

Growth Characteristics and Changes in the Active Ingredients of *Salvia mirzayanii* Essential oil under Foliar Application of Humic Acid and Amino Acid

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ABSTRACT

Salvia mirzayanii is a medicinal plant from the Lamiaceae family, which has long been of interest to the industry due to its medicinal properties and various metabolites, including terpenoids, phenolic compounds, flavonoids, fatty acids, and sterols. This study was conducted to investigate the effect of foliar spraying of amino acid with concentrations of 0, 0.5, 1, and 1.5 ml/l and humic acid with concentrations of 0, 5, 10, and 15 gr/l along with irrigation water on essential oil compounds and plant growth. The results showed that humic acid (15 ml/l) and amino acid (0.5 ml/l) increased fresh weight, dry weight of leaves, number of inflorescences, and weight of inflorescences, and a further increase in the amino acid level caused a decrease in the measured agricultural characteristics. The use of humic acid 15 mgr/l caused the increase of 5-neo-sedranol, α -terpinyl acetate, bicyclogermacrene, and linalyl acetate as the main components of the essential oil to the extent of 37.5, 20.6, 32.5, 94.1 and 22.7% compared to the control respectively. The amount of 5-neo-sedranol and bicyclogermacrene decreased with the increase of amino acid. Amino acid treatment of 1.5 ml/l increased the percentage of linalyl acetate (40.98%), α -terpinyl acetate (18.06%), and linalool (10.71%) compared to the control. According to the trend of changes in the percentage of essential oil compounds and the measured agricultural characteristics, it is suggested to use the level of 0.5 mg amino acid and 15 mg of humic acid to reach the most desirable quantitative and qualitative yield of *Salvia mirzayanii*.

Keyword: *Salvia mirzayanii*. Essential oil, Fertilizer. 5-neo-sedranol, Linalyl acetate,

INTRODUCTION

The genus Lamiaceae is one of the largest plant genera. It grows in almost all parts of the world, especially in the Mediterranean region [1] and consists of about 236 genera and 6900 to 7200 species of fragrant bushes and short shrubs. *Salvia* L. is the largest genus of Lamiaceae and has about 1000 species that are mostly distributed in Central and South America and West and East Asia [3]. Many species of this genus grow in Iran, of which 17 are native [4]. *Salvia* has long been considered due to its many medicinal properties, including various metabolites such as terpenoids, phenolic compounds, flavonoids, fatty acids, and sterols. It is widely used in the pharmaceutical, food, health, and cosmetic industries [5]. In a study conducted by Mohammadian et al. [6] on the phytochemical compositions of *S. sahndica* at different stages of growth (vegetative, flowering and seeding), they observed that the total level of phenol in various stages varies from 0.13 to 0.32 mmol of gallic acid per mg of extract and total level of flavonoid varied from 3.12 to 5.52 mmol of quercetin per mg of extract and its antioxidant activity level using AP method varied from 10.1 to 21.27 mmol/mg of extract given environmental considerations, various organic acids have been extensively used to improve the quantity and quality of crops and horticultural products in recent years.

Humic acid is extracted from various sources such as soil, humus, oxidized peat lignite, coal, etc. [7]. One of the beneficial effects of humic compounds is increasing plant growth. Low concentrations of humic acid significantly increase plant growth [8]. Different results on the effect of humic acid on the essential oil of medicinal plants have been reported. In investigating the essential oil yield and height of *Thymus vulgaris*, humic acid caused a decrease in these two indices but significantly increased the fresh and dry weight of the plant and its antioxidant activity [9]. Investigations revealed that humic acid increases essential oil, growth and biomass indices of

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Hyssopus officinalis [10]. Increasing the level of humic acid foliar application also increased the growth indices and yield of *Nigella sativa* [11]. Amino acids are essential components in the early stages of protein synthesis. About 20 important amino acids are involved in each protein synthesis process in a cell. Studies in recent years have indicated that amino acids can significantly directly affect vital activities and plant structures. An amino acid is an organic form of nitrogen that improves various plants' yield and growth. Amino acid is used as soil and as a foliar application [12].

