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Effects of Compound Fertilization on Growth and Alkaloids of Datura (*Datura innoxia* Mill.) Plants.

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Abstract
The effect of 0, 100, 200, 400, 600 and 800 kg ha^{-1} of Sangral, a complex chemical fertilizer at rates, on growth, alkaloid content, drug yield and nutrient uptake of datura (*Datura innoxia* Mill.) plants was studied during two successive seasons. The plant height, the number of branches and leaves/plant, the fresh and the dry weights increased with increasing fertilizer rates up to 800 kg ha^{-1}; however, the maximum increase was recorded at 600 kg ha^{-1}. Total alkaloid and drug (hyoscyamine + scopolamine) contents also increased with increasing the fertilization level to a peak value of 600 kg ha^{-1}. It then, decreased at 800 kg ha^{-1} level. Plant leaves and fruits were the most valuable organs for alkaloid and drug accumulation followed by stems, roots and crowns, respectively. N, P and K in the leaves were linearly increased by increasing fertilizer level. It seems that compound fertilizers increase the availability of essential nutrient elements necessary for datura growth and metabolism, causing vigorous vegetation and high chemical production.

Keywords: Datura, NPK fertilization, alkaloids

1 Introduction

Datura is an annual wild plant belonging to the family Solanaceae and it is considered one of the most important medicinal plants, known as a main source of a variety of alkaloids required for pharmaceutical industries. Several compounds, important for drug manufactory, are present in datura plants. Daturine, hyoscyamine, atropine, scopolamine and essence materials used as antispasmodic, narcotic, neuro-sedative and anti-asthmatic drugs were found in datura (Chiej, 1984). Therefore, a wide range of studies to improve the growth and productivity of datura was made.

The effects of environmental factors such as temperature and light have been extensively examined (Sivastava and Luthra, 1993). Cultural practices to improve growth, yield and chemical compounds such as watering and fertilization have also been investigated but on a more limited scale (El-Kady *et al.*, 1980; Mazrou *et al.*, 1988; El-Masry *et al.*, 1996). Most studies were done in clay soils which are less permeable to water and can hold the nutrients for relatively long time. Moreover, most fertilizers used in

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these studies were generally highly water-soluble single fertilizers consisting of only one or two of N, P, or K; therefore, fertilization recommendations were inconsistent.

Under Al-Qassim conditions, the major problem in using single fertilizers is that the dry sandy soil is highly permeable to water and nutrients, due to low organic matter and clay contents as well as low cation exchange capacity (CEC). This often causes a great leaching and a huge loss of nutrients and may also create a potential groundwater contamination problem. In this regard, PETROVIC (1990) reported that around 80% of the nutrient elements found in single-fertilizers can be leached in sandy soils.

Micronutrients are often neglected in using the single-fertilizer form, although vigorous plant growth and crop production require an adequate supply and balanced amounts of all nutrients (MENGEL and KIRKBY, 1987) in order to maximize plant health and vigor by optimizing the plant nutrient-uptake. This can be only achieved if the nutrient content of the fertilizer is appropriate to the needs of the plants. Compound-fertilizers, containing both macro- and micro elements, may possess this characteristic and in several studies have been found to escape leaching losses of nutrients when applied in chelating form (BROWN *et al.*, 1982; SNYDER *et al.*, 1984; MANCINO, 1991).

Sangral compound fertilizer is frequently supplied with 20% - 20% - 20% N-P-K and with other macro and nearly all micro elements necessary for plant growth in adequate, balanced and chelating form. Not enough research is available regarding the effect of "Sangral" fertilizer on datura plants or of any specific factors that might be relevant to its requirement for the medicinal plants. Besides, very limited information is available concerning fertilization requirements for field production of datura in Saudia Arabia. An early article by MAZROU *et al.* (1988) described the influence of nitrogen nutrition on field datura grown under conditions different from those prevailing in Al-Qassim provided limited guidelines for datura production.

The present study was undertaken to determine how the compound fertilizer "Sangral" affects growth, chemical composition and nutrient uptake of datura (*Datura innoxia* Mill.) plants grown under the environmental conditions of the Al-Qassim region, Saudi Arabia.

