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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Phenological and Morphological Response of Chickpea (*Cicer arietinum* L.) *Rhizobia* and *Azotobacter* Inoculation

Nohutun (*Cicer arietinum* L.) Rhizobia ve Azotobacter İnokülasyonuna Fenolojik ve Morfolojik Tepkisi

Engin TAKIL[1*], Nihal KAYAN[2]

Abstract

Excessive use of nitrogen has become a threat to human health and the environment due to high concentrations of nitrate and nitrite accumulating in surface and ground waters. Biological dinitrogen fixation (N_2) is a very important natural process in world agriculture. *Rhizobia* is a common name for a certain Gram-negative group of Alphaproteobacteria and Betaproteobacteria that can form nodules on the root and fix nitrogen in symbiosis with legumes as their host plants. *Azotobacter* spp. is a free-living microorganism that has the ability to fix atmospheric nitrogen into the soil. Field trials were carried out of Eskişehir Osmangazi University Field Crops Department experiment areas during the production season of 2017 and 2019. The study was evaluated the effects on morphological and phenologocial characters of two N doses (0 and 25 kg ha^{-1} N), four bacteria inoculations (control, *Rhizobia*, *Azotobacter*, *Rhizobia* + *Azotobacter*) on chickpea cultivars (Azkan, Akca, Cakir, Isik). Experiment arranged in split split plot experimental design with three replications. Nitrogen application positively affected number of days to emergence, number of branches, branch diameter and grain yield. Phenological and morphological properties affected by climate conditions. The high temperature caused the number of days to emergence, number of days to flowering and number of days to maturity to be earlier in the second year. Plant height, first pod height and grain yield were higher first year than second year due to high precipitation. While the response of the cultivars was different in terms of phenological properties, Azkan cultivar gave the best results in terms of morphological properties. *Rhizobia* + *Azotobacter* inoculation gave the best results in terms of phenological and morphological characteristics except for number of days to maturity. *Rhizobia* + *Azotobacter* inoculation can lead to additional income generation of the farming community in Turkey. The use of biofertilizers may reduce the application of chemical fertilizers and we get healthy, pollution-free production for a better future for our increasing populations.

Keywords: Rhizobia, Azotobacter, Morphological properties, Nitrogen, Phenological stages.

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Öz

Azotun aşırı kullanımı, yüzey ve yer altı sularında biriken yüksek konsantrasyonlardaki nitrat ve nitrit nedeniyle insan sağlığını ve çevreyi tehdit eder hale gelmiştir. Biyolojik azot fiksasyonu dünya tarımında çok önemli doğal bir süreçtir. *Rhizobia*, kök üzerinde nodüller oluşturabilen ve konukçu bitkileri olarak baklagillerle simbiyozda azotu sabitleyebilen belirli bir Gram-negatif Alphaproteobacteria ve Betaproteobacteria grubunun ortak adıdır. *Azotobacter* spp., atmosferik nitrojeni toprağa sabitleme yeteneğine sahip serbest yaşayan bir mikroorganizmadır**.** Tarla denemeleri 2017 ve 2019 üretim sezonunda Eskişehir Osmangazi Üniversitesi Tarla Bitkileri Bölümünde yürütülmüştür. Araştırmada iki azot dozu (0 ve 25 kg/ha N) dört nohut çeşidi (Azkan, Akça, Çakır ve Işık) ve dört bakteri uygulamasının (kontrol, *Rhizobia, Azotobacter* ve *Rhizobia + Azotobacter*) nohutun fenolojik ve morfolojik özellikleri üzerine etkisi değerlendirilmiştir. Araştırma bölünen bölünmüş parseller deneme deseninde üç tekerrürlü olarak yürütülmüştür. Ana parsellerde azot dozları, alt parsellerde nohut çeşitleri ve altın-altı parsellerde ise bakteri uygulamaları yer almıştır. Fenolojik ve morfolojik özellikler iklim koşullarından etkilenmiştir. Yüksek sıcaklık, ikinci yılda çıkış, çiçeklenme ve olgunlaşmanın daha erken olmasına neden olmuştur. Bitki boyu, ilk bakla yüksekliği ve tane verimi, yüksek yağış nedeniyle birinci yıl ikinci yıla göre daha yüksek olmuştur. Azot uygulamaları çıkış zamanı, dal sayısı, dal çapı ve tane verimini olumlu yönde etkilemiştir. Fenolojik özellikler bakımından çeşitlerin tepkileri farklı olurken, morfolojik özellikler bakımından en iyi sonuçları Azkan çeşidi vermiştir. Olgunluk süresi dışında fenolojik ve morfolojik özellikler açısından *Rhizobia + Azotobacter* uygulaması en iyi sonuçları vermiştir. *Rhizobia + Azotobacter* uygulaması Türkiye'deki çiftçilerin ek gelir elde etmesine yol açabilir. Biyogübre kullanımı kimyasal gübre kullanımını azaltabilir. Biyogübreler ile artan nüfusumuza daha iyi bir gelecek sağlamak için sağlıklı ve kirlilik içermeyen temiz bir üretim elde edebiliriz.

Anahtar Kelimeler: Rhizobia, Azotobacter, Morfolojik özellikler, Azot, Fenolojik evreler.

1. Introduction

Application of mineral fertilizers is a way to re-fertilize soils used in agricultural production (Basal and Szabo, 2020). Nitrogen is the most important limiting factor for high yield in the world (Namvar et al., 2011). Nitrogen is included in the structure of important organic compounds such as protein, amino acid, amide, nucleic acid, chlorophyll in the plant. On the other hand, excessive use of nitrogen has become a threat to human health and the environment due to high concentrations of nitrate and nitrite accumulating in surface and ground waters. With nitrogen fixation, molecular nitrogen is reduced to form ammonia for use by living organisms, and this fixed nitrogen can be directly absorbed by plants. (Cheng, 2008).

