BIOCHEMICAL EVALUATION OF THE INFLUENCES OF INDOL ACETIC ACID (IAA), KINETIN, ETHREL AND ALAR AS GROWTH REGULATORS ON OIL AND PROTEIN CONSTITUENT'S IN DATURA SEEDS

SHEHATA, SH. M.

Soils, Water and Environment Res. Inst., ARC, Giza, Egypt

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Abstract

Phytochormones are chemical messengers playing an important role in regulation of the growth and development of higher plants. The role of growth regulators in directing the translocation and accumulation of nutrients in plants was detected. Datura Stramonium L. plants were sprayed separately with IAA (50, 100 and 200 ppm), Kinetin (5, 10 and 20 ppm), Ethrel (50, 100 and 200 ppm) and Alar (1000, 2000 and 4000 ppm). The predominant fatty acids of the seed oil were $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ Also traces of remaining even-number and odd number saturated and unsaturated fatty acids were present. IAA, kinetin, Ethrel and Alar at different concentration caused an increase in the percent of stearic, palmitic, oleic and behenic acids. On the other hand, the percent of lenoleic acid was decreased.

Amino acids and protein contents were determined in seeds of *Datura stramonium* L. plants, which were separately developed, under the effect of the different concentrations of IAA, kinetin, Ethrel and Alar. The results revealed that the seed protein comprised 17 common amino acids. The major amino acids were glycine, alanine, aspartic acid and glutamic acid. Amino acids such as methionine, arginine, leucine, phenylalanine, lysine, valine and threonine were present in normal concentrations. Other essential and non-essential amino acids were present also in minor concentrations, except serine. The protein content of all treatments was higher than the control. The activity rates of these plant growth regulators on protein biosynthesis was in the decreasing order: Kinetin > Alar> IAA> Ethrel. Total free amino acids were present in low concentration in all treatments.

INTRODUCTION

Datura Stramonium L. plant is a member of the family solanacia and one of the important medicinal plant grown Bnouham *et al.* (2002). Rawia Zayeda *et al.* (2006), Shehata and Elbanna (1999) and Berkov *et al.* (2003) reported that *Datura Stramonium* is cultivated mainly for its alkaloids. Abd el-Samad *et al.* (1980) also reported that the seed oil percent is approximately similar to cotton seed oil. The effect of growth regulators on the oil content of seeds of some plants was studied by several investigators. In this connection, Bodrov (1973) reported that spraying sunflower plants with GA_3 at the beginning of flowering stage gave a lower yield of

seeds oil content. Izzo *et al.* (1977) stated that treatment of *Vicia sative* L with GA_3 or CCC increased the triglyceride content of the seeds oil. Latti *et al.* (1977) mentioned that spraying *Vicia Sativa* L with GA_3 or CCC increased stearic, oleic, linolenic and the free fatty acids contents. Arana et al (2006) found that there is a GA response in *Datura ferox* seeds plants. Khalil (1992) reported that IAA and Ethrel at various concentrations (10^{-7} to 10^{-3}) had a pronouned stimulatory effect on photosynthesis, protein synthesis, RNA synthesis and the other studied process. Casal *et al.* (1991) found that IAA at hight concentration, severely inhibited the different processes in *Datura ferox* Concentration. Rimoozi and Vetter (1974) found that regarding *Agaricus bisporus* Ethrel at low concentration decreased the level of individual amino acids, while it was increased at high concentration of Ethrel. They also found that Ethrel at all concentration decreased protein levels of the plant . Abd el-Rahim *et al.* (2000) stated that spraying kinetin at 10 ppm on Datura plants increased the protein and free amino acid contents of young leaves. Suryanaraycena *et al.* (1978) showed that the formation of protein in leaf and stem of mango was promoted by Alar (5000 ppm) treatment .

The aim of the present work was to study the role of some plant growth regulators (IAA, kinetin, Ethrel and Alar) on the oil contents and fatty acids composition as well as protein levels and amino acids composition of *Datura stramonium* seeds.