2 Materials and Methods

Two field experiments were initiated during two successive years to determine the response of datura plants to the compound-Sangral fertilizer applied at 6 rates (0, 100, 200, 400, 600 and 800 kg ha^{-1}). The chemical composition of Sangral (Sinclair Horticulture LTD, England) compound-fertilizer is as follows: N, 20%; P, 20%; K, 20%; S, 0.4%; Mg, 0.02%; Fe, 70 ppm; Zn, 14 ppm; Cu, 16 ppm; Mn, 42ppm; B, 22 ppm and Mo, 14 ppm.

By the end of December, 2000 and 2001, seeds of Al-Qassim locally growing *Datura innoxia* were planted in pans and, ten weeks later, the seedlings with three leaves were transplanted 50 cm apart in 2×4 m plots in sandy soil. The chemical and physical properties of the soil at the field site are shown in Table 1. At the beginning of April, plants were fertilized four times with four equal portions of the proposed fertilizer rate, in monthly intervals, as side dressing treatments. Both experiments were designed in a complete randomized block design with three replications. During the experimental period, all normal agricultural practices were performed.

Table 1: Chemical and Physical Properties of the Soil.

Chemical Properties		
pH		8.2
ECe (mS)		2.6
Soluble Cations (meq L^{-1})		
	Na^{+}	11.00
	Ca^{2+}	4.35
	Mg^{2+}	2.50
Soluble Anions (meq L^{-1})		
	${CO_3}^{2-} + {HCO_3}^{-}$	2.99
	${SO_4}^{2-}$	11.70
	Cl^{-}	7.60
	$CaCO_3$	4.00 %
	O. M.	0.23 %

Physical Properties		
Fractions (%):		
	Sand	95.30
	Silt	3.60
	Clay	1.10
	Texture	Sandy Soil

Throughout the growth period, the plant height, the number of the branches and leaves, the fresh and dry weights of leaves, stems, roots, crowns, flowers, and fruits/plant were determined.

At the fruiting stage (1^{st} of Sept.), plants were harvested and their parts were separated and dried individually at 70°C to a constant weight. Then the following chemical analysis was performed using finely powdered materials: (a) total alkaloid percent was determined according to KARAWYA *et al.* (1975), (b) hyoscyamine and scopolamine were measured according to MIRALDI *et al.* (2001), (c) total nitrogen was measured using Micro-Kjeldahl method as described by CHAPMAN and PRATT (1978), (d) phosphorus was measured colorimetrically using the Stannus chloride method described by FRIE *et al.* (1964), (e) and potassium using the Flame Photometry method described by JONES and STEYN (1973).

All statistical analyses were performed according to SNEDECOR and COCHRAN (1980) with the aid of the COSTAT computer program for statistics. Differences among treatments were tested with LSD at a 5% level of significance.

3 Results and Discussion

3.1 Vegetative Growth Analysis

Data recorded during the two experimental seasons showed that fertilization with the compound fertilizer "Sangral" in a suitable dose is very important to produce healthy and vigorous datura plants with sufficient foliage, branches, flowers, fruits and roots (Tables 2 & 3).

Table 2: Effects of fertilization regime on plant height, number of branches and leaves of datura plants during the two experimental seasons.

Fertilizer Rate (kg ha^{-1})	*Plant Height (cm)*	*No. Branches per Plant*	*No. Leaves per Plant*	*No. Leaves per Branch*
		First Experimental Season		
00	060.5	3.63	095.33	26.26
100	071.6	4.04	112.14	27.75
200	085.2	5.75	132.60	23.06
400	097.4	6.50	164.50	25.30
600	111.4	7.25	180.24	24.86
800	072.0	4.00	106.15	26.54
L.S.D (5%)	4.9	1.03	21.15	1.45
		Second Experimental Season		
00	058.4	3.72	087.15	23.42
100	065.5	4.66	108.11	23.20
200	077.8	6.94	121.07	17.45
400	093.5	8.37	147.50	17.62
600	107.6	9.17	160.27	17.48
800	067.6	4.87	100.30	20.59
L.S.D (5%)	6.11	1.42	15.12	1.10

