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Cytological Effect of the Herbicide 2,4-D isooctylester 48% on Root Mitosis of *Allium cepa*

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Summary The effect of the herbicide 2,4-D isooctylester 48% (Esteran 48) has been studied on root mitosis of *Allium cepa.* Root tips of *Allium cepa* were treated with a series of concentrations, ranging from 50 ppm to 50,000 ppm for 3, 6, 12, 24 h. Examinations of roots were done in permanent root tip squash preparations stained by the Feulgen technique. Esteran 48 effects the relative duration of each mitotic stage as compared with the control. It also caused reduction in the mitotic index, indicating mitotic inhibition, and increased in the frequency of abnormal mitosis.

The type of the abnormalities induced: chromosome stickiness, C-metaphase, tetraploid cells, bridges, laggards, tripolar anaphases-telophases and micronuclei.

The effect of Esteran 48 on root mitosis simulates that of colchicine in the type of abnormal metaphase (C-mitosis) and induction of polyploidy cells as well as the accumulation of metaphases.

Key words 2,4-D isooctylester, Chromosome abnormalities.

It is now widely known that herbicides are capable of inducing genetic effects on plants. Many studies have demonstrated the mutagenic action of herbicidal solutions on seeds, seedlings and growing plants. Plant assay systems have been found useful for the detection of environmental mutagens (De Serres 1978). It is ironical that although herbicides are used in modern technology of crop production, they are sources of potentially hazardous substances in the human environment. Many cytological studies have been carried out to detect the harmful effect of different pesticides and herbicides on different plants (Soriano 1984, Njagi and Goplan 1981, Mousa 1982, Amer and Farah 1987, El-Khodary *et al.* 1989). The use of chromosomal aberrations, induced by pesticides in crop plants, is there fore, being accepted as indicators of genetic damage (Ma 1982).

The herbicide 2,4-D isooctylester 48% is also known as Esteran 48 and it is actively control of many broad leaf weeds. The present investigation was carried out to study the cytological effect of the herbicide Esteran 48 on the process of mitosis in the root tips of *Allium cepa.*

Material and methods

The bulbs of *Allium cepa* was used as plant test material. Roots while intact, were treated with different concentrations of the herbicide, Esteran 48 ranging from 50 ppm to 50,000 ppm for 3, 6, 12 and 24 h. Roots were fixed in acetic alcohol (1 : 3) for 24 h.

Examination of the roots was done in permanent root tip squash preparations stained by the Feulgen technique. Three replicates were performed for each treatment and the control, and scoring was made from the 3 roots of each replicate. The mitotic index was calculated for each treatment as a number of dividing cells/100 cells.

Results and discussion

The used concentrations of Esteran 48 caused distinct decrease in the mitotic index when com-

pared with the control (Table 1). In all the treatments the decrease in MI values in the root meristems of *Allium cepa* with increasing concentrations is attribute to mitotic inhibitions. A drop in mitotic activity was clearly observed when the roots were treated with high concentrations. Such a drop in the mitotic index indicates that Esteran 48 interferes in the normal sequence of mitosis thus preventing a number of cells entering the prophase, state at interphase. Such reduction in the mitotic activity could be due to the inhibition of DNA synthesis (Schneiderman *et al.* 1971). Beau *et al.* (1976) also showed that exposing the root tips of *Vicia faba* to high concentrations of the herbicide (Paraquate), led to inhibition of DNA synthesis. This may suggest that Esteran 48 could have the same effect. The reduction of mitotic activity seems to be common effect of most herbicides tested for their action on mitosis (Amer and Farah 1980, 1983, Tomkins and Grant 1972, Badr 1983, Badr and Ibrahim 1987). Mitotic inhibition by herbicides has been attributed to blocking of mitotic cycle during interphase which may result from prolonged G_2 period or to the inhibition of DNA synthesis (Chand and Roy 1981).

