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Efficiency of Nano-chitosan and Azotobacter on growth and yield of kohlrabi plant

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ABSTRACT

The research was conducted in one of the fields of the Department of Plant Production in the desert region (tomato development project) affiliated with the Directorate of Agriculture of the Holy Karbala Governorate for the purpose of studying the effect of spraying the shoots of the Kohlrabi plant (hybrid) with the Nano biopolymer (Chitosan) and the *Azotobacter* bio inoculum on the roots of the plant seedlings in terms of growth indicators and yield for the autumn agricultural season. 2023-2024 ,The means were compared according to the Duncan multiple ranges test at significant to level of 0.05.with R.C.B.D . The first factor was spraying the shoots with Nano-Chitosan at a concentration of (0, 1, and 2) g L^{-1}, two weeks after transplanting, and two sprays every 14 days. The second factor has three levels, the first is planting without a vaccine, the second is inoculating the seedling roots with *Azotobacter* at a level of 5 g , and the third level is inoculating with 10 grams of the biological factor. The results showed a significant superiority of the interaction treatment between the bio-addition of *Azotobacter* at a level of 5 g .and a concentration of 2 g of Nano-chitosan in the characteristics of each of the plant length 60 cm, the number of leaves 21.67 leaves. $plant^{-1}$the leaf content of chlorophyll 77.59 mg 100 g $^{-1}$, The Weight of the knobs cm $plant^{-1}$ 278.9 g , the total yield 14.87 ton . h $^{-1}$, content of nitrogen 3.43% and the knobs content of protein 21.48% compared to the control treatment, which gave the lowest value.

كفاءة الكيتوسان النانوي و الازوتوباكتر في نمو وإنتاجية نبات الكل

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الخلاصة

أُجري البحث في أحد حقول قسم الإنتاج النباتي في المنطقة الصحراوية (مشروع تنمية الطماطة) التابع لمديرية زراعة محافظة كربلاء المقدسة لغرض دراسة تأثير رش نبات الكلم (الهجين) بالبوليمر الحيوي النانوي (الكيتوسان) والملقح الحيوي الازوتوباكتر على جذور شتلات النبات في مؤشرات النمو والحاصل للموسم الزراعي الخريفي 2023-2024، وقورنت المتوسطات وفق اختبار دنكن متعدد المدى عند مستوى معنوي 0.05 ، وكان العامل الأول هو رش النباتات بالنانو كيتوسان بتركيز (0، 1، و2) غم لتر$^{-1}$، بعد أسبوعين من الزراعة، ورشتين كل 14 يومًا. العامل الثاني له ثلاثة مستويات، الأول الزراعة بدون لقاح، والثاني تلقيح جذور الشتلات الازوتوباكتر بتركيز 5 غم، والثالث تلقيحها بـ 10 غم من العامل الحيوي. وقد أظهرت النتائج تفوقًا معنويًا لمعاملة التفاعل بين الإضافة الحيوية للآزوتوباكتر بتركيز 5 غم وتركيز 2 غم من النانو كيتوسان في صفات كل من طول النبات 60 سم، وعدد الأوراق 21.67 ورقة نبات$^{-1}$، ومحتوى الأوراق من الكلوروفيل 77.59 ملغم/100 غم$^{-1}$، ووزن الكورمات غم نبات$^{-1}$ 278.9 غم، والمحصول الكلي 14.87 طن/هـ$^{-1}$، ومحتوى النيتروجين 3.43%، ومحتوى الكورمات من البروتين 21.48%، وذلك مقارنةً بمعاملة المقارنة التي أعطت أقل قيمة.

الكلمات المفتاحية: السماد الحيوي ، ازوتوباكتر ، الكايتوسان النانوي ، الكلم .