Nitrogen is given to the soil as fertilizer in inorganic or organic forms (Belliturk and Saglam, 2005). Biological dinitrogen fixation (N_2) is a very important natural process in world agriculture (Herridge et al., 2008). Biological nitrogen fixation can be explained as the reduction of atmospheric nitrogen to ammonium nitrogen and making it available to plants. The fixation of elemental nitrogen in the atmosphere takes place by microorganisms living symbiotically and non-symbiotically. Biological nitrogen fixation, catalyzed by the nitrogenase enzyme, is a process found only in some prokaryotes. Due to their inceptive role in the nitrogen cycle, diazotrophs are present in virtually all ecosystems, with representatives in environments as varied as aerobic soils (e.g., *Azotobacter* species), the ocean surface layer (*Trichodesmium*) and specialized nodules in legume roots (*Rhizobium*) (Halbleib and Ludden, 2000). Environmentally friendly biological fertilizers contain beneficial microorganisms and do not pollute the soil and water. These fertilizers are widely used in many countries.

It was understood at the end of the 19th century that legume plants assimilate atmospheric nitrogen through nodules. The isolation of root nodule bacteria was reported by Beijerinck in 1888 and it was reported that these bacteria are responsible for nitrogen fixation. (Willems, 2006). *Rhizobia* is a common name for a certain Gram-negative group of Alphaproteobacteria and Betaproteobacteria that can form nodules on the root and fix nitrogen in symbiosis with legumes as their host plants (Sprent, 2008). Most legumes have the ability to associate with *Rhizobia* bacteria to fix nitrogen. However, leguminous plants will be able to achieve this symbiosis when they encounter compatible and efficient *Rhizobia* that can form effective nodules. Therefore, the *Rhizobia* population in the soil plays an important role in legume productivity (Amarger, 2001). Chickpea can provide 42-70% of its nitrogen need symbiotically depending on the environmental conditions (Beck, 1988).

Many bacterial species such as *Bacillus, Azotobacter, Azospirillum, Beijerrinckia* and *Pseudomonas* can fix nitrogen (Dobereiner, 1997). *Azotobacter* spp. is a free-living microorganism that has the ability to fix atmospheric nitrogen into the soil. Besides their roles in the production of plant growth elements, they also have the ability to synthesize antibiotics and vitamins, and their anti-pathogenic properties. Therefore, they are very important in agriculture (Wani et al., 2016). *Azotobacter* spp. provides biomass increase by improving the nitrogen, phosphorus and potassium values of the soil. It has been determined that they are especially effective in the increase of antioxidant enzymes, carotenoids, chlorophyll pigments, soluble protein and dry matter (Karaboz and Ozcan, 2005). In uncultivated soils and in the rhizosphere of plants, the population of *Azotobacter* is generally low. For nitrogen fixation and inoculation of plants, fast-growing and highly nitrogen-fixing *azotobacteria* are used. Although there is substantial data showing that *Azotobacters* stimulate plant growth, the way in which *Azotobacter* increases plant growth is not yet fully understood (Wani et al., 2013).

The objectives of the present study were to evaluate the effects of *Azotobacter* and *Rhizobia* on the phenology and morphology of different chickpea varieties.

2. Materials and Methods

2.1. Experimental site

Field trials were carried out in the fields of Eskişehir Osmangazi University Field Crops Department during the production season of 2017 and 2019 (39°48' N; 30°31' E, 798 m above sea level). Climatic data for long-term and experimental years were shown in Figure 1 and Figure 2. Total precipitation was 218.6 and 163.9 mm in the experimental years, respectively and long-term total precipitation was 165.6 mm. The annual average temperature was recorded as 15.96 °C in 2017 and 16.13 °C in 2019 years. In the experimental area, the organic matter content was 1.41%, the lime was 2.09% and the pH was 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.

