RESEARCH ARTICLE

Cytogenetic effect of Systemic Fungicide Calixin on root meristem Cells of *Allium cepa* L.

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ABSTRACT

The cytotoxic effect of Calixin, a fungicide was investigated in the mitotic cell division in root tip cells of *Allium cepa* L. The seeds of *Allium cepa* were treated with different concentrations (0.02%, 0.04%, 0.06% and 0.08%) of Calixin for 3, 6, 9, 12 h treatment periods. The obtained results indicate that Calixin had the ability to cause production of a large number of mitotic abnormalities. These abnormalities appeared in varying degrees depending on the dose. Various abnormalities on chromosomes like lagging early anaphase, chromosomal bridges, c-metaphase, sticky metaphase, multipolarity, fragment, vagrant etc were seen among mitotic divisions treated with Calixin.

Keywords : *Allium cepa*, mitotic index, chromosomal aberrations, cytotoxic effect, fungicides,

INTRODUCTION

Fungicides are most commonly used against diseases of agricultural crops in many countries of the world. Fungicides produce a diverse range of products with novel modes of action. The extensive use of fungicides in plant protection against fungal disease generates long term residues in food and in the environment (Petit et al., 2008).

Fungicides may also influence to change plant genetic system due to their mutagenicity and carcinogenicity. Constant use of these chemicals may result in changing the hereditary constitution of an organism (Wuu and Grant, 1967, Wuu and Grant, 1982). Cytogenetic studies have been carried out to detect harmful effects of different pesticides on different plant species (Rank et al., 2002, Marcano et al., 2004). Mutation breeding has become increasingly popular in present times as an effective tool for crop improvement (Siddiqui and Khan, 1999).

There are several studies aiming to explain and to understand the effects of fungicides in plant systems. Rayburn et al., (1993) stated out that amount of nuclear DNA is decreased by the fungicide, captan and this fungicide has been mutagenic, carcinogenic and teratogenic effects on many organisms. Celik (2006) used two fungicides in his experiment, Derosol and Korsikol and examined by cytogenetic effects on barley root tip meristem cells. The effect of these two fungicides effect on chromosome fragments, bridge, stickiness and polar deviation is evident.

Tridomorph are systemic fungicidal derivatives which are systemic and show eradiact action against powdery mildew of barley. Tridomorph is available under the commercial name Calixin as 25% E.C Syngenta and is used extensively in the agricultural area. The fungicide is also reported to be highly effective against Ascomycetes such as *Mycospaerella musicola* and *Erysiphae graminis.* It has also shown direct fungitoxic action against various phytopathogenic fungi including *Botrytis cinerea*, *Phomopsis citri, Diplodia hetalensis, Penicillum digitatum, Cladosporium cucumerinum.*

There is no literature available on the cytogenetic effects of this chemical in the plant systems. The aim of this study was to investigate the chromosomal aberration induced by fungicide Calixin in the root tips of *Allium cepa* L. and also to determine the relation between mitotic chromosomal aberrations with mitotic index.

MATERIAL AND METHODS

Healthy and dry seeds of untreated *Allium cepa* L var. N-53 were obtained from National Horticultural Research and Foundation (NHRDF) Chitegong, Nasik. Seeds (1,500) were pre-soaked in tap water for 12 hours and then treated with four different concentrations (0.02%, 0.04%, 0.06%, 0.08%) of tilt fungicide for 3, 6, 9 and 12 hours at room temperature (22+2^{o}C). Conical flasks containing the seeds and solution were periodically shaken for 2-3 min. during the treatment. After treatment, the seeds were thoroughly washed with running tap water to remove the excess amount of fungicide from the seeds, if any. One set of seeds was kept untreated to act as control for comparison. Both the treated and controlled seeds were transferred to the petri dishes having moist filter papers for germination. Hundred seeds were used for each dose and control. The embryonic roots were reached 1.5 - 2 cm in length (both experimental and control) were excised and fixed with glacial acetic acid: ethanol (3:1) solution and kept for 24 hours. After 24 hours the root tips were transferred to 70% ethanol and stored in a refrigerator. The concentrations were chosen according to their dose of application in agricultural field to control different diseases. For mitotic studies, the root tips were hydrolyzed in 1 (N) HCl at 60^{0}C for 5 minutes, followed by staining with 2% aceto-orcein following the method described by Sharma and Sharma (1980). After 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. The mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells. Cytological abnormalities were also observed and scored. Abnormalities were photographed using Carl Zeiss Axiostar Plus microscope mounted with Canon camera model, Power Shot G12. All experiments were conducted with five replicates and average results were taken

