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Response of Datura innoxia Mill plants to Jasmonic Acid Application

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Abstract

Two experiments were carried out during the 1994/1995 and 1995/1996 seasons to study the effect of Jasmonic acid[1] on the growth, cholorophyll content, the levels of endogenous hormones, alkaloid contents and the yield of Datura innoxia Mill plants. JA was appled at three levels of concentration, i.e. 200, 400 and 800 ppm, and distilled water as a control group.

The results indicate that the application of JA at different concentrations significantly decreased plant height and increased the number of both leaves and branches as well as the dry weight of the different organs of Datura plants.

Plants treated with JA showed a decrease in transpiration rate and total Chl a + b, with an increase in the concentration of the JA.

Results also showed that the alkaloid content of the seeds was higher than that of the different organs of Datura innoxia. Seed yield and alkaloid level, as well as the oil content, of the seeds were significantly increased by JA treatments. The effect was more pronounced with JA at 800 ppm during both seasons. On the other hand, JA treatments decreased growth promoters and increased growth inhibitors with an increasing concentration of JA.

1 Introduction

Datum innoxia Mill is one of the most important solanaceous plants. which is considered as a main sourse of tropane alkaloids needed for the pharmaceutical industries. In the last few years, great attention has been given to improving the yield and quality of Datura.

Application of a wide variety of both naturally occurring and synthetic chemical growth regulators have been extensively used in order to ascertain their beneficial effects upon

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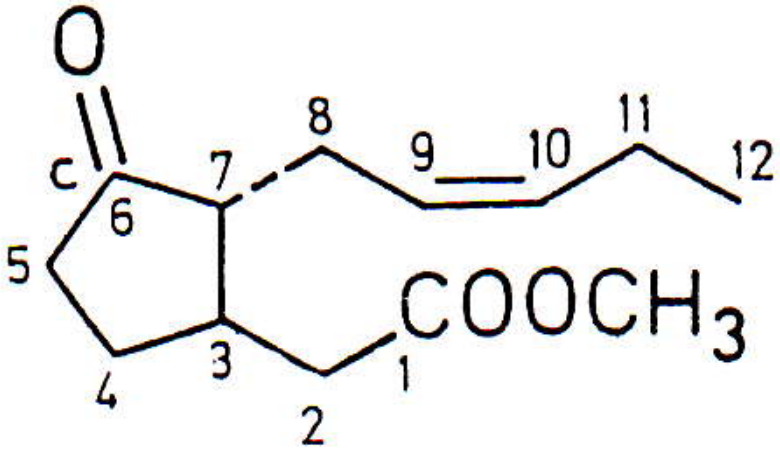
[1] Jasmonic acid was a generous gift from Dr. 0. Miersch of Institute of Plant Biochemistiy, D-060 18 Halle (Saale), Germany.

the growth and development of plants. A number of synthetic growth retardants have been discovered and proved to be of considerable importance in agriculture (YAMANE et al. 1990; YAMANE et al., 1981; SEMBDNER AND KLOSE, 1985).

Jasmonic acid (JA) and its methyl ester (JA-Me) (Fig. 1) are endogenous physiologically active compounds with a phytohormone-like action and are widely distributed in higher plants (UEDA et al, 1931; SEMBDNER AND KIOSE, 1985 and PARTHIER, 1990). They cause growth inhibition (MIERSCH et at, 1986 and POPOVA et al, 1988).

According to these contradictory opinions, we carried out this investigation in order to determine the optimum JA level which may lead to an increase in the drug and alkaloid yields from Datura innoxia Mill plants under our experimental and environmental conditions.



R = H, Jasmonic acid R = CH_3, Methyl Jasmonate

Fig. 1: Structure/formula of Jasmonic acid and methyl ester (VICK AND ZIMMERMANN, 1986)

2 Materials and Methods

Two field experiments were carried out at the Experimental Farm of the Faculty of Agriculture at Shibm El-Kom, Mmufiya University during two successive seasons of 1994/1995 and 1995/1996 to study the effect of Jasmonic acid (JA) on the growth, phytosynthetic pigments, photohonnone concentrations, drug yield and alkaloids content of the different plant organs of Datura innoxia Mill plants.

The seeds from the local plants were planted in seed pans on the 1st of October 1994 and 1995 in the nursery. The seedlings of 2-3 pair of leaves were transplanted at mid December in the seasons of 1994/1995 and 1995/1996 into plots of 2 x 4 m with 40 cm between the plants. During the soil preparation, chemical fertilizers, calcium superphosphate (15% P205) and potassium sulphate (50% K20), were added to the soil at rates of 130 and 85 Kg/fed. respectively. From the nitrogen fertilizer the plants received urea (46% N) at rate of 85 Kg/fed. in three equal side dressings on the 10th of January,

February and March in the two seasons. All other cultural practices were performed as usual. The study was conducted in a randomized complete block design with three replicates.

