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EFFECT OF PUTRESCINE AND PHENYLALANINE ON GROWTH, AND ALKALOID PRODUCTION OF SOME *Datura species.*

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ABSTRACT

Two field experiments were carried out at the Experimental Farm of Cultivation and Production of Medicinal and Aromatic Plants Dept. National Research Centre at Giza, during two successive seasons, to study the effect of foliar application of two tropane alkaloid precursors, phenylalanine (0, 100 and 200 ppm) and/or putrescine (0, 50 and 100 ppm) on some growth parameters and alkaloid contents of *D. stramonium* and *D. innoxia* plants that originated from Bulgaria. *Datura stramonium* showed the superiority of all growth parameters, except root weight at flowering stage, while at fruiting stage, *Datura innoxia* showed visible increment of all growth parameters. Application of phenylalanine 100 ppm+ putrescine 50 ppm was sufficient to accelerate the growth parameters of *D. stramonium* at flowering and fruiting stages and *D innoxia* at fruiting stage. At flowering stage, significant promotion in all growth parameters of *D. innoxia* was observed due to foliar application of phenylalanine 100+ putrescine 100 ppm. *D. stramonium* with high level of phenylalanine and putrescine separately significantly increased crude protein in leaves at flowering stage, while *D. innoxia* had an opposite trend. The highest value of free amino acids in leaves at flowering stage was obtained from *D. stramonium* plants treated with putrescine 50 ppm, while the maximum percentage for *D. innoxia* was observed in plants treated with phenylalanine 100+ putrescine100 ppm. Hyoscine and hyoscyamine alkaloids were determined in leaves and fruits at flowering and fruiting stages using HPLC. Most of phenylalanine and putrescine treatments tended to increase hyoscine and hyoscyamine contents (mg/g dry matter and mg/plant) of two *Datura spp*, throughout the growing seasons. Foliar spraying with phenylalanine and putrescine connectively increased greatly hyoscine, hyoscyamine and total alkaloids contents in *Datura spp* more than individually ones, in most cases. Application of phenylalanine 100 + putrescine 50 ppm gave the highest values of hyoscyamine mg/plant, while treatment of phenylalanine 100 + putrescine100 gave the highest levels of total alkaloids mg /plant in both *Datura spp*.

Keywords: *Datura stramonium*, *Datura innoxia*, Polyamines, Putrescine, Phenylalanine, Growth, Yield, Alkaloids, Hyoscine, Hyoscyamine

INTRODUCTION

Datura stramonium and *Datura innoxia* are considered the most important medicinal plants containing alkaloids. Most organs of these species are rich in two alkaloids, hyoscine and hyoscyamine, which are reputed for their use aspre-anaethetics in surgery, prevention of motion sickness and mania. Tropane alkaloids are formed in the roots and transported to the aerial parts of the plant (Shoji *et al.*, 2000). Datura alkaloids are biosynthetically derived from putrescine and phenylalanine (Medina-Bolivar and Flores,

1995). The biosynthetic steps for tropane alkaloids start from putrescine, which has converted by putrescine N-methyltransferase to N-methylputrescine to tropinone and ultimately for accumulation of hyoscyamine and hyoscine (Sato *et al.*, 2001).

The triamine spermidine and the tetraamine spermine are present in all plant cells, together with their diamine precursor putrescine. These polyamines have been suggested to play an exclusive role in plant physiological processes, including growth, differentiation, senescence and responses to stress (Schuber, 1989; Galston and Sawhney, 1990; Chibi *et al.*, 1994 and Bouchereau *et al.*, 1999). Polyamines are biologically active compounds involved in various physiological processes. They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation, related to aging and senescence, and usually involved in plant responses to stress (Flores and Galston 1982, Friedman *et al.* 1989). Also Wolukau *et al.*, (2004) stated that at 25 °C, putrescine stimulated all pollen germination in *Prunus mume* plant.

Several studies have been carried out to enhance the growth criteria and accumulation of hyoscine and hyoscyamine through application of amino acids foliar spray (Davies, 1982). In this connection Moursy et al. (1988) established callus lines of *Datura stramonium L.* and found that phenylalanine and ornithine increased both fresh and dry weights of callus compared with control. Moreover, Aziz *et al.*, (2002) illustrated the effect of biochemical precursors i.e. phenylalanine and putrescine on the tropane alkaloid (hyoscine and hyoscyamine) in *D. metal.* They found that putrescine and phenylalanine at 0.05 and 0.1 mM enhanced root growth and production of hyoscine and hyoscyamine.

The present work was carried out to illustrate the effect of two alkaloid precursors, putrescine (diamine) and phenylalanine (amino acid) on growth criteria and alkaloids production in *Datura stramonium* and *Datura innoxia* through physiological plant stages to replace these natural substances instead of other synthetic plant growth regulators, which have not desired in the recent trend. .

MATERIALS AND METHODS

Two-field experiments were carried out at the Experimental Farm of Cultivation and Production of Medicinal and Aromatic Plant Dept., National Research Centre, Giza, Egypt, during two consecutive seasons (2001 / 2002 and 2002 / 2003). Seeds of *Datura stramonium* and *Datura innoxia* originated from Bulgaria (provided by Prof. Dr. Liuba Evstatieva Botany Institute, Bulgarian Academy of Sciences) were seeded in seedbeds in the same farm on 25th of October in both seasons. The experimental soil was clay loam soil of the following characteristics: coarse sand, 4.5 %; fine sand 32.4 %; clay 23.8 %; silt 39.3 %; organic matter 1.78 %; total N (mg/100g) 5.31; P (mg/100g) 0.27; K (mg/100g) 1.38; pH 8.0; E.C. (dS/m) 0.8 and $CaCO_3$ 3.7 %. The experimental area was arranged in a complete randomized block design with four replicates. Plots area were 9 m^2 (3X3), with 5 rows, the distance between plants 60 cm., only 25 plants in every plot. After 60 days

from sowing, the seedlings were transplanted into the open field immediately after irrigation. During soil preparation, 150 kg/fed. super phosphate (15.5 % P_2O_5) was applied. After transplanting, 200 kg ammonium nitrate (33.5 % N) and 100 kg potassium sulphate (48 % K) per fed. were added. The mineral fertilization was conducted through two equal portions during the growing season; the first portion was added after one month of transplanting, while the second one was applied after two weeks of the first portion.

All agricultural practices were carried out as usually recommended for *Datura species* cultivation in Egypt. Putrescine was used as foliar application at rates of zero, 50 and 100 ppm. as well as, phenylalanine was sprayed with levels, zero, 100 and 200 ppm and their combination were applied. Both of them were sprayed two times during a growing season. After one month of transplanting, the first one was applied, while the second one was sprayed after two weeks in both seasons. The spraying treatments were carried out at the early of morning and spreading agent "Masrol" was added to solutions (1ml/l) to reduce solution surface tension.