INTRODUCTION

Plants of the vegetable crop family gain the utmost importance in the human diet because of the nutritional and health value they represent, including the Kohlrabi ***Brassica oleracea var gongylodes*** , which belongs to the *Brassicaceae* family, as the edible part of the plant is the swollen stem, and the plant contains mineral elements, vitamins, effective compounds beneficial to public health, and antioxidants. the plant has several varieties, including local ones that are elongated and other types that are balled. Its uses are in cooking, salads, or making pickles (Ali and Khalid, 2023).

The steady increase in population growth in the world has led to an increase in demand for food products to meet the actual need (Al-Mosawy, 2023), which has led agricultural producers to excessive use of chemical fertilizers in addition to the use of intensive agriculture by exploiting the entire land area, which has led to the deterioration of the soil's nutritional stock by a rate approaching between (5-50) %. The increase in levels of chemical fertilizers has led to the accumulation of harmful compounds in the human body as a result of consumption and the emergence of health problems as a result of that accumulation (Turk *et al*, 2020). Also, the use of bio fertilizers, which depends on the use of natural biological systems to transfer important nutrients to the plant without resorting to harmful chemical fertilizers, with the aim of maintaining the level of production so that the food becomes healthy and safe (Mohammed *et al*, 2017).

The need for the emergence of biological alternatives, including bio fertilizers, aims to reduce the use of traditional fertilizers. Organic farming reduces environmental pollution

problems and works to sustain agriculture by increasing metabolic activities to revive soil microbiology (Ahmed, 2016), which contributes to providing the plant with elements such as phosphorus, which also plays a major role in the synthesis of energy-rich compounds that the plant acquires to build carbohydrates and enzymes (Taiz and Zeiger, 2014). Microorganisms provide a suitable environment for activating the vital functions of plants, which increases vegetative growth , These organisms are more beneficial to the soil than modern fertilizers, as they contribute to increasing the soil's mineral and ion content and improving the chemical condition, in addition to the metabolic activities they perform. (Al-Sharia, 2022). They also contribute to increasing the concentration of nitrogen in the soil content and thus accelerating plant growth. *Azotobacter* bacteria contribute to improving the root environment and work to provide the root zone with important nutrients for the purpose of increasing the plant's absorption capacity through the readiness of these ions in the soil solution (Al-Bayati *et al*, 2023).

Clean agriculture works to manage natural biological systems in the transfer of important nutrients to plants without resorting to harmful chemical fertilizers, with the aim of maintaining the level of production and keeping plants free of pollutants so that food becomes healthy and safe (Sabalpara and Lalit, 2016). It's also achieves increased production and environmental protection to produce healthy and safety food (Turk., 2023).

The *Azotobacter chroococcum* bacteria It is characterized by its ability to fix nitrogen, in addition to acting as growth promoters, as it has a role in increasing the growth and productivity of plants (Turan *et al*., 2006). Bio fertilizers are used to reduce the addition of chemical fertilizers by no less than 25%, in addition to their role in reducing Environmental pollution problems and works to sustain agriculture (Al-Juthery *et.al*, 2020). Diversifying the sources of organic fertilizers has prompted specialists in the agricultural fields to search for alternatives that can be used in organic agriculture, and among those alternatives are the waste of mollusks and other aquatic organisms and the exoskeletons of crustaceans through the production of organic material (AL- Malikshah.2023.)

Chitosan has been used in several agricultural experiments and has shown clear superiority in vegetative indicators and yield, and these The material is Chitosan polymer (Sathiyabama *et al*, 2014).which is one of the substances found in the cell walls of the exoskeletons of arthropods, fungi, crustaceans, crabs, shrimp, and fish scales. Nano technology was used to produce this compound in its Nano form, which works to increase efficiency and effectiveness while increasing the surface area of the material so that it is added in a slow and easy release method, which provides a continuous supply of nutrients (Vurukonda *et al* , 2018). it has been widely used in the fields of biomedicine, biotechnology and agricultural engineering (Hidangmayum *et al* , 2019). The polymer also works to resist dryness through control It helps close stomata and reduce the water level in the leaves (Naeem *et al*, 2020) This substance helped induce resistance to diseases and stimulate the plant to produce effective, useful enzymes. For plants (Chun and Chandrasekaran, 2019),. It is also used to combat rot on fruit during canning and exporting from by covering the fruits with a thin layer of it (pan *et al* 2022). It is a biopolymer that can be obtained from Chitin (Abdulrasool and Al-Malikshah, 2022) Chitosan is known as a straight-chain copolymer

