

Increased Concentration Of Active Substances That Kill Cancer Cells In Rosemary Plants (*Rosmarinus Officinalis* L.) By Spraying Growth Regulators

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Abstract:

The study was carried out in the Department of Horticulture, Faculty of Agriculture, University of Baghdad, Abu Ghraib. Rosemary seedlings, six months old and sourced from a private nursery in Baghdad, were selected based on uniformity in vegetative growth and height. The seedlings were transferred to plastic containers with a diameter of 25 cm and a weight of 10 kilograms, utilizing a soil mixture of river sand and peat moss in a ratio of three to one. Optimal conditions were maintained, including continuous irrigation, hoeing, and weeding. Samples from the agricultural medium were collected to assess the effects of salicylic acid and cytokinin for measurement purposes, with the experiment designed according to a completely randomized block design. RCB as an empirical study comprising 48 treatments $4 \times 4 \times 3$, where the initial factor comprised four concentrations of volatile oil extraction, involved the application of growth regulators (cytokinin and salicylic acid) at cytokinin concentrations of (0, 50, 100, 150) mg per soil. The third factor comprised four amounts of salicylic acid: 0, 100, 200, and 300 mg. The most significant findings indicated that the triple interference resulted in a notable change across all features, with cytokinin at a concentration of 150 mg/l and salicylic acid at 300 mg/l. The maximum output of volatile oil from the plant reached 317 mg per plant in contrast to the control treatment.

Keywords: Rosemary, Plant growth regulators, Cancer

INTRODUCTION

The rosemary plant is a perennial evergreen shrub characterized by its herbaceous, semi-dendric nature and extensive branching. It thrives in various soil types, exhibits resilience to low temperatures, and prefers warm climates with temperatures ranging from 20 to 35 degrees Celsius. The plant typically reaches a height of 90 cm but can grow up to approximately two meters. Its leaves emit a distinct fragrance, with the upper surface displaying a light green hue and the lower surface exhibiting a silvery color, complemented by a potent aromatic scent. either beautiful flowers exhibit blue, purple, or white hues and take the form of clustered inflorescences, which are rich in nectar and attract bees [1]. Rosemary is renowned for its unique aromatic scent, making its oils a crucial fundamental component. Additionally, the plant encompasses various compounds that have demonstrated efficacy in individual studies. Notably, significant research has validated the leaves as a primary source of phenolic compounds containing inorganic substances [2]. Scholars concur that the aerial parts of the rosemary plant, particularly the leaves, are utilized in the treatment of ailments [3]. The rosemary herb has demonstrated medical applications in eradicating cancer cells as of Friedman [4] and exhibits impacts on metabolism and combating free radicals. Numerous research indicates its significant significance in cancer therapies. Blood and prostate have anti-cancer properties because to the presence of Mark acid and caronic acid, which function to eliminate cancer cells. Ghanbari et al. [5] research has elucidated the function of several secondary metabolites utilized as food preservatives, cancer preventatives, and treatments for many chronic diseases, including type II diabetes and cardiovascular conditions, as well as their applications as colorants, oils, fibers, or flavoring compounds [6]. It is posited that certain compounds are generated as a defensive mechanism to safeguard plants from external threats, including pests and pathogens, while simultaneously serving as attractive agents for pollinators [7]. The essential oils of the rosemary plant are significant from a medical perspective, exhibiting antiviral, antibacterial, anti-inflammatory, and anticancer properties, as noted by [8]. In recent years, the most widely produced pharmaceuticals globally are generated from secondary plant metabolites, notably include volatile oils, phenols, terpenes, and alkaloids. Two thousand twenty-two Fazili, It contains cytokinin, a plant growth regulator that plays a

crucial role in various biological processes, stimulating cell growth during key stages of plant development. Cytokinin enhances cell division, promotes plant growth, and mitigates leaf senescence by preserving proteins and chlorophyll from degradation. Additionally, it facilitates the distribution of salts, plant hormones, and other substances among plant cells. [9] and [10] referred that salicylic acid is a phenolic acid comprising 11% salicin. Experiments have demonstrated that this acid functions as a plant hormone, exhibiting high efficacy in regulating physiological processes, mitigating stress, and enhancing the plant's resistance response [11].

MATERIALS AND METHODS

A field study was conducted in the shade of the Department of Horticulture at the College of Agriculture, University of Baghdad Abu Ghraib during the winter season of 2022-2023, from November 5, 2022, to May 5, 2023. Rosemary seedlings, six months old and sourced from a private nursery in Baghdad Governorate, were selected, ensuring uniformity in size, vegetative growth, and height. The seedlings were then transferred to plastic sandals with a diameter of 25 cm and a weight of 10 kilograms, and the soil was it comprises a mixture of river mixed and peat moss in a ratio of three to one, with suitable conditions maintained through ongoing irrigation, hoeing, and weeding operations. Samples were collected from the agricultural medium to assess the effects of salicylic acid and cytokinin. For measurement purposes, the experiment was structured based on the complete random sectors of RCPG, comprising 48 treatments in a $4 \times 4 \times 3$ configuration. The first factor consisted of four concentrations of cytokines (0.50, 100, 150 mg) in soil, while the second factor included four concentrations of salicylic acid (0, 100, 200, 300 mg). Volatile oils were extracted using the steam distillation method via the Cleavinger distillation apparatus, which involved passing water vapor through the blended plant components, facilitating the extraction process. Evaporation of active substances during its passage on the components of the model, for example, 100 grams of mashed leaves were taken, then the evaporated compounds pass on a condenser, then they settle and turn into a liquid state and the liquid mixture runs down and meets in an oil collection vessel, after which the oil is separated from the hydrosol, which is a liquid with pure water molecules, where two layers are formed, then the oil layer separates by measuring the size of the volumetric cylinder, as it represents the percentage of oil in 100 grams of leaves and was ranging between 3.5 To 4.4 grams, as 20 ml was taken and injected on the HPLC device at ideal conditions for separation according to the [12], where the active compounds of the samples under study were diagnosed based on the standard models, as after the samples were processed by injection with the HPLC device by dissolving the standard models in its solvents prepared according to their properties and melting temperature, where eight microliters of each model were injected independently, after that they were injected The compounds are categorized to assess their overlap under identical separation and diagnostic conditions.

