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GENOTOXIC EFFECTS OF CARBENDAZIM (FUNGICIDE) ON THE ROOT APICAL MERISTEMS OF *ALLIUM CEPA L.*

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ABSTRACT

Higher plants are recognized as excellent genetic models to detect environmental mutagens, and are therefore, frequently used in monitoring studies. The genotoxic potential of carbendazim (fungicide) was investigated by using chromosome aberration in *Allium cepa L.* root tip cells. In this study, the effects of Carbendazim, a systemic fungicide were investigated in the mitotic cell division in onion *(Allium cepa L.)* root tip cells during germination. *Allium cepa L.* roots were treated with 1g/L, 2g/L and 3g/L concentrations of Carbendazim and distilled water as control at 6 hours, 12 hours and 18 hours duration. All the concentrations used, caused several abnormalities in mitotic cell divisions and the Mitotic Index in the onion root tip cells decreased when the concentrations of Carbendazim solution is increased.The total percentage of aberrations generally increased in a dose and time dependent manner.

INTRODUCTION

In agriculture, plant diseases are controlled primarily by chemicals (pesticides, bactericides, nematicides, *etc*) P. C. Garciäa *et al.*, 2002.As many as 400 chemicals are being used as pesticides (Grover and Tyagi, 1980).Helsel (1987) estimated that about 17% of applied pesticides are fungicides.

Pesticides when used in small amounts have several advantages. However, in high concentrations they act on DNA, plant metabolism and regular cell division (Tripathy *et al.* 1993). Many genotoxic studies have been carried out to detect the harmful effect of different pesticides have some hazardous effects in addition to their benefits. Their undesirable residues in water, food and in environment may cause health problems.

Chromosomal anomalies induced by some of these compounds were found to be linked with their capacity to induce mutations (Wuu and Grant 1966, Panda and Sharma 1979, Gichner *et al.*, 1982). Chromosomal anomalies produced by pesticides, therefore, have been regarded as reliable evidence of the genotoxicity (Grant 1982, Ma 1982).

Fungicides are used to control fungal diseases by killing the fungus that causes the disease. They are most commonly used against diseases of agricultural crops in many countries of the world. The fungicide is used to control plant diseases in cereals and fruits like citrus, bananas, strawberries, pineapples, and pome etc. Constant use of these chemicals may result in changing the hereditary constitution of an organism (Wuu and Grant, 1966 & 1967). When some chemicals accumulated within food chain to a toxic level, these chemicals affect directly the public health (Fisun and Rasgele, 2009).

Fungicides are among the least investigated pesticides for their genotoxic activities. The few investigated fungicides were found to exert C-mitotic activity and induce chromosomal abnormalities in a number of crop plants (Fiskesjo 1969, Ahmed and Grant 1972, Spasojevic 1974). Some fungicides were also found to induce chromosomal stickiness, bridges and lagging (Bielecki, 1974, Al-Najjar and Soliman, 1980). The interest in the impact of fungicides is mainly related to their toxicity. Like all pesticides, fungicides also affect human health and the environment, hence the need for assessing their effects (Adams and Moss 2008).

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 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 (Kovalchuk *et al.*, 1998, Fisun and Rasgele, 2009).

Carbendazim, a systemic fungicide has extensive application world-wide (WHO/FAO, 1994). There is only limited number of study available on the genotoxic effects of this chemical in the plant systems.

Onion *(Allium cepa L.)* is very suitable for genotoxic studies (Peter Firbas and Tomaž Amon, 2013). Let us list some of its advantages:

(i) The root growth dynamics is very sensitive to the pollutants

(ii) The mitotic phases are very clear in the onion

(iii) It has a stable chromosome number

(iv) Diversity in the chromosome morphology

(v) Clear and fast response to the genotoxic substances

(vi) Spontaneous chromosomal damages occur rarely.

Therefore, onion plant is selected for the study. The present study was designed to examine the effect of Carbendezim on cell-divisions in the root apical meristems of *Allium cepa L.*,to reveal the genotoxic effects induced by this chemical.

MATERIALS AND METHODS

1.1 Chemistry of Carbendazim

Carbendazim ($C_9H_9N_3O_2$) (IUPAC name: Methyl 2- benzimidazolecarbamate) (Fig. 1) is a broad spectrum benzimidazole carbamate fungicide with molecular weight=191.187 g/mol.

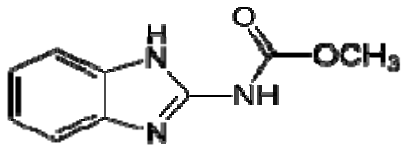

Fig. I- Carbendazim- Structure

1.2 Preparation of onion bulbs

The plant used as test material was *Allium cepa L.*The root meristems of *Alliumcepa* consist of diploid (2n= 16) set of chromosomes. 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 carbendazim dissolved with distilled water (1g/L., 2 g/L. and 3g/L.) for 6, 12 and 18 hours. Similarly, distilled water is used as Control.