Jasmonic acid (JA) was applied as a foliar application at the rates of 200, 400 and 800 ppm. Distilled water was used for the control plants. Plants were sprayed twice at 35 days from transplanting and 7 days later by means of a hand atomizer until run-off. Tween 20 was used a wetting agent at a concentration of 0.5%.

At the full flowering stage, plant height, number of both leaves and branches and dry weights of leaves, stems, flowers and roots were measured. The different plant organs were dried at 70°C and ground in preparation for chemical analysis. The chlorophyll, and transpiration rate were measured at the full flowering stage.

1) Chlorophyll a, Chl b and carotenoids were determined spectrophotometrically as described by Wettstein (1957).

2) The transpiration rate (g water/cm2/h) was determined as according to Kreeb (1990).

3) The total alkaloid percentage in the different dried plant organs was determined according to the method described by Karawya et al. (1975).

Determination of endogenous growth hormones

Extraction of endogenous growth hormones was carried out according to the method of Shindy and Smith (1975). 30 grams fresh weight of the leaves at full flowering stage were used for the determination of auxin and their inhibitors, gibberellins and cytokinins. Plant material was extracted three times with 80% cold methanol. The combined alcohol extracts were evaporated under reduced pressure and the aqueous residue was partially purified by partition with ethyl acetate. The acidic ethyl acetate fraction was then collected and dried under vacuum at 37°C to dryness to determine auxin levels and their inhibitors and gibberellins, whereas the alkaline fraction was used to determine cytokinins. Separation was carried out by paper chromatography using a solvent composed of isopropanol : ammonia : water (10:1:1). Bioassay techniques were followed using wheat coleoptile straight assay (Bently and Housley, 1954) for auxins and their inhibitors, lettuce hypocotyl assay (Frankland and Wareing, 1960) for gibberellins and cucumb cotyledons assay (Fletcher et al., 1982) for cytokinins. The results of the phytohormones were statistically analyzed according to Tukay (1953).

At harvest, the seed yield, oild and alkaloid yield were recorded. The oil percentage in Datura seed was determined using Soxhlet continuous extraction apparatus according to A.O.A.C (1980). All data were subjected to a statistical analysis of variance (Snedecor and Cochran, 1969).

3 Results and Discussion

3.1 Growth analysis

Data in Table (1) indicated clearly that plant heights significantly decreased with increasing Jasmonic acid concentrations. The highest value in this respect was obtained at 800 ppm at which plant heights were decreased by about 13% and 15% in the first and second seasons, respectively, when compared to untreated plants. It is clear from the same results that all levels of JA caused a significant increase in the number of both leaves and branches per plant, and the drug weight of the different parts of Datura innoxia Mill plants (Table 2). The highest value in this respect was obtained by 800 ppm of JA. These results were true for both 1994/1995 and 1995/1996 seasons.

The dwarfing effect of Jasmonic acid may be due to the influences of JA on preventing cell elongation and/or stopping cell division - it acts as an antigibberellin (Fig. 2). In this regard, several investigators reported that JA shortened the height of many plants species (Dathe et al., 1981; Sembdner and Gross, 1986; Parthier 1990; Gendy and Schilling, 1990; Gendy and Selim, 1994)

The increase in drug weights of treated plants (Table 2) might be attributed to the positive effect of JA on the number of both leaves and branches and dry matter deposition. This increase also might be due to the enhancement effect of JA on CO_2 fixation and/or the increase in the anabolic metabolism (Sembdner and Parthier, 1993).

Table 1: Effect of Jasmonic acid on growth parameters of Datura plants during 1994/95 and 1995/96 seasons

JA levels (ppm)	Plant height cm/plant	No. of leaves/plant	No. of branches/plant
1994/95 season			
Control	90.2	89.2	15.2
200	87.4	95.4	17.6
400	80.6	96.8	18.3
800	78.4	80.2	15.0
L.S.D 5%	5.2	18.1	4.2
1995/96 season			
Control	86.4	84.8	14.2
200	82.1	89.9	16.3
400	80.2	92.4	19.4
800	73.4	81.4	14.8
L.S.D 5%	4.8	16.4	3.4

Table 2: Effect of Jasmonic acid on the drug yield in gins/plant of Datura innoxia Mill plants during the seasons of 1994195 and 1995/96

