

Effect of herbicides on mitosis of *Hibiscus cannabinus* Linn.

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(Received: March 28, 2007; Accepted: May 24, 2007)

ABSTRACT

The decreases in the mitotic index were observed after treatment of 2,4-D, Oxyfluorfen and Glyphosate. The mitotic index decreased from 8.45 to 3.56 at 1000 to 5000 ppm of *Hibiscus cannabinus* Linn, 8.26 to 2.84 at 1000 to 8000 ppm of Oxyfluorfen and 9.44 to 4.62 at 5000 to 25000 ppm of Glyphosate. The percentage of abnormalities in root tips cells increased gradually with increase in concentrations of herbicides. The percentage of abnormalities in root tips cells were 0.33 to 3.11 at 2000 to 5000 ppm of 2,4-D, 1.07 to 2.92 at 2000 to 8000 ppm of Oxyfluorfen and 0.62 to 2.54 at 15000 to 25000 ppm of Glyphosate. The abnormalities like binucleolate cells and clumping of chromosomes were observed.

Key word: Mitosis, *Hibiscus cannabinus*, 2, 4-D, Oxyfluorfen and Glyphosate.

INTRODUCTION

The growth of the plants can be described in term of cell division, cell enlargement and cell differentiations. Due to spray application of herbicides some irregularities were induced in *Hibiscus cannabinus* Linn. These irregularities have thus imbalance the metabolic activities of plants, which then could not give rise to the mature tissue.

The present study deals with the inhibition and the behavior of the dividing cells in mitosis from the following treatment of 2, 4-D, Oxyfluorfen and Glyphosate.

MATERIALS AND METHODS

A large number of seeds of *Hibiscus cannabinus* Linn. were treated with 50 ml of various concentrations of 2,4-D (1000 to 5000 ppm), Oxyfluorfen (1000 to 8000 ppm) and Glyphosate (5000 to 25000 ppm) prior to lethal dose for germination up to 24 hours. The seed soaked in distilled water for the same period were used as control. After treatment they were washed thoroughly in distilled water and allowed to

germinate in petridishes lined with double layered moistened filter paper under laboratory conditions. Similarly, seeds soaked in distilled water were kept for germination under similar condition and used as control.

Root tips of seedlings were fixed in freshly prepared carnoy's fluid (3: 1, ethanol: glacial acetic acid) for 24 hours. Root tips then washed thoroughly with distilled water and were stored in 70 % alcohol. The root tips were hydrolyzed for 7 to 10 minutes in 1 N HCL at 60°C. Slides were prepared by squash method using iron alum as mordant and hematoxylin stain. The slides were made permanent by using acetic-acid- butanol grades and mounts in D.P.X.

The dividing cells in metaphase and anaphase were scored for chromosomal aberration prior to lethal dose. The mitotic index was calculated by applying the following formula.

$$\text{Mitotic index} = \frac{\text{Total no. of dividing cells observed}}{\text{Total no. of meristematic cells observed}}$$

RESULTS AND DISCUSSION

In control root tips, the mitosis was normal in *Hibiscus cannabinus* Linn. The mitotic index was 14.21 in 24 hours treated seedlings.

2,4-D

2,4-D induced some abnormalities such as binucleolate cells at all concentrations and chromosomal bridges at 4000 and 5000 ppm of herbicide. The percentage of abnormalities at 1000, 2000, 3000, 4000 and 5000 ppm was 0.0, 0.83, 1.30, 3.0 and 3.71, respectively (Table 1, fig. 1). The frequency of abnormalities increases with increasing concentrations of herbicide.

This herbicide also affected the division of meristematic cells in root tips cells. The rate of mitosis decreased with increased in concentrations of herbicide. The mitotic index at 1000, 2000, 3000, 4000 and 5000 ppm was 8.45, 6.72, 6.12, 4.64, and 3.56, respectively as against 14.71 in control (Table 1, fig. 2).

The cells at resting stage had single nucleolus in nucleus. But, however, some of the cells showed binucleolate condition with various percentages. The cells with binucleolate condition showed nuclei were mostly equal in size.

