


The effect of Rhizobium, Azotobacter and microbial consortium (Rhizobium/Azotobacter) on some growth parameters and nodulation of chickpeas (*Cicer arietinum* L.)

Rhizobium, Azotobakter ve mikrobiyal konsorsiyumun (Rhizobium/Azotobakter) nohutta (*Cicer arietinum* L.) bazı büyüme parametreleri ve nodulasyon üzerine etkisi

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ARTICLE INFO	ABSTRACT
<p>Article history: Recieved / Geliş: 08.05.2024 Accepted / Kabul: 14.07.2024</p> <p>Keywords: Inoculation Azotobacter Chickpea Rhizobium Yield components</p> <p>Anahtar Kelimeler: Aşılama Azotobakter Nohut Rhizobium Verim unsurları</p> <p>✉Corresponding author/Sorumlu yazar: Ummahan ÇETİN KARACA ucetin@selcuk.edu.tr</p> <p>Makale Uluslararası Creative Commons Attribution-Non Commercial 4.0 Lisansı kapsamında yayınlanmaktadır. Bu, orijinal makaleye uygun şekilde atıf yapılması şartıyla, eserin herhangi bir ortam veya formatta kopyalanmasını ve dağıtılmasını sağlar. Ancak, eserler ticari amaçlar için kullanılamaz. © Copyright 2022 by Mustafa Kemal University. Available on-line at https://dergipark.org.tr/tr/pub/mkutbd This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.</p> 	<p>The adoption of microbial fertilizers such as rhizobium and azotobacter can reduce the requirement for chemical fertilizers and their negative impact on the environment. Overuse of chemical fertilizers to increase productivity has been shown to increase costs, reduce the microorganism population of the soil, and cause serious human and animal health problems by accumulating in plants and entering groundwater. For this purpose, a greenhouse experiment was conducted under controlled conditions with treatments of control, nitrogenous control, rhizobium, azotobacter, and the rhizobium/azotobacter consortium. Seeds inoculated with bacteria were planted on media containing sterile sand + perlite. Plants were harvested at 50% flowering, and some yield and yield components were determined. Inoculation of chickpea seeds with rhizobium, azotobacter, and rhizobium/azotobacter combinations of bacteria had different effects, and these differences were found to be statistically significant. In the experiment, rhizobium/azotobacter treatments were effective on the wet and dry weight of plant upper parts, the number of nodules, the weight of nodules, the nitrogen content of plant upper parts, and the nitrogen content of the root of the chickpea plant. In addition, rhizobium inoculation was effective on the plant's upper part and root length, and azotobacter inoculation was effective on the wet and dry weight of chickpea roots.</p> <p>ÖZET</p> <p>Rhizobium ve azotobakter gibi mikrobiyal gübrelerin kullanımı, kimyasal gübrelere olan ihtiyacı ve olumsuz çevresel etkileri azaltılabilir. Verimliliği artırmak için gereğinden fazla kullanılan kimyasal gübrelemenin ekonomik olmadığı, toprağın mikroorganizma popülasyonunu olumsuz olarak etkilediği, ayrıca bitkilerde depolanarak ve yeraltı sularına karışarak insan ve hayvan sağlığı açısından ciddi sorunlara sebep olduğu belirlenmiştir. Bu amaçla araştırmada; kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter'in konsorsiyum uygulamaları ile kontrollü şartlarda sera denemesi kurulmuştur. Bakterilerle aşılamanın tohumlar, steril kum+perlit içeren ortamlara ekilmiştir. Bitkiler çiçeklenmenin %50'sini geçtiği dönemde hasat edilmiş ve bazı verim ve verim unsurları ölçülmüştür. Nohut bitkisinin tohumlarına rhizobium, azotobakter ve rhizobium/azotobakter kombine bakterilerinin aşılamanın etkileri üzerine farklılıklar istatistiksel olarak önemli bulunmuştur. Denemede nohut bitkisinin bitki üst aksam yaş ve kuru ağırlık, nodül sayısı, nodül ağırlığı, bitki üst aksam azot ve kökte azot içeriğine rhizobium/azotobakter kombine aşılması etkili olmuştur. Bunun yanı sıra bitki üst aksam ve kök uzunluğuna rhizobium aşılması, nohut bitkisinin kök yaş ve kuru ağırlığına ise azotobakter aşılamanın etkili olduğu belirlenmiştir.</p>
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INTRODUCTION