JA levels (ppm)	Leaves	Stems	Flowers	Total herb	Roots
1994/95 season					
Control	80.2	455	9.4	135.1	15.8
200	82.4	48.2	9.8	140.4	18.2
400	86.9	50.4	10.2	147.5	19.6
800	88.1	52.8	10.3	151-2	19.5
L.S.D 5%	4.2	4.2	0.8	78	2.4
1995/96 season					
Control	79.2	46.2	9.2	134.6	14.2
200	80.4	49.4	9.6	139.4	15.4
400	83.2	50.8	10.0	144.0	18.4
800	84.6	53.7	10.2	148.5	19.6
L.S.D 5%	3.1	4.6	0.6	6.6	2.6

3.2 Transpiration rate

Data recorded in Table (3) showed clearly that all levels of JA caused a significant decrease in the transpiration rate. The most effective treatments to decrease the transpiration rate were at the highest levels of JA. This decrease may be attributed to the stomata closure by JA (SATLER AND THIMANN, 1981; HORTON, 1991; GENDY AND SELIM, 1994). Jasmonic acid activities seem to be similar to abscisic acid inthis respect (SEMBDNER AND PARTHIER, 1993).

3.3 Photosynthetic pigments

Data in Table (3) shows clearly that all JA levels decreased Chi a, Chi b and total Chi (a+b) as well as carotenoids in both seasons. On the contrary, the carotenoids significantly increased with increasing JA levels. The lowest value of total Chl a+b resulted from plants sprayed at the highest levels of JA.

The decrease in photosynthetic pigments as a result of JA treatments has been reported in other investigations by Parthier et al. (1987a), Ueda and Kato (1982), Parthier (1990), Gendy and Schilling (1990), Gendy and Selim (1994). The role of JA of inhibiting the chlorophyll content was attributed to the capability of JA to reduce chioroplast development during leaf growth and to promote the rate of leaf senescence as well as to stimulate the degradation of chlorophyll (SEMBDNER AND PARTHIER, 1993). The reduction in CM content may be due to several factors: 1) inhibition of endogenous hormonal activity, 2) suppression of rRNA incorporation into plastid nucleic acid and its synthesis,

3) inhibition of GA-dependent DNA biosynthesis which decreases the protein content necessary for Chl biosynthesis, and/or 4) increasing chlorophyllase synthesis (PARTHIER, 1991).

Table 3: Effect of Jasmonic acid on chlorophyll concentration and transpiration rate in leaves of Datura plants (1994/1995 and 1995/1996 seasons)

