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Evaluation of the Effect of a Hormonal Combination with Amino Acids on the Chemical Content and Antioxidant Activity in the Rosemary Plant

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ABSTRACT: During the spring season of 2023-2024, a factorial experiment was carried out at the research station unit of the College of Agricultural Engineering Sciences/ University of Baghdad - Jadriyah, using a complete randomized block design (RCBD), inside an open field (site A), with the aim of evaluating the effect of a combination of growth regulators with amino acids on the growth, yield, chemical content, and antioxidant activity of the local variety of rosemary (*Rosmarinus officinalis* L.). The experiment included two factors, each with three levels and all interactions, with three replications, and the experimental unit contained 16 plants, totaling 432 plants. Homogeneous saplings with a height of 8–10 cm were used, planted in 8-liter plastic pots filled with gypsum soil, and the plants were acclimatized prior to treatment application. The first factor included growth regulators (control, Kin, BA) at a rate of 50 mg L^{-1}, while the second factor included amino acids (control, Glutamic acid, Aspartic acid) at the same concentration, and the treatments were sprayed three times during different growth stages. Statistical analysis of the means revealed significant differences between most of the traits. Kinetin treatment was superior in chemical and qualitative traits, especially with regard to percentage yield of essential oil, leaf carbohydrate content, total flavonoid contents and improvement the composition of some volatile components and physical characteristics. The Benzyladenine treatment gave the highest total phenolic content and free radical scavenging activity, along with improved oil traits. Glutamic acid significantly increased essential oil percentage and the corresponding yield, carbohydrate content, phenolics, flavonoids and antioxidant activity whereas aspartic acid was more effective in improving essential oil percentage, some volatile oil constituents and physical properties of the oils. The interaction between growth regulators and amino acids had a prominent synergetic effect which was evident in yield, chemical composition, and antioxidants activity. These results verified that co-application of growth regulators and amino acids is a successful approach to promote the yield as well as oil quality of rosemary.

INTRODUCTION

The medicinal and aromatic plants have always been a crucial component of human life because from nutrition to treatment, they formed the fundamental basis of ancient medicine in dealing with disease and improving public health. While today's big medical advances and great strides forward are changing this, some things never change: traditional medicine-using people continue to depend heavily upon these plants for their primary health care needs (El-gabal, 2007).

Due to their importance and richness in biologically active compounds that can be used directly or after extraction for incorporation into pharmaceutical industries (Karim and Qar'an, 1986 ; Yesmin, 2015), it is essential to monitor the cultivation stages of medicinal and aromatic plants in general and rosemary in particular, to enhance sustainable agricultural knowledge. These plants are considered among the alternative economic methods that have recently expanded to develop sustainable

production patterns, and to monitor their climatic requirements compared to current climatic variables and their effects. Therefore, encouraging interest and developing the cultivation of these plants is necessary due to their important role in future agricultural development.

One of the most important plants used nutritionally and medicinally is rosemary, (*Rosmarinus officinalis* L.) The plant, also called the mint family, is an aromatic, perennial, evergreen, semi-shrubby herbaceous plant that belongs to the Lamiaceae family. This family is considered one of the large families, containing 180 genera and 3500 species.

The original habitat of the rosemary plant is the Mediterranean basin and Southern Europe, where it also grows wild. Its growth was introduced to many countries from there. France, Spain, and Tunisia are the primary producers as they grow oil extracted from this plant (Peter, 2004). The plant has an extensive range, and contains more than one hundred compounds, such as Camphor, Camphene, Cineol and Borneol (Roa *et al.,* 1998). Asides from being utilized as an herb, the leaves and oil of Rosemary are often included in the food industry for their volatile content that imparts some desired taste. The essential oil of rosemary is used in various fields because its properties are antioxidant, antibacterial and anti-fungal (Tawfeeq, 2016). It is also used in pesticides products. It is also widely used in cosmetics and pharmaceutical industries as well (Maistor et al., 2010). Due to its numerous bioactive components it is an important under-utilized plant species that deserves exploration, cultivation development of its growth and improvement in traits and oils (Wiedenhoeft, 2006).

A number of studies reported that, the factors impacting the yield and quality of volatile oil in medicinal and aromatic plants are not independent on each other as are correlated to each other's such as abiotic (climate and soil), genetic factors, some agronomical practices, fertilization practices (Hussain, 2009 Esmaeilian et al., 2021).

Cytokinins are considered to play an essential role in stimulating the process of cell division and specialization in participation with auxins. Their importance is also evident in many other physiological processes, such as in breaking apical dominance, which affects the branching process of plants. The first discovered cytokinin compound is Kinetin. This compound does not exist naturally in plants; rather, it is one of the products of the thermal degradation of adenine. The discovery of Kinetin encouraged the use of industrial synthesis for hundreds of similar compounds. However, Kinetin is one of the most common and used substances in studies related to the physiological effects of cytokinins (Wareing and Phillips, 1981). Kinetin works to inhibit apical dominance in the plant, activate the growth of lateral buds and transverse expansion of cells, form the phenotype of the plant, delay senescence, and maintain the green color (Al-Asadi, 2019). The plant growth regulator Benzyladenine (BA), also promoting cell division, enlargement and delaying senescence on plants, has the same effect. On the other hand, cell division needs access to plenty of nutrients. This is when organic foliar fertilizers become increasingly important to serve this purpose, as they are a safer alternative for chemical ones, more so with medicinal plants. The utilization of biostimulants has also been increased in agriculture production in recent years and contribute to declining the use of chemical compounds (Hassan and Zuhwan, 2014).

