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INFLUENCE OF MICROBIAL FERTILIZERS ON MORPHOLOGICAL, PHYSIOLOGICAL AND VALUABLE ECONOMIC CHARACTER OF CHICKPEA (CICER ARIETINUM L.)

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Abstract
A field experiment was conducted to study the effects of Rhizobium 3 , Rhizobium 9 and PlantaStim microbial fertilizers on the morphological, physiological and valuable economic characters of chickpea (Cicer arietinum L.). All samples treated with microbial fertilizers showed positive results compared to the control variation. Efficiency of Rhizobium 3, among the microbial fertilizers, was higher. In the chickpea samples, treatment with Rhizobium 3 increased the total chlorophyll content by 14.91%, carotenoid content by 20%, plant biomass by 31.39%, grain number by 19.37%, productivity by 27.25%, total protein content by 25.29%, and amount of nodule by 2.07 times, compared to the control variation.

Keywords Chickpeas, microbial fertilizer, chlorophyll, carotenoid, transpiration rate, productivity elements.

INTRODUCTION

Global climate change is causing water scarcity in many territories of the world. Ensuring the food security of the population requires the wide implementation of high caloric crop types which are resistant to drought. Chickpea plant is considered as one of such crops (Field CB, 2014). The annual production of chickpeas worldwide reaches up to 15.0 million tons. Herein India's makes up 73% (FAOSTAT). There are mainly two types of chickpeas in the market. They are Kabuli and Desi.

Desi is cultivated in 75% of the whole chickpea cultivation area in the world, and 25% of the area belongs to Kabuli ecotype (Kassie M., 2009). Chickpea plant has types with large, small and medium sized seeds, with different colors. According to the size of seeds, they are divided into 2 groups: large-grained (12 x 9 mm) and small-grained types. The kabuli type of chickpeas has large white grains, while the grains of desi type plant are small, dark in colour (A.Frimpong, 2009).

Chickpea plant grain occupies an important place in the human diet. It contains protein, carbohydrate, folate, β-carotene, minerals, vitamins and fatty acids necessary for human health (A.K.Jukanti, 2012).

A number of microbial fertilizers are used in the cultivation of legumes. One of these are microbial fertilizers based on Rhizobium strains.

Rhizobium bacteria is effective in increasing the yield of leguminous plants and soil fertility . It is advisable to use bacterial fertilizers with the most active strains of the nodule bacteria on leguminous (mung bean, soybean, bean, lentil, pea) crops (H.H.Zahran, 1991).

When microbial fertilizers are used in the cultivation of leguminous crops, the microflora that mobilizes nitrogen, phosphorus and potassium in the soil is activated. As a result, the plant will be able to get the nutritional minerals it needs, not only during the application period, but also during the entire vegetation period (D.M.Sytnikov 2012).

Growth and development of leguminous plants is improved and productivity increases when they are treated with plant growth-accelerating rhizobacteria compared to the treatment of leguminous plants with Rhizobium bacteria separately (M.S.Dardanelli, 2008; M.Yadegari 2010). Microbial fertilizers obtained on the basis of azotobacters increase the germination of seeds and absorption of nutrients in plants . Moreover, it accelerates the synthesis of protein and amino acids, benefits the growth of crops and helps restore soil fertility (A.D.Jnawali, 2015).

Application of Rhizobium and Azotobacter strains together on chickpea plants increased the amount of nitrogen in the roots and grain yield as well (A. Abdiev, 2019; A. Siddiq, 2014).

In addition, there are microbial fertilizers obtained on the basid of Trichoderma fungi types. Trichoderma species are symbiotically associated with the apoplastic part of plant roots and directly affect the plant. It has a positive effect on the germination of seeds, absorption of substances and increases productivity (B.N.Singh 2015).

Microbial fertilizers affect physiological and biochemical processes in plants, improve plant growth and development, and serve to increase crop productivity. Based on this, the aim of the research is to study the effect of microbial fertilizers on the change of the morphophysiological and valuable economic characteristics of the chickpea plant.

MATERIALS AND METHODS

Malkhotra variety of Chickpea (Cicer arietinum L.) seed was used in the field experiments. Rhizobium 3 and Rhizobium 9 preparations were obtained from the collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan. PlantaStim (Trichoderma Lignorum) was obtained from private company of AnGuzal Agroservice, Uzbekistan.

