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Research Article

EFFECTS OF BIOPOLYMER COATING ON BACTERIA INOCULATED SEED ON SOME GROWTH PARAMETERS AND NODULATION VALUES OF CHICKPEA PLANT

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ABSTRACT. Recent studies have not overlooked the importance of microorganisms in sustainable agriculture. However, biological fertilizers are limited due to the sensitivity of using these microorganisms as fertilizers. To increase the use of microbial fertilizers, there is a need to maintain the viability of microorganisms to avoid these limitations. For this purpose, chickpea (Azkan) seeds were surface sterilized with sodium hypochlorite solution (0.5%) and inoculated by *Rhizobium ciceri* bacteria in a liquid solution ($1x10^8$ CFU/mL). Seeds inoculated with rhizobium bacteria were coated with sodium alginate and chitosan solutions prepared at 1% concentration. After coating the seed surface, it was stored in a place away from light for a certain period (3 months). At the end of this period, seeds were planted in a sterile culture substrate (sand and perlite) and transplanted to controlled greenhouse conditions. In addition, seeds inoculated and covered with biomaterials were planted as a control on the same day (day 0). Chickpea plants were harvested at the end of 60–65 days of the vegetative period, and some growth parameters (length of upper parts and roots, the upper part portions' wet and dry weight, roots' wet and dry weight, nodules' number and weight, nitrogen concentration in leaves and roots) were determined. The effect of coating the surface of bacteria-inoculated seeds with sodium alginate and chitosan was statistically significant at p-values of less than 0.05 and 0.01. When compared to the plants under control, the coating with sodium alginate was more effective in increasing some of the growth parameters and the bacterial population than the coating with chitosan.

Keywords: *Chickpea, chitosan, coating, inoculation, Rhizobium ciceri, sodium alginate*

INTRODUCTION

One significant health issue is the overuse of harmful agricultural pesticides in the agriculture sector. The adverse impacts of inappropriate and irregular use of chemical fertilizers in agriculture have led to a rise in the global utilization of microbial fertilizers [1; 2]. Using carefully calibrated and controlled distribution of chemical agents and microorganisms is one strategy to lessen the overuse of pesticides in agriculture. In sustainable agriculture, encapsulation has proven to have positive results in providing nutrients for crop production. Encapsulating biological and chemical agents offers key advantages such as sustained and regulated release, increased efficiency, and absence of environmental contamination [3]. Recently, there have been several polymer-based studies in the literature, and these studies have shown that experimental formulations have been produced [4] and that they are potential bacterial transporters [5]. It has been observed that polymers used for the encapsulation of soil microorganisms protect the bacteria against biotic and abiotic stresses by releasing the bacteria into the environment when they degrade [4]. Research showed that encapsulating chickpea and soybean seeds in alginate microspheres following inoculation with *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 notably enhanced the number and size of nodules [6]. Coating seeds with chitosan biopolymer has shown significant increases in stimulating

germination, growth, and yield [7; 8]. Furthermore, it has emerged as an important strategy for managing abiotic stresses, stimulating innate plant defenses toward infectious agents, and controlling disease in various crop species [9; 10; 11; 12; 13]. Alginate is a widely commonly used biopolymer for microorganisms, as indicated by researchers [14]. They studied a seed-coating substance using carboxymethyl chitosan as the main component to coat the seeds of soybean plants. Compared to the control, the coated soybean seeds increased yield by 18% at a lower cost (26%) [15]. Rocha et al. [16] observed that the impact of utilizing encapsulated seeds varies based on criteria such as plant species and growth situations. Because natural biopolymers have good film-forming ability, they can form a tough protective film on the surface layer, allowing seeds to absorb more nutrients. Since biopolymers have good permeability, they can enhance certain enzyme activities of seeds. The coating method is thought to help increase bacterial cultures survival rate and spread. It also protects bacterial cells from harmful environments, thereby reducing cell loss. Recent studies have shown that many experimental polymer-based formulations have been prepared [4] and are potentially bacterial carriers [5]. The ability to coat bacterially inoculated seeds with natural, inexpensive, and widely available biomaterials with high solubility may lead to increased usage of microbial fertilizers in agriculture. Our objective is to maintain the population and viability of bacteria for a longer period after coating the inoculated seed surface with chitosan and sodium alginate biopolymers. This research hypothesizes that bacteria-inoculated and coated seeds can enhance the effective nodules in the plant root and improve nitrogen fixation.

The study examined the impact of chitosan and the use of sodium alginate as biomaterials on chickpea plants' growth parameters and modulation values by coating compounds on the seed surface after inoculating rhizobium bacteria on the seeds.

MATERIALS AND METHODS

Test Plant

The Azkan chickpea variety from the Anatolian Agricultural Research Institute in Eskişehir, Türkiye, was used as the plant material in the greenhouse experiment.

Bacteria Cultivation

The *Rhizobium ciceri* used in the experiment was obtained from the biological laboratory of Ankara Soil, Fertilizer, and Water Resources Central Research Institute. The rhizobium strain is cultivated on yeast mannitol agar (YMA) medium in the laboratory and inoculated at a concentration of $1x10^8$ CFU/mL [17].

Biomaterials

Sodium alginate solution was prepared at 1% (w/w) in distilled water, and the chitosan solution was produced at a concentration of 1% (w/w) in distilled water by adding 1 ml of 1% acetic acid solution and dissolving the solution in a mechanical mixer [18; 19; 20]. After the biopolymers were prepared, the seeds were surface sterilized and inoculated with bacteria. Bacteria-inoculated seeds were coated by inoculating them in biomaterial solutions for 5 minutes. The coated seeds were kept in a place away from light for 1-2 hours and then packaged.

