Pak.J.Bot., 26(1): 69-74, 1994.

CYTOTOXIC EFFECTS OF HERBICIDE RONSTAR ON MERISTEMATIC CELLS OF *ALLIUM CEPA* L.

SADIA K. BUTT AND AHSAN A. VAHIDY

Department of Genetics,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Actively growing root tips of onion (*Allium cepa* L.) were used to evaluate cytotoxic effects of herbicide, Ronstar. The dividing cells exposed to 1000, 2000, 3000 and 4000 ppm of Ronstar for 3, 6, 9 and 12 h durations showed a significant reduction in mitotic index especially in treatments of higher concentrations and longer durations. Stickiness and fragmentation of chromosomes were the most frequent aberrations observed, bi- and multi-nucleate cells were noted as well. Abnormalities in lesser frequencies included C-mitosis and polyploidy. Such chromosomal abnormalities may effect adversely the vigor, fertility, yield or competitive ability of the plants exposed to the herbicide.

Introduction

Many herbicides have mutagenic and/or carcinogenic effects on biological systems inducing chromosomal damages in the crop plants. The link between chromosomal anomalies produced by herbicides and gene mutation has been demonstrated by Panda & Sharma (1979). The use of chromosomal aberrations induced by herbicides in crop plants may, therefore, be accepted as indicator of genetic damages.

The herbicide Ronstar available in liquid form is used as an effective weed killer for rice crop. It contains 120 g/l oxadiazon, which is 5-tert-Butyl-3- (2,4-dichloro-5-isopropoxy phenyl) -1, 3, 4- oxadiazol- 2- one with molecular formula: $C_{15}H_{18}Cl_2N_2O_3$. Ronstar is applied to the rice crop during pre-emergence or post-emergence. It has low vapor pressure and low solubility in water, thus tends to persist in the soil for a long time. This herbicide causes inhibition of protoporphy rinogen oxidase, which results in the uncontrolled autoxidation of protoporphyrinogen to protoporphyrin IX. The latter is a potent photosensitizer, thus its massive accumulation causes rapid photobleaching of green tissues in plants (Matring & Scalla, 1988). In the present study cytotoxic effects of the herbicide Ronstar were evaluated on the dividing cells of the root tips of onion (*Allium cepa* L).

Materials and Methods

Locally obtained onion bulbs of equal size were cleaned, rooting area shaved and placed on beakers with basal portion dipped in water. After four days, actively growing root tips of about 2-3 cm length were harvested and treated with Ronstar @ 1000, 2000, 3000 and 4000 ppm for 3, 6, 9 and 12-h. The experiment was performed in a Randomized Complete Block Design with 3 replications. After treatments the root tips were transferred to 1.8% aceto-orcein for 24 h. Root tips fixed and without any treatment of herbicide transferred directly to aceto-orcein were kept as control. The stained material was squashed for examining abnormalities and mitotic index.

Table 1. Mitotic index, percentage of normal and abnormal mitotic cells out of total dividing cells after treating root tips of *Allium cepa* with herbicide Ronstar in varying concentrations for different durations. All original readings were transformed by arcsine transformation.

Concentration in ppm	Duration in hours	Mitotic index	Normal cells	Abnormal cells	Stickiness & bridges	Fragmentation & polyploidy	Star- & c-metaphase anaphase	Laggards & multipolar	Ring chromosomes & bi/multi nucleate cells	Miscellaneous abnormalities
Control	-	19.9±1.9	90.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1000	3	21.0±3.0	43.7	46.3±0.5	19.4	6.4	0.0	0.0	0.0	7.8
	6	16.3±1.3	43.1	46.9±1.7	17.1	10.5	5.8	0.0	1.9	5.2
	9	16.7±0.9	40.8	49.1±2.4	29.0	1.8	1.8	0.0	3.6	2.0
	12	18.4±0.8	38.5	51.4±3.2	26.6	4.3	3.8	0.0	0.0	6.1
2000	3	16.2±3.1	37.5	52.5±2.0	19.0	18.5	4.9	0.0	0.0	0.0
	6	16.1±1.8	35.8	53.3±3.9	20.2	20.4	0.0	3.9	0.0	1.7
	9	16.4±1.5	35.6	54.4±1.3	25.0	12.1	2.2	2.2	0.0	0.0
	12	17.4±3.8	33.9	56.1±0.5	25.0	4.7	8.5	0.0	0.0	5.6
3000	3	16.2±1.7	33.8	56.2±5.2	25.6	12.0	3.4	0.0	0.0	4.3
	6	19.1±1.4	31.6	58.3±3.4	22.6	7.8	1.9	1.9	1.6	15.6
	9	18.4±1.3	31.0	59.0±1.0	21.5	11.9	5.5	0.0	1.5	10.6
	12	17.4±3.4	29.2	60.8±1.0	25.9	4.5	2.0	0.0	2.0	9.0
4000	3	16.6±1.7	29.4	60.6±1.2	20.1	17.6	2.0	6.0	1.9	5.4
	6	21.3±3.1	26.3	63.6±0.8	21.2	18.6	4.1	5.9	0.0	8.6
	9	21.9±4.8	24.6	65.4±2.1	27.8	16.6	5.2	6.2	1.8	9.3
	12	16.7±3.1	23.3	66.7±1.7	25.5	10.5	3.4	4.3	6.3	12.2

