of beans seed

Sources	DF	N		P		K		Ca		Mg	
		MS	F	MS	F	MS	F	MS	F	MS	F
Inoculation	1	0.279	18.41**	0.0018	3.00	0.033	0.58	0.0002	0.67	0.0003	1.64
Dose	2	0.237	15.65**	0.0055	9.07**	0.146	2.57	0.0014	5.73*	0.0003	1.56
Ino. x Dose	2	0.193	12.77**	0.004	6.55*	0.064	1.13	0.0002	0.96	0.0005	2.27
Error	12	0.015	---	0.0006	---	0.057	---	0.0028	---	0.0002	---
Total	17	---	---		------	---	---	---	---	---	---

Table 2 (continuation)

Sources	DF	Fe		Cu		Mn		Zn	
		MS	F	MS	F	MS	F	MS	F
Inoculation	1	547.25	18.68**	3.467	15.49**	1.191	1.29	64.592	29.94**
Dose	2	242.69	8.28**	2.814	12.57**	2.254	2.45	80.91	37.50**
Ino. x Dose	2	140.34	4.79*	1.919	8.57**	0.588	0.64	24.96	11.57**
Error	12	29.30	---	0.224	---	0.922	---	2.16	---
Total	17	---	---	---	---	---	---	---	---

* : $P<0.05$, ** : $P<0.01$

The effect of the application of inoculation and molybdenum on the nitrogen content of the bean stem was found to be statistically significant. The nitrogen content of bean ranges from 3.59 to 4.39%. Inoculation and molybdenum application increased the nitrogen content of the plant. The phosphorus contents (0.36%) of the plants which were not inoculated were lower than the inoculated plants (0.46%). The effect of the inoculation on the grain potassium content was different from the non-inoculated conditions, and this difference was statistically significant ($p<0.05$). The K content of non-inoculated plants was determined to be 1.83%, and the potassium content of the grafted plants was found to be higher (2.33%). By inoculation, the magnesium and calcium levels increased slightly and this increase was found to be statistically significant. The calcium and magnesium content of the bean ranges from 0.17 to 0.20%.

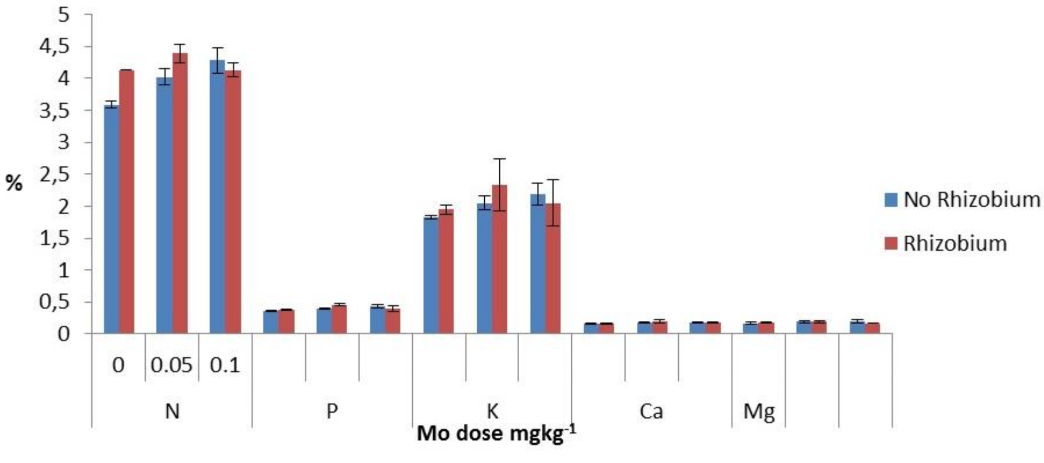

Figure 1
Effect of Rhizobium Inoculation and Mo Application on the Macro Nutrient Element Content of Beans Seed

According to the results obtained from the research, the effect of molybdenum application and micronutrient on micronutrient elements in bean was different and statistically significant. It was found that micronutrients generally increase in micronutrients in the bean by enhancing the molybdenum doses of this effect. The content of Fe in beans ranged from 37.59 to 65.25 mg/kg, and the application of grafting and molybdenum increased the content of iron in the grain. The copper content (7.69 mg/kg) of the bean grains of the grafted plants was found to be higher than the grains content of uninoculated plants (5.27 mg/kg). In addition, the effect of molybdenum doses on the copper content of the plant was also found to be statistically significant. Manganese content of bean was determined between 14.98-16.96 mg/kg, and the manganese contents of plants vaccinated were higher. The zinc content of the plant increased by grafting and molybdenum application and this increase was found statistically significant. The amount of zinc after non-inoculated plants was determined to be 14.77 mg/kg, which increased to 27.28 mg/ kg by inoculation.