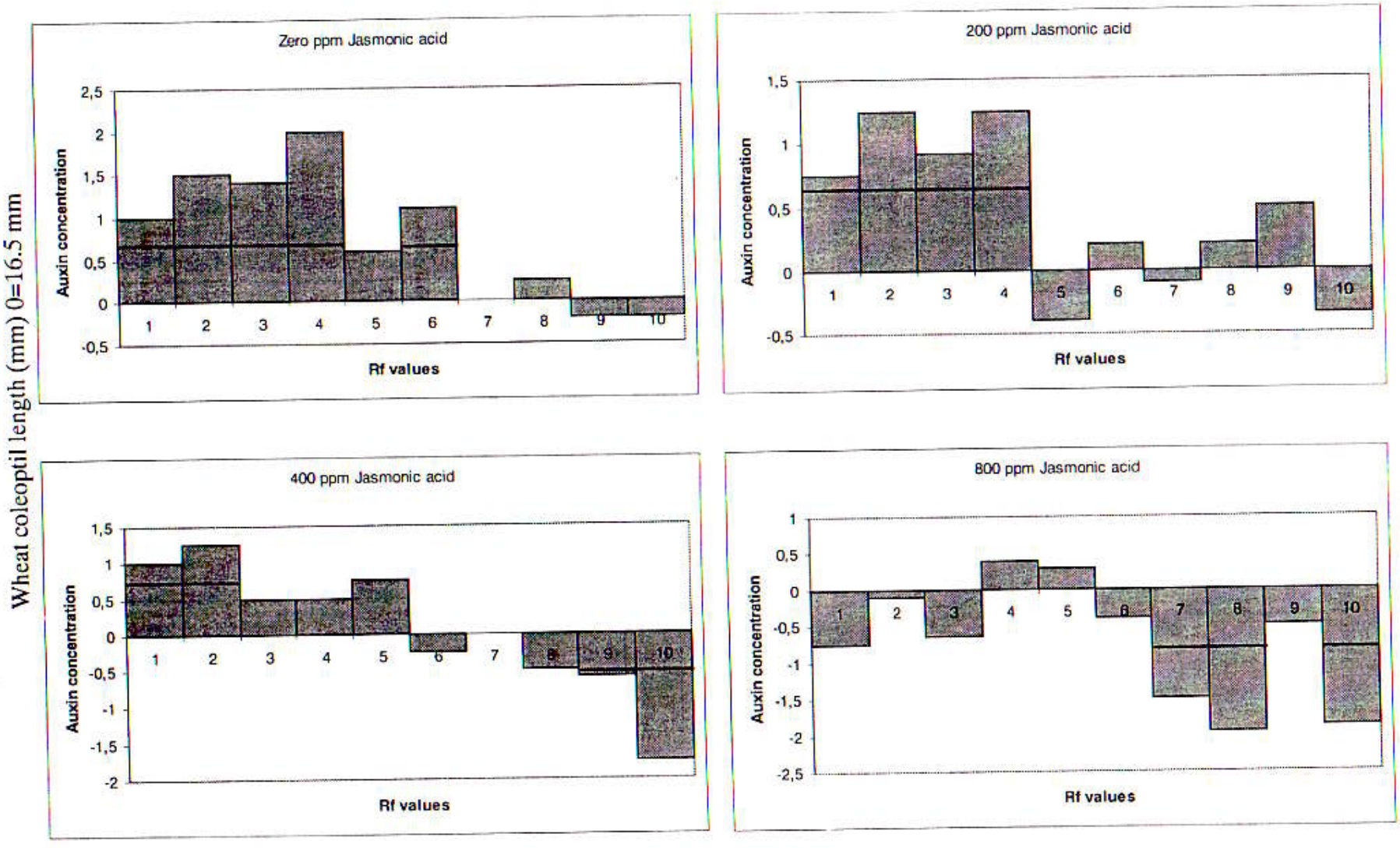
JA levels (ppm)	Chlorophyll concentration (mg/g.f.wt)				Transpiration rate (mg/cm2/h)
	Chl a	Chl b	Chl a + b	Carotenoid	
1994/1 995 season					
Control	6.32	2.18	8.50	2.77	5.8
200	6.12	2.12	8.24	2.80	5.0
400	5.80	2.08	7.88	2.90	4.2
800	5.42	2.00	7.42	2.98	3.2
L.S.D 5%	0.85	0.12	0.89	0.21	1.0
1995/1996 season					
Control	5.12	2.00	7.12	2.30	4.9
200	5.00	1.90	6.90	2.45	4.6
400	4.82	1.82	6.64	2.54	3.9
800	4.34	1.68	6.02	2.62	3.2
L.S.D 5%	0.67	0.16	0.94	0.32	1.2

