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Cytological Effects of Blitox on Root Mitosis of *Allium cepa* L.

Anirban Paul, Sudipa Nag and Kaushik Sinha

Department of Botany, Rampurhat College, Rampurhat, Birbhum, West Bengal, India

Abstract **-** The genotoxic potential of blitox (fungicide) was investigated by using chromosome aberration in *Allium cepa* root tip cells. *Allium cepa* roots were treated with 2g/lt., 3g/lt. and 4g/lt. concentrations of blitox and distilled water as control at 4 hours, 8 hours and 12 hours duration. The results indicated that blitox significantly increased the genetical abnormalities at all concentrations and treatment periods when compared with their controls and this increase was dose-dependent for the 4, 8 and 12 hours treatments. On the other hand, blitox significantly decreased the mitotic index (MI) in all treatments when compared with their controls. This study indicates that blitox decreased the mitotic index and produced clastogenic and aneugenic types of abnormalities in *Allium cepa* root tip cells. The data obtained in this study showed that chromosomal aberrations assay can be used as an important test battery to detect possible genotoxicity of chemicals in *Allium cepa*.

Index Terms **-** *Allium cepa*, blitox, chromosomal aberrations, fungicide, genotoxic effect, Mitotic index

I. INTRODUCTION

Fungicides are most commonly used against diseases of agricultural crops in many countries of the world. Although fungicide application results in quick and high control of the diseases, the widespread use of these chemicals may cause environmental and food contaminations (Fisun and Rasgele, 2009; Tort and Turkyilmaz, 2003). Pollution is a major problem which lowers the quality of life in various aspects. Environmental pollutions may be mutagenic or toxic for all living organisms (Yuzbasioglu et al., 2008; Grover, 1999). Constant use of these chemicals may result in changing the hereditary constitution of an organism (Wuu and Grant, 1967; Wuu and Grant, 1966). When some chemicals accumulated within food chain to a toxic level, these chemicals affect directly the public health (Fisun and Rasgele, 2009). In context, Dryanowska (1987) and Cantor et al. (1992) showed that the frequency of cancer increases among people who have been exposed directly or indirectly to pesticides or fungicides. So those should be screened before the use in order to select which are least toxic (Mann, 1977). Generally, toxic effects of environmental pollutants cause genetic damage on plant cells. But toxicity is not always correlated with genotoxicity (Fisun and Rasgele, 2009; Kovalchuk et al., 1998)

The fungicide blitox is a commercial form of copper oxychloride 50 and is used extensively in the agricultural area. Blitox is effective in control of bacterial blight, leaf spot, early and late blight, brown rot, bacterial canker, leaf curl, downy mildew and powdery mildew diseases. There is no study available on the cytogenetic effects of this chemical in the plant systems. Induction of mitotic abnormalities on root tip cells of plants may cause a decrease in mitotic index (Panneerselvam et al., 2012; Bushra et al., 2002; Kovalchuk et al., 1998).

The aim of this study was to investigate the chromosomal aberration induced by fungicide blitox in the root tips of *Allium cepa* L. and also to determine the relation between mitotic chromosomal aberration with mitotic index.

II. MATERIAL AND METHODS

The fungicide used in this work was copper oxychloride 50 contains a group of M^2 fungicide, whose trade name is blitox. Its molecular formula is $CuCl_2 \bullet 3Cu(OH)_2$ and molecular weight is 427.2 .

The plant used as test material was *Allium cepa* L. (2n= 16). Ten clean and healthy bulbs of *A. cepa* were chosen for each treatment group. Before starting the experiments, dry scales of bulbs were removed and then the onion bulbs were induced to root by placing them on culture tubes filled with distilled water with the base of the onion touching the surface of the water at room temperature. When the roots reached 1.5 - 2 cm in length, they were treated with different concentrations of fungicide blitox dissolved with distilled water (2 g/lt., 3 g/lt. and 4 g/lt.) for 4, 8 and 12 hours. Controls were also treated with distilled water for the same time periods. The concentrations were chosen according to their dose of application in agricultural field to control different diseases.

For mitotic studies, the root tips of *A. cepa* were fixed in 1:3 acetic acid – ethyl alcohol mixture for overnight, followed by 5-7 minutes treatment in 45% acetic acid. Then root tips were hydrolyzed in 1 (N) HCl at 60^0C for 5 minutes, followed by

staining with 2% aceto-orcein following the methods described by Sharma and Sharma (1980).

After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control. Effect of chemical treatment and control on different chromosome plates were observed under light microscope. To determine the effects of this chemical on mitotic index, 2000 cells were scored in control group and in each treated group. The mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells. Cytological abnormalities were also observed and scored.

