Enhancement of alkaloids production in suspension cultures of *Datura stramonium* L. and *Datura metel* L.

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ABSTRACT

Cell suspension cultures were induced from different explant cultures of D. stramonium L. and D. metel L. Influence of different concentrations of various growth regulators, i.e., 2,4-D, Kin, NAA and BAP on callus production was investigated. Effect of various concentrations of different precursors, i.e., phenyealanine, ornithine and hyocine on alkaloids production were determined. The optimum value of cell growth parameters as well as alkaloids production were obtained from leaf, stem and root cell cultures. D. Stramonium showed better results as compared with D. metel. The best supplementation to the liquid MS medium was 1 mg/l of each of NAA and BAP. Phenylalanine at 20 mg/l gave the best results of cell growth and alkaloids accumulation in different types of Datura cell cultures. Chemical analysis of the different cell lines of Datura was identified by HPLC.

Key words: *Datura sp., cell culture, precursors, alkaloids, HPLC.*

INTRODUCTION

The genus *Datura* is a pharmaceutical important medical plant, as it is an important source of the medical alkaloids. One of the main alkaloids synthesized in several *Datura* species is scopolamine and hyoscyamine (Tailang *et al.*,1997). Many pharmaceutical products are based on plant products, and much effort has been invested in the biotechnology production of secondary metabolites by plant cell cultures (Wink,1987). Several cell culture systems, which are better producers than the respective plants, are now available (Zenk, 1982; Staba, 1985; Collin, 1987; Di Cosmo, 1990).

Secondary metabolism in higher plants is strongly influenced by environmental factors (Sakuta and Komamine, 1987). In suspension cultures, plant growth regulators and nutritional factors affect the production of secondary metabolites as well as growth (Bohm, 1980). Different types and concentrations of growth regulators, i.e., auxins and cytokinins, are known to show different effects on plant growth and production of secondary metabolites (Sakuta and Komamine, 1987). The growth and alkaloids content of leaf and stem calli cultures of *D. stramonium* were increased when 1 mg/l of 2,4-D , NAA, Kin and BAP were added to the culture media alone or in a combination (El-Bahr *et al.*, 1989). Supplementation of culture medium with 1 mg/l of 2,4-D and Kin enhanced callus growth and hyoscyamine production in *Datura stramonium* and *D.*

innoxia cultures (Tailang, 1997). Similarly, Nussbaumer *et al.* (1998) examined a clone of *Datura candida* x *D. aurea* for its growth and hyoscyamine and scopolamine content under various culture conditions. They reported that half-strength B5 medium supplemented with 1 mg/l of each of NAA and BAP gave the best value of root culture growth. Meanwhile, full-strength B5 medium supplemented with the same concentrations of NAA and BAP gave the best results of hyoscyamine and scopolamine production.

Attempts to induce or increase the production of metabolite formation in cultured tissues by supplying precursors or intermediate compounds gave encouraging results. In this respect, ornithine is a precursor of tropane alkaloid and tropic acid is derived from phenylalanine, the second step, estrification of tropane and tropic acid led to produce hyoscyamine in different living tissues (Trease and Evans, 1978). Also, ornithine is a precursor of both scopolamine and hyoscyamine. On this basis, Chan and Staba (1965) indicated that ornithine, arginine, proline, glutamic acid, phenylalanine and phenylacetic acid were the most precursors for tropane alkaloid. The addition of dihydroquercetin to carrot cell cultures restored pigment formation, which was formerly repressed under the influence of 2,4-D (Ozeki and Komamine, 1985).

Demeyer *et al.* (1992) obtained calli from leaf, stem and root explants of five *D. stramonium* varieties when cultured on MS medium supplemented with BAP. The highest values of alkaloids (hyoscyamine and scopolamine) contents were produced from leaf explant callus. Addition of chloramphenicol (1-2 ppm) to leaf explant callus cultures of *D. stramonium* and *D. innoxia* enhanced callus growth as well as hyoscyamine content up to 112% (Tailang *et al.*, 1997).

