

Evaluation of the effect of a hormonal combination with amino acids on growth parameters and yield in rosemary plants

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ABSTRACT

A factorial experiment was carried out using Randomized Complete Block Design layout (RCBD) at the experimental research station, College of Agriculture Engineering Science – University of Baghdad- Al-Jadriyah, in an open greenhouse during the spring season 2023–2024. The aim was to determine the influence of a hormone combination with amino acids on some growth parameters, yield and chemical composition as well as antioxidant activity of rosemary plant (*Rosmarinus officinalis* L.), local variety. The experiment included two factors, each with three levels, and three replicates, where plants were sprayed with the growth regulators benzyl adenine (BA) and kinetin (Kin) at a rate of 50 mg L⁻¹, In addition to glutamic acid and aspartic acid amino acids at equal concentrations, in three successive sprays throughout growth stages. Statistical analysis revealed significant differences for most of the traits studied. The kinetin application showed significant amelioration in some vegetative growth parameters; such as leaf area, leaf width, dry weight of the vegetative total, leaf content of total chlorophyll, in addition to the fresh and dry leaf yield of the plant per unit area. On the other hand, benzyl adenine significantly enhanced the number of branches and leaf thickness, as well as total fresh weight and dry weights of vegetative parts, percentage of total vegetative mass and dry leaf yield. Among amino acids, glutamic acid was the most superior with respect to plant height, leaf thickness, dry vegetative biomass and its percentage as well as fresh and dry leaf yield. Conversely, aspartic acid increased the number of branch and leaf width and thickness, blade fresh weight, and chlorophyll content. Most visible effects on growth traits, yield, chemical composition and antioxidant status including the improvement in synthetic production of volatile oil compounds are the result of the combined activity of growth regulators with amino acids. These results support the use of this mixture to enhance growth and quality of rosemary plants.

KEYWORDS: Rosemary, growth regulators, amino acids, vegetative traits, chlorophyll.

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تقييم تأثير توليفة هرمونية مع الأحماض الأمينية على معالم النمو والمحصول في نبات إكليل الجبل

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المخلص

أجريت تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة (RCBD) في وحدة المحطات البحثية التابعة لكلية علوم الهندسة الزراعية / جامعة بغداد – الجادرية، داخل البيت المكشوف خلال الموسم الزراعي الربيعي 2023–2024، بهدف دراسة تأثير التوليفة الهرمونية مع الأحماض الأمينية في مؤشرات النمو والحاصل والمحتوى الكيميائي والفعالية المضادة للأوكسدة لنبات إكليل الجبل (*Rosmarinus officinalis* L.) الصنف المحلي. اشتملت التجربة على عاملين بثلاثة مستويات لكل منهما، وبثلاثة مكررات، إذ رُشّت النباتات بمنظمات النمو البنزويل أدنين (BA) والكينيتين (Kin) بتركيز 50 ملغم لتر⁻¹، إضافة إلى الأحماض الأمينية حمض الكلوتاميك وحمض الأسبارتيك بالتركيز نفسه، وذلك بثلاث رشّات متتابعة خلال مراحل النمو. أظهرت نتائج التحليل الإحصائي وجود فروق معنوية في معظم الصفات المدروسة. تفوقت معاملة الكينيتين في تحسين عدد من صفات النمو الخضري، تمثلت بزيادة المساحة الورقية، عرض الورقة، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلورفيل الكلي، إضافة إلى حاصل الأوراق الطري والجاف للنبات ووحدة المساحة. في حين أظهرت معاملة البنزويل أدنين تأثيراً معنوياً في زيادة عدد الأفرع، سمك الورقة، الوزن الطري والجاف للمجموع الخضري، والنسبة المئوية للمجموع الخضري، فضلاً عن حاصل الأوراق الجاف. أما بالنسبة للأحماض الأمينية، فقد سجلت معاملة حمض الكلوتاميك تفوقاً معنوياً في ارتفاع النبات، سمك الورقة، الوزن الجاف للمجموع الخضري، النسبة المئوية له، وحاصل الأوراق الطري والجاف، بينما أظهرت معاملة حمض الأسبارتيك تفوقاً في عدد الأفرع، عرض وسمك الورقة، الوزن الطري للمجموع الخضري ومحتوى الكلورفيل. أسهم التداخل المشترك بين منظمات النمو والأحماض الأمينية في تحسين معنوي لصفات النمو والحاصل والمحتوى الكيميائي والفعالية المضادة للأوكسدة، مع تعزيز المركبات الفعالة للزيت الطيار، مما يؤكد أهمية اعتماد هذه التوليفة لتحسين إنتاجية وجودة نبات إكليل الجبل.

الكلمات المفتاحية: إكليل الجبل، منظمات النمو، الأحماض الأمينية، الصفات الخضريّة، الكلوروفيل.

INTRODUCTION

The medicinal and aromatic plants are some of the oldest perennial plants utilized by humans for food and medicine throughout time. Studies have revealed that a considerable population in developing countries continue to depend largely on these plants for their primary healthcare requirements. Herbal medicine is greatly appreciated even in the face of advancement of western medicine (Elgabal, 2007). These plants are rich sources of medicinally active compounds which emphasized the potential use of these plants for therapeutic purposes due to their curative properties in various diseases more than synthesized drugs and besides, they also contain vitamins and nutrients beside the bioactive ingredients (Yesmin, 2015).

