Phytochemical and Morpho-physiological Responses of Lemon Balm (*Melissa officinalis* L.) to Biostimulants Application

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Abstract

Background: **Biostimulants are amino acids and organic components, which can play main role in the plant growth and dry matter accumulation.**

Objective: **Determine the influence of foliar application of active amino acids on quality and quantity yield of *Melissa officinalis* L.**

Methods: **This study was conducted in a research farm at the institute of medicinal plants on the base of randomized complete blocks design (RCBD) with three replications. The treatments were control (foliar application with distilled water) and commercial formulations of aminolforte, kadostim, fosnutren, and humiforte (each of them 2 L ha^{-1}), and chemical fertilizer (70 kg ha^{-1} N.P.K).**

Results: **The results indicated that a significant increase in the content of citronellal, neral, delta-cadinene, germacrene, and geranial, were found in response to aminolforte and fosnutren application. The essential oil content was also increased through using kadostim and chemical fertilizer. A significant improvement in the plant height, number of flowers per stem, and SPAD value were observed in response to the foliar application of aminolforte. Morover, foliar application of fosnutren improved leaf length, leaf number, leaf area, leaf dry weight, and shoot dry weight, accordingly. There was no variation in the number of branches per plant among the treatments of kadostim and aminolforte; both of these treatments improved the attribute.**

Conclusion: **The foliar application of free amino acids based on commercial formulations including aminolforte, kadostim, humiforte, and fosnutren can be effective on the quality and quantity yield of lemon balm.**

Keywords: ***Melissa officinalis*** **L., Amino acids, Essential oil, Foliar application, Quality and quantity yield**

Introduction

Growth is a multifactor dependent rhythmic phenomenon. Besides the availability of nutrients, plants require growth regulators for their growth and development and these are available/ synthesized endogenously in the plant system. Under favorable conditions, the levels of endogenous growth promoting substances are higher than that of inhibiting substances which, indeed, accelerate growth and development of the plant [1, 2]. Several organic products called "biostimulants" are now available in the market to make agriculture more sustainable [3]. Agricultural biostimulants include diverse formulations of compounds, substances and micro organisms that are applied to plants or soils to improve crop vigour, yields and quality [4].

The crop life cycle from seed germination to plant maturity can influence by biostimulants in some certain ways, that include increase plant metabolism efficiency, improve plant tolerance to abiotic stresses, facilitating nutrient assimilation, translocation and use, enhancing quality attributes of produce, including sugar content, colour, fruit seeding, etc., increasing water use efficient, enhancing certain physicochemical properties of the soil and support to the development of complementary soil microorganisms. Commonly used biostimulants are amino acid formulations, mixtures of nutrients, hydrolyzed proteins, triacontanol, humic acids, seaweed extracts and brasinolides [2, 5, 6]. Some studies expressed that foliar application of these chemicals, are able to enhance plant productivity [7, 8]. Cerdan *et al*., (2009) and Ertani *et al*., (2009) reported nutrient uptake in particular nitrogen and iron increased in corn and tomato plants with using of protein hydrolysates. Also they stated that increasing nitrogen and iron uptake, lead to enhancing activities of nitrate reductase and glutamine synthetase, and Fe (III)-chelate reductase activity, respectively [9, 10].

Amino acids are biologically important organic compounds which composed of amine and carboxylic acid functional groups. The amino acids have various functions in plants. So that they are vital for the synthesis of proteins or used to precursors for various metabolites with multiple functions in plant growth and development such as hormones, cell wall components, and a large group of secondary metabolites. For example methionine is a precursor for ethylene and polyamines such as spermine and spermidine, which synthesized from *S*–adenosyl methionine. Tryptophan is a precursor for auxin synthesis. Glycine and glutamic acid are fundamental for chlorophyll synthesis. Proline helps to pollen fertility or lysine, methionine, glutamic acid is essential amino acids for pollination. Also, amino acids have a chelating effect on micronutrients and when applied together with micronutrients, the absorption and transportation of micronutrients inside the plant are easier. Amino acids exudated from the plant's root and they help in improving the microflora of the soil, thereby facilitating the nutrient uptake [11, 12, 13].