The aim of the present investigation is to study the effect of some growth regulators (2,4-D, NAA, Kin and BAP) and different precursors (phenylalanine, ornithine and hyocyne) on callus growth and total alkaloid content of different cultures of *Datura stramonium* and *D. metel.*

MATERIALS AND METHODS

Plant material

Seeds of *Datura stramonium* and *D. metel* were secured from the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt. Seeds were surface sterilized by immersion in 70% ethanol for 10 sec, followed by three washes using sterile distilled water, then immersed in 50% Clorox solution (5.25 Cl_2) containing a drop of Twin 20 for 15 min and then rinsed several times with sterile water. The seeds were then germinated aseptically on solid basal MS medium Murashige and Skoog, 1962). Cultures were solidified by 0.7% agar added prior to autoclaving at 1.2 kg/cm^2 for 15 min. The pH of the culture medium was adjusted to 5.8 by addition of 0.1 N HCl or 0.1 N KOH. Seed germination took place within 7-10 days. After one month from germination, different segments of leaves, stems and roots were excised from mother plantlets of the two Datura species.

Callus production

First experiment: The three types of explants were cultured on the following media:

1- Basal MS medium (hormone-free).
2- MS + 0.0 mg/l 2,4-D + 1.0 mg/l Kin
3- MS + 1.0 mg/l 2,4-D + 0.0 mg/l Kin
4- MS + 1.0 mg/l 2,4-D + 1.0 mg/l Kin
5- MS + 1.0 mg/l 2,4-D + 2.0 mg/l Kin
6- MS + 2.0 mg/l 2,4-D + 1.0 mg/l Kin

Second experiment: The three types of explants were cultured on the following media:

1-MS + 0.0 mg/l NAA + 1.0 mg/l BAP
2-MS + 1.0 mg/l NAA + 0.0 mg/l BAP
3-MS + 1.0 mg/l NAA + 1.0 mg/l BAP
4-MS + 1.0 mg/l NAA + 2.0 mg/l BAP
5-MS + 2.0 mg/l NAA + 1.0 mg/l BAP

Cultures of all treatments were maintained at 26 ± 1°C under light conditions (16 hr/day photoperiod, 3000 Lux cool light fluorescent lamps) for 30 days. Calli were subcultured twice with 30 days interval. Each treatment was replicated five times.

Determination of callus growth

Growth parameters of calli produced from different types of cultures were scored after 30 days from incubation as follows:

- Fresh weight (g/Jar).
- Dry weight (g/Jar).
- Dry matter content (%).

Cell cultures

According to Torres (1988), suspension cultures were initiated by placing pieces of friable callus of leaf, stem and root explants of the two Datura species. After sieving, the obtained cells were maintained in an agitated liquid MS medium supplemented with the best growth regulators at the optimum concentrations. The following liquid MS media containing 1 mg/l of different growth regulators were used:

1-MS medium + 1 mg/l 2,4-D + 1 mg/l Kin
2-MS medium + 1mg/l NAA + 1 mg/l BAP

Scoring of cell culture growth

The growth parameters of the cell suspension cultures were scored during the growth cycle as follows:

-The cell number, calculated according to Neumann (1966).
-Packed cell volume (PCV), determined according to Patrick (1984).
-Dry weight (g/ml).

Alkaloid precursors

The effect of different precursors, i.e., phenylalanine, ornithine and hyocyne with *D. stamonium* and phenylalanine and hyocyne with *D. metel* at different concentrations (0, 5, 10, 20 and 50 mg/l) on growth dynamics and alkaloid production from different types of cell cultures were studied.

Chemical analysis

Hyoscyamine was determined as total alkaloids by HPLC (high pressure liquid chromatography) in different cultures of *Datura sp.*, according to the method described in British Pharmacopoeia (1998).

Statistical analysis

All experiments were carried out in a completely randomized design, and the data were statistically analyzed according to the method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Callus production

Effect of 2,4-D and Kin:

The effect of 2,4-D as an auxin in combination with Kin as a cytokinin at different concentrations on callus production from different explants, i.e., leaf, stem and root of *D. stramonium* and *D. metel* is presented in Table (1). Basal MS medium, free of growth regulators, failed to initiate and produce callus from the different explants of both of Datura species. Supplementation of MS medium with 2,4-D and Kin alone gave positive results depending on the genotype, type of explant and the concentration of the growth regulator Generally, the highest values of callus production were recorded with *D. stramonium* as compared with *D. metel.*

Table (1): Effect of different combinations (mg/l) of 2,4-D and Kin added to MS medium on fresh weight (g), dry weight (g) and dry matter content (%) of callus cultures of leaf, stem and root explants of Datura stramonium and Datura metel cultured under light conditions.