Amino acids act as stimulants of quantitative and qualitative plant growth. These compounds play a major role in the biosynthesis of secondary and hormonal metabolites [13]. In general, amino acids are substances that stimulate metabolism and metabolic processes to increase plant yield [14]. Thus, bio-stimulants can be one of the most important factors in the successful cultivation of a medicinal plant since actors in the successful cultivation of a medicinal plant, in addition to quantitative indices, also affect the quality indices of the medicinal plant. This effect is due to the amino acids used in the formulation of these bio-stimulants, which increase mRNA transcription by 2.5 times, activate hormones that are effective in reproductive growth, activate the process of carbohydrate formation, increase the uptake and transport of elements and increase the content of protein in plants, improve quantitative and qualitative characteristics in a shorter period, especially under environmental stress [13,15]. Positive effects of amino acids on plants include improving the physiological indices and biochemical composition of tea (*Thea Sinensis* L.) [15], increasing quantitative and qualitative yield in chamomile (*Matricaria chamomilla* L.) [16], and increasing the number of grains in wheat ears [17]. Environmental challenges such as contamination of water resources, reduced quality of crops, reduced biodiversity, resistance to pests and reduced soil fertility have made it essential to move from conventional cropping systems toward sustainable agricultural systems [18]. Given the importance of sustainable agricultural development and medicinal plant products as natural medicines without problematic compounds for human health, the present study was conducted to investigate the effect of humic acid and amino acid foliar application on growth characteristics and essential oil of *S. mirzayanii* in Shiraz climate conditions.

MATERIAL AND METHODS

This research was conducted in 2019 on the farm of Besat station in Shiraz, affiliated with the Research and Education Center for Agriculture and Natural Resources of Fars province, with an average altitude of about 1486 meters above sea level, mean rainfall of 337 mm and an average temperature of 18°C. Physical and chemical analysis of the field soil is in the table 1.

Table 1 Results of physical and chemical analyses of experimental soil before the experiment.

clay	silt	sand	Cu	Mn	Zn	Fe	K	P	N	OC	pH	Ec
%			PPM							%		dS.m ⁻¹
28.3	45.1	31.6	1.52	19.8	1.55	7.6	449	15.4	0.07	0.68	7.52	7.6

The experiment was conducted as a factorial in a randomized complete block design with two factors, including 4 levels of amino acid foliar application (0, 0.5, 1, and 1.5 gr/l) and 4 levels of humic acid foliar application (0, 5, 10 and 15 ml/l) of commercial composition (Table 2) in three replications.

Table 2 Characteristics of humic acid and amino acids used in the experiment.

Compounds	Trade name	Common name
Humic Acid: 14%		
Fulvic Acid: 1%		
Potassium: 6%	Agrihumic (Raha Andish Kavan)	Natural humic acid
Organic matter: 69%		
Total amino acids: 52%		
Free amino acids: 48%	Pigma Amin 50	Free Amino Acid
Total Organic matter: 38%		
Total nitrogen: 7.5%		

Before planting and preparing the seedlings in March, they were planted in pots with an opening diameter of 20 cm, and the necessary care was taken until the transfer. At the same time, the land was prepared for planting. After three leaves sprouted in the seedling, they were transferred to the main field in May and planted in rows at a distance of 50 and 30 cm from each other. The use of humic acid twice along with irrigation water and foliar spraying of the treatments twice after the establishment of the plant in the main land was done at two weeks intervals. The plants were irrigated once a week until full flowering. After the full flowering of the plants (12 weeks after the establishment of seedlings) and harvesting at this time, fresh and dry weight the plant, the number and weight of inflorescences, and the percentage of leaf dry matter were calculated based on the following equation.

Percentage of leaf dry matter= $100 - (\text{Fresh leaf weight} - \text{dry leaf weight}) \times 100$

In this study, no fertilizers or herbicides were used, and weeding was done continuously by hand during the growth period

Essential Oil Extraction and Measurement of Essential Oil

Immediately after harvesting, the fresh weight of the plant was measured with a digital scale and plants were separated from each plot to calculate the dry weight. After placing them in an oven at 75°C until reaching a constant weight, their dry weight was calculated [19]. The EOs of the dried samples (30 gr of the plant material) were hydro-distillate for 3 hr, using a glass Clevenger-type apparatus according to the method outlined by the European Pharmacopoeia The Eos were dried over anhydrous sodium sulfate, and calculated the average of EO yields (w/w%). Essential oil constituents were determined using a gas chromatograph and gas chromatograph/mass spectrometer (GC, GC/MS).

