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Original Article

The Effect of Biostimulants based on Free Amino Acids on some Growth and Physiological Parameters of *Dracocephalum moldavica* L. under Salinity Stress

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ABSTRACT

In arid and semi-arid areas salinity reduces agricultural productivity. Biostimulants based on amino acids promote plant growth and yield while significantly reducing abiotic stresses-related injuries. As a result, current study assessed the effect of biostimulants based on free amino acids on Moldavian balm under salinity stress. A factorial experiment using a randomized complete block design with three replications was done. The plants were imposed with four levels of salinity (0, 20, 40 and 60 mM NaCl) and were treated using four formulations of amino acids (Kadostim, Fosnutren, Humiforte and Aminolforte). The results revealed that increasing the salinity level significantly reduced growth parameters such as fresh and dry weight of biomass, root length, shoot length, secondary branch length, crown diameter, internode number, internode length, secondary branch number, leaf number and biochemical parameters such as RWC, chlorophyll *a*, *b*, total and carotenoids while increased some osmolytes such as soluble sugar and proline in root and shoot. Leaf spraying of the bio-stimulates based on amino acids, significantly improved the negative effects of salinity stress on growth and biochemical parameters in Moldavian balm, and among them Kadostim had the most significantly positive effects on morphological and biochemical parameters and followed by Aminolforte.

INTRODUCTION

Today, the increasing tendency to consume medicinal plants and their products has highlighted the role of these plants in the global economic cycle of both developing and developed countries [1]. Overuse of chemical fertilizers in agriculture due to rising costs, soil degradation, unbalanced soil pH, and loss of the structure of soil microorganisms will endanger the environment and human health [2]. It is critical to find sustainable and environmentally friendly alternatives to chemical fertilizers. Biostimulants regulate and promote biological processes as well as plant growth [3]. These products are ecologically friendly and help to the production of crops for sustainable agricultural purposes at a low cost and high yield. Their consumption reduces the use of chemical inputs [4]. Several of these biostimulants are based on amino acids [4]. Studies have shown that amino acids both directly and indirectly affect the physiological activities and growth of the plant [4]. Amino acids as organic nitrogen compounds are the building units of proteins [5]. These compounds play a critical role in the biosynthesis of hormones and yield by increasing resistance to environmental stresses [6]. Moldavian balm is an annual herbaceous plant in the Lamiaceae family. All of the plant's organs contain essential oils, including as geranyl acetate, geranial, geraniol, and neral, which have antimicrobial and antibacterial properties that are used to treat wounds, facilitate digestion, and reduce symptoms of constipation and palpitations [7]. This research was carried out in order to investigate the effect of biostimulants based on free amino acids (Kadostim, Fosnutren, Humiforte and Aminolforte) on the negative effects of salinity stress on Moldavian balm plants.

MATERIALS AND METHODS

This research was conducted in a greenhouse at Shahid Bakri Higher Education Center in Miandoab, Iran. The average temperature at night/day was around 20/30±5 ºC with a relative humidity of 60-80%. A factorial experiment was carried out based on a completely randomized design with three replications to evaluate the effect of four kinds of bio stimulants based on free amino acids (Kadostim, Fosnutren, Humiforte and Aminolforte) on some growth and physiological parameters of Moldavian balm (*Dracocephalum moldavica* L.) under salinity (0, 20, 40 and 60 mM NaCl or EC= 0, 1.8, 3.6 and 5.4 dS/m) conditions. Table 1 shows the compounds of Kadostim, Fosnutren, Humiforte and Aminolforte.

Moldavian balm seeds were taken from the local population of Miandoab, Iran. Initially, 20 Moldavian balm seeds were sown in four liter pots filled with an equal mixture of (v:v:v) garden soil, sand and rotted manure. In the two true leaves stage, the plants were thinned, and five plants were kept in each pot. Plants were irrigated three times each week. Salinity treatments were implemented two months after the start of the experiment. To avoid osmotic stress, the irrigation water's salt content was progressively increased (was started with 20 mM NaCl in irrigation water and increased by 20 mM each time to reach the final concentration of 40 and 60 mM). Throughout the test, salinity levels containing control, 20, 40, and 60 mM, describe normal, low, moderate, and severe salinity conditions, respectively. To prevent excessive accumulation of salt in the root zone, the plants were watered weekly with tap water (about 25% more than the water requirement of the plant until the pot drainage salinity reached the required EC), and then the immediately treated with the saline water. Six times (with a two-week interval) the plants sprayed with the bio stimulants. To evaluate the physiological and biochemical parameters, leaf samples were taken from mature leaves on the middle branch.

Relative water content (RWC) of the leaves was measured according to the method of Ritchie *et al*. [8]. Chlorophyll and carotenoids were measured by Arnon's method [9]. Soluble sugar was measured by method of Dubois *et al*. [10]. Proline was measured according to the method of Bates *et al*. [11].