Growing Media

A 1:1 mixture of sterilized sand and perlite was added to 2 kg of pots as the growth medium for the experiment conducted under greenhouse conditions. The sand and perlite were sterilized in an autoclave at 121 °C under 1 atmosphere pressure for 120 minutes.

Nutrient Solution

An adaptable nutrient solution for chickpea plants was prepared, and 1 milliliter of each microelement solution was added for every liter. Throughout the experiment, the plants were watered with a nutrient solution that was diluted to 1/5 with distilled water [21].

Establishment of the Experiment

The experiment was conducted in computer-controlled greenhouses (temperature was maintained at 25±3 °C, solar radiation at 1750±50 kcal.m^{-2}, and relative humidity at 60±10% during the experiment) with 4 replicates according to the randomized plot experimental design. The experimental pots were randomly distributed. Pots were changed daily to ensure homogeneity in the greenhouse. Chickpea seeds were treated with 0.5% NaClO solution for surface sterilization before incubation with bacteria in the study. Following inoculation, chitosan, and sodium alginate solutions were prepared at 1% concentration and applied to the seeds' surface for five minutes. The treated seeds were dried in a dark area for 1-2 hours, wrapped in a bag, and maintained for 90 days. The seeds that were stored for 90 days were planted in a growth medium of sterile sand and perlite for a greenhouse experiment that was carried out under controlled conditions. Likewise, seeds treated with bacteria and encapsulated with biopolymers were planted as a control treatment. Plants were harvested at 50% flowering, and some measurements (plant and root length, plant wet and dry weight, root wet and dry weight, nodule number and weight, leaf and root nitrogen content.) were evaluated

Statistical Analysis

The data obtained from the greenhouse experiment, conducted according to the randomized plot experimental design, were submitted to the analysis of variance according to the Minitab19 statistical program. The treatments that were significantly important according to the F-test have been grouped in Duncan's multiple comparison test.

RESULTS

After the seeds were inoculated and coated, they were kept in a sealed container for 3 months to check the viability of the bacteria. This period was chosen as a preliminary test because the population of microorganisms is usually higher between 3 and 6 months. After the inoculated seeds were covered with biomaterials, the bacteria's effectiveness was studied when they could remain in the package. The seeds inoculated with bacteria and covered with biomaterials were kept at room temperature in a sealed package and protected from light during storage. As a result of the analysis of variance (ANOVA) performed on the chickpea plant length data, it was found that the differences between treatments (inoculation, inoculation+chitosan, inoculation+sodium alginate) and storage periods (0 and 90 days) on the length of the plants were not statistically significant. It was found that the length of the chickpea plants varied between 22.67 and 26.66 cm (Figure 1). When evaluating the data obtained from the experiment, the highest plant upper part length was 26.66 cm in the rhizobium bacteria-inoculated seeds that were planted immediately, without waiting, and without being coated with chitosan or sodium alginate biopolymers. The lowest chickpea plant height, at 22.67 cm, was achieved by seeds that were covered with sodium alginate biopolymer, incubated with bacteria, and then planted without a waiting time. The study revealed that there were statistically significant ($p<0.01$) differences in the root lengths of chickpea plants between the bacteria incubation and the chitosan and sodium alginate treatments, but an insignificant difference between the storage times. The root lengths of Azkan chickpea plants ranged from 27.33 to 30.78 cm (Figure 1). Incubated seeds coated with chitosan biopolymer and planted

without storage had the shortest root length of 27.33 cm. Seeds treated with sodium alginate and planted after 90 days of storage had the highest root length.

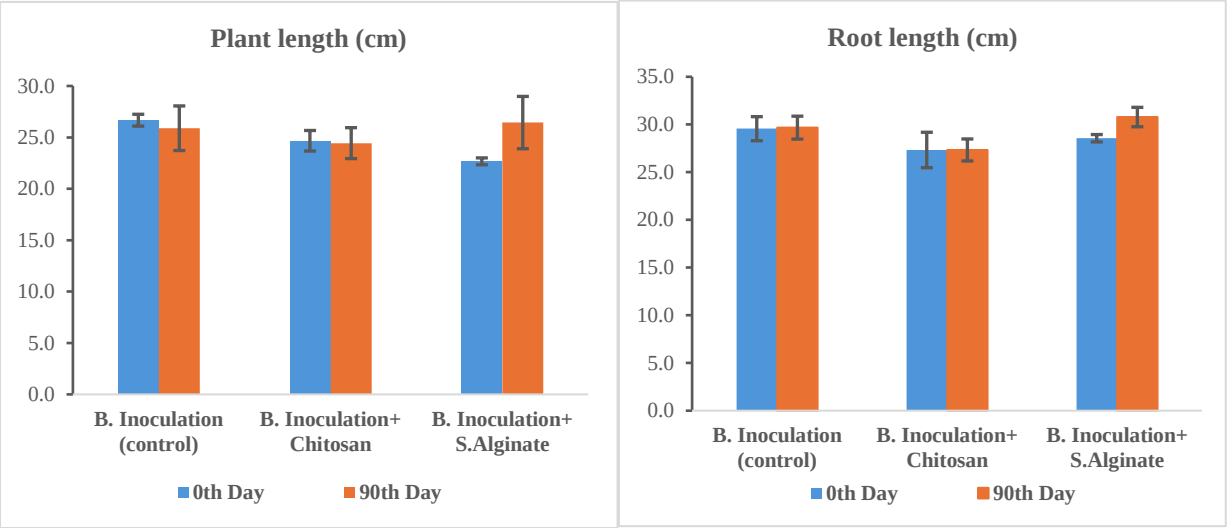

Figure 1. *Effects of storage time (0 and 90 days) and coating with chitosan and sodium alginate biopolymers on the plant and root lengths of bacteria-inoculated seeds of chickpea (Azkan).*

