

RESEARCH PAPER

Response of Growth, Yield and Active Compounds of Rosemary to Spraying of Nano-Zinc and Benzyl Adenine

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ABSTRACT

An experiment was implemented during the 2023-2024 year at the research station of the College of Education for Pure Science - Ibn Al-Haitham - University of Baghdad. The investigation aimed to evaluate the effects of nano zinc and benzyl adenine (BA) spraying on the growth, yield, and active compound content of rosemary. The factorial experiment conducted based on a randomized complete block design (RCBD) at three replications. The first factor consisted of three concentrations of nano-zinc (0, 10 and 20 mg L⁻¹), while the second factor contains three concentrations of BA (0, 3 and 6 mg L⁻¹). Spraying nano-zinc at 20 mg L⁻¹ considerably enhanced the height of the plant, the branches number for each plant, branches length, plant content of nitrogen and phosphorous, chlorophyll and volatile oil content in leaves, and leaves yield (1086.7 Kg ha⁻¹). Conversely, nano-zinc at 10 mg L⁻¹ concentration was substantially effective in the leaves content of rosmarinic acid (681.1 mg g⁻¹ dry weight). For BA, spraying at a concentration of 6 mg L⁻¹ significantly excelled in the number of branches per plant (13.22 mg L⁻¹), plant content of nitrogen and phosphorous, leaves content of chlorophyll, volatile oil and rosmarinic acid (720.1 mg g⁻¹ dry weight) and leaves yield (1117.2 Kg ha⁻¹). The correlation between studied factors influenced most of the studied traits.

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INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.), a perennial and woody herb of the Lamiaceae family, is renowned for its versatile applications across the pharmaceutical, cosmetic, and hygiene industries [1]. While often used as a culinary spice, aromatic qualities of rosemary are frequently incorporated into personal care products. Traditionally, rosemary has been employed in herbal medicine to support memory enhancement, alleviate muscle cramps

and myalgia, promote hair growth, and improve circulatory as well as nervous system function [2]. The medicinal value of the plants is because of their leaves and content of volatile oil. The leaves of rosemary consist of 1.0-2.5% aromatic oils, and such construction can differ based on the chemo type and the growth phase. The essential oil is nearly uncolored to faint yellow liquid with a distinctly fresh and appealing smell [3]. The essential oil (volatile oil) is therapeutically used

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and contains many important active compounds, such as borneol, bornyl acetate, camphene, cineol, pinene and camphor. Also, rosmarinic acid as plant leaves content is a natural phenolic compound with many biological activities; which show key role in growth promotion and defense mechanisms in plants. Rosmarinic acid has antiangiogenic, antioxidant, anti-inflammatory and antimicrobial activities. Moreover, it is used to reduce the risk of various types of cancer by preventing cell damage [4]. Despite the medicinal importance of the plant, its cultivation is limited to the experimental scale, which requires introducing the necessary improvements in cultivation technology by applying crop service operations, including spraying of microelements like zinc [5]. Zinc as most vital micronutrients impacting growth and efficiency involve the creation of tryptophan, which oversees the auxin biosynthesis. Furthermore, it is a building block, regulator and cofactor of a large array of enzymes, such as dehydrogenase, alkaline phosphatase and phospholipase, as well as function in the metabolism of nucleic acids and the protection of proteins [6]. Zinc has been found to significantly diminish the impact of sodium chloride under salt stress through the inhibition of absorption and transfer of such toxicants. [7]. The existing focus in agriculture is on adopting innovative technologies, like nano fertilizers, which provide significant advantages due to their distinctive characteristics. Their ultra-small diameter improves chemical efficacy and provides an extensive surface area for metabolic processes [8]. Furthermore, these fertilizers exhibit rapid absorption by plant tissues, attributed to their superior solubility, high stability, slow-release properties, and exceptional efficiency. Most importantly, eco-friendly nature makes them a sustainable choice for modern agriculture [9]. Nano-fertilizers serve as an efficient delivery mechanism for transporting nutrients to specific plant parts by utilizing the porous surfaces of plant tissues. This targeted approach increases nutrient efficiency and addresses deficiencies essential for plant growth [10]. Specifically, applying nano-zinc has shown significant potential in enhancing plant benefits, as it positively impacts photosynthesis efficiency and stimulates vegetative growth [11]. This makes nano-fertilizers a promising innovation for improving agricultural productivity while maintaining environmental sustainability [5]. It was observed that nano-zinc treatment at a