consisting of D-glucosamine and N-acetyl -D-glucosamine linked by β-type bonds (Al-Temimi, 2020)

Therefore, the research aimed to: test the efficiency of the best combination of Chitosan Nano fertilizer with the ground biological addition of *Azotobacter* bacteria for the purpose of increasing the vegetative indicators and yield of Kohlrabi plants.

MATERIALS AND METHODS

An experiment was conducted during the fall semester of 2023 in one of the fields of the Plant Production Department in the desert region (the Tomato Development Project) of the Agriculture Directorate of the Holy Karbala Governorate. The seeds of the (hybrid) variety were planted on September 20, 2023, and care continued until seedlings with four true leaves were obtained. Kohlrabi seedlings were transferred to the field on October 25, 2023. The purpose of the research is to study the effect of two factors. The first factor is contamination of the roots of the plant seedlings with three levels of the *Azotobacter* biological additive (5and10)grams. A plant added to the no-addition treatment (Ahmad,2024). The second factor is spraying the plant's foliage afterb14bdays of transplantation with Nano-Chitosan at a concentration of (0, 1, and 2) grams. L^{-1}. After two weeks of transplanting , two sprays every 14 days until complete wetness, using a portable sprayer with a capacity of 16 liters, drops of Sodium Tripolyphosphate were added as a dispersing agent to reduce the phenomenon of surface tension. The experiment followed a Randomized Complete Block Design (RCBD) with two factors and three different replications. with three replications and two factors (AL-Rawi and Khalf Allah,2000). The number of transactions reached 27 experimental units. The means were compared according to Duncan's multiple range test at the probability level of 0.05. The seedlings were planted on terraces, each terrace width 75 cm and the distance between each terrace was 50 cm to isolate the treatments. The planting distance was 60 cm between one seedling and another and on both sides of the terrace to adopt dense planting . The length of each experimental unit was 3 m. The irrigation process was carried out using the drip irrigation method with 12 plants in the experimental unit.. The irrigation process was carried out using the drip irrigation method. Five samples of field soil were taken from different places before planting, at different depths between 10-30 cm, then mixed well and a sample was taken to one of the private scientific laboratories for analyses. The results were as shown in Table (1).

Table (1) Some physical and chemical characteristics of field soil

Type of analysis	Unit of measurement	measurement
pH	—	7.8
EC	$ds^{-1}.m$	3.41
N	$mg.kg^{-1}$	17
P	$mg.kg^{-1}$	4.3
K	$mg.kg^{-1}$	59.9
Na^{+}	$mg.l^{-1}$	298.8
SO_3	$meq.l^{-1}$	573.7
Cl^{-}	$mg.l^{-1}$	339
Ca^{++}	$mg.l^{-1}$	229.42

The research included measuring the following indicators

1- The height of the plant (cm)

The lengths of the plants were measured from the soil surface to the end of the growths with a metric ruler and the average was taken.

2- Number of leaves per sheet. Plant^{-1}

The number of leaves per plant was calculated by averaging five plants.

3- Total Chlorophyll content of leaves (mg 100 g $^{-1}$)

Five plants were randomly selected. The leaves were taken from them and after washing them well from the dust (Al-Sahaf, 1989) . Using with acetone mash, a clear, white extract was obtained. Using a Spectrophotometer, optical absorption was measured at two wavelengths: 645 and 663, and the amount of chlorophyll was calculated according to the following equation.

