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EFFECTS OF *RHIZOBIA* AND *AZOTOBACTER* ON YIELD AND YIELD COMPONENTS OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Biofertilizers have long been assessed as a powerful technology to obtain sustainable enhanced crop production. *Azotobacter* has been universally accepted as a major inoculum used in biofertilizers to restore the nitrogen level in a cultivated field. This study was carried out in dry farming areas in 2017 and 2019 to examine the effects of nitrogen application and different bacterial applications on the yield and yield components of chickpea cultivars. In the study, the effects on chickpea grain yield and yield components were evaluated by using two nitrogen doses (0 and 25 kg ha^{-1}), four chickpea cultivars (Azkan, Akca, Cakir, Isik) and four bacteria applications (control, *Rhizobia, Azotobacter*, *Rhizobia* + *Azotobacter*). The experimental design was split split plot with three replicates. Nitrogen doses were in main plots, chickpea cultivars in subplots and bacteria application in sub-sub plots. All of the investigated characters except for hundred kernel weight and harvest index were higher during the first year than in the second year due to higher precipitation. All of the investigated characters increased with nitrogen application except for seed germination and hundred kernel weight. In terms of the examined cultivars, Azkan variety gave the highest values, followed by Akca variety. In general, co-inoculation of *Rhizobia* and *Azotobacter* enhanced all of the investigated characters except for seed germinations. Co-inoculation may be a technique essential for chickpea production but intensive further work is needed to develop this technology for commercial use by farmers.

Keywords: *Azotobacter;* nitrogen; *Rhizobia;* yield components; yield.

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INTRODUCTİON

Edible legumes, which are an important food source for many developing countries, have an important place in daily consumption in Turkey as well (FAO, 2022). Chickpea (*Cicer arietinum* L.) is one of the most important legume species and its grain is an important source of proteins for human food consumption.

Nitrogen (N) is the most limiting nutrient for crop production and hence, is the fertilizer nutrient that is applied in the greatest amount (Liu *et al.*, 2019). Although N is found in high concentrations as a gas in the atmosphere, dinitrogen (N_2) form cannot be used as a food source by most organisms (Postgate, 1978). In an agro-ecosystem, water is the most important factor for plant growth, with N in second place. Under natural conditions plants use N in the form of urea, ammonium and nitrates from industrial fertilizers, decaying biological matter and biological nitrogen fixation (BNF) (Nag *et al.,* 2019). The microbial conversion of atmospheric N_2 gas to ammonia is defined as BNF. Due to high-priced chemical fertilizers ecological and health hazards, alternative nutrient sources need to be found (Lallawmkima**,** 2018**).** BNF is very important because it can reduce chemical fertilizers in agricultural systems and improves environmental sustainability. *Azotobacter* and *Rhizobia* are two important bacterial species that play an active role in BNF.

Due to the ability of leguminous plants to fix the free nitrogen in the air to the soil, their importance in terms of environment and sustainability is increasing (Gulumser, 2016; Adak, 2021). The N obtained by the rotation of legumes has two benefits. 1) N fertilizer applications are reduced 2) N accumulated into root and shoot residues can be mineralized for uptake by a subsequent crop in the rotation (Liu *et al.,* 2019). Chickpea, like other legumes, is reported to benefit the subsequent cereal crop. Inoculation with *Rhizobium* species in chickpea was effective in increasing the seed yield from 21% to 35% (Khaitov *et al*., 2016). Plant-based protein utilization may be increased by improving BNF in chickpeas.

The legumes reduce the need for the application of inorganic fertilizers. Because they have the symbiosis with *Rhizobia* to fix the N. The legumes supply all the essential nutrients and sugar to *Rhizobia* (Udvardi and Poole, 2013). *Rhizobia* form nodules and set up a symbiosis with the roots of legumes. Later *rhizobia* reduce atmospheric N into a form directly assimilated by plants (ammonium) (Akter *et al.,* 2018). Plant–microbe mutualism can be affected by many factors and *Rhizobia*–legume symbiosis is an important plant–microbe mutualism type (Han *et al.,* 2020). Rhizobial infection of the legume roots, nodule development, nodule functioning and nodule senescence stages is a complex process and it is named *rhizobia*–legume symbiosis (Schumpp and Deaki, 2010; Laranjo*et al.,* 2014).

Azotobacter sp. is used as a biofertilizer in the cultivation of many agricultural crops. Biofertilizer is an alternative fertilizer that is useful for agricultural production. *Azotobacters* are to play multiple rolesin sustainable agriculture, yield and soil health and their optimum growth potential (Hariyono *et al.,* 2017). Free-living bacteria are phototrophs and they live in soil, water, rhizosphere and leaf surfaces. Heterotrophic N bacterias are an important component of diazotropes. But their fixation capacity is limited because organic substrates for energy production are lacking (Russelle, 2008; Cooper and Scherer, 2012). *Azotobacter* is a heterotrophic free-living N_2-fixing bacteria. It can fix atmospheric N, as well as synthesize substances such as B vitamins, gibberellins and indoelacetic acid (Sharma, 2005). Mirza *et al*., (2007) and Mishra *et al*., (2009) reported that bacteria increase grain yield.

This study was carried out to determine whether less nitrogen fertilizer would be sufficient by using two different bacteria separately and together. *Azotobacter* used in the study has recently started to be used by farmers in our country. The most effective use in field applications is not yet known. For this purpose, *Rhizobia* and *Azotobacter*, nitrogen application and different chickpea cultivars were evaluated in terms of yield and yield components under field conditions.