1.3 Squash preparation

For mitotic studies, the root tips of *Allium cepa L.*were fixed in Acetic acid – Ethyl alcohol1:3 (v/v) mixture for overnight, followed by 5-7 minutes treatment in 45% acetic acid. Then root tips were hydrolyzed in 1N HCl at 60ºC for 5 minutes, followed by staining with 2% Aceto-orcein, following the methods described by Sharma and Sharma (1980). The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant, 1982. After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control.

1.4 Scoring of slides

Effects of chemical treatment and control on different slides were observed under light microscopy. Photomicrographs of cells showing chromosomal aberrations as well as showing normal mitosis were taken using Olympus microscope.The mitotic index (MI) was calculated anddifferent types of chromosomalaberrations were also observed and scored.

RESULTS AND DISCUSSION

Mitotic Index (M. I)

According to Smaka kinel *et.al.* (1996) mitotic index is an acceptable measure of cytotoxicity for all living organisms. Mitotic index and chromosomal aberration analysis of *A. cepa* root tip assay are used to detect potential genotoxicity of chemical substances (Kumar and Panneerselvam, 2007; Abu and Mba, 2011).Induction of mitotic abnormalities on root tip cells of plants may cause a decrease in mitotic index (Panneerselvam *et al.*, 2012). In the present study, Carbendazim 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 L.* by using fungicide Raxil. The decrease of mitotic index was dose dependent. At all treatment periods, the highest concentration of carbendazim decreased mitotic Index more than other used concentrations (Fig-II) (Table- I).Sudhakar *et.al* (2001) the decrease in mitotic index may be due to inhibition of DNA synthesis at S- phase. Since it decreased the M. I in root tip cells of *Allium cepa L.* Carbendazim can be accepted as a toxic agent in this study.

Chromosomal aberration

Carbendazim significantly increased the percentage of aberrated 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 aberrations in different plants (Badr, 1998; Pandy *et al.*, 1994; Armbruster *et al.*, 1991; Badr, 1983; Behera *et al.*, 1982 and Mann, 1977). In this study, the most common aberrations were fragments, bridges, C-Mitosis, stickiness, ring chromosome, disturbed anaphase, metaphase and telophasein cell division (Fig- III& IV) (Table- II). The genotoxic effects were noticed in the form of chromatin bridges, chromatin fragments and ring chromosomes. Ring chromosomes are the result of loss of chromosomes from the telomeric side. Chromatin bridges could happen during the translocation of the unequal chromatid exchange and cause structural chromosome mutation. This type of aberration was also observed in the mitosis of *Vicia faba* and *Allium cepa*after treatments with food additives (Gomurgen, 2005 and Turkoglu 2007). Disturbed metaphase, anaphase and telophase might be due to the disturbance of the spindle apparatus. The chromosomal damage produced by chemicals may to their effect on DNA (Grant, 1978).

CONCLUSION

Genotoxic effects produced by Carbendazim were investigated in root meristems of *Allium cepa L.*Higher concentration and longer duration of treatment is toxic to cells.The present study revealed genotoxic effects of Carbendazim.

The outcome of this study suggests, safety measures to farmers avoid direct contact with high concentration of Carbendezim contaminated mud while working in the fields or in fields irrigated with Carbendazim contaminated surface/ ground water and increase public awareness about ill effects of fungicides in water, food and the environment. Meanwhile the use of this fungicide should be under control in agricultural fields.

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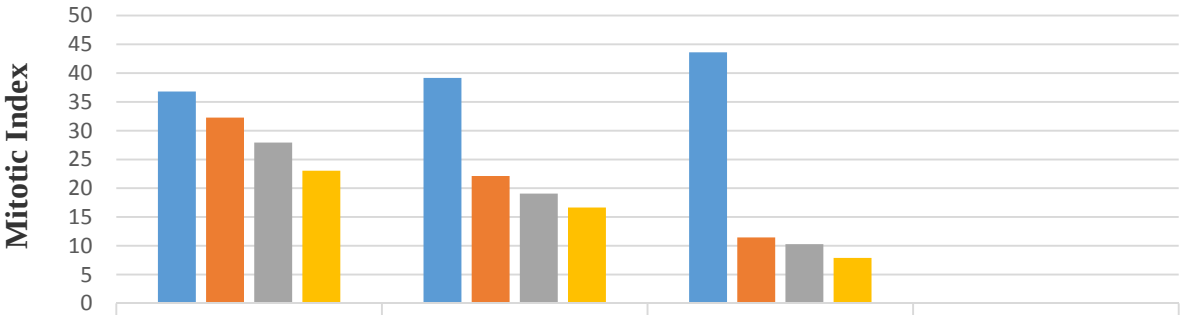
Table- I: Mitotic indices of the root apical meristems of *Allium cepa L.* treated with Carbendazim