3.4 Phytohormones concentration

Auxin and their inhibitors

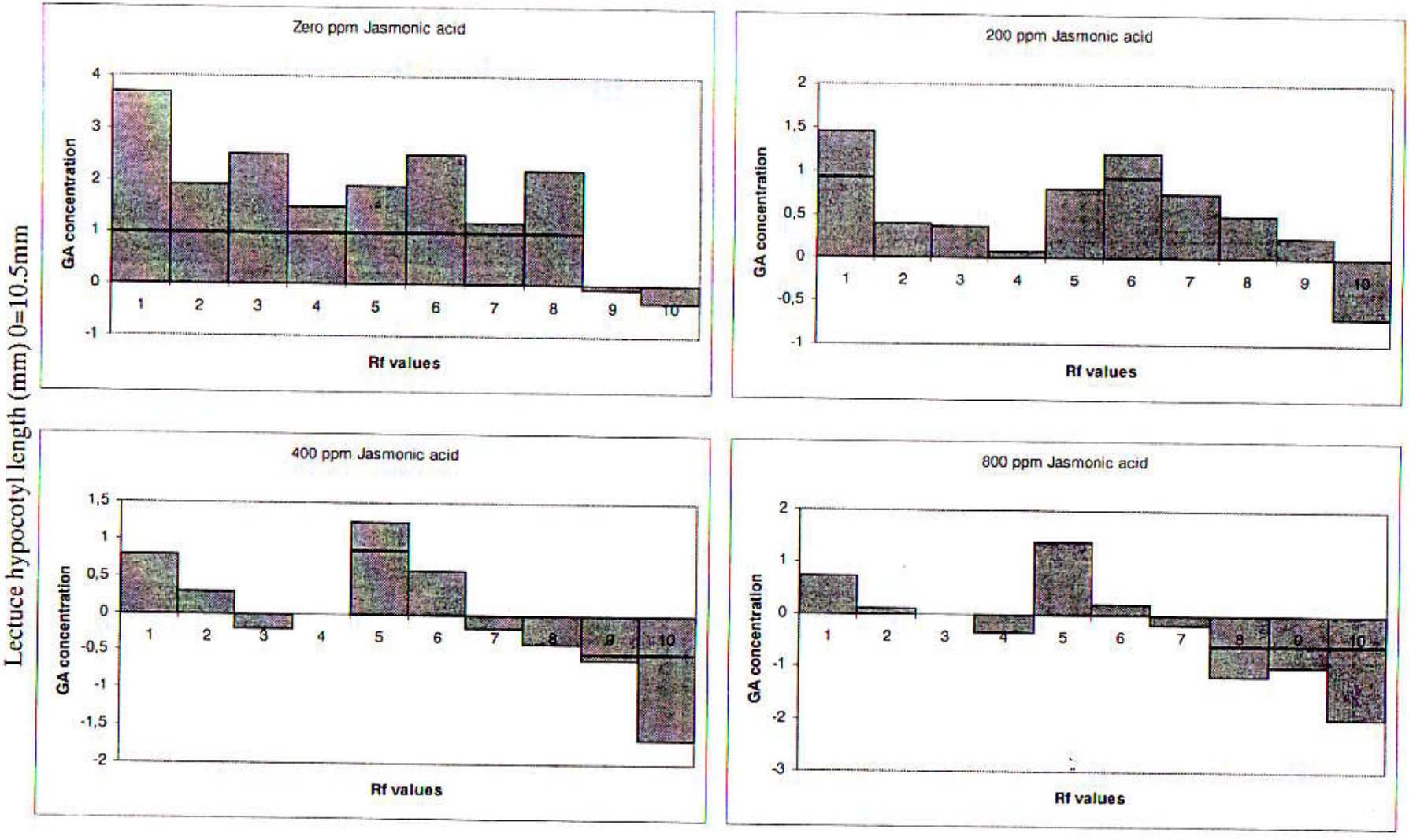
From the results obtained from the leaf extract of the wheat straight growth assay (Fig. 2) it can be seen that the control plants recorded significant levels of auxin at R_f (s) 0.0-0.4 and 0.5-0.6 at full-flowering stage. On the contrary, less content of auxin inhibitors was shown at the same stage.

From the effect of JA treatments on the endogenous levels of auxin like substances and their inhibitors of full-flowering stage of Datura innoxia Mill plants it can be seen that both levels of JA (200 and 400 ppm) induced significant levels of auxin activity in Datura leaves at R_f(s) 0.0-0.4, 0.0-0.2 and 0.4-0.5 respectively. Whereas no significant amounts of auxin was detected with the application of JA at 800 ppm (small peaks of activity were found at R_f 0.3-0.5). On the other hand, plants treated with 200 ppm JA showed insignificant levels of auxin-inhibitors although significant levels of auxin inhibitors at R_f(s) 0.8-1.0 and 0.0-0.1 and 0.6-0.8 and 0.9-1.0 were recorded for the application of JA at 400 and 800 ppm respectively.