MATERIALS AND METHODS

Field trials were carried out in the fields of Eskisehir Osmangazi University Field Crops Department (39º48' N; 30º31' E, 798 m above sea level) during the production season of 2017 and 2019. Climate data is presented in Figure 1. The soil of the experimental area has organic matter content of 1.41%, with lime 2.09% and pH of 7.58. In the first year, available P_2O_5 and K_2O contents were 108.9 kg ha^{-1} and 1944.6 kg ha^{-1}, respectively. pH of 7.78, with lime 5.60%, organic matter 0.93%, available P_2O_5 23.4 kg ha^{-1} and K_2O 2729.8 kg ha^{-1} in the second year.

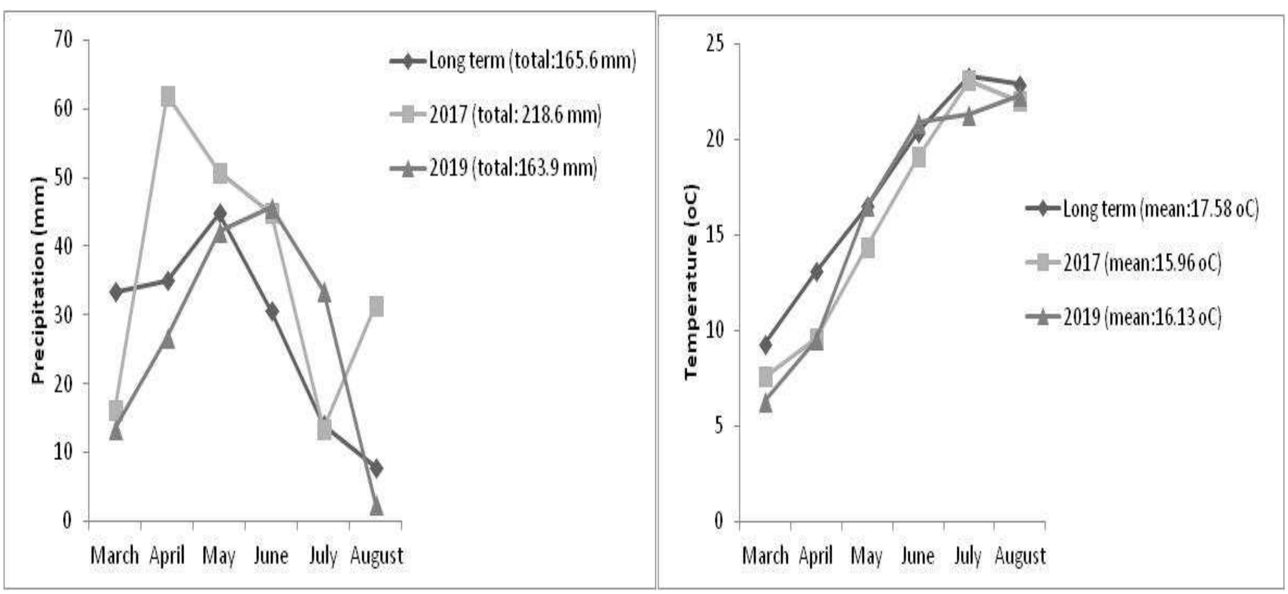

Figure 1. Total precipitation and mean temperature of the research area(Eskisehir meteorology directorate)

The study evaluated using two N doses (0 and 25 kg ha^{-1}), four chickpea cultivars (Azkan, Akca, Cakir, Isik) and four bacterial applications (control, *Rhizobia, Azotobacter*, *Rhizobia* + *Azotobacter*). Split split plot experimental design was used research and it was three replicates. Main plots included N doses, subplots included chickpea cultivars and sub-sub plots included bacteria application.

Experimental areas were processed with plows in autumn; Before sowing, the seed bed was prepared with a cultivator. Wheat was planted as a pre-plant in both years. Seeds were sown in 30 cm row spacing with a sowing rate of 48 seeds per square meter. Each sub-sub plot was 7.2 m^2 (4 m × 1.8 m). N fertilization (21% ammonium sulfate) was applied in doses of 25 kg ha^{-1} in N plots at the sowing time (starter N doses). Chickpea cultivars were provided by the Transitional Zone

Agricultural Research Institute. Chickpea was spring sowed in both years (11 April 2017 and 26 April 2019) and at the time of sowing, basal fertilizer application of 60 kg P_2O_5 ha^{-1} was given to each plot. Before sowing, seeds were inoculated recommended rate (100 kg seed to 1 kg peat inoculant) with *Mesorhizobium muleiense* (formed colonies at 10^{-8} level) bacteria in *Rhizobia* plots. Bacterial inoculation was done with water containing 2% sugar. Inoculation material was obtained from the Soil, Fertilizer and Water Central Research Institute. The refrigerator at +4 ºC was used to store the material. Vitormone (which contains *Azotobacter chroococcum and Azotobacter vinelandii*) was applied to *Azotobacter* plots at vegetative periods. *Rhizobia* and *Azotobacter* were performed in *Rhizobia* + *Azotobacter* plots as described above. Control plots weren't made for any treatment of any bacteria inoculation. Plots were hand-weeded two times when needed each year. Chickpeas were harvested on 16 August 2017 and 31 August 2019.

Seed germination was determined in all the sub-sub plots. Ten plants were selected from each sub-sub plot randomly and grain yield per plant (g), seeds per plant, pods per plant and biological yield per plant (g) were recorded. After harvesting of each sub-plot, harvest index (%), hundred grain weight (g), grain yield (kg ha^{-1}) and biological yield (kg ha^{-1}) were determined (Tosun and Eser, 1975).

The variance analysis was subjected based on General Linear Model using the Statview package (SAS Institute). Means were compared by Least Significant Differences (LSD) test.

