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## EFFECT OF PINCHING AND KINETIN WITH HUMIC ACID ON THE VEGETATIVE GROWTH OF THE ROSEMARY PLANT (*ROSMARINUS OFFICINALIS* L.)

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### SUMMARY

The study, conducted in a lath house in the autumn of 2022–2023 at the College of Agriculture, Al-Qasim Green University, Al-Qasim, Iraq, determined the humic acid effect on the vegetative growth of the rosemary plant (*Rosmarinus officinalis* L.). The experiment included two factors: the first factor was pinching, where half of the plants received pinching and the other half was without pinching. The second factor was adding humic acid at concentrations of 0, 4, and 8 ml/L and spraying with kinetin at concentrations of 0.50 and 100 mg/L. The experiment layout was in a randomized complete block design (RCBD) with factorial arrangement (2 × 3 × 3) and three replications. The triple interaction treatment of pinching, humic acid at 0 concentration, and kinetin at 0 concentration gave significant highest values for plant sprouting (29.67 cm). Meanwhile, for the concentration of 50 mg/L, the number of vegetative branches is 5.33 branches plant<sup>-1</sup>, and the treatments (P1), humic (8 ml/L), and kinetin (50 mg/L) gave the topmost values, significantly affecting the traits, stem diameter (5.98 mm), and the number of leaves (696.00 leaves plant<sup>-1</sup>).

**Keywords:** Rosemary plant (*R. officinalis* L.), pinching, humic acid, kinetin, sprouting, branches per plant, stem diameter, number of leaves

**Key findings:** The triple interaction treatment of pinching P1, humic acid at 0 concentration, and kinetin at 0 concentration significantly gave the highest values for sprouting (29.67 cm). Similarly, the concentration of 50 mg/L resulted in the number of vegetative branches at 5.33 branches per plant in the rosemary plant (*R. officinalis* L.).

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## INTRODUCTION

The rosemary plant (*Rosmarinus officinalis* L.) is one of the perennial and evergreen shrubs of the Lamiaceae family. Its original home is the Mediterranean basin and Southern Europe, where it grows wild there, from which its cultivation and spread expanded to most countries of the world. Spain, France, and Tunisia are among the most important countries that produce the oil extracted from this plant (Peter, 2004).

The cultivation of medicinal and aromatic plants, in general, and rosemary, in particular, increases. It is one of the alternative economic crops whose cultivation has expanded on a large scale recently due to the suitability of the natural conditions and circumstances. The environmental benefits of its cultivation add to the good economic returns resulting from it. Therefore, attention and development in cultivating these plants are essential for their important role in future agricultural development. Especially, they contain tannins, B1, B2, B3, B6, and B9, and vitamin C and minerals, such as iron, calcium, zinc, and potassium.

The rosemary plants also contain antioxidants and volatile oils that comprise the compounds borneol, cineol, camphor, camphene, and linalool. Moreover, they contain flavonoids and rosmarinic acid. Rosemary often serves as a spice, appetizer, and taste enhancer. It is an ingredient in salads, desserts, meat, and their products, as well as in fish, vegetables, and soups, and in preserving canned meat and fish products. Its pure oil is also desirable in perfumery, cosmetics, and shampoos to strengthen hair, and in skin care products for the face, hands, and body. It is effective in the manufacture of soap and other detergents due to its distinctive and pleasant aroma. Modern agriculture is moving towards avoiding all pollutants by relying on the use of natural materials, such as organic and biofertilizers, instead of chemical fertilizers (EL-Akabawy, 2000).

The process of pinching (pinching the growing top) is one of the important agricultural operations that leads to an

increase in the number of plant branches and breaking the apical dominance, which significantly stimulates the growth of lateral shoots (Ahmed *et al.*, 2007; Rao *et al.*, 2008). It also affects the shape of the plant and increases the number of branches and leaf area; hence, many plant breeders perform pinching to increase the density of the plant. Cytokinins play a fundamental role in stimulating the process of cell division and specialization in partnership with auxins. Their importance also appears in many other physiological processes, such as in the phenomenon of apical dominance, which influences the process of plant branching. The first cytokinin compound discovered is kinetin, which is not found naturally in plants. Kinetin is actually a product formed during the thermal degradation of adenine.