In this study a statistical analysis was done to estimate standard error (SE) of the results. Photomicrographs of cells showing chromosomal aberrations as well as showing normal mitosis were taken using Olympus microscope.

III. RESULTS

Microscopic examination of squashed *Allium cepa* L. root tip meristem cells showed that blitox treatments induced a number of mitotic abnormalities when compared with control. The increase of mitotic abnormalities was dependent on the increasing treatment periods and concentrations (Figure-2). The most common chromosomal abnormalities were stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, clumping and fragmentation (Figure-3).

Blitox caused a decrease in mitotic index (MI) at all the treatment groups. MI decreased in treated plants with different concentrations and treatment periods (Table-1, Figure-1).

IV. DISCUSSION AND CONCLUSION

Fungal diseases cause extensive crop losses each year. The fungicide blitox is widely used to control fungal diseases in onion and other crops. According to Pest Control Products Act (23-Dec-2008-4320) copper oxychloride 50 is a warning poison. There is no published data available on the cytogenetic effects of copper oxychloride in plant systems. Chromosomes of *Allium cepa* L. can be used for testing the potential poison in mitotic cells (Yuzbasioglu, 2003; Celik, 2006; Smaka-Kinel et al., 1996; Grant, 1982).

Mitotic index is an acceptable measure of cytotoxicity for all living organisms (Smaka-Kinel et al., 1996). The cytotoxicity level can be determined by the decreased rate of mitotic index. A decrease of mitotic index below 50% usually has lethal effects (Panda and Sahu, 1985). If mitotic index decreases below 22% of control, that it causes sub lethal effects on test organism (Antonsie-Wiez, 1990). According to many investigators, abnormalities due to inhibition of spindle formation such as c-mitosis, multipolarity, stickiness reflects high toxicity of pollutants (Lazareva et al., 2003; Kovalchuk et al., 1998; Haliem 1990; Amer and Ali, 1974).

In the present study, blitox decreased the mitotic index at all concentrations and at all treatment periods when compared with control. Similar type of result is also found by Fisun and Rasgele (2009) on *Allium cepa* by using fungicide raxil. The decrease of mitotic index was dose dependent. At all treatment periods, the highest concentration of blitox decreased mitotic activity more than other used concentrations. The percentage of mitotic index decreased with the increase of cells with c-mitosis, stickiness, laggards, anaphase and telophase bridges etc. Since it decreased the MI in root tip cells of *Allium cepa* L. Blitox can be accepted as a toxic agent in this study.

Blitox significantly increased the percentage of abnormal cells at all concentrations and treatment periods in mitotic cell divisions when compared with control. It has been shown by many investigators that several other fungicides induce chromosomal abnormalities in different plants (Badr, 1998; Pandy et al., 1994; Armbruster et al., 1991; Badr, 1983; Behera et al., 1982; Mann, 1977). In this study, the most common abnormalities were stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, clumping and fragmentations in cell division.

Chromosomal stickiness is characterized by chromosomal clustering during any phase of the cell cycle. Stickiness and clumping may be caused by genetic and environmental factors. Several agents have been reported to cause chromosomal stickiness (Panneerselvam et al., 2012; Caetano-Pereira et al., 1998; Badr and Ibrahim, 1987). Gaulden (1987) postulated that sticky chromosomes result from the defective functioning of one or two types of specific non-histone proteins involving chromosome organization which are needed for chromatid separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them or by the direct action of mutagens (Turkoglu, 2007). The primary cause and biochemical basis of chromosomal stickiness are still unknown (Pagliarini, 2000). C-mitosis is one of the consequences of inactivation of spindle apparatus connected with delay in the division of centromere (Mann, 1977). Disturbed metaphase, anaphase and telophase may be due to disturbance of spindle apparatus which allows that the chromosomes to spread irregularly over the cell; results c-mitosis, star-anaphase and star-telophase respectively (Amer and Ali, 1974).

In this study, occurrence of several types of chromosomal abnormalities, such as stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, clumping and fragmentation of *Allium cepa* L. root tip cells clearly shows that the accumulated effect of blitox results inactivation of spindle formation, deformation of non-histone chromosomal proteins and mutation of the structural genes.

As a result, the present study shows that blitox, commercial formula of copper oxychloride 50, reduced mitotic index of cells because of its cytotoxic activity. Blitox also

induced chromosomal abnormalities in mitotic cell division. A linear relationship was observed between the percentage of mitotic abnormalities and mitotic index. These results indicated that blitox should be regarded as an mutagenic agent for plants. Hence, the use of this fungicide should be under control in agricultural fields.