Samples of *Datura stramonium* and *Datura innoxia* were collected during flowering stage at 15th April and fruiting one at 15th June. Sample of 5 plants were taken randomly from the middle two rows of each plot for measuring root weight, stem weight, number of leaves, leaves weight, plant fresh weight, plant dry weight, fruit fresh weight and fruit dry weight. Represented samples from the roots, leaves and fruits of each species were subjected for chemical analysis. Determination of crude protein by modified micro-Kjeldahl method as described by A.O.A.C. (1980) and free amino acids content was determined according to Rosen (1957).

Analysis of hyoscine and hyoscyamine:

Hyoscine and hyoscyamine were analyzed by the method of Medina-Bolivar and Flores (1995). Ethanolic extract of dried samples was evaporated to dryness at 40°C by rotary evaporator and then 5ml of 0.5 M sulphuric acid and 20ml chloroform were added to the residue. The aqueous phase was adjusted to pH 10 with ammonia solution, chloroform layer was separated by separatory funnel, dried with sodium sulphate anhydrous and then evaporated to dryness. The residues were dissolved in methanol and filtered through a 0.2-µm filter. Extracts were subjected for separation using Shimadzu-HPLC with Nova-Pak C18 (Waters) steel column (3.9 x 150 mm) using a mobile phase of 12.5% (v/v) acetonitrile and 87.5% aqueous phosphoric acid (0.3%) adjusted to pH 2.2 with triethylamine at a flow rate 0.8 ml/min. using UV-detector (260 nm). Identification and quantitification of hyoscine and hyoscyamine in the samples were done by reference to the retention time of authentic standards (Sigma).

Data obtained (means of both growing seasons) were subjected to factorial 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).

RESULT AND DISCUSSION

1- Growth parameters.

Growth parameters data of *Datura stramonium* and *Datura innoxia* at flowering and fruiting stages as affected by Putrescine and Phenylalanine application are shown in Tables (1 and 2).

a- Species effects.

Both species were significantly different for root weight, stem weight, number of leaves, and plant weight at flowering stage. *Datura stramonium* showed superiority of all these parameters except root weight at flowering stage. On the other hand, the opposite trend was observed at fruiting stage in *Datura innoxia* that showed visible increment of all growth parameters.

b- Phenylalanine and putrescine effects

Growth parameters were fluctuated in their responses to different phenylalanine and putrescine treatments. Most of phenylalanine and putrescine treatments tended to decrease values of growth characters, but the consistent significant decreases, throughout the growing season were recorded for putrescine at 50 ppm (ranged between 4.2 % and 20.6%, relative to control). Application of 100 ppm phenylalanine+100 ppm putrescine gave the highest values of all growth parameters of *Datura spp* at flowering stage (Table 1) comparing with untreated plants (control), followed by phenylalanine (100 ppm) + putrescine (50 ppm). On the other hand, at fruiting stage application of 100 ppm phenylalanine+50 ppm putrescine gave the highest increases in growth parameters that constituted about 40% and 56% above control for fresh weights of leaves and whole plant, respectively. Increasing phenylalanine from 100 to 200 ppm significantly increased root weight at flowering stage and leaves fresh weight, fruit fresh weight, plant fresh weight, fruit dry weight and plant dry weight at fruiting stage as well as stem weight and plant fresh weight at flowering stage. At fruiting stage, leaves dry weight insignificantly increased, while number of leaves insignificantly decreased and leaves weight not responded. Increasing putrescine from 50 to 100 ppm significantly increased root weight, stem weight, leaves weight and plant fresh weight at flowering stage. On the other hand, number of leaves at flowering stage and leaves fresh weight, leaves dry weight and plant dry weight at fruiting stage insignificantly increased, while fruit dry weight was not responded. The positive effect of phenylalanine on fresh and dry weight may be due to its effect on growth stimulation of plant cells.

Enhancement effects of phenylalanine and putrescine on growth parameters are in line to the results obtained by Goss, (1973) reported that amino acids can serve as a source of carbon and energy, as well as protect the plant against pathogens and also in the synthesis of other organic compounds such as protein, amines, purines and pyrimidines , alkaloids, vitamins and others. As for polyamine enhancement effects, Evans and Malmberg (1989) reported that polyamine involvement in various growth and developmental phases: cell division, embryogenesis, rooting, flowering, and pollen tube growth. These results are going also in the same line with those

obtained by Thom et al., (1981) who reported that amino acids provide plant cells with an immediately available source of nitrogen that generally can be taken by cells more rapidly than inorganic nitrogen. The results also agree with those reported by Moursy *et al.*, (1988) established callus lines of *Datura stramonium* L. and found that phenylalanine and ornithine increased both fresh and dry weights of callus compared with control. Plant height, number of leaves and branches, leaf area, as well as fresh and dry weights of different organs of *D. metel* were significantly increased by soaking the seeds with different levels of adenine, cytosine and thiamine (Hussein *et al.*, 1992).

c- Interaction effects.

With respect to the response of growth parameters at flowering and fruiting stages of *D. stramonium and D. innoxia* to foliar application with phenylalanine and putrescine (Table 1 and 2), No clear trend was observed in the interaction treatment at flowering stage. Application of phenylalanine 100 ppm + putrescine 50 ppm to *D. stramonium* recorded the highest values in all growth parameters compared with control and other treatments except stem weight that reached the maximum values (127.7 g/plant) by application with phenylalanine 100+putrescine 100 ppm compared with control (86.5 g/plant). On the other hand, foliar application with phenylalanine (100 ppm) +putrescine (100ppm) to *D. innoxia* plants significantly promoted all growth parameters at flowering stage. Meanwhile, at fruiting stage (Table, 2), all growth parameters in both *Datura spp* gave the highest values by application with phenylalanine 100ppm+ putrescine 50ppm followed by phenylalanine 100+ putrescine 100 except fruit dry weight in *D. innoxia* that showed the highest values with phenylalanine 200 ppm followed by phenylalanine 100ppm +putrescine 50 ppm. These results are going in the same line with those obtained by El-Fawakhry and El-Tayab (2003) who found that foliar spray with amino acids improved the vegetative growth and produced a high quality inflorescences of Chrysanthemum. Several authors also indicated the promotion effect of amino acids on *Datura spp* plants including, Trofimova *et al.*, (1973) on *Datura innoxia*, El-Sherbeny and Hassan (1987) on *D. stramonium* and Hussein *et al.*, (1992) on *D. metel.*

2- Crude protein and free amino acids contents.

a- Species effects

Crude protein and free amino acids contents (g/100 g dry matter) in leaves of *D. stramonium* and *D. innoxia* as affected by phenylalanine and putrescine at flowering and fruiting stages are presented in Tables (3 and 4). Data reveal that *D. Stramonium* showed insignificant increased in crude protein at flowering stages, while it recorded highly significant at fruiting stage. Crude protein in fruits showed the same pattern at fruiting stages. Total free amino acids significantly increased in leaves of *D. stramonium* reached 5.03 (g/100g dry matter) at flowering stages. On the other hand, at fruiting stages, no significant difference was observed. The highest recorded value of total free amino acids was obtained in the fruits of *D. stramonium* that reached 3.49, while *D. innoxia* reached only 2.52 g/100g dry matter.