The data obtained from the different treatments were statistically analysed on computer using SPSS/PC$^+$ programme. Data, pertaining to several classifying and dependent variables, were recorded through Data Entry II (DE II). The mitotic index was computed as "Number of dividing cells/total number of cells x 100". The abnormalities were also expressed as the percent of total dividing cells. All such readings were subjected to arcsin transformation. Since the experiment was conducted in a 4 x 4 factorial plan in a Randomized Complete Block Design with three replications, the analysis was conducted through a command Manova (Multivariate Analysis of Variance) as exemplified below:

MANOVA TRMITIND BY REP (1,3) CON (1,4) DUR (1,4) / DESIGN = REP, CON, DUR, CON BY DUR

where REP = replications, CON = concentrations and DUR = durations.

Results and Discussion

The exposure of herbicide Ronstar at various concentrations for different durations to the dividing cells of onion altered the mitotic indices (Table 1). It was slightly higher than the control on treatments of 1000 ppm for 3 h and 4000 ppm for 6 and 9 h. ANOVArevealed that with respect to different concentrations the decrease in mitotic index was significant while with respect to durations it was non-significant (Table 2). Significant decrease in mitotic index suggests mitodepressive action of the compound, indicating that Ronstar interferes in the normal sequence of mitosis. Such reduction in mitotic activity could be due to inhibition of DNA synthesis. Several other herbicides like isoproturon (Chauhan & Sundararaman, 1990b), Garlon-4 (El-Khodary *et al.*, 1989), Glean (Badr & Ibrahim, 1987), carbamates (Ennis, 1948; Amer & Farah, 1974; 1976), trifluralin (Lingnowski & Scott, 1972), carbetamex and paradone plus (Badr, 1983) also induced mitotic inhibition.

The frequency of normal prophase, metaphase and anaphase cells decreased with an increase in concentration and duration of the treatment. Short term treatment of 3

Table 2. Analyses of variance for mitotic index and abnormal dividing cells in root tips of *Allium cepa* exposed to Ronstar at different concentrations and durations.

Sources of variation	Degrees of freedom	Mean Squares	
		Mitotic index	Abnormal dividing cells
Replication (R)	2	9.62	19.15
Concentration(C)	3	25.54*	528.46**
Duration(D)	3	2.02$^{n.s.}$	51.45**
CXD	9	12.66$^{n.s.}$	1.34$^{n.s}$
Error	30	6.54	4.69

*Significant at 0.05 level of probability. **Significant at 0.01 level of probability. $^{n.s.}$Non significant.