Wheat coleoptil length (mm) 0=16.5 mm

Figure 2: Effect of Jasmonic acid levels on auxin content in Datura



Lectuce hypocotyl length (mm) 0=10.5mm

Figure 2a: Effect of Jasmonic acid levels on GA content in Datura

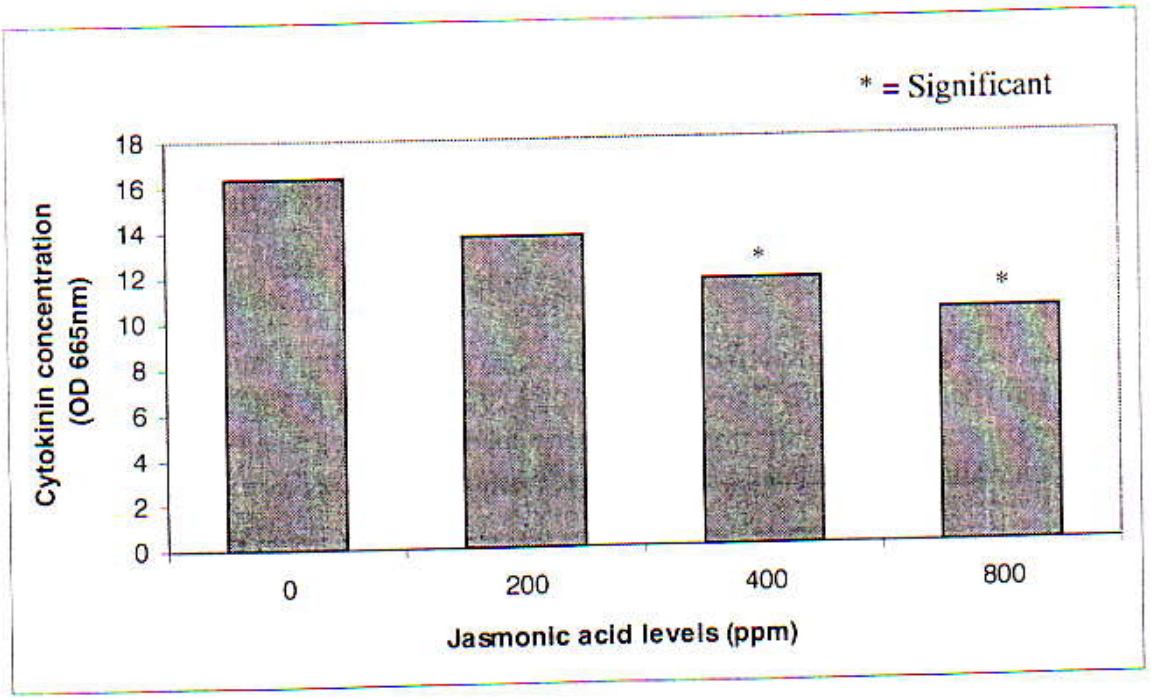


Figure 3: Effect of Jasmonic acid levels on cytokinin content in Datura leaves

Gibberellins and their inhibitors

The results obtained from the lettuce hypocotyl assay for the leaf extract of the control plants Fig. (2a) showed significant amounts of gibberellin activity at R_f(s) from 0.0 to 0.8 and insignificant levels of gibberellin-inhibitors at the full flowering stage.

It can be seen from Fig. (2) that the leaf extract from Datura innoxia Mill plants treated with JA at 200 and 400 ppm achieved significant levels of gibberellins at R_f(s) 0.0-0. 1, 0.5-0.6 and 0.4-0.5 respectively,m while no significant levels of gibberellins were detected with the JA treatment at 800 ppm. On the contrary, significant levels of gibberellin-inhibitors were recorded at Rf(s) 0.8-1.0 and 0.7-1.0 for JA treatments at 400 and 800 ppm respectively. These results agree with those of Gendy and Selim (1994), who reported that the gibberellin concentration in faba bean was decreased after leaf treatment with JA.

Cytokinin

From Fig. (3) it can be seen that the control plants achieved the highest level of cytokinin, estimated by the cucumber cotyledons assay for the leaf extract from Datura plants at full flowering stage. On the other hand, the cytokinin levels were reduced gradually by the application of JA at 200, 400 and 800 ppm plants plants plants . Similar findings were reported by Ueda et al. (1981), Ueda and Kato (1982), who found that the application of JA decreased the concentration of cytokinin.

3.5 *Total alkaloids*

alkaloid percentage

The data shown in Table (4) show clearly that JA application on Datura innoxia Mill plants caused a slight decrease in the alkaloid concentration in the dried leaves, stems and roots when compared to the untreated plants, whereas the total alkaloid percentage in the flowers showed an increase for both seasons.

The decrease in the alkaloid percentages as a result of JA application in the leaves, stems and roots of Datura innoxia Mill plants could be due to the dilution effect of alkaloid concentrations in the previously mentioned organs as a result of the increase in drug yield. Similar results were obtained by Mostafa et al. (1984) on Datura plants.

alkaloid content

The data presented in Table (4) indicate that JA application on Datura innoxia Mill plants at its different concentrations increased the alkaloid yield/plant in flowers incomparison to the control plants. The best results in this respect were obtained by spraying the plants with 400 and 800 ppm of JA in the two experimental seasons.

It seems from these results (Tables 3 and 4) that the application of JA was more effective in increasing the alkaloid yield of datura flowers when compared to untreated plants. These results are in agreement with the findings of Parthier (1991).

Table 4: Effect of Jasmonic acid on the alkaloids content of the different Datura plant organs during the seasons of 1994/95 and 1995/96

JA levels (ppm)	Leaves		Stems		Flowers		Total herb		Roots	
	%	g/plant	%	g/plant	%	g/plant	%	g/plant	%	g/plant
1994/95 season										
Control	0.86	0.69	0.56	0.25	0.89	0.08	0.79	0.59	0.40	0.06
200	0.74	0.61	0.52	0.25	0.96	0.09	0.70	0.56	0.31	0.06
400	0.72	0.63	0.45	0.23	1.20	0.12	0.62	0.57	0.24	0.05
800	0.70	0.62	0.36	0.19	1.42	0.15	0.60	0.42	0.20	0.04
L.S.D 5%	-	0.06	-	0.02	-	0.02	-	0.08	-	0.02
1995/96 season										
Control	0.58	0.54	0.48	0.22	0.72	0.07	0.61	0.43	0.31	0.04
200	0.60	0.48	0.40	0.20	0.84	0.08	0.58	0.49	0.28	0.04
400	0.52	0.43	0.36	0.18	0.99	0.10	0.50	0.47	0.21	0.04
800	0.48	0.41	0.30	0.16	1.20	0.12	0.48	0.33	0.18	0.04
L.S.D 5%	-	0.09	-	0.04	-	0.04	-	0.09	-	N.S.