RESULTS AND DISCUSSION

The effects of year, varieties and applications were significant for seed germination but N doses did not affect seed germination. Seed germination was lower in the second year (Table 1). The mean temperature was lower during the first year but higher temperatures in April, when experiments were set up in the first year, resulted in more seed germination. Germination rate is usually low at low temperatures (Karakurt *et al.,* 2010; Dadasoglu and Ekinci, 2013). With 29.70 number/m^2, the highest seed germination was determined in the Cakir variety (Table 1). In the control plots, the highest seed germination was determined. *Rhizobia* plots were in the same statistical group as control plots (Table 1). The responses of applications to N and varieties were different. Therefore, the interaction of year × N doses × varieties × bacterial application was significant for seed germination (Figure 2A).

The effects of years, N doses, varieties and applications were significant for biological yield per plant (Table 1). Biological yield per plant was lower in the second year than in the first year. While the total precipitation was 218.6 mm in the first year, it was 163.9 mm in the second year (Figure 1). Chickpeas are grown in dryland areas. High precipitation in the first year, especially in April and May caused higher biological yield per plant. Biological yield per plant was increased with N application (Table 1). Datt *et al.* (2003) reported that N application increased photosynthetic activity by encouraging vegetative growth in plants. Increased photosynthetic activity may have resulted in higher biological yield per plant. Kagan (2012) reported that the biological yield per plant, increased with N application for chickpea. While the highest yield components were determined in Azkan variety, it was followed by Akca variety (Table 1). This differences originated from genotipic differences (Dogan *et al*. 2015). Biological yield per plant is highest in *Rhizobia* + *Azotobacter* applications. It was followed by *Rhizobia* plots (Table 1). The beneficial effects of *Azotobacter* and *Rhizobia* are enhancing N fixation. The sufficiency of N will be able to increase the biological yield per plant. With bacterial symbiosis, nutrient uptake increases and this is beneficial for the nitrogenase enzymeactivity. As a result, N fixation increases and positively affect root and shoot development (Abdiev *et al*., 2019). Co-inoculation with *Azotobacter* and *Rhizobia* positively affected biological yield per plant compared to single inoculation. Co-inoculation of legumes with *Rhizobia* and PGPR can increase plant growth and grain yield, as it improves the nodulation process compared to inoculation with *Rhizobia* alone (Peix *et al*. 2015). Biological yield per plant increased with increasing nutrient uptake due to BNF with inoculation applications. While biological yield per plant was higher in the first year, it was lower in the second year. Therefore, the interaction of year × N applications × varieties × application was significant (Figure 2B).

The effects of years, N doses, varieties and applications were significant for pods per plant (Table 1). Pods per plant was lower in the second year than in the first year. Since higher precipitation received in the first year, plant growth and growing period extended. Consequently, plants produce more pod (Figure 1). Pods per plant was increased with N application (Table 1). Nitrogen cause higher photosynthetic activity. Therefore plants produce more pod (Datt *et al.,* 2003). Bahr (2007) indicated that N application increased the pods per plant. Kagan (2012) reported that the pods per plant increased with N application for chickpea. While the highest pods per plant was determined in Azkan variety, it was followed by Akca variety (Table 1). This differences originated from genotipic differences (Dogan *et al*. 2015). Pods per plant is highest in *Rhizobia* + *Azotobacter* applications. It was followed by *Rhizobia* plots (Table 1).With bacterial symbiosis, nutrient uptake increases and this is beneficial for the nitrogenase enzymeactivity (Abdiev *et al*., 2019). Co-inoculation with *Azotobacter* and *Rhizobia* positively affected pods per plant compared to single inoculation. Co-inoculation of legumes with *Rhizobia* and PGPR can increase plant growth and grain yield compared to inoculation with *Rhizobia* alone (Peix *et al*. 2015). Ucar (2019) indicated that pods per plant increased with *Rhizobia* applications. While pods per plant were higher in the R^+, 25 kg ha^{-1} N and Azkan varieties, the same yield components showed lower values in other plots. Therefore N applications × varieties × application interaction was significant (Figure 3A). While pods per plant were lower in control plots in 2019 for Cakir variety, the same yield component showed higher values in other plots. Cakir cultivar may have been more affected by drought. Cultivars that perform well in arid conditions are genetically stronger cultivars. Therefore year × varieties × application interaction was significant (Figure 3B). While pods per plant were higher in the first year, the same yield components were lower in the second year. Higher precipitation in the first year may have caused the results in chickpeas to be like this. Therefore interaction of year × N applications × application was significant (Figure 4A).

The effects of years, N doses, varieties and applications were significant for seeds per plant (Table 1). Seeds per plant was lower in the second year than in the first year. High precipitation in the first year caused higher seeds per plant (Figure 1). Seeds per plant was increased with N application (Table 1). Datt *et al.* (2003) reported that N application increased photosynthetic activity. Higher photosynthetic activity result in more seeds per plant. Kagan (2012) reported that the, seeds per plant increased with N application for chickpea. While the highest seeds per plant was determined in Azkan variety, it was followed by Akca variety (Table 1). This differences originated from genotipic differences (Dogan *et al*. 2015). Seeds per plant is highest in *Rhizobia* + *Azotobacter* applications. It was followed by *Rhizobia* plots (Table 1). The beneficial effects of *Azotobacter* and *Rhizobia* are enhancing N fixation. The sufficiency of N will be able to increase the seeds per plant. Co-inoculation with *Azotobacter* and *Rhizobia* positively affected seeds per plant compared to single inoculation. Co-inoculation of legumes with *Rhizobia* and PGPR can increase plant growth and grain yield (Peix *et al*. 2015). Bulut (2013) indicated that seeds per plant increased with *Rhizobia* applications. While seeds per plant was higher in the first year, the same yield components were lower in the second year. Therefore, the interaction of year × N applications × varieties × application was significant (Figure 4B).

