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The Effect of Gamma-Aminobutyric Acid on Growth and Phytochemical Characteristics of Lemon Balm (*Melissa officinalis* L.) under Salinity Stress

Mahsa Hamidinia[1], Marzieh Ghanbari Jahromi[2*], Fereidon Ghasemkhan-Ghajar [3]

1. M. Sc. student, Department of Horticultural Science, SR.C., Islamic Azad University, Tehran, Iran
2*. Assistant Professor, Department of Horticultural Science, SR.C., Islamic Azad University, Tehran, Iran
3. Assistant Professor, Department of Agronomy, SR.C., Islamic Azad University, Tehran, Iran

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*Corresponding Author E-

ghanbari@iau.ac.ir

ABSTRACT

γ-aminobutyric acid (GABA) is a biochemical elicitor that can function as an endogenous signaling molecule. Nowadays, the use of GABA to mitigate the effects of environmental stresses and enhance the production of bioactive compounds in plants has become common. This study aimed to investigate the effect of gamma-aminobutyric acid (0, 0.5, 1.5, and 3 mM) on the growth, physiological, and biochemical characteristics of lemon balm (*Melissa officinalis*) under salinity stress (0, 60, and 120 mM) using a factorial experiment based on a completely randomized design with three replications. The results showed that 120 mM salinity stress significantly reduced morphological traits such as plant height, fresh weight of aerial parts and roots, and photosynthetic pigment content in the plant. The application of 3 mM gamma-aminobutyric acid resulted in the highest levels of phenols, total flavonoids, and antioxidant activity, which were positively correlated. Under moderate salinity stress (60 mM), the essential oil content of the plant increased, but under severe salinity stress (120 mM), the percentage and yield of essential oil decreased. In summary, while lemon balm demonstrates sensitivity to salinity stress, GABA application effectively mitigates its adverse effects by enhancing growth and stimulating production of valuable secondary metabolites. Foliar treatment with 3 mM GABA is recommended as a practical strategy to improve antioxidant capacity and essential oil yield under moderate saline conditions up to 60 mM NaCl, supporting sustainable cultivation of this medicinal plant in affected regions.

1- Introduction

Lemon balm (*Melissa officinalis*) is a perennial medicinal plant from the mint family (Lamiaceae), known for its sedative, anti-anxiety, and antimicrobial properties [1]. This plant is native to Mediterranean regions but is cultivated in many parts of the world. Its leaves contain adequate amounts of vitamins (such as vitamin C and vitamin A) and minerals (such as calcium, magnesium, and potassium), making it a vegetable with significant nutritional value [2]. The leaves of lemon balm contain bioactive compounds like phenolic acids, flavonoids, and terpenoids, which are responsible for its therapeutic properties [3]. The essential oil of lemon balm also contains compounds such as citral, geraniol, and linalool, which impart a lemony aroma and flavor. This characteristic has led to the use of this plant in cooking and especially in the food industry as a natural seasoning and flavoring agent in beverage production [4]. In traditional medicine, lemon balm is used to treat digestive disorders, insomnia, and anxiety. Additionally, studies have shown that this plant possesses strong antioxidant and anti-inflammatory properties [5].

Salinity stress is one of the most significant limiting factors for plant growth and production, especially for medicinal plants, in arid and semi-arid regions [6]. Increased salt concentration in the soil reduces soil water potential and impedes water uptake by the roots. These conditions lead to osmotic stress in the plant, which in turn causes reduced growth, decreased photosynthesis, and disruption of plant metabolism [7]. Additionally, the accumulation of toxic ions such as sodium and chloride in plant tissues can cause ionic toxicity, leading to cellular damage and cell death [8]. Medicinal plants are more sensitive to salinity stress due to their production of valuable secondary metabolites. This stress can reduce the production of bioactive compounds such as alkaloids, flavonoids, and terpenoids, which play a key role in the therapeutic properties of these plants [9]. Furthermore, salinity stress can alter the activity of antioxidant enzymes and increase the production of reactive oxygen species (ROS), resulting in oxidative damage to cell membranes and DNA. Consequently, the reduction in both the quality and quantity of active compounds in medicinal plants under salinity stress poses serious challenges for the pharmaceutical and agricultural industries [10].

GABA is a four-carbon, non-proteinaceous compound naturally found in plants, animals, and microorganisms [11]. It is recognized as an inhibitory neurotransmitter in the central nervous system of mammals, but it also plays significant roles in plants. In plants, GABA acts as a signaling molecule in response to environmental stresses such as salinity [12], drought [13], temperature extremes [14], and mechanical damage [15]. Under stress conditions, GABA levels in plants rapidly increase, aiding in the regulation of ionic balance, maintenance of cell membrane potential, and reduction of oxidative damage [16]. Furthermore, GABA is involved in plant carbon and nitrogen metabolism and can serve as a precursor for the synthesis of other important compounds such as proline and polyamines [17]. Studies have shown that GABA accumulation in plants can enhance their resistance to environmental stresses [18], and therefore, the use of GABA as a growth stimulant in agriculture has garnered significant attention.

Researchers have reported in an experiment that in German chamomile, the application of GABA, methyl jasmonate, salicylic acid, and humic acid treatments under severe drought stress conditions led to an increase in essential oil content. Comparison among treatments showed that the highest essential oil content in German chamomile was obtained from the GABA treatment [19].