Nowadays, nitrogen (N) fertilizers play a crucial role in crop production and are considered the primary external inputs to improve production from agriculture. Nevertheless, nitrogen fertilizers substantially contribute to environmental contamination and can profoundly affect all living organisms (Verma et al., 2014). Hence, it is necessary to give precedence to advancing new developing methodologies that are both ecologically and financially sustainable. Rana et al., (2012) suggest that utilizing azotobacter as a biofertilizer is crucial for enhancing the nutrient composition of plants and soil, increasing crop yields, and protecting the environment. Moreover, applying azotobacter bacteria significantly enhanced maize grain production, with an increase of up to 35% compared to plants not treated with the bacteria (Bandhu & Parbati, 2013). In 2009, İşler and Çoşkan discovered that adding different types of bacteria to soybeans, such as a control, seed inoculation, upper inoculation, upper inoculation twice, pulverized inoculation on the seed bed, and inoculation with peat, all worked to improve nitrogen fixation. These techniques can be successfully implemented in practical applications. In a study conducted by Rodelas et al., in 1999, it was found that a combination of Z25 *Rhizobium Leguminosarium* with five different strains of *Azotobacter chroococcum* or *Azotobacter vinelandii* had a notable impact on nodulation, plant growth, and nitrogenase activity in the roots of common bean plants during flowering. The strains *A. chroococcum* H23, *A. vinelandii* ATCC 12837, and Dv42 had a significant positive effect on dry matter accumulation and total nitrogen content in all parts of the plant that contained nodules, as well as promoting plant growth. Their report revealed a strong correlation between the impact of each nitrogenobacter strain and the number of living cells used as inoculum. In 2014, Andjelkovic et al., conducted a study that investigated the impact of preplant inoculation with individual and mixed cultures of rhizobium (*Rhizobium meliloti*), azotobacter (*Azotobacter chroococcum*), and actinomycete (*Streptomyces* spp.) on the growth parameters of alfalfa plants. The studied parameters showed that the interaction between the cultivar and inoculation had a beneficial impact compared to the control. When Rodelas et al., (1999b) inoculated common bean plants with different combinations of rhizobium/azospirillum and rhizobium/azotobacter, they observed significant differences in the distribution, concentration, and total content of macro- and microelements compared to plants inoculated with rhizobium alone. The selection of azotobacter and azospirillum strains for combination inoculation showed significant differences in their effects. The study (2007-2008) aimed to investigate the effects of inoculation with *Rhizobium leguminosarium* (Rh), *Azotobacter chroococcum* (AZ1), and *Bacillus megaterium* var *phosphaticum* (BM3) on various aspects of dry bean plants. These aspects included nodulation, nitrogen fixation, rhizosphere microbial population, NPK content, yield, and pod quality. The study focused specifically on two dry bean varieties, Bronco and Paulista, and used a dosage of chemical NPK fertilizer that was 25% below the recommended amount. The results showed that the application of a combination of biofertilizers had an important effect on growth parameters, nodulation, and N₂ fixation of dry beans (Gharib et al., 2015). In 1983, Iruthayathas et al., conducted a study to investigate the effects of combination inoculation of different strains of rhizobium and azospirillum on winged bean and soybean growth under hot and tropical conditions. Some combinations of these bacteria resulted in significant enhancements in nodulation, N₂ fixation, shoot dry matter production, and N reception. The result of the combined inoculation was primarily determined by the genotypes of these bacteria. Dashadi et al., (2011) evaluated the impact of adding natural *Rhizobium leguminosarium* F46 and *Azotobacter chroococcom* AGO11 strains in combination with nitrogen fertilizer on the growth and growth indexes of common bean plants in the presence of water stress conditions. Results demonstrated that the combined application of rhizobium and azotobacter resulted in significant improvements in various growth parameters, including the number of nodules, nodulation, total nitrogen content, relative water content, root dry weight, average day germination, and day germination rate. The combined addition of rhizobium and azotobacter bacteria enhanced the absorption of water and nutrients in the presence of limited water availability, thereby reducing the impact of water restriction. Ibrahim et al., (2022) conducted a study to investigate

the impact of co-inoculation with *Azotobacter chroococcum* and *Rhizobium leguminosarium* on the growth, yield, and quality parameters of pea plants. The study included evaluating the effects before planting and after emergence. Compared to plants only inoculated with rhizobium, the addition of *A. chroococcum* and *R. leguminosarium* significantly improved plant biomass, nutrient absorption, and the concentration of leaf photosynthetic pigments. Additionally, adding *A. chroococcum* led to better nodulation values, such as more nodules, larger nodules, and higher dry weight nodules, as well as higher nitrogenase enzyme activity compared to plants in the control group. Furthermore, the co-inoculation with *A. chroococcum* resulted in a significant increase in seed production and improved the levels of ascorbic acid, protein, and carbohydrates in the seeds. This study evaluated the effect of rhizobium, azotobacter, and their combined application on the yield components and nodulation values of chickpea plants in controlled conditions.

MATERIALS and METHODS

A 1:1 mixture of sand + perlite was used as the medium in the study. The sand provided for the experiment was washed and sterilized in an autoclave at 121°C under 1 atm pressure for 120 minutes and then used. Similarly, we sterilized the perlite in the environment before use. The experiment used brown pots with a capacity of 2 L. The pots were sanitized before use.

Biological fertilizers used in the experiment

Azotobacter spp.: Obtained from a company that produces organic fertilizer.

