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NITROGEN INHIBATORS AND RHIZOBIA CAN IMPROVE YIELD AND YIELD COMPONENTS IN BEAN

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ABSTRACT

The aim of this study is investigation of the effects of rhizobia and different nitrogen fertilizer types and doses on yield and some yield components for bean. The field experiment was conducted during the 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey. The experiments were laid out as a randomized complete block design in a factorial arrangement and replicated four times per treatment. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-1} DMPP). Bean varieties Goynuk-98 was used research material. The effects of year were significantly for all of the investigated characters but differences between the rhizobia and nitrogen fertilization were significant were all of the investigated characters except for plant emergence number. All of the investigated characters were higher in first year than second year due to climatic conditions. Rhizobia inoculation was increased yield and yield components. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided highest values for investigated characters and grain yield. Inoculation with rhizobia and 50 kg ha^{-1} DMPP application can be recommended for beans in Eskişehir conditions.

Keywords: DMPP, *Phaseolus vulgaris* L., yield, yield components.

INTRODUCTION

To maintain crops yield, agricultural soils need to be supplemented with nitrogen (Ozdemir et al., 2019; Rodrigues et al., 2019). However, sub-optimal or over-fertilization have led to an increase of N losses through ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions from soil (IPCC, 2007), which cause severe environmental and ecological problems in water, air and soil (Vries et al., 2013).

The use of fertilizers in agriculture, both organic and synthetic, in agriculture, is considered the most important anthropogenic source of N_2O emissions (c. 70% of the total worldwide), mainly produced as a by-product or intermediate product of microbial processes (e.g. nitrification and denitrification) (Ussiri and Lal, 2013). Nitrification inhibitors can delay the microbial oxidation of NH_4^+ to nitrite (NO_2^-) for a certain period (several weeks or months) and are therefore very effective at blocking microbial nitrification and subsequent denitrification (Weiske et al., 2001; Zerulla et al., 2001). A proposed strategy to minimize N losses is the use of nitrification inhibitors (NIs), which delay the oxidation of NH_4^+ to NO_3^- via nitrification (Fuertes-Mendizabal et al., 2019). The most regularly used and best understood nitrification inhibators are the synthetic dicyandiamide (DCD), 2-chloro-6-(trichloromethyl)-pyridine (nitrapyrin) and 3,4-dimethylpyrazole phosphate (DMPP). The nitrification inhibitor, 3,4-dimethylpyrazole phosphate (DMPP), is already used extensively, as it can slow down the ammonia-oxidizing process by inhibiting the activity of the ammonia monooxygenase enzyme, thereby reducing N losses (Di and Cameron, 2012). Pasda et al. (2001) found that the yield-increasing effect of DMPP was stronger the lighter the soil and the higher the amount of precipitation in the period January-July. This relationship gives an indication of the risk of potential NO_3^- leaching at a site. The higher this risk, the greater the chance that the addition of a nitrification inhibitor will result in improved N efficiency and thus in a yield increase.

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Soil bacteria, Rhizobium, Bradyrhizobium, Sinorhizobium and Azorhizobium provide 80% of the total nitrogen required by pulses, a group of plants that provide 25-35% of useful proteins to the world (Medeot et al., 2010). One of the efforts contribute to increase the yield of beans was the inoculation with suitable *Rhizobia* bacteria (Graham et al., 2003; Slattery et al., 2004). Karaca (2010) and Altunkaynak and Ceyhan (2018) reported that rhizobia application and nitrogen fertilization increases the yield in beans. Bildirici (2003) indicated that rhizobia bacteria inoculation and nitrogen fertilization increases the pod number per plant, seed number per plant and yield in beans. Pirbalouti et al. (2006) reported that different bacteria inoculation increases the biological yield per plant, pod number per plant, seed number per plant, harvest index and yield in beans. Kucuk (2011) indicated that rhizobia application and nitrogen fertilization increases the hundred kernel weight and yield in beans.

In recent years, researchers have focused on 3,4-dimethylpyrazole (DMPP), one of the new nitrification inhibitors, which has attracted the attention of many scientists. The aim of this study is investigation of the effects of rhizobia and different nitrogen fertilizer types and doses on yield and some yield components for bean.

