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Research Article

# The Effects of Bacterial Inoculation and Molybdenum Applications on Yield and Yield Components of Bean

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**Abstract**

This study was planned as a two-year field trial so as to determine the differences in beans made by bacterial inoculation and molybdenum doses which are likely to have effects on yield and yield components. In this study, Akman 98 bean variety which has a widespread cultivation area as a plant material, three doses of molybdenum element ($Mo_0$=without molybdenum, $Mo_1$=0.05 ppm, $Mo_2$=0.10 ppm) which is effective in nodule and enzyme mechanism were used. For inoculation material, *Rhizobium tropici* (CIAT899) bacteria which belongs to the bean was used. The study was planned according to factorial randomized block experimental designs and set up with four replications. Some agronomic features such as plant height, number of beans per plant, grain yield, thousand grain weight and beans were investigated. Looking at the two-year combined data, it was demonstrated that molybdenum application and inoculation have a significant effect on the properties examined in bean plant. While increases in molybdenum doses generally resulted in increases in yield and yield components, the highest yields (350 kgda$^{-1}$) were achieved in the case of co-administration of molybdenum and inoculation

## INTRODUCTION

Boosting vegetative production in Turkey has a great importance in terms of adequate and balanced nutrition of the increasing population, enhancing the income level of our citizens who make a living from agriculture and make up 40-50% of the population of the country and in terms of national economy accordingly. Edible grain legumes have great importance among dry bean, chickpea, lentil, green pea in ensuring adequate and balanced nutrition of the country's population.

Increasing vegetative production, in other words, the purchase of more quality products from the unit area, depends primarily on the productivity levels of the land, besides other applications and measures. The availability of nutrients in the soil is affected by many factors such as the physical and chemical structure of the soil, usage, the amount of organic matter, and the ratio of other nutrients. Even if the nutrients needed for growth and development are sufficient in the soil, the plants are not always able to make full use of them. For example, liming reduces the availability of nutrients such as iron and zinc. In addition, if inadequate nutrient content in the soil limits plant growth, the plant will not be able to make full use of them even if other nutrients are found to be of sufficient size Bayraklı [1].

Molybdenum deficiency is seen in plants grown in soil with molybdenum below 0.025 kg per decare Marschner [2]. Some plants have higher requirements for molybdenum. However, in terms of nitrogen determination, legumes and cruciferae family vegetables such as cauliflower and cabbage need molybdenum more than the others.

Molybdenum is one of the micronutrients required for plant growth and development. It constitutes part of the enzyme nitrogenase which is essential for the conversion of atmospheric $N_2$ to ammonia $NH_3$. Mo deficiencies are therefore much more pronounced in legumes Bailey and Laidlaw [3], as compared to non-leguminous plants.

Akçin [4] found in some of his studies on bean plants that nitrogen detection of the plants, consequently the amount of the products, increased as a result of inoculation with rhizobium cultures. The application of molybdenum in deficient soil encouraged nodule formation and Nfixation Rahman et al, [5].

Regarding this issue, Şehirali et al. [6], determined in their studies performed in Ankara conditions that the yields of the grains in the parcels with bacterial inoculation and in the parcels with urea equivalent to 5 kgda$^{-1}$ pure nitrogen were similar to each other and this yield was 98% more than the control group.

Likewise, in their studies on beans performed in Samsun Bozoğlu et al. [7], determined that bacterial inoculation and manure application have an effect on increasing grain yield. In their study to determine the effects of zinc and molybdenum from micro elements, together with bacterial inoculation, on some quality properties, Mut and Gülümser [8], applied zinc (0–0.28–0.70 mg kg$^{-1}$ Zn) and 3 doses of molybdenum (0-0.025-0.050 mg kg$^{-1}$ Mo) to Damla-89 chickpea with and without bacterial inoculation. In this study, zinc and molybdenum were applied to leaves 10-20 cm in length. As a result of the combined analysis of variance over the years, it was determined that the application of inoculation, zinc and molybdenum was effective in P, Zn, Mn and Fe in the grains.