Specifications of the Devices used

GC and GC-MS Analysis

Agilent Technologies gas chromatograph (A 7890), HP-5 column with 30m in length and 0.32 mm in diameter, static phase layer thickness of 0.25 μm , column temperature programming from 60 to 210°C with increasing temperature of 30°C per minute, type of detector: FID at 290°C, carrier gas: nitrogen at a rate of 1 ml/min, injection temperature 280°C

Gas chromatograph connected to a spectrometer

Gas chromatograph connected to Agilent technologies mass spectrometer (A5975), HP-5MS column with 30 mm in length and 0.25 mm in diameter, static phase layer thickness 0.25 μm , column thermal programming from 60 to 210°C with increasing temperature of 30°C per minute, and 210 to 240 with an increase in temperature of 20°C per minute, injection chamber temperature: 280°C, ionization energy: 70 volts, carrier gas: helium.

Identifying the Compounds of the Essential Oil

The percentage of compounds of each essential oil was calculated after isolation using the inhibition index. Mass spectra of compounds in essential oils were obtained for qualitative analysis (identification). The spectra were identified by calculating the Kovats inhibition indices by injecting normal hydrocarbons (C7-C25) under the same conditions as injecting essential oils and comparing them with the values published in different references. Mass spectra were also examined to identify the compounds and the identifications were confirmed using the mass spectra of standard compounds and various references. The relative percentage of each of the constituents essential oils was obtained according to the area under its curve in the gas chromatography spectrum and was compared with the values published in different references considering the Kovats index [20, 21, 23].

Statistical analysis of data was performed using SAS and Excel software. Duncan's test compared the means obtained after analysis of variance at 5%.

RESULTS AND DISCUSSION

Investigation of Morphological Traits

Based on the analysis of variance, the effect of foliar application of amino acids and humic acid on the vegetative traits of *S. mirzayanii* was according to Table 3.

Table 3 Analysis of variance for leaf fresh and leaf dry weight, number of inflorescences and weight of inflorescences and leaf moisture ratio based on dry weight at different levels of foliar application of amino acid and humic acid.

Mean of Squares						
S.V	D.F	leaf dry weight	leaf fresh weight	number of inflorescences	weight of inflorescences	leaf moisture ratio (based on dry weight)
Replication	2	0.28 **	0.013 **	66.42 **	0.22 **	2.40 **
Humic acid (H)	3	2.47 **	0.080 **	975.25 **	1.37 **	8.09 **
Amino acid (A)	3	0.57 **	0.082 **	302.75 **	0.50 **	16.33 **
H × A	9	0.11 **	0.003 **	14.58 **	0.22 **	6.12 **
Error	30	0.01	0.001	0.88	0.01	0.03
CV (%)		10.07	14.1	14.2	15.9	12.3

**Significant at the 1% probability level

According to the results of the analysis of variance, the simple effect of foliar application of amino acids and humic acid and the interaction effect of these two factors on fresh weight, dry weight, and plant moisture ratio based on dry weight, height and weight of inflorescence were significant at 1% level ($P \leq 0.01$).

Leaf Fresh Weight and Leaf Dry Weight

According to the results of comparing the means, fresh leaf weight increased by 2.3 times due to using humic acid with a concentration of 15 gr/l compared to the control treatment without humic acid. This increase was 1.8 times for leaf dry weight. Studies show a positive effect of humic acid on vegetative characteristics in *Hyssopus officinalis*, *Ocimum basilicum*, and *Nigella sativa* [23,10, 24]. Organic fertilizers increase plant growth by improving soil pH, soil water holding capacity, and plant availability of nutrients. Humic acid increases shoot weight by improving soil granulation, more uptake of water and nutrients [25], and more photosynthesis activity of the plant due to increased activity of the RuBisCO enzyme [26]. The amount and weight of inflorescences were significantly affected by the amount of humic acid (Table 3). The highest number of inflorescences per plant at a concentration of 15 gr was 28, which increased 3.5 times compared to the control and the average weight of inflorescences in this treatment was 2.5 times higher than the control (Table 4).

Table 4 Simple and interaction effects of different amino acid and humic acid doses on morphological characteristics of *S. mirzayanii*.