The discovery of kinetin encouraged the industrial synthesis of hundreds of similar compounds. However, kinetin is one of the most common and used substances in studies on the physiological effects of cytokinins (Wareing and Phillips, 1981). Kinetin works on inhibiting apical dominance in the plant, stimulating the growth of lateral branches, the occasional expansion of cells, forming the apparent appearance of the plant, delaying senescence, and preserving the green color (Al-Asadi and Al-Khikani, 2019). One of the organic fertilizers used is humic acid, which is considerably a complex substance that results from the decomposition of plant and animal materials through the humidification process.

These substances consist mainly of humic acid, fulvic acid, and humin. These substances have an essential role in soil fertility and plant nutrition, and humic acids have a positive effect on growth, as they enhance nutrient uptake and stimulate physiological activity within plant cells (Pettit, 2008). When spraying the rosemary plant with humic acid, it will significantly improve the growth of shoots (Jalayerinia *et al.*, 2017). The presented study aimed to determine the effect of pinching, kinetin, and humic acid on the vegetative growth and some secondary metabolites of rosemary plants.

## MATERIALS AND METHODS

The experiment, conducted in a randomized complete block design, was a factorial experiment ( $2 \times 3 \times 3$ ) with three replications. Each replicate contained 18 treatments with five seedlings for each experimental unit. The folds represented the main panels, and the humic concentrations represented by kinetin concentrations and their interactions were secondary panels. The means comparison used the least significant difference (LSD) test at the 5% level. The experiment included a study of two factors. The main factor was pinching—the growing top of the rosemary seedlings was cut off, with half of the plants cut off, denoted by the symbol P1, and leaving the rest of the plants uncut, denoted by the symbol P0. The second factor was adding humic acid and spraying kinetin. In spraying with kinetin, the treatment comprised spraying the leaves until they were completely wet (control without using kinetin, symbolized by K0). The use of kinetin at a concentration of 50 mg/L had the symbol K1, and the kinetin at a concentration of 100 mg/L had the symbol K2. The spraying with kinetin twice had the first spraying done immediately after the renovation on October 26, 2022. The second spraying ensued a month after the first spraying, on November 27, 2022, with the humic acid added to the soil, according to the following concentrations: without addition (symbolized as H0); adding humic acid at 4 ml/liter (symbolized as H1); and adding humic acid at 8 ml/liter (symbolized as H2). The addition of humic acid was in three batches. The first was after the pinching process, cutting off the growing tip of the plant on October 12, 2022. The second was a month after the first addition, on November 12, 2022, and the third addition was on December 12, 2022. Measurements of the studied traits continued on all experimental plants for five plants in each experimental unit and included the following traits: plant height (cm), main stem diameter (mm), the number of vegetative branches (branch plant<sup>-1</sup>), leaf area (cm<sup>2</sup> plant<sup>-1</sup>), the number of leaves (leaf plant<sup>-1</sup>), and plant spreads (cm).

## RESULTS

### Plant height

The results revealed significant differences in the binary interaction between the treatment of pinching and humic acid (Table 1). The treatment P0H2 excelled with the highest average rosemary plant height of 31.39 cm compared with the treatment P1H0, which gave the lowest average plant height of 28.67 cm. The bi-interactions between the treatments of spraying with kinetin and pinching led to a significant superiority among the plants, as the comparison treatment P0K0 was distinct in giving the highest height. It amounted to 30.89 cm, remarkably excelling the treatment P1K2, which gave the lowest height at 28.28 cm. It is also noteworthy from the table that significant differences occurred between the treatments. In the triple interaction between the study factors, treatment P0H2K2 provided the highest average plant height of 32.33 cm compared with treatments P1H0K1 and P1H0K2, with the lowest plant height average of 27.33 cm.

