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Impact of Amino Acid Sprays on *Rosmarinus officinalis* L. Vegetative Growth, Oil Production, and Quality in Soilless Culture Systems

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ABSTRACT
For two seasons in a row (2022 and 2023), this study was conducted at the Central Laboratory for Agricultural Climate Research Centre (CLAC) in Dokki, Giza, Egypt, beneath an unheated plastic house. This study investigated the effects of foliar sprays containing amino acids on the vegetative growth, oil output, and quality of *Rosmarinus officinalis* L. Plants were given aqueous solutions containing 100 and 200 parts per million of the amino acids glutamine and tryptophan three weeks after transplanting. Two harvests of the plant were made: one in mid-May and another three months later. For both harvests, the essential oil % was calculated and important growth and yield metrics were assessed. Gas Chromatography (GC) was used to study the composition of essential oils. The findings showed that foliar spraying with amino acids greatly increased yield, essential oil percentage, and vegetative development. Important constituents of the essential oil profile comprised 1–8 Cineol, Camphor, α-Pinene, and β-Pinene; significant compositional variances were ascribed to various amino acid treatments.

Keywords: tryptophan, glutamine, soilless cultivation, amino acid, and *Rosmarinus officinalis*.

Introduction
A member of the Lamiaceae family, rosemary (*Rosmarinus officinalis* L.) is notable for its wide range of medicinal and industrial uses. Native to the Mediterranean, rosemary has long been valued for its powerful therapeutic effects in addition to its culinary uses (Golkar *et al.,* 2020; Aziz *et al.,* 2022). Bioactive chemicals including α-pinene, camphor, and linalool are abundant in the plant's essential oil, contributing to its anti-inflammatory, antibacterial, and antioxidant properties (Borges *et al.,* 2018; Bajalan *et al.,* 2017). The plant's wide range of medicinal benefits, such as antifungal, anti-inflammatory, antioxidant, and antibacterial properties, as well as its importance in a variety of sectors, such as food and cosmetics, are confirmed by its adaptability (González-Minero *et al.,* 2020; Nieto *et al.,* 2018).

Its secondary metabolites, including flavonoids, phenolic acids, and terpenoids, are principally responsible for these effects. Furthermore, because of its strong antibacterial properties, rosemary essential oil has been recognized as a possible natural preservative source, which makes it useful for prolonging the shelf life of a variety of goods (Al-Fraihat *et al.,* 2023). Beyond its conventional usage, rosemary has a wide range of medicinal applications. Current studies demonstrate its promise for treating a variety of illnesses, such as diabetes, cancer, and neurological disorders. For example, in both human and animal investigations, rosemary extracts have demonstrated strong neuroprotective and anticancer effects (Petiwala *et al.,* 2013; Hou *et al.,* 2012). More study and interest in rosemary as a source of bioactive compounds with possible health benefits has grown as a result of this growing body

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of information. This growing body of information has sparked more study and commercial exploitation of rosemary as a source of bioactive compounds with possible health advantages (Najar *et al.,* 2021).

The importance of natural bio-stimulants, such as amino acids, in promoting plant growth and development is becoming increasingly recognized as the usage of medicinal plants, such as rosemary, continues to develop. Essential chemical substances that are involved in many physiological functions, amino acids have become powerful growth promoters. According to Talaat et al. (2005) and Abo Sedera et al. (2010), they are essential for the synthesis of proteins, enzymes, and other compounds required for plant health. It has been demonstrated that their use in agriculture enhances plant quality overall, yield, and stress tolerance. Tryptophan and glutamine are two examples of amino acids that are essential for plant development and stress response. Melatonin, which affects development and helps plants resist environmental stressors including drought and salt, is derived from tryptophan (Arnao and Hernández-Ruiz, 2019; Farooq *et al.,* 2015). Similar to this, glutamine promotes growth and aids in the synthesis of proteins and other amino acids, which enhances plant function and nutrient absorption (El-Kassas *et al.,* 2020). It has been shown that applying amino acids as foliar sprays improves plant development characteristics and yield, making it a useful strategy for maximizing agricultural output (Khattab *et al.,* 2012; Wassel *et al.,* 2015). Aside from their function in plant development, amino acids also act as chelating agents, which make it easier for nutrients to be absorbed. According to Popko et al. (2018), they aid in the formation of complexes with metal ions, increasing their availability to plants and boosting nutrient absorption in general. The effectiveness of amino acids as natural biostimulants is further supported by this chelating function, which is especially helpful in controlling nutritional shortages and maximizing plant health (Sowmya *et al.,* 2023).

The use of amino acids and medicinal herbs in agriculture reflects a larger movement toward effective and sustainable farming methods. Soilless culture is one such technique that uses inert substrates or nutrient solutions instead of conventional soil to grow plants. This method offers several advantages over conventional soil-based cultivation, including higher crop yields, more efficient use of water and nutrients, and reduced environmental impact (Joshi *et al.,* 2022). Soilless culture techniques, such as hydroponics and substrate culture, have gained prominence for their ability to provide controlled growing conditions and address challenges associated with soil-based farming. Substrate culture, which utilizes inert media such as sand, peat moss, and perlite, provides mechanical support for plants and improves root development (Deepagoda *et al.,* 2013). Plant growth, yield, and quality can all be greatly impacted by the substrate selection, underscoring the significance of choosing the right media for the best outcomes (Alsmairat *et al.,* 2018; Fussy and Papenbrock, 2022). In addition to providing the benefits of increased yields and cleaner production, these soilless growing techniques support sustainable agriculture by tackling problems with soil deterioration, water shortages, and climate change (Manukyan *et al.,* 2004; Udagawa, 1995). All things considered, combining natural biostimulants like amino acids, medicinal herbs like rosemary, and cutting-edge production methods like soilless culture offers a comprehensive strategy for raising agricultural sustainability and productivity. It is feasible to solve the issues of global food security while reducing the negative effects on the environment by using the medicinal qualities of plants, maximizing growth with biostimulants, and implementing sophisticated farming techniques. Our knowledge and use of these important agricultural resources will only grow as a result of the continuous study and development in these fields. The purpose of this research is to improve knowledge of biostimulants in sustainable agriculture and optimize rosemary growth techniques.