concentration of 10 mg L⁻¹ significantly enhanced leaf dry weight as compared to other treatments, namely, flavonoid content and essential oil percentage at 5 mg L⁻¹. Also, spraying of growth regulators, like benzyl adenine (BA), is one of the variables that can stimulate the development and growth of the plant [12]. BA has ability to cause a change in the plant pattern by increasing cellular division, elongation and opening of lateral buds through reducing the phenomenon of apical dominance controlled by auxin. In addition to, they influences the activation of RNA and essential enzymes for vital reactions, increases the accretion of chlorophyll proteins, and delays leaf aging, which elevates the transport of nutrients to active tissues [13]. This experiment aimed to assess the effects of spraying nano-zinc and BA on the yield, growth, and active compound content of rosemary. The study was carried out to explore how these treatments influence the physical and chemical processes of the plant, ultimately contributing to enhanced productivity and enhanced quality of rosemary.

MATERIAL AND METHODS

A field experiment was conducted during the 2023-2024 crop year at the research station of the College of Education for Pure Science - Ibn Al-Haitham - University of Baghdad, in a clayey silt, sandy clay loam, to gain insight into the response of growth, yield and active compounds of rosemary to spraying of nano-zinc and BA. Optimal soil conditions were created thorough plowing, disking and levelling. The experimental field was then divided into 27 uniform units, each unit covering an area of 1 m². Nitrogen and phosphorus fertilizer were applied according to the established scientific recommendations to improve soil fertility. Six-month-old rosemary seedlings were carefully selected to ensure similarity in size, vegetative growth, and height. These seedlings were transplanted into the field on March 15, 2024, they were planted with a separation of 50 cm between rows and 25 cm between individual plants to ensure proper spacing and resource allocation. A factorial experiment was conducted using a randomized complete block design (RCBD) with three replications to ensure statistical reliability. The experiment were examined two factors: the first was nano-zinc, applied at three concentrations (0, 10, and 20 mg L⁻¹), and the second was BA, also

administered at three concentrations (0, 3, and 6 mg L⁻¹). The seedlings of rosemary were sprayed two times with concentrations of both nano-zinc and BA, the first one after 30 days of seedling transplanting and the second one was after 60 days. Spraying was performed in the morning with a 200 L portable sprayer until the vegetative mass was completely wet. Rosemary seedlings were sprayed with the assigned concentrations of nano-zinc and BA: the first application occurred 30 days after transplanting, and the second followed one month later. Spraying was conducted in the early morning using a 200 L hand sprayer, ensuring complete wetting of the vegetative mass. Routine agricultural practices were performed as required to support plant growth.

Studied traits

The following traits were measured at the late of June 2024 as follows:

1. Plant height (cm): It was measured as an average height of five plants picked randomly out of each experimental unit from the surface of the soil to the highest growing tip of the plant.
2. The number of branches per plant: It was assessed by calculating the number of main lateral branches from five plants in each experimental unit.
3. Branch length (cm): It was measured as an average length of five branches from five plants from the point of contact of the branch with the stem to tip.
4. Nitrogen content of plant (%): It was determined by Micro-kjeldhal based on the Haynes method [14].
5. Phosphorous content of plant (%): It was assessed using a spectrophotometer at a wavelength of 880 nm, following the Haynes method [14].
6. Chlorophyll content of leaves (mg g⁻¹ fresh

weight): It was measured using the Mackinney method [15].

7. Leaves yield (Kg ha⁻¹): It was calculated using the Eq. 1.

8. Volatile oil content of leaves (%): The volatile oil was extracted from 15 g of dried leaf through hydro-distillation for 3 h using a clevenger-type apparatus. The extracted oil was then dried using anhydrous sodium sulphate to remove residual moisture [16]. The volatile oil content of the leaves was calculated using the Eq. 2 [17].