Traits	Leaf	Leaf	Number	Weight	% leaf dry	
Treatment	fresh weight (gr)	Dryweigh (gr)	of inflorescence per plant	of inflorescences (gr)	matter	
Humic acid(gr/l)						
0	0.82 d	0.21 c	8 d	0.73 d	26.78 a	
5	1.16 c	0.23 c	20 c	1.14 c	20.15 c	
10	1.45 b	0.31 b	26 b	1.28 b	21.15 b	
15	1.89 a	0.39 a	28a	1.54 a	20.41 bc	
Amino acid (ml/l)						
0	1.09 c	0.25 c	14 d	1.01 c	25.07 a	
0.5	1.50 a	0.39 a	26 a	1.41 a	25.51 a	
1	1.53 a	0.31 b	23 b	1.28 b	20.58 c	
1.5	1.21 b	0.19 d	19 c	1.00 c	17.33 d	
Humic acid× Amino acid						
H.A	A.A					
0	0	0.48 j	0.16 j	0 i	0.00 j	33.33 a
	0.5	1.2 gh	0.29 f	12 g	1.12 f	24.17 d
	1	1.12 h	0.27 g	10 h	1.04 g	24.11 d

5	1.5	0.47 j	0.12 k	9 h	0.75 i	25.53 c
	0	0.74 i	0.19 i	12 g	1.31 de	25.68 c
	0.5	1.31 ef	0.32 e	25 d	1.30 de	24.43 d
	1	1.34 de	0.28 fg	24 d	1.12 f	20.90 ef
10	1.5	1.25 fg	0.12 k	20 f	0.84 h	9.60 i
	0	1.18 gh	0.25 h	20 f	1.35 cde	21.19 e
	0.5	1.54 c	0.43 b	30 b	1.40 c	27.92 b
	1	1.69 b	0.31 e	30 b	1.28 e	18.34 g
15	1.5	1.4 d	0.24 h	22 e	1.09 fg	17.14 h
	0	1.94 a	0.39 c	24 d	1.36 cd	20.10 f
	0.5	1.96 a	0.5 a	35 a	1.80 a	25.51 c
	1	1.95 a	0.37 d	28 c	1.69 b	18.97 g
	1.5	1.7 b	0.29 f	25 d	1.30 de	17.06 h

Means with at least one common letter do not have a statistically significant difference (Duncan 5%)

Results of other studies also show the positive effect of humic acid on the reproductive properties of plants. These substances improve the uptake of nutrients by mobilizing ions and plant physiological metabolism, which increases plant reproductive growth [27]. Reproductive characteristics are improved by increasing humic acid compared to the control treatment and also under the effect of optimal concentration of humic acid in nutrient solution due to increased root growth, increased photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll), and leaf area or increased photosynthetic capacity [28, 29] and increased nutrient uptake as a result of adding of humic acid. The amino acid foliar application significantly affected vegetative and reproductive characteristics measured in the plant (Table 2). Foliar application with a 1 ml/l concentration increased leaf fresh weight by 40.3%. Leaf fresh weight from foliar application of amino acid with this concentration and treatment of 0.5 ml/l of amino acids were included in one group without significant differences. With increasing its foliar application to 1.5 ml, the fresh weight of the leaves decreased. Increasing the foliar concentration of amino acid more than this amount reduced leaf dry weight (Table 3). Using amino acids provides the nutrients the plant needs, increases the vegetative body, and produces plant biomass. In other words, leaf weight increase as a result of using amino acids was observed through the improvement of growth and development in the vegetative yield of *S. mirzayanii*, which resulted in the allocation of more photosynthetic substances, followed by an increase in fresh and dry weight of the leaves and other aerial organs.

