

# Effect of Some Amino Acids on Growth and Essential Oil Content of Chamomile Plant

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## ABSTRACT

The effects of foliar application of different concentrations (0, 50, 100, 150 mgL<sup>-1</sup>) of three amino acids (ornithine, proline, phenylalanine) on the vegetative growth, essential oil and some metabolite of chamomile (*Matricaria chamomilla* L., Rausch.) were studied. The study was conducted in pot experiments in complete randomized block design with three replicates in two successive seasons (2001/2002 and 2002/2003). All treatments of ornithine, proline and phenylalanine led to significant increases in the plant height, number of branches, number of flowers per plant, fresh weight and dry weight of herb and flowers, the effect was more pronounced with 50 mgL<sup>-1</sup> ornithine, 100 mgL<sup>-1</sup> proline or phenylalanine. Essential oil percent and yield increased by all treatments of the three amino acids at all cuttings, more so with the second cut. The three amino acids treatments increased total phenols and total indoles but decreased total amino acids and total carbohydrates (except 100 mgL<sup>-1</sup> proline). Gas liquid chromatographic analysis revealed that the main identified components of essential oil were farnesene, bisabolol oxide B,  $\alpha$ -bisabolol, chamazulene and bisabolol oxide A. Amino acid treatments resulted in quantitative differences in these components of the essential oil.

**Key Words:** Amino Acids; Growth; Essential oil; Chamomile

## INTRODUCTION

Chamomile (*Matricaria chamomilla* L. Rausch., Composite) is one of the important medicinal herb native to southern and eastern Europe. Egyptian chamomile is famous by its high quality and, therefore, large quantities of this plant exported to west Europe, especially Germany. Chamomile, in particular their flower-head contained several groups of compounds having important therapeutic values especially sesquiterpene essential oil. The terpenes,  $\alpha$ -bisabolol oxides and chamazulene, are the most important compounds (Reichling & Beiderbeck, 1991). Chamomile can be used as a drug to treat inflammations of the skin and the mucous membranes as well as bacterial diseases of the skin (Reichling & Beiderbeck, 1991).

Aberg (1961) indicated that amino acids can act as growth factors of higher plants since they are the building blocks of protein synthesis, which could be enzymes important for metabolic activities. There is evidence that ornithine is the precursor of polyamines that are essential in the regulation of plant growth and development (Smith, 1985; Martin-Tangy, 2001). Proline has been shown to accumulate in plant tissues under various conditions (Yang *et al.*, 1999; Mansour, 2000). The proposed function of the accumulated proline are osmoregulation, maintenance of membrane and protein stability, growth, seed germination and provisions of a store of carbon, nitrogen and energy (Mansour, 2000; Hare *et al.*, 2001; Hare *et al.*, 2003). However, the exact role of proline still needs more documentation (Stewart & Larher, 19809; Mansour, 2000).

Moursy *et al.* (1988) working on *Datura stramonium* L. indicated that phenylalanine or ornithine increased the fresh and dry weights of callus explants. Gamal El-Din *et al.* (1997) reported an increase in vegetative growth of lemongrass as a result of ornithine and phenylalanine treatments. In addition, phenylalanine application significantly increased fresh and dry weight of *Datura* during vegetative and flowering stages (Youssef *et al.*, 2004). Talaat and Youssef (2002) similarly found a pronounced increase in vegetative growth of basil plant as a result of Lysine and ornithine treatments.

The present study was carried out to investigate the effect of different concentrations of ornithine, proline and phenylalanine on vegetative growth, essential oil and some biochemical constituent contents of chamomile plant.

## MATERIALS AND METHODS

Pot experiments were conducted during two successive seasons (2001/2002 & 2002/2003) at the experimental farm of National Research Center, Dokki, Giza, Egypt to study the effect of foliar application of amino acids on growth and essential oil content of chamomile plant. Chamomile seeds were grown on pots filled with loamy soil in October 2001 and October 2002. Then, they were thinned to four seedlings per pot. Fertilization of the plants was carried out at the rate of 2 g calcium superphosphate (16 % P<sub>2</sub>O<sub>5</sub>), 2 g calcium nitrate (15.5 % N) and 1 g potassium sulphate (48 – 52 % K<sub>2</sub>O) per pot. Each pot was irrigated with one liter of tap water twice weekly.

The plants were grown under natural conditions: 15-35°C and 10-20°C day and night temperatures, respectively, with 12–13 h photoperiod.

**Amino acids treatments.** The plants were sprayed with amino acids (ornithine, proline or phenylalanine) at concentrations 50, 100 and 150 mgL<sup>-1</sup>. The plants were sprayed twice with amino acids: the first spray was applied after 30 days of sowing and the second spray was applied one week after the first one. All treatments were replicated three times (three plants/replicate). For determination of growth parameters, two samples of plants were harvested, the first one at full vegetative stage (70 days old) and the second one at full flowering stage (100 days old). Plant height, number of branches/plant, number of flowers/plant, fresh and dry weights of the herb/plant, fresh and dry weights of flowers/plant were determined for each harvest.