9. Rosmarinic acid content of leaves (mg g⁻¹ dry weight): The rosmarinic acid content in the leaves was estimated according to Guenther [17].

Estimation and diagnosis of the rosmarinic acid using HPLC

The rosmarinic acid was separated by HPLC device under the conditions shown in Table 1. The qualitative identification of rosmarinic acid was carried out by comparing its retention time in the crude sample with that of the standard compound. The concentrations of rosmarinic acid were then calculated using the Eq. 3.

Where, Ac is area of the active ingredient in the crude. As is area of the active ingredient in the standard compound. C is concentration of standard compound. D is number of dilution times.

Following data collection and tabulation, statistical analysis was conducted using Genstat software in accordance with the experimental design. Treatment means were compared using the least significant difference (LSD) test at the 0.05 probability level [18].

RESULTS AND DISCUSSION

Plant height (cm)

Nano-Zn effect

The data presented in Table 2 demonstrate a significant effect of nano-zinc (nano-Zn) spraying

$$\text{Seed yield (ton ha}^{-1}\text{)} = \frac{\text{Plant leaves yield (g plant}^{-1}\text{)} \times \text{Plant density (plant ha}^{-1}\text{)}}{10^3} \quad (1)$$

$$\text{volatile oil (\%)} = \frac{\text{volatile oil weight (g)}}{\text{Sample weight (g)}} \times 100 \quad (2)$$

$$\text{Concentration of active compounds} = (\text{Ac/As}) \times \text{C} \times \text{D} \quad (3)$$

on plant height. The highest concentration (20 mg L⁻¹) of nano-Zn resulted in the greatest average plant height, measuring 41.44 cm, whereas the control treatment (without nano-Zn spraying) recorded the lowest average, at 34.56 cm. The superior performance of plants treated with nano-Zn can be attributed to the enhanced chemical efficacy of this fertilizer, owing to nanoparticle size. The small particle size increases surface area, thereby enhancing physiological activity [8]. This mechanism likely promoted the biosynthesis of tryptophan, a precursor for indole-3-acetic acid (IAA) synthesis, which plays a critical role in cell elongation [6].

BA effect

The findings in Table 2 indicate that there was a significant effect of BA spraying in this trait. Control treatment (without spraying of BA) showed the highest average of plant height (40.89 cm) in comparison to spraying of BA at a concentration of 3 and 6 mg L⁻¹, which resulted in the lowest average of 39.00 and 33.33 cm, respectively. The

decreasing the plant height during spraying BA may be due to its antagonism effects on the action of auxins accumulated in the terminal bud, thus destroying the apical dominance of the main stem [13].

Interaction effect

The interaction between the studied variables had a significant effect on this trait (Table 2). This interaction results from diverse relative responses of the trait to different nano-Zn and BA concentrations. Spraying nano-Zn at 20 mg L⁻¹ along with BA at 3 mg L⁻¹ yielded the highest value (44.00 cm), which was not significantly different from the combination of 20 mg L⁻¹ nano-Zn without BA spraying (42.33 cm). In contrast, the combination of BA at 6 mg L⁻¹ with no nano-Zn resulted in the lowest value (29.33 cm).

Branches number per plant

Nano-Zn effect

The results presented in Table 3 indicate that the branches number of plant was significantly

Table 1. Separation conditions.

Separation column	C-18 DB Colum (50 x 2.0)
Mobile phase	Phosphoric acid (0.1%) + methanol 88: 12 (V: V)
Flow rate	1.2 ml min ⁻¹
sample volume	25 ug ml ⁻¹
Detector	UV at 285 nm wavelength and 20°C

Table 2. Response of plant height (cm) to spraying nano- Zn and

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	39.00	35.33	29.33	34.56
10	41.33	37.67	32.67	37.22
20	42.33	44.00	38.00	41.44
Average	40.89	39.00	33.33	
Lsd 0.05	Nano-Zn = 1.33	BA = 1.33	Interaction = 2.31	

