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Effect of Nano-zinc EDTA, Methods of Application and Salicylic Acid, on Growth Characteristics and Productivity of Broad Bean (*Vicia faba* L.) in Calcareous Soil.

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ABSTRACT

This investigation was conducted in Biology Department, Faculty of Science, Soran University, located in Soran District, Erbil Governorate, in Kurdistan Region of Iraq. The research investigated the influences of five concentrations of Nano-zinc EDTA, which contains 12% Nano-zinc (0, 60, 120, 180 and 240 mg L^{-1}), applied via foliar and soil application methods, along with two concentrations (0 and 100 mg L^{-1}) of Salicylic acid and their combinations on growth, yield components and total yield of broad bean plant (*Vicia faba* L.) cultivated in calcareous soil.

The factorial pot experiment was carried out using Randomized Complete Design (CRD) with four replications. The results showed that Nano-zinc concentrations, methods of application, salicylic acid concentration and their interactions significantly influenced most of the growth and productivity characteristics. The highest leaf area values were observed in treatments with Zn_2 (60 mg kg^{-1}), soil application, and SA_1 (100 mg L^{-1}) with the values of (16.38, 15.32 and 15.34 cm^2) respectively. In contrast the lowest values were obtained from Zn_3 (120 mg L^{-1}), foliar application and SA_0 (0 mg L^{-1}) with the mean values of (12.98, 14.83 and 14.81 cm^2) respectively. The maximum and minimum leaf area values (18.21 and 11.60 cm^2) were obtained from interaction treatments of (Zn_2 (60 mg kg^{-1}) × soil application × SA_1 and Zn_3 × Foliar × SA_0) respectively. Similarly, the highest pod weight values (134.81, 107.63 and 107.88 g $plant^{-1}$) were associated with treatments Zn_4, soil application, and SA_1 respectively. The lowest values (81.08, 96.00, and 95.76 g $plant^{-1}$) were noted from Zn_1, foliar application, and SA_0 treatments respectively. Interaction treatments also revealed significant differences, with the highest pod weights (143.02, 161.06 and 115.18 g $plant^{-1}$) were obtained from (Zn_4 (180 mg kg^{-1}) × soil application, Zn_3 × SA_1, and soil application × SA_1) respectively, while the lowest values (79.75, 75.73, and 91.30 g $plant^{-1}$) were found from (Zn_1 (0 mg kg^{-1}) × soil application, Zn_1 × SA_1, and foliar application × SA_0) respectively.

***Keywords*:** Broad bean, Calcareous soil, Nano-zinc, Salicylic acid, Yield parameters**.**

INTRODUCTION

Broad beans (*Vicia faba* L.) are nutrient-rich legumes valued for their high protein content and their ability to enhance soil fertility through nitrogen fixation. They thrive in cool climates, contribute to sustainable agriculture, and play a vital role in ensuring food security. Successful cultivation requires appropriate seedbed preparation and optimal plant spacing. Broad beans are widely cultivated in regions such as the Middle East and Kurdistan [1].

Zinc is a vital micronutrient required for key physiological processes in plants, including enzyme activation, hormone synthesis, and the facilitation of photosynthesis. Despite its importance, zinc availability is less than plant requirement's in calcareous soils due to its precipitation in unavailable form of $ZnCO_3$, which causes growth inhibition, nutrient imbalances, and reduced productivity. In particular. Legumes like beans are very sensitive to zinc disorder, where both deficiency and excess can negatively impact their growth and yield, this challenges can be solve by zinc [2]. Recent advancements in nanotechnology have introduced Nano-zinc as an innovative solution for sustainable agriculture, due to its nanoscale size and high surface area, Nano-zinc demonstrates superior nutrient delivery and uptake efficiency compared to traditional zinc sources. Studies have consistently shown that zinc oxide nanoparticles can more effectively support plant growth and soil health, while also contributing to improved crop yields. However, the potential toxicity at elevated concentrations necessitates careful management. As a component

of modern strategies like biofortification, Nano-zinc offers a more targeted and environmentally efficient approach to mitigating micronutrient deficiencies in cropping systems [3].
Zinc is commonly applied to crops through foliar sprays or soil fertilization, the foliar application often proving more effective during critical growth stages due to its rapid absorption and direct delivery to plant tissues. However, zinc availability in the soil can be significantly decrease in slightly alkaline to alkaline soil, and in calcareous soil growth media, which limits its absorption by plants. In response to this challenge, zinc oxide nanoparticles have emerged as a promising alternative, providing more efficient enhancement of plant growth, yield, and grain zinc concentration compared to conventional zinc fertilizers [4]. A field experiment indicated that the foliar application of zinc sulfate significantly improved broad bean growth and yield. The highest concentration (75 mg Zn L^{-1}) led to the greatest increases in plant height, pod number, seed weight, and overall yield [5]. Some other investigations indicated that, zinc oxide nanoparticles (ZnO-NPs) and other zinc fertilizers significantly improved vegetative growth, yield traits, and seed zinc content in beans plants, especially with twice foliar application. It has also increased soil and grain zinc levels, yield, and antioxidant activity in soybean, highlighting their potential to enhance crop productivity and decrease zinc deficiency [4].
Salicylic acid (SA) is a key plant hormone that plays a significant role in promoting plant growth, increasing yield, and enhancing the nutritional quality of crops, including their protein and carbohydrate content. Improving nutrient availability had significant influences on physiological processes, SA contributes to the development of the best plant quality and more productive plants. Its positive impact on both crop quantity and quality makes it a valuable agricultural tool for improving overall productivity and nutritional outcomes [6]. Numerous investigations have confirmed that, foliar SA application at 100–150 ppm improved faba bean growth, yield components, and seed quality [7]. On the other hand, another field experiment on effects of Salicylic acid was done in Babylon during the winter season of 2022–2023. The results indicated that foliar spraying with 200 mg L^{-1} salicylic acid significantly enhanced broad bean growth and yield, increasing plant height, leaf area, pod number, seed yield, and harvest index [8].
Zinc and salicylic acid are recognized as synergistic agents in supporting plant growth, particularly under challenging environmental conditions. When applied as foliar sprays, salicylic acid enhances photosynthetic activity and facilitates improved nutrient uptake, while zinc causes increasing water use efficiency and plant tolerance to various stresses. The combined application of zinc and SA has been shown to produce complementary effects, resulting in healthier plant development, improved yields, and enhanced seed quality. This integrated approach presents a practical and sustainable strategy for promoting crop productivity, especially in nutrient-poor or drought-affected soils [9, 10].
Most of the soils in the Iraqi Kurdistan region suffer from a zinc deficiency since the concentration of available zinc is below the critical concentration of 0.69 mg kg^{-1} soil [11]. Plants in our region often face micronutrient deficiencies as a result of calcareous soils high in calcium carbonate, which limits nutrient availability and reduces plant growth and yield.
This study aims to assess how Nano-zinc and salicylic acid, applied through leaves and roots, affect the growth, nutrient uptake, and yield of broad beans in calcareous soil, with the goal of improving productivity and sustainability.

