



RESEARCH ARTICLE

# Effect of probiotics 'Bio-health' and foliar fertilization with zinc sulfate on growth and yield of okra (*Abelmoschus esculentus* (L.) Moench)

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## Abstract

The experiment was conducted in a private field in Jaflawiya, Al-Mishkhab District, Najaf Governorate, for the spring season 2021, to study the effect of soil application with probiotics Bio health and foliar fertilization with aqueous zinc sulfate on some vegetative growth characteristics and yield of okra Husainawia variety. The experiment was a factorial experiment according to Randomized Complete Block Design (R.C.B.D.) with 2 factors and 3 replicates. The first factor was ground application with probiotics Bio health at 3 levels (0, 2500, 5000 g ha<sup>-1</sup>) and the second factor is the foliar spraying of zinc in (ZnSO<sub>4</sub>.7H<sub>2</sub>O) using 3 concentrations (0, 75 and 150 mg L<sup>-1</sup>). Both factors were applied 3 times during the growing season. The first application was in the stage of 4 true leaves and zinc was sprayed a week later. The second and third were 30 and 60 days after the first addition respectively. Analysis of variance (ANOVA) was performed and the averages were compared using the least significant difference test. Least Significant Difference Test (L.S.D.) at 0.05 probability level. The highest results were recorded in the treatment of adding the Bio health at a level of 5000 g ha<sup>-1</sup>, which led to the highest rates of vegetative growth indicators, quantitative and qualitative yield and chemical indicators, including plant height, leaf area, knot ratio, number of pods, total yield, nitrogen and phosphorous ratio in pods, zinc content in pods and protein % in pods. In general, the ground application of Bio health at 5000 g ha<sup>-1</sup> with zinc 150 mg L<sup>-1</sup> significantly outperformed most of the single and interaction treatments. It gave the highest significant increase in vegetative growth indicators, yield, chemical and qualitative indicators.

## Keywords

fertilization; foliar spray; micro-elements; pod yield

## Introduction

Okra plant (*Abelmoschus esculentus* (L.) Moench) belongs to the family Malvaceae. The regions of Egypt, Sudan, Eritrea and Ethiopia are believed to be its original homeland, from which it spread to all temperate regions of the world (1, 2). Okra is one of the important summer vegetables in Iraq, it is grown in order to obtain its pods rich in nutrients such as magnesium, phosphorous and calcium. It also contains vitamin A and vitamin C as well as riboflavin and thiamine. Okra leaves and pods and vegetative parts are used in many industries and are used as a raw material in the paper industry. An adhesive substance is extracted from the pods, roots and stems and is also used to filter sugarcane juice (3).

Ripe okra seeds are a rich source of high-quality protein when compared to other vegetable crops. They also contain significant amounts of oil, carbohydrates, dietary fiber, vitamins and minerals (4). These seeds are utilized in the coffee industry by being mixed with coffee seeds and they have an oil content estimated at around 20 %. The oil extracted from the seeds is used in edible oil (1). Okra is cultivated on a global scale across an area exceeding 20 million ha, yielding 9872826 tons. In Iraq, the crop covers an area of approximately 12128 ha with a productivity of 68451 tons (5).

In agriculture, biotechnology significantly enhances the productivity of crops overall (6). The use of probiotics is crucial for increasing production, improving quality, reducing reliance on chemical fertilizers and establishing an environmentally friendly agricultural approach (7). Additionally, humic acids, seaweed and microorganisms within the stimulator enhance soil properties through biological and metabolic activities as well as by making elements more accessible to plants (8). Furthermore, humic acids improve soil structure, texture and acidity levels.

It was previously demonstrated that probiotics improve the vegetative growth characteristics of potato plants (9). Foliar nutrition serves as an effective and necessary aid alongside fertilizers to enhance production (10) and plants require zinc, a crucial yet trace nutrient, for completing the plant's life cycle and growth (11). Zinc plays a vital role in cell divisions, division orientation and the synthesis of natural growth regulators within the plant (12). This element can be supplied to the plant through foliar feeding, by spraying it on the vegetative system (3) as well as through soil treatment (13).

Zinc plays a crucial role in enhancing the activity of several enzymes, particularly carbonic anhydrase, carboxylase and dehydrogenases (14). Plants require zinc for the production of the amino acid tryptophan, which serves as the fundamental building block for acetic acid, an important hormone for plant growth. Consequently, a deficiency in zinc leads to significant alterations in plant growth by reducing the production of the hormone Indole Acetic Acid, which is essential for cell division and elongation. As such, zinc is essential in addressing the physiological dwarfing of plants (Rosset) by promoting the elongation of internodes (15). Therefore, this study seeks to assess how the probiotics Bio health and foliar fertilization with aqueous zinc sulphate impact the growth and production traits of okra plants.