**Determination of some biochemical constituents.** These were determined at full vegetative stage as described in the following section. Total carbohydrate content was determined according to Dubois *et al.* (1956). The method of Danial and George (1972) was used to determine phenols. Total indoles were determined according to Bentley (1961). Total nitrogen and protein pattern were determined based on A.O.A.C. (1970).

Essential oil was determined in the flower heads of each treatment which were collected twice a week at early morning. Then, the flowers were air dried in shade. The flower heads were collected during three months from the start of flowering. The flower heads of each month were pooled together to determine the essential oil. The samples collected in the three months were designed stages I, II and III, respectively. Essential oil was determined in the flower heads of each treatment by steam hydrodistillation according to Guenther (1961) and British Pharmacopoeia (1980). Gas liquid chromatography (Hewlett, Packed, HP6890 series) was used to determine essential oil constituents for the obtained oil from different treatments.

**Statistical analysis.** The obtained data were subjected to one way analysis of variance (ANOVA) according to Snedecor and Cochran (1980). From which the least significant difference (L.S.D.) at 0.05 level was calculated for comparison between different treatments.

## RESULTS AND DISCUSSION

**Vegetative growth.** Data presented in Table I showed that all treatments caused significant increase in plant height, number of branches, fresh and dry weights of aerial vegetative parts. The more pronounced effect on these growth criteria were obtained at treatment 50 mgL<sup>-1</sup> ornithine and 100 mgL<sup>-1</sup> proline or phenylalanine. All amino acid treatments significantly increased plant height, number of branches, number of flower head, fresh and dry weights of aerial parts and flower head per plant (Table II). Foliar application of 50 mgL<sup>-1</sup> ornithine and 100 mgL<sup>-1</sup> proline or phenylalanine resulted in the greatest effect as

compared with other treatments. Similar findings were obtained in tobacco (Darwish & Reda, 1975), *Datura* (El-Bahr *et al.*, 1990), Lemon-grass (Gamal El-Din *et al.*, 1997), *Hyoscyamus muticus* L. (Reda *et al.*, 1999) and *Iberis amara* L. (Attoa *et al.*, 2002), where different amino acids enhanced plant growth. This regulatory effect of amino acids on growth could be explained by the notion that some amino acids (e.g. phenylalanine, ornithine) can affect plant growth and development through their influence on gibberellin biosynthesis (Waller & Nawacke, 1978). In addition, Bidwell (1972) and Fowden (1973) reported that amino acids acting as the building blocks of proteins can serve in number of additional functions in regulation of metabolism, transport and storage nitrogen.

**Biochemical constituents.** Total phenol and total indole contents in vegetative aerial parts significantly increased by all treatments of amino acids, 150 mgL<sup>-1</sup> ornithine, proline or phenylalanine was more effective (Table III). Proline or phenylalanine at 50 or 150 mgL<sup>-1</sup> significantly decreased the total carbohydrates, whereas 150 mgL<sup>-1</sup> of ornithine only had such effect (Table III). However, 100 mgL<sup>-1</sup> proline increased total carbohydrates which was unclear. Comparable results were obtained by Gamal El-Din *et al.* (1997) on lemon-grass, El-Sherbini and Hassan (1987) on *Datura stramonium* L., and Tarraf (1999) on lupine who reported that application of amino acids decreased soluble sugar and carbohydrate contents. All amino acids treatments increased total nitrogen and crude protein except (except 100 and 150 mgL<sup>-1</sup> ornithine, Table III). These results are in agreement with those of Gamal Eldin *et al.* (1997) and Tarraf (1999). The effect of tested amino acids on the previous biochemical contents could be through plant protection from ammonia toxicity as they remove amide formation, serving as a source of carbon and energy as well as protection of plants against pathogens, functioning as buffers and biosynthesis of other organic compounds such as protein, amines, purines, pyrimidines, vitamins, enzyme, terpenoids (Goss, 1973).

**Essential oil percent.** Table IV indicate that the greatest oil percent in flower heads was obtained at full flowering stage. The greatest increases in oil percent and yield were obtained at treatments 50 mgL<sup>-1</sup> of ornithine and 100 mgL<sup>-1</sup> of proline or phenylalanine. Gamal El-Din *et al.* (1997) reported that foliar application of amino acids significantly increased essential oil percent and yield on lemongrass plant. Talaat and Youssef (2002) obtained similar results on basil plants. Stieber *et al.* (1979) interpreted such response as that flowers of chamomile have pro-chamazulene bearing and pro-chamazulene free glandular hairs which increase during flowering and reach a maximum at full-flowering and then declined. Reda *et al.* (1999) and Salamon and Honcariv (1994) have reached similar conclusion.

