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Growth and chemical composition of *Urtica pilulifera* L. plant as influenced by foliar application of some amino acids

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Abstract
Two field experiments were carried out at the Experimental Farm of Faculty of Agriculture, Cairo University during two successive seasons 2009 and 2010, to study the effect of foliar amino acids application (tryptophan, tyrosine and glutamic) at different doses (0, 50, 100 and 150 ppm) on growth and yield parameters as well as chemical composition of *Urtica pilulifera* plants. The results showed that application of all amino acids forms significantly increased the determined parameters (plant height, number of branches, fresh and dry weight of herb , and yield of seeds) as well as chemical composition(total carbohydrate, total lipid content and total caffeic acid derivatives) in herb and seeds of plant as compared to untreated plants.The most effective treatment was application of tryptophan at 100ppm dose.

Key words: *Urtica pilulifera*, amino acids, caffeic acid, lipids.

1. Introduction
Urtica pilulifera L. is a member of family *Urticaceae. Urtica* plant*s* are used as active principle with other ingredient as anhydrous lanolin and mint oil in preparation of wound–healing antimicrobial ointment [1]. The plant extract used in ointment as agent for skin regeneration [2]. *Urtica* sp. was reported as one of the most effective medicinal plant to treat benign prostate hyperplasia [3-4].
The *Urtica pilulifera* is widely used in folk remedy to treat hyperglycemia, hypertension and inflammation of some organs such as uvula and uterus, uterus bleeding, anemia, wound healing, as purifier and as toner tea. *Urtica* is used for various immune disorder and applied either topically or taken orally as an herbal tea. Herb extract of *Urtica* plant is useful for bladder disorder, it reduced postoperative blood loss, bacteriuria, and prevented hemorrhagic and purulent inflammation following adenomectomy[5]. Amino acids as organic nitrogenous compounds are the building blocks in the process of protein biosynthesis. Amino acids are particularly important for cell growth stimulation. They act as buffers which help to maintain favorable pH value within the plant cell, since they contain both acid and basic groups. They remove ammonia from the cell, so they protect plants from ammonia toxicity [6].
Amino acids has function in the biosynthesis of otherorganic compounds i.e., pigments, vitamins, alkaloids, enzymes, terpenoids, coenzymes, purine and pyrimidine bases [7]. Amino acids have the greatest importance in plant nutrition for obtaining of higher yields and quality and shortening of the productive cycle with better dry material. It gave more abundant and more uniform flowering [8]. Previous studies have proved that amino acids can directly or indirectly influence the physiological activities of the plant. As well as amino acids help in improving the microflora of the soil thereby facilitating the assimilation of nutrients. Glycine and Glutamic acids are fundamental metabolites in the process of chlorophyll synthesis. Amino acids are precursors or activators of phytohormones and growth substances. [9].While, Hass[10] stated that the biosynthesis of cinamic acids which are the starting materials for the synthesis of phenols are derived from phenylalanine and tyrosine. Tyrosine is the hydroxyl phenol acid that is used to build hormones. In the recent years many researchers