In a study investigating the effects of GABA and salinity stress on the characteristics of apple mint (*Mentha suaveolens* Ehrh.), it was stated that generally, the extremely severe NaCl stress (i.e., exceeding 100 mM), which resulted in a sharp decline in yield component values, was beyond the tolerance range of *M. suaveolens* mint. Therefore, it was logical to compensate for the reduced yield of medicinal metabolites by foliar application of GABA at low concentrations (i.e., 0.1–0.2 mM) under 100 mM NaCl stress or lower levels [20].

Researchers examining the effects of GABA and salicylic acid on the growth and physiology of tomatoes reported that the highest concentration of GABA (10 mg/L) and the highest concentration of salicylic acid (1.5 mM) produced the most favorable results in tomato seedling production in terms of growth characteristics and quality [21].

Given that lemon balm is a medicinal plant with high nutritional value and diverse applications, both in its fresh and processed forms, and that no report has yet been published on investigating the growth and phytochemical performance of this plant with the aim of mitigating salinity stress using GABA, this research was conducted for this purpose.

2- Materials and Methods

This experiment was conducted to investigate the effects of different concentrations of GABA and salinity stress on the quantitative and qualitative characteristics of lemon balm under greenhouse conditions, using a factorial arrangement in a completely randomized design. First, four-leaf lemon balm seedlings were prepared and planted in plastic pots (17 cm diameter) filled with a cocopeat and perlite substrate (2:1 ratio). After seedling establishment, salinity treatments were applied using sodium chloride (0, 60, and 120 mM) through irrigation water. GABA was obtained from Tehran Shimi Saz Co. (Tehran, Iran). Different GABA concentrations (0, 0.5, 1.5, and 3 mM) were applied as foliar sprays four times at two-week intervals. The stress period lasted 40 days. The electrical conductivity (EC) of the drainage water was measured weekly. Plants were sampled seven days after the end of the treatment period (day 50), and their growth, physiological, and biochemical traits were measured and analyzed.

Plant height was measured using a ruler. To measure the fresh weight of the aerial parts and roots, three plants from each pot were cut at soil level (the crown area). After being transferred to the laboratory, their fresh weight was measured.

2-1- Measurement of Plant Pigment Content: To determine pigment content, 0.5 g of plant material was ground in a porcelain mortar with liquid nitrogen. Twenty milliliters of 80% acetone were added to the sample, which was then centrifuged at 6000 rpm for 10 minutes. The supernatant was collected and transferred to a glass flask. An aliquot of the supernatant was transferred to a spectrophotometer cuvette, and absorbance was measured at wavelengths of 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids using a Visible/UV-45 Lambda model spectrophotometer. Finally, the amounts of chlorophyll a, chlorophyll b, and carotenoids, expressed in milligrams per gram of fresh sample weight, were calculated using the following formulas [22].

(1) Chlorophyll a = (19.3 × A663 - 0.86 × A645) V / 100W

(2) Chlorophyll b = (19.3 × A645 - 3.6 × A663) V / 100W

(3) Carotenoids = [100(A470) - 3.27(mg chl. a) - 104(mg chl. b)] / 227

V = Volume of the filtered solution (supernatant from centrifugation)

A = Light absorbance at wavelengths of 663, 645, and 470 nm

W = Fresh weight of the sample in grams

2-2- Determination of Total Phenol Content: 0.5 mL of the extracted solution was thoroughly mixed with 5 mL of Folin-Ciocalteu reagent (which had been diluted 10-fold with distilled water) and 4 mL of a 1 M sodium carbonate solution. The mixture was left to stand at room temperature for 15 minutes. Subsequently, the absorbance of the solution was measured using a Visible/UV-45 Lambda model spectrophotometer at a wavelength of 765 nm [23]. Additionally, the colorimetric method (Folin-Ciocalteu) was performed on standard gallic acid solutions of different concentrations, and a standard curve was plotted against the absorbance of gallic acid (Y = 0.00114X + 0.01062, where Y is the absorbance value and X is the concentration in ppm). To determine the phenol concentration of the samples, the absorbance values were converted to ppm (X).

2-3- Determination of Total Flavonoid Content: The aluminum chloride colorimetric method was used to determine flavonoid content. For each of the methanolic plant extracts (0.5 mL of a 1:10 g/mL dilution), 1.5 mL of methanol, 0.1 mL of aluminum chloride (10% in methanol), 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water were combined separately. The solutions were then left at room temperature for 30 minutes. The absorbance of each reaction mixture was measured at 415 nm using a Visible/UV-45 Lambda model spectrophotometer. A standard curve was prepared using methanolic solutions of quercetin (Quercetin, Sigma Chemical Co.) at concentrations ranging from 250 to 1000 µg/mL, and the curve was plotted using Excel software to obtain the linear equation y = bx + a. The absorbance values measured for the samples were then substituted for y to calculate x, which represents the corresponding concentration [24].

2-4- Measurement of Antioxidant Activity: To measure the antioxidant activity, the free radical DPPH (2,2-Diphenyl-1-Picrylhydrazyl) was used. First, methanolic extracts of the plant samples were prepared at different concentrations, ranging from 5×10^{-2} mg/100 µL to 5×10^{-6} mg/100 µL, in pure methanol. A mixture in a 1:1 ratio of DPPH solution (8 mg/100 mL) and the plant extracts at varying concentrations was then prepared. The absorbance of the samples was measured at 517 nm after 30 minutes at laboratory temperature using a Visible/UV-45 Lambda model spectrophotometer.

