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Phytochemical-growth Responses of Lemon Balm (*Melissa officinalis* L.) to Gibberellic Acid and Benzyladenine under Different Nutritional Conditions

Marjan Varmazyar, Reza Shahhoseini*, Hossein Salehi-Arjmand and Morteza Akramian

Department of Medicinal Plants, Arak University, Arak, Iran

*Corresponding Author: Email: Reza.shahhoseini@gmail.com, r-shahhoseini@araku.ac.ir

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ABSTRACT

The present study aimed to assess the effects of gibberellic acid and benzyladenine on growth and some phytochemical characteristics of *Melissa officinalis* L. under different nutritional conditions in the hydroponic culture system. The experiment was conducted in a factorial split plot based on a completely randomized design with three replications. The main plot included nutrient solution type (Arnon Hoagland, Shi et al, and He et al that were named 1 to 3 throughout the text, respectively type 1, type 2, and type 3) and the subplot included the factorial combination of gibberellic acid (0 and 100 ppm) and benzyladenine (0, 50 and 100 ppm) as foliar spraying. The results showed that the effects of nutrient solution type on different plant growth traits, chlorophyll contents, antioxidant capacity and plant essential oil content were significant. Furthermore, different traits of the plant were affected by benzyl adenine (BA) solution spraying. The highest amounts of chlorophylls a (1.07 mg g^{-1} FW) and b (0.33 mg g^{-1} FW) and carotenoids were observed in plants treated with 50 ppm BA solution. The highest amounts of phenolic compounds (48.13 mg g^{-1} DW) and antioxidant capacity (154.62 mg Ascorbic acid g^{-1} DW) were observed at 100 ppm BA. The use of gibberellic acid (GA_3) led to significant increases in plant height, stem diameter, internode length, shoot dry weight, chlorophylls a and b contents and plant essential oil content. However, a significant reduction in volume and dry weight of roots, and total phenol content was observed in response to GA_3 treatment. BA increased the content of phenolic compounds and the antioxidant capacity of the plants.

Keywords: Antioxidant capacity, Essential oil, Nutrient solution, Total phenol

INTRODUCTION

Growth regulators are a group of natural or synthetic substances that regulate the growth, development and various physiological processes of plants at very low concentrations. This group of substances can affect the growth of medicinal plants as well as the production of essential oils. The growth and production of essential oils in medicinal plants are influenced by endogenous and exogenous growth regulators [1]. Currently, 125 different types of gibberellins (GAs) are known in higher plants and gibberellin-producing fungi, only a few of which have biological activity. GAs are involved in many physiological processes such as stem growth, flowering, seed germination, dormancy, emergence of sexual organs, senescence delay, parthenocarpy, as well as fruit formation and development [2]. The most famous GA is gibberellic acid (GA_3), whose presence has been reported in many higher and lower plants. Commercially, the most widely used GAs are usually related to GA_3, GA_4 and GA_7. There is a close and direct relationship between GA_3 and primary and secondary metabolism, especially the production of essential oils and aromatic compounds [3]. Another important group of plant hormones is cytokinins (CK) which play various roles in plants including controlling cell division, affecting seed germination, reducing terminal dominance and the growth of lateral buds. These compounds improve root formation at very low concentrations [4]. Benzyladenine (AB) is an adenine-type cytokinin, as well as an efficient and safe regulator that plays essential roles in many aspects of medicinal plant development and stimulates anabolic processes in plant cells [5]. Benzyladenine is mainly used in plant production to promote non-meristem differentiation and lateral bud outgrowth [6]. The results of various studies suggest that the application of BA in different plant species has different effects, such as bud and shoot regeneration, stimulating plant growth and metabolism, regulating plant adaptation to various environmental stresses, improving flowering development, fruit set and seed yield and fruit quality [7].

Soilless culture is a type of cultivation method in which the roots of plants are placed in a medium without soil containing nutrient solution, air or a solid substrate. Besides the high efficiency of water consumption in this method, soilless culture also resulted in a higher crop yield. Production in a soilless culture system entails supplying essential elements for plant growth so that the quantity and quality of cultivated plants in this cultivation method closely depend on the nutrient solution used [8]. One of the important factors affecting the production of plants in soilless culture is nutrition management. In addition to providing sufficient amounts of elements in the nutrient solution, optimal nutrition also requires a balance between the different elements. Otherwise, the increase in the amount of nutrients not only does not lead to an increase in yield but also creates disturbances in the growth of the plant and finally the yield of the crop reduces [9].