Table (1): Effect of phenylalanine and putrescine on growth characters of *Datura stramonium* and *Datura innoxia* at flowering stage (mean values of two successive seasons).

Parameters / Treatments		Root weight (g/plant)			Stem weight (g/plant)			No. of leaves / plant			leaves weight (g/plant)			Plant weight (g/plant)		
		Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control		14.6	34.7	24.7	86.5	87.2	86.9	90	85	87.5	168	131	149	269	253	261
Pha 100		17.0	29.9	23.5	110.0	62.1	86.1	102	75	88.5	179	106	143	306	198	253
Pha 200		12.0	42.0	27.0	72.5	106.7	89.6	87	87	87	145	141	143	230	290	260
Put.50		14.5	25.5	20.0	78.8	59.1	69.0	86	74	80	137	104	121	230	189	210
Put.100		13.8	40.0	26.9	74.6	86.5	80.6	83	80	81.5	146	139	143	234	266	251
Pha100+put.50		24.4	39.5	32.0	119.4	94.0	106.7	116	82	99	220	148	184	364	282	323
Pha100+put.100		20.2	49.6	34.9	127.7	108.3	118	114	106	110	212	156	184	360	314	337
Pha.200+put.50		10.0	32.2	21.1	71.3	68.6	70.0	79	79	79	135	113	124	216	214	215
Pha200+put.100		10.6	41.9	26.3	74.2	84.7	79.5	73	98	85.5	137	143	140	222	270	246
Mean		15.2	37.3		90.6	84.1		92.2	85.1		164	131		270	252	
LSD 5%	Species	1.7			2.4			1.0			5			6		
	Treatment	2.7			4.44			3.4			10			18		
	Interaction	3.8			6.2			4.8			15			26		

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

Table (2): Effect of phenylalanine and putrescine on growth characters of *Datura stramonium* and *Datura innoxia* at fruiting stage (mean values of two successive seasons).

Parameters / Treatments		Leaves fresh weight (g/plant)			Fruit fresh weight (g/plant)			Plant fresh weight (g/plant)			leaves dry weight (g/plant)			Fruits dry weight (g/plant			Plant dry weight (g/plant)		
		Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control		217	269	243	150	234	192	905	1500	1203	29.2	35.3	32.3	21.2	32.5	26.8	100	145	127
Pha 100		213	263	238	156	232	194	925	1363	1144	28.6	36.2	32.4	21.5	31.1	26.3	102	129	119
Pha 200		170	359	264	167	300	234	950	1813	1382	23.5	43.1	33.3	24.1	41.1	32.6	105	169	137
Put.50		167	275	221	147	221	184	810	1391	1101	23.4	32.7	28.1	20.8	28.0	24.4	92	131	111
Put.100		173	276	225	149	235	192	850	1375	1113	23.2	37.0	30.1	20.5	30.6	24.5	95	131	112
Pha100+put.50		289	390	340	254	305	280	1425	2325	1875	39.9	46.3	43.1	37.2	40.2	41.2	160	193	179
Pha100+put.100		225	387	306	252	235	244	1221	1600	1411	31.2	43.0	37.1	36.8	28.8	32.8	130	147	138
Pha.200+put.50		230	240	235	231	202	217	1199	1300	1250	31.7	31.0	31.4	31.8	23.7	27.8	124	122	123
Pha200+put.100		166	268	217	163	215	189	902	1375	1139	23.1	34.7	28.9	23.5	25.7	25.6	93	128	111
Mean		206	303		185	242		1021	1560		28.2	37.7		26.7	31.3		111	144	
LSD 5%	Species	11			5			20			0.5			2.8			5		
	Treatment	12			18			7^			2.2			3.3			6		
	Interaction	17			26			110			3.3			4.7			8		

Table(3): Effect of phenylalanine and putrescine on crude protein and free amino acids contents in the leaves of *Datura stramonium* and *Datura innoxia* at flowering stage (mean values of two successive seasons).

Character / Treatments		Crude protein (g/100g dry matter)			Total free amino acid (g/100g dry matter)		
		Str.	Inox	Mean	Str.	Inox	Mean
Control		23.6	22.8	23.20	3.36	4.12	3.74
Pha 100		21.9	25.7	23.80	5.82	2.82	4.32
Pha 200		26.9	21.9	24.40	5.15	2.84	4.00
Put.50		21.3	24.1	22.70	6.36	3.28	4.82
Put.100		26.6	23.8	25.20	5.42	3.30	4.36
Pha100+put.50		27.2	19.7	23.45	4.05	3.80	3.93
Pha100+put.100		24.4	21.6	23.00	5.86	6.50	6.18
Pha.200+put.50		27.7	25.4	26.55	4.65	5.97	5.31
Pha200+put.100		24.3	26.6	25.45	4.60	6.38	5.49
Mean		24.88	23.51		5.03	4.33	
LSD 5%	Species	NS			0.23		
	Treatment	1.81			0.31		
	Interaction	2.57			0.44		

Str.= *Datura stramonium* Inox.= *Datura innoxia*
Pha= Phenylalanine Put. = Putrescine

b- Phenylalanine and putrescine effects

Data in Tables (3 and 4) show also the effect of Phenylalanine and putrescine on crude protein and free amino acids contents in leaves of both *Datura spp.* Foliar application with Phenylalanine at 100 ppm was insignificant increased comparing with untreated plants. Increment of putrescine from 50 to 100 ppm significantly increased crude protein in the leaves and reached 8.6% over control. The highest recorded values were obtained in plants treated with phenylalanine 200+ putrescine 50 ppm that reached 14.4% over control, followed by phenylalanine 200 + putrescine 100 ppm reached 9.7% compared with control at flowering stage. Meanwhile at fruiting stage (Table 4), foliar application of phenylalanine100+putrescine100 recorded the highest values of crude protein in leaves followed by phenylalanine 200 + putrescine 100 ppm and putrescine 100 reached 19.7%, 15.4% and 11.8% over control, respectively. Most treatments significantly augmented crude protein in fruits except application of phy 100 that insignificantly decreased it. The highest value of crude protein was obtained in fruits of plants received phenylalanine 200 + putrescine 100ppm followed by plants received phenylalanine 200 + putrescine 100 ppm. Foliar application with Phenylalanine 100 ppm was insignificant increased free amino acids in fruits comparing with untreated plants. The more phenylalanine was applied the more free amino acids in fruits was observed. On the other hand, with putrescine application, no significant difference was observed. The highest value of free amino acids was obtained in fruits of plants received high levels of phenylalanine with high levels of putrescine followed by plants received phenylalanine 100+ putrescine 100 ppm. Davies,(1982) reported that amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins, which formed by a process in which ribosome catalyze the polymerization of amino acids.

