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INFLUENCE OF SOME ANTIOXIDANTS ON GROWTH, FLOWER-HEADS AND ESSENTIAL OIL CONTENT OF *Matricaria chamomilla*, L. PLANTS

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ABSTRACT

A field experiment was carried out during the two successive seasons of 2000/2001 and 2001/2002 at the Experimental Farm of Medicinal and Aromatic Plants Department, Giza, Egypt to study the effect of ascorbic acid and thiamine each at 00, 100, 200 mg/l and their combinations on growth, flower yield, essential oil yield and composition during the harvesting seasons of chamomilla plants.

The obtained results showed that foliar application of ascorbic acid at 100 and 200 mg/l as well as thiamine at 100 mg/l had a significant effect in increasing plant growth and flower heads production, especially with 100 mg/l thiamine combined with 200 mg/l ascorbic acid. The same treatment also caused the maximum increase in oil percentage and yield in flower-heads. All treatments decreased chamazulene percent except with plants treated by thiamine 100 mg/l combined with ascorbic acid 200 mg/l. The same treatment gave the highest essential oil yield as compared with other treatments and control. All treatments increased 1,8-cineol, farnesene and α-bisabolol and decreased bisabolol oxide and chamazulene, except foliar application of 100 mg/l thiamine combined with 200 mg/l ascorbic acid which gave the maximum content of chamazulene. Thiamine treatments did not affect bisabolol oxide A while ascorbic acid increased the relative percentage of bisabolol oxide and the highest content was recorded from 200 mg/l ascorbic acid combined with 200 mg/l thiamine.

INTRODUCTION

Matricaria chamomilla L. Family Asteraceae is an annual herb with an erect, much-branched, glabrous stem with sparse, finely divided (two or three times pinnate) leaves. The flower-heads are used medicinally. Their constituents include up to one per cent of an essential oil with azulene (chamazulene) that turns blue on distillation, and bisabolol and farnesene; also flavonoid, coumarin glycosides, mucilage and fatty acids. These substances give German Chamomile anti-inflammatory, antiseptic, carminative, diaphoretic, sedative and antispasmodic properties. It is one of the most widely used herbal medicines, particularly for children's ailments (Stodola and Volák, 1992).

Ascorbic acid (vitamin C) is known as growth regulating factor that influence many biological processes in plant. Price, (1966) reported that ascorbic acid (AsA) increased nucleic acid content, especially RNA. It also influenced the synthesis of enzymes, and proteins, in addition, it acts as co-enzyme in metabolic changes (Patil and Lall, 1973 and Reda *et al.*, 1977). Talaat (1998) reported that foliar application of ascorbic acid to lavander plants significantly increased growth parameters. Similar results were obtained by Tarraf *et al.* (1999) on lemongrass (*Cympopogon citratus* L.). They reported that foliar application of 50 mg/l ascorbic acid significantly promoted vegetative growth expressed as plant height, number of leaves and tillers, fresh and dry weights of herb as well as total carbohydrate, crude protein, essential oil % and oil yield/plant.

Thiamine (vitamin B_1) is needed for decarboxylation of keto acids, involving pyruvic and ketoglutamic acids, hence its importance was reported in the metabolism of carbohydrates and fats (Robinson, 1973 and Bidwell, 1979) as well as in developmental regulation (Belanger *et al.*, 1996). Pronounced increments in essential oil of lemongrass at 40 mg/l thiamine + 0.15 % microelements mixture was obtained by Refaat and Balbaa (2001). Foliar application of thiamine to various plants increased the vegetative growth, crude protein and amino acids of sesame (Zaki and Tarraf, 1999), fixed and essential oil content of *Nigella sativa* L. (Naguib and Khalil, 2002).

The aim of this investigation was to study the effect of ascorbic acid and thiamine on plant growth, flower-heads yield as well as essential oil content of chamomile plants.

MATERIAL AND METHODS

Field experiment was carried out during the two successive seasons of 2000/2001 and 2001/2002 at the Experimental Farm of Medicinal and Aromatic Plants Department, National Research Centre, Giza, Egypt. Chamomile seeds were secured from Institute of Horticulture Research, Agricultural Research Centre, Ministry of Agriculture, A.R.E.

The seeds were sown directly in the field in plots (2x2 m^2) at 15^{th}, 18^{th} September, 2000, 2001 respectively. After three weeks from sowing the plants were thinned leaving one plant per hill with 50 cm in between, and 60 cm between rows. The factorial experiments were laid-out in a complete randomized block design with three replications (plots). Each plot contained 3 rows, (12 plants per plot) i.e., 12000 plants/feddan.

