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ORIGINAL ARTICLE

Cytotoxicity Effect Of Dizeb M45 On Meristematic Cells Of Allium Cepa

Swapnil Kamble

Dept .of Botany
Rajiv Science and Commerce College, Zari Jamni
Email:Skkamble56@gmail.com

Abstract:

The enormous use of fungicides produce an adverse effect on crop production in agricultural. Such crop with genetic mutation is also shows their negative impact on life of human beings and animals. As well as the larger use of these chemicals causes pollution, this is hazardous to environment and human beings.
The effect of different concentration of Dizeb M45 on mitosis in Allium cepa test system show increase in the mitotic index as well as the frequency of abnormal cells get increased with increase in the concentration of Dizeb M45.This study was conducted during September 2011 to march 2012.

KEY WORDS:

Mitotic index, Cytotoxicity, Chromosomal aberration.

INTRODUCTION

Hurtado in 1987 described that agrochemicals such as fungicide and insecticide will certainly affects the growth and development of non-targeted host. Malode,(1990) investigated effect of cyprocanzol on growth and mitotic chromosomes in Triticum aestivum. Zusti and Kaul (1975) studied cytogenetic activity of some common fungicide in higher plants. In 1991 Berger and Cwick , gives some adverse nutritional aspects of insecticides. In 1995 two Pakistani botanists studied that Topsin-M fungicide showed significant increase in chlorophyll protein and phenolic content of Hibiscus esculentous and Capsicum annum and carbohydrate and phenolic content of Solanum melongena and Avina sativa (Siddiqui 1997). Earlier in 1990 Coman et al. described that Alchoholar metaxyl induced sharp decrease in cell division. In 1991 Harichand et al. worked out that carbendazim produced chromosomal aberration in somatic and germ cells of pearl millet and sunflower.

In 2008, Ozlem et al. studied mitotic changes in root apical meristems of Lens culinaris treated with Fusilade (Fluzifop-p-butyl) herbicide. The scientists Jyophish et al.(2008) published their work on “Cytotoxicity effect of endosulfon on root apical meristem of Allium cepa”. These mutations seen in prophase, metaphase, anaphase and telophase. The chromosomal abnormalities were studied in the form of

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sticky chromosome, laggard, bridge, clumped metaphases and fragmented chromosomes.

MATERIALS AND METHODS:

Method of treatment to onion bulb:

To study the cytological effect of fungicides, the germinated onion bulb with 2-3 cm long roots were treated with 100ml 0.5% DizebM45,1%DizebM45, 1.5% DizebM45, 2% DizebM45 solutions. These all coupling jars including germinated onion bulb placed in incubator at 12-240c for 3hours. After the completion of treatments the root tips was thoroughly washed under tap water for 5-6 times to remove excess of chemical sticking to roots. An individual control was also maintained in distilled water root tips of different length were taken and freshly prepared Carnoys fluid (3:1), at different times to determine the frequency of highest mitotic activity for 24hrs. For further studies the material was preserved in 70% alcohol at 8oC.

Hydrolysis, staining and squashing

Preserved root tips about 8-10 in number wise taken in cavity block and hydrolyzed in 1N HCl at 60oC in oven for 10 to 12 minutes and stained in 2% acetocarmine for 25 to 30 minutes. Deeply stained meristematic region of root tips i.e. 0.5 to 1mm in length were squashed. Squashes were made according to method of Darlingtion and La Cour (1962). For cytological observations about 20 slides per concentrations were screened and results were recorded. After observations photographs were taken with CCD camera and slides which were important cytologically were made permanent with N-butanol, acetic acid series. The duration of genotoxicity test is 3-4 weeks including initial toxicity testing, scoring of aberrations statistics . All experiments were done thrice.

PARAMETER STUDIED:

Following parameters have been selected to study the cytological effect of Dizeb M45 on root of Allium cepa in solvent distilled water.

1) Mitotic index:

Mitotic index in terms of percentage frequency of dividing cells was taken into consideration. Active mitotic index was calculated by scoring only metaphase and from the total dividing cells. It was computed as follows

$$\text{Mitotic index} = \frac{\text{No. of dividing stages}}{\text{Total No. of cells observed}} \times 100$$

$$\text{Active mitotic index} = \frac{\text{No. of metaphse} + \text{No. of anaphase}}{\text{Total No. of cells observed}} \times 100$$

2) Chromosomal Aberrations:

Chromosomal aberrations in mitosis were calculated by scoring about 1800to 2200 meristematic cells for each treatment.