Statistical Analysis

Statistical analysis of the data was performed with SPSS (version 24) software. Mean comparison of groups were done by Duncan's method at 5% probability level. Slicing was done based on salinity in parameters where the interaction between factors was significant.

RESULTS AND DISCUSSION

Growth Parameters

The interaction of salinity and bio stimulants significantly affected growth parameters such as fresh and dry weight of biomass, root length and shoot height, crown diameter, leaf number, internode number, internode length, secondary branch length, leaf length, leaf width and RWC. In addition, simple effect of salinity and amino acids significantly affected secondary branch number (Table 2). The foliar application of the bio stimulants, biased on amino acids, increased fresh and dry weight of biomass compared to the control plants (Fig.1). Bio stimulants such as amino acids by increasing the absorption of water and nutrients, especially nitrogen, increase photosynthesis and this causes the production of more assimilates and the improvement of plant growth and height [12].

Table 1 The compounds of biostimulants in this study

Biostimulants (based on amino acid)	Free amino acids (mg/L)	Organic matters (%)	Nitrogen (%)	P_2O_5 (%)	K_2O (%)
Kadostim	3750	2	-	3	
Fosnutren	3750	2	3.8	6	-
Humiforte	3750	2	6	5	3
Aminolforte	3750	2	1.1	-	-

The biostimulants are the same in amino acids such as Glysin (11.2%), Valine (5.1%), Proline (8.3%), Alanine (13.2%), Aspartic acid (4.4%), Arginine (8.3%), Glutamic acid (0.9%), Lysine (5.1%), Leucine (16.4%), Isoleucine (4.4%), Phenylalanine (5.1%), Methionine (4.2%), Serine (3.9%), Threonine (0.3%), Histidine (0.3%), Tyrosine (1.5%), Glutamine (0.9%), Cysteine (0.3%), Asparagine (0.4%), and Tryptophan (0.4%), and also in organic matter content, while they are different in NPK content.

Under low, moderate and severe salinity stress, root length decreased by 13, 14 and 19% compared to normal, respectively. Under severe salinity stress, shoot length significantly decreased by 19% compared to control. Foliar application of amino acids under all salinity levels significantly improved root and shoot length compared to untreated plants with amino acids. Maximum root length, under control and low salinity stress conditions belonged to the treated plants with Kadostim, followed by Aminolforte and under moderate and severe salinity stress belonged to the treated plants with Aminolforte. Maximum shoot length under all salinity levels belonged to the treated plants with Kadostim. Under normal and severe salinity stress, there was no a significant different between shoot length in the treated plants with Kadostim and Aminolforte (Fig. 2). According to Table 2, Kadostim had 3% P_2O_5, and phosphorus (P) is an essential element that improved root system [13]. Because supplied P by Kadostim increased chlorophyll *b* concentration (Fig. 6) and subsequently enhanced the absorption of indicate light energy, so that the net photosynthetic rate increases the shoot length and biomass of Moldavian balm with the foliar application of Kadostim. Kadostim contains 2% organic matter, 3% P_2O_5, nitrogenous compounds and various amino acids, specially glycine (Table 1), which increases nitrogen absorption by the plant, and thereby increase factors related to plant growth, including root and shoot length. Amino acids and nitrogen in the initial stages of growth increase the elongation shoots and roots. Similar results showed that foliar application of bio stimulant application caused significant increase growth parameters of lettuce plants grown under deficit irrigation conditions [14]. Among the amino acids compounds, tryptophan is a precursor to the production of the auxin hormone, which increases the elongation of cells and, as a result, increases the plant height [15].

With an increase in the salinity concentrations, gently decreased internode number and internode height and foliar application of all amino acids significantly increased them compared to untreated plant. Under normal, low, moderate and severe salinity stress, Kadostim increased internode number by 68, 68, 64 and 92% and Aminolforte increased internode number by 63, 75, 100 and 23%, compared to untreated plant, respectively. Under normal, low, moderate and severe salinity stress, Fosnutren increased in internode length by 57, 62, 52 and 52% compared to untreated plant, respectively (Fig. 3). Biological stimulants such as amino acids increase the absorption of water and nutrients, especially nitrogen, increase photosynthesis, and as a result, produce more assimilates and plant growth and increase plant height [16].

Kadostim and Humiforte significantly improved leaf number and under severe salinity stress, Humiforte and Aminolforte increased significantly leaf number compared to other treatments (Fig. 4). Salinity caused to leaf fall and reduced number of leaves; in fact, reducing leaves number is a mechanism to resist salinity. By accelerating the absorption of water and nutrients and increasing vegetative growth, amino acids have caused the development of the number of plant leaves. This result was in agreement with the results of the effect of amino acids on medicinal mint [17]. Severe salinity stress significantly decreased crown diameter by 44% compared to control and application the Kadostim, Fosnutren, Aminolforte and Humiforte in all salinity concentration significantly increased crown diameter compared to untreated plants. Fosnutren increased crown diameter under normal, low and moderate salinity stress by 68, 87 and 68% compared to control plants, respectively, and followed by Kadostim. Under severe salinity stress condition Kadostim increased crown diameter by 128% compared to control (Fig. 4). Previous research showed that the crown diameter of *Thuja orientalis* increased with the increase in the concentration of the amino acids [18]. In addition, the use of these bio stimulants in *Mentha spicat* increased crown diameter under salinity stress conditions [17].