In a study by Önder et al. [9], performed with bacterial inoculation on determination of symbiotic nitrogen fixation in some beans and chickpeas grown in Konya region and on obtaining active *Rhizobium* sp. Isolates, it was determined that natural *Rhizobium* sp. Isolates in beans were under the threat of excessive nitrogen manuring. They also stated that inoculating the seeds with effective *Rhizobium* sp. isolates in bean planting increases grain yield.

Likewise, Nadeem et al. [10], investigated the effects of seed inoculation and different nitrogen manuring level on yield and development of mung bean and stated that the number of branches and grains, thousand grain weight, grain yield and protein content were significantly affected by seed inoculation.

In parallel with these studies, in a study by Kaçar et al. [11], the effects of inoculation and nitrogenous manuring on yield and yield components were determined in Bursa conditions and they concluded that inoculation did not have an effect on the characteristics examined among the varieties in ecological conditions in Bursa, increasing manure doses generally enhanced yield and yield components and Şahin 90 variety reached the highest yield with the application of 9 $kgda^{-1}$ N. Gök et al [12], investigated the effects of inoculating by various *Rhizobium* sp strains (B0: without bacteria, B1: 19+380, B2: 377+379, B3: 19+380+377+379) on nodule formation, biomass formation and $N_2$-fixation of peanut plant (NC7) in different doses of iron (0, 15 and 30 $mgkg^{-1}$ Fe) and molybdenum (0, 0.5 ve 1.0 $mgkg^{-1}$ Mo) application in field conditions. At the end of the study, according to the flowering time data, the effects of the applications generally did not cause significant statistical differences. While iron applications did not have a significant effect on N contents of root and nodules, according to harvest time data, bacterial applications increased grain yield, N contents in the grain and N intake parameters.

In another study, the effect of *Rhizobium* sp inoculation on the bean seeds in Turkey was investigated and the researchers set up a field trial by inoculating three kinds of bean seeds (Akman 98, Göynük 98, Şehirali 90) with *Rhizobium* sp isolate. According to the results of the experiment, it was determined that inoculation and nitrogen manuring increased seed quality (seed yield, protein ratio, seed weight) significantly Küçük and Kıvanç [13].

This research was planned with the aim of determining the effect of bacterial inoculation and different doses of molybdenum on the yield and yield components of the bean plant.

## MATERIAL AND METHOD

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) which was provided from the biological laboratories of Soil, Fertilizer and Water Resources Central Research Institute Directorate, Ankara. The test was set up according to randomized block experimental designs with four replications and it was carried out in field conditions as a two-year study. In the experiment, each parcel 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 leves: inoculated and non inoculated) and factor B (3 levels: $Mo_0$= without molybdenum, $Mo_1$=0.05 ppm, $Mo_2$=0.10 ppm).

**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 | mg $kg^{-1}$ | 5.88 |
| % $CaCO_3$ | | 22.42 | Mn | mg $kg^{-1}$ | 36.18 |
| % O.M. | | 1.20 | Zn | mg $kg^{-1}$ | 2.46 |
| % Clay | | 24.2 | B | mg $kg^{-1}$ | 3.92 |
| % Silt | | 32.6 | Ca | me/100 g | 1.8 |
| % Sand | | 43.2 | Mg | me/100 g | 1.6 |
| Class | | Loam | Na | me/100 g | 0.36 |
| N | mg $kg^{-1}$ | 111.23 | K | me/100 g | 0.99 |
| P | mg $kg^{-1}$ | 13.60 | | | |

After these parcels were made ready for planting and after the surface sterilization of the seeds were made with 0.5% sodium hypochlorite, they were planted being inoculated by *Rhizobium tropici* bacteria developed in YMB (Yeast mannitol broth). The study was carried out on a total of 24 parcels including 3 molybdenum dozes, 2 inoculation factors and 4 replications. The amount of seed was calculated for each parcel, 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. In order 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 dressing was applied to all parcels during planting 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 dressing was given as 5 mg $kg^{-1}$ Fe, 12 mg $kg^{-1}$ Mn, 2 mg $kg^{-1}$ Zn, 1 mg $kg^{-1}$ Cu.