Oxyfluorfen

In Oxyfluorfen, treated root tips of seedlings were showed clumping of chromosomes at 4000, 6000, and 8000 ppm and precocious movement at all concentrations of herbicide except lower most ones. The percentage of abnormalities was 0.0, 1.07, 1.6, 2.79, and 2.92 at 1000, 2000, 4000, 6000 and 8000 ppm, respectively (Table 1, fig.3). The percentage of abnormalities was increased as the concentrations of herbicide increase. The mitotic index decreased with increasing concentrations of herbicide. The mitotic index at 1000, 2000, 4000, 6000 and 8000 ppm was 8.26, 6.48, 4.77, 3.41 and 2.84, respectively (Table 1, fig. 4).

Glyphosate

This herbicide induced abnormalities like binucleolate cells and grouping of chromosomes at 15000, 20000 and 25000 ppm in root tips. The

percentage of abnormalities at 5000, 10000, 15000, 20000 and 25000 ppm was 0.0, 0.0, 0.62, 0.99, and 2.54, respectively (Table 1, fig. 5).

The percentages of abnormalities were increased as the concentrations of herbicide increase. The mitotic index also reduced as the concentrations of herbicide increased. The mitotic index was 9.44, 5.82, 5.04, and 4.62 at 5000, 10000, 15000, 20000 and 25000 ppm, respectively (Table 1, Fig. 6).

DISCUSSION

Mitosis was found to be normal in control seedlings of *Hibiscus cannabinus* Linn.

2, 4-D

This herbicide induced chromosomal aberration like binucleolate cells. The binucleolate cell formation might have been due to the constriction of fully formed nucleus in two pieces and failure of plate formation. This herbicide affects the number of dividing cells and decreased mitotic index. Similar results were reported by Doxey and Rhodes (1949) on *Allium cepa*, Nygren (1949) on *Allium cepa*, Crocker (1953) on Effect of herbicides 2, 4-D Sawamura (1964) on *Allium triticum*, *Tradescantia spp.* And *Vicia faba*, Alekperer *et al.*(1964) on *Allium fistulosa*, Mohandas and Grant (1972) on some weeds, Rojik *et al.*(1973) on *Vicia faba*, Strove (1973) on barley, Dharurkar and Dnyansagar (1974) on *Eichhornia crassipes*, Sikka and Sharma (1976) on *Allium spp.* Tomkins and Grant (1976) on some weeds, Bayliss (1977) on *Daucus corota*, Prasad and Das (1977) on *Vicia faba*, Mauras and Pareyre (1977) on *Allium sativum*, Kolhe (1979) on some weeds, Hadke (1980) on *Psoralea corylifolia*, Bakale and Hadke (1981) on *Euphorbia geniculata*, Deshmukh (1981) on *Cassia occidentalis*, *Lagasca mollis* and *Corchorus olitorious*, Bakale *et al.* (1981) on *Malvastrum coromendelianus*, Bakale and Kolhe (1981) on *Solanum xanthocarpum*, Sheleg and Deeva (1987) on barley, Dzhelepov (1988) on wheat, Bakale and srinivasu (1989) on *Psoralea corylifolia*, Grover *et al.* (1990) on *Allium cepa*, and Barely, Trivedi and Alok (1991) on *Mecardonia procumbens*, Jain (1993) on *Chenopidium album*, Bobde (1993) on *Crotolaria juncea*, Gopal (1993)

Table - 1: Showing the mitotic index and total percentage of abnormalities in the seedlings of *Hibiscus cannabinus* Linn. at different concentrations of herbicides.

