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RESEARCH ARTICLE

THE EFFECT OF NITROGEN FIXING ASYMBIOTIC BACTERIA FERTILIZER ON NUTRIENT CONTENTS AND SOME AGRONOMIC PROPERTIES OF CORN PLANT

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ABSTRACT

This study aims to examine the effects of biological fertilizer consists of microorganisms, which bind the free nitrogen (N_2) of air in an asymbiotic way, like *Azospirillum* sp., *Azorhizobium* sp. and *Azoarcus* sp. on the properties of soil and nutrient contents of the plant and some agronomic properties of corn. The experiment was set up in a randomized block design with 3 repetitions. In the experiment, 2 doses of biological fertilizer (0–0,5 g ha^{-1}; BF_0-BF_1) and 3 doses of nitrogen fertilizer (0 – 125 – 250 kg N ha^{-1};N_0-N_1- N_2) were applied. Consequently, the effects of the applications on the electrical conductivity of soil, NO_3-N were statistically significant, and in the second period, the effects on available Mn in the soil, and iron and copper in the plant, and agronomic properties like cob diameter, height of the first cob, stem thickness were determined. The biological fertilizer enabled the corn plant to make better use of the Fe element in the soil.Compared to the control parcel (BG_0N_0), an increase of 22,3 g cob-1 (24,03%) in grain weight and 183 kg da^{-1} (24,04%) in grain yield was detected with biological fertilizer application (BG_1N_0). These results Show the effectiveness of biological fertilizer. It was concluded that for environmentally friendly and economical corn production 125 kg N ha^{-1} nitrogen may be recommended along with a 0,5 kg ha^{-1} biological fertilizer.

Keywords:*Maize, Biofertilizer, Nutrition, Yield, Soil Properties.*

1. INTRODUCTION

Population growth causes increasing nutritional requirements. Agricultural areas are gradually decreasing. For this reason, it is necessary to get more crop from per unit. This is also a degradation problem i.e. pressure on natural resources due to exceeding carrying capacity. Since using fertilizers is important to crop increase more chemical fertilizers are using by the day. Using chemical fertilizers causes soil deterioration (Salinization, heavy metal accumulation, nutrient imbalance, deterioration of microorganism activity), water eutrophication and nitrate accumulation, air pollution by nitrogen and sulfur and greenhouse effect [1]. Human health is threatened, and environmental pollution is increasing [2]. These negative situations guided researchers to work on alternative agricultural production techniques and plant nutrition strategies and new production systems like “organic farming” and “good farming practices” have been put into practice. These of biological fertilizers in plant nutrition and protection and sustainability of the ecological balance are aimed in these systems [3].

It is recommended to use microorganisms selected from the rhizosphere in order to protect and increase soil fertility and to obtain maximum product from plants. For this purpose, plant growth-promoting rhizobacteria (PGPR) are used as biological fertilizers (BF) due to their beneficial effect on plant growth. Biofertilizers are of great importance in sustainable agricultural production [4]. PGPR are bacteria which are colonized in plant root. PGPR provide mineralization of soil organic matter, immobilization of mineral nutrients, dissolution of phosphates, balanced nitrification, nitrogen fixation and plant hormone production, acquisition of nutrients that encourage plant growth and reduction of heavy metal toxicity, and protect the plant against root pathogens. As a result, they contribute to the increase of agricultural production by promoting plant growth [5].

Corn (*Zea mays* L.) is a plant which can grow under different climatic conditions. It has high potential of yield, is called the "king of grains" and ranks third in the world in terms of production area [6]. Due to its high yield potential, it requires a high amount of nutrients and a good nutritional management strategy [7]. Corn plant has a high need for nitrogen among nutrients [8]. Nitrogen is a factor which limits productivity in corn cultivation most. The nitrogen need of plants is supplied by chemical fertilizers, mineralization of organic matter and biological fixation of atmospheric nitrogen. Nitrogen given by chemical fertilizers is lost by leaching, turning into gas and volatization, and its effectiveness decreases. It also causes environmental pollution. Plants benefit more effectively from nitrogen which is biologically bound to the soil. Especially bacteria, algae and fungi are used individually and in combination as biological fertilizers [4]. Some of the biological fertilizers include *Rhizobiums* pp. species which live in symbiosis with legumes, some include *Azospirillum* sp., *Azotobacter* sp., *Acetobakter* sp, *Azoarcus* sp. which are free-living species [10].