## Materials and Methods

This section should be written well defined to understand the steps of investigation done, which allows other researchers to reproduce the result. Can be given in a single heading or may be presented in separate headings depending on the requirement and need of author(s). The study was conducted in the spring growing season of 2021 in a private field located in the Al-Jaflawiya area of Najaf Governorate, Al-Mishkhab district. The aim was to

investigate the impact of soil treatment using the bio-stimulant Bio health and foliar fertilization with water zinc sulfate on various characteristics of vegetative growth and yield of the Hussainawiya variety of okra plants. Prior to planting, 10 random soil samples were collected from the experimental field for analysis of certain chemical and physical properties (Table 1).

**Table 1.** Some of the (pre-planting) chemical and physical properties of the experimental field soil.

Item	Quantity	Unit
Clay	302.80	%
Silt	480.30	%
Sand	216.9	%
<b>Texture</b>		<b>Silt-clay</b>
EC	2.34	ds. m <sup>-1</sup>
O M	1.4	%
pH	6.89	-----
soluble nitrogen	157.143	mg. kg <sup>-1</sup>
soluble phosphorous	175.91	mg. kg <sup>-1</sup>
soluble potassium	223	mg. kg <sup>-1</sup>
Magnesium	13.284	mg. kg <sup>-1</sup>
Na	11.923	mg. kg <sup>-1</sup>
SO <sub>4</sub>	21.946	mg. kg <sup>-1</sup>
Zinc	0.98	mg. kg <sup>-1</sup>
Ca	189.6	mg. kg <sup>-1</sup>

The tillage and preparation of the field soil were performed and the organic manure (buffalo waste) compost was added and mixed with the furrow's soil. The experiment field was divided into 3 replications with 9 experimental units (3.2 m<sup>2</sup> each) and the plant spacing was 40 cm, as each experimental unit contains 20 plants. The sowing process was carried out on 1/4/2021 using the seeds of the local variety produced locally in the province of Babylon.

The experiment included 2 factors, the first was the addition of the bio-stimulant Bio health (at 3 levels 0, 2500, 5000 g ha<sup>-1</sup>) by injecting the bottom of each plant with a distance of approximately 10 cm through a 10 mL medical syringe, the application was conducted for 3 periods, the first was at 4 true leaf appearance while the second and third applications were after 30 and 60 days from the first application respectively, where the control was treated with water only. The second factor included foliar fertilization with aqueous zinc sulphate, the treatment was at concentrations of 0, 75 and 150 mg L<sup>-1</sup> for each experimental unit. The spraying was carried out 1 week after the addition of the bio-stimulant (16) and 2 other treatments after an interval of 30 days. Watering was done as needed and insects were controlled several times using the insecticide castal benzoate. The experiment was factorial according to RCBD with 3 replicates containing 27 experimental units. Vegetative and quantitative plant growth indices were measured including plant height (cm plant<sup>-1</sup>), shoot dry weight (g plant<sup>-1</sup>) (17), leaf area (cm<sup>2</sup> leaf<sup>-1</sup>) using the scanner and the Imagej program. Measurements also included the number of pods (pod plant<sup>-1</sup>) and the total yield (Kg ha<sup>-1</sup>). Leaf content of nitrogen content was estimated %

according to Kjeldahl method using the Micro - (Kjeldahl) device (18) and the % of phosphorous (%) by a spectrophotometer at a wavelength of 430 nm according to the standard method (19). The zinc content in the leaf ( $\text{mg kg}^{-1}$  dry weight) was also estimated by an Atomic Absorption Spectrophotometer (20). Data was taken at the end of the experiment and statistical analysis was performed using the GenStat, 2010 program. ANOVA was also analysed and differences between means were tested according to the least significant difference test at a probability level of 0.05 (21).

## Results and Discussion

In case of the vegetative growth and yield, the results (Table 2) showed significant differences between the treatments of adding the probiotics Bio-health and spraying with zinc in the indicators of vegetative growth and yield including plant height, leaf area, knot %, number of pods, total yield, nitrogen and phosphorous ratio, zinc content in pods and protein % in pods. In general, treatment of the Bio health at a level of  $5000 \text{ g ha}^{-1}$  led to the highest rates of vegetative growth indicators and yield compared to the lowest concentration ( $2500 \text{ g ha}^{-1}$ ). Similarly, the higher concentration of zinc  $150 \text{ mg L}^{-1}$  resulted in higher rates of the same indicators than the of the concentration of  $75 \text{ mg L}^{-1}$  and the control. The interaction between the 2 experimental factors was higher in its significant effect in all the indicators under study on vegetative growth and the highest rates were in the interaction of Bio health at  $5000 \text{ g ha}^{-1}$  and spraying with zinc sulfate  $150 \text{ mg L}^{-1}$ .