2. Materials and Methods

2.1 Experimental Site and Duration

The Central Laboratory for Agricultural Climate Research Centre (CLAC) in Dokki, Giza Governorate, Egypt, was the site of the experiment. During two consecutive growth seasons (2022 and 2023), an unheated net house was used.

2.2 Plant Composition and Growing Environment

Rosmarinus officinalis L. seedlings were acquired from the Medicinal and Aromatic Plants Section at El-Kanater El-Khairia, Qalubia Governorate, Horticulture Research Institute, A.R.C. They were then moved into plastic pots (5 liters in volume) that were filled with two distinct substrate media: sand with distinct physical and chemical properties (table 1) and peat moss mixed with perlite (1:1 v/v). Early in

March, the seedlings were put on greenhouse terraces that could drain. Based on local water analysis, they were watered with a balanced nutrient solution that was modified from Cooper's solution (Cooper, 1979; El-Behairy, 1994). In Table 2, the pH was maintained between 6.0 and 6.5 and the electrical conductivity (EC) of the nutrient solution was maintained between 2.0 and 2.2 mS/cm.

Table 1. Physical and chemical characteristics of two commercial and the total volume was made up to 100 ml with substrates

Substrate	Physical				Chemical	
	Bulk density g/l	%Total pore space	% Water holding capacity	% Air porosity	E.C mm-hos-1	pH
Peat moss : Perlite	392.6	63.1	45.7	15.3	0.44	7.4
Sand	1656	23	18.56	3.29	0.87	7.6

Table 2. The chemical composition of nutrient solutions

Elements	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
Concentration (ppm)	205	73	288	187	55	4.89	0.99	0.04	0.042	0.18

Climatic data in the study area was obtained daily from transplanting until end of the experiment over the two study seasons using the weather station located in the greenhouse at the Central Laboratory for Agriculture Climate as shown in (Table A).

Table A: Climatic data of the experimental site during (2022 and 2023) seasons

Month	Soil temperature [°C]		T Min.		T Max.		RH		Wind		SRAD	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Mar	11.47	20.89	10.51	13.32	23.31	27.37	53.42	52.97	182.03	190.63	0.28	0.34
Apr	17.48	24.59	16.86	15.63	32.25	31.08	45.66	45.61	59.17	86.83	0.32	0.43
May	20.24	26.96	19.37	19.47	34.21	34.23	43.31	46.02	40.65	76.13	0.41	2.42
Jun	24.00	30.09	23.10	22.88	37.73	36.83	49.70	48.74	113.67	354.17	0.37	98.23
Jul	24.65	32.17	23.87	24.73	37.98	39.70	52.75	53.93	192.42	400.32	0.30	87.26

TMIN	Temperature at 2 Meters Minimum (°C)	RH	Relative Humidity Average at 2 Meters (%)
TMAX	Temperature at 2 Meters Maximum (°C)	WIND	Wind Speed at 2 Meters (m/s)
SRAD	Solar Radiation (MJ/m^2/day)		

2.3. Fertilization and Foliar Application

Three weeks after transplanting, the plants were sprayed with aqueous solutions of amino acids tryptophan (100 and 200 ppm) and glutamine (100 and 200 ppm). Foliar applications were performed three times: the first after new leaf emergence, the second one week later, and the third one week after the second application. Additional foliar applications were made two weeks after the first harvest.

2.4. Experimental Treatments

Control (peat moss + perlite) only, 100ppm tryptophan with (peat moss + perlite), 200ppm tryptophan with (peat moss + perlite), 100 ppm Glutamine with (peat moss + perlite), 200 ppm

Glutamine with (peat moss + perlite), control (sand) only, 100ppm tryptophan with(sand), 200ppm tryptophan with (sand), 100 ppm Glutamine with (sand) and 200 ppm Glutamine with (sand).

2.5. Measurements of Harvesting and Growth

Two times, in May and three months later, the plants were harvested. The herb was cut five centimeters above the ground. Among the vegetative growth metrics noted were: According to A.O.A.C. (1990), the number of branches per plant, plant height, fresh weight (g/plant), and dry weight (%) were calculated.

2.6. Herbage's Chemical Composition Analysis

2.6.1. Quantification and Extraction of Essential Oils

Essential oil was extracted from 100 g of fresh herbage using hydro distillation. Distillation was maintained for 2.5 to 3.0 hours until no further rise in oil output was detected. The extracted oil was allowed to settle, and its amount was measured calculated according to (Clevenger, 1928). The following formula was used to determine the proportion of essential oil:

$$\text{Oil (\%)} = \left(\frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}}\right) \times 100$$

The average fresh weight of the plant was multiplied by the average oil % to get the oil production per plant:

$$\text{Oil yield/plant} = \text{Plant fresh weight (g)} \times \text{Oil (\%)}$$

2.6.2. NPK Analysis

A 500 ml Kjeldahl flask was filled with 0.1g of the dried sample after the plants were harvested 80 days after transplanting and samples were dried for 24 hours at 70°C. The solution was then digested till it became colorless after adding 10 milliliters of concentrated sulfuric acid (H2SO4). After cooling, the solution was employed for further chemical analysis after being diluted with around 25 milliliters of distilled water (Solution 1).

2.6.2.1. Nitrogen Determination

Total nitrogen was quantified following the Kjeldahl method and modified by Watanabe and Olsen (1965). In accordance with A.O.A.C. (1990).

2.6.2.2. Phosphorus

Murphy and Riley's (1962) ascorbic acid reductant technique was used to colorimetrically assess total phosphorus.

2.6.2.3. Potassium: a flame photometer was used to determine the total potassium in Solution 1.

2.6.3. Total Carbohydrates

A glucose standard curve was used to determine the total carbs in accordance with A.O.A.C. (1990).

2.6.4. Identification of pigments in plants:

Chlorophyll a, b and carotene were extracted and quantified in accordance with Wettstein (1957) and A.O.A.C. (1990).