The percentage of DPPH free radical inhibition for the samples was calculated using the following formula:

$R\% = (A_D - A_S) / A_D \times 100$ (4)

Where:

R% = Inhibition percentage

A_D = Absorbance of DPPH at 517 nm

A_S = Absorbance of the samples at 517 nm

To compare the activity of the extracts, the IC50 parameter (the concentration of extract that inhibits 50% of the free radicals) was used [25].

2-5- Essential Oil Percentage and Yield: The essential oil percentage was measured by hydrodistillation using a Clevenger apparatus.

To obtain the essential oil yield, the dry matter weight was multiplied by the essential oil percentage for each treatment.

2-6- Statistical Analysis: The data obtained from measuring the variables of interest were first recorded in Excel and then subjected to statistical analysis using the SAS statistical software version 9.4. The comparison of data means at the 1% or 5% significance level was performed using Duncan's multiple range test. Graphs and figures were prepared using Excel 2019 software.

3- Results and Discussion

3-1- Morphological Traits: The results of the variance analysis showed that the plant height and the fresh weight of the aerial parts and roots of lemon balm were significantly affected by the main effect of salinity stress at the 1% probability level. The simple effect of GABA on plant height was significant at the 1% level, and on the fresh weight of aerial parts and roots at the 5% level. Furthermore, the interaction effect of salinity stress and GABA foliar application was significant on plant height at the 1% probability level and on the fresh weight of aerial parts and roots at the 5% level (Table 1).

Table 1 Analysis of variance of salinity and γ-aminobutyric acid (GABA) on the morphological traits of *Melissa officinalis*

S.O.V.	D.f.	M.S.		
		Height	Fresh Weight of Aerial Parts	Fresh Weight of Roots
Salinity stress (S)	2	1155.19**	2481.69**	2508.86**
γ-aminobutyric acid (G)	3	153.14**	103.52*	151.44**
S × G	6	0.75**	44.32*	68.05*
Experimental error	24	1.78	23.31	35.58
Coeff of variation (%)	-	4.30	7.18	11.44

*, and **: significant at 5, and 1% probability levels, respectively.

3-1-1- Plant Height: Based on the results of comparing the means of the interaction effects between salinity stress and GABA foliar application treatments, the highest plant height of lemon balm (44.33 cm) was observed in the control treatment with the application of 3 mM GABA. The lowest plant height (18.67 cm) was recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 1).

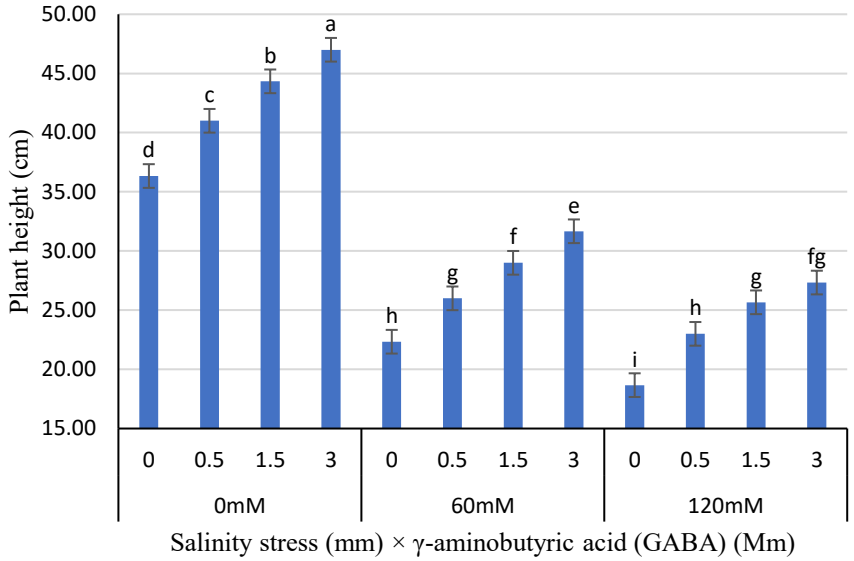

Fig 1 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the plant height of *Melissa officinalis*

3-1-2- Fresh Weight of Aerial Parts and Roots: The highest fresh weight of aerial parts (85.67 g) and fresh root weight (71.67 g) in lemon balm were observed in the control treatment with the application of 3 mM GABA. The lowest fresh weight of aerial parts (49.00 g) and fresh root weight (32.67 g) were recorded in the severe salinity stress treatment (120 mM NaCl) combined with the application of 0.5 mM GABA (Figure 2).