Vegetative growth, represented by plant height, number of branches and leaves per plant, fresh and dry weights of plant parts increased linearly by increasing Sangral rate of application and reached their maximum values at 600 kg ha^{-1} (Tables 2 & 3) then, all parameters tended to decline at 800 kg ha^{-1} level. The 93% and 98% increases in the dry weights of the aerial part observed at 600 kg ha^{-1} during the first and second season, respectively, were met by 51% and 24% increases in the dry weight of the roots, as compared with the control (Table 3). While at 800 kg ha^{-1}, the corresponding increases in the aerial part were 12.5% and 16%, respectively, the roots increased by

Table 3: Effects of fertilization regime on fresh and dry weights (g) of datura plant organs during the two experimental seasons.

Fertilizer Rate (kg/ha)	*Leaves/Plant*		*Stems/Plant*		*Crowns/Plant*		*Flowers/Plant*		*Fruits/Plant*		*Aerial Part/Plant*		*Roots/plant*	
	f.wt	d.wt	f.wt	d.wt	f.wt	d.wt	f.wt	d.wt	f.wt	d.wt	f.wt	d.wt	f.wt	d.wt
First Experimental Season														
00	140.8	42.55	130.5	45.44	40.5	09.85	06.55	1.88	169.4	42.33	0487.7	142.1	045.4	15.75
100	155.5	44.08	220.2	70.58	52.5	10.00	07.00	1.40	205.7	50.65	0640.9	176.8	050.6	16.00
200	167.2	46.82	272.4	87.11	61.6	11.74	08.65	1.55	350.5	77.42	0860.3	224.5	072.4	18.61
400	195.7	48.11	299.6	90.24	82.5	17.54	10.44	1.73	436.5	87.20	1024.6	224.6	089.5	19.58
600	240.4	49.12	375.8	93.70	88.7	24.62	12.35	1.82	537.2	105.51	1254.6	274.7	119.3	23.81
800	156.3	47.70	262.7	78.24	62.9	19.45	09.88	1.54	325.2	97.66	0819.9	244.2	056.8	16.85
LSD(5%)	11.54	1.88	38.50	12.45	9.16	1.12	1.22	0.25	45.15	7.11	95.45	28.40	12.15	1.11
Second Experimental Season														
00	118.5	33.24	125.8	40.24	36.7	09.11	06.12	1.75	160.6	39.45	0203.4	123.8	051.5	17.05
100	137.5	37.50	179.5	55.20	40.4	09.65	07.24	1.50	210.2	45.50	0574.8	149.5	063.2	17.20
200	152.4	39.67	251.4	74.65	55.4	11.22	08.52	1.66	280.4	59.54	0588.6	145.8	070.5	17.55
400	175.3	42.07	277.5	82.45	75.5	16.61	09.70	1.82	329.5	66.80	0818.5	199.5	085.4	18.75
600	210.4	62.61	314.2	86.72	82.3	21.42	11.55	1.91	467.4	93.26	1014.4	241.7	102.7	21.06
800	147.7	43.71	212.5	74.70	40.2	17.52	09.52	1.45	272.6	70.75	0846.9	238.9	050.6	14.88
LSD(5%)	22.15	2.05	26.50	12.60	6.65	1.11	0.85	0.22	33.20	6.95	101.4	32.18	9.16	0.98