Table 3. Response of branches number to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	7.33	10.00	13.33	10.22
10	8.67	12.33	15.00	12.00
20	11.67	17.33	16.67	15.22
Average	9.22	13.22	15.00	
Lsd 0.05	Nano-Zn = 0.79	BA = 0.79	Interaction = 1.36	

influenced by nano-Zn concentrations. The 20 mg L⁻¹ concentration resulted in the highest average (15.22 branches per plant), in contrast to the control treatment (without nano-Zn spraying), which recorded the lowest average (10.22 branches per plant). The enhancement in branch number can be attributed to the unique properties of nano-Zn fertilizer, which provide a large surface area for various metabolic processes in the plant due to the small particle size [8]. Additionally, zinc plays a crucial role as a regulator of numerous enzymatic activities within the plant [6].

BA effect

The data in Table 3 also indicate that spraying BA at a concentration of 6 mg L⁻¹ significantly outperformed other treatments, resulting in the highest average for this trait (15.00 branches per plant). In contrast, the control treatment (without BA spraying) recorded the lowest average (number of branches per plant). The production of branches in plants is related to apical dominance, which is regulated by plant hormones, particularly auxin. BA plays a role in overcoming apical dominance by reducing the levels of tryptophan, a non-polar amino acid essential for auxin biosynthesis. Additionally, BA may increase the activity of IAA-oxidase and peroxidase enzymes, which reduce auxin levels in the plant, thereby promoting the development and growth of lateral buds. This is facilitated by the expansion of vascular tissues, contributing to the increase in the number of branches [13].

Interaction effect

The interaction between the two factors had a significant effect on this trait (Table 3). This interaction may be attributed to the diverse responses of the trait towards different concentrations of nano-zinc combined with BA. Spraying nano-Zn at 20 mg L⁻¹ along with BA at 3 mg L⁻¹ resulted in the highest value (17.33 branches

per plant), whereas the control combination recorded the lowest value (7.33 branches per plant).

Branch length (cm)

Nano-Zn effect

The results in Table 4 show a significant effect of nano-Zn spraying on branch length. High concentration (20 mg L⁻¹) of nano-Zn resulted in the long average branch length, measuring 29.5 cm, while the control treatment (without nano-Zn application) recorded the short average at 23.6 cm. The enhancement in branch length following nano-Zn application may be attributed to the biochemical activity of zinc nanoparticles, which stimulate tryptophan biosynthesis, enhance IAA synthesis and promote cell elongation [6]. Alternatively, the increased branch length could be linked to the positive effect of nano-Zn on plant height.

BA effect

The results in Table 4 show that spraying BA at 3 mg L⁻¹ significantly outperformed the other treatments, resulting in high average for this trait (28.6 cm), with no significant difference from the control treatment (without BA), which had the low average (27.5 cm). In contrast, spraying BA at 6 mg L⁻¹ resulted in the low average (22.9 cm). The reduction in branch length with the higher BA concentration may be due to the antagonistic effects of BA on auxin activity, as well as the increased activity of IAA-oxidase and peroxidase enzymes [19].

Interaction effect

The interaction between the factors studied had a significant effect on this trait (Table 4). This may be due to the different responses of the trait towards different concentrations of nano-Zn and BA. Spraying nano-Zn at 20 mg L⁻¹ without BA resulted in the highest value (31.8 cm), while

Table 4. Response of branch length (cm) to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	24.5	26.8	19.7	23.6
10	26.4	28.8	22.4	25.9
20	31.8	30.1	26.7	29.5
Average	27.5	28.6	22.9	
Lsd 0.05	Nano-Zn = 1.2 BA = 1.2 Interaction = 2.0			

spraying BA at 6 mg L⁻¹ without nano-Zn resulted in the lowest value (19.7 cm).

Nitrogen content of plant

Nano-Zn effect

Spraying nano-Zn at a concentration of 20 mg L⁻¹ significantly increased the plant's nitrogen content, achieving the highest average of 2.524%, as compared to 2.458% at 10 mg L⁻¹ of nano-Zn, and the lowest average of 2.253% in untreated plants (Table 5). Zinc is an essential micronutrient for plant growth and development, acting as a cofactor and regulator for various enzymes, including dehydrogenase, alkaline phosphatase, and phospholipase [6]. The enhancement in nitrogen content may be attributed to the role of zinc nanoparticles in enhancing the plant's critical metabolic processes. In this regard, Fageria et al. [20] noted that zinc improves the cation-exchange capacity of roots, thereby facilitating the uptake of essential nutrients.