Field experiments were conducted in 2021 and 2022 to study the effect of Rhizobium 3, Rhizobium 9 and PlantaStim on growth, nodulation and yield of Chickpea (Cicer arietinum L.) at the Institute of Genetics and Plant Experimental Biology, Kibray, Tashkent region, Uzbekistan. In the experiments randomized block design was used with three replications, in a plot of 10 m2 with a row spacing of 30 cm and a plant spacing of 10 cm was used. Experimental treatments included a non-inoculated control, inoculated with Rhizobium 3, Rhizobium 9 and PlantaStim.

The soil of the area where the field experiments were conducted is considered to be irrigated gray soil, the humus content is 0.8-1.2%, and the level of availability of mobile phosphorus is on average 30-38 mg/kg (D.E.Qulmamatova, 2022).

In the Malkhotra variety of chickpea, physiological indicators were determined during the period of general flowering of plants.

Extraction and determination of pigment concentration: Amount of pigments in leaves of chickpea plant samples were determined in the experiment, i.e. 3-4 leaves, calculating from the glineth point. Fifty milligrams of each leaf sample, placed in a test tube, obtained homogenization in 5 ml of 95% ethyl alcohol solution (Lichtenthaler and a Wellburn, a 1983). The homogenization underwent centrifugation at a speed of 5000 rpm for 12 min. The amount of chlorophyll a, b and carotenoids in the resulting extract attained determination by an Agilent Cary 60 UV-Vis spectrophotometer at 664, 649 and 470 nm. Based on these indicators, the following equations were used in calculating the amounts of chlorophyll a, b and carotenoids in chickpea leaves (Nayek et al., 2014):

Ch-a=13.36A664 – 5.19 A649

Ch-b=27.43A649 – 8.12 A664

C x+c=(1000A470 – 2.13Ca – 97.63Cb)/209

Transpiration rate was determined according to Ivanov's method [Ivanov A.A. et.al, 1950], water holding capacity was determined with the method by Kushnirenko (Kushnirenko M.D. et al, 1970), and Tretyakov's method (Tretyakov N.N. et.al, 1990) was used in determining the total amount of water in leaves.

Harvest index was calculated according to the following formula:

$$Harvest\ index(\%) = \frac{Seed\ yield}{Biological\ yield} \times 100$$

Determination of nitrogen and total protein contents. Kjeldahl is one of the methods used to determine the amount of total proteins. According to this method, the total calculated protein has the amount of nitrogen determining it. The essence of the process is to hydrolyze the organic substances in the sample with the help of concentrated sulfuric acid (amine groups in the protein) to form ammonium sulfate salts.

$$\text{nitrogenous organic matter} + H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$$

After completing the hydrolysis, the ammonium sulfate formed receives treatment with sodium hydroxide to convert it to ammonia.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2NH_4OH \begin{cases} \nearrow 2H_2O \\ \searrow \mathbf{NH_3} \end{cases}$$

Ammonia or ammonium hydroxide formed, after neutralization, assimilates into the sulfuric acid solution. The remaining acid gets titrated with an alkaline solution. Estimating the amount of nitrogen from the amount of ammonia calculated followed. An accurate sample for analysis came from the average crushed homogeneous specimen of the studied material in a test tube; the error rate should not exceed 0.1%. The sampling progressed to quantitate in a Kjeldahl flask. Later, the experiment advanced according to the protocol and instructions of Metody Kontrolya (Chemical Factors, 2004).

Processing of the obtained results. The mass fraction of nitrogen (X) in the analyzed sample for calculation used the formula as a percentage of the mass of the specimen by the volume after the titration of the amount of ammonia that has passed through the diluted sulfuric acid.

$$X = \frac{(V_1 - V_0) \times K \times 0{,}0014 \times 100}{M}$$

The volume of 0,1 mol/l sodium hydroxide solution served to titrate the remaining 0.1 mol/l sulfuric acid solution in sample experiment vo, ml.

The obtained data were analyzed on the statistical software ANOVA STATGRAPHICS-18 (www.statgraphics.com).

The research was carried out in order to study the effect of microbial fertilizers on changes in photosynthetic pigments, water balance, morphological characters, productivity elements in chickpea plants.

RESULTS AND DISCUSSIONS

When photosynthetic pigments in leaves were analyzed, the chickpea plants, which were inoculated with microbial fertilizers, showed higher results than the control variation (Figure 1). In this case, the amount of chlorophyll a increased by 7.29 (10.94%), the amount of chlorophyll b by 12.05 (24.10%), the total amount of chlorophyll by 7.72 (14.91%) and the amount of carotenoids by 15, 55 (20%) as well. No difference was observed between the amounts of photosynthetic pigments in samples treated with Rhizobium 9 and PlantaStim microbial fertilizers. The samples, which were treated with Rhizobium 3, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid, showed the highest indicators.