Table 5: Effect of Jasmonic acid on seed yield, seed oil and seed alkaloids yields of Datura plants

JA levels (ppm)	Seed yield g/plant	Seed oil		Seed alkaloid	
		%	g/plant	%	g/plant
1994/95 season					
Control	50.1	14.6	7.31	1.40	0.70
200	55.3	15.2	8.41	2.10	1.16
400	64.2	16.8	10.79	3.30	2.12
800	60.4	16.0	9.66	2.80	1.69
L.S.D 5%	2.5	1.2	1.45	1.12	0.62
1995/96 season					
Control	48.2	14.2	6.84	1.30	0.63
200	52.4	15.0	7.86	2.20	1.15
400	66.8	16.2	10.82	3.28	2.19
800	61.6	15.8	9.73	2.60	1.60
L.S.D 5%	2.9	1.4	1.66	1.20	0.75

The beneficial effects of growth regulators on the biosynthesis of alkaloids and other secondary metabolites in many medicinal plants were also reported by Moskova-Simenova et al. (1987); Moftah and Attia (1992). The increase in N uptake and amino nitrogen content resulted from these growth regulators and, as reported by Abdou (1987), might be reasons for stimulating alkaloid biosynthesis.

3.6 The yield

As shown in Table 5 seed yield was effected positively and significantly by Jasmonic acid application. The highest seed yield was obtained at the moderate rate of JA. The biggest increase in seed yield reached about 63% and 56% in the first and second seasons, respectively, when compared to the control group.

It can be also seen from Table 5 that foliar spray of JA to Datura plants leads generally to an increase in oil and alkaloid percentages and yields in the seeds when compared to untreated plants. The alkaloid content of seeds reached its maximum value when plants were sprayed JA at 400 ppm.

From the aforementioned discussion it could be concluded that the application of JA modifies the growth of Datura plants. The modifications are characterized by significantly shorter plants. Moreover, the seed yield and alkaloid yield were increased.

4 References

1 Abdou, M.A.H. (1987): Study of some factors effecting seed germination and seedling growth of some trees. Ph.D. Diss. Fac. of Agric. Minia Univ., Egypt.

2 A.O.A.C. (1980): Association of Official Agricultural Chemists: Official Methods of Analysis, Washington D.C., U.S.A.

3 Bentley, J.A. and S. Housley (1954): Bioassay of plant growth hormones. Plant Physiol. 1: 405.

4 Dathe, W.; H. Ronsch; A. Preiss; W. Schade; G. Sembdner and K. Schreiber (1981): Endogenous plant hormones of the broad bean (Viciafaba L.) (-) Jasmonic acid, a plant growth inhibitor in pericarp. Planta 153: 530-535.

5 Fletcher, R.A.; V. Kallidumbil and P. Steele (1982): An improved bioassay for cytokinins assay cucumber. Plant Physiol. 69: 675-677.

6 Frankland, B. and P.F. Wareing (1960): Effect of gibberellic acid on hypocotyl growth of lattuce seedlings. Nature 185: 255-265.

7 Gendy, A.A. and G. Schilling (1990): Effects of Jasmonic acid (JA) and Beta Hydroxy Ethyihydrazine (BOH) on growth, paddy yield and grain chemical composition of rice. Menofiya J. Agric. Res. Vol. 15(2): 1379- 1400.

8 Gendy, A.A. and A.H. Selim (1994): Broad Bean Response to Jasmonic Acid (JA) Application. Menofiya J. Agric. Res. Vol. 19 No. 6(1): 2903-2918.

9 Horton, R.F. (1991):. Methyl Jasmonate and transpiration in barley. Plant Physiol. 96: 1376-1378.

10 Karawya, M.S.; S.M. Abdel-Wahab; M.S. Hifnawy and M.G. Ghourab (1975): Colorimetric and gas-liquid chromatographic determination of atropin, Hyoscyamine and Hyoscine (Scopolamine) in Solanaceous plants. J. Assoc. of Agric. Chem. 58: 884-887.