applied foliar spray of amino acids and proved remarkable enhancement in plant growth and chemical composition on some medicinal plant i.e., Reda [11] found that used of three amino acids were significantly increased vegetative growth at 50mg/ ornithin and at 100mg/L of both proline and cysteine of *Hyoscyamus muicus* L. Hendawy [12] stated that amino acids such as asparatic, glutamic, tryptophan and phenylalanine at 50, 100 and 150ppm enhanced *Echinacea* growth parameters and the most effective amino acid on plant weight, aerial parts weight, branches number and weight of root was glutamic acid followed by asparatic then tryptophan at medium rate of application.Wahba [13] using tryptophan and asparatic acid as foliar application at 0, 25, 50 and 75ppm they reported that, all treatments increase the vegetative growth, flowering parameters and yield of corms of *Antholyza aethiopica*.Talaat [14] investigated the effect of lysine at (25, 50,100ppm) on growth and yield of *Ocimum basilicum* L. and they found that the maximum mean values of plant growth parameters, plant height, fresh and dry weight of herb as well as number of branches were recorded by application of 25mg/L lysineon *Ocimum basilicum* L. Effect of phenylalanine and methionine on the growth of *Catharanthus roseus* were studied by Naguib [15] and stated that, the application of two amino acids phenylalanine and methionine produced significant increment in plant height, no. of branches, fresh and dry weight of plant as well as herb yield. The high rate of application (100ppm) was more effective than the low rate (50ppm) for both amino acids.Youssef [16] showed that, application of tyrosine at 50ppm on *Ocimum basilicum* resulted in maximum values of plant height, plant diameter, number of branches, plant fresh and dry weight, leaves fresh and dry weight as well as flowers fresh and dry weight. El-Gengaihi [17] reported that, tryptophan has the higher effect on the production of aerial parts, flowers yield and seeds yield than phenylalanine on *Anchusa italica*. Talaat [18] found that foliar spray with tryptophan increased plant growth, photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids, soluble and total insoluble sugars, total proteins and total alkaloids in the leaves of periwinkle plant (*Catharanthus roseus* L.). Mahgoub [19] on *Pelargonium graveolens* plant and Nahed [20] on *Salvia farinacea* plants, they found that amino acids significantly increased vegetative growth. Karima [21] on chamomile plants (*Chamomilla recutita* L.), revealed that all treatments of ornithine, proline and phenylalanine led to significant increases in the plant height, number of branches, number of flowers per plant, fresh and dry weight of herb and flowers, the effect was more pronounced with 50 mgL^{-1} ornithine, 100 mgL^{-1} proline or phenlalanine. Moreover, the same authors found that, essential oil percentand yield increased by all treatments of the three amino acids at all cuttings. Also, Abdel-Aziz [22] mentioned that foliar application of *Syngonium podophyllum* plants with100 ppm ascorbic acid and 50 ppm thiamine gave the highest levels of total carbohydrates content.In this respect Mahgoub [23] reported that spraying *Dahliapinnata* L. plants with thiamine significantly increased plant height, number of branches, number of leaves, fresh and dry weight of leaves, stem diameter and fresh and dry weight of stem.

The aim of this study was investigate the effect of amino acids on the quantitative and qualitative of *Urtica piluiifera* plant production under Egyptian conditions.

2. Materials and methods

2.1. Plant material

The present study was carried out during two successive seasons of 2009 and 2010 at the Experimental Farm of Faculty of Agriculture, Cairo University. This study aims to the role of amino acids on the vegetative growth, yield and chemical composition of *Urtica pilulifera* plant.

Seeds of *Urtica pilulifera* were obtained from Borg El- Arab location in April 2008. Borg El-Arab is a city follows the Alexandria Governorate in Egypt. Borg El-Arab is located at kilometer 34 of Alexandria-Matroh coastal.

The seeds were propagated in Faculty of Agriculture Experimental Farm and then the collected seed were used as plant material in this experiment in during two successive seasons (2009 and 2010). *U. pilulifera* seeds were sown directly in the experimental plots at the end of September in both seasons in rows 60cm apart at 40cm in between plants. The plants were harvested at full bloom stage.