The effects of years, N doses, varieties and applications were significant for grain yield per plant (Table 1). Grain yield per plant was lower in the second year than in the first year. High precipitation in the first year caused higher grain yield per plant (Figure 1). Grain yield per plant was increased with N application (Table 1). Increased photosynthetic activity may have resulted in higher grain yield per plant (Datt *et al.,* 2003). Yagmur and Engin (2005) reported that increasing N doses increased grain yield per plant. Kagan (2012) reported that the grain yield per plant increased with N application for chickpea. While the highest grain yield per plant was determined in Azkan variety, it was followed by Akca variety (Table 1). This differences originated from genotipic differences (Dogan *et al*. 2015). Grain yield per plant is highest in *Rhizobia* + *Azotobacter* applications. It was followed by *Rhizobia* plots (Table 1). With bacterial symbiosis, nutrient uptake increases and this is beneficial for the nitrogenase enzymeactivity (Abdiev *et al*., 2019). Co- inoculation with *Azotobacter* and *Rhizobia* positively affected grain yield per plant compared to single inoculation. Co-inoculation of legumes with *Rhizobia* and PGPR can increase plant growth and grain yield (Peix *et al*. 2015). While grain yield per plant was higher in the first year, the same yield components were lower in the second year. Therefore, the interaction of year × N applications × varieties × application was significant (Figure 5A).

Years, varieties and applications's effects were significant for hundred kernel weight but differences between N doses were not significant for these characters. Hundred kernel weight was lower in the first year (Table 1). In the second year, July precipitation is very high in our study (Figure 1). The high precipitation during the grain filling period had a positive effect on the hundred kernel weight (Uzun *et al.,* 2012). Therefore, the hundred kernel weight is higher in the second year. While the highest hundred kernel weight was determined in Cakir variety, Azkan and Akca varieties followed it. These three cultivars were included in the same statistical group (Table 1). Hundred kernel weight may vary depending on genotypes (Aydogan *et al.,* 2020). Hundred kernel weight is highest in *Rhizobia* + *Azotobacter* applications (Table 1). Hundred kernel weight was improved with co-inoculation with *Azotobacter* and *Rhizobia*. Increased nutrient uptake due to BNF with inoculation treatments increased hundred kernel weight. The responses of applications to N and varieties were different. Therefore interaction of year × N application × varieties × application was significant for hundred kernel weight (Figure 5B).

The effects of N application, varieties and applications were significant for harvest index but differences between years were not significant for these characters (Table 2). Nitrogen generally increased harvest index. While the harvest index was 48.07% in the 0 kg ha^{-1} N, it was 48.71 % in the 25 kg ha^{-1} N (Table 2). Nitrogen increases the number of flowers in the first stage of development. The increase in the number of pods per plant may have caused an increase in the harvest index. Karasu *et al.* (2009) indicated that the N fertilization increased harvest index. Akca variety showed the highest harvest index (Table 2). The harvest index varies according to the genetic characteristics of the plants, environmental conditions and cultivation method (Soysal and Erman, 2020). The highest harvest index was observed in the *Rhizobia* + *Azotobacter* plots. *Rhizobia* and *Azotobacter* plots were in the same statistical group as *Rhizobia* + *Azotobacter* plots (Table 2). Bacteria application increased the harvest index in our study. Bacteria increase plant nutrient uptake by changing enzyme and hormone synthesis (Sahin *et al* 2004; Yolcu *et al* 2012). This may have caused the harvest index to be high. It is indicated that bacteria increased harvest index by affecting on sharing of the dried weight of the plant and allocating more dry matter to the grain (Naseri *et al.,* 2013). Cig (2010) reported that the harvest index increased with dual bacteria applications. While harvest index was lower in control plots in 2019 for Azkan variety and 0 kg ha^{-1} N, harvest index showed the higher values in other plots. Therefore interaction of year × N application × varieties × application was significant for harvest index (Figure 6A).

Differences between years, N application, varieties and applications are important for biological yield (Table 2). In the first year, biological yield was higher. Higher precipitation in the first year caused plants to photosynthesize longer. Thus, more dry matter accumulated and the biological yield was higher. Biological yield increased with N application. While the biological yield at 0 kg ha^{-1} N application was 3710 kg ha^{-1}, the biological yield was 4660 kg ha^{-1} with 25 kg ha^{-1} N application (Table 2). The increase in biological yield with N application may be due to the effect of N application on the vegetative growth of chickpeas. Nitrogen may increase biological yield with an effect on plant vegetative growth. Total dry matter production is increased by N (Caliskan *et al.,* 2008). Total dry matter can increase more biological yield per plant, pods and seeds which finally resulted in a high grain and biological yield. Some researchers have reported that N fertilizer applications increase biological yield compared to control (Eker, 2019). The highest biological yield was determined in Azkan variety (4740

Table 1. Different N, varieties and applications effects on some traits of chickpea.

