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Cytotoxic Effects Induced By The Fungicide Dithane M-45 To Gram (*Cicer Arietinum* L.)

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ABSTRACT:

The cytotoxic effects of Dithane M-45, a fungicide were investigated in the mitotic cell division in gram (*Cicer arietinum* L.) root tip cells. The gram grains were treated with different concentrations of fungicide at room temperature. For this aim, the gram seeds were treated with four different concentrations (5%, 10%, 15% and 20%) of Dithane M-45 for 4, 8, 12 and 16 hours treatment periods. About 1- 1.5 cm length of root tip were cut,stained according to aceto-orcein squash procedure. About 400 cells were scored for each treatment and classified into normal and aberrant division stage. Calculate the mitotic index (MI) and the number of abnormal cells were counted in each phase of cell division. It produces several chromosomal abnormalities in mitotic divisions and the MI is reduced when the concentrations of Dithane M-45solution is increased.The obtained results indicate that Dithane M-45 had the ability to cause production of a large number of mitotic abnormalities. The chromosomal abnormalities were found to be increased as the concentration and treatment periods of the fungicide increased when compared to control. Various abnormalities on chromosomes like fragmentation, condensed chromatin, chromatin granulation, c-metaphase, chromosomal bridges, lagging chromosome, sister chromatin distaining etc. were seen among mitotic divisions treated with Dithane M-45. This result suggests that Dithane M-45 has some negative effects on mitotic divisions in *Cicer arietinum* L. root tip cells.

INTRODUCTION:

The chickpea (*Cicer arietinum* L.) is an annual legume of the family Fabaceae. Itis the important food grain legume and rich in protein. Chickpeas (*Cicer arietinum* L.) are one of the most widely consumed pulses in the world and protein content varies from 21.7 to 23.4% (El-Adawy, 2002). It is a pulse commonly used in Indian cuisine. Chickpea protein is rich in lysine and arginine but most deficient in the sulfur-containing amino acids, methionine and cystine (Manan *et al.*, 1984). Chickpea contains twice the amount of protein than that of cereals, hence it can balance the amino acid and may improve the nutritive value of a cereal-based diet (Singh *et al.*, 1988). In the last decades, the use of fungicides in agriculture for fungal diseases control has become crucial. Large amount of these chemicals is released into the environment and many of them affect non-target organisms, being a potential hazard to human health. When some chemicals accumulated within food chain to a toxic level, these

chemicals affect directly the public health (Fisun and Rasgele, 2009).

Fungicides produce a diverse range of products with novel modes of action. The extensive use of these compounds in the agriculture system raises public concern because of the harmful potential of such substances in the environment and human health (Mendes *et al.*, 2005). Pesticide exposure is ubiquitous, due not only to agricultural pesticide use and contamination of foods, but also to the extensive use of these products in and around residences (Pastor *et al.*, 2003).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 mutagenecity and carcinogenicity. 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). 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. The present study has been carried out to investigate the influence of Dithane M-45 in *Cicer arietinum* root tip cells during mitotic cell division.

MATERIALS AND METHODS:

Healthy and dry seeds of *Cicer arietinum* L. were pre-soaked in tap water and then treated with Dithane M-45 at four different concentrations (5, 10, 15 and 20%) for 4, 8, 12 and 16 hours. After treatments, the seeds were thoroughly washed with running tap water to remove the excess amount of fungicide from the seeds. One set of seedswere kept untreated to act as control for comparison. Both the treated and controlled seeds were transferred to the Petridishes having the moist filter papers for germination. Forty seeds were used from each dose and control. The Petridishes were kept at room temperature (28-30^{0}C) for three days.

After three days the root tips of germinated seeds (both experimental and control) having a length in about 1.0-1.5 cm were excised and pretreated with aqueous para-dichlorobenzene for three hours, washed with distilled water, 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. For examination, the root tips were first treated with 2% aceto-orcein and 1(N) HCl (9:1) and just warmed over a flame of sprit lamp.

After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control. Effects of fungicide treatment and control on different slides were observed under light microscope and 400 cells were counted from each treatment. The mitotic index (MI) was calculated and different types of chromosomal aberrations were also observed and scored. Mitotic index was expressed in terms of divided cells/total number of cells x100. These four different concentrations were chosen according to their dose of application in cultivated field to control different diseases.