[20.2 x D (645)] + [8.02 x D (663)] (v / w x 1000) x100 = Total chlorophyll

4- The Weight of the knobs g plant^{-1}

The knobs of five plants in each experimental unit were weighed and the average was taken

5- Total marketable yield

Small, infected and deformed knobs were excluded from the plant yield, and the remaining knobs were then calculated as a marketable yield according to the following:

$$\text{Total marketable yield (tons.h}^{-1}) = \frac{\text{Experimental unit yield} \times 10{,}000\ m^2}{\text{Experimental unit area}}$$

6- Percentage of nitrogen content in leaves: %

Total nitrogen (N) was determined by distillation using a Microkaldal.(Jackson,.1958)

$$N\% = \frac{\text{The volume of acid consumed by elution x the standard of acid x 14 x the volume of dilution}}{\text{Volume of sample taken upon distillation x weight of digested sample} \times 1000} \times 100$$

7- Protein of knobs %

The percentage of protein in the knobs was calculated on the basis of dry weight according to the following equation:

Protein percentage based on dry weight = percentage of nitrogen in knobs × 6.25

Then it was calculated based on the wet weight as in the following equation:

$$\text{\% of protein based on wet weight} = \frac{\text{\% of protein based on dry weight} \times \text{\% of dry matter in knobs}}{100}$$

(A.O.A.C, 1980)

RESULTS AND DISCUSSION

Table (2) shows the superiority of the treatment of inoculating the roots of the Kohlrabi seedlings with the *Azotobacter* at a level of 10 g in the plant height trait, as it gave the highest height of 47.33 cm. As for the effect of the single factor, spraying Nano chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the rate of plant height, reaching 54.56 cm compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi seedlings with the biological vaccine at a level of 5 g

and spraying nano chitosan at a concentration of 2 g L^{-1} in the plant height trait, as it gave the highest height of 60 cm compared to the untreated plants, which gave 34.33 cm .

Table (2) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant seedlings with the biological vaccine at a level of 5 g in the number of leaves in the plant, as it gave the largest number 17 leaves . As for the effect of the single factor, spraying Nano chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the average number of plant leaves, reaching 19.56. compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi plant seedlings with the biological vaccine with spraying Nano chitosan in the number of leaves trait, as it gave the largest number of 21.67 . compared to the untreated plants, which gave the least number of plant leaves 9.33.

Table (2) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant seedlings with the bio- vaccine at a level of 10 g in the characteristic of the leaf content of chlorophyll as it gave the highest content 67.68. mg. 100 g $^{-1}$ As for the effect of the single factor, spraying Nano-chitosan at a concentration of 2g L^{-1}, it showed a significant superiority in the leaf content of chlorophyll mg. 100 g $^{-1}$ 74.16 compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi plant seedlings with the bio-inoculate at a level of 5 g and spraying Nano-chitosan at a concentration of 2 g L^{-1} in the characteristic of the leaf content of chlorophyll as it reached 77.59 mg. 100 g $^{-1}$ compared to the untreated plants, which gave the lowest values 51.84 .

Table (3) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant seedlings with the biological vaccine at a level of 10 g In terms of plant weight, as it gave the largest corm weight 197.8 g As for the effect of the single factor, spraying Nano-chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the corm weight reaching 251.3 g. compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi plant seedlings with the biological vaccine at a level of 5 g and spraying Nano-chitosan at a concentration of 2 g L^{-1} in the corm weight trait, as it reached 278.9 g . compared to the untreated plants, which gave the lowest values 78.4 g.

Table (3) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant with the biological vaccine at a level of 10 g in the total yield characteristic of , as it gave the highest yield of 10.54 ton. ha^{-1}. As for the effect of the single factor, spraying Nano-chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the total yield of , as it gave the highest yield of 13.40 ton. ha^{-1} compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi plant with the biological vaccine at a level of 5 g and spraying Nano-chitosan at a concentration of 2 g L^{-1} in the total yield characteristic of as the yield reached 14.87 ton. ha^{-1}compared to the untreated plants, which gave the lowest values 4.18 ton. ha^{-1} .