It has been determined that the use of chemical nitrogen fertilizers can be reduced by 50% by using biological fertilizers [2]. The use of 200 ml ha^{-1}*Azospirillum brasilense* containing biological fertilizers in corn plants has proved to be effective and has been shown as an alternative to the use of chemical fertilizers [11]. The positive effects of biological fertilizer and urea application on corn yield were determined, and it was stated that planting corn seeds after inoculating with biological fertilizers would decrease urea application rate [12]. İnoculation with *Azospirillum brasilense* has been reported to increase the efficiency of nitrogenous fertilizers and corn yield [13].

Many researches have been carried out to demonstrate the effectiveness of biological fertilizers in corn cultivation. However, biological fertilizers can be produced to contain microorganism species for single or multiple purposes. Biological fertilizers may consist of imported or native species in terms of the microorganism they contain. In the application of these fertilizers, the problem of adaptation in biological fertilizers consisting of imported species, and the competition and resistance potentials which may occur

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in biological fertilizers containing native species should be taken into account. Therefore, it is important to determine the suitability of these fertilizers in different climate, soil and plant conditions.

Along with investigating the efficiency of a freeze-dried commercial preparation containing asymbiotic bacterias *Azospirillum sp.*, *Azorhizobium sp.* And *Azoarcus sp*. in corn cultivation, this study will also help to determine whether the effectiveness of chemical nitrogen fertilizer may be increased with the use of biological fertilizers or not.

2. MATERIAL and METHOD

2.1. Material

The research was carried out on the experimental field of Manisa Celal Bayar University Alaşehir Vocational School. The experimental area is located at 38° 22 '24 "north latitude; 28° 31' 35" east longitude and is 152 meters above sea level. The climate in Alaşehir district is warm in winter, and very hot and dry in summer (Mediterranean climate). The average annual precipitation is 598 mm, and most of the precipitation occurs in the winter months. Annual average temperature is 16,3 °C, with a temperature of 25,4 °C, July is the hottest month of the year. [14].
The materials of the experiment are the field soil in which the research was conducted, chemical fertilizers used in the research, biological fertilizer (BF) and corn (*Zea mays* L.var. Indentada) cultivated as test plant. Some physicochemical properties of the soil belonging to the experimental area are given in Table 1.

Table 1. Some physicochemical properties of the soil.

Parameter 0 – 20cm20 – 40cm				
pH	7,77*	(0.05)**	7,81	(0,04)
Electrical conductivity (µScm^{-1})	716	(152)	610	(66)
Lime (%)	4,17	(0.10)	4,4	(0,45)
texture	Loam		Loam	
Sand (%)	50,10	(1,11)	48,74	(1,66)
Silt (%)	20,26	(0,87)	20,76	(1,00)
Clay (%)	29,64	(0,36)	30,50	(2,18)
Organic Matter (%)	2,13	(0,31)	2,07	(0,21)
NH_4-N (mg 100g^{-1})	18,83	(2,64)	21,27	(1,47)
NO_3-N (mg 100g^{-1})	1,74	(0,59)	1,54	(0,22)
Total-N (%)	0,13	(0,01)	0,13	(0,01)
Available-P (mg kg^{-1})	37,69	(1,57)	38,21	(4,87)
Extractable-Na (mg kg^{-1})	34,23	(14,8)	28,77	(9,04)
Available-K (mg kg^{-1})	501	(76)	486	(58)
Available-Ca (mg kg^{-1})	2150	(30)	2034	(140)
Available-Mg (mg kg^{-1})	743	(64)	714	(63)
Available-Fe (mg kg^{-1})	6,07	(0,14)	6,79	(0,74)
Available-Cu (mg kg^{-1})	1,95	(0,11)	1,70	(0,07)

Available-Zn (mg kg^{-1})	4,26	(0,22)	4,18	(0,16)
Available-Mn (mg kg^{-1})	13,35	(0,97)	8,49	(0,44)

* All values are the average of 4 repetitions and are calculated on the oven-dry weight.
**The numbers in parentheses give the standard deviation of the mean.

The experimental soil has "slightly alkaline" reaction [15] and is non-saline [16]. The soil texture is in the loamy, "medium humus" [17] and medium calcareous class [18]. According to the macro plant nutrient content in the experimental soil; nitrogen was identified as “good" [19], phosphorus as "good" [20], potassium as "very high" [21]. Calcium as "good" and magnesium as "very high" [19]. The microelements (Fe, Zn, Mn and Cu) were found to be at a "sufficient" level [22] in the experimental soil.