Leaf length and leaf width significantly affected by the interaction of salinity concentration and different amino acids. Rising salinity concentration significantly decreased leaf length and leaf width. The all amino acids increased leaf length and leaf width compared to untreated plants (Fig.5). By increasing in salinity concentrations as 20, 40 and 60 mM, secondary branch number gently decreased by 12, 44 and 24% compared to the control, respectively (Fig. 5).

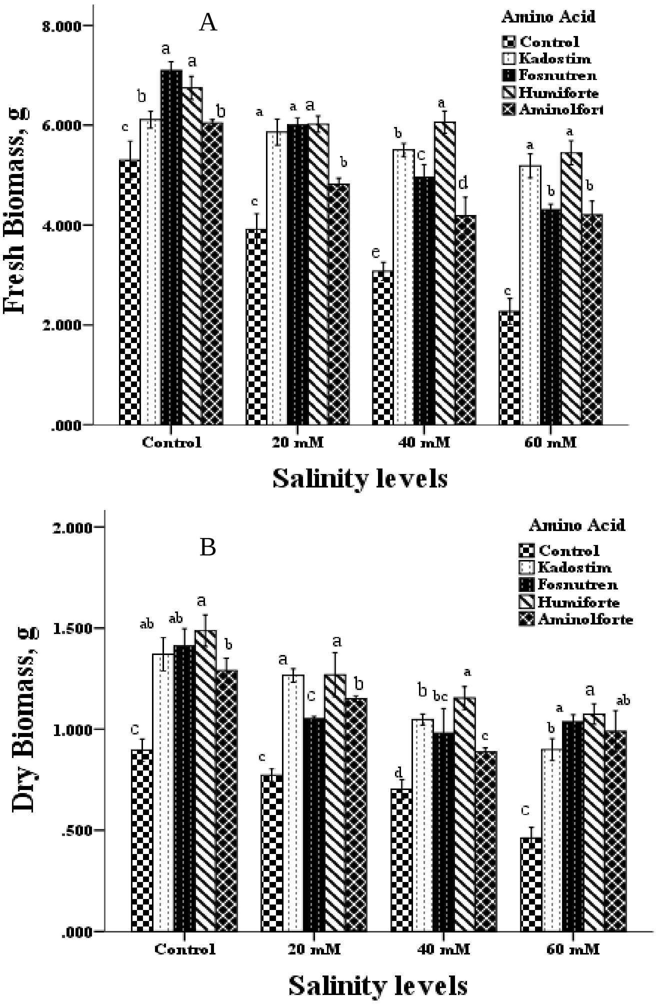


Fig. 1 Slicing the interaction effects between salinity levels and different amino acid compounds on shoot fresh weight (left) and shoot dry weight (right) in *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