All experiments were conducted with five replicates and average results were taken.

RESULTS:

The mitotic activity was normal in control roots of *Cicer arietinum* L. A wide spectrum of chromosomal abnormalities were noted in fungicide treated roots (Table 1). The increase of mitotic abnormalities and decrease of mitotic index (MI) was dependent on the increasing concentrations and treatment periods of Dithane M-45 fungicide. The mitotic index in control was observed to be maximum with no chromosomal abnormalities. All concentrations of fungicide cause a decrease in MI when the different division stages were examined. The percentage of abnormal mitotic stages was seen to increase respectively with increasing fungicide concentration. The treated root tips showed various types of metaphasic and anaphasic aberrations at each dose of treatment. Mitotic index of control set was 38.98 in 4h, 38.73 in 8 h, 39.55 in 12 h and 38.67 in 16 hrespectively. At lowest concentration of Dithane M-45 (5%), the mitotic index is reduced to 36.50 in 4htreatment period and further increase in concentration, resulted in decline in mitotic index. When the seeds were treated with 20% of Dithane M-45, the mitotic index was greatly reduced and found to be 16.43 in 16htreatment period. The fungicide Dithane M-45 was found to reduce the MI, irrespectively of the concentration or exposure time, compared to the control. We can infer that there is a direct correlation between the increase of exposure time, fungicide concentration and mitotic activity reduction (Figure 4).

Dithane M-45 significantly increased the percentage of aberrated cells at all concentrations and treatment periods in mitotic cell divisions when compared with control. In this study, the most common cytotoxic abnormalities like chromosomalfragmentation (Fig. 1), condensed chromatin, chromatin granulation, c-metaphase, chromosomal bridges (Fig. 2), lagging chromosome (Fig.3), sister chromatin distainingetc were observed. The treated root tips showed various types of aberrations at each dose of treatment. Increase in concentration of Dithane M-45 significantly increased the mitotic inhibition and ensured the harmful effect on mitotic cycle. The most prevalent aberration caused by Dithane M-45was 4fragments at metaphase in 16 h treatment period (20%), 5bridges at anaphase in 12 h treatmentperiod (20%) and 4 stickiness at anaphase 4in 16 h treatmentperiod (15%) respectively (Table 1).At all treatment periods, the highest concentration of Dithane M-45 (20%) decreased mitotic activity more than other used concentrations. The highest chromosomal anomalies were recorded at a higher concentration and longer exposure (48.49 at 20% concentration in 16 h treatment period).The fungicide Dithane M-45 was found to reduce the MI, irrespectively of the concentration or exposure time, compared to the control. We can conclude that there is a direct correlation between the increase of exposure time, fungicide concentration and percentage of total chromosomal aberration increase (Figure 5)

DISCUSSION:

The mitotic index is a reliable predictor of the cell proliferation in the tissue or organ. Decrease the mitotic index in root tip meristemsof *Cicer arietinum* L were observed in treated seeds when compared to the control and it decreased gradually in all the treatments with increasing concentrations and treatment periods of Dithane M-45. Similar type of results were also found on *Allium cepa* by using fungicide tilt (Pulate and Tarar, 2014) and the root tip cells of *Helianthus annuus* with copper chloride (Inceer *et al*., 2003). In the present study, the chromosomal aberrations induced by the fungicide Dithane M-45 included sticky metaphase, anaphase bridge and fragments may also be observed. Similar results have also been reported in *Trigonella* sp. by Abbasi and Anis (2002); Jabee *et al*., (2008). 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 (Pulate and Tarar, 2014). 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). The chromosome bridges were recorded at all the concentrations of the treated fungicide and it produced due to chromosomal breakage and joining of incorrect ends (Gill *et al*., 2000). However, Dithane M-45 fungicide has different effects on cell division mechanism. It may be concluded that as has been stated above, Dithane M-45 fungicide has harmful effects on the root tip meristem cells of *Cicer arietinum* L and it acts almost like a mutagen.

CONCLUSION:

Cytogenetic activities of fungicide Dithane M-45 were investigated in root meristems of *Cicer arietinum* L. Higher concentration and longer duration of treatment is toxic to cells. The present investigation clearly showed that there was a significant reduction in the mitotic index of the dividing cells and the chromosomal abnormalities were found to be increased as the concentration of the fungicide increased. These results indicated that Dithane M-45 should be regarded as a mutagenic agent for plants.