Melissa officinalis L., known as lemon balm, belongs to the Labiatae family and is one of the most widely used medicinal plants in the world due to its essential oil and phenolic compounds. The vegetative part of this plant has a lemon-like aroma. The aerial organs and especially the leaves contain essential oils that are rich in citronellol, citral, geraniol, linalool and eugenol acetate. The extract of *M. officinalis* contains different phenolic compounds such as rosmarinic acid and flavonoids [10]. The essential oil of *M. officinalis* L. with antimicrobial, antiviral and digestive effects is widely used in the treatment of nervous ailments and disorders related to the stomach and gut [11]. Due to the high capacity of *M. officinalis* in medicinal and cosmetic industries [12], it is necessary to apply appropriate production methods to improve the quantity and quality of plant materials of this species.

Nutritional management, optimal nutrition, and balanced nutrition are among the most important factors in enhancing biomass and yield of medicinal plants. This is more important in soilless and hydroponic systems and can be precisely controlled. On the other hand, growth regulators such as gibberellins and cytokinins, in addition to stimulating growth and development, play a significant role in increasing the synthesis of metabolites. The use of regulators alongside nutrient solutions can provide a promising approach to the quantitative and qualitative production of medicinal species. Considering the increasing use of hydroponic cultivation methods for the production of medicinal plants, as well as the important effects of the type of nutrient solution and growth regulators on the quantitative and qualitative characteristics of the produced plant materials, the present study aimed to assess the effect of GA_3 and BA on some morphological and phytochemical characteristics of *M. officinalis* under different nutritional conditions.

MATERIAL AND METHODS

Preparation of Plant Materials

The present experiment was conducted to evaluate the effects of nutrition solution, gibberellic acid (GA_3) and benzyladenine (BA) on the growth and physiological traits of *M. officinalis* as a factorial split plot based on a completely randomized design with three replications in the laboratories of Arak University. The main plot included nutrient solution type and the subplot included the factorial combination of GA_3 at 0 and 100 ppm and BA at 0, 50 and 100 ppm as foliar spraying. The three nutrient solution types used included Arnon Hoagland (1950), Shi et al. (2007) and He et al. (2009) were named 1 to 3 throughout the text, respectively (type 1, type 2, and type 3) (Table 1). To experiment, the plant seedlings of *M. officinalis* were produced in seedling trays and then transferred to plastic containers with a diameter of 25 cm and height of 30 cm containing perlite substrate. The plants were automatically treated with nutrient solutions from the tank through the pipe and nozzle. To prevent salt accumulation, the culture media were washed once a week. GA_3 and BA were applied three times (twice in the vegetative growth phase and once at the beginning of the flower emergence phase) as foliar spraying. Control plants were also sprayed with distilled water. The morphological and physiological traits of the treated plants were assayed 130 days after planting.

Table 1 Different nutrient solutions used in the experiment

Chemical composition	Nutrient solution compounds	Type 1 (Hoagland and Arnon, 1950)	Type 2 (Shi et al., 2007)	Type 3 (He et al., 2009)
	Macroelements ($mmol.L^{-1}$)			
Potassium nitrate	KNO_3	5	4	5
Calcium nitrate	$NO_3)_2.4H_2O$(Ca	5	4	5
Magnesium sulphate	$MgSO_4..7H_2O$	2	2	2
Potassium dihydrogen phosphate	KH_2PO_4	1	2.5	2.5
	Microelements ($mg\ kg^{-1}$)			
Boric acid	H_3BO_3	2.86	2.96	2.96
Manganese sulfate	$MnSO_4$	-	10	10
Manganese chloride	$MnCl_2.4H_2O$	1.81	-	-
Zinc sulfate	$ZnSO_4.5H_2O$	0.22	1	1
Copper sulfate	$CuSO_4.5H_2O$	0.08	0.95	0.95
Molybdic acid	$H_2MoO_4.H_2O$	0.02	0.05	0.05
Ferric-EDTA	Fe-EDTA	5	5	5

Assay of Growth Traits

For determination of dry weight, the plants were first harvested at the full flowering stage and separated into two parts, including aerial parts and roots, then rinsed with deionized water and blotted with filter paper. The dry weight was determined

after oven drying at 80 ˚C for 48 h. The volume of the roots was measured by immersing them in distilled water inside a one-liter graduated cylinder.