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

Table (4): Effect of phenylalanine and putrescine on crude protein and free amino acids content (g/100g dry matter) in leaves and fruits of *Datura stramonium* and *Datura innoxia* at fruiting stage (mean values of two successive seasons).

Organs		Leaves						Fruits					
Treatmentsr		Crude protein			Free amino acids			Crude protein			Free amino acids		
		Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control		24.3	21.2	22.8	3.25	3.83	3.54	16.4	12.5	14.5	2.85	2.42	2.64
Pha 100		28.2	17.3	22.8	3.39	2.97	3.18	15.0	12.8	13.9	3.84	1.57	2.71
Pha 200		25.3	18.4	21.9	4.01	4.08	4.05	19.0	11.1	15.1	3.52	2.90	3.21
Put.50		23.0	22.5	22.8	3.74	4.12	3.93	17.5	12.8	15.2	2.90	2.45	2.68
Put.100		25.8	25.2	25.5	2.45	3.29	2.87	20.1	12.5	16.3	2.65	2.59	2.62
Pha100+put.50		26.3	24.0	25.2	4.35	3.53	3.94	18.7	12.4	15.6	3.02	2.50	2.76
Pha100+put.100		30.2	24.3	27.3	2.71	4.09	3.40	18.9	12.6	15.8	5.02	2.87	3.95
Pha.200+put.50		30.3	20.3	25.3	3.15	2.96	3.06	18.8	15.9	17.4	2.50	2.01	2.26
Pha200+put.100		27.9	24.7	26.3	3.66	2.84	3.25	20.2	15.0	17.6	5.11	3.34	4.23
Mean		26.8	22.0		3.41	3.52		18.3	13.1		3.49	2.52	
LSD 5%	Species	0.1			NS			0.1			0.08		
	Treatment	0.3			0.14			0.9			0.19		
	Interaction	0.5			0.19			1.1			0.26		

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

foliar application of amino acids. `Polyamines can influence the transcriptional and translational stages of protein synthesis (Pegg, 1986), stabilize membranes (Schuber, 1989) and alter intracellular free calcium levels (Khan *et al.*, (1993).

c- Interaction effects

Effect of the interaction between Phenylalanine and putrescine and both D. *stramonium and D. innoxia* on crude protein and free amino acids contents in leaves and fruits are shown in Table (3 and 4). Treating *D. stramonium* with high level of phenylalanine and putrescine separately significantly increased crude protein in leaves at flowering stage. On the other hand, *D. innoxia* have opposite trend. The highest values of leaves crude protein at flowering stage was observed in *D. stramonium* treated with phenylalanine 200 + putrescine 50 ppm that reached 17.7% over control while the maximum value of *D. innoxia* was observed with plants treated with phenylalanine 200 + putrescine 100 that reached 16.6% compared with untreated plant. At fruiting stage, the maximum values of crude protein were recorded with *D. stramonium* plants treated with phenylalanine 200 + putrescine 100 ppm and putrescine 100ppm that reached 23.2% and 22.6% over control, respectively. On the other hand, the maximum values of crude protein were recorded with *D. innoxia* plants treated with phenylalanine 200 + putrescine 50 ppm and phenylalanine200+putrescine100 ppm that reached 27.2% and 20% over control, respectively.

The highest value of free amino acids in leaves at flowering stage was obtained of *D. stramonium* plants treated with putrescine 50 ppm that reached 89.3% over untreated plants, while the maximum percentage (57.8% over control) was observed in *D. innoxia* plants treated with phenylalanine100+putrescine100 ppm. The first unequivocally established function for polyamines at the molecular level is the donation of a 4-aminobutyl moiety by spermidine to the eukaryotic initiation factor 5A [eIF-5A] precursor protein to form the amino acid hypusine (Park *et al*, 1993). Application of phenylalanine 100 + putrescine 50 ppm gave the highest values (4.35 g/100g dry matter) of free amino acids in leaves at fruiting stage followed by plants received phenylalanine 200 ppm (4.01 g/100g dry matter) comparing with control (3.25 g/100g dry matter) for *D. stramonium*. Meanwhile *D. innoxia* showed the maximum values with plants treated with putrescine 50 ppm (4.12 g/100g dry matter) followed by plants received phenylalanine 100 + putrescine 100 (4.09 g/100g dry matter) and phenylalanine 200 pm (4.08 g/100g dry matter). The highest value of free amino acids in fruits was obtained of *D. stramonium* and *D. innoxia* plants received phenylalanine 200 + putrescine 100ppm that calculated 79.3% and 38% over untreated plants, respectively followed by plants received phenylalanine 100 + putrescine 100 ppm that reached 76.1% and 18.6% over control plants, respectively. These results are supported by those obtained by Hussein *et al.*, (1992) who found that adenine, cytosine and thiamine as basic nitrogen compounds showed a stimulatory effect on organic materials such as protein and alkaloids content in all organs of Datura plants. Also, polyamines can influence the transcriptional and translational stages of protein synthesis (Pegg, 1986), stabilize membranes (Schuber, 1989) and

alter intracellular free calcium levels (Khan *et al.*, 1993). These findings are in accordance with Cohen, (1998) who reported that the diamine putrescine and the polyamines spermidine and spermine are small aliphatic amines found in all plant cells. These basic molecules, which are positively charged at physiological pH because of ubiquitous in nature and the positive charge, polyamines are known to bind to negatively charged molecules, e.g. nucleic acids, acidic phospholipids and various types of proteins.