Growth substances (mg/l)		*Datura stramonium* L.			*Datura metel* L.		
		Leaf	Stem	Root	Leaf	Stem	Root
2,4-D	Kin	Fresh weight (g)					
0	1	2.35±0.025	2.17±0.0268	2.05±0.0546	2.23±0.0452	1.95±0.0983	1.37±0.0687
1	0	1.76±0.024	1.35±0.0453	1.15±0.0436	1.42±0.0832	1.07±0.0435	0.99±0.0089
1	1	10.43±0.283	8.74±0.183	6.46±0.342	9.51±0.362	7.82±0.523	5.17±0.231
1	2	9.83±0.145	6.94±0.546	5.22±0.372	7.65±0.245	5.32±0.735	2.74±0.0198
2	1	7.26±0.261	4.83±0.0582	3.15±0.0159	5.27±0.632	4.15±0.324	2.35±0.282
		Dry weight (g)					
0	1	0.18±0.0025	0.16±0.0024	0.14±0.0056	0.17±0.0034	0.14±0.0025	0.08±0.0009
1	0	0.12±0.0012	0.09±0.0008	0.07±0.0005	0.10±0.0045	0.08±0.0017	0.05±0.0006
1	1	0.98±0.0054	0.61±0.0032	0.42±0.0067	0.78±0.0036	0.54±0.0037	0.33±0.0053
1	2	0.87±0.0098	0.47±0.0055	0.32±0.0058	0.53±0.0066	0.33±0.0015	0.15±0.0068
2	1	0.63±0.0063	0.39±0.0014	0.27±0.0055	0.43±0.0015	0.30±0.0017	0.12±0.0088
		Dry matter content (%)					
0	1	7.64	7.37	6.82	7.62	7.18	8.76
1	0	6.82	6.67	6.08	7.04	7.47	5.05
1	1	9.86	6.95	6.54	8.27	6.32	6.12
1	2	8.85	6.77	6.13	6.92	6.20	5.47
2	1	8.67	6.60	8.57	8.15	6.12	3.58

Mean ± S. E.of 5 replicates.

Among the different treatments, the highest values of callus production were obtained from the addition of 1 mg/l of each of 2,4-D and Kin to MS medium. The maximum values of calli fresh weight were 10.43, 8.74 and 6.46 g for leaf, stem and root explants of *D. Stramonium*, respectively (Fig. 1). Meanwhile, they were 9.51, 7.82 and 5.17 g in leaf, stem and root cultures of *D. metel*. Similarly, dry weights of calli of the three explants were 0.98, 0.61 and 0.42 g in leaf, stem and root of *D. Stramonium*, respectively. Fresh weights were 0.78, 0.54 and 0.33 g in leaf, stem and root, derived calli of *D. metel* respectively. The presence of 2,4-D or Kin alone in the culture medium gave a little effect on callus production with the different explants of the two types of *Datura sp*. The descending order of the dry matter content (%) was 9.86, 6.95 and 6.54 in leaf, stem and root callus of *D. Stramonium*, respectively. It was 8.27, 6.32 and 6.12 % in leaf, stem and root callus of *D. metel*, respectively.

Fig. (1): Callus production from leaf, stem and root explants of D. stramonium (from left to right) after 4 weeks of cultivation on MS medium supplemented with 1 mg/l 2,4-D +1 mg/l Kin.

Effect of NAA and BAP

Data presented in Table (2) show that the addition of NAA alone or in combination with BAP gave varied effects on callus production. No callus induction was observed in the absence of both growth regulators. Supplementation of MS medium with NAA or BAP alone gave a small effect on callus production. On other hand, the highest values of callus production were obtained with MS medium supplemented with NAA and BAP. Also, the best results of callus production were observed with the different explants of *D. stramonium*, as compared with *D. metel*. Calli of *D. stramonium* (Fig. 2) gave the maximum values, which were 12.75, 9.77 and 8.32 g for leaf, stem and root explants, respectively. The optimum supplementation was 1 mg/l NAA + 1 mg/l BAP, which enhanced callus production from the different Datura explants. Also, leaf, stem and root of *D. metel* explants showed the best results of callus production, i.e., 10.38, 9.12 and 7.28 g, respectively. Dry weights of calli were 1.27, 0.83 and 0.65 g for leaf, stem and root explants of *D. Stramonium*. They were 0.94, 0.77 and 0.45 g for leaf, stem and root of calli of *D. metel*, respectively. The descending order of dry matter content (%) was 9.96, 8.49 and 7.81 in leaf, stem and root callus of *D. Stramonium*, respectively. Meanwhile, they were 9.15, 8.44 and 6.18 % in leaf, stem and root calli of *D. metel* , respectively.