Herbicides	Conc. (ppm)	Total no. of cells observed	Total No. of dividing cells	Mitotic Index	Binucleolate Cells	Chromosomal Bridges	Clumping of Chromosomes	Grouping of Chromosomes	Precocious Movement	Abnormalities Percentage
-	Control	700	103	14.71	-	-	-	-	-	-
2,4-D	1000	603	51	8.45	-	-	-	-	-	0.83
	2000	595	40	6.72	0.83	-	-	-	-	1.30
	3000	565	35	6.19	1.30	-	-	-	-	3.0
	4000	581	27	4.64	1.65	1.35	-	-	-	3.71
	5000	589	21	3.56	1.91	1.8	-	-	-	-
Goal	1000	593	49	8.26	-	-	-	-	-	-
	2000	599	41	6.84	-	-	-	-	1.07	1.07
	4000	586	28	4.77	-	-	0.51	-	1.09	1.6
	6000	589	20	3.41	-	-	0.64	-	2.15	2.79
	8000	597	17	2.84	-	-	0.73	-	2.19	2.92
Glyphosate	5000	625	59	9.44	-	-	-	-	-	-
	10000	635	37	5.82	-	-	-	-	-	-
	15000	613	34	5.54	0.44	-	-	0.18	-	0.62
	20000	615	31	5.04	0.69	-	-	0.30	-	0.99
	25000	605	28	4.62	0.98	-	-	1.56	-	2.54



Fig. - 1: Graph showing percentage of abnormalities in root tips cells at different concentrations of 2,4-D.

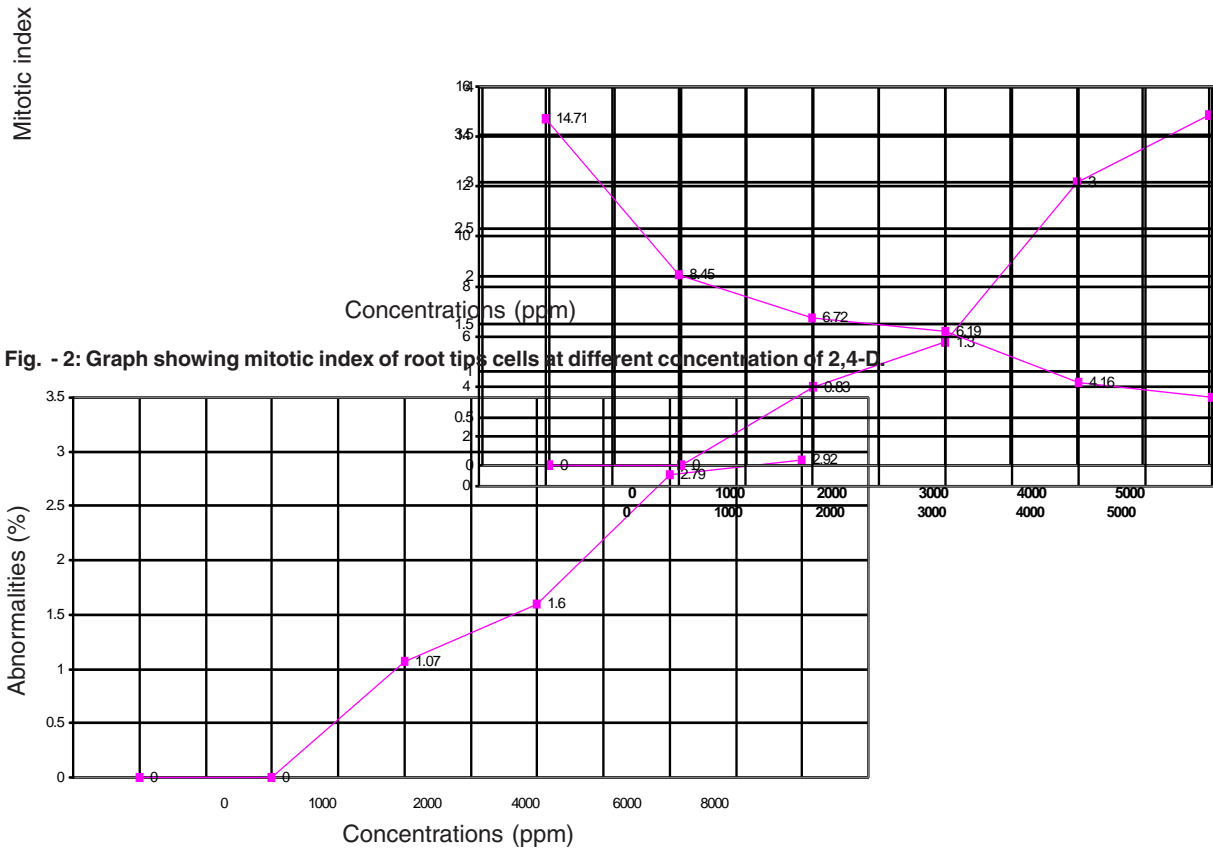


Fig. - 2: Graph showing mitotic index of root tips cells at different concentration of 2,4-D.

