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PHYSIOLOGICAL RESPONSE OF *Lavandula angiostifolia* L. PLANTS TO CYSTEINE AND NICOTINEAMIDE

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ABSTRACT

A pot experiment was conducted in the screen of the National Research Centre to study the effect of foliar application of cysteine and nicotineamide on the growth and chemical constituents of lavender plants (*Lavandula angiostifolia* L.). The results indicated that foliar application of cysteine, nicotineamide or their combinations enhanced the vegetative growth of lavender plants, especially when plants were sprayed with cysteine (100 mg/l) combined with nicotineamide (100 mg/l). Data also show that foliar application of cysteine (100 mg/l) combined with nicotineamide (100 mg/l) significantly increased total sugars, total proteins, essential oil percentage and yield. On the other hand, cysteine (50 mg/l) combined with nicotineamide (100 mg/l) resulted in the highest content of linalool, linalyl acetate and geraniol in lavender volatile oil.

INTRODUCTION

A growing interest in the use of the natural substances instead of synthetic products for flavouring results in a large demand for aromatic plants which are sources of natural compounds. Aromatic plants and their essential oils rich in aromatic and flavouring chemicals are used in cosmetics and pharmaceuticals and as flavour ingredients in food products (Morton, 1977; Simon *et al.,* 1984 and Duke, 1985).

Lavender (*Lavandula angiostifolia* L.), Family *Lamiaceae*, is one of the most important aromatic plants grown in Egypt. Essential oil extracted from lavender plants is one of the most important volatile oils, which is widely used for the scenting of soaps. It is used in many cosmetics and technical preparations: bath salts, room sprays and disinfectants (Guenther, 1973). Lavender is an evergreen subshrub with a much-branched woody stem, the square green shoots thickly covered with entire, linear leaves, which at first are white-felted, later green. All parts of the plant are aromatic. The flowering stems or the flowers alone are used medicinally. Garden Lavender has mild sedative, carminative, antispasmodic, rubefacient and tonic properties. In herbalism it is still used internally for headache, nervous disorders and insommia, as a cough suppressant and for flatulence, but mostly it is used externally as a skin freshener. The essential oil, obtained from the fresh plants by steam distillation, is a component of various proprietary preparations. The oil´s chief use, however, is in perfumes, colognes and toilet articles. It is also used to mask unpleasant odours in medicines (Stodola and VolǗk, 1992).

The amino acid cysteine is a component of the antioxidant glutathione (Buwalda *et al*., 1990; Strohm *et al.,* 1995; Noctor *et al*., 1996, 1997). The influence of cysteine availability on glutathione levels reflects the importance of glutathione as a reservoir of reduced sulfur (Buwalda *et al.,* 1990) and as the principal form in which organic sulfur is transported in many plants (Bergmann and Rennenberg, 1993).

Little information is available about the role of vitamins in the regulation of the biosynthesis of essential oils in plants. Nicotineamide (niacin) is considered as one of growth regulating substances which, in minute quantities, can alter some physiological aspects of plants (Bearder, 1980). Robinson (1973) reported that nicotineamide acts as coenzyme in the enzymatic reactions by which carbohydrates, fats and proteins are metabolized and involved in photosynthesis and respiration.

The aim of the present work was to enhance vegetative growth of *Lavandula angiostifolia* by using cysteine and nicotineamide, and studying their effects on metabolism, especially on volatile oils.

MATERIAL AND METHODS

A pot experiment had been conducted at the Experimental Farm of National Research Centre, Dokki, Cairo during two successive seasons of 1999/2000 and 2000/2001. Uniform terminal cuttings of lavender (5-6 buds) were kindly supplied from the Experimental Farm of Pharm. Sci. Dept., Giza, Egypt. All cuttings were treated for 60 seconds with 1000 mg/l IBA before sowing to stimulate rooting. Two uniform cuttings were planted in each pot (30 cm in diameter) at 18^{th} and 21^{st} November for the two successive seasons, respectively.

Each pot was fertilized with 4 g calcium superphosphate (P_2O_3) and 1 g potassium sulphate (48% K_2O) before planting and 1 g ammonium nitrate (33.5% N) at 30 and 45 days from planting. Fertilization was repeated after collecting the first cutting. All plants were irrigated as needed.

