ROLE OF CHROMIUM IN GROWTH AND ALKALOID CONTENT OF DATURA STRAMONIUM

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THESIS

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By
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D E D I C A T I O N

To those who fixed the milestones of my career.

To the love, affection and caress.

To the candle lights watching patiently the road to my destiny.

To the passion, sacrifice and adoration

To father, Mother and Husband.

ACKNOWLEDGEMENT

This investigation has been carried out as a joint problem between the Botany Department, Faculty of Science, and the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

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Thanks are also due to staff members of the Experimental station, and colleagues, in both Botany and Pharmacognosy Departments who have helped me in producing this thesis in its appropriate form.

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TO WHOM IT MAY CONCERN

This is to certify that Maymona Abd-El-Aziz Kord has attended and passed succesfully the following post graduate courses as a partial fulfilment of the requirements for the degree of master of science, during the academic year 1975-1976.

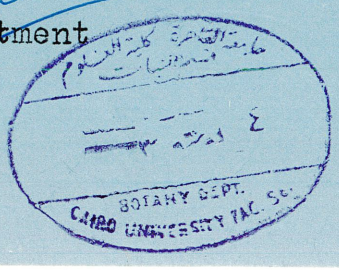
1- Enzymology.
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5- Growth regulators.
6- Soil microbiology.
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This thesis has not been previously submitted for any degree at this or any other university.

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PREFACE

Inspite of the fact that heavy metal pollution received great attention, within the last few decades, by most of the scientists of the globe yet chromium received little attention compared with other metals viz. lead, mercury, zinc or tin that induced a high range of contamination affecting the health and well-fare of all living organisms including man.

Chromium is not yet introduced to industry of biocides, thus it played little or no role in the economy of cultivated soils except for being consi-dered as a trace nutrient element.

According to the American Standards Association, the maximum permissible dose of chromium must not exceed 0.1 mg/1 m^3 of air. As a water pollutant, hexavalent chromium could be removed by adsorption using calcinated coke (Huang and Wu, 1975) whereas divalent chromate and dichromate apparently have slight affinity towards the calcium surface and consequently could not be removed by these procedures. The health hazards, reported for chromium, include chronic bron-chitis, tubercular lesions, ulcers and in few cases dermatitis in the neck (Krishna et al., 1976). Being

an air pollutant, it also causes carcinogenic hazards (Papadoublore et al., 1976) but it alleviated death, if administered to mice, as chromium chloride, 1 hour before challenging them with Salmonella typhasa (Snyder et al., 1977).

It is interesting to note that chromium is one of the heavy metals reported in the human body of Japannese people (Sumino et al., 75). It showed higher concentrations in tissues exposed to environment and accumulated in larger levels in males than in females.

This investigation is an endeavour to elucidate the possible effects of anionic and cationic chromium ions on growth morphology and alkaloid content of, the medicinal plant, Datura stramonium.

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PART I

I N T R O D U C T I O N

INTRODUCTION

A- HISTORICAL REVIEW

In the last decades chromium received great attention because of its wide spread use in industrial and domestic products although several of its chemical forms are very toxic. Industrial production and handling of certain Cr(VI) products is associated with respiratory injury, cancer, skin ulceration and induction of allergic chromium dermatitis. Land dumping of wastes from industrial Cr(VI) production and electroplating operations are mainly responsible for ground water contamination. Discharge of Cr(VI) into streams and rivers caused damage to aquotic ecosystems.

Sharkhnazarov et al. (1974) pointed out that workers in potassium dichromate industry (up to 5 years service had a high glycogen, acid and alkaline phosphatase activity in their leukocytes. Those working more than 10 years were short of glycogen in their leukocytes and both phosphatases were inhibited.

In his report; Jaworski (1976) mentioned that most of the Cr(III), present in water, is adsorbed on particulate matter whereas Cr(VI) remains in solution where it

is a powerful oxidising agent. Cr (VI) is reduced to Cr(III) when it reacts with animal or vegetable material in water. Hutcheson et al. (1975) reported that 20 ppm chromium did not affect the vitality or metabolic functions of rats.

Apart from the wide application of radioactive chromium in medical diagnosis (Dunn and Goldwein, 1975; Schiffer et al. 1975; Lapchenkov et al., 1973; Suemasu et al., 1970) chromium, as an element, interferes with the metabolic activities of living organisms. Its poisoning effect depends upon its valency (Fuoco and Papoff, 1975). Juvenile fish were poisoned by 53 and 24 mg/Litre tri- and hexavalent chromium respectively (Negilski, 1976).

According to Kryshtab (1971), exposure of animals to chromium biride dust induced fibrosis accompanied by disorders in protein metabolism. 6×10^{-5} mM chromium (III) did not increase the swelling of rat liver mitochondria but formed a complex with the intrachain disulphid of insulin (Campbell and Mertz, 1963). In his opinion (Anghileri, 1969), the mechanism of chromium accumulation in tumor cells is not a simple phosphomonesterase action but the interaction of chromium with calcium and/or nucleic acid metabolism was the main cause of such accumulation (Anghileri, 1971). Absorption and accumulation

of chromium in blood platelets or erythrocytes sharply decreased with age (Burger et al., 1975; Tsukoda et al., 1971; Glomski et al., 1971; Talskaya and Shitikova, 1964).

5 p.p.m. chromium, in drinking water, lowered serum cholesterol and glucose levels in rats (Schroeder et al., 1971). Shakhnazarov (1973) showed that prolonged oral intake of small doses of dichromate, by dogs, (1 mg/kg) decreased the total glycogen and inhibited the activity of the oxidation reduction and hydrolytic enzymes, with a drops in the - SH groups and ascorbic acid. At higher doses acid phosphatase activity and RNA synthesis as well as mono amino oxidase were highly increased. According to Hirsch and Pakuts (1975) chromium chloride did not elicit nephrotoxicity or stimulation of N-methyl nicotinamide uptake. The enhancement of renal N-methyl nicotinamide accumulation or retention by potassium dichromate appears to be associated with the nephrotoxicity produced by this compound.

Very little had been published on the role of chromium in the physiology and metabolism of plants. In some instances it is considered an indepensable micro element (Bertrand and De Wolf, 1965, 1968). As early as 1957, 1958, Dobrolyuoskii and Slavvo reported that application of 0.6 kg/hectare of chromium sulphate

to vines, prior to mass blooming, improved the quality of grapes. The weight and size increased by 21% and 18% respectively. The sugar content and the gluco acidiometric coefficient were also high. Lower doses (0.15 kg/hectare) were more effective. Similar observations were reported by Bertrand and De Wolf (1968) experimenting on potato. In acid soils, chromium sulphate was very toxic to wheat and tomato (Moulinier and Mazoyer, 1968). Rusko (1968 a,b) noticed that application of molybdenum, chromium or zinc to grapes, before flowering, raised the total nitrogen of the grapes and chlorophyll content of the leaves.

Usually the chromium content of plants is very low but some species can accumulate appreciable amount of the metal(Lyon et al. 1968). The effect of chromium on mineral nutrition have already been discussed (Hewitt, 1948, 1953). Translocation of ionic chromium from roots is blocked through chelation by proteins (Dekock, 1956). On the other hand, Lyon et al. (1968) demonstrated the presence of chromate ions in the xylem sap, indicating that the metabolism of this ion took place in the leaves.

Water culture experiments with glycine max (Turner and Rust, 1971) showed that 0.5 p.p.m. anionic chromium lowered the uptake of calcium, potassium, iron, phosphorus,

iron and manganese in roots. In soils 0.60 p.p.m. chromium intefered with accumulation of calcium, potassium, magnesium, phosphorus, boron and copper of tops at 5 p.p.m. or above. Symptoms of toxicity appeared as severe wilting of tops of the treated plants. Chromate was reported to be absorbed freely by barley roots (Shewry and Peterson, 1973). But unless the concentration was high (0.1 m.M) less than 1% of the absorbed anion was transported to the shoot. Most of the absorbed ion was found in a non-particulate form in the vacuoles. Skeffington et al. (1976) Further reported that chromate was more toxic to growth of barley than chromic though apparent uptake of the latter was much faster. In the meantime, chromate uptake was active whereas chromic uptake was passive, demonstrating that the two forms did not share a common uptake mechanism.

B- MATERIAL AND METHODS

This investigation was carried out in the Experimental Station annexed to the Faculty of Pharmacy, Cairo University. Seeds of Datura stramonium were kindly supplied by staff members of the Station, to whom the author is greatly endebted for their kind help and supervision during the laying out, preparation and agrotechnical manipulation during planting and growth of plants. All facilities offered for manuring, irrigation holing ... etc are greatly acknowledged.

In this investigation, each treatment was repeated in 10 plots. Each plot contained 4 rows of 3 meters length each, in which seeds were sown at 25 cm distance. After emergence, seedlings were thinned to two per spot.

45 days after sowing, an initial sample was taken haphazardly (one plant from each plot) before spraying the plants (10 mls for each plant) with a solution containing 0, 0.05, 0.1 or 0.5 mM of either potassium chromate, ammonium dichromate or chromic chloride. One week later, another sample was taken, from each treatment, before a second spray, with the same amount of solution,

was performed on each plant. A third spray was carried out, one week after, then the harvest was collected when the plants reached 66 days of age. Several plants were left in each plot to obtain ripen seeds.

In all samples, the length of main axis and all laterals, number of leaves, area of leaves, fresh and dry weight of stems and leaves as well as pigmentations were determined. In the meantime, the number of flowers, fresh and dry weight of young fruits as well as air dry and oven dry weight of 100 seeds was obtained. Another aliquot of samples was simultaneously collected for alkaloid assay.

The chromium concentrations were chosen on the basis of a preliminary investigation studying the effect of a wide range of concentrations on the rate of germination and length of protruding radicle. In such investigation 1 mM concentration arrested germination.

Before alkaloid estimation, a preliminary investigation was carried out on 30 day old potted plants in order to find out the most suitable method of drying the leaves to yield the maximum amount of alkaloids. The results showed that drying in the shade yielded more

alkaloids than drying in the sun whereas at 50°C, oven drying caused a remarkable loss of alkaloids. Accordingly all alkaloid estimations were performed on shade-dried samples, then their moisture content was determined for further calculations.

Usually spraying was performed very early in the morning after collecting the samples and before sunrise.

Pigment Estimation:

The procedures reported by Metzner et al. (1965) were followed in this investigation. Several samples, haphazardly collected from leaves, at different positions on the plant stem, ranging from 0.2 - 0.5 gm fresh weight were taken from the middle part of the leaf, few millimeters far from the midrib. These were immediately dipped in few mls redistilled acetone containing little magnesium carbonate (0.1%) to prevent decomposition of chlorophyll. The containers were kept in the dark, at a cold temperature then homogenized, diluted and left again in the dark for further extraction in the cold. When the debris appeared free of colour, the mixture was centrifuged, made up to the appropriate volume and the colour intensity was immediately recorded at 663, 647 at 452.5 nm wave length. The following equations record the pigments in mgm per litre

from which further calculations could be easily obtained.

Chlorophyll a = $11.78\ E_{663} - 2.29\ E_{647}$

Chlorophyll b = $24.05\ E_{647} - 4.77\ E_{663}$

Carotenes = $4.75\ E_{452.5}$ - (Chl.a + Chl.b) x 0.226

Alkaloid Estimation:

The procedures reported in the Egyptian Pharmocopia (1972) were followed.20 gm powdered leaves were moistened with little 50% ammonium hydroxide then alcohol extracted under reflux. The alcohol extract was evaporated under reduced pressure to about 15 ml then 10 ml N/10 sulphuric acid were added. The mixture was filtered quantitatively into a separating funnel using distilled water. The filterate was then 2-3 times extracted with chloroform (ca 20 ml for each extraction) and the combined extracts were rendered alkaline with little dilute ammonia. Keep the solution standing till the chloroform layer clearly separated then remove the water layer.

Repeat extraction of the acid solution with chloroform and test for alkaloids in a portion of the chloroform extract by the Mayer's reagent. Wash the combined extracts with water several times then keep over anhydrous sodium sulphate, in a desiceator, overnight. The chloroform was evaporated, under reduced pressure and the residue was

taken up in little neutral alcohol. The alcohol was evaporated on a boiling water both, and the residue was kept at 100°C for 15 minutes. The process was repeated twice to ensure removal of all volatile bases. The residue was then taken up in 2 ml chloroform for G.L.C. assay.

The temperature of the column was kept isothermal at 150°C for 3 minutes and then programmed by increasing the temperature at the rate of 40°C per minute to 250°C then the maximum temperature was kept isothermal for 3 minutes.

The rate of flow of the nitrogen gas carrier was 50 ml per minute. The hydrogen flow rate was 50 ml per minute; air flow rate 390 - 400 ml per minute. The chart speed was 1 cm per minute whereas the injected sample was 0.1 microleter .

PART II

EFFECT OF VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON GERMINATION OF DATURA STRAMONIUM SEEDS

EFFECT OF VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON GERMINATION OF DATURA STRAMONIUM SEEDS

In this preliminary investigation 100 seeds of Datura stramonium were spread in 15 cm diameter by 2.5 cm height Petri dishes, layered by a filter paper. Each dish received 15 ml of either 0, 0.05, 0.1, 0.5, 1.0 and 2.5 mM chromate, di-chromate or chromic salts. All dishes were kept in the dark for 7 days, at 27°C ± 0.1°C, during which the number of germinated seeds was counted daily starting from the third day. On the 7th day, the length of roots of all germinated seeds was determined.

Results

Table 1 and Fig. 1 reveal the fact that with progress of age, the number of germinated seeds progressively increased except at 1.0 and 2.5 mM concentration of all salts where no germination was recorded till the 7th day. Under all conditions, the chromic ion ($CrIII^{+}$) almost induced the same number of germinated seeds as the control samples, particularly by the end of the experimental period. Even the small number of germinated seeds, observed, at the early part of the

Table 1 : Effect of various concentrations of chromium salts on the percentage germination of Datura Stramonium seeds during seven days at 27°C in the dark.