Assay of Photosynthetic Pigments

Extraction and measurement of chlorophyll and carotenoids were also performed according to the method described by Lichtenthaler and Wellburn [13]. For this purpose, 0.1 g of fresh leaves was ground in a Chinese mortar with 5 ml of 80% acetone, and the resulting mixture was centrifuged for 10 min at 10,000 rpm. The absorbance of the upper solution was measured at wavelengths of 663, 646 and 470 nm, and the amount of photosynthetic pigments was calculated in mg per g fresh weight.

$Chlorophyll_a$ (Chl_a) = 12.25 A_{663} - 2.79 A_{646} × V/W

$Chlorophyll_b$ (Chl_b) = 21.50 A_{646} - 5.1 A_{663} × V/W

Carotenoid = (1000 A_{470} - 1.82 Chl_a - 85.02 Chl_b) / 198 × V/W

Assay of Total Phenol

The total phenolic compounds of the extracts were measured using the Folin-Ciocalteau reagent. For this purpose, 2 mL of Folin-Ciocalteau reagent (1:10) was added to 400 µL of the diluted extract, and after 5 min, 1.6 mL of sodium carbonate 7.5% was mixed with the extraction solution. After 30 min, the absorbance of the mixture was measured at a wavelength of 765 nm using a spectrophotometer (UNICO model UV/Vis 2150, USA) [14] (Singleton & Rossi, 1965). Gallic acid was used as a standard and the results were expressed in mg GA_3 equivalents per g dry weight.

Assay of Antioxidant Activity

The 2,2 diphenyl-1-picrylhydrazyl (DPPH) was used to assay the antioxidant activity. So 2 mL of DPPH solution 4% was added to 2 mL of the methanolic extract of samples. After incubation in the darkness for 30 min, the absorbance of the samples was read at 517 nm. The inhibition percentage of DPPH free radicals was calculated using the following formula [15].

Percentage of inhibition of free radicals = $\frac{(AC-AS)}{AC} \times 100$

Where AC is the absorption rate of the control and AS is the absorption rate of the plant extract. Finally, the antioxidant activity of the extracts was calculated using ascorbic acid as a standard based on mg ascorbic acid equivalent per g dry weight.

Assay of Essential Oil Content

Essential oil from the dried aerial part was extracted by water distillation method using Clevenger (model of British Pharmacopoeia) for 2.5 h and its amount was calculated in terms of volume-weight (v/w).

Statistical Analysis of Data

The statistical analysis of the obtained data was performed using SAS software (version 9.1). To compare the means, Duncan's multiple range test was used at a 5% probability level. Moreover, Excel software (2010 series) was used for drawing the graphs.

RESULTS AND DISCUSSION

Plant Growth Traits

According to the analysis of variance (Table 2), only the effect of GA_3 on plant height was significant. Foliar application of GA_3 caused a significant increase in plant height compared to the untreated plants (Table 3). Stem diameter was significantly influenced by GA_3 and BA treatments (Table 2). Use of GA_3 enhanced stem diameter (Table 3). Furthermore, stem diameter significantly increased with increasing BA concentration, so the highest stem diameter (0.45 cm) was related to the treatment of 100 ppm of BA (Table 4). According to the results of variance analysis (Table 2), the effects of GA_3 and BA on internode length were significant. Increasing GA_3 concentration increased the internode length (Table 3). Furthermore, an increase in the concentration of BA significantly decreased the internode length so that the shortest internode length was obtained at the concentration of 100 ppm BA (Table 4). Among all the studied treatments, the dry weight of the aerial part was only affected by GA_3 (Table 2). Foliar spraying of 100 ppm GA_3 increased the dry weight of the aerial parts of *M. officinalis* by 23.97% (Table 3).

Table 2 Variance analysis of the effect of nutrition solution and foliar application of gibberellic acid (GA_3) and benzyladenine (BA) on some morphological and physiological traits of *M. officinalis*

