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Effect of mineral fertilizers and inoculation with nitrogen-fixing bacteria on yield and biomass of *Cicer arietinum* L.

Jonita Perfanova, Hristo Valchovski*, Gergana Kuncheva, Lev Tribis

Institute of Soil Science, Agrotechnology and Plant Protection "Nikola Pushkarov" - Sofia, Agricultural Academy
Corresponding author*: h_valchovski@abv.bg

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Abstract

The study evaluates the grain yield and green biomass of chickpea (*Cicer arietinum* L.) at four levels of nitrogen and phosphorus fertilizers, with sole inoculation with strain *Bradyrhizobium* sp. (cicer) 3352 and combined effect. The plants were grown until the full maturity of grain in pot experiment on two soil types Alluvial meadow soil (Eutric Fluvisol) and Smolnitsa (Pellic Vertisols). Both inoculation with strain *Bradyrhizobium* sp. (cicer) 3352 and mineral fertilizers have strong positive effect of chickpea production and biomass. As a result of these researches, the optimal norm for mineral nutrition of chickpea ($N_{70}P_{160}$ mg/kg) was determined.

Key words: nitrogen-fixing bacteria, chickpea, soil microbiology, fertilizers

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most ancient legume crops. There is archaeological evidence that it was used for food as early as 7,500 years ago. It originates from the region of Asia Minor. Its carbohydrate- and protein-rich seeds, as well as its relatively easy to see, are prerequisites for its rapid spread. The largest producer of chickpea in the world is India - gives 80 - 90% of the world production, followed by Turkey, Pakistan and Iran. The production of chickpea in the USA and Canada is increasing rapidly (Mc Kenzie et al., 2001, Walley et al., 2005). Chickpea is particularly important in the agriculture of the Mediterranean basin. This territory covers seven countries in Africa, eight in Asia and five in Europe, representing about 1.06 million hectares of area and 0.87 million tons of chickpea production. Although Mediterranean acreage is relatively small compared to the world one, chickpea is the most important leguminous crop in Western Asia, North Africa, Turkey, Spain, Portugal, Italy and Greece (Saxena, 1990). For Bulgaria, chickpea is an old, traditional legume crop. In recent years, the interest of producers in its cultivation has returned. According to the Ministry of Agriculture, it occupied 35% of the total area leguminous crops (58,102 da), and it occupied the second place (17,670 da), after the beans.

Chickpea is mainly grown for human food, but this crop is an alternative source of protein and energy for farm animals as well. Its seeds contain 12.6% to 31.2% crude protein, 47.0% to 71.2% nitrogen-free extractives, 2.1% to 7.2% fat, 1.5% to 12.8% cellulose, 2.0% to 5.0% ash and 9.1% to 14.2% water. This chemical composition, compared with the chemical composition of other legume crops, shows that, with the exception of soybean, chickpea exceed all other crops in terms off at content. Its seeds contain on average 2.5 times more fat than beans, 3-5 times more than peas, lentils and broad beans and 1.5-2 times more than vetch (Christodoulou et al., 2006).

Another advantage of this culture is that it is undemanding to soil characteristics. It can be grown on all soil types except acidic soils. It develops successfully and gives a good yield even on such soils like sandy and saline soils on which other leguminous crops can not develop. An important quality of the chickpea, especially in the current conditions of global warming, is its great drought resistance. Chickpea is drought tolerant because of their deep root system, which draws water from deep in the soil profile. Many authors found that in areas with lower rainfall, quality chickpea production is obtained (Singh, 1997, Pu-hai et al., 2003, Sabaghpour et al., 2006, Al-Rifaee et al., 2007, Erler et al., 2009).