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Table-1: Mitotic Index (MI), type and percentage of mitotic abnormalities in the root tip cells of *Allium cepa* L. exposed to blitox

Time of Treatment (hrs)	Conc. (g/lt.)	Mitotic Index (Mean ± SE)	Mitotic abnormilities %										% Total Abnormalities
			S	L	C-M	B	M	P	S-A	S-T	CL	F	
	Control	18.17 ± 2.4	0	0	2.45	0	0	0	0	0	0	0	2.45 ± 1.1
	2	15.23 ± 2.2	0	0	2.72	0	0	0	1.8	0	0.04	0	4.56 ± 2.0
4	3	11.33 ± 2.1	0.05	1.5	2.98	1.2	0.05	2.4	3.5	0.68	4.6	0	16.96 ± 6.7
	4	9.92 ± 1.3	1.06	0.65	3.5	0.4	0.43	0	4.3	0.71	5.5	4.2	20.75 ± 7.3
	Control	15.37 ± 1.6	0	0	0	0	0	0	0	0	0	3.5	3.5 ± 1.52
	2	10.91 ± 2.1	3.6	0.3	4.2	1.2	0	3.2	2.4	0.69	1.2	1.1	17.89 ± 3.5
8	3	9.91 ± 2.4	4.1	1.2	4.9	3.3	2.3	4.1	2.75	1.48	3.4	2.7	30.23 ± 9.2
	4	8.74 ± 2.1	5.2	2.5	5.1	3.9	3.2	4.8	3.9	5.05	3.9	3.6	41.15 ± 8.4
	Control	15.42 ± 1.3	0	0	4.5	0	0	0	0	0	0	0	4.5 ± 2.03
	2	9.28 ± 2.0	2.0	1.2	2.9	2.44	1.43	2.3	4.2	1.7	2.4	1.6	22.17 ± 8.1
12	3	8.52 ± 1.9	4.9	2.3	5.8	4.8	4.7	2.9	2.5	2.6	4.9	3.75	39.15 ± 9.7
	4	6.24 ± 1.8	7.2	3.5	6.2	5.46	5.1	3.24	3.9	4.1	6.44	5.6	50.74 ± 9.8

abbreviations: S: Stickiness; L: Laggards; C-M: C-mitosis; B: Bridges; M: Multipolarity; P: Picnosis; S-A: Star-Anaphase; S-T: Star-Telophase; CL: Clumping; F: Fragmentations.

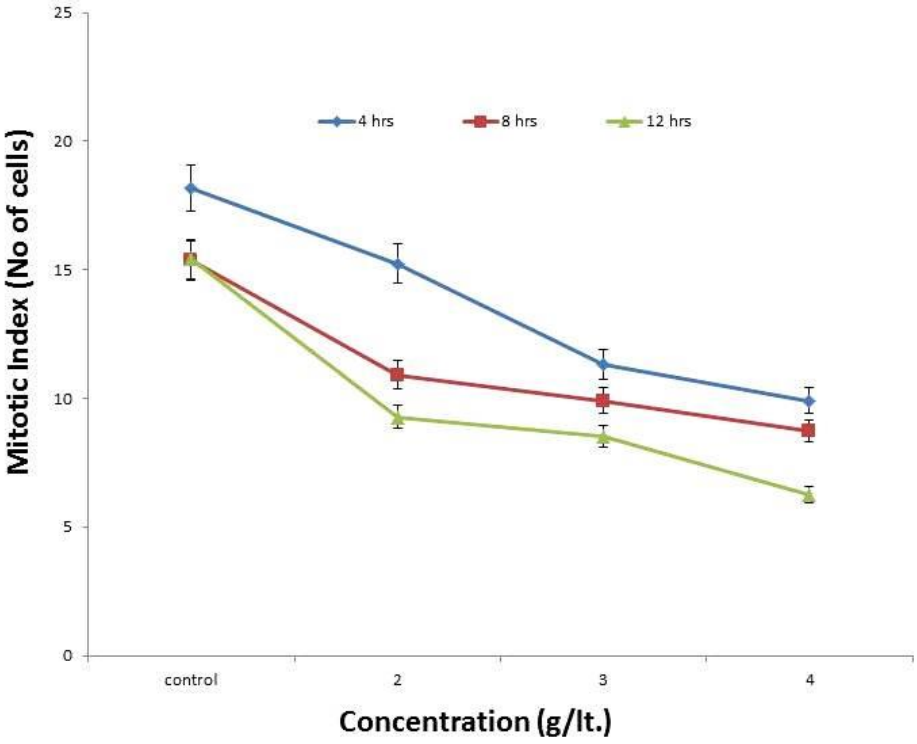

Figure-1: Mitotic Index of *Allium cepa* L. root meristem cells treated with blitox at different times and concentrations