a.Aberrations in various metaphases such as clumped metaphase were scored.
b.Aberrations in various anaphases such as bridge were scored.
Aberrations in various telophases such as arrested telophase were scored. Percentage of aberrant metaphases, anaphases and telophase of each type of irregularity were calculated by using formula.

$$\text{Percentage of aberrant stages} = \frac{\text{Total No. of aberrant stages}}{\text{Total No. of dividing cells}} \times 100$$

RESULTS AND DISCUSSION:

The inhibitory effect of Dizeb M45 (Mencozeb) in distilled water as solvent was evaluated on the mitotic activity of Allium cepa root meristem and effect was compared with distilled water as control. The mitotic cells were counted in root meristems in above groups at 3 hours of treatment with each concentration of Dizeb M45 (Mencozeb) were 0.5% DizebM45, 1% DizebM45, 1.5% DizebM45, 2% DizebM45 respectively used which produce indefinite variability in a mitotic index. The data on the effect of Dizeb M45 fungicide on mitotic index and active mitotic index is tabulated in Table I. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase, metaphase anaphase and telophase were recorded.

In table I, the mitotic index frequency and active mitotic index frequency in control were found 18.28% and 7.86% while in 0.5% DizebM45, 1% DizebM45, 1.5% DizebM45 and 2% DizebM45 shows mitotic index frequencies and active mitotic index frequencies were 18.09% and 7.88%, 22.02% and 9.77%, 19.00% and 8.35%, 17.12% and 7.80% respectively. It indicates the indefinite variability's between them in comparison with control.

In 1% DizebM45 and 1.5% DizebM45 shows more variability in mitotic index frequency as well as in active mitotic index frequency than present in control. The cytological abnormalities are scored in mitotic cells and results are shown in table II. The treatment with Dizeb M45 fungicide resulted an observable cytological changes. These were in the form of fragmented chromatid laggards, chromatid bridges, polar metaphases and clumped metaphases. The frequency of chromosomal abnormalities found in (table II) 0.5% DizebM45, 1% DizebM45, 1.5% DizebM45 and 2% DizebM45 were 5.51%, 4.45%, 5.90% and 5.48% respectively. In control it was only 0.098%.

Table I :- Effect of Dizeb M45 on mitotic and active mitotic index frequency in Allium cepa

Treatment	Total no. of cells scored	No. of mitotic dividing stages				Total no. of dividing stages	Mitotic index frequency (%)	Active mitotic index frequency (%)
		P	M	A	T			
Control	2034	164	89	71	48	372	18.28	7.86
0.5 %	2067	172	95	68	39	374	18.09	7.88
1 %	1821	182	102	76	41	401	22.02	9.77
1.5 %	1915	159	82	78	45	364	19.00	8.35
2 %	2114	161	85	80	36	362	17.12	7.80

P:- Prophse, M-Metaphase,A-Anaphase,T-Telophase .

The frequencies of chromosomal abnormalities given in table (II) indicates that with increase in the concentration of fungicide i.e. Dizeb M45 there is increase in chromosomal abnormalities were observed. It means that chromosomal abnormalities are directly proportional to the concentrations of fungicides. The comparative data revealed that Dizeb M45 (Mencozeb) were cause a more effects on somatic chromosome of Allium cepa. These have been statistically significant differences between control

and treated group in mitotic index, active mitotic index and chromosomal abnormalities. Now a day there is a high consumption of fungicides and insecticides in agricultural field, which directly used in inappropriate concentration to cure the fungal seed borne diseases which shown their adverse impact on seed germination, seedling height and on somatic chromosomes.

Table II :- Effect of different concentration of Dizeb M45 for 3 hrs. on mitosis in Allium cepa .

Treatment	Total no. of cells scored	Type of mitotic abnormalities					Total no.of Abnormal cells	Frequency of mitotic abnormal cells (%)
		F	L	B	P.M.	C.M.		
Control	2034	---	---	---	---	2	2	0.098
0.5 %	2067	12	27	34	---	41	114	5.51
1 %	1821	17	20	13	02	31	83	4.45
1.5 %	1915	15	31	26	04	37	113	5.90
2 %	2114	19	23	36	03	35	116	5.48

F-Fragments ,L-Laggard ,B- Bridge, P.M.-Polar Metaphase, C.M.-Clumped Metaphase .