The research results indicated that some biopolymers applied to the seeds contributed to the different wet weights of the upper parts of Azkan chickpea plants. These differences were statistically significant ($p<0.01$) between treatments (inoculation, inoculation+chitosan, inoculation+sodium alginate) and storage time (0 and 90 days). The wet weights of Azkan chickpea plants varied between 8.93 and 13.29 g (Figure 2). The highest wet weight of the chickpea plant was determined to be 13.29 g in plants cultivated from seeds that were coated with chitosan biopolymer after being inoculated with bacteria and maintained for 3 months.

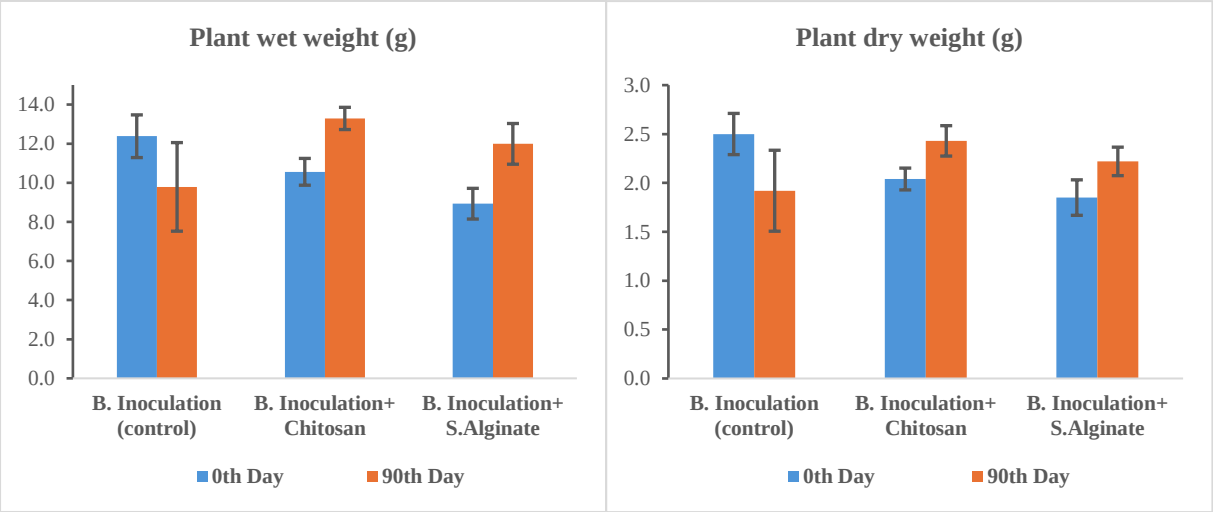

Figure 2. *Effects of storage time (0 and 90 days) and coating with biopolymers of chitosan and sodium alginate on the wet and dry weight of upper parts (g/plant) of bacteria-inoculated seeds of chickpea (Azkan).*

The analysis of variance revealed statistically significant differences ($p<0.01$) in the dry weights of chickpea plants inoculated with rhizobium bacteria and treated with chitosan and the sodium alginate biopolymers in the different applications (inoculation, inoculation+chitosan, inoculation+sodium alginate) and storage durations. The dry weights of the upper parts of Azkan chickpea plants ranged from 1.85 to 2.50 g (Figure 2). The highest dry weight of the plants was 2.50 g in the seeds treated with rhizobium bacteria rather than coated with chitosan and sodium alginate biomaterials and without undergoing a storage period. The upper part of the plant had the lowest dry weight of 1.85 g when the seeds were incubated with rhizobium bacteria and coated with sodium alginate biomaterial directly without any storage time. The investigation demonstrated statistically significant differences at a 1 % level in the root wet weights of chickpea plants when coated with chitosan and sodium alginate biopolymers on the bacteria-inoculated seeds. At the same time, the effect of the application periods of the seeds coated with biopolymers on the root wet weights of the plants was found to be statistically significant ($p<0.01$). After 90 days of storage and planting of the seeds coated with the sodium alginate biopolymer and incubated with bacteria, the highest wet weights of the plant roots were obtained at 51.31 g. The lowest plant root wet weights of 28.39 g were determined in the chickpea seeds inoculated with bacteria and coated with chitosan (Figure 3). On the other hand, coating the surface of the inoculated seeds with sodium alginate biopolymer was more effective on plant root wet weight than the seeds coated with chitosan.

A research study was carried out to investigate how covering biomaterials (chitosan and sodium alginate) influences the shelf life of rhizobium bacteria in Azkan chickpea seeds. The study showed statistically significant variations ($p<0.05$) in the root dry weights of chickpea plants across different treatments (inoculation, inoculation+chitosan, inoculation+sodium alginate) and storage durations (0 and 90 days). The plant root dry weight varied between 1.79 and 3.40 g. Seeds treated with bacteria and coated with sodium alginate biopolymer had the highest root dry weight of 3.40 g after 3 months of storage.