Rosemary (*Rosmarinus officinalis* L.) is a type of evergreen woody that belongs to the family Lamiaceae. The species is native to the Mediterranean region and Southern Europe, although it grows in a wild state and cultivation occurs in almost all the countries of the world; main producers of its oil extraction are Spain, France and Tunisia (Peter, 2004). Medicinal and aromatic plants, in particularly rosemary, are an alternate economic crop to be considered with notable development during recent years based on the adaptation of natural and environmental conditions to its cultivation as well as for giving a good economics return. Thus, it was necessary to stimulate research aimed at improving the cultivation of these species for its important contribution in sustainable agricultural development. Nutritionally, rosemary is rich in nutrients including vitamins B1, B2, B3, B6 and B9 as well as vitamin C and minerals such as iron, calcium, zinc or potassium. It's also filled with antioxidants, as well as volatile oils that are made up of borneol, cineole, camphor and camphene and linalool, along with a number of flavonoids and rosmarinic acid. Rosemary is utilized as seasoning and as it is added to a salad, desserts, meats, fish, and vegetables, and is used for preparing soups and preserving items. Pure oil is used in making perfumes, cosmetics, shampoos & conditioners to strengthen the hair and even in soaps & detergents due to its perfume as well. In the present agriculture industry, there is an inclination to replace chemical fertilizers with organic and bio fertilizers for reduction of pollution hazards and environment conservation (EL-Akabawy 2000).

Rosemary grows widely everywhere in the world, it has over 100 chemical constitutions among which are camphor, camphene, borneol and cineole. The essential oils of rosemary are used in a number of industries and also sold for a wide range of applications such as antimicrobial, antioxidant, antibacterial or antifungal uses. They are as well used in pest control agents (Tawfeeq, 2016). Rosemary oil is also used in the preparation of cosmetics and medicines (Maistor et al., 2010). Importance of several active compounds in rosemary emphasizes the importance that attention must be given, and more priority should be placed to develop its growth and enhance its characteristics to increase oils yield obtained from it (Wiedenhoeft, 2006). Many factors influence the level and quality of essential oils content in medicinal and aromatic plants and often these factors are difficult to

separate from one another as the environmental such as climate, soil, interacts with genetic factors and some agricultural practices together with the type of fertilizer inputted (Hussain 2009; Esmailian et al., 2021).

Plant growth regulators, in particular cytokinins, are known to be critical for the improvement of growth properties. They also stimulate cell division and differentiation, overcome apical dominance, promote the expansion of lateral bud, postpones senescence via maintaining greenness (Wareing & Phillips 1981; Al-Asadi et al., 2019). Of all those compounds, two are considered to be very important, namely kinetin and Benzyladenine since they induce cell division, the sizes of cells as well as enhancing vegetative branching (Hassan & Zuhwan, 2014).

Amino acids are also essential for the promotion of growth and amelioration of damage due to environmental stresses, such as dryness or low temperature, as they act like biostimulants (Nur et al., 2006; Hassanein et al., 2010). One of the most abundant of these acids is Glutamine, which acts as one of the major nitrogen donors in plants, and it contributes to enhanced root system development, photosynthesis performance, enzymatic activity and resistance mechanism against different environmental stresses (Sim et al., 2022; Han et al., 2022). Aspartic acid is essential in metabolic pathways related to nitrogen and carbon metabolism and plays a role in protein, nucleotide, and hormone synthesis, as well as in enhancing plant tolerance to abiotic stresses such as salinity, drought, and cold (Han & Zhang, 2021).

Accordingly, combining plant growth regulators with amino acids may represent an effective approach to improving vegetative and physiological growth of rosemary plants and increasing their content of volatile oils of medical and economic importance.

MATERIALS AND METHODS

A factorial trial was conducted according to RCBD in an open greenhouse of location 'A' which belongs to Research Stations Unit, College of Agricultural Engineering Sciences, University of Baghdad - Al- Jadriyah in spring growing season 2023/2024. The main purpose of this experiment was to evaluate the impact of a hormonal combination with amino acids on growth parameters, yield, chemical content, and antioxidant activity in rosemary plants (*Rosmarinus officinalis L.*). The experiment comprised two factors and their interactions, with three replicates, resulting in 27 experimental units. Each experimental unit consisted of 16 pots per treatment, bringing the total number of plants in the experiment to 432. Newly formed offshoots of the local cultivar were selected, small in size and homogenous in terms of branching and overall size. The plants possessed fibrous root systems and were uniform in height, ranging from 8 to 10 cm. These plants were obtained from specialized nurseries in Baghdad Governorate (Al-Krayaat area) after being classified at the Iraqi National Herbarium for seed testing and certification, in order to verify and confirm their identity as

Rosmarinus officinalis L., as described in the British Herbal Pharmacopoeia. The plants were moved to spacious plastic containers with a volume of 8 liters (23 cm in height and 26 cm in diameter), filled with gypsiferous soil obtained from the college fields by excavating to a depth of 30 cm. The pots were placed under open-field conditions, and the plants were acclimatized for one week inside the open field to ensure suitable environmental conditions for all plants prior to initiating the experiment (from March until mid-November). Routine plant maintenance operations were continued, including irrigation by spraying and weeding as needed, with care taken to cover the greenhouse with shade cloth during periods of extreme summer heat.