MATERIAL AND METHODS

The field experiment was conducted during the 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39°48’ N; 30°31’ E, 798 m above sea level). Climatic data for long term and experimental years are shown in Figure 1. Long term annual total precipitation is 104.1 mm and it was 143.4 and 170.2 mm in the experimental years, respectively. Annual average temperature was 19.64°C in 2017 and 20.1°C in 2018. The soil of experimental area was organic matter content of 1.44%, with lime 2.50% and pH of 7.61. Corresponding available P_2O_5, K_2O and N contents were 108.9 kg ha^{-1}, 1944.6 kg ha^{-1} and 0.07% in the first year, respectively. In the second year, it was organic matter 1.65%, with lime 7.56%, pH of 7.71, available P_2O_5 177.5 kg ha^{-1} K_2O 2450.0 kg ha^{-1} and N 0.08%.

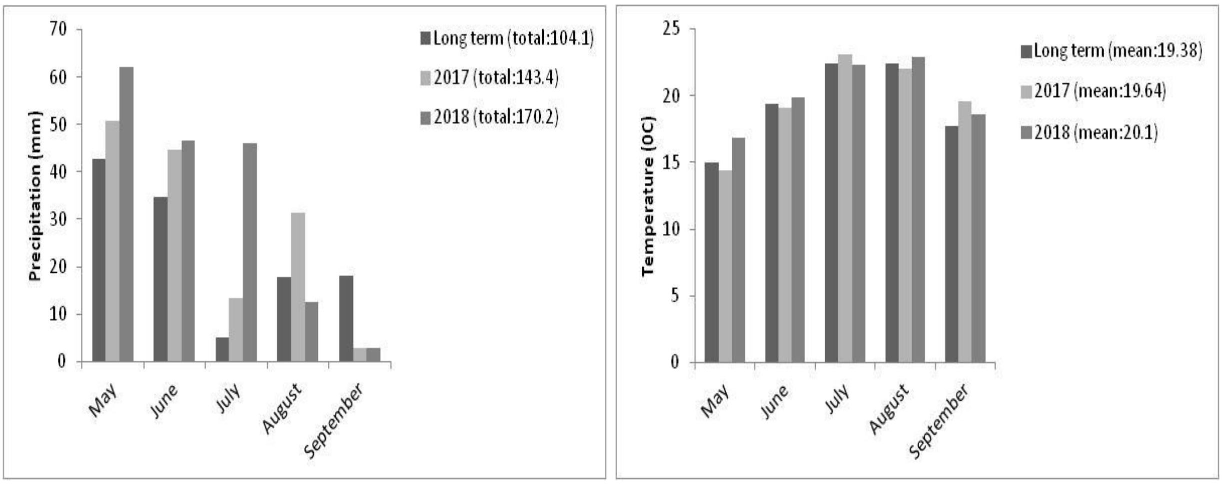

Figure 1. Climatic data of the research area

The experiments were laid out as a randomized complete block design in a factorial arrangement and replicated four times per treatment. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-}1 DMPP). Bean varieties Goynuk-98 was used research material. Each plot was 7.2 m^2 (4 m x 1.8 m) and bean was sown 45 cm row spacing and seeding rate was 26 seeds m^{-2}. The sowing time was 03 May and 04 May in 2017 and 2018, respectively. All of the nitrogen fertilizer were applied at sowing time. Seeds were inoculated with *Rhizobium endophyticum* (formed colonies at 10-8 level) bacteria at the recommended rate (100 kg seed to 1 kg peat inoculant) before sowing in rhizobia plots. Application of the peat inoculant on the seeds was carried out by water, which contains 2% sugar. Peat inoculation was provided by the Soil, Fertilizer and Water Central Research Institute. It was kept in refrigerator at +4^{o}C until use**.** The basal fertilizer application of 60 kg ha^{-1} TSP [triple super phosphate (43-44 $P_2O_5$$^{\%}$)] was given to each plot at the sowing. Harvest time of chickpea was on 13 September 2017 and 27 August 2018 in the first and second year, respectively.

Plant emergence number was determined all the plots. The biological yield per plant (g), number of pod per plant, number of seed per pod, number of seed per plant and grain yield per plant (g) were evaluated on 5 randomly selected plants in each plot. Each plot was harvested, blended and harvest index (%), hundred kernel weight (g) and grain yield (kg ha^{-1}) were estimated.