Materials and Methods

Experimental site and duration

The experiment was conducted in a covered field of the Biology Department at Soran University, Kurdistan Region, Iraq, during the winter growing season of 2024–2025. The area is characterized by a Mediterranean climate with moderate rainfall and seasonal temperature variation (Table 1).

Experimental design and layout

The study followed a completely randomized design (CRD) in a factorial arrangement with four replications, totaling 80 pots. Three factors were tested and then treatment combinations were randomly assigned to pots:

- First Factor: Nano-zinc application at five levels: 0, 60, 120, 180, and 240 mg Kg^{-1} (for soil application) or mg L^{-1} (for foliar application), which donated by Zn_1, Zn_2, Zn_3, Zn_4, and Zn_5, respectively.
- Second factor: two methods of Nano-zinc application, either foliar application or soil fertilization, which donated by F and S, respectively.
- Third factor: foliar Salicylic acid at two levels: 0 and 100 ppm, with symbols of SA_0 and SA_1, respectively. That means half of experimental units were sprayed with salicylic acid (SA_1), while others were not sprayed (SA_0).

The total number of experimental units = Levels of factor 1 × levels of factor 2 × levels of factor 3 × number of replications, (5×2×2×4=80 pots).

Planting and growing conditions

Broad bean (Vicia faba) seeds were sown in plastic pots (30 cm diameter × 30 cm height), each pot filled with equal amounts of homogenized soil (15 kg pot-1). The physiochemical properties of experiment soil is exposed in Table 2. Five seeds were sown per each pot on October 22, 2024. After emergence of seedlings, on 10th November 2024, the number of plants were thinned to three healthy plants per pot.

Preparation and application of treatments

Zinc fertilizer containing 12% Nano-Zn was applied to broad bean plants through both soil and foliar methods. For soil application, the fertilizer was incorporated into the soil at concentrations of 0, 60, 120, 180, and 240 mg kg^{-1}. Each pot contained 13.6 kg of soil, corresponding to fertilizer doses of 0, 816, 1632, 2448, and 3264 mg per pot, respectively. These doses equated to actual Nano-Zn applications of 0, 97.92, 195.84, 293.76, and 391.68 mg per pot. For foliar application, solutions with concentrations of 0, 60, 120, 180, and 240 mg L^{-1} of zinc fertilizer were prepared, and 250 ml of each solution was sprayed per pot. Consequently, the amount of fertilizer applied per pot was 0, 15, 30, 45, and 60 mg, which corresponded to 0, 1.80, 3.60, 5.40, and 7.20 mg of actual Nano-Zn per pot, respectively.

The amount of actual Nano-Zn applied through soil application is roughly 54 times higher per pot compared to the foliar application at all concentration levels. Soil zinc was applied once to the soil before flowering (40 days after seed sowing). However, foliar application was done twice — before flowering and during flowering — by dissolving Nano-zinc fertilizer in distilled water.

Salicylic acid was applied twice as a foliar spray at a concentration of 0 and 100 mg L^{-1}: once after each foliar zinc application. Tween was added as an adjuvant to all foliar sprays to improve leaf adherence and absorption.

Measured parameters

Physiological parameters

Leaf area (cm^2):

Leaf area was measured by determining leaf length and width using a ruler. The area was calculated using the following standard leaf area formula:

$$Leaf\ Area\ (cm^2) = 0.919 + 0.6821 \times Leaf\ Length\ (cm) \times Leaf\ Width (cm)$$ [12, 13].

Number of leaves per plant:

Number of leaves was counted manually from one plant selected from each pot.

Relative Water Content (RWC, %):

Fresh leaves were collected from the upper, middle, and lower canopy of one plant per pot. Fresh weight was recorded immediately. Leaves were then placed in distilled water to obtain turgid weight. Afterward, the leaves were oven-dried and dry weight was recorded. Relative water content was calculated using these measurements with following formula:

Table 1. Climatic conditions during the growing season which recorded from meteorological station of Soran city.

Months	Year	Climatic Conditions in Soran City Air Temp. °C		Relative moisture %	Rainfall (mm)
		Minimum*	Maximum		
October	2024	6	39	76.9	0.20
November	2024	-1	25	89.5	80.2
December	2024	-4	21	90.0	32.4
January	2025	-5	18	86.3	38.2
February	2025	-8	19	93.5	59.5
March	2025	0	30	79.6	15.1
April	2025	5	36	74.8	83.9

*Minimum temperatures (e.g., -8 °C in February) reflect the lowest values recorded on specific days, not throughout the entire month.

$$RWC\ (\%) = \frac{Fresh\ Weight - Dry\ Weight}{Turgid\ Weight - Dry\ Weight} \times 100$$ [14].

Leaf chlorophyll content (SPAD):
Leaf chlorophyll content was measured five times, the period between two successive measurements was seven days, by using a portable SPAD meter. The chlorophyll was measured from upper, middle, and lower leaves of targeted plant from each pot.
Seed protein percentage (%): Seed nitrogen content was measured using the Chlorophyll meter by slicing the seeds and recording the Nitrogen value directly. Then converting it into protein percentage using following formula:

$$Protein\ \% = Nitrogen\ \% \times 6.25$$ [15].