Some physical and chemical properties of the research soil are given in (Table 1). After 90 days of vegetation, plant samples were harvested by hand. Following the harvest, nitrogen analysis of plant leaves was performed using method of wet decomposition Bayraklı [1], with sulphuric acid Lindsay and Norwell [14]. Nitrogen content in grain was determined in LECO in dried and grounded samples by passing through pure water and the value of grain protein was calculated by multiplying these values by 6.25 factor Lindsay and Norwell [14]. Other measurements and observations made on bean plants are plant height, the number of beans per plant, the number of grains in the bean, harvest, thousand grain weight and grain yield Akçin [15].

## STATISTICAL ANALYSIS

Data were analyzed as a factorial experiment in a completely randomized manner with four replication using the JMP statistical software version 5.1 (SAS Institute INc., Cary, NC,

**Table 2:** Effect of Rhizobium Inoculation and Mo Application on Yield and Yield Components of Akman-98 Bean Plant.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.year** | **Non Inoculated** | Control | 260.7 | 24.6 | 293.0 | 26.0 | 9.3 |
| | | Dose1 | 309.3 | 23.9 | 310.9 | 33.0 | 12.6 |
| | | Dose2 | 325.7 | 24.1 | 304.5 | 29.7 | 12.3 |
| | **Inoculated** | Control | 261.3 | 25.8 | 297.7 | 26.0 | 10.5 |
| | | Dose1 | 344.7 | 26.3 | 339.9 | 29.7 | 12.2 |
| | | Dose2 | 356.7 | 28.9 | 363.7 | 32.0 | 12.6 |
| **2.year** | **Non Inoculated** | Control | 251.2 | 21.5 | 313.7 | 56.9 | 11.3 |
| | | Dose1 | 252.9 | 23.9 | 316.3 | 64.5 | 10.8 |
| | | Dose2 | 294.6 | 25.0 | 328.8 | 65.3 | 11.1 |
| | **Inoculated** | Control | 253.4 | 24.0 | 308.4 | 55.8 | 11.1 |
| | | Dose1 | 350.9 | 24.8 | 335.0 | 63.4 | 10.8 |
| | | Dose2 | 363.7 | 26.5 | 343.8 | 70.7 | 11.2 |
| **Variance Resource** | | **D.F.** | **ANOVA** | **ANOVA** | **ANOVA** | **ANOVA** | **ANOVA** |
| **Year** | | 1 | NS | * | NS | * | * |
| **Inoculation** | | 1 | * | * | * | NS | NS |
| **Dose** | | 2 | * | * | * | * | * |
| **Year x Inoculation** | | 1 | * | NS | * | NS | NS |
| **Year x Dose** | | 2 | NS | NS | NS | NS | * |
| **Inoculation x Dose** | | 2 | * | NS | * | NS | NS |
| **Year x Inoculation x Dose** | | 2 | NS | * | NS | NS | NS |
| *: p<0.05 NS: Not Significant | | | | | | | |