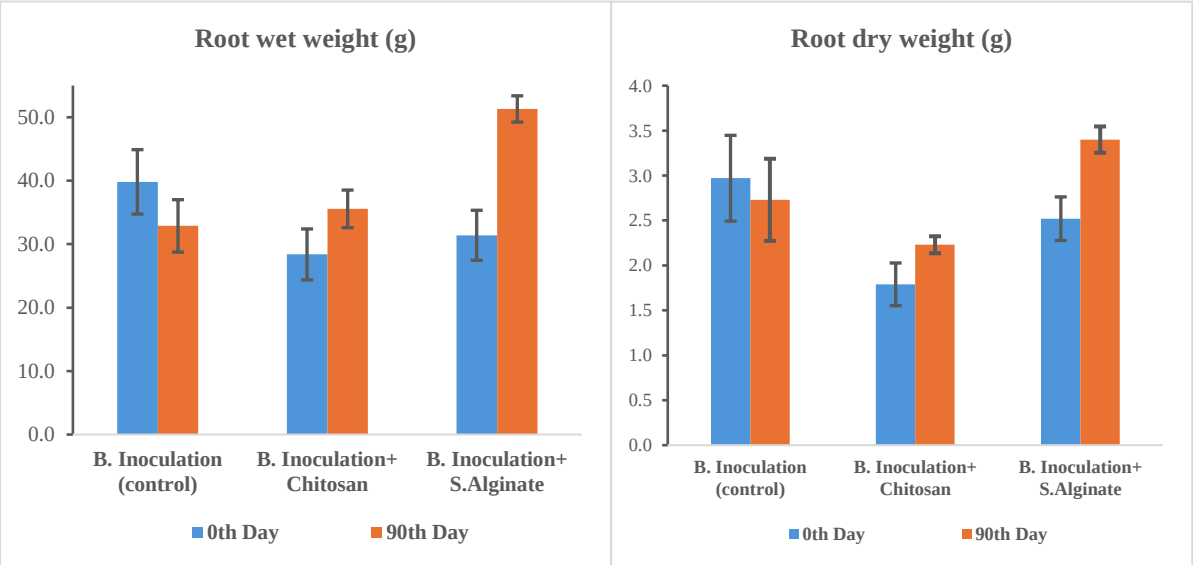

Figure 3. *Effects of storage time (0 and 90 days) and coating with biopolymers of chitosan and sodium alginate on the root wet and dry weight (g/plant) of bacteria-inoculated seeds of chickpea (Azkan).*

Chickpea seeds coated with chitosan biopolymer, incubated with bacteria, and planted without a waiting time obtained the lowest plant root dry weight (1.79 g). It was found that there was a statistically significant difference ($p<0.01$) in the number of nodules in the roots of the chickpea plant of chitosan and sodium alginate biopolymers coated on seeds inoculated with rhizobium bacteria between treatments (inoculated, inoculated+chitosan, and inoculated+sodium alginate) and the duration of storage (0 and 90 days). The number of nodules ranged from 8 to 27.33, and the highest number of nodules was 27.33 in the seeds inoculated with bacteria that were not coated with biopolymers and planted on the same day. Seeds inoculated with bacteria, coated with chitosan biopolymer, and planted after 90 days showed the lowest nodule numbers with 8 pcs. Applying chitosan and sodium alginate biopolymers to bacteria-inoculated chickpea seeds reduced bacterial viability and caused fewer nodules compared to the inoculated control treatment.

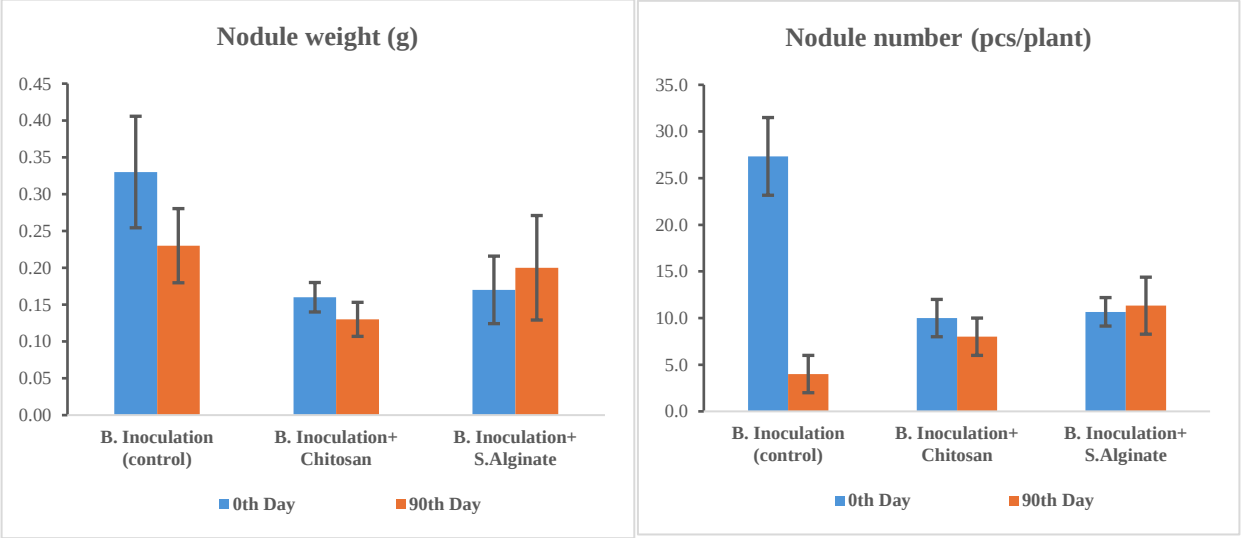

Figure 4. *Effects of storage time (0 and 90 days) and coating with biopolymers of chitosan and sodium alginate on the number (pcs/plant) and weight (g/plant) of nodules in the root of bacteria-inoculated seeds of chickpea (Azkan).*