only 7% at the first season and even decreased at the second season, compared with the control. Therefore, at the 800 kg ha^{-1}, the shoot/root ratio suddenly increased by about 60% of the control (on dry weight bases), during the 1st and exceeded 100% at the 2nd season (Fig 1). It is interesting to notice that the shoot/root ratio recorded at 800 kg ha^{-1} increased by about 16% over that recorded at 600 kg ha^{-1}, in spite of the negative effect of the former fertilizer dose, on all growth parameters, when compared with the later dose. Two reasons may explain this phenomenon. The first, it seems that the high fertilization ratio affected the roots much more negatively than it did to the aerial parts. In this connection, at 800 kg ha^{-1} root dry weight decreased by 41% of that recorded at 600 kg ha^{-1}, while the corresponding decrease in the shoot dry weight was only 12%. The second reason might be related to the relatively higher RWC% of the roots than that recorded for the shoots at the 800 kg ha^{-1} (Fig 2). This high amount of water within root tissues might affect their dry matter content, upon drying, much more than the effect on the shoots, leading to higher dry weight of the shoots than the roots. In this respect, an early study by TISDALE *et al.* (1992) showed that the excess amount of fertilizers caused burning and death of the root hairs, affecting negatively the root growth by inhibiting the elongation and enlargement of the root cells, consequently limited the extension of the roots in the soil as they became week, short, and fluffy. In another study, MENGEL and KIRKBY (1987) found that, the overloaded dose of fertilization increased the soil osmotic pressure and thus, soil water became tightly held within the soil granules causing dehydration of the plant organs, particularly the aboveground parts because of the less water uptake and translocation to the stems, leaves and other aerial plant parts. In addition, nitrogen fertilization was found to increase the growth, yield and alkaloid content in *Datura innoxia* (RUMINSKA and EL-GAMAL, 1978) at moderate fertilizer doses, while at high doses these variables were decreased. In a further study, BARKER and COREY (1990) reported that the extreme fertilization regime may enhance ethylene evolution and affect the plant growth negatively. Data recorded in this study confirm these findings since the highest dose of fertilization caused substantial reductions in the plant height, number of branches and leaves per plant (Table 2) as well as fresh and dry weights of leaves, stems, crowns, flowers and fruits of treated plants (Table 3). On the other hand, maximum values of growth variables were positively correlated (R^2=92%) with the 600 kg ha^{-1} dose of Sangral.

It is not surprising that plants without or with low fertilizer application grew very slowly and showed poor characteristics because of the essential role that nutrient elements play in plant structure and biology. The increase in the vegetative and reproductive growth with the optimum ratio of Sangral fertilization (600 kg ha^{-1}) might be due to the effective role of the balanced amounts of the nutrient elements in enhancing plant growth and physiological activity. In this regard, it is well known that the root and shoot morphogenesis of plant species is affected by the level and form of a fertilizer (ALBREGTS *et al.*, 1991). The significant roles of nitrogen and sulfur in amino acid formation, protein synthesis and phytohormone production (WAGNER and MICHAEL, 1971; MENGEL and KIRKBY, 1987; LING and SILBERBUSH, 2002); phosphorus in the nucleic acids formation, cell membrane structure and ATP generation (Garton and Widders, 1990; Ling and Silberbush, 2002), potassium and calcium in enzyme activity, stomata movement, meristematic zone formation, cell-membrane integrity, and plant water relations (Salisbury and Ross, 1992; Kaya *et al.*, 2002) made it very important for these elements to be found in suitable quantities in the fertilization practices and that was the case for Sangral fertilizer added to datura plants. In this regard, FeiBo *et al.* (1998) and Sawan *et al.* (2001), reported that N, P, and K are the nutrients that have the largest effect on the physiology and yield of crops. They are essential for photosynthesis and dry matter production. Moreover, the micro-elements found in a reasonably balanced level and chelated form gave the fertilizer a good opportunity to play an important role in the physiological activities of a plant. In this concern, the majority of the micro elements were found to enhance the activity of many enzymes within plant cells (Liptay and Arevalo., 2000). In addition, Fe, Mn and Zn somehow participate in chlorophyll formation (Salisbury and Ross, 1992). Mo is essential for N fixation in the soil and for nitrate reductase enzyme in the plant (Mengel and Kirkby, 1987). Cu and B are essential factors for phytohormone synthesis, carbohydrate translocation, and nucleic acid formation (Bernier, 1988).

Figure 1: Effects of fertilization rate on the shoot/root ratio of datura plants.