### Stem diameter

From Table 2, significant differences appeared between the humic treatments of the rosemary plant, where treatment H0 gave the largest stem diameter of the plant, reaching 5.54 mm compared with treatment H1. It gave the smallest stem diameter, reaching 5.01 mm. In the interaction among study factors, one can note considerable differences between the treatments, as treatment P1H2K1 gave the highest values for the average stem diameter, reaching 5.98 mm. Meanwhile, treatment P1H2K2 recorded the lowest average stem diameter at 4.63 mm.

### Branches per plant

The findings disclosed significant differences in the triple interaction between the treatments for the rosemary plant (Table 3). The treatment P1H1K1 gave the most vegetative

**Table 1.** Effect of pinching, humic acid, kinetin, and their interactions on plant height (cm) of rosemary plants for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	29	31	31.83	30.61
	K1	31	31.67	30	30.89
	K2	30	27.17	32.33	29.83
P1	K0	30	30	29.83	29.94
	K1	27.33	31.17	31.17	29.89
	K2	27.33	29	28.5	28.28
LSD <sub>0.05</sub>		4.02			2.32
P×H					P
P0		30	29.94	31.39	30.44
P1		28.22	30.06	29.83	29.37
LSD <sub>0.05</sub>		2.32			N.S.
K×H					K
K0		29.50	30.50	30.83	30.28
K1		29.17	31.42	30.58	30.39
K2		28.67	28.08	30.42	29.06
LSD <sub>0.05</sub>		N.S.			N.S.
H		29.11	30.00	30.61	
LSD <sub>0.05</sub>		N.S.			

**Table 2.** Effect of pinching, humic acid, kinetin, and their interactions on stem diameter (mm) of rosemary plants for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	5.36	4.71	4.88	4.98
	K1	5.70	4.90	4.88	5.16
	K2	5.64	5.01	5.66	5.44
P1	K0	5.48	5.03	4.94	5.15
	K1	5.46	5.35	5.98	5.60
	K2	5.61	5.04	4.63	5.09
LSD <sub>0.05</sub>		1.27			N.S.
P×H					P
P0		5.57	4.87	5.14	5.19
P1		5.52	5.14	5.19	5.28
LSD <sub>0.05</sub>		N.S.			N.S.
K×H					K
K0		5.42	4.87	4.91	5.07
K1		5.58	5.13	5.43	5.38
K2		5.63	5.03	5.15	5.27
LSD <sub>0.05</sub>		N.S.			N.S.
H		5.54	5.01	5.16	
LSD <sub>0.05</sub>		0.52			

**Table 3.** Effect of pinching, humic acid, kinetin, and their interactions on the number of vegetative branches plant<sup>-1</sup> of rosemary plants for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	4.67	4.67	5.00	4.78
	K1	5.00	4.33	5.00	4.78
	K2	4.83	5.00	5.00	4.94
P1	K0	4.67	4.33	5.00	4.67
	K1	4.83	5.33	5.00	5.06
	K2	4.67	4.83	4.33	4.61
LSD <sub>0.05</sub>		0.84			N.S.
P×H					P
P0		4.83	4.66	5	4.83
P1		4.72	4.83	4.77	4.78
LSD <sub>0.05</sub>		N.S.			N.S.
K×H					K
K0		4.67	4.50	5.00	4.72
K1		4.92	4.83	5.00	4.92
K2		4.75	4.92	4.67	4.78
LSD <sub>0.05</sub>		N.S.			N.S.
H			4.75	4.88	
LSD <sub>0.05</sub>		N.S.			

branches of the plant, reaching 5.33 branches plant<sup>-1</sup>, while treatments P1H1K0 and P1H2K2 recorded the fewest branches. The vegetative rate of the plant reached 4.33 branches plant<sup>-1</sup>.