The variance analysis were subjected to 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 were significantly for all of the investigated characters but differences between the rhizobia and nitrogen fertilization were significant were all of the investigated characters except for plant emergence number (Tables 1, 2). While plant emergence number higher Rh^{+} on 50 kg ha^{-1} DMPP plots in 2017, Rh^{-} showed the lower values on same plots in 2017 (Figure 2A). Biological yield per plant, pod number per plant, seed number per plant, grain yield per plant had higher values all of the plots in Rh^{+} but these traits showed the lower values in Rh^{-} (Figures 2B, 3A, 3B, 4B). While seed number per pod and harvest index higher Rh^{+} on control plots in 2018, Rh^{-} showed the lower values on same plots in 2018 (Figures 4A, 5A). Grain yield higher Rh^{+} on 50 kg ha^{-1} DMPP plots in 2017 but Rh^{+} showed the lower values on control plots in 2018 (Figure 5B). For this reason, year x rhizobia x nitrogen fertilization interactions was significantly.

Table 1. Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	PEN	BYP (g)	PNP	SNP	SN Pod	GYP (g)
2017	13.39 A	64.55 A	20.77 A	68.23 A	3.37 A	27.42 A
2018	13.21 B	42.90 B	16.26 B	43.22 B	2.71 B	16.91 B
Mean	**13.30**	**53.72**	**18.51**	**55.72**	**3.04**	**22.16**
Rh^{-}	13.33	39.51 B	14.28 B	41.12 B	2.90 B	16.65 B
Rh^{+}	13.27	67.93 A	22.75 A	70.33 A	3.19 A	27.68 A
Mean	**13.30**	**53.72**	**18.51**	**55.72**	**3.04**	**22.16**
Control (0)	13.45	34.92 E	12.65 E	34.64 E	2.76 C	13.55 E
25 kg ha^{-1} AS	13.30	42.84 D	15.28 D	47.01 D	3.09 AB	18.73 D
25 kg ha^{-1} DMPP	13.23	54.97 C	17.69 C	55.19 C	3.15 A	22.18 C
50 kg ha^{-1} AS	13.28	60.74 B	21.04 B	62.93 B	3.03 B	24.98 B
50 kg ha^{-1} DMPP	13.25	75.15 A	25.92 A	78.86 A	3.17 A	31.38 A
Mean	**13.30**	**53.72**	**18.51**	**55.72**	**3.04**	**22.16**
Year	**	**	**	**	**	**
Rhizobia	ns	**	**	**	**	**
Nitrogen fertilization	ns	**	**	**	**	**
Year x rhizobia	ns	**	ns	**	*	**
Year x nitrogen fertilization	*	**	**	**	**	**
Rhizobia x nitrogen fertilization	**	**	**	**	**	**
Year x rhizobia x nitrogen fertilization	**	**	**	**	**	**

ns: non-significant, *: p≤0.05, **: p≤0.01. Means in the same column with different letters are significant. PEN: Plant emergence number, BYP: Biological yield per plant, PNP: Pod number per plant, SNP: Seed number per plant, SN Pod: Seed number per pod, GYP: Grain yield per plant.

All of the investigated characters were higher in first year than second year (Tables 1, 2). Important yield components and grain yield was lower due to total high temperature especially flowering time (June) and grain filled period (August) in the second experimental year (Figure 1, Tables 1, 2). Flowering and pollination were affected negative due to high temperatures in generative period and therefore yield and important yield components were lower in second year for bean. Warland et al. (2006) reported that grain yield was reduced when temperature is increased 1.5^{o}C. Aytekin and Caliskan (2015) reported that any stress in generative period for bean were created lower yield compared to other periods. Lower number of pod per plant, number of seed per pod and lower hundred kernel weight are caused this lower grain yield. Bozoglu and Gulumser (2000), and Ulker (2008) indicated that the number of pods per plant was affected by environmental conditions. Kacar et al. (2004) reported that high temperature causes the small grain in beans. Rodríguez et al. (2005) and Ashraf and Hafeez (2004) reported that high temperatures were decreased assimilation and therefore plant growth was decreased.