Fig. - 3: Graph showing percentage of abnormalities in root tips cells at different concs. of oxyfluorfen (goal).

Mitotic index

Concentrations (ppm)

Fig. - 4: Graph showing mitotic index of root tips cells at different concentrations of oxyfluorfen (goal).

Abnormalities (%)

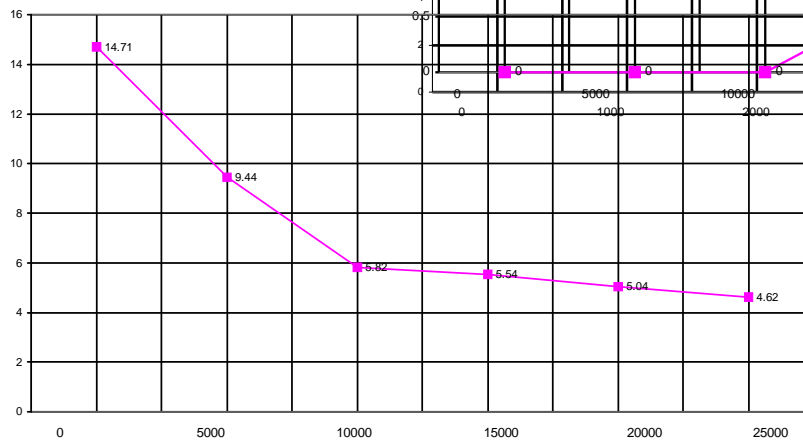
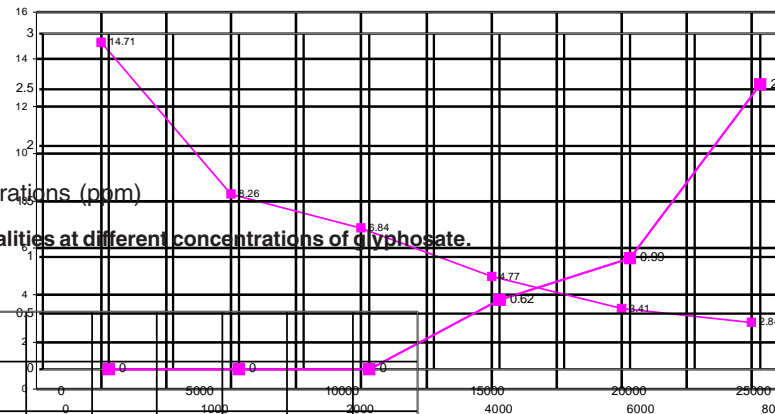
Concentrations (ppm)

Fig. - 5: Graph showing percentage of abnormalities at different concentrations of glyphosate.

Mitotic index

Concentrations (ppm)

Fig. - 6: Graph showing mitotic index of root tips cells at different concentrations of glyphosate.



on *Medicago sativa* and Kulkarni (1998) on *Crotalaria medicaginea* var. *luxurians*.

The chromosomal bridges were observed in the present study. They were found when chromosome fail to separate at the time of anaphase. Similar results were reported by Dharurkar and Dnyansagar (1974) on *Eichhornia crassipes*, Bakale and Hadke (1981) on *Euphorbia geniculata*, Bakale et al.(1981) on *Malvastrum coromendelianus*, Bakale and Kolhe (1981) on *Solanum xanthocarpum*, Jain (1993) on *Chenopodium album*, Gopal (1993) on *Medicago sativa* and Kulkarni (1998) on *Crotalaria medicaginea* due to applications of 2,4-D.