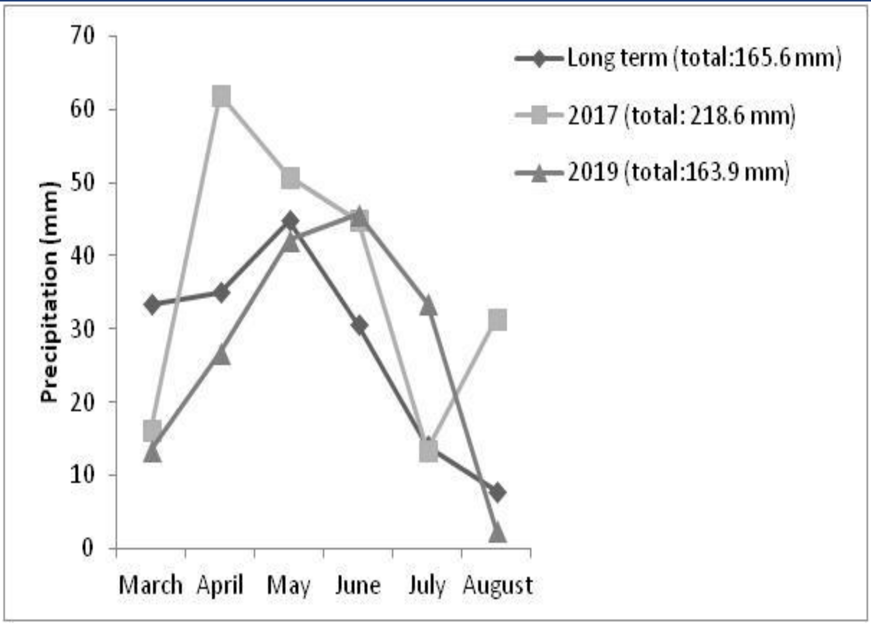

Figure 1. Total precipitation of the research area

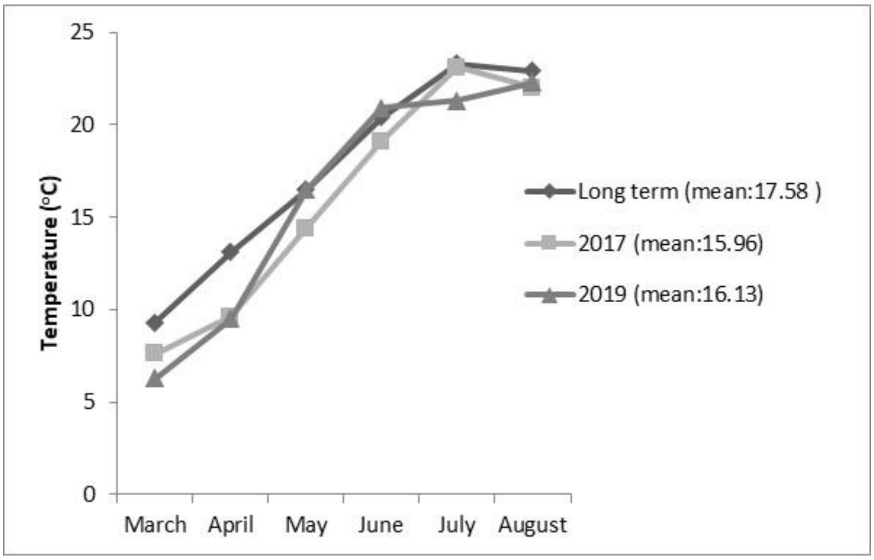

Figure 2. Mean temperature of the research area

2.2. Trial Management

The study was evaluated the effects on morphological and phenologocial characters of two N doses (0 and 25 kg ha^{-1} N), four bacteria inoculations (control, *Rhizobia*, *Azotobacter*, *Rhizobia* + *Azotobacter*) on chickpea cultivars (Azkan, Akca, Cakir, Isik). Experiment arranged in split split plot experimental design with three replications. Main plots subplots and sub-sub plots included N doses, chickpea cultivars and bacteria inoculation, respectively.

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 x 1.8 m). Nitrogen fertilization (21% amonium sulfate) were applied in doses of 25 kg ha^{-1} in N sub-sub plots at the sowing time (starter nitrogen doses). Chickpea cultivars were provided by the Transitional Zone Agricultural Research Instutute. Chickpea was sown spring in both years (11 April 2017 and 26 April 2019) and the basal fertilizer application of 60 kg P_2O_5 ha^{-1} was given to each sub-sub plot at the time of sowing. 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 (contains *Azotobacter chroococcum* *and Azotobacter vinelandii*) was applied *Azotobacter* plots at vegetative periods. *Rhizobia* and *Azotobacter* were performed in *Rhizobia* + *Azotobacter* plots as described above. Control plots weren't made any treatment of any bacteria inoculation. Plots were hand-weeded two times when needed in each year. Plants were harvested was on 16 August 2017 and 31 August 2019 respectively.

Number of days to emergence, number of days to flowering, number of days to pod seeting and number of days to maturity were determined all the sub-sub plots. The plant height (cm), first pod height (cm), number of branches and branch diameter (mm) were evaluated on 10 randomly selected plants in each sub-sub plot. Each sub-sub plot was harvested, blended and grain yield (kg ha^{-1}) were estimated (Tosun and Eser, 1975).

2.3. Statistical Analysis

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

3. Results and Discussion

3.1. Number of Days to Emergence

Variance analysis and mean values for trait were given *Table 1*. The effects of years, nitrogen, variety and inoculation were significant for number of days to emergence. Bacteria inoculations had a positive effect on the number of days to emergence. The earliest emergence was observed in only Rhizobia applied plots, followed by Rhizobia + Azotobacter applied plots. Pathak et al. (2013) and Mahato et al. (2009) reported that Azotobacter and PSB were significant in increasing seed germination. Nitrogen application was affected the number of days to emergence and lower number of days to emergence was observed in the nitrogen applied plots. The earliest emergence was observed in the Isik variety with 9.87 days and the latest emergence was observed in the Azkan variety with 13.89 days. Number of days to emergence was lower in second year than first year. Emergence was completed earlier in the second year. The high temperature in May caused the emergence to be earlier in the second year. The temperature is lower in April, when emergence is completed in the first year (*Figure 2*). Photoperiod, day length and climatic factors are effective on the phenological properties of plants. Especially the temperature factor is the most important factor determining the phenological characteristics (Gupta et al., 1994; Bange et al., 1998). While the first year number of days to emergence are high in both of the nitrogen doses but the second year is low. Therefore, year x nitrogen interaction was significantly for number of days to emergence (*Figure 3A*). The first year number of days to emergence are high in all of the varieties but the second year is low. For this reason, year x varieties interaction was significantly for number of days to emergence (*Figure 3B*). While the first year number of days to emergence are high in all of the bacteria inoculation but the second year is low. The high temperature in May caused the emergence to be earlier in the second year. For this reason, year x inoculation interaction was significantly for number of days to emergence (*Figure 4A*). 0 kg ha^{-1} N in *Rhizobia* plots showed very low values, the same nitrogen dose showed very high values in other bacterial inoculations. For this reason, nitrogen x inoculation interaction was significantly for number of days to emergence (*Figure 4B*). While Cakir variety in *Rhizobia* + *Azotobacter* plots showed low values, the same variety showed high values in other bacterial inoculations. For this reason, variety x inoculation interaction was significantly for number of days to emergence (*Figure 5A*).

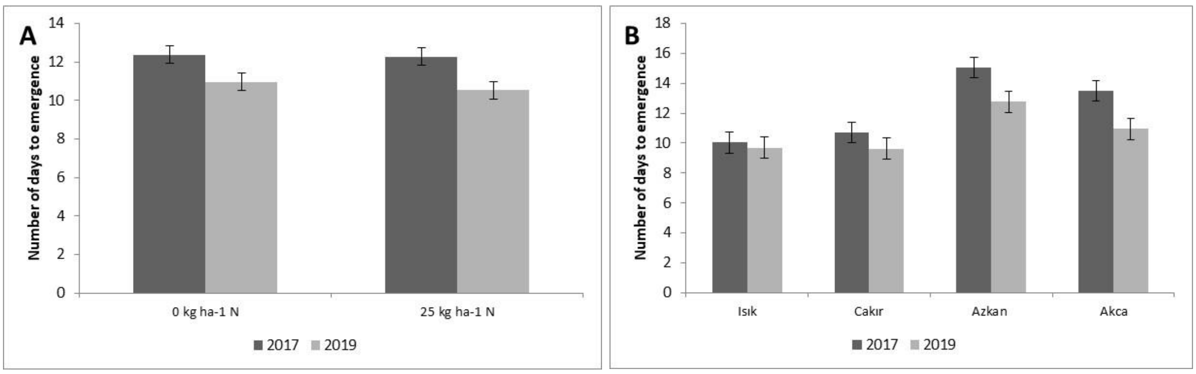

Figure 3. Number of days to emergence (A) year x nitrogen interaction and number of days to emergence (B) year x varieties interaction [LSD 5%: 0.147 (A);5%: 1.117(B)]

Table 1. Different nitrogen, varieties and inoculations effects on some traits of chickpea.