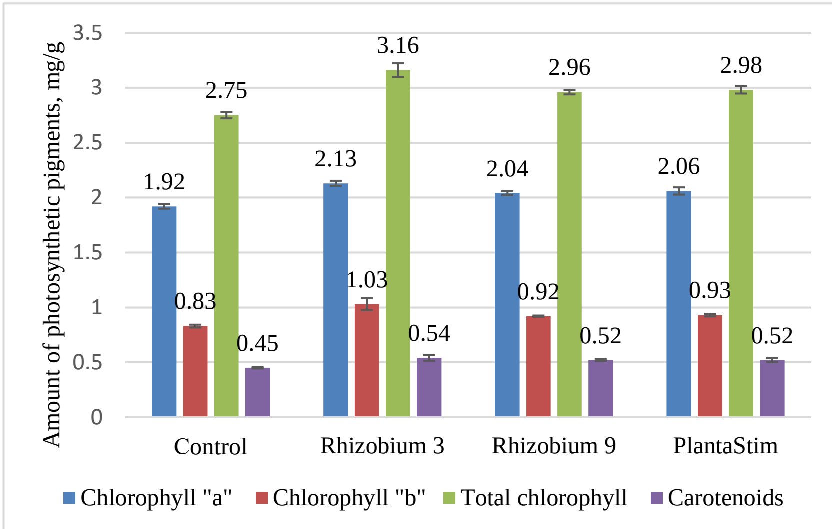

Figure 1. The effect of microbial fertilizers on changes in the amount of photosynthetic pigments in chickpea plants

Changes in the rate of photosynthesis are directly related to the photosynthetic pigments in chloroplasts (Maisura, et al., 2014). These pigments affect the growth and development of the plant (L.Taiz, et al., 2006). A number of abiotic factors influence the change in the amount of photosynthetic pigments. The decrease in the amount of chlorophyll in the leaves of plants is mainly observed in conditions of salinity stress (Z.A.Saqib, 2012), heat stress (S.Sangwan, 2012), water stress (soil waterlogging) (Amri M., 2014) and water shortage (Y.Alaei, 2011).

Mineral fertilizers, especially nitrogen fertilizers, play an important role in increasing the amount of chlorophyll in plant leaves. A high chlorophyll content results in a high yield (I.Skudra, 2017). Moreover, a number of microbial fertilizers also cause changes in the amount of photosynthetic pigments in leaves. In soybean, Rhizotorphin microbial fertilizer (Yu.Tsvetkova, 2020), in green pea (Pisum sativum L.), microbial fertilizer obtained from T42 strain of Trichoderma asperellum (B.N.Singh, 2015) and in mung bean Rhizobium 3 and Rhizobium 9 (Matkarimov, 2024) increased chlorophyll and carotenoids content up to 10.91%-14.08% respectively.

Influence of microbial fertilizers on the physiological processes as transpiration rate, total amount of water in leaves, water retention property, which are related to water exchange in chickpea plant, was comparatively analyzed (Table 1). According to the results of the analysis, the transpiration rate in the leaves of chickpea plants, treated with Rhizobium 3 and Rhizobium 9, did not differ significantly from the control variations. It helped to partially increase the total amount of water in leaves and water retention property.

In the samples, treated with PlantaStim, the transpiration rate increased by 9.95%, and the total amount of water in leaves and water retention property decreased partially. In the process of transpiration, the increase of absorption of water and mineral substances, with the help of root hairs, and evaporation from the leaves, lead to decrease in temperature and prevent overheating of the leaves (Sagdiev M.T., 2007).

Table 1

The effect of microbial fertilizers on physiological processes related to water exchange in chickpea plants

	Transpiration rate (mg H_2O /1 g. wet leaf x 1 hour)	Total amount of water in leaves,%	Water retention property,%
Monitor	277.33 ± 5.48	80.87 ± 0.33	31.09 ± 0.28
Rhizobium 3	279.75 ± 7.16	81.37 ± 0.28	30.16 ± 0.18
Rhizobium 9	280.26 ± 5.25	80.99 ± 0.31	30.23 ± 0.22
PlantaStim	304.93 ± 8.16	79.21 ± 0.20	32.37 ± 0.52

Height of one plant 83.47±0.91 cm in control variations, and under variations treated with Rhizobium 3, Rhizobium 9 and PlantaStim was 86.47±0.57 cm, 85.33±0.88 and 84.07 cm respectively (Fig. 2). There wasn't noted significant difference in plant height among the variations. However, the highest value was detected under the variation treated with Rhizobium 3, and it was 3.59% higher than control variation.

