The Effect Of Herbicides On Root Tip Mitosis In Waterhyacinth

R. D. DHARURKAR and V. R. DNYANSAGAR

Lecturer in Botany, Biology Department, E.S.A. College of Science, Bassein Road, Dist. Thana (M.S.) India, and Professor and Head of the Botany Department, Nagpur University Campus, Nagpur-10 India

ABSTRACT

A gradual decrease in the percentage of waterhyacinth (*Eichhornia crassipes* (Mart.)Solms.) cells undergoing mitosis occurred as the concentration of 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), acrolein (acrolein), and NaPCP (sodium pentachlorophenate) was increased. Mitotic activity occurred in only 1% of the cells with silvex and 2% in the case of other two herbicides. Complete inhibition of mitosis was observed with 350 ppm of acrolein and 300 ppm of silvex and sodium pentachlorophenate. Various types of abnormalities such as fragmentation, stickiness, bridges, precocious movement of chromosomes, and abnormal shapes of nuclei were observed. Clumping of meristematic cells was noticed after treatment with sodium pentachlorophenate. Mitosis was not evident with 150 ppm of silvex and sodium pentachlorophenate or with 200 ppm of acrolein.

INTRODUCTION

Herbicides have become an important method for the control of waterhyacinth. Levan (10), Ryland (15), Deyson and Rollen (5), and Compton (2) reported the supressing action of (2,4-dichlorophenoxy)acetic acid (2,4-D) on the frequency of nuclear divisions in the cells of *Allium cepa* L. and *Pisum sativum* L. root tips. Ennis (7), Muhling et al. (12) and Bingham (1) observed various types of mitotic abnormalities induced by different herbicides.

A perusal of available literature indicates no information on the action of silvex, acrolein and sodium pentachlorophenate on the root tip mitosis. The present paper deals with the effect of these herbicides on root tip mitosis of waterhyacinth.

METHODS AND MATERIALS

Solutions of silvex, sodium pentachlorophenate, and acrolein were prepared with distilled water at concentrations from 50 to 400 ppm. Roots of four young waterhyacinth plants were exposed to each concentration of herbicide. Control plants remained in tap water. The root tips in each concentration were fixed after 24, 48 and 72 hr. and subsequently stored in 70% alcohol.

The root tips were hydrolized in 1N HCl for 15 minutes at 60 C, washed in tap water, and mordanted with iron-alum for 10 minutes. They were then washed thoroughly, stained with haematoxylin, and squashed in 45% acetic acid. The micropreparations were made permanent with n-butyl alcohol and mounted in euparal. The mitotic index was obtained by dividing the total number of cells undergoing mitosis by the total number of cells observed.

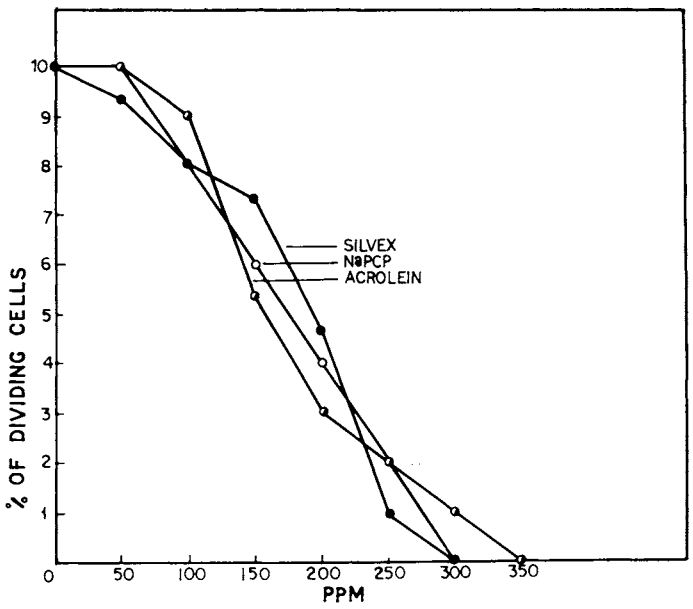

Figure 1. Decrease in the percentage of dividing root tip cells of waterhyacinth plants, 24 hr after treatment with silvex, sodium pentachlorophenate and acrolein.