2.6.5. Phenolic substances (mg/100g FW) as catechol were determined

To determine phenolic compounds concentration, we utilized the colorimetric Folin-Denis method as described by Shahidi and Naczk (1995). The necessary reagents included Folin-Ciocalteu phenol reagent, which was outsourced from Sigma Company, Germany was one of the required reagents.

2.7. Economic assessment

The sum of the costs for the metal table, tank, plastic, irrigation and drainage equipment, pot, and substrate is the total fixed cost, and the sum of the costs for the chemical nutrient solutions, seedlings, and amino acids is the total operational cost (L.E/m2). The combination of the herb dry weight for the two seasons, the number of plants per square meter, and the average market price (L.E./kg) was used to determine the total income. The market price of the experiment's components, expressed in Egyptian pounds, as of 2022 and 2023 is shown in the table (3).

Table 3: Price of the experiment constituents (L.E.)

Item	Cost (L.E.)	Item	Cost (L.E.)
Pot	2.5	**Seedling**	3.75
Sand (L)	2	**Nutrient Solution (L)**	15
Perlite (L)	3	**Power (m^2)**	30
Irrigation system (m^2)	100	**Irrigation (m^2)**	30
Tank	75	**Amino acid (1g)**	10
N. plant/m2	16		
Rosemary Market prices kg/DW	350		

2.8. Design of Experiments

30 treatments, comprising two soilless medium (sand and peat moss/perlite), two amino acid concentrations, and three replicates, were used in the fully randomized trial.

The study was conducted using a completely randomized design with 30 treatments arranged in a 2 × 5 × 3, including two soilless media (peat moss/perlite and sand), five amino acid concentrations, and three replicates.

2.9. Statistical analysis system

The SAS (1996) statistical analysis system application was utilized. In this investigation, meaningful comparisons between means were made using Duncan range tastes.

3. Results and Discussion

3.1. Growth Parameters

When compared to untreated plants, the growth characteristics of cultured rose marital plants increased dramatically when amino acids were added, according to the data shown in Tables 4 and 5. In the first and second cuts, plants treated with 200 ppm tryptophan grew in peat moss and perlite substrate, and in the first and second cuts, plants treated with 200 ppm glutamine grew in peat moss and perlite substrate, achieving the highest value for plant height in comparison to the control treatment. In terms of plant height, plants grown in sand or peat moss without any additions had the lowest values. According to plant fresh weight data, plants growing in sand substrate treated with 200 ppm tryptophan recorded the highest plant fresh weight in the first and second cuts, followed by plants growing in sand substrate treated with 100 ppm glutamine. However, plants growing in peat moss and perlite substrate treated with 100 ppm glutamine had the fewest branches/plant in the first and second cuts. When compared to the other treatments, plants growing in sand with 200 ppm or 100 ppm tryptophan recorded the best value in terms of number of branches/plants. Plants growing in sand substrate treated with 200 ppm tryptophan had the highest fresh weight in the first and second cuts, followed by plants growing in sand substrate treated with 100 ppm tryptophan. In contrast, plants growing in peat moss and perlite substrate without any additions had the lowest fresh weight in the first and second cuts.

Plants growing on sand substrate treated with 200 ppm tryptophan had the greatest results for dry weight in both cuttings, according to data on plant dry weight. Plants grown in peat moss and perlite substrate treated with 200 ppm glutamine followed, with no discernible difference. Additionally, the plants that were developing without any additions had the lowest plant dry weight value in both cuts. A part of (water content) Plants growing in sand culture treated with 200 ppm tryptophan had the lowest water content of any of the other treatments, according to the data in (table 4 and 5). In both cuts, the plants growing in peat moss with perlite substrate without any additives had the lowest water content,

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Table 4: Effect of foliar spray with tryptophan and glutamine application on the morphological characteristics of rosemary (*Rosmarinus officinalis* L.) Plant in first season

Treatment		Height (cm)		No. of branches/plant		Fresh weight/plant		% Dry weight		% Moisture	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	64^{d}	65.67^{ed}	4.33$_{cd}$	7^{c}	114^{j}	256.33^{g}	20.286^{d}	18.32^{e}	79.71^{a}	81.68^{a}
	Tryp. 100	65.33^{cd}	70.3^{cd}	7.33$_{ab}$	12^{ab}	141.7^{g}	273.33^{e}	22.71^{bcd}	20.85^{ed}	77.29^{abc}	79.14^{ab}
	Tryp 200	100^{a}	99^{a}	6.33$_{bcd}$	10^{bc}	135.67^{h}	261.43^{f}	22.25^{cd}	21.39^{cde}	77.75^{ab}	78.61^{abc}
	Glutamine 100	68^{cd}	73^{cbd}	6.67$_{bc}$	12^{ab}	152.33^{d}	287^{d}	23.505^{bc}	23.11^{bcd}	76.5^{bc}	76.89^{bcd}
	Glutamine 200	80^{b}	82^{cb}	4$_{d}$	8.67^{c}	154.67^{c}	292^{c}	23.8^{abc}	22.25^{bcde}	76.2^{bcd}	77.53^{abcd}
Sand	Control	65^{d}	73.3^{cbd}	7.33$_{ab}$	13^{ab}	124.5^{i}	256.17^{g}	21.27^{cd}	19.31^{ed}	78.73^{ab}	80.68^{ab}
	Tryp. 100	76.5^{cb}	84^{b}	9.33$_{a}$	14.33^{a}	163.67^{b}	302.78^{b}	25.27^{ba}	25.61^{abc}	74.72^{dc}	74.39^{cde}
	Tryp 200	69.833^{cbd}	76.5^{cbd}	9.33$_{a}$	13.67^{a}	177.5^{a}	315.4^{a}	26.74^{a}	28.49^{a}	73.26^{d}	71.51^{e}
	Glutamine 100	50.5^{e}	54.5^{e}	7.67$_{ab}$	12.33^{ab}	149^{e}	285^{d}	23.48^{bc}	26.05^{ab}	76.52^{bc}	73.95^{de}
	Glutamine 200	59^{ed}	68.27^{d}	5.67$_{bcd}$	10^{bc}	144.83^{f}	273.17^{e}	23.23^{bcd}	23.53^{bcd}	76.76^{abc}	76.46^{bcd}