3- Hyoscine, Hyoscyamine and total alkaloids contents in leaves.

a- Species effects

The data presented in table 5 and 6 reveal that hyoscine, hyoscyamine and total alkaloids in leaves at flowering and fruiting stages, measured both as mg/g dry matter and mg/plant of *D. stramonium* was very much higher when compared to *D. innoxia* except hyoscine content (mg/plant) that showed significant increased in *D. innoxia* at fruiting stage than *D. stramonium*. Moreover, leaves of *D. stramonium* accumulated much higher amounts of hyoscyamine than in *D. innoxia* (approximately 2 fold). *D. stramonium* also achieved over two-fold higher hyoscyamine (mg/plant) than *D. innoxia*. Hyoscyamine was considered the main alkaloids in leaves of *D. stramonium,* while hyoscine considered the main ones in *D. innoxia*. Quantities of hyoscine, hyoscyamine and total alkaloids in leaves at flowering stages (young plants) of *D. stramonium* and *D. innoxia* are higher than that at fruiting stages. These results are going in the same line with those obtained by Miraldi *et al.*, (2001) who reported that atropine and scopolamine content of *D. stramonium* depend on both the plant part considered and the stage of plant growth. They also added that, in leaves of young *D. stramonium* plants, scopolamine content is higher than atropine, also hyoscyamine being always the predominant component. Stems of young plant, not the seeds as generally held are the plant parts with the highest content of tropane alkaloids.

b- Phenylalanine and putrescine effects

The obtained results (table 5 and 6) demonstrated that most of phenylalanine and putrescine treatments significantly increased hyoscine content (mg/g dry matter and mg/plant) of two *Datura spp* at flowering and fruiting stages. Foliar application with phenylalanine or putrescine at low levels increased hyoscine content, while increasing concentrations decreased it. The comparison between phenylalanine and putrescine revealed the superior effect of putrescine on the hyoscine and total alkaloids contents (mg/g dry matter). The highest values of hyoscine content in leaves of two *Datura spp* under investigation at flowering stage were obtained with plants received phenylalanine 100+putrescine 100 ppm followed by plants treated with putrescine 50 ppm. In leaves at fruiting stage (Table 6), hyoscine content was showed the same pattern, but the highest values of hyoscine content were observed with plants received phenylalanine 200 + putrescine 50 ppm followed by plants treated with putrescine 50 ppm.

The highest values of hyoscyamine mg/g dry matter at flowering stage was detected in *Datura spp* plants treated with putrescine 50 ppm and this increment reached 26.2%, followed by phenylalanine 100+putrescine 50 that reached 22.4%, while phenylalanine 100+ putrescine 50ppm gave the

highest values of hyoscyamine mg/plant and phenylalanine 100+ putrescine100 gave the highest one in total alkaloids mg /plant. Application of phenylalanine200+putrescine 50ppm significantly decreased hyoscyamine and total alkaloids contents at flowering stage that gave the lowest values, while this treatment significantly increased hyoscyamine and total alkaloids contents at fruiting stages and gave also the highest amount of hyoscyamine (1.50) and total alkaloids (3.05) mg/g dry matter, and hyoscyamine (47.2) and total alkaloid (95.6)mg/plant. Trofimova *et al.*, (1973) reported that the addition of certain amino acids to the nutrient medium stimulated alkaloids synthesis and adenosine triphosphatase activity of *D. innoxia*. On the other hand, Staba and Jindra (1968) found that amino acid precursors had no significant effect upon the production of alkaloids in *D. stramonium*.

c- Interaction effects

Data in Table (5) clearly indicated that foliar application with putrescine 50 ppm increased greatly the biosynthesis of hyoscine and total alkaloids contents (mg/g dry matter) in *D. stramonium* at flowering stage, which reached 32.2% and 32.1% over control followed by application with phenylalanine100+putrescine100 ppm that reached 29.5% and 21% over control, respectively. On the other hand, *D. innoxia* showed more responded in hyoscine and total alkaloids accumulation by application with phenylalanine 100 + putrescine 100 ppm that reached 34.3% and 30.3% over control followed by putrescine 50 ppm that reached 21.9% and 19% over control, respectively. Meanwhile, the highest amount of hyoscyamine (1.86 mg/g dry matter) in *D. stramonium* recorded in case of application of putrescine 50 ppm followed by application with phenylalanine 200 ppm that reached (1.82) and phenylalanine 100 + putrescine 50, while phenylalanine 100 ppm gave the highest amount of hyoscyamine content (0.92) followed by phenylalanine 100 +putrescine 100 (0.91).

Regarding to the hyoscine, hyoscyamine and total alkaloids content (mg/plant) in *D. stramonium*, the most pronounced effect was obtained in case of application with phenylalanine 100 +putrescine 50 followed with phenylalanine 100 + putrescine 100. Hyoscyamine content in *D. innoxia* had the same trend, while hyoscine and total alkaloid gave the highest values due to foliar application with phenylalanine 100+ putrescine 100 followed by phenylalanine 200+ putrescine 100, respectively. Dealing with the fruiting stage (Table 6), data clearly indicate that in most cases spraying with phenylalanine and putrescine connectively increased greatly the biosynthesis of hyoscine, hyoscyamine and total alkaloids contents more than individually ones.

4- Hyoscine, Hyoscyamine and total alkaloids contents in fruits.

a- Species effect

Data presented in table (7) show that no significant differences between *D. stramonium* and *D. innoxia* were observed in hyoscine content mg/g dry matter, while hyoscine mg/plant showed highly significant increased

Table(5): Effect of phenylalanine and putrescine on alkaloids content in the leaves of *Datura stramonium* and *Datura innoxia* at flowering stage (mean values of two successive seasons)..

Characters / Treatments	Hyoscine (mg/g dry matter)			Hyoscyamine (mg/g dry matter)			Total alkaloids (mg/g dry matter)			Hyoscine (mg/plant)			Hyoscyamine (mg/plant)			Total alkaloids (mg/plant)		
	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control	1.49	1.37	1.43	1.41	0.74	1.07	2.90	2.11	2.50	33.6	25.2	29.4	31.7	13.6	22.6	65.2	38.8	52.0
Pha 100	1.53	1.45	1.49	1.56	0.92	1.24	3.09	2.37	2.73	36.7	21.7	29.2	37.5	13.8	25.6	74.2	35.5	54.8
Pha 200	1.52	1.27	1.39	1.82	0.76	1.29	3.34	2.03	2.68	29.5	25.2	27.4	35.4	15.1	25.3	64.9	40.4	52.6
Put.50	1.97	1.67	1.82	1.86	0.84	1.35	3.83	2.51	3.17	36.2	24.5	30.4	34.2	12.3	23.3	70.5	36.8	53.6
Put.100	1.60	1.50	1.55	1.49	0.84	1.16	3.09	2.34	2.71	31.3	29.4	30.3	29.1	16.5	22.8	60.4	45.9	53.1
Pha100+put.50	1.65	1.33	1.49	1.80	0.83	1.31	3.45	2.16	2.80	48.7	27.8	38.2	53.0	17.3	35.2	101.7	45.1	73.4
Pha100+put.100	1.93	1.84	1.88	1.59	0.91	1.25	3.51	2.75	3.13	54.8	40.5	47.6	45.0	20.0	32.5	99.8	60.5	80.1
Pha.200+put.50	1.39	1.66	1.53	1.34	0.71	1.03	2.73	2.37	2.55	25.1	26.5	25.8	24.2	11.4	17.8	49.4	37.9	43.6
Pha200+put.100	1.63	1.81	1.72	1.58	0.58	1.08	3.20	2.42	2.81	29.9	36.6	33.2	29.0	11.7	20.4	58.9	48.3	53.6
Mean	1.63	1.54		1.60	0.79		3.24	2.34		36.2	28.6		35.5	14.6		71.7	43.2	
LSD 5% Species	0.04			0.01			0.03			0.2			0.5			0.7		
LSD 5% Treatment	0.04			0.03			0.05			1.6			1.4			1.7		
LSD 5% Interaction	0.06			0.04			0.06			2.2			2.0			2.4		

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

Table(6): Effect of phenylalanine and putrescine on alkaloids content in the leaves of *Datura stramonium* and *Datura innoxia* at fruiting stage (mean values of two successive seasons).