Sources of variations	DF	MS											
		Plant height	Stem diameter	Internode length	Aerial dry weight	Root volume	Root dry weight	Chlorophyll a	Chlorophyll b	Carotenoids	Total phenol	Antioxidant capacity	Essential oil content
Nutrient solution (A)	2	40.79 ns	0.028 ns	0.272 ns	166.55 ns	24385.18 *	837.91 *	0.298 **	0.017 **	0.011 ns	5.19 ns	1273.85 *	0.005 **
Main error	6	6.44	0.008	0.023	121.39	2864.81	80.97	0.008	0.001	0.002	115.26	193.005	0.001
GA_3 (B)	1	3424.07 **	0.39 **	1.072 **	4978.56 **	217868.51 **	2939.01 **	0.593 **	0.032 **	0.001 ns	543.33 *	618.84 ns	0.016 **
BA (C)	2	32.78 ns	0.058 *	0.456 *	130.77 ns	8379.63 ns	309.64 *	0.306 **	0.015 **	0.019 **	478.22 *	1661.09 **	0.003 ns
A×B	2	19.85 ns	0.0001 ns	0.45 ns	114.42 ns	3674.07 ns	69.60 ns	0.095 **	0.005 **	0.007 ns	91.59 ns	3773.08 **	0.009 **
A×C	4	22.18 ns	0.013 ns	0.067 ns	109.15 ns	3440.74 ns	46.77 ns	0.074 **	0.005 **	0.01 *	24.05 ns	3564.71 **	0.001 ns
B×C	2	22.31 ns	0.025 ns	0.183 ns	67.70 ns	5079.63 ns	195.91 ns	0.09 **	0.008 **	0.005 ns	288.73 ns	1225.81 *	0.007 *
A×B×C	4	8.21 ns	0.006 ns	0.07 ns	70.80 ns	3151.85 ns	45.13 ns	0.054 **	0.003 **	0.007 ns	106.34 ns	1790.06 **	0.0004 ns
Error	30	14.50	0.011	0.092	95.25	3160.37	79.95	0.007	0.001	0.003	96.03	266.07	0.002

ns means non-significant difference, * and ** show significant differences at the probability levels of 5 and 1%, respectively.

Table 3 The effect of foliar application of gibberellic acid (GA_3) on some morphological traits of *M. officinalis*

GA_3 (ppm)	Plant height (cm)	Stem diameter (cm)	Internode length (cm)	Root volume (cm^3)	Root dry weight (g)	Aerial dry weight (g)
0	21.68 b	0.31 b	2.14 b	425.00 a	32.01 b	80.09 b
100	37.61 a	0.48 a	2.43 a	317.11 b	47.26 a	99.29 a

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

Table 4 The effect of foliar application of benzyladenine (BA) on some morphological traits of *M. officinalis*

BA (ppm)	Stem diameter (cm)	Internode length (cm)	Root dry weight (g)
0	0.34 b	2.45 a	58.38 a
50	0.40 ab	2.29 ab	54.95 ab
100	0.45 a	2.13 b	50.56 b

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

Root Growth

The results of variance analysis showed that the effects of nutrition solution type and GA_3 paying on root volume were significant (Table 2). The highest root volume (400.55 cm^3) was obtained in plants treated with nutrient solution type 2, which was 22.41% higher than the lowest value of this trait in plants treated with nutrient solution type 3 (Fig. 1a). Root volume of *M. officinalis* was significantly decreased in response to GA_3 treatment (Table 3).

The results showed that root dry weight was significantly affected by nutrient solution and applied plant growth regulators (Table 2). Regarding the nutrient treatments, the highest and lowest root dry weights were obtained in plants grown in nutrient solution types 1 and 3, respectively (Fig. 1b). Application of GA_3 (Table 3) and BA (Table 3) decreased root dry weight so that the highest value of this trait was obtained in the untreated plants.

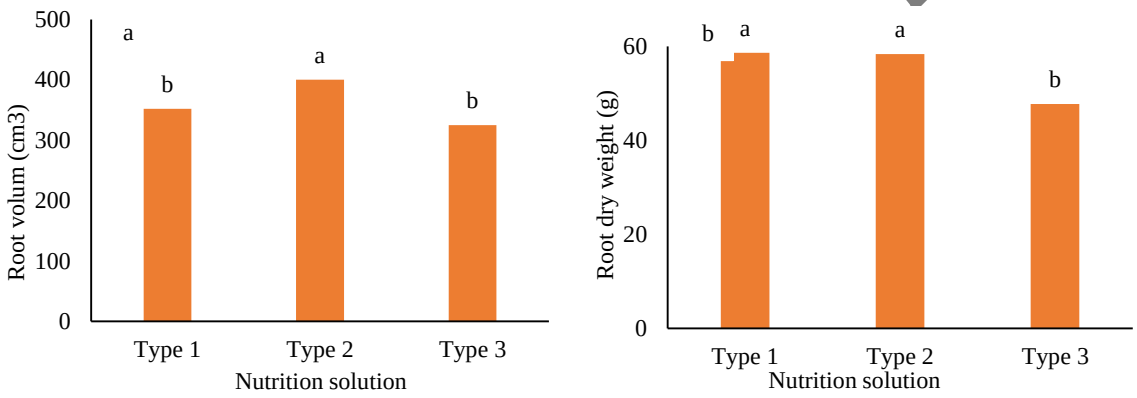

Fig. 1 The effect of different nutrition solutions on root volume (a) and root dry weight (b) of *M. officinalis*: Type 1: Arnon Hoagland 1950, Type 2: Shi et al. 2007, Type 3: He et al. 2009