In addition, a lot of these acids also stimulate cell division and increase enzyme activity (Nur et al., 2006; Hassanein et al., 2010). They can help make plants more resistant to stresses like drought and heat, some of them. Consequently, the exogenous application of amino acids to stressed plants has an essential role in the reopening of stomata, enhanced water uptake, better respiration rate and delayed senescence and wilting (Shafeek et al., 2012). Glutamic acid, A necessary amino acid involved in the growth of a plant. It is a biostimulant that alleviates the damage by low temperature stress through triggering biosynthesis pathways going the cells involve freezing tolerance development (Sim et al., 2022). It is an important nitrogen source for plant growth where inorganic nitrogen, e.g. nitrate or ammonium are assimilated into glutamine. Glutamic acid is crucial to the growth and development of plants, promotes seed germination, improves root construction, increases photosynthesis and promote nitrogen metabolism-related enzymes and metabolic substances. It also causes ample accumulation of amino acids and starch in the plant. Glutamic acid provides a dynamic balance of nitrogen and carbon as a result of senescence (Han et al., 2022).

Aspartic acid (Asp) has also been described as one of the most important acids in the aspartate metabolic pathway. It is a crucial amino acid necessary for protein production, playing a key role as a foundational element in nitrogen and carbon metabolism across various metabolic processes. It is associated with numerous metabolic pathways including protein synthesis, nucleotide metabolism, the TCA cycle, glycolysis, and hormone production. Aspartate also plays an important role in plant resistance to abiotic stress, such as cold stress, drought stress, salt stress, or heavy metal stress (Han, M.; Zhang, 2021).

MATERIALS AND METHODS

A factorial experiment with RCBD was carried out in the open plastic house, Site A, belonging to the Research Stations Unit of the College of Agricultural Engineering Sciences / University of Baghdad - Al-Jadriya, during the spring growing season 2023-2024 to evaluate the effect of a hormonal combination with amino acids on growth parameters, yield, chemical content, and oxidative activity in the rosemary plant (*Rosmarinus officinalis* L). For two factors and their interactions, with three replicates of 27 experimental units, each unit comprising 16 observations (16 pots per treatment), the total number of experimental plants was 432. Newly formed and growing offshoots (basal vegetative shoots) of the local variety were selected. They were small, uniform in branching and size, had a fibrous root system, and were of similar length, reaching a height of 8-10 cm. These offshoots were sourced from specialized nurseries in Baghdad Governorate/Al-Krayat area after being sorted at the Iraqi National Herbarium for seed testing and certification. This confirmed the species *Rosmarinus officinalis* L., described in the British Pharmacopoeia. The plants were planted in large 8-liter plastic pots measuring 23 cm in length and 26 cm in diameter, filled with gypsum soil from the college fields after removing the top 30 cm layer. The pots were then placed in a greenhouse for a week of acclimatization to provide suitable environmental conditions for all plants before the start of the experiment (From March to mid-November) with continued service operations for the plants, including sprinkler irrigation and weeding as needed, taking care to cover the house with tarpaulins during the extreme summer heat.

STUDIED TRAITS

1. PERCENTAGE OF OIL:

The weight of the extracted oil was calculated using a sensitive balance, and the percentage was determined according to the following equation: (Habibullah, 2012)

$$\text{Essential oil percentage}\% = \frac{\text{Volume of extracted oil from the sample (g)}}{\text{Weight of leaf sample (g)}} \text{x}100$$

2. PLANT OIL YIELD (G PLANT^{-1}):

The oil yield was estimated by adding the leaf yield and the percentage of volatile oil, as explained in the following equation, as mentioned in Guenther (1972):

Volatile oil yield g plant^{-1} = Leaf yield g plant^{-1} × percentage of volatile oil

3. Leaf carbohydrate content (mg g^{-1}):

Estimated using the Phenol-Sulfuric Acid Modification (PSACM) Colorimetric Method, according to the method of Dobois *et al.* (1956).

3. LEAF TOTAL PHENOLIC CONTENT (MG/KG^{-1} DRY WEIGHT)

The total phenolic content of the leaves was determined using the method of Liu et al. (2011). "This involved taking 0.5 ml of the extract prepared above for each treatment and placing it in a test tube. Then, 3 ml of Folin-Ciocatlue reagent (0.1%) was added to each tube, followed by 2.5 ml of sodium carbonate (Na2CO3) (0.2% w/v). The mixture was thoroughly mixed, and the test tubes were left to stand for three minutes at room temperature. The optical absorption was then measured using a spectrophotometer at a wavelength of 750 nm. The blank tube was prepared using the same method, but without adding the plant extract".

4. TOTAL FLAVONOID CONTENT OF THE LEAVES (MG/KG^{-1} DRY WEIGHT)

The total flavonoid content of the leaves was determined using the method of Liu et al. (2011). "This involved taking 0.5 ml of the extract prepared above and placing each in a A test tube was prepared, and 2.5 ml of ethanol was added to each tube. The mixture was thoroughly mixed, and then 3 ml of 0.01 mol/L aluminum chloride (AlCl3) was added. The test tubes were then left at room temperature for 10 minutes, and the light absorption was measured using a spectrophotometer at a wavelength of 400 nm. Blank was prepared using the same method, except for the addition of the plant extract".