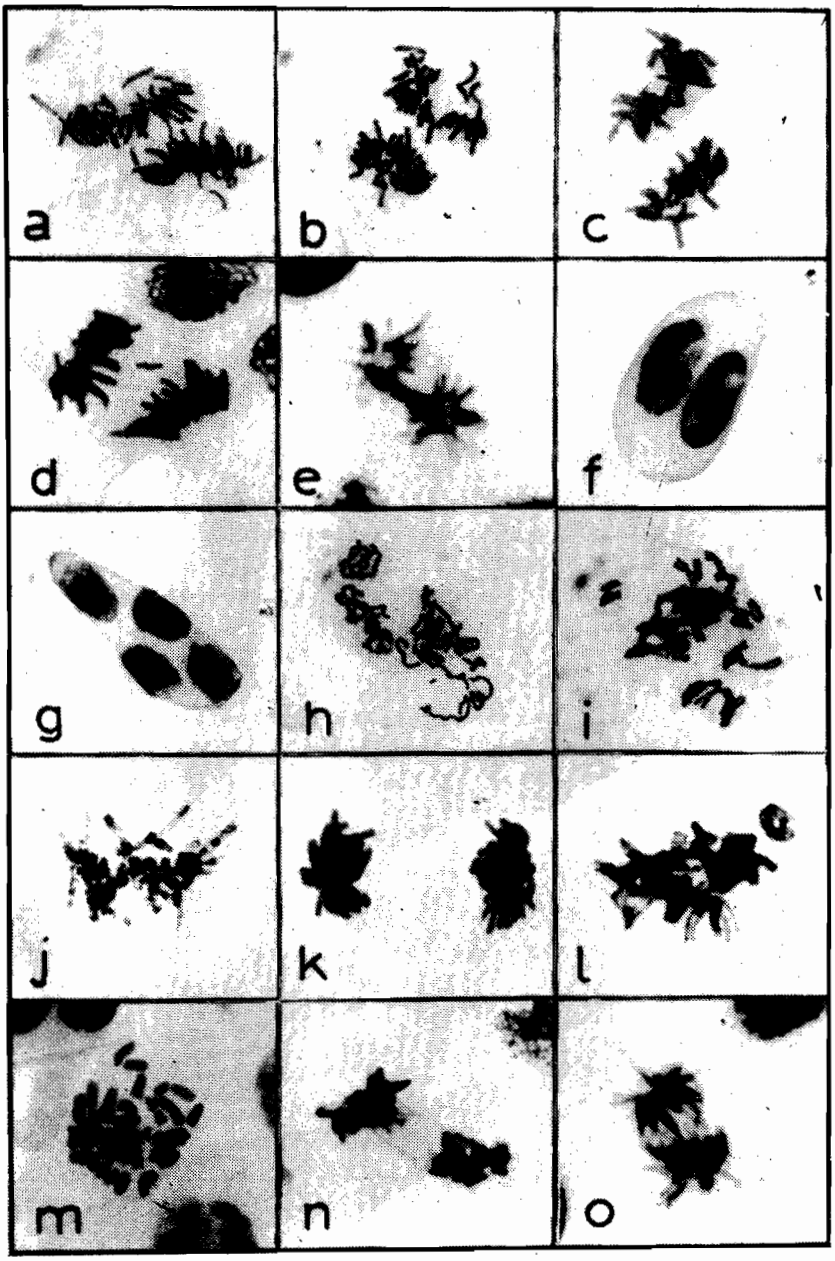

Fig. 1. Some mitotic abnormalities in dividing cells of *Allium cepa* exposed to different treatment combinations of herbicide Ronstar. (a) Polyploidy at anaphase. (b) Tripolar anaphase. (c) Tetrapolar anaphase. (d) Laggard formation. (e) Star formation. (f) Binucleate cell. (g) Multinucleate cell. (h) Disturbed prophase. (i) Disturbed metaphase. (j) Stickiness at metaphase. (k) Stickiness at anaphase. (l) Ring chromosome formation. (m) Chromosome breaks at metaphase. (n) Double bridges at anaphase. (o) Multiple bridges at anaphase.

and 6 h revealed low frequency while long term treatments of 9 and 12 h revealed high frequency of prophase cells. ANOVA for abnormal dividing cells (Table 2) revealed that with respect to different concentrations and durations of Ronstar treatments increase in cytological aberrations was highly significant while with respect to interaction between concentrations and durations cytological aberrations were nonsignificant. Cytological aberrations produced by Ronstar are as follows:

1. Mitotic abnormalities include C-metaphase, polyploidy, multipolar anaphase, laggards, star metaphase, bi- and multinucleate cells and disturbed mitotic stages. C-metaphase was frequently produced at 3000 and 4000 ppm. It is produced as a result of inhibition of spindle fiber formation (Deysson, 1968). These cells after restitution give rise to polyploid cells (Fig. 1a) which were most frequently found in treatments of 2000 and 4000 ppm concentrations, such cells lead to the formation of multipolar anaphase (Figs. 1b & 1c). Lagging chromosomes (Fig. 1d) were produced due to impairment of mitotic apparatus, treatments of 4000 ppm gave high frequency of laggards. Star metaphase (Fig. 1e) was frequently found in 1000 and 3000 ppm. It is considered as being fore step of complete disturbance of spindle.

Formation of bi-nucleate (Fig. 1f) and multi-nucleate (Fig. 1g) cells were observed in some treatments of 1000, 3000 and 4000 ppm. Ronstar interferes in the process of cytokinesis by affecting the functions of microtubules and golgi complex, which leads to the formation of bi-nucleate cells. Multi-nucleate cells are formed as a result of proceeding multipolar mitosis and failure of cell plate formation. Bi- and multi-nucleate cells lead to the aneuploid cells in subsequent mitotic divisions (Chauhan & Sundararaman, 1990a). Concentrations of 3000 and 4000 ppm induced a considerable percentage of disturbed prophase (Fig. 1h), metaphase (Fig. 1i) and anaphase. These are formed as a result of inhibition of spindle formation (El-Khodary *et al.*, 1990).