Chitosan and sodium alginate biomaterials were believed to have initially decreased bacterial viability because of their antibacterial characteristics. It was noted that the seeds retained bacterial viability after being coated with biomaterials and were stored for 90 days. In the study to evaluate the effect of some coating biomaterials (chitosan and sodium alginate) and storage periods on the viability of bacteria inoculated on the surface of seeds, biopolymers applied to chickpea seeds and inoculated with bacteria caused important variations in nodule weights in the plant roots and these differences were statistically significant ($p<0.01$) through treatments although they were not significant between storage periods. The highest nodule weight recorded was 0.33 g because seeds containing bacteria were planted without a biopolymer that was covered and without pre-storage. The smallest nodule weight of 0.13 g was obtained from seeds treated with rhizobium bacteria, coated with chitosan biopolymer, and stored for 90 days. The application of chitosan and sodium alginate biopolymers to rhizobium inoculated seeds resulted in a reduction in bacterial viability and a decrease in the number of nodules present in the roots of plants grown from non-pre-stored seeds; consequently, nodule weights were found to be lower compared to the control treatment.

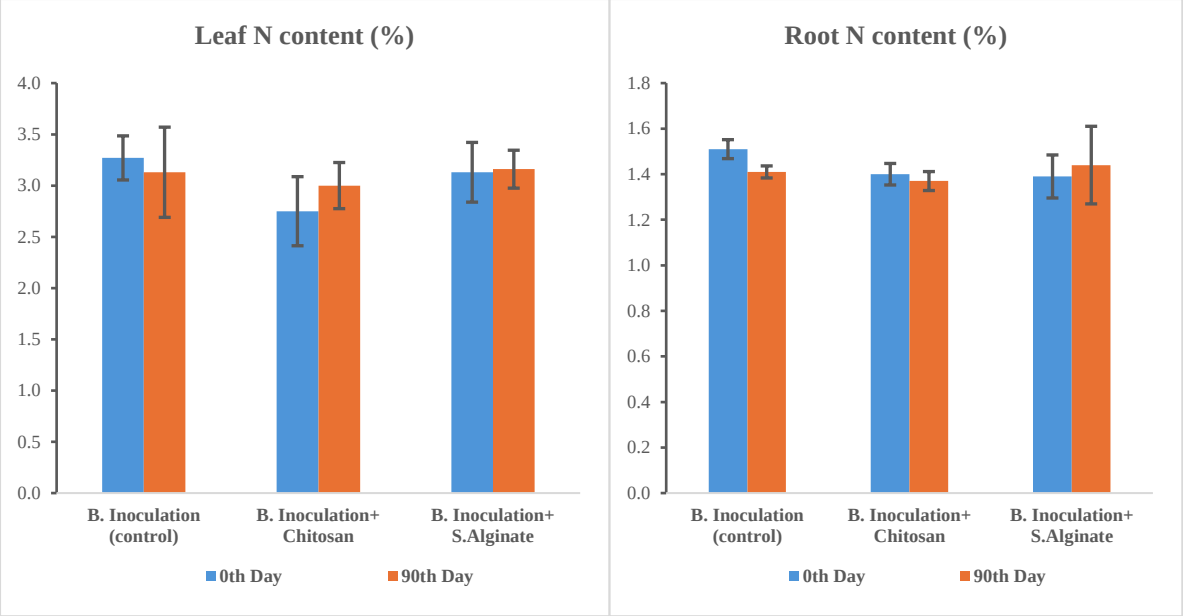

Figure 5. *The effect of chitosan and sodium alginate biopolymers coated on inoculated seeds, as well as the storage periods (0 and 90 days), on nitrogen content (%) in the upper part and root of chickpea (Azkan) plants*

In studies, biopolymers used in coatings enabled slow release of bacteria into the soil when degraded by soil microorganisms and protected microorganisms from biotic and abiotic stresses. Coating the seed surface with inoculated bacteria can increase cell viability during storage, and the coated bacterial cells can be released into the target environment slowly and controllably with long-term improvement effectiveness. This can have positive effects on chickpea plant development and nodulation values. An experiment was conducted to investigate the effect of chitosan and sodium alginate biomaterials on the surface of chickpea (Azkan) seeds inoculated with rhizobium bacteria, as well as the effect of these biomaterials on the shelf life of bacteria after three months of storage, and the nitrogen content in chickpea leaves. According to the data obtained from the research, the differences in nitrogen content were observed between treatments (inoculation, inoculation+chitosan, inoculation+sodium alginate), and these differences were found to be statistically insignificant. However, the effect of the storage duration on the nitrogen level in the plant leaves showed a variation, but it was not statistically significant. As can be seen in Table 1, the nitrogen content of Azkan chickpea plants varied between 2.75 to 3.27%. In this study, the highest nitrogen content of the chickpea plant was found to be 3.27% when the bacteria-inoculated seeds were planted without biopolymer coating and a storage period. In addition, 3.16% nitrogen content in chickpea plants was obtained by sowing seeds that had been inoculated with rhizobium bacteria, covered with sodium alginate, and then stored for 90 days.