Table 2. Some Physio-chemical of the Soran city soil used in pot experiment. *

Soil Physiochemical Properties			
Soil properties	Units		Values
Particle size distribution (PSD)	Sand	$(g\ kg^{-1})$	370
	Silt		250
	Clay		380
	Soil texture		clay loam
	Soil pH		7.53
Bulk density	$g\ cm^{-3}$		1.44
Soil water content at	Saturation point (S.P.)	%	52,00
	Field capacity (F.C.)		26.60
	Wilting point (W.P.)		13.80
Ece	$dS\ m^{-1}$		0.37
CEC	CmolC kg Soil		26.96
Organic matter content	$(g\ kg^{-1}\ soil)$		11
Calcium carbonate			309.20
Active calcium carbonate			48.90
Available nitrogen	$(mg\ kg^{-1} soil)$		59.67
Available Phosphorous			2.01
Available potassium			64.10
Available Fe			2.00
Available Zn			0.49
Available Cd			0.40

Yield parameters
Number of pods per plant (pod $plant^{-1}$):

*The soil was analyzed at Department of Natural Resources, Faculty of Agriculture, Suleimani University.

The pods were counted manually from each pot at harvest.
Pod Length (cm):
The length of pods was measured using a flexible ruler.
Number of seeds per pod (seed pod^{-1}):
Seeds were counted from all pods from each pot.
Pod Weight (g):
The weight of pods was recorded using an electronic balance.

Statistical analysis
The entire collected data were analyzed using univariate analysis of variance (ANOVA). Treatment means were compared using the Duncan test at a 1% significance level ($p < 0.01$). The tables were used to display results clearly.

Results and Discussion

Physiological parameters

The following physiological traits were recorded to evaluate the internal responses of broad bean plants under various treatments:

Leaf area

Leaf area was significantly affected by Nano-zinc levels, application methods, salicylic acid concentrations, and their interactions at P- value ≥ 0.01, as shown in Table 3.

The results of statistical analysis indicated that the levels of applied Zn, methods of application, and SA concentration were affected significantly on leaf area, the highest values were recorded from Zn2, soil application, and SA1 with the values of (16.38, 15.32 and 15.34 cm^2) respectively, while the lowest values were obtained from Zn3, foliar application and SA0 with the mean values of (12.98, 14.83 and 14.81 cm^2) respectively. The mentioned results may be because of Zinc role in plant growth and its low concentration in the soil (Table 1) that is low than the critical concentration of 0.69 mg Zn Kg^{-1} soil [11]. The reason that soil application method superior on foliar application method may be due to cold stress which reached to (-8 °C) as shown in Table 2, but in general in cold stress the soil temperature is higher than air temperature. Salicylic acid also promotes plant growth and causes increase in leaf area, similar results was recorded by [16].

The di factor interactions (Nano-Zn × application methods, and Nano-Zn × Salicylic acid levels) influenced significantly on leaf area. The highest values (17.23 and 16.66 cm^2) were observed from interaction treatments of (Zn2 × soil application, Zn2 × SA1) respectively, while the lowest values of them (12.13 and 11.90 cm^2) were recorded from interaction treatments of (Zn_5 × foliar application, $Zn_3 \times SA_0$) respectively. In contrast, the interaction between application methods and salicylic acid levels showed no significant impact on leaf area of broad bean plants.

In the triple factor interactions among Nano-zinc levels, application methods, and salicylic acid concentrations (Zn × Method × SA), was statistically significant. The highest and lowest leaf area values (18.21 and 11.60 cm^2) was observed from interaction treatments of (Zn_2 × soil application × SA_1, and Zn_3 × Foliar × SA_0) respectively.

The di and triple interactions causes creating different growth conditions for plants which caused obtaining different values for leaf area [17, 18]. This results indicate that the combined use of Nano-Zn and salicylic acid, as well as their proper application methods, play a key role in enhancing leaf area development, while high zinc doses or the absence of SA may limit this growth, matching results were recorded in [9].

Table 3. The influences of levels of Nano-Zn, methods of application, levels of Salicylic acid and their combinations on Leaf area (cm^2) *.

Leaf area (cm^2)

Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application: SA_0	Level of SA (mg L^{-1}) application: SA_1	Nano Zn–Methods Interaction		Nano-Zn–SA Interaction
Zn_1	Foliar	13.96^{b}	15.23^{a}	14.60^{ab}	SA_0	15.26^{ab}
Zn_2		15.97^{a}	15.11^{a}	16.36^{ab}		16.11^{ab}
Zn_3		11.60^{b}	12.65^{b}	15.54^{ab}		11.90^{b}
Zn_4		15.57^{a}	15.02^{a}	17.23^{a}		15.14^{ab}
Zn_5		16.09^{a}	17.15^{a}	12.13^{b}		15.63^{ab}
Zn_1	Soil	16.57^{a}	16.16^{a}	13.83^{ab}	SA_1	15.70^{ab}
Zn_2		16.26^{a}	18.21^{a}	15.29^{ab}		16.66^{a}
Zn_3		12.20^{b}	15.46^{a}	13.62^{ab}		14.06^{ab}
Zn_4		14.71^{a}	12.54^{a}	16.62^{a}		13.78^{ab}
Zn_5		15.16^{a}	15.92^{a}	15.54^{ab}		16.53^{a}

SA-Zn Method Interaction

Level of SA (mg L^{-1}) application	Foliar Method (F)	Soil Method (S)	SA means
SA_0	14.64^{a}	15.03^{a}	14.81^{b}
SA_1	14.98^{a}	15.66^{a}	15.34^{a}
Methods mean	14.81^{b}	15.32^{a}	

Nano-Zn means

Zn_1	Zn_2	Zn_3	Zn_4	Zn_5
15.48^{ab}	16.38^{a}	12.98^{b}	14.46^{ab}	16.08^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Number of leaves per plant

Analysis of variance revealed that Nano-Zn concentration, application method, SA level, and their interactions significantly affected the number of leaves per plant at P- value ≤ 0.01, see Table 4.