Studied Traits

Plant height (cm plant⁻¹)

Plant height Last measurement of the experiment was made on August 11, 2024 using a measuring tape. Measured from the point where it attaches to the soil surface to the highest growing apex. Mean value was based on data from eight plants of each treatment.

Number of branches (branch plant⁻¹)

Branches joined to the main stem were counted at experiment end on August 11 2024. This score started at the bottom branch close to soil level and ended in the highest point of the plant. Eight plants were assessed for all treatments, and the average number of branches was then determined.

Leaf area per leaf (cm² leaf⁻¹)

Leaf area was measured at the end of the experiment on 9/11/2024 using Digimizer leaf area measurement software. Eight newly developed leaves were sampled from the middle third of each plant, and measurements were taken for all plants within the experimental unit, with the mean value recorded.

Leaf thickness

Leaf thickness of the main leaf was measured for plants in each experimental unit by sampling eight leaves from the middle third of each plant. Measurements were taken using a Vernier caliper, and the mean value was calculated.

Leaf width

Leaf width of the main leaf was measured for plants in each experimental unit by sampling eight leaves from the middle third of each plant. Measurements were taken using a Vernier caliper, and the mean value was calculated.

Fresh vegetative biomass (g plant⁻¹)

At the finish of the experimental season, eight plants were collected per treatment. The complete plant, comprising both above-ground and below-ground components, was uprooted for each treatment separately. After removing adhering soil particles, the plants were weighed immediately to determine fresh vegetative biomass (g plant⁻¹) using a sensitive balance, and the mean value was

calculated.

Dry vegetative biomass (g plant⁻¹)

The same eight plant samples were air-dried in a shaded, well-ventilated area away from direct sunlight, without the use of artificial heat, until constant weight was reached. The samples were then re-weighed, and the final values were recorded as dry vegetative biomass (g plant⁻¹), and the mean was calculated.

Dry matter percentage of vegetative biomass (%)

The fresh weight of the entire vegetative biomass was determined using a sensitive balance after sampling eight plants from each treatment and drying them until constant weight was achieved (Al-Sahhaf, 1989). The dry matter percentage was calculated using the following equation:

$$\text{Dry matter percentage} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Total Chlorophyll Content in Leaves (mg g⁻¹ fresh weight):

Chlorophyll content was measured using 80% acetone, where 0.2 g of leaf was weighed and 2 ml of acetone was added, and measured using a Spectrophotometer according to the following equation (Goodwin, 1979):

$$\text{Total Chlorophyll (mg/L)} = 20.2 D(645) + 8.02 D(663)$$

D = wavelength

D (663) = absorbance reading at a wavelength of 663 nanometers.

D (645) = absorbance reading at a wavelength of 645 nanometers.

V = final volume of the extract (10 ml)

W = weight of the leaf tissue (0.5 g)

$(100 \times (L) \text{ for the final extract volume} \times L.\text{mg}) / (\text{g}) \text{ sample weight} = \text{g } 100 / \text{mg}$

$$\text{mg} / 100\text{g} = \frac{\text{mg} \cdot L \times \text{final extract volume} L \times 100}{\text{sample weight g}}$$

Fresh leaf yield per plant (kg ha⁻¹)

Eight fresh leaves were collected from eight plants per treatment, and leaf weight per plant was measured using a sensitive balance, with the mean value recorded. Fresh leaf yield per plant was calculated using the following equation:

$$\text{Fresh leaf yield (kg ha}^{-1}\text{)} = \frac{\text{Fresh leaf weight per plant (g)} \times \text{Number of plants per hectare}}{1000}$$

Dry Leaf Yield per Plant (kg ha⁻¹):

Eight leaves were taken from eight plants for each treatment and dried until weight stabilization, and calculated for each plant using a sensitive scale based on their average. The dry leaf yield was extracted according to the following equation:

$$\text{Dry Leaf Yield (kg ha}^{-1}\text{)} = \frac{\text{Average Dry Weight of Plant Leaves (g)} \times \text{Number of Plants per Hectare}}{1000}$$

Fresh and dry leaf yield per unit area (g m⁻²): The fresh and dry leaf yield per unit area were calculated by dividing the fresh and dry biomass by the extraction area. After the fresh and dry leaf yield of each plant was determined, the values were converted to that per unit area, and observed means are presented.

