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Cytological Effects of Fungicide Topsin in *Allium cepa*

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The biocides of the environment are known to induce cytological abnormalities in plant and animal cells. Especially the somatic cell abnormalities induced by fungicides and insecticides have been worked out by many workers (Sohier and Enaam 1968, Reddy and Rao 1969, Taramohan 1975, Kumar *et al.* 1977 and Sahu *et al.* 1981). In many cases the cytological abnormalities caused are very similar to those induced by mutagenic agents and hence screening of biocides for their mutagenic potential is gaining more importance. The present communication is a report on the cytological abnormalities induced by a fungicide Topsin in case of *Allium cepa.*

Materials and methods

Topsin [1, 2 bis (3-methoxycarbonyl-2-thiouride) benzene] developed by Nippon Soda Company Ltd., Tokyo, Japan, a systemic fungicide widely used in the agricultural field has been screened for its mutagenic and carcinogenic potential in the present study. Actively growing young roots of *Allium cepa* were treated with 100, 300, 500 and 1000 μg/ml of Topsin (70% WP) for 6, 24 and 48 hrs and recovery experiments were also carried out. Controls were maintained in all the cases. The treated as well as control roots were excised and fixed in aceticalcohol, after 24 hrs were transferred to 70% alcohol. The root tips were squashed in 0.5% haematoxylin. In each case 3000 cells were examined to record the data on mitotic indices and aberrant mitosis.

Results and discussion

Mitotic index, mitotic depression and % abnormalities observed are given in Table 1. The untreated cells showed normal division and no abnormalities have been recorded.

Cytotoxic effects of the fungicide such as total mitodepression, nuclear pycnosis and chromosome clumping was observed in considerable number. The chemical also induced C-metaphase and sticky metaphase (Fig. A). This was followed by disturbed anaphase giving rise to tripolar and tetrapolar cells (Fig. B). Sticky anaphase was also recorded in considerable number. Clastogenic effects such as

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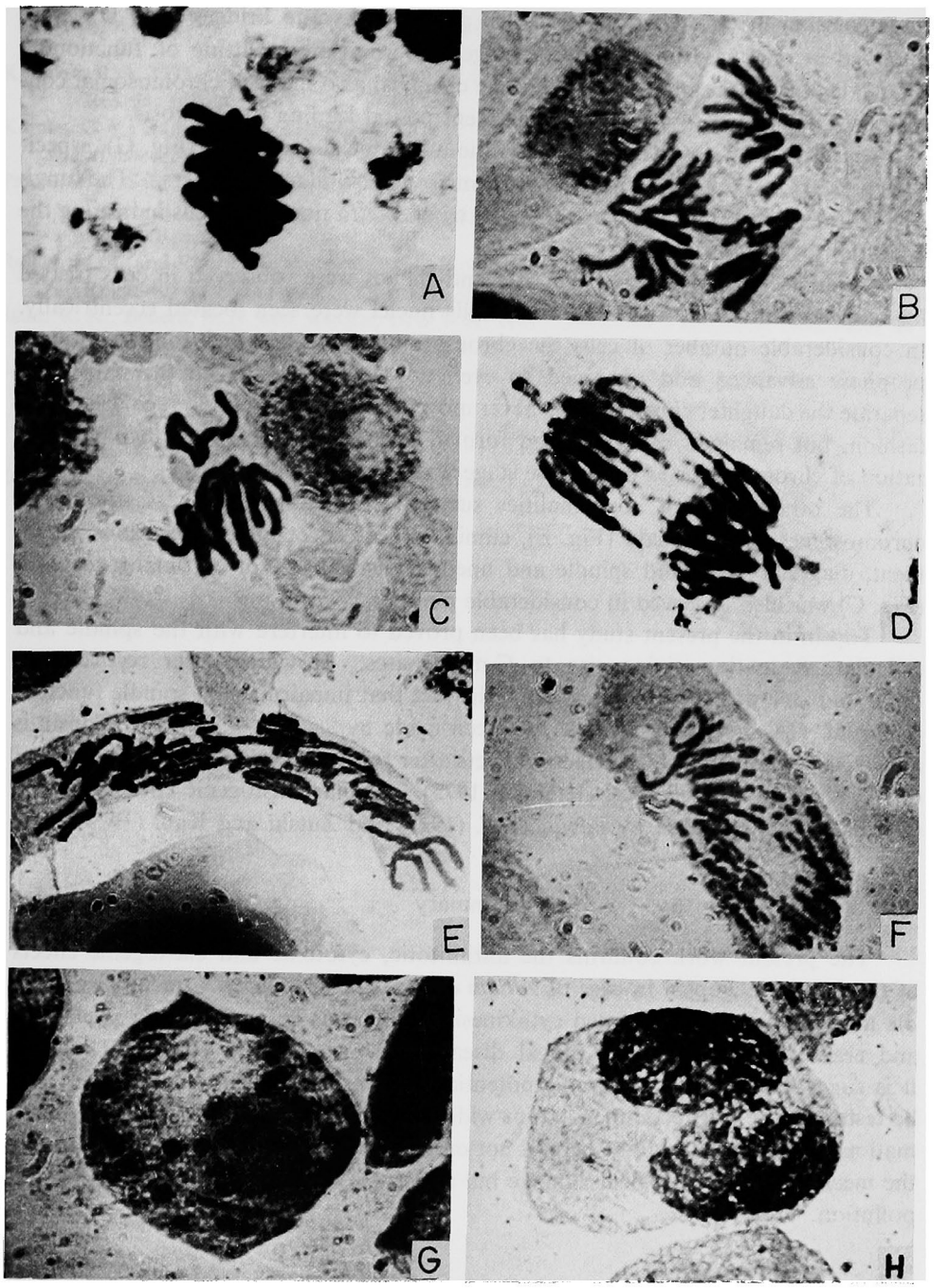
Table 1. Mitotic index, mitodepression and % abnormalities observed