Nitrogen fixation in chickpea could reach 176 kg/ha depending on the method of determination, the variety, the presence of naturally occurring nitrogen-fixing bacteria and other environmental conditions (Beck and Douglas, 1992). According to Walley et al., (2005), estimates of nitrogen derived from nitrogen fixation vary widely. For example, under dry conditions chickpea receives an average of 57% nitrogen from nitrogen fixation, and when grown under irrigated conditions nitrogen fixation is high and can reach 85%. Doughton et al., (1993) found a negative correlation between chickpea nitrogen fixation and soil nitrogen at a depth of 1.2 m, with an estimated value of nitrogen fixation ranging from 17 to 90%. Herridge et al., (1995), concluded that chickpea fixed more than 70% nitrogen at available soil nitrate levels of 50 kg/ha at 1.2 m. In experiments with chickpea, differences were found in their responsiveness to nitrogen and phosphorus (Walley et al., 2005). The starting dose of nitrogen reduces nitrogen fixation. The use of nitrogen and phosphorus does not affect the grain yield, but it is necessary to optimize the fertilization of chickpea with low doses of nitrogen and phosphorus (respectively 30 kg N/ha and 20 kg P/ha). In a field experiment with five chickpea cultivars, Solaiman et al., (2007) investigated the effect of inoculation and mineral nitrogen on tuber formation, nitrogen fixation, yield and yield quality. They found that two of the cultivars inoculated with nitrogen-fixing bacteria and without the use of mineral nitrogen obtained the highest values of the investigated parameters compared to the use of 100 kg N/ha in another cultivar. According to Gan et al., (2004), phosphorus increases the number of tubers by 1.5 to 2 times. Subba Rao et al., (1986) found that inoculating chickpea seeds with nitrogen-fixing bacteria and applying 50 kg/ha P_2O_5 in the form of superphosphate significantly increased the nitrogen content of the grain.

The aim of the study is to contribute to the knowledge about relationship between soil bacteria and chickpea.

Materials and methods

The experiment was carried out in pots with capacity of 1.5 L with four replicates. Two soil types were used for the study: Alluvial meadow soil (Eutric Fluvisol) and Smolnitsa soil (Pellic Vertisols) (table 1). The yield and biomass were measured with mean value of three repetitions from each variant.

In order to establish an optimal concentration of fertilizers for chickpea production improvement, three increasing levels of nitrogen (35, 70 and 140 mg/kg soil) and three levels of phosphorus (80, 160 and 240 mg/kg soil) and the combinations between them.

<u>Scheme of the variants in the experiment</u>

1. Control
2. Inoculation with strain *Bradyrhizobium* sp. (cicer) 3352
3. $P_{80}N_0$ mg/kg soil
4. $P_{160}N_{35}$ mg/kg soil
5. $P_{80}N_{70}$ mg/kg soil
6. $P_{80}N_{140}$ mg/kg soil
7. $P_{160}N_0$ mg/kg soil
8. $P_{160}N_{35}$ mg/kg soil
9. $P_{160}N_{70}$ mg/kg soil
10. $P_{160}N_{140}$ mg/kg soil
11. $P_{240}N_0$ mg/kg soil
12. $P_{240}N_{35}$ mg/kg soil
13. $P_{240}N_{70}$ mg/kg soil
14. $P_{240}N_{140}$ mg/kg soil

Phosphorus was applied as KH_2PO_4 and nitrogen as NH_4NO_3. Chickpea seeds were inoculated with a suspension of a seven day old culture of strain 3352 with a titer of 10^8 cells per ml. Soil moisture was maintained at 60% of the total soil humidity. In the maturity phase, above-ground biomass and grain yield are recorded.

The effect on the monitored parameters was assessed by application of ANOVA. Differences were considered statistically significant at level of $p < 0.05$.

Table 1. Agrochemical properties of studied soil types

Soil type	pH $_{(KCl)}$	Soil organic matter %	Total nitrogen %	Total phosphorus %	Available nitrogen, mg/1000 g	Available phosphorus, mg/100 g	Available potassium mg/100g
Alluvial meadow soil (Eutric Fluvisol)	5.7	1.36	0.049	0.070	7.6	8.4	24.3
Smolnitsa soil (Pellic Vertisols)	6.9	5.5	0.14	0.07	14.2	17.8	27.3