RESULTS AND DISCUSSION:

1- Plant Height (cm/plant):

The results revealed that rosemary plants showed a non-significant response to amino acid treatment in terms of plant height (Table 1), particularly with glutamic acid (50 mg L⁻¹), with mean height of 40.21, followed by the treatment without the addition with an average height of 39.87 cm/plant, while plants treated with aspartic acid (50 mg L⁻¹) showed an average height of 38.53 cm/plant. The results indicate that rosemary plants treated with growth regulators had varying effects on plant height. The treatment without the addition showed an average height of 40.01 cm/plant, followed by plants treated with BA (100 mg L⁻¹) with an average height of 39.44 cm/plant, while plants treated with kinetin (100 mg L⁻¹) showed the lowest average height. The average plant height was 39.11 cm (plant-1). These results also indicate that plants not treated with growth regulators showed better growth. The results revealed a significantly two-way interaction, as the treated with (50 mg L-Glutamic acid with 100 mg L-Kinetin), showed an increase of 15.50% compared to the treatment without the addition. Conversely, plants treated with (50 mg L-Aspartic acid with 100 mg L-Kinetin) showed the lowest average plant height, with a decrease of 13.42% compared to the previous treatment.

Table 1. Effect of amino acids, growth regulators, and their interaction on plant height (cm)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	41.29	38.50	39.83	39.87
Glutamic (B1)	38.92	42.25	39.46	40.21
Aspartic (B2)	39.96	36.58	39.04	38.53
Mean Growth regulators	40.06	39.11	39.44	
LSD 5%	Amino acids	Growth regulators	Interaction	
	N.S	1.33	2.29	

2- Number of Branches:

The statistical analysis results in Appendix 1 and Table 3 indicated that the plants treated with amino acids significantly outperformed in the number of branches for rosemary plants. The treatment with

(50 mg L⁻¹ Aspartic acid) achieved the highest average number of branches at 42.05 branches plant⁻¹ while the treatment with (50 mg L⁻¹ Glutamic acid) achieved an average of 41.22 branches plant⁻¹, whereas The control treatment (no addition) yielded the lowest average number of branches at 37.71 branches plant⁻¹. It is clear that the general behavior of the plants treated with growth regulators varied in terms of the number of branches; the control treatment (no addition) achieved an average of 41.88 branches plant⁻¹, closely followed by treatment with (100 mg L⁻¹ BA) averaging 41.44 branches plant⁻¹. Treatment with (100 mg L⁻¹ Kinetin) yielded the lowest average number of branches at 37.66 branches plant⁻¹.

Concerning the Two-way interaction of the studied factors, the results revealed that the number of branches was positively affected by the spray treatments. Plants treated with aspartic acid at 50 mg L⁻¹ combined with no growth regulator, as well as those treated with glutamic acid at 50 mg L⁻¹ combined with Benzyladenine at 100 mg L⁻¹, recorded increases of 49.29% and 41.18%, respectively, compared with the untreated control.

Table 2. Effect of amino acids, growth regulators, and their interaction on number of branches (plant⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	32.46	35.25	45.42	37.71
Glutamic (B1)	44.71	33.12	45.83	41.22
Aspartic (B2)	48.46	44.62	33.08	42.05
Mean Growth regulators	41.88	37.66	41.44	
LSD 5%	Amino acids	Growth regulators	Interaction	
	2.34	2.34	4.05	

3- Leaf Area (cm² plant⁻¹):

The statistical analysis results in Table 1 and Table 4 indicated that the plants treated with amino acids did not show a significant response in leaf area for rosemary plants. The control treatment showed the highest mean at 1.84 cm² plant⁻¹, while the treatment with (50 mg L⁻¹ Glutamic acid) had an average of 1.64 cm² plant⁻¹, which was not far from the plants treated with (50 mg L⁻¹ Aspartic acid) that showed an average of 1.58 cm² plant⁻¹. The results from the same table show that the leaf area of rosemary plants was affected by growth regulators in equal proportions, as the treatment with (100 mg L⁻¹ Kinetin) recorded an average of 1.71 cm² plant⁻¹, and the treatment with (100 mg L⁻¹ BA) recorded an average of 1.70 cm² plant⁻¹, both following the control treatment which obtain mean of 1.64 cm² plant⁻¹. Regarding the two-way interaction of the study factors, the results indicate that

the treatment (without adding acid and without adding growth regulators) recorded the greatest leaf area which achieved a significant increase of 28.18%, while the treatment (without adding and with 100 mg L⁻¹ Kinetin) recorded a significant increase of 22.81% compared to the former.

Table 3. Effect of amino acids, growth regulators, and their interaction on leaf area (cm²plant⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	1.91	1.83	1.80	1.84
Glutamic (B1)	1.49	1.70	1.73	1.64
Aspartic (B2)	1.53	1.62	1.59	1.58
Mean Growth regulators	1.64	0.130	1.70	
LSD 5%	Amino acids	Growth regulators	Interaction	
	N.S	0.070	4.05	

4- Leaf Thickness:

The results revealed that the plants treated with amino acids did not differ significantly in leaf thickness in rosemary plants (Table 4). The treatment with (50 mg L⁻¹ Glutamic acid) resulted in an average leaf thickness of 0.746 inches, while the treatment with (50 mg L⁻¹ Aspartic acid) yielded an average of 0.744 inches. The treatment without addition had an average of 0.674 inches. The results implies that the leaf thickness in rosemary plants varied in response to growth regulators, with the treatment without addition achieving an average of 0.756 inches, whereas plants treated with Benzyladenine at 100 mg L⁻¹ had an average of 0.708 inches, which was close to the treatment (100 mg L⁻¹ Kinetin) that achieved an average of 0.701 inches. Regarding the interaction effect between the study factors, the leaf thickness of rosemary plants was significantly affected by the different spraying factors, specifically the plants sprayed with (50 mg L⁻¹ Aspartic acid along with 100 mg L⁻¹ Benzyladenine) and the plants treated with (50 mg L⁻¹ Glutamic acid with 100 mg L⁻¹ Kinetin), with increases of 32.32% and 28.05% respectively for both treatments as compared to (without addition with 100 mg L⁻¹ BA) treatment.