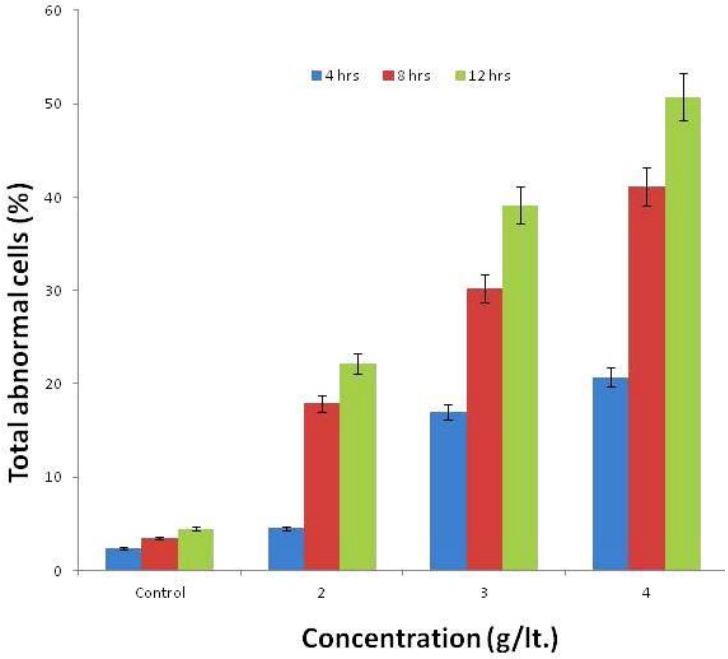

Figure-2: Cytotoxic effects of blitox at different times and concentrations in *Allium cepa* L. root tip cells

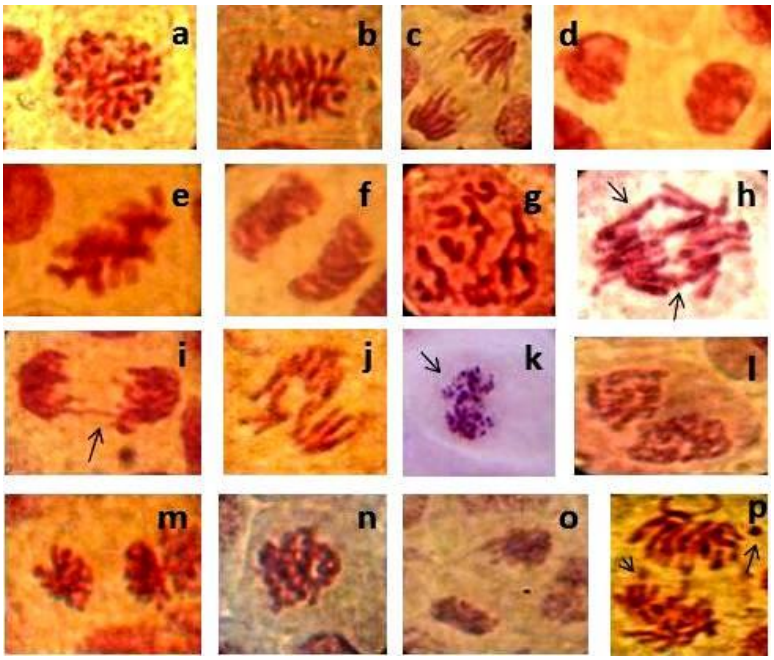

Figure-3: Normal and Abnormal Stages of Mitosis in the Root Tip Cells of *Allium cepa* L. treated with blitox. a-p: a: Normal Prophase; **b:** Normal Metaphase; **c:** Normal Anaphase; **d:** Normal Telophase; **e:** Stickiness; **f:** Telophase laggard; **g:** c-mitosis; **h:** Anaphase bridge; **i:** Telophase bridge; **j:** Multipolarity; **k:** Picnosis; **l:** Star-anaphase; **m:** Star-telophase; **n:** Metaphase clumping; **o:** Telophase clumping; **p:** Fragmentations.

AUTHORS

First Author – Anirban Paul, M.Sc. in Botany, Part-Time Lecturer, Department of Botany, Rampurhat College, Rampurhat, Birbhum, West Bengal; and Ph. D. scholar, Department of Botany, Visva-Bharati, Santiniketan, Birbhum, West Bengal, India.
(email address: anirbanpaul2@gmail.com)

Second Author – Dr. Sudipa Nag, Ph.D. in Botany, Assistant Professor, Department of Botany, Rampurhat College, Rampurhat, Birbhum, West Bengal, India.
(email address: s_m_2003@rediffmail.com)

Third Author – Kaushik Sinha, M.Sc. in Botany, Guest Lecturer, Department of Botany, Rampurhat College, Rampurhat, Birbhum, West Bengal, India.
(email address: kaushiksinhabotany@gmail.com)

Correspondence Author – Dr. Sudipa Nag, email address: s_m_2003@rediffmail.com, contact number- +91-9475852075.