Among the significant impact of zinc concentration on number of leaves per plant, the highest means (92.94, 83.66, and 83.25 leaves plant^{-1}) were observed from treatments of (Zn_5, soil application, and SA_1) respectively, while the lowest means (69.37, 80.25, and 80.25 leaves plant^{-1}) were recorded at treatments of (Zn_1, foliar application, and SA_0) respectively.

The two-way interactions between (Nano-Zn levels × application methods and Nano-Zn levels × SA levels) were statistically significant. The combination of (Zn_5 × soil application and Zn_5 × SA_1) yielded the highest number of leaves (97.00 and 96.38) respectively. While (Zn_1 × foliar application and Zn_1 × SA_0) resulted in the lowest means (64.88 and 65.25) respectively. The combined effect of all three factors—zinc level, application method, and salicylic acid level—was statistically significant, the treatment (Zn_5 × soil application × SA_1) recorded the highest number of leaves per plant (97.00), while the lowest mean (64.50) was observed in (Zn_1 × foliar application × SA_1).

These findings highlight the critical role of factor interaction in enhancing vegetative growth. The superior performance of the high zinc dose applied through soil in combination with salicylic acid suggests a synergistic effect that promotes leaf development and entire plant growth. Similar results were recorded in previous studies by [19].

Table 4. The influences of levels of Nano-Zn, application methods, Salicylic acid levels and their interactions on Number of leaves per plant *.

Number of leaves per plant

Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application: SA_0	Level of SA (mg L^{-1}) application: SA_1	Nano Zn–Methods Interaction		Nano-Zn–SA Interaction
Zn_1	Foliar	65.25^{f}	64.50^{f}	64.88^{g}	SA_0	68.75^{d}
Zn_2		76.00^{de}	82.00^{c-e}	73.88^{f}		77.00^{c}
Zn_3		80.00^{c-e}	83.50^{b-d}	79.00^{ef}		79.75^{c}
Zn_4		84.25^{b-d}	88.25^{a-c}	79.25^{d-f}		83.14b^{c}
Zn_5		87.50^{a-c}	91.50^{ab}	81.75^{c-e}		91.63^{a}
Zn_1	Soil	72.25^{ef}	75.50^{de}	82.13^{c-e}	SA_1	70.00^{d}
Zn_2		78.00^{c-e}	80.50^{c-e}	86.25^{bc}		81.25^{bc}
Zn_3		79.50^{c-e}	84.75^{b-d}	86.00^{b-d}		84.13^{bc}
Zn_4		84.00^{b-d}	88.00^{a-c}	89.50^{b}		88.13^{ab}
Zn_5		95.75^{a}	97.00^{a}	96.38^{a}		94.25^{a}

SA-Zn Method Interaction

Level of SA (mg L^{-1}) application	Foliar Method (F)	Soil Method (S)	SA means
SA_0	78.60^{a}	81.95^{a}	80.25^{b}
SA_1	81.90^{a}	85.15^{a}	83.52^{a}
Methods mean	80.25^{b}	83.66^{a}	

Nano-Zn means

Zn_1	Zn_2	Zn_3	Zn_4	Zn_5
69.73^{d}	79.13^{c}	81.94^{bc}	86.13^{b}	92.94^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Relative Water Content (RWC %)

Analysis of variance revealed that Nano-zinc concentration, application method, salicylic acid (SA) level, and their interactions showed significant effect on the leaf relative water content (RWC%) at $P \leq 0.01$ (Table 5). This may due to their role in enhancing osmotic adjustment, membrane integrity, or antioxidant activity, which can enrich the physiological contexts [20, 21].

No significant effect was observed from individual treatments (Nano-Zn levels and SA levels) on relative water content, except application methods, that significantly affected the RWC. The highest mean (80.76 %) were obtained from treatment of soil application, while the lowest mean (77.30 %) were observed from treatments of foliar application method.

The dual interaction between (Nano-zinc levels × application method, and application methods × SA levels) were statistically significant. The highest RWC means (82.97% and 81.04%) were recorded from (Zn_2 × soil application, and soil application × SA_0) respectively, whereas (Zn_3 × foliar application, and foliar application × SA_0) yielded lowest means (74.72% and 75.47%) respectively. Meanwhile, the interaction between Nano-Zn levels and SA levels showed no significant effect on RWC.

The combined effect of all three factors—zinc level, application method, and salicylic acid level—was statistically significant. The highest (83.49%) and lowest (71.38%) means were observed in the (Zn_5 × soil application × SA0, and Zn3 × foliar application × SA_0) respectively.

These findings imply that, high levels of Nano-zinc and salicylic acid, combined with effective application methods, enhance plant water status under drought conditions. In addition, the data revealed the potential of Nano-zinc and SA in improving water use efficiency and regulating physiological responses, thereby mitigating the adverse effects of drought and promoting improved plant performance in water-limited environments [20, 21].

Chlorophyll content (SPAD)

The univariate analysis of variance (ANOVA) revealed that, no statistically significant effects ($p > 0.01$) were observed from any of the main factors—zinc level, application method, and salicylic acid concentration—on leaf chlorophyll content. Additionally, no significant two-way or three-way interactions were observed among these factors, see Table 6. These findings indicate that, under the tested conditions and treatment levels, neither the

individual factors nor their combinations had a measurable impact on chlorophyll accumulation in the leaves.
Although salicylic acid and zinc are widely reported to enhance chlorophyll biosynthesis by improving nutrient assimilation, stimulating photosynthetic enzyme activity, and reducing oxidative damage, the absence of a significant effect on chlorophyll content in this study may be due to several interrelated factors. First, the plants may have been grown under non-stress or near-optimal conditions, where chlorophyll synthesis was not notably impaired and therefore less responsive to further stimulation. Second, the combined application of Nano-zinc and SA may have resulted in complex interactions—such as altered nutrient dynamics, antagonistic signaling, or feedback inhibition—that neutralized the expected positive effects of each treatment [22].
Furthermore, the lack of a significant response may be linked to the relatively low concentration of SA (100 mg L^{-1}) used in this experiment. Numerous investigations have provided evidence that the beneficial effects of SA on chlorophyll content are dose-dependent, with more pronounced increases observed at higher concentrations, such as 200 mg L^{-1}. This suggests that the concentration applied in the current study may have been insufficient to trigger measurable physiological responses under the given conditions [23].