OBSERVATIONS

Cells of waterhyacinth root tips were found with all stages of mitosis in the control and in roots exposed to 50 to 200 ppm of all the herbicides for 24 hr. A gradual decrease in the percentage of cells undergoing mitosis occurred as the concentration of the herbicides increased. At 250 ppm mitotic activity was seen only in 1% of the cells of root tips treated with silvex and in 2% of the cells exposed to sodium pentachlorophenate and acrolein (Figure 1). Complete inhibition of mitosis was observed at the 350 ppm of acrolein and 300 ppm of silvex and sodium pentachlorophenate.

Various types of abnormalities such as stickiness of chromosomes, fragmentation, abnormal shapes of the nuclei, precocious movement of chromosomes, multinucleolate condition, and bridges resulted in mitosis due to the action of the herbicides (Figure 2). Clumping of meristematic cells was noticed after treatment with sodium pentachlorophenate (Figure 3).

After the 48-hr treatment, mitosis was not observed at 50 to 150 ppm concentration for any of the herbicides. The mitotic index was 0.023, 0.033, and 0.042 for 150 ppm of silvex, sodium pentachlorophenate, and acrolein, respectively. The number of dividing cells were few in the treated roots and their percentage decreased at even higher concentrations (Figure 4). Mitosis, however, was not seen at the 200, 250, and 300 ppm treatments for silvex, sodium pentachlorophenate, and acrolein, respectively.

Mitotic abnormalities similar to those observed 24 hr after treatment were found in root tips 48 hr after treatment with any of the herbicides.

Normal mitosis was also observed in the cells of the control, 50 ppm of silvex, and 50 to 100 ppm of sodium pentachlorophenate and acrolein 72 hr after treatment. A decrease occurred in the percentage of cells undergoing mitosis as the concentration of the herbicides was increased (Figure 5). The mitotic index was 0.013, 0.021 and 0.038 for 100 ppm of silvex, sodium pentachlorophenate, and acrolein, respectively. Mitosis could not be observed in the cells treated with silvex or sodium pentachlorophenate at 150 ppm or more, nor with the 200 ppm or higher concentrations of acrolein.

DISCUSSION

Mallah and Dawood (11) observed a reduction in the number of cells undergoing mitosis in the roots of *Vicia marbonensis* L. treated with sodium arsenite ($NaAsO_2$). They explained the cause of reduction due to pre-prophase poisonous effects of the chemicals. Similar mitotic inhibiland (15) and Rojas-Garceduenas and Kommedhal (14).

Sawamura (17) observed that cells of staminal hairs of *Tradescantia reflexa* in early prophase treated with a 0.05% solution of 2,4-D did not show any further mitotic stages. The mitotic activity was also found to be delayed in his experiment. Such a delay in mitotic activity was also found in root tip cells of waterhyacinth.

Apparently these herbicides either prevent or limit spindle formation in the root tip cells of waterhyacinth. Gentner and Burr (8) reported that 4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline (nitralin) resulted in a multinucleolate condition in *Zea mays* L. root tips, which implies chromosome duplication and irregularity in size of nuclei and suggest unequal separation in terms of numbers. Ennis (7) using isopropyl carbanilate (propham) found mitotic aberrations in certain root and shoot cells of *Avena sativa* L. and *Allium cepa* L. He noticed anaphase bridges, fragments, binucleate cells, and increased number of chromosomes. However, in the present study an increase in chromosome number was not observed.

In waterhyacinth stickiness of chromosomes and compact masses of chromatin were seen mostly during the metaphase stage. Unrau and Larter (19) observed stickiness of chromosomes in large number of mother cells of barley (*Hordeum vulgare* L.). They explained that this condition of stickiness and also corrosion of chromosomes material might result from exchange in the chemical or electrostatic properties of nucleic acids of chromosomes.

Sawamura (18) studied the effect of non-hormonic herbicides on staminal hairs of *Tradescantia reflexa* Raf., stipular cells of *Vicia faba* L., petal cells of onion (*Allium cepa* L.) and pollen grains of *Tradescantia*, sp. and found that these herbicides induced various types of mitotic abnormalities. Darlington and Koller (4) expressed the opinion that maleic hydrazide (MH) induced chromosome breakage might be due to similar interphase breakage of chromosome thread, or due to stickiness of the chromosomes. The stickiness might be caused by the dissolution of the chromosome pellite or an excess of nucleic acid charge on the chromosome.

Irregular shapes of nuclei were observed in waterhyacinth due to herbicide treatment. Possible changes in the permeability or in the structure of the nuclear membrane resulted in irregular shapes and ovate nuclei.