Nutritional spray on plants can decrease the delay between absorption and consumption of elements of plant that is very important for fast growth stage of plant [14]. Follet *et al*., (1981) reported that chlorophyll content is related to the amount of nutrient uptake by plants from the soil [15]. Khan *et al*., (2002) reported foliar application of chitosan for enhanced physiological efficiency of plants [7]. Mustafa *et al*., (2009) studied the effect of Plant Growth Regulators (PGRs) for improvement of ornamental plants [16]. There are several reports on the effect of growth promoters on crop with particular reference to increased vegetative growth characters, the quantity and quality yield [17]. Increased values of biochemical constituents strengthened the role of biologically active amino acids in the metabolism of tea plants (*Camellia* sp.) [2].

Lemon balm (*Melissa officinalis* L.) belongs to the family Lamiaceae is an aromatic perennial herb, up to about 1 m hight. It is native to the eastern Mediterranean region and western Asia is also widely cultivated in Europe [18, 19, 20]. It has long been used for its soothing effects and herbal aromatic properties [21]. Oil of balm has also been shown to have antiviral, antibacterial and antispasmodic activity [19, 22]. The highest levels of essential oil have been extracted in late summer from the lower parts of the plant [23]. Lemon balm has traditionally been employed against catarrh, fever [24], flatulence and headache [25].

Knowledge of medicinal plant production, particularly biological active compounds, is important when developing effective management programs. Comprehension of the effects and possible mode of actions of biostimulant based on amino acids spray on lemon balm production are fragmentary. An understanding of biostimulant induction based on amino acids application might help develop an optimum cultivation program or achieve uniform stands for research. Therefore, the present research was aimed to document the influence of foliar applied active formulations of amino acids on the quality and quantity of lemon balm.

Materials and Methods

Field experiment

To investigate the effects of biostimulants on growth and phytochemical characteristics of *Melissa officinalis* L., a field experiment was conducted at the Medicinal Plants Institute (MPI) affiliated with the Academic Center for Education, Culture and Research (ACECR) in 2014. The geographical and climatic characterizations of experimental field as well as properties of the soil are shown in Table 1. This study was done on the base of randomized complete blocks design (RCBD) with three replications. The treatments were commercial formulations of aminolforte, kadostim, fosnutren, and humiforte (each of them at 2 L ha^{-1}), chemical fertilizer (70 kg ha^{-1} N.P.K), and control treatment (foliar application with distilled water). The details of formulations are shown in Table 2.

The seeds with a suitable quality of germination were prepared from the seed bank (175-MPISB) of the institute of medicinal plants. The seeds were sown in the rows with 30 cm distance from each other, and 30 cm inter-row spacing at January 2014. The space between replications was 1.5 m and distance from each plot side was considered 1 m. The irrigation and other field practices were done as needed. Three foliar replications of the biostimulants and chemical fertilizer were carried out during the month April-June. The biostimulants diluted with distilled water and were sprayed three times during growth stages with fifteen day intervals on the aerial parts of the plant. The first spray 45 days after sowing was performed and other foliar application were done 60 and 75 days after sowing, respectively. To increase the absorption of solutions by plants, foliar application of biostimulants were performed under without wind or rain conditions and before sunrise when plant stomata are open. The sampling of the plant in the onset of flowering was conducted.

Table 1- Characterization of the experimental field

I. Geographical and climatic properties of the experimental field				
Longitude	Latitude	Altitude (m)	Average annual rainfall (mm)	The annual mean temperature (°C)
50◦ 53' 7"	35◦ 54' 17"	1461	263	13.21

II. Soil properties of the experimental field					
Depth of soil (cm)	EC* (dS m^{-1})	pH	N (%)	P (ppm)	K (ppm)
0-30	0.93	7.9	0.08	36.2	498

*Electrical Conductivity

Table 2- The formulation of biostimulants used in the experimental treatments.

Biostimulants*	Formulation of compounds**
Aminolforte	3750 mg L^{-1} free amino acids, 2 % organic components, and 1.1 % total N (0.8 % urea N, and 0.3 % organic N)
Kadostim	3750 mg L^{-1} free amino acids, 2 % organic components, and 4.2 % total N (0.8 % ammonia N, 3.1 % nitric N, and 0.3 % organic N), and 6 % potassium (K_2O)
Humiforte	3750 mg L^{-1} free amino acids, 2 % organic components, and 6 % total N (1.4 % ammonia N, 3.7 % urea N, 0.5 % nitric N, 0.3 % and organic N), 5 % potassium (K_2O), and 3 % phosphorous (P_2O_5)
Fosnutren	3750 mg L^{-1} free amino acids, 2 % organic components, and 3.8 % total N (2.1 % ammonia N, 1.4 % nitric N, and 0.3 % organic N), and 6 % phosphorous (P_2O_5)