Nine treatments had been done i.e., ascorbic acid and thiamine each at (00, 100, 200 mg/l) and their combinations. Aqueous solution of each substance was prepared using distilled water. The growth regulating substances were sprayed two times at 45 and 55 days after sowing at the previously mentioned concentrations.

All plants received a basal dose of calcium super-phosphate (15.5 % P_2O_5) at a rate of 150 kg/fed, during soil preparation and 200 kg/fed nitrogen fertilizer as ammonium nitrate (33.5 % N) and potassium fertilizer as potassium sulphate (48 % K_2O) at a rate of 50 kg/fed were supplied in two equal doses, the first one at one month after sowing and the second another month later.

Vegetative growth criteria i.e., plant height, number of branches per plant, fresh and dry weights of herb per plant were recorded at 3rd April, 2001 and 2002, respectively. Flower yield (g/plant and Ton/Fed.) were collected at 29th January, 13th February, 27th February and 11th March 2001, 2002.

Essential oil percentage in the flower-heads was determined four times during the harvesting season (29th January, 13th February, 27th February and 11th March 2001, 2002) using Clevengers apparatus according to the Egyptian Pharmacopoeia (1984), and the essential oil yield (ml/plant & l/fed.) were calculated.

Qualitative and quantitative determination of the different main constituents of chamomile oil, obtained from the second sample (13th Feb.) from each treatment had been carried out. Gas liquid chromatographic apparatus HEWLET PACKARD, equipped with FID, HP 6890 series GC System, USA was used for the separation of chamomile oil fractions of the samples. The analysis conditions were as follows: The chromatography was fitted with splitless injector (initial temp. 250°C) and capillary column HP-INNOWAX Polyethylene Glycol (30 m length, 250 µm diameter, 0.15 µm film thickness, initial flow 2.0 ml/min). The column was operated, using a temperature program, a linear increase with rate of 4°C/min, from (70°C to 190°C); with nitrogen at 30 ml/min, as a carrier gas. The flow rates for hydrogen and air were 30 and 300 ml/min, respectively. Detector temperature was 280°C. Chart speed was 0.5 cm / min. The standard material was injected with the samples of chamomile oil under the same conditions.

Data obtained (mean of the two growing seasons) were subjected to standard analysis of variance procedure. The values of LSD were obtained whenever F values were significant at 5 % level as reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect on vegetative growth

Data presented in Table (1) indicate that plant height, number of branches, fresh and dry weights of herb of ascorbic acid and thiamine-treated plants were significantly affected, being highly pronounced when plants treated with thiamine at 100 mg/l combined with 200 mg/l ascorbic acid.

Table (1): Effect of ascorbic acid and thiamine on the growth of chamomile plants. (Average of two seasons)

Treatments (mg/l)	Plant height (cm)	Number of braches /plant	Fresh weight of herb (g/plant)	Dry weight of herb (g/plant)
Effect of ascorbic acid (mean)				
Control	79.78	21.33	562.50	93.75
AsA 100	84.33	22.44	754.17	125.69
AsA 200	91.67	27.56	835.39	139.23
LSD	2.74	1.62	55.44	9.24
Effect of thiamine (mean)				
Control	77.17	21.11	725.00	120.83
Th 100	98.61	26.33	850.00	141.67
Th 200	80.00	23.89	577.06	96.18
LSD	2.74	1.62	55.44	9.24
Effect of interaction				
Control	78.50	16.67	562.50	93.75
Th 100	93.33	25.67	600.00	100.00
Th 200	67.50	21.67	525.00	87.50
AsA 100	71.50	19.00	800.00	133.33
AsA 200	81.50	27.67	812.50	135.42
AsA 100 + Th 100	99.00	25.00	875.00	145.83
AsA100 + Th 200	82.50	23.33	587.50	97.92
AsA 200 + Th 100	103.50	28.33	1075.00	179.17
AsA 200 + Th 200	90.00	26.67	618.67	103.11
LSD	4.75	2.80	96.02	16.00

AsA = Ascorbic acid; Th = Thiamine

These results are in agreement with those obtained by Talaat (1998) who reported that foliar application of ascorbic acid to lavander plants significantly increased growth parameters. Similar results were obtained by Tarraf *et al.* (1999) on lemongrass (*Cympopogon citratus* L.). It was also reported that foliar application of thiamine to various plants increased growth criteria: (Zaki and Tarraf, (1999) on sesame; Refaat and Balbaa, (2001) on lemongrass and Naguib and Khalil, (2002) on *Nigella sativa* L.).