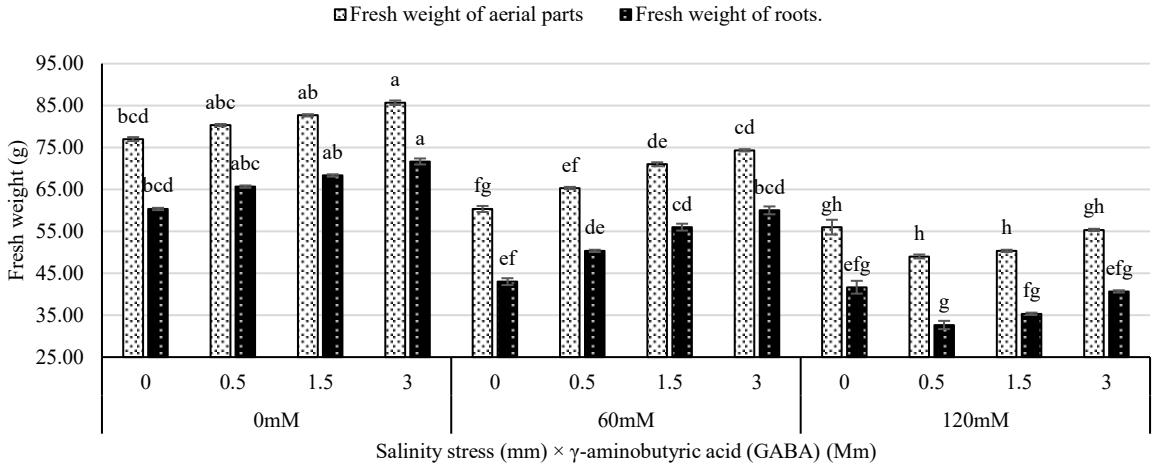

Fig 2 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Fresh weight of aerial and root of *Melissa officinalis*

At higher salinity concentrations, growth reduction was observed due to the strong toxic properties of salt minerals. Typically, the most sensitive response to salinity is a decrease in growth, which is reflected in reduced plant height and biological yield. The decrease in plant height results from reduced longitudinal cell growth. Salinity stress initially affects plant cell growth by influencing turgor pressure (turgor). A reduction in turgor pressure due to salinity is recognized as the most important inhibitory factor for plant growth under saline conditions [26]. A growth medium that is saline and contains high amounts of ions disrupts

the metabolism of other essential nutrients. Competition from Na^+ ions with K^+ and Cl^- ions with NO_3^- interferes with the uptake of these elements, leading to a decrease in plant growth and biomass. One of the key characteristics influencing salinity tolerance is the maintenance of cell turgor, with osmotic regulation occurring through the uptake of salts (ionic salts) and the synthesis of organic compounds. Plants expend considerable energy to synthesize organic compounds (such as glycine, betaine, proline, mannitol, and sorbitol). Due to the consumption of this energy for osmotic regulation, the growth of aerial parts and, consequently, plant weight show a significant decline [27].

GABA has positive effects on plants in response to environmental stresses. This substance functions as both an amino acid and a signaling molecule in plants and can help modulate the plant's physiological responses to stress [28]. GABA leads to increased production of nutrients, thereby enhancing plant weight and height [29]. It influences the production of hormones such as auxin and cytokinin, aids in plant growth and development, and contributes to increased weight of both aerial parts and roots [30]. GABA assists in improving root growth, which enhances access to water and nutrients and leads to increased root mass [31]. Additionally, this compound possesses antioxidant properties, reduces damage caused by oxidative stress in plants, and helps maintain plant health and growth under challenging conditions [32].

3-2- Photosynthetic Pigments: The results of the variance analysis showed that chlorophyll a, chlorophyll b, and carotenoids in lemon balm exhibited a significant difference at the 1% probability level under both the main and interactive effects of salinity stress and GABA foliar application (Table 2).

Table 2 Analysis of variance of salinity and γ-aminobutyric acid (GABA) on Photosynthetic pigments of *Melissa officinalis*

S.O.V.	D.f.	M.S.		
		Chlorophyll a	Chlorophyll b	Carotenoid
Salinity stress (S)	2	9.77**	7.48**	4.19**
γ-aminobutyric acid (G)	3	3.61**	1.67**	0.87**
S × G	6	0.45**	0.12**	0.07**
Experimental error	24	0.04	0.01	0.01
Coeff of variation (%)	-	5.27	4.25	4.32

**: significant at 1% probability levels, respectively.

3-2-1- Chlorophyll a and b: Based on the results of comparing the means of the interaction effects between salinity stress and GABA foliar application treatments, the highest chlorophyll a (5.18 mg per gram fresh weight) and chlorophyll b (3.48 mg per gram fresh weight) were observed in the control treatment (absence of salinity stress) combined with the application of 3 mM GABA. The lowest chlorophyll a (1.83 mg per gram fresh weight) and chlorophyll b (1.10 mg per gram fresh weight) were recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 3).

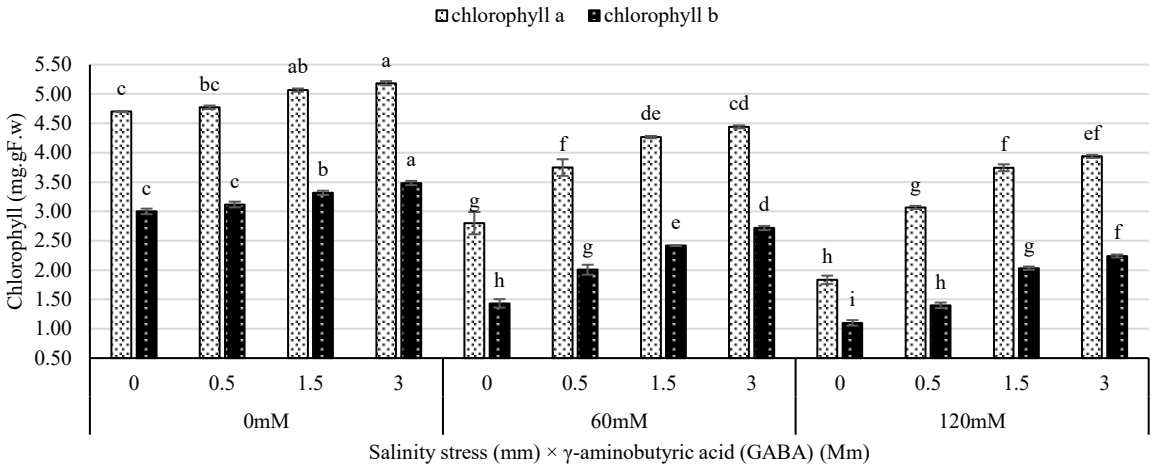

Fig 3 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Chlorophyll a and b of *Melissa officinalis*