Fig. (2): Callus production from leaf, stem and root explants of D. Stramonium (from left to right) after 4 weeks of cultivation on MS medium supplemented with 1 mg/l NAA+ 1 mg/l BAP.

Table (2): Effect of different combinations (mg/l) of NAA and BAP supplemented to MS medium on fresh weight (g), dry weight (g) and dry matter content (%) of calli derived form leaf, stem and root explants of Datura stramonium and Datura metel cultured for 30 days under light conditions.

Growth substances (mg/l)		*Datura stramonium* L.			*Datura metel* L.		
		Leaf	Stem	Root	Leaf	Stem	Root
NAA	BAP	Fresh weight (gm)					
0	1	4.57±0.125	3.63±0.253	2.57±0.324	3.25±0.456	3.11±0.143	2.15±0.128
1	0	2.87±0.235	2.13±0.143	2.00±0.173	2.35±0.256	1.92±0.128	1.53±0.146
1	1	12.75±0.567	9.77±0.468	8.32±0.624	10.38±0.562	9.12±0.269	7.28±0.368
1	2	10.22±0.568	8.59±0.259	7.25±0.463	8.24±0.635	7.42±0.258	4.54±0.273
2	1	8.73±0.189	7.25±0.246	6.13±0.453	6.87±0.125	5.63±0.345	3.92±0.135
		Dry weight (gm)					
0	1	0.35±0.0012	0.27±0.0024	0.22±0.0035	0.25±0.0078	0.20±0.0012	0.17±0.0025
1	0	0.25±0.0011	0.18±0.0024	0.15±0.0019	0.18±0.0022	0.16±0.0017	0.12±0.0015
1	1	1.27±0.0185	0.83±0.0025	0.65±0.0017	0.94±0.0023	0.77±0.0045	0.45±0.0035
1	2	0.94±0.0058	0.72±0.0015	0.47±0.0024	0.73±0.0026	0.45±0.0037	0.25±0.0016
2	1	0.85±0.0025	0.61±0.0011	0.35±0.0012	0.61±0.0028	0.38±0.0027	0.22±0.0025
		Dry matter content (%)					
0	1	7.56	7.45	8.56	7.69	6.43	7.90
1	0	8.71	8.45	7.50	7.56	8.33	7.80
1	1	9.96	8.49	7.81	9.15	8.44	6.18
1	2	9.19	8.38	6.48	8.85	6.76	5.50
2	1	9.14	8.31	5.70	8.81	6.75	5.41

Mean ± S.E.of 5 replicates.

From the above results, one may conclude that supplementation of MS medium with 1 mg/l of each of NAA and BAP is more suitable for callus production from different explants of both *Datura sp.* than 2,4-D and Kin. In this respect, such results are in agreement with those of Nussbaumer *et al.* (1998). They reported that supplementation of B5 medium with 1 mg/l from each of NAA and BAP gave the best results of growth value for *Datura candida* x *D. aurea.* Also, our results are close to those of El-Bahr *et al.* (1989). They reported that the addition of 1 mg/l of each of BAP and NAA was more suitable for callus production from *D. stramonium* than the addition of NAA and Kin. In this respect, Torres (1988) reported that callus initiation and production are due to the presence of auxins and cytokinins that stimulated cell division and cell enlargement.

Cell cultures

Cell number

The highest values of cell number ($x10^5$) were recorded after 24 days from cultivation Figs. 3-a and b). Generally, it was observed that different cell cultures take a relatively similar trend with higher values in case of *D. stramonium* compared with those of *D. metel.* The maximum cell number was 5.825 ($x10^5$ cells / ml) in leaf cell cultures of *D. stramonium,* when MS medium was supplemented with 1 mg/l of each of NAA and BAP. In case of *Datura metel,* the supplementation of MS medium with 1 mg/l of each of NAA and BAP induced a number of 5.455 ($x10^5$ cells / ml) for leaf cell cultures.