Table 5: Effect of foliar spray with tryptophan and glutamine application on the morphological characteristics of rosemary (*Rosmarinus officinalis* L.) Plant in Second Season

Treatment		Height (cm)		No. of branches/plant		Fresh weight/plant		% Dry weight		% Moisture	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	61.33^{e}	69.57^{f}	5.67^{de}	10.67^{cd}	119.33^{i}	217.67^{j}	20.43^{d}	19.23^{e}	80.7^{a}	82.03^{a}
	Tryp. 100	67.33^{cd}	72.17^{ef}	9^{abc}	13.33^{abc}	142.33^{f}	257.33^{g}	23.1^{bcd}	21.53^{cde}	77.8^{b}	79.6^{abc}
	Tryp 200	102.33^{a}	98.17^{a}	7.67^{cde}	12.67^{abcd}	136^{g}	240.4^{h}	23.2^{bcd}	22.5^{cd}	78.37^{ab}	79.07^{bcd}
	Glutamine 100	65.33^{ed}	75.17^{edf}	9^{abc}	11^{bcd}	165.67^{c}	270.3^{d}	24.33^{abc}	23.83^{bc}	77.17^{bc}	76.6^{def}
	Glutamine 200	82.67^{b}	84^{cb}	5.33^{e}	9.67^{d}	164.63^{c}	280.67^{c}	24.5^{abc}	22.83^{cd}	76.87^{bc}	78.57^{bcde}
Sand	Control	63^{ed}	78.67^{ced}	8.67^{bc}	14^{ab}	130.33^{h}	219.17^{i}	21.63^{cd}	20.5^{de}	78.83^{ab}	81.33^{ab}
	Tryp. 100	78^{b}	88^{b}	10.67^{ab}	15^{a}	177^{b}	287.53^{b}	25.9^{ab}	26.6^{ab}	75.1^{cd}	75.7^{f}
	Tryp 200	71.33^{c}	80.97^{bcd}	11.33^{a}	13.67^{abc}	182^{a}	296.67^{a}	27.13^{a}	29.17^{a}	74^{d}	72.1^{g}
	Glutamine 100	53.8^{e}	56.9^{g}	10^{abc}	11.67^{bcd}	154^{d}	265.67^{e}	23.6^{bc}	27.47^{a}	77.33^{bc}	76.1^{ef}
	Glutamine 200	61.41^{e}	73.1^{ef}	8^{cd}	11^{bcd}	146.8^{e}	261.67^{f}	23.9^{bc}	23.77^{bc}	77.47^{bc}	77.2^{cdef}

followed by the plants growing in sand culture with a non-significant difference. The use of tryptophan or glutamine applications may have increased the various vegetative growth features under study because they naturally improve growth parameters, increase the soil's capacity to hold water and fertilizer, chelate nutrient elements and make them more accessible for plant roots to absorb, promote root growth, and provide a source of growth regulators. According to Sedibe and Allemann (2012) and Eiasu (2009), fertilizer application and environmental conditions have an impact on rose geranium production and oil composition. Additionally, tryptophan or glutamine acid considerably improved rosemary growth characteristics over the course of two seasons in most treatments as compared to the untreated plants.

According to Sedibe and Allemann (2012) and Eiasu (2009), fertilizer application and environmental factors have an impact on the yield and oil composition of rose geranium. Additionally, in most treatments, tryptophan or glutamine acid significantly improved rosemary growth parameters over the course of two seasons when compared to the untreated plants. Ahmad H. Al-Fraihat et al. 2023 found that some of the effective actions of L-tryptophan have been confirmed, even though its physiological effects on plants have not yet been fully clarified. Tryptophan management on an exogenous base raises auxin levels in plant tissues. In the two cuts during both seasons.

3.2. Chemical Composition of Plant Leaves:

As shown in (Tables 6 and 7), it was discovered that plants treated with glutamine or tryptophan had the greatest plant N (%), P%, and K% values. Additionally, during the two experimental seasons, the addition of tryptophan statistically promoted the greatest values of plant NPK, total carbohydrates (%), and phenol (%). Even in peat moss and perlite or plants growing in sand, the untreated plants obtained the lowest values of plant chemical basic parameters during the course of the two seasons, regardless of the control treatment.

The data in Table (6, 7) regarding the nitrogen content of leaves revealed that plants treated with 200 ppm tryptophan and growing in sand had the highest nitrogen percentage when compared to the other treatments. These plants were followed by plants treated with 100 ppm tryptophan growing in sand culture and plants treated with 200 ppm glutamine growing in peat moss and perlite, with no discernible differences between them. Conversely, even when grown in sand or peat moss and perlite culture, the untreated plants had the lowest nitrogen concentration. The first and second cuts showed the same pattern. According to the P% data in the same table, plants treated with 200 ppm tryptophan grown in sand culture had the highest P% value, followed by plants treated with 100 ppm tryptophan grown in sand culture and plants treated with 100 ppm glutamine in peat moss and perlite culture. The difference between these plants was not statistically significant. Conversely, the untreated plants cultivated in perlite culture or sand or peat moss had the lowest percentage P value; this pattern was also seen in the second cut. According to the data regarding the %K, plants treated with 200 ppm tryptophan in sand culture had the highest percentage of any treatment. These were followed by plants treated with 100 ppm tryptophan grown in sand culture and plants grown in peat moss treated with 200 ppm glutamine. In both cuts, we saw the same pattern. Additionally, data showed that, in terms of potassium content in leaves in first and second cuttings, untreated plants in sand culture or peat moss and perlite culture had the lowest value among the other treatments.