Oxyfluorfen

This herbicide induced chromosomal aberration like precocious movement of chromosomes in root tips cells. It might be due to sudden contraction of some spindle fibers due to the toxic effects of herbicide. Similar results were reported by Gopal (1993) on *Medicago sativa* and Kulkarni (1998) on *Crotalaria medicaginea* var. *luxurians* due to application of oxyfluorfen.

In present study, the root tip cells showed

clumping of chromosomes. This may be due to metaphase chromosomes became thick and finally stick together forming a compact clump. Gopal (1993) on *Medicago sativa* and Kulkarni (1998) on *Crotalaria medicaginea* reported clumping of chromosomes due to application of oxyfluorfen.

Glyphosate

In present study, some chromosomal abnormalities induced due to application of glyphosate. Binucleolate cells and grouping of chromosomes observed at higher concentrations of herbicide. The grouping of chromosomes might be due to the effect of herbicide on the condensation cycle of chromosomes. Upchurch and Baird (1972) Mosher et al. (1976) on *Crotalaria juncea*, Vaughn and Duke (1986) on *Glycin max*, Ditomasa (1988) on Pea, Jain (1993) on *Chenopodium album*, Bobde (1993) on *Crotalaria juncea*, and Kulkarni (1998) on *Crotalaria medicaginea* var. *luxurians* reported grouping of chromosomes as well as binucleolate cells due to application of glyphosate.

Of the three herbicides used in the present study bring about physiological and structural effects which ultimately lead to cassation of growth of plants and finally resulting in to its death.

REFERENCES

1. Alekperov, U. K., Kolomnots, A. F. and Scherbakor, Antimutagenic activity of paraquat. *Dokl. Akad. Nauk. USSR* **176**: 99 (1967).
2. Bakale, V. L. and Hadke, S.N. Effect of herbicides 2, 4-D, sodium arsenate, and lasso on mitosis in *Euphorbia geniculata* Orteg. *Perspective in Cytology and Genetics*. **3**: 295 – 299 (1981).
3. Bakale, V.L. and Kolhe, R. L. Mitotic abnormalities induced by herbicides in *Solanum xanthocarpum*, Schard and Wendl. *Perspective in Cytology and Genetics*. **3**: 299 – 303. (1981).
4. Bakale, V.L. and Srinivasu, T. Impact of weedicides on Cytology of *Psoralea corylifolia*. *Proc. Conf. of Cytology and Genetics*. **2**: 193 – 196 (1981).
5. Bakale, V.L., Deshmukh, S. B. and Deshmukh, V. R. Effect of herbicides 2, 4-D on Cytology of *Malvastrum coromendelianus*. *Perspective in Cytology and Genetics*. **3**: 268 – 293 (1981).
6. Bayliss, M. W. The Effect of herbicides 2, 4-D on growth and mitosis in re-suspension cultures of *Daucus corota*, Lant. *Sci. Letters*. **8(2)**: 99 – 103 (1977).
7. Bobde, S. N. Comparative effect of herbicides on *Crotalaria juncea* L. *Ph. D. Thesis*, Nagpur Univ., Nagpur. (1993).
8. Crocker, B. H. 1953. Effect of 2, 4-D and 2, 4, 5-T on mitosis in *Allium cepa*. *Bot. Gaz*. **114**: 274 – 283.
9. Deshmukh, V. R. Effects of weedicides on cytomorphology of weeds. *Ph. D. Thesis*, Nagpur Univ., Nagpur.(1981).