Plants were foliarly sprayed with cysteine or nicotineamide at zero, 50 and 100 mg/l and interaction treatments of the different concentrations of the two factors had been also carried out. Cysteine treatments had been applied at 5^{th} & 6^{th} March 2000, 2001 and nicotineamide at 6^{th} and 7^{th} March, 2000, 2001 respectively. The experimental design was complete randomized with three replicates, each replicate represented by three pots. The volume of the spraying solution was maintained just to cover completely the plant foliage till drip. The first cut was collected at 5^{th} & 6^{th}, June 2000 and 2001, respectively. Foliar spray with cysteine and nicotineamide was repeated one month after the first cutting. The second cut was collected at 10^{th}, 9^{th} Oct. 2000 and 2001, respectively. The plant herbage was harvested, by cutting 10 cm above the soil surface, and plant growth characters in terms of plant height, number of branches, and herbage fresh and dry weights were recorded.

Total sugars in the dried material were determined according to Dubois *et al.* (1956). Total protein content was determined using the method described by Bradford (1976), while essential oil percentage in the fresh herbage of each treatment was determined by hydro-distillation according to the Egyptian Pharmacopoeia (1984).

Qualitative and quantitative determination of the different main constituents of lavender oil, obtained from the first cut from each treatment had been carried out in parallel with authentic samples of different oil components by GLC technique. The qualitative identification of the main oil fractions was carried out by comparing the relative retention time of different peaks with those of the pure authentic samples. The quantitative determination was achieved by the peak area percentage, which was measured for each fraction; to study the changes in the constituents of lavender oil as a result of the effect of different treatments used.

For this purpose, gas liquid chromatographic apparatus HEWLET PACKARD, equipped with FID, HP 6890 series GC System, USA was used for the separation of lavender oil fractions. 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 lavender oil under the same conditions.

Data obtained (mean of two growing seasons), were subjected to standard analysis of variance procedure. The values of LSD were calculated at 5 % level according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect on vegetative growth. Data presented in Table (1) show that cysteine treatments significantly promoted the number of branches, fresh and dry weights of herb in both cuttings, especially in plants treated with 100 mg/l cysteine. Plant height was also significantly promoted as a result of foliar application of cysteine, especially at 100 mg/l. These findings were in agreement with those obtained by Reda *et al*., (1999) who reported that vegetative growth criteria of *Hyoscyamus muticus* L. were significantly increased under cysteine effect, especially at treatment 100 mg/l cysteine.

The present results emphasized that nicotineamide significantly increased plant height, number of branches, fresh and dry weights of herb in both cuttings (Table, 1). The highest recorded results were obtained in plants treated with 100 mg/l nicotineamide. These results are in accordance with those obtained by Foda (1987) and Deyab (1989) on wheat plants. In addition, Hathout *et al.* (1993a,b) found that the application of 10, 40 and 80 mg/l nicotineamide as foliar spray on tomato plants caused stimulatory effects on growth, yield and endogenous promoters (auxins and gibberellins). Similar findings were also obtained by Tarraf *et al*., (1999) who reported that foliar application of nicotineamide on lemongrass plants significantly promoted vegetative growth.

Data presented in Table (1) show also that foliar spray of lavender plants with cysteine (100 mg/l) combined with nicotineamide (100 mg/l) significantly influenced plant height. This combined treatment resulted in the highest pronounced effect on number of branches as well as fresh and dry weights of herb in both cuttings. These results coincided with those obtained by Abdel-Halim (1995), Reda *et al.* (1999) and Tarraf *et al*., (1999). This stimulatory effect of nicotineamide on growth was found to be correlated with the increase in content and activity levels of endogenous promoters particularly GA_3 and IAA which are known to promote linear growth of plant organs (stoddart, 1986).