Mesorhizobium cicer: The *Mesorhizobium cicer* used in the study was obtained from the biological laboratories of the Ankara Soil, Fertilizer, and Water Resources Central Research Institute.

Establishment of the experiment

The Azkan chickpea variety, which was registered by the Geçit Kuşığı Agricultural Research Institute in 2009, was used in the study. The study was conducted in a computer-controlled greenhouse (temperature was maintained at 25±3°C, solar radiation at 1750±50 kcal m⁻², and relative humidity at 60±10% during the experiment) according to the completely randomized design with 4 replications. Sterilized sand and perlite were added to the disinfected pots in a ratio of 1:1 by volume. In the study, rhizobium bacteria isolated from peat culture were propagated on yeast mannitol agar (YMA) medium and then grown on a liquid growth medium (YMB) (Somasegaran and Hoben 1994). After the bacterial concentration was adjusted to 1x10⁸ CFU/mL, the seeds were inoculated. We obtained *Azotobacter* in a liquid growth medium and adjusted its concentration to 1x10⁸ CFU/ml. The inoculated seeds were planted, and the greenhouse experiment was established under controlled conditions. The Jensen nutrient solution was used in the study (Vincent 1970). Once we prepared the nutrient solution, we added 1 ml of each of the microelement solutions per liter. We diluted the prepared nutrient solution to 1/5 before using it. The pots were watered with the nutrient solution prepared according to the macro- and micronutrient needs of the plants until the end of the experiment. The plants were harvested after 50% of the flowering period, and some measurements were made on the plants.

Table 1. Content of the nutrient solution used in the experiment

Çizelge 1. Denemede kullanılan besin solüsyonunun içeriği

Nutrient solution (L)		Micro element solutions	
KNO ₃	0.5 g	500 mgkg ⁻¹ B	H ₃ BO
CaHPO ₄	1.0 g	500 mgkg ⁻¹ Mn	MnSO ₄
K ₂ HPO ₄	0.2 g	50 mgkg ⁻¹ Zn	ZnCl ₂
MgSO ₄ .7H ₂ O	0.2 g	50 mgkg ⁻¹ Mo	MoO ₃
NaCl	0.2 g	20 mgkg ⁻¹ Cu	CuSO ₄ .5H ₂ O
FeCl ₃	0.1 g		

Statistical analyses

The data obtained from the greenhouse experiment, which was established with 4 replicates according to the completely randomized design, were subjected to analysis of variance according to the Minitab 19 statistical program. Significant treatments according to the F test were grouped in the Duncan multiple comparison test.

RESULTS and DISCUSSIONS

According to the results of the analysis of variance performed on the data related to the parameters measured in Azkan chickpea plants, it was determined that the differences between the control, nitrogen control, rhizobium, azotobacter, and rhizobium x azotobacter combined treatments were statistically significant (Table 1). The differences in the lengths of the plant tops and roots between the treatments were found to be statistically significant ($p < 0.01$). The highest aerial parts and root lengths were found in the rhizobium treatment. The rhizobium bacteria inside the nodule provide the nitrogen necessary for the plant, and the plant provides the bacteria with the glucose and energy that they need to fix the free nitrogen in the atmosphere. Nitrogen, which supports plant growth, is an essential nutrient for plant physiology. Plants use a significant amount of nitrogen during the developmental period. Nitrogen supports the development of roots, leaves, stems, branches, shoots, and fruits. In addition to these benefits, nitrogen also enhances flowering (Kacar and Katkat, 2007). The lowest aerial parts and root lengths were recorded in the control treatment. The root lengths of Azkan chickpea plants varied between 24-31.33 cm (Figure 1; Table 1).

In analyzing the values of plant top length, it was found that single and combined bacterial treatments had higher values than the control and nitrogen control treatments. In general, a consortium of microorganisms can potentially perform better than the inoculation of single strains of microorganisms. A microbial consortium can significantly improve crop and soil productivity under extreme stress conditions compared to single-species inoculants (Behera et al. 2021). It was found that rhizobium and rhizobium x azotobacter combined inoculations obtained higher values than the data obtained from the nitrogen control application on the upper limb length of chickpea plants. Azotobacteria have the ability to convert atmospheric nitrogen in the soil into a form that plants can easily absorb, therefore providing a natural nitrogen fertilizer. Furthermore, these bacteria naturally create hormones that promote root growth. Herridge et al. (2008) noticed that applying effective rhizobia to legumes with inoculation can enhance legume yield and yield components while also preserving soil health. Moreover, environmentally friendly practices used to improve N fixation resulted in increased shoot growth, pod number, and pod grain yield (Siczek & Lipiec, 2016). Meral et al., (1998) investigated the effects of bacterial inoculation and different nitrogen doses on some yield components in chickpea plants. They found that there was no nodulation, root weight, plant height, plant weight, number of fruits in the plant, or grain weight increase in treatments without bacterial