**The major components of chamomile oil.** Since the formation of the important components of chamomile oil was related to flower formation stage, full-flowering stage was chosen to identify the constituents of the oil as it had

**Table I. Effect of ornithine, proline or phenylalanine treatment on vegetative growth of chamomile plant at full vegetative stage. Values are means of two seasons (2001/2002 and 2002/2003)**

Amino acids treatments	Concentration (mgL <sup>-1</sup> )	Plant height (cm)	No. of branches /plant	Fresh weight of aerial vegetative part (g/plant)	Dry weight of aerial vegetative part (g/plant)
Ornithine	50	32.3	19	35.40	4.10
	100	31.3	18	28.77	3.42
	150	29.0	17	25.46	3.07
Proline	50	31.0	18	28.21	3.40
	100	31.0	18.7	38.65	4.54
	150	31.0	17	34.37	4.03
Phenylalanine	50	26.0	17	38.94	4.25
	100	31.0	21	39.78	4.59
	150	26.7	18	31.61	3.65
Control (Untreated)	0	24.0	15	24.45	2.90
L.S.D. (0.05 level)		2.0	1.72	3.47	0.50

**Table II. Effect of ornithine, proline or phenylalanine treatment on vegetative growth and flower heads of chamomile at full flowering stage. Values are means of two seasons (2001/2002 and 2002/2003)**

Amino acids treatments	Concentration (mgL <sup>-1</sup> )	Plant height (cm)	Vegetative parts			Flower-heads		
			No. of Branches	Fresh wt. (g/plant)	Dry wt. (g/plant)	No. of flower heads/plant	Fresh wt. (g/pant)	Dry wt. (g/plant)
Ornithine	50	58.0	24.5	77.26	13.31	32.5	4.17	0.79
	100	56.7	23.2	72.04	12.41	32.0	3.38	0.64
	150	55.8	22.5	68.38	11.78	27.8	3.00	0.57
Proline	50	52.5	21.2	56.42	10.24	53.3	2.95	0.56
	100	65.7	25.3	76.55	12.37	57.0	3.97	0.75
	150	54.7	19.0	53.89	8.71	28.0	2.82	0.54
Phenylalanine	50	59.0	23.3	78.75	14.04	41.5	4.08	0.78
	100	67.3	25.3	81.69	14.22	44.1	4.94	0.94
	150	64.0	24.8	73.51	13.51	29.90	3.35	0.64
Control (Untreated)	0	46.0	16.5	43.89	7.09	24.3	2.11	0.40
L.S.D. (0.05 level)		5.9	1.83	7.52	1.22	3.45	0.70	0.13

**Table III. Effect of ornithine, proline or phenylalanine treatment on biochemical constituents in aerial vegetative parts of chamomile determined at full-vegetative stage. Values are means of two seasons (2001/2002 and 2002/2003)**

Amino acids treatments	Concentration (mgL <sup>-1</sup> )	Total phenols (mg/g)	Total indoles (mg/g)	Total amino acids (mg/g)	Total carbohydrates (%)	Total nitrogen (%)	Crude protein (%)
Ornithine	50	22.1	9.9	21.5	15.9	3.4	21.3
	100	22.2	10.0	17.6	15.8	3.00	18.8
	150	23.7	10.0	18.5	13.8	2.8	17.5
Proline	50	23.1	10.0	21.7	13.4	4.0	25.0
	100	22.1	10.0	21.4	17.8	4.5	28.1
	150	26.8	10.8	24.0	11.7	4.3	26.9
Phenylalanine	50	24.0	10.9	18.4	13.6	4.3	26.9
	100	25.4	10.3	17.2	16.5	4.5	28.1
	150	25.8	11.0	24.0	13.7	4.0	25.0
Control (Untreated)	0	20.8	9.8	30.3	15.4	2.9	18.1
L.S.D. (0.05 level)		2.84	0.42	9.95	1.5	0.25	1.60

the maximum level of essential oil in this study. The major components of chamomile oil were farnesene,  $\alpha$ -bisabolol oxide B,  $\alpha$ -bisabolol, chamazulene and  $\alpha$ -bisabolol oxide A (Table V). The area-percent of  $\alpha$ -bisabolol oxide A was the highest terpenoid in the flower heads of the control and treated plants (Table V). This was followed by Chamazulene percent. This result is consistent with those reported in chamomile type of Egypt and chamomile imported from other countries (Piccaglia & Marotti, 1993; Reda *et al.*, 1999). Our data indicated that all amino acid

treatments increased area percent of farnesene,  $\alpha$ -bisabolol oxide B and  $\alpha$ -bisabolol (except ornithine at 150 mgL<sup>-1</sup> & proline at 150 mgL<sup>-1</sup>).