	Seed germination (number/m^2)	Biological yield per plant (g)	Pods per plant (number)	Seeds per plant (number)	Grain yield per plant (g)	Hundred kernel weight(g)
2017	29,66A	14,375A	20,43A	18,51A	6,93A	37,49B
2019	29,01B	11,574B	15,36B	14,09B	5,63B	39,94A
0 kg ha^{-1} N	29,30	11,46B	15,58B	14,29B	5,52B	38,82
25 kg ha^{-1} N	29,38	14,48A	20,20A	18,31A	7,04A	38,61
Isik	29,39B	12,55C	17,45C	15,92C	6,07C	38,38B
Cakir	29,70A	11,81D	16,16D	14,68D	5,70D	38,89A
Azkan	29,25B	14,76A	20,06A	18,40A	7,11A	38,86A
Akca	29,01C	12,76B	17,90B	16,20B	6,23B	38,73A
Control	29,60A	10,58D	14,86D	13,15D	5,03D	38,50B
Rhizobia	29,51A	13,47B	18,79B	17,04B	6,55B	38,65B
Azotobacter	29,05B	12,16C	16,67C	15,39C	5,90C	38,54B
R+A	29,19B	15,66A	21,25A	19,62A	7,64A	39,17A
Year (Y)	**	**	**	**	**	**
N application (A)	ns	**	**	**	**	ns
Year × N app.	**	**	*	**	**	ns
Varieties (B)	**	**	**	**	**	**
Year × varieties	**	**	**	**	**	**
N app. × varieties	**	**	**	**	**	**
Year × N app. × var.	**	**	ns	**	**	**
Application (C)	**	**	**	**	**	**
Year × application	*	**	**	**	**	**
N app. × app.	**	**	ns	**	**	*
Varieties x app.	**	**	**	**	**	**
Year × N app.× app.	**	**	*	**	**	*
Year × var. × app.	**	**	*	**	**	**
N app. × var. × app.	**	**	**	**	**	**
Year × N app. × var. × app.	**	**	ns	**	**	**

ns:non-significant, *: p≤0.05, **: p≤0.01.

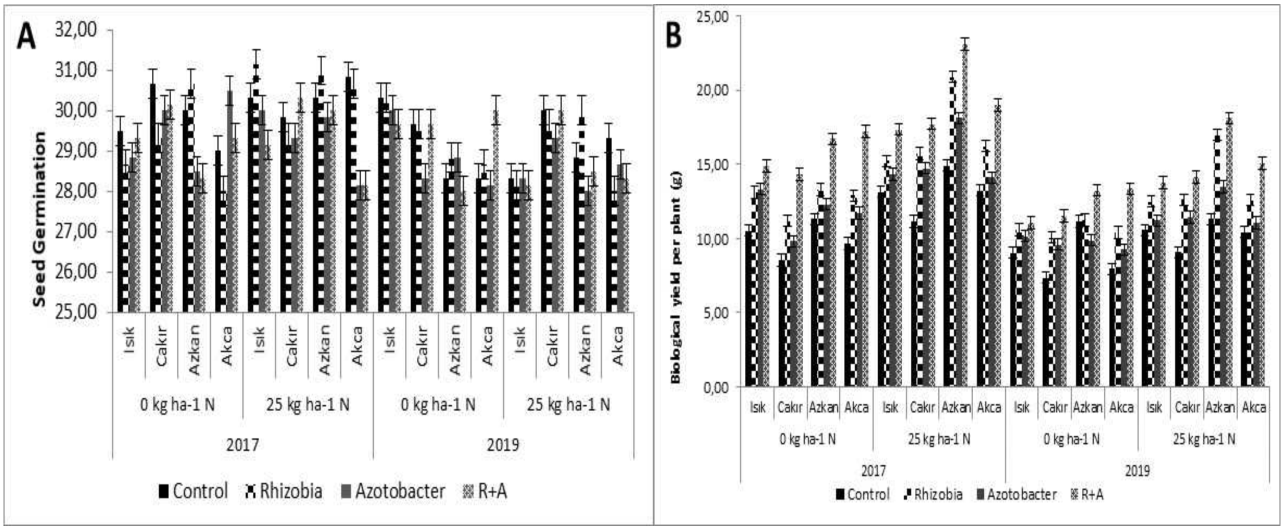

Figure 2. Seed germination (A) and biological yield per plant (B) interactions between year, N application, varieties and application [LSD: 0.806 (1%) (A); 0.373 (1%) (B)]