* Biostimulants supplied by Inagrosa Industries Agro Biological are compatible with the climate of Iran.
** Quantity and kind of free amino acids applied in the formulation of bio-stimulators in this experiment based on the percent of total amino acids are as follows: 11.2 % glycine, 5.1 % valine, 8.3 % proline, 13.2 % alanine, 4.4 % aspartic acid, 8.3 % arginine, 0.9 % glutamic acid, 5.1 % lysine, 16.4 % leucine, 4.4 % isoleucine, 5.1 % phenylalanine, 4.2 % methionine, 3.9 % serine, 0.3 % treonine, 0.3 % histidine, 1.5 % tyrosine, 0.9 % glutamine, 0.3 % cysteine, 0.4 % asparagine, 0.4 % tryptophan.

Studied traits

The measured traits are as follows: plant height, shoot dry and fresh weight, plant dry weight, leaf dry and fresh weight, leaf length and width, leaf number, number of flowers per stem, SPAD value (SPAD), leaf area, and phytochemical traits including of essential oil percent and its components. To measure leaf area, a portable leaf area meter was used with manual scan. For measurement of SPAD value, 10 leaves above the stem in each plant were selected and the mean of the leaf SPAD value was measured by the SPAD apparatus (Minolta, 502) [26].

Essential oils analysis

The aerial parts of the plants were harvested at the beginning of flowering stage. The harvested materials were air-dried in a shaded place at a convenient temperature and in an air-flow during 7 days. The samples were transferred to phytochemical analysis laboratory, for determine the percentage of essential oils according to the European Pharmacopoeia method [27]. Essential oils of the aerial parts were extracted by hydro-distillation method for 3 h using Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and kept on 4 °C until it was analyzed [27]. The extracted essential oils were identified by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) analysis. GC/MS analyses were carried out in Agilent GC/MS-6890 gas chromatograph. The operating condition was as follows: Carrier gas: helium with a flow rate 0.8 ml min^{-1}; column temperature, 5 min at 50 $^{\circ}$C, 3 min at 240 $^{\circ}$C and finally 3 min at 300 $^{\circ}$C; injector temperature: 290 $^{\circ}$C; detector temperature: 290 $^{\circ}$C, the volume injected: 1 ml of the oil in chloroform (5%); split ratio, 1:53. The mass spectra operating parameters are as follows: electron ionization (EI): 70 eV; ion source temperature: 220 $^{\circ}$C; Solvent delay: 3.0 min, scan speed: 2000 amu s^{-1}. Identification of components of the essential oil, citronellal, neral, delta-cadinene, germacrene, and geranial extracts was based on direct comparison of the retention times and mass spectra data with those for standard compounds and computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of fragmentation patterns of the mass spectra with those reported in the literature [28]. Each extraction and the compound percentages were replicated three times.

Statistical analysis

All data were subjected to the statistical analysis based on a randomized complete block design (RCBD) with six treatments and three replications. Analysis of variance of the results was done using the SPSS software (ver. 17), and the treatments means were compared using a Duncan's multiple range test at a probability level of 0.05.

Results

Morpho-physiological traits

The results indicated that the application of plant biostimulants based on amino acids on the lemon balm had a significant effect on plant height, leaf length, leaf width, leaf number, stem dry and fresh weight, leaf fresh weight, shoot dry and fresh weight, and number of flowers ($p \leq 0.01$), and also, leaf area, and SPAD value ($p \leq 0.05$). But, the number of branches was not affected with various treatments (Table 3). The mean comparisons of traits showed that the maximum plant height (64.5 cm), and number of flowers (55.3) was observed in aminolforte, while the minimum rate of them (47.7 cm, and 16, respectively) was obtained in the control treatment. Among the various treatments, plant height was increased significantly with the use of chemical fertilizer, aminolforte, and humiforte. Although, the number of flowers under chemical fertilizer and control treatments were not significantly different, it significantly enhanced by biostimulants application (Table 4).

The highest leaf length (52.6 mm) was observed in fosnutren treatment, and the lowest value (42.2 mm) was obtained in the control treatment. Leaf area of lemon balm was increased by applying aminolforte, chemical fertilizer, and fosnutren, compared to the control treatment (11.1, 12.2, and 32.9%, respectively), while, it was not significant differences between kadostim, humiforte, and control treatment. The maximum and minimum leaf numbers were observed in the fosnutren application (74.3), and control treatment (39.3), respectively. The mean comparison also showed that the number of branches had no significant difference between control treatment (6.3), chemical fertilizer (6), humiforte (7.7), and fosnutren (7). The highest number of branches was obtained to aminolforte, and kadostim (8) (Table 4).