MATERIALS AND METHODS

Datura stramonium L. was conducted to study the effect of the growth regulators on the chemical composition of the harvested seeds. The seeds Of *Datura stramonium* were obtained from pharmacognosy Dept. Fac. Pharm., Cairo Univ., Pot experiments were conducted during two successive seasons 2002 and 2003 to study the impact of four growth regulators on the chemical composition of the harvested seeds. The experimental treatments were arranged in a complete randomized design three rephicates. The experiment comprised of 132 pots, each 12 pots were assigned for one treatment. The experimental pots were irrigated regularly with tap water and received two sprays of the different doses of the growth regulators as follows:

1- Indol acetic acid (IAA) at 50 ppm
2- Indol acetic acid (IAA) at 100 ppm
3- Indol acetic acid (IAA) at 200 ppm
4- Kinetin at 5 ppm
5- Kinetin at 10 ppm
6- Kinetin at 20 ppm

7- Ethrel at 50 ppm
8- Ethrel at 100 ppm
9- Ethrel at 200 ppm
10- Alar at 1000 ppm
11- Alar at 2000 ppm
12- Alar at 4000 ppm
13- Control treatment plants received spray of distilled water.

The *Datura stramonium* seeds (10 seeds for eash pot) were sown in earthenware pots, 40 cm in diameter, was filled with 15 kg of clay soil which obtained from aforementioned farm . It was air dried , softly crushed, sieved through a 2 mm sieva, mixed thoroughly. The two experiments were done at the National Research center (NRC), Kanater during 2002 – 2003 at the first of April the growth regulators at the above mentioned concentrations were applied twice by means of an atomizer sprayer. The first application was at tillering stage, i.e. about 45 days from sowing (number of plants in each pot was thinned then singled to one plant pot) while the second application was carried out after 30 days from the first application (about 75 days from sowing).

At the harvest time (after about four months from sowing maturity stage) the mature seeds of treated and control Datura plants were separated and ground for the chemical determinations. The oil content of the ground seeds of *Datura Stramonium* was extracted with petroleum ether (b.P. 60 – 80°C) in a soxhlet apparatus according to A.O.A.C. (1980).

Identification and determination of the fatty acids by gas liquid chromatographic techniques:

The total fatty acids were prepared from the seeds oil by the method recommended by A.O.A.C. (1980) and their methyl esters were prepared by diazomethane method (Abd El-Samad *et al.*, 1980). Fatty acid methyl esters were analyzed by gas liquid chromatography (GLC) using pye Unicam model 104 gas liquid chromatograph equipped with a flame ionization detector and coiled glass column (1.5 m x 4 mm) packed with 10% PEGA supported on acid washed diatomite C (100-120 mesh). The gas chromatographic conditions used for isothermal work were as follows:-

Column temperature	: 170°C
Detector temperature	: 220°C
Range	: x 100
Attinuator	: 5 x 122
Hydrogen flow rate	: 45 ml/min
Nitrogen flow rate	: 45 ml/min
Air flow rate	: 500 ml/min.
Chart speed	: 1 Cm/2 min

The area of eash peak, representing the fatty acid methyl ester, was measured and the percentage composition of fatty acids was calculated.

Determination of Total Individual Amino Acids:-

Qualitative and quantitative determination of the individual amino acids (Free amino acids and protein) in datura seeds, were carried out using thin layer chromatographic technique as described by Bhushan (1991).

Determination of true Protein:

The true protein of datura seeds was determined as follows:-

A sample of datura seeds flour (0.50) was carefully milled with 2 ml of 2% of trichloroacetic acid solution.

The precipitated protein was separated by centrifugation and washed several times by the same reagent to remove the low molecular weight of nitrogenous compounds which may interfere with protein determination. The isolated protein was estimated by the usual micro-Kjeldahl method (A.O.A.C., 1980).

RESULTS AND DISCUSSION

Extracted Datura seeds oil percentage (Table 1) reached its highest % by spraying plants with IAA at 100 ppm, kinetin at 5 ppm, Ethrel at 200 ppm and Alar at 2000 ppm.