Data shown in Table (2) indicate that fresh and dry weights of flower-heads (g/plant & ton/fed.) significantly increased as a result of ascorbic acid treatments at (100 or 200 mg/l) as well as thiamine at 100 mg/l. On the other hand, foliar application of thiamine at 200 mg/l significantly decreased fresh and dry weights of flower-heads. Plants treated with 100 mg/l thiamine combined with 200 mg/l ascorbic acid recorded the highest fresh and dry weights of flower-heads. The flower-heads production increased gradually as the plant age advanced during the harvesting seasons and the greatest fresh weight of flower-heads yield (2.36 ton/fed.) and dry weight (0.45 ton/fed.) were recorded in plants treated with 100 mg/l thiamine combined with 200 mg/l ascorbic acid as compared with other treatments and control.

Table (2): Effect of ascorbic acid and thiamine on fresh and dry weights of flower-heads (g/plant & Ton/Fed.) of chamomile plants. (Average of two seasons)

Treatments (mg/l)	First sample (29 Jan.) (g/plant)	Second sample (13 Feb.) (g/plant)	Third sample (27 Feb.) (g/plant)	Fourth sample (11 Mar.) (g/plant)	Total yield of flower-heads (Ton/feddan)
Fresh weight					
Effect of ascorbic acid (mean)					
Control	10.14	26.42	65.05	82.16	1.23
AsA 100	17.06	42.43	78.45	134.21	1.69
AsA 200	19.76	54.15	101.73	147.48	1.89
LSD	0.35	3.62	5.07	4.61	0.01
Effect of thiamine (mean)					
Control	12.88	36.70	55.08	126.60	1.34
Th 100	20.59	52.33	134.84	141.73	2.07
Th 200	13.49	33.98	55.31	95.51	1.39
LSD	0.35	3.62	5.07	4.61	0.01
Effect of interaction					
Control	11.27	31.32	45.85	78.79	1.16
Th 100	13.90	36.25	117.91	110.53	1.82
Th 200	5.27	11.70	31.39	57.17	0.70
AsA 100	12.47	34.13	54.98	143.91	1.39
AsA 200	14.90	44.64	64.43	157.11	1.47
AsA 100 + Th 100	23.50	60.31	135.60	155.56	2.04
AsA100 + Th 200	15.20	32.85	44.77	103.17	1.63
AsA 200 + Th 100	24.37	60.42	151.02	159.12	2.36
AsA 200 + Th 200	20.00	57.41	89.76	126.21	1.83
LSD	0.60	6.27	8.78	7.99	0.02
Dry weight					
Effect of ascorbic acid (mean)					
Control	2.01	5.61	11.40	16.91	0.24
AsA 100	2.77	7.47	17.62	22.86	0.34
AsA 200	3.81	10.56	19.86	25.97	0.38
LSD	0.10	0.98	1.54	1.48	0.004
Effect of thiamine (mean)					
Control	2.75	6.88	14.16	20.63	0.28
Th 100	3.76	9.75	20.32	26.17	0.40
Th 200	2.09	7.01	14.40	18.95	0.28
LSD	0.10	0.98	1.54	1.48	0.004
Effect of interaction					
Control	1.94	5.53	12.74	16.84	0.23
Th 100	3.21	6.00	13.47	22.68	0.33
Th 200	0.89	5.31	8.00	11.22	0.16
AsA 100	2.14	7.15	13.77	22.36	0.28
AsA 200	4.16	7.97	15.97	22.68	0.34
AsA 100+Th 100	3.63	9.78	23.28	26.74	0.41
AsA100 + Th 200	2.55	5.48	15.81	19.49	0.32
AsA 200 + Th 100	4.43	13.46	24.22	29.08	0.45
AsA 200 + Th 200	2.84	10.25	19.39	26.15	0.37
LSD	0.17	1.70	2.67	2.57	0.008

AsA = Ascorbic acid; Th = Thiamine

These results are in accordance with those obtained by Talaat (1998) who reported that ascorbic acid significantly promoted growth of lavender plants. Similar results were obtained by Tarraf *et al.* (1999) on lemongrass plants. In this respect, Smirnoff (1996) indicated that ascorbic acid had a possible role in cell division and expansion.