The degree of cytological aberrations either in mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of mutagen (Reddi and Reddi 1985). Esteran 48 exerted a marked mitodepressive action on mitosis and induced a number of chromosomal aberrations. These abnormalities are shown in Table 2.

The colchicine C-metaphase (Fig. 1) configurations as the major mitotic abnormality produced in roots treated with high concentrations and/or prolonged periods of treatments. C-mitosis is one of the consequences of inactivation of spindle apparatus connected with the delay in the division of centromere. Deysson (1968) suggested that this might lead to the polyploid cells thus formed degenerate without further division. Esteran 48 also induced sticky metaphase (Fig. 2). Other mitotic abnormalities induced by this herbicide are poliploidy (Fig. 3), tripolar anaphases (Fig. 4) and tripolar telophases (Fig. 5), bridges (Fig. 6) and lagging chromosomes (Fig. 7) and micronuclei (Fig. 8). Aberrations involving the chromosomal structure as elegant indicators of chromosomal mutations.

In the present study a remarkable correlation exists between stickness and bridges produced. These suports the hypothesis that bridges induced by Esteran 48 is due to the stickness of the chromosomes. Stickiness is regarded as physiological effect exerted by herbicides in plants, which has been considered of the chromosomes. On the other hand stickiness has been attributed to the improper folding of chromosome fibers, which makes the chromatids connected by means of subchromatid bridges (McGill *et al.* 1974, Klasterska *et al.* 1976). The phenomena of chromosome stickiness dominant indicate that the toxicity of Esteran 48 and its low mutagenic potential.

This herbicide tested on this study produced anaphase bridges (Table 2). Bridges were found to be the result of stickiness of the chromosomes. The irregular spreading of chromosomes may be attributed to the disturbance of spindle apparatus (Amer and Ali 1974). It is well known that fragments and bridges lead to structural changes in the chromosomes. Breaking up chromosomes followed by proximal chromatid reunion evidently results in dicentric chromosomes which form characteristic anaphase bridges (Tomkins and Grant 1972, Grant 1978), or these anaphase bridges can be attributed to the general stickiness of chromosomes (Abraham and Koshy 1979).

Lagging chromosomes induced this herbicide are acting only on spindle apparatus resulting in scattered anaphases and laggard chromosomes. Tripolar and trinuclaeted cells are indicating the inhibition of cytokinesis (Somashekar and Godwa 1984).

The presence of micronuclei observed in the present study is not a deviant phenomenon (Amer and Ali 1974, Amer and Farah 1980, 1983), these are evidently the resultants of the chromosome fragments of the cells concerned. The formation of micronuclei, may be the result of lagging chromosome or chromosome segment produced during a preceding division. Sparrow and Singleton (1953) mentioned that micronuclei are a fair index of fragment production.