Table 1: Mitotic Index (MI), type and percentage of mitotic abnormalities in the root tip cells of *Cicer arietinum* L. exposed to Dithane M-45.

Treatment		No. of cells examined	No.of division cells examined	Mitotic Index (%)	Types and percentage of abnormalities.			Total aberration (%)
Time	Concentration				Fragment	Bridge	Sticky chromosome	
4h	Control	21.06	8.21	38.98	1	0	0	4.75
	5%	18.38	6.71	36.50	1	1	-	10.88
	10%	17.94	6.05	33.72	0	1	1	11.12
	15%	18.05	5.34	29.58	1	2	0	16.62
	20%	18.66	5.12	27.43	1	1	2	21.44
8h	Control	21.25	8.23	38.73	1	0	0	4.70
	5%	20.54	7.21	35.10	0	3	0	14.60
	10%	18.33	6.34	34.59	1	1	2	21.82
	15%	17.45	5.81	33.29	2	2	1	28.65
	20%	18.15	5.25	28.92	2	2	2	33.06
12h	Control	18.33	7.25	39.55	1	0	0	5.45
	5%	19.51	5.90	30.24	0	3	2	25.63
	10%	19.05	5.53	29.03	2	2	3	36.74
	15%	17.34	4.59	26.47	1	5	1	40.36
	20%	16.71	4.02	24.06	1	**5**	2	47.87
16h	Control	21.54	8.33	38.67	0	1	0	4.64
	5%	18.41	4.85	26.34	2	2	3	38.02
	10%	18.73	4.10	21.89	3	3	2	42.71
	15%	20.21	4.23	20.93	2	3	**4**	44.53
	20%	18.56	3.05	16.43	**4**	2	3	48.49