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5. ESTIMATE OF THE ANTIOXIDANT ACTIVITY OF THE PERCENTAGE OF FREE RADICAL SCAVENGING

the method described by Mashkor et al. (2014) was adopted " The method for estimating the percentage of free radical scavenging was as follows: 40 mg of Diphenyl-1-picrylhydrazyl (DPPH) was dissolved in 100 mL of methanol with continuous stirring. After complete dissolution, additional amounts of methanol were added, and the optical absorption of the reagent was measured at a wavelength of 516 nm until the optical absorption of the reagent reached 0.7 ± 0.01 nm. One hundred µL of the grain extract was taken, 4 mL of DPPH reagent was added, and the solution was left to stand for 24 hours at laboratory temperature. The optical absorption readings of the samples were taken at a wavelength of 516 nm, and the following equation was applied":

$$\text{Oxidative Activity \%} = \frac{[(\text{Optical Absorption of the Empty Sample} - \text{Optical Absorption of the Plant Sample}}{\text{Optical Absorption of the Empty Sample}} \text{x} 100$$

6. OIL QUALITY (GC-MC TECHNIQUE):

Oil Extraction: Replace the Take (20 g) of the fresh sample and place it in a flask. Add (100 ml) of distilled water and place it in a claving apparatus for 3 hours. Collect the oil and add 20 ml of hexane to separate the oil from the water droplets that had accumulated with it. The collected oil was then stored in a refrigerator until analysis.

Analytical Method: The test was conducted at the laboratories of the Scientific Research Authority / Environmental and Water Research Center utilizing a Shimadzu 2010 gas chromatograph of Japanese origin equipped with an ionizing flame detector (FID) and a DM-5Ms capillary column measuring 30 meters. The sample size was 0.25 micrometers by 0.25 millimeters, with injection and detector temperatures set at 280°C and 340°C, respectively. The separation column temperature was incrementally raised from 100°C to 300°C at a rate of 10°C per minute. Inert nitrogen gas was employed as the carrier gas at a pressure of 100 kPa.

7. REFRACTIVE INDEX OF VOLATILE OIL:

This represents the ratio of the sine of the angle of incidence of light to the sine of the angle of refraction at a specific temperature (Tayfour and Rashid, 1990). The refractive index of all volatile oil samples was estimated using an Abbe Refractometer at 20°C (Chen et al., 1993). Volatile Oil Density (mg/µL)

The density of each oil sample was calculated by taking 200 µL of oil per treatment using a micropipette at room temperature (25°C), then weighing it, and applying the following equation:

$$\text{Volatile Oil Density} = \frac{(\text{weighing oil sample})\ (\text{mg})\ (100\mu\text{L volume})}{\text{Volume of Oil Sample (}\ \mu\text{L)}}$$

8. SPECIFIC GRAVITY

The specific gravity of the oil represents its degree of saturation (Tayfour et al., 1990). It was estimated by taking 100 µL of the volatile oil using a micropipette and a sensitive four-decimal balance at 20°C for three measurements from each sample. The weight of that volume of oil was then divided by the weight of the same volume of distilled water at the same temperature (Chen et al., 1993).

RESULTS AND DISCUSSION:

1. OIL PERCENTAGE (% OIL CONTENT)

The results in Table 1 show that the oil percentage in rosemary plants was significantly affected by amino acid treatments. The treatment with (50 mg L^{-1} Aspartic acid) recorded a mean of 3.193%, while the treatment with (50 mg L^{-1} Glutamic acid) recorded a mean of 2.927%. This was followed by the control treatment (without addition), which recorded a mean of 2.223%.

The results also indicate that the oil percentage increased in conjunction with treating rosemary plants with growth regulators. The treatment with (100 mg L^{-1} Kinetin) recorded a mean for this trait of 2.943%, while the treatment with (100 mg L^{-1} BA) recorded a mean of 2.890%. Plants in the control treatment (without addition) recorded a mean of 2.510%.

Regarding the two-way interaction effect, it was observed that the treatments did not significantly differ from each other. The treatment combining (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin) and the treatment combining (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA) recorded an increase in the oil percentage trait by 270.5% and 264.5%, respectively, compared to the control A0B0.

Table 1. Effect of amino acids, growth regulators, and their interaction on oil percentage (%)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	2.000	2.420	2.250	**2.223**
Glutamic (B1)	2.650	3.000	3.130	**2.927**
Aspartic (B2)	2.880	3.410	3.290	**3.193**
Mean Growth regulators	**2.510**	**2.943**	**2.890**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.138	0.138	N.S	

2- OIL YIELD PER PLANT (L HA^{-1})

The results of the statistical analysis in Table 2 indicate that the oil yield of the rosemary plant was not significantly affected by treating the plants with amino acids. The treatment (50 mg L^{-1} Glutamic acid) achieved a mean of 0.0347 L ha^{-1}, while the plants treated without addition achieved a mean of 0.292 L ha^{-1}, which was close to the treatment (50 mg L^{-1} Aspartic acid) in achieving a mean of 0.277 L ha^{-1}.

It may appear from the results that the oil yield was significantly affected when treating rosemary plants with growth regulators. The treatment (100 mg L^{-1} Kinetin) showed a mean for the trait of 0.319 L ha^{-1}, followed by the treatment (100 mg L^{-1} BA) in achieving a mean of 0.314 L ha^{-1}, then the plants treated without addition that gave a mean of 0.0282 L ha^{-1}.

