

THE ROLE OF AMINO ACIDS ORNITHINE AND PHENYLALANINE IN GROWTH, CHEMICAL CONSTITUENTS AND ALKALOIDAL CONTENTS IN *Datura innoxia* Mill

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ABSTRACT

An experiment was conducted in two successive seasons to study the effect of ornithine and phenylalanine (100,200 and 400 ppm) on growth, carbohydrates, pigments and alkaloidal content of *Datura innoxia* Mill leaves at flowering stage. The highest value of plant height and number of branches or leaves were obtained from plants treated with 200 ppm ornithine and phenylalanine. Plants treated with 400 ppm ornithine and 200 ppm phenylalanine gave the highest value of leaf area and dry weight of leaves.

The application of amino acids, ornithine and phenylalanine resulted in low levels of soluble as well as, non soluble sugars and total carbohydrate of *Datura* leaves as compared to the control. While the same treatment gave the highest level of chlorophyll a and b, whereas carotenoids were decreased due to applications of low concentrations of ornithine and phenylalanine.

Ornithine and phenylalanine showed a stimulatory effect on leaf chemical compositions such as nitrogen, protein, RNA, DNA and alkaloidal content. The highest values of these contents were obtained from ornithine at 400 ppm and phenylalanine at 200 or 400 ppm.

INTRODUCTION

Datura innoxia Mill. is one of the most important medicinal plants containing alkaloids. The accumulation of the alkaloids attains its maximum in the leaves during the flowering stage. The leaves are rich in two alkaloids: hyoscyne and hyoscyamine, which are reputed for their use as pre-anaesthetics in surgery, prevention of motion sickness and mania (Hilal and Youngken, 1984). The alkaloids in several *Datura* species, are mostly formed in the roots, but are rapidly translocated to the leaves. The plant produces hyoscyamine ($C_{17}H_{22}NO_3$) and the valuable drug hyoscyne ($C_{17}H_{20}NO_3$).

The six amino acids regarded as the starting points for alkaloids biosynthesis, namely, phenylalanine, tryptophan, ornithine, lysine, histidine and anthranilic acid. Feeding experiments with labelled ornithine, have revealed that this amino acid is incorporated stereospecifically to form the pyrrolidine ring of tropine. Also, phenylalanine, is the precursor of tropic acid. Esterification of tropic acid with tropine produce hyoscyamine (Balbaa *et al.*, 1976).

The similarity of some alkaloid structures to plant growth hormones (Nowacki *et al.*, 1965), stimulated the idea that at least some alkaloids or their precursors can play a role in growth regulation. However, exposure of an alkaloid rich plant to treatments with alkaloid precursors could either exceed the optimal level and prove to be inhibiting, or the level may still below the critical point and however, be stimulating. Trofimova *et al.*, (1973), reported that the addition of certain amino acids to the nutrient medium stimulated alkaloid synthesis and adenosin triphosphatase activity of *Datura innoxia*.

Ahmed and Leete, (1970) and Evans and Woolley, (1976) recorded that the development of arginine, ornithine, proline and glutamic acid groups played apart in the biogenesis of tropane alkaloids of *Datura innoxia*. Hence, the presentwork was conducted aiming to study the effect of these compounds on growth and yield of *Datura innoxia*, and its alkaloidal content.

MATERIAL AND METHODS

The present work conducted in two successive seasons 1998 and 1999 at the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University. The seeds of *Datura innoxia* Mill were planted on the 25th of March in the two seasons in the plot area 2m² contained 8 plants similarly spaced. The design of the experiments was a complete randomized block design of three replicates.

The object of the study was to investigate the effect of ornithine and phenylalanine on the growth of *Datura innoxia*, as well as its chemical compositions. The treatments were divided into two groups; at the first group the plants were sprayed with three concentrations (100, 200 and 400 ppm) of ornithine, and the second group, the plants were sprayed with the same concentrations of phenylalanine, additional control treatment was sprayed with distilled water. Plant were sprayed twice with the above mentioned concentrations after 50 and 65 days after planting.