The experimental plots were irrigated with artesian water in the experimental area. Irrigation water quality characteristics were determined by analyzing water samples taken from this artesian at the beginning, middle and end of the irrigation season for 3 times. The results obtained are shown in Table 2. The irrigation water has mild alkaline reaction and is in the C_2S_1 irrigation water class. It does not cause any salinity and alkalinity problems under normal conditions [16]. Concentrations of Na^+, Cl^- and boron, which are toxic to plants, are suitable [23].

Table 2. Quality properties of irrigation water.

Parameter		
pH	7,71	(0,23)*
Electrical conductivity (EC) (µScm^{-1})	600	(27,09)
Na^+ (me l^{-1})	0,95	(0,05)
K^+ (me l^{-1})	0,11	(0,01)
Ca^{++}+ Mg^{++} (me l^{-1})	4,85	(0,25)
Total cation (me l^{-1})	5,91	
Cl^-(me l^{-1})	0,97	(0,16)
$CO_3^=$ (me l^{-1})	0,00	
HCO_3^-(me l^{-1})	3,86	(0,13)
$SO_4^=$ (me l^{-1})	1,16	(0,05)
Total anion (me l^{-1})	5,99	
Sodium adsorption ratio(SAR)	0,61	
B (mg l^{-1})	0,17	(0,02)
Class	C_2S_1	

* The numbers in parentheses give the standard deviation of the mean.

The biological fertilizer used in the study is a commercial preparation consisting of endophyte bacterias of *Azospirillum* sp., *Azorhizobium sp.* and *Azoarcus* sp., which have the ability to bind atmospheric nitrogen (N_2) to soils in an asymbiotic way. It is in 0.5 g packages and the number of live bacteria is reported as 10^{11}cfu / gr.

In the study, single hybrid and mid-late Helen corn variety (*Zea mays* L. var. Indendata) with a vegetation period of 130-135 days was used as a test plant. The variety has no soil selectivity and has high adaptability. It is a variety with high hectoliter weight, low grain moisture at harvest and high yield. It was preferred in the experiment as it is a variety cultivated in the Aegean region because of these features.

2.2. Method

In the research, biological fertilizer and chemical nitrogen fertilizer were considered as independent variables. The experiment was created in a randomized block design with 3 replications. Biological fertilizers were tested at doses of 0 – 0,5 g ha^{-1} (BF_0 – BF_1), and nitrogen at 0 – 125 – 250 kg N ha^{-1} (N_0 – N_1 – N_2). 3 nitrogen doses without biological fertilizers ((BF_0N_0, BF_0N_1, BF_0N_2) and 3 nitrogen doses with biological fertilizers (BF_1N_0, BF_1N_1, BF_1N_2) constituted the application subjects. Parcel dimensions are 3,0m x 3,4m and a parcel area is 10,2 $m^{2.}$ In order to reduce the edge effects in the experiment, 2 m space was left between the blocks and 1 m between the parcels. Considering the previous studies and regional conditions, the nutrient needs of the corn plant were determined as 250 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5 and 200 kg ha^{-1} K_2O in the study.

Nitrogen applications considered as independent variable are zero nitrogen dose ($N_{0)}$, half nitrogen dose ($N_{1)}$ and full nitrogen dose ($N_{2).}$ While planting all of the N_1 and half of the N_2 doses were applied with ammonium sulfate fertilizer (21%N) as an amount of 595 kg ha^{-1} on May 21, 2015. 41 days later (on June 6.2015) half of the N_2 was applied with ammonium nitrate fertilizer (33%N) as an amount of 379 kg $ha^{-1.}$ Phosphorus and potassium fertilizers were applied to all parcels with triple super phosphate (42-44% P_2O_5) in the amount of 349 kg $ha^{-1}$and with 400 kg ha^{-1} potassium sulfate ((50%K_2O). Then the fertilizers applied to the parcels were mixed with a cultivator to a depth of 10-12 cm soil.

After chemical fertilizer applications, 5 rows of 68cm x 18cm row spacing and two seeds in each row in each plot, 32 seeds in each row and 160 seeds in each parcel were planted (May 21. 2015). When the plants reached a shoot length of about 10 cm, a single plant was left with 18 cm row spacing by singling. When the plants reached a shoot length of about 14-30 cm (June 17. 2015), biological fertilizer was applied at a dose of 0,5 g ha^{-1} by following the fertilizer manufacturer's recommendations. Biological solutions of 5 liters prepared for each parcel on which biological fertilizer was used were applied homogeneously to the root of the plant rows in the evening in cool hours with a ridge sprayer. Immediately after the application, irrigation was made with the drip irrigation system, thereby enabling the microorganisms to reach the root area. The development of the test plant in the experimental field was precisely observed from seed planting to harvest. All cultural processes were carried out in a way that would not harm the microbial population in the soil. No pesticides or herbicides were used during the experiment.