Concentration and treatment period		Mitotic index	Mitodepression	C-metaphase	Bi and multi nucleate cells	Tri and tetra nucleate cells	Chromosomal fragments	Bridges	Chromosomal contraction	Chromatin erosion	Unequal distribution	Breaks and gaps	Diagonal pole and spindle	Micronuclei
100	6h	0.8	96.8	—	—	0.3	0.8	—	0.4	—	—	—	—	—
300	6h	0.8	96.8	—	—	0.3	0.9	—	0.8	—	0.4	—	0.3	—
500	6h	1.2	95.2	0.4	—	0.8	1.3	0.4	0.8	—	0.5	—	0.4	0.8
1000	6h	0.8	96.8	0.8	0.3	0.8	1.8	0.8	1.0	0.6	0.8	0.3	0.9	1.3
100	24h	1.2	95.2	1.6	0.6	1.0	2.3	1.0	1.0	0.9	1.7	0.3	1.3	1.9
300	24h	2.8	88.8	1.9	1.0	1.2	2.9	1.8	1.8	0.6	3.1	0.8	1.5	3.4
500	24h	3.2	87.2	2.3	1.2	1.8	3.0	2.0	1.9	1.4	3.8	0.6	1.8	6.8
1000	24h	4.0	84.0	6.6	1.4	2.0	3.8	2.9	1.8	1.9	5.3	0.9	2.0	8.1
100	48h	4.0	84.0	8.2	1.8	2.3	4.9	1.9	2.0	2.0	8.9	1.3	4.8	5.3
300	48h	4.2	83.2	9.0	2.5	2.8	5.6	3.8	2.8	2.8	10.9	1.8	6.9	5.8
500	48h	4.6	82.8	10.3	3.6	3.9	7.9	4.8	3.4	2.8	11.4	3.4	8.1	6.1
1000	48h	3.2	87.2	11.4	4.8	4.9	10.4	6.9	5.4	3.6	13.8	6.1	9.6	6.6
Control		25	—	—	—	—	—	—	—	—	—	—	—	—



Figs. A–H. A, sticky metaphase. ×1800. B, a tetrapolar anaphase. ×2000. C, cell showing unequal distribution of chromosomes. ×1700. D, anaphase bridge. ×1800. E, cell showing scattered chromosomes at meta-anaphase. ×2100. F, cell with fragmentation of chromatids. ×2300. G, cell with micronuclei. ×2100. H, a binucleate cell. ×2000.

fragmentation of chromosomes (Fig. F), gaps, breaks and bridges (Fig. D) were observed at anaphase and telophase stages. Some times splitting of functioning spindle lead to multipolar divisions. The chemical also induced chromosomal contraction at all concentrations and treatment period leading to C-mitosis.