The highest number and weight of inflorescence were obtained from foliar application of amino acid with a concentration of 0.5 ml/l. Foliar application of amino acids increased these two indices by 85.7% and 39.6% compared to the control without amino acids. With increasing the amino acid concentration of more than 0.5 ml/l, the number and weight of inflorescence also decreased. Some studies have shown that plants can uptake nitrogen in amino acids without relying on mineralization. However, increasing the level of amino acids due to increased stress resulting from increasing nitrogen content can also reduce plant shoots' weight [30]. The use of amino acids increases transpiration in a plant. It seems that increased photosynthesis is the cause of increased transpiration. Accordingly, increasing the demand for minerals and water for photosynthesis increases the flow of raw sap to leaves, leading to the opening of pores and thus an increased rate of transpiration [31]. An unbalanced transpiration increase reduces plant biomass weight and consequently affects reproductive organs [32]. Reduced plant shoot growth due to increased use of amino acids in soybeans and tobacco has also been reported [33, 34]. In each level of humic acid, the highest level of studied traits was obtained using 0.5 ml/l of the amino acid (Table 4). Investigating leaf dry matter levels showed that increasing humic acid reduced leaf dry matter. Although an increase in humic acid increased the dry weight of the plant, the level of dry matter compared to the level of leaf fresh weight showed a decreasing trend. Increasing humic acid and amino acids increased the water storage level in the plant tissue and, as a result, decreased plant dry matter. The highest level of dry matter was obtained when humic acid was used. Changes in the level of plant dry matter showed that using amino acids up to the level of 0.5 ml/l increases plant dry matter and decreases it. Also, in the interaction of humic acid and amino acid, the highest level of dry matter belonged to the zero level of these two substances. By affecting plant cell metabolism

and chelating power and increasing water and nutrient uptake, humic acid increases growth and interstitial water uptake [35].

Essential Oil Compounds

Sixty-two compounds of *S. mirzayanii* essential oil were isolated under experimental treatments (Table 5).

Table 5 Number of compounds isolated from essential oil (*S. mirzayanii* affected by different levels of humic and amino acid.

Amino acid (ml lit ⁻¹)	Humic acid (gr lit ⁻¹)			
	0	5	10	15
0	51	53	36	47
0.5	24	51	49	45
1	39	51	29	29
1.5	35	62	44	44

The number of compounds isolated from each treatment was as follows:

In this study, different levels of amino acids and humic acid with different levels were identified, of which 18 had similar compounds in all treatments, and the mean total percentage of it in essential oil was based on table 6.

Table 6. Similar compounds isolated from essential oil of *S. mirzayanii*.

No.	Compound name	RI	Total percentage	No.	Compound name	RI	Total percentage
1	Myrcene	990	0.78	10	Geranyl acetate	1385	1.59
2	Limonene	1029	0.54	11	β-Elementene	1393	1.79
3	1,8-Cineole	1031	3.42	12	(E)-Caryophyllene	1422	1.42
4	Linalool	1099	5.22	13	Bicyclogermacrene	1498	4.68
5	α-Terpineol	1190	3.12	14	α-Cadinene	1536	0.29
6	Linalyl acetate	1258	16.52	15	Spathulenol	1579	2.39
7	δ-Elementene	1338	1.1	16	β-Eudesmol	1651	1.19
8	α-Terpinyl acetate	1348	14.28	17	α-Cadinol	1654	1.5
9	Neryl acetate	1363	0.67	18	5-neo-Cedranol	1688	20.11

These compounds together accounted for 60.8% of the essential oil compounds.

The five major compounds of *S. mirzayanii* essential oil were 5-neo-sedranol (20.1%), linalyl acetate (16.25%), and α-terpinyl acetate (14.28%), linalool (5.22%) and bicyclogermacrene (4.64%) which made up 60.8% of the essential oil compounds. The analysis of the variance of the percentage of these five compounds in the essential oil of *S. mirzayanii* as a result of using different levels of amino acids and humic acid is according to Table 7.

Table 7 Summary of analysis of variance of percentage of essential oils compounds of *S. mirzayanii* in different levels of amino acid and humic acid.

S.O.V	DF	5-neo-Cedranol	Linalyl acetate	α-Terpinyl acetate	Linalool	Bicyclogermacrene
rep	2	64.69 **	43.67 **	32.64 **	4.37 **	3.51 **
Humic Acid	3	81.00 **	21.14 **	37.33 **	47.66 **	2.86 **
Amino Acid	3	126.68 **	99.67 **	17.84 **	3.61 **	25.03 **
humic x amino	9	4.12 **	38.58	22.20 **	10.68 **	8.05 **
Error	30	0.15	0.16	0.08	0.06	0.03
C.V. (%)		19.98	12.40	19.94	14.5	13.9