The lowest nitrogen content of 2.75% was obtained when the bacteria-inoculated seeds were coated with chitosan biopolymer and then planted without a storage duration. The study found that covering bacteria-inoculated seeds with chitosan biopolymer and the storage duration had a lower effect on the nitrogen content of the upper portion of the plant compared to other biopolymers. According to the data obtained from the study, in the study investigating the effect of some coating biomaterials (chitosan and sodium alginate) on the shelf life of rhizobium bacteria inoculated into the seeds of the chickpea plant (Azkan), chitosan and sodium alginate biopolymers coated on chickpea seeds incubated with rhizobium bacteria

revealed differences in nitrogen content in the plant roots throughout different treatments and storage durations, although these differences were determined to be statistically insignificant. Nitrogen contents in the roots of Azkan chickpea plants were found to vary between 1.37 and 1.51%. The seeds inoculated with rhizobium bacteria had the highest nitrogen content (1.51%) in the roots of chickpea plants when planted without storage period and without biopolymer coating. On the other hand, this value was found in the seeds planted after the inoculated seed was coated with sodium alginate biopolymer and after 90 days of storage. The lowest nitrogen content in chickpea roots was 1.37% in bacterially inoculated chitosan-coated seeds planted after 90 days of storage.

DISCUSSION

According to the results obtained from the study, the results of the analysis of variance on some yield components and nodulation values of the chickpea plant of chitosan and sodium alginate biopolymers applied on chickpea seeds inoculated with rhizobium bacteria showed statistically significant ($p<0.05$ and 0.01) differences between treatments (inoculation, inoculation+chitosan, inoculation+sodium alginate) and storage periods for the length of the root, root wet and dry weight, plant wet and dry weight, number and weight of nodules in the Azkan chickpea variety, whereas the upper plant part length and the leaf and root nitrogen contents were found to be insignificant. Shcherbakova et al. [6] showed that treating chickpea and soybean seeds with *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 encapsulated with alginate microspheres significantly increased the number and weight of nodules. Costales et al. [22] investigated the effectiveness of a chitosan polymer on *Bradyrhizobium japonicum* seeds and its effects on seed viability, bacterial viability, and nodulation in soybean inoculation-polymer treatment throughout various storage durations. The polymer did not impact seeds' germination or bacteria's survival through the seeds. Chakraborty [23] the seeds coated with biodegradable biopolymer (chitosan or sodium alginate) after bacterial inoculation on the seed surface and stored for a long time (90 days) partially increased some yield components of the plants and nodulation of the root zone. Since chitosan and sodium alginate have antibacterial properties, it was thought that they may have initially reduced bacterial viability. However, chickpea seeds were found to maintain bacterial viability within 3 months of storage after coating with biomaterials. Jarecki [24] conducted a study demonstrating the efficiency of an advanced coating (chitosan/alginate/PEG) and a commercial inoculant applied alone or in combination with soybean seeds. The study revealed that planting seeds with a coating alone was ineffective and did not lead to the development of the required number of nodules on soybean roots. Encapsulation using degradable chitosan/alginate microparticles achieved bacterial protection in the experiment. Encapsulated samples maintained bacterial bioactivity. Alginate/chitosan components are increasingly being used in agriculture due to their availability and cost-effectiveness. Chitosan effectively aids pest control, fertilization, and nutrient absorption [25].

Table 1. Effect of coating chitosan and sodium alginate biopolymers onto seeds inoculated with rhizobium bacteria and storage periods on some yield elements and nodulation in chickpea variety Azkan.