BA effect

Regarding the effect of BA on rosemary, the results in Table 5 show that spraying BA at a concentration of 6 mg L⁻¹ outperformed other treatments, resulting in high average nitrogen content of 2.554%. This was followed by BA at 3 mg L⁻¹, which resulted in 2.424%, while untreated plants recorded the low average of 2.257% (Table

5). The role of BA in promoting cell division and expansion, stimulating lateral bud formation, and directing the distribution of photosynthetic products to growth regions contributed to the increased nitrogen content in the leaves [13]. Additionally, Davies [21] reported that spraying cytokinins enhances the absorption and transfer of nutrients from soil solutions.

Interaction effect

It was observed that there was a significant interaction between the studied factors. Spraying nano-Zn at 20 mg L⁻¹ and BA at 6 mg L⁻¹ resulted in high value (2.637%), with no significant difference as compared to spraying nano-Zn at 10 mg L⁻¹ and BA at the same concentration (2.597%). In contrast, untreated plants recorded low value of 2.053%.

Phosphorous content of plant

Nano-Zn effect

Table 6 shows that spraying nano-Zn at 10 mg L⁻¹ provided a considerable advantage, resulting in the high percentage of this trait (0.425%) as compared to the control treatment, which recorded the low percentage (0.312%). The activity of zinc nanoparticles, when applied at 20 mg L⁻¹, was also effective in increasing the phosphorus content in rosemary leaves. This may be because of its function in enhancing the cellular division

Table 5. Response of nitrogen content of plant to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	2.053	2.277	2.430	2.253
10	2.303	2.473	2.597	2.458
20	2.413	2.523	2.637	2.524
Average	2.257	2.424	2.554	
Lsd 0.05	Nano-Zn = 0.040		Interaction = 0.069	

Table 6. Response of phosphorous content of plant to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	0.248	0.332	0.356	0.312
10	0.258	0.357	0.393	0.336
20	0.351	0.452	0.471	0.425
Average	0.286	0.380	0.407	
Lsd 0.05	Nano-Zn = 0.039		Interaction = N. S	

and stimulating the vegetative growth (Tables 2, 3 and 4), which was beneficially exhibited in the ability of the plants in increasing the absorption of nutrients and thus elevating their concentration in the plant tissues.

BA effect

The results shown in Table 6 explain that spraying BA at a 6 and 3 mg L⁻¹ reached 0.407 and 0.380%, respectively with non-significant difference as compared with reference treatment which achieved the low percentage 0.286%. The increasing the phosphorous content of plant with the application of BA at a 6 mg L⁻¹ could be because of the role of BA in enhancing the cell division of lateral buds and boosting the plant number of branches.

Interaction effect

According to the results in Table 6, the interaction between the two experimental factors had no significant effect on this trait.

Chlorophyll content of leaves (mg g⁻¹ fresh weight) Nano-Zn effect

The findings in Table 7 indicate that the chlorophyll content in leaves was significantly affected by nano-Zn concentrations. The 20 mg L⁻¹ concentration achieved high average (1.527 mg g⁻¹ fresh weight), in contrast to the control treatment (without nano-Zn spraying), which recorded low average (1.283 mg g⁻¹ fresh weight). This enhancement can be attributed to the chemical activity of the nano-Zn fertilizer, when applied at an optimal concentration. This led to an enhancement in the activity of enzymes is responsible for chlorophyll biosynthesis [22]. Alternatively, the observed superiority may be due to the positive role of nano-Zn in increasing the plant's nitrogen content (Table 5).

BA effect

Table 7 also shows that spraying BA at a concentration of 6 mg L⁻¹ significantly outperformed the other treatments, achieving high average for this trait (1.568 mg g⁻¹ fresh weight). In contrast, the control treatment (without BA) recorded low average (1.290 mg g⁻¹ fresh weight). This could be because of the role of BA in the development of chloroplasts and stimulating their construction, as well as delaying the loss and decomposition of chlorophyll [23]. In this regard, many studies indicated that cytokinins encourage chloroplast differentiation, chlorophyll construction and reduce their destruction, in addition to delaying leaf aging [19].