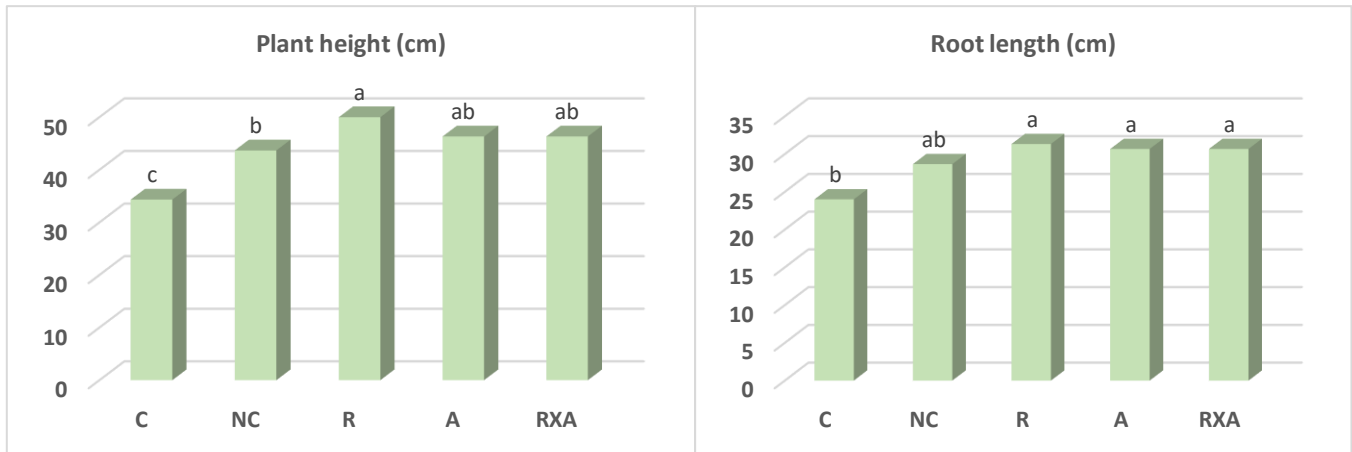


Figure 1. Effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on plant upper parts and root length ($p < 0.01$) (C: Control, NC: Nitrogenous control, R: Rhizobium, A: Azotobacter)

Şekil 1. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılımlarının bitkinin üst aksam ve kök uzunluğu üzerine etkileri ($p < 0.01$) (C: Kontrol, NC: Azotlu kontrol, R: Rhizobium, A: Azotobakter)

inoculation. Andjelkovic et al., (2014) investigated the effects of pre-sowing inoculation with individual and combined cultures of rhizobium (*R. meliloti*), nitrogenobacter (*A. chroococcum*), and actinomycete (*Streptomyces* spp.) on the growth parameters of alfalfa plants. For the parameters tested, the interaction between cultivar and inoculation resulted in a positive effect compared to the control. The highest plant height was obtained in the Synteza 1 cultivar using an inoculum containing all varieties of microorganisms. The highest values for root and green mass per plant were obtained in the K-28 cultivar using this microbial inoculum. The highest values for root mass were obtained in this cultivar (K-28) using combined cultures of rhizobium and actinomycetes.

This study found that adding rhizobium or rhizobium x azotobacter together had statistically significant effects ($p < 0.01$) when compared to the control treatments. It was determined that the wet weight of the upper parts of chickpea plants varied between 5.17 and 7.98 g (Figure 2; Table 1). The dry weights of the upper parts of chickpea plants varied between 0.65 and 1.26 g (Figure 2; Table 1).