3-2-2- Carotenoids: The highest carotenoid content in lemon balm (2.64 mg per gram fresh weight) was observed in the control treatment (without salinity stress) combined with the application of 3 mM GABA. The lowest carotenoid content (0.90 mg per gram fresh weight) was recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 4).

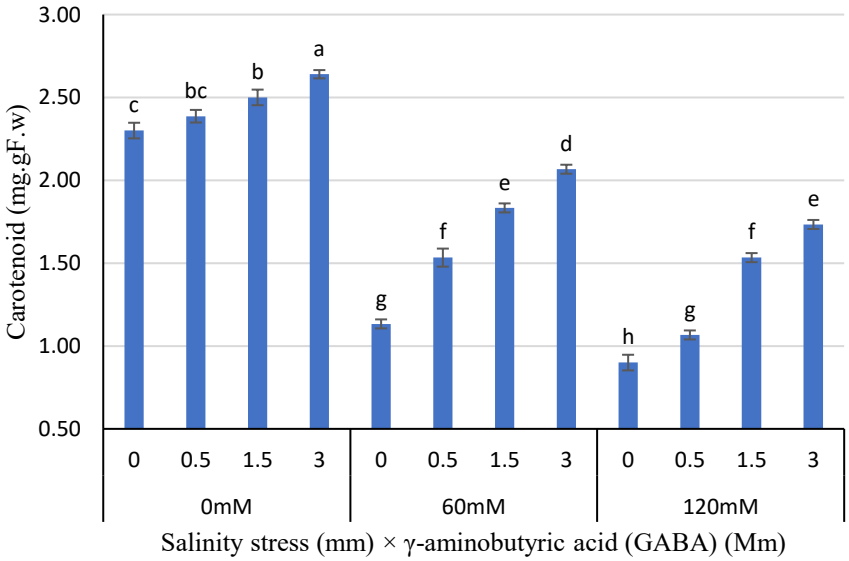

Fig 4 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Carotenoid of *Melissa officinalis*

The reduction in chlorophyll content under stress conditions is likely due to the activation of chlorophyll catabolic pathways, and chlorophyll levels indicate plant resistance to stress [33]. The degradation of chlorophyll and carotenoids under salt stress conditions can result from increased activity of the enzyme chlorophyllase, changes in the structure and function, and reduced content of proteins—particularly plant membrane proteins and enzymes involved in chlorophyll biosynthesis pathways, such as 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, and protochlorophyllide oxidoreductase [34]. Salinity stress reduces plant growth and yield by disrupting the balance in the uptake of essential elements,

water, and by inducing oxidative stress. Although plant growth is the result of regular and complete physiological processes, and the inhibition of plant growth by environmental factors cannot be attributed solely to one specific physiological process, the predominant physiological phenomenon is photosynthesis [35]. Plant growth and biomass production depend on the net photosynthesis rate, and salinity stress, depending on its intensity, affects photosynthesis [36].

GABA positively affects the activity of enzymes involved in photosynthesis. By increasing the activity of these enzymes, chlorophyll synthesis is also enhanced [31]. This compound acts as an antioxidant, helping to reduce damage caused by oxidative stress. This action contributes to the protection of chloroplasts and chlorophyll-synthesizing proteins [37]. GABA helps improve stomatal status, which enhances gas exchange and consequently improves photosynthesis and chlorophyll synthesis [38]. Additionally, this substance increases protein synthesis, which aids in the production of chlorophyll and carotenoids [39]. With increased chlorophyll levels, the plant gains a greater ability to absorb sunlight. This, in turn, can contribute to increased carotenoid production, as carotenoids play an important role in protecting the plant from intense light [40].

3-3- Phytochemical Traits: The results of the variance analysis showed that total phenol and total flavonoids, antioxidant activity, essential oil percentage, and essential oil yield of lemon balm were significantly affected by the main effects of salinity stress and GABA foliar application at the 1% probability level. The interaction effect of the treatments was significant for total phenol, antioxidant activity, essential oil percentage, and essential oil yield at the 1% probability level, and for total flavonoids at the 5% level (Table 3).

Table 3 Analysis of variance of salinity and γ-aminobutyric acid (GABA) on phytochemical traits of *Melissa officinalis*

S.O.V.	D.f.	M.S.				
		Total Phenol	Total Flavonoid	Anti-Oxidant Activity	Essential Oil Percent	Essential Oil Yield
Salinity stress (S)	2	2178.95**	2342.33**	2646.03**	0.58**	1010.04**
γ-aminobutyric acid (G)	3	645.45**	318.85**	785.00**	0.16**	158.62**
S × G	6	49.62**	9.18*	59.47**	0.01**	7.12**
Experimental error	24	6.30	3.44	7.67	0.003	3.17
Coeff of variation (%)	-	4.84	4.11	4.85	6.11	8.52

*, and **: significant at 5, and 1% probability levels, respectively.