Concerning the function of thiamine in plants, Robinson (1973) and Bidwell (1979) reported that diphosphothiamine is the biologically active form of thiamine and acts as coenzyme involved in oxidative and nonoxidative decarboxylations and it is essential for the metabolism of pyruvic and α-ketoglutaric acids.

Effect on essential oil

Table (3) show the obtained distilled essential oil from flower-heads collected during four stages from each treatment throughout the flowering period.

It is clear from the obtained data that thiamine treatments especially at 100 mg/l combined with ascorbic acid at 200 mg/l caused the maximum increase in oil percentage in flower-heads. Data also revealed that essential oil percentage increased in the second, third and fourth samples in comparison with the first one and the greatest increase was obtained in the second sample. The highest essential oil yield (27.02 ml/plant & 3.89 l/fed.) were recorded in plants treated with 100 mg/l thiamine combined with 200 mg/l ascorbic acid.

In support of these results, Stieber *et al.* (1979) interpreted that flowers of chamomile had prochamazulene-bearing and prochamazulene free glandular hairs and these latter increased during flowering reached a maximum at full-flowering and then declined. Reda *et al.* (1999) reported that the maximum content of essential oil was obtained at full-flowering stage of chamomile. In addition, Solomon and Honcariv (1994) obtained similar results at Poland for chamomile. It was also verified that the formation of the important therapeutical constituents of chamomile oil was related to flowering stages (Franz *et al.*, 1986 and Repcak *et al.*, 1993). It could be suggested that both vitamins might indirectly regulate terpenoid pathway. Chamomile essential oil, belongs to sequiterpenes, which could be biosynthesized in the plant cytosol from mevalonic acid pathway via isopentenyl pyrophosphate (IPP), which is the basic building block of the isoprenoids (Galili *et al.*, 2002).

Data presented in Table (4) indicate that the essential oil of Matricaria chamomilla was characterized by the high amounts of bisabolol oxide A (44.78-65.87%) followed by chamazulene (8.92-22.49%) and α-bisabolol (6.26-11.94%). The obtained data show that all treatments increased 1,8-cineol, farnesene and decreased bisabolol oxide and chamazulene, except foliar application of 100mg/l thiamine combined with 200 mg/l ascorbic acid which gave the maximum content of chamazulene. Application of thiamine at 200 mg/l gave the highest content of 1,8-cineol (1.17%), farnesene (0.49%) and bisabolol oxide B (0.69%). All treatments increased α-bisabolol and the highest relative percentage was recorded from treatment of 100 mg/l ascorbic acid combined with 200 mg/l thiamine.

Table (3): Effect of ascorbic acid and thiamine on chamomile essential oil (%, ml/plant and yield Liter/ Feddan).

Treatments (mg/l)	First sample		Second sample		Third sample		Fourth sample		yield	
	Effect of interaction									
	%	ml/ plant	%	ml/ plant	%	ml/ plant	%	ml/ plant	Total yield ml/ plant	Total yield Liter/ Fed
Effect of ascorbic acid (mean)										
Control	0.63	0.55	0.51	1.24	0.52	3.02	0.50	3.60	8.41	1.21
AsA 100	0.71	1.04	0.74	2.71	0.64	4.53	0.57	6.47	14.75	2.12
AsA 200	0.72	1.20	0.74	3.37	0.71	6.40	0.63	7.75	18.72	2.70
LSD	0.02	0.03	0.04	0.31	0.02	0.21	0.02	0.34	0.41	0.06
Effect of thiamine (mean)										
Control	0.64	0.69	0.69	2.13	0.58	2.68	0.55	5.80	11.31	1.63
Th 100	0.79	1.37	0.80	3.59	0.76	8.69	0.67	7.98	21.63	3.11
Th 200	0.63	0.73	0.50	1.60	0.52	2.57	0.49	4.05	8.95	1.29
LSD	0.02	0.03	0.04	0.31	0.02	0.21	0.02	0.34	0.41	0.06
Effect of interaction										
Control	0.61	0.57	0.58	1.51	0.56	2.14	0.54	3.53	7.76	1.12
Th 100	0.72	0.83	0.64	1.92	0.60	5.90	0.59	5.45	14.10	2.03
Th 200	0.57	0.25	0.31	0.30	0.39	1.02	0.38	1.81	3.38	0.49
AsA 100	0.67	0.70	0.79	2.24	0.59	2.69	0.49	5.88	11.51	1.66
AsA 200	0.64	0.80	0.71	2.64	0.60	3.22	0.61	7.99	14.65	2.11
AsA 100 + Th 100	0.81	1.59	0.86	4.31	0.78	8.81	0.70	9.07	23.78	3.42
AsA100 + Th 200	0.66	0.84	0.58	1.59	0.56	2.07	0.52	4.47	8.97	1.29
AsA 200 + Th 100	0.84	1.70	0.90	4.54	0.91	11.37	0.71	9.41	27.02	3.89
AsA 200 + Th 200	0.67	1.11	0.61	2.92	0.62	4.61	0.56	5.86	14.50	2.09
LSD	0.04	0.05	0.07	0.54	0.04	0.37	0.03	0.59	0.70	0.1