During the flowering stage, the mean value of plant height, number of branches per plant, number of leaves per plant, leaf area and dry weights of leaves were recorded. Total carbohydrates % in leaves, soluble and non soluble sugars were determined according to Dubois *et al*, (1956) The photosynthetic pigments of fresh leaves, chlorophyll a, b and carotenoids were determined and calculated according to the standard method of Moran (1982). Total nitrogen was determined by Kjeldahl method (A.O.A.C., 1984), the crude protein was calculated by multiplying the total nitrogen by 6.25. Total nucleic acids was determined spectrophotometrically according to the method of Nieman and Poulson (1963 and 1967) in the fresh leaves. Total alkaloids were extracted according to the method of Balbaa *et al*, (1964) and then determined according to the colorimetric method of Karawya *et al*, (1975).

Data obtained were subjected to standard analysis of variance procedure, Snedecor and Cochran, (1980).

RESULTS AND DISCUSSION

1- Growth characters :

Data presented in Table (1) showed that the application of ornithine and phenylalanine at different concentrations had a significant positive effect on growth characters of *Datura innoxia*, such as plant height, number of branches and leaves, leaf area as well as dry weight of leaves, compared to

corresponding control. Concerning plant height and number of branches, the data showed that foliar application of ornithine and phenylalanine especially 200 ppm, showed the highest value of plant height and number of branches comparing with other treatments and control. Number of leaves per plant generally increased with all concentrations of ornithine. The most effective treatment in this respect was that of 200 ppm ornithine and phenylalanine for number of branches.

Table (1): Plant height, number of branches, number of leaves, leaf area and dry weight of leaves of *Datura innoxia* Mill. as affected by ornithine and phenylalanine.

(Average of two seasons).

Treatments		Plant height (cm)	number of branches/plant	number of leaves/plant	leaf area/plant (cm ²)	dry weight of leaves/plant
Control		95.89	28.25	129.35	200.75	29.66
ornithine (ppm)	100	97.76	29.64	134.41	197.33	27.05
	200	101.31	32.28	137.87	199.95	28.62
	400	93.54	28.13	133.25	239.23	30.34
phenylalanine (ppm)	100	96.14	30.96	128.75	198.48	30.16
	200	102.4	31.85	134.18	206.40	32.23
	400	87.96	26.64	127.82	197.25	25.25
L.S.D. at 5%		1.91	1.97	1.93	1.78	1.84

It is clear from the same table that ornithine (400 ppm) significantly increased leaf area and dry weight of leaves. While the maximum significant value for these characters were obtained as a result of phenylalanine at 200 ppm.

From the above mentioned data, it can be concluded that ornithine and phenylalanine at 200 ppm had a favourable effect on growth characters of *Datura innoxia*, compared with other treatments and control.

Concerning the effect of amino acids on vegetative growth, the present results are in agreement with those obtained by Salonen (1980), Moursy *et al.*, (1988) and El-Bahr *et al.*, (1990), they reported pronounced enhancement of some vegetative growth criteria of different species under the effect of amino acids application. These results are also supported by those obtained by Gamal El-Din *et al.*, (1997) and Reda *et al.*, (1999), who reported that foliar application of amino acids on lemon-grass and *Hyoscyamus muticus* plants significantly promoted plant growth.

2- Chemical composition:

(a) Carbohydrate :

Data presented in Table (2) show the effect of ornithine and phenylalanine on soluble as well as non soluble sugars and total carbohydrates of *Datura* leaves. All treatments led to significant reduction in soluble sugar contents of *Datura* leaves in comparison with control. Similar trend as soluble sugars was recorded for non soluble sugars, except that the highest concentration of phenylalanine (400 ppm) favoured the highest content. Also the same results were obtained on total carbohydrate of *datura* leaves.

Generally, it seems from the above mentioned data that ornithine and phenylalanine, when used at lower concentrations, reduced the soluble, non soluble sugars and total carbohydrates of *Datura innoxia* leaves. These results are in harmony with related literature, where El-Sherbeny and Hassan (1987), also, Gendy and Rabie (2000), found that carbohydrates content in *Datura* sp. were decreased due to amino acids application.

Table (2): Soluble sugars, non soluble sugars and total carbohydrate percentage of dry leaves of *Datura innoxia* Mill. as affected by ornithine and phenylalanine (Average of two seasons).