Table (3) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant with the bio-vaccine at a level of 5 g in the characteristic of the nitrogen content of the leaves, as it gave the highest content of 2.74 % . As for the effect of the single factor, spraying Nano-chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the nitrogen content of the leaves, as it gave the highest content of 3.10 %. compared to the comparison treatment. While the interaction between inoculating the roots of

the Kohlrabi plant with the bio-inoculate at a level of 5 g and spraying Nano-chitosan at a concentration of 2 g L^{-1} in the characteristic of the nitrogen content of the leaves, as it reached 3.43 %. compared to the untreated plants, which gave lowest values 1.92% .

Table (3) shows the superiority of the treatment of inoculating the roots of the Kohlrabi plant with the biological vaccine at a level of 5 g in the characteristic of the protein content of the knobs %, as it gave the highest content of 17.14%. As for the effect of the single factor, spraying Nano chitosan at a concentration of 2 g L^{-1}, it showed a significant superiority in the protein content of the knobs as it gave the highest content of 19.42% . compared to the comparison treatment. While the interaction between inoculating the roots of the Kohlrabi plant with the biological vaccine at a level of 5 g and spraying Nano chitosan at a concentration of 2 g L^{-1} in the characteristic of the protein content of the knobs 21.48 %, as it reached compared to the comparison treatment, which gave 12.04 %.

Table (2) The effectiveness of spraying with Nano-Chitosan and inoculating the roots of Kohlrabi seedlings with *Azotobacter* on Growth traits of plant and chlorophyll

Treatment			height of plant cm^{-1}		Number of leaves Leaves. Plant $^{-1}$		Chlorophyll content of leaves (mg 100 g^{-1})	
Average of Azotobacter g $plant^{-1}$		0	41.67	b	14.67	b	61.51	c
		5	47.11	a	17.00	a	66.33	b
		10	47.33	a	16.44	a	67.68	a
L.S.D			1.407		0.813		0.803	
Average of Chitosan g L^{-1}		0	37.11	c	11.56	c	55.37	c
		1	44.44	b	17.00	b	65.99	b
		2	54.56	a	19.56	a	74.16	a
L.S.D			1.407		0.813		0.803	
Azotobacter g $plant^{-1}$ X Chitosan g L^{-1}	0	0	34.33	g	9.33	f	51.84	h
		5	36.67	g	11.67	e	54.89	g
		10	40.33	f	13.67	d	59.38	f
	1	0	42.67	ef	15.67	c	61.85	e
		5	44.67	de	17.67	b	66.50	d
		10	46.00	cd	17.67	b	69.61	c
	2	0	48.00	c	19.00	b	70.84	c
		5	60.00	a	21.67	a	77.59	a
		10	55.67	b	18.00	b	74.06	b
L.S.D			2.436		1.408		1.391	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

Table (3) Effectiveness of spraying with Nano-Chitosan and inoculating the roots of Kohlrabi seedlings with *Azotobacter* on quality traits and plant yield