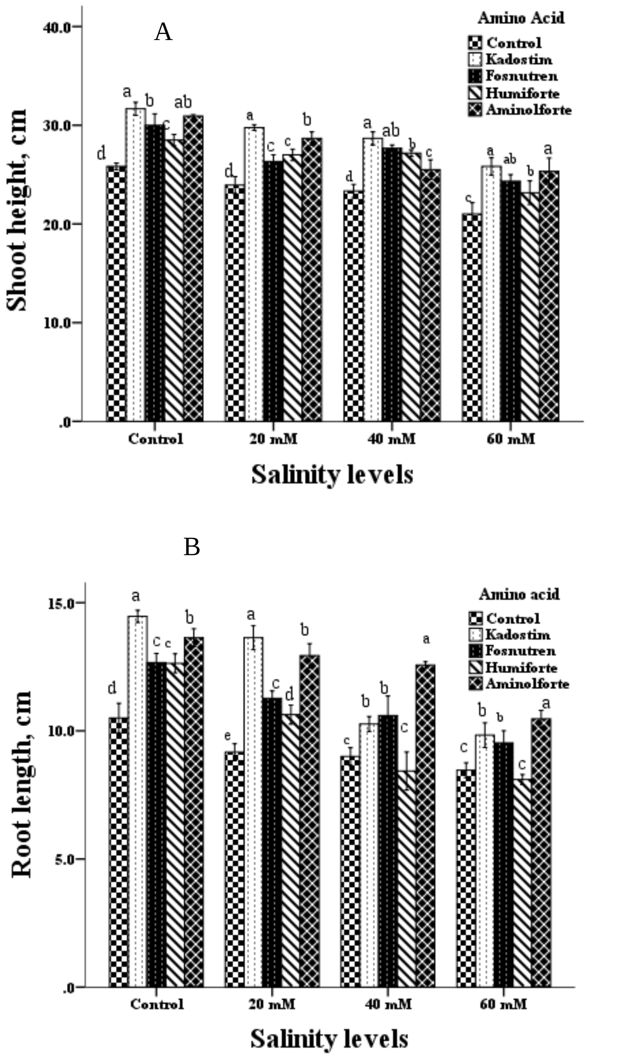


Fig. 2 Slicing the interaction effects between salinity levels and different amino acid compounds on root length (left) and shoot height (right) in *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

Table 2 Variance analysis of experimented factors affect growth parameters and RWC.

Source of variation	df	Fresh biomass	Dry biomass	Shoot length	Root length	Internode number	Internode length
Salinity (S)	3	10.82 **	0.47 **	75.58 **	35.37 **	20.51 **	2.22 **
Amino Acid (AA)	4	11.11 **	0.51 **	48.96 **	21.28 **	27.39 **	3.84 **
S × AA	12	0.57 **	0.02 **	2.18 **	1.86 **	1.94 **	0.08 **
Error	40	0.04	0.003	0.46	0.13	0.37	0.02
CV (%)	-	23.31	23.91	10.53	17.61	24.07	17.45
Source of variation	df	Leaf number	Crown diameter	Secondary branch number	Leaf length	Leaf width	RWC
Salinity (S)	3	271.11 **	1.09 **	26.37 **	307.18 **	119.38 **	354.48 **
Amino Acid (AA)	4	154.28 **	2.07 **	31.69 **	222.23 **	73.73 **	89.32 **
S × AA	12	8.88 **	0.09 **	0.93 ns	23.85 **	4.29 **	14.84 **
Error	40	1.75	0.004	0.50	1.01	0.47	0.45
CV (%)	-	10.41	22.72	21.95	18.83	14.88	6.73

ns, and **, non- significant and significant at 1% level of probability. RWC, Relative water content

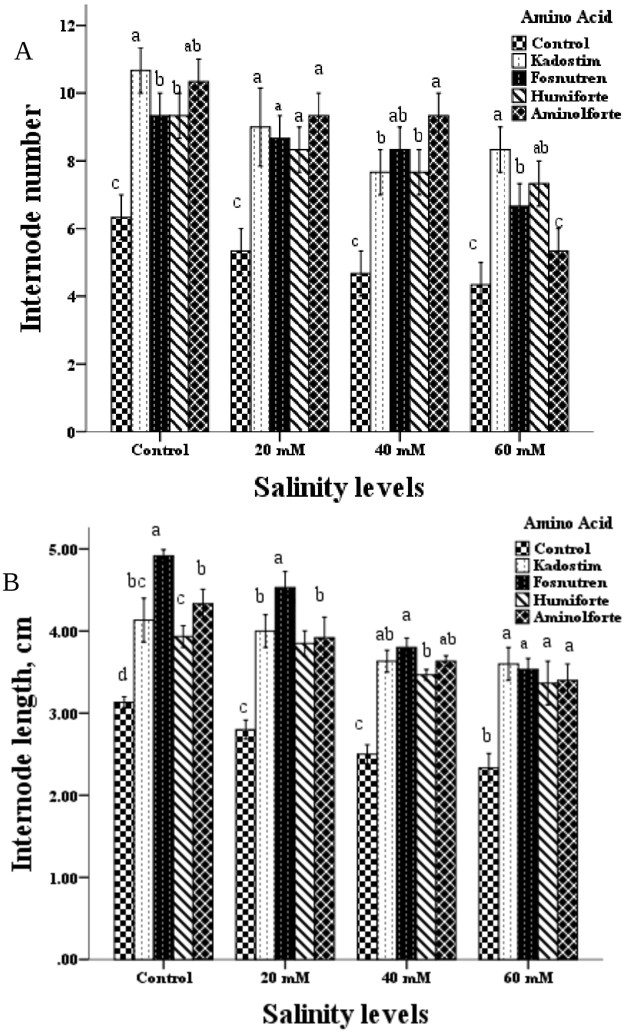


Fig. 3 Slicing the interaction effects between salinity levels and different amino acid compounds on internode number (left) and internode length (right) in *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

Foliar application of all amino acids significantly increased the secondary branch number and among them Kadostim had the most positive effect and increased secondary branch number by 68% compared to control plants (Fig. 5).