The same table shows that plants treated with 200 ppm tryptophan grown in sand had the greatest value, followed by plants treated with 100 ppm tryptophan cultivated in sand. Even in sand, peat moss, and perlite cultures, the untreated plants performed noticeably worse than the other treatments, as seen by the first and second cuttings. Data regarding the percentage of carbohydrates in leaves revealed that, despite the lowest value observed in untreated plants in sand culture of peat moss and perlite culture, the highest amount of carbohydrates was significantly obtained in plants treated with 200 ppm of tryptophan grown in sand culture, followed by plants treated with 100 ppm of tryptophan grown in sand culture. This pattern was noted in the initial and subsequent cuts. The same chart for the amount of phenols in leaves revealed that, when compared to the other treatments, plants treated with 200 ppm tryptophan had the greatest phenol content, followed by plants treated with 100 ppm tryptophan in sand culture. The untreated plants in sand culture or peat moss and perlite culture had the notably lowest value. in each of the two seasons' cuts.

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Table 6: Effect of foliar spray with tryptophan and glutamine application on the chemical composition of plant leaves of rosemary (*Rosmarinus officinalis* L.) Plant in first season

Treatment		% N		% P		% k		% Carbohydrate		% Phenol	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	1.64_{e}	1.88_{e}	0.38_{i}	0.64_{i}	1.49_{h}	1.86_{j}	5.27_{f}	5.6_{j}	4.35_{h}	5.5_{i}
	Tryp. 100	2_{d}	2.05_{de}	0.49_{g}	0.76_{f}	1.59_{g}	2.15_{g}	6.271_{d}	6.14_{g}	4.95_{f}	6.53_{f}
	Tryp 200	1.72_{e}	2.02_{de}	0.46_{g}	0.73_{g}	1.57_{g}	2.03_{h}	5.7_{e}	5.19_{h}	4.74_{g}	6.1g
	Glutamine 100	2.14_{d}	2.4_{bc}	0.59_{d}	0.87_{c}	1.74_{d}	2.33_{d}	7.301_{c}	7.59_{d}	5.2_{d}	7.64_{d}
	Glutamine 200	2.42_{c}	2.54_{bc}	0.63_{c}	0.92_{b}	1.79_{c}	2.44_{c}	7.680_{b}	7.94_{c}	5.4_{c}	7.96_{c}
Sand	Control	1.67e	1.9_{e}	0.41_{h}	0.69_{h}	1.51_{h}	1.98_{i}	5.725_{e}	5.72_{i}	4.71_{g}	5.82_{h}
	Tryp. 100	3_{b}	2.61_{b}	0.69_{b}	0.96_{a}	1.88_{b}	2.58_{b}	8.573_{a}	8.33_{b}	5.78_{b}	8.72_{b}
	Tryp 200	3.29_{a}	2.97_{a}	0.72_{a}	0.98_{a}	1.96_{a}	2.62_{a}	8.818_{a}	8.59_{a}	5.94_{a}	8.93_{a}
	Glutamine 100	2.13_{d}	2.29_{cd}	0.55_{e}	0.84_{d}	1.69_{e}	2.27_{e}	6.922_{c}	7.15_{e}	5.11_{de}	7.61_{d}
	Glutamine 200	2.12_{d}	2.26_{cd}	0.52_{f}	0.79_{e}	1.66_{f}	2.2_{f}	6.402_{d}	6.88_{f}	5.06_{e}	6.94_{e}

Table 7: Effect of foliar spray with tryptophan and glutamine application on the chemical composition of plant leaves of rosemary (*Rosmarinus officinalis* L.) Plant in Second cut season

Treatment		% N		% P		% k		% Carbohydrate		% Phenol	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	2.058^{g}	1.763^{g}	0.534^{g}	0.607^{h}	1.547^{g}	1.623^{j}	5.86^{h}	5.88^{i}	4.06^{g}	6.43^{i}
	Tryp. 100	2.273^{f}	2.02^{f}	0.571^{f}	0.67^{f}	1.686^{e}	1.84^{g}	6.82^{d}	6.65^{f}	4.09^{g}	7.249^{f}
	Tryp 200	2.353^{e}	2.031^{f}	0.59^{ef}	0.64^{g}	1.651^{f}	1.735^{h}	6.24^{g}	6.25^{g}	4.32^{f}	7.054^{g}
	Glutamine 100	2.663^{c}	2.604^{c}	0.663^{bc}	0.723^{e}	1.81^{c}	2.126^{d}	6.72^{d}_{e}	8.71^{c}	5.11^{d}	8.034^{d}
	Glutamine 200	2.952^{b}	2.728^{c}	0.65^{c}	0.827^{c}	1.82^{c}	2.237^{c}	7.2^{c}	8.81^{c}	5.25^{c}	8.35^{c}
Sand	Control	2.119^{g}	2.027^{f}	0.533^{g}	0.62^{gh}	1.577^{g}	1.674^{i}	5.99^{h}	6.07^{h}	4.05^{g}	6.773^{h}
	Tryp. 100	3.015^{b}	2.881^{b}	0.686^{b}	0.973^{b}	1.913^{b}	2.377^{b}	8.67^{b}	9.37^{b}	5.9^{b}	8.967^{b}
	Tryp 200	3.133^{a}	3.088^{a}	0.726^{a}	1.013^{a}	1.957^{a}	2.447^{a}	9.2^{a}	9.55^{a}	6.42^{a}	8.977^{a}
	Glutamine 100	2.589^{c}	2.403^{d}	0.62^{d}	0.754^{d}	1.736^{d}	2.061^{e}	6.58^{ef}	8.18^{d}	4.91^{e}	7.809^{e}
	Glutamine 200	2.473^{d}	2.267^{e}	0.603^{de}	0.703^{e}	1.753^{d}	1.977^{f}	6.53^{f}	7.06^{e}	4.83^{e}	7.853^{e}

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3.3. Contents of leaf pigments

Information tabulated in table (8, 9) documented the impact of treating rosemary plants grown in sand or peat moss and perlite culture with tryptophan or glutamine on the pigments of the leaves. Data indicated that adding glutamine or tryptophan increased the color level of the leaves. During both seasons in the two cuts in comparison to the other treatments, plants treated with 200 ppm tryptophan and grown in sand culture showed the highest levels of chlorophyll A, chlorophyll B, and carotenoids in their leaves, while untreated plants grown in sand or peat moss with perlite showed the lowest levels of these pigments in their leaves.