In the experiment, composite soil samples were taken from each plot in two periods, before biological fertilizer application (17 June 2015) and at harvest (19 October 2015). The samples taken were made ready for analysis in accordance with the rule.Also, leaf samples were taken from the first leaves opposite and below the cob while the corn plant was in the full detasking stage, representing the plots. The samples cleaned according to the methots were dried at 65^{o}C and made ready for analysis by grinding [24]. In order to determine the yield and agronomic characteristics, the edge rows of each plot were excluded from the sampling at harvest, and randomly 10 plants from the three rows in the middle were cut from the soil surface.

pH of soil samples was analyzed according to Jackson [25], electrical conductivity [26], Rayment and Higginson, organic matter according to Rautenberg and Kremkus [27], calcium carbonate according to Schlichting and Blume [28], the texture analysis of the soil according to Bouyoucos [29], total N according to Bremner [30] , NH_4-N according to Kandeler and Gerber [31], NO_3-N according to Scharpt and Wehrmann [32], available P according to Olsen and Sommers [20], available K, Ca, Mg according to Pratt [33], available Fe, Zn, Mn, Cu according to Lindsay and Norwell [22], respectively.

Plant samples prepared for analysis were subjected to wet combustion with concentrated nitric-perchloric acid mixture. Potassium and calcium flame photometric, magnesium, iron, copper, zinc, manganese elements were determined by Atomic Absorption Spectrophotometric methods in extracts obtained by wet combustion [24]. Phosphorus contents of the samples were measured in a colorimeter using the Vanado-Molybdo phosphoric acid (yellow colour) method [34]. Total nitrogen analysis in plant samples was made via the modified Kjeldahl method [30].

10 plant samples were taken randomly from each plot during the harvest. In the samples taken, measurements were made regarding planth eight, first cob height, stem thickness in plant, cob height, cob diameter, number of rows on the cob, grain weight on the cob, single stem weight, thousand-grain weight and grain yield. The results of the measurements are given as mean values. All the data obtained in the study were evaluated using the "SPSS 20.0" software, using the randomized blocks experimental design in factorial order according to multivariate ANOVA (MANOVA) variance analysis technique. Differences between group averages were determined via Duncan test.

3. RESULTS and DISCUSSION

3.1. The Effects on Some Physicochemical Properties of Soils

The effects of biological fertilizer and nitrogen applications on properties of soil are given in Table 3. While the effects ($P<0,05$) of the applications on electrical conductivity (EC) and NO_3-Nin the 1^{st} period and on available–Mn ($P<0,01$) in the 2^{nd} period were determined to be significant, the effects of applications on other properties were found to be insignificant (Table 3). Soil reaction (pH) decreased due to nitrogenous fertilizer applied in the 1^{st} period and EC values, which are indicators of salinity, increased. The reason for these changes is that the nitrogen fertilizer (Ammonium sulphate) used is a salt with physiological acid character. In the second period, an increase in pH values and a decrease in EC values were observed in all parcels with and without biological fertilizers (Table 3). The decrease in biological activity in the second period may be interpreted as the reason for the increase in pH, and rainfall and leaching of the salts through irrigation as the reason for the decrease in EC values. These results show that BF application has no significant effect on pH and EC values. Goutami et al. [35] stated that inorganic fertilizer, BG and organic farm manure applications do not significantly affect pH and EC values. Mulyani et al. [36] found that the pH value of the soil was affected by the BF application in their study to reveal the effect of BF application on the chemical properties of the soil. Orhan et al. [37] reported that PGPR application significantly affected the pH value. In the first period, the amount of organic matter in the soil was not affected by the practices. In the second period, there was a decrease in the amount of organic matter compared to the first period (Table 3). Second period soil samples were taken approximately 4 months after the first period. This period is the time when microbiological activity is most intense. Due to the high microorganism activity during this period, the organic matter has been mineralized and its amount has decreased.