Photosynthetic Pigments

According to the analysis of variance (Table 2), the effects of all the applied treatments (nutrient solution, GA_3, BA and their interaction) on the content of chlorophylls a and b of lemon balm plants were significant. The results showed that the highest contents of chlorophylls a (1.067 mg g^{-1} fresh weight) and b (0.329 mg g^{-1} fresh weight) were recorded in plants treated with nutrient solution type 3, 100 ppm GA_3 and 50 ppm BA. However, the lowest content of chlorophylls was obtained from the plant grown under nutrient solution type 1 without any treatments of growth regulators (Table 5).

Table 5 The effect of foliar application of gibberellic acid (GA_3) and benzyladenine (BA) on chlorophyll content and antioxidant activity of *M. officinalis* under different nutrition solution

Nutrition solution	GA_3 (ppm)	BA (ppm)	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Antioxidant activity (mg Ascorbic acid g^{-1} DW)
1	0	0	0.136 g	0.089 e	65.34 g
		50	0.287 fg	0.142 cde	102.51 d-g
		100	0.206 fg	0.120 de	154.62 ab
	100	0	0.375 def	0.174 cd	91.14 d-g
		50	0.387 c-f	0.168 cd	98.50 d-g
		100	0.373 def	0.156 cde	142.23 abc
2	0	0	0.295 efg	0.125 de	126.33 bcd
		50	0.564 cd	0.192 cd	115.58 cde

		100	0.279 fg	0.159 cde	73.66 fg
		0	0.578 c	0.202 bc	106.75 c-f
	100	50	0.532 cd	0.180 cd	128.48 bcd
		100	0.295 efg	0.136 cde	128.14 bcd
		0	0.321 efg	0.166 cd	125.24 bcd
	0	50	0.483 cde	0.181 cd	122.37 b-e
3		100	0.295 efg	0.135 cde	142.17 abc
		0	0.838 b	0.270 ab	87.86 efg
	100	50	1.067 a	0.329 a	110.49 c-f
		100	0.306 efg	0.133 cde	118.30 b-e

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

The results of variance analysis showed that the carotenoid content was only affected by foliar application of BA. Furthermore, the interaction effect of the nutrient solution type and different levels of BA was significant in this trait (Table 2). According to the obtained results, the highest content of carotenoids was obtained in the plants treated with nutrient solution type 3 and the application of BA at 50 ppm. The lowest content of carotenoids was related to the treatment of nutrition solution type 1 and 100 ppm BA (Fig. 2).

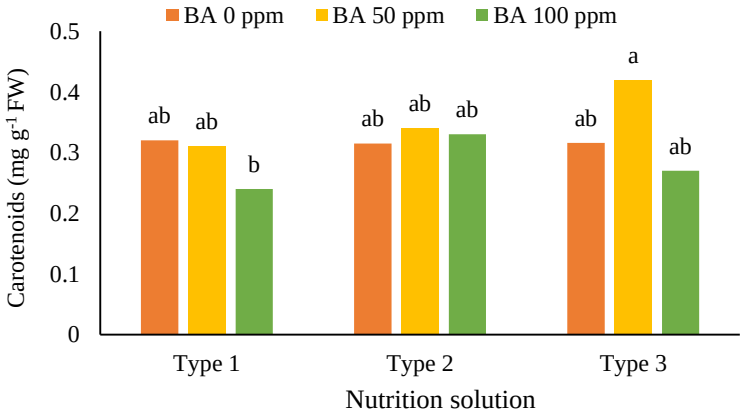

Fig. 2 The effect of foliar application of benzyl adenine on carotenoids content of *M. officinalis* under different nutrition solutions
Type 1: Arnon Hoagland 1950 Type 2: Shi et al. 2007 Type 3: He et al. 2009

Total Phenol Content

The results showed that the total phenol content of *M. officinalis* was affected by foliar application of GA_3 and BA (Table 2). Treatment with GA_3 at a concentration of 100 ppm caused a reduction in phenolic compounds compared to the untreated plants (Fig. 3a). However, increasing the concentration of BA resulted in a significant increase in the phenolic content of lemon balm plants so that the highest content (48.13 mg GA_3 per gram dry weight) was obtained at 100 ppm BA concentration (Fig. 3b).