	Number of days to emergence	Number of days to flowering	Number of days to pod seeting	Number of days to maturity	Plant height (cm)	First pod height (cm)
Inoculation						
Control	12.08A	63.25A	73.60A	123.97D	49.79	29.47B
Rhizobia	10.93B	62.70B	73.20B	125.47B	49.63	29.19B
Azotobacter	12.04A	63.14A	73.43AB	124.89C	49.71	29.73B
R+A	11.10B	62.50B	73.16B	126.08A	50.74	30.58A
Mean	**11.54**	**62.89**	**73.35**	**125.10**	**49.96**	**29.74**
Nitrogen						
0 kg ha^{-1} N	11.67A	62.66B	73.08	124.25B	49.63	29.48
25 kg ha^{-1} N	11.40B	63.13A	73.62	125.96A	50.31	30.00
Mean	**11.54**	**62.89**	**73.35**	**125.10**	**49.97**	**29.74**
Varieties						
Isik	9.87C	61.95B	72.22B	124.68B	49.06B	28.56C
Cakir	10.16C	62.31B	72.70B	123.68C	51.31A	30.48B
Azkan	13.89A	65.58A	76.22A	126.20A	51.47A	31.22A
Akca	12.22B	61.75B	72.25B	125.85A	48.03B	28.70C
Mean	**11.54**	**62.89**	**73.35**	**125.10**	**49.97**	**29.74**
Years						
2017	12.32A	64.37A	74.89	127.35A	58.19A	33.43A
2019	10.76B	61.42B	71.81	122.86B	41.75B	26.05B
Mean	**11.54**	**62.89**	**73.35**	**125.10**	**49.97**	**29.74**
General Mean	11.54	62.89	73.35	125.10	49.96	29.74
Year (Y)	**	**	ns	**	**	**
Nitrogen (N)	**	*	ns	**	ns	ns
Yx N	*	ns	ns	ns	ns	ns
Varieties (V)	**	**	**	**	**	**
Y x V	*	ns	ns	ns	**	**
N x V	ns	ns	ns	**	**	ns
Y x N x V	ns	ns	ns	ns	*	**
Inoculation (I)	**	**	*	**	ns	**
Y x I	*	*	ns	ns	**	*
N x I	**	ns	ns	**	ns	**
V x I	*	*	*	**	**	**
Y x N x I	ns	ns	ns	ns	**	ns
Y x V x I	ns	ns	ns	ns	ns	**
N x V x I	ns	**	**	**	**	**
Y x N x V x I	ns	ns	ns	ns	*	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$.

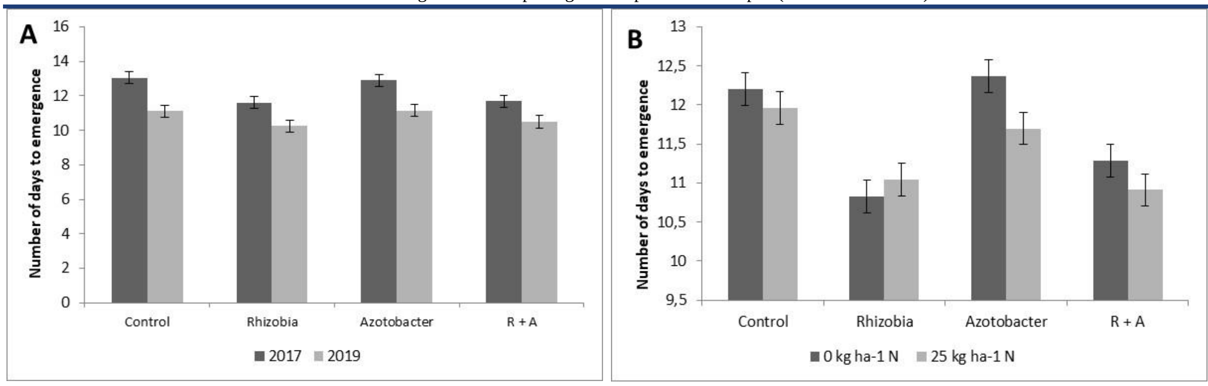

Figure 4. Number of days to emergence (A) year x inoculation interaction and number of days to emergence (B) nitrogen x inoculation interaction [LSD 5%: 0.326 (A);1%: 0.326(B)]

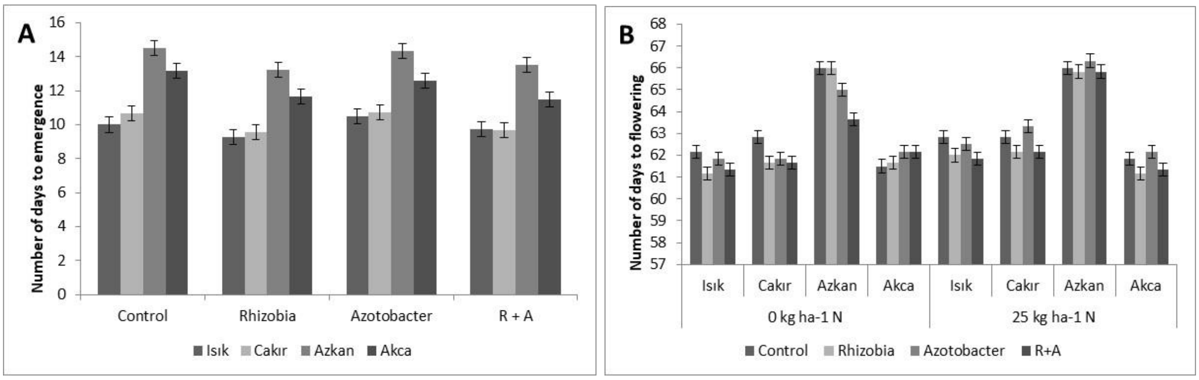

Figure 5. Number of days to emergence (A) varieties x inoculation interaction and number of days to flowering (B) nitrogen x varieties x inoculation interaction [LSD 5%: 0.461 (A);1%: 0.854(B)]

3.2. Number of days to flowering

Variance analysis and mean values for trait were given *Table 1*. The effects of years, nitrogens, varieties and inoculations were significantly for number of days to flowering. Bacteria inoculations had a positive effect on number of days to flowering. The earliest flowering was observed in *Rhizobia + Azotobacter* plots, followed by only *Rhizobia* plots (*Table 1*). Zaman et al. (2011) and Ndlovu (2015) reported that *Rhizobia* inoculation was caused earlier flowering. Nitrogen application did not affect number of days to flowering and earlier flowering was observed in control plots. Turk and Alagoz (2020) reported that they observed earlier flowering in the control plots when applied nitrogen. The earliest flowering was seen in the Akca variety with 61.75 days, followed by the Isik variety with 61.95 days. The latest flowering is 65.58 days in Azkan variety. Holt and Campell (1984) stated that these differences in number of days to flowering and number of days to maturity may be due to genetic characteristics of varieties. Number of days to flowering was lower in second year than first year. Flowering was completed earlier in the second year. High temperatures during the second year of number of days to flowering (June) resulted in earlier flowering (*Figure 2*). Photoperiod, day length and climatic factors are effective on the phenological properties of plants. Especially the temperature factor is the most important factor determining the phenological characteristics (Gupta et al., 1994; Bange et al., 1998). Azkan variety had later number of days to flowering but other varieties had earlier number of days to flowering. For this reason, nitrogen x variety x inoculation interaction was significantly for number of days to flowering (*Figure 5B*). While the first year number of days to flowering are high in all of the bacteria inoculation but the second year is low. For this reason, year x inoculation interaction was significantly for number of days to flowering (*Figure 6A*).

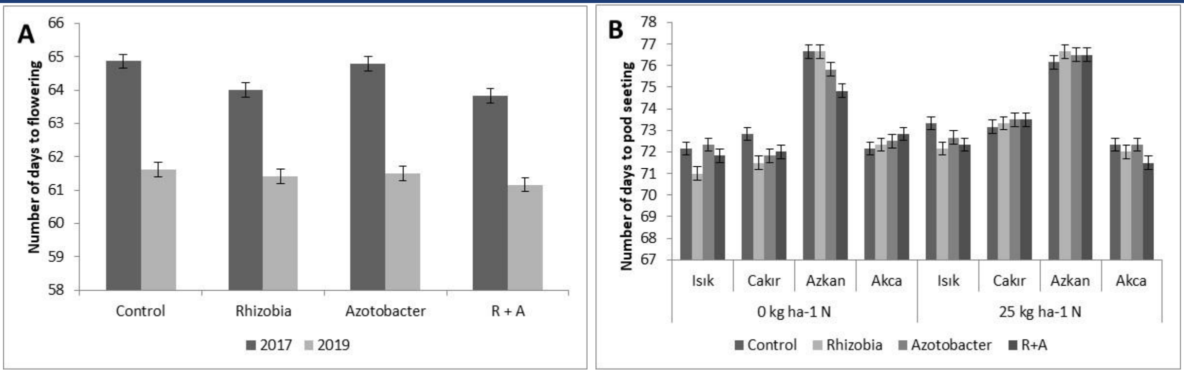

Figure 6. Number of days to flowering (A) year x inoculation interaction and number of days to pod seeting (B) nitrogen x varieties x inoculation interaction [LSD 5%: 0.427 (A);1%: 0.848(B)]

3.3. Number of days to pod seeting

Variance analysis and mean values for trait were given *Table 1*. The effects of varieties and inoculations were significantly for number of days to pod seeting but differences between years and nitrogen was not significantly for this character. Bacteria inoculations had a positive effect on number of days to pod seeting. *Rhizobia* + *Azotobacter* had the earliest number of days to pod seeting, followed by *Rhizobia* plots. Number of days to pod seeting was 72.22 days in Isik variety and 76.22 days in Azkan variety. Azkan variety had later number of days to pod seeting but other varieties had earlier number of days to pod seeting. For this reason, nitrogen x variety x inoculation interaction was significantly for number of days to pod seeting (*Figure 6B*).

3.4. Number of days to maturity

Variance analysis and mean values for trait were given *Table 1*. The effects of years, nitrogens, varieties and inoculations were significantly for number of days to maturity. Bacteria inoculation had no effect on number of days to maturity. The earliest maturity was observed in the control plots, and the latest maturity in the *Rhizobia* + *Azotobacter* plots. Nitrogen application did not affect number of days to maturity and earlier maturity was observed in control plots. The earliest maturity was seen in the Cakir variety with 123.68 days, followed by the Isik variety with 124.68 days. The latest maturity is 126.20 days in Azkan variety. Azkan and Akca varieties were included in the same statistical group. Holt and Campell (1984) stated that these differences in number of days to flowering and number of days to maturity may be due to genetic characteristics of varieties. Number of days to maturity was lower in second year than first year. While the mean temperature was 15.96 ºC in the first year, it was 16.13 ºC in the second year (*Figure 2*). High temperature in the second year caused earlier number of days to maturity. Photoperiod, day length and climatic factors are effective on the phenological properties of plants. Especially the temperature factor is the most important factor determining the phenological characteristics (Gupta et al., 1994; Bange et al., 1998). While number of days to maturity was higher at *Rhizobia*, 0 kg ha^{-1} N and Isik variety plots, Isik variety showed the lower values other plots. Therefore, nitrogen x varieties x inoculation interaction was significantly (*Figure 7A*).

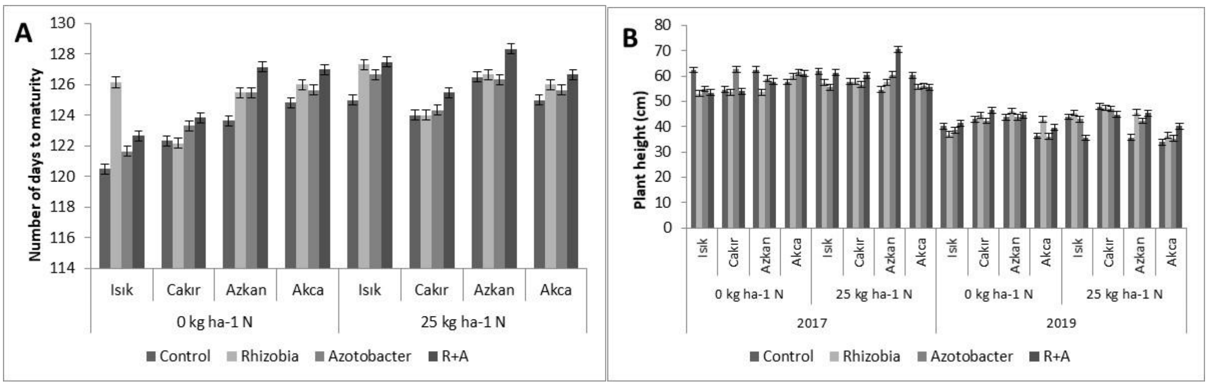

Figure 7. Number of days to maturity (A) nitrogen x varieties x inoculation interactions and plant height (B) year x nitrogen x varieties x inoculation interaction [LSD 1%: 0.999 (A);5%: 5.311(B)]

3.5. Plant height

Variance analysis and mean values for trait were given *Table 1*. The effects of years and varieties were significantly for plant height but differences between nitrogen and inoculations were not significantly for this character. The highest plant height was observed in Azkan variety with 51.47 cm. Azkan variety was followed by Cakir variety with 51.31 cm and these two cultivars were included in the same statistical group. Akca variety has the lowest plant height with 48.03 cm. Plant height is genotypic property and are affected by environmental conditions. With increasing plant height, leaf area per plant, number of leaves and assimilation increased (Vartanli and Emeklier, 2007). Plant height was higher first year than second 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 dry farming areas. High precipitation in the first year, especially in April and May caused higher plant height. High precipitation in spring encourages the vegetative development of the plant. Therefore, the plant height can be longer. While plant height was higher at *Rhizobia* + *Azotobacter*, 25 kg ha^{-1} N and Azkan variety plots in first year, the same variety showed the lower values other plots. Therefore, year x nitrogen x varieties x inoculation interaction was significantly (*Figure 7B*).

3.6. First pod height

Variance analysis and mean values for trait were given *Table 1*. The effects of years, varieties and inoculation were significantly for first pod height but differences between nitrogen was not significantly for this character. First pod height are highest in *Rhizobia* + *Azotobacter* inoculations. The other three bacterial treatments were included in the same statistical group. First pod height is genotypic property and are affected by environmental conditions. While the highest first pod height was measured in Azkan variety with 31.22 cm, the lowest value was measured in Isik variety with 28.50 cm. First pod height was higher first year than second year (*Table 1*). High precipitation in the first year, especially in April and May caused higher first pod height (*Figure 1*). While first pod height was higher in first year, it was lower in second year. Therefore, year x nitrogen x varieties x inoculation interaction was significantly (*Figure 8A*).

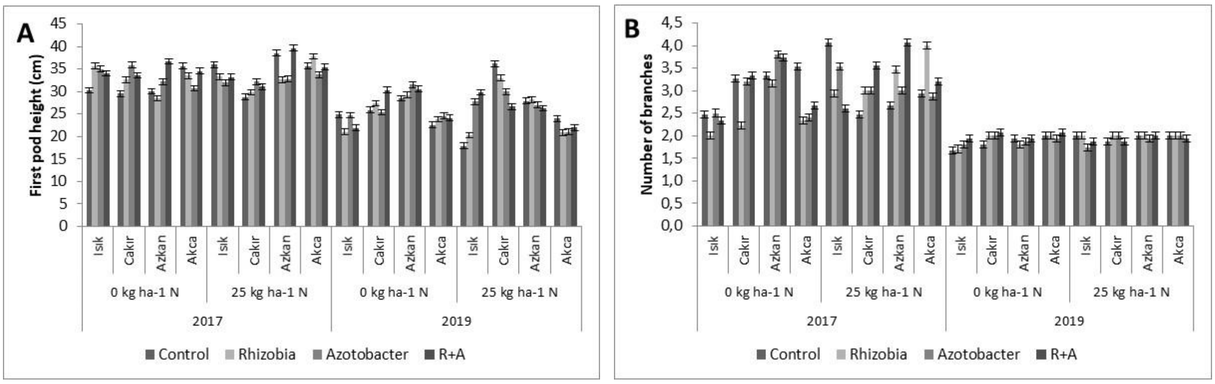

Figure 8. First pod height (A) years x nitrogen x varieties x inoculation interaction and number of branches (B) year x nitrogen x varieties x inoculation interaction [LSD 1%: 2.499 (A);1%: 0.301(B)]

3.7. Number of banches and branch diameter

Variance analysis and mean values for traits were given *Table 2*. The effects of years, nitrogens, varieties and inoculations were significantly for number of branches and branch diameter. The number of branches and branch diameter are highest in *Rhizobia* + *Azotobacter* plots. While the lowest number of branches was seen in *Rhizobia* plots, the lowest branch diameter was observed in control plots. Yasari and Patwardhan (2007) reported that *Azotobacter* + *Azosprillum* inoculation increased the number of branches. Nitrogen application had a positive effect on the number of branches and branch diameter. With the application of nitrogen fertilizer, the vegetative growth increased, and as a result number of branches was increased too (Tuncturk et al., 2011). The highest number of branches and branch diameter were determined in the Azkan variety, while the lowest was determined in the Isik variety. Number of branches and branch diameter are genotypic property and are affected by environmental conditions. Number of banches and branch diameter were higher first year than second year. Number of branches is formed under the influence of genetic structure and environmental factors. High temperatures have a negative effect on number of branches (Erkovan et al., 2020). The higher temperatures in the second year may have caused the lower number of branches (*Figure 2*). While number of branches and branch diameter were higher in first year, it was lower in second year. Therefore, year x nitrogen x varieties x inoculation interaction was significantly (*Figure 8B, 9A*).

Table 2. Different nitrogen, varieties and inoculations effects on some traits of chickpea.

	Number of branches	Branch diameter (mm)	Grain yield (kg ha^{-1})
Inoculation			
Control	2.50AB	4.81C	1.510,0D
Rhizobia	2.41C	4.92B	1.960,0B
Azotobacter	2.47BC	4.88B	1.770,0C
R+A	2.57A	5.26A	2.290,0A
Mean	**2.48**	**4.97**	**1.880,0**
Nitrogen			
0 kg ha^{-1} N	2.40B	4.72B	1.650,0B
25 kg ha^{-1} N	2.58A	5.21A	2.110,0A
Mean	**2.48**	**4.97**	**1.880,0**
Varieties			
Isik	2.32C	4.64D	1.820,0C
Cakir	2.47B	5.03B	1.710,0D
Azkan	2.66A	5.38A	2.130,0A
Akca	2.49B	4.81C	1.870,0B
Mean	**2.48**	**4.97**	**1.880,0**
Years			
2017	3.05A	5.97A	2.080,0A
2019	1.92B	3.97B	1.680,0B
Mean	**2.48**	**4.97**	**1.880,0**
General Mean	2.48	4.97	1.880,0
Year (Y)	**	**	**
Nitrogen (N)	**	**	**
Yx N	**	ns	**
Varieties (V)	**	**	**
Y x V	**	**	**
N x V	**	**	**
Y x N x V	**	**	**
Inoculation (I)	**	**	**
Y x I	**	**	**
N x I	**	**	**
V x I	**	**	**
Y x N x I	**	**	**
Y x V x I	**	**	**
N x V x I	**	**	**
Y x N x V x I	**	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$.

3.8. Grain yield

Variance analysis and mean values for trait were given *Table 2*. The effects of years, nitrogens, varieties and inoculations were significantly for grain yield. The highest grain yield was observed in the *Rhizobia* + *Azotocacter* plots. Bacteries had a positive effect on grain yield. Single and combined use of bacteria resulted in higher above-ground plant biomass with simultaneous root growth. Nutrient uptake in plant is increased by inoculation and this is due to increased biological nitrogen fixation in inoculanted plants. Also, interaction effect of chemical and bio-fertilizers caused increasing grain yield. Using nitrogen application and dual inoculation with *Rhizobia* + *Azotobacter* had highest grain yield (*Figure 9B*). Grain yield have been increased by co-inoculation with bacteria (Mirza et al., 2007; Mishra et al., 2009). Grain yield increased with nitrogen application. While the grain yield at

0 kg ha^{-1} nitrogen application was 1.650 kg ha^{-1}, the grain yield was 2.110 kg ha^{-1} with 25 kg ha^{-1} nitrogen application. Nitrogen is involved in photosynthesis, DNA synthesis, protein formation, respiration and N_2 fixation would directly influence plant groowth and development (Caliskan et al., 2008; Erman et al., 2011). Soysal and Erman (2020) reported that chemical fertilizers increase grain yield in chickpea. 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. Grain yield was higher first year than second year. While the total precipitation was 218.6 mm in the first year, it was 163.9 mm in the second year (*Figure 1*). Plant growing is carried out depending on precipitation in dry farming areas. Chickpea is the plant of the dry farming areas and the high precipitation in the first year, especially April and May, affected the grain yield positively. While grain yield was higher in first year, it was lower in second year. Therefore year x nitrogen x varieties x inoculation interaction was significantly (*Figure 9B*).

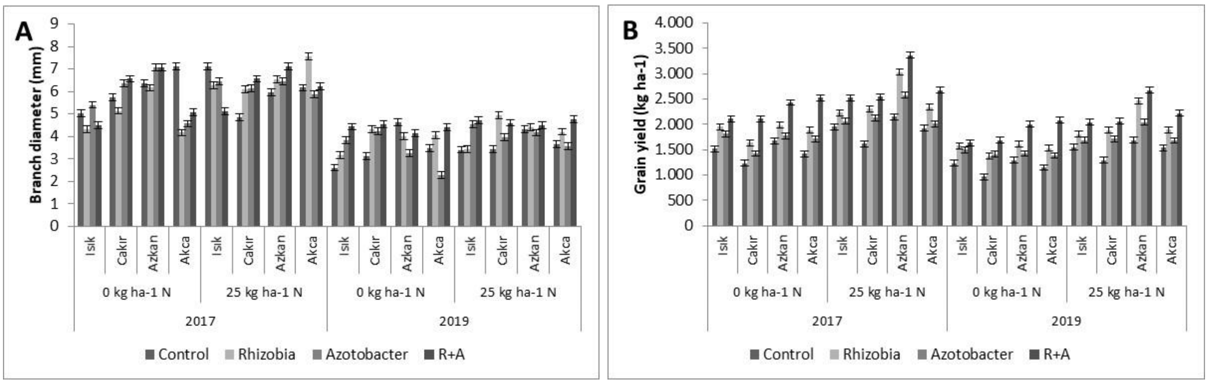

Figure 9. Branch diameter (A) years x nitrogen x varieties x inoculation interaction and grain yield (B) year x nitrogen x varieties x inoculation interaction [LSD 1%: 0.268 (A);1%: 4.348(B)]

4. Conclusions

Phenological and morphological properties affected by climate conditions. The high temperature caused the emergence, flowering and maturity to be earlier in the second year. Plant height, first pod height and grain yield were higher first year than second year due to high precipitation. Nitrogen application positively affected number of days to emergence, number of branches, branch diameter and grain yield. While the response of the cultivars was different in terms of phenological proporties, Azkan cultivar gave the best results in terms of morphological proporties. *Rhizobia + Azotobacter* inoculation gave the best results in terms of phenological and morphological characteristics except for number of days to maturity. *Rhizobia + Azotobacter* inoculation can lead to additional income generation of the farming community in of Turkey. Use of biofertilizers may reduce the application of chemical fertilizers and we get healthy, pollution free production for better future of our increasing populations.

References

Amarger, N. (2001). Rhizobia in the field. *Advances in Agronomy,* 73:109-168.

Bange, M. P., Hammer, G. L. and Ricket, K. G. (1998). Temperature and sowing date affect the linear increase of sunflower harvest index. *Agronomy Journal,* 90(3): 324-328.

Basal, O. and Szabo, A. (2020). Yield and quality of two soybean cultivars in response to drought and N fertilization. *Journal of Tekirdag Agricultural Faculty*, 17(2): 203-210.

Beck, D. P. (1988). Biological nitrogen fixation studies. Food Legume Improvement Program. *Annual Report, Icarda*, p.177-183.

Belliturk, K. and Saglam, M. T. (2005). Tekirdag Ili topraklarinin mineralize olan azot miktarlari ile mineralizasyon kapasiteleri uzerinde bir arastirma. *Journal of Tekirdag Agricultural Faculty,* 2(1): 89-101.

Caliskan, S., Ozkaya, I., Caliskan, M. E. and Arslan, M. (2008). The effect of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in Mediterranean type soil. *Field Crops Research* 108: 126-132.

Cheng, Q. (2008). Perspectives in biological nitrogen fixation research. *Journal of Integrative Plant Biology,* 50(7): 786–798.

Dobereiner, J. (1997). Biologial nitrogen fixation in the tropics: Social and economic contributions. *Soil Biology and Biochemistry,* 29: 771–774.

Erkovan, S., Ileri, O., Erkovan, H. I. ve Koc, A. (2020). Eskişehir ekolojisinde uygun ekim zamanı ve ekim sıklığının yem bezelyesinin yaş ot verimi ve bazı özelliklerine etkisi. *ÇOMÜ Ziraat Fakültesi Dergisi,* 8 (1): 225–232.

Erman, M., Demir, S., Ocak, E., Tufenkci, S., Oguz, F. and Akkopru, A. (2011). Effects of Rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions 1-Yield, yield components, nodulation and AMF colonization. *Field Crops Research*, 122(1): 14-24.

Gupta, S., Subrahmanyam, D. and Rathore, V. S. (1994). Influence of sowing dates on yields and oil quality in sunflower. *Journal of Agronomy and Crop Science,* 172(2): 137-144.

Halbleib, C. M. and Ludden, P. W. (2000). Regulation of biological nitrogen fixation. *Journal of Nutrition* 130: 1081–1084.

Herridge, D. F., Peoples, M. B. and Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil*, 311:1–18.

Holt, N. W. and Campbell, S. J. (1984). Effect of plant density on the agronomic performance of sunflower on dry land. *Canadian Journal of Plant Science,* 64: 599-605.

Karaboz, I. ve Ozcan, N. H. (2005). İzmir ve Aydin yoresindeki topraklardan izole edilen *Azotobacter chroococcum* (Beijerinck, 1901) izolatlarinin tuz, sicaklik ve bazi agir metallere toleranslarinin belirlenmesi. *Orlab On-Line Microbioloji Dergisi,* 3: 2-10.

Mahato, P., Badoni, A. and Chauhan, J. S. (2009). Effect of Azotobacter and nitrogen on seed germination and early seedling growth in tomato. *Researhcer,* 1(4): 62-66.

Mirza, B. S., Mirza, M. S., Bano, A. and Malik, K. A. (2007). Coinoculation of chickpea with Rhizobium isolates from roots and nodules and phytohormone producing Enterobacter strains. *Australian Journal of Experimental Agriculture,* 47 (8): 1008–1015.

Mishra, P. K., Mishra, S., Selvakumar, G., Kundu, S. and Shankar, G. H. (2009). Enhanced soybean (*Glycine max* L.) plant growth and nodulation by Bradyrhizobium japonicum-SB1 in presence of *Bacillus thuringiensis*-KR1. *Acta Agriculture Scandinavica, Sec. B - Plant Soil Science*, 59: 189–196.

Namvar, A., Seyed Sharifi, R., Sedghi, M., Asghari Zakaria, R., Khandan, T. and Eskandarpour, B. (2011). Study on the effects of organic and inorganic nitrogen fertilizer on yield, yield components and nodulation state of chickpea (*Cicer arietinum* L.). *Communication Soil Science and Plant Analysis,* 42(9): 1097-1109.

Ndlovu, T. J. (2015). Effect of *Rhizobium phaseoli inoculation and phosphorus application on nodulation, growth and yield components of two drybean (Phaseolus vulgaris) cultivars*. (MSc thesis) University of Limpopo (unpublished).

Pathak, D. V., Singh, S. and Saini, R. S. (2013). Impact of bio-inoculantas on seed germination and plant growth of Gauva (*Psidium guajava*). *Journal of Horticulture and Forestry*, 5(10): 183-185.

Soysal, S. ve Erman, M. (2020). Siirt ekolojik koşullarında mikrobiyolojik ve inorganik gübrelemenin nohut (*Cicer arietinum* L.)'un verim, verim öğeleri ve nodülasyonu üzerine etkilerinin araştırılması. *ISPEC Tarım Bilimleri Dergisi*, 4(3): 649-670.

Sprent, J. I. (2008). 60Ma of legume nodulation. What's new? What's changing? *Journal of Experimental Botany,* 59: 1081–1084.

Tosun, O. ve Eser, D. (1975). Nohut (*Cicer arietinum* L.) üzerinde ekim sıklığı çalışmaları, I. ekim sıklığının verim üzerine etkileri. *Ankara Universitesi Ziraat Fakültesi*, 25 (1): 171-180.

Tuncturk, R., Tuncturk, M. ve Turkozu, D. (2011). Van ekolojik koşullarında değişik azot ve fosfor dozlarının rezene (*Foeniculum vulgare* Mill.)' de verim ve kalite üzerine etkisi. *YYU Tarım Bilimleri Dergisi,* 21(1): 19-27.

Turk, M. ve Alagoz, M. (2020). Farklı azot dozlarının arı otu (*Phacelia tanacetifolia* Bentham.)'nun tohum verimi ve bazı tarımsal özellikleri üzerine etkisi. *Türk Bilim ve Mühendislik Dergisi*, 2(2): 53-56.

Vartanli, S. and Emeklier, H. Y. (2007). Determination of the yield and quality characteristics of hybrid maize varieties under Ankara conditions. *Journal of Agricultural Science* 17(3): 195-202.

Wani, S. A., Chand, S. and Ali, T. (2013). Potential use of *Azotobacter chroococcum* in crop production: An overview. *Current Agriculture Research Journal,* 1(1): 35-38.

Wani, S. A., Chand, S., Wani, M. A., Ramzan, M. and Hakeem, K. R. (2016)**.** *Azotobacter chroococcum* – A potential biofertilizer in agriculture: An overview, K.R. Hakeem et al. (eds.), Springer.

Willems, A. (2006). The taxonomy of rhizobia: an overview. *Plant and Soil,* 287:3–14.

Yasari, E. and Patwardhan, A. M. (2007). Effects of (*Azotobacter* and *Azosprillum*) inoculants and chemical fertilizers on growth and productivity of canola (*Brassica napus* L.). *Asian Journal of Plant Science*, 6(1): 77-82.

Zaman, S., Mazid, M. A. and Kabir, G. (2011). Effect of rhizobium inoculant on nodulation, yield and yield traits of chickpea (*Cicer arietinum* L.) in four different soils of greater Rajshahi. *Journal of Life and Earth Science,* 6: 45- 50.