Table 1. The oil percent of Datura seeds treated with different concentration of the growth regulators.

Growth regulators treatments	Concentration (ppm)	Seeds oil Content %
Control	Distilled water	14.60 ± 1.20
IAA	50	18.94 ± 1.11
	100	20.38 ± 1.09
	200	17.90 ± 1.32
Kinetin	5	22.19 ± 2.01
	10	14.47 ± 1.21
	20	14.29 ± 1.31
Ethrel	50	14.69 ± 1.00
	100	16.29 ± 1.52
	200	18.18 ± 1.22
Alar	1000	13.82 ± 1.01
	2000	18.87 ± 1.51
	4000	14.16 ± 1.17

Values were average of 12 plants.

The total fatty acids were isolated from the oil and their methyl esters were prepared. Table 2 showed that, the predominant fatty acids are even number C_{16}, $C_{18:1}$ and $C_{18:2}$ (13.17, 14.43 and 71.01% respectively). Also traces of even-numbered C_{10}, C_{12}, C_{14}, C_{18} and C_{22} (0.46, 0.05, 0.07, 0.33 and 0.48% respectively) were detected. The high percentage of even numbered fatty acids in Datura seeds reflects their biosynthetic pathway from acetate by condensation. Also the high percentage of unsaturated fatty acids proved the efficiency of desaturation.

It is observed from Table 2 that the Datura seeds oil contained a high amount of the essential fatty acid ($C_{18:2}$ linoleic acid). It is also clear from Table 2 that spraying with IAA, kinetin, Ethrel and Alar with different doses decreased the percentage of linoleic acid. The interpretation of these results is based on the hypothesis that the defferent regulators promote the desaturase enzymes in the seeds. All doses of IAA, kinetin, Ethrel and Alar (except IAA at at 100 ppm, kinetin at 5 ppm and Alar at 2000 and 4000 ppm) decreased the oleic acid percent. On the other hand all doses of IAA, kinetin, Ethrel and Alar (except Ethrel at 200 ppm) increased C_{22} Fatty acid.

These results are in agreement with those of Abd el-samed *et al.* (1980), they found that Alar as a growth regulator increased C22 fatty acid content of Roselle seeds oil. Also Diab *et al.* (1981) and Abd el-Rahim *et al.* (2000) observed similar findings.

Table 2. The percent of the individual fatty acids in seeds oil of Datura as affected by IAA, kinetin, Ethrel and Alar.

Hormon	Con.	Fatty acids									
	ppm	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	C_{22}
Control	00	0.46	-	0.05	-	0.07	13.17	0.33	14.43	71.01	0.48
IAA	50	0.80	-	0.29	-	0.12	15.39	0.27	13.30	63.66	6.17
	100	0.09	-	0.12	-	0.07	13.04	0.27	16.50	67.32	2.59
	200	0.27	0.23	0.91	0.10	0.30	12.24	1.42	8.50	41.25	34.78
kinetin	5	0.29	-	0.25	-	0.11	22.02	0.50	19.30	47.33	10.20
	10	0.36	-	0.16	0.09	0.14	11.60	0.38	11.51	61.80	13.96
	20	4.63	0.07	0.11	0.10	0.16	16.15	0.56	14.38	57.78	5.62
Ethrel	50	0.38	0.16	0.13	0.06	0.06	14.60	2.16	12.80	56.11	13.54
	100	9.25	-	0.55	0.82	6.57	10.07	0.55	9.94	49.64	12.61
	200	15.79	8.06	1.22	1.23	33.27	3.61	0.23	7.91	28.45	0.23
Alar	1000	0.02	-	0.02	-	0.02	24.38	0.34	13.69	60.57	0.78
	2000	0.48	-	0.02	-	0.18	19.24	0.35	20.28	54.11	5.16
	4000	0.85	-	0.18	-	0.32	11.80	0.30	28.76	51.71	6.08

By spraying Datura plants with IAA at 100, kinetin at 5 ppm, Ethrel at 200 ppm and Alar at 2000 ppm produced the highest percentage of Datura seeds oil (Table 1). These four treatments oil observed different constituents.