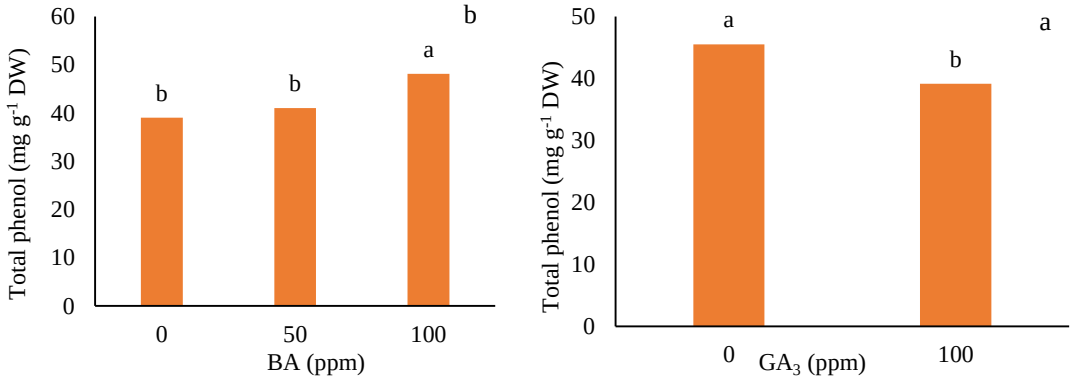

Fig. 3 The effect of foliar application of gibberellic acid (a) and benzyladenine (b) on total phenol content of *M. officinalis*

Antioxidant Activity

The antioxidant activity of *M. officinalis* extract was significantly influenced by the interaction effects of applied nutrient solutions, GA_3 and BA (Table 2). Among the assayed treatments, the highest antioxidant activity was obtained under nutrient

solution type 3 and untreated plants with any growth regulators. The other treatments did not show significant differences with each other (Table 5).

Essential Oil Content

The results of the analysis of variance (Table 2) showed the significant effect of the nutrient solution type and GA_3 application on the essential oil content of *M. officinalis*. In addition, the interaction effects of the nutrient solution × GA_3 and GA_3 × BA on the essential oil content were significant. According to the obtained results, the highest essential oil content (0.26%) was related to Hoagland nutrient solution (nutrition solution type 1) with the use of GA_3 at 100 ppm (Fig. 4a) The interaction effect of GA_3 and BA showed (Fig. 4b) that the highest content of *M. officinalis* essential oil (0.24%) was extracted from the plants treated with the highest concentrations of GA_3 (100 ppm) and BA (100 ppm).

Based on the results of the current study, GA_3 foliar application increased the height of the lemon balm plant. Similar results have been reported regarding the effect of this substance on the height of lavender plants [3], lettuce [16] and dill [17]. GA_3 increases the absorption of nutrients by increasing cell division and the development of terminal and lateral buds. During cell division, there is a greater need for nutrients, and GA_3 increases the height, number of leaves, leaf area, and number of branches by increasing the absorption of nutrients [3]. In the present study, the use of GA_3 and BA caused a significant increase in stem diameter compared to the untreated plants. BA, by increasing cell division, and GA_3, by increasing the elasticity of the cell wall and the longitudinal growth of the cell and through the improving cell sap concentration and the hydrolysis of starch to sugar, cause a decrease in the water potential of the plant cell so that more water enters the cell and consequently stem diameter increases [1]. The present study showed that the application of GA_3 decreased the volume and dry weight of the roots in the lemon balm plant. The use of GA_3 has also been reported to increase the dry weight of roots in dill [17], and mung bean [18]. According to the results of Atteya et al. [19], the use of GA_3 in *Tagetes patula* has also increased the dry weight of aerial parts. GA_3 is involved in various physiological activities of the plant, including cell division and elongation, and as a result improves some growth parameters, such as the elongation of the internodes in the stem, which leads to an increase in the dry weight of the aerial parts of the plant [20].