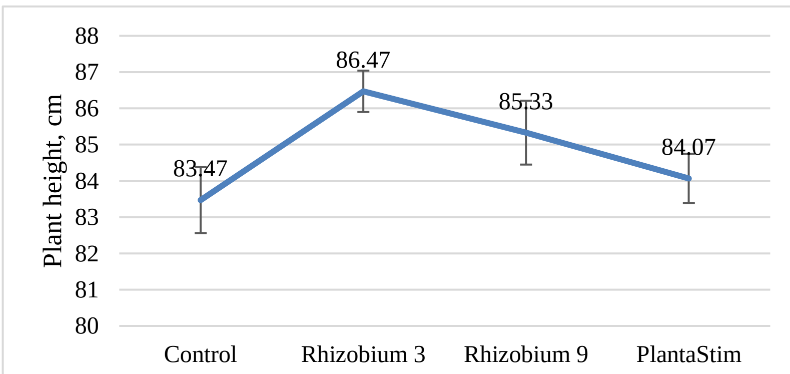

Figure 2. Effect of microbial fertilizers on plant height of pea plants

Plant height and branching are important morphological characteristics of chickpea plant (N.S.Taspaev, 2018). Primary and secondary branching is considered to be one of the main morphological characters in chickpea plant. It was observed that the number of main branches of one plant was from 3.20±0.14 to 3.33±0.13, and the number of side branches was from 14.87±0.40 to 15.47±0.31. The highest rate was found under the variation treated with Rhizobium 3 (Fig. 3).

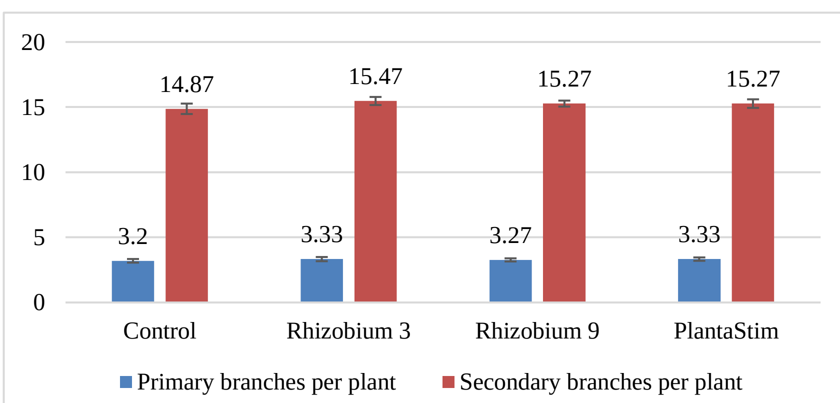

Figure 3. Effect of microbial fertilizers on branching of chickpea plants

Chickpea is a plant with a deep and strong root system, which forms nodules in the roots together with Rhizobium bacteria. These nodules have the property of fixing atmospheric nitrogen in a useful form for the plant. In chickpea, nodulation begins approximately one month after germination (P.M.Gaur, 2010). The number of nodules was higher in chickpea plant samples treated with microbial fertilizers as Rhizobium 3 and Rhizobium 9 compared to control variation and PlantaStim microbial fertilizer (Fig. 4). In this case, the nodule mass per plant during the general flowering phase was 2.22±0.05 g. and 2.21±0.05 g. in the control variation and plants treated with PlantaStim, respectively, and in the samples treated with Rhizobium 3 and Rhizobium 9 the nodule mass was 4.60±0.17 g and 3.84±0.09 g, respectively. The root nodule mass in the treated samples with Rhizobium 3 and Rhizobium 9 was 2.07 and 1.73 times higher than in the control variety, respectively (Fig. 5). An increase in nodule mass in the root of a chickpea plant leads to an increase in nitrogen fixation (Wadisirisuk, 1985). It has been found that treating mung bean plants with Rhizobium 3 and Rhizobium 9 microbial fertilizers has a positive effect on the formation of root nodules, growth and development (F.Matkarimov 2019).

Rhizobium 9

Rhizobium 3

PlantaStim

Control

Figure 4. Formation of nodules in chickpea plants as a result of the effect of microbial fertilizers.