Regarding the two-way interaction effect of the study factors, it was observed that there was a significant effect for the plants treated (50 mg L^{-1} Glutamic acid with no addition), followed jointly by the plants of the two treatments (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) and (no addition with 100 mg L^{-1} Kinetin) in achieving an increase amounting to 334.26% for the first and 310.67% for the two treatments jointly compared to the treatment (50 mg L^{-1} Aspartic acid with no addition)

Table 2. Effect of amino acids, growth regulators, and their interaction on Oil yield per plant **(**L ha^{-1})

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	0.0250	0.0375	0.0250	**0.0292**
Glutamic (B1)	0.0417	0.0250	0.0375	**0.0347**
Aspartic (B2)	0.0178	0.0333	0.0318	**0.0277**
Mean Growth regulators	**0.0282**	**0.0319**	**0.0314**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	N.S	0.0039	0.0068	

3. LEAF CARBOHYDRATE CONTENT (MG G^{-1})

The results of statistical analysis in Table 3 indicate that leaf carbohydrate content in rosemary plants did not show a significant difference in effect when treated with amino acids. The control treatment (without addition) gave a mean of 104.95 mg g^{-1}. Meanwhile, the treatment with (50 mg L^{-1} Glutamic acid) gave a mean of 103.77 mg g^{-1}, comparable to the treatment with (50 mg L^{-1} Aspartic acid), which gave a mean of 103.62 mg g^{-1}.

The results shows that leaf carbohydrate content varied in its effect when rosemary plants were treated with growth regulators. The control treatment (without addition) gave a mean of 107.75 mg g^{-1}. In contrast, the treatment with (100 mg L^{-1} Kinetin) achieved a mean of 102.41 mg g^{-1}, comparable to the treatment with (100 mg L^{-1} BA), which gave a mean of 102.18 mg g^{-1}.

Regarding the interactive effect of the study factors, the result indicates a variation in the effect of the interaction treatments. The two treatments combining (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) and (control with 100 mg L^{-1} Kinetin) showed an increase of 250.27% and 241.44%, respectively, compared to the treatment combining (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA).

Table 3. Effect of amino acids, growth regulators, and their interaction on leaf carbohydrate content (mg g^{-1})

Amino acids	**Growth regulators**			**Mean Amino acid**
	No addition (A0)	**Kinetin (A1)**	**BA (A2)**	
No addition (B0)	94.61	112.24	107.99	**104.95**
Glutamic (B1)	105.71	86.38	119.21	**103.77**
Aspartic (B2)	122.92	108.62	79.33	**103.62**
Mean Growth regulators	**107.75**	**102.41**	**102.18**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	N.S	2.642	4.576	

4. TOTAL PHENOLIC CONTENT (MG KG^{-1} DRY WEIGHT)

The results in Table 4 revealed that amino acids had a varying effect on the total phenolic content in rosemary leaves. The control treatment (without addition) achieved a mean for this trait of 887.033 mg kg^{-1} dry weight. The treatment with (50 mg L^{-1} Glutamic acid) achieved a mean of 824.367 mg kg^{-1} dry weight, followed by the treatment with (50 mg L^{-1} Aspartic acid), which achieved a mean of 770.033 mg kg^{-1} dry weight.

It is noted that growth regulators also has varying impact on the total phenolic content in rosemary leaves. Plants in the control treatment (without addition) gave a mean for this trait of 939.367 mg kg^{-1} dry weight. Plants treated with (100 mg L^{-1} BA) achieved a mean for this trait of 828.367 mg kg^{-1}, followed by the treatment with (100 mg L^{-1} Kinetin), which achieved a mean of 713.700 mg kg^{-1}.

The results indicate a significant interactive effect of the study factors on the total phenolic content in leaves. The treatments combining (50 mg L^{-1} Glutamic acid with control) and (50 mg L^{-1} Aspartic acid with control) achieved increase percentages of 257.28% and 254.67%, respectively, compared to the treatment combining (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} Kinetin).

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Table 4. Effect of amino acids, growth regulators, and their interaction on total phenolic content (mg kg^{-1} dry weight)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	906.70	866.70	887.70	**887.03**
Glutamic (B1)	963.70	612.70	896.70	**824.37**
Aspartic (B2)	947.70	661.70	700.70	**770.03**
Mean Growth regulators	**939.37**	**713.70**	**828.37**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	9.46	9.46	16.38	

5. TOTAL FLAVONOID CONTENT (MG KG^{-1} DRY WEIGHT)

The results in Table 5 implies that amino acids had a significant impact on the total flavonoid content in rosemary leaves. The treatment with (50 mg L^{-1} Glutamic acid) recorded a mean for this trait of 65.313 mg kg^{-1} dry weight. In comparison, the two treatments (control) and (50 mg L^{-1} Aspartic acid) recorded means of 61.807 mg kg^{-1} dry weight and 61.457 mg kg^{-1} dry weight, respectively.

It is observed that foliar application with growth regulators achieved a significant increase in the chemical content of total flavonoids. Plants treated with (100 mg L^{-1} Kinetin) recorded a mean for this trait of 70.103 mg kg^{-1} dry weight. Meanwhile, the two treatments (control) and (100 mg L^{-1} BA) recorded means of 59.447 mg kg^{-1} dry weight and 59.027 mg kg^{-1} dry weight, respectively.

Furthermore, the results indicate a significant interactive effect of the study factors on the total flavonoid content in leaves. The two treatments combining (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} Kinetin) and (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin) recorded increase percentages of 251.95% and 219.54%, respectively, compared to the treatment combining (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA).