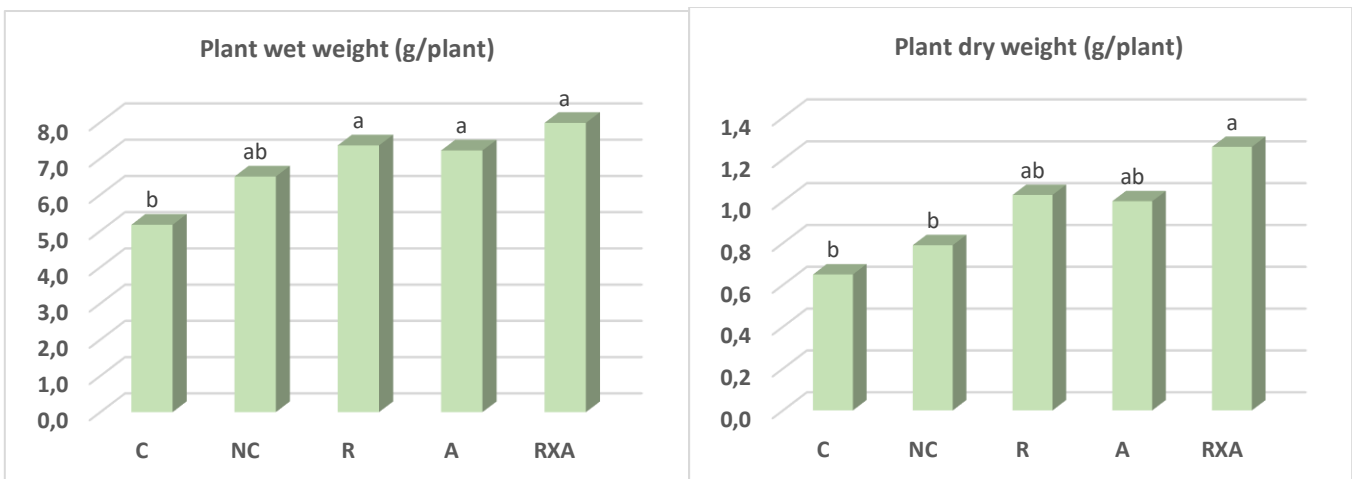


Figure 2. Effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on the wet and dry weight of plant upper parts ($p < 0.01$) (C: Control, NC: Nitrogenous control, R: Rhizobium, A: Azotobacter)

Şekil 2. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılımlarının bitkinin üst kısımlarının yaş ve kuru ağırlığı üzerine etkileri ($p < 0.01$) (C: Kontrol, NC: Azotlu kontrol, R: Rhizobium, A: Azotobakter)

Therefore, the use of a consortium of bacteria with different beneficial characteristics and microbial metabolites with different biological potencies can offer a simple and inexpensive sustainable strategy to improve plant performance, quality, and yield (Ju et. al. 2019). Plants inoculated with rhizobium x azotobacter combined bacteria yielded the highest wet and dry weights of their upper parts, while the control treatment yielded the lowest wet and dry weights. The plants that grew from seeds that were inoculated with rhizobium and rhizobium x azotobacter bacteria together had higher wet weights than the nitrogen control group. They studied the effect of inoculation with plant growth-promoting rhizobacteria (PGPR) and rhizobium on the yield and yield components of bean cultivars under field conditions for two consecutive years. The rhizobium strain inoculation resulted in a significant variation in plant growth. PGPR inoculated significantly increased the number of pods per plant, number of seeds in pods, 100-grain weight, seed weight per plant, seed yield and protein content, and total dry matter content in R6 (Yadegari & Rahmani, 2010).

According to the research data, it was determined that the effects of control, nitrogenous control, rhizobium, azotobacter, and rhizobium x azotobacter combined inoculations on chickpea plants' root wet and dry weights were statistically significant ($p<0.01$). The Azkan variety chickpea plants' root wet weights ranged from 2.99 to 7.21 g (Figure 3; Table 1). The plants inoculated with azotobacter isolate yielded the highest root wet and dry weights, while the control treatment yielded the lowest wet and dry weights. Among the treatments, the root dry weights of plants of the chickpea variety Azkan ranged from 0.21 to 0.51 g. The plants obtained from seeds inoculated with rhizobium bacteria had the highest root dry weight (0.43 g) (Figure 3; Table 1).

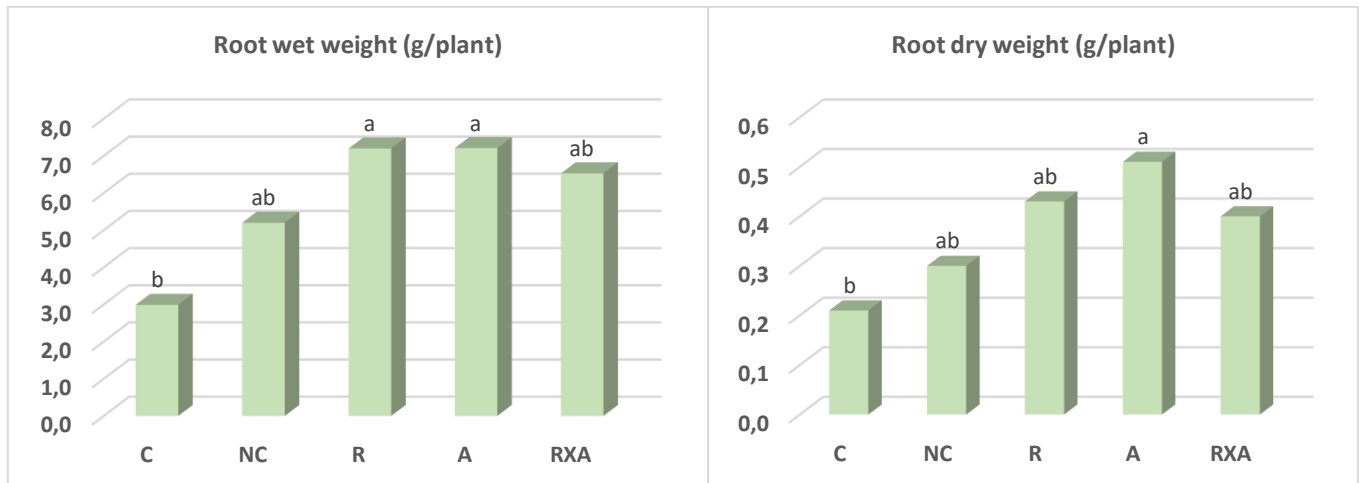


Figure 3. Effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on plant root wet and dry weight ($p<0.01$) (C:Control, NC: Nitrogenous control, R: Rhizobium, A: Azotobacter)