Table 8: Effect of foliar spray with tryptophan and glutamine application on the Contents of leaf pigments of Plant Leaves of rosemary (*Rosmarinus officinalis* L.) plant in first season.

Treatment		Chl. A		Chl. B		Carotenoids	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	0.49h	0.51i	0.176f	0.169h	0.2g	0.273g
	Tryp. 100	0.55f	0.59g	0.243de	0.205f	0.24f	0.309ef
	Tryp 200	0.53g	0.56h	0.233e	0.203f	0.25ef	0.313de
	Glutamine 100	0.63d	0.68d	0.3c	0.244d	0.34c	0.367b
	Glutamine 200	0.66c	0.71c	0.323b	0.283c	0.35c	0.378b
Sand	Control	0.51h	0.52i	0.194f	0.182g	0.21g	0.292f
	Tryp. 100	0.74b	0.73b	0.331b	0.316b	0.39b	0.405a
	Tryp 200	0.78a	0.76a	0.378a	0.35a	0.43a	0.416a
	Glutamine 100	0.58e	0.64e	0.281c	0.221e	0.29d	0.329cd
	Glutamine 200	0.57e	0.62f	0.26d	0.206f	0.27e	0.343c

Table 9: Effect of foliar spray with tryptophan and glutamine application on the Contents of leaf pigments of plant leaves of rosemary (*Rosmarinus officinalis* L.) plant in second season

Treatment		Chl. A		Chl. B		Carotenoids	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	0.528h	0.6f	0.17hi	0.186g	0.307h	0.305e
	Tryp. 100	0.597f	0.66e	0.2g	0.205ef	0.35e	0.371d
	Tryp 200	0.588f	0.65e	0.18h	0.202f	0.337f	0.344e
	Glutamine 100	0.663d	0.72bc	0.25d	0.267d	0.378d	0.380d
	Glutamine 200	0.696c	0.74b	0.29c	0.288c	0.396c	0.399c
Sand	Control	0.547g	0.61f	0.17i	0.188g	0.321g	0.307f
	Tryp. 100	0.716b	0.77a	0.32b	0.31b	0.411b	0.413b
	Tryp 200	0.747a	0.79a	0.34a	0.33a	0.43a	0.438a
	Glutamine 100	0.635e	0.71c	0.23e	0.214e	0.369d	0.393c
	Glutamine 200	0.623e	0.69d	0.22f	0.205ef	0.35e	0.376d

3.4. Plant productivity

Using tryptophan or glutamine on rose marital oil yield/plant data revealed that it enhanced oil yield/plant in the two cuttings throughout both seasons as compared to the untreated plants, as indicated in Table (10, 11). Overall, the most productive plants were those growing in sand culture treated with 200 ppm tryptophan, followed by those growing in sand culture treated with 100 ppm tryptophan, and finally, those growing in peat moss and perlite treated with 200 ppm glutamine. However, research showed that untreated plants grown on perlite and peat moss produced the least amount of oil per plant. The oil yield/m^2 showed a similar pattern. Regarding dry weight/m2 yield, data indicated that plants treated with 200 ppm tryptophan grown in sand culture produced the most in both cuts during both seasons, closely followed by plants treated with 100 ppm tryptophan growing in sand culture. On the other hand, plants treated with 200 ppm glutamine grown in peat moss and perlite culture produced significantly more than plants treated with 200 ppm tryptophan grown in peat moss and perlite culture.

Significantly the lowest yield was produced by the untreated plants in the sand or peat moss and perlite culture.

Table 10: Effect of foliar spray with tryptophan and glutamine application on the plant productivity of rosemary (*Rosmarinus officinalis* L.) plant in first season

Treatment		Oil yield/plant		Oil yield /m^2		Yield of dry weight/m^2	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	0.249^{j}	0.69^{g}	3.99^{j}	10.97^{h}	370.02^{g}	751.6^{d}
	Tryp. 100	0.411^{g}	0.97^{e}	6.58^{g}	15.54^{e}	514.41^{cd}	912.1^{cd}
	Tryp 200	0.369^{h}	0.9^{f}	5.91^{h}	14.4^{f}	483.02^{ef}	894.8^{cd}
	Glutamine 100	0.526^{d}	1.08^{cd}	8.41^{d}	17.36^{cd}	492.9^{cd}	1061.5^{bc}
	Glutamine 200	0.553^{c}	1.12^{c}	8.85^{c}	17.94^{c}	589.1^{c}	1039.7^{bc}
Sand	Control	0.295^{i}	0.73^{g}	4.71^{i}	11.6^{g}	423.67^{fg}	791.5^{d}
	Tryp. 100	0.631^{b}	1.27^{b}	10.09^{b}	20.39^{b}	661.56^{b}	1240.8^{ab}
	Tryp 200	0.777^{a}	1.37^{a}	12.43^{a}	21.89^{a}	759.49^{a}	1437.6^{a}
	Glutamine 100	0.476^{e}	1.06^{d}	7.62^{e}	16.94^{d}	559.96^{cd}	1188^{b}
	Glutamine 200	0.446^{f}	0.1^{e}	7.13^{f}	15.92^{e}	538.31^{cde}	1028.5^{bc}

Table 11: Effect of foliar spray with tryptophan and glutamine application on the Plant productivity of rosemary (*Rosmarinus officinalis* L.) Plant in Second season