Period of Germination	0 mM	0.05 mM			0.1 mM			0.5 mM		
		Chromate	Dichromate	Chromic	Chromate	Dichromate	Chromic	Chromate	Dichromate	Chromic
3 days	61	27	37	62	13	32	58	7	2	44
4 days	67	30	41	69	19	36	61	14	4	51
5 days	79	38	49	73	29	47	71	22	11	68
6 days	82	42	53	77	33	50	76	27	17	72
7 days	88	48	57	81	37	54	80	32	21	78

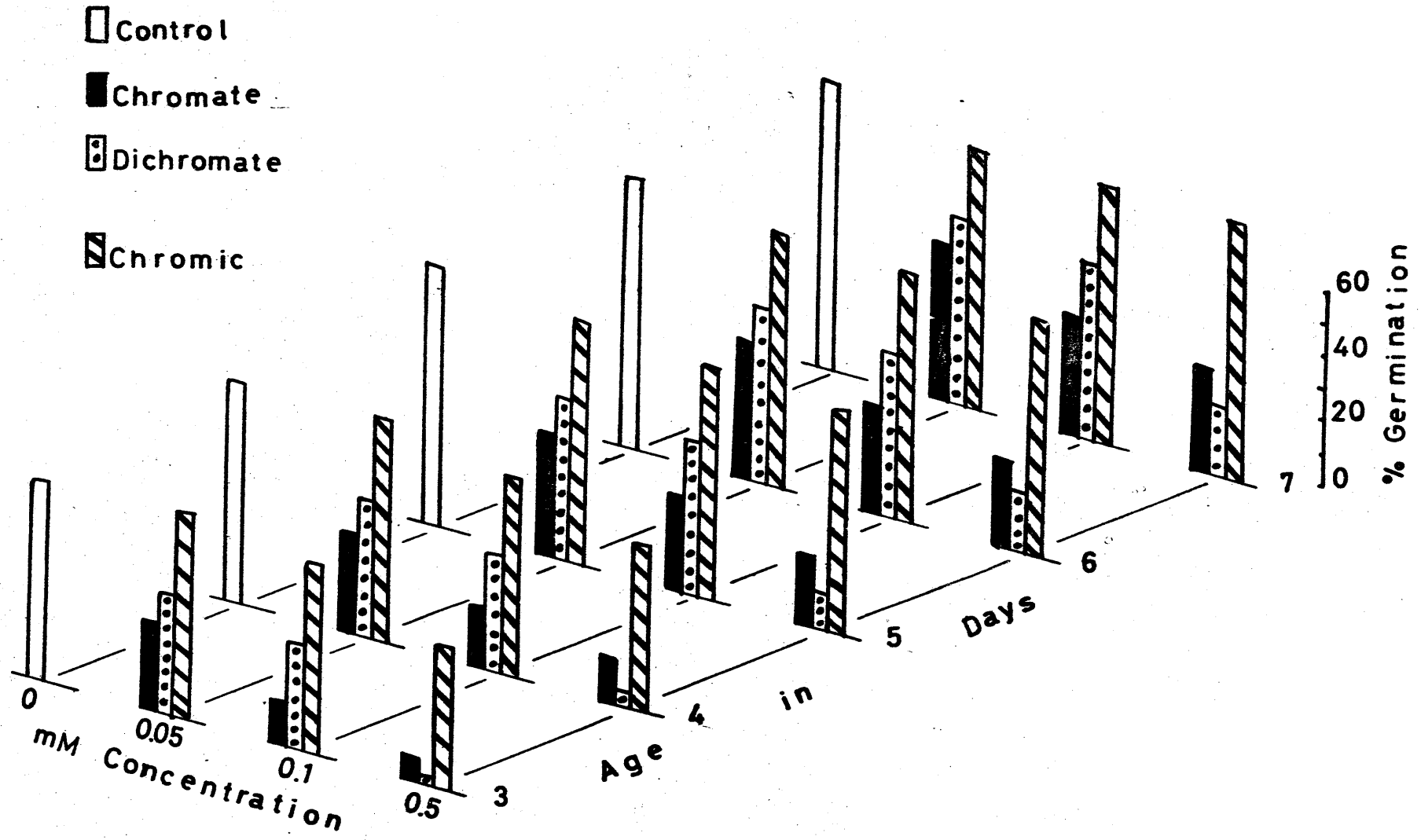


FIG. 1: EFFECT OF VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON THE PERCENTAGE GERMINATION OF DATURA STRAMONIUM SEEDS

experiment, with the high dose of chromic ion was almost overcome with progress of time.

Chromate (CrO_4^{--}) was more effective in lowering the number of germinated seeds than dichromate($Cr_2O_7^{--}$). But with lapse of time and increasing the concentration, particularly at 0.5 mM, the number of germinated seeds in the dichromate was far less than that in chromate.

Table 2 and Fig. 2 show that, within the range of experimental error, the root length of all treatments sharply decreased; a phenomenon that was furthered with concentration but partially alleviated with dichromate application; chromic ion exerted the least effect.

Accordingly, in the following experiments, only three concentrations were used as spray (10 mls per plant). Leaf spraying was preferred since several despütes are still at hand, concerning absorption and translocation of various ions via the root. Personal practice by the first supervisor, during his stay in Department of Botany and Microbiology, Technical University, Munich, showed that toxicity of dichromate to leaves was highly and rapidly apparent when supplied via the cut ends of stems rather than through roots of intact seedlings.

Table 2 : Effect of various concentrations of chromium salts on root length of 7-days old seedlings of Datura Stramonium .

(cm length per root of seedling)

No.	0 mM			0.05 mM									0.1 mM									0.5 mM								
				Chromate			Dichromate			Chromic			Chromate			Dichromate			Chromic			Chromate			Dichromate		Chromic		
1	9.4	8.0	7.3	2.0	4.3	4.7	5.4	4.6	5.3	6.8	5.9	6.4	2.5	3.2	2.7	5.6	5.0	3.3	6.8	5.1	3.9	2.9	0.9	0.5	2.8	1.1	5.7	5.0	3.9
2	7.9	9.0	9.7	3.1	4.9	4.1	4.8	5.3	4.7	6.3	6.4	5.9	3.0	0.3	3.0	5.3	3.7	4.2	5.9	6.8	6.7	0.3	1.7	2.1	2.7	1.6	3.6	3.4	4.4
3	9.6	7.6	7.6	4.2	3.6	5.0	4.4	4.4	5.1	6.0	6.3	5.9	0.9	1.7	1.1	3.6	3.9	3.6	6.6	5.9	3.9	0.9	1.5	2.3	0.2	2.1	3.1	3.4	3.7
4	9.8	7.4	8.3	3.4	3.6	4.9	5.1	4.9	3.9	6.2	6.1	6.1	1.3	0.7	2.0	2.9	4.0	5.5	4.8	4.5	5.4	1.1	1.3	2.9	1.4	2.4	5.0	3.6	3.6
5	8.4	9.9	8.8	4.9	2.7	2.1	3.8	4.4	3.9	6.1	6.0	6.3	1.9	3.1	3.0	2.9	5.5	4.1	3.5	4.1	4.8	1.3	2.2	1.4	0.3	1.6	3.8	4.8	3.9
6	7.9	8.2	9.7	5.1	3.3	4.9	3.9	4.1	3.9	6.0	6.0	6.4	3.1	1.3	3.1	5.5	4.4	3.5	3.5	3.8	4.1	2.6	2.4	2.8	1.8	1.9	4.2	5.4	3.3
7	8.4	9.3	8.0	5.1	4.1	3.8	4.6	5.4	3.9	6.1	5.9	5.9	3.8	1.4	1.2	3.9	3.9	5.4	3.5	6.7	3.4	2.3	2.1	1.3	0.4	0.9	5.4	3.7	3.1
8	7.2	7.6	9.1	2.1	3.8	2.7	4.1	5.3	5.3	6.3	6.2	6.4	0.8	1.9	0.4	2.9	5.6	3.7	6.1	4.0	3.1	2.4	1.8	1.9	1.1	1.8	5.2	4.9	4.0
9	9.3	9.1	7.8	3.2	2.8	3.4	4.3	4.7	3.9	6.8	6.0	6.2	2.4	3.4	0.9	4.8	4.7	4.6	5.6	3.9	4.6	1.6	2.7	2.1	2.3		3.7	4.5	3.2
10	3.8	9.4	9.2	4.6	3.3	4.6	5.2	4.2	3.9	6.5	5.9	6.0	2.8	3.5	1.7	4.0	4.4	5.0	4.4	5.2	3.9	1.9	2.5	2.4	1.4		5.1	3.8	4.3
11	7.9	8.7	8.9	2.7	3.6	2.7	4.7	3.8	5.0	5.9	6.6	6.6	3.7	1.4	1.1	2.9	3.9	3.1	4.3	6.4	4.9	1.8			2.6		5.6	4.8	4.6
12	7.6	9.8	7.8	5.3	4.3	2.7	4.3	5.3	4.1	6.6	6.5	6.7	2.7			4.8	4.4	3.7	4.0	6.1	6.3	1.7			2.8		5.2	4.9	4.0
13	7.7	8.4	7.4	3.8			4.0	4.9	3.8	6.3	6.7	6.0	1.6			5.2	3.9	3.1	4.1	3.8	6.8				1.7		4.4	5.5	4.7
14	7.6	7.1	10.0	3.4			4.8	4.1	4.0	6.7	6.4	6.2	1.6			5.5	3.1	4.7	4.7	5.0	5.8						3.2	5.3	4.8
15	9.7	9.2	9.1	3.8			4.4	4.9	4.4	6.1	6.6	6.3	1.9			5.5	5.3	4.6	3.4	4.2	4.3						3.4	4.7	5.0
16	7.8	9.4	9.2	3.8			4.3	4.4	4.2	6.2	6.4	6.7				3.7	4.8	4.0	6.7	5.3	6.0						5.9	5.2	4.3
17	8.5	8.4	7.4	2.3			5.2	5.1	3.9	6.4	6.6	6.1				4.7		3.4	3.1	4.4	4.9						4.1	3.5	3.3
18	6.7	9.0	7.9	4.7			3.8			6.0	6.7	6.1				4.9		6.7	3.9	3.6	6.5						5.1	4.2	3.3
19	7.8	8.5	9.3	2.7			3.8			6.8	6.7	6.4				4.7		3.1	4.1	6.1	6.3						3.5	3.7	4.5
20	6.8	8.0	9.0	3.8			4.8			6.3	6.0	6.1				5.0		3.9	3.3	4.3	5.4						5.3	3.6	5.0
21	7.8	9.6	8.8	2.9			5.0			5.9	6.6	6.2				4.1		4.1	6.3	6.3	4.7						4.1	3.7	3.2
22	9.3	8.9	9.0	2.9			5.0			6.6	6.6	5.9				4.8			3.8	3.7	3.0						5.3	5.0	3.2
23	9.0	9.3	9.1	4.2			3.9			6.3	6.5	6.1							4.6	6.2	6.3						3.3	3.9	5.2
24	8.1	7.7	9.8	2.6						6.7	6.5	6.5							4.9	6.1	4.2						3.6	4.3	4.2
25	8.5	7.7	8.8							6.2	6.6	6.1							5.2	4.7	3.9						5.1		
26	7.5	9.2	6.1							6.1	6.3								6.2								3.7		
27	7.5	9.4	8.1							6.4									4.3								3.1		
28	7.3	9.8	8.9							6.6									6.2								3.5		
29	9.2	6.7								6.1									5.7								4.9		
30	9.6	9.6								6.1									6.7								3.2		
Mean	8.6			3.6			4.5			6.3			2.1			4.3			4.9			1.9			1.6		4.2		

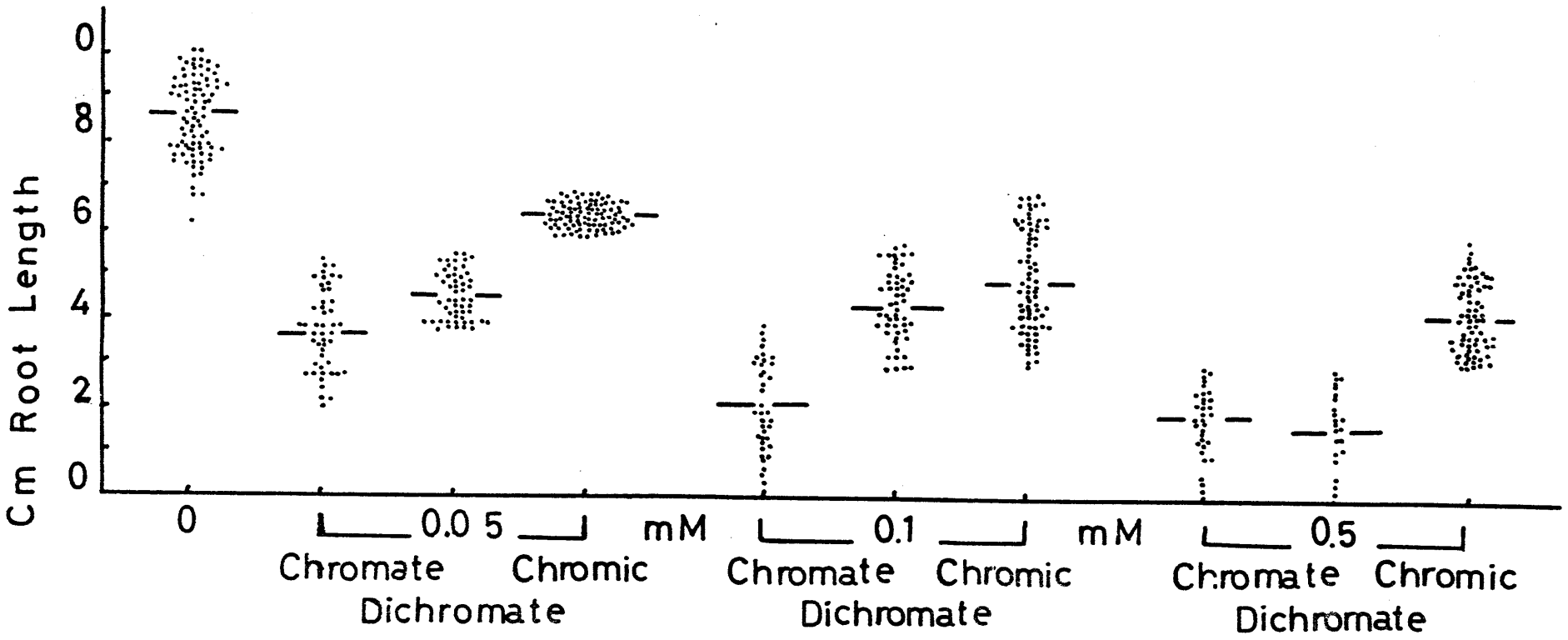


FIG. 2 : EFFECT OF VARIOUS CONCENTRATIONS OF DIFFERENT CHROMIUM IONS ON THE ROOT LENGTH OF 7 DAYS OLD SEEDLINGS OF DATURA STRAMONIUM .

PART III

EFFECT OF WEEKLY SPRAY WITH VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON GROWTH MORPHOLOGY, FLOWERING, FRUITING AND PIGMENTATION OF DATURA STRAMONIUM DURING THE GROWTH SEASON

EFFECT OF WEEKLY SPRAY WITH VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON GROWTH MORPHOLOGY, FLOWERING, FRUITING AND PIGMENTATION OF *DATURA STRAMONIUM* DURING THE GROWTH SEASON

45 days old plants were sprayed, in the early morning, each with 10 mls of 0, 0.05, 0.1 or 0.5 mM of either chromate, dichromate or chromic salts. Plant samples were collected weekly before and/or after the following spray. Morphological criteria of growth as well as pigmentation of leaves were performed on the initial as well as the sprayed plants. Unfortunately, samples of 45 days old plants, kept for pigment analysis, were lost during transport from the field to the labs.

Results

Table 3 and Fig. 3,4,5,6,7 show that growth, during the first 45 days, was very feeble. Within one week, almost all growth criteria were doubled or even more, and they progressively increased with further progress of age.

Growth of Stems and Leaves:

Fig. 3 shows that one spray with $Cr\ III^{+}$ remarkably decreased stem length; a phenomenon that was alleviated

Table 3 : Effect of consecuitive weekly sprays by various concentrations of chromium salts on the growth morphology of Datura Stramonium during the growth season .