Table 5. The influences of levels of Nano-Zn, methods of application, levels of Salicylic acid and their combinations on relative water content (%) *.

Relative Water Content (%)						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application SA_0	Level of SA (mg L^{-1}) application SA_1	Nano Zn–Methods Interaction		Nano-Zn – SA Interaction
Zn_1	Foliar	74.71^a	82.08^a	78.40^a	SA_0	78.48^a
Zn_2		79.24^a	74.95^a	77.10^a		81.18^a
Zn_3		71.38^a	78.06^a	74.72^a		75.67^a
Zn_4		74.94^a	77.38^a	76.16^a		75.65^a
Zn_5		77.06^a	83.15^a	80.11^a		80.28^a
Zn_1	Soil	82.25^a	81.94^a	82.10^a	SA_1	82.01^a
Zn_2		83.12^a	82.82^a	82.97^a		78.89^a
Zn_3		79.97^a	82.65^a	81.31^a		80.35^a
Zn_4		76.37^a	79.20^a	77.78^a		78.29^a
Zn_5		83.49^a	75.92^a	79.71^a		79.54^a
SA-Zn Method Interaction						
Level of SA (mg L^{-1}) application		Foliar Method (F)		Soil Method (S)		SA means
SA_0		75.47^b		81.04^b		78.26^b
SA_1		79.12a^b		80.51a^b		79.82^a
Methods mean		77.30^b		80.76^a		
Nano-Zn means						
Zn_1	Zn_2	Zn_3		Zn_4		Zn_5
80.24^a	80.03^a	78.01^a		76.97^a		79.91^a

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Table 6. The influences of levels of Nano-Zn, methods of application, Salicylic acid levels and their combinations on chlorophyll content (SPAD)*.

Chlorophyll Content (SPAD)						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application		Nano Zn–Methods Interaction		Nano-Zn–SA Interaction
		SA_0	SA_1			
Zn_1	Foliar	46.09^{a}	46.79^{a}	46.44^{a}	SA_0	46.22^{a}
Zn_2		45.92^{a}	46.24^{a}	46.08^{a}		46.43^{a}
Zn_3		47.42^{a}	45.78^{a}	46.60^{a}		47.66^{a}
Zn_4		45.51^{a}	47.56^{a}	46.54^{a}		46.45^{a}
Zn_5		47.14^{a}	45.11^{a}	46.13^{a}		46.62^{a}
Zn_1	Soil	46.36^{a}	47.64^{a}	46.97^{a}	SA_1	47.21^{a}
Zn_2		46.95^{a}	46.33^{a}	46.64^{a}		46.28^{a}
Zn_3		47.90^{a}	47.80^{a}	47.85^{a}		46.79^{a}
Zn_4		47.40^{a}	49.44^{a}	48.42^{a}		48.60^{a}
Zn_5		46.10^{a}	46.92^{a}	46.51^{a}		46.02^{a}

SA-Zn Method Interaction

Level of SA (mg L^{-1}) application	Foliar Method (F)	Soil Method (S)	SA means
SA_0	46.24^{a}	46.94^{a}	46.59^{a}
SA_1	46.30^{a}	47.62^{a}	46.96^{a}
Methods mean	46.27^{a}	47.28^{a}	

Nano-Zn means

Zn_1	Zn_2	Zn_3	Zn_4	Zn_5
46.72^{a}	46.36^{a}	47.23^{a}	47.48^{a}	46.32^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Seed protein content (%)

The statistical analysis of seed protein percentage (%) revealed that the effects of Nano-zinc levels, application methods, salicylic acid treatments, and their interactions had significantly affected the protein concentration in broad bean seeds at P-Value ≤ 0.01, see Table 7.

The effect of each Nano-zinc levels, application methods, and salicylic acid levels had significant impact on the seed protein concentration. The highest means (32.80, 30.30, and 30.40 %) were obtained from treatments (Zn_5, soil application, and SA_1) respectively, while the lowest means (25.42, 28.88, and 28.76 %) were recorded at the (Zn_1, foliar application, and SA_0) respectively.

The interaction between (Nano-zinc levels × application methods, Nano-zinc levels × SA levels, and application methods ×salicylic acid) had significantly affected seed protein concentration. The highest protein means (33.56, 33.58, and 31.16 %) observed in (Zn_5 × soil application, Zn_5 × SA_1, and soil application × SA_1) respectively, while the lowest means (24.62, 25.15, and 28.09 %) were recorded from treatments (Zn_1 × foliar application, Zn_1 × SA_0, and foliar application × SA_0) respectively.

The combined effect of all three factors—zinc level, application method, and salicylic acid level—was statistically significant. The highest and lowest means (34.73 and 24.66) were observed in the (Zn_5 × soil application × SA_1, and Zn_1 × foliar application × SA_0) respectively.

Zinc and Salicylic Acid significantly enhance protein and nitrogen content in bean seeds through distinct but complementary mechanisms. Zinc improves enzymatic processes related to nitrogen assimilation, while SA modulates stress responses and protein synthesis pathways. When applied together, they can synergistically enhance seed nutritional quality, especially in legumes grown under stress-prone or nutrient-deficient conditions [7, 24, 25].

Table 7. The influences of levels of Nano-Zn, methods of application, Salicylic acid levels and their combinations on Seed protein content (%)*.