RWC

Relative water content of leaves decreased in proportion to increasing of salinity concentrations and different amino acids tended to increase relative water content compared to the control. However, foliar application of Aminolforte at 0 and 20 mM, as well as 60 mM salinity treatment resulted in significantly higher relative water content of leaves compared to the control plant (Fig. 7). The decrease in RWC in the leaves under salinity stress is related to negative osmotic potential. Saline condition prevents water absorbing, and the plant suffers from dehydration and causes the stomata to close. With increasing salinity levels in the soil, decrease the soil water potential, leading to reduce relative water content compared to the control samples [19].

Physiological Parameters

The interaction of salinity and bio stimulants significantly affected physiological parameters such as chlorophyll *a*, *b*, *a*/*b*, total chlorophyll, carotenoids in leaf, and proline and soluble sugar in root and shoot (Table 3).

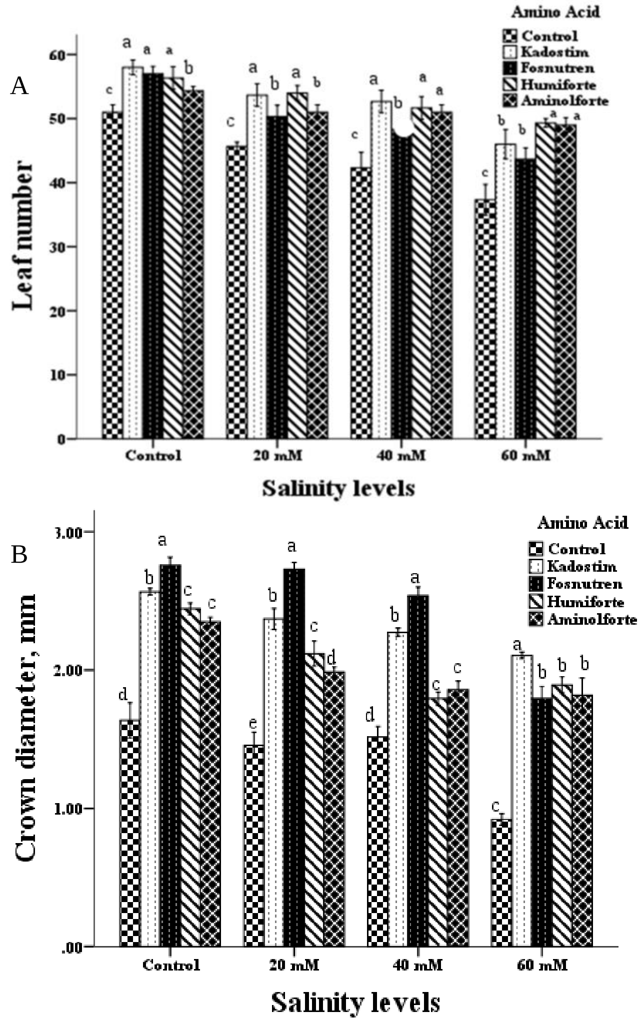
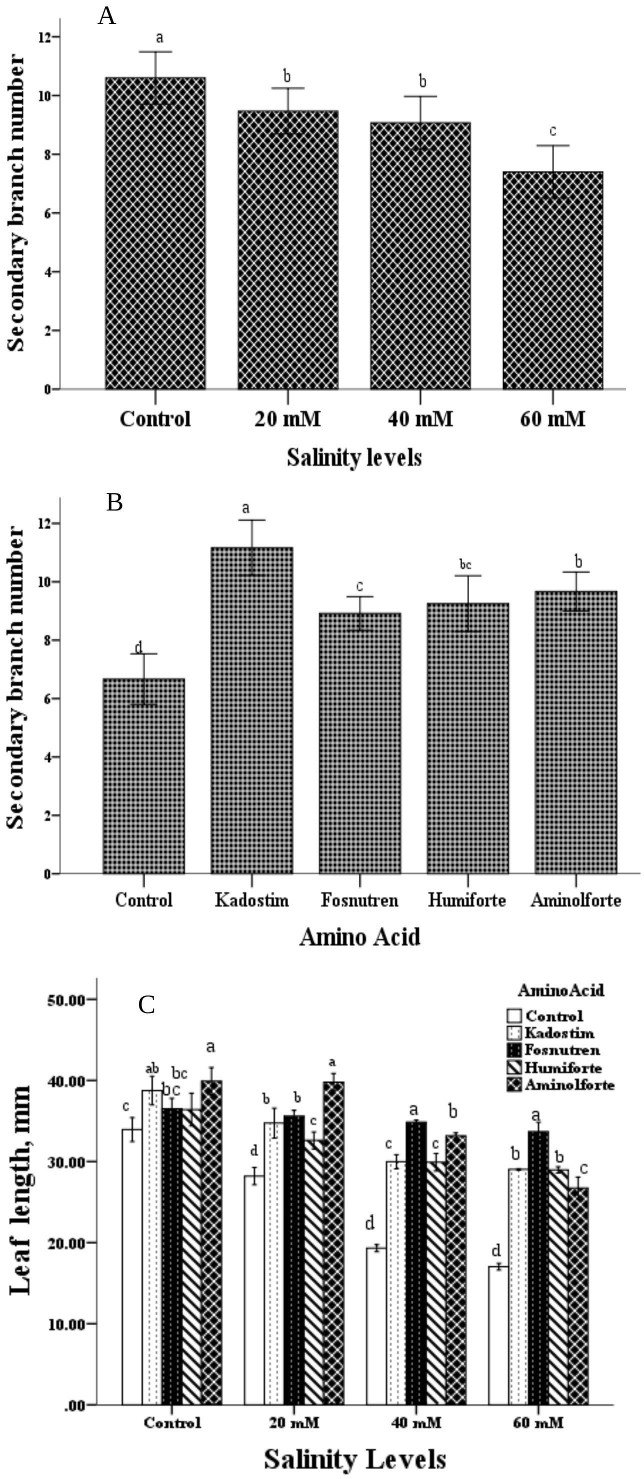