2.2 Soil analysis

The mechanical and chemical analysis of the soil were carried out before planting in Soil Science Department, NRC according to Chapman [24] and the obtained results were as follow: **Mechanical analysis:** sand 55%, silt 29.75% ,

clay14.93%, soil texture (sandy loam). **Chemical analysis:** pH 8.23, E.C. 2.8/mmohs, organic matter 0.23%, total N 480ppm, total P 37.8ppm, total K 35ppm , Cation Na^+ 9.5, K^+ 0.7, Ca^{++} 14.0, Mg^{++} 8.2. Anion, HCO 4.4, SO 25.0, Cl 13.0

2.3 Cultivation procedures and maintenance

This experiment was carried out to study the role of amino acids, trypotphan, tyrosine and glutamic at 0, 50, 100 and 150ppm as foliar applications. All plants received the applied treatment at three times, the first one after sixty days from planting, the second and third were performed after 30 days from the first one. Spraying treatments were done in early morning till the solution run off using Misrule as wetting agent at 1%, control plants were sprayed with distilled water containing the same wetting agent. All treatments were fertilized with 150 kg/fed. ammonium sulphate, 100 kg/fed calcium super phosphate and 100 kg/fed potassium sulphate, calcium super phosphate was added during the preparation of soil, while both amounts of ammonium and potassium sulphate were added to the plants at two portions as side dressing at intervals two months starting one month from planting.

2.4 Data recorded

Recorded parameters at full bloom were plants height, number of branches, fresh and dry weights of herb g/plant were measured at the end of growth (end of May at 8 months age. Seeds yield was determined as g/plant, and kg/fed. at the end of growth.

2.5. Chemical analyses

2.5.1. Preparation of samples

From each treatment, plants were collected at full bloom as well as seeds at the end of growth. Each treatment of herb was ground in a mill to fine powder while, in case the seeds which immediately powdered before chemical investigation then kept in plastic bags in a desiccators over $CaCl_2$**.**

2.5.2. Determination of total carbohydrates was carried using the phenol sulphuric acid method according to Dubois [25]. A standard curve was prepared using different concentrations of pure glucose.

2.5.3.Determination of caffeic acid derivatives content in the plant parts of *Urtica pilulifera* was determined as chicoric acid, since, it was noticed that the commercial proportions are evaluated based on their content of chicoric acid as mentioned by Bauer [26].

2.5.4. Determination of total lipids content in the dried herb and seeds were determined according to A.O.A.C [27].

2.5.5 Statistical analysis

Each treatment of experiment consisted of three replicates contained eighteen plants in a complete randomized design and all obtained data were subjected for statistical analysis according to Snedecor [28].

3. Results and discussion

3.1. Vegetative growth.

3.1.1. Plant height

Urtica pilulifera plant treated with tryptophan, tyrosine or glutamic acid at different concentrations caused a significantly increase in plant height (cm) compared to untreated plants (Table,1).The maximum level of both tryptophan and glutamic acid were more effective than tyrosine treatment but the differences between them were insignificant. The best treatment for plant height was resulted with glutamic acid at150ppm. The same trend was reported by Naguib [15]stated that, application of two amino acids (phenylalanine and methionine) on *Catharanthus roseus* plant produced significant increment in plant height.Youssef [16]showed that, application of tyrosine at 50ppm on *Ocimum basilicum* resulted in maximum values of plant height. Nahed [20
] on chamomile plants (*Chamomilla recutita* L.), revaled that, all treatments of ornithine, proline and phenylalanine led to significant increases in the plant height.

3.1.2.Number of branches/plant

It is obvious from data in Table (1) that, all amino acids (tryptophan, tyrosine and glutamic) significantly increased number of branches/plant at medium and high concentration (100 and 150ppm) in both seasons.. The amino acid source and its concentration showed that glutamic acid at 150ppm produced the most promising effect on branching of *Urtica pilulifera* plants. The above results are in harmony with Hendawy[12] on *Echinacea purpurea* who concluded that, application of tryptophan and glutamic acid significantly increased number of branches, also with Talaat [14]on *Ocimum* plants and El-Gengaihi [17]on *Anchusa italica* they stated that application of tryptophan significantly increased the production of aerial parts such as number of branches.

Table (1): Effect of amino acids on growth parameters of *Urtica pilulifera* plants during 2009 and 2010 seasons.