Şekil 3. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılamalarının bitki kökünün ıslak ve kuru ağırlığı üzerine etkileri ($p<0.01$) (C: Kontrol, NC: Azotlu kontrol, R: Rhizobium, A: Azotobakter)

Examining the dry weights of the plants from seeds inoculated with rhizobium and rhizobium x azotobacter combined bacteria revealed higher values than in the nitrogen control group. Several studies indicate that, overall, a microbial consortium is more effective at performing functions than individual strains. Ju et al., 2019 found that bacterial consortiums enhance beneficial characteristics in plants by providing a variety of promotion mechanisms for plant growth, compared to individual strains and biological control mechanisms. Utilizing this consortium is an appropriate strategy to improve drought conditions (Joshi et. al. 2020), reduce salinity (Nawaz et. al. 2020), enhance nutrient absorption (Rana et. al. 2012), and manage pests and plant diseases in crops (Rodriguez et. al. 2019). It was found by İşler and Çoşkan (2009) that different ways of adding bacteria (control, seed inoculation, upper inoculation, upper inoculation twice, pulverized inoculation on the seedbed, and inoculation with peat) increased

nitrogen fixation in soybeans and that these methods could be used in real life. In a study, Andjelkovic et al., (2014) discovered that pre plant inoculation with single and combined cultures of rhizobium (*R. meliloti*), azotobacter (*A. chroococcum*), and actinomycetes (*Streptomyces* spp.) had a positive effect on growth parameters (height, number of stems, and plant weight) of alfalfa plants when comparing the interaction between variety and inoculation with the control.

They studied the effects of inoculation with natural strains of *R. leguminosarum* F46 and *A. chroococcum* AGO11 and nitrogen fertilizer on the growth and growth indices of common bean plants under water stress conditions. The results showed that inoculation alone and co-inoculation of rhizobium and azotobacter increased most of the growth indices, such as the number of nodules, nodulation, total nitrogen content, relative water content, root dry weight, mean day germination, and day germination rate. Co-inoculation of rhizobium and azotobacter increased water and nutrient uptake under water stress conditions, thus alleviating the effect of water shortages (Dashadi et al., 2011).

The different treatments (control, nitrogenous control, rhizobium, azotobacter, and rhizobium x azotobacter) had a statistically significant ($p < 0.01$) effect on the number and weight of nodules in chickpea plants grown in a controlled greenhouse. Plants inoculated with a combination of rhizobium and azotobacter bacteria showed the highest number of nodules, ranging from 0 to 38 (Figure 4; Table 1). The control, nitrogen control, and azotobacter treatments all had zero nodules, indicating the lowest count across all treatments. The nodules' weights varied between 0 and 0.59 g across the different treatments. The nodules in the root of the plants inoculated with a combination of rhizobium and azotobacter had the maximum weight, measuring 0.59 g (Figure 4; Table 1). The control, nitrogenous control, and azotobacter treatments all had nodules weighing 0 g, which was the lowest observed weight.

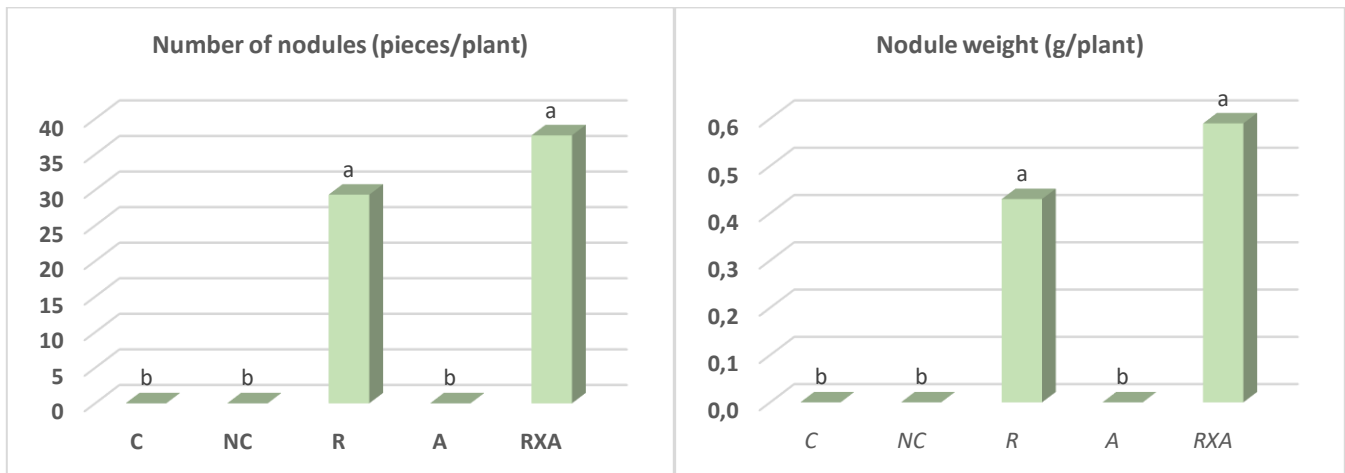


Figure 4. Effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on nodule number (pcs/plant) and nodule weights ($p < 0.01$) (C:Control, NC: Nitrogenous control, R: Rhizobium, A: Azotobacter)