The chemical usually indicates the stress condition developed by the use of agrochemicals such as insecticides and fungicides suffer from the chemical stress (Siddiqui, 1997). It was also noted that the systematic fungicides and phenolic compound causes the chemical stress can inhibit the seed germination and seedling growth (Helsy 1990; Datta and Sinha-Roy 1973; Friendman et al., 1977, Einhely 1998; Siddiqui and Ahmed 1996). It has also been reported that phenolic compounds are responsible for limiting growth, respiration, photosynthesis and disruption of cell membrane (Mucias et al., 1992). Also some compounds act as cytotoxic, genotoxic, mutagenic and anticarcinogenic, there by affecting plant and animals. In present study systematic fungicide used and Dizeb M45 (Mencozeb) its effectivity has been studied in Allium cepa test system to determine compound action whether these compounds acts as a cytotoxic, genotoxic, mutagenic or antimitotic property.

In present study the result of normal mitosis showed the number of dividing cells i.e. prophase, metaphase, anaphase and telophase. Prophase having chromosome become visibly distinct as long thin threads divided into chromatid, each sister chromatid is attached to other in region of the centromere, nucleolus and nuclear membrane are present.

In metaphase, disappearance of nuclear membrane and nucleolus occurred; spindle formation takes place which results into movement of chromosome at equatorial regions. In anaphase, centromere divides, become functionally double, chromatid converted into independent chromosomes that separates and move to opposite poles and in telophase, spindle disappears and reconstruction of nuclear envelops about the two groups of offspring chromosomes begins, chromosomes uncoiled to become like slender thread and nucleoli reappears.

Result of normal mitosis indicated that in the fungicide Dizeb M45 (Mencozeb) there will be the indefinite variation seen in number of dividing cell when compared with control.

The result shows that very negligible abnormalities were seen in control also. From result it was shown that there will be decrease in the mitotic index frequency and active mitotic index frequency and active mitotic frequency with single variability seen when concentration of Dizeb M45 (Mencozeb) get increased.

In certain study the effect of Boron on the mitotic index of A. cepa root meristematic cells investigated by Muhsin et al. (2007). By using growth inhibition test LD 50 value was determined first and

then different doses of varied concentrations introduced. Application process was carried out at 12, 24 and 48 hrs. The most observed abnormalities were
C-metaphase, prometaphase and disturbed anaphase-telophase. In addition to this anaphase bridge, polyploidy and late chromosome was observed in A. cepa.

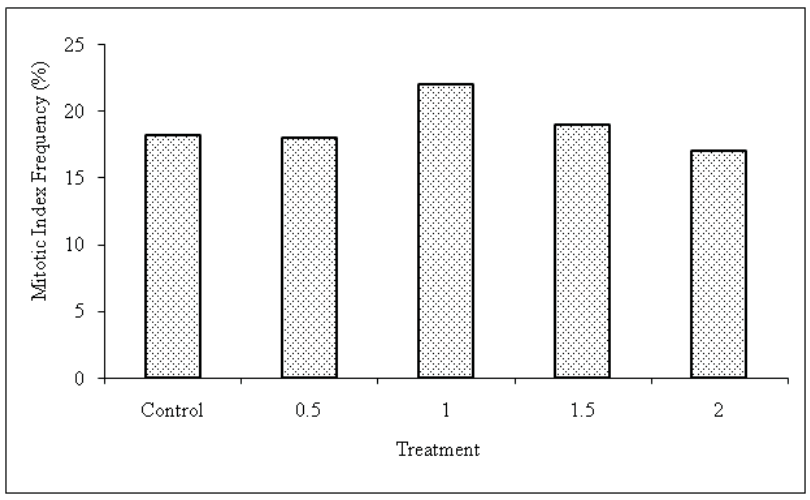
The most frequent aberrations are clumped metaphase in which induction of cell cycle arrest at metaphase, subsequent apoptosis occur and chromosomes are intermingled with each other. In single chromatid bridge one of the chromatid become fragmented during anaphase and centromere of chromosome goes to the opposite pole and central position remains in between the two poles forming a bridge, laggards occurs and reconstruction of nuclear envelop about two groups of offspring's chromosomes was arrested because of arrested telophase.

A. cepa root tip meristems have been widely used for evaluation of cytotoxic and anti-mitotic activity of compounds (Shehab, 1980; Williams and Omoh, 1996 and Al-Meshal, 1987). The data present in table II showed the genotoxic effect of Dizeb M45 (Mencozeb) fungicide respectively. The numbers of chromosomal aberrations are concentration and time dependent.

The frequencies of chromosomal aberrations increases with increase in the concentrations of Dizeb M45 [Table (II)] when compared with control. It means that chromosomal aberration is directly proportional to concentrations of fungicide.