Interaction effect

Regarding the interaction between experimental factors, the findings in Table 7 indicate that the interaction had a substantial effect on this trait. Diverse relative responses of this trait to different concentrations of nano-Zn and BA levels may explain the reason for this interaction. Spraying nano-Zn at 20 mg L⁻¹ and BA at 6 mg L⁻¹ recorded high value (1.620 mg g⁻¹ fresh weight), with no significant difference as compared to spraying nano-Zn at 10 mg L⁻¹ and BA at the same concentration (1.589 mg g⁻¹ fresh weight). In contrast, the control treatment combination recorded low value of 1.118 mg g⁻¹ fresh weight.

Leaves yield (Kg ha⁻¹)

Nano-Zn effect

Yield of rosemary leaf was significantly increased for spraying nano-Zn at 20 mg L⁻¹, with improvement of 5.10% and 19.79% as compared to spraying nano-Zn at 10 mg L⁻¹ and the control treatment (Table 8). The enhancement in leaf yield with application of nano-Zn at 20 mg L⁻¹ can be attributed to the enhanced plant height (Table 2),

Table 7. Response of chlorophyll content of leaves (mg g⁻¹ fresh weight) to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	1.118	1.238	1.495	1.283
10	1.312	1.465	1.589	1.455
20	1.441	1.519	1.620	1.527
Average	1.290	1.407	1.568	
Lsd 0.05	Nano-Zn = 0.050	BA = 0.050	Interaction = 0.087	

number of branches per plant (Table 3), branch length (Table 4), and chlorophyll content in leaves (Table 7). These results are consistent with those of Mehraban et al. [5].

BA effect

The results in Table 8 indicate that spraying BA at 6 mg L⁻¹ significantly outperformed other treatments, achieving the high average yield (1117.2 kg ha⁻¹) as compared to spraying BA at 3 mg L⁻¹, which resulted in 998.7 kg ha⁻¹. The control treatment (spraying distilled water only) recorded low average of 912.0 kg ha⁻¹. The enhancement in leaf yield can be attributed to the effect of BA at 6 mg L⁻¹, which enhanced the number of branches per plant (Table 3) and chlorophyll content in leaves (Table 7).

Interaction effect

Regarding the interaction between experimental factors, the findings in Table 8 indicate that the interaction had a significant impact on this trait. Diverse relative responses of this trait to different concentrations of nano-Zn and BA levels may explain the observed interaction. Spraying nano-Zn at 20 mg L⁻¹ combined with spraying BA at 6 mg L⁻¹ recorded high value of 1155.5 kg ha⁻¹, with no significant difference as compared to spraying nano-Zn at 10 mg L⁻¹ and BA at the same concentration (1132.9 kg ha⁻¹). In contrast, the control combination recorded low value of 784.8

kg ha⁻¹.

Volatile oil content of leaves

Nano-Zn effect

Spraying nano-Zn at 20 mg L⁻¹ significantly outperformed other treatments, achieving high percentage of volatile oil content in leaves (4.10%), as compared to spraying 10 mg L⁻¹ of nano-Zn (3.85%) and the untreated plants, which recorded low average of 3.69% (Table 9). The enhancement in volatile oil content could be attributed to the role of zinc nanoparticles in enhancing the plant's nitrogen and phosphorus content (Tables 5 and 6) and chlorophyll content (Table 7). This stimulated the activity of multiple enzymes, improving the efficiency of photosynthesis and increasing its metabolic byproducts. These results are consistent with the findings of Mehraban et al. [5].