(mean of 10 plants, haphazardly collected from the various plots)

Age in Days	Treatment	Salt Concentration mM		Stems			Total Leaves				Mean Leaf		
				Length(cm)	F.wt. (gm.)	D.wt. (gm)	Number	Area (cm^2)	F.wt. (gm.)	D.wt. (gm)	Area (cm^2)	F.wt. (gm)	D.wt.(gm.)
45	Initial	0	0	27.5	14.59	1.256	7	217.6	6.88	0.856	31.2	0.98	0.108
52	One week after 1st spray	0	0	62.3	38.82	3.521	31	1426.1	35.47	4.318	46.0	1.14	0.139
		0.05	Chromate	39.0	20.50	1.350	22	803.2	12.86	1.869	36.5	0.58	0.085
			Dichromate	32.0	17.92	1.800	10	556.2	7.85	0.911	55.6	0.79	0.091
			Chromic	35.5	15.43	1.248	12	367.4	9.11	1.008	30.2	0.76	0.084
		0.1	Chromate	40.7	18.17	1.674	14	442.3	9.64	1.536	31.6	0.69	0.110
			Dichromate	39.2	11.52	1.944	16	630.8	12.47	1.608	39.4	0.78	0.101
			Chromic	48.8	22.92	2.585	19	922.5	19.51	2.119	48.6	1.02	0.112
		0.5	Chromate	55.0	18.85	1.550	24	1017.1	23.95	2.899	42.4	1.00	0.121
			Dichromate	28.5	15.76	1.252	10	383.6	7.57	0.773	38.4	0.76	0.077
			Chromic	71.0	42.37	3.902	50	1431.1	43.64	5.711	28.6	0.87	0.114
59	One week after 2nd spray	0	0	94.6	71.65	6.656	40	2017.5	31.27	6.197	50.4	0.78	0.155
		0.05	Chromate	64.3	46.16	3.347	28	1350.2	28.74	3.553	48.2	1.03	0.127
			Dichromate	43.3	28.05	2.179	12	587.4	7.45	2.232	48.9	0.62	0.186
			Chromic	58.0	26.57	2.953	19	756.1	13.12	3.447	39.8	0.69	0.181
		0.1	Chromate	77.0	39.82	3.916	25	966.5	23.56	3.795	38.7	0.94	0.152
			Dichromate	58.7	35.84	3.156	32	1078.3	23.02	4.387	33.7	0.72	0.137
			Chromic	75.6	41.97	4.889	36	1256.7	31.74	5.789	34.9	0.88	0.161
		0.5	Chromate	77.7	31.73	3.724	25	1077.9	26.82	3.355	43.1	1.07	0.134
			Dichromate	61.0	44.68	4.505	26	1548.8	38.75	5.753	59.6	1.49	0.221
			Chromic	94.3	61.97	6.889	50	1656.7	47.74	6.789	33.1	0.95	0.136
66	One week after 3rd spray	0	0	140.5	150.33	16.199	83	3950.5	100.15	11.555	47.6	1.21	0.139
		0.05	Chromate	77.8	52.45	3.938	37	1424.3	26.59	2.691	38.5	0.73	0.073
			Dichromate	83.8	37.48	3.880	28	963.5	22.82	4.508	34.4	0.82	0.161
			Chromic	77.3	33.10	3.156	22	977.0	24.63	4.037	44.4	1.12	0.184
		0.1	Chromate	89.2	53.02	4.903	30	1041.0	32.12	3.328	34.7	1.07	0.111
			Dichromate	69.0	46.08	3.777	37	997.4	31.50	4.834	27.0	0.85	0.131
			Chromic	80.0	55.05	5.843	46	1338.8	39.37	5.913	29.0	0.86	0.128
		0.5	Chromate	97.3	80.92	8.689	30	1155.6	47.11	6.014	38.5	1.57	0.201
			Dichromate	74.7	67.32	5.921	36	1625.1	41.11	5.407	45.1	1.142	0.150
			Chromic	114.3	73.20	16.229	86	2731.0	80.88	15.460	31.6	0.940	0.180

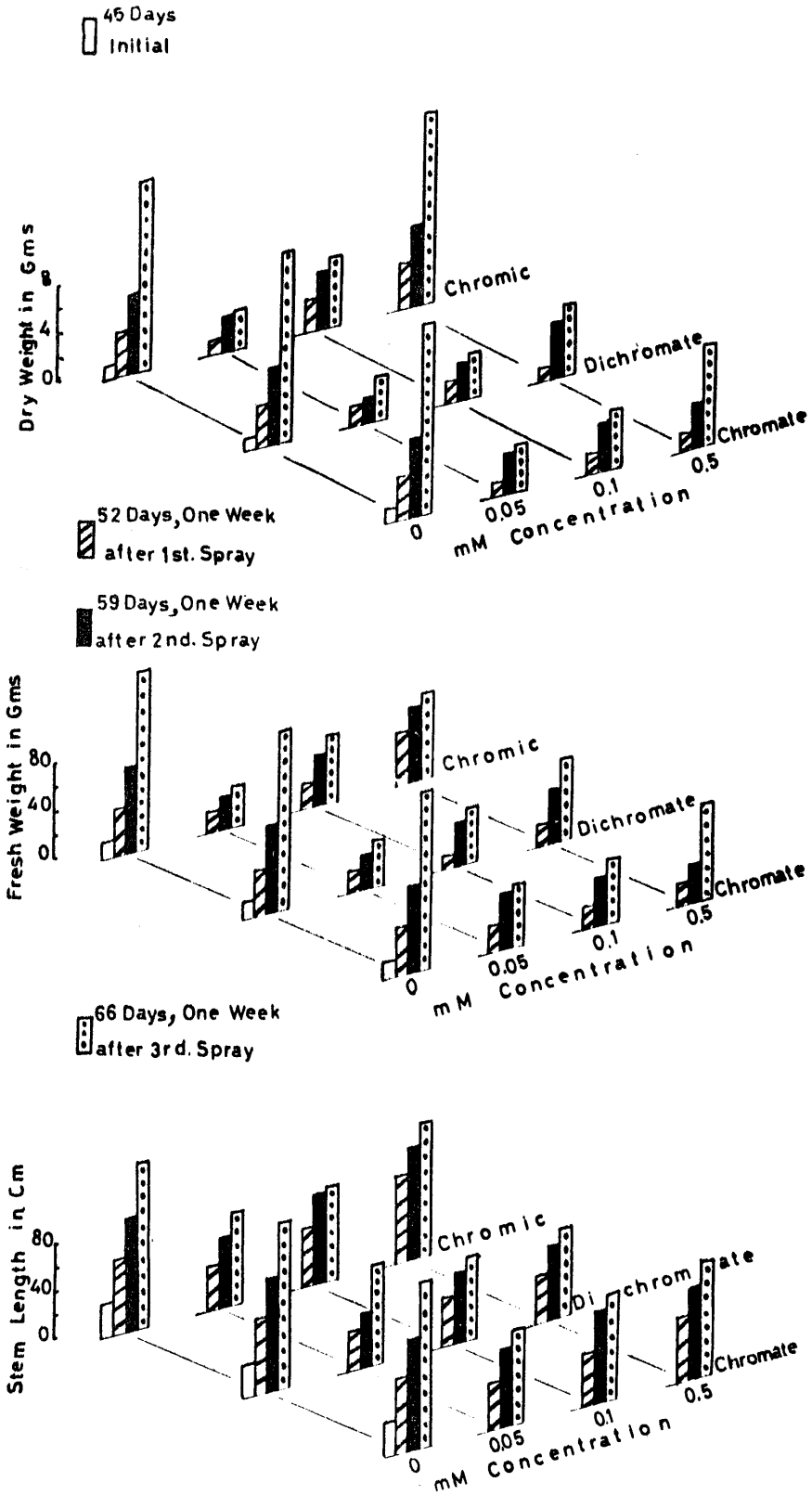


FIG.3 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON STEM LENGTH, FRESH AND DRY WEIGHT OF *DATURA STRAMONIUM* STEMS DURING THE GROWTH SEASON

with increased concentration. Dichromate had almost the some suppressive effect irrespective of concentration whereas the rise of chromate dose, not only counteracted the inhibitory effects but noticeably increased the stem length at 0.5 mM level. These fluctuations were accompanied by a noticeable consistents drop in fresh and dry weight of stems under all treatments, except 0.5 mM chromate that caused minor fluctuations.

Compared with the control, the second spray with $Cr_2O_7^{--}$ highly suppressed stem length and its fresh and dry weight, but rise of concentration partially alleviated this effect. Both chromic and chromate had almost the same lesser inhibitory effects that were persistent with rise of concentration of the latter and alleviated by the former ion, with rise of concentration. The third spray seemed to have the same effects except for the fact that inspite of the very low fresh weight of stems, sprayed with 0.5 mM chromic yet their dry weight was almost similar to the control.

Fig. 4 also demonstrates the fact that the first spray with dichromate was more effective than chromate in reducing the number of leaves, a phenomenon that was unaffected by rise of concentration. The remarkable

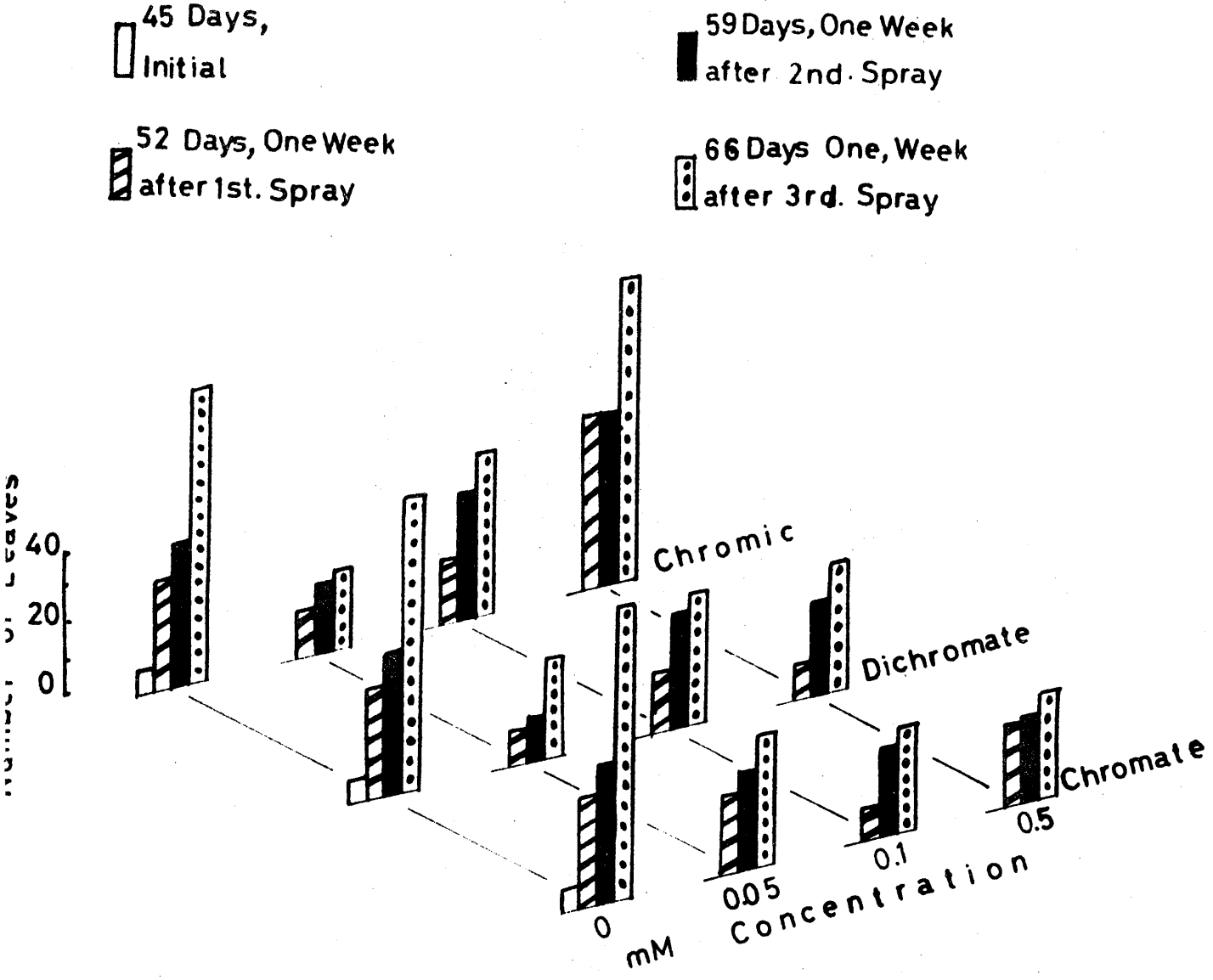


FIG. 4: EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON NUMBER OF LEAVES OF DATURA STRAMONIUM DURING THE GROWTH SEASON

decrease in leaf number by chromic ion was reversed by raising the concentration to 0.5 mM. The second spray was still suppressive to leaf number of all treated plants. Compared with the first spray, 0.5 mM chromic ion apparently did not favour the formation of new leaves during this period. On the other hand, with the third spray, the number of leaves of all treated plants was still lagging far bebyond the control plants, with minor differences between treatments except for 0.5 mM chromic that had almost the same number of leaves as the controls.