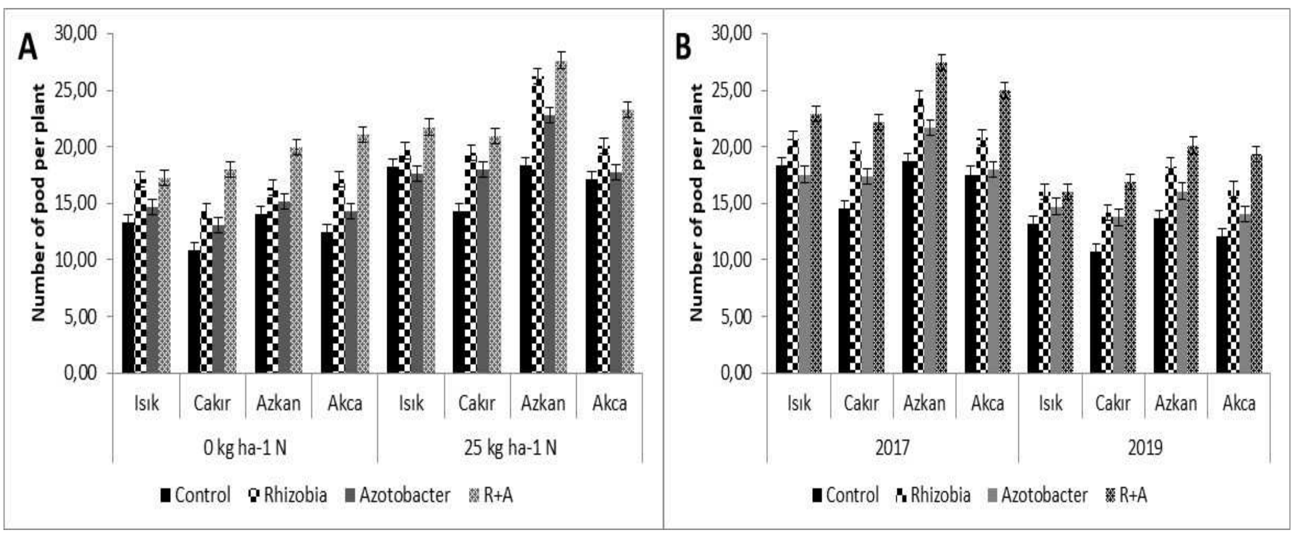

Figure 3 Pods per plant (A) interactions between N application, varieties and application and pods per plant (B)interactions between year, varieties and application [LSD: 0.998 (1%) (A); 0.998 (5%) (B)]

kg ha^{-1}), followed by Akca variety (4130 kg ha^{-1}) and Isik variety (4040 kg ha^{-1}). The lowest biological yield was determined in Cakir (3830 kg ha^{-1}) variety (Table 2). Biological yield is related to the weight of the above-ground part of the plant, and it varies according to the genotype, climatic conditions and cultural practices (Kaplan and Senkal, 2021). *Rhizobia* + *Azotobacter* plots obtained the highest biological yield (Table 2). Bacteries had a positive effect on biological yield. The plants co-inoculated with *Rhizobium* and *Azotobacter* strains produced taller plants, higher root and plant biomass, more primary and secondary branches and straw weight as compared to the single inoculation (Abdiev *et al.,* 2019). Co-inoculation of *Rhizobia* with *Azotobacter* may have contributed to Biological Nitrogen Fixation of the crop. Also, an interaction effect of chemical and bio-fertilizers caused increasing biological yield. Using N application and dual inoculation with *Rhizobia* + *Azotobacter* had the highest biological yield (Figure 6B). Many researchers reported that bacteria inoculation and chemical fertilization provide increases in biological yield (Sultana *et al*., 2016; MeCarty *et al*., 2017; Sood *et al*., 2018). While biological yield was higher in the first year, it was lower in the second year. Bacteria work better when there is enough moisture in the soil. The fact that the precipitation was more suitable in the first year had a positive effect on the bacteria. Therefore interaction of year × N application × varieties × application was significant (Figure 6B).

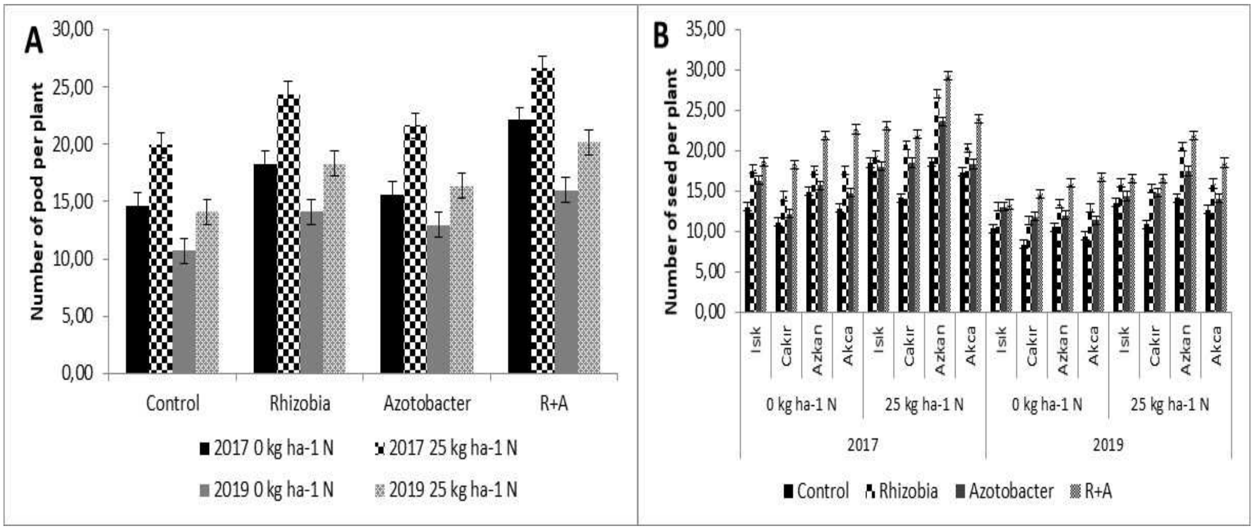

Figure 4. Pods per plant (A) interactions between year, N application and application and seeds per plant (B)interactions between year, N application, varieties and application [LSD: 0.705 (5%) (A): 0.618 (1%) (B)].