Table 5. Effect of amino acids, growth regulators, and their interaction on total flavonoid content (mg kg^{-1} dry weight**)**

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	906.70	866.70	887.70	**887.03**
Glutamic (B1)	963.70	612.70	896.70	**824.37**
Aspartic (B2)	947.70	661.70	700.70	**770.03**
Mean Growth regulators	**939.37**	**713.70**	**828.37**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	9.46	9.46	16.38	

6. FREE RADICAL SCAVENGING PERCENTAGE (DPPH % INHIBITION)

The results from Appendix 2 and Table 20 show a variation in the effect of foliar spraying with amino acids. The control treatment (without addition) showed a mean for this trait of 11.063%. In contrast, treating plants with (50 mg L^{-1} Glutamic acid) showed a mean of 8.963%, which was not significantly different from the treatment with (50 mg L^{-1} Aspartic acid), which

showed a mean of 7.953%. Significant variation in the effect of foliar application with growth regulators on the free radical scavenging activity is observed. The control treatment (without addition) gave a mean for this trait of 10.927%, followed by the treatment with (100 mg L^{-1} Kinetin), which showed a mean of 9.783%, and then the treatment with (100 mg L^{-1} BA), which showed a mean of 7.270%.

The increase is attributed to the increased seed content of total flavonoids (Table 5), which positively affected the increase in free radical scavenging activity. From the same appendix and table, a significant interactive effect between the experimental factors was observed. The two treatments combining (control with 100 mg L^{-1} BA) and (50 mg L^{-1} Glutamic acid with control) showed increase percentages of 416.26% and 383.00%, respectively, compared to the treatment combining (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} Kinetin).

Table 6. Effect of amino acids, growth regulators, and their interaction on total flavonoid content (mg kg^{-1} dry weight**)**

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	9.600	10.560	13.030	**11.063**
Glutamic (B1)	11.660	4.120	11.110	**8.963**
Aspartic (B2)	11.520	7.130	5.210	**7.953**
Mean Growth regulators	**10.927**	**7.270**	**10.927**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.254	0.254	0.440	

7. PERCENTAGE OF CINEOLE COMPOUND (CINEOLE %)

The results show that the percentage of the active compound Cineole in rosemary plants was affected by foliar spraying with amino acids (Table 7). The treatment with (50 mg L^{-1} Aspartic acid) gave a noticeable increase in the peak area percentage of the active compound, reaching 34.693%. This was followed by the treatment with (50 mg L^{-1} Glutamic acid), which gave a peak area percentage of 33.793%. In contrast, plants in the control treatment (without addition) gave a peak area percentage of 31.823%. The results indicate that foliar spraying with growth regulators did not have a significant effect. The treatments (100 mg L^{-1} Kinetin), (100 mg L^{-1} BA), and the control treatment gave peak area percentages for the active compound of 34.010%, 33.787%, and 32.513%, respectively.

Regarding the interactive effect of the study factors, the results showed no significant interaction between the experimental factors. The two treatments combining (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin) and (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA) showed peak area percentages for the Cineole compound of 35.66% and 34.97%, respectively, compared to the control treatment, which was close to them and showed a peak area percentage for the Cineole compound of 31.11%.

Table 7. Effect of amino acids, growth regulators, and their interaction on percentage of cineole compound (cineole %)

Amino acids	Growth regulators			Mean Amino
	No addition (A0)	Kinetin	BA	
No addition (B0)	31.110	32.470	31.890	**31.823**
Glutamic (B1)	32.980	33.900	34.500	**33.793**
Aspartic (B2)	33.450	35.660	34.970	**34.693**
Mean Growth regulators	**32.513**	**34.010**	**33.787**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.616	0.616	N.S	

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8. PERCENTAGE OF CYMENE COMPOUND (CYMENE %)

It is observed that the rosemary plant responded to the foliar spraying factor with amino acids (Table 8). Plants treated with (50 mg L^{-1} Aspartic acid) recorded a peak area percentage for the active compound Cymene of 3.833%, which was close to plants treated with (50 mg L^{-1} Glutamic acid), which recorded a peak area percentage for the active compound Cymene of 3.460% (Table 8). Then, plants in the control treatment (without addition) recorded a peak area percentage for the active compound Cymene of 2.410%.

The results from Figure 3 indicate that the plants also responded to foliar spraying with growth regulators. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) recorded peak area percentages for the active compound Cymene of 3.540% and 3.407%, respectively, while the control treatment (without addition) recorded a peak area percentage for the active compound Cymene of 2.757%.

Regarding the interactive effect treatments, the figure shows no significant interaction between the study factors. It was observed that the interaction treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) recorded higher peak area percentages for the active compound Cymene, reaching 4.250%, 4.000%, and 3.800%, respectively.

Table 8. Effect of amino acids, growth regulators, and their interaction on percentage of cymene compound (cymene %)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	2.030	2.780	2.420	**2.410**
Glutamic (B1)	2.990	3.590	3.800	**3.460**
Aspartic (B2)	3.250	4.250	4.000	**3.833**
Mean Growth regulators	**2.757**	**3.540**	**3.407**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.261	0.261	N.S	

9. PERCENTAGE OF LIMONENE COMPOUND (LIMONENE %)

The results revealed that the rosemary plant responded to foliar spraying with amino acids (Table 9). The treatment with (50 mg L^{-1} Aspartic acid) showed a peak area percentage for the active compound Limonene of 5.400%, followed by the treatment with (50 mg L^{-1} Glutamic acid), which showed a peak area percentage for the active compound Limonene of 4.897%. Then, the control treatment (without addition) showed a peak area percentage for the active compound Limonene of 3.610%.

The results indicate that the plant also responded to foliar spraying with growth regulators. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) showed peak area percentages for the active compound Limonene of 4.930% and 4.890%, respectively, followed by plants in the control treatment (without addition), which showed a peak area percentage for the active compound Limonene of 4.087%.