Table 3- Analysis of variance for effects of biostimulants on morphological parameters of *Melissa officinalis* L.

S.O.V	df	Mean Square										
		Leaf number	Leaf area	Leaf lenght	Leaf width	Height	Number of flowers	Number of branches	Leaf dry weight	Stem dry weight	Shoot dry weight	SPAD value
Treatment	5	628.22**	83.05*	45.5**	18.4**	95.85**	749.52**	2.23ns	0.144**	0.065**	0.27**	6.59*
Replication	2	1.39	12.59	1.25	0.273	11.78	17.55	0.167	0.014	0.003	0.001	1.380
Error	10	11.98	24.41	4.14	2.29	6.43	13.42	0.7	0.010	0.004	0.009	1.631
CV	-	2.29	9.76	4.27	4.77	4.45	9.93	11.68	6.18	7.01	3.76	4.08

Ns: non significant differences; *: significant at $p<0.05$; **: significant at $p<0.01$

Table 4- Mean comparison for the effects of biostimulants application on morphological traits of lemon balm (*Melissa officinalis* L.)

Treatment	Plant height (cm)	Leaf number	Leaf area (cm^2)	Leaf lenght (mm)	Leaf width (mm)	Number of flowers	Number of branches
Control	47.7^{c}	39.3^{e}	45.3^{c}	42.2^{c}	27.7^{c}	16^{d}	6.33
Chemical fertilizer	60.4^{ab}	47^{d}	51^{b}	48.3^{b}	32.2^{b}	20.3^{d}	6
Aminolforte	64.5^{a}	68.3^{b}	50.33^{b}	50^{ab}	30.9^{b}	55.3^{a}	8
Kadostim	56.9^{bc}	73.3^{ab}	47.8^{bc}	48.7^{b}	35.2^{a}	48.3^{b}	8
Humiforte	54.5^{b}	57.3^{c}	47.2^{bc}	43.7^{c}	30.1^{bc}	35.7^{c}	7.66
Fosnutren	57.3^{bc}	74.3^{a}	60.2^{a}	52.6^{a}	31.1^{b}	45.7^{b}	7

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability

Table 4- Continued

Treatment	Stem dry weight ($t\ ha^{-1}$)	Leaf dry weight ($t\ ha^{-1}$)	Shoot dry weight ($t\ ha^{-1}$)	SPAD value (SPAD)
Control	0.877^{b}	1.457^{c}	2.334^{c}	29^{b}
Chemical fertilizer	1.105^{a}	1.638^{b}	2.744^{b}	32.44^{a}
Aminolforte	0.994^{ab}	1.618^{b}	2.612^{b}	32.83^{a}
Kadostim	0.757^{c}	1.537^{b}	2.294^{c}	30.8^{ab}
Humiforte	0.717^{c}	1.516^{b}	2.233^{c}	30.43^{ab}
Fosnutren	0.96^{b}	1.934^{a}	2.894^{a}	32.31^{a}

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability

Shoot dry weight was improved by chemical fertilizer, aminolforte, and fosnutren, but humiforte and kadostim had no significant effect on this trait in compare with control treatment. The maximum shoot dry weight (2.99 t ha^{-1}) was observed in fosnutren, and its minimum (2.23 t ha^{-1}) was obtained in humiforte treatment. Also, leaf dry weight was enhanced with biostimulants and chemical fertilizer application. The highest and lowest amount of leaf dry weight were observed in fosnutren (1.93 t ha^{-1}) and control treatment (1.45 t ha^{-1}), respectively. A comparison between control treatment and various biostimulants revealed that the stem dry weight was enhanced (26%) with chemical fertilizer, but it was reduced with humiforte, and kadostim application (13.7, and 18.2%, respectively) (Table 6). The highest and lowest leaf width (35.2 mm, and 27.7 mm) were obtained by kadostim and control treatments, respectively. The maximum SPAD value (32.83 SPAD) was gained in aminolforte treatment, but there was no significant different among all amino acid formulations treatments (Table 4).

