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CYTOGENETIC ACTIVITIES OF FUNGICIDE ON THE ROOT APICAL MERISTEMS OF ONION PLANT (*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.When this study, thecytogenetic activities of systemic fungicide (Benomyl) were investigated in the mitotic cell division in onion plant (*Allium cepa* L.).Onion roots were treated with 1g/L, 2g/L and 3g/L concentrations of benomyl and distilled water as control at 3 hours, 6 hours and 12 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 benomyl solution is increased. Based on our findings, it is reported that benomyl has some negative effects on mitotic divisions in onion root tip cells.

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 anomaliesinduced 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 metabolic inhibitors and their modes of action can be classified into different groups; inhibitors of electron transport chain, inhibitors of enzymes, inhibitors of nucleic acid metabolism, protein synthesis and sterol synthesis (WHO, 1994). When they 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.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 cytogenetic activities.There is not much information on their effects on different plants.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).

Benomyl was first reported as a fungicide in 1968 and introduced onto the market in 1971 by the U.S Company Du Pont (Tomlin, 1994). It is a systemic, benzimidazole fungicide that is selectively toxic to microorganisms and to invertebrates, especially earthworms. It is used against a wide range of fungal diseases of field crops, fruits, nuts,ornamentals, mushrooms, and grasses. In Turkey, benomyl is used especially in the treatment of *Pyriculariaoryzae* Cav. in rice. Although the field use of pesticides has now become a common practice in rice cultivation.

The aim of this study is to determine the influence of benomyl in onion (*Allium cepa* L.)root tip cells during mitosis.

MATERIALS AND METHODS

1.1 Chemistry of Benomyl

Benomyl ($C_{14}H_{18}N_4O_3$) is a colorless crystalline solid (pure compound).It is a broad spectrum benzimidazolecarbamate fungicide with molecular weight=290.32 g/mol.(Fig. 1)

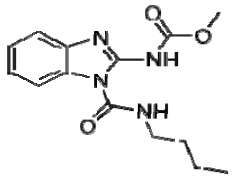


Fig. I-BenomylStructure

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 onionwere 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 benomyl dissolved with distilled water (1g/L., 2 g/L. and 3g/L.) for 3, 6 and 12 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 alcohol 1:3 (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. Mitotic index (MI) was calculated and different types of chromosomalaberrations were also observed and scored.

RESULTS AND DISCUSSION

Mitotic Index (M. I.)

According to Smakakinel*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, benomyl 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 benomyl 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.*Benomyl can be accepted as a toxic agent in this study.

Chromosomal aberration

Benomyl 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; Pandyet al., 1994; Armbruste, 1991; Badr, 1983; Behera, 1982, Mann, 1977).

In our study we observed some abnormalities in meristematic cells of *Allium cepa L.* The most common aberrations were fragments, bridges, C- Mitosis, stickiness, ring chromosome, disturbed anaphase, metaphase and telophasein cell division(Fig- III) (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 *Viciafaba* 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

Cytogenetic activities of fungicide (benomyl) were investigated in root meristems of *Allium cepa* L.Higher concentration and longer duration of treatment is toxic to cells.In our opinion, more detailed studies should be done on different types of the chemicals, which are used as pesticides. The outcome of this study suggests, safety measures to farmers avoid direct contact with high concentration of benomyl contaminated mud while working in the fields or in fields irrigated with benomyl 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.

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

Duration (Hours)	Concentration (g/ L)	Total No. of Cells analyzed (N)	Total No. of divided cells (n)	Mitotic Index (M. I) = $\frac{n \times 100}{N}$
3	Control	538	215	39.96
	1	522	196	37.54
	2	526	181	34.41
	3	524	166	31.67
6	Control	526	224	42.58
	1	531	142	26.74
	2	514	112	21.78
	3	518	97	18.72
12	Control	541	260	48.05
	1	512	76	14.84
	2	520	58	11.15
	3	516	32	06.20

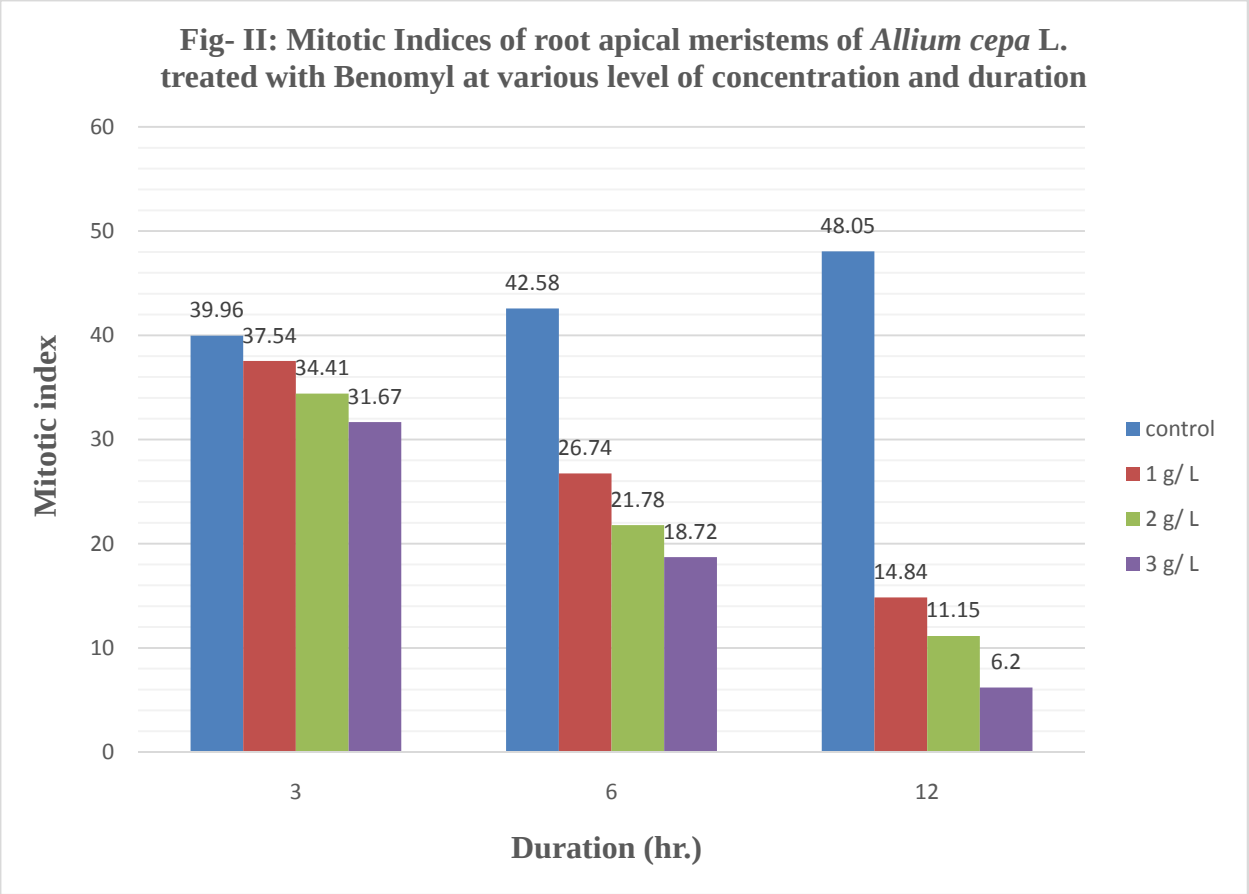