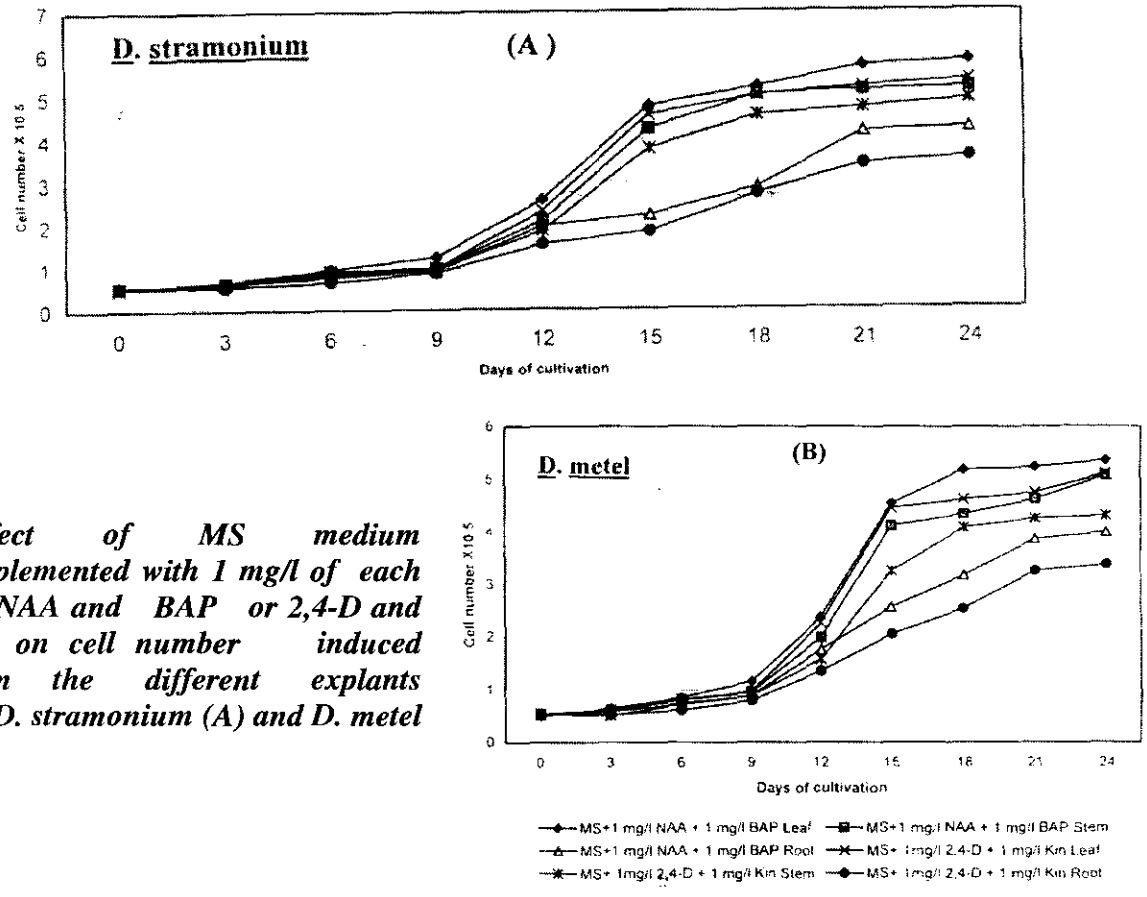
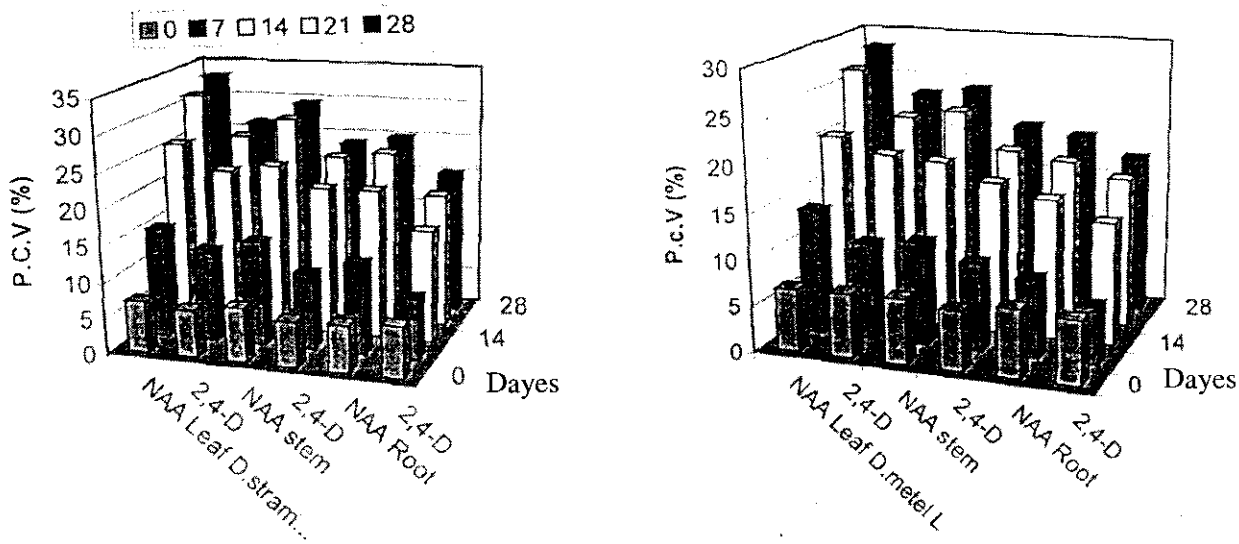