Treatment		Oil yield g/plant		Oil yield g/m^2		Yield of dry weight g/m^2	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Peat moss + Perlite	Control	0.37^{g}	0.59^{h}	5.921^{g}	9.5^{g}	389.55^{e}	710.567^{g}
	Tryp. 100	0.499^{e}	0.8^{f}	7.982^{e}	12.806^{f}	526.83^{cd}	951.621^{de}
	Tryp 200	0.504^{e}	0.803^{f}	8.06^{e}	12.844^{f}	504.28^{cd}	891.789^{ef}
	Glutamine 100	0.684^{c}	0.957^{d}	10.938^{c}	15.304^{d}	645.01^{b}	1051.812^{cd}
	Glutamine 200	0.701^{c}	1.085^{c}	11.213^{c}	17.364^{c}	645.099^{b}	1099.952^{bc}
Sand	Control	0.436^{f}	0.638^{g}	6.972^{f}	10.209^{g}	451.382^{de}	759.122^{fg}
	Tryp. 100	0.812^{b}	1.182^{b}	12.996^{b}	18.904^{b}	733.177^{a}	1190.504^{ab}
	Tryp 200	0.852^{a}	1.279^{a}	13.636^{a}	20.46^{a}	790.782^{a}	1288.792^{a}
	Glutamine 100	0.572^{d}	0.961^{d}	9.158^{d}	15.371^{d}	581.782^{bc}	1003.945^{cde}
	Glutamine 200	0.51^{e}	0.904^{e}	8.167^{e}	14.458^{e}	561.072^{c}	999.96^{cde}

3.5. Volatile Oil Constituents Identified by CG

Using glutamine or tryptophan affected the volatile oil components, according to the findings shown in Table (12). A GC chemical analysis might reveal the profile of the rosemary volatile oil subjected to glutamine or tryptophan. Furthermore, the GC results showed that the unidentified compounds from the separated compounds ranged from 0% to 2.88% and from 0% to 2.41% in the first and second seasons, respectively, while the identified compounds ranged from 88.52% to 99.91% and 91.81% to 99.9%. Thirteen compounds in all were discovered, and the diagnostic information was compiled. The main constituents were α-Pinene, Camphene, β-Pinene, limonene, Camphore, 1,8-Cineol, α-Terpineol, Borneol, β-Caryophyllene, Bornyl acetate, and Eugenol. Plants treated with 100 ppm tryptophan cultivated in sand culture had the greatest 1,8-cineol content, whereas untreated plants grown in peat moss and perlite culture had the lowest value. Moreover, plants treated with 100 ppm glutamine and cultivated in sand culture exhibited the highest value of α-Terpineol, whereas untreated plants grown in peat moss and perlite culture had the lowest value.

3.6. Impact of Amino Acids and Substrates on Dry Yield and Revenue

The economic effects of different pot substrates and amino acid concentrations (ppm) on the dry yield (kg/m^2) of rosemary plants for the 2022 and 2023 growing seasons are shown in Table (13). This

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Table 12: Effect of amino acids on essential oils composition of rosemary leaves during the two seasons in soilless culture systems

Treatment	Control	Tryp 100ppm	Trypt 200ppm	Glut 100ppm	Glut 200ppm	Control	Tryp 100ppm	Trypt 200ppm	GLut 100ppm	Glut 200ppm
Components%	**Peat moss + Perlite**					**Sand**				
α-Pinene	4.95	3.39	3.92	3.81	3.75	3.33	3.81	3.8	3.90	3.50
Camphene	2.27	1.85	2.27	2.28	2.18	2.37	2.27	2.455	2.40	2.45
β -Pinene	2.25	0.91	1.13	0.76	1.06	1.01	0.86	1.21	1.55	1.29
limonene	1.69	1.335	1.235	1.6	2.16	1.35	1.73	2.08	2.43	2.95
Camphore	12.13	11.04	11.52	11.60	9.57	9.71	10.84	10.68	11.34	11.53
1,8-Cineol	**47.68**	**51.44**	**53.35**	**54.56**	**53.26**	**48.71**	**51.48**	**54.14**	**54.97**	**54.79**
α-Terpineol	10.65	12.65	12.04	14.26	14.19	14.94	15.25	15.61	15.45	17.56
Borneol	2.91	1.09	2.38	2.52	1.41	2.33	2.72	2.63	1.62	1.21
Bornyl acetate	1.83	4.21	3.61	1.81	3.15	1.47	1.58	1.21	1.47	1.66
Eugenol	3.38	1.21	1.98	1.21	1.35	1.61	1.56	1.53	1.53	1.46
β -Caryophyllene	4.72	3.32	2.94	2.60	3.26	3.93	3.145	1.84	1.59	1.33
U.K	0.94	1.93	0.59	0.82	0.98	0.59	0.83	1.58	0.76	0
U.K	0.27	0.52	0.14	0	0.16	0.14	0	0	0	0

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Table 13: The economic impact of substrate and amino acids concentration (ppm) of rosemary plant as an average dry yield kg/m^2 for two growing seasons 2022 and 2023.

Substrates cultures	Amino acids concentration (ppm)	Operated cost (LE)/m^2	Total cost (LE)/m^2	dry yield kg/m^2	Price (LE/kg)	Return (LE)/m^2	Net return (LE)/m^2	B/C ratio	Treatment Order
Peat moss + Perlite	**Control**	160	460	2.22	350	777.61	317.6	**0.69**	**9**
	Tryp 100 ppm	220	520	2.91	350	1019.9	499.9	**0.96**	**6**
	Tryp 200 ppm	280	580	2.77	350	970.86	390.9	**0.67**	**9**
	Glutamine 100 ppm	240	540	3.25	350	1137.9	597.9	**1.11**	**4**
	Glutamine 200 ppm	320	620	3.37	350	1180.8	560.8	**0.90**	**8**
Sand	**Control**	160	410	2.43	350	848.99	439.0	**1.07**	5
	Tryp 100 ppm	220	470	3.83	350	1339.1	869.1	**1.85**	1
	Tryp 200 ppm	280	530	4.28	350	1496.8	966.8	**1.82**	2
	Glutamine 100 ppm	240	490	3.33	350	1166.8	676.8	**1.38**	3
	Glutamine 200 ppm	320	570	3.13	350	1094.7	524.7	**0.92**	7

• Net return = Revenue (L.E/m2)- Total cost (L.E/$^{m2)}$
• The revenue to total Cost (B/C) ratio was calculated to represent the profit percentage

analysis's main focus is on each treatment's fixed costs, operating expenses, total costs, dry yield, revenue, net return, and benefit-to-cost (B/C) ratio. It is clear from the data that the rosemary yield and ensuing income were greatly impacted by various substrates and amino acid concentrations. The tryptophan treatments (100 ppm and 200 ppm) produced the highest values for the sand substrate, measuring 3.82 kg/m² and 4.27 kg/m², respectively. Compared to the other treatments, these yields produced greater revenues and net returns. With the greatest B/C ratio of 1.82 and a net return of 966.83 LE/m2, the tryptophan 200 ppm treatment in particular demonstrated the most profitable use of resources.