AsA = Ascorbic acid; Th = Thiamine

Thiamine treatment did not affect bisabolol oxide A while ascorbic acid increased the relative percentage of bisabolol oxide and the highest content (65.97%) was recorded from 200 mg/l ascorbic acid combined with 200 mg/l thiamine.

In this concern, Franz *et al.* (1986) reported that full-flowering stage was the most suitable one to study components of chamomile essential oil. In support Hölzl and Demuth (1975), Piccaglia and Marotti (1993) and Reda *et al.* (1999) reported the same pattern of oil constituents in chamomile type from Egypt.

Table (4): Effect of ascorbic acid and thiamine on essential oil composition of flower-heads of chamomile plants.

Treatment (mg/l) / Compound %	Control	Th 100	Th 200	AsA 100	AsA 200	AsA 100 + Th 100	AsA 100 + Th 200	AsA 200 + Th 100	AsA 200 + Th 200
1,8-cineol	0.30	0.71	1.17	0.80	0.38	0.64	0.72	0.55	0.86
Farnesene	0.07	0.18	0.49	0.15	0.13	0.12	0.25	0.15	0.10
Bisabolol Oxide B	0.03	0.17	0.69	0.22	0.26	0.25	0.32	0.17	0.15
Bisabolol Oxide	0.26	0.04	0.25	0.04	0.05	0.08	0.18	0.02	0.03
α-bisabolol	6.26	8.81	9.18	7.01	7.12	7.34	11.94	6.92	8.21
Bisabolol oxide A	58.55	58.15	55.62	64.20	65.81	57.74	57.38	44.78	65.87
Chamazulene	22.49	15.83	12.03	14.83	11.66	18.01	18.12	35.92	8.92
Total identified	87.96	83.89	79.43	87.25	85.41	84.17	88.92	88.50	84.14

AsA = Ascorbic acid; Th = Thiamine

Reichling and Beiderbeck (1991) also reported that the most important compounds of pharmaceutical value in chamomile flower-heads are α-bisabolol, α-bisaboloxides and matricine (decomposed to chamazulene during distillation). Supporting the present data, Pino *et al.*, (2002) identified the volatile compounds in the oil of chamomile from Iran using GC/MS. They found that the main components were α-bisabolol oxide A and chamazulene.

It could be concluded that the present results support the role of ascorbic acid in plant growth, metabolism and important processes reported by Smirnoff (1996) and Blokhina *et al.* (2003).

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تأثير بعض مضادات الأكسدة على نمو وتزهير ومحتوى الزيت الطيار لنبات شيح البابونج

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أجريت تجربة حقلية خلال الموسمين الزراعيين ٢٠٠٠/ ٢٠٠١-٢٠٠١/٢٠٠٢ بمزرعة قسم زراعة وإنتاج النباتات الطبية والعطرية، المركز القومي للبحوث بالجيزة و ذلك لدراسة تأثير المعاملة بالرش بحمض الأسكوربيك و الثيامين على نمو نبات شيح البابونج وكذلك على محتوى الأزهار من الزيوت الطيارة.

وقد أوضحت النتائج أن معاملة النباتات بالرش بحمض الأسكوربيك عند تركيز ٢٠٠ مجم/لتر بالإضافة إلى الثيامين ١٠٠ مجم/لتر أدت إلى أفضل النتائج بالنسبة لنمو النبات ومحتوى الأزهار من الزيوت الطيارة. كما أدت جميع المعاملات إلى خفض محتوى الزيت الطيار من الكمازولين ما عدا معاملة النباتات بالثيامين عند تركيز ١٠٠ مجم/لتر + حمض الأسكوربيك عند ٢٠٠ مجم/لتر. كما أوضحت النتائج أن الثيامين لم يؤثر على محتوى الزيت من باي سابولول أوكسيد أ بينما أدى حمض الأسكوربيك إلى زيادة نسبته في الزيت الطيار