Incubation period	Treatments	PUPL	RL	PUWW	PUDW	RWW	RDW	NW	NN	PUNC	RNC
		cm		g/plant					pcs	%	
0th Day	B. Inoculation (control)	26.67±0.58 n.s	29.56±1.26 n.s	12.38±1.09 ab	2.50±0.21 a	39.82±5.08 b	2.97±0.48 ab	0.33±0.08 n.s	27.33±4.16 a	3.27±0.22 n.s	1.51±0.04 n.s
	B. Inoculation+ Chitosan	24.67±1.00 n.s	27.33±1.86 n.s	10.56±0.69 bc	2.04±0.11 bc	28.39±4.01 d	1.79±0.24 d	0.16±0.02 n.s	10.00±2.00 bc	2.75±0.34 n.s	1.40±0.05 n.s
	B. Inoculation+ S.Alginate	22.67±0.33 n.s	28.56±0.38 n.s	8.93±0.78 c	1.85±0.18 c	31.40±3.95 cd	2.52±0.24 bc	0.17±0.05 n.s	10.67±1.53 bc	3.13±0.29 n.s	1.39±0.09 n.s
Mean		**24.67±1.83 n.s**	**28.48±1.49 n.s**	**10.62±1.67 n.s**	**2.13±0.33 n.s**	**33.20±6.39 B**	**2.43±0.59 b**	**0.22±0.10 n.s**	**16.00±8.85 A**	**3.051±0.34 n.s**	**1.43±0.08 n.s**
90th Day	B. Inoculation (control)	25.89±2.17 n.s	29.67±1.20 n.s	9.79±2.27 c	1.92±0.41 c	32.88±4.13 cd	2.73±0.46 bc	0.23±0.05 n.s	4.00±2.00 b	3.13±0.44 n.s	1.41±0.03 n.s
	B. Inoculation+ Chitosan	24.44±1.50 n.s	27.33±1.15 n.s	13.29±0.57	2.43±0.16 ab	35.56±2.97 bc	2.23±0.09 cd	0.13±0.02 n.s	8.00±2.00 c	3.00±0.23 n.s	1.37±0.04 n.s
	B. Inoculation+ S.Alginate	26.44±2.55 n.s	30.78±1.02 n.s	11.99±1.04 ab	2.22±0.15 abc	51.31±2.07 a	3.40±0.15 a	0.20±0.07 n.s	11.33±2.19 bc	3.16±0.19 n.s	1.44±0.17 n.s
Mean		**25.6±2.04 n.s**	**29.30±1.81 n.s**	**11.69±2.00 n.s**	**2.19±0.32 n.s**	**39.91±9.05 A**	**2.79±0.57 a**	**0.19±0.06 n.s**	**11.11±3.33 B**	**3.10±0.27 n.s**	**1.41±0.09 n.s**
LSD (5% & 1%)		**2.80 n.s**	**2.18 n.s**	**2.16 ****	**0.40 ****	**6.80 ****	**0.55 ***	**0.09 n.s**	**4.65 ****	**0.53 n.s**	**0.15 n.s**
CV %		**6.27**	**4.24**	**10.87**	**10.46**	**10.45**	**11.94**	**25.52**	**19.28**	**9.60**	**6.06**
General Mean		**25.13**	**28.87**	**11.16**	**2.15**	**36.55**	**2.61**	**0.20**	**13.56**	**3.08**	**1.42**
Inoculation		26.28±1.48 n.s	29.61±1.10 A	11.10±2.13 n.s	2.21±0.44 n.s	36.35±5.62 B	2.85±0.44 A	0.28±0.08 A	20.67±7.87 A	3.20±0.32 n.s	1.46±0.06 n.s
Chitosan		24.56 ±1.15 n.s	27.33±1.38 B	11.93±1.60 n.s	2.24±0.25 n.s	31.98±5.03 B	2.01±0.29 B	0.15±0.02 B	9.00±2.10 B	2.88±0.29 n.s	1.39±0.04 n.s
S. Alginate		24.56 ±2.63 n.s	29.67±1.40 A	10.46±1.87 n.s	2.04±0.25 n.s	41.35±11.26 A	2.98±0.52 A	0.18±0.07 B	11.00±2.19 B	3.15±0.22 n.s	1.42±0.13 n.s

* $p<0.05$, ** $p<0.01$: the differences between groups at the 5% and 1% levels according to the LSD test. N.S: Not Significant.
S.Alginate: Sodium Alginate, PUPL: Plant upper part length, RL: Root length, PUWW: Plant upper part wet weight, PUDW: Plant upper part dry weight, RWW: Root wet weight, RDW: Root dry weight, NN: Nodule number, NW: Nodule weight, PUNC: Plant upper part nitrogen content, RNC: Root nitrogen content

Alginate is a polyuronic acids isolated from the cell walls of brown seaweed species (*Phaeophyceae*) and synthesized as an extracellular matrix by certain soil bacteria (*Azotobacter vinelandii* and *Pseudomonas aeruginosa*) [26]. Alginate is the most widely used biopolymer for microorganisms [14]. As a result, it was observed that inoculating Azkan chickpea plants with rhizobium bacteria, coating them with some biopolymers (chitosan and sodium alginate), and storing the inoculated and coated seeds were effective in increasing the yield components of the plants, providing nitrogen requirements, and preventing nodule formation. Because natural biopolymers have the good film-forming ability, they can form a dense protective film on the surface layer, allowing seeds to absorb more nutrients. Since the polymers have good permeability, they can increase the activity of certain enzymes in the seeds.

CONCLUSION

The results indicated that biopolymers significantly decreased bacterial viability compared to the inoculated control, which was obtained after inoculation with chickpea seeds, chitosan, and sodium alginate. Bacterial viability decreased as storage time increased in the chitosan treatment, whereas it increased significantly in the sodium alginate treatment depending on storage time. Our study's results suggest that applying chitosan and sodium alginate biopolymers onto inoculated chickpea seeds led to differences in certain yield components and nodulation values of the plants. Furthermore, these biopolymers were responsible for both increases and decreases in these plant components. Therefore, the application of a biodegradable biopolymer coating (chitosan or sodium alginate) to chickpea seeds after bacterial inoculation on the seed surface and extended storage (90 days) led to a partial increase in certain yield components and root zone nodulation. Since alginates are extracted from brown seaweed species, they may have shown microbial interactions with rhizobium bacteria. In addition, it was observed that after coating with biomaterials, especially the sodium alginate biopolymer, the population of rhizobium bacteria was maintained by about 50% within 3 months of storage.

REFERENCES

[1] Malusa, E., Vassilev, N. (2014): A contribution to set a legal framework for biofertilizers. Applied Microbiology Biotechnology, 98:6599.

[2] Kaur, R., Kaur, S. (2018): Biological alternates to synthetic fertilizers: Efficiency and future scopes. Indian Journal of Animal Research, 52(6), 587–595. https://doi.org/10.18805/IJARe.A-5117.

[3] John, R.P., Tyagi, R.D., Brar, S.K., Surampalli, R.Y., Prevost, D. (2011): Bio-encapsulation of microbial cells for targeted agricultural delivery. Critical Reviews in Biotechnology, 31 (3):211–226.

[4] Bashan, Y. (1998): Inoculants of plant growth-promoting bacterium for use in agriculture, Biotechnology Advances, 4: 729–770.