11 Kreeb, K.-H. (1990): Methoden Zur Pflanzenokologie und Bioidikation. Gustave Fisher, Jena PP. 327, Germany.

12 Miersch, 0.; A. Meyer; S. Vorkefeld and G. Sembdner (1986): Occurrence of (±) 7-iso-Jasmonic acid in Viciafaba L. and its biological activity. J. Plant Growth Regul. 5: 9 1-100.

13 Moftah, A.E. and F.A. Attia (1992): Response of periwinkle (Catharanthus roseus G. Don) plants to foliar application of growth regulators. MiiaJ. Agric. Res. and Dev. Vol. 14 No.2:404-421.

14 Moskova-Simenova D.; L. Atanasova and A. Ivanova (1987): Kinetin influence in the phenolic metabolism in young tomato plants. Proc. Inter. Symp. Plant Growth Regulators III: 197-200.

15 Mostafa M.B.; A.E. Awad, H.M. Owais and A.K. Dawii (1984): Physiological studies on growth, chemical composition and alkaloids of Datura innoxia Mill. 2- Effect of growth regulators. Ann. of Agric. Sci. Moshtohor, Vol. 21.

16 Parthier, B.; G. Herrmann; J. Lehmann and R.A. Weidhase (1987a): Actions of methyl jasmonate in barley leaf senescence. In: Schuster G. (ed.) Neue Erkenntnisse und Trends in der Pflanzenphysiologie. Univ. Press. Leipzig, PP. 64-68, Germany.

17 Parthier, B. (1990): Jasmonates: Hormonal Regulators or Stress Factors in leaf Senescence? J. Plant Growth Regul. 9:1-7.

18 Parthier, B. (1991): Jasmonates, new regulators of plant and development: many facts and few hypothesis on their actions. Bot. Acta 104: 446-454.

19 Popova, L.P.; TS.D. Tsonev and G. Vaklinova (1988): Changes in some photosynthetic and photorespiratory properties in barley leaves after treatment with Jasmonic acid. J. Plant Physiol. 132: 257-26 1.

20 Satler, S.O. and K.V. Thimann (1981): Le Jasmonate de methyle neuveau et puissant promoteur da la senescence des feuilles. Compt Rend Acad Sci. Paris Ser. III 293: 735-740.

21 Sembdner, G. and C. Klose (1985): Jasmonic acid, one new phytohormone. Biol. Rdsch. 23, PP. 29-40.

22 Sembdner, G. and D. Gross (1986): Plant growth substances of plant and microbial origin. In Plant Growth Substances (1985), Ed. M. Bopp. PP. 139-49, Berlin, Heidelberg, New York, Tokyo, Springer Verlag, pp. 139-147.

23 Sembdner, G. and B. Parthier (1993): The biochemistry and the physiological and molecular actions of Jasmonates. Ann. Rev. Plant Physiol. 44:569-589.

24 Shindy, W.W. and O.U. Smith (1975): Identification of plant hormones from cotton ovules. Plant Physiol. 55: 550-554.

25 Snedecor, G.W. and W.G. Cochran (1969): Statistical methods. 6th Ed. Iowa State Univ. Press, Iowa, U.S.A., PP. 120-145.

26 Tukay, J.W. (1953): Some selected quick and assay methods of statistical analysis. Trans. N.Y. Acad. Sci., 16: 88-97.

27 Ueda, J.; J. Kato; H. Yamane and N. Takahashi (1981): Inhibitory effect of methyl Jasmonate and its related compounds on Kinetin-induced retardation of oat leaf senescene. Physiol. Plant 52: 305-309.

28 Ueda, J. and J. Kato (1982): Inhibition of cytokinine induced plant growth by Jasmonic acid and its methyl ester. Physiol. Plant 54: 249-252.

29 Vick, B.A. and D.C. Zimmermann (1986): Characterization of 12-oxo-phytodienoic acid reductase in corn. The Jasmonic acid pathway. Plant Physiol. 80: 202-205.

30 Wettstein, D. (1957): Chiorophyll-Letale und Submikroskopische Formwechsel der Plastiden. Exptl. Res. 12, PP. 427-433.

31 Yamane, H.; J. Sugawara; Y. Suzuki; E. Shimamura and W. Takahashi (1980): Synthesis of Jasmonic acid related compounds and their structure activity relationships on the growth of rice seedlings. Agric. Biol. Chem. 44: 2857-2864.

32 Yamane, H.; H. Takagi; T. Abe; T. Yokota and N. Takahashi (1981): Identification of Jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol. 22: 689-697.