RESULTS

Yield of volatile oil (mg per plant)

The results of table (1) demonstrate significant effects on the kernel yield of volatile oil, with plants treated with a concentration of 150 mg/l of cytokine achieving the highest yield at 288 mg/plant, in contrast to the control treatment, which yielded the lowest at 190 mg/plant. Additionally, spraying with salicylic acid resulted in a notable increase in volatile oil yield, with the highest concentration of 300 mg/l producing a yield of 279 mg/plant compared to the control treatment. The comparison yielding the lowest rate of 219 mg per plant. The overlap produced a notable effect, with cytokinin levels at 150 mg/l and salicylic acid at 300 mg/l. The maximum production of volatile oil from the plant was 317 mg per plant, whereas the control treatment yielded the lowest quantity at 153 mg per plant.

Table (1): Effect of salicylic acid and cytokinin on the oil content of rosemary plant

	Cytokinin		Salicylic acid		mean
	0	100	200	300	
0	153	168	202	237	190
50	232	237	236	256	240
100	239	243	280	304	266

150	249	288	298	317	288
mean	219	234	254	279	
L.S.D. 0.05			43.98		

Concentration of the active compound Rosmarinic acid in mg per plant:

The results of table (2) indicate substantial variations in the concentration of the active compound Rosmarinic acid in plants. A cytokinin concentration of 150 mg/l yielded the highest level of the active substance at 1137 mg/plant, in contrast to the control treatment, which produced the lowest concentration at 608 mg/plant. Additionally, the application of salicylic acid resulted in a significant increase in the leaf concentration of the active substance, with a dosage of 300 mg/l achieving the highest level at 1056 mg/plant. In comparison to the treatment that yielded the lowest rate of (725) mg per plant.

The bilateral overlap among the research workers yielded a substantial effect, with cytosine measuring 150 mg/l and salicylic acid at 300 mg/l, resulting in a peak value of 1210 mg/plant, in contrast to the comparison factors, which recorded a minimum rate of 418 mg/plant.

Table (2) Impact of salicylic acid and cytokinin on Rosmarinic acid in the rosemary plant

Cytokinin	Salicylic acid				mean
	0	100	200	300	
0	418	478	728	817	618
50	602	802	857	978	811
100	892	1061	1173	1222	1086
150	988	1156	1193	1203	1137
mean	725	874	986	1055	
L.S.D. 0.05			72.22		

Concentration of the active component Linalool in mg per plant:

The statistical analysis results of table (3) indicated a marked superiority in the plant content of the active compound Linalool, with a cytokinin level of 150 mg/l yielding the highest concentration at 771 mg/plant, in contrast to the control treatment, which produced the lowest concentration at 250 mg/plant. Furthermore, the data reveal that plants treated with salicylic acid exhibited a higher content of the active ingredient Linalool. The concentration was measured at 300 mg/liter with a rate of 617 mg/plant, in contrast to the control treatment, which yielded a lower rate of 380 mg/plant. However, significant variations were only observed in the bilateral interaction between the two study components. Documented a cytokine level of 150 mg/l and a salicylic concentration of 300 mg/l, with the maximum value reaching 1004 mg/plant, in contrast to the comparative treatment, which exhibited a minimum value of 186 mg/plant .

Table (3) Impact of salicylic acid and cytokinin on linalool in the rosemary plant

Cytokinin	Salicylic acid				mean
	0	100	200	300	
0	186	229	279	303	250
50	244	390	432	498	392
100	463	591	593	661	577
150	629	648	803	1004	771
Mean	380	464	527	617	
L.S.D. 0.05			55.61		

DISCUSSION

The application of SA enhanced the percentage and output of volatile oil in the rosemary plant. This may be associated with the enhanced development of plants induced by salicylic acid, alterations in the population of oil glands in leaves, and the beneficial influence of salicylic acid on the production of mono- or sesquiterpenes. [13]. These results corroborate the findings of [14], which indicated that the treatment

of SA greatly enhanced the volatile oil of rosemary, as presented in the following table. Cytokinin participate in processes including cell division, bud growth initiation, aging delay, photosynthesis, chloroplast division and growth regulation, metabolic regulation in response to environmental stimuli, and abiotic stress protection. Cytokinin plays a function in preserving proteins in the treated areas, which exhibit prolonged viability.

CONCLUSIONS

Response of rosemary plants to cytokine, which had a positive effect on the yield of active compounds and gave the best results when sprayed at a concentration of 150 mg/L.

Rosemary plant response to salicylic acid, which had a direct and positive effect on the synthesis of active substances at a concentration of 300 mg/l. The interaction between the concentration of cytokinin and salicylic acid led to a state of nutritional and regulatory integration in affecting plant behaviour, which positively affects the increase of the active substance.

RECOMMENDATIONS

Using cytokinin in greater concentrations to give the best vegetative growth and the best amount of active ingredient. Spraying rosemary plants with concentrations higher than 300 mg/l of salicylic acid and watching its effect on the active substance. Conducting experiments and studies of different plants to know their behavior under the influence of these organizations because of their importance in increasing growth, the amount of yield and the medically active substance.

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