Total-N contents of soils were not affected much by the subjects applied in both periods and were determined to be at the same levels. NH_4-N values generally increased with nitrogen application in the 1st period. In the second period, NH_4-N values were determined at approximately the same levels. In both periods, the highest NH_4-N values were reached with BF and half dose nitrogen application. NO_3-N amounts increased in the first period due to nitrogen applications. The reason for this may be the conversion of nitrogen in the form of NH_4 to NO_3 by nitrification. In the second period, there was a decrease in NO_3-N values due to leaching compared to the first period (Table 3). In a study conducted, NH_4-N and NO_3-N were found to be higher in control and bio-fertilizer applications than chemical fertilizer applications in the soil [2].

The effects of the discussed subjects on available P, K, Ca, and Mg in the soil were not found to be statistically significant (Table 4).Available P was higher in parcels where BF and nitrogen were not applied (control parcels) than all other parcels. Since BF and N applications increase microorganism activities, it is thought that the use of available P also by microorganisms causes this decrease. In the second period, the amount of available K increased in other applications compared to the control. Organic and inorganic acids which pass into the soil as a result of increased microbial activity with microorganism and nitrogen applications may be the reason for this increase. In the second period, available Mg increased in the N_1 and N_2 parcels compared to the N_0 parcel where BF was not applied. The reason for this may be the physiological acid character of the nitrogen fertilizer applied. In the parcels where BF was applied, an increase occurred only in the N_2 dose. However, in general, the amounts of these macro nutrients tended to decrease in parcels N_1 and N_2 compared to the N_0 dose in plots treated with BF. This tendency to decrease was observed more clearly in N_1 parcels (Table 4). Similar results were obtained with the amounts of available micronutrients (Fe, Zn, Mn, Cu) (Table 5.). Goutami et al. (2015) reported that inorganic fertilizer, BF and organic farm manure applications cause significant effects on available N and P among macro elements in the soil and on Fe among micro elements. In their study which was conducted by applying organic fertilizer, different N doses and BF containing *Azotobacter* and *Azospirillum*, Tiyagi et al. [38] found that the N, P, K content in plants and soil increased significantly in all BF applied combinations. Orhan et al. [37] found that PGPR application significantly affected the total-N, available P, K, Ca, Mg, Fe, Mn, Zn contents in the soil. These results show that the application of BF and chemical nitrogen fertilizer together increases the utilization rates of the plant in nutrients. A better observation of this effect at the N_1 dose compared to the N_2 dose proves that the application of BF may reduce the amount of chemical nitrogen fertilizer to be used.

Table 3. The effects of biological fertilizer application on some chemical properties of the soil, total –N, NH_4-N and NO_3N contents.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**pH**		**EC (µScm^{-1})**		**OM (%)**		**Total-N (%)**		**NH_4-N (mg N 100 g^{-1})**		**NO_3-N (mg N 100 g^{-1})**	
		I	II	I	II	I	II	I	II	I	II	I	II

	0	7,89	8,00	522c	467	2,49	1,88	0,120	0,130	9,03	9,36	0,87b	0,47
0	**125**	7,80	8,04	712ab	533	2,67	2,25	0,128	0,120	9,34	9,17	1,44a	0,57
	250	7,82	8,07	666abc	511	2,16	1,95	0,120	0,117	8,81	9,81	1,14ab	0,44
	0	7,88	8,05	573bc	492	2,11	1,90	0,115	0,113	8,49	9,77	0,95b	0,50
0,5	**125**	7,75	8,01	796a	447	2,04	1,74	0,120	0,117	11,27	10,35	1,54a	0,49
	250	7,78	8,00	722ab	508	2,17	1,87	0,116	0,113	9,42	9,20	1,37a	0,68
Significance		0,053	0,201	<0,050	0,899	0,159	0,825	0,792	0,543	0,375	0,697	<0,050	0,597

EC = Electrical conductivity, OM = Organic matter.

Table 4. The effects of biological fertilizer application on macronutrient contents.

Biological fertilizer (g ha^{-1})	Nitrogen dose (kg N ha^{-1})	Available-P (mg kg^{-1})		Available -K (mg kg^{-1})		Available-Ca (mg kg^{-1})		Available-Mg (mg kg^{-1})	
		I	II	I	II	I	II	I	II
	0	41,20	32,10	304,44	265,88	2168	1811	790	802
0	**125**	50.64	25,90	330,69	375,66	2228	2291	779	820
	250	51,37	26,51	304,44	300,27	2235	2211	827	828
	0	38,47	27,16	335,93	357,14	2155	2237	799	795
0,5	**125**	31,19	26,90	288,69	292,33	2242	1891	782	752
	250	31,75	26,51	304,44	328,04	2195	2211	813	815
Significance		0,12	0,808	0,739	0,874	0,455	0,84	0,623	0,6

Table 5. The effects of biological fertilizer application on micronutrient contents.