Table 4. Effect of amino acids, growth regulators, and their interaction on leaf area (cm²plant⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	0.729	0.663	0.631	0.674
Glutamic (B1)	0.775	0.808	0.657	0.746
Aspartic (B2)	0.765	0.633	0.835	0.744

Mean Growth regulators	0.756	0.701	0.708
LSD 5%	Amino acids	Growth regulators	Interaction
	0.038	0.038	0.066

5- Leaf Width (caliper units)

Results in Table (5) show that the average leaf width was not significantly affected by amino acid treatments. Plants in the control treatment (no addition) recorded the highest average of 3.66 caliper units, followed by plants treated with (50 mg L⁻¹ Aspartic acid) which recorded an average of 3.57 caliper units. This was close to plants treated with (50 mg L⁻¹ Glutamic acid), which recorded an average of 3.49 caliper units. The results revealed that leaf width in rosemary plants did not differ significantly in response to growth regulators. Treatment (100 mg L⁻¹ Kinetin) achieved the highest average of 3.69 caliper units, while the control treatment (no addition) achieved an average of 3.55 caliper units, which was close to treatment (100 mg L⁻¹ BA) that achieved an average for the trait of 3.49 caliper units. Regarding the two-way interaction effect of the study factors, results showed not significantly affected by the different spray factors.

Table 5. Effect of amino acids, growth regulators, and their interaction on leaf width (caliper units)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	3.77	3.84	3.37	3.66
Glutamic (B1)	3.28	3.67	3.52	3.49
Aspartic (B2)	3.59	3.55	3.57	3.57
Mean Growth regulators	3.55	3.69	3.49	
LSD 5%	Amino acids	Growth regulators	Interaction	
	N.S	N.S	N.S	

6- Fresh Biomass (g plant⁻¹)

Statistical analysis results in Table 6 indicate that plants treated with amino acids differed significantly in average fresh vegetative biomass per plant. The control treatment (no addition) recorded the highest average of 72.35 g plant⁻¹, while plants treated with (50 mg L⁻¹ Aspartic acid) recorded an average of 66.30 g plant⁻¹, followed by plants treated with (50 mg L⁻¹ Glutamic acid) which recorded an average of 66.27 g plant⁻¹. The results from the same table show that treating plants with growth regulators, specifically (100 mg L⁻¹ BA), achieved the greatest mean of 71.05 g plant⁻¹, followed by plants treated with (100 mg L⁻¹ Kinetin) with mean of 69.34 g plant⁻¹, compared to the control treatment (no addition) which recorded the minimum average for the trait at 64.53 g plant⁻¹. Regarding the interactive effect, results from the same table show that fresh vegetative biomass was

significantly affected by the spray treatments (50 mg L⁻¹ Glutamic acid along with 100 mg L⁻¹ Kinetin) and (No amino acid addition + 100 mg L⁻¹ BA), with increases of 48.85% and 43.38%, respectively, for both treatments compared to the treatment (50 mg L⁻¹ Glutamic acid + No growth regulator addition).

Table 6. Effect of amino acids, growth regulators, and their interaction on fresh biomass (g plant⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	72.61	69.90	74.53	72.35
Glutamic (B1)	50.64	75.38	72.80	66.27
Aspartic (B2)	70.33	62.75	65.81	66.30
Mean Growth regulators	64.53	69.34	71.05	
LSD 5%	Amino acids 4.11	Growth regulators 4.11	Interaction 7.12	

7- Dry Biomass

Results in Table 7 showed that the dry biomass of rosemary plants was not significantly affected when the plants were treated with amino acids. The treatment without addition achieved an average of 25.47 g, while the plants treated with (50 mg L⁻¹ glutamic acid) achieved an average of 23.86 g, which was not far from the treatment with (50 mg L⁻¹ aspartic acid), which achieved an average of 21.92 g. The results revealed that the dry biomass increased significantly when rosemary plants were treated with growth regulators. The treatment with (100 mg L⁻¹ BA) achieved an average of 24.09 g, while the treatment with (100 mg L⁻¹ kinetin) achieved an average of 23.76 g, and the plants treated without addition achieved an average of 23.39 g. Regarding the combined effect of the study factors, the plants sprayed with 50 mg L⁻¹ glutamic acid and 100 mg L⁻¹ BA and plants sprayed with 50 mg L-glutamic acid and 100 mg L⁻¹ Kinetin) exhibited superior dry vegetative mass, with increases of 60.85% and 60.38%, respectively, compared to plants treated with 50 g L-glutamic acid alone.