3-3-1- Total Phenol and Total Flavonoids

Based on the results of comparing the means of the interaction effects between salinity stress and GABA foliar application treatments, the highest total phenol (73.45 mg gallic acid per gram dry matter) and total flavonoids (66.00 mg quercetin per gram dry matter) were observed in the mild salinity stress treatment (60 mM NaCl) combined with the application of 3 mM GABA. The lowest total phenol (28.18 mg gallic acid per gram dry matter) and total flavonoids (28.67 mg quercetin per gram dry matter) were recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 5).

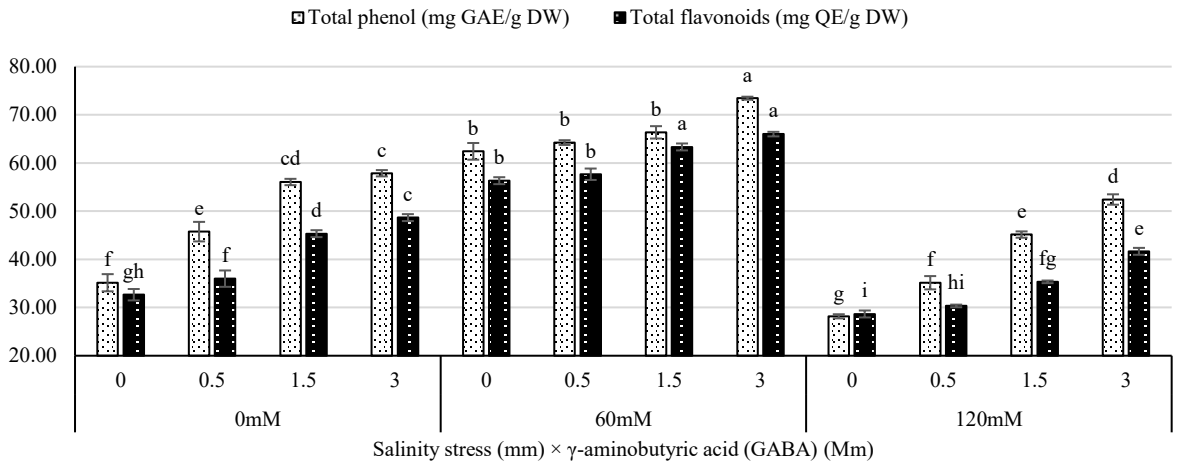

Fig 5 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Total Phenol and Total flavonoids of *Melissa officinalis*

The free hydroxyl groups attached to the aromatic ring of phenols reduce oxidative damage by scavenging free radicals and through other defense mechanisms such as quenching singlet oxygen, chelating metals, and binding toxic ions. In this way, they protect cellular structures from the negative effects of stress [41]. Phenolic compounds increase rapidly in response to stress and accumulate in large quantities in the epidermal layer of plant tissue. The accumulation of phenolic compounds in trichomes, vacuoles, and the cell walls of epidermal cells protects the underlying mesophyll cells from damage. These compounds, with their antioxidant and radical-detoxifying capabilities, enhance plant resistance [42].

GABA, as a metabolic regulator, stimulates the biosynthesis processes of secondary metabolites such as phenols and flavonoids. These compounds act as natural antioxidants in plants and are produced in response to environmental stresses [43]. GABA influences the production of plant hormones such as auxins, cytokinins, and gibberellins. These hormones play a crucial role in regulating the production and metabolism of flavonoids and phenols [44].

3-3-2- Antioxidant Activity: The highest antioxidant activity in lemon balm (81.00%) was observed in the mild salinity stress treatment (60 mM NaCl) combined with the application of 3 mM GABA. The lowest antioxidant activity (31.00%) was recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 6).

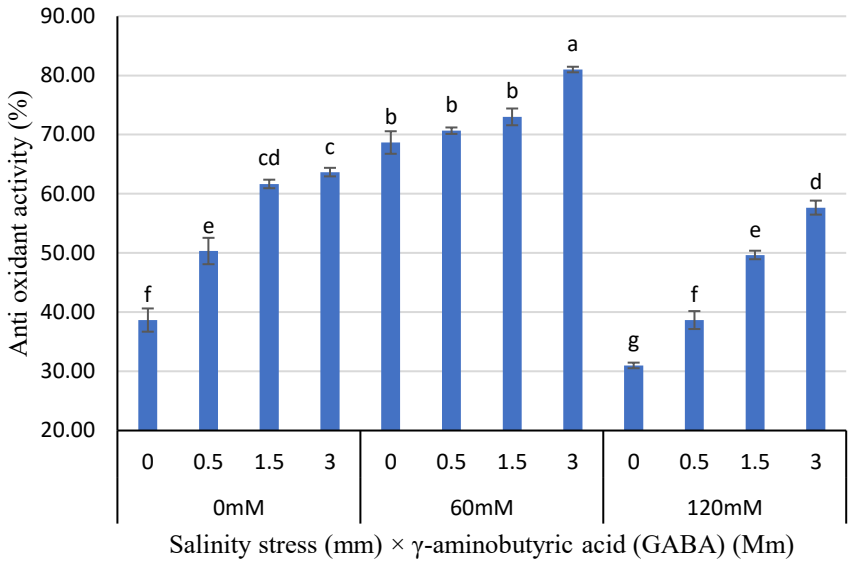

Fig 6 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Anti-oxidant activity of *Melissa officinalis*

3-3-3- Essential Oil Percentage: Based on the results of comparing the means of the interaction effects between salinity stress and GABA foliar application treatments, the highest essential oil percentage in lemon balm (1.62%) was observed in the mild salinity stress treatment (60 mM NaCl) combined with the application of 3 mM GABA. This was followed by a percentage of 1.22% in the mild salinity stress treatment (60 mM NaCl) combined with the application of 1.5 mM GABA. These two treatments belonged to the same statistical group. The lowest percentage (0.52%) was recorded in the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 7).