Data recorded in table 2 indicate that treating Datura plants with Ethrel at 200 ppm increased C_{10}, C_{11}, C_{13} and C_{14} (15.79, 8.06, 1.22 and 33.27% respectively).

Mean while there was a higher decrease in the other fatty acids C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, C_{22} (3.61, 0.23. 7.91, 28.45 and 0.23% respectively). Also, kinetin at 5 ppm and Alar at 2000 ppm treatments on Datura plants showed nearly the same effect in which, C_{14}, C_{16}, C_{18}, $C_{18:1}$ and C_{22} contents were increased but the content of $C_{18:2}$, was decreased relative to control. Mean –while, C_{10} and C_{12} contents were decreased by kinetin 5 ppm and Alar 2000 ppm respectively but C_{10} and C_{12} were increased by Alar 2000 ppm and kinetin 5 ppm respectively. On the ether hand, the effect of IAA at 100 ppm nearly unchanged the fatty acids content of Datura seeds oil except that of C_{12} and C_{22} was increased but of C_{10} fatty acid was decreased.

From the previous data it can be concluded that the using of growth regulators changed the biosynthesis of saturated and unsaturated fatty acids. The interpretation of the above results is that Ethrel at 200 ppm Alar at 2000 ppm and Kinetin at 5 ppm treatments promoted the acetate moiety condensation and inhibits the desaturase enzymes (Lotti *et al.,* 1977).

The amino acids and protein content in seeds of *Datura stramonium* L. Plants sprayed with different concentrations of IAA (50, 100 and 200 ppm), kinetin (5, 10 and 20 ppm), Ethrel (50, 100 and 200 ppm) and Alar (1000, 2000 and 4000 ppm) were determined. The results are shown in table 3.

It is evident from data in table 3 that the content of total protein and amino acids contents were affected by the different concentrations of the aforementioned plant growth regulators. On the other hand, it is found that, its maximum level was attained at 100 ppm of IAA, 5 ppm kinetin, 200 ppm Ethrel and 2000 ppm Alar. Almost no change in the content of total amino acids at concentration of IAA 50 ppm, Alar 1000 ppm and kinetin 20 ppm was recorded, while Ethrel at 50 ppm treatment produced the minimum level. (similar to control) relative to the other treatments. However, all treatments decreased the levels of the total free amino acids relative to control. It must be noticed also that the content of total amino acids is always greater in seeds which were developed under influence of most of the concentrations

By examining data in Table 3 it is apparent that the acid hydrolysate of the datura seeds contained 17 common amino acids. Furthermore, it is possible to classify the detected amino acids into three groups. The first group comprising the major amino acids, these are glycine, alanine, aspartic acid, glutamic acid (non -- essential amino acids), whereas the second group contained: methionine, arginine, phenylalanine, leucine, serine, lysine, threonine and valine (essential amino acids except serine) in moderate quantity and the third group, comprised the amino acids histidine, tyrosine, cysteine, proline and isoleucine (essential amino acids except cysteine and proline) in minor concentrations (Table 3).

Table 3. Amino Acid Composition of protein of Datura stramonium seeds as affected by Indol acetic acid (IAA), Ethrel, kinetin and Alar of plant growth regulators