Phytochemicals traits

The results showed that the application of amino acid compounds had a significant effect ($p \leq 0.01$) on the content of plant essential oil and its major components (germacrene, delta-cadinene, neral, and citronelle) (Table 5). Among the biostimulants, only kadostim and fosnutren increased the percent of essential oil. The highest essential oil was observed in kadostim and chemical fertilizer, while the lowest content was obtained in control, aminolforte, and humiforte treatments. Use of chemical fertilizer and fosnutren led to the reduction of germacrene, when it compared with the control treatment. The highest and lowest amount of germacrene was observed in aminolforte (2.89%) and chemical fertilizer (0.37%), respectively. In comparison with control, the content of citronelle and neral were increased with the biostimulants application. However, they were decreased by using chemical fertilizer. Hence, the highest content of citronelle and neral was observed in aminolforte, and the least content of them was acquired in chemical fertilizer. The applied biostimulants and chemical fertilizer reduced delta-cadinene content in compare to the control treatment, excepted aminolforte. The maximum and minimum content of delta-cadinene was observed in aminolforte (4.15%) and chemical fertilizer (1.23%), respectively. Although, the application of chemical fertilizer increased the content of essential oil but, it reduced the major components of essential oil. Though, the essential oil content was reduced under aminolforte treatment, but its major components was enhanced (Table 6).

Table 5- Analysis of variance for the effects of biostimulants application on phytochemical traits of lemon balm (*Melissa officinalis* L.)

S.O.V.	df	Mean Square					
		Germacrene	Delta-Cadinene	Neral	Citronellal	Geranial	Essential oil
Treatment	5	2.527**	3.257**	278.08	7.616**	146.662**	0.00725**
Replication	2	0.130	0.134	8.615	0.295	24.58	0.0004
Error	10	0.160	0.150	7.487	0.247	5.28	0.0004
CV%	-	29.30	13.78	13.57	23.22	11.95	15.21

Ns: non significant differences; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$

Table 6- Mean comparison for the effects of biostimulants application on phytochemical traits of lemon balm (*Melissa officinalis* L.)

Treatment	Essential oil (%)	Germacrene-D (%)	Neral (%)	Citronellal (%)	Geranial (%)	Delta-cadinene (%)
Control	0.1[c]	1.47[bc]	14.87[c]	0.98[cd]	19.9[b]	3.76[a]
Chemical fertilizer	0.2[a]	0.37[e]	4.78[d]	0.21[d]	6.06[c]	1.23[c]
Aminolforte	0.1[c]	2.89[a]	33.12[a]	4.23[a]	22.54[ab]	4.15[a]
Kadostim	0.2[a]	1.83[b]	25.43[b]	3.57[ab]	20.13[b]	2.59[b]
Humiforte	0.1[c]	0.98[cd]	21.69[b]	2.69[b]	19.93[b]	2.36[b]
Fosnutren	0.15[b]	0.65[d]	21.1[b]	1.18[cd]	26.86[a]	2.81[b]

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability

Discussion

This study showed that the biostimulants increased some of morphological and phytochemical traits of lemon balm in comparison with control. Similar results found by Thomas *et al*. (2009), on tea crop [2], Shahriari *et al*. (2011), on wheat [29], and Rahimi *et al*. (2013), on basil plants [30]. Amino acids are considered as precursors and constituents of proteins, which are important for stimulation of cell growth [29]. Many studies showed that the amino acids which were used for plant fertilization, promoted the processes of a plant respiration, photosynthesis, and water cycle. In addition, the amino acids increased the concentration of ascorbic acid, accelerated protein synthesis, moreover, promoted the plant growth and yield formation [31, 32, 33]. Saeed *et al*. (2005), on soybean found that treatments of amino acids significantly improved growth traits of shoots and fresh weight as well as yield of pod [34].

The maximum plant height (64.5 cm), and number of flowers (55.3) were observed in aminolforte. Some amino acids such as phenylalanine, ornithine can influence on gibberellins biosynthesis [35], as well increasing the height of lemon balm may be was due to this reason. Also, the highest leaf length (52.6 cm), and leaf area of plant (60.2 cm^2) were observed in fosnutren treatment. Some of biostimulants are composed of various amino acid and they promote the development of root system and growth of the aboveground plant part [36]. One of the biostimulants functions is included increasing the plant nutrition uptake. Therefore, they can improve use efficiency of nutrients [37]. Positive effect of amino acids on leaf and yield of lemon balm might be due to stimulating the interaction effects of amino acids and nutrients on the plant cells growth. However, amino acids were introduced by Rahimi (2013) as a source of energy during lack of carbohydrates [30]. In general, these compounds can be supply essential nutrients like nitrogen, phosphorous and potassium and affect on morphological growth of the plant.