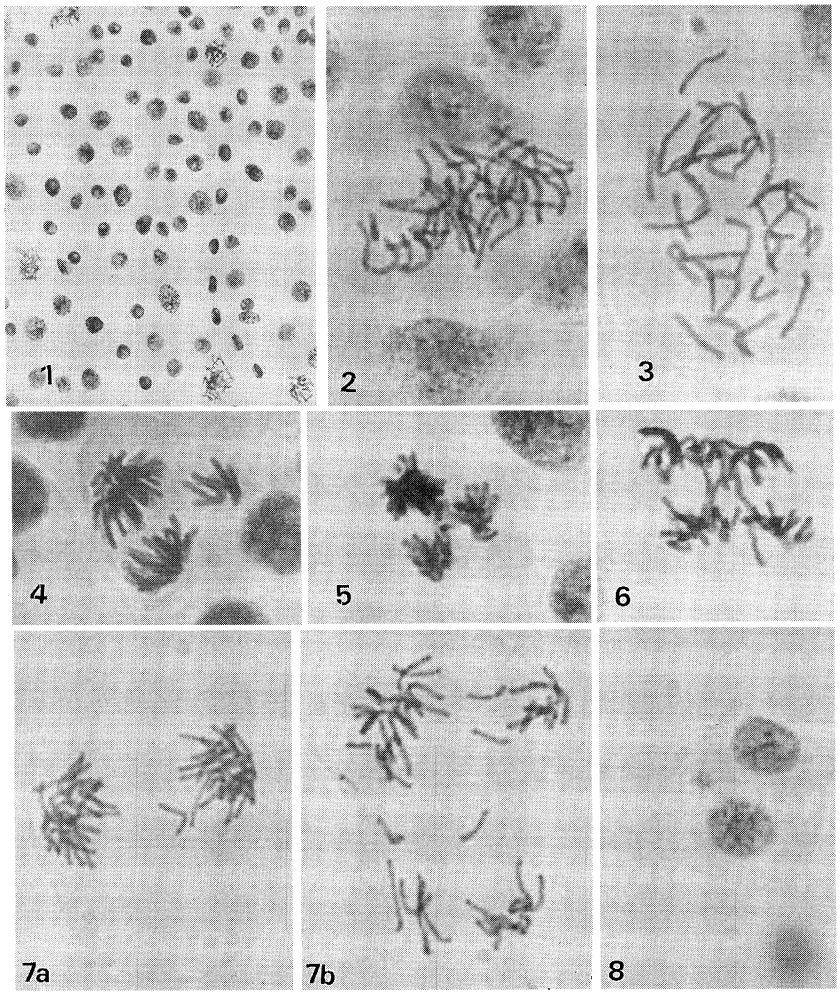
Table 1. Number of total cells examined and total mitosis, percentage of total and abnormal mitotic phases, mean of mitotic index after treating *Allium cepa* root tips with different concentrations of Esteran 48 for different periods

Time of treatment (h)	Conc. (ppm)	Total cells examined	Total mitosis	Prophase (%)		Metaphase (%)		Anaphase (%)		Telophase (%)		MI means±SE
				Total	Abn.	Total	Abn.	Total	Abn.	Total	Abn.	
3	control	4888	156	19.23	0	28.21	0	25.64	0	26.92	0	2.82±0.60
	50	7540	235	26.80	1.28	20.43	14.47	19.15	1.28	16.59	0	3.11±0.29
	100	7041	132	28.79	5.30	18.94	9.85	13.64	1.51	21.97	0	1.84±0.29
	500	4020	94	47.87	0	2.13	20.21	15.96	1.06	12.77	0	2.67±0.52
	1000	6434	177	41.82	2.82	18.64	12.43	9.04	10.73	4.52	0	2.77±0.40
	5000	7649	136	36.76	0	16.18	40.44	0	2.21	4.41	0	1.81±0.31
	10000	3993	30	16.68	3.33	30.00	40.00	3.33	3.33	3.33	0	0.77±0.13
	50000	7306	114	23.68	7.02	4.38	26.32	11.40	7.02	15.79	4.39	1.54±0.29
6	control	2698	91	36.26	0	34.07	0	12.09	1.10	16.48	0	3.26±0.81
	50	2364	85	20.00	1.18	7.06	20.00	4.71	11.76	31.76	3.53	1.96±0.37
	100	4678	80	23.75	0	8.75	30.00	12.50	11.25	12.50	1.25	1.71±0.17
	500	4492	92	32.61	5.43	4.35	30.43	6.52	3.26	14.14	3.26	0.87±0.35
	1000	1405	3	33.33	0	0	0	0	0	66.67	0	0.21±0.00
	5000	6541	71	18.31	0	1.41	66.20	1.41	7.04	0	5.63	1.27±0.27
	10000	5436	75	12.00	1.33	12.00	16.00	17.34	17.34	14.66	9.33	1.25±0.45
	50000	4598	18	0	5.56	16.66	50.00	0	11.11	11.11	5.56	0.56±0.06
12	control	5138	57	26.32	0	71.93	0	1.75	0	0	0	1.11±0.26
	50	6261	45	26.67	0	42.22	24.44	6.67	0	0	0	0.84±0.14
	100	4427	32	40.63	3.13	12.50	21.87	18.74	0	3.13	0	0.71±0.04
	500	1131	11	27.27	0	18.18	36.37	18.18	0	0	0	0.71±0.70
	1000	4771	28	64.29	0	3.57	28.57	0	0	3.57	0	0.60±0.03
	5000	3265	21	4.76	0	9.52	85.72	0	0	0	0	0.64±0.64
	10000	6726	51	7.85	1.96	1.96	70.59	3.92	11.76	1.96	0	0.76±0.01
	50000	4152	22	18.18	0	13.64	50.00	0	13.63	4.55	0	0.54±0.16
24	control	3076	122	16.39	0	40.17	0	24.59	0	18.85	0	3.75±0.52
	50	5770	50	18.00	0	6.00	32.00	12.00	20.00	12.00	0	0.86±0.05
	100	2682	15	13.33	0	26.67	20.00	20.00	6.67	13.33	0	0.59±0.07
	500	4367	26	30.77	3.85	7.69	57.69	0	0	0	0	0.57±0.10
	1000	6561	37	13.52	2.70	16.22	51.35	2.70	2.70	10.81	0	0.57±0.04
	5000	3910	53	20.75	5.66	15.09	11.32	18.87	1.89	22.64	3.78	0.57±0.03
	10000	7442	57	10.53	1.75	0	38.60	12.28	1.75	24.56	10.53	0.77±0.15
	50000	6794	46	10.87	4.35	15.21	21.74	17.39	8.70	15.22	6.52	0.68±0.25