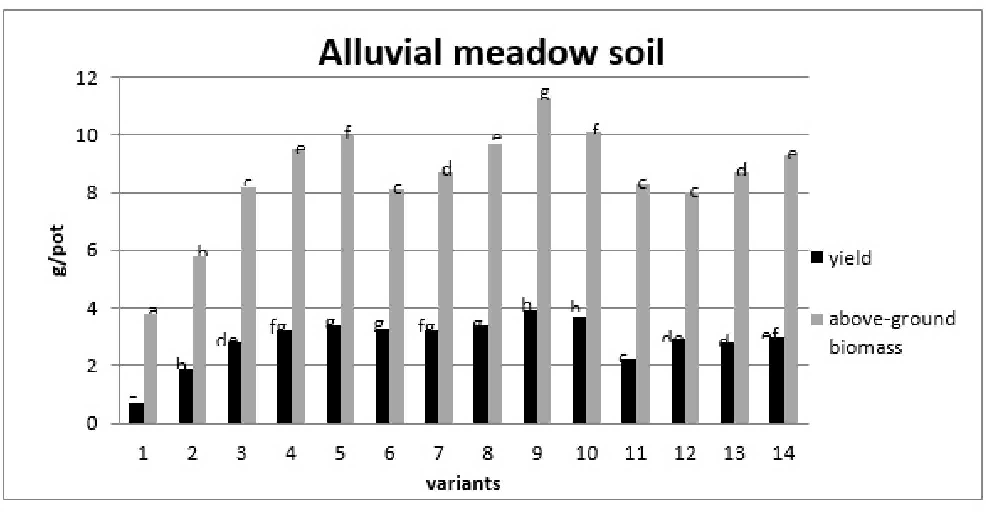
Results and discussion

The influence of nitrogen and phosphorus fertilizers on the performance of strain *Bradyrhizobium* sp. (cicer) 3352 in the two studied soils is presented in figures 1 and 2. As can be seen, strain *Bradyrhizobium* sp. (cicer) 3352 was effective – the grain yield of the inoculated control was increased twice that of the non-inoculated control.

The application of phosphorus fertilizer to the Alluvial-meadow soil has a positive effect on plant growth in all variants. At all three doses phoshorus, grain yield increased over the control and inoculated variant. Phosphorus fertilizer addition increased number of seeds/pod in the loamy sand soil and number of pods/plant in the clay soil, reported Lusiba et al., (2018). The combined application of nitrogen and phosphorus leads to a significant increase in grain yield, which varies from 0.6 g to 2 g per pot or from 133 to 211% compared to the control, in the variants $P_{80}N_0$ mg/kg soil and $N_{70}P_{160}$ mg/kg, respectively. The maximum increase compared to all tested variants of the experiment was shown by the $N_{70}P_{160}$ mg/kg - 2 g/pot variant (211%). The increase in yield under the influence of nitrogen and phosphorus indicates the insufficient supply of the Alluvial-meadow soil with these nutrients. Also, Gebremariam and Tesfay, (2021) suggested that the use of phosphorus along with rhizobium inoculation could be used as a nutrient management strategy for increased and sustained chickpea production.

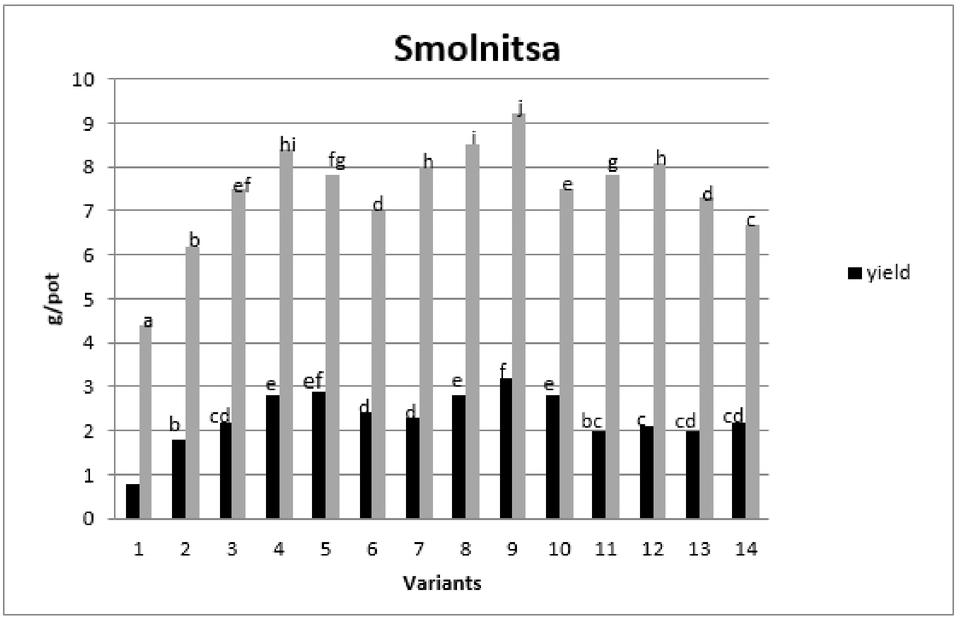
The effect of the introduction of nitrogen and phosphorus fertilizers in Smolnitsa soil (PellicVertisols) is smaller than that in the Alluvial-meadow soil, which is due to the better agrochemical properties of the Smolnitsa soil. Phosphorus application increased grain yield compared to the inoculated variant and control with a maximum at the medium dose (140%).