The results showed the SPAD value was increased under application of biostimulants based on amino acids. SPAD value is an indicator of leaf chlorophyll content that the improvement of leaves. SPAD value will lead to produce high energy and will increase the quality and quantity of plant yield eventually [38]. Shehata *et al*. (2011), expressed the chlorophyll *a* and *b* content were increased with amino acids using, but carotenoid content was reduced [35]. Thomas *et al*. (2009), reported that the stomatal conductance and leaves chlorophyll content of tea (*Camellia* sp.) were increased with growth regulators application [2]. Fraser and Percival (2003) reported an improvement in chlorophyll content due to the foliar application of commercial biostimulants [39].

This finding indicated that the biostimulants application resulted to increase leaf area and SPAD value. Therefore, the absorption of light radiation, rate of photosynthesis, and biomass production were increased by these factors, which eventually, shoot dry weight improved. Although, leaf dry weight was enhanced under humiforte and kadostim in compared to control, but stem dry weight was decreased. Consequently, shoot dry weight was reduced to the point that there was no significant difference between their and control treatment.

Regarding the phytochemical traits, application of amino acid compounds showed a significant effect on the content of plant essential oil and its components (germacrene, delta-cadinene, neral, and citronelle). These results are in a good line with the findings of Golzadeh *et al*. (2011), on *Matricarai recutita* [40], and Rafiee *et al*. (2013), on *Calendula officinalis* L. [41]. Ardebili *et al.* (2012), indicated that the foliar application of amino acids at suitable concentrations had positive effects on the content of secondary metabolites, antioxidants, and antioxidant activity. They reported that increasing the phytochemical constituents of *Aloe vera* plants by using amino acids can demonstrate which these compounds involved to stimulate plant metabolic pathways [42]. El-Sharabasy *et al*. (2012), reported amino acids have a significant effect on the production of secondary metabolites. They stated that the steroid biosynthesis have a positive correlation with increasing the glutamine concentration, so that the highest steroid content was recorded in the usage of glutamine at 500 mg L^{-1} [43].

The phenylpropanoid pathway serves as a rich source of metabolites in the plants and some secondary metabolite derived from this pathway. The phenylpropanoids are a diverse family of organic compounds that are synthesized by plants from the amino acid phenylalanine [44]. Phenylalanine is an end product of the shikimate pathway, which also gives rise to the aromatic amino acids tyrosine and tryptophan [45]. Also, was reported the biosynthesis of cinamic acids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine [46]. Therefore, the use of biostimulants such as fosnutren and kadostim may be due to increasing the content of aromatic amino acids that resulted in activation of phenylpropanoid pathway and eventually enhanced essential oil content. However, the results of biostimulants application on phytochemicals traits of medicinal plants are related to plant species. Our previous studies showed that the biostimulants application had not significant effect on essential oil content of *Satureja hortensis* L., but these compounds increased essential oil content of the rosemary plant [26, 46]. Biostimulants have a lot of active compounds, amino acids and interaction effects with other elements in its formulations. However, biostimulants have a wide range of effects on plants including enhancing biotic and abiotic stress resistance, and increasing the plant nutrient uptake. Foliar application of biostimulant formulations significantly enhanced plant nutrient uptake from the soil in the treated plants. These can be one of the reasons for the high content of phytochemical yield [47].

Conclusion

The results of the tested different biostimulants lead us to conclude that they had a positive and significant effect on the quality and quantity of lemon balm. The best results were obtained by application of aminolforte and fosnutren treatments. Use of aminolforte had a significant effect on the number of flowers, SPAD value, citronellel, neral, germacrene, and delta-cadinen. Foliar application of fosnutren caused an improvement in the leaf number, leaf area, leaf dry weight, and shoot dry weight. Also, the application of humiforte and chemical fertilizer had a positive effect on the essential oil content.

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References

1. Gensheng P. Study on the relations between growth of tea and endogenous hormones IAA and ABA. *J. Tea Sci.* 1991; 11: 25 - 28.

2. Thomas J, Mandal AKA, Raj Kumar R and Chordia A. Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camellia* sp.). *International Journal of Agricultural Research* 2009; 4: 228 - 236.

3. Colla1 G, Rouphael Y, Canaguier R, Svecova E and Cardarelli M. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Front Plant Sci*. 2014; 5: 448.

4. Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie J, Norrie J and Prithiviraj B. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regulation* 2009; 28: 386 - 399.