Figs. 5,6,7 illustrate the fact that, within one week the mean area, fresh and dry weight of control leaves appreciably increased then remained almost constant till the end of the experimental period. On the other hand, the total area, fresh and dry weight of the same leaves remarkably and progressively increased with age. One spray with dichromate caused a noticeable drop in leaf area that was furthered with rise of concentration to 0-5 mM. This reflected itself on fresh and dry weight of total leaves. The mean leaf area of such treatment was apparently higher but dropped with concentration whereas the mean fresh or dry weight of

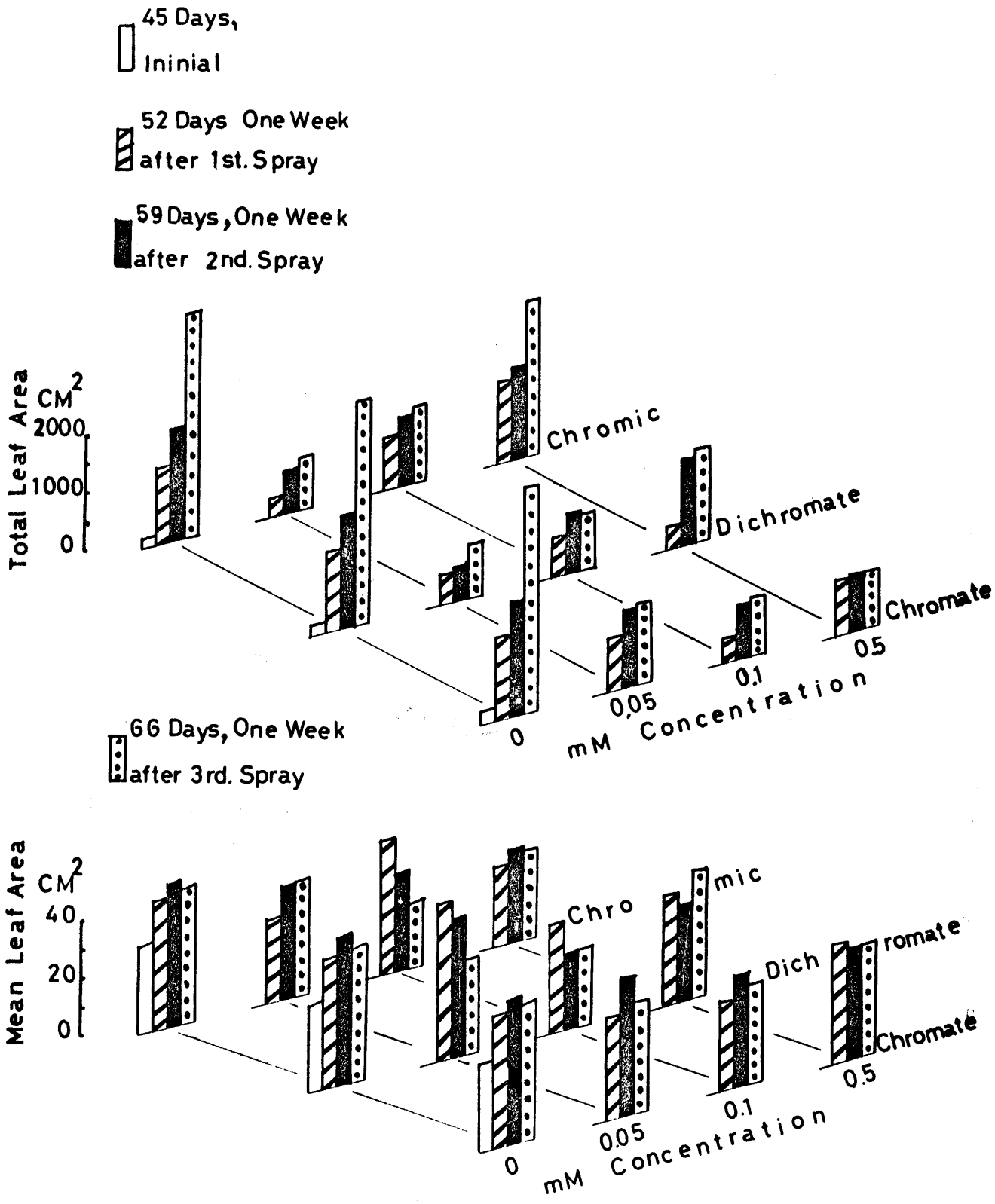


FIG. 5 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON LEAF AREA OF DATURA STRAMONIUM DURING THE GROWTH SEASON

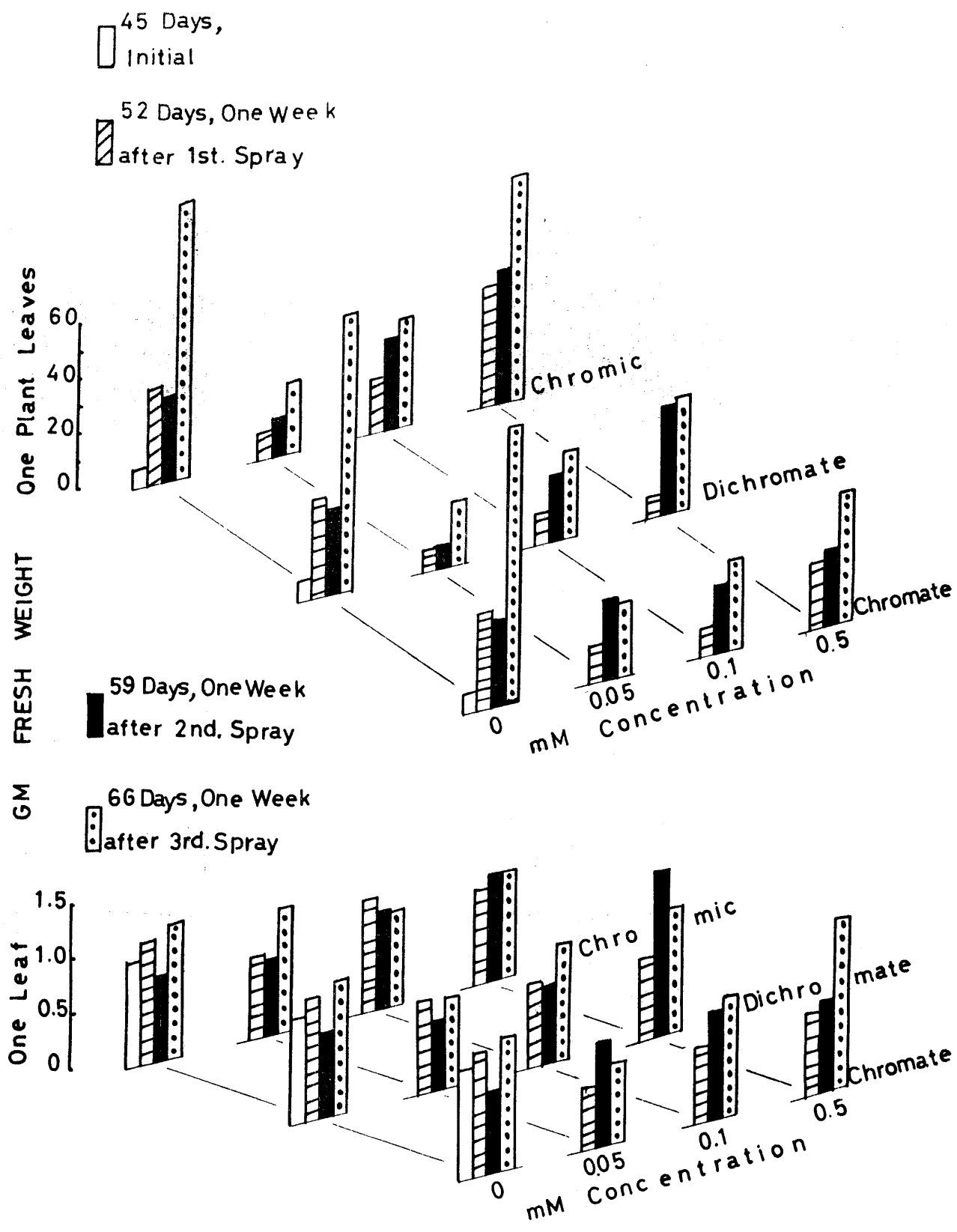


FIG. 6 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON FRESH WEIGHT OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON

leaves remained at a consistent low level irrespective of concentration. The mild inhibitory effects of chromate ion on total area, fresh and dry weight of leaves, though were furthered by rise of concentration to 0.1 mM yet the high concentration partially counteracted such effect. On the other hand, the severe drop in total area, fresh and dry weight of leaves by chromic ion treatment were totally alleviated at 0.5 mM with appreciable increase in fresh and dry weight. The mean area, fresh and dry weight of chromate-treated plants followed the sequence of their totals but chromic treatment was suppressive to such criteria;particularly at the least and highest concentrations.

Compared with the controls, the second spray was highly suppressive to total leaf area of all treatments but the high dose of dichromate or chromic partially alleviated such effect. On the other hand, the fluctuations in the mean leaf area were minor with chromate or dichromate spraying at 0.05 or 0.5 mM level but chromic ion remarkably lowered the mean leaf area. The total fresh weight was slightly lowered by chromate treatment whereas the total dry wieght severely dropped to a constant level irrespective of concentration. On

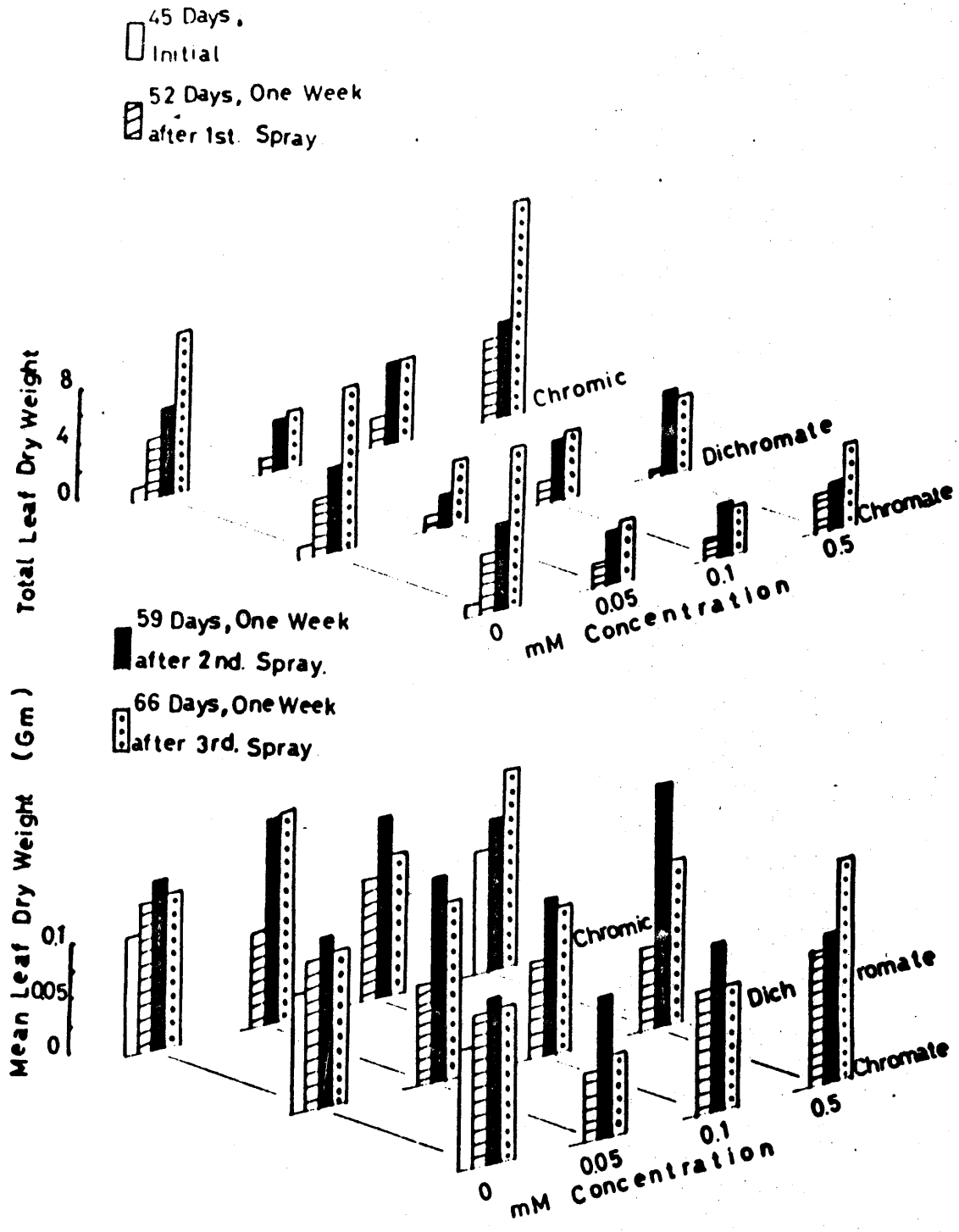


FIG. 7 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON THE LEAF DRY WEIGHT OF DATURA STRAMONIUM DURING THE GROWTH SEASON

the other hand, the appreciable drop in fresh and dry weight following dichromate or chromic treatment was alleviated or reversed (under the latter condition) with rise of concentration. The mean fresh weight was almost unaffected by chromate but severely dropped by the other treatments. The mean dry weight of leaves, mostly, dropped by all treatments except dichromate that showed a remarkable increase in mean fresh and dry weight of leaves at 0.5 mM.

The third spray was still suppressive to mean area, fresh and dry weight of leaves except at 0.5 mM where a noticeable rise in dry weight was highly apparent; more prominently with chromate. The same applies to the total leaf area but the rise in chromate level furthered whereas that of dichromate or chromic partially alleviated such effect on total leaf area. Such inhibitory effects on total fresh and dry weight of leaves were also partially alleviated with rise of concentration of the chemicals except chromic that induced a noticeable increase in total dry weight of leaves.

Flowers Fruits and Seeds:

Table 4 and Fig. 8 show that one spray with either chromium salts severely reduced the number of flowers;

Table 4 : Effect of consecuitive weekly sprays by various concentrations of chromium salts on flowering and fruiting of Datura Stramonium during the growth season.

Age in Days	Treatment	Salt concentration m.M.		No.of flowers	Total fruits (gm.)		100 seeds (mgm.)	
					F. wt.	D. wt.	Air dry	oven dry
		0 0		12	10.01	0.973	-	-
52	One week after 1st spray	0.05	Chromate	9	1.09	0.144	-	-
			Dichromate	4	-	-	-	-
			Chromic	1	-	-	-	-
		0.1	Chromate	5	-	-	-	-
			Dichromate	7	-	-	-	-
			Chromic	8	2.04	0.224	-	-
		0.5	Chromate	12	1.06	0.132	-	-
			Dichromate	4	00	00	-	-
			Chromic	23	5.94	1.106		
		0 0		15	21.32	2.284	-	-
59	One week after 2nd spray	0.05	Chromate	16	16.19	2.181	-	-
			Dichromate	5	4.93	0.556	-	-
			Chromic	7	16.31	1.516	-	-
		0.1	Chromate	12	11.56	0.913	-	-
			Dichromate	16	13.13	1.447	-	-
			Chromic	26	19.91	2.23	-	-
		0.5	Chromate	13	15.86	1.452	-	-
			Dichromate	18	17.76	2.192	-	-
			Chromic	20	19.75	2.576	-	-
		0 0		35	63.36	10.559	601.9	545.6
66	One week after 3rd spray	0.05	Chromate	11	19.98	2.803	616.8	573.8
			Dichromate	14	10.53	1.533	597.8	549.1
			Chromic	12	8.10	1.535	589.8	532.8
		0.1	Chromate	14	20.614	2.357	614.3	567.3
			Dichromate	12	19.92	2.285	608.4	559.8
			Chromic	15	21.61	3.927	593.8	547.1
		0.5	Chromat	14	37.85	4.822	588.3	545.7
			Dichromate	18	27.60	4.329	600.1	549.9
			Chromic	38	42.42	8.381	563.8	516.4