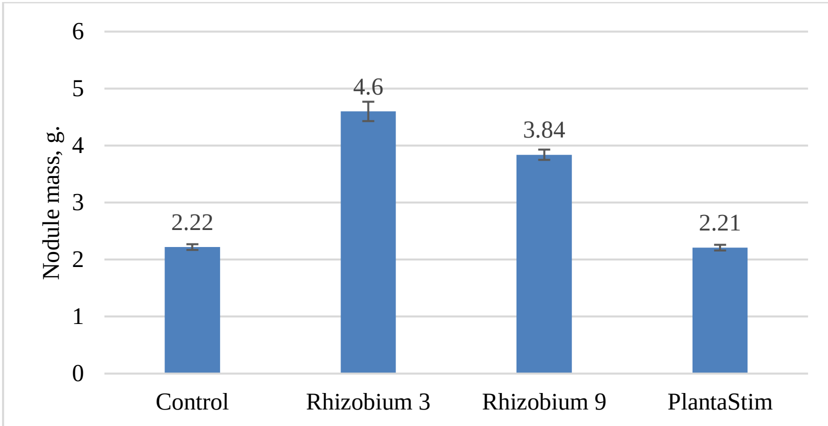

Figure 5. Effect of microbial fertilizers on changes in nodule mass in chickpea plants

Increasing crop productivity in agriculture is of great importance. In the framework of the research, valuable economic characteristics such as biomass per plant, number of pods per plant, number of grains per plant, pods weight per plant, grain weight per plant, 100-seed weight, grain yield (1 m2/g), harvest index and total protein quantity were analyzed (Table 2).

Table 2

Effect of microbial fertilizers on valuable economic characteristics of chickpea plant

	Control	*Rhizobium 3*	*Rhizobium 9*	PlantaStim
Biomass per plant, g.	53.74±1.79	70.61±1.65	63.68±1.19	58.57±1.57
Number of pods per plant	68.20±2.05	76.80±1.46	73.40±0.86	70.67±1.46
Number of grains per plant	75.73±1.80	90.4±2.09	88.20±1.47	84.87±1.3
Pod weight per plant, g.	35.93±1.06	40.12±1.02	38.04±0.80	37.36±0.70
Grains weight per plant, g.	24.04±0.56	30.31±0.68	28.30±0.66	28.33±0.43
100-seed weight	33.07±0,54	33.68±0,41	33.15±0,48	33,41±0,61
Grain yield, g. (1 m^2)	315.16±9,1	401.04±8,6	376.48±9,4	377.88±10,7
Total protein content, %	21.43	26.8 5	24.13	23.37
Harvest index	45.08	43.06	44,42	44.60

The above-ground dry biomass indicator of the chickpea in one plant was 53.74±1.79 g in control variation, and in the samples treated with Rhizobium 3, Rhizobium 9 and PlantaStim, this indicator showed 70.61±1.65 g, 63.68±1.19 g and 58.57±1.57 g, respectively. The main yield elements as the number of pods per plant, the number of grains, the weight of pods and the weight of grains per plant were 68.20±2.05 pcs, 75.73±1.80 pcs, and 35.93±1.06 g, and 24.04±0.56 g, respectively. These indicators were higher in all variations treated with microbial fertilizers than the control variation. The grain weight of the plant is of great importance in obtaining high yield (S.V. Bulyntsev, 2015). The main components that improve grain yield in chickpea plants are plant biomass, grain number and grain weight (Qulmamatova, D.E. 2023). According to the results of the experiment the weight of 100 grains made up 33.07 - 33.68 g. Grain weight of the samples treated with microbial fertilizers increased slightly. The grain yield per 1 m2 under the control variety was 315.16 g, whereas, in the samples treated with Rhizobium 3, Rhizobium 9 and PlantaStim was 401.04 g, 376.48 g, and 377.88 g, respectively. The highest rate was observed in the samples treated with Rhizobium 3 and the yield was 27.25% higher than in the control variation. The total amount of protein in the grain was between 21.43 and 26.85%. Also, the highest rate was observed in the samples treated with Rhizobium 3, and the yield was 25.29% higher than in the control variety. The harvest index was 43.06 - 45.08. Higher biomass in the samples treated with microbial fertilizers led to a partial decrease in the yield index.

In the chickpea samples grown under field conditions, higher total water amount, number of photosynthetic pigments, root nodules and yield elements in the leaves treated with Rhizobium 3 and Rhizobium 9 microbial fertilizers, as well as increased transpiration rate, photosynthetic pigment content and yield elements in the samples treated with PlantaStim had a significant positive effect on the productivity of the plant, compared to the control variation.

During the selection process, in cultivation of chickpea, Rhizobium 3 , Rhizobium 9 and PlantaStim microbial fertilizers have a positive effect on the morphological, physiological characteristics of the plant, as well as the valuable economic characteristics, and serve to increase crop productivity.

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