Table (1): Effect of cysteine and nicotineamide on vegetative growth of *Lavandula angiostifolia* plants. (average of the two seasons)

Treatments	Plant Height (cm)		Number of Branches / plant		Fresh weight of herb (g/plant)		Dry weight of herb (g/plant)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Effect of Cysteine (mean)								
Control	38.11	33.67	8.89	8.56	94.22	68.56	23.68	17.14
C 50	39.67	36.44	9.78	10.11	103.92	81.09	25.41	20.27
C 100	41.89	38.78	13.44	11.11	116.93	86.59	29.68	21.65
LSD (5%)	0.76	0.68	0.73	0.62	2.47	2.07	0.62	0.52
Effect of Nicotineamide (mean)								
Control	36.44	33.67	9.78	8.89	94.73	69.06	23.55	17.27
NA50	40.11	37.00	10.44	9.11	101.64	80.21	25.98	20.05
NA100	43.11	38.22	11.89	11.78	118.71	86.97	29.23	21.74
LSD (5%)	0.76	0.68	0.73	0.62	2.47	2.07	0.62	0.52
Effect of Interaction								
Control	35.33	29.00	8.00	7.33	84.68	58.86	21.17	14.71
C 50	36.33	34.33	9.33	8.67	96.88	69.58	24.22	17.40
C 100	37.67	37.67	12.00	10.67	102.61	78.75	25.65	19.69
NA50	38.67	34.67	9.00	8.33	93.43	69.64	23.36	17.41
C50+NA50	39.00	37.00	9.67	9.33	103.59	82.24	25.90	20.56
C100 +NA50	42.67	39.33	12.67	9.67	107.89	88.75	26.97	22.19
NA100	40.33	37.33	9.67	10.00	104.55	77.18	26.14	19.30
C50 +NA100	43.67	38.00	10.33	12.33	111.28	91.46	27.82	22.87
C100 NA100	45.33	39.33	15.67	13.00	140.29	92.28	35.07	23.07
LSD (5%)	1.32	1.18	1.27	1.07	4.29	3.59	1.07	0.90

C= Cysteine, NA= nicotineamide

Effect on chemical constituents. Data presented in Table (2) show that foliar application of cysteine to lavender plants significantly increased total sugars % in the herb, especially at 100 mg/l treatment. Total protein followed the same trend. These increases hold true for both cuttings. These results are in agreement with those obtained by Reda *et al*., (1999) who reported that total nitrogen percentage in *Hyoscyamus muticus* L. was significantly increased in the leaves as a result of cysteine foliar application.

Data presented in Table (2) show that foliar application of nicotineamide on lavender plants significantly increased total sugars %, especially at 100 mg/l. Total protein was also increased as a result of nicotineamide application at 100 mg/l concentration. These results could be explained by the findings obtained by Robinson (1973) who reported that nicotineamide acts as coenzyme in the enzymatic reactions by which carbohydrates, fats and proteins are metabolized and involved in photosynthesis and respiration.

Data presented in Table (2) show also that foliar spray of lavender plants with cysteine (100 mg/l) combined with nicotineamide (100 mg/l) significantly increased total sugars % and total protein in both cuttings.

Table (2): Effect of cysteine and nicotineamide on the chemical constituents of *Lavandula angiostifolia* L. plants.

Treatments	Total sugars %		Protein (µg/g FW)		Oil %		Oil yield (ml/plant)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
ffect of Cysteine (mean)								
Control	14.08	16.53	1645.59	1352.94	0.20	0.17	18.88	12.19
C 50	15.48	16.98	1702.39	1393.91	0.22	0.22	22.86	18.36
C 100	17.06	18.16	1727.33	1414.18	0.24	0.25	29.06	21.60
LSD (5%)	0.40	0.34	8.81	3.84	0.01	0.01	0.86	0.60
ffect of Nicotineamide (mean)								
Control	14.25	16.02	1666.81	1366.65	0.20	0.19	18.61	13.12
NA50	15.91	17.52	1691.21	1385.35	0.21	0.22	21.13	17.59
NA100	16.47	18.13	1717.30	1409.03	0.26	0.24	31.07	21.43
LSD (5%)	0.40	0.34	8.81	3.84	0.01	0.01	0.86	0.60
ffect of Interaction								
Control	13.22	15.23	1613.52	1328.75	0.17	0.12	14.40	7.16
C 50	14.10	15.52	1685.23	1380.33	0.20	0.21	19.37	14.56
C 100	15.43	17.30	1701.68	1390.87	0.22	0.22	22.06	17.65
NA50	14.52	16.62	1641.81	1347.92	0.20	0.19	18.21	12.94
C50+NA50	15.50	17.63	1706.45	1395.67	0.21	0.22	21.41	18.08
C100 +NA50	17.70	18.32	1725.36	1412.47	0.22	0.25	23.75	21.75
NA100	14.52	17.74	1681.45	1382.16	0.23	0.21	24.05	16.47
C50 +NA100	16.83	17.79	1715.49	1405.72	0.25	0.25	27.79	22.45
C100 +NA100	18.05	18.88	1754.97	1439.21	0.30	0.28	41.36	25.39
LSD (5%)	0.69	0.58	15.27	6.65	0.01	0.01	1.49	1.04