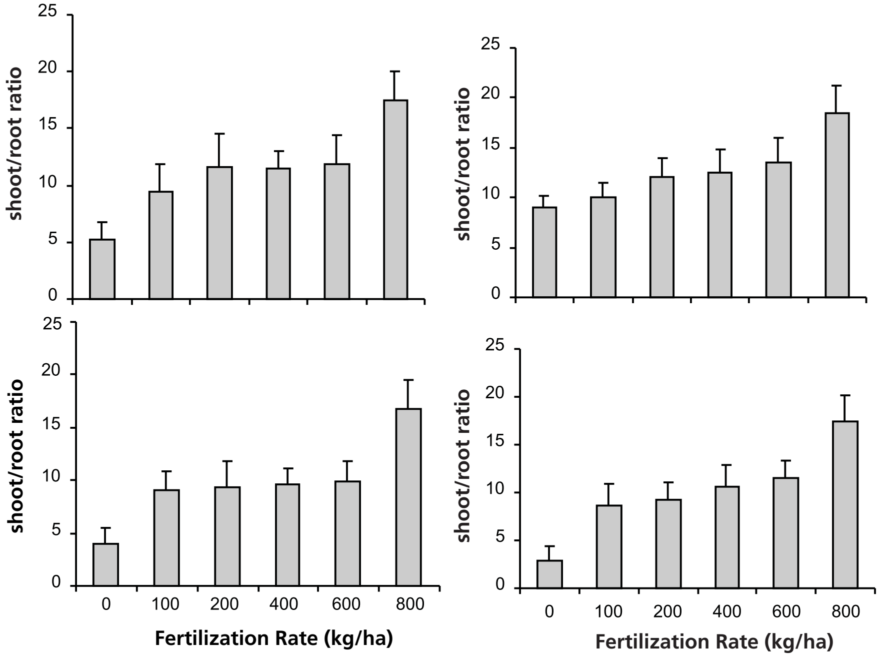
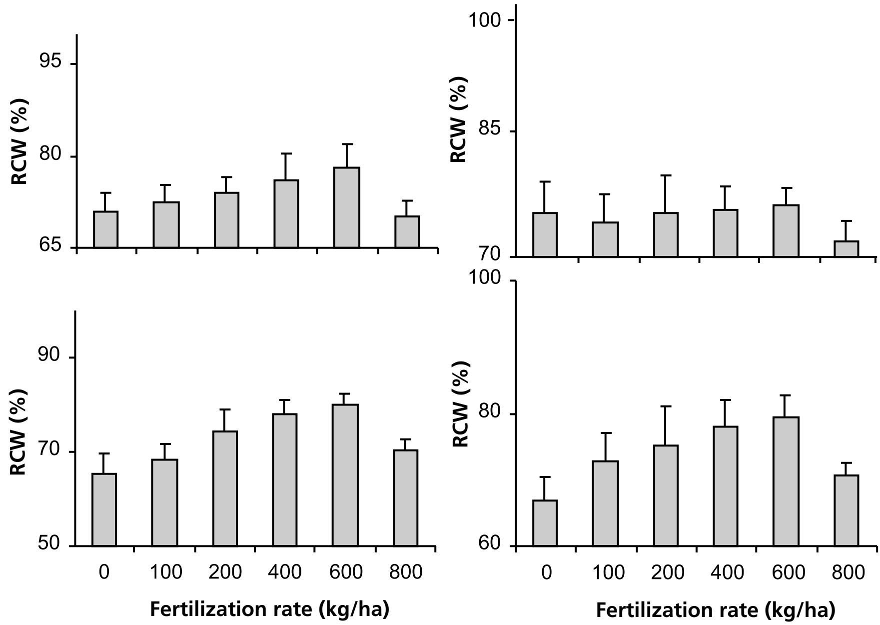


Figure 2: Effects of fertilization rate on the relative water content (RWC (%) of datura plants.