The increase in yield of treatments, treated with inoculation and higher rates of phosphorus application was due to increase in yield components of crop of these plots (Raut and Kohire, 1991; Jain et al., 1999). In the variants with P_{80} mg/kg the largest increase (132%) was given by



LSD – 0.24, p <0.05 (yield) LSD – 0.25, p <0.05 (above-ground biomass)

Fig. 1. Effect of nitrogen and phosphorus fertilizers and inoculation on Alluvial meadow soi1: 1. Control. 2. Inoculation with strain *Bradyrhizobium* sp. (cicer) 3352, 3. $P_{80}N_0$ mg/kg soil, 4. $P_{160}N_{35}$ mg/kg- soil, 5. $P_{80}N_{70}$ mg/kg soil, 6. $P_{80}N_{140}$ mg/kg soil, 7. $P_{160}N_0$ mg/kg soil, 8. $P_{160}N_{35}$ mg/kg soil, 9. $P_{160}N_{70}$ mg/kg soil, 10. $P_{160}N_{140}$ mg/kg soil, 11. $P_{240}N_0$ mg/kg soil, 12. $P_{240}N_{35}$ mg/kg soil, 13. $P_{240}N_{70}$ mg/kg soil, 14. $P_{240}N_{140}$ mg/kg soil



LSD – 0.24, p <0.05 (yield) LSD – 0.25, p <0.05 (above-ground biomass)

Fig. 2. Effect of nitrogen and phosphorus fertilizers and inoculation onSmolnitsa. 1. Control. 2. Inoculation with strain *Bradyrhizobium* sp. (cicer) 3352, 3. $P_{80}N_0$ mg/kg soil, 4. $P_{160}N_{35}$ mg/kg- soil, 5. $P_{80}N_{70}$ mg/kg soil, 6. $P_{80}N_{140}$ mg/kg soil, 7. $P_{160}N_0$ mg/kg soil, 8. $P_{160}N_{35}$ mg/kg soil, 9. $P_{160}N_{70}$ mg/kg soil, 10. $P_{160}N_{140}$ mg/kg soil, 11. $P_{240}N_0$ mg/kg soil, 12. $P_{240}N_{35}$ mg/kg soil, 13. $P_{240}N_{70}$ mg/kg soil, 14. $P_{240}N_{140}$ mg/kg soil

the medium dose nitrogen, followed by the low dose nitrogen (128%) and the high dose nitrogen (110%). On the P_{160} mg/kg soil background, the increase in yield from the medium dose nitrogen is also the largest - 138%, and the smallest - from the high dose nitrogen (117%). On the $P_{240}N_{140}$ mg/kg soil background, the highest yield increase was obtained from the high nitrogen dose (111%). The same trend was observed in the yield from above-ground mass of chickpea grown on the two studied soils. Similar results published Madzivhandila et al. (2012). The greater grain yield at high phosphorus levels was associated with greater shoot biomass. Of all the variants of the experiment, the yield increase was the greatest in the $N_{70}P_{160}$ mg/kg – 203% in the Alluvial-meadow soil and 148% in the Smolnitsa soil. The middle rate of N fertilization 75 kg N/ha combined with biofertilizer inoculation had of superior effect on chickpea, producing 73.2% more yield (1.68 mg/ha), (Khaitov and Abdiev, 2018).

Conclusion

The apply of nitrogen and phosphorus fertilizers in increasing doses has a positive effect on the efficiency of the studied strain *Bradyrhizobium* sp. (cicer), which depends on the dose and the ratio between the introduced nutrients.The maximum efficiency of the strain in both soils is shown by the $N_{70}P_{160}$ mg/kg variant. In the pots with Alluvial meadow soil (Eutric Fluvisol), the grain yield was increased by 211% compared to the control, and that of above-ground mass – by 203%. In the variants with the Smolnitsa (Pellic Vertisols), the increase in grain yield was 194%, and in the above-ground plant - 148%.

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Conflict of interest: The authors declare there are no conflicts of interest.

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