Treatments Plant Stimulant	Concn (ppm)	Protein Mg/g of Dry seeds	Amino Acids (mg/g of dry seeds)								
			Glycine	Alanine	Aspartic acid	Glutamic acid	Methionine	Arginine	Phenylalanine	Leucine	Serine
Control	00	295.59	56.80	41.4	27.02	16.80	20.41	20.24	17.60	12.84	20.26
IAA	50	331.66	58.20	45.02	32.20	20.22	22.05	22.66	17.64	16.40	19.26
	100	354.67	58.45	45.47	35.45	23.68	22.05	24.62	23.40	13.86	14.21
	200	349.24	58.45	47.04	33.65	23.62	22.60	25.65	21.80	15.88	16.84
Ethrel	50	301.68	57.40	37.62	25.64	20.23	19.09	23.80	14.68	16.04	15.26
	100	345.35	58.59	46.09	30.20	22.42	23.44	25.69	18.20	16.82	17.43
	200	296.50	57.40	38.02	32.20	24.29	17.61	18.44	17.64	10.80	11.26
kinetin	5	374.88	58.80	48.80	40.10	39.61	25.88	25.95	20.21	21.28	14.13
	10	338.56	57.90	37.61	34.65	39.08	20.89	24.25	16.62	20.64	14.84
	20	324.91	57.85	41.83	33.60	30.83	20.45	23.69	16.26	15.26	20.40
Alar	1000	326.69	57.05	40.80	25.65	23.67	24.07	19.47	23.40	18.68	19.05
	2000	362.84	58.20	46.40	33.60	39.48	24.40	20.29	23.46	19.62	21.26
	4000	349.29	58.45	32.45	32.50	35.04	24.29	22.87	25.08	20.24	22.21

P values were calculated by the t test, were < 0.01 for all treatments compared to controls

Table 3. Cont.

Treatments Plant Stimulant	Concn (ppm)	Amino Acids (mg/g of dry seeds)								Total Amino Acids (mg/g)	Total free Amino Acids (mg/g)
		Lysine	Threonine	Valine	Histidine	Tyrosine	Cysteine	Proline	Iso-Leucine		
Control	00	13.86	6.54	11.86	7.86	7.86	7.47	8.20	5.40	303.91	16.32
IAA	50	13.86	13.86	14.21	10.60	8.80	7.92	6.63	5.86	335.39	3.73
	100	16.43	21.42	14.27	12.29	9.47	9.82	7.20	7.56	359.65	4.98
	200	16.18	18.85	12.07	10.60	8.23	9.59	6.40	6.80	354.25	5.01
Ethrel	50	19.01	12.40	10.86	7.21	7.20	7.42	5.40	5.48	304.74	3.06
	100	19.66	17.85	13.36	8.48	8.80	9.59	5.87	7.42	350.11	4.76
	200	15.89	16.74	10.01	7.14	7.13	7.42	5.37	6.28	303.64	7.14
kinetin	5	19.67	12.85	11.40	12.62	9.03	5.02	6.28	5.40	377.03	2.15
	10	17.40	12.65	10.81	8.05	8.67	5.85	5.69	7.36	342.76	4.20
	20	13.85	11.69	10.09	8.08	7.87	7.84	5.69	6.28	331.56	6.65
Alar	1000	15.43	14.87	13.14	7.84	9.60	7.84	6.07	6.04	332.67	5.98
	2000	16.47	12.89	15.04	9.81	9.60	5.64	6.63	6.49	369.28	6.44
	4000	14.80	12.77	13.24	12.40	11.27	5.23	6.07	7.20	356.11	6.82

P values were calculated by the t test, were < 0.01 for all treatments compared to controls

It is noticed from the date in Table 3 that no great variations were observed among the different stimulants on the amino acids contents of Datura seed. It was therefore suggested that the level and not the source of the plant stimulants may be the main factor which affected the formation of most amino acids during the seed development.

It is of interest to note that the content of threonine was increased by at least two fold in all treatments as refered to the control. This trend was also true for glutamic acid by treatments of kinetin and Alar as well as Ethrel at 200 ppm.

As indicated in Table 3, the concentrations of most amino acids of Datura seeds were increased or decreased within certain limits parallel with the increasing or decreasing their protein contents but were not by increasing the concentration of the stimulants. In conniction, the relative percentages of these amino acids were changed. These results indicated that the present plant growth regulators (IAA at 100, Ethrel at 200, kinetin at 5 and Alar at 2000 ppm) which elevated the protein content of Datura seed, in different order may be stimulated the enzymes concerned with essential and non-essential amino acids. In this respect, proline content was decreased in all treatments as refered to the control. These results are in accordance with those of Hamama *et al.* (1981) on Datura and in some extent with those obtained on tomato by Sharma and Pande (1975) and disagree with the data obtained on *Agaricus bisporus*, except of proline by Rimoezi and Vetter (1974).