C= Cysteine, NA= nicotineamide

These results could be explained by the findings obtained by Reda *et al*., (1999) who emphasized that the promotive effect of cysteine availability might be due to its effect on glutathione levels which is important as a reservoir of reduced sulfur (Buwalda *et al.,* 1990) and as the principal form in which organic sulfur is transported in many plants (Bergmann and Rennenberg, 1993). Supporting this suggestion, Buwalda *et al*. (1990), Strohm *et al,* (1995), Noctor *et al*. (1996) & (1997) reported that the amino acid cysteine is a component of the antioxidant glutathione.

In addition, Sana and Ota (1977), Bearder (1980), Foda, (1987) Sharaf El-Din *et al*. (1987) and Hathout *et al*. (1993a,b) reported that nicotineamide plays an important role in increasing pigments, carbohydrates, nitrogen, RNA and DNA contents in plants. Tarraf *et al*., (1999) also reported that foliar application of nicotineamide to lemongrass plants significantly increased total carbohydrates and crude proteins.

Effect on essential oil. Data presented in Table (2) show that oil % and total oil yield/plant were significantly increased as a result of foliar spray of cysteine, nicotineamide or their combinations. It is clear from the obtained data that the highest recorded value of essential oil was obtained from plants treated with 100 mg/l nicotineamide combined with 100 mg/l cysteine treatment. These results hold true for essential oil % and total oil yield/plant in both cuttings.On the other hand, the interaction effect of cysteine combined with nicotineamide show that the highest recorded values of essential oil % and yield were obtained in plants treated with 100 mg/l cysteine combined with 100 mg/l nicotineamide (Table, 2).

In this concern, Tarraf *et al*., (1999) reported that foliar application of nicotineamide to lemongrass plants significantly increased essential oil percentage and yield per plant.

The changes in the oil percentage might be attributed to cysteine effect on metabolism and enzyme levels responsible for mono or sesqueterpene biosynthesis as reported by Lawrence (1978). Furthermore, it was found that the enzymes responsible for the biosynthesis of higher terpenes arised in plastids as reported by Amelunxen and Arbeiter (1967), and these enzymes could cause an increase in the essential oil.

The essential oil of lavender herb from different treatments in addition to that of the untreated control were subjected to fractionation using gas liquid chromatography (GLC) and the data are represented in Table (3). Thirteen hydrocarbons and oxygenated terpenes were markedly identified. Accordingly it is clear from the obtained data that 1,8-cineol and linalool represent the major compounds which ranged from 11.54 to 59.25 and 9.11 to 16.86%, respectively.

Hydrocarbon terpenes ranged from 5.50 to 19.95%, while the oxygenated compounds ranged from 57.76 to 83.81%. It is also clear from data presented in Table (3) that plants treated with cysteine (100 mg/l) combined with nicotineamide (50 mg/l) recorded the highest level of total oxygen compounds (83.81%). Meanwhile, plants treated with cysteine at 50 mg/l combined with 50 mg/l nicotineamide recorded the lowest content of total oxygenated compounds (57.76 %).

Foliar application of cysteine (50 mg/l) combined with nicotineamide (100 mg/l) possessed the best quality of the essential oil because of its low content of 1,8-cineol and a relatively high content of linalool, linalyl acetate and graniol which is a desirable character (Table, 3).

These results are in agreement with those obtained by Piccaglia and Marotti (1993) who reported that the main components in *Lavandula angustifolia* P. Miller x *L. latifolia* (L. fill.) Medikus (lavendin) oil are 1,8-cineol, linalool, camphor and linalyl acetate which define the oil quality. There are also some minor compounds such as nerol, geraniol, neryl acetate and geranyl acetate which are important because they form the so called “rhodinol fraction” which provides a rose scent that confers a sweet and pleasant note to the oil.