Treatments		Soluble sugars %	Non soluble sugars %	Total carbohydrate %
Control		4.87	10.24	15.11
ornithine (ppm)	100	4.49	9.74	14.25
	200	4.66	9.16	13.82
	400	4.80	9.06	13.66
phenylalanine (ppm)	100	4.15	9.97	14.12
	200	4.01	10.01	14.02
	400	4.14	11.18	15.32
L.S.D. at 5%		0.08	0.06	0.07

(b) Photosynthetic pigments :

The influence on photosynthetic pigment obtained for ornithine and phenylalanine treated *Datura innoxia* M. leaves is presented in Table (3). It could be noticed that all treatments significantly increased chlorophyll a, chlorophyll b and chlorophyll a+b values over the control. The highest increases were obtained with ornithine at 100 ppm and phenylalanine at 200 ppm concentration (Table 3).

From the same Table, carotenoids content of *Datura* leaves showed insignificant responses to alkaloid precursor treatments, except ornithine and phenylalanine at (100 ppm). The other concentrations of ornithine and phenylalanine slightly decreased carotenoids content in *Datura* leaves. It could be, however, noted that only chlorophyll a and b responded significantly to the alkaloid precursor treatments. On the other hand, an inhibitory effect of certain alkaloids precursor was detected on carotenoids synthesis in plants. Similar results were obtained on *Datura* plants by El-Sherbeny and Hassan, (1987), Hussein *et al.*, (1992), who stated that application of amino acid treatment resulted in significant effect on chlorophyll a and b in *Datura* leaves, while carotenoids decreased significantly. The accumulation of photosynthetic pigments chlorophyll a, b, as a result of these nitrogen compounds may be due to the important role of nitrogen in the biosynthesis of chlorophyll molecules, Mayer *et al.*, (1968).

Table (3): photosynthetic pigments content mg/g dry leaves of *Datura innoxia* Mill. as affected by ornithine and phenylalanine. (Average of two seasons).

Treatments	Chlorophyll a	Chlorophyll b	Chl. a+b	carotenoids	
Control	4.85	1.53	6.38	2.31	
ornithine (ppm)	100	5.94	2.47	8.41	2.36
	200	5.34	1.96	7.30	2.04
	400	5.33	2.04	7.37	1.48
phenylalanine (ppm)	100	5.24	2.13	7.37	2.47
	200	6.00	2.05	8.05	1.96
	400	5.22	1.95	7.17	2.08
L.S.D. at 5%	0.06	0.04	0.08	0.05	

(c) Nitrogen, protein, RNA, DNA and total alkaloids:

Total nitrogen and crude protein contents (%) of *Datura* leaves are shown in Table (4). It could be concluded that all concentrations of treatments excepting phenylalanine (400 ppm) significantly increased total nitrogen or crude protein in *Datura* leaves. The highest recorded values of total nitrogen and protein percent were obtained in the leaves of plants treated with 400 ppm ornithine (4.01 and 25.06 %) followed by 200 ppm phenylalanine (3.49 and 21.81 %) respectively. These results agree with those obtained by El-Bahr *et al.*, (1990) on *Datura metel* and Talaat and Youssef (2002) on Basil plant. They concluded that some alkaloid precursors treatment resulted in an increase of total nitrogen or crude protein.

The effect of ornithine and phenylalanine on RNA and DNA (mg/g) in *Datura* leaves is presented in the same Table (4). It could be noticed that all treatments significantly increased RNA and DNA compared with control. Increasing the concentration of ornithine and phenylalanine caused a gradual increase in both RNA and DNA mg/g in the leaves. The maximum increase was obtained with higher concentrations of ornithine and phenylalanine (400 ppm). On the other hand, all treatments of foliar spray at different rates of ornithine and phenylalanine induced reduction in RNA/DNA ratio. These results are in agreement with those obtained by Drumm and Walter (1982) and Hassanein *et al.*, (1998) who found that the nucleic acid contents (DNA and RNA) increased progressively during their active growth reaching their maximum levels when the leaves approach their full expansion.

Table (4) : Nitrogen, protein, RNA, DNA and total alkaloid content of *Datura innoxia* Mill. leaves as affected by ornithine and phenylalanine. (Average of two seasons).