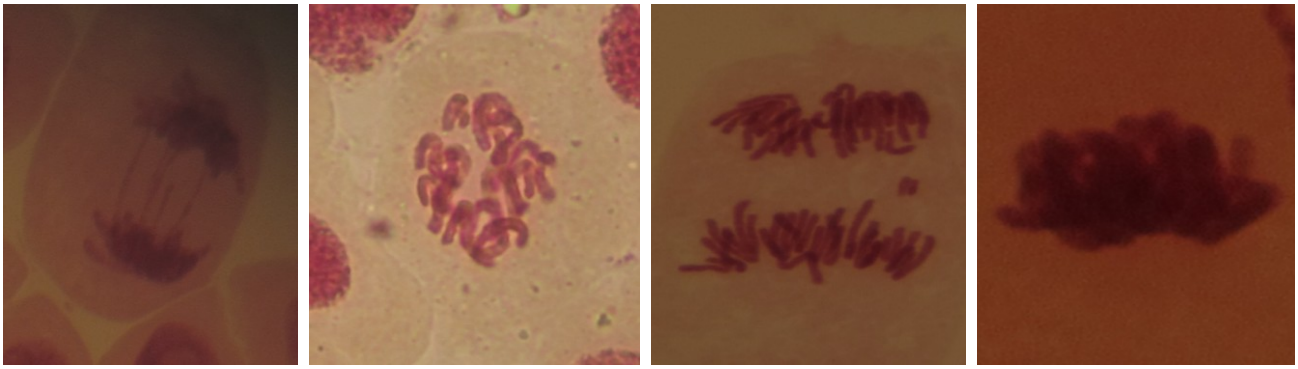
RESULTS AND DISCUSSION

Mitotic index is an acceptable measure of Cytotoxicity for all living organisms. The mitotic index of control set with a little reduction was noted as the time of treatment prolonged. The Cytotoxicity level can be determined by the decreased rate of mitotic index. The increase of mitotic abnormalities was dependent on the increasing treatment periods and concentrations. Careful screening in mitotic index was noticed in the root tip cells as concentration and duration of treatment. Mitotic index of control set was 15.23 ± 2.2 in 3h, 15.37 ± 1.6 in 6h, 15.42 ± 1.3 in 9h and 15.42 ± 1.3 in 12h. It declined to 9.70 ± 0.37 3h (0.02%) to 2.19 ± 0.16 12h (0.08%). The MI of the other concentration was sharply decreased as the time of treatment increased recording value of 2.19 ± 0.16 12h (0.08%) treatment (Table-1). The highest value was recorded at a higher concentration and longer exposure (40.58 ±1.59 at 0.08% concentration in 12 h treatment).

The most common abnormalities were Anaphase Bridge [Fig-1(a)], C-Metaphase [Fig-1(b)], Laggards [Fig-1(c)] Stickiness [Fig-1(d)], also observed.

Table 2: Mitotic Index (MI), type and percentage of mitotic abnormalities in the root tip cells of *Allium cepa* L. exposed to Calixin

Treatment		Mitotic index (% ± SE)	No. of cells examined	Types and percentage of abnormalities.						Total aberration (% ± SE)
Time	Concentration			Stickiness	Bridge	Vagrant	C Anaphase	Multipolarity	Fragment	
	Control	15.23 ±2.2	500	0	1.8	0	2.72	0	0.04	4.56 ± 2.0
	0.02%	9.70 ± 0.37	500	0	11	3	2	0	0	3.20 ±0.37
3h	0.04%	8.09 ± 0.41	500	13	18	8	11	3	0	10.60 ±1.96
	0.06%	7.86 ± 0.33	500	23	16	20	9	1	1	13.60 ±0.75
	0.08%	3.48 ± 0.86	500	31	26	22	23	0	1	20.60 ±2.01
	Control	15.37 ±1.6	500	0	0	0	0	0	3.5	3.5 ± 1.52
	0.02%	7.27 ± 0.32	500	22	27	6	6	4	0	13.00 ±0.45
6h	0.04%	7.52 ± 0.36	500	25	19	7	17	1	0	13.80 ±1.39
	0.06%	7.01 ± 0.26	500	54	21	13	13	0	0	20.00 ±1.22
	0.08%	3.35 ± 0.51	500	68	6	11	2	0	1	27.16 ±1.35
	Control	15.42 ± 1.3	500	0	0	0	4.5	0	0	4.5 ± 2.03
	0.02%	9.12 ± 0.46	500	0	9	1	2	0	0	2.40 ±0.40
9h	0.04%	8.66 ± 0.43	500	23	12	12	7	3	0	11.40 ±0.51
	0.06%	5.15 ± 0.44	500	23	20	13	13	0	0	13.80 ±0.86
	0.08%	3.35 ± 0.51	500	68	6	11	2	0	1	27.16 ±1.35
	Control	15.42 ± 1.3	500	0	0	0	4.2	0	0	4.2 ± 2.03
	0.02%	8.43 ± 0.26	500	0	6	4	2	1	1	2.80 ±0.37
12 h	0.04%	5.84 ± 0.22	500	30	33	15	4	0	1	16.60 ±0.87
	0.06%	5.04 ± 0.47	500	25	32	21	16	1	0	19.00 ±1.14
	0.08%	2.19 ± 0.16	69	24	2	0	2	0	0	40.58 ±1.59