Treatment			The Weight of the knobs cm plant^{-1}		The total yield (ton. h^{-1})		Percentage of nitrogen content in leaves: %		Protein content of knobs %	
Average of Azotobacter g plant^{-1}	0		156.9	c	8.36	c	2.45	c	15.33	c
	5		191.5	b	10.21	b	2.74	a	17.14	a
	10		197.8	a	10.54	a	2.64	b	16.54	b
L.S.D			1.161		0.064		0.092		0.577	
Average of Chitosan g L^{-1}	0		107.7	c	5.74	c	2.03	c	12.72	c
	1		187.1	b	9.97	b	2.70	b	16.88	b
	2		251.3	a	13.40	a	3.10	a	19.42	a
L.S.D			1.161		0.064		0.092		0.577	
Azotobacter g plant^{-1} x Chitosan g L^{-1}	0	0	78.4	i	4.18	h	1.92	f	12.04	f
		5	109.2	h	5.82	g	1.98	f	12.37	f
		10	135.6	g	7.23	f	2.20	e	13.75	e
	1	0	157.6	f	8.40	e	2.52	d	15.77	d
		5	186.3	e	9.93	d	2.81	bc	17.56	bc
		10	217.4	d	11.59	c	2.76	c	17.29	c
	2	0	234.8	c	12.52	b	2.91	bc	18.19	bc
		5	278.9	a	14.87	a	3.43	a	21.48	a
		10	240.3	b	12.81	b	2.97	b	18.58	b
L.S.D			2.011		0.110		0.160		0.999	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

We note from the tables (2) that spraying with the Nano-polymer chitosan significantly improves the characteristics (Tall of plant , number of leaves, chlorophyll content of the leaves). This may be attributed to the biological and Nano-components of the polymer, and this may contribute to the integration of cell division and recovery. Photocells reflect positive signals (Al-Khafaji , 2023) . The results showed a clear effectiveness of adding the bio fertilizer *Azotobacter*, which is attributed to the effectiveness of these organisms in the soil and the provision of a suitable environment in the area around the roots, in addition to the abundance of nutrients as a result of their metabolic processes. This has encouraged an increase in the efficiency of the roots and has been reflected in the growth of the root system and the size of the corm, and this is consistent with (Ahmad, 2024).

Tables 3 show a clear increase in the nitrogen content of the leaves and protein content of knobs. This may be due to the effectiveness of the biopolymer and its Nano property, as the polymer penetrates the cellular membranes easily and slowly. It has contributed to an increase in the synthesis of the chlorophyll molecule (Elshamy,2019), the basis of which is the porphyrin ring, thus increasing the efficiency of metabolism. Photosynthesis in plants, and the effectiveness and activity of microorganisms may have helped provide phosphorus to the roots, and this reflected positively on growth and yield (Fanaei *et al*., 2015 ,Caddell,

2019). The interaction between the two factors may have contributed to pushing the plant to grow and increasing yield because of their effect on the physiological aspect of the plant through the accumulation of organic matter and the availability of nutrients in the soil solution and the plant, and thus affects the biomass of the plant, and it reflects positively on the rest of the growth characteristics and yield. (Ahmad *et al*., 2024).

Treatment with polymer nanoparticles has contributed to an increase in most vegetative growth indicators (plant height, number of leaves, leaf area, corm diameter). The reason for this may be attributed to the role of the Nano polymer because it contains the main and secondary elements, being an organic chitin us compound that contributes to the plant's vital activities and resistance to conditions. Environmental conditions, and may have stimulated the production of plant hormones such as cytokinin, gibberellin, and auxin (Mady, 2014). These compounds cause the plant to divide and elongate cells, thus leading to an increase in growth, and this in turn reflects positively on increased branching and the size of vegetative growth .

The Nano state of the compound is one of the factors of slow and continuous release, and this may have contributed to the longer-term supply of micronutrients, as nanoparticles are involved in the formation of proteins and amino acids and in the porphyrin ring that forms the chlorophyll molecule, which is one of the most important factors for plant growth and health, (Singh and Singh. 2008).

The use of chitosan polymer in its Nano form may contribute to improving the availability of phosphorus to the plant, which improves the condition Nutritional status of the plant and prevents The loss of nutrients in the soil also contributes to an increase in the activity of microorganisms in it.(Al-Malikshah**,** 2023).

CONCLUSIONS

The sprayed with Nano-chitosan and inoculating the roots of the Kohlrabi plant with the biological vaccine (*Azotobacter*) with their interaction showed clear superiority in growth indicators, effectiveness in plant growth and improving soil parameters as a result of being organic materials first and being in the Nano state second.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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