All amino acids treatments decreased the area percent of chamazulene while treatment 150 mgL<sup>-1</sup> phenylalanine increased it. Treatments of ornithine at 50 mgL<sup>-1</sup>, proline at 100 and 150 mgL<sup>-1</sup> and phenylalanine at 50 mgL<sup>-1</sup> caused pronounced increases in  $\alpha$ -bisabolol oxide A, while other treatments decreased it. Similar results were obtained by Talaat and Youssef (2002) who report that different

**Table IV. Effect of ornithine, proline or phenylalanine treatment on essential oil percent and yield of chamomile. Values are means of two seasons (2001/2002 and 2002/2003).**

Amino acids treatments	Concentration (mgL <sup>-1</sup> )	Essential oil percent						Essential oil yield (ml/flower heads/plant)					
		1 <sup>st</sup> stage		2 <sup>nd</sup> stage		3 <sup>rd</sup> stage		1 <sup>st</sup> stage		2 <sup>nd</sup> stage		3 <sup>rd</sup> stage	
		a	b	a	b	A	B	a	b	a	b	A	B
Ornithine	50	0.69	156.8	0.87	133.8	0.53	126.2	0.029	322.2	0.036	257.1	0.022	244.4
	100	0.62	140.9	0.84	129.2	0.48	114.3	0.021	233.3	0.028	200.0	0.015	166.7
	150	0.46	104.5	0.72	110.8	0.45	107.1	0.014	155.6	0.022	157.1	0.014	155.6
Proline	50	0.59	134.1	0.72	110.8	0.48	114.3	0.017	188.9	0.021	150.0	0.014	155.6
	100	0.81	184.1	0.84	129.2	0.79	188.1	0.032	355.6	0.033	235.7	0.031	344.4
	150	0.73	165.9	0.77	118.5	0.61	145.2	0.021	233.3	0.022	157.1	0.017	188.9
Phenylalanine	50	0.70	159.1	0.72	110.8	0.56	133.3	0.029	322.2	0.029	207.1	0.023	255.6
	100	0.82	186.4	0.84	129.2	0.71	169.0	0.041	455.6	0.041	292.9	0.035	388.9
	150	0.69	156.8	0.71	109.2	0.49	116.7	0.023	255.6	0.024	171.4	0.016	177.8
Control (Untreated)		0.44	-	0.65	-	0.42	-	0.009	-	0.014	-	0.009	-
L.S.D. (0.05 level)		0.02		0.04		0.02		0.001		0.006		0.001	

1<sup>st</sup> sample: Flowers collected during one month after start of flowering stage; 2<sup>nd</sup> sample: Flowers collected during full-flowering stage; 3<sup>rd</sup> sample: Flowers collected during three month after start of flowering stage; a: % of total weight of collected flowers; b: as percent of the control

**Table V. Major component percentage of chamomile oil produced from of chamomile flower heads as affected by ornithine, proline or phenylalanine treatment. Values are means of two seasons (2001/2002 and 2002/2003)**

Amino acids treatments	Concentration (mgL <sup>-1</sup> )	Major components of chamomile oil					
		Farnesene	$\alpha$ -Bisabolol B	oxide $\alpha$ -Bisabolol	Chamazulene	$\alpha$ -Bisabolol A	oxide
Ornithine	50	6.98	5.52	7.33	8.12	64.60	
	100	8.72	3.63	5.01	9.79	56.13	
	150	9.48	6.02	4.79	11.27	54.25	
Proline	50	12.70	7.43	5.78	10.72	46.01	
	100	6.80	5.52	7.76	8.11	58.74	
	150	7.46	5.46	3.82	9.07	59.86	
Phenylalanine	50	7.11	2.89	9.12	9.98	60.27	
	100	9.97	3.99	14.84	11.48	48.36	
	150	7.05	4.39	10.98	12.30	53.92	
Control (Untreated)		6.55	2.51	5.77	11.78	57.81	
L.S.D. (0.05 level)		0.07	0.04	0.05	0.08	0.03	

concentrations of ornithine and lysine decreased the oil components of basil. It is concluded that foliar spray of amino acids (ornithine, phenylalanine & proline) within the range from 50 to 150 mgL<sup>-1</sup> caused only quantitative in the components of chamomile oil, while its major components remained the same. It could be, therefore, recommended that foliar application of ornithine at 50 mgL<sup>-1</sup>, proline at 150 mgL<sup>-1</sup> or phenylalanine at 50 mgL<sup>-1</sup> before the starting of flowering increased the essential oil contents, in particular the valuable components as  $\alpha$ -bisabolol oxide A.

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