Fig. 4 Slicing the interaction effects between salinity levels and different amino acid compounds on leaf number (left) and crown diameter (right) of *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

Photosynthetic Pigments

With increasing in salinity concentration chlorophyll, *a*, *b*, total chlorophyll and carotenoid significantly decreased (Fig.7). Under low, moderate, and severe salinity stress decreased chlorophyll *a* by 12, 26 and 53%, chlorophyll *b* by 34, 36 and 55% and total chlorophyll by 15, 24 and 49%, respectively, compared to control. Leaf spraying of all amino acids significantly increased the contents of chlorophyll *a*, *b*, total chlorophyll and carotenoid for example Aminolforte under normal conditions, low, moderate and severe salinity stress increased chlorophyll *a* by 41, 37, 38 and 27%, respectively, compared to untreated plants and followed by Kadostim.



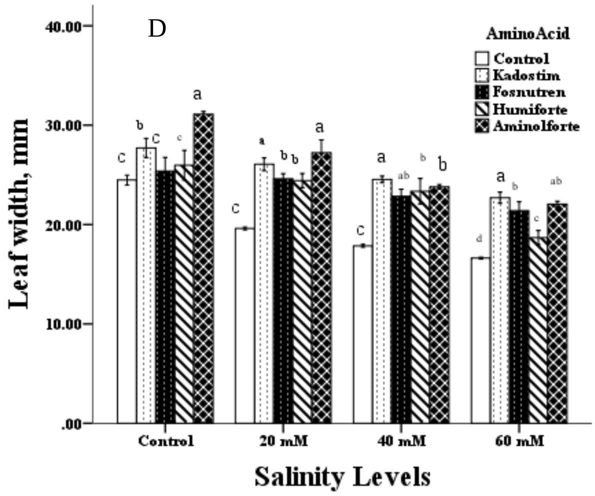


Fig. 5 Mean comparison of salinity levels (above-left) and different amino acid compounds (above-right) on secondary branch number and slicing the interaction effects between salinity levels and different amino acid compounds on leaf length (bottom-left), leaf width (bottom-right) water content of *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