Şekil 4. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılamaalarının nodül sayısı (adet/bitki) ve nodül ağırlıkları üzerine etkileri ($p < 0.01$) (C: Kontrol, NC: Azotlu kontrol, R: Rhizobium, A: Azotobakter)

Rodelas et al., (1999a) showed that using a mix of biofertilizers had a big effect on the quantities of growth, nodulation, and N_2 -fixation in dry beans. Siddiqui et al., (2014) found in their study that inoculating chickpea (*Cicer arietinum*) seeds with *A. chroococcum* and an effective rhizobial strain significantly improved nodulation, root nitrogen content, and grain yield compared to non-inoculated seeds. They declared that the introduction of microorganisms to the plant can lead to the production of certain chemicals that enhance growth.

The study found that the nitrogen content in the upper parts and roots of chickpea plants changed significantly when they were treated with different types of bacteria, such as nitrogenous control, rhizobium, azotobacter, and rhizobium x azotobacter combined. The study revealed that the nitrogen levels in the upper parts of Azkan chickpea plants ranged from 2.15% to 3.84% (Figure 5; Table 1). When infected with a combination of bacteria (rhizobium x azotobacter), the upper part of the chickpea plant showed the highest nitrogen content, with a value of 3.84%. In contrast, the control treatment had the lowest nitrogen content in the upper part, determining 2.15%. Furthermore, several bacterial consortiums demonstrate the ability to perform nitrogen fixation. Microorganisms convert certain unavailable elements into easily absorbed forms, produce phytohormones, and synthesize iron chelates, all of which are crucial for maintaining soil quality and overall health. Additionally, they can decrease the negative effects associated with certain traditional, non-sustainable agriculture practices (Gosal and Kaur, 2017). The highest nitrogen content in the upper part of the plants was 3.71%, followed by the nitrogen content in the plants treated with nitrogen control. The analysis showed that the nitrogen concentration in the roots of Azkan chickpea plants ranged from 1.66% to 2.35%. The plants inoculated with a combination of rhizobium and azotobacter had the highest nitrogen content of 2.35%, whereas the control treatment exhibited the lowest nitrogen content of 1.66%.

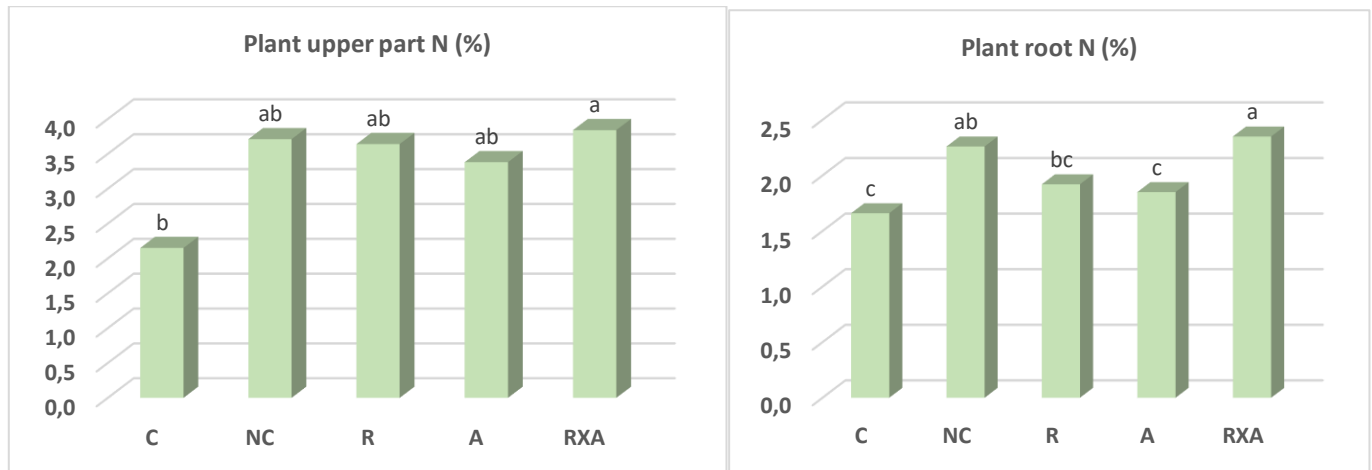


Figure 5. The effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on nitrogen content in plant upper parts and root ($p < 0.01$) (C: Control, NC: Nitrogenous control, R: Rhizobium, A: Azotobacter)