3.2 Chemical Analysis

3.2.1 Total Alkaloid Content

The production of alkaloids in *Datura innoxia* has been investigated in the different plant parts at different fertilization ratios (Table 4). Total alkaloid in most plant parts were consistent and positively correlated with the ratio of fertilizer application up to 600 kg ha^{-1}, and then tended to decrease at 800 kg ha^{-1}. The maximum contents of total alkaloids were found in the leaves and fruits as compared to other organs. The maximum value was recorded at 600 kg ha^{-1}, at which total alkaloids in the aerial part increased by about 79% and 60% of the control in the first and second season, respectively. These increases were due to the increases in alkaloid contents of different plant parts particularly fruits a 120% increase as compared to the control.

Roots and crowns showed also some increase in their total alkaloids with fertilizer application; however, their alkaloids were less than those recorded in the other plant parts. Concerning fruits, early studies by Robins and Abraham (1997) and, Miraldi *et al.* (2001) showed that the pericarp of either closed or mature capsules of *Datura stramonium* contained a very low alkaloid content, while in the seeds, the alkaloid content strongly increased during maturation. In the present study, the increase in the alkaloid content with fertilizer application might be attributed to the increase in seed yield within fruits.

Table 4: Effects of fertilization regime on total alkaloid content (mg/plant) and concentration (mg/(g DM)) in datura plant organs during the two experimental seasons.

Fertilizer Rate (kg/ha)	*Leaves*		*Stems*		*Crowns*		*Fruits*		*Aerial Part*		*Roots*	
	mg/plant	*mg/g DM*	*mg/plant*	*mg/g DM*	*mg/plant*	*mg/g DM*	*mg/plant*	*mg/g DM*	*mg/plant*	*mg/g DM*	*mg/plant*	*mg/g DM*
First Experimental Season												
00	171.3	4.02	50.3	1.12	7.63	0.77	92.4	2.17	321.6	2.30	09.12	0.58
100	186.2	4.22	67.4	1.25	7.76	0.78	148.2	2.19	409.6	2.34	11.23	0.70
200	195.3	4.17	74.4	1.36	12.13	1.04	162.2	2.19	444.0	1.99	16.20	0.87
400	228.1	4.75	109.9	1.38	16.05	0.92	188.1	2.16	542.2	2.23	17.02	0.87
600	230.5	4.68	122.2	1.61	17.22	0.70	206.2	2.96	576.1	2.11	19.78	0.83
800	207.6	4.34	104.2	1.33	17.33	0.89	114.5	2.05	443.6	1.83	11.50	0.68
LSD	11.5	NS	10.6	0.14	3.53	0.11	19.8	NS	32.6	0.26	1.44	0.14
Second Experimental Season												
00	166.3	4.99	40.4	1.01	5.68	0.62	75.8	1.90	288.2	2.36	11.00	0.64
100	170.7	4.53	55.2	1.21	5.86	0.58	116.4	2.55	348.2	2.35	13.12	0.76
200	180.4	4.54	74.6	1.40	6.94	0.62	142.2	2.39	404.1	2.18	14.09	0.80
400	214.2	5.09	82.4	1.69	9.01	0.54	155.5	2.32	461.1	2.22	16.11	0.86
600	219.0	4.85	86.7	1.84	9.80	0.46	167.3	2.79	482.8	1.83	18.20	0.87
800	197.6	3.15	74.7	0.75	10.13	0.58	121.5	2.18	403.9	1.95	12.46	0.83
LSD	10.8	0.62	9.5	0.20	2.72	0.09	26.4	0.14	40.5	0.22	1.62	0.11

As was expected, suitable rates of fertilization enhanced the alkaloid formation, since alkaloids are aromatic nitrogenous compounds containing nitrogen on their carbon skeletons. In addition, fertilization provides the elements that contribute in the synthesis of various amino acids, the precursor of a variety of alkaloids (SALISBURY and ROSS, 1978). Thus alkaloid concentrations increased within plant parts.

The positive effect of fertilization on the growth of plant parts, discussed above, may be another reason of an increasing alkaloid content per plant. In this regard, nitrogen fertilization was found to increase the growth, yield and alkaloid content in *Datura innoxia* (RUMINSKA and EL-GAMAL, 1978) at moderate fertilizer doses, while at high doses, these variables were decreased. In the same connection, DEMEYER and DEJAEGERE (1993) explained the increase in the alkaloid as a result of increasing fertilization as that, nitrogen and other elements of the fertilizer are incorporated into amino acids which are the main precursor of the alkaloids in the roots before they translocate to the upper plant parts.