Treatments		Plant height cm		Number of Branches/plant	
		S_1	S_2	S_1	S_2
Control		117.0	107.7	17.0	18.8
Trytophan (ppm)	50	126.7	124.7	18.3	20.7
	100	137.0	146.3	19.0	22.1
	150	141.7	150.7	17.7	21.0
Tyrosine (ppm)	50	127.7	137.3	16.7	21.0
	100	132.7	138.3	17.7	21.3
	150	131.1	133.7	16.0	20.6
Glutamic (ppm)	50	126.0	138.3	17.3	20.7
	100	134.0	141.0	18.3	22.1
	150	142.3	152.0	22.7	24.3
LSD at 0.05		6.4	7.4	2.4	2.3

S_1: First season S_2: Second season

3.1.3.Fresh and dry weights of herb

Fresh weight of herb, significantly responded to tryptophan, tyrosine and glutamic acid foliar application at different concentrations compared with untreated plants. Glutamic acid was superior to the other two amino acids in biomass production giving the highest mean values (277 0.3g and 3344.0 g/ plant) at high concentration in the first and second seasons, respectively (Table 2). Results of herb dry weight g/plant gave the same trend as mentioned for fresh weight.

The mentioned results are in accordance with those of Hendawy [12] on *Echinacea* plants, using tryptophan and glutamic at 100 and 150ppm, reported that. application of tryptophan and glutamic significantly enhanced aerial parts weight and glutamic acid was superior in this concern. Naguib**[**15]stated that, the application of two amino acids phenylalanine and methionine produced significant increment in plant height, number of branches, fresh and dry weight of plant as well as herb yield. The same authors found that,The high rate of application (100ppm) was more effective than the low rate (50ppm) for both amino acids. Youssef [16] showed that, application of tyrosine at 50ppm on *Ocimum basilicum* resulted in maximum values of plant fresh and dry weight, leaves fresh and dry weight as well as flowers fresh and dry weight.

3.1.4 .The seeds yield:

Table (3) used all amino acids at all applied concentrations (50, 100 & 150ppm) significantly increased seed yield/plant and the highest mean values were produced with glutamic acid followed with tryptophan while tyrosine exhibited the least mean values in this concern. As for effect of applied concentrations, seed yield was gradually increased with increasing of amino acid concentration. The foliar application of glutamic acid at 150ppm produced the highest seed yield, 336kg/fed and 346.8kg /fed for first and second seasons, respectively,

followed by tryptophan at the same concentration which produced 303.6kg/ fed and 327.6kg /fed for first and second seasons, respectively.

Table (2): Effect of amino acids on the yield of herb of *Urtica pilulifera* plants during 2009 and 2010 seasons

Treatments		Herb weight g/plant			
		Fresh		Dry	
		S_1	S_2	S_1	S_2
	Control	2254.8	2424.0	587.6	307.8
Tryptophan (ppm)	50	2508.7	2601.7	315.1	333.8
	10	2563.3	2786.3	335.4	363.9
	150	2693.5	3048.2	367.9	419.8
Tyrosine (ppm)	50	2478.0	2561.3	313.6	330.7
	10	2566.3	2855.3	326.4	347.1
	150	2705.7	2921.3	329.5	374.2
Glutamic (ppm)	50	2628.7	2901.0	331.2	403.8
	10	2682.4	3135.6	361.5	432.3
	150	2770.3	3344.0	373.2	467.6
LSD at 0.05		59.2	62.3	19.4	17.2

. S_1: First season S_2: Second season

Table (3): Effect of amino acids on the yield of seeds of *Urtica pilulifera* plants during 2009 and 2010 seasons