5. Mandal AKA, Kumar R and Thomas J. An overview of PGR trials in UPASI TRF. *Planters Chron*. 2007; 103: 12 - 16.

6. Calvo P, Nelson L and Kloepper JW. Agricultural uses of plant biostimulants. *Plant Soil*. 2014; 383: 3 - 41.

7. Khan WM, Prithiviraj B and Smith DL. Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. *Photosynthetica* 2002; 40: 621 - 624.

8. Biles SP, and Cothren JT. Flowering and yield response of cotton to application of mepiquat chloride and PGR-IV. *Crop Sci*. 2001; 41: 1834 - 1837.

9. Cerdan M, Sanchez-Sanchez A, Oliver M, Juarez M and Sanchez-Andreu JJ. Effect of foliar and root applications of amino acids on iron uptake by tomato plants. *Acta Hortic*. 2009; 830: 481 - 488.

10. Ertani A, Cavani L, Pizzeghello D, Brandellero E, Altissimo A, Ciavatta C and Nardi S. Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *J. Plant Nutr. Soil Sci.* 2009; 172: 237 - 244.

11. Tempone AG, Sartorelli P, Mady C and Fernandes F. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. *Cell Mol. Biol*. 2007; 15: 15 - 25.

12. Less H and Galili G. Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. *Plant Physiol.* 2008; 147: 316 - 330.

13. Sharma SS and Dietz KJ. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot*. 2006; 57 (4): 711 - 726.

14. Taiz L and Zeiger E. Plant physiology. 4th Edition. Sinauer Associates. Sunderland. 2002, pp: 690 - 691.

15. Follet RH, Murphy LS and Donalua RL. Soil-fertilizer-plant relationship. *Fertilizer Soil.* 1981; 16: 478 - 481.

16. Mustafa SG, Kaukaband M and Ahmad A. Foliar application of plant growth regulators (PGRs) and nutrients for improvement of lily

flowers. *Pakistan. J. Botany* 2009; 41: 233 - 7.
17. Hampton MO, Schaffer B and Bryan H. Nutrient concentration, growth and yield of tomato and squash in municipal solid-waste amended soil. *Hortic. Sc.* 1994; 29: 785 - 788.
18. Wichtl M and Bisset NG. Herbal drugs and phytophamaceuticals. Second edition. CRC press. 1994, pp: 468 - 469.
19. Leung AY and Foster S. Encyclopedia of Common Natural Ingredients used in Food, Drugs and Cosmetic. 2nd ed. John Wiley and Sons Press. USA. 1886.
20. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. France. 1995, pp: 423 - 425.
21. Sari AO and Ceylan A. Yield characteristics and essential oil composition of lemon balm (*Melissa officinalis* L.) grown in the Aegean Region in Turkey. *Turkish J. Agriculture & Foresty* 2002; 26 (4): 217 - 224.
22. Schultze W, Zaglein A, Hose S, Kubeczka K and Czygan F. Volatiles in flowers of balm (*Melissa officinalis* L.). The Royal Botanic Gardens Press. UK. 1992, pp: 57 - 366.
23. Kennedy D, Little OW, Haskell CF and Scholey AB. Anxiolytic effect of combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. *Phytotheraphy R.* 2006; 20 (2): 96 - 102.
24. Kabala-Dzik A, Stojke R, Szaflarska- Stojko E, Wroblewska- Adamekl T, Stojko A, Stojko J and Stawiarska-Pieta B. Influence of honey balm on the rate of scare formation during experimental burn wound healing in PLGS. *Bull. Vet. Inst. Pulley* 2004; 48: 311 - 16.
25. Weiman Z, Alkrinawi S, Golfarb D and Bitran C. Efficiency of herbal tea preparation in infantile colic. *The J. Pediatr.* 1993; 122: 650 - 652.
26. Foroutan Nia A, Mehrafarin A, Hadavi E and Naghdi Badi H. The influences of bio-stimulators compounds on growth traits and essential oil content of rosemary (*Rosmarinus officinalis* L.). *J. Med. Plants* 2014; 13 (51): 51 - 61.
27. European Pharmacopoeia. *Melissa officinalis* L. Council of Europe, Committee on Herbal Medicinal Products (HMPC), Strasbourg, France. 2005, (01): p. 213.
28. Adams RP. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Allured Publishing. Carol Stream, IL, USA; 2004, pp: 456.
29. Shahryari R and Khayatnezhad M. Humiforte application for production of wheat under end seasonal drought stress. *Advanced Environmental Biological* 2009; 5 (1): 141 - 44.
30. Rahimi A, Mehrafarin A, Naghdi Badi H and Khalighi-Sigarooodi F. Effects of biostimulators and bio-fertilizers on morphological traits of basil (*Ocimum basilicum* L.). *Annals of Biological. Res.* 2013; 4 (5): 146 - 151.
31. Alaru M, Laur U, and Jaama E. Influence of nitrogen and weather conditions on the grain quality of winter triticale. *Agronomy Res.* 2003; 1: 3 - 10.
32. Meijer AJ. Amino acids as regulators and components of nonproteinogenic pathways. *The Journal of Nutrition* 2003; 39: 2057 - 2062.