Characters / Treatments	Hyoscine (mg/g dry matter)			Hyoscyamine (mg/g dry matter)			Total alkaloids (mg/g dry matter)			Hyoscine (mg/plant)			Hyoscyamine (mg/plant)			Total alkaloids (mg/plant)		
	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control	1.39	1.17	1.28	1.18	0.61	0.90	2.57	1.78	2.18	40.59	41.30	40.94	34.5	21.5	28.0	75.1	62.8	68.9
Pha 100	1.64	1.20	1.42	1.37	0.94	1.16	3.01	2.14	2.58	46.90	43.48	45.19	39.2	34.1	36.6	86.1	77.5	81.8
Pha 200	1.23	1.19	1.21	1.58	0.75	1.16	2.81	1.94	2.38	28.91	51.29	40.10	37.1	32.3	34.7	66.0	83.6	74.8
Put.50	1.59	1.36	1.48	1.50	0.66	1.08	3.09	2.02	2.56	37.21	44.51	40.86	35.2	21.6	28.4	72.4	66.1	69.2
Put.100	1.26	1.13	1.20	1.73	0.56	1.15	2.99	1.69	2.34	29.23	41.80	35.52	40.1	20.7	30.4	69.3	62.5	65.9
Pha100+put.50	1.38	1.26	1.32	1.16	0.59	0.88	2.54	1.83	2.19	55.10	58.34	56.72	46.3	27.3	36.8	101.4	85.7	93.5
Pha100+put.100	1.73	1.17	1.45	1.36	0.59	0.98	3.09	1.76	2.43	53.99	50.26	52.13	42.4	25.3	33.9	96.4	75.6	86.0
Pha.200+put.50	1.66	1.43	1.55	1.92	1.08	1.50	3.58	2.51	3.05	52.62	44.33	48.48	60.9	33.5	47.2	114.0	77.8	95.6
Pha200+put.100	1.57	1.25	1.41	1.47	1.04	1.26	3.04	2.29	2.67	36.27	43.43	39.85	34.0	36.1	35.1	70.3	79.6	74.9
Mean	1.49	1.24		1.48	0.76		2.97	2.00		42.31	46.53		41.1	28.1		83.5	74.6	
LSD 5% Species	0.01			0.01			0.03			0.75			0.4			1.8		
LSD 5% Treatment	0.02			0.03			0.03			1.26			1.1			1.8		
LSD 5% Interaction	0.03			0.04			0.04			1.78			1.6			2.5		

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

Table (7): Effect of phenylalanine and putrescine on alkaloids content in the fruits of *Datura stramonium* and *Datura innoxia* at fruiting stage (mean values of two successive seasons)..

Character		Hyoscine (mg/g dry matter)			Hyoscyamine (mg/g dry matter)			Total alkaloids (mg/g dry matter)			Hyoscine (mg/plant)			Hyoscyamine (mg/plant)			Total alkaloids (mg/plant		
Treatment		Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean	Str.	Inox	Mean
Control		1.48	1.63	1.56	1.34	0.95	1.15	2.82	2.58	2.7	31.4	53.0	42.2	28.4	30.9	29.6	59.8	83.8	71.8
Pha 100		1.55	1.76	1.66	0.98	0.90	0.94	2.53	2.66	2.60	33.3	54.8	44.0	21.1	28.0	24.5	54.4	82.8	68.6
Pha 200		1.42	1.46	1.44	0.99	1.21	1.10	2.41	2.67	2.54	34.2	60.0	47.1	23.9	49.7	36.8	58.1	109.7	83.9
Put.50		1.78	1.95	1.87	1.31	1.23	1.27	3.09	3.18	3.14	37.0	54.6	45.8	27.2	34.4	30.8	64.3	89.0	76.7.
Put.100		1.90	1.64	1.77	1.40	0.92	1.16	3.39	2.56	2.98	39.0	46.6	42.8	28.7	26.1	27.4	67.6	72.7	70.2
Pha100+put.50		1.55	1.67	1.61	1.30	1.10	1.20	2.85	2.77	2.81	57.7	75.5	66.6	48.2	49.8	49.0	105.9	125.3	115.6
Pha100+put.100		1.97	1.91	1.94	1.60	0.90	1.25	3.57	2.81	3.19	72.5	55.0	63.8	58.9	25.9	42.4	131.4	80.9	106.2
Pha.200+put.50		1.77	1.86	1.82	1.50	0.96	1.23	3.27	2.82	3.05	56.3	44.1	50.2	47.7	22.8	35.2	104.0	66.8	85.4
Pha200+put.100		1.82	1.82	1.82	1.00	1.36	1.18	2.82	3.18	3.00	46.2	46.8	46.5	25.4	35.0	30.2	71.6	81.7	76.7
Mean		1.69	1.74		1.27	1.06		2.97	2.80		45.3	54.5		34.4	33.6		79.7	88.1	
LSD 5%	Species	NS			0.04			0.08			0.8			0.4			0.1		
	Treatment	0.09			0.09			0.11			1.4			1.1			1.7		
	Interaction	0.13			0.13			0.16			'2.0			1.6			2.5		

Str.= *Datura stramonium* Inox.= *Datura innoxia* Pha= Phenylalanine Put. = Putrescine

in *D. innoxia*. Moreover, *D. stramonium* showed the superiority of hyoscyamine and total alkaloid contents mg/g dry matter. Hyoscyamine accumulation mg/plant showed the same trend, while total alkaloid mg/plant have opposite trend.

b- Phenylalanine and putrescine effects

As shown in Table (7) alkaloid contents were affected significantly by phenylalanine and putrescine. Foliar application with phenylalanine100 +putrescine 100 ppm and putrescine 50 ppm gave the highest increase in hyoscine, hyoscyamine and total alkaloids contents mg/g dry matter in fruits of *D. stramonium*, while application of phenylalanine 100 + putrescine 50 ppm caused the highest increments in hyoscine, hyoscyamine and total alkaloids accumulation (mg/plant) followed by application with phenylalanine 100+ putrescine 100 ppm. The most common polyamines studied in plants are the diamine putrescine (Put), the triamine spermidine (Spd), the tetramine spermine (Spm). Polyamines are also part of the overall metabolism of nitrogenous compounds (Rowland *et al*,. 1988). Also Koriesh (1984) and Harridy (1986) reported that the highest values of fresh and dry weight as well as alkaloids content of *Catharanthus roseus* plants were observed when sprayed by tryptophan at 100 ppm.