Fig.(3):Effect of MS medium supplemented with 1 mg/l of each of NAA and BAP or 2,4-D and Kin on cell number induced from the different explants of D. stramonium (A) and D. metel (B).

Packed cell volume (%)

The packed cell volume (%) (P.C.V) (Figs. 4-a and b) of different types of cell cultures was estimated during and at the end of 28 days of cultivation. The best results were obtained from leaf, stem and root cell cultures; 31.4, 28.6 and 24.3 %, respectively for *Datura stramonium* when MS medium was supplemented with 1 mg/l NAA + 1 mg/l BAP. In case of *Datura* metal, records with MS medium containing 1 mg/l of each of NAA and BAP in which leaf, stem and root packed cell volumes were 26.7, 22.8 and 18.1%, respectively.



(A) **(B)**

Fig. (4): P.C.V of the different cell types of D. stramonium (A) and D. metel (B) cultured on MS medium supplemented with 1 mg/l 2,4-D+1mg/l Kin or 1mg/l NAA+1 mg/l BAP.

From the above results, it may be concluded that *D. stramonium* showed the optimum cell number and the P.C.V in the different culture types as compared with *D. metel.* Similarly, leaf explants gave better results than the other explants. Also, supplementation of MS medium with 1 mg/l NAA + 1mg/l BAP gave the best results of cell number and P.C.V compared with the other supplementations of growth regulators.

The obtained results reveal the stimulating effect of the combinations of cytokinin and auxin, added to culture medium, on callus initiation or mass cell production. This may be due to the effect of cytokinin on cell division and auxins on cell enlargement (Torres, 1988). In this connection, Skoog and Schmitz (1972) reported that cytokinins are generally added to culture media aiming to promote cell division in calli cultures of plant tissue, and stimulating the rate of protein synthesis in tobacco cell cultures.

Effect of precursors on cell growth and alkaloids accumulation

The effect of different precursors, i.e, phenylalanine, ornithine and hyocyne with *D. stramonium* and phenylalanine and hyocyne with *D. metel* at different concentrations (0, 5, 10, 20 and 50 mg/l) on cell growth and

Table (3a): Effect of different precursors (phenylalanine, ornithine and hyocyne) at different concentrations added to MS medium supplemented with 1 mg/l of each of NAA and BAP on growth and a lkaloids accumulation (mg/ 100 mg dry weight) in the different types of D. stramonium and D. metel cultures.

Precursors conc., (mg/l)	*Datura stramonium*						*Datura metel*			
	Phenylalanine		Hyocyne		Ornithine		Phenylalanine		Hyocyne	
	Cell growth	Total Alkaloids	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)
					Leaf					
0	--	40.54	--	40.54	--	40.54	--	13.25	--	13.25
10	++	50.22	++	44.82	++	42.35	+	20.14	+	18.57
20	+++	54.89	+++	47.56	+++	45.48	++	22.27	++	20.46
50	+	39.67	--	38.56	--	33.7	--	16.54	--	15.43
					Stem					
0	--	35.82	--	35.82	--	35.82	--	12.65	--	12.65
10	+	42.56	+	40.38	+	39.45	+	16.33	+	14.62
20	++	47.60	+	45.06	+	44.01	++	18.64	+	16.52
50	+	30.24	+	35.00	--	33.2	--	14.33	--	11.48
					Root					
0	--	29.73	--	29.73	--	29.73	--	9.56	--	9.56
10	+	33.64	+	34.82	+	35.59	+	11.28	+	11.54
20	++	39.69	+	39.55	+	39.19	+	15.23	+	13.25
50	+	25.87	--	24.82	--	22.56	--	10.58	--	9.25