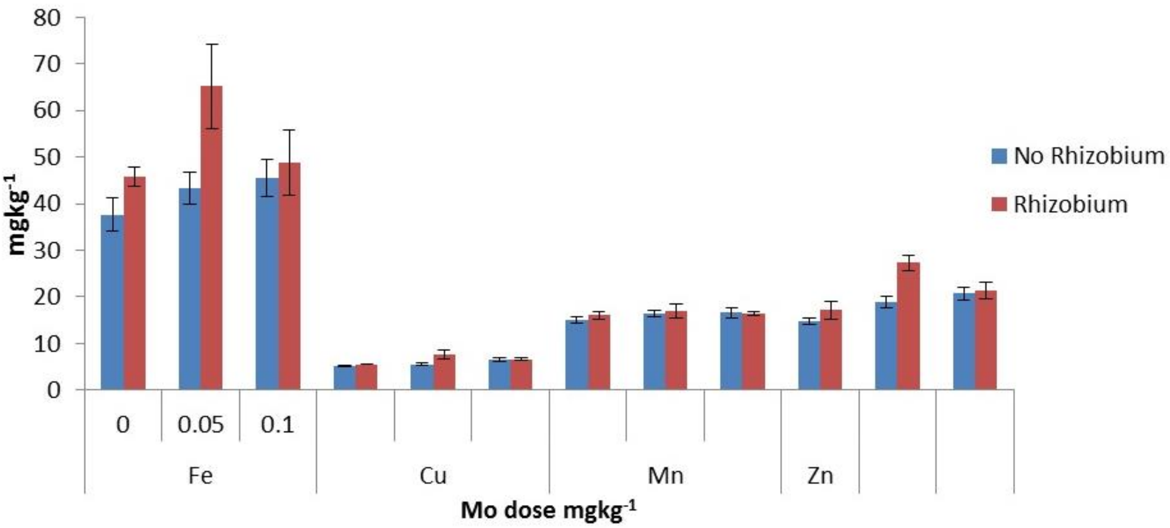

Figure 2
Effect of Rhizobium Inoculation and Mo Application on the Micro Nutrient Element Content of Beans Seed

Molybdenum is found in the structure of nitrogenase and every bacterium that binds to nitrogen needs molybdenum during fixation. Molybdenum nitrogenase is a necessary component of two important enzymes such as nitrate reductase. It was determined that nodules did not detect nitrogen even though they were formed in plants grown in soil with molybdenum deficiency. Manganese deficiency inhibits chlorophyll formation of the plant (Kızıloğlu 1995). On the other hand, Şehirali et al. (1981), Aydemir (1985), Akçin 1976, Bozoğlu et al. (1997), Gür (2002), Mut and Gülümser (2003), Nadeem et al. (2004), Önder et al. (2003), Kacar et al. (2004), Gök et al. 2003, Doğan et al., (2007), Küçük and Kıvanç (2008) have obtained similar results in their studies on this subject.

4. Conclusion

As a result, the common nitrogen of the leguminous *Rhizobium* bacterium is presented to the free nitrogen plant in the air. In this way, nitrogen binding is an important resource for agriculture. At the same time, environmental pollution from chemical fertilizers will be reduced. However, many factors such as temperature, soil moisture, salinity, soil reaction, nutrients in the soil and strains affect the nitrogen amount bound in this way, in other words, the successful *Rhizobium* legume plant symbiosis. Depending on these factors, the amount of nitrogen that is fixed is significantly affected. According to the obtained data, seed inoculation and micro fertilization increased the amount of the crop and the nutrient content of the grains. For this reason, it is necessary to apply molybdenum with inoculation and to apply 0.05 mg kg^{-1} Mo as the optimum dose to increase the content of nutrients in the bean grains.

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