Another study the systematic fungicides active constituent carbendazim also induced chromosomal aberrations in somatic and reproduction of Pearl and Sunflower (Grover and Sharma, 1991). The studies about the effects of different pesticides found out some of genotoxic effects of pesticides on plants (Sinha 1989; Basic et al., 1991, Aktac et al., 1994; Tartar et al., 2006). In our study chromosomal bridges, lagging chromosomes, stickness and change in the plane of the cell division were observed. Similar abnormalities were recorded with treatment conducted with the herbicide Logran on root tip cells of T. aestivum L. and Hordeum vulgare L. (Kayamak and Muranli, 2006). The types of chromosomal abnormalities observed after treatment with Linuron in mitosis of Helianthus annus L. included fragments disturbed metaphase, C-metaphase, lagging chromosome and chromatid bridge (Inceer et al., 2004).

Fig. 1: Effect of Different Concentrations of Dizeb M45 on Mitotic and Active Mitotic Index



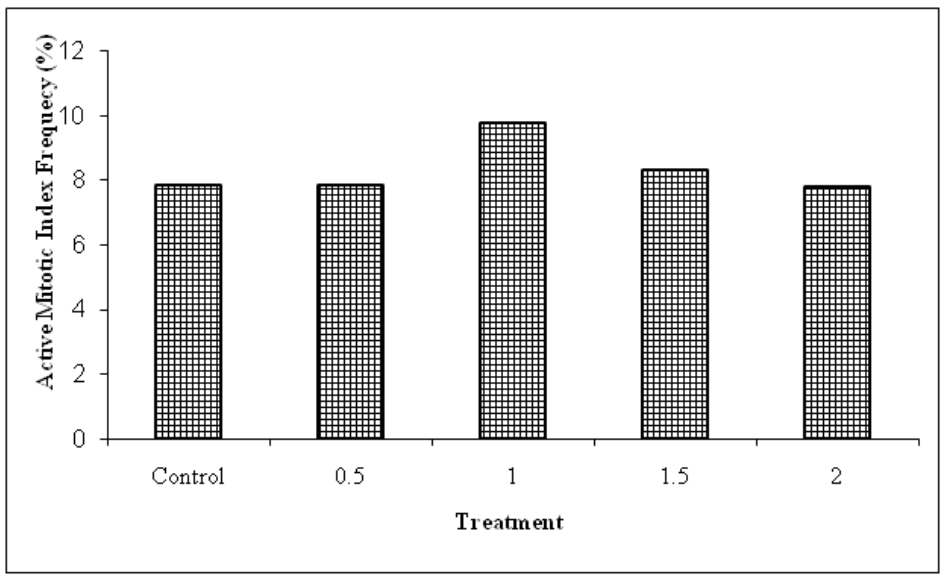
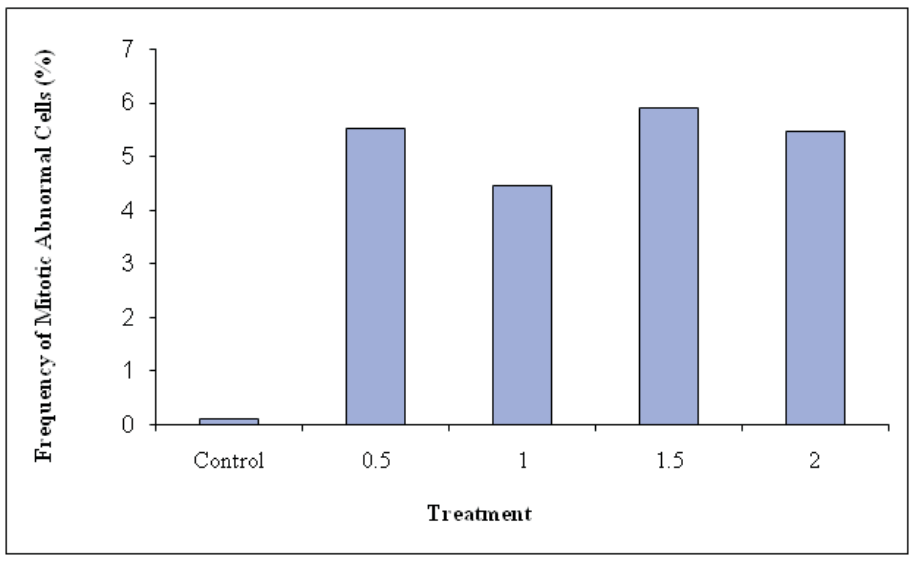


Fig. 2: Effect of Different Concentrations of Dizeb M45 on Somatic Chromosome in Allium cepa.



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