### Leaf area

The study factors and their bi-interactions between pinching and humic acid expressed a notable effect on the average leaf area (Table 4). The treatment P0H2 gave the highest average leaf area of 678 cm<sup>2</sup>, while plants treated with P1H2 had the lowest average leaf area of 631.89 cm<sup>2</sup>. One can also note from the table significant differences between the coefficients in the three-way interaction between factors. The study showed treatments P0H2K0 and P1H2K2 provided the highest average leaf area, amounting to 822.67 cm<sup>2</sup>, while treatment P1H0K1 gave the lowest average leaf area, which amounted to 525.33 cm<sup>2</sup>.

### Leaves per plant

The results revealed a significant superiority among plants existed in the binary interactions between kinetin and humic (Table 5). The

H1K2 treatment was exceptional by giving the highest average number of leaves per plant, which amounted to 639.50 leaves plant<sup>-1</sup> versus the H1K0 treatment. It gave the lowest average number of leaves for the plant, amounting to 458.50 leaves plant<sup>-1</sup>. In the triple interaction between the study factors, we noticed a remarkable supremacy between the treatments, as the P1H2K1 treatment gave the most average number of leaves per plant, which reached 696.00 leaves plant<sup>-1</sup>. Meanwhile, the P1H1K0 treatment recorded the lowest average number of leaves per plant, which amounted to 434.33 leaves plant<sup>-1</sup>.

### Plant spread

The findings enunciated that significant differences emerged between plants in bi-interactions (Table 6). The P1K0 treatment has characteristics of giving the largest plant spreads, reaching 26.28 cm in the control. The P1K2 treatment indicated the lowest plant sprouting, amounting to 23.39 cm, and the combined effect between spraying with kinetin and adding humic acid had a significant effect on the rate of plant spreads. Treatment H0K0 had the largest plant spread, reaching 27.25

**Table 4.** Effect of pinching, humic acid, kinetin, and their interactions on the leaf area  $\text{cm}^2\text{plant}^{-1}$  of rosemary plants for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	583.33	583.33	822.67	663.11
	K1	663.67	566.33	608.67	612.89
	K2	753.33	834.00	602.67	730.00
P1	K0	699.00	527.33	534.00	586.78
	K1	525.33	749.33	789.33	688.00
	K2	583.33	583.33	822.67	663.11
LSD <sub>0.05</sub>		295.39			N.S.
P×H					P
P0		666.78	661.22	678	668.67
P1		644.22	659.44	631.89	645.18
LSD <sub>0.05</sub>		170.54			N.S.
K×H					K
K0		641.17	555.33	678.33	624.94
K1		594.50	657.83	699.00	650.44
K2		730.83	767.83	587.5	695.39
LSD <sub>0.05</sub>		208.87			N.S.
H		655.50	660.33	654.94	
LSD <sub>0.05</sub>		N.S.			

**Table 5.** Effect of pinching, humic acid, kinetin, and their interactions on the number of leaves  $\text{plant}^{-1}$  of the rosemary plant for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	549	482.67	589.67	540.45
	K1	549	474	479.00	500.67
	K2	580.33	635.67	558.33	591.44
P1	K0	617.67	434.33	510.00	520.67
	K1	485.67	600.33	696.00	594.00
	K2	566.33	643.33	495.33	568.33
LSD <sub>0.05</sub>		235.38			N.S.
P×H					P
P0		559.44	530.78	542.33	544.1833
P1		556.56	559.33	567.11	561
LSD <sub>0.05</sub>		N.S.			N.S.
K×H					K
K0		583.33	458.50	549.83	530.55
K1		517.33	537.17	587.50	547.33
K2		573.33	639.50	526.83	579.89
LSD <sub>0.05</sub>		135.89			N.S.
H		558.00	545.06	554.72	
LSD <sub>0.05</sub>		N.S.			

**Table 6.** Effect of pinching, humic acid, kinetin, and their interactions on plant spreads (cm) of rosemary plants for the autumn of 2022–2023.