**Significant at the 1% probability levels

The analysis of variance showed a significant effect of humic acid, amino acids, and their interaction on the studied compounds of essential oil. The concentration of humic acid increased the level of studied compounds. The highest compound level was obtained using a concentration of 15 gr/l humic acid. The level of linalool in this humic acid concentration was 2.94 times higher than that of the control treatment and had the highest change among the studied compounds. The rate of change in 5-neo-sedranol, α-terpinyl, bicyclogermacrene, and linalyl acetate was 37.5%, 22.5, 32.7 and 20.59%, respectively. Although the level of secondary metabolites in plants is

under the control of genes, their accumulation and concentration are greatly influenced by environmental conditions [36]. Since the level of essential oils increases due to various humic acid treatments, terpenoid compounds and the biosynthesis of their constituent units (isoprenoids) require ATP and NADPH. Elements such as phosphorus play a major role in the structure of constituent units of essential oils, including Isopentenyl pyrophosphate (IPP) and Dimethylallyl pyrophosphate (DMAPP) [37]. Humic acid increases the level of essential oil in the vegetative body by providing more phosphorus and nitrogen present in the components of the essential oil. Nitrogen also increases photosynthetic efficiency by increasing the number of leaves and surfaces and providing appropriate conditions for receiving sunlight energy and being involved in the structure of chlorophyll and enzymes involved in photosynthetic carbon metabolism and plays a key role in increasing the level of essential oil [38]. In other studies on the medicinal plant *Dracocephalum*, humic acid increased the composition of essential oils in this plant [39]. Also, it increased the level of essential oil and its compounds as a result of using humic acid in the medicinal plants of Moldavian dragonhead [40], basil [41] and Hyssop [10]. In studies conducted by Rowshan and Tarakemeh [42], Haghighat et al. [43], and Ziaei et al. [44], higher levels of 5-neocedranol than other compounds were reported. Investigating the essential oil of 12 samples of *S. mirzayanii* showed that the main constituent of the essential oil of this plant is monoterpene. In the essential oil compounds of these 12 samples, cineole and α -Terpinyl acetate were among the important compounds in all samples. Also, linalool and linalyl acetate were reported as the most important compounds in several samples of *S. mirzayanii* oil [45], which is consistent with the results of the present study. The origin, cultivation conditions growth stage, and method of preparation and extraction greatly impact the quantitative and qualitative characteristics of essential oils [46, 47]. With increasing amino acid concentration, the concentrations of 5-neocedranol and bicyclogermacrene decreased. The highest reduction rate in these two compounds belonged to a concentration of 1.5 ml/l amino acid (26.97 and 51.92, respectively) compared to the control treatment without foliar application. Increasing the concentration of foliar application to the level of 1.5ml/l of amino acids caused an increase in the level of linalyl acetate (40.98%), alpha-terpinyl (18.06%), and linalool (10.71%). Foliar application of amino acid at a concentration of 0.5 ml/l had no significant effect on any of these compounds, and the concentration of these compounds was placed in one statistical group without significant differences from the control (Table 8).

Table 8 Simple and interaction effects of different levels of amino acid and humic acid on some of the *S. mirzayanii* essential oil compounds.

Concentration	5-neo-Cedranol	Linalyl acetate	α -Terpinyl acetate	Linalool	Bicyclogermacrene	
Humic acid (gr lit ⁻¹)						
0	16.68 d	15.25 c	12.68 c	2.39 d	4.41 b	
5	20.00 c	16.18 b	13.73 b	5.51 c	4.41 b	
10	20.80 b	16.28 b	13.93 b	5.97 b	4.50 b	
15	22.94 a	18.39 a	16.80 a	7.03 a	5.41 a	
Amino acid (ml lit ⁻¹)						
0	23.06 a	14.74 c	13.12 b	5.32 b	5.74 a	
0.5	22.74 a	14.77 c	13.36 b	5.14 b	5.85 a	
1	17.79 b	15.80 b	15.17 a	4.56 c	4.39 b	
1.5	16.84 c	20.78 a	15.49 a	5.89 a	2.76 c	
Humic acid× Amino acid						
H.A	A.A					
0	0	20.10 e	10.30 i	13.14 d	3.12 e	5.50 b
	0.5	17.80 f	10.10 i	14.23 c	2.35 f	5.74 b
	1	15.85 h	15.28 g	12.46 e	2.10 f	3.49 f
	1.5	12.99 i	25.30 a	10.89 f	2.00 f	2.90 g
5	0	23.18 c	14.84 g	14.00 c	6.07 c	4.96 c
	0.5	24.10 b	14.90 g	14.00 c	6.08 c	4.69 cd
	1	16.79 g	16.00 f	13.10 d	5.00 d	4.07 e
	1.5	15.93 h	18.97 d	13.80 c	4.87 d	3.93 e
10	0	23.95 b	14.03 h	13.00 d	6.90 b	4.63 d