33. Dromantiene R, Pranckietiene I, Sidlauskas G and Pranckietis V. Changes in technological properties of common wheat (*Triticum aestivum* L.) grain as influenced by amino acid fertilizers. *Zemdirbyste-Agriculture* 2013; 100 (1): 57 - 62.

34. Saeed MR, Kheir AM, and Al-Sayed AA. Supperssive effect of some amino acids against *Meloidogyne incognita* on soybeans. *J. Griculture Science* 2005; 30 (2): 1097 - 1103.

35. Shehata SM, Abdel-Azem HS, El-Yazied AA and El-Gizawy AM. Effect of foliar spraying with amino acids and seaweed extract on growth chemical constitutes, yield and its quality of celeriac plant. *European J. Scientific Res.* 2011; 58 (2): 257 - 65.

36. Nikiforova VJ, Bielecka M, Gakiere B, Krueger S, Rinder J, Kempa S, Morcuende R, Scheible WR, Hesse H and Hoefgen R. Effect of sulfur availability on the integrity of amino acid biosynthesis in plants. *Amino Acids* 2006; 30: 173 - 83.

37. Vernieri P, Borghesi E, Ferrante A and Magnani G. Application of biostimulants in floating system for improving rocket quality. *J. Food, Agriculture and Environment* 2005; 3: 86 - 88.

38. Li YC, Stofella PJ, and Bryan H. Management of organic amendments in vegetables crop production system in Florida. *Soil. Crop. Sci. Soc. Proc.* 2000; 59: 17 - 21.

39. Fraser GA and Percival GC. The influence of biostimulants on growth and vitality of three urbon tree species following transplanting. *Aboricul.* 2003; 27: 43 - 57.

40. Golzadeh H, Mehrafarin A, Naghdi Badi H, Fazeli F, Qaderi A and Zarinpanjeh N. Effect of biostimulators compounds on quantitative and qualitative yield of German chamomile (*Matricaria recutita* L.). *Seasonal Publication of Medicinal Plants* 2011; 8 (1): 195 - 208.

41. Rafiee H, Mehrafarin A, Qaderi A, Kalate Jari S and Naghdi Badi H. Phytochemical, agronomical and morphological responses of pot marigold (*Calendula officinalis* L.) to foliar application of bio-stimulators (bioactive amino acid compounds). *J. Med. Plants* 2013; 12 (47): 48 - 61.

42. Ardebili ZO, Moghadam ARL, Ardebili NO and Pashaie AR. The induced physiological changes by foliar application of amino acids in *Aloe vera* L. plants. *Plant Omics J.* 2012; 5(3): 279 - 84.

43. El-Sharabasy S, Farag MA, El-Emerym, GAE, Safwat G and Diab A. Effect of Amino acids on the growth and production of steroids in date palm using tissue culture technique. *Researcher* 2012; 4 (1): 75 - 84.

44. Vogt T. Phenylpropanoid biosynthesis. *Mol. Plant* 2010; 3: 2 - 20.

45. Rippert P, Puyaubert J, Grisollet D, Derrier L and Matringe M. Tyrosine and phenylalanine are synthesized within the plastids in *Arabidopsis*. *Plant Physiology* 2009; 149: 1251 - 60.

46. Mehrabi S, Mehrafarin A and Naghdi Bad H. Clarifying the role of methanol and amino acids application on savory (*Satureja hortensis* L.). *Annals of Biological Research* 2013; 4 (4):

190 - 95.

47. Foroutan Nia A, Bahman S, Naghdi Badi HA, Mehrafarin A and Labbafi MR. Morpho-physiological and phytochemical traits of gazania (*Gazania rigens*) affected by foliar application of bio-stimulants. *Eur. Asian J. Biosciences* 2015; 9: 21 - 28.