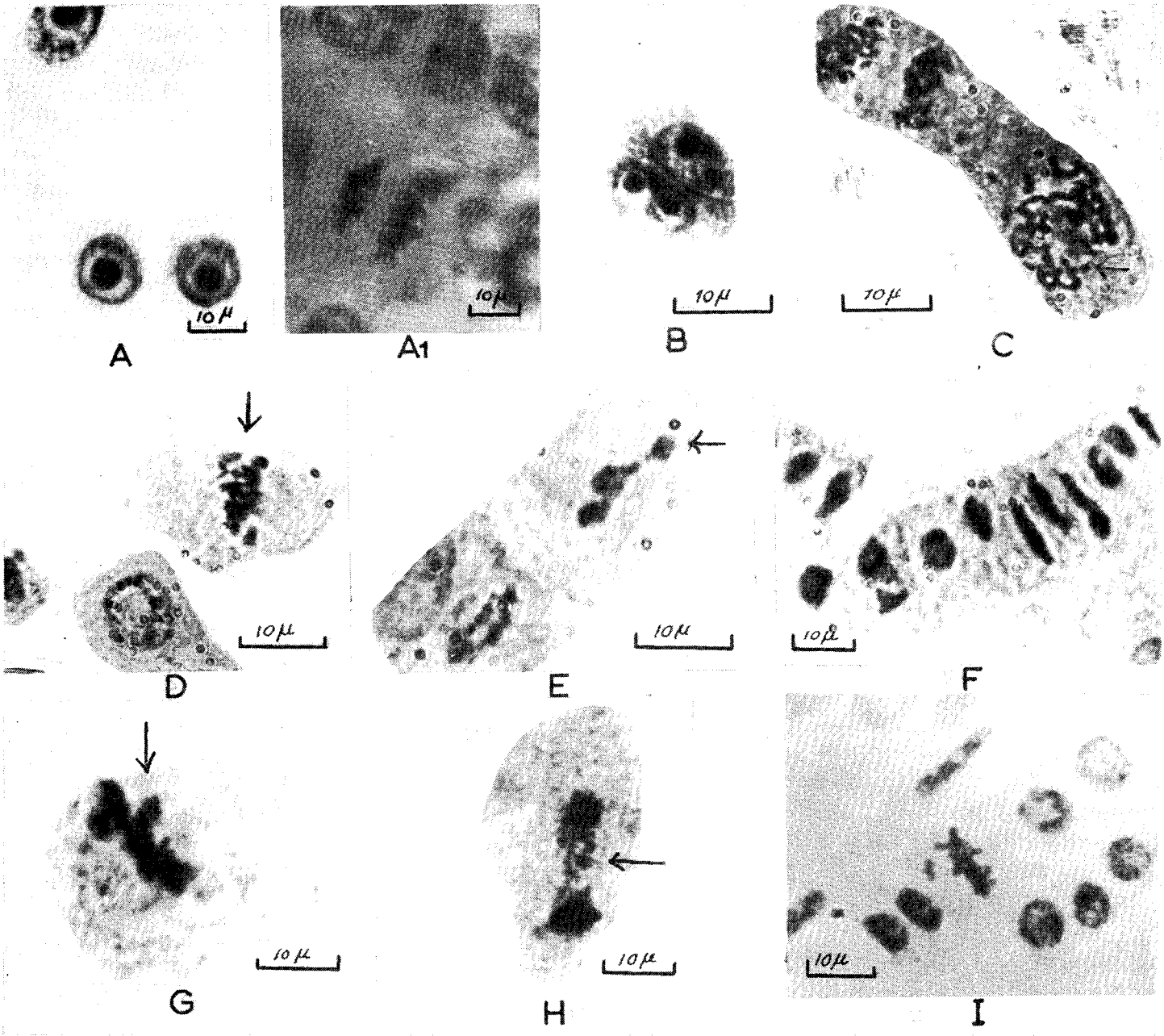

Figure 2. Waterhyacinth root tip cells after treatment with silvex, sodium pentachlorophenate and acrolein, showing: A. Single nucleolate condition, A_1. Anaphase of control plants. B. Multinucleolate condition, C. Fragmentation, D. Clumping of chromosomes, E. Grouping of chromosomes, F. Nuclei of various shapes, G. Stickiness of chromosomes, H. Formation of bridges, I. Precocious movement of chromosome. (Arrows indicate location of abnormality).