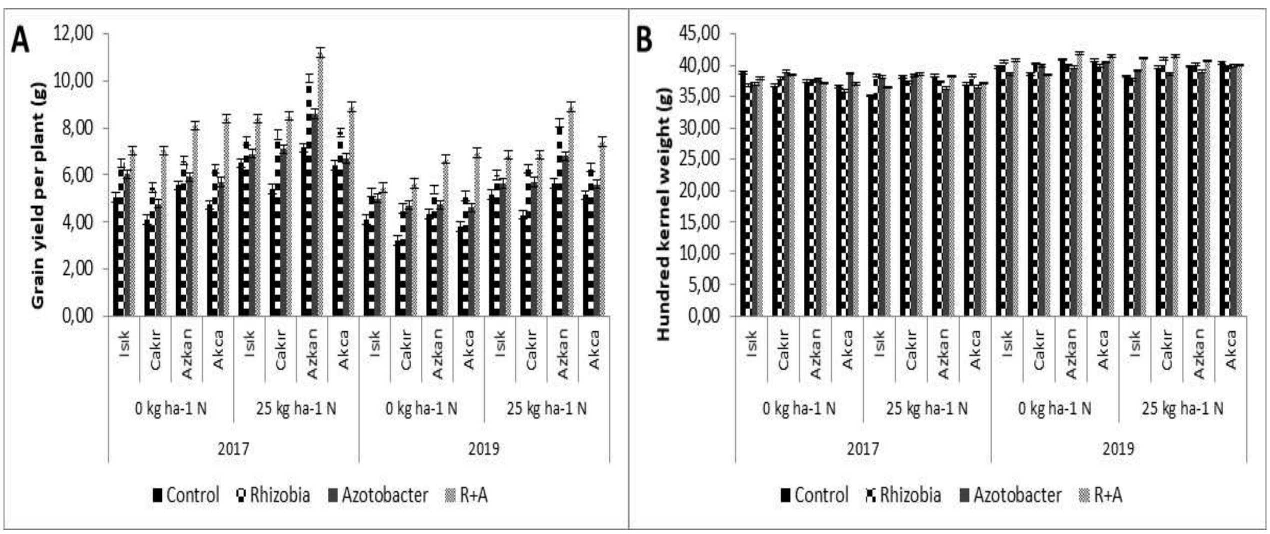

Figure 5. Grain yield per plant (A) and hundred kernel weight (B) interactions between year, N application, varieties and application [LSD: 0.148(1%) (A);1.194 (1%) (B)]

The effects of all the examined factors on grain yield were significant (Table 2). Grain yield is higher in the first year. Plant growing is carried out depending on precipitation in dryland areas. Chickpea is the plant of the dry farming and the high precipitation in the first year, especially in April and May, affected the grain yield positively. Grain yield increased with N application. While the grain yield at 0 kg ha^{-1} N application was 1650 kg ha^{-1}, the grain yield was 2110 kg ha^{-1} with 25 kg ha^{-1} N application (Table 2). Nitrogen directly influences plant growth and development. N is involved in DNA synthesis, respiration, photosynthesis, N_2 fixation and protein formation (Erman *et al.,* 2011). Soysal and Erman (2020) reported that chemical fertilizers increase grain yield in chickpea. Grain yield is higher nitrogen applied plots in the first year (Figure 7). When soil is sufficient moisture, plant nutrient uptake increases. The highest grain yield was determined in Azkan variety (2130 kg ha^{-1}), followed by Akca variety (1870 kg ha^{-1}) and Isik variety (1820 kg ha^{-1}). The lowest grain yield was determined in Cakir (1710 kg ha^{-1}) variety (Table 2). Grain yield is the result of the combined effects of the genetic potential of the plant, environmental factors and breeding techniques. The differences in grain yield are largely due to the genetic characteristics of the cultivars (Anil, 2000). The highest grain yield was observed in the *Rhizobia* + *Azotobacter* plots (Table 2). Bacteria positively affect grain yield. Qureshi *et al*. (2013) reported, increase in chlorophyll content, photosynthesis and transpiration through inoculation with *Rhizobia*. Increased photosynthesis with

Table 2. Different N applications, varieties and applications effects on some traits of chickpea.

	Harvest index (%)	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})
2017	48,27	4600A	2080A
2019	48,51	3770B	1680B
0 kg ha^{-1} N	48,07B	3710B	1650B
25 kg ha^{-1} N	48,71A	4660A	2110A
Isik	48,42B	4040C	1820C
Cakir	48,10C	3830D	1710D
Azkan	48,10C	4740A	2130A
Akca	48,95A	4130B	1870B
Control	47,44B	3440D	1510D
Rhizobia	48,64A	4340B	1960B
Azotobacter	48,62A	3920C	1770C
R+A	48,87A	5040A	2290A
Year (Y)	Ns	**	**
N application (A)	*	**	**
Year × N app.	**	**	**
Varieties (B)	**	**	**
Year × varieties	**	**	**
N app. × varieties	**	**	**
Year × N app. × var.	**	**	**
Application (C)	**	**	**
Year × application	**	**	**
N app. × app.	**	**	**
Varieties x app.	**	**	**
Year × N app.× app.	**	**	**
Year × var. × app.	**	**	**
N app. × var. × app.	**	**	**
Year × N app. × var. × app.	**	**	**

ns:non-significant, *: p≤0.05, **: p≤0.01.