Table 2. Frequencies of different types of metaphase and ana-telophase abnormalities after treating

Time of treatment (h)	Conc. (ppm)	% of pro-metaphase abnormalities				% of ana-telophase abnormalities			
		laggard	sticky metaphase	C-mitosis	tetraploid cells	bridge	micro nuclei	tripolar anaphase	early anaphase
3	control	0	0	0	0	0	0	0	0
	50	0	8	92	0	66	0	34	0
	100	0	30	70	0	100	0	0	0
	500	5	0	85	10	0	0	0	0
	1000	0	8	82	11	37	0	10	53
	5000	0	2	93	6	34	0	0	66
	10000	0	92	8	0	0	0	0	100
	50000	2	71	21	6	62	0	15	23
6	control	0	0	0	0	0	0	0	100
	50	0	45	40	15	27	0	9	64
	100	0	48	48	4	44	0	0	56
	500	11	54	32	4	36	9	19	36
	1000	0	0	0	0	0	0	0	0
	5000	0	0	100	0	36	0	7	57
	10000	1	67	22	10	27	0	20	53
	50000	0	46	46	8	0	0	0	0
12	control	0	0	0	0	0	0	0	0
	50	0	18	64	18	0	0	0	0
	100	0	0	100	0	100	0	0	0
	500	0	0	100	0	0	0	0	0
	1000	0	100	0	0	0	0	0	0
	5000	0	5	95	0	0	0	0	0
	10000	0	100	0	0	80	0	0	20
	50000	0	45	55	0	100	0	0	0
24	control	0	0	0	0	0	0	0	0
	50	0	56	44	0	60	0	0	40
	100	0	0	100	0	0	0	0	100
	500	0	6	88	6	0	0	0	0
	1000	0	19	81	0	100	0	0	0
	5000	50	50	0	0	0	60	40	0
	10000	4	84	8	4	60	40	0	0
	50000	6	44	31	19	66	0	0	34

Allium cepa root tips with different concentrations of 2,4-D isooctylester 48% for different periods.



Figs. 1–8. Cytological abnormalities found in the root tip meristems of *A. cepa.* 1) C-mitosis, 2) sticky metaphase, 3) poliploidy, 4) tripolar anaphase, 5) tripolar telophase, 6) bridges, 7) lagging chromosomes, 8) micronuclei.

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