Duration (Hours)	Concentration (g/ L)	Total No. of Cells analyzed (N)	Total No. of divided cells (n)	Mitotic Index (M. I) = n x 100 / N
6	Control	1326	488	36.80
	1	1224	395	32.27
	2	1221	341	27.92
	3	1311	302	23.03
12	Control	1333	522	39.15
	1	1302	288	22.11
	2	1322	252	19.06
	3	1224	204	16.66
18	Control	1286	561	43.62
	1	1328	152	11.44
	2	1322	136	10.28
	3	1315	104	07.90

Table- II: Carbendazim induced aberrations in root apical meristems of *Allium cepa L.*

Duration (Hours)	Concentration (g/ L)	Total No. of divided cells (N)	Total No. of aberrant cells (n)	% of aberrant cells = n X 100 / N
6	Control	488	0	0
	1	395	12	3.03
	2	341	29	8.50
	3	302	46	15.23
12	Control	522	0	0
	1	288	55	19.09
	2	252	61	24.20
	3	204	70	34.31
18	Control	561	0	0
	1	152	77	50.65
	2	136	83	61.02
	3	104	88	84.61

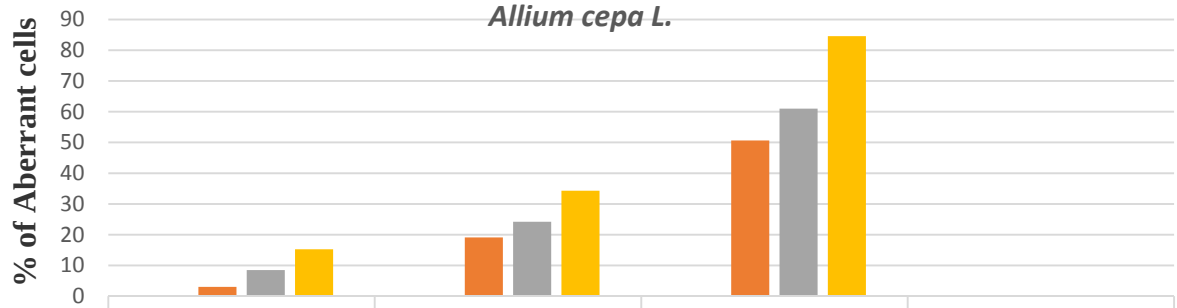
Fig- II Mitotic indices of root apical meristems of *Allium cepa L.* treated with Carbendazim at various levels of concentration and duration



	6 hr	12 hr	18 hr
Control	36.8	39.15	43.62
1 g/L	32.27	22.11	11.44
2 g/L	27.92	19.06	10.28
3 g/L	23.03	16.66	7.9

Duration

Fig- III Carbendazim induced aberrations in root apical meristems of *Allium cepa L.*

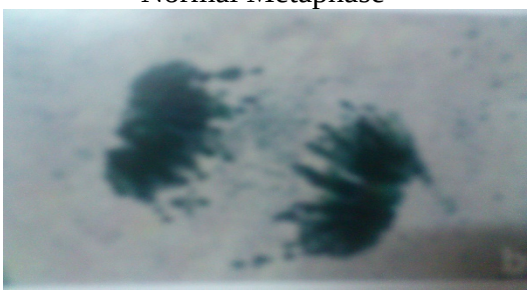


	6 hr	12 hr	18 hr
Control	0	0	0
1 g/ L	3.03	19.09	50.65
2 g/ L	8.5	24.2	61.02
3 g/ L	15.23	34.31	84.61

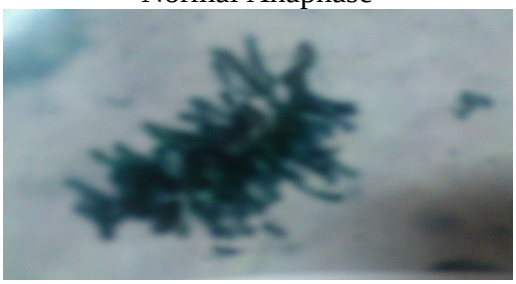
Duration

Fig- IV. Root meristems of *Allium cepa L.* showing normal Mitotic stages and selected Mitotic anomalies

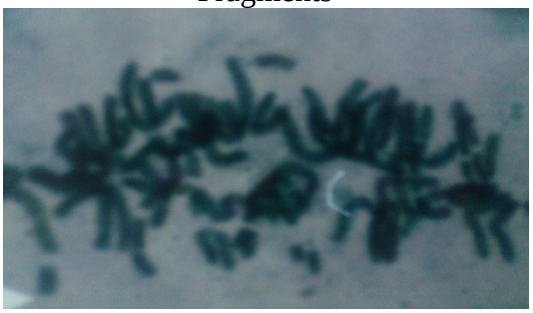
Normal Metaphase



Normal Anaphase



Fragments



Anaphase Bridge

A. Ring chromosome