**Table 3:** Impact of 2-year average values of Rhizobium Inoculation and Mo Application on Yield and Yield Components of Akman-98 Bean Plant.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AVERAGE** | **Non Inoculated** | Control | 255.92 | 23.08 | 303.34 | 41.46 | 10.30 |
| | | Dose1 | 281.11 | 23.91 | 313.63 | 48.75 | 11.73 |
| | | Dose2 | 310.12 | 24.54 | 316.61 | 47.46 | 11.67 |
| | **Inoculated** | Control | 257.36 | 24.92 | 301.57 | 40.92 | 10.82 |
| | | Dose1 | 347.77 | 25.55 | 337.43 | 46.54 | 11.52 |
| | | Dose2 | 360.17 | 27.70 | 353.77 | 51.33 | 11.90 |
| **Variance Resource** | | **D.F.** | **ANOVA** | **ANOVA** | **ANOVA** | **ANOVA** | **ANOVA** |
| **Inoculation** | | 1 | * | * | * | NS | NS |
| **Dose** | | 2 | * | * | * | * | * |
| **Inoculation x Dose** | | 2 | * | NS | * | NS | NS |
| *: p<0.05 NS: Not Significant | | | | | | | |

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.

## RESULTS AND DISCUSSION

During two-year trial period, at the maturity period of the plant and at the end of the second year, observations and measurements were made on 5 plants randomly selected from each parcels. Two year combined data and variance analysis results of this data were given in (Table 2) and (Table 3) about the properties investigated in this study which was carried out to determine the effects of bacterial inoculation, molybdenum applications and the years on yield and yield components of Akman 98 bean variety.

As seen in the tables, the effect of bacterial inoculation was different on among the properties in the plant such as yield, protein and thousand grain weights, and these differences were found to be statistically significant at 5% level. On the other hand, the effect of application time (year) was different on protein %, number of beans per plant, and pod length and this difference was found to be statistically significant at $p<0.05$ level.

In addition, the effect of molybdenum doses on the yield and yield components of the plant was also found to be statistically significant. When the relations between the applications were considered, the effect of year x inoculation was found statistically significant on yield and thousand-grain weight. Besides, it is seen that the effect of year x dose interaction on pod length, the effect of inoculation x dose on yield and thousand grain weight and the effect of year x inoculation x dose on protein were statistically significant ($p<0.05$).

### Yield (kg)

As seen in table 2, the maximum yield value in Akman 98 bean variety was obtained from the second dose of molybdenum in inoculated group with 357 kg $da^{-1}$ in the first year, according to two-year combined data. The lowest value, on the other hand, was obtained from the inoculated and non-inoculated control groups respectively in the first and second years. Yield values obtained in terms of inoculation had a statistical difference. In other words, inoculation with *Rhizobium* had a significant effect on the yield of Akman 98 bean variety. However, the molybdenum doses increased the yield statistically (Table 2). Inoculation and interactions were statistically significant and the plant yield values varied between 251-364 kgda$^{-1}$. In other respects, the results showed similarities or differences to the results of some researchers Gök et al, Kaçar et al, Bozoğlu et al [7,11,12]. Actually, in one of their studies, Küçük and Kıvanç [13], investigated the effect of inoculation and *Rhizobium* sp on the quality of bean seeds and they set up a field trial and inoculated three varieties of bean seeds (Akman 98, Göynük 98, Şehirali 90) with local *Rhizobium* sp. isolate. As a result of the test, they determined that inoculation and nitrogenous manure increased seed quality significantly (seed yield, protein ratio, seed weight).

### Pod length (cm)

As can be seen in (Table 2), the highest value of pod length was determined as 12.6 cm and the lowest value was determined as 9.3 cm according to the data obtained in the first and second years. The values of pod length obtained in terms of year and molybdenum doses were statistically significant. As is statistically significant, the value of the pod length obtained as a result of bacterial inoculation varied from 9.3 to 12.6 cm. On the other hand, according to the first and second year averages, only molybdenum dose was found statistically significant ($p < 0.05$). In other words, the inoculation and inoculation dose interaction did not have any effect on the pod length (Table 3).