BA effect

Regarding the effect of BA, the findings in Table 9 show that spraying BA at 6 mg L⁻¹ significantly outperformed the other treatments, resulting in high percentage of volatile oil content (4.06%), followed by spraying BA at 3 mg L⁻¹, which yielded 3.99%. Untreated plants recorded low percentage of 3.59%. The role of BA in increasing the plant's nitrogen and phosphorus content (Tables 5 and 6) and chlorophyll content in leaves (Table 7) likely contributed to enhanced photosynthetic activity, improving the biosynthesis pathways responsible

Table 8. Responses of leaves yield (Kg ha⁻¹) to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	784.8	873.7	1063.0	907.2
10	928.0	1041.1	1132.9	1034.0
20	1023.2	1081.3	1155.5	1086.7
Average	912.0	998.7	1117.2	
Lsd 0.05	Nano-Zn = 37.0 BA = 37.0 Interaction = 64.1			

Table 9. Responses of volatile oil content of leaves to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L ⁻¹)	BA Conc. (mg L ⁻¹)			Average
	0	3	6	
0	3.37	3.77	3.93	3.69
10	3.61	3.88	4.05	3.85
20	3.80	4.32	4.20	4.10
Average	3.59	3.99	4.06	
Lsd 0.05	Nano-Zn = 0.06 BA = 0.06 Interaction = 0.11			

Table 10. Responses of rosmarinic acid content of leaves (mg g^{-1} dry weight) to spraying of nano-Zn and BA concentrations and their interaction.

Nano-Zn Conc. (mg L^{-1})	BA Conc. (mg L^{-1})			Average
	0	3	6	
0	440.7	593.0	701.6	578.4
10	507.3	748.5	787.5	681.1
20	576.5	704.4	671.3	650.7
Average	508.2	681.9	720.1	
Lsd 0.05	Nano-Zn = 25.4	BA = 25.4	Interaction = 43.9	

for volatile oil production and its accumulation in oil glands.

Interaction effect

The interaction between the studied factors had a significant impact on this trait (Table 9). Plants sprayed with nano-Zn at 20 mg L^{-1} and BA at 3 mg L^{-1} exhibited high volatile oil content (4.32%), while the control combination recorded low value of 3.37%.

Rosmarinic acid content of leaves (mg g^{-1} dry weight)

Nano-Zn effect

The results reveal that the rosemary plants sprayed with nano-Zn at 10 mg L^{-1} significantly outperformed other treatments, achieving high average of rosmarinic acid content in leaves (681.1 mg g^{-1} dry weight), as compared to the control treatment, which recorded low average of 578.4 mg g^{-1} dry weight (Table 10).

BA effect

Table 10 show that spraying BA at 6 mg L^{-1} significantly outperformed other treatments, resulting in high average of rosmarinic acid content (720.1 mg g^{-1} dry weight). This was in comparison with spraying BA at 3 mg L^{-1} , which gave 681.9 mg g^{-1} dry weight, and the control treatment, which recorded low average of 508.2 mg g^{-1} dry weight. The superiority of plants sprayed with BA at 6 mg L^{-1} in terms of nitrogen and phosphorus content (Tables 5 and 6) and chlorophyll content (Table 7) may explain the enhanced rosmarinic acid content observed in these plants.

Interaction effect

The results in Table 10 reveal that the interaction between the two factors significantly impacted this trait. The plants sprayed with nano-Zn at 10 mg L^{-1} and BA at 6 mg L^{-1} exhibited high rosmarinic

acid content of 787.5 mg g^{-1} dry weight, with no significant difference from plants sprayed with the same concentration of nano-Zn and 3 mg L^{-1} of BA (748.5 mg g^{-1} dry weight). In contrast, the control combination recorded low value of 440.7 mg g^{-1} dry weight.

CONCLUSION

The research findings demonstrated a remarkable response of rosemary plants to zinc nanoparticle spraying. Applying nano-zinc at a concentration of 20 mg L^{-1} substomatal stimulation of the vegetative growth and enhanced the plant's chemical composition, resulting in increased leaf yield per unit area and higher essential oil content. Meanwhile, spraying nano-zinc at 10 mg L^{-1} notably elevated the rosmarinic acid content of leaf. Similarly, rosemary plants showed a clear response to BA application. Spraying BA at a concentration of 6 mg L^{-1} enhanced the branches number of plant and improved the plant's essential chemical elements. This treatment also boosted leaf yield and the byproducts of the photosynthesis process, particularly the leaves' volatile oil content and rosmarinic acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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