The date in Table 3 clearly show that the content of true protein was increased in the seeds of all treatment . The highest level of protein contents were in seeds of datura plant treated with Ethrel at 200 ppm followed by kinetin at 5 ppm then Alar at 2000 ppm and IAA at 100 ppm. However, these results may be due to the stimulatory effects of the aloremention plant growth regulators on the biosynthesis of RNA and protein (Beaudoin *et al.,* 2006 and Meir *et al.,* 2006).

The results reported in this investigation are in accordance with these reported by Abdel-Samed *et al.* (1980) and Mella *et al.* (2004). However, the present results indicated also that the protein contents of all subjected seeds were higher in its amount of certain amino acids than the control. This may be due to the accelaration of the rate of incorporation of methionine, phenylalanine, leucine and threonine into protein fraction by the applied stimulants. These findings are in agreement in some extent, with those reported by Yakovleva *et al.*. (1975). They reported that in vitro, phytohormones and abscisic acid slightly affect the protein synthesis, as well as Chrocomo and Lee (1975), they showed that kinetin and IAA had some effects on protein biosynthesis of *Phaseolus valgaris coleaptiles*.

It is of interest to note that the concentration of the total free amino (Table 3) was decreased by less than 50% in all subjected seeds as compared with the control.

Also, the increases of protein and amino acids content by the growth regulators treatment are paralleled with the content of total seeds oil, in which, the increasing of seeds oil by IAA, kinetin, Ethrel and Alar (100, 5, 200 and 2000 ppm respectivety) was accompanied by the increasing of total protein and amino acids content by the same treatments.

These results indicated that free amino acids are synthesized and directly incorporated into protein chains of the enzymes which are responsible for the biosynthesis of fatty acids then incorporated into triglycerides (oil of seeds). This fact may be due to the enhancement of the aminoacyl – T- RNA synthetase, which catalyses the linkage between an amino acid and its specific t RNA in the process of protein biosynthesis (Biswas *et al.,* 1973 and Mella *et al.,* 2004).

In addition, the increasing of ribosomal RNA by the present stimulants promoted the incorporation of the activated amino acids (t RNA.AA) into protein chains and then into the responsible enzymes. Furthermore, the binding energy required to form a new peptide linkage was dervied from GTP, which increased also by the growth regulators (Meir *et al.,* 2006 and Beaudoin *et al.,* 2000).

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التقييم الكيميائى الحيوى عن تأثيرأندول حامض الخليك والكينيتين و الايثيرل والألار كمنظمات للنمو على مكونات زيت وبروتين بذور الداتورا

شعبان محمد شحاته

معهد بحوث الاراضى والمياه والبيئة – مركز البحوث الزراعية – جيزة – مصر

أجرى رش نباتات الداتورا ببعض منظمات النمو مثل أندول حامض الخليك بتركيز (٥٠، ١٠٠، ٢٠٠ جزء فى المليون) والكينتين بتركيز (٥، ١٠، ٢٠ جزء فى المليون) والايثيريل بتركيز (٥٠، ١٠٠، ٢٠٠ جزء فى المليون) وكذلك الألار بتركيز (١٠٠٠ ، ٢٠٠٠، ٤٠٠٠ جزء فى المليون) بالإضافة الى المقارنة التى تم رشها بالماء المقطر.

أجرى تقدير نسبة الزيت فى كل معاملة واتضح ان نسبة الزيت ارتفعت فى حالة رش النباتات بواسطة الهرمونات الآتية الاندول حامض الخليك والكينيتين والايثيريل والالار بالتركيزات (١٠٠ ، ٥، ٢٠٠، ٢٠٠٠ جزء فى المليون) على التوالى.