--- Control, + low increase, ++ medium increase, +++ High increase

Table (3b): Effect of different precursors (phenylalanine, Ornithine and Hyocyne) at different concentrations added to MS medium supplemented with 1 mg/l of each of 2,4-D and Kin on growth and alkaloids accumulation (mg/ 100 mg dry weight) in the different types of D. stramonium and D. metel cultures.

Precursors conc., (mg/l)	*Datura stramonium*						*Datura metel*			
	Phenylalanine		Hyocyne		Ornithine		Phenylalanine		Hyocyne	
	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)	Cell growth	Total Alkaloids (mg)
Leaf										
0	--	36.20	--	36.20	--	36.20	--	11.25	--	11.25
10	++	38.40	++	35.45	+	38.45	+	15.63	+	18.57
20	+++	41.90	+++	41.01	++	39.56	+	20.25	+	20.46
50	+	35.20	--	32.55	--	33.25	--	11.65	--	15.43
Stem										
0	--	33.85	--	33.84	--	33.84	--	12.22	--	12.22
10	++	38.52	+	37.56	+	33.48	+	14.23	--	13.52
20	+++	41.69	++	40.52	++	35.98	+	16.58	+	14.37
50	--	29.78	--	24.56	--	30.48	--	11.59	--	11.08
Root										
0	--	30.82	--	30.82	--	30.82	--	9.12	--	9.12
10	++	35.76	--	32.45	+	29.56	+	11.22	--	12.33
20	+++	37.81	+	37.70	++	33.86	+	14.83	+	14.25
50	+	29.35	--	29.88	+	28.57	--	9.66	--	9.02