Regarding the interactive effect treatments, the figure shows a significant interaction between the study factors. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) recorded peak area percentages for the active compound Limonene of 5.800%, 5.660%, and 5.360%, respectively

Table 9. Effect of amino acids, growth regulators, and their interaction on percentage of limonene compound (limonene %)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	3.190	3.990	3.650	**3.610**
Glutamic (B1)	4.330	5.000	5.360	**4.897**
Aspartic (B2)	4.740	5.800	5.660	**5.400**
Mean Growth regulators	**4.087**	**4.930**	**4.087**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.195	0.195	0.337	

10. PERCENTAGE OF TERPINENE COMPOUND (%)

The results from Table 10 indicate that the peak area percentage of the active compound Terpinene was significantly affected by the foliar spraying factor with amino acids. The treatment with (50 mg L^{-1} Aspartic acid) showed a peak area percentage for the active compound Terpinene of 8.517%, followed by the treatment with (50 mg L^{-1} Glutamic acid), which showed a peak area percentage for the active compound Terpinene of 7.830%. Then, the control treatment (without addition) showed a peak area percentage for the active compound Terpinene of 6.460%.

Results also illustrates that the peak area percentage of the active compound Terpinene was significantly affected by the foliar spraying factor with growth regulators. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) showed peak area percentages for the active compound Terpinene of 8.040% and 7.863%, respectively, followed by the control treatment (without addition), which showed a peak area percentage for the active compound Terpinene of 6.903%.

Regarding the interactive effect between the study factors, the results show a significant interaction between the study factors. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) recorded peak area percentages for the active compound Terpinene of 9.250%, 8.800%, and 8.350%, respectively.

Table 10. Effect of amino acids, growth regulators, and their interaction on percentage of terpinene compound (terpinene %)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	6.050	6.890	6.440	**6.460**
Glutamic (B1)	7.160	7.980	8.350	**7.830**
Aspartic (B2)	7.500	9.250	8.800	**8.517**
Mean Growth regulators	**6.903**	**8.040**	**7.863**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.174	0.174	0.302	

11. PERCENTAGE OF CAMPHOR COMPOUND (CAMPHOR %)

The results show that the peak area percentage of the active compound Camphor was significantly affected by the foliar spraying factor with amino acids (Table 11). The treatment with (50 mg L^{-1} Aspartic acid) showed a peak area percentage for the active compound Camphor of 14.407%, followed by the treatment with (50 mg L^{-1} Glutamic acid), which showed a peak area percentage for the active compound Camphor of 13.827%. Then, the control treatment (without addition) showed a peak area percentage for the active compound Camphor of 12.607%.

Table 11 also illustrates that the peak area percentage of the active compound Camphor was significantly affected by the foliar spraying factor with growth regulators. The two treatments (100 mg L^{-1} BA) and (100 mg L^{-1} Kinetin) showed peak area percentages for the active compound Camphor of 13.900% and 13.897%, respectively, followed by the control treatment (without addition), which showed a peak area percentage for the active compound Camphor of 13.043%.

Regarding the interactive effect between the study factors, the result shows a significant interaction between the study factors. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) recorded peak area percentages for the active compound Camphor of 14.890%, 14.680%, and 14.330%, respectively.

Table 11. Effect of amino acids, growth regulators, and their interaction on percentage of camphor compound (camphor %)

Amino acids	**Growth regulators**			**Mean Amino acid**
	No addition (A0)	**Kinetin (A1)**	**BA (A2)**	
No addition (B0)	12.230	12.900	12.690	**12.607**
Glutamic (B1)	13.250	13.900	14.330	**13.827**
Aspartic (B2)	13.650	14.890	14.680	**14.407**
Mean Growth regulators	**13.043**	**13.897**	**13.900**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.138	0.138	0.239	

12. PERCENTAGE OF A-PINENE COMPOUND (A-PINENE %)

The results indicate that the peak area percentage of the active compound α-Pinene was significantly affected by the foliar spraying factor with amino acids (Table 12). The treatment with (50 mg L^{-1} Aspartic acid) showed a peak area percentage for the active compound α-Pinene of 9.350%, followed by the treatment with (50 mg L^{-1} Glutamic acid), which showed a peak area percentage for the active compound α-Pinene of 8.867%. Then, the control treatment (without addition) showed a peak area percentage for the active compound α-Pinene of 7.467%.

Table 12 also illustrates that the peak area percentage of the active compound α-Pinene was significantly affected by the foliar spraying factor with growth regulators. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) showed peak area percentages for the active compound α-Pinene of 8.943% and 8.803%, respectively, followed by the control treatment (without addition), which showed a peak area percentage for the active compound α-Pinene of 7.937%.

Regarding the interactive effect between the study factors, the figure shows a significant interaction between the study factors. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) recorded peak area percentages for the active compound α-Pinene of 9.890%, 9.600%, and 9.360%, respectively.

Table 12. Effect of amino acids, growth regulators, and their interaction percentage of α-pinene compound (α-pinene %)

Amino acids	**Growth regulators**			**Mean Amino acid**
	No addition (A0)	**Kinetin (A1)**	**BA (A2)**	
No addition (B0)	7.000	7.950	7.450	**7.467**
Glutamic (B1)	8.250	8.990	9.360	**8.867**
Aspartic (B2)	8.560	9.890	9.600	**9.350**
Mean Growth regulators	**7.937**	**8.943**	**8.803**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.195	0.195	0.337	

13. REFRACTIVE INDEX

The results from Table 13 show a significant effect of foliar spraying with amino acids on the refractive index trait of the essential oil extracted from rosemary plants. The treatments (50 mg L^{-1} Aspartic acid), (50 mg L^{-1} Glutamic acid), and the control treatment (without addition) showed close values of 1.255, 1.247, and 1.228, respectively.