Table 7. Effect of amino acids, growth regulators, and their interaction on dry biomass (g plant⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	27.02	26.34	23.05	25.47
Glutamic (B1)	16.99	27.33	27.25	23.86
Aspartic (B2)	26.17	17.62	21.97	21.92
Mean Growth regulators	23.39	23.76	24.09	

	Amino acids	Growth regulators	Interaction
LSD 5%	N.S	1.50	2.60

8- Percentage of Dry Matter in the Vegetative Mass:

The results of Table 8 showed that the plants treated with amino acids differed significantly in the average percentage of dry matter in the vegetative mass, as the treatment (50 mg L⁻¹ Glutamic acid) recorded an average of 38.243%, while the plants treated without addition recorded an average of 35.283%, followed directly by the plants treated with (50 mg L⁻¹ Aspartic acid) which recorded an average of 33.510%. The results from the same table indicate that rosemary plants responded to treatment with growth regulators, as the treatment without addition recorded an average of 38.600%, followed by the treatments (100 mg L⁻¹ benzyl adenine) and (100 mg L⁻¹ Kinetin) which recorded averages of 34.423% and 34.013%, respectively. Regarding the combined effect of the study factors, it was observed that the interaction treatments (50 mg L⁻¹ Glutamic acid without addition) and (50 mg L⁻¹ Glutamic acid with 100 mg L⁻¹ BA) resulted in increases of 243.46% and 231.90%, respectively, for both treatments compared to the treatment (50 mg L⁻¹ Aspartic acid with 100 mg L⁻¹ Kinetin).

Table 8. Effect of amino acids, growth regulators, and their interaction on percentage of dry matter in the vegetative mass (%)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	37.650	37.380	30.820	35.283
Glutamic (B1)	40.960	36.110	37.660	38.243
Aspartic (B2)	37.190	28.550	34.790	33.510
Mean Growth regulators	38.600	34.013	24.423	
	Amino acids	Growth regulators	Interaction	
LSD 5%	1.267	1.267	2.195	

9. Total chlorophyll content in leaves (mg g⁻¹)

The results in Table (9) revealed a significant effect of amino acid spraying on total chlorophyll content in rosemary plants. The treatment with aspartic acid at 50 mg L⁻¹ recorded a mean value of 0.0866 mg g⁻¹, which was close to that obtained by plants treated with glutamic acid at 50 mg L⁻¹, which recorded a mean of 0.0838 mg g⁻¹. This was followed by the untreated treatment, which recorded a mean value of 0.0803 mg g⁻¹.

The results also revealed that total chlorophyll content varied significantly when rosemary Plants

received treatment with growth regulators. The untreated treatment, kinetin at 100 mg L⁻¹, and benzyladenine at 100 mg L⁻¹ recorded values of 0.0859, 0.0829, and 0.0819 mg g⁻¹, respectively.

The results showed a significant interaction between study factors. The superiority of plants treated with aspartic acid at 50 mg L⁻¹ combined with no growth regulator, as well as plants treated with aspartic acid at 50 mg L⁻¹ combined with kinetin at 100 mg L⁻¹, in total chlorophyll content. These treatments recorded increases of 215.80% and 209.53%, respectively, compared with the treatment without amino acid combined with kinetin at 100 mg L⁻¹, which recorded the lowest values among the studied treatments for this trait.

Table 9. Effect of amino acids, growth regulators, and their interaction on total chlorophyll content in leaves (mg g⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	0.0813	0.0797	0.0800	0.0803
Glutamic (B1)	0.0840	0.0817	0.0857	0.0838
Aspartic (B2)	0.0923	0.0873	0.0800	0.0866
Mean Growth regulators	0.0859	0.0829	0.0819	
LSD 5%	Amino acids 0.0021	Growth regulators 0.0021	Interaction 0.0036	

10- Fresh leaf yield in the plant (kg ha⁻¹)

The results in Table 10 indicated that the plants treated with amino acids significantly differed in the average fresh leaf yield, where the treatment without addition achieved an average of 1.444 kg ha⁻¹, while the treatments (50 mg L⁻¹ Glutamic acid) and (50 mg L⁻¹ Aspartic acid) achieved averages of 1.367 kg ha⁻¹ and 1.349 kg ha⁻¹, respectively. The results shows that the fresh leaf yield in the plant significantly increased when rosemary plants were treated with growth regulators, where the treatment (100 mg L⁻¹ Kinetin) achieved an average of 1.560 kg ha⁻¹, while the treatment (without addition) achieved an average of 1.344 kg ha⁻¹. The plants treated with (100 mg L⁻¹ BA) achieved an average of 1.256 kg ha⁻¹.

The results revealed a significant two-way interaction between study factors. An increase in the average fresh leaf yield specifically for the plants (without addition with 100 mg L⁻¹ Kinetin) and (50 mg L⁻¹ Glutamic acid with without addition), where the increase amounted to 250% and 225%, respectively, for both treatments compared to the lowest values achieved by the treatments (without addition with 100 mg L⁻¹ BA) and (50 mg L⁻¹ Aspartic acid with without addition) and (50 mg L⁻¹ Glutamic acid with 100 mg L⁻¹ BA).

