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Effect of nanochitosan and *Trichoderma harzianum* on some growth parameters of *Borago officinalis* L.

J. H. Muttair , F.h.R. Al-miahy

[1,2,] Department of Horticulture, College of Agriculture and Marshlands, University of Dhi Qar, Iraq.

[1]*E_mail: jaber.post@utq.edu.iq*

[2]*E_mail: Falah-ha@utq.edu.iq*

Abstract

A field experiment was conducted in one of the fields of Al-Rifai District - Thi Qar Governorate during the 2024-2025 season to estimate the influence of both nanotechnologies (such as nanochitosan) and Biofertilizers (such as *Trichoderma harzianum*) on some growth parameters of *Borago officinalis* L. . The experiment was designed according to a split-plot design and RCBD design with three replicates. The main factor included the addition of three levels of *Trichoderma harzianum* (0, 5, 10) g. plant-1, and the secondary factor included three levels of nanocytosan (0, 100, 200, 300) mg.L-1. The results showed that there is a positive and significant effect of the two-way interaction between Nanochitosan (300) mg.L-1 and *Trichoderma harzianum* (10) g. plant-1 on most growth indicators, including the trait of increase in plant height at a rate of (65.70) cm. plant-1, as well as the trait of total chlorophyll content of leaves at a rate of 55.00 SPAD units, as well as the trait of the number of branches at a rate of (15.67) branches. plant-1, as well as the trait of the number of flowers at a rate of (3596) flowers. plant-1. As well as the leaf area trait at a rate of (140.62) cm2, as well as the number of leaves trait at a rate of (92) leaves. plant-1, as well as the stem diameter trait at a rate of (2.137) cm. plant-1, the results also indicated that the nano fertilizer treatment and the biofertilizer treatment, each separately, significantly increased most of the measured plant traits compared to the control treatment. From the results, it can be concluded that the use of nanocytosan and Trichoderma harzianu together was effective in increasing the growth indicators of the borage plant.

Keywords. nano-chitosan, Trichoderma, borage plant, Borago officinalis L.

I.Introduction

Borage is a medicinal plant belonging to Boraginaceae family. United Kingdom, New Zealand, and Canada are among the world's largest producers of borage. The plant is native to the Mediterranean region, the plant's various parts are used in traditional medicine to treat coughs and infections such as pneumonia and others, and also as a sedative. Borage infusion treats bronchitis, colds, rheumatism, rashes, and urinary tract infections(Asadi et al., 2014 ; Navaey et al., 2014). Nano-stimulants enhance agricultural production by improving nutrient use efficiency, which increases crop growth and productivity. Nano-stimulants are also less expensive than traditional chemicals,When nano-fertilizer was added to fava beans (*Vicia faba* L.), all studied plant growth traits were superior (Hasan , 2024; Al-Rumaiydh et al., 2021).Recently, the world has turned to finding alternatives that reduce the use of chemical fertilizers, including the use of biofertilizers (Chandini et al., 2019).The amount of nutrients absorbed by plants can be increased through the use of the fungal inoculum *Trichoderma harzianum* (Yasir et al., 2025).Borage was chosen for its medicinal importance and the limited availability of information about its cultivation in Iraq. Therefore, been known the impact of environmentally friendly treatments (nanochitosan and *Trichoderma harzianum*) on borage, and . in addition to understanding the mechanisms of plant interaction with external stimuli and directing them to serve growth of Borago

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officinalis L.

Figure 1. Photo of borage plant during a field experiment.

1. Materials and Methods

The experiment was carried out according to a split plot design in Randomized Complete Blocks Design (RCBD) as a factorial experiment with two factors. The first factor (main plots) included adding the fungus (Trichoderma harzianum) to the soil at a concentration of (10, 5, and 0 g plant-1) as recommended by (Yasir et al., 2025). The second factor (secondary plots) included spraying the plants with nanocytosan at four concentrations (0, 100, 200, and 300 mg L-1), with a total number of experimental units (3*4*3=36).