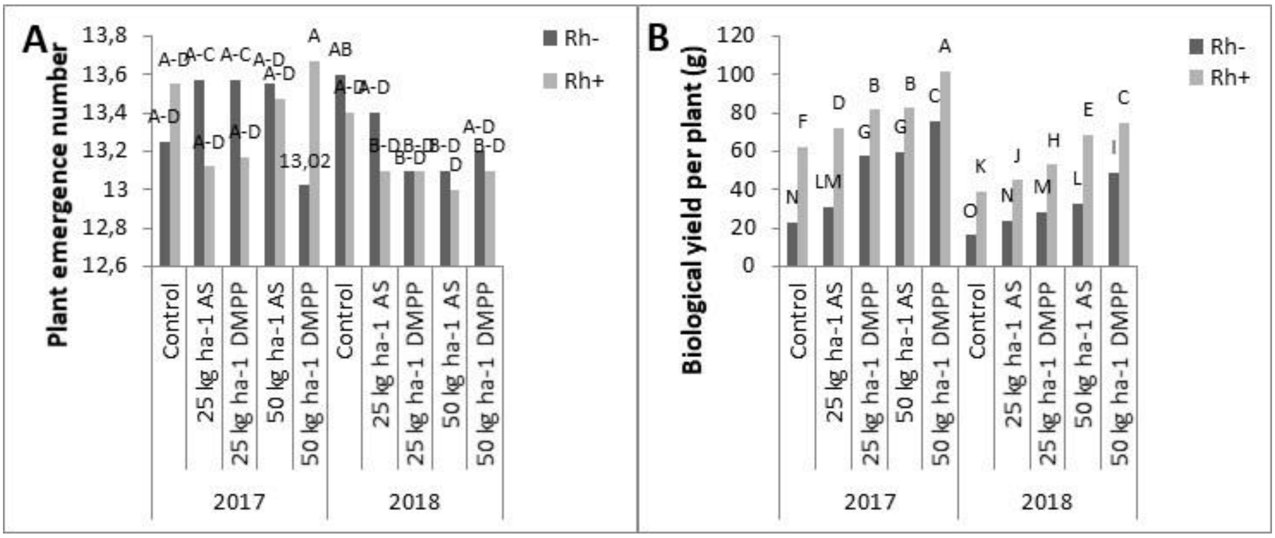

Figure 2. The interaction between rhizobia and different nitrogen fertilization on plant emergence number (A) and biological yield per plant (B) of bean. Letters on each bar represent significance level at $P < 0.05$.

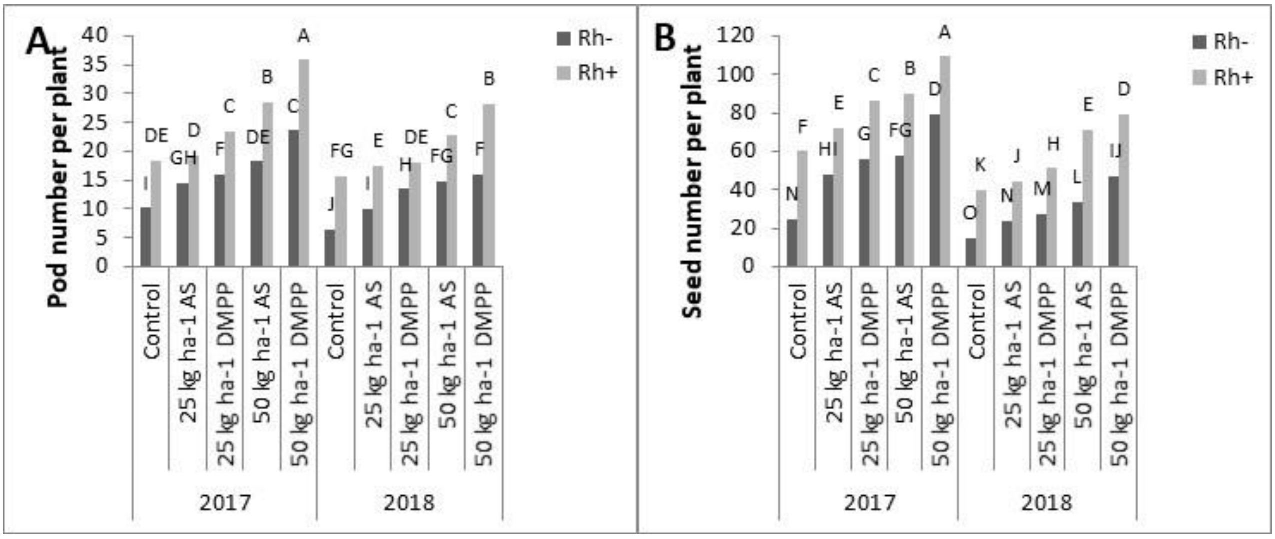

Figure 3. The interaction between rhizobia and different nitrogen fertilization on pod number per plant (A) and seed number per plant (B) of bean. Letters on each bar represent significance level at $P < 0.05$.