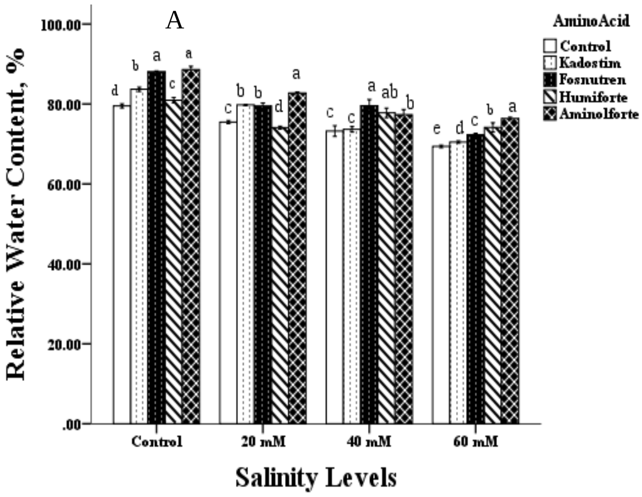
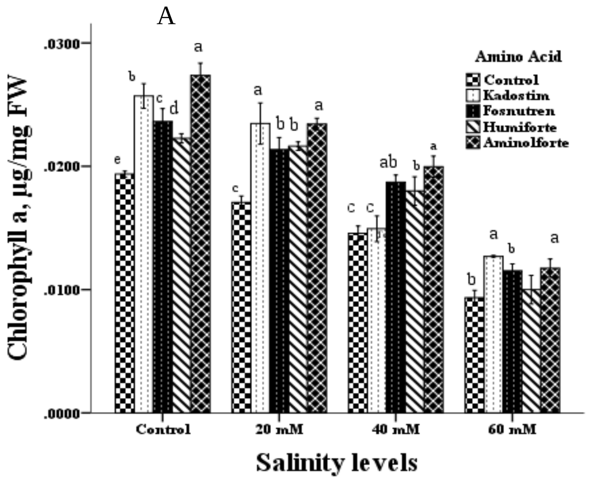
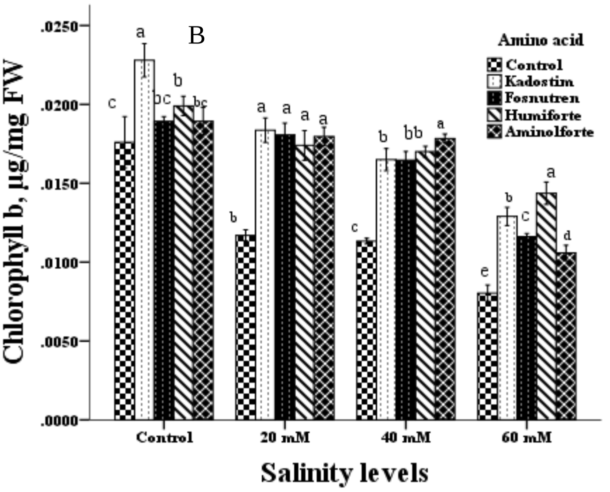
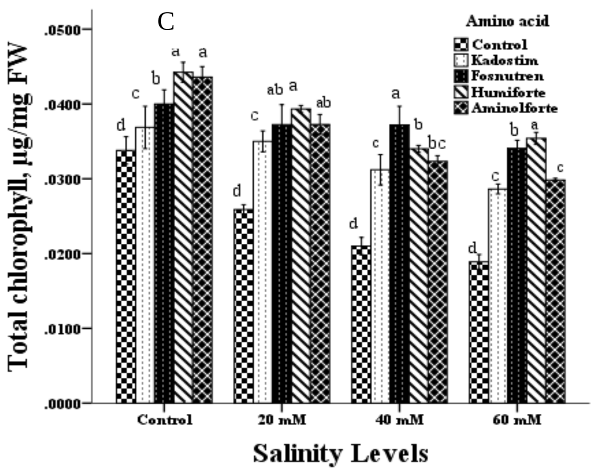


Fig. 6 Slicing the interaction effects between salinity levels and different amino acid compounds on relative water content of *D. moldavica* L. of leaves. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

Salinity stress leads to an increase in some growth regulators such as abscisic acid, and as a result, the activity of the chlorophyllase enzyme increases and plant chloroplasts are destroyed [20]. Also, the increase of reactive oxygen species under salinity stress leads to the damage of the chlorophyll structure, reducing its content and preventing the synthesis of new chlorophyll [21].







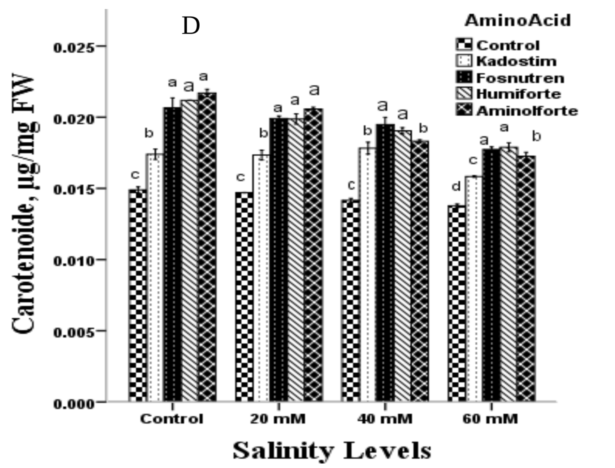


Fig. 7 Slicing the interaction effects between salinity levels and different amino acid compounds on chlorophyll *a* (above-left), chlorophyll *b* (above-right), total chlorophyll (bottom-left) and carotenoid (bottom-right) at *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at p<0.01).

Since glutamate is a precursor for the synthesis of proline and chlorophyll, and in stress conditions, this precursor is used for proline synthesis, as a result, the amount of chlorophyll decreases [22]. Amino acids by increasing in absorption of water and nutrient mineral increase synthesis of chlorophyll and duo to increase in photosynthesis [16]. An increase in leaf pigments, due to application of amino acids, has also been reported in other studies [17; 23]. In this research, it was found that soluble sugars and proline in shoots and roots increased with increasing salinity stress and amino acids application significantly increased soluble sugar and proline in shoots and roots compared to control in the all salinity levels (Fig. 8).

Table 3 Variance analysis of experimented factors effect on physiological parameters.