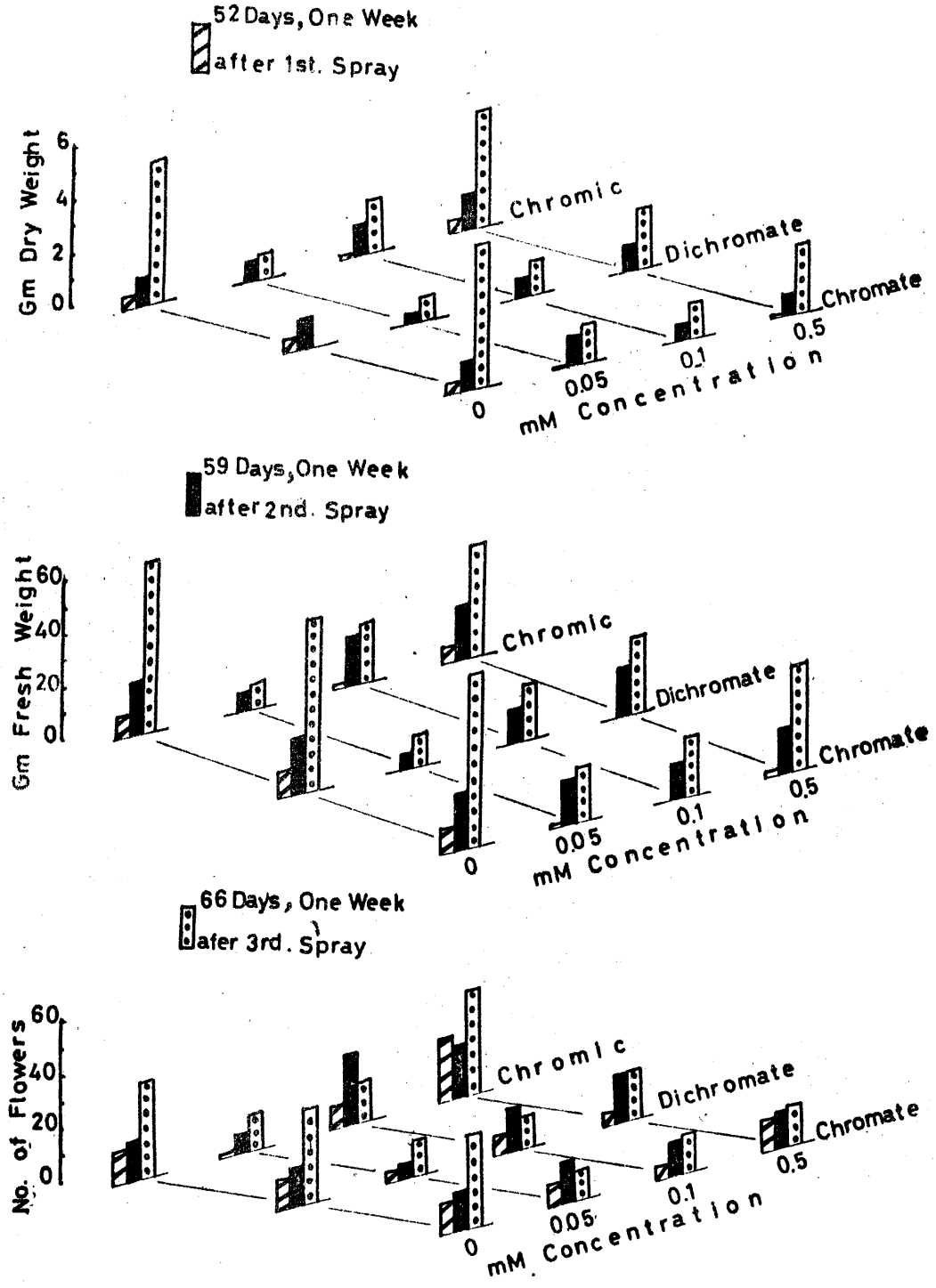


FIG. 8 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON NUMBER OF FLOWERS, FRESH AND DRY WEIGHT OF DEVELOPING FRUITS OF DATURA STRAMONIUM DURING THE GROWTH SEASON .

a phenomenon that continued with increased concentration of either chromate or dichromate but was reversed at 0.5 mM chromic. With the second spray still dichromate and chromic reduced the number of flowers but the rise of concentration of the former abolished while the increased concentration of the latter reversed such effect.

The second spray with chromate seemed with little or no effect on the number of flowers. Compared with the first spary, 0.5 mM chromic slightly decreased the number of flowers. The third spray caused a sharp decline in flower number but 0.5 mM chromic that seemed without appreciable effect.

The first spray also reduced the number and weight of young fruits. Dichromate-treated plants did not bear any fruits whereas 0.5 mM chromic produced larger fresh and dry weight of fruits compared with chromate. The second spray with dichromate still induced a severe drop in fresh and dry weight of green fruits, a phenomenon that was gradually abolished with increased concentration.

Chromate and chromic were less effective but the high level of the latter, not only abolished such deleterious effects but also slightly increased the dry weight of fruits. The third spray severely attenuated the fresh and dry weight of the formed fruits though chromic was least effective particularly at 0.5 mM.

Since the number of dry capsules and number of seeds per capsule were liable to erroneous determination we only compared the air and oven dry weight of the produced seeds. Table 4 showed that such criteria per 100 seeds were slightly, if at all, affected by spraying once or several times with the various concentrations of the chromium ions.

Pigmentation of Leaves:

Table 5 and Fig. 9 show that on fresh weight basis, one or several sprays with either chromium salts at 0.05 mM increased chlorophyll a and b with a concomitant drop in carotenoids. Further rise in concentation abolished the effects on chlorophyll a or carotenoids but only attenuated those on chlorophyll b. The stimulatory effect of dichromate or chromic lasted longer with increased concentration to 0.1 mM, though at lower or higher concentrations, the three ions had almost the same effect.

Table 5 : Effect of consecuitive weekly sprays by various concentrations of chromium salts on pigmentation of Datura Stramonium leaves during the growth season .

(mean , in mgm , of haphazard samples collected from 10 plants from each plot)

Age in Days	Treatment	Salt concentration mM		Chlorophyll a per				Chlorophyll b per				Carotens per			
				1 gm F.wt.	Leaf area	100 cm^2	1 gm D.wt.	1 gm F.wt.	leaf area	100 cm^2	1 gm D.wr.	1 gm F.wt.	leaf area	100 cm^2	1 gm D.wt.
45	Initial	0 0													
52		0 0		3.59	4.11	8.94	29.51	0.82	0.93	2.03	6.70	0.93	1.07	2.32	7.66
	One week after the 1st spray	0.05	Chromate	7.07	4.13	11.32	48.60	3.59	2.10	5.75	24.70	0.11	0.07	0.18	0.79
			Dichromate	6.90	5.42	9.74	59.48	2.58	2.03	3.65	22.26	0.23	0.18	0.33	1.99
			Chromic	6.88	5.23	17.31	62.24	2.17	1.65	5.46	19.63	0.33	0.25	0.83	2.99
		0.1	Chromate	3.94	2.71	8.57	24.70	3.96	2.73	8.63	24.87	0.56	0.39	1.22	3.51
			Dichromate	3.57	2.78	7.05	27.65	3.52	2.74	6.95	27.26	0.63	0.49	1.24	4.85
			Chromic	3.56	3.66	7.54	32.81	2.62	2.69	5.54	24.14	0.73	0.74	1.53	6.68
		0.5	Chromate	3.66	3.65	8.61	30.22	2.70	2.70	6.36	22.31	0.76	0.76	1.79	6.27
			Dichromate	3.12	2.37	6.17	30.78	1.90	1.44	3.76	18.75	1.02	0.78	2.02	10.06
			Chromic	3.18	2.78	9.68	24.26	1.40	1.24	4.28	10.73	1.09	0.95	3.32	8.31
59		0 0		4.45	3.47	6.89	22.29	1.96	1.53	3.03	9.86	0.67	0.52	1.04	3.35
	One week after the 2nd spray	0.05	Chromate	8.24	8.49	17.60	66.83	5.63	5.80	12.02	45.64	0.42	0.43	0.89	3.39
			Dichromate	8.25	5.12	10.40	27.50	4.01	2.48	5.07	13.33	0.28	0.17	0.42	0.93
			Chromic	7.69	5.31	13.35	29.28	2.74	1.89	4.76	10.44	0.31	0.22	0.54	1.19
		0.1	Chromate	4.16	3.92	10.13	25.77	4.47	4.21	10.89	27.71	0.57	0.54	1.40	3.55
			Dichromate	7.29	5.25	15.57	38.31	3.20	2.30	6.84	16.81	0.17	0.13	0.36	0.92
			Chromic	6.48	5.70	16.34	35.40	2.64	2.32	6.65	14.41	0.29	0.26	0.73	1.58
		0.5	Chromate	4.60	4.92	11.42	36.70	3.78	4.04	9.38	36.10	0.78	0.83	1.94	6.22
			Dichromate	3.93	5.85	9.83	26.47	3.37	3.62	8.43	22.68	0.72	0.78	1.31	4.88
			Chromic	4.23	4.02	12.14	29.54	2.34	2.23	6.74	16.41	1.08	1.03	3.09	7.57
66		0 0		4.69	5.66	11.89	40.67	2.88	3.48	7.30	24.97	0.71	0.85	1.80	6.14
	One week after the 3rd spray	0.05	Chromate	8.48	6.09	24.39	83.81	6.61	4.75	19.00	65.30	0.51	0.37	1.48	5.09
			Dichromate	8.86	7.22	20.99	44.87	4.78	3.89	11.31	24.18	0.07	0.06	0.16	0.35
			Chromic	8.20	9.18	20.67	50.03	4.45	4.88	11.22	27.14	0.08	0.09	0.20	0.49
		0.1	Chromate	4.53	3.34	9.63	30.11	4.92	3.63	10.45	32.70	0.61	0.45	1.30	4.06
			Dichromate	8.21	6.98	25.85	53.27	4.38	3.72	13.78	28.39	0.13	0.11	0.41	0.85
			Chromic	7.25	6.24	21.50	48.71	3.52	3.03	10.44	23.67	0.09	0.08	0.27	0.63
		0.5	Chromate	4.23	6.65	17.25	33.15	4.44	6.97	18.10	34.78	0.61	0.95	2.48	4.76
			Dichromate	4.36	3.77	11.08	25.10	4.21	3.52	10.35	23.45	0.56	0.48	1.41	3.20
			Chromic	4.56	4.29	13.51	23.87	4.01	3.77	11.87	20.97	0.96	0.90	2.84	5.01

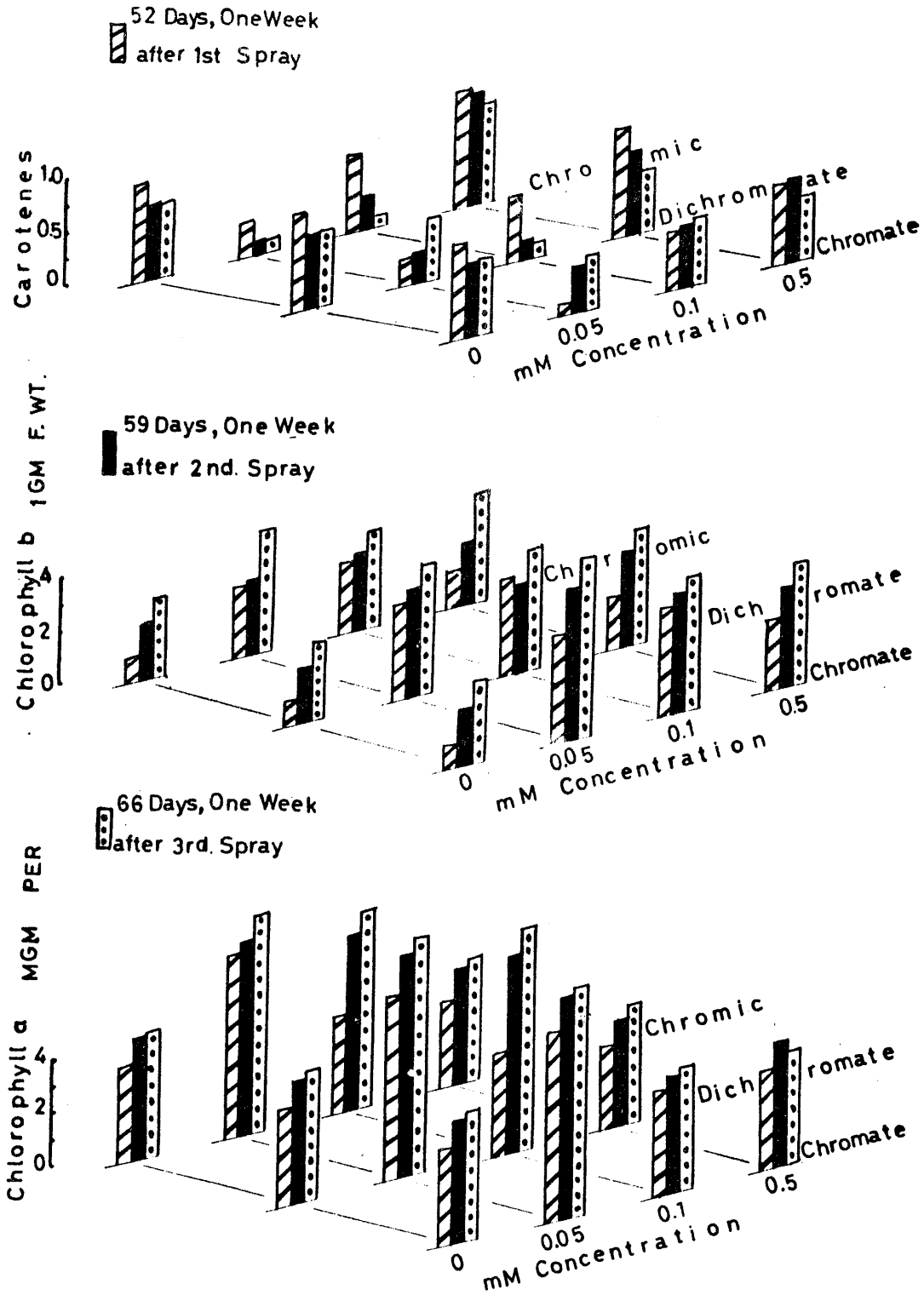


FIG. 9 : EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON PIGMENTATION OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON

Chromate showed the maximum stimulatory effects on chlorophyll b and maximum inhibitory effects on carotenoids.

Based on mean leaf area (Fig. 10), though one spray with low chromate was without effect while with the other salts, it was stimulatory to chlorophyll a content, yet the rise of concentration of either salts was inhibitory. Chlorophyll b also increased but was furthered with rise of concentration to 0.1 mM followed by an appreciable decline particularly with dichromate and chronic. On the contrary, the leaves were almost depleted of their carotenoids but the rise of concentration of the salts almost caused the regain of carotenoids to almost the control level particularly with the chromic ion. The second and third spray were highly stimulatory to chlorophyll a or b accumulation but still inhibitory to carotenoid accumulation. Both phenomena were attenuated with further rise of concentration to 0.5 mM.