(a) (b) (c) (d)

Fig. 1(a) Anaphase bridge (b): C- Metaphase (c): Laggards (d): Sticky Metaphase

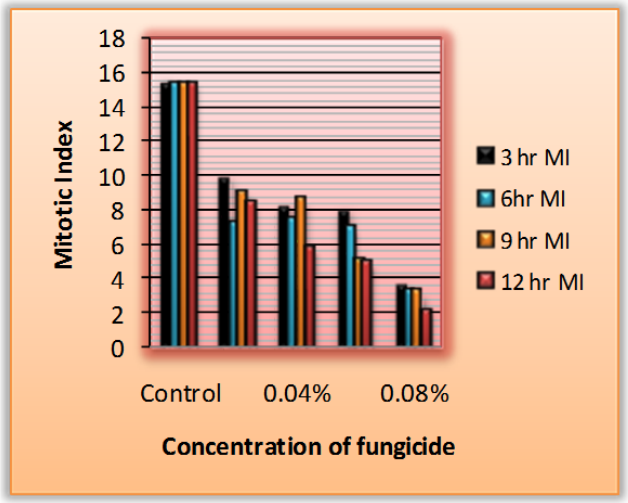


Fig. 2: Mitotic Index of *Allium cepa* L. root meristem cells treated with Calixin at different times and concentrations

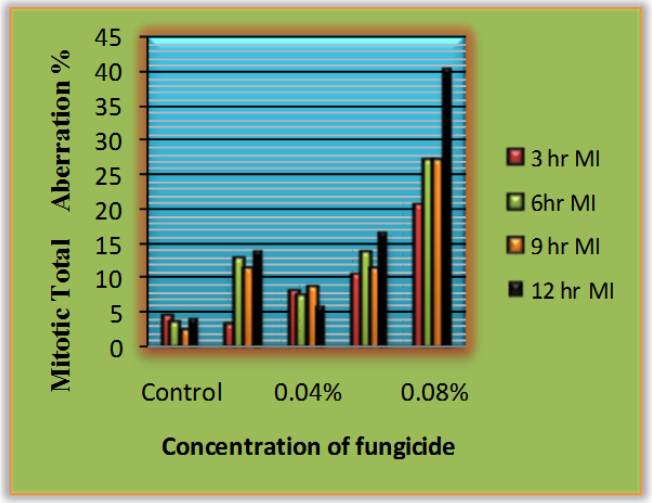


Fig. 3: Cytotoxic effects of Calixin at different times and concentrations in *Allium cepa* L. root tip cell

According to many investigators, abnormalities due to inhibition of spindle formation such as c-mitosis, multipolarity, stickiness reflects high toxicity of pollutants (Amer and Ali, 1974; Haliem, 1990; Lazareva et al., 2003). In the present study, Calixin decreased the mitotic index at all concentrations and at all treatment periods when compared with control. Similar type of result is also found by (Pulate and Tarar, 2014) on *Allium cepa* by using fungicide tilt.

The decrease of mitotic index was dose dependent. At all treatment periods, the highest concentration of Calixin 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. Calixin can be accepted as a toxic agent in this study. Calixin 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 (Behera et al., 1982; Badr, 1983; Armbruster et al., 1991; Pandy et al., 1994; Badr, 1998). In this study, the most common abnormalities were stickiness, laggards, c-mitosis, bridges, vagrant, Multi polarity 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) C-mitosis is one of the consequences of inactivation of spindle apparatus connected with delay in the division

of centromere (Mann, 1977). Sıng (1992) mentioned that univalent and laggard formation may be due to the failure of pairing and lagging to the failure of moving apart. Bridges and fragments are clastogenic effects, both resulting from chromosomal and chromatid breaks (Kovalchuk et al., 1998). The induction of vagrant chromosomes leads to separation of unequal number of chromosomes in the daughter nuclei and subsequently formation of daughter cells with unequal sized or irregularly shaped nuclei at interphase (ElGhamery et al., 2003).

CONCLUSION

These results indicated that Calixin should be regarded as a mutagenic agent for plants. Hence, the use of this fungicide should be under control in agricultural fields.

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