--- Control, + low increase, ++ medium increase, +++ High increase

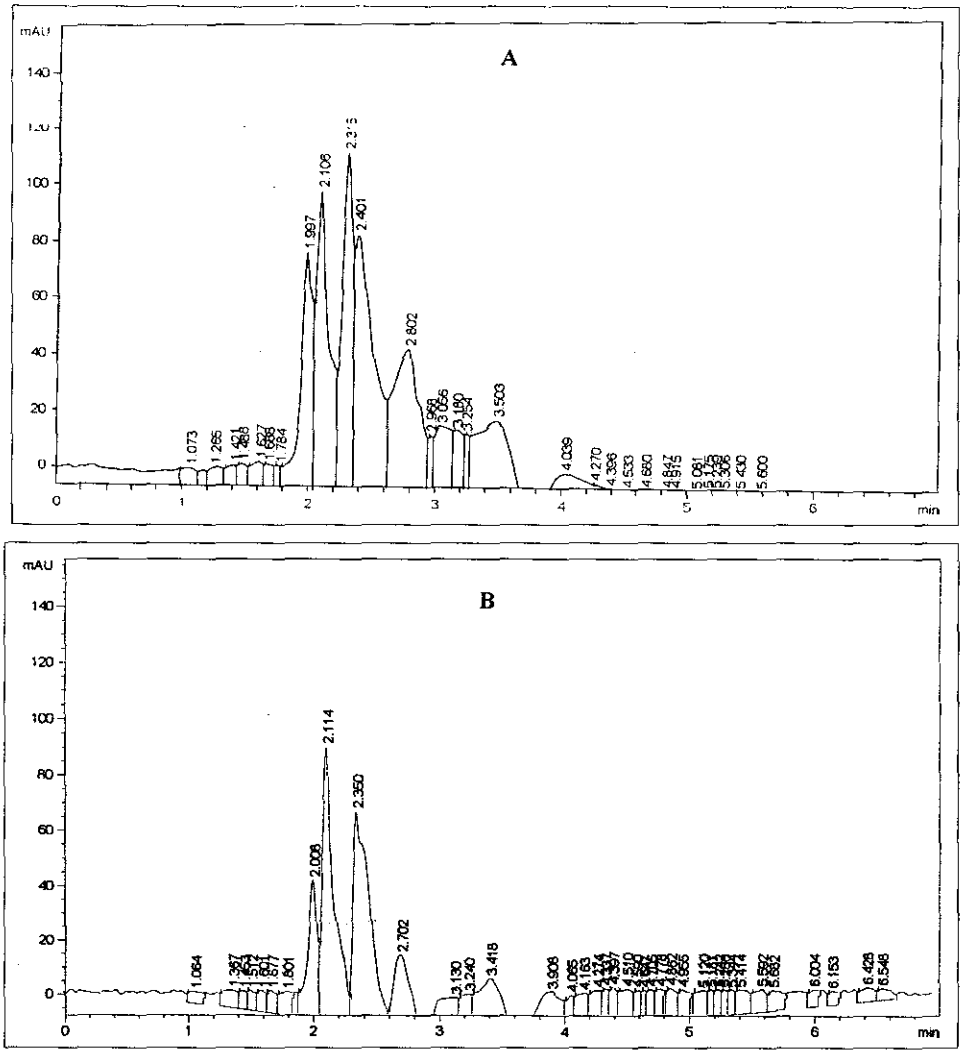


Fig. (5): HPLC of leaf cell suspension culture of D. stramonium(A) and D. metel (B), cultured on MS medium supplemented with 1mg/l NAA + 1 mg/l BAP and modified with 20 mg/l with phenilalanine.

alkaloid production from the different types of cell cultures were studied. Data presented in Table (3a and b) revealed that leaf-derived cells showed the best response of alkaloids accumulation, calculated as scopolamine (mg/100 mg dry weight) using HPLC (Figs. 5a and b). MS medium supplemented with 1 mg / l NAA + 1 mg/l BAP (Table 3a) showed the best results than the addition of 1 mg/l 2,4-D + 1 mg/l Kin (Table 3b). Phenylalanine was found to be the suitable precursor for alkaloids accumulation and growth parameters of the different types of cell cultures. On the other hand, the optimum concentration of phenylalanine was 20 mg/l compared with the other concentrations. The high concentration of different precursors showed insignificant results for cell growth and alkaloids accumulation.

The obtained results are in agreement with Tiburcio *et al.* (1985), who indicated that total alkaloids content in tobacco cell cultures

can be increased to 3.7 % on a dry weight basis by the addition of organic acids to the medium. In this respect, formation of tropane alkaloids in cell cultures of *Datura sp.* is caused mainly by strong repression in the biosynthesis of tropic acid; the acidic moiety of hyosyamine and scopolamine (Lindsey and Yeoman, 1983). Similarly, Koumba and Macheix (1981) reported that supplying precursors or intermediate compounds produced encouraging results and the addition of phenylalanine has been reported to increase the accumulation of hydroxycinnamoyl esters in apple cell cultures.

Form the present results it may be concluded that culturing leaf-derived cells of *Datura stramonium* on MS medium containing (1 mg/l) NAA + BAP with the addition of (20 mg/l) phenylalanine stimulated total alkaloids production.

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الملخص العربي

تنشيط إنتاج القلونيات فى مزارع خلايا الداتورا استرامونيم والداتورا ميتل

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تم دفع الإنتاج الخلوى من الإجزاء النباتية المختلفة للداتورا استرامونيم والداتورا ميتل. تم دراسة تأثير التركيزات المختلفة من منظمات النمو مثل داى كلوروفينوكسى حمض الخليك، نفتالين حمض الخليك وبنزيل امينوبيورين على إنتاج وتكوين الكالس. وتم تقدير التركيزات المختلفة من البادئات (precursors) المختلفة مثل فينيل الالنيل، الأورنثين والهيوسين على النمو الخلوى وكذلك إنتاج القلونيات الكلية فى المزارع الخلوية لكل من الورقة، والساق والجذر علي التوالي. اظهرت الداتورا استرامونيم أفضل النتائج مقارنة بالداتورا ميتل. كانت أفضل إضافة لبيئة موراشيج – سكوج المغذية هى 1 ملى جرام/ لتر من كل من نفتالين حمض الخليك وبنزيل امينو بيورين، حيث أعطى تركيز الفينل الالنيل عند 20 ملى جرام / لتر أفضل النتائج لمزارع النمو الخلوى وتراكم القلونيات الكلية فى المزارع المختلفة نوعي صنفى الداتورا. وقد تم استخدام جهاز HPLC فى تقدير القلونيات الكلية فى المزارع المختلفة.