	0.5	24.06 b	14.06 h	12.10 e	7.00 b	4.97 c
	1	17.60 f	15.11 g	14.11 c	5.00 d	4.21 e
	1.5	17.60 f	21.90 b	16.50 b	4.98 d	4.19 e
15	0	25.00 a	19.80 c	12.33 e	5.18 d	7.85 a
	0.5	25.00 a	20.00 c	13.09 d	5.11 d	8.00 a
	1	20.92 d	16.79 e	21.00 a	6.13 c	5.80 b
	1.5	20.85 d	16.96 e	20.77 a	11.70 a	1.8 h

The means with at least one common letter do not have a statistically significant difference (Duncan 5%)

Amino acid metabolism can affect the biosynthetic pathway of secondary metabolites in medicinal plants. One of the ways to change the biosynthesis of secondary metabolites in medicinal plants (such as metabolites of essential oil components) is to cope with any stimulus that may affect the biosynthesis pathway.

The present study revealed that foliar application of amino acids might affect the biosynthesis pathway. As a production stimulant, secondary metabolites increased or decreased the level (%) of essential oil compounds in *S. mirzayanii*. The trend of changes in essential oil compounds due to foliar application of plants with amino acids may be due to changes in vegetative growth, more nutrient uptake by roots and photosynthetic activities of plants, and changes in the population of essential oil-producing glands in leaves and flowers [48]. The highest level of 5-neo-sedranol and bicyclogermacrene was obtained in the interaction of 15 gr/l of humic acid and 0 and 1.5 ml/l of amino acids. In each level of humic acid, the level of this compound decreased with increasing the level of amino acid. The results showed that increasing the level of humic acid made this compound less effective than increasing the amino acid concentration. The highest level of α -terpinyl acetate belonged to the interaction of 15 gr/l of humic acid and 1.5 ml/l of amino acids. The highest percentage of linalool in the essential oil was obtained from 15 gr/l of humic acid and 1.5ml/l of amino acid at 11.7%. The percentage of bicyclogermacrene compound as a result of using 15 mgr/l humic acid and 10, 0, and 0.5 ml/l amino acids were 7.85 and 8%, respectively, which included the highest statistical group without statistically significant difference from the other. With increasing humic acid levels, the percentage of 5-neo-sedranol ($r^2 = 0.95^{**}$), linalyl acetate ($r^2 = 0.86^{**}$), bicyclogermacrene ($r^2 = 0.87^{**}$), α -terpinyl acetate ($r^2 = 0.87^{**}$), and linalool ($r^2 = 0.67$) increased significantly based on a positive and significant linear relationship (Figure 1).