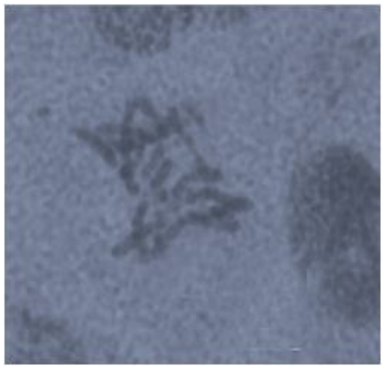

Fig. 1. Chromosome fragmentation

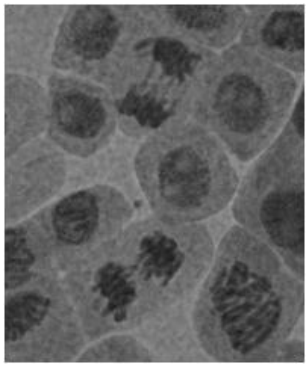

Fig. 2. Chromosomal bridges

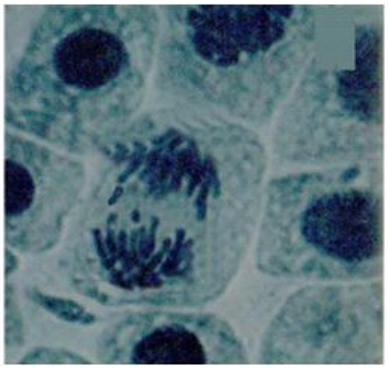

Fig.3. Lagging chromosome

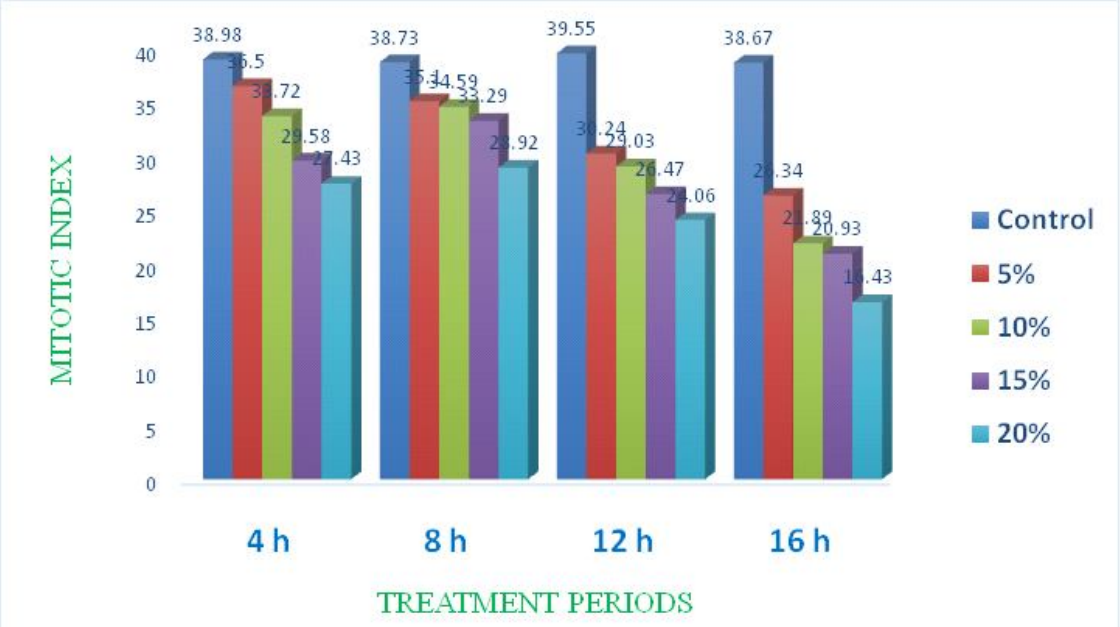

Figure 4: Mitotic index of C*icer arietinum* L. root meristem cells treated with Dithane M-45 at different times and concentrations.

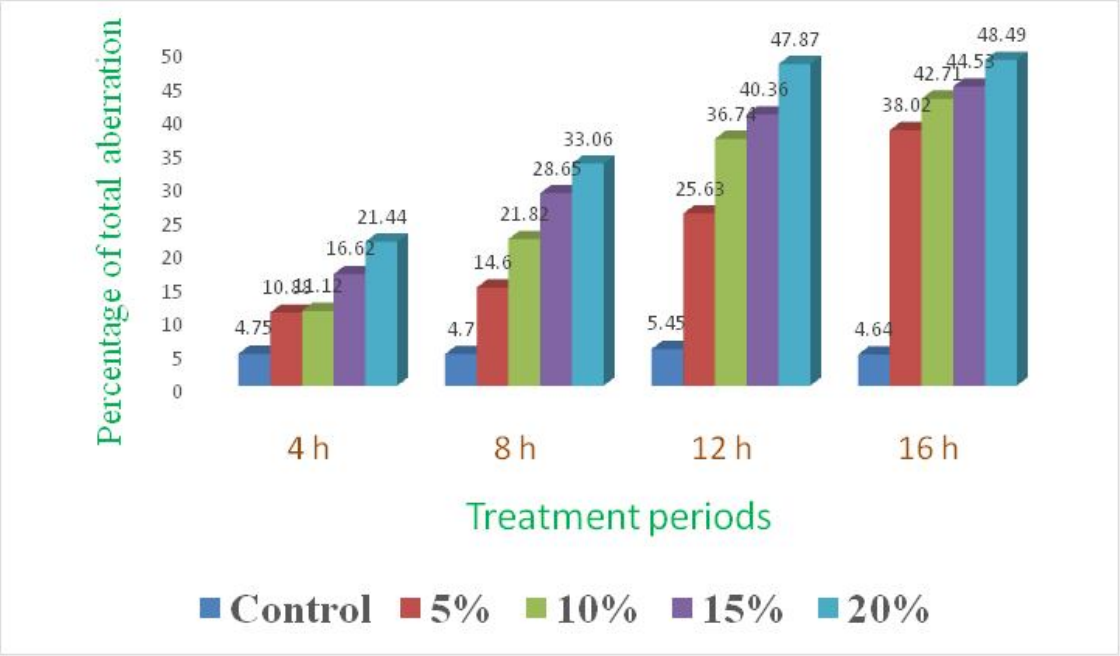

Figure 5: Percentage of total chromosomal aberrationsof C*icer arietinum* L. root meristem cells treated with Dithane M-45 at different times and concentrations.

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