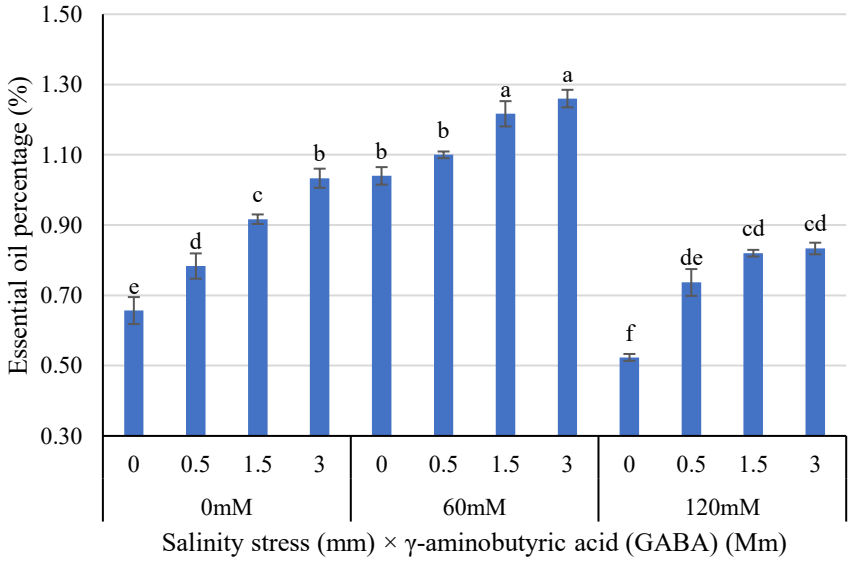

Fig 7 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Essential oil percent of *Melissa officinalis*

3-3-4- Essential Oil Yield: The highest essential oil yield in lemon balm (36.11 grams per plant) was calculated for the mild salinity stress treatment (60 mM NaCl) combined with the application of 3 mM GABA. The lowest essential oil yield (9.65 grams per plant) was recorded for the severe salinity stress treatment (120 mM NaCl) without GABA application (Figure 8).

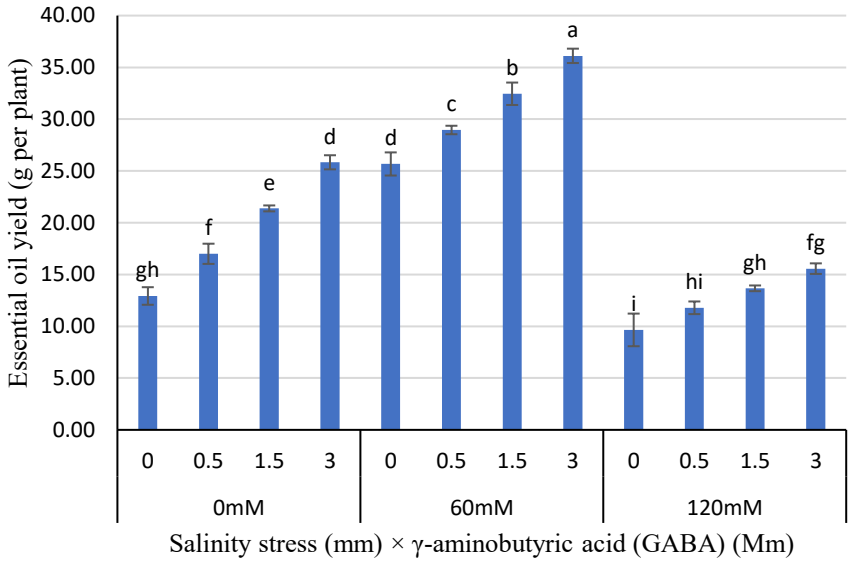

Fig 8 Results of the mean comparison of the interactive effects of salinity stress treatments × γ-aminobutyric acid (GABA) foliar application on the Essential oil yield of *Melissa officinalis*

One of the plant responses to salinity stress is the increased activity of the antioxidant system and alterations in the production of secondary metabolites such as essential oils [6]. Under salinity stress, water uptake by the roots is reduced, and ionic balance within plant cells is disrupted. These conditions lead to the overproduction of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•). At high concentrations, ROS can damage membrane lipids, proteins, and DNA [13]. To counteract this damage, plants activate their antioxidant system. This system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), which neutralize ROS. In addition to enzymes, non-enzymatic compounds like glutathione, ascorbate, and flavonoids also play a role in mitigating oxidative stress. Enhanced antioxidant activity helps plants prevent damage caused by ROS and maintain their survival under saline conditions [12].