The control treatments (without amino acid addition) had the lowest dry yield and, hence, the lowest returns for the peat moss + perlite and sand substrates. The glutamine 100 ppm treatment produced the maximum yield from the peat moss + perlite substrate, 3.25 kg/m². This resulted in a profitable situation with a net return of 597.92 LE/m² and a B/C ratio of 1.11.

3.7. Profitability and Cost Analysis

Sand substrates typically displayed reduced total costs as compared to the peat moss + perlite treatments, however the overall prices varied considerably throughout treatments. The greater dry yield, particularly in the tryptophan 100 ppm and 200 ppm treatments, led to significantly larger returns even though the sand-based treatments were less expensive overall. This implies that, especially when contrasted with the more economical sand-based substrates, the greater expenses related to the peat moss + perlite treatments did not always result in increased profitability.

3.8. Advantageous to Ratio of Cost (B/C)

One important measure of profitability is the B/C ratio. The tryptophan 100 ppm on sand substrate had the greatest B/C ratio of 1.85 among all treatments, meaning that for every LE used, about 1.85 LE was returned in profit.

Conversely, treatments with lower B/C ratios like the control groups, which consisted of peat moss with perlite and sand were less profitable, with values at or below 1, suggesting a negligible return on investment.

The study's findings clearly show that using amino acids to plant development is economically feasible, which is crucial for farmers (Kocira *et al.,* 2020; Fatma *et al.,* 2021; Mohammed *et al.,* 2021).

4. Discussion

The data show that plants treated with 200 ppm tryptophan in sand substrate exhibited the highest growth parameters, particularly in terms of plant height and fresh weight. This suggests that tryptophan, especially at higher concentrations, significantly enhances vegetative growth. The results align with previous studies that highlight the role of amino acids in promoting plant growth and stress tolerance (Talaat *et al.,* 2005; Abo Sedera *et al.,* 2010).

Many amino acids, including those that are not involved in protein synthesis, have been found to play active roles in plant growth and the plant's reaction to environmental stressors in addition to serving as building blocks for protein synthesis. Furthermore, as building blocks for several primary and secondary metabolites, amino acids play crucial roles in human nutrition, either as necessary dietary components or as a source of nutraceutical substances (Trovato *et al.,* 2021)

The productivity data in this table indicate that plants treated with 200 ppm tryptophan in sand substrate produced the highest dry weight and oil yield per plant. This is consistent with the growth and chemical composition data, suggesting that tryptophan enhances both biomass production and essential oil synthesis. The increased oil yield is economically significant, as rosemary essential oil is a high-value product with applications in the food, cosmetic, and pharmaceutical industries (González-Minero *et al.,* 2020).

Notwithstanding the many roles that amino acids play in plant growth and stress resistance, nitrogen is essential for the manufacture of amino acids, and knowledge of how nitrogen is absorbed, stored, and transported in plants is crucial to the field of amino acid biology (Neill and Lee, 2020).

Al-Fraihat *et al.* (2023) according to their findings, the use of tryptophan or glutamine amino acids greatly increased the growth of rosemary, the proportion of volatile oil, and some volatile oil constituents. The major volatile oil constituents identified by GC, including α-Pinene, Camphene, β-Pinene, and 1,8-Cineol. The data show that amino acid treatments, particularly tryptophan, influenced the composition of the essential oil. For example, plants treated with 100 ppm tryptophan in sand substrate had the highest 1,8-Cineol content, which is a key component of rosemary oil with antimicrobial and anti-inflammatory properties (Borges *et al.,* 2018). Since glutamate eventually shows up in glutamine (Gln), protein, and glutathione, it is evident that cells take glutamate more quickly than they breakdown it.

Furthermore, even though the physiological effects of functional L-tryptophan on plants are still unclear, some of its activities have been shown. Auxin levels in plant tissues are increased by exogenous Trp treatment. In Arabidopsis, IAA is similarly generated from tryptophan via indole-3-acetaldoxime (Rosa *et al.,* 2023). chamomile plants' levels of chlorophylls a and b were significantly increased when they were sprayed with amino acids (Gendy *et al.,* 2016).

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The tryptophan 200 ppm treatment in sand substrate yielded the highest net return making it the most economically viable option. The control treatments (without amino acids) had the lowest returns, highlighting the economic benefits of amino acid supplementation in rosemary cultivation. These findings are consistent with previous studies that emphasize the role of biostimulants in improving crop productivity and profitability (Kocira *et al.,* 2020; Fatma *et al.,* 2021).

5. Conclusion

Amino acids play a crucial role in plant growth and development. They serve as building blocks for proteins and enzymes, which are essential for various physiological processes. Amino acids like tryptophan and glutamine are particularly important as they contribute to stress tolerance, nutrient uptake, and the synthesis of growth regulators. Tryptophan is a precursor for auxin, a plant hormone that promotes growth, while glutamine aids in protein synthesis and nutrient assimilation. Foliar application of amino acids has been shown to improve plant vigor, yield, and resistance to environmental stresses, making them valuable tools in sustainable agriculture.

Additionally, the use of tryptophan in a sand substrate was found to be the most effective in improving plant productivity and economic quality but glutamine was better with peat moss and perlite substrate. These findings suggest that amino acids, particularly tryptophan, can be used as effective biostimulants to enhance the growth and quality of rosemary in soilless cultivation systems.

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