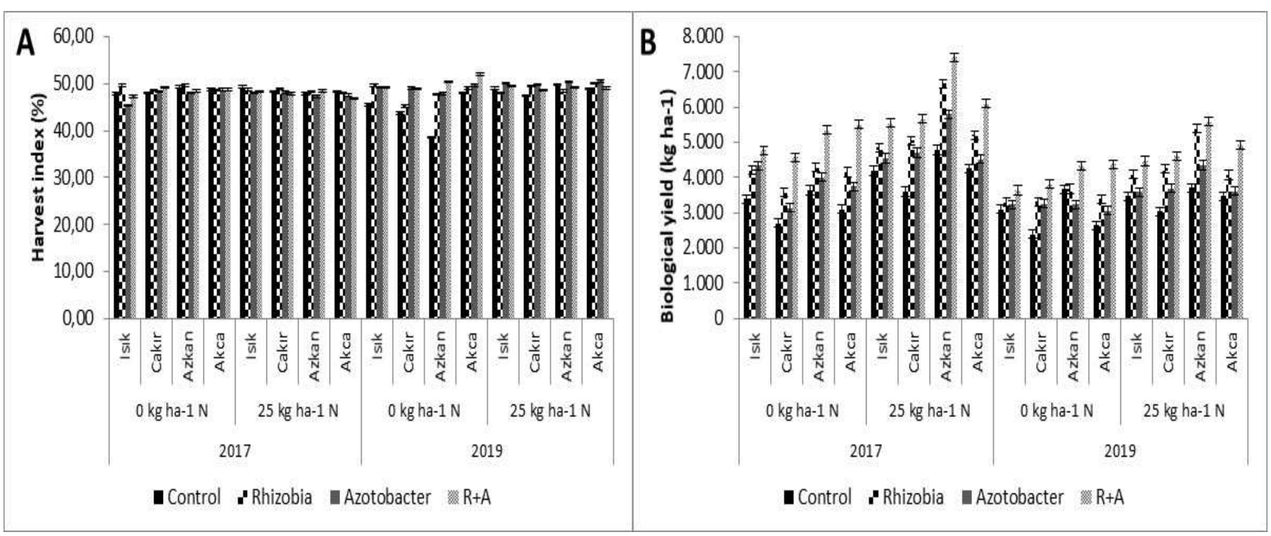

Figure 6.Harvest index (A) and biological yield (B) interactions between year, N application, varieties and application [LSD: 1.148 (1%) (A): 8.651(1%)(B)]

bacteria positively affected grain yield. Single and combined use of bacteria resulted in higher above-ground plant biomass with simultaneous root growth. Nutrient uptake in the plants is increased by inoculation and this is due to increased BNF in inoculated plants. Co-inoculation with *Rhizobia* and *Azotobacter* significantly increased the hundred-seed weight, the grain yield per plant, seeds per plant, pods per plant and the biological yield per plant compared to single inoculation. Grain yield increased due to the increase in yield components. Also, the interaction effect of chemical and bio-fertilizers caused an increasing grain yield. Using N application and dual inoculation with *Rhizobia* + *Azotobacter* had the highest grain yield (Figure 7). Grain yield has been increased by co-inoculation with bacteria (Mirza *et al.,* 2007; Mishra *et al.,* 2009). While grain yield was lower in the second year, it was higher in the first year. Therefore interaction of year × N application × varieties × application was significant (Figure 7).

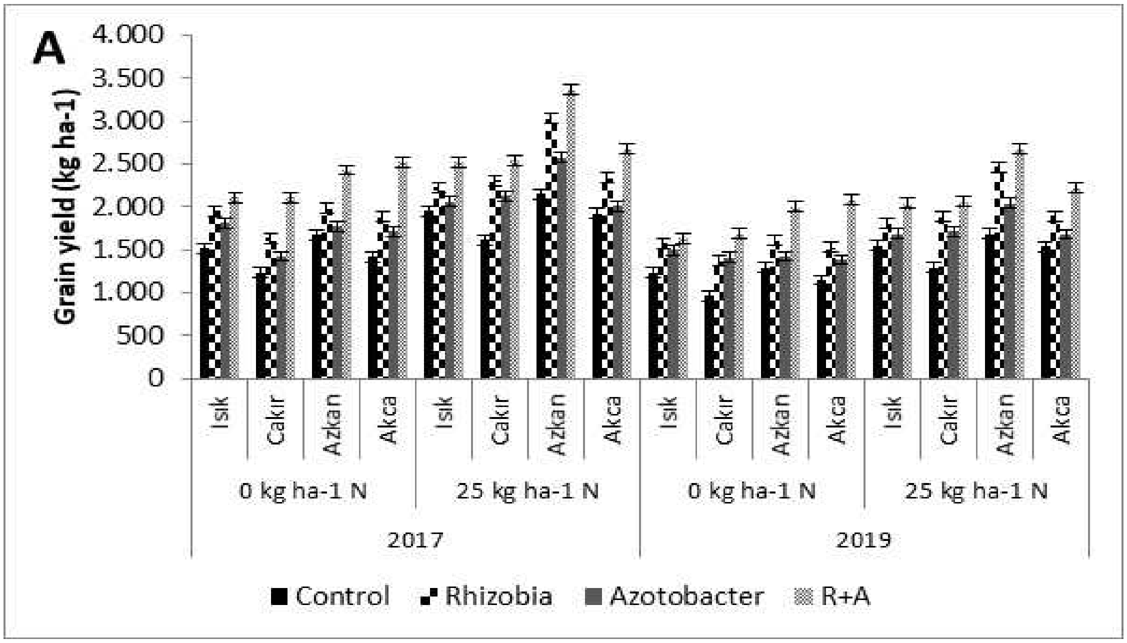

Figure 7. Grain yield interactions between year, N application, varieties and application [LSD: 4.348 (1%)]

Conclusions: Currently, the use of ecofrendly inputs such as *Rhizobia* and *Azotobacter* is one of the known solutions to reduce the consumption of agrochemical inputs. Co-inoculation of the chickpea seeds *Rhizobia* and *Azotobacter* caused the enhancement of yield components and grain yield in four chickpea varieties. Yield and yield components decreased at low N levels and in uninoculated plots. Nitrogen application and inoculated plants obtained the highest values of these characters. In general, co-inoculation of *Rhizobia* and *Azotobacter* enhanced the yield and yield components. Co-inoculation may be a technique essential for chickpea production but intensive further work is needed to develop this technology for commercial use by farmers. According to the results of this study, Azkan cultivar, 25 kg ha^{-1} N dose and *Rhizobia* + *Azotobacter* inoculation are recommended for Central Anatolia and similar ecological conditions for chickpea.

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