[5] Jung, G., Mugnier, J., Diem, H.G., Dommergues, Y.R. (1982): Polymer entrapped rhizobium as an inoculant for legumes, Plant Soil 65: 219–231.

[6] Shcherbakova, E.N., Shcherbakov, A.V., Rots, P.Y., Gonchar, L.N., Mulina, S.A., Yahina, L.M., Chebotar, V.K. (2018): Inoculation technology for legumes based on alginate encapsulation. Agronomy Research, 16(5), 2156–2168.

[7] Lizárraga-Paulín, E.G., Torres-Pacheco, I., Moreno-Martínez, E., Miranda-Castro, S.P. (2011): Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level. African Journal of Biotechnology, 10: 6439-6446.

[8] Salachna, P., Zawadziñska, A. (2014): Effect of chitosan on plant growth, flowering and corms yield of Potted freesia. Journal of Ecological Engineering, 15 (3): 97–102.

[9] Al Tawaha, A.R.M., Al-Ghzawi, A.L.A. (2013): Effect of chitosan coating on seed germination and salt tolerance of lentil (*Lens culinaris* L.). Research on Crop, 14:489-491.

[10] Mahdavi, B., Rahimi, A. (2013): Seed priming with chitosan improves the germination and growth performance of ajowan (*Carumc opticum*) under salt stress. EurAsian Journal of BioSciences, 7: 69-76.

[11] Pongprayoon, W., Roytrakul, S., Pichayangkura, R., Chadchawan, S. (2013): The role of hydrogen peroxide in chitosan-induced resistance to osmotic stress in rice (*Oryza sativa* L.). Plant Growth Regulation, ISSN 0167-6903.

[12] Suvannasara, R., Boonlertnirun, S. (2013): Studies on appropriate chitosan type and optimum concentration on rice seed storability. Asian Research Publishing Network (ARPN) Journal of Agricultural and Biological Science, 8. ISSN 1990-6145.

[13] Mahdavi, B. (2016): Effects of priming treatments on germination and seedling growth of anise (*Pimpinella anisum* L.). Journals Agriculture Science Developments, 5 (3): 28-32.

[14] Cassidy, M. B., Lee, H., Trevors, J.T. (1996): Environmental applications of immobilized microbial cells: a review. Journal of Industrial Microbiology, 16(2), 79–101.

[15] Zeng, D.F., Zhang, L. (2010): A novel environmentally friendly soybean seed-coating agent. Acta Agricultural Scandinavica, Section B. Soil & Plant Science, 60, 545–551.

[16] Rocha, I., Ma, Y., Souza-Alonso, P., Vosátka, M., Freitas, H., Oliveira, R.S. (2019): Seed coating: A tool for delivering beneficial microbes to agricultural crops. Frontiers in Plant Science, 10(November). https://doi.org/10.3389/fpls.2019.01357.

[17] Somasegaran, P., Hoben, H.J. (1994): Handbook for Rhizobia: Methods in Legumes-Rhizobium Technology. Springer-Verlag, New York, Inc., 450. https://doi.org/10.1007/978-1-4613-8375-8.

[18] Meng, X., Tian, F., Yang, J., He, C.N., Xing, N., Li, F. (2010): Chitosan and alginate polyelectrolyte complex membranes and their properties for wound dressing application. J. Mater. Sci. Mater. Med. 21, 1751–1759.

[19] Gierszewska, M., Ostrowska-Czubenko, J., Chrzanowska, E. (2018): pH-responsive chitosan/alginate polyelectrolyte complex membranes reinforced by tripolyphosphate. Eur. Polym. J. 101, 282–290.

[20] Florczyk, S.J., Kim, D., Wood, D.L., Zhang, M. (2011): Influence of processing parameters on pore structure of 3D porous chitosan–alginate polyelectrolyte complex sca_olds. Hournal Biomater. Med. Res. Part A. 98, 614–620.

[21] Sonneveld, C., Straver, N. (1989): Nutrient solution for vegetables and flowers grown in water or substrates. Serie: Voedingsoplossingen glastuinbouw, Proefstation voor Tuinbouw onder Glas, Naaldwijk, Proefstation voor Bloemisterij, Aalsmeer, Consulentschap voor de Tuinbow, vol. 8, 35 pp.

[22] Costales, D., Nápoles, M.C., Alejandro, F.R., Gustavo, G.A., Cecilia, P., Susana, S., Perrig and Diego Group of Bioactive Products, De. (2019): Effect of chitosan polymer and inoculated with *Rhizobium japonicum* on soybean germination survial of seedling, nodulation and bacteria viability on seeds. Legume Research, 42(2), 265–269. https://doi.org/10.18805/LR-410.

[23] Chakraborty, A.P. (2020): Carrier Based Bioformulations of PGPR- Characteristics, shelf life and application in ımproving health status of crop plants- A mini review. 7(April), 88–98.

[24] Jarecki, W. (2021): Soybean response to seed coating with chitosan + alginate/PEG and/or inoculation. Agronomy, 11, 1737. https://doi.org/10.3390/ agronomy11091737.

[25] Juri´c, S. (2020): Bioencapsulation as a sustainable delivery of active agents for plant nutrition/protection and production of functional foods. Doctoral Thesis, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia.

[26] Khong, T.T., Aarstad, O.A., Skjåk-Bræk, G., Draget, K.I., Vårum, K.J. (2013): Gelling concept combining chitosan and alginate proof of principle. Biomacromolecules,14, 2765−2771.