Seed Protein Content (%)						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application		Nano Zn – Methods Interaction		Nano-Zn–SA Interaction
		SA_0	SA_1			
Zn_1	Foliar	24.66^{e}	24.61^{e}	24.63^{d}	SA_0	25.15^{e}
Zn_2		26.55^{ef}	26.01^{ef}	26.28^{cd}		26.76^{e}
Zn_3		28.77^{c-e}	32.45^{a-c}	30.61^{ab}		29.70^{cd}
Zn_4		28.89^{b-e}	32.79^{ab}	30.83^{ab}		30.19^{b-d}
Zn_5		31.60^{a-c}	32.45^{a-c}	32.03^{a}		32.01^{a-c}
Zn_1	Soil	25.63^{ef}	26.77^{ef}	26.20^{cd}	SA_1	25.69^{e}
Zn_2		26.96^{b-f}	29.36^{b-e}	28.16^{bc}		27.69^{de}
Zn_3		30.63^{a-c}	32.33^{a-c}	31.47^{a}		32.40^{a-c}
Zn_4		31.52^{b-d}	32.65^{a-c}	32.08^{a}		32.72^{ab}
Zn_5		32.43^{a-c}	34.73^{a}	33.56^{a}		33.58^{a}

SA-Zn Method Interaction

Level of SA (mg L^{-1}) application	Foliar Method (F)	Soil Method (S)	SA means
SA_0	28.09^{a}	29.44^{ab}	28.77^{b}
SA_1	29.66^{ab}	31.17^{b}	30.41^{a}
Methods mean	28.89^{b}	30.30^{a}	

Nano-Zn means

Zn_1	Zn_2	Zn_3	Zn_4	Zn_5
25.42^{c}	27.23^{b}	31.03^{a}	31.44^{a}	32.80^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Yield Parameters

The yield performance of broad bean plants was evaluated using the following components:

Number of pods per plant

The statistical evaluation of the number of pods per plant indicated that only Nano-zinc levels affected significantly on number of pods plant^{-1}, while methods of application and salicylic acid treatments had non-significant effects, as shown in Table 8.

Regarding to the Nano-zinc levels that significantly affected the pod numbers, the highest mean (7.19 pod plant^{-1}) was recorded from (Zn_4), while the lowest mean (5.25 pod plant^{-1}) was occurred at (Zn_2). It is appeared that there is significant differences only between Zn_4 and other levels of applied Nano-Zn, or increase in concentration of Nano-Zn from Zn_4 to Zn_5 appears to reduce number of pods, suggesting a possible toxicity or nutrient imbalance at higher concentrations of Nano-Zn. Although Nano-Zinc applications enhanced the concentration of several nutrients, such as Zn, Ca, and Fe, but high doses led to reductions in essential elements like phosphorus, and in some cases, no change in nitrogen uptake [26].

The di interaction treatments of (Levels of Nano-Zn × methods of application, and Levels of Nano-Zn × levels of SA) were influenced significantly on number of pods the highest values (7.38 and 8.13 pod plant^{-1}) were observed from interaction treatments of (Zn_4 × soil application, and $Zn_4 \times SA_1$) respectively, while their lowest values (5.38 and 5.25 pod plant^{-1}) were obtained from (Zn_2 × foliar application, and $Zn_2 \times SA_0$) respectively. The interaction between the application methods and SA levels indicated no significant effect. This may be due to the single effects of the studied factors or the interaction between levels of the studied factors created different growth media for plant growth [22].

The reduced number of pods per broad bean plant demonstrated in certain treatments may be attributed to a complex interplay of environmental, physiological, and agronomic factors. Environmental stresses such cold stress (Table 2), temperature fluctuations, and inadequate pollination due to a scarcity of pollinators can significantly impair reproductive development. These conditions may lead to physiological stress, resulting in blossom drop and ultimately reducing pod set, particularly in cultivars that are not fully self-fertile [27, 28]. In addition to environmental influences, agronomic practices such as planting density play a critical role in pod formation and overall yield. Research has demonstrated that wider planting distances (e.g., 60 × 30 cm) significantly enhance plant architecture by promoting branching, increasing pod number, and improving seed development, whereas closer spacing (e.g., 40 × 20 cm) tends to favor vegetative growth at the expense of reproductive success [29]. Thus, suboptimal spacing may exacerbate the effects of environmental stressors, compounding reductions in pod number, and finally results of experiments are differing depending on amount of soil per pod, type of soil, climatic conditions…etc.

Table 8. The influences of levels of Nano-Zn, methods of application, levels of Salicylic acid and their interactions on Number of Pods per Plant*.

Number of pods per Plant						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application		Nano Zn – Methods Interaction		Nano-Zn –SA Interaction
		SA_0	SA_1			
Zn_1	Foliar	5.50^{a}	6.25^{a}	5.87^{a}	SA_0	5.63^{b}
Zn_2		5.50^{a}	5.25^{a}	5.38^{a}		5.25^{b}
Zn_3		5.50^{a}	6.25^{a}	5.88^{a}		5.88^{ab}
Zn_4		6.50^{a}	7.50^{a}	7.00^{a}		6.25^{ab}
Zn_5		6.25^{a}	6.75^{a}	6.50^{a}		6.00^{ab}
Zn_1	Soil	5.75^{a}	5.25^{a}	5.50^{a}	SA_1	5.75^{ab}
Zn_2		5.00^{a}	6.50^{a}	5.75^{a}		5.88^{ab}
Zn_3		6.25^{a}	6.50^{a}	6.38^{a}		6.37^{ab}
Zn_4		6.00^{a}	8.75^{a}	7.38^{a}		8.13^{a}
Zn_5		5.75^{a}	6.25^{a}	6.00^{a}		6.50^{ab}
SA-Zn Method Interaction						
Level of SA (mg L^{-1}) application		Foliar Method (F)		Soil Method (S)		SA means
SA_0		5.85^{a}		5.75^{a}		5.80^{a}
SA_1		6.40^{a}		6.65^{a}		6.53^{a}
Methods mean		6.13^{a}		6.20^{a}		
Nano-Zn means						
Zn_1	Zn_2		Zn_3	Zn_4		Zn_5
5.69^{a}	5.56^{a}		6.13^{a}	7.19^{a}		5.25^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Pod length

The statistical analysis showed that, all of the study factor and their combinations were not influenced significantly on pod length, as recorded in Table 9.

The previous results underscore the critical role of appropriate Nano-Zinc dosage and timing in optimizing nutrient assimilation and boosting crop productivity [30]. While the absence of a significant response in this study may be attributed to the relatively low concentration of salicylic acid (100 ppm), it is important to note— as previously discussed— that the effects of SA are dose-dependent. Prior studies have consistently shown more pronounced physiological responses at higher concentrations, particularly around 200 ppm [23].