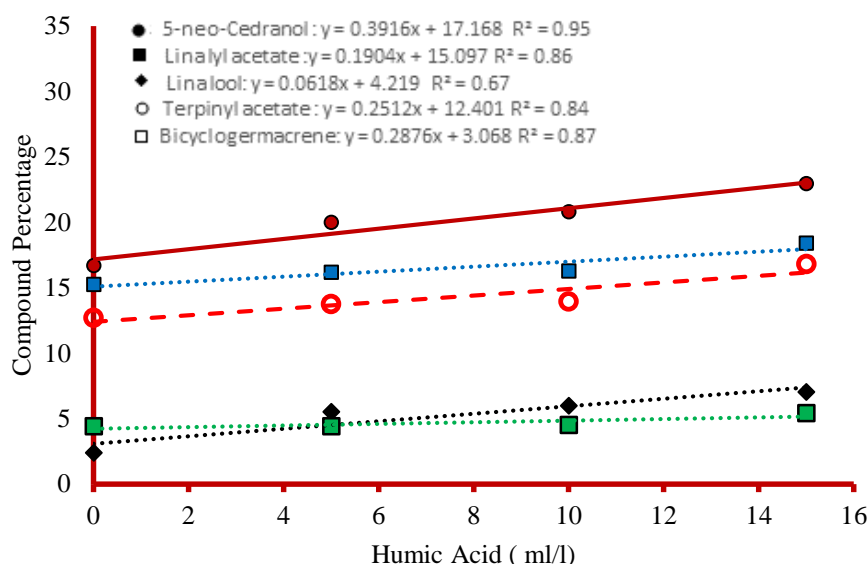


Fig. 1 The relationship between some essential oil compounds and different levels of humic acid.

The relationship between amino acid levels and the percentage of essential oil compounds in 5-neo-sedranol ($r^2 = 0.88^{**}$) and bicyclogermacrene ($r^2 = -0.86$) was negative and significant, and the relationship between linalyl acetate ($r^2 = 0.73$) and α -terpinyl acetate ($r^2 = 0.89^{**}$) was positive and significant, but no significant relationship was observed between the amino acid level and percentage of linalool in the essential oil (Figure 2).

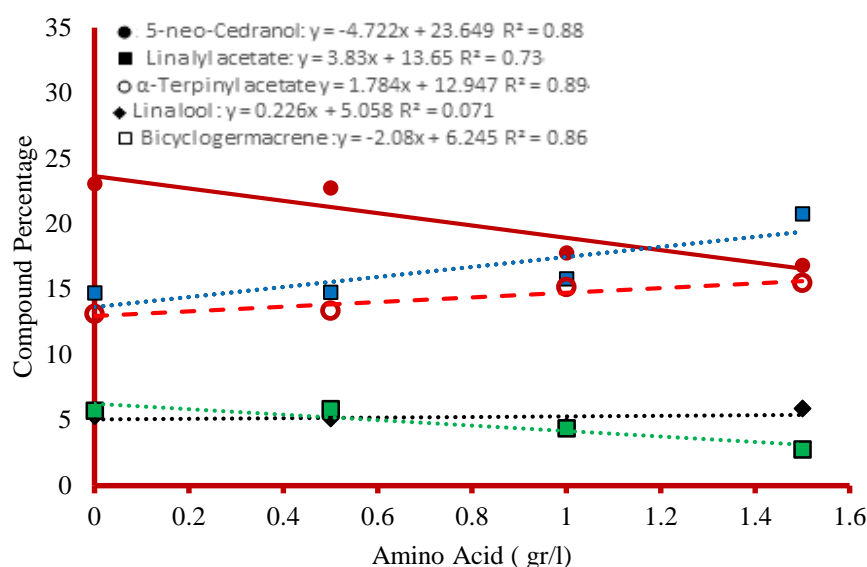


Fig. 2 The relationship between some essential oil compounds and different levels of amino acid.

CONCLUSION

Environmental factors greatly impact plant growth, and some of them are under the control of humans. Some of these factors are using organic fertilizers and amino acid compounds that are very effective in increasing yield and improving plant quality. The use of organic fertilizers is more important than chemical fertilizers. Chemical fertilizers provide only one or more elements needed for plant growth. In contrast, organic fertilizers improve the physicochemical properties of the soil and provide an appropriate environment for better growth and, finally, plants with higher yield and quality, in addition to providing conditions for the availability of many low-consumption and high consumption elements. Since active compounds in medicinal plants may be directly influenced by some high consumption and micronutrient elements, humic acid fertilizers that contain most of these elements can be used in the large-scale and economical cultivation of medicinal plants. Based on the results of the present study, it can be stated that the use of humic acid fertilizer had a positive effect on the characteristics of the measured plant of *S. mirzayanii* and levels of essential oil compounds. The results also show the highest fresh weight and dry weight and percentage of leaf dry matter, and the number and weight of *S. mirzayanii* inflorescences were obtained at 0.5 ml/l of amino acid. With increasing amino acid concentration, these indices decreased. In general, soil use of humic acid up to 15 gr/l and foliar application of amino acid up to 0.5 gr/l is recommended to increase the quantity and quality of *S. mirzayanii*.

Competing Interests

The authors declare none.

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