Biological fertilizer (g ha^{-1})	Nitrogen dose (kg N ha^{-1})	Available -Fe (mg kg^{-1})		Available-Zn (mg kg^{-1})		Available -Mn (mg kg^{-1})		Available -Cu (mg kg^{-1})	
		I	II	I	II	I	II	I	II
	0	5,68	5,75	4,07	3,52	6,28	5,10a	1,59	1,53

0	**125**	6,13	6,50	4,34	3,91	5,98	5,09a	1,74	1,65
	250	7,10	6,85	3,98	3,24	5,52	4,44ab	1,45	1,48
0,5	**0**	6,42	6,73	3,41	4,28	5,25	3,69bc	1,55	1,40
	125	6,45	6,67	3,22	3,37	5,35	3,53c	1,47	1,32
	250	6,06	5,86	3,50	3,72	5,72	4,12bc	1,57	1,55
Significance		0,211	0,853	0,133	0,514	0,168	<0,010	0,529	0,359

3.2. The Effects of Biological Fertilizer Application on Nutrient Content of Corn Plant

The effects of biological fertilizer and nitrogen doses on the nutrient contents of the corn plant are given in Table 6. While the effects of the discussed subjects in the study on N, P, K, Ca, Mg, Zn and Mn contents in the corn plant were not found statistically significant, the effect on Fe and Cu was($P<0,05$) significant (Table 6). When the amount of nutrients determined in the corn plant in the study were compared with the limit values suggested by Jones et al. [39], the N, P, K, Mg and Mn contents of the corn grown as test plant were found to be at the limit of "deficient" level and "sufficient" level, while Ca, Fe, Zn and Cu contents were "sufficient".

Nitrogen contents of plant samples taken from BF applied parcels were found to be higher than the plant samples taken from the unapplied parcels. It was determined that BF and nitrogen applications do not affect the phosphorus content of the plant. Similar results were found in all applications. In the examination of the effect of bio-fertilizer applications on the potassium content of the plant, it was found that the plant potassium was lower in the parcels where BF was applied, except for the N_2 dose. Calcium content of the plants taken from the parcels where BF applied was found to be higher compared to the unapplied parcels. Magnesium contents of plants were higher in N_0 doses where nitrogen was not applied, compared to the nitrogen doses of N_1 and N_2, in parcels with and without BF (Table 6.).İn their study investigating the effects of inoculation of corn seeds with diazotrophic bacteria and nitrogen doses applied as sprinkling, Longhini et al. [40] reported that N application up to 120 kg N ha^{-1} increased the nutrient content of the leaves, and also that N, P, K and S increased, Ca and Mg decreased through BF application.

Iron as one of the micronutrients of the plant samples increased through BF application. This increase was 10,3% for the N_0, 14,4% for the N_1 and 21,2% for the N_2, compared to the control respectively. In the parcels where BF was not applied, there was no increase in parallel with the nitrogen doses, even a decrease in the N_2 dose occurred (Table 6). These results show that BF application significantly affects the iron uptake of the plant and consequently the iron content of the plant. İpek and Eşitken [41] stated that Some PGPR increase the Fe availability in soil by decreasing pH by releasing organic acids or synthesizing low-molecular-weight iron-chelating agents (siderophores). While the BF application had no effect on the zinc and manganese contents of the corn plants, an increase in copper content was observed. Copper content of the plants did not change depending on nitrogen doses in parcels where BF not applied.

The increase in copper content of plant samples taken from BF applied parcels was 13,3% in the N_0, 31,3% in the N_1 and 26,7% in the N_2 compared to the control (Table 6).

Table 6. Theeffect of biological fertilizer application on nutrient contents of corn plant.

Biological fertilizer (g ha^{-1})	Nitrogen dose (kg N ha^{-1})	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
	0	2,59	0,19	1,65	0,24	0,23	117bc	26	21	15c
0	**125**	2,43	0,19	1,55	0,27	0,21	118abc	29	19	16bc
	250	2,54	0,18	1,49	0,24	0,20	113c	25	19	15c
	0	2,63	0,20	1,60	0,30	0,23	129abc	27	16	17bc
0,5	**125**	2,58	0,18	1,44	0,29	0,19	135ab	24	18	21a
	250	2,57	0,19	1,60	0,30	0,21	137a	28	18	19ab
Significance		0,722	0,302	0,182	0,056	0,200	<0,050	0,623	0,814	<0,050

3.3. The Effects of Biological Fertilizer Application on Some Agronomic Properties of Corn Plant
Effects of BF application and different nitrogen doses on height of the first cob ($p<0,01$), stem thickness ($p<0,05$) and cob diameter ($p<0,05$) were found to be important. Although the effects on other agronomic properties were not statistically significant, efficiency of BF application was observed (Table 7).