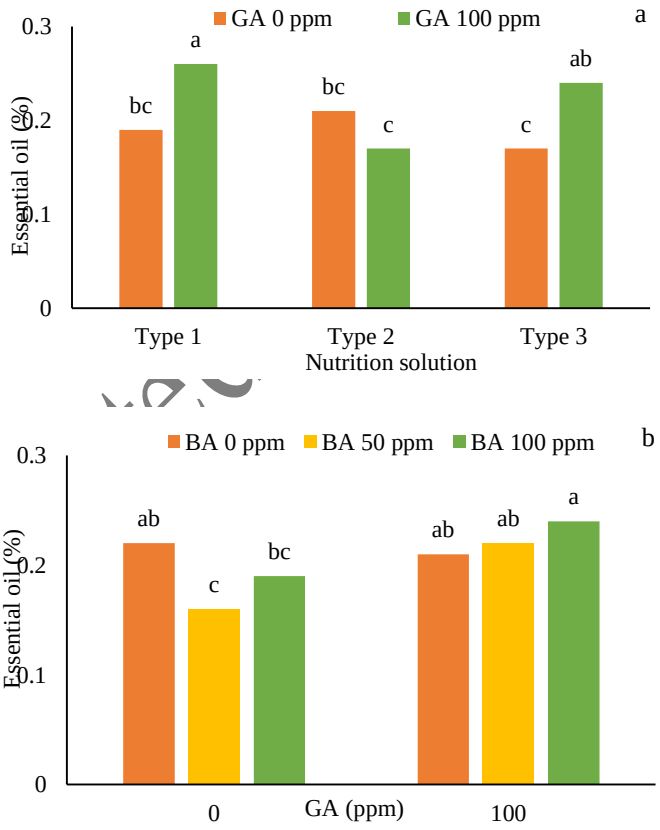

Fig. 4 The interaction effect of gibberellic acid × nutrition solution (a) and gibberellic acid × benzyladenine (b) on essential oil content of *M. officinalis* Type 1: Arnon Hoagland 1950 Type 2: Shi et al. 2007 Type 3: He et al. 2009

The use of hydroponic systems has an effective role in improving yield and yield components (root dry weight, shoot dry weight, leaf dry weight, and total dry weight) and even secondary metabolites such as essential oil content in medicinal and aromatic plants. The application of plant growth regulators such as gibberellins and cytokinins can strengthen this system [21]. Gibberellins can stimulate the growth and elongation of roots in hydroponic conditions, as they stimulate the growth of other organs. In a study, the application of gibberellin increased root growth and yield of *Lactuca sativa* L. var. *Crispa*

and *Eruca sativa* L. are grown in a hydroponic system [22]. GA_3 in hydroponic systems and under salinity stress conditions in *Saccharum officinarum* L., in addition to reducing the effects of salinity stress, caused an increase in root mass [23]. These studies showed that GA_3 regulated the root growth of plants with changes in cell wall components in hydroponic systems [24]. In the same way, the results of the positive effect of cytokinins such as BA on root growth in hydroponic conditions have been reported [25, 26].

The results of this study showed that the contents of chlorophyll a and b and carotenoids in plants treated with nutrient solutions 2 and 3 were higher than the plants grown in nutrient solution 1. Nitrogen is one of the main involved elements in chlorophyll structure and therefore the chlorophyll content of plants can be affected by the nitrogen content of nutrient solution. A positive correlation has been shown between the chlorophyll content of plants and the nitrogen concentration of the nutrient solution [27]. It has been previously reported that the application of nitrogen in lavender [28] increased chlorophyll content. Another reason for the increase in the content of photosynthetic pigments in plants subjected to nutrient solutions 2 and 3 compared to nutrient solution 1 can be attributed to the increase in the concentration of phosphorus in the nutrient solution. Phosphorus deficiency can reduce the ability of nitrogen absorption by restricting the root growth. It has been reported that an increase in nitrogen and phosphorus concentrations was accompanied by an increase in the relative amount of chlorophyll and photosynthesis in the leaves [29]. On the other hand, when most of the nitrogen in the nutrient solution is in the form of nitrate, the concentrations of potassium, calcium and magnesium in the plant increase. Due to the involvement of magnesium in the structure of chlorophyll, increasing the concentration of magnesium is another factor in increasing the content of photosynthetic pigments [30].

Considering the role of chlorophyll in the absorption and use of light energy in photosynthesis, the effect of plant growth regulators on the processes of biosynthesis and breakdown of chlorophyll is directly effective on photosynthesis [1]. The positive effect of GA_3 on the increase of chlorophylls a and b contents has also been reported in coriander [16]. Cytokinins do not completely prevent senescence, even if a small portion of leaves are treated with cytokinin, they remain green while the surrounding tissues of the same leaf begin to senesce. In the case of treatment with cytokinin, nutrients are preferably transferred to the treated tissues and accumulate in those parts. In this regard, it is thought that growth regulators cause the transfer of nutrients by creating a new relationship between the source and the sink. Metabolism in the treated area may be stimulated by the application of hormones and cause the movement of nutrients to this area [31].