Significant effect of foliar spraying with growth regulators on this trait in the plant is observed. The treatments (100 mg L^{-1} Kinetin), (100 mg L^{-1} BA), and the control treatment (without addition) gave close values for the trait of 1.248, 1.247, and 1.235, respectively.

Furthermore, results revealed no significant effect was observed in the values of the interactive effect between the experimental factors. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) showed values of 1.263, 1.259, and 1.253, respectively.

Table 13. Effect of amino acids, growth regulators, and their interaction on refractive index

Amino acids	**Growth regulators**			**Mean Amino acid**
	No addition (A0)	**Kinetin (A1)**	**BA (A2)**	
No addition (B0)	1.221	1.233	1.229	**1.228**
Glutamic (B1)	1.239	1.249	1.253	**1.247**
Aspartic (B2)	1.244	1.263	1.259	**1.255**
Mean Growth regulators	**1.235**	**1.248**	**1.247**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.014	0.014	N.S	

14. OIL DENSITY (G/CM³)

The results from Table 14 show the plant's response to foliar spraying with amino acids regarding the essential oil density trait. The treatments (50 mg L^{-1} Aspartic acid), (50 mg L^{-1} Glutamic acid), and the control treatment (without addition) showed values for the trait of 1.009 g/cm³, 1.000 g/cm³, and 0.989 g/cm³, respectively.

From the same table, a variation in the effect of foliar spraying with growth regulators on the plant's oil density is observed. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) gave a mean for the trait of 1.003 g/cm³, respectively, followed by the control treatment (without addition), which showed a mean for the trait of 0.992 g/cm³.

Regarding the interactive effect between the study factors, the results indicate that the oil density of rosemary plants was not significantly affected. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) showed values of 1.019, 1.012, and 1.008 g/cm³, respectively

Table 14. Effect of amino acids, growth regulators, and their interaction on oil density (g/cm³)

Amino acids	**Growth regulators**			**Mean Amino acid**
	No addition (A0)	**Kinetin (A1)**	**BA (A2)**	
No addition (B0)	0.986	0.992	0.989	**0.989**
Glutamic (B1)	0.994	0.998	1.008	**1.000**
Aspartic (B2)	0.996	1.019	1.012	**1.009**
Mean Growth regulators	**0.992**	**1.003**	**1.003**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.008	0.008	N.S	

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SPECIFIC GRAVITY (G/ML)

The results from Table 15 indicate a significant effect of foliar spraying with amino acids. The two treatments (50 mg L^{-1} Aspartic acid) and (50 mg L^{-1} Glutamic acid) showed a mean for the specific gravity trait of the essential oil in rosemary plants of 0.894 g/mL and 0.882 g/mL, respectively, followed by the control treatment (without addition), which showed a mean of 0.861 g/mL. No significant effect of foliar spraying with growth regulators on the specific gravity trait in the plant is observed. The two treatments (100 mg L^{-1} Kinetin) and (100 mg L^{-1} BA) showed a mean for the trait of 0.885 g/mL and 0.884 g/mL, respectively, while the control treatment (without addition) showed a mean for the trait of 0.869 g/mL.

Regarding the interactive effect between the study factors, the results indicate that the specific gravity of the essential oil in rosemary plants was not significantly affected. The treatments (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} Kinetin), (50 mg L^{-1} Aspartic acid with 100 mg L^{-1} BA), and (50 mg L^{-1} Glutamic acid with 100 mg L^{-1} BA) gave values of 0.905, 0.899, and 0.892 g/mL, respectively.

Table 15. Effect of amino acids, growth regulators, and their interaction on specific gravity (g/ml)

Amino acids	Growth regulators			Mean Amino acid
	No addition (A0)	Kinetin (A1)	BA (A2)	
No addition (B0)	0.856	0.866	0.860	**0.861**
Glutamic (B1)	0.871	0.884	0.892	**0.882**
Aspartic (B2)	0.879	0.905	0.899	**0.894**
Mean Growth regulators	**0.869**	**0.885**	**0.884**	
LSD 5%	**Amino acids**	**Growth regulators**	**Interaction**	
	0.019	N.S	N.S	

DISCUSSION:

Based on the results and previous results obtained regarding the chemical and qualitative properties of rosemary plants, the plant growth regulators Kin + BA, the amino acid treatment Glutamic acid + Aspartic acid, and the non-sprayed treatment of each had a clear effect on improving the quality of the oil and active ingredients, whether individually or collectively, in the characteristics of the leaf carbohydrate content, the chemical content of total phenols and flavonoids, and the percentage of oil. In addition, they affected the proportions of the main components of the volatile oil in rosemary plants, such as Cineole, p-Cymene, Limonene, Terpinen, Camphor, and α-Pinene. The results indicated that the best values were achieved when combining BA with the amino acids, which demonstrates a synergistic effect that enhanced the physiological pathways responsible for the formation of the active ingredients. Regarding the effect of single spray treatments of plant growth regulators, the Kin treatment was observed a notable rise in the percentage of oil characteristics, plant yield of oil, leaf carbohydrate content, chemical content of total flavonoids, oil quality (GC technology), refractive index, specific gravity, and specific weight. This may be due to the role of cytokinins in stimulating the Phenylalanine Ammonia-Lyase (PAL) pathway as a result of raising the biological efficiency of the leaves, which increases carbon metabolism and the production of secondary compounds. This aligns with what was mentioned by Taiz & Zeiger (2015) and Singh et al. (2017) that cytokinins increase the production of secondary compounds in aromatic plants and enhance their accumulation, in addition to the polar nature of cationic compounds that possess positive groups increasing polarity. The greater the polarization, the more light refraction increases due to the interaction of the light field with more responsive charges, which explains the noticeable increase in the refractive index. Additionally, surface compounds often have a higher molecular weight, contributing to increased density and specific weight (Atkins & de Paula, 2010). The BA treatment showed A substantial rise in the proportion of oil, the oil yield from the plant, the chemical content of total phenols, and the quality of the oil (GC technique) with compounds such as Cineole, Limonene, Cymene, and the percentage of DPPH free radical scavenging, as well as the refractive index, specific density, and specific weight due to its active Playing a part in enhancing cell replication and promoting protein production and enzymes associated with secondary pathways such as the Mevalonate Pathway (MVA) and the the methylerythritol phosphate