Table 10. Effect of amino acids, growth regulators, and their interaction on fresh leaf yield in the plant (kg ha⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	1.333	1.800	1.200	1.444
Glutamic (B1)	1.500	1.400	1.200	1.367
Aspartic (B2)	1.200	1.480	1.367	1.349
Mean Growth regulators	1.344	1.560	1.256	
LSD 5%	Amino acids 0.068	Growth regulators 0.068	Interaction 0.117	

11. Dry Leaf Yield per Plant (kg ha⁻¹):

The results revealed that the dry leaf yield per plant in rosemary was not significantly affected by amino acid treatment (Table 11). Treatments (50 mg L⁻¹ Glutamic acid), (no addition), and (50 mg L⁻¹ Aspartic acid) gave averages of 0.833 kg ha⁻¹, 0.700 kg ha⁻¹, and 0.644 kg ha⁻¹, respectively. In addition, results shows that dry leaf yield per plant increased significantly when rosemary plants were treated with growth regulators. Treatments (100 mg L⁻¹ Kinetin), (100 mg L⁻¹ BA), and (no addition) gave values of 0.767 kg ha⁻¹, 0.711 kg ha⁻¹, and 0.700 kg ha⁻¹, respectively. Significant two-way interaction was also observed. The treatments (50 mg L⁻¹ Glutamic acid + No addition), (50 mg L⁻¹ Glutamic acid + 100 mg L⁻¹ BA), and (No addition + 100 mg L⁻¹ Kinetin) achieved increases of 300% and 280%, respectively, compared to the lowest values recorded for treatment (50 mg L⁻¹ Aspartic acid + No addition).

Table 11. Effect of amino acids, growth regulators, and their interaction on dry leaf yield in the plant (kg ha⁻¹)

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	0.600	0.900	0.600	0.700
Glutamic (B1)	1.000	0.600	0.900	0.833
Aspartic (B2)	0.500	0.800	0.633	0.644
Mean Growth regulators	1.344	0.174	1.256	
LSD 5%	Amino acids N.S	Growth regulators 0.100	Interaction 0.117	

12. Fresh Leaf Yield per Unit Area (g m^{-2}):

The results revealed that the fresh leaf yield per unit area in rosemary was significantly affected by amino acid treatment (Table 12). Treatments (no addition), (50 mg L^{-1} Glutamic acid), and (50 mg L^{-1} Aspartic acid) gave values of 0.060 g m^{-2} , 0.057 g m^{-2} , and 0.054 g m^{-2} , respectively. The results shows that fresh leaf yield per unit area was significantly affected when rosemary plants were treated with growth regulators. Treatment (100 mg L^{-1} Kinetin) achieved the highest average for the trait at 0.065 g m^{-2} , followed by the control treatment (no addition) which achieved an average of 0.056 g m^{-2} , then treatment (100 mg L^{-1} BA) which achieved an average of 0.051 g m^{-2} . Regarding the interactive effect of the study factors, the results shows a significant effect for plants treated with (No addition + 100 mg L^{-1} Kinetin), followed closely by plants from the two treatments (50 mg L^{-1} Glutamic acid + No addition) and (50 mg L^{-1} Aspartic acid + 100 mg L^{-1} Kinetin), in recording fresh leaf yield per unit area with increases of 250%, 226%, and 224%, respectively, compared to the lowest values achieved by treatments (No addition + 100 mg L^{-1} BA), (50 mg L^{-1} Aspartic acid + No addition), and (50 mg L^{-1} Glutamic acid + 100 mg L^{-1} BA).

Table 12. Effect of amino acids, growth regulators, and their interaction on fresh leaf yield per unit area (g m^{-2})

Amino acids	Growth regulators			Mean
	No addition (A0)	Kinetin (A1)	BA (A2)	Amino acid
No addition (B0)	0.054	0.075	0.050	0.060
Glutamic (B1)	0.063	0.058	0.050	0.057
Aspartic (B2)	0.050	0.062	0.052	0.054
Mean Growth regulators	0.056	0.065	0.051	
LSD 5%	Amino acids 0.003	Growth regulators 0.003	Interaction 0.006	

13. Dry Leaf Yield per Unit Area (g m^{-2}):

The results revealed that the dry leaf yield per unit area in rosemary differed significantly when plants were treated with amino acids (Table 13). Treatment (50 mg L^{-1} Glutamic acid) gave the highest average of 0.035 g m^{-2} , followed by treatments (50 mg L^{-1} Aspartic acid) and (no addition), which achieved averages of 0.030 g m^{-2} and 0.029 g m^{-2} , respectively. The results show that dry leaf yield per unit area was not significantly affected when rosemary plants were treated with growth regulators. Treatments (A2), (A1), and (A0) showed averages for the trait of 0.033 g m^{-2} , 0.032 g m^{-2} , and 0.029 g m^{-2} , respectively. Regarding the interaction of study factors, results implies that plants treated with (B1 + A0), (B0 + A1), (B1 + A2), (B2 + A2), and (B2 + A1) were affected in the trait of dry leaf

yield per unit area, with increases of 200%, 180.95%, 171.42%, and 161.90%, respectively, compared to the interaction treatments with the lowest values (A0B2).