According to Sosnowski et al. [32], cytokinins accelerate the production of photosynthetic proteins and can increase cell development in some tissues and organs. Furthermore, cytokinins prevent the destruction of chlorophyll. Cytokinins increase the absorption of amino acids, strengthen the maintenance of proteins in the plant and prevent aging by stimulating cell division [33]. An increase in chlorophyll content as a result of cytokinins application has also been reported in lemongrass [34], which is consistent with the results of the current experiment.

Phenylpropanoid compounds lead to certain colors, tastes and physiological characteristics in plants and protect the plant against biotic and abiotic stresses, especially herbicides, and ensure the survival of the plant by attracting pollinators [35]. In this study, foliar spraying of GA_3 and BA, respectively, caused a decrease and increase in the content of plant phenolic compounds compared to untreated plants. The increase in the production of phenolic compounds as a result of cytokinin application has also been shown in previous studies. It has been reported in *Scutellaria altissima* plant that the application of different cytokinins increased the number of phenolic compounds in the plant [36]. In stevia, the maximum content of phenolic compounds has been obtained in the combination of spermidine, BA and GA_3 [37]. It has been reported that cytokinin increases the production of phenolic compounds in tissues by inducing cell differentiation [38].

In this study, the antioxidant activity of extracts in plants treated with BA increased, which is in line with the results reported regarding the use of different cytokinins in dill, stevia, and lemongrass [17, 34, 37]. On the other hand, plants subjected to nutrient solutions 1 and 3 and treated with GA_3 showed the highest percentage and yield of essential oil. Furthermore, BA together with GA_3 could increase the percentage of essential oil in lemon balm. The increase found in essential oil production in the present study is consistent with the increase reported in the content and yield of *Tagetes patula* essential oil as a result of GA_3 application [19]. In the dill plant, foliar spraying of GA_3 caused an increase in essential oil yield [17]. Moreover, it has been reported in geranium plants that the use of BA increased the major compounds of the essential oil [39]. Growth regulators play a key role in the primary metabolism of plants, so the growth and development of medicinal plants and the production of effective substances in them can depend on these compounds. Growth regulators can improve the yield of essential oils in medicinal plants by affecting growth, essential oil biosynthesis and the number of essential oil storage structures [31, 34, 39]. The growth responses and phytochemical production of *Melissa officinalis* L. can be significantly influenced by nutritional management, particularly through the manipulation of ammonium (NH_4^+) to nitrate (NO_3^-) ratios and the application of growth regulators such as gibberellic acid and benzyladenine. Recent studies have demonstrated that optimizing these nutritional conditions can enhance both the morpho-physiological traits and the phytochemical profiles of various Lamiaceae species, including lemon balm [40, 41].

CONCLUSION

The application of plant growth regulators such as gibberellic acid and benzyladenine can enhance the growth and biochemical responses of medicinal species including *Melissa officinalis*. Gibberellic acid is known to stimulate cell elongation and division. Therefore, it can increase plant height and canopy, while benzyladenine, a cytokinin, can improve cell differentiation and shoot proliferation. The synergistic effects of these plant growth regulators combined with optimal nutritional conditions could potentially lead to a significant increase in the synthesis of phytochemicals, including terpenoids and phenolic composition, which are essential for the medicinal properties of *M. officinalis*. Controlled nutritional conditions led to enhanced phytochemical content and subsequently enhanced the therapeutic properties of *M. officinalis*. Research suggests that the manipulating of nutrient ratios not only affects growth, biomass, and yield but also the synthesis and accumulation of beneficial and valuable secondary metabolites. The increased content of terpenoid compounds and phenolic composition is certainly associated with the antioxidant properties of medicinal plants such as lemon balm, which are essential for its medicinal efficacy. Therefore, the integration of nutritional control with plant growth regulators offers a promising approach to maximize both the growth and phytochemical potential of lemon balm.

Declarations

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

All the authors contributed to the performed experiments. **RSH** and **HS** contributed to designing experiment, analyzing the data and performing statistical analyses, writing, revising and improving the manuscript. **MV** performed the experiments and interpreted the results. **MA** conceptualized the idea, contributed to designing experiment. All the authors have read and approved the final manuscript.

Data Availability

The data presented in this study are available on request from the corresponding authors.

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