pathway (MEP), which are the two primary routes responsible for terpene and essential oil biosynthesis. BA activates terpene biosynthesis-related enzymes, as reported by Davies (2010); and Hussein (2020) found that foliar application of BA to medicinal plants was associated with enhanced EO production and accumulation in of key terpene constituents.

Concerning glutamic acid, its conspicuous impact on the enhancement of leaf carbohydrates and essential oil%, oil yield/plant, total content of phenolics and flavonoids as well as oil quality expressed from GC analysis particularly cineole, limonene and cymene along with free radical scavenging activity (DPPH) may be due to its pivotal function in carbon and nitrogen metabolism. . Glutamic acid being one of the major amino acids synthesis precursor, facilitates photosynthesis products which are redirected toward secondary metabolites by initiating reductive biosynthetic pathway. These results support those by Fawzy et al. (2012) reported that glutamic acid contributes to the stimulation of secondary metabolite synthesis in medicinal plants, and are complemented by the results obtained from Al-Hadithi (2018).

On the other hand, aspartic acid was apparently involved in increasing oil percentage, oil yield per plant and quality of oil estimated by GC. This impact can be explained by the indispensable nature of this element in biosynthesis of essential amino acids like methionine, threonine and lysine as well as its role in amino acid-phenolic pathways. These functions altogether are responsible for enhanced secondary metabolite production and increased antioxidant potential in medicinal plants. Scientific studies have indicated that aspartic acid acts as a key activator of the enzymes responsible for synthesizing phenols and terpenes, and that the presence of amino acids with carboxyl groups capable of forming strong hydrogen bonds with oil molecules increases internal cohesion and molecular polarizability, which reflects on the chemical and qualitative properties of the oil (Taiz & Zeiger, 2015; Gunstone, 2002; Swern, 1979). Some local studies, such as Al-Khafaji (2021), have shown an increase in the effectiveness of antioxidant compounds and a rise in polyphenol content when aspartic acid is applied, supporting the practical results of this experiment. Regarding the effect of the combined treatments, it was observed from the previous results and the analysis of variance supplements that the plant growth regulators Kin + BA and the treatment with amino acids Glutamic acid + Aspartic acid, along with the non-sprayed treatment for each, played an effective role in stimulating most traits, including the carbohydrate content of the leaves, the chemical content of phenols and total flavonoids, the percentage of oil, the oil yield, and the oil quality (GC technique) such as Cineole, Cymene, Limonene, α-Pinene, Camphor, the percentage of DPPH free radical scavenging, refractive index, specific density, and specific weight. This may be due to the cumulative effect between them in stimulating cell division and the formation of enzymes responsible for secondary pathways, and the contribution of amino acids to enhancing the biosynthesis of those compounds through increased carbon and nitrogen assimilation. Several studies, such as Hussein (2020) and Fawzy et al. (2012), have indicated the significant polarity and hydrogen bonding caused by amino acids B1 and B2. This synergistic relationship provides a chemically coherent environment, and the most favorable effect on the essential oil yield of an alone spraying treatment is due to amino acid enables the cultivation since it boosts growth regulators.

Consequently, we can say that the best treatments of improving the chemical properties of rosemary leaves were those receiving BA together with amino acids (B2) as they resulted in a clear improvement in oil content, phenolic and flavonoid contents, major terpenoids content refract index, specific density and specific gravity. This means that this interaction improved the biological pathway of bioactive compound formation. These characteristics are significantly affected by changes in chemical structure or polarity (Nielsen, 2017), thus emphasizing their relevance to applied and agronomic research (compared with single treatments or unsprayed control). Combined effect of growth regulators and amino acids might have resulted in integrative manner for complete stimulation of metabolic activity, new buds formation, better nutrient uptake and enhancement of physiological/chemical attributes in medicinal plants. This increases the plants adaptability against stressful conditions and results in an improved productivity and oil quality comparing with individual applications, rendering it an efficient strategy (Rouphael & Colla, 2020; Khan et al., 2018; Calvo et al., 2014).

CONCLUSION

The present study showed that foliar application of plant growth regulators PGRs combined with amino acids had a stimulating effect on the chemical composition, essential oil yield and antioxidant activity of rosemary plants. Out of the treatments applied, combination treatment of Benzyladenine along with amino acids mainly aspartic and glutamic acids gave excellent enhancement for essential oil percentage, phenolics; flavonoids concentration in addition to enhancement of major terpenoid contents and oil physicochemical properties. As for Kinetin, it played a specific role in promoting carbohydrate storage and oil quality whereas amino acids were involved helpful in inducing the biosynthesis of secondary metabolites and increasing antioxidant capacity. The combination of growth regulators with amino acids was more effective than individual application, formulating

the additive roles of these compounds in triggering metabolic pathways of oil biosynthesis and quality improvement. Thus, foliar co-application of growth regulators and amino acids may be suggested as an alternative efficient and economic agronomic approach to increase productivity and quality of essential oil in rosemary cultivation.

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