II.Results and Discussion

The results of the statistical analysis in Table No. (1) show that the study factors have a significant effect on the plant height trait, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving it the highest average height of 63.33 cm, compared to the concentration (0 g. plant-1) which gave the lowest average height of 55.64 cm. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average height of 61.82 cm, compared to the concentration treatment (0 mg.L-1) which gave the lowest average height of 56.90 cm. As for the binary interaction between nanochitosan and

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(Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest height of 65.70 cm. While the control treatment gave the lowest height of 53.70 cm.

Table 1. Effect of nanochitosan and Trichoderma combinations on the height characteristic of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
55.64	**57.97**	**55.00**	**55.90**	**53.70**	**0**
58.83	**61.80**	**59.30**	**57.80**	**56.40**	5
63.33	**65.70**	**64.20**	**62.80**	**60.60**	10
	61.82	59.50	58.83	56.90	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
5.871	**3.765**				**2.387**

The results of the statistical analysis in Table No. (2) show that the study factors have a significant effect on the total chlorophyll characteristic of the plant, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving the highest total chlorophyll average of 52.83, compared to the concentration (0 g. plant-1) which gave the lowest total chlorophyll average of 47.08. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average total chlorophyll, reaching 51.87, compared to the concentration treatment (0 mg.L-1) which gave the lowest average total chlorophyll, reaching 48.10. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest total chlorophyll, reaching 55.00. While the control treatment gave the lowest total chlorophyll, reaching 45.80.

Table 2. Effect of nanochitosan and Trichoderma combinations on the Total chlorophyll property of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
47.08	**48.90**	**47.10**	**46.50**	**45.80**	**0**
49.90	**51.70**	**50.20**	**49.50**	**48.20**	5
52.83	**55.00**	**53.60**	**52.40**	**50.30**	10

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	51.87	50.30	49.47	48.10	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
4.725	3.135				0.718

The results of the statistical analysis in Table No. (3) show that the study factors have a significant effect on the trait of the number of plant branches, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving the highest average number of branches, which reached 16.00, compared to the concentration (0 g. plant-1), which gave the lowest average number of branches, which reached 13.83. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average number of branches, reaching 15.67, compared to the concentration treatment (0 mg.L-1) which gave the lowest average number of branches, reaching 14.33. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest number of branches per plant, reaching 17.00. While the combination treatment (0 g. plant-1) + (200 mg.L-1) gave the lowest number of branches, reaching 13.00.

Table 3. Effect of nanochitosan and Trichoderma combinations on the Number of branches of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
13.83	**14.67**	**13.00**	**14.00**	**13.67**	**0**
14.83	**15.33**	**15.00**	**14.67**	**14.33**	5
16.00	**17.00**	**16.00**	**16.00**	**15.00**	10
	15.67	14.67	14.89	14.33	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
1.434	0.904				0.681

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The results of the statistical analysis in Table No. (4) show that the study factors have a significant effect on the number of flowers trait, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving it the highest average number of flowers, which reached 3396, compared to the concentration (0 g. plant-1), which gave the lowest average number of flowers, which reached 3005. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average number of flowers, reaching 3330, compared to the concentration treatment (0 mg.L-1) which gave the lowest average number of flowers, reaching 3049. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest number of flowers, reaching 3596, while the comparison treatment gave the lowest number of flowers, reaching 2923.

Table 4. Effect of nanochitosan and Trichoderma combinations on the Number of flowers of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
3005	**3139**	**2946**	**3012**	**2923**	**0**
3142	**3255**	**3168**	**3098**	**3049**	5
3396	**3596**	**3461**	**3349**	**3176**	10
	3330	3192	3153	3049	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
163.5	93.7				114.4

The results of the statistical analysis in Table No. (5) show that the study factors have a significant effect on the leaf area characteristic of the plant, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving the highest average leaf area of 135.36, compared to the concentration (0 g. plant-1) which gave the lowest average leaf area of 118.03. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average leaf area of 133.86, compared to the concentration treatment (0 mg.L-1) which gave the lowest average leaf area of 116.04. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest leaf area of 140.62, while the control treatment gave the lowest leaf area of 105.21.