Leaper and Bishop (9) found that having two unsubstituted positions on the benzene ring opposite each other increased the effectiveness of the chlorophenoxy herbicides. So in silvex and sodium pentachlorophenate the substitution (especially with chlorine) at both the position may be playing a major role in inducing mitotic abnormalities.

Densely stained chromatin granules were observed in the waterhyacinth root tip cells. These granules probably suggest an inbalanced nucleic acid supply. Similar granules were seen by Crocker (3) in the root tip cells of onion after treatment with 2,4-D and (2,4,5-trichlorophenoxy)-acetic acid (2,4,5-T). However, origin of these granules is not known. Furthermore, he observed an increase in the number of cells during prophase apparently due to inhibition of spindle formation. This resulted in stickiness of chromosomes. Sax (16) thought that the stickiness and tendency of the chromosomes to clump at metaphase is a primary effect of X-ray treatment and regarded them as indicating an excess change of nucleic acid on the chromosomes. Sax (16), Doxey and Rhodes (6), and Nygren (13) stated that mitotic abnormalities induced by many herbicides were similar to those caused by X-rays and musturd gas.

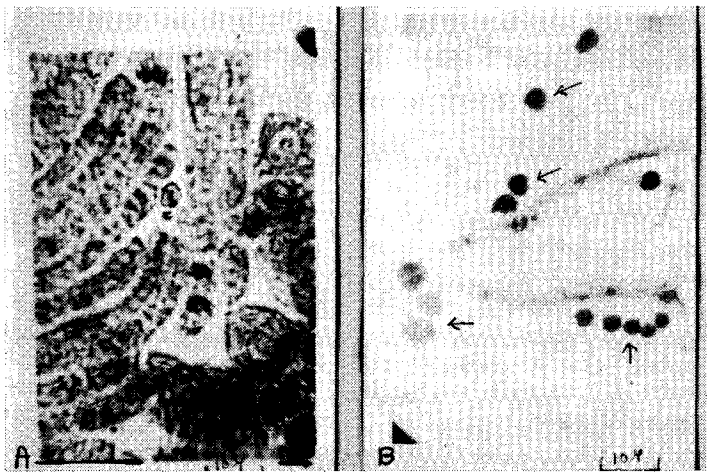

Figure 3. Root tip cells of waterhyacinth plants treated with sodium pentachlorophenate showing A. Control, B. Grouping of meristematic cells as indicated by arrow.

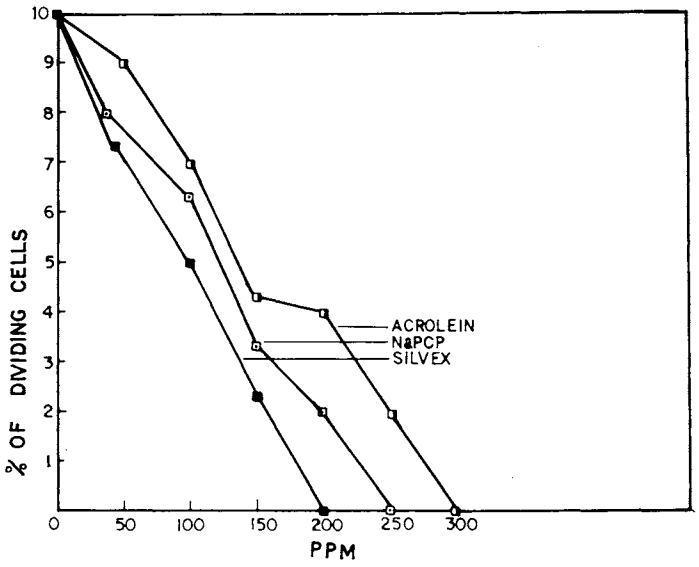

Figure 4. Decrease in the percentage of dividing root tip cells of waterhyacinth plants 48 hr after treatment with silvex, sodium pentachlorophenate and acrolein at different concentrations.