Based on unit leaf area (Fig. 11), one spray induced a remarkable increase in chlorophyll a and b;

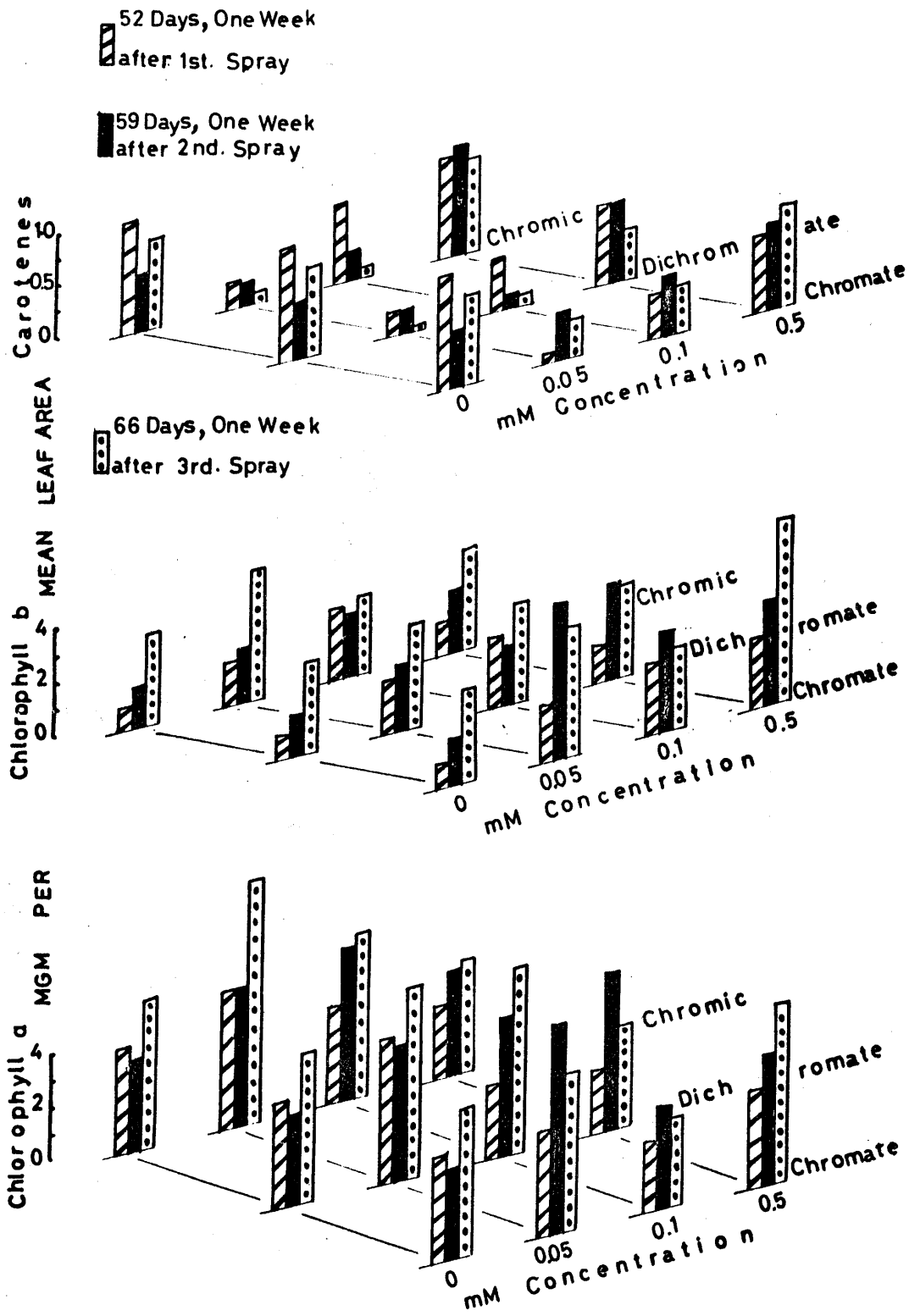


FIG.10: EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON PIGMENTATION OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON

more prominently with chromic ion, accompanied by a severe drop in carotenoids; more prominently with chromate ion. Further rise in concentration slightly attenuated the stimulatory effects on chlorophyll a or b but partially alleviated the inhibitory effect on carotenoids or stimulated its accumulation at 0.5 mM chromic. The same trends were also observed with the second or third spray with chromate showing the highest chlorophyll a and b at 0.5 mM concentration.

On dry weight basis (Fig. 12), one spray with 0.05 mM of either salts remarkably increased chlorophyll a and b and decreased carotenoid contents. Chromate provoked maximum chlorophyll b and least chlorophyll a and carotenoids whereas chromic induced the highest chlorophyll a or carotenoids and least chlorophyll b. Further rise of concentration abolished such effects.

The second or third spray with 0.05 mM chromate remarkably increased chlorophyll a and b but was almost without effect on the carotenoids. The other salts were less effective on chlorophyll a or b but severely attenuated carotenoid accumulation. Further rise of concentration attenuated the stimulatory effects of chromate on chlorophyll a or b but had little or slight

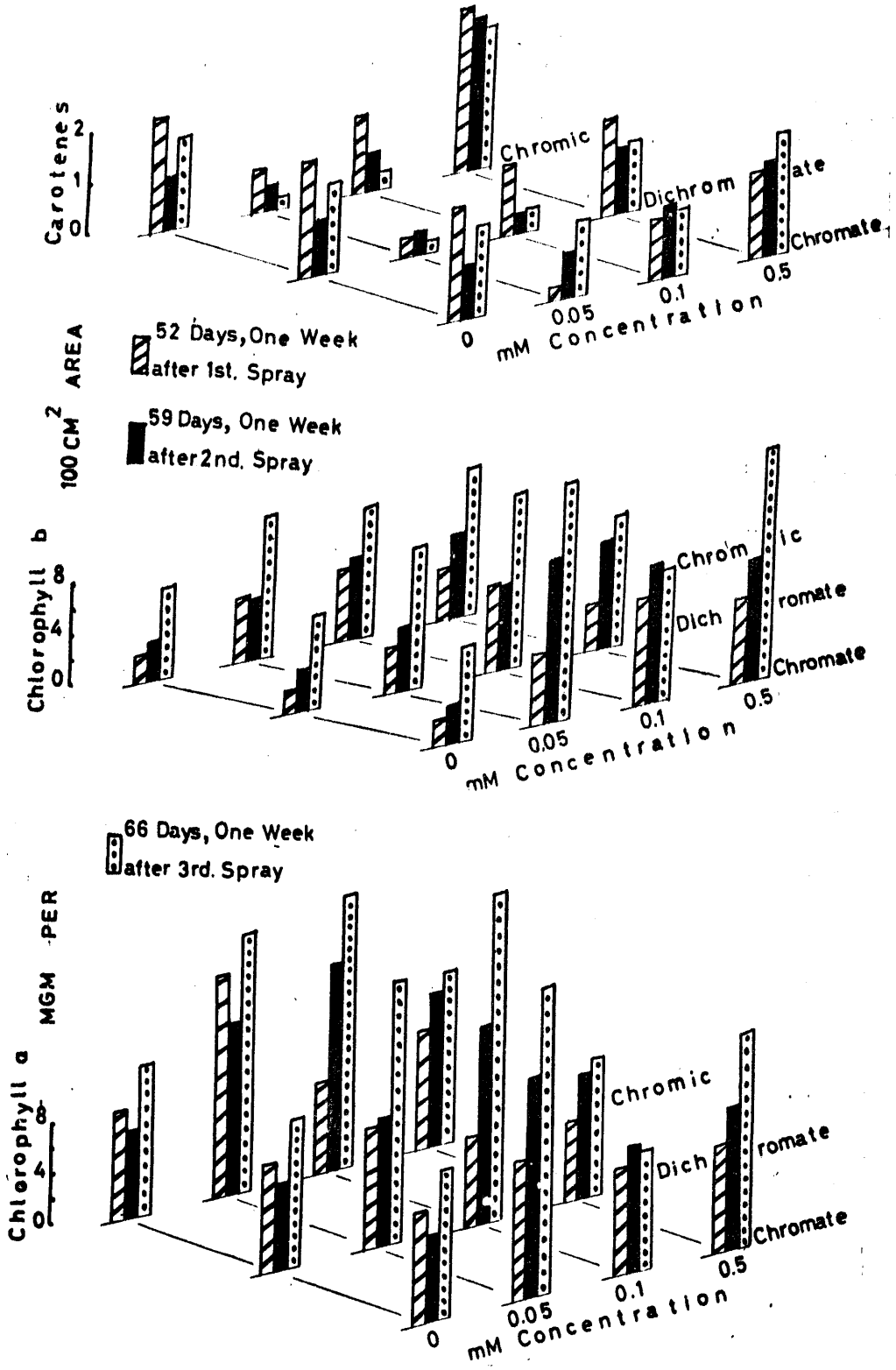


FIG. 11 EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON PIGMENTION OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON.

inhibitory affect on carotenoids. The high doses of the other salts slightly, if at all affected the original response of the pigments except at 0.5 mM where chlorophylla severely dropped.

The second spray with 0.5 mM of these two salts inhibited carotenoid formation whereas the third spray partially alleviated their inhibitory effects on such pigments.

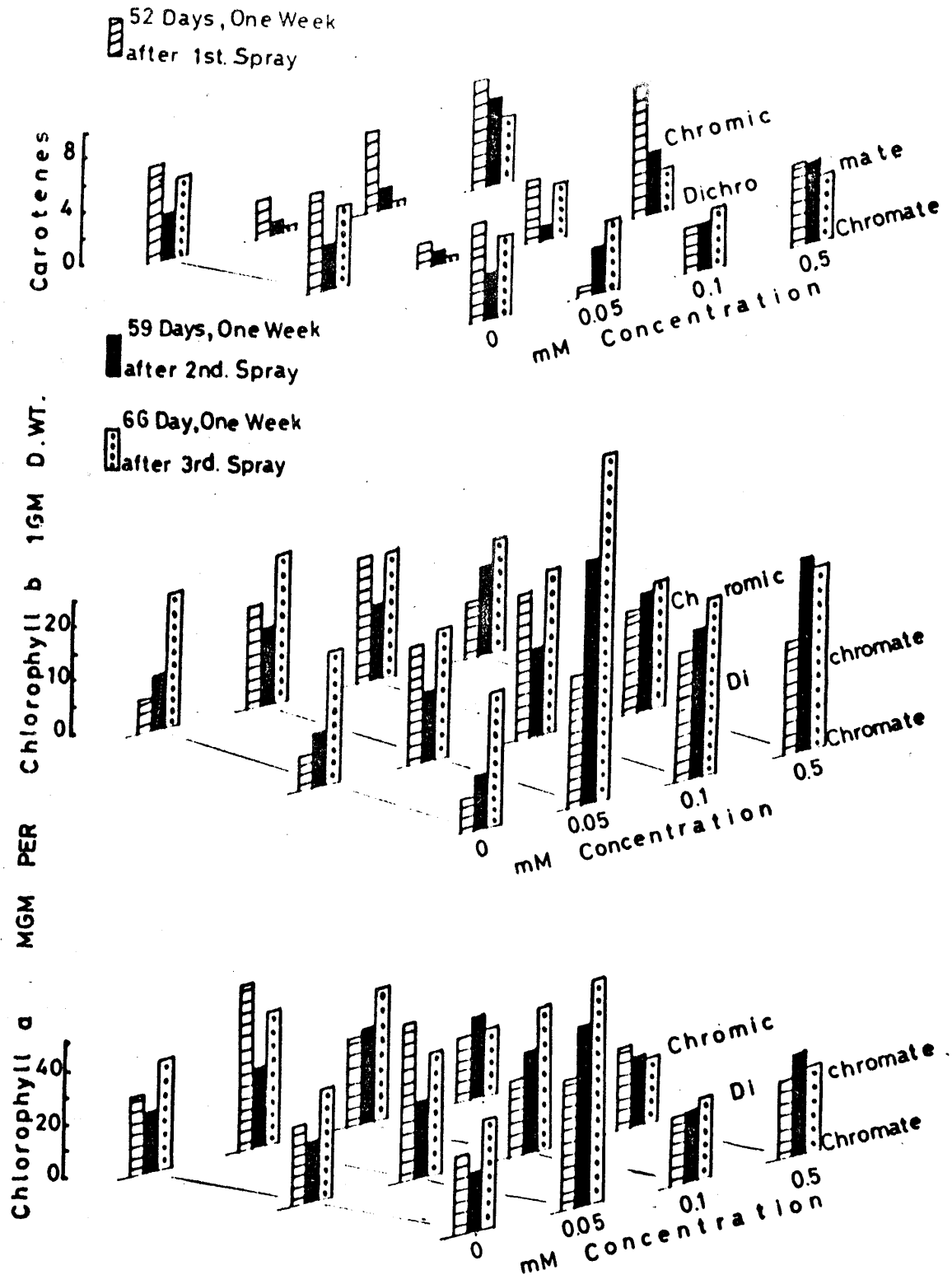


FIG.12: EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON PIGMENTATION OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON

PART IV

EFFECT OF WEEKLY SPRAY WITH VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON THE ALKALOID CONTENT OF DATURA STRAMONIUM

EFFECT OF WEEKLY SPRAY WITH VARIOUS CONCENTRATIONS OF CHROMIUM SALTS ON THE ALKALOID CONTENT OF *DATURA STRAMONIUM* LEAVES DURING THE GROWTH SEASON

Samples from the various treatments were dried, in the shade, till constant weight. The leaves were finely ground, extracted then quantitatively assayed for their alkaloid content using the gas liquid chromatographic methods. The accuracy and fineness of the resolution of the various alkaloid components is apparent from Fig. 13 representing the alkaloid picture of 66 days old control sample .