The average plant height measured in the study varied between 246 cm and 261 cm (Table 7). Plant height decreased in N_1 and N_2 parcels where biological fertilizer was not applied, compared to control (N_0). In the parcels where biological fertilizer was applied, plant height increased by 1,6% in N_2 parcels and 5,2% in N_1parcels, compared to N_0 First cob binding height amounts varied between 98 cm and 107 cm (Table 7). The highest cob binding height was achieved with N_0 (BF not applied) with height of 107 cm. The next highest cob forming was achieved with N_1(BF applied) with a height of 105 cm. Both of them were statistically in different groups. While nitrogen application generally decreased the first cob forming height, an increase occurred with only half dose of nitrogen and BF application. Stem thickness amounts in the plants varied between 16mm and 18 mm (Table 7). The highest stem thickness (18 mm) was achieved with N_1 dose (BF applied). The average cob height rates varied between 20 cm and 21 cm (Table 7). Cob length increased one unit in N_1 and N_2 parcels compared to N_0 parcels where BF was applied on the other hand, in those where BF was not applied, while there was an increase in N_1 parcels, it remained the same in N_2 parcels. Cob diameter rates varied between 42 mm and 46 mm (Table 7). The highest cob diameter (46 mm) was achieved with N_2 dose (BF not applied). It is seen that nitrogen application

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increases the diameter of the cob. Same results in the samples taken from N_1 and N_2 parcels where BF was applied shows that half dose nitrogen with BF is sufficient. The number of rows in the cob varied between 15 and 16 (Table 7). It can be suggested that BF application has no effect on the number of rows in cob. Llamelo et al. [42] found that different BF created significant differences in plant height, cob height, cob length, cob diameter, thousand grain weight and calculated yield. Marngar and Dawson [43] found that the application of *Azospirillum* inoculation with 150 kg ha^{-1} of nitrogen and 15 kg $ha^{-1}$of $ZnSO_4$ increased plant height, dry weight, amount of grains and grain yield.

Average weight of grain in cob varied between 87,0g $cob^{-1}$and 147,5 g cob^{-1} (Table 7). Grain weights in the cob were determined approximately at the same levels in $N_{0.}$ N_1 and N_2 where BF was not applied. Nitrogen application had no effect on weight of grain in cob. In BF applied parcels at the N_0 an increase of 24% in weight of grain in cob, 70% in the $N_1$and 20% in the N_2 were observed compared to the parcels where BF was not applied. When parcels where BF applied were evaluated within themselves, an increase of 28% in the N_1 and a decrease of 5% in the N_2 were observed compared to the N_0. Those rates show that BF application positively affects the weight of grain in cob and this effect is clearer at the $N_1$with BF application. It may be suggested that the fact that asymbiotic bacteria in biological fertilizer content are more effective with N_1 nitrogen dose caused this significant increase. In their study investigating the effects of BF, mineral fertilizer and BF+ mineral fertilizer applications on corn agriculture, Namazari et al. [44] determined that BF application positively affected 100 grains weight, cob weight and grain yield, and BF+ mineral fertilizer combination increased the yield components.

Average single cob weight varied between 123,3 g and 182,5 g (Table 7). In $N_{0.}$ N_1 and N_2 applications where BF was not applied, rates of single cob weight were determined at approximately the same levels by being parallel with the rates of grain weight in the cob. In the BF applied parcels, an increase of 17% in the N_0 dose, 48% in the N_1 dose and 15% in the N_2 dose were observed compared to parcels where BF was not applied. When parcels where BF applied were evaluated within themselves, an increase of 22% in the N_1 and a decrease of 2% in the N_2 were observed compared to N_0 dose. These results show that BF applications positively affects single cob weight as well as grain weigh in the cob. BF and half dose nitrogen application caused the highest increase in single cob weight.