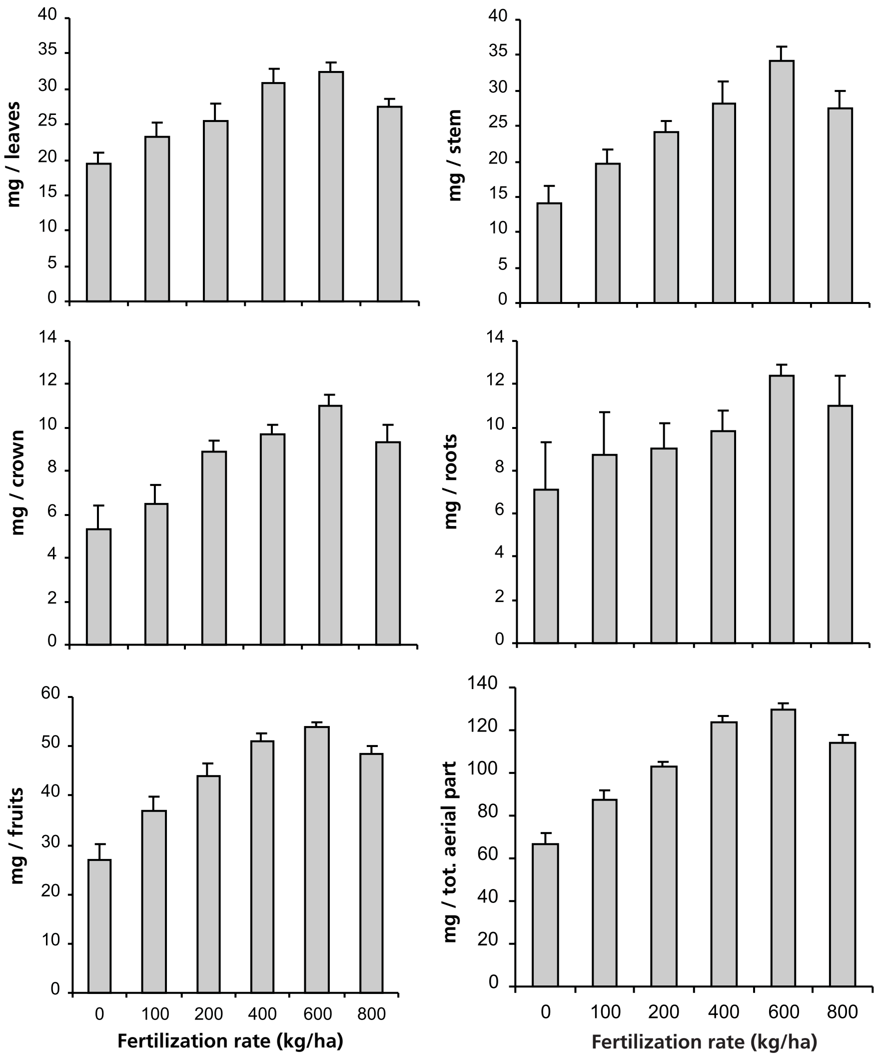
The drop in alkaloid content recorded at 800 kg ha^{-1}, on the other hand, might be explained mainly through the negative effect of extreme doses on roots in which alkaloids are synthesized (ROBINS and ABRAHAM, 1997). In this regard, MIRALDI *et al.* (2001) reported that the root is the principle site of alkaloid synthesis and that secondary modifications of alkaloids occur in the aerial parts. They added that, at plant maturity, alkaloids were absent in roots of adult plants. This study shows that alkaloid concentrations in the roots were very low in any treatment, compared with other plant parts.

3.3 Hyoscyamine and Scopolamine

The production of the most abundant alkaloids in datura, hyoscyamine (atropine) and scopolamine, has been investigated in different plant parts and at different rates of Sangral fertilization as they are included in many official pharmacopoeias because of their anti-cholinergic activities.