Source of variation	df	Chl *a*	Chl *b*	Total Chl	Carotenoid
Salinity (S)	3	0.001 **	0.002 **	0.002 **	4. 90 **
Amino Acid (AA)	4	5.07 **	5.67 **	0.000 **	15.31 **
S × AA	12	6.99 **	5.22 **	1.248 **	0.41 **
Error	40	5.27	3.72	1.725	0.06
CV (%)	-	21.48	23.32	19.40	13.21
Source of variation	df	Root soluble sugar	Shoot soluble sugar	Root proline	Shoot proline
Salinity (S)	3	76.79 **	164.26 **	12.09 **	23.37 **
Amino Acid (AA)	4	70.12 **	221.26 **	2.22 **	34.12 **
S × AA	12	3.18 **	17.31 **	0.21 **	1.49 **
Error	40	1.15	0.53	0.02	0.03
CV (%)	-	6.94	8.55	24.22	23.31

**, significant at 1% level of probability. Chl, chlorophyll.

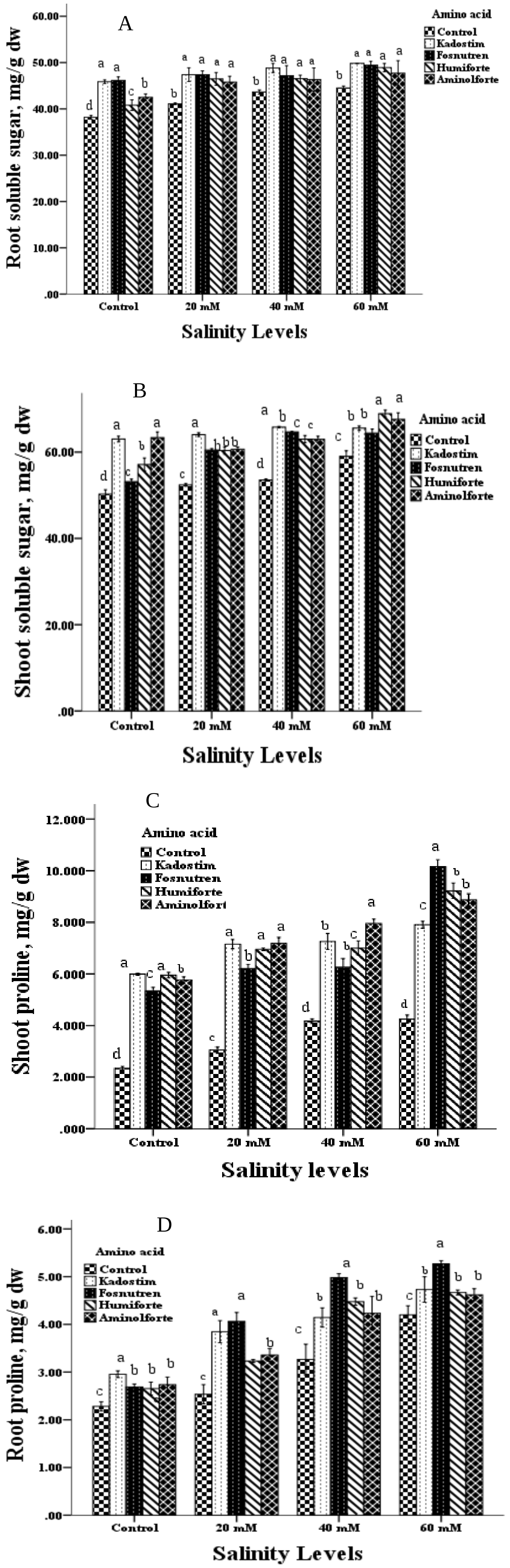


Fig. 8 Slicing the interaction effects between salinity levels and different amino acid compounds on soluble sugar in root (above-right) and shoot (above-left), and proline in root (bottom-left) and shoot (bottom-right) in *D. moldavica* L. Means with different letters indicate significant differences (using Duncan's multiple range test at $P< 0.01$).

Under low, moderate and severe salinity stress increased soluble sugars by 4, 7 and 17% in shoots and 8, 15 and 17% in roots, as well as proline increased by 30, 78 and 82% in shoots and by 11, 43 and 84% in roots, respectively, compared to control. Soluble sugars regulate osmosis and maintain the integrity of membranes and the stability of proteins in the cell [24]. Proline plays a role in protecting the structure of macromolecules and hydroxyl-proline in cell wall synthesis. In conditions of salinity stress, the plant increases its osmotic potential compared to the osmotic potential of the root environment by increasing osmolytes such as proline in order to absorb the required water and salts [25, 26].

CONCLUSION

To overcome the negative effects of salinity stress in the plants can use bio stimulant based on amino acids can. In this research, with increasing in the concentration of salinity significantly reduced growth parameters and biochemical parameters while increased some osmolytes such as soluble sugar and proline in root and shoot. Leaf spraying of the bio-stimulates based on amino acids (Kadostim, Fosnutren, Humiforte and Aminolforte), significantly improved the negative effects of salinity stress on growth and biochemical parameters in Moldavian balm, and among them Kadostim has the most significantly positive effects on morphological and biochemical parameters and followed by Aminolforte.

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