Şekil 5. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılımalarının bitkinin üst kısmı ve kökteki azot içeriği üzerine etkileri ($p < 0.01$) (C: Kontrol, NC: Azotlu kontrol, R: Rhizobium, A: Azotobakter)

According to Rana et al., (2012), the use of azotobacteria as a biofertilizer is of great importance in improving the nutrient profile of plants and soil, increasing yield, and protecting the environment. Furthermore, inoculation with azotobacteria increased grain yield in maize by up to 35% compared to non-inoculated plants (Bandhu & Parbati, 2013). A study by Rodelas et al., (1999a) showed that planting broad bean seeds with Z25 *R. leguminosarium* and five different strains of *A. chroococcum* or *A. vinelandii* had a big impact on nodulation, plant growth, and the nitrogenase activity of nodulated roots during the flowering stage. Compared to plants only inoculated with rhizobium, the combination of *A. chroococcum* and *R. leguminosarum* significantly improved plant biomass, nutrient absorption, and the concentration of leaf photosynthetic pigments. Additionally, adding *A. chroococcum* led to better nodulation data, such as more nodules, larger nodules, and higher dry-weight nodules, as well as higher nitrogenase enzyme activity compared to plants in the control group. Furthermore, our results showed that there was no significant difference between the treatments administered before and after planting (Ibrahim et al., 2022).

Table 2. The effects of Rhizobium, Azotobacter, and Rhizobium x Azotobacter combined inoculations on some growth parameters and nodulation values of chickpeas plant

Çizelge 2. Rhizobium, Azotobacter ve Rhizobium x Azotobacter kombine aşılamalarının nohut bitkisinin bazı büyüme parametreleri ve nodülasyon değerleri üzerine etkileri

Applications	PH (cm)	RL (cm)	PWW (g/ plant)	PDW (g/plant)	RWW (g/plant)	RDW (g/plant)	NN (pieces /plant)	NW (g/plant)	PUN (%)	PRN (%)
Control	34.33 c	24.00 b	5.17 b	0.65 b	2.99 b	0.21 b	0.00 b	0.00 b	2.15 b	1.6 6 c
Nitrogenous control	43.67 b	28.67 ab	6.50 ab	0.79 b	5.20 ab	0.30 ab	0.00 b	0.00 b	3.71 ab	2.2 6 ab
Rhizobium	50.00 a	31.33 a	7.36 a	1.03 ab	7.20 a	0.43 ab	29.33 a	0.43 a	3.64 ab	1.9 2 bc
Azotobacter	46.33 ab	30.67 a	7.22 a	1.00 ab	7.21 a	0.51 a	0.00 b	0.00 b	3.38 ab	1.8 5 c
Rhizobiumx Azotobacter	46.33 ab	30.67 a	7.98 a	1.26 a	6.53 ab	0.40 ab	37.67 a	0.59 a	3.84 a	2.3 5 a
LSD	*	*	*	*	*	**	*	*	*	*
CV (%)	3.39	4.84	7.89	11.29	15.46	20.13	5.23	6.81	11.06	5.1 0

PH: Plant height, RL: Root length, PWW: Plant wet weight, PDW: Plant dry weight, RWW: Root wet weight, RDW: Root dry weight, NN: Number of nodules, NW: Nodule weight, PUN: Plant upper part N, PRN: Plant root N (*: $p < 0.01$ **: $p < 0.05$).

In conclusion, this controlled greenhouse study looked at how different types of bacteria (control, nitrogenous control, rhizobium, and azotobacter), as well as the use of these bacteria together, affected different parts of chickpea plants' yield and nodulation values. Compared to other control and nitrogen control treatments, applying both symbiotic and asymbiotic bacteria to seeds resulted in higher yield components and nodulation values. A microbial consortium is more effective than single-species inoculations in enhancing crop and soil fertility, especially in extreme conditions. Microbial fertilizers and worldwide consortiums are the best solutions for achieving sustainable agriculture practices. Hence, the use of microbiological fertilizers in agriculture can enhance plant growth by increasing the microbe population in the soil. Implementing microbial fertilizers will enhance the use of low-chemical fertilizers, reduce the dependence on pesticides during treatment, and improve soil productivity. In the context of plants, it has the potential to enhance plant resistance to environmental stresses and promote growth in roots, stems, and crop production. Microbial fertilizers typically do not serve as a complete substitute for chemical fertilizers. Instead, they minimize the dependence on chemicals and provide advantages to ecological agriculture. Fertilizers that contain microorganisms play a crucial role in encouraging sustainable agriculture.

STATEMENT OF CONFLICT OF INTEREST

The authors of the manuscript declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

The authors declare that they have contributed equally to the work.

STATEMENT OF ETHICS CONSENT

Ethical approval is not required as this manuscript does not contain any studies with human or animal subjects.

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