Results

Total Alkaloids:

Table 6 and Fig. 14 show that the first spray with 0.05 mM chromium salts very severely suppressed the total alkaloid content of the growing plant; a phenomenon that was partially alleviated with rise of concentration of chromate, to a lesser extent with dichromate but remarkably increased at 0.5 mM chromic. The second spray was less deleterious but further increase of chromate suppressed whereas that of dichromate and chromate favoured alkaloid accumulation;

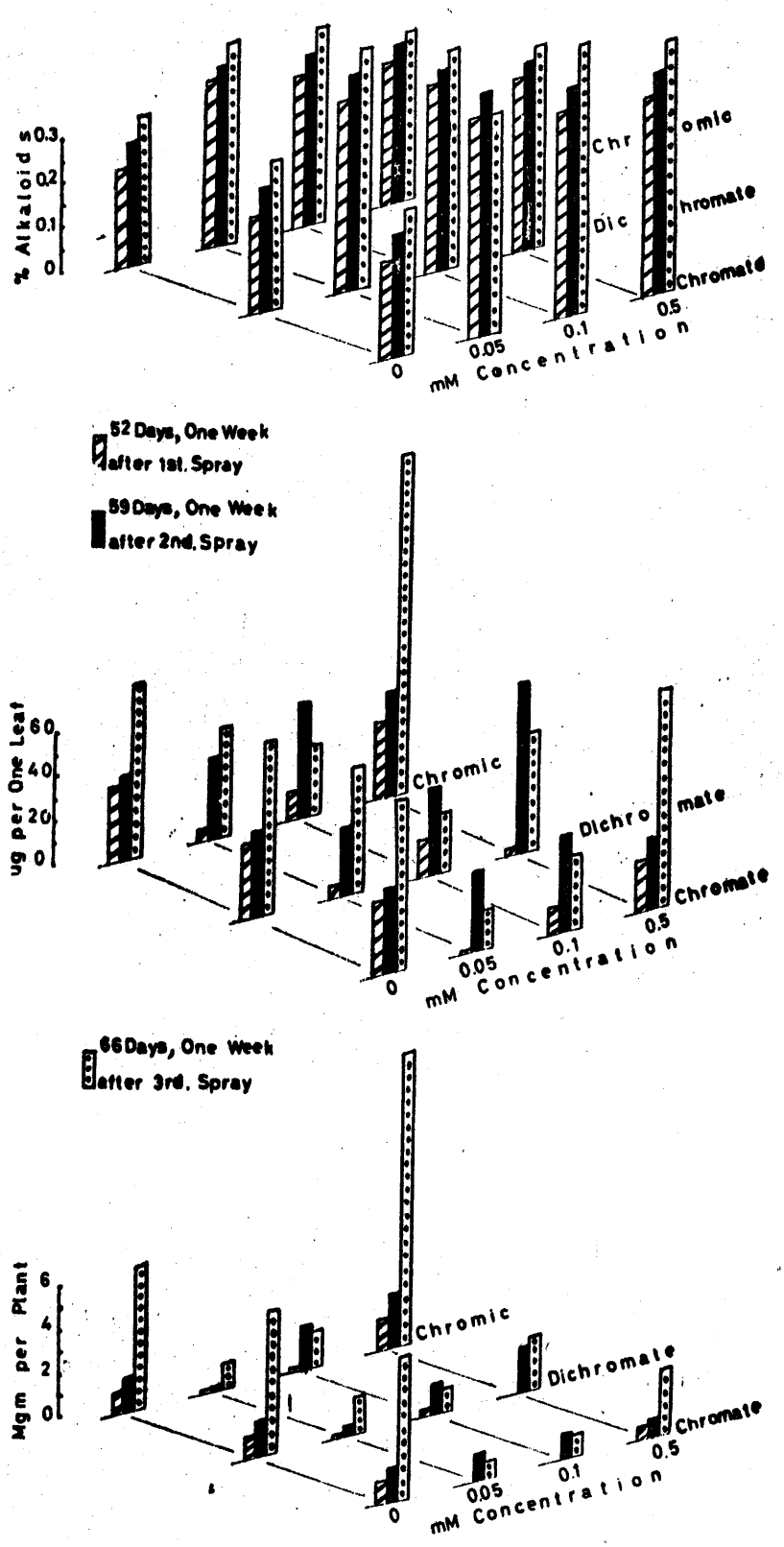


FIG.13: EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON THE TOTAL ALKALOID YIELD BY DATURA STRAMONIUM DURING THE GROWTH SEASON

more prominently by the latter ion. The third spray was suppressive but the increased concentration of the ions did not alleviate such effect except 0.5 mM chromic that almost doubled the alkaloid content of the plant.

On the basis of one plant leaf (Fig. 14), one spray with 0.05 chromate or chromic was severely suppressive to alkaloid accumulation but was slightly alleviated with rise of concentration, under the former, and completely abolished, under the latter treatment. Inhibition by dichromate was partially alleviated at 0.1 mM followed by another decline with further rise.

The second spray with chromate or chromic seemed without effect but the rise of chromic ion concentration slightly raised the total alkaloid content. On the other hand, dichromate slightly reduced the alkaloid content but the higher doses were slightly stimulatory. The third spray severely attenuated the total alkaloid content but 0.5 mM chromate or chromic initiated a rapid increase in alkaloids; more prominently with the latter ion.

Table : 6 Effect of consecuitive weekly sprays with different concentrations of various chromium salts on yield of total alkaloids from Datura Stramonium during the growth season.

Age in Days	Treatment	Salt concentration m.M		Leaves of one plant mgm	One plant leaf ug	Percentage
52	One week after 1st spray	0 0		1.031	33.0	0.232
		0.05	Chromate	0.025	1.1	0.505
			Dichromate	0.052	5.2	0.439
			Chromic	0.056	4.6	0.383
		0.1	Chromate	0.160	11.4	0.470
			Dichromate	0.253	15.8	0.431
			Chromic	0.221	11.6	0.344
		0.5	Chromate	0.560	23.3	0.463
			Dichromate	0.034	3.4	0.404
			Chromic	1.762	35.2	0.329
59	One week after 2nd spray	0 0		1.539	38.0	0.278
		0.05	Chromate	1.012	36.1	0.555
			Dichromate	0.351	29.3	0.491
			Chromic	0.069	36.4	0.404
		0.1	Chromate	1.080	43.2	0.520
			Dichromate	1.259	39.3	0.456
			Chromic	1.869	51.9	0.387
		0.5	Chromate	0.802	32.1	0.497
			Dichromate	2.009	77.3	0.422
			Chromic	2.404	48.1	0.367

Table: 6 (Cont.)

Age in Days	Treatment	Salt concentration m.M		Leaves of one plant mgm	One plant leaf ug	Percentage
		0 0		6.478	78.0	0.337
66	One week after 3rd spray	0.05	Chromate	0.669	18.0	0.642
			Dichromate	1.602	57.2	0.548
			Chromic	1.076	48.9	0.459
		0.1	Chromate	0.979	32.6	0.613
			Dichromate	0.981	26.5	0.497
			Chromic	1.455	31.6	0.436
		0.5	Chromate	2.988	99.6	0.573
			Dichromate	1.928	53.6	0.458
			Chromic	13.344	155.2	0.388

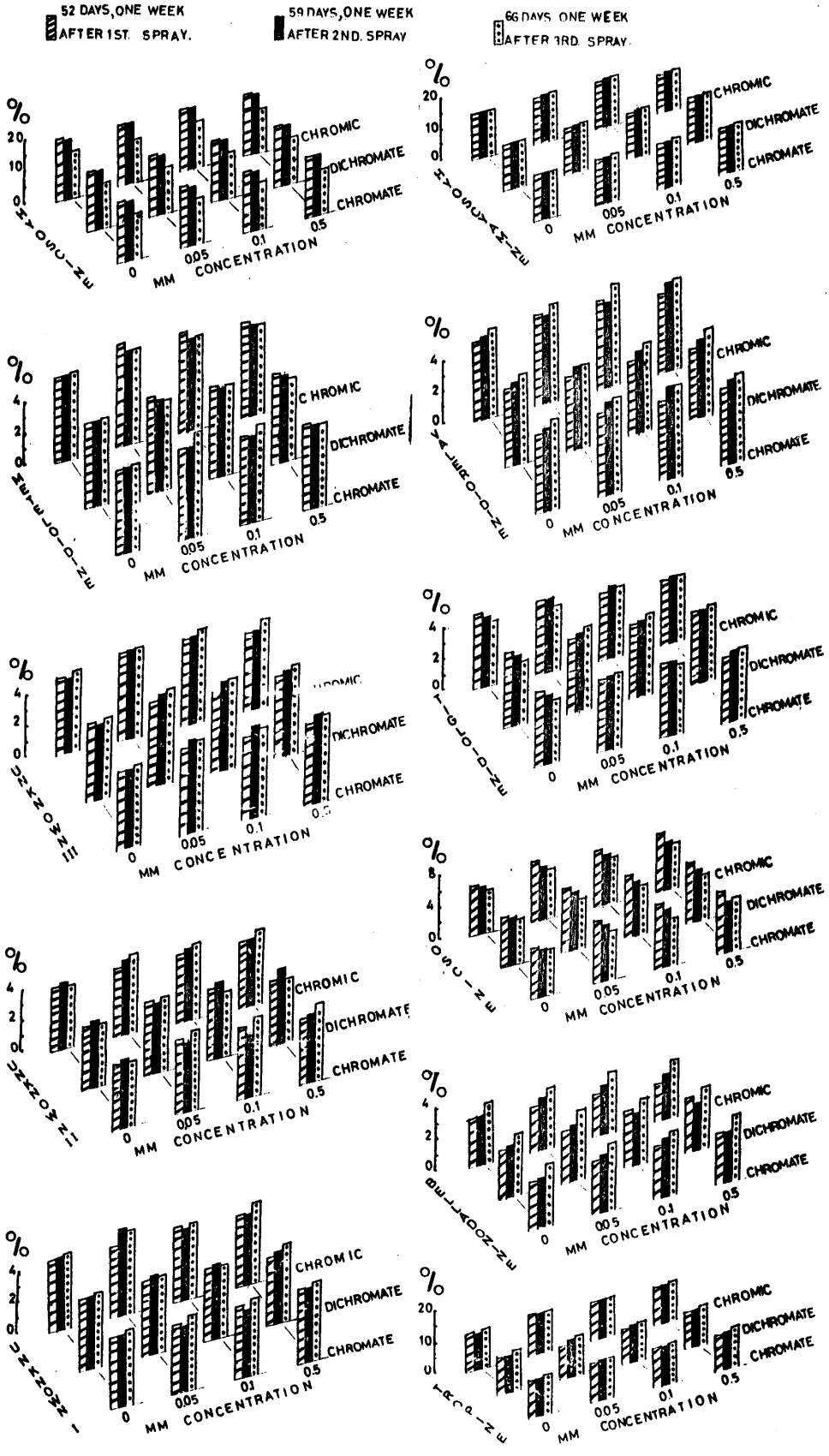


FIG 15 EFFECT OF WEEKLY SPRAY WITH DIFFERENT CONCENTRATIONS OF CHROMIUM IONS ON THE PERCENTAGE COMPONENT ALKALOIDS OF TOTAL ALKALOID CONTENT OF DATURA STRAMONIUM LEAVES DURING THE GROWTH SEASON

Irrespective of these fluctuations, the percentage alkaloid remarkably increased following chromium ions application; more prominently by chromate and to a lesser extent by chromic ion. Such stimulation was slightly attenuated by increased dose of each individual ions and furthered with increased number of sprays.

Alkaloid Components:

Table 7 and Fig. 15 record the percentage of the most important known and unidentified components of the total alkaloid content of the various samples. It appears that neither tropine, unknown I, belladonine nor tigloidine percentage was affected by the various treatments. The first spray by all chromium salts were highly initiative to oscine accumulation; a phenomenon that continued with the second and third spray with either chromate or dichromate but gradually faded or even abolished following the second and third spray with chromic particularly at its high concentration. Increased percentage of unknown II was highly apparent following the third spray with chromate or chromic. Similar effects were observed for unknown III following dichromate or chromic application though chromate was stimulatory following its second application.

Table 7 : Effect of consecuitive weekly sprays of different concentrations of chromium salts on percentage of the various alkaloids component of Datura Stramonium leaves during the growth season.

R.T. (Oscine)	Age / alkaloid	52 Days										59 Days								
		0 0	0.05 mM			0.1 mM			0.5 mM			0 0	0.05 mM			0.1 mM			0.5 mM	
			a	b	c	a	b	c	a	b	c		a	b	c	a	b	c	a	b	c
0.16	Tropine	12.3	12.8	10.3	11.3	12.5	11.4	12.9	12.3	11.9	12.4	12.4	13.4	12.1	13.3	12.8	12.0	12.9	12.6	11.9	12.5
0.22	Unkown (1)	4.7	4.6	4.9	4.7	4.8	4.7	4.9	4.9	4.5	4.7	4.7	4.4	5.1	5.6	4.5	4.9	4.6	5.1	4.7	4.6
0.30	Belladonine	3.2	3.5	3.4	2.9	3.4	3.7	2.8	3.2	3.5	2.4	3.3	3.8	3.6	3.4	3.7	3.4	3.2	3.2	3.2	2.9
0.92	Unkown (2)	4.4	4.9	4.8	4.5	4.7	4.7	4.4	4.3	4.3	4.3	4.4	4.6	4.6	4.9	4.3	4.5	4.6	4.4	4.5	4.3
1	Oscine	6.7	8.2	8.3	7.8	8.4	8.1	7.2	8.2	8.0	7.6	6.2	7.4	7.3	6.9	7.1	7.0	6.6	6.8	6.8	6.2
1.43	Tigloidine	4.9	4.8	4.9	4.8	4.8	4.8	4.4	4.3	4.8	4.2	4.6	4.9	5.1	4.9	4.8	5.0	4.8	4.6	4.8	4.3
1.60	Unkown (3)	5.1	5.6	5.6	5.8	5.4	5.3	5.7	5.3	5.4	5.1	5.0	6.1	6.0	5.9	6.0	5.9	5.7	5.8	5.6	5.1
1.88	Valeroidine	5.1	5.4	4.9	5.9	5.2	4.8	5.8	5.1	4.6	5.2	5.4	6.1	5.3	5.9	6.0	5.1	5.7	5.4	5.2	5.7
2.08	Meteloidine	5.5	5.9	6.3	6.8	5.8	6.1	6.6	5.6	5.9	6.2	5.6	5.9	6.1	6.3	5.7	5.9	6.1	5.5	5.7	5.8
2.21	Hyoscyamine	14.8	15.3	15.1	15.5	15.1	15.0	15.3	15.2	14.9	15.1	15.1	15.4	15.5	16.1	15.2	15.5	16.0	15.3	15.2	15.8
2.88	Hyoscine	19.8	20.3	19.9	20.3	19.8	19.8	20.1	19.6	19.8	19.9	18.6	19.4	19.3	20.1	19.1	18.8	18.8	18.8	18.6	18.9
		86.5	91.3	88.4	90.3	89.9	88.4	90.1	88.0	87.6	87.1	85.3	91.4	90.0	93.3	89.2	88.0	89.0	87.5	86.2	86.1

* Non reported alkaloids are unknown of minor importance and very small insignificant percentage compared with those recorded in this table.

a = Chromate
b = Dichromate
c = Chromic

Table 7 : (Cont.)

66 Days

R.T. (Oscine)	Age / alkaloid	0 0	0.05 m.M.			0.1 m.M.			0.5 m.M.		
			Chromate	Dichromate	Chromic	Chromate	Dichromate	Chromic	Chromate	Dichromate	Chromic
0.16	Tropine	12.9	13.6	12.8	13.4	13.6	12.6	13.2	13.3	12.6	11.0
0.22	Unkown (1)	4.9	4.9	4.9	5.4	5.1	4.7	4.9	5.1	5.1	5.3
0.30	Belladonine	4.0	4.3	4.4	3.9	4.1	4.2	3.9	4.1	4.0	3.7
0.92	Unkown (2)	4.1	5.3	4.9	5.1	5.2	4.3	4.9	5.1	4.4	4.8
1	Oscine	5.9	6.4	6.6	6.3	6.1	6.1	6.0	5.9	6.0	5.9
1.43	Tigloidine	4.2	4.9	5.4	4.3	4.6	5.2	4.6	4.6	4.9	4.3
1.60	Unkown (3)	5.3	5.9	6.2	5.8	5.8	5.9	6.1	5.6	5.8	5.7
1.88	Valeroidine	6.8	6.2	5.2	6.3	5.9	5.7	6.1	5.7	5.6	5.9
2.08	Meteloidine	5.7	6.8	5.9	6.2	6.3	5.9	6.1	5.4	5.4	5.8
2.21	Hyoscyamine	15.3	15.9	16.1	16.4	15.7	15.9	16.0	16.1	15.3	15.3
2.88	Hyoscine	14.8	14.8	15.3	14.9	14.7	14.7	14.8	14.7	14.8	14.0
		83.9	89.0	87.7	88.0	87.1	85.2	86.6	85.6	83.9	81.7

* Non reported alkaloids are unknown of minor importance and very small insignificant percentage compared with those recorded in this table .