It did not differ much in terms of chemical and qualitative indicators. The results showed in Table 3 the significant effect of the treatments of adding the probiotic and spraying with zinc on the chemical and qualitative indicators, represented by the % of nitrogen, the % of phosphorous, the zinc content and the % of protein in the pods. It is noted that there is a significant effect of spraying with zinc sulphate on the chemical and qualitative indicators, including the % of nitrogen, the % of phosphorous, the content of zinc and the % of protein in the pods and that these chemical and qualitative indicators increase with the increase in the concentration of the spray. The superiority of zinc concentration  $150 \text{ mg L}^{-1}$  and the Bio healthiest  $5000 \text{ g ha}^{-1}$  led to the highest rates of yield indicators and its components compared to the control plants (sprayed with water only). The interaction between Bio health and zinc sulphate had a significant effect on the chemical and qualitative indicators. The treatment of the interaction of Bio health at  $5000 \text{ g ha}^{-1}$  with zinc sulphate  $150 \text{ mg L}^{-1}$  spraying had the highest values than the other interaction treatments.

Bio-health is characterized by its high nitrogen content of humic acid. This is for the role of nitrogen in increasing the metabolism process and increasing the content of chlorophyll in the leaf (the formation of the quaternary porphyrin ring, which enters the composition of the chlorophyll molecule), which increases the efficiency of the dye, as nitrogen is necessary for division and cellular expansion for growth (22). Humic acid also increases the permeability of elements in cell membranes and stimulates growth through its contents of organic

**Table 2.** Effect of soil injection with probiotics Bio health and foliar spray with zinc

Treatment		Plant height cm	Shoot DW g. plant <sup>-1</sup>	Leaf area cm. plant <sup>-1</sup>	Fruit formation %	No. of pods (pod. plant <sup>-1</sup> )	Total yield (Ton. ha <sup>-1</sup> )	
<b>Bio health g. ha<sup>-1</sup></b>	0	128.18	161.20	187.09	63.09	66.00	1.05	
	2500	143.85	179.74	210.66	73.36	100.18	2.01	
	5000	161.39	200.69	236.20	73.54	119.50	2.70	
<b>L.S.D. 0.05</b>		1.542	1.476	1.345	1.166	1.296	0.014	
<b>Zinc mg. L<sup>-1</sup></b>	0	134.46	171.98	200.60	66.18	84.60	1.59	
	75	145.72	178.23	213.41	70.74	97.70	1.99	
	150	153.24	191.41	220.75	73.06	103.38	2.19	
<b>L.S.D. 0.05</b>		1.542	1.476	1.345	1.166	1.296	0.014	
<b>Bio health X Zinc</b>	0	121.30	153.42	175.39	57.68	52.74	0.69	
	0	75	127.69	158.53	192.03	63.07	68.23	1.09
		150	135.55	171.63	196.29	68.51	77.03	1.39
		0	135.65	172.07	199.02	67.15	85.67	1.57
	2500	75	143.97	180.60	214.74	74.99	104.46	2.14
		150	151.93	186.57	218.23	77.94	110.43	2.34
		0	146.44	190.47	227.39	73.73	115.40	2.53
	5000	75	165.49	195.57	233.41	74.16	120.43	2.75
		150	172.24	216.03	247.73	72.72	122.68	2.84
<b>L.S.D. 0.05</b>		2.670	2.557	2.330	2.020	2.245	0.024	

**Table 3.** Effect of soil injection with probiotics bio health and foliar spray with zinc sulfate on the chemical and qualitative indicators of okra pods.

			Pod's content of				
Treatments			N (%)	P (%)	Zinc mg. kg <sup>-1</sup>	Protein (%)	
<b>Bio health</b> <b>g. ha<sup>-1</sup></b>		0	0.37	0.329	56.56	2.32	
		2500	0.69	0.437	59.72	4.30	
		5000	0.91	0.512	64.51	5.70	
<b>L.S.D. 0.05</b>			0.028	0.0023	0.848	0.181	
<b>Zinc</b> <b>mg. L<sup>-1</sup></b>		0	0.51	0.386	54.56	3.23	
		75	0.66	0.424	62.30	4.17	
		150	0.78	0.467	63.93	4.91	
<b>L.S.D. 0.05</b>			0.028	0.0023	0.848	0.181	
<b>Bio health X</b> <b>Zinc</b>	0	0	0.24	0.263	54.35	1.55	
		75	0.36	0.323	56.81	2.27	
		150	0.50	0.402	58.52	3.14	
	2500	0	0.61	0.413	54.37	3.85	
		75	0.69	0.433	61.31	4.33	
		150	0.76	0.364	63.47	4.74	
	5000	0	0.69	0.484	54.96	4.30	
		75	0.95	0.516	68.78	5.93	
		150	1.10	0.535	69.79	6.86	
	<b>L.S.D. 0.05</b>			0.049	0.0040	1.469	0.313