An interesting observation made is the micronuclei formation (Fig. G), especially the cells at 24 hrs treatment showed micronuclei in large numbers. The fungicide treatment also resulted in bi, (Fig. H) tri and tetra nucleated cells indicating the inhibition of cytokinesis.

Differences in nuclear pattern, size and shapes were observed in cells treated for longer duration of time. In many cells nuclei were seen located eccentrically. In considerable number of cells the chromosomes never showed condensation as prophase advanced and remained so even at metaphase. When the chromatid separate the daughter chromosomes never moved to opposite poles in regularly order fashion, but remained entangled and formed restitution nuclei. Non synchronization of chromosomes at metaphase stage was observed frequently.

The other types of abnormalities such as chromatin erosion, scattering of chromosomes at metaphase (Fig. E), elimination of chromatid, precautious movement, diagonal pole and spindle and unequal distribution of chromatin material (Fig. C) was also observed in considerable number.

Topsin in the present study has been proved to interfere with the spindle and cell plate formation giving rise to C-metaphases. However, after recovery no tetraploid cell was recorded indicating the fact that impairment of spindle function is partial. A similar observation has been made by Sahu *et al.* (1981). Topsin is also recorded to produce clastogenic effects after recovery period indicating its effect on G_2 phase of the cell cycle (Kihlman 1975). Similar clastogenic effects in plant cells have been recorded by Panda *et al.* (1977) and Zutshi and Kaul (1975).

Summary

The present work confirms the antimitotic, cytotoxic and clastogenic effects of the fungicide Topsin in case of *Allium cepa*. It also induced C-metaphase, spin dle abnormalities and inhibited cytokinesis. Topsin is being used as a protectant and eradicant in controlling fungal diseaese of various crop plants. Therefore it is suggested that the mutagenic potential of Topsin on such crop plants should be tested before it is recommended for wide use in the agricultural field. The information on crop plants will be helpful not only from the view point of understanding the mechanism of cytological damage but also for its implication on environmental pollution.

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References

Kihlman, B. A. 1975. Root tips of *Vicia faba* for the study of chromosomal aberrations. Mutat. Res. **31**: 401.

Kumar, C. V., Ramiah, N. J. and Subadra, J. 1977. Cytological effects of fungicide Nystatin in *Allium cepa*. Indian J. Exp. Biol. **15**: 1071–1072.

Panda, B. B., Sahu, R. K. and Sharma, C. B. S. R. 1977. Cytogenetic hazards from fen sulfothion, Dexon (DAPA) and its degradation product dimethyl phenylene diamine (IMPDA). Mutat. Res. 53: 247.

Reddy, M. A. and Rao, B. V. R. 1969. The cytological effects of insecticides (Dimecron-100 and Rogar-40) on *Vicia faba* L. Cytologia **34**: 408–417.

Sahu, R. K., Behera, B. N. and Sharma, C. B. S. R. 1981. Effects of a fungicide, Dexon and its derivatives on root tip systems. The Nucleus **24** (1, 2): 60–65.

Sohier, A. M. and Enaam, A. M. 1968. Cytological effects of pesticides II. Meiotic effects of some phenols. Cytologia **33**: 21–33.

Taramohan, S. 1975. Cytological effects of fungicide plantvax and vitavax on *Allium cepa*. Curr. Sci. **44**: 813–814.

Zutshi, U. and Kaul, B. L. 1975. Cytogenetic activity of some common fungicides in higher plants. Cytobios **12**: 61.