P	K	H			K x P
		H0	H1	H2	
P0	K0	24.83	26.33	21.67	24.28
	K1	24.5	24.5	26	25.00
	K2	23.83	25	25.17	24.67
P1	K0	29.67	23.67	25.5	26.28
	K1	24.5	23.17	26.67	24.78
	K2	23.17	23.83	23.17	23.39
LSD <sub>0.05</sub>		3.91			2.25
P×H					P
P0		24.39	25.28	24.28	24.65
P1		25.78	23.56	25.11	24.82
LSD <sub>0.05</sub>		N.S.			N.S.
K×H					K
K0		27.25	25.00	23.58	25.28
K1		24.50	23.83	26.33	24.89
K2		23.5	24.42	24.17	24.03
LSD <sub>0.05</sub>		2.76			N.S.
H		25.08	24.42	24.69	
LSD <sub>0.05</sub>		N.S.			

cm, a substantial difference from treatment H0K2, which reached 23.5 cm. The triple interaction between the study factors proved significantly excellent between the treatments, as treatment P1H0K0 gave the widest average of plant sprouting, reaching 29.67 cm, while treatment P0H2K0 had the lowest average of plant seedlings, which reached 21.67 cm.

## DISCUSSION

The natural concentration of auxin at the top of the plant makes the plant grow taller, and the pinching process will reduce the concentration of this auxin, thus breaking the apical dominance, allowing the lateral shoots to grow. On the other hand, in certain species, the absence of pinching may limit optimal vegetative growth due to a persistent apical dominance. As a result, pruned plants tend to be shorter than unpruned ones due to the loss of apical dominance (Habibullah, 2012).

The decrease in plant height in pruned plants could be mainly due to the elimination of apical dominance, the production of appropriate levels of cytokinins in the roots of plants, and their transfer to the sites of lateral

shoots. The latter contributes to the processes of division through the formation of lateral branches. They work in an interconnected or antagonistic manner with other hormones, such as auxins and gibberellins, and convert the plant's metabolic materials from vertical growth to horizontal growth, forming more branches for each plant. When the removal of apical dominance happens, the axillary buds grow better, hence, converting them into branches (Sunitha *et al.*, 2006; Dorajeerao and Mokashi, 2012).

The reason for the significant increase achieved in most vegetative growth traits after spraying with kinetin may be due to its role in increasing cell elongation and stimulating cell division. Its role also includes transporting and distributing solutes toward the sources of growth and consumption. Likewise, it could refer to its role in stimulating the growth of leaf stems through cell division. A study initially distinguished this finding, revealing such development (Yassin, 2001). Kinetin also stimulates lateral bud growth, which endogenous auxins usually suppress, as inhibited by the effect of internal auxins. Consequently, this ends apical dominance by stimulating the tissues adjacent to the vascular

tissue of the buds and stems of lateral buds and, thus, facilitates the transfer of water and nutrients, encouraging bud emergence. Side effects on their development (Devlin and Francis, 2000) may be the role of kinetin in increasing cell division in the apical meristems and cambium and adding new cells to the plant (Mazher *et al.*, 2011).

In addition to kinetin, humic acid also plays a significant role in enhancing vegetative growth, resulting from the decomposition of organic matter. The increase in vegetative growth characteristics when using humus fertilizers (humic acid) is because of the rich nitrogen and phosphorus contents, which are part of the composition of proteins and enzyme cofactors. These contribute to increasing cell division and construction and stimulating the plant's vital activities. It is essential in plant nutrition, which is evident in improved growth through its effect on the processes of photosynthesis and respiration, as it works to activate certain enzymes and inhibit other enzymes.

Moreover, humic acid increases the plant's resistance to harsh environmental conditions during the growing season, raises the permeability of cellular membranes, and stimulates many vital reactions in the plant (Moghadam *et al.*, 2014). Canellas *et al.* (2015) explained that humic acid boosts resistance to pests, diseases, and unsuitable environmental conditions by regulating the absorption of phosphorus and potassium and improving soil composition. It has a wide range of increasing plant production and has an effective effect in enhancing the efficiency of photosynthesis and increasing the percentage of chlorophyll.

## CONCLUSIONS

The triple interaction between the three factors, pinching P1, adding humic acid at a concentration of zero, and spraying with kinetin at zero concentration gave significantly maximum values in all traits in the rosemary plant (*R. officinalis* L.).

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