Salinity stress stimulates the biosynthetic pathways of secondary metabolites such as terpenoids, flavonoids, and alkaloids. These compounds play a crucial role in plant defense against environmental stresses. In medicinal plants, salinity stress leads to increased production of essential oils. Essential oils are volatile, aromatic compounds primarily composed of terpenoids and phenolic derivatives [10]. Studies have shown that salinity stress is associated with increased expression of key genes in the terpenoid biosynthesis pathway. This upregulation of gene expression results in higher essential oil production [18].

Salinity stress activates signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway and increases the levels of plant hormones, such as abscisic acid (ABA). These signaling changes regulate the expression of genes related to the antioxidant system and the biosynthesis of secondary metabolites [9]. Additionally, salinity stress can lead to the accumulation of compatible solutes such as proline and GABA. These compounds not only act as osmolytes but also play a role in regulating the activity of antioxidant enzymes and the biosynthesis of essential oils [45].

With its antioxidant properties, GABA helps reduce the negative effects of oxidative stress. By mitigating cellular damage caused by free radicals, the plant's antioxidant activity is enhanced, and in turn, the production of antioxidant compounds

increases [46]. The use of this substance strengthens the plant's defense responses. The production of phenols and flavonoids, as part of the plant's defense system against pests and diseases, also contributes to improving essential oil performance [47].
GABA increases the activity of enzymes involved in the biosynthesis of phenols and flavonoids and helps improve nutrient uptake in plants [43]. Nutrients such as phosphorus and potassium play a role in the biosynthesis of phenols and flavonoids, and better availability of these elements contributes to increased production of these compounds [48]. GABA influences sugar and lipid metabolism, acts as an energy source for the production of secondary metabolites like essential oils, and leads to higher essential oil percentage and yield in plants [49]. By affecting pH and cellular environmental properties, it optimizes conditions for the synthesis of phenolic and flavonoid compounds [46].

4- Conclusion

This study demonstrated that foliar application of 3 mM GABA significantly mitigated the adverse effects of salinity stress on lemon balm plants. This treatment significantly improved growth indices (plant height, fresh weight of aerial parts and roots) and increased essential oil percentage. The highest essential oil yield, photosynthetic pigment content, and secondary metabolite accumulation (total phenol and flavonoids) were observed under 60 mM salinity combined with 3 mM GABA. Therefore, foliar application of 3 mM GABA is recommended for lemon balm cultivation in moderately saline areas (≈60 mM NaCl) to improve essential oil yield and overall plant quality. This approach offers a sustainable management strategy to maintain economically viable production of lemon balm under saline conditions.

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Author Contributions

All activities were carried out by the author.

Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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مقاله علمی_پژوهشی

تاثیر گاما-آمینوبوتیریک اسید (GABA) بر رشد و ویژگی‌های فیتوشیمیایی گیاه بادرنجبویه (*Melissa officinalis* L.) در شرایط تنش شوری

مهسا حمیدی‌نیا[۱]، مرضیه قنبری‌جهرمی[۲*]، فریدون قاسم‌خان قاجار[۳]

۱- دانشجوی کارشناسی ارشد، گروه علوم باغبانی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۲*- استادیار، گروه علوم باغبانی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۳- استادیار، گروه علوم زراعت، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

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* مسئول مکاتبات:

ghanbari@iau.ac.ir

چکیده

گاما-آمینوبوتیریک اسید (GABA) یک الیسیتور بیوشیمیایی است که می‌تواند به عنوان یک مولکول سیگنال‌دهنده درون‌زا فعالیت کند. امروزه استفاده از GABA به منظور کاهش اثرات تنش‌های محیطی و افزایش تولید مواد موثره در گیاهان متداول شده است. تحقیق حاضر با هدف بررسی تاثیر GABA (صفر، ۰/۵، ۱/۵، ۳ میلی‌مولار) بر رشد، ویژگی‌های فیزیولوژیکی و بیوشیمیایی گیاه بادرنجبویه (*Melisa officinalis*) تحت تنش شوری (صفر، ۶۰ و ۱۲۰ میلی‌مولار) به صورت فاکتوریل بر پایه طرح کاملا تصادفی در سه تکرار انجام شد. نتایج نشان داد که تنش شوری ۱۲۰ میلی‌مولار سبب کاهش معنی‌دار صفات مورفولوژیک ارتفاع بوته، وزن تر اندام هوایی و ریشه و میزان رنگیزه‌های فتوسنتزی در گیاه گردید. با کاربرد GABA ۳ میلی‌مولار بیشترین مقدار فنول، فلاونوئید کل و فعالیت آنتی‌اکسیدانی که با هم همبستگی مثبت دارند، مشاهده شد. در شرایط افزایش شدت تنش شوری تا ۶۰ میلی‌مولار میزان اسانس گیاه افزایش پیدا کرد، اما با افزایش شدت تنش شوری تا ۱۲۰ میلی‌مولار، درصد و عملکرد اسانس کاهش یافت. به طور خلاصه، در حالی که بادرنجبویه به تنش شوری حساسیت نشان می‌دهد، محلول‌پاشی GABA به طور موثری اثرات نامطلوب آن را با افزایش رشد و تحریک تولید متابولیت‌های ثانویه ارزشمند کاهش می‌دهد. محلول‌پاشی با ۳ میلی‌مولار GABA به عنوان یک استراتژی عملی برای بهبود ظرفیت آنتی‌اکسیدانی و عملکرد اسانس در شرایط شوری متوسط تا ۶۰ میلی‌مولار NaCl توصیه می‌شود و از کشت پایدار این گیاه دارویی در مناطق شور حمایت می‌کند.