10. Dharurkar, R. D. and Dnyansagar, V. R. Effect of herbicides on the root tips mitosis in *Eichhornia crassipes*. *Hyacinth control Journal*. **12**: 26 – 29 (1974).
11. Ditomaso, J. M. Herbicide induces polyamine accumulation. *42nd Annual meet. of Northeastern weed Science. Soc.* 52 – 53 (1988).
12. Doxey, D. and Rhodes, A. Effect of plant regulator. 2-methyl, 4-chlorophenoxy acetic acid on mitosis in *Allium cepa*. *Ann. Bot.* (N. S.) **13**: 105 – 111 (1949).
13. Dzheleпов, K. Cytogenetics effect of some chemical compound used in agriculture. 1. Herbicides. *Genetika 2. Seleksiya*. **21(4)**: 296 – 300. (1988).
14. Gopal, K. R. Herbicidal effects on cytomorphology of weed *Medicago sativa* Linn. . *Ph. D. Thesis*, Nagpur Univ., Nagpur. (1993).
15. Grover, I. S., Dhingra, A. K. Adhikari, N. and Ladhar, S.S. Genotoxicity of pesticides and plant system. *Progress in Clinical and Biological Research*. **340**: 91 – 106 (1990).
16. Hadke, S. M. Effect of herbicide on cytomorphology of weed *Psoralea corylifolia*. *Ph. D. Thesis*, Nagpur Univ., Nagpur. (1980).
17. Jain, S. B. Cytomorphological effects of weedicides on weed *Chenopodium album*. *Ph. D. Thesis*, Nagpur Univ., Nagpur. (1993).
18. Kolhe, R. R. Effect of herbicide on cytomorphology of farm weeds. *Ph. D. Thesis*, Nagpur Univ., Nagpur. (1979).
19. Kulkarni, G. B. Effects of agro-chemicals on *Crotalaria medicaginea* var. *luxurians*. *Ph. D. Thesis*, Dr. Babasaheb Ambedkar Marathwada Univ., Aurangabad. (1998).
20. Mauras, V. and Pareyre, C. Compared cytotoxicity of mixture of simazine, and paraquat in commercially prepared form. *Plant. Med. Phytother.* **11**: 53 – 57 (1977).
21. Mohandas, T. and Grant, W. F. Cytogenetics effect of 2, 4-D and amintrale in relation to nuclear volume and DNA contain in some higher plants. *Can. J. Genet. Cytol.* **14**: 773 – 783 (1972).
22. Moshier, L. Turgeon, A. J. and Penner, D. Effect of Glyphosate and siduron on turf-grass establishment. *Weed Sci.* **24**: 445 – 448 (1976).
23. Nygren, A. Cytomorphological studies of the Cytological, MCPA, 2, 4, 5-T, on *Allium cepa* . *Ann. Royal. Coll. Sweden* **16**: 723 – 728. (1949).
24. Prasad, G. and Das, K. Effect of some growth substances. *Cytol.* **42**: 232 – 239 (1977).
25. Rojik, I., Horrath, M. and Lonthal, I. A herbicide effect in the mitosis in *Vicia faba*. *Acta. Bot. Acad. Sci. Hung* **18**: 163-169 (1973).
26. Sawamura, S, Cytological studies on the effect of herbicides on plant cell *in vitro*. *Cytol.* **29**: 86 – 102 (1964).
27. Sheleg, Z. I. And Deeva, V. P. Cytogenetic following systematic application in barbely stands. *Dakalady Akademii Nauk.* **31(4)**: 365 – 367(1987).
28. Sikka, A. Sharma, A.K. Effect of some herbicides on plant chromosomes (*Allium spp.*) *Proc. Indian National Sci. Acad.* **42**: 299-307 (1976).
29. Strove, V. S. The cytogenetic activities of the herbicide atrazine, chloro-IPC and paraquat. *Spr. Genet.* **6**: 239-297 (1973).
30. Tomkins, D. J. and Grant, W. F. Monitoring natural vegetation for herbicides induced chromosomal aberration. *Mut. Res.* **36**: 73 – 83 (1976).
31. Trivedi, M. P. and Alok, A. K. Cytological effect of 2, 4-D *Mecardonia procumbens* (Miller) Small. *Acta. Bot. Indica.* **19(1)**: 84 – 86. (1991).
32. Upchurch, R. P. and Baird, D. D. Herbicidal action of Mono-573 as influenced by light and soil. *Proc. Western Soc. Weed Sci.* **25**: 44 (1972).
33. Vaughn, K. C. and Duke, S. O. Ultra structural effect of Glyphosate on *Glycin max* seedlings. *Pesticide Biochem and Physiol.* **26**: 56 – 65 (1986).