Likewise, in a study performed in Samsun conditions on beans, Bozoğlu et al. [7], stated that the values obtained in terms of plant height as a result of inoculation and nitrogenous manure applications were not statistically different compared to the control parcels. In Trakya conditions, Karahan and Şehirali [16], detected that the plant height was 33.56 cm in non-inoculated parcels and between 212 and 35.62 cm in inoculated parcels; and they also stated that the parcels with nitrogen applications had higher values than in control parcels. In addition, in a study performed in Konya conditions, Önder et al. [9], reported that bean height varied between 8 and 12,3 cm.

### The number of pods per plant

Inoculation and Molybdenum applications at different doses resulted in an increase in the number of pods of Akman 98 bean variety compared to the control parcel. The effect of the year on the number of pods was statistically significant. As a matter of fact, according to the results of the experiment performed in the 1st year, it was determined that the number of the pods obtained from inoculated and non-inoculated parcels was lower than the results of the tests in the 2$^{nd}$ year. The lowest bean number was obtained from the control parcels in one year with 26 ones, while the highest bean number was obtained from Mo applications. On the other hand, the highest bean number in the second year was obtained from the parcels with inoculation and molybdenum applications. Taking into account the average of two years, it is seen that only molybdenum doses are effective on the number of the pods per plant (Table 3).

### Thousand grain weight

When the test results were evaluated in terms of thousand grain weight, it is seen that the highest value was obtained from the second dose of inoculated Mo application with 363.7 g in the first year; however, the lowest value was obtained from the non-inoculated control group with 293.0 g. It is understood from the results of variance analysis that the year factor has no statistically significant effect on thousand grain weight. Also, as inoculation and dose interaction are statistically significant, this varied between 293.0 and 363.7 g in the first year; and between 313.7-343.8 g in the second year respectively (Table 3). Similarly, as Hernandez and Hill, Akçin and Işık, Pekşen and Gülümser, and Meral et al. [15,17-19], mentioned, inoculation made a difference in thousand grain weight statistically. On the other hand, according to the variance analysis results of the bean obtained by combining years, the interactions of inoculation and doses were found statistically significant.

### Protein (%)

As a result of the analysis of variance, it was seen that the years and the applications of the experiment were statistically effective on protein %. While the protein was 24.54 % in the first year, this value increased 27.70% in the second year. Higher grain yield in the second year contributed this increase. The effect of molybdenum doses on protein was found to be statistically significant. Nadeem et al. [10], found out in a study that branch number in the plant, grain number, thousand grain weight, grain yield and protein content were significantly affected by the inoculation of the seed. In the same way, fertilizer application significantly increased the protein content of grain and grain yield. In a study performed on 482 Southern Yellow chickpeas, Pekşen and Gülümser [18], reported that crude protein yield per decare was between 9.26 - 14.64 kg; and Karahan (1999), in his study on the same chickpea variety, stated that crude protein yield per decare was between 2.74 - 8.74 kg. Yield values of crude protein obtained from this trial are higher than the other values reported by many researchers.

## CONCLUSION

According to the results of the research, the effect of doses of Mo application on Akman 98 bean variety varied depending on the doses. All Mo treatments led to significant improvement of pod and seed weight compared to the control. The combination treatment (Rhizobium+Mo) significantly magnified all the bean yield components compared to the control treatment. Applying mineral nitrogen fertilizers instead of doing crop rotation for legumes, which play an important role in increasing nitrogen amount of the soil so as to meet nitrogen demands of the plants at the present time, leads to enormous energy losses for this production made through industry. It is not possible for the plant to make use of the fertilizer optimally not only because of energy costs but also because some of the mineral nitrogen fertilizers are washed away and some are removed from the soil by denitrification. As a consequence of the use of nitrogenous fertilizers, together with the high amount in yield, we are confronted with environmental problems arising from the mixing of excess nitrogenous fertilizers and nitrogenous wastes into ground water and drinking water. According to the results, the plantation of the seeds by bacterial inoculation as well as microbial fertilizers increased the amount of the product and the protein content of the grains. Balanced and conscious biological and micro fertilization, as well as the quality of the product, is also important for the positive impact on people’s health who consume this product.

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