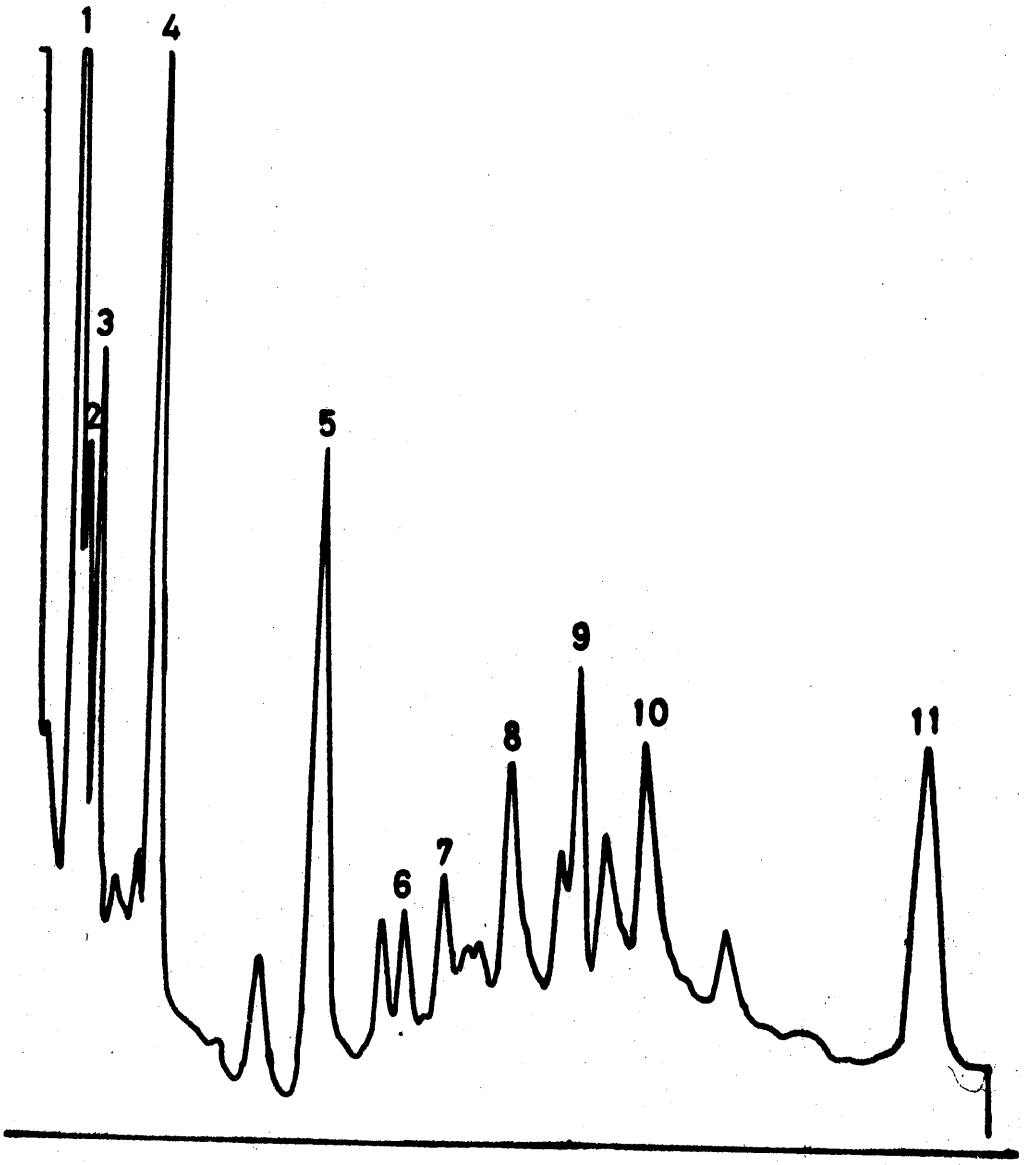


FIG. 14: RESOLUTION OF ALKALOIDS BY GAS LIQUID CHROMATOGRAPHY

1 : Tropine
2 : Unknown I
3 : Belladonine
4 : Unknown II
5 : Oscine
6 : Tigloidine
7 : Unknown III
8 : Valeroidine
9 : Meteloidine
10 : Hyoscyamine
11 : Hyoscine

Noticeable increases were also observed in valeroidine and hyoscine following the second spray with chromate; a phenomenon that was apparent for metelordine after the third spray. Meteloidine percentage also increased by the first and second spray with low doses of dichromate but the third dose was stimulatory to hyoscyamine. The first spray with chromic favoured a rise in valeroidine and meteloidine; a phenomenon that continued for the latter component following the second spray. The second spray with chromic also stimulated hyoscyamine and hyoscine; a phenomenon that continued for the former alkaloid with the third spray.

PART V

- D I S C U S S I O N
- S U M M A R Y
- R E F E R E N C E S
- A R A B I C S U M M A R Y

D I S C U S S I O N

The results of this investigation clearly establish the fact that anionic chromium is much more effective in retarding or inhibiting growth phenomena when applied at endurable concentrations. The three chromium ions were severely toxic to Datura stramonium at 1.0 mM concentration or higher. In most instances, particularly at the lower levels of the ions, chromate was a better inhibitor to germination than dichromate; more prominently at the lower doses.

These deduction are highly apparent from the observed low percentage germination and root length of the seedlings following application of anionic chromium compounds compared with cationic chromium. Even the inhibitory effects of comparatively high doses of chromic ion, at the early stages of germination, were almost overcome with duration but not highly apparent with root length.

These observations are in full agreement with those of Hewitt (1953) and Skeffington et al. (1976). They might be attributed to the rapid absorption of chromate and non-or limited absorption of chromic ion by the developing roots as already concluded by Bourque et al.(1967),

Eckert and Blincoe (1970), and Blincoe (1974).

Since Skeffington et al. (1976) proved that uptake of chromate by barley roots is active while that of chromic is passive and, in the meantime, Myttenaere and Mousny (1974), working with rice, concluded that chromic was absorbed more rapidly than chromate, we thought it more feasible to apply the chromium ions as leaf spray in order to avoid these discrepancies and to eliminate any stumbling blocks against transport of these ions to the metabolising leaves.

The results of spraying 45 days old Datura stramonium plants with various doses of the different chromium ions showed that they had already stunted the plants shortening their stem length, reducing the number , total and mean leaf area and lowering the fresh and dry weight of both organs. Still anionic chromium is more effective than the cationic form; more pronouncedly the dichromate ion. Such inhibitions were partially alleviated with increased concentration and/or number of sprays. Chromate, in particular, was stimulatory to most of these criteria of growth, especially the gain in dry weight.

These observations might be attributed to inhibition of cell division and/or cell expansion and development at the apical and intercallary meristems as well as leaf primordia, as a result of spraying.

Furthermore, they are in accordance with the early observation that chromic is less toxic than chromate.

Skeffington et al. (1976) attributed the low toxicity of chromic ion to the multiplicity of the cation binding sites in the cell and cell walls that would adsorb the ion whereas most of the chromate exists in the soluble fraction of the cell. Still blockage of chromic transport (Pratt, 1966; Shewry and Peterson, 1974) and the rapid movement of chromate; being held up, like calcium, by ion exchange in the xylem vessel walls of the leaves (Bell and Bedulph, 1963) might also participate in the difference in toxicity of both ions.

The lesser toxicity of chromate than dichromate might be attributed to the possibility of reduction of chromate by the cells (Myttenaere and Mousny, 1974); a phenomenon already confirmed by Skeffington et al. (1976) who noticed that barley root cells were able to

transform part of the absorbed chromate to chromic ions. As far as the author is aware, reduction of dichromate by living cells was not reported by previous investigators.

Such inhibitory effects were extended to flower primordia where the same trends of inhibition were reported for the number of flowers, fresh and dry weight of developing fruits. Still the third spray with 0.5 mM chromic significantly alleviated such inhibition. But such deleterious effects were not apparent on the seed quality as observed from the minor differences in air and oven dry weight of 100 seeds of the control and chromium-sprayed plants.

It should be recalled here, that Jawarsky (1976) attributed the low toxicity of Cr III, to its poor membrane permeability and non-corrosive nature whereas Cr VI, being a strong oxidizing agent can easily cross the biological membranes and thus is highly toxic.

In this connection, it may be mentioned that Mortvedt and Giordono (1975) found that chromic sulphate was less toxic than sodium dichromate at 80 p.p.m. chromium but both were very toxic at 320 p.p.m. According

to Myttenaere and Mousny (1974), chromium ion specifically binds to different proteins. Malek et al., (1976) confirmed the formation of complexes of Cr^{2+} with histidine glycine or arginine.

Still chromic ion is capable of binding with cysteine derivatives (De Meester and Hodgson, 1976); a phenomenon that is regarded of great significance in chelation therapy for metal poisoning.

Despite these inhibitory effects on growth and development of stems and leaves, 0.05 mM of these chromium ions remarkably initiated chlorophyll a and b accumulation with reduced content of carotenoids that were mostly alleviated or reversed with rise of concentration of the ions; more prominently on dry weight basis. Further spray with either chemicals did not significantly alter the initial effects produced by the first spray.

It is interesting to note that pigmentation was denser, by all treatments, when calculated per unit surface; an indication of increased capacity of photosynthesis irrespective of other responses.

Active chlorophyll accumulation might be attributed to increasedrate of synthesis and/or longivity of the

produced pigments in presence of chromium. Rusko (1968b) reported increased chlorophyll content in grape leaves following chromium application. He attributed such increase to more stability of the plastids, in presence of the ion that exerted a protective action against chlorophyll degradation.

The decreased total and mean leaf area, fresh and dry weight reflected themselves on the total alkaloid content per plant or unit leaf of the plant particularly following the first spray. The second spray with the high dose of dichromate or chromic accelerated while the other doses and/or other ion were suppresive; aphenomenon that further extended to the third spray where only chromic ion was a very effective activator. Inspite of such inhibitory effects of chromium (VI) compared with chromium (III) on alkaloids per plant or per unit leaf yet the percentage alkaloid, following chromium (VI) treatment was higher than chromium (III); the former increased while the latter decreased with increased number of sprays.

These and the previous observations denote that the deletrious effects of chromium ions are persistant on growth criteria particularly at the lower levels and/or

first spray, and to a lesser extent by the cationic form (Cr III). Increased number of sprays and/or concentration, particularly Cr^{+3}, totally alleviated or even enhanced growth and metabolic activities.

These phenomena may be attributed to the interferance of chromium with energy transfer since several investigators (Danenberg and Gleland, 1975; Gupta et al., 1976) revealed the fact that chromium binds with ATP and thus interferes with hexokinase and pyruvic kinase. According to the first author, Cr-ATP binds tightly with the active centre of hexokinase and glucose/fructose or mannose and very slowly releases Cr-ADP-hexose-6-phosphate. The second author showed that Cr-ATP increased the rate of enolization of pyruvic acid. Such shifts may lead to deviations in metabolic pathways that might lead to increased alkaloid content.

It should be mentioned that chromium ions not only increased the percentage yield of alkaloids and/or total alkaloid per plant or leaf but also interfered with the biosynthesis of the individual alkaloids; increasing the rate of synthesis of some of them. Such alternations dependended on type of ion and/or number of sprays. These

observations differed from those obtained from pre-sowing irradiation with Co^{60} (Balbaa et al., 1967) that inhibited most of the growth criteria but had little or no effect on the nature and proportion of individual alkaloids in several Datura species.

S U M M A R Y

In retarding or inhibiting growth phenomena when applied at endurable concentrations. The three chromium ions were severely toxic to Datura Stramonium at 1.0 mM concentration or higher. In most instances, particularly at the lower levels of the ions, chromate was a better inhibitor to germination than dichromate; more prominently at the lower doses.

Spraying 45 days old Datura Stramonium plants with various doses of the different chromium ions stunted the plants shortening their stem length, reducing the number and total and mean leaf area and lowering the fresh and dry weight of both organs. Still anionic chromium is more effective than the cationic form; more pronouncedly the dichromate ion. Such inhibitions were partially alleviated with increased concentration and/or number of sprays. Chromate, in particular, was stimulatory to most of these criteria of growth especially the gain in dry weight.

Despite these inhibitory effects on growth and development of stems and leaves, 0.05 m M of these chromium

ions remarkably initiated chlorophyll a and b accumulation with reduced content of carotenoids that were mostly alleviated or reversed with rise of concentration of the ions; more prominently on dry weight basis. Further spray with either chemicals did not significantly alter the initial effects produced by the first spray.

Inspite of inhibitory effects of chromium (VI) compared with chromium (III) on alkaloids per plant or per unit leaf yet the percentage alkaloid , following chromium (VI) treatment was higher than chromium (III); the former increased while the latter decreased with increased number of sprays. Chromium ions interfered with the biosynthesis of the individual alkaloids; increasing the rate of synthesis of some of them. Such alterations depended on type of ion and/or number of sprays.

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الملخص العربي

عند دراسة ظاهرة تثبيط نمو النباتات بتعريضها لتركيزات مختلفة من أملاح الكروم ، لوحظ أن ايونات الكروم الثلاثة لها تأثير سام على نبات الداتوره سترا مونيوم عند تركيز ١ مل جزيئى او أكثر .

وفى معظم الحالات لوحظ ان ايون الكرومات له تأثير على تثبيط الانبات اكبر من ثنائى الكرومات خاصة عند الجرعات المنخفضة .

وعند رش نبات الداتوره سترا مونيوم (٤٥ يوم) بجرعات مختلفة من ايونات الكروم فان ذلك يسبب قصر الساق ويقلل عدد ومساحة اوراق النبات ، وكذلك يقلل الوزن الطازج والجاف لكلا الساق والورقة .

وقد وجد أن أيون الكروم السالب له تأثير أكبر من الموجب ويلاحظ ذلك بوضوح فى حالة ثنائى الكرومات .

مثل هذا التثبيط يقل جزئيا مع زيادة التركيز وعدد الرشات . وقد وجد ان الكرومات له تأثير منشط لمعظم هذه الحالات من النمو خاصة فى محصول الوزن الجاف .

وبالرغم من تأثيرات تثبيط النمو للساق والاوراق لوحظ ان التركيز ٥ ر٠ مل جزيئى يسبب زيادة فى كمية الكلوروفيل a ، b ونقص فى كمية الكاروتين ومن الملاحظ ان الرشات الاخرى لاتسبب التأثير الواضح الناتج من الرشة الاولى .

ومقارنة تأثير الكروم سداسى التكافؤ وثلاثى التكافؤ على القلوانيات لكل نبات او ورقة وجد ان الاول تأثيره اقوى من الاخير •

كما لوحظ ان ايونات الكروم تتداخل مع تخليق القلوانيات المختلفة مسببه زيادة فى بعضها •

ومثل هذا التغير يعتمد كلية على نوع الايون وعدد الرشات •

تأثير الكروم على النمو والمحتوى الكلى للفلوانيات فى نبات الداتوره سترامونيوم

رسالة مقدمة إلى كلية العلوم — جامعة القاهرة
للحصول على درجة ماجستير فى النبات (فسيولوجى)
كجزء من متطلبات الحصول على درجة الماجستير

مقدمة من
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جامعة القاهرة
كلية العلوم
قسم النبات

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