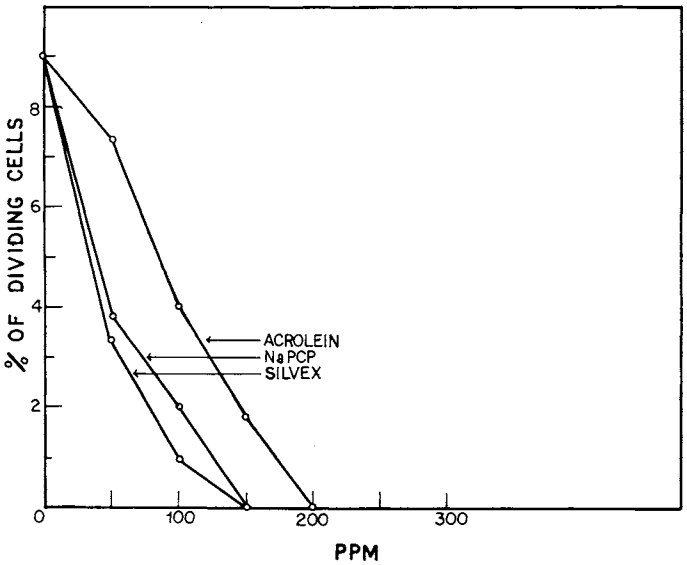

Figure 5. Decrease in the percentage of dividing root tip cells of waterhyacinth plants 72 hr after treatment with silvex, sodium pentachlorophenate, and acrolein at various concentration.

LITERATURE CITED

1. Bingham, S. W. 1968. Effect of DCPA on anatomy and cytology of roots. Weed Sci. 16:449-452.
2. Compton, W. 1952. The effect of maleic hydrazide on growth and cell division in *Pisum sativum*. Bull. Torrey. Bot. Club. 79:205-211.
3. Crocker, B. 1953. Effect of 2,4-dichlorophenoxy and 2,4,5-trichlorophenoxy acetic acid on mitosis in *Allium cepa*. Bot. Gaz. 115:274-280.
4. Darlington, C. D. and P. C. Koller. 1947. The chemical breakage of chromosomes. Heredity 1:187-221.
5. Deyson, G. and A. Rollen. 1951. Surl'action antimitotique an l'hydrazide. Compt. Rend. Acad. Sci. Paris. 233:820-821.
6. Doxey, D. and A. Rhodes. 1949. Effect of plant growth regulator 2-methyl, 4-chlorophenoxy acetic acid on mitosis in onion (*Allium cepa*). Ann. Bot. 13:105-111.
7. Ennis, W. B. 1948. Some cytological effects of O-isopropyl N-phenyl carbamate upon *Avena sativa*. Amer. J. Bot. 35:21-35.
8. Gentner, W. A. and L. G. Burr. 1968. Gross morphological and cytological effects of nitralin on corn roots. Weed Sci. 16:259-260.
9. Leaper, J. M. T. and J. R. Bishop. 1951. Relation of halogen position to physiological properties in the monoditrichlorophenoxy acetic acids. Bot. Gaz. 112:250-258.
10. Levan, A. 1939. Cytological phenomenon connected with the root swelling caused by the growth substances. Hereditas 21:87-96.
11. Mallah, G. S. and M. N. Dawood. 1956. Cytological studies of sodium arsenite on *Vicia narbonesis* roots. Alexandria. J. Agri. Res. 4:91-105.
12. Muhling, G. N., J. Van't Hof., G. B. Wilson and B. H. Griasbv. 1960 Cytological effects of herbicidal substituted phenols. Weeds 8:173-181.
13. Nygren, A. 1949. Cytological studies of the effect of 2,4-D, MCPA and 2,4,5-T on *Allium cepa*. Ann. Roy. Agric. Coll. Sweden. 16:723-728.
14. Rojas-Graceduenas, M. and T. Kommedhal. 1958. The effect of 2,4-D acid on radicle development and stem anatomy of soybean. Weeds 6:49-51.
15. Ryland, A. G. 1948. A cytological study of the effect of colchicine, I.A.A.; KCN and 2,4-D on plant cells. Elisha Mitchell Sci. Soc. 64:117-125.
16. Sax, K. 1941. The behaviour of X-rays induced chromosomal aberration in *Allium* root tip cells. Genetics 24:418-425.
17. Sawamura, S. 1964 Cytological studies on the effect of herbicides on plant cells in vivo I. Hormonic herbicides. Cytologia 29.86-102.
18. Sawamura, S. 1965. Cytological studies on the effect of herbicides on plant cells in vivo II. Non-hormonic herbicides. Cytologia. 30:325-348.
19. Unrau, T. and E. N. Larter. 1952. Cytogenetical responses of cereals to 2,4-D. A study of meiosis of plants treated at various stages of growth. Canadian J. Bot. 30:22-27.