The non-significant differences in pod length across some treatments may be attributed to limited plant height, which caused pods to reach the soil surface and restricted their ability to elongate further. Broad beans naturally form large clusters and require sufficient spacing—typically 30 to 40 cm between rows and between individual plants—to develop fully [31]. Another reason may be because of temperature fluctuations as we mentioned before, especially frequent very low temperature, significantly harm broad bean growth by affecting key stages like pod formation and development that reduces entire yield [28].

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Table 9. The influences of levels of Nano-Zn, application methods, Salicylic acid levels and their combinations on Pod length (cm)*.

Pod Length (cm)						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application SA_0	Level of SA (mg L^{-1}) application SA_1	Nano Zn–Methods Interaction		Nano-Zn–SA Interaction
Zn_1	Foliar	12.99^{a}	12.79^{a}	12.89^{a}	SA_0	13.01^{a}
Zn_2		11.60^{a}	12.27^{a}	11.93^{a}		12.68^{a}
Zn_3		12.41^{a}	11.01^{a}	11.71^{a}		12.69^{a}
Zn_4		12.89^{a}	12.66^{a}	12.77^{a}		13.32^{a}
Zn_5		11.61^{a}	12.13^{a}	11.87^{a}		12.21^{a}
Zn_1	Soil	13.04^{a}	12.41^{a}	12.72^{a}	SA_1	12.60^{a}
Zn_2		13.77^{a}	12.35^{a}	13.06^{a}		12.31^{a}
Zn_3		12.98^{a}	13.17^{a}	13.07^{a}		12.09^{a}
Zn_4		13.76^{a}	14.42^{a}	14.09^{a}		13.54^{a}
Zn_5		12.81^{a}	13.33^{a}	13.07^{a}		12.73^{a}
SA-Zn Method Interaction						
Level of SA (mg L^{-1}) application		Foliar Method (F)		Soil Method (S)		SA means
SA_0		12.30^{a}		13.27^{a}		12.78^{a}
SA_1		12.17^{a}		13.14^{a}		12.65^{a}
Methods mean		12.23^{a}		13.20^{a}		
Nano-Zn means						
Zn_1	Zn_2	Zn_3		Zn_4		Zn_5
12.81^{a}	12.50^{a}	12.39^{a}		13.43^{a}		12.47^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Number of seeds per pod

The statistical analysis revealed that none of the individual treatments—Nano-zinc level, application method, or salicylic acid concentration—had a significant effect on the number of seeds per pod, see Table 10.

The absence of a significant effect on the number seeds per pod observed in this study may be related to the timing and frequency of the foliar applications (Zn and SA). While previous research has shown that salicylic acid at 100 ppm can improve seed quality and production, its effectiveness is closely linked to optimal timing and repeated applications at key developmental stages [23]. Similarly, studies on Nano-zinc have highlighted that dual applications during flowering and pod development stages are critical for enhancing seed formation [32]. Within the scope of this research, the absence of such targeted application strategies (mainly during pod filling stage) may have limited the potential impact of SA and Nano-zinc on seed number per pod.

Pod weight

The statistical analysis of pod weight (g $plant^{-1}$) revealed that the effects of Nano-Zn levels, methods of application, and salicylic acid levels, as well as their interactions, were statistically significant at $p \leq 0.01$, as it's shown in Table 11. The highest pod weight values (134.81, 107.63 and 107.88 g $plant^{-1)}$ were recorded from treatments Zn_4, soil application, and SA_1 respectively, while the lowest values (81.08, 96.00, and 95.76 g $plant^{-1}$) were noted from Zn_1, foliar application, and SA_0 treatments respectively.

The interaction treatments between two factors caused significant increase in in pod weight per plant the highest values (143.02, 161.06 and 115.18 g $plant^{-1}$) were recorded from interaction treatments of (Zn_4 × soil application, $Zn_3 \times SA_1$, and SA_1 × soil application) respectively, while their lowest values (79.75, 75.73, and 91.30 g $plant^{-1}$) were recorded from interaction treatments of (Zn_1 × soil application, $Zn_1 \times SA_1$, and foliar application × SA_0) respectively.

The triple interaction among the factors had significant effect of pod weight per plant, the highest and lowest values (182.35 and 70.30 g $plant^{-1}$) were recorded from interaction treatments of (Zn_4 × soil application × SA_1, and Zn_1 × soil application × SA_1) respectively.

The significant effects observed on pod weight—particularly in relation to the method of Nano-Zn application and its interaction with Nano-Zn levels and salicylic acid—are consistent with earlier findings on pod length and seed number. These findings indicate that broad bean pod weight is closely associated with both pod length and the number of seeds it contains; longer pods with more seeds tended to have greater individual pod weight, whereas shorter pods with fewer seeds contributed to lower pod weight [33].

Table 10. The influences of levels of Nano-Zn, methods of application, Salicylic acid levels and their combinations on Number of seeds per pod*.

Number of Seeds per Pod						
Levels of Nano Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application		Nano Zn–Methods Interaction		Nano-Zn–SA Interaction
		SA_0	SA_1			
Zn_1	Foliar	3.00^{a}	3.25^{a}	3.13^{a}	SA_0	3.50^{a}
Zn_2		3.50^{a}	3.25^{a}	3.38^{a}		3.38^{a}
Zn_3		3.75^{a}	3.50^{a}	3.63^{a}		3.63^{a}
Zn_4		3.75^{a}	3.50^{a}	3.63^{a}		3.75^{a}
Zn_5		3.75^{a}	3.75^{a}	3.75^{a}		3.63^{a}
Zn_1	Soil	4.00^{a}	3.75^{a}	3.88^{a}	SA_1	3.50^{a}
Zn_2		3.25^{a}	3.25^{a}	3.25^{a}		3.25^{a}
Zn_3		3.50^{a}	3.50^{a}	3.50^{a}		3.50^{a}
Zn_4		3.75^{a}	3.50^{a}	3.62^{a}		3.50^{a}
Zn_5		3.50^{a}	3.00^{a}	3.25^{a}		3.38^{a}
SA-Zn Method Interaction						
Level of SA (mg L^{-1}) application		Foliar Method (F)		Soil Method (S)		SA means
SA_0		3.55^{a}		3.60^{a}		3.57^{a}
SA_1		3.45^{a}		3.40^{a}		3.43^{a}
Method means		3.50^{a}		3.50^{a}		
Nano-Zn means						
Zn_1	Zn_2		Zn_3	Zn_4		Zn_5
3.50^{a}	3.31^{a}		3.56^{a}	3.62^{a}		3.50^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Table 11. The influences of levels of Nano-Zn, methods of application, Salicylic acid levels and their combinations on Pod weight (g)*.