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From the above mentioned data, it could be concluded that cysteine and nicotineamide, might play a role in plant phytochemical mechanisms through their effect on the metabolism of terpenes, essential oil, carbohydrates and proteins, but further studies are needed to learn more about these mechanisms.

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الاستجابة الفسيولوجية لنبات اللافندر للسيستيين و النيكوتين أميد

إيمان محمود طلعت
قسم النبات ، المركز القومى للبحوث ، الدقى ، القاهرة.
الرقم البريدي: ١٢٦٢٢

أجريت تجربة أصص فى الصوبة السلكية بالمركز القومى للبحوث و ذلك لدراسة تأثير الرش بالحمض الأمينى سيستيين والنيكوتين أميد على النمو الخضرى والمحتوى الكيماوى لنبات اللافندر. و قد أثبتت الدراسة أن رش النباتات بالسيستيين أو النيكوتين أميد أدى الى تشجيع النمو الخضرى و زيادة محتوى العشب من الزيت الطيار ، كما أدت المعاملة المشتركة بالسيستيين ١٠٠ مجم/لتر + النيكوتين أميد ١٠٠ مجم/لتر الى أفضل النتائج بالنسبة للنمو الخضرى و محتوى العشب من السكريات الكلية و البروتين الكلى و الزيت الطيار و محصول الزيت الطيار.

أدت معاملة العشب بالرش بالسيستيين (٥٠ مجم/لتر) + النيكوتين أميد (١٠٠ مجم/لتر) الى أفضل النتائج بالنسبة لصفات الزيت الطيار حيث أدت الى إنخفاض محتوى الزيت من ١,٨ - سنيول وزيادة محتوى الزيت من الليناالول و ليناليل اسيتات والجيرانيول.

Table (3): Effect of cysteine and nicotineamide on the essential oil components of *Lavandula angiostifolia* L. plants.

Component %	control	C 50	C 100	NA50	NA100	C50+ NA50	C50+ NA100	C100+ NA50	C100+ NA100
α - Pinene	1.62	0.91	5.70	2.07	8.58	1.15	2.05	2.78	2.20
β - Pinene	1.88	0.65	3.60	2.40	0.69	1.58	2.72	2.00	2.52
α - Terpinene	2.45	1.12	5.70	2.27	0.72	1.71	0.92	2.80	1.36
α- Caryophyllene	1.85	0.81	1.78	1.76	0.38	1.17	0.48	0.00	1.04
Sesquterpenes	1.28	1.05	2.53	1.20	0.46	1.82	4.34	0.00	1.20
β - Bisabolene	0.98	0.95	0.64	0.71	0.36	1.04	1.77	0.00	1.00
Total hydrocarbons	*10.07*	*5.50*	*19.95*	*10.42*	*11.18*	*8.48*	*12.27*	*7.58*	*9.32*
1,8 – Cineol	39.66	47.42	17.69	38.00	59.25	27.47	11.54	44.69	39.84
Linalool	10.03	13.25	15.67	9.62	11.85	9.11	12.35	15.57	16.86
Camphor	8.03	4.88	5.56	3.02	3.26	3.37	2.43	9.37	3.15
Linalyl acetate	5.51	4.66	5.70	3.39	2.51	8.53	11.29	4.09	6.15
Geraniol	0.53	5.78	7.34	5.76	0.71	5.66	16.46	3.07	1.58
Lavandulyl acetate	2.82	0.87	6.23	2.48	0.46	2.53	7.75	5.55	3.11
Geranyl acetate	1.56	0.81	1.81	1.64	0.31	1.09	1.71	1.47	1.92
Total oxygen compounds	*68.13*	*77.68*	*59.99*	*63.91*	*78.35*	*57.76*	*63.52*	*83.81*	*72.61*
Identified	*78.21*	*83.18*	*79.94*	*74.32*	*89.54*	*66.25*	*89.54*	*91.39*	*81.92*
Not Identified	*21.79*	*16.82*	*20.06*	*25.68*	*10.46*	*33.75*	*10.46*	*8.61*	*18.08*

C= Cysteine, NA= nicotineamide