Treatments		Yield of seeds			
		g/plant		Kg/fed.	
		S_1	S_2	S_1	S_2
	Control	17.5	18.1	210.0	217.2
Tryptophan (ppm)	50	20.2	20.2	242.2	242.9
	10	24.2	25.9	295.2	310.8
	150	25.3	27.3	303.6	327.6
Tyrosine (ppm)	50	18.8	19.2	225.6	230.5
	10	21.3	21.6	255.6	254.2
	150	21.8	22.4	261.6	268.8
Glutamic (ppm)	50	21.6	23.1	259.2	277.6
	10	27.4	26.1	328.8	313.8
	150	28.0	28.9	336.0	346.8
LSD at 0.05		2.1	1.5	17.4	15.6

. S_1: First season S_2: Second season

3.2. Chemical composition:

3.2.1. Total carbohydrate content%.

3.2.1.1.In herb

Data in Table (4) demonstrated that total carbohydrate percentages were affected due to spraying of *Urtica pilulifera* plants with different concentrations of amino acids. The three amino acids enhanced the accumulation of carbohydrate in herb as compared to unsprayed plants. The most effective amino acid was tryptophan followed by glutamic then tyrosine in total carbohydrate content. It obvious from the same data that tryptophan at the medium level of application produced the highest total carbohydrate content, followed with glutamic acid at 150ppm during two seasons.

3.2.1.2. In seed

Foliar application of different amino acids significantly increased total carbohydrate percentages in seed as compared to unsprayed plants. In regard to the effect of different concentrations of amino acids on total carbohydrate, the data in Table(4) show that total carbohydrate content were increased gradually with increasing the concentrations from 50ppm to 100ppm and reached their maximum with foliar application of tryptophan at 150ppm followed by glutamic at 150ppm, while tyrosine produced the least mean values.

Table (4): Effect of amino acids on the total carbohydrate % on herb and seeds of *Urtica pilulifera* pants during 2009 and 2010 seasons.

Treatments		Total carbohydrate %			
		Herb		Seeds	
		S_1	S_2	S_1	S_2
	Control	11.7	12.8	12.7	12.8
Trytophan (ppm)	50	14.2	14.7	13.5	14..5
	100	16.3	17.8	17.8	19.5
	150	16.2	16.7	16.6	10.5
Tyrosine (ppm)	50	14.5	13.5	13.6	13.8
	100	15.3	16.6	16.0	17.2
	150	15.2	15.9	13.4	15.2
Glutamic (ppm)	50	15.3	15.6	13.4	13.8
	100	14.8	15.9	16.0	16.9
	150	16.2	16.8	17.7	19.2
LSD at 0.05		1.3	1.3	1.3	1.2

S1: First season S2: Second season

3.2.2. Total caffeic acid derivatives (TCAD)

It is obvious from the data tabulated in Table (5) that, the seeds are rich with caffeic acid and derivatives (0.68% and 0.66%) more than herb (0.52% and 0.77%) in the two seasons, respectively.It could be noticed from the same Table (5) that spraying plants with tryptophan, tyrosine or glutamic acid at different concentrations increased the total caffeic acid derivatives as compared to untreated plants.These increments were significant in most cases. Spraying plants with tyrosine increased the mean values of TCAD in herb and seeds during both seasons comparing to tryptophan and glutamic acid treatments. Tyrosine at high concentration (150ppm) showed a promising effect on total caffeic acid derivatives in herb and seeds during two growing seasons. The above mentioned results showed that, the best amino acid for enhancing caffeic acid derivatives production is tyrosine that due to the role of tyrosine as a member of shikimic acid pathway that ended by phenolic compounds synthesis including caffeic acid derivatives [9].

3.3. Fixed oil content in seeds.

It is obvious from the data of both seasons in Table (6) that the seeds of *Urtica pilulifera* plants are richest organ in lipid (14.69 and 14.75%) for the two seasons respectively, followed by herb (0.92 and 0.75%) regardless of the applied treatments of amino acids. Foliar application of tryptophan, tyrosine and glutamic acid significantly increased the total lipid content in herb and seeds comparing to untreated plants.