Table 13. Effect of amino acids, growth regulators, and their interaction on dry leaf yield per unit area (g m^{-2})

Amino acids	Growth regulators			BA (A2)	Mean Amino acid
	No (A0)	addition (A1)	Kinetin (A1)		
No addition (B0)	0.025		0.038	0.025	0.029
Glutamic (B1)	0.042		0.025	0.038	0.035
Aspartic (B2)	0.021		0.034	0.036	0.030
Mean Growth regulators	0.029		0.032	0.033	
LSD 5%	Amino acids		Growth regulators	Interaction	
	0.004		0.004	0.007	

DISCUSSION

Regarding the previous results for the vegetative growth parameters of rosemary and its components, and, it was observed that, in general, the plant responded to treatment with the plant growth regulators Kin + BA, treatment with the amino acids Glutamic acid + Aspartic acid, and the unsprayed treatment of each. The effect was evident in most growth characteristics, whether individually or through interaction between them. In general, the best vegetative growth was achieved when combining the growth regulators and the amino acids, indicating a synergistic effect between them, which was reflected in the characteristics of plant height, leaf area, number of branches, and leaf chlorophyll content. Regarding the reason for the significant increase in vegetative characteristics achieved in single spray treatments using the Kin-treated plant growth regulator compared to the unsprayed treatment—specifically leaf area, leaf width, total chlorophyll content, and dry leaf yield per unit area—this may be due to the role of cytokinins in stimulating apical cell division, increasing protein synthesis, and activating meristematic tissues. This interpretation aligns with Sharma and Singh's (2015) research, which demonstrated that kinetin promotes vegetative growth and postpones senescence in medicinal plants. Similarly, Al-Saidy's (2019) study on rosemary plants supported these results, showing that cytokinins notably boosted plant height and leaf chlorophyll levels.

Similarly, the benzyladenine (BA) treatment had a notable and wide-ranging impact on vegetative characteristics, such as leaf size, leaf thickness, percentage of dry matter in vegetative biomass, dry leaf yield per plant and per unit area, as well as fresh and dry vegetative biomass. BA outperformed kinetin in many of these traits in terms of significance, likely due to BA's strong cytokinin properties in promoting cell division, stimulating lateral bud growth, and increasing leaf size.

Davies (2010) suggested that BA has a high effectiveness in enhancing plant growth by increasing the production of RNA and proteins. Hussein (2020) similarly showed that applying BA on medicinal plants' leaves resulted in notable enhancements in leaf size, overall chlorophyll levels, and plant biomass. This aligns with the results of the current study.

Regarding individual amino acid spray applications, glutamic acid demonstrated a significant impact on enhancing vegetative growth characteristics in comparison to the control treatment that did not receive treatment. These characteristics encompassed plant height, fresh and dry leaf yield per plant and per unit area, dry vegetative biomass, leaf thickness, and total chlorophyll content within the leaves. This phenomenon could be ascribed to the function of glutamic acid in improving nutrient absorption efficiency, enhancing photosynthetic performance, participating in chlorophyll production, and facilitating the synthesis of other amino acids. This ultimately results in increased availability of nutrients. Evidence supporting these findings can be found in the studies by Fawzy et al. (2012) and Al-Hadithi (2018), which demonstrated the beneficial impact of applying glutamic acid to medicinal plants' foliage.

Similarly, aspartic acid treatment resulted in promoting the various vegetative growth parameters such as number of branches/plant, leaf thickness and width, fresh and dry leaf yield per unit area, fresh vegetative biomass/plants, total chlorophyll content in leaves. This may be due to the fact that it can contribute to stimulating photosynthesis activity, stimulating vegetative growth and enzyme associated with process of growth or biosynthetic processes in leaves of crops and its importance as a precursors for several essential amino acids such as methionine, lysine and threonine, traits enhancement in chlorophyll content and antioxidant potential. These results are consistent with the reports of Taiz and Zeiger (2015) and Al- Khafaji (2021), who established response of medicinal plants to applications of amino acids.

The interaction treatments, as shown in the previous results and analysis of variance, demonstrate that the combination of plant growth regulators (Kin + BA), amino acid treatments (glutamic acid + aspartic acid), and the untreated control significantly enhanced various vegetative growth traits. This could be because Kin and BA play complementary roles in guiding growth and promoting cell division, as well as the contributions of glutamic acid and aspartic acid in enhancing the plant's overall physiological condition and enhancing its effectiveness, since they are essential components for synthesizing proteins and chlorophyll compounds. Several studies, including Hussein's research in 2020 and the work of Fawzy et al. (2012), have suggested that the combination of growth regulators and amino acids produces better outcomes compared to using each one separately.

As a result, it can be inferred that treatments that involve benzyladenine paired with amino acids prove to be the most effective in enhancing the overall growth characteristics of rosemary plants in challenging environmental conditions. These treatments improve nutrient absorption, increased

protein and enzyme production crucial for growth, ultimately providing the maximum benefit to the plants when compared to separate treatments or untreated controls.

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