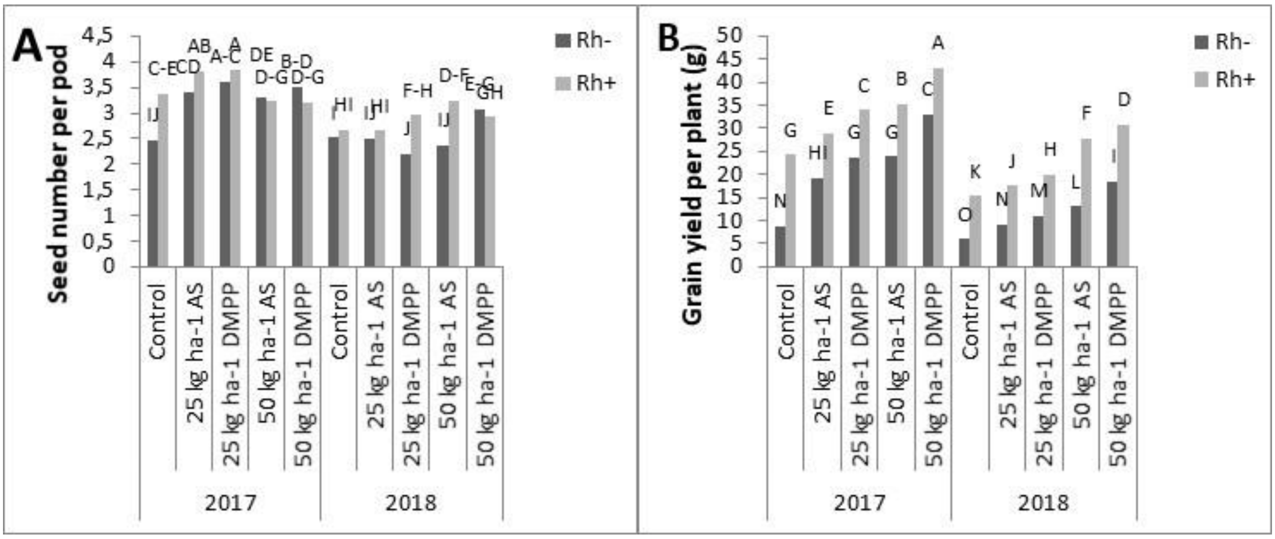

Figure 4. The interaction between rhizobia and different nitrogen fertilization on seed number per pod (A) and grain yield per plant (B) of bean. Letters on each bar represent significance level at $P < 0.05$.

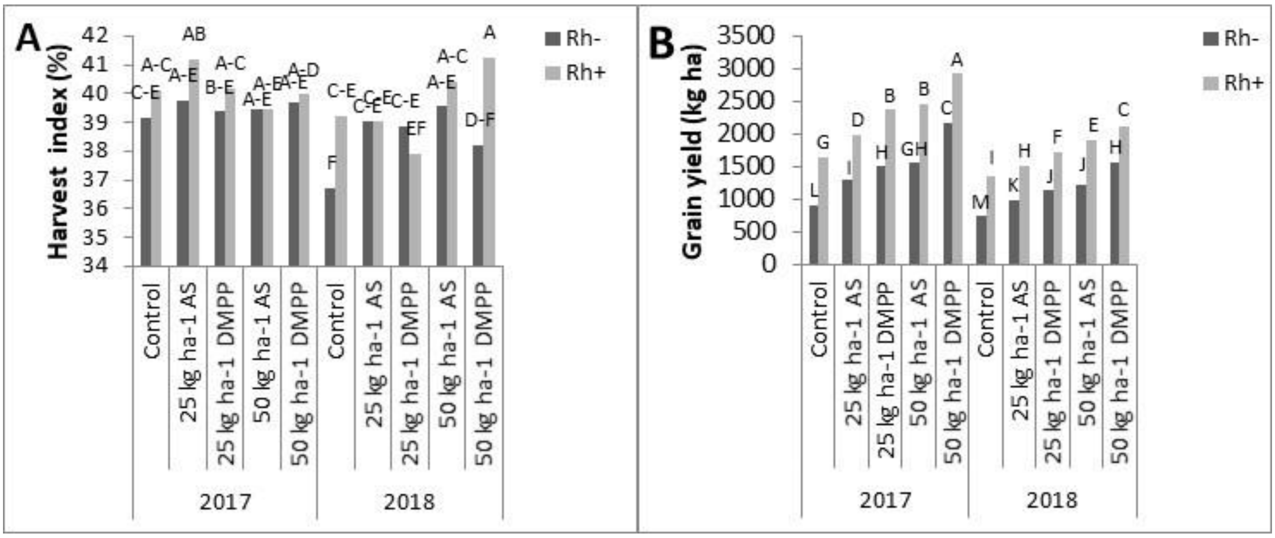

Figure 5. The interaction between rhizobia and different nitrogen fertilization on harvest index (A) and grain yield (B) of bean. Letters on each bar represent significance level at $P < 0.05$.