اجرى تفريد الاحماض الدهنية المكونة للزيت بواسطة الغاز كروماتوجرافى واتضح من تفريد عينه الكنترول ان الأحماض الدهنية السائدة هى حامض الاستياريك والاوليك واللينوليك (١٣,٧، ١٤,٤٣، ٧١,٠١%) على التوالى وهذا يوضح إرتفاع النسبة المئوية للاحماض الدهنية الضرورية فى الزيت (أوليك + لينوليك).

ووجد أنه عند رش النباتات بواسطة كل الهرمونات السابقة وبمختلف التركيزات أدى الى تناقص النسبة المئوية لحامض اللينوليك وأيضا وجد أن عند الرش بواسطة الاندول حامض الخليك والكينيتين والايثيريل بالتركيزات (١٠٠ ، ٥ ، ٢٠٠ جزء فى المليون) على التوالى أدى الى تناقص النسبة المئوية لحامض الاوليك . ويمكن تفسير هذه الظاهرة السابقة بأن الهرمونات السابقة تؤدى الى تثبيط الإنزيمات المسئوله عن تخليق مثل هذه الأحماض.

لقد أمكن ملاحظة ظاهرة هامة أنه عند رش النباتات بواسطة الاثيريل بتركيز ٢٠٠ جزء فى المليون أدى الى إرتفاع النسبة المئوية للأحماض الدهنية المشبعة وقصيرة السلسلة (حتى ك١٤) وعلى عكس ذلك وجد أن بنفس المعاملة تناقصت النسبة المئوية للأحماض الدهنية بصفة عامة المشبعة والغير مشبعة وهذا يوضح أن هذا الهرمون وعند نفس التركيز يعمل على تثبيط الانزيمات المسئولة عن تخليق الأحماض الدهنية وقد تم أيضا دراسة مكونات البذور من الاحماض الأمينية والبروتين الحقيقى لجميع المعاملات مستعينا بطرق التحليل الكروماتوجرافى الورقى وقد أمكن التوصل للأتى نتيجة المعاملة بهذه الهرمونات.

يحتوى ناتج التحليل المائى الحامضى لبروتين بذرة الداتورا على سبعة عشر من الأحماض الأمينية الشائعة والتى أمكن تقسيمها ضمنيا الى ثلاثة مجموعات الاولى: تشمل على الاحماض التى توجد بكميات كبيرة وهى : جليسين – الانيين – اسبارتك – جلوتاميك.

اما المجموعة الثانية فتحتوى على الأحماض التى توجد بكميات متوسطة وهـى : المثيـونين – أرجينين – ليوسين – فنايل الانين سيرين – ليسين – فالين ثربونين – والمجموعـة الثالثـة يتبعهـا الأحماض التى توجد بكميات صغيرة نسبيا وهى : الهستيرين – تيروسين – سيستئين – بـرولين – ايروليوسين – وقد سجلت إختلافات واضحة بين كميات هذه الأحماض بين التركيزات المختلفة لنفس منظم النمو كذلك فيما بين جميع المنشطات والتى ظهر منها تفوق الينتين ثم اندول حمض الخليك فى التركيزات المعتدلة على باقى المعاملات.

إتضح من تقدير كمية البروتين الحقيقى أن نسبته المئوية كانت أعلى فى جميع المعاملات بالنسبة لتجربة المقارنة الا أن التركيزات المنخفضة والمتوسطة من المنشطات المستخدمة كان لها أكبر الأثر على هذه الزيادة أما التركيزات المرتفعة قد أدت الى تثبيط واضح لعملية تخليق البروتين فـى هـذه البذور وعموما فأنه يمكن ترتيب هذه المنشطات تنازليا حسب درجة تأثيرها على التخليـق الحيـوى للبروتين فى بذور الداتورا: الكينتين> الألار > اندول حمض الخليك > الايثيرل.

ظهر من حساب نسبة الأحماض الامينية الحره الكلية أنها توجد بكميات منخفضة جدا ، لا تتعدى فى أى معامله على نصف نسبة نظيرتها بتجربة المقارنة وهذه النتيجة تدل على انها تنشط انزيميـا فور تكونها لترتبط مع t RNA ثم تدخل فى تكوين جزئيات البروتين.