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Table 5. Effect of nanochitosan and Trichoderma combinations on the Paper space of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
118.03	**126.07**	**126.22**	**114.65**	**105.21**	**0**
125.30	**134.90**	**127.52**	**123.49**	**115.28**	5
135.36	**140.62**	**137.04**	**136.13**	**127.63**	10
	133.86	**130.26**	**124.76**	**116.04**	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
4.458	2.225				3.810

The results of the statistical analysis in Table No. (6) show that the study factors have a significant effect on the number of leaves trait, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving the highest average number of leaves, which reached 86.42, compared to the concentration (0 g. plant-1), which gave the lowest average number of leaves, which reached 71.83. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average number of leaves reaching 86.11, compared to the concentration treatment (0 mg.L-1) which gave the lowest average number of leaves reaching 74.67. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest number of leaves reaching 92.00. While the combination treatment (0 g. plant-1) + (200 mg.L-1) gave the lowest number of leaves reaching 66.00.

Table 6. Effect of nanochitosan and Trichoderma combinations on the Number of papers of borage plant

Average combinations Trichoderma	**nanochitosan (mg/L-1)**				**Trichoderma**
	300	200	100	0	**)gm(**
71.83	**82.67**	**66.00**	**70.33**	**68.33**	**0**
80.50	**83.67**	**82.00**	**80.33**	**76.00**	5
86.42	**92.00**	**88.67**	**85.33**	**79.67**	10
	86.11	78.89	78.67	74.67	**Average combinations nanochitosan**
LSD 0.05					

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Interference	nanochitosan	Trichoderma
8.571	5.415	4.014

The results of the statistical analysis in Table No. (7) show that the study factors have a significant effect on the stem diameter characteristic of the plant, as the results showed that the factor (Trichoderma harzianum) was superior to the concentration (10 g. plant-1) by giving the highest average stem diameter of 2.043 cm, compared to the concentration (0 g. plant-1) which gave the lowest average stem diameter of 1.767 cm. The levels of the biostimulant nanochitosan were superior to the concentration (300 mg.L-1) by giving the highest average stem diameter of 1.986 cm, compared to the concentration treatment (0 mg.L-1) which gave the lowest average stem diameter of 1.793 cm. As for the binary interaction between nanochitosan and (Trichoderma harzianum), the combination (10 g. plant-1) + (300 mg.L-1) was superior by giving the highest stem diameter of 2.137 cm. While the control treatment gave the lowest stem diameter of 1.673 cm.

Table 7. Effect of nanochitosan and Trichoderma combinations on the height trait of borage plant

Average combinations Trichoderma	nanochitosan (mg/L-1)				Trichoderma harzianum)gm(
	300	200	100	0	
1.767	**1.863**	**1.813**	**1.717**	**1.673**	**0**
1.906	**1.957**	**1.937**	**1.893**	**1.837**	5
2.043	**2.137**	**2.090**	**2.077**	**1.870**	10
	1.986	**1.947**	**1.896**	**1.793**	**Average combinations nanochitosan**
LSD 0.05					
Interference	**nanochitosan**				**Trichoderma**
0.2076	0.1305				0.1007

III.Conclusions

The study concluded that the combination of nanochitosan and *Trichoderma harzianum* enhanced plant growth, consistent with what (Yasir et al. 2025; Hasan , 2024) demonstrated regarding the role of Trichoderma harzianum in promoting plant growth, in addition to its role in increasing mineral absorption. This calls for further field experiments to study the compatibility between nanochitosan and Trichoderma harzianum and determine their effects on different plants.

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Trichoderma fungi enhance the decomposition of organic fertilizers, increase the availability of essential acids and nutrients for plant growth, and stimulate root hair growth with the hormones they produce. They also promote cell division and elongation, improving root health and increasing nutrient absorption (Salim et al., 2021).

Trichoderma is a beneficial fungus capable of penetrating root tissue, extending its mycelium to act as nutrient-transporting tubes. It also produces acids and enzymes that reduce the reaction rate and increase the availability of nutrients. It is highly efficient at decomposing organic waste, providing nutrients, including nitrogen, ready for plant absorption(Kudury et al., 2024).

In addition, many other studies have confirmed that the use of nanochitosan and Trichoderma harzianum had a positive effect on plant growth, which is consistent with the results of the experiment.

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