2. Chromosomal stickiness (Figs. 1j & 1k) was induced by almost all the treatments. It is a chromatid type aberration (Klasterska *et al.*, 1976), and is induced by the effect of herbicide on chromosomal proteins attributed to the improper folding of chromosome fibers, which render the chromatids connected by means of subchromatid bridges (Badr & Ibrahim, 1987).

3. Chromosomal anomalies include chromosomal breaks, bridges and formation of ring chromosomes (Fig. 1l). Chromosome breaks (Fig. 1m) were produced by all treatments except the one of 1000 ppm for 3h duration. Herbicides exert a clastogenic action on chromosomes, which is generally regarded to involve an action on DNA. Formation of anaphase bridges (Figs. 1n & 1o) may be due to chromosome breaks and reunion (Tomkins & Grant, 1972; Ahmad & Yasmin, 1992), or due to chromosomal stickiness (Kabarity *et al.*, 1974).

Chromosomal aberrations induced by Ronstar are similar to those induced by other herbicides, chemical mutagens and radiations. Such chromosomal irregularities may affect adversely the vigor, fertility and yield of exposed plant. Pesticides with such actions can also alter the genetic constitution of crop and other living organisms, resulting in mutational changes which could be very dangerous. There is need to study the morphological and biochemical effects of pesticides on agricultural crops. The present results of herbicide Ronstar weigh against wide application of such chemicals in weed control and suggest the need for mutagenicity testing of herbicides using cytogenetic and other systems.

References

Ahmad, S. and R. Yasmin. 1992. Effects of methyl parathion and tri-miltox on the mitosis of *Allium cepa*. *Cytologia*, 57: 155-160.

Amer, S.M. and O.R. Farah. 1974. Cytological effects of pesticides VII. Mitotic effects of isopropyl-N-phenyl carbamate and Duphar. *Cytologia*, 40: 21-29.

Amer, S.M. and O.R. Farah. 1976. Cytological effects of pesticides VIII. Cytological effects of carbamate pesticides IDC, Rogor and Duphar. *Cytologia*, 41: 597-606.

Badr, A. 1983. Mitodepressive and chromotoxic activities of two herbicides in *Allium cepa*. *Cytologia*, 48: 451-457.

Badr, A. and A.G. Ibrahim. 1987. Effect of herbicide Glean on mitosis, chromosomes and nucleic acids in *Allium cepa* and *Vicia faba* root meristem. *Cytologia*, 52: 293-302.

Chauhan, L.K.S. and V. Sundararaman. 1990a. Effect of substituted ureas on plant cell. I. Cytological effects of iso-proturon on root meristem cells of *Allium cepa*. *Cytologia*, 55: 91-98.

Chauhan, L.K.S. and V. Sundararaman. 1990b. Effect of substitute ureas on plant cell. II. Ultrastructural effects of iso-proturon on the root meristem cells of *Allium cepa*. *Cytologia*, 55: 99-105.

Deysson, G, 1968. Antimitotic substances. *Intern. Rev. Cytol.*, 24: 99-148.

El-Khodary, S., A. Habib and A. Haliem. 1989. Cytological effect of herbicide Garlon-4 on root mitosis of *Allium cepa*. *Cytologia*, 54: 465-472.

El-Khodary, S., A. Habib and A. Haliem. 1990. Effect of herbicide tribunil on root mitosis of *Allium cepa*. *Cytologia*, 55: 209-215.

Ennis, W.B. 1948. Responses of crop plants to isopropyl-N-phenyl carbamate. *Bot. Gaz.*, 109: 473-493.

Kabarity, A., A. El-Bayoumi and A.A. Habib. 1974. Effect of morphine sulphate on mitosis of *Allium cepa* L., root tips. *Biol. Plant.*, 16: 275-282.

Klasterska, I., A.T. Natarajan and C. Ramel. 1976. An interpretation of the origin of subchromatid aberrations and chromo some stickiness as a category of chromatid aberration. *Hereditas*, 83: 153-162.

Lingnowski, E.M. and E.G. Scott. 1972. Effect of trifluralin on mitosis. *Weed Sci.*, 20: 367-370.

Matring, M. and R. Scalla. 1988. Studies on the mode of action of acifluorfen methyl in non-chlorophyllous soybean cells: accumulation of tetrapyrols. *Plant Physiol.*, 86: 619-622.

Panda, B.B. and C.B.S.R. Sharma. 1979. Organophosphate induced chlorophyll mutations in *Hordeum vulgare*. *Theor. Appl. Genet.*, 55: 253-255.

Tomkins, O.J. and W.F. Grant. 1972. Comparative cytological effects of pesticides menzon, metrobromuron and tetra chloroisophthalonitrile in *Hordeum* and *Tradescantia*. *Can. J. Genet. Cytol.*, 14: 245-256.

(Received for Publication 10 August 1993)