Tryptophan was the most effective amino acid on accumulation of lipid in these parts followed with glutamic acid then tyrosine. The data in the same table show that the application of tryptophan at 100ppm produced high percentage of total lipid in all parts followed by glutamic acid at 150ppm. It is obvious from obtained results that the most effective amino acid to enhancement total carbohydrate and total lipid was tryptophan at 100ppm while, the best one for accumulation of caffeic acid derivatives is tyrosine at high concentration (150ppm).

Table (5): Effect of amino acids on total caffeic acid derivatives of *Urtica pilulifera* plants during 2009 and 2010 seasons.

Treatments		Total caffeic acid derivatives %			
		Herb		Seeds	
		S_1	S_2	S_1	S_2
Control		0.52	0.77	0.68	0.66
Tryptophan (ppm)	50	0.68	1.31	1.11	1.17
	10	0.87	1.20	1.07	1.12
	15	1.04	1.13	0.90	0.94
Tyrosine (ppm	50	0.98	1.18	1.20	1.15
	10	1.05	1.34	1.25	1.28
	15	1.28	1.83	1.41	1.47
Glutamic (ppm)	50	0.66	0.92	0.98	0.99
	10	0.89	1.11	1.15	1.09
	15	1.03	1.31	1.26	1.19
LSD at 0.05		0.10	0.12	0.13	0.17

S_1: First season S_2: second season

Table (6): Effect of amino acids on total fixed oil content in herb and seeds of *Urtica pilulifera* plants during 2009 and 2010 seasons.

Treatments		Total lipid content (%)			
		Herb		Seeds	
		S_1	S_2	S_1	S_2
	Control	0.92	0.82	14.69	14.75
Tryptophan (ppm)	50	1.71	1.35	21.00	22.04
	10	1.86	1.88	22.89	22.78
	15	1.56	1.60	20.77	21.32
Tyrosine (ppm	50	1.11	1.20	17.28	16.33
	10	1.76	1.66	19.9	19.11
	15	1.28	1.58	18.44	18.17
Glutamic (ppm)	50	1.28	1.19	19.88	18.91
	10	1.79	1.75	20.84	21.51
	15	1.89	1.86	22.45	22.95
LSD at 0.05		0.12	0.11	0.15	0.11

S_1: First season S_2: second season

Amino acids were known as growth factors of higher plants and as constituents of the protein part of enzymes also their important role for metabolic processes as they are components of enzymes [29] On the other hand, the secondary plant substances such as terpenoids, phenols and alkaloids are derived from the metabolism of carbohydrate, fats and amino acids and the caffeic acid derivatives and phenols are derived from phenyl alanine and tyrosine [10]. Also the role of tryptophan is well known, it has an indirect role on the growth via auxin synthesis. Several alternative routs of IAA synthesis in plants starting from tryptophan, thus when tryptophan is supplied to most plant tissues IAA was formed [30]. It is obvious from the above explanation the important role of amino acids in growth and in production of different plant substances which is in accordance with our results, also our results are in accordance with Hendawy [12] on *Echinacea* plants, who reported that the application of amino acids (tryptophan, tyrosine asparatic and glutamic acids) significantly increased total carbohydrate percentage, caffeic acid derivatives production and total lipid content and the best one was glutamic acid. The same trend was reported by Maryam [31], stated that spraying of amino acids successfully manipulates the yield and yield components of *Ocimume basilicum* plant. Also, Moslem [32] mentioned that the results indicated that the yield of radish (*Raphanus sativus* L.) affected by amino acids significantly.

Conclusion

Application of all amino acids on *Urtica* plants significantly enhanced all growth parameters , plant height , number of branches , fresh and dry weight of herb and flowers,yield of seeds and all determined chemical compositions ,total carbohydrate ,total lipid content and the total caffeic acid derivatives. Foliar application of glutamic acid gave the best effect on growth parameters at 150ppm, while the application of tryptophan at the medium dose 100ppm produced the highest effect on chemical composition.

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