c- Interaction effects

Foliar application of phenylalanine100+putrescine 100 showed the highest increments of hyoscine, hyoscyamine and total alkaloids mg/g dry matter in fruits of *D. stramonium*, while *D. innoxia* show high response to foliar application with putrescine 50 ppm followed by phenylalanine200+ putrescine 100 ppm. The highest recorded values of hyoscine, hyoscyamine and total alkaloids accumulation (mg/plant) in fruits of *D. stramonium* was obtained by spraying phenylalanine 100 + putrescine 100 followed by phenylalanine 100+putrescine 50 ppm. On the other hand hyoscine, hyoscyamine and total alkaloids in fruits of D. innoxia showed highly significant increment in case of phenylalanine 100+putrescine 50 ppm followed by spraying with individually phenylalanine at high level.

Polyamines are biologically active compounds involved in various physiological processes. They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation, related to aging and senescence, and usually involved in plant responses to stress (Flores and Galston 1982, Friedman *et al.* 1989). Also, Zabetakis *et al.*, (1999) reported that the origin of the tropane ring skeleton in *D. stramonium* is recognized to arise from ornithine, acetate and methionine that convert to Putrescine so, putrescine is consider a precursor of tropane ring.

In conclusion, It is clear that 100 ppm of phynylalanine+50 ppm putrescine is sufficient to accelerate the growth parameters of *D. stramonium* at flowering and fruiting stages. *D innoxia* had the same pattern except at flowering stage that showed significantly promoted all growth parameters due to foliar application of phenylalanine 100+ putrescine 100 ppm. Most of phenylalanine and putrescine treatments tended to increase hyoscine and hyoscyamine contents (mg/g dry matter and mg/plant) of two *Datura spp*, throughout the growing seasons.

In most cases spraying with phenylalanine and putrescine connectively increased greatly the biosynthesis of hyoscine, hyoscyamine and total alkaloids contents more than individually ones. Foliar spray with phenylalanine100 + putrescine 50ppm gave the highest values of hyoscyamine mg/plant and phenylalanine 100 + putrescine100 gave the highest one in total alkaloids mg /plant in both *Datura spp.*

REFERENCES

A.O.A.C. (1980). Official Methods Of Analysis Of Association Of Official Analytical Chemists. 12th Ed. Washington, D.C.

Aziz, A.; M. Marziah; M. M. Radzali and MS. Halimi (2002). The influence of precursors on hyoscyamine and scopolamine production by transformed root cultures of *Datura metel* L. Jurnal of tropical medicinal plants, 3 (1), 105-111.

Bouchereau, A.; A. Aziz; F. Larhre and J. Martin-Tanguy (1999). Polyamines and environmental challenges: recent development. Plant Sci.,140, 103-125.

Chibi, F.; A. J. Matila; T. Angosto and D. Garrido(1994). Changes in polyamine synthesis during anther development and pollen germination in tobacco (Nicotiana tabacum). Physiol. Planta. 92, 61-68.

Cohen, S.S. (1998). A Guide to the Polyamines. *Oxford: Oxford University Press.*

Davies, D. D. (1982). Physiological Aspects of Protein Turn Over. Encycl, Plant Physiol. New Series, 14 A (Nucleic acids and proteins: structure biochemistry and physiology of proteins). Pp. 190-288-Ed., Boulter, D. and Parthier, B., Spring-Verlag, Berlin, Heidelberg and New York.

El-Fawakhry, F. M. and H. F. El-Tayeb (2003). Effect of some amino acids and vitamins on chrysanthemum production. J. Adv. Agric. Res. Alex. Univ. 8(4), 755-766.

El-Sherbeny, S. E. and E. A. Hassan (1987). Physiological studies on *Datura stramonium* " The effect of some alkaloid precursors on growth, chemical composition and leaf alkaloids. Bull. N. R. C. Egypt. 12, 101.

Evans, P. T. and R. L. Malmberg (1989). Do polyamines have roles in plant development? Ann. Rev. Plant Physiol. Plant Biol. 40, 235-269.

Flores, H. E. and A. W. Galston (1982). Polyamine and plant stress: activation of putrescine biosynthesis by osmotic shock. Science 217, 1259-61.

Friedman, R.; Altman, A. and Levin, N. (1989). The effect of salt stress on polyamine biosynthesis and content in mung bean plants and halophytes. Physiol. Plant. 76: 295-302.

Galston, A. W. and R. Sawhney (1990). Polyamines in plant physiology. Plant Physiol. 944, 406-410.

Goss, J. A. (1973). Amino Acid Synthesis and Metabolism. Physiology of plants and their cells. P. 202. Pergamon Press INC, New York-Toronto, Oxford, Sydney, Braunscweig.

Harridy, I. M. A. (1986). Physiological studies on Periwinkle plant *Catharunthus roseus*. G. don. Ph. D. Thesis, Faculty of Agric. Cairo Univ. Egypt.

Hussein, M. S.; S. E. El-Sherbeny and B. H. Abou Leila (1992). Effect of some basic nitrogen compounds on the growth, photosynthetic pigment and alkaloid contents in *Datura metel* L. Egypt. J. Physiol. Sci., 16 (1-2), 141-150.

Khan, N.A.; A. Sezan; V. Quemener and J-P. Moulinoux (1993). Polyamine transport regulation by calcium and calmodulin: role of Ca^2+-ATPase. Journal of Cell Physiology 157: 493-501.

Koriesh, E. M. (1984). Effect of indole acetic acid, sodium acetate and tryptophan on growth and total alkaloids of *Catharanthus roseus*. G. don. The 2nd general Conference of Agric. Res. Cent. Cairo, Egypt.

Medina-Bolivar,F. and H. E. Flores (1995). Selection for hyoscyamine and cinnamoyl putrescine overproduction in cell and root cultures of *Hyoscyamus muticus*. Plant Physiol. 108: 1553-1560.

Miraldi, E.; A. Masti; S. Ferri and I. B. Comparini (2001). Distribution of hyoscyamine and scopolamine in *Datura stramonium*. Fitoterapia 72, 644-648.

Moursy, H. A. ; Hussein, S. M. and El-Bahr, K. M.(1988). Effect of some alkaloid precursors on the growth and alkaloid production of *Datura stramonium* L. cultured *in Vitro*. Egypt, J. Bot. 31 : 153-165.