Regardless of the fertilization treatments, the recorded data indicated that maximum drug contents were found in the flowers followed by the leaves then stems, fruits and finally roots (Fig. 3), with hyoscyamine being always the predominant component. It is obvious that the response of these drugs to the fertilization was very much similar to that of alkaloids. In this regard, several studies indicated that suitable amounts of mineral nutrition enhanced the hyoscyamine and scopolamine synthesis in datura, as a result of influencing the formation of amino acids that are converting to the drug components (ROBINS and ABRAHAM, 1997; DEMEYER and DEJAEGERE, 1993; PINOL *et al.*, 1999). Recently, MIRALDI *et al.* (2001) reported that the levels of hyoscyamine and scopolamine within datura plant parts depend on several factors, the nutritional state is one of them, and atropine is often formed from hyoscyamine racemization and the hyoscyamine content is approximately two fold that of scopolamine.

Figure 3: Effect of fertilization on total hyoscyamine and scopolamine in datura plant parts as mg/dry weight of plants.



3.4 N P K Concentrations

The increase in the concentration of the nutrient elements within datura leaves as a result of fertilizer application was found to be significant during the two growing seasons as compared with the control (Table 5). It is evident that the linear increase in the concentration of the nutrient elements was positively correlated (R^2= 0.89) with the increase in the fertilization ratio. The most increase in the elements was recorded at 600 kg ha^{-1} ratio, at which the N, P, and K percentages were raised by about 80%, 60% and 8% of control, respectively, during the first season. The same enhancement trend was recorded at the second growth season. As was expected, at the suitable 600 kg ha^{-1} ratio roots tend to extend to a large volume (KATAYAMA *et al.*, 1999) and most, if not all, physiological activities of fertilized plants greatly increase, particularly the absorption of water and uptake of nutrients from the soil (BOYLE *et al.*, 1991).

Table 5: Effects of fertilization regime on N, P, and K concentration (%) and content (mg/ tot dry weight) of datura leaves during the two experimental seasons.

Fertilizer Rate (kg ha^{-1})	*Concentration (%)*			*Content (mg/plant)*
	N	P	K	
First Experimental Season				
00	0.91	0.45	2.39	1595
100	1.05	0.55	2.81	1945
200	1.08	0.58	2.44	1919
400	1.10	0.59	2.76	2136
600	1.65	0.71	2.59	2426
800	1.22	0.63	2.02	1846
Second Experimental Season				
00	1.29	0.38	2.04	1232
100	1.25	0.42	2.25	1470
200	1.35	0.44	2.67	1771
400	1.38	0.50	2.65	1907
600	1.93	0.56	2.70	3249
800	1.58	0.54	2.48	2010

On the other hand, the harmful effect of the extreme amounts of fertilization (800 kg ha^{-1}) on root growth and branching, as discussed above, lowered their ability to adsorb the nutrients sufficiently. These findings were in harmony with those reported by MAZROU (1985) on *Atropa belladonna* and MAZROU and AL-HUMAID (2000) on gladioli plants.

4 Zusammenfassung

Düngungseinfluss auf Wachstum und Alkaloidgehalt von Datura (Datura innoxia M.)

Der Einfluss von 0, 100, 200, 400, 600 und 800 kg/ha von Sangral, einem Mischdünger, der alle essentiellen Makro- und Mikronährstoffe für Pflanzen enthält, auf Wachstum, Alkaloidgehalt und Nährstoffaufnahme von *Datura innoxia* Mill wurde während zweier Vegetationsperioden untersucht. Pflanzenhöhe, Anzahl der Zweige und Blätter pro Pflanze sowie Frisch- und Trockengewicht und N-P-K- Gehalt der Blätter haben ihr Maximum bei 600 kg/ha, ebenso der Alkaloid- und Drogen (Hyoscyamin und Scopolamin) - Gehalt. Blätter und Früchte waren die wichtigsten Pflanzenorgane für einen hohen Alkaloid- und Drogengehalt, gefolgt von Stengeln, Wurzeln und Kronen.

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