Table 7. The effects of biological fertilizer application on some agronomic properties of corn plant.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**Plant height (cm)**	**First cob height (cm)**	**Stem thickness (mm)**	**Cob length (cm)**	**Cob diameter (mm)**	**Number of rows in cop (piece)**	**Weight of grain in cop (g cob^{-1})**	**Weight of a single cob (g)**	**Weight of thousand grain (g)**	**Grain yield (kg da^{1})**
	0	258	107a	16c	20	43bc	15	92,8	127,3	343	761
0	**125**	246	98c	16c	21	44abc	16	87,0	123,3	350	714
	250	253	99c	17ab	20	46a	15	91,6	127,5	343	751

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0,5	**0**	248	101bc	16c	20	42c	16	115,1	149,2	347	944
	125	261	105ab	18a	21	45ab	16	147,5	182,5	370	1209
	250	252	98c	17ab	21	45ab	16	109,6	146,2	377	899
Significance		0,114	<0,01	<0,05	0,632	<0,050	0,743	0,182	0,183	0,075	0,182

Thousand grain weight values varied between 343 g and 377 g (Table 7) and determined at the same levels in the $N_{0.}$ N_1 and N_2 applications where BF was not applied. There was a 2% increase at the N_1 dose alone. On the other hand, in the parcels where BF was applied, there was an increase of 1,2% in the N_0 nitrogen dose, 5,7% in the N_1 dose and 9,9% in the N_2 dose compared to the parcels where BF was not applied. When the parcels BF applied were evaluated within themselves, there was an increase of 6,6% in the N_1 dose and 8,7% in the N_2 dose compared to the N_0 dose. These results show that BF application has a positive effect on thousand grain weight.

Grain yield rates varied between 714 kg da^{-1} and 1209 kg da^{-1}(Table 7). According to the yield rates obtained from the N_0 parcels where BF was not applied, there was a decrease of 6,2% in the N_1 dose and 1,3% in the N_2 dose. In the BF applied parcels, the grain yield increased by 24% in the N_0 nitrogen dose, 69% in the N_1 dose and 20% in the N_2 compared to the parcels where BF was not applied. When BF applied parcels were evaluated within themselves, an increase of 28% in the N_1 dose and 5% in the N_2 dose was observed compared to the N_0 dose. These results show that if nitrogen is sufficient in the soil, the effects of nitrogenous fertilizer on the yield are negative, but the nitrogenous fertilizer application with BF affects the yield positively up to a certain dose. Sarajuoghi et al. [45] reported that the application of nitrogenous and phosphorous fertilizers in different combinations with BF containing *Azotobacter* and *Mycorrhiza* increased the grain yield in corn by 15,78%, and that the applied BF reduced the need for phosphorus and nitrogen fertilizers.

4. CONCLUSIONS and SUGGESTIONS

In this study the efficacy of biological fertilizer at different nitrogen doses was shown by applying 0- 125 – 250 kg ha^{-1} nitrogen doses to the parcels without biological fertilizer (BF_0) and 0,5 g ha^1 dose of biological fertilizer (BF_1). In the evaluation of the studied soil properties, the effect ($P<0,05$) of application subjects on electrical conductivity (EC) and NO_3-N in the first period and the effect on the available Mn in the second period were found to be statistically significant. While the effects on N, P, K, Ca, Mg, Zn, Mn were found to be insignificant in the statistical evaluation of the analysis results on plant samples, it was determined that the effects on Fe and Cu were significant ($P<0,05$). Especially the positive effect of BF application on Fe is remarkable. In all the parcels where BF was applied, significant increases occurred in the Fe content of the plant compared to the parcels without BF. This shows that the applied biological fertilizer increases the availability of the iron element in the soil.

The effects of biological fertilizer application and different nitrogen doses on such agronomic properties as first cob height (P<0,01), stem thickness (P<0,05) and cob diameter (P<0,05) were determined to be significant. Although the effects on other agronomic properties were not statistically significant, the efficiency of the BF application was observed. When the data obtained from the biological fertilizer applied (BF_{1}) and non-applied (BF_0) parcels, where nitrogen was not applied, were compared, (BF_0), increases in the grain weight of the cob were found to be 22,3 g $cob^{-1,}$ single cob weight 21,8 g cob^{-1}, thousand grain weight 4 g and grain yield 183 kg da^{-1} depending on BF application.

In evaluating the results of this study as a whole, it was determined that the best results were obtained from the parcels where half of the biological fertilizer and recommended nitrogen were applied. Therefore, it was concluded that biological fertilizer containing asymbiotic microorganisms may be recommended with the application of 125kg N ha^{-1} nitrogen in corn cultivation, thereby preventing soil degradation and environmental pollution which may occur with excessive use of fertilizers.

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