Park, M.H., Wolff, E.C. & Folk, J. E. (1993). Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential role in cellular regulation. BioFactors 4: 95-104.

Pegg, A.E. (1986). Recent advances in the biochemistry of polyamines in eukaryotes. Biochemical Journal 234: 249-262.

Rosen, H. (1957). A modified ninhydrin colormetric analysis for amino acids. Arch. Biochem. Biophys., 67, 10.

Rowland, A.J.; Borland, A.M. and Lea, P.J. (1988). Changes in amino acids, amines and proteins in response to air pollutants. In: Schulte-Hostede, S. N.M.

Sato, F.; T. Hashimoto; A. Hachiya; K. Tamura; K-B. Choi; T. Morishige ; H. Fujimoto and Y. Yamada (2001). Metabolic engineering of plant alkaloid biosynthesis. Proceedings of the National Academy of Sciences, USA 98, 367-372.

Schuber, F. (1989). Influence of polyamines on membrane functions. Biochemical Journal 260: 1-10.

Shoji, T. ; Y. Yamada and T. Hashimoto (2000). Jasmonate induction of putrescine N-methyltransferase genes in the root of *Nicotiana sylvestris*. Plant and Cell Physiology 41, 831-839.

Snedecor, G.M. and Cochran , W.G. (1980). Statistical Methods. 7th Ed. Iowa State College Press, Iowa, USA.

Staba, E. J. and A. Jindra (1968). Datura tissue cultures: production of minor alkaloids from chlorophyllous and nonchlorophyllous strains. J. Pharm. Sci., 57, 701.

Talaat I. M. and A. A. Youssef (2002). The role of the amino acids lysine and ornithine in growth and chemical constituents of basil plants. Egypt. J. Appl. Sci., 17, (5) 83-95.

Thom, M. ; A. Maretzki; E. Kprmer and W. S. Sokai (1981). Nutrient uptake and accumulation by sugar cane cell culture in relation to growth cycle. Plant Cell Tissue and Organ Culture. (1), 3-14.

Trofimova, N. A.; I. F. Gusev and N. N. Doshchinskaya (1973). Trails on the tissue culture of *Datura innoxia* and the alkaloid formation in the tissues. Rastitel,nye Resursy 9, 168.

Wolukau, J. N.; S. Zhang; G. Xu and D. Chen (2004). The effect of temperature, polyamines and polyamine synthesis inhibitor on *in vitro* pollen germination and pollen tube growth of Prunus mume. Scientia Horticulturae 99, 289-299.

Zabetakis, I.; R. Edwards and D. O,Hagan (1999). Elicitation of tropane alkaloid biosynthesis in transformed root cultures of *Datura stramonium*. Phtochemistry, 50, 53-56.

تـأثيرالرش الورقى بالبترسين والفينايل الانين علـى النمـو وانتـاج القلويـدات لنوعين من جنس الداتورة

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** قسم النبات- المركز القومى للبحوث- القاهرة- الدقى

تم إجراء دراسة حقلية فى المزرعة التجريبية للمركز القومى للبحوث ببولاق الدكرور خـلال موسـمين متتاليين (٢٠٠١ - ٢٠٠٢ / ٢٠٠٢- ٢٠٠٣) وذلك بهدف دراسة تاثير الرش الورقى بالبترسين بتركـيزات مختلفـة (٠ و ٥٠ و ١٠٠ جزء فى المليون) والحامض الامينى فينايل الانين بتركيزات مختلفة (٠ و١٠٠ و ٢٠٠ جـزء فـى المليون) وكذلك التداخل بينهما على بعض مؤشرات النمو ومحتوى النبات من القلويدات لنباتين من جنس الداتورة وهما *Datura stramonium, Datura innoxia* والمستوردة من بلغاريا وقد توصلت الدراسة للنتائج التالية:

-اظهر نبات *Datura stramonium* تفوق واضح خلال مرحلة النمو الزهرى فى جميع صفات النمو ماعدا وزن الجذور بينما خلال مرحلة النمو الثمرى اظهرت *Datura innoxia* زيادة واضحة فى جميع صفات النمو

-وقد ادى الرش الورقى بالحامض الامينى فينايل الانين ١٠٠ + بترسين ٥٠ جزء فى المليون افضـل النتـائج فـى الاسراع بمؤشرات النمو لنبات *Datura stramonium* فى خلال الطور الزهرى والطور الثمرى وقد اعطـى نبات *Datura innoxia* نفس التاثير فى الطور الثمرى فقط بينما اعطى فى الطور الزهرى اعلى زيادة معنويـة فى النمو عند استخدام الرش بالمعاملة بالحامض الامينى فينايل الانين ١٠٠+ بيترسين ١٠٠ جزء فى المليون

-أدت معاملة نبات *Datura stramonium* بالتركيز المرتفع من الحامض الامينى فينـايل الانيـن او البترسـين منفردا الى زيادة معنوية فى محتوى الاوراق من البروتين فى مرحلة النمو الزهرى بينمـا سـلك نبـات *Datura innoxia* اتجاه معاكس.

-أدت معاملة نبات *Datura stramonium* بالبترسين منفردا بتركيز ٥٠ جزء فى المليـون الـى أعلـى قيـم للاحماض الامينية الحرة فى مرحلة النمو الزهرى بينما أدت المعاملة بالحامض الامينى فينايل الانين ١٠٠+ بترسين ١٠٠ جزء فى المليون الى الحصول على اعلى قيم من الاحماض الامينية الحرة فى نبات *Datura innoxia*.

-تم تقدير قلويدات الهيوسين والهيوسيامين فى الاوراق والثمار فى مرحلتى النمو الزهرى والثمرى باسـتخدام جـهاز الكروماتوجرافى ذو الأداء العالى (HPLC) وقد أدت معظم معاملات الحامض الامينى فينايل الانين والبترسين الى زيادة محتوى الاوراق والثمار من القلويدات عند تقديرها سواء ملللجرام/ جرام او بتقديرها ملللجـرام/نبـات لكـلا النوعين من الداتورة أثناء مرحلتى الازهار والاثمار.

-أدى الرش فى معظم المعاملات بالحامض الامينى فينايل الانين والبترسين معا الى زيادة كبيرة فى التخليق الحيـوى للهيوسين والهيوسيامين وكذلك القلويدات الكلية من إضافتهما منفردا فى كلا نوعى الداتورة .

-وقد أدى الرش الو رقى بالحامض الامينى فينايل الانين ١٠٠ + بترسين ٥٠ جزء فى المليون الى الحصـول علـى اعلى نسبة لقليد الهيوسيامين بينما اعطت المعاملة بالحامض الامينى فينايل الانين ١٠٠+ بترسين ١٠٠ جزء فـى المليون اعلى محتوى من القلويدات الكلية فى كلا نوعى الداتورة.