compounds, amino acids and mineral elements, especially potassium, which plays an important role in many important functions of plants such as enzyme activation, protein synthesis, photosynthesis, regulation of osmosis, energy transfer and transmission through the phloem (23). Hence, the nutrients in the leaves increased and this will push the plant to increase the efficiency of metabolism and manufacture carbohydrates and proteins, and this will reflect positively on the increase in the characteristics of vegetative growth, which is represented in increasing the area of leaf metabolism and the height of the plant and increasing its weight and this is consistent with what was indicated Mechanism (24).

On the other hand, the microorganisms in the rhizosphere, *Bacillus subtilis* strains, produce auxin-like compounds IAA or GA. These substances stimulate the plant to increase cell division and elongation, which is reflected in an increase in the vegetative plant parts and an increase in plant heights and stored nutrients (25). The probiotic also contains the fungus *Trichoderma* sp. which secrete enzymes around the root area that stimulate the plant to raise the nutritional status of the plant and its growth (26). This is reflected in the increase in plant height and leaf area and this in turn is reflected in the increase in dry matter in the plant and thus the number of pods in total production (27). The Bio-enhancer contains many growth regulators (auxins, cytokines and gibberellins) that play an important role in plant growth and development, increase the speed of cell division and elongation and the formation of leaves. In addition to the role played by auxins in increasing flowering rates and the formation of pollen, which increases the rate of fruit formation (28).

It is also due to the positive effect of zinc in the influx of K<sup>+</sup> into the guard cells, which led to an increase in the efficiency of the photosynthesis process, which leads to an increase in the activity of nutrient metabolism in the plant (29, 30), which reflects the increase in plant growth

and thus the increase in yield. In addition to its effect on the formation of a strong root system, as it works to increase the ability of the plant to absorb and accumulate nutrients in the plant, which reflects the increase in the height of the plant and the dry weight of the vegetative total and the leaf area, which in turn reflects the increase in the % of knots and the number of pods in the total production of the plant. Also, zinc has a role in increasing the percentage of knots, the number of pods and the total production through its physiological effect on the growth and development of the plant by stimulating it to produce auxin and gibberellin and reduce the effect of abscisic acid, cellulose and increase the transmission of photosynthesis products. Also, zinc has a direct role in the elongation of cells and causes an increase in plant height, an increase in leaf area, an increase in the dry weight of the vegetative complex and an increase in the percentage of fruit it may increase production and increase the length of the pods (31). Zinc also has a positive effect in activating the enzymes responsible for building protein and chlorophyll, which leads to an increase in chlorophyll content. This, in turn, helps in increasing the activity of the cell on division and growth, as it works to equip it with the food necessary to complete all its physiological activities and this leads to an increase in the size of the cell and thus increase the characteristics of vegetative, syphilitic and yield growth (4, 32).

The nitrogen-rich humic in the probiotic helps to increase the readiness of the elements, which stimulates an increase in nitrogen absorption and its transfer from the leaf, storage and assimilation (33, 34). Protein and potassium in the bio stimulator increase photosynthesis (23). Consequently, the chemical and qualitative characteristics increased as well as the role of biology in the biostimulant that increases the readiness for absorption of elements by the plant, which led to an increase in the elements inside the plant and thus an increase in the qualitative characteristics (24, 35-36).

The higher values when spraying with zinc is attributed to the fact that zinc is a catalyst for the absorption of nitrogen by the plant, as it influences nitrogen metabolism, thus increasing the vegetative and root growth, which is reflected in the increase in flowering and yield growth. Spraying zinc on the plant causes its accumulation within the tissues of the plant and thus increases within the pods (29, 37). The reason for the increase in pod's content of nitrogen and phosphorous is mostly attributed to the fact that the efficiency of the photosynthesis process increased due to spraying zinc with the appropriate concentrations, as it increased the processed nutrients in the leaves and thus increased the proportion of protein content (2). While, zinc has a role in activating some enzymes in chloroplasts and this is reflected in the increase in the content of chlorophyll in the plant (30, 38).

### Authors' contributions

ZHT designed the concept of study, HMM and ZHT performed the field work, data analysis and writing of manuscript.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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