Treatments	N %	Protein %	RNA mg/g	DNA mg/g	RNA/DNA	total alkaloids content mg/g dry weight	total alkaloids content mg/total dry weight	
Control	3.12	19.50	0.77	0.58	1.33	3.26	96.69	
Ornithine (ppm)	100	3.26	20.38	0.86	0.67	1.28	4.02	108.74
	200	3.33	20.81	0.90	0.73	1.23	4.19	119.92
	400	4.01	25.06	0.99	0.76	1.30	4.33	131.37
phenylalanine (ppm)	100	3.32	20.75	0.85	0.75	1.13	4.17	125.77
	200	3.49	21.81	0.93	0.79	1.18	4.95	159.54
	400	3.20	20.00	0.95	0.81	1.17	5.25	132.56
L.S.D. at 5%	0.11	0.65	0.05	0.08	0.10	0.07	0.10	

The data presented in the same Table (4) reveal that the application of alkaloid precursors ornithine and phenylalanine, resulted in significant increase in the alkaloidal content (mg/g) and /or (mg per total dry weight) in the leaves of *Datura innoxia*, in comparison to the control. Best results were obtained with phenylalanine at (400 ppm). While, ornithine at 100 ppm gave the lowest increase of total alkaloids (mg/g). On the other hand, application of phenylalanine (200 ppm) recorded the highest values of alkaloidal contents (mg/total dry weight). Generally, it may be concluded from the abovementioned results that phenylalanine treatment had a stimulatory effect in increasing the alkaloidal content, compared to ornithine. These results are in harmony with those of Koul *et al.*, (1983), Nyman *et al.*, (1993), Ali (1998) and Reda *et al.*, (1999). They all came to a conclusion that, ornithine application lead to a significant increase in alkaloid production of *Hyoscyamus muticus* and *Atropa belladonna*. There is a strong correlation between alkaloid formation and ornithine, This incorporation of the precursors enhanced the production of alkaloid. Also, from the abovementioned data it could be concluded that, alkaloids content increased in *Datura* leaves by application of phenylalanine treatments. These results agreed with the findings of Ghosal *et al.*, (1985) and Kafarski *et al.*, (1995). They reported that alkaloids were derived from aromatic acids phenylalanine and tyrosine. In this connection El-Sherbeny and Hassan (1987) and Moursy *et al.*, (1988) on *Datura stramonium* and Ali and Ahmed (1998) on *Atropa belladonna*, reported that the application of phenylalanine, caused significant increases in alkaloids content of the leaves.

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دور الأحماض الأمينية الأورنيثين والفينيل الالين في النمو والمحتوى الكيماوى
والقلويدات في نبات الداتورا
إبراهيم الأباصيرى حبة
المركز القومى للبحوث - قسم النبات - الدقى - القاهرة

يهدف البحث إلى دراسة تأثير الأورنيثين والفينيل الالين بتركيزات ١٠٠، ٢٠٠، ٤٠٠ جزء في المليون على النمو، الكربوهيدرات، الصبغات ومحتوى القلويدات في أوراق الداتورا إنوكسيا في مرحلة الإزهار خلال موسمين متتاليين. وأظهرت النتائج أن أعلى معدل لطول النبات وعدد الأفرع وعدد الأوراق نتج من معاملة النباتات بالأورنيثين والفينيل الالين عند تركيز ٢٠٠ جزء في المليون، بينما أعطت معاملة النباتات بالأورنيثين والفينيل الالين عند تركيز ٤٠٠، ٢٠٠ جزء في المليون أعلى زيادة في مساحة الورقة والوزن الجاف للأوراق.

أعطت المعاملة بالأحماض الأمينية الأورنيثين والفينيل الالين أقل محتوى من السكريات الذائبة، والغير ذائبة والسكريات الكلية في أوراق الداتورا مقارنة بالكنترول، بينما أعطت نفس المعاملات زيادة في محتوى الكلورفيل أ، ب ونقص في الكاروتين مع التركيزات المنخفضة.

أنت المعاملة بالأورنيثين والفينيل الالين إلى زيادة كل من النيتروجين، السبروتين، الأحماض النووية وكذلك القلويدات بأوراق نبات الداتورا وكانت أعلى القيم مع المعاملة بالأورنيثين عند تركيز ٤٠٠ جزء في المليون والفينيل الالين عند تركيز ٢٠٠، ٤٠٠ جزء في المليون.