Pod Weight (g plant^{-1}.)						
Levels of Nano-Zn (mg kg^{-1})	Methods of Nano-Zn application	Level of SA (mg L^{-1}) application		Nano Zn–Methods Interaction		Nano-Zn – SA Interaction
		SA_0	SA_1			
Zn_1	Foliar	82.67^{a}	80.94^{a}	82.12^{a}	SA_0	86.25^{a}
Zn_2		80.41^{a}	79.07^{a}	79.84^{a}		90.35^{a}
Zn_3		88.83^{a}	91.50^{a}	90.49^{a}		94.84^{a}
Zn_4		113.43^{a}	140.93^{a}	126.84^{a}		110.50^{a}
Zn_5		92.88^{a}	115.43^{a}	103.87^{a}		97.20^{a}
Zn_1	Soil	89.82^{a}	70.30^{a}	79.75^{a}	SA_1	75.73^{a}
Zn_2		99.05^{a}	107.45^{a}	104.48^{a}		92.85^{a}
Zn_3		100.63^{a}	120.64^{a}	110.57^{a}		105.74^{a}
Zn_4		107.52^{a}	182.35^{a}	143.02^{a}		161.06^{a}
Zn_5		100.80^{a}	108.19^{a}	104.52^{a}		111.80^{a}
SA-Method Interaction						
Level of SA (mg L^{-1}) application		Foliar Method (F)		Soil Method (S)		SA means
SA_0		91.38^{a}		99.99^{a}		95.76^{a}
SA_1		100.54^{a}		115.18^{a}		107.88^{a}
Method means		96.00^{a}		107.63^{a}		
Nano-Zn means						
Zn_1	Zn_2	Zn_3		Zn_4		Zn_5
81.08^{a}	91.74^{a}	100.29^{a}		134.81^{a}		87.68^{a}

*Mean for the factors and their combination separately having same letter (letters) means there is no significant differences among them at $p \leq 0.01$and vice versa.

Conclusion

This study demonstrates that the combined application of Nano-zinc, salicylic acid, and optimized delivery methods significantly improves physiological traits and yield components in broad bean plants. Soil application was more effective than foliar treatment in enhancing leaf development, water retention, and seed nitrogen content. While chlorophyll content and seed number per pod were unaffected, integrated treatments notably increased pod number, length, and weight. These results underscore the synergistic effect of Nano-zinc and SA in promoting growth and productivity, emphasizing the importance of appropriate application methods.

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تأثير نانو-زنك، طرائق الإضافة، وحمض الساليسيليك على صفات النمو وإنتاجية نبات الفول العريض (*Vicia faba L.*) في التربة الكلسية .

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الخلاصة

أُجري هذا البحث في قسم علوم الحياة، كلية العلوم، جامعة سوران، الواقعة في قضاء سوران بمحافظة أربيل، إقليم كردستان، العراق. تضمن البحث دراسة تأثير خمسة تراكيز من الزنك النانوي المخلّب بالـ***EDTA*** ، والذي يحتوي على 12% من الزنك النانوي: 0، 60، 120، 180، و240 ملغم لتر$^{-1}$، باستخدام طريقتي الإضافة الورقية والتربة، بالإضافة إلى رش مستويين من حمض الساليسيليك: 0 و100 ملغم لتر$^{-1}$، وتداخلاتهم على بعض صفات النمو ومكونات الحاصل والحاصل الكلي لنبات الفول العريض المزروع في تربة كلسية. نُفذت التجربة بطريقة الأصص وفق تصميم عشوائي كامل ***CRD*** وبأربعة مكررات**.**

أظهرت النتائج أن لمستويات الزنك النانوي، وطريقة الإضافة، وتركيز حمض الساليسيليك، وتداخلاتهم، تأثيراً معنوياً في معظم صفات النمو والإنتاجية. إذ سُجلت أعلى القيم للمساحة الورقية من المعاملات Zn_2 ، وطريقة الإضافة عبر التربة، والمعاملة SA_1 ، وبلغت 16.38، 15.32، و15.34 سم^2 على التوالي، في حين سُجلت أدنى القيم من المعاملاتZn_3 ، والإضافة الورقية، والمعاملة SA_0 وبلغت 12.98، 14.83، و14.81 سم^2 على التوالي. كما أن أعلى وأدنى قيم للمساحة الورقية 18.21 و11.60 سم^2 ظهرت في معاملات التداخل Zn_2 × إضافة تربة × SA_1 ، و Zn_3 × إضافة ورقية × SA_0 على التوالي **.**أما بالنسبة لوزن القرن، فقد سُجلت أعلى القيم 134.81، 107.63، و107.88 غم نبات$^{-1}$ من المعاملات Zn_4 ، الإضافة عبر التربة، و SA_1 على التوالي، بينما كانت أدنى القيم 81.08، 96.00، و95.76 غم نبات$^{-1}$ في المعاملات Zn_1 ، الإضافة الورقية، و SA_0 على التوالي. أما التداخلات فقد أظهرت أن أعلى أوزان للقرون 143.02، 161.06، و115.18 غم نبات$^{-1}$ سُجلت من المعاملات Zn_4 × إضافة تربة، SA_1 × Zn_3، و SA_1 × إضافة تربة على التوالي، بينما أدنى القيم 79.75، 75.73، و91.30 غم نبات$^{-1}$ كانت من معاملات Zn_1 × إضافة تربة، SA_1 × Zn_1، و إضافة ورقية × SA_0 على التوالي**.**

الكلمات المفتاحية: الباقلاء، نانو-زنك، حمض الساليسيليك، التربة الكلسية، مؤشرات الإنتاج.