All of the investigated characters were higher in Rh+ than the Rh- except for the hundred kernel weight (Tables 1, 2). Rhizobia is one of plant growth promoting rhizobacteria. Biological nitrogen fixation is an alternative source of fertilizer for the development of sustainable agriculture. PGPR increases plant growth by producing hormonal substances and provides the intake of soil plant nutrients (Cakmakci, 2005). Rhizobia increases the growth and yield of crops (Ucar, 2019). Grain yield and important yield components increased with the effect of rhizobia in our study. Karadavut and Ozdemir (2001) indicated that bacteria inoculation was increased pod number per plant and grain yield. Rahman et al. (2008) reported that rhizobia inoculation were increased pod number per plant, seed number per pod and grain yield per plant. Altunkaynak and Ceyhan (2018) reported that bacteria was increased grain yield. Sahin (2018) indicated that rhizobia bacteria inoculation was increased biological yield per plant and grain yield per plant but hundred kernel weight was decreased bacteria inoculation. Sen (2018) reported that pod number per plant, seed number per plant, harvest index and grain yield were increased inoculation.

Table 2. Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	HKW (g)	HI (%)	GY (kg ha^{-1})
2017	41.05 A	39.83 A	1885 A
2018	39.26 B	39.02 B	1425 B
Mean	**40.15**	**39.42**	**1655**
Rh$^-$	40.49 A	38.98 B	1312 B
Rh$^+$	39.83 B	39.87 A	1999 A
Mean	**40.15**	**39.42**	**1655**
Control (0)	40.88 A	38.80 B	1162 E
25 kg ha^{-1}AS	40.10 AB	39.74 A	1446 D
25 kg ha^{-1}DMPP	40.40 AB	39.08 AB	1693 C
50 kg ha^{-1}AS	39.70 B	39.72 A	1783 B
50 kg ha^{-1}DMPP	39.70 B	39.78 A	2193 A
Mean	**40.15**	**39.42**	**1655**
Year	**	**	**
Rhizobia	**	**	**
Nitrogen fertilization	**	**	**
Year x rhizobia	ns	ns	**
Year x nitrogen fertilization	ns	**	**
Rhizobia x nitrogen fertilization	ns	**	**
Year x rhizobia x nitrogen fertilization	ns	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$. Means in the same column with different letters are significant. HKW: Hundred kernel weight, HI: Harvest index, GY: Grain yield.

All of the investigated characters were higher in 50 kg ha^{-1} DMPP except for the hundred kernel weight (Tables 1, 2). Gokmen et al. (2008) reported that nitrogen inhibitors were increased grain yield and harvest index but thousand kernel weight was decreased in wheat. Liu et al. (2013) indicated that DMPP was increased grain yield in wheat-corn crop rotation systems. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions (especially precipitation and temperature), soil moisture, pH, soil texture and mineral N content. May, June and July were very rainy in both years of our study. Excessive precipitation causes nitrogen losses in soil and thus benefits of DMPP are more observed. Therefore, important yield components and grain yield may be higher in 50 kg ha^{-1} DMPP plots in our study. Pasda et al. (2001), positive effect of DMPP on yield is more clear when precipitation was higher.

CONCLUSIONS

All of the investigated characters were higher in first year than second year due to climatic conditions. Yield and important yield components were effected rhizobia and nitrogen fertilization application. Rhizobia inoculation was increased yield and yield components. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided highest values for investigated characters and grain yield. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions. Excessive precipitation causes nitrogen losses in soil and thus benefits of DMPP might be more observed. The results indicated that DMPP is appropriate for bean. However, the effect of inhibitor fertilizers may depending on ecological conditions, application time, crop rotation, application dose. Inoculation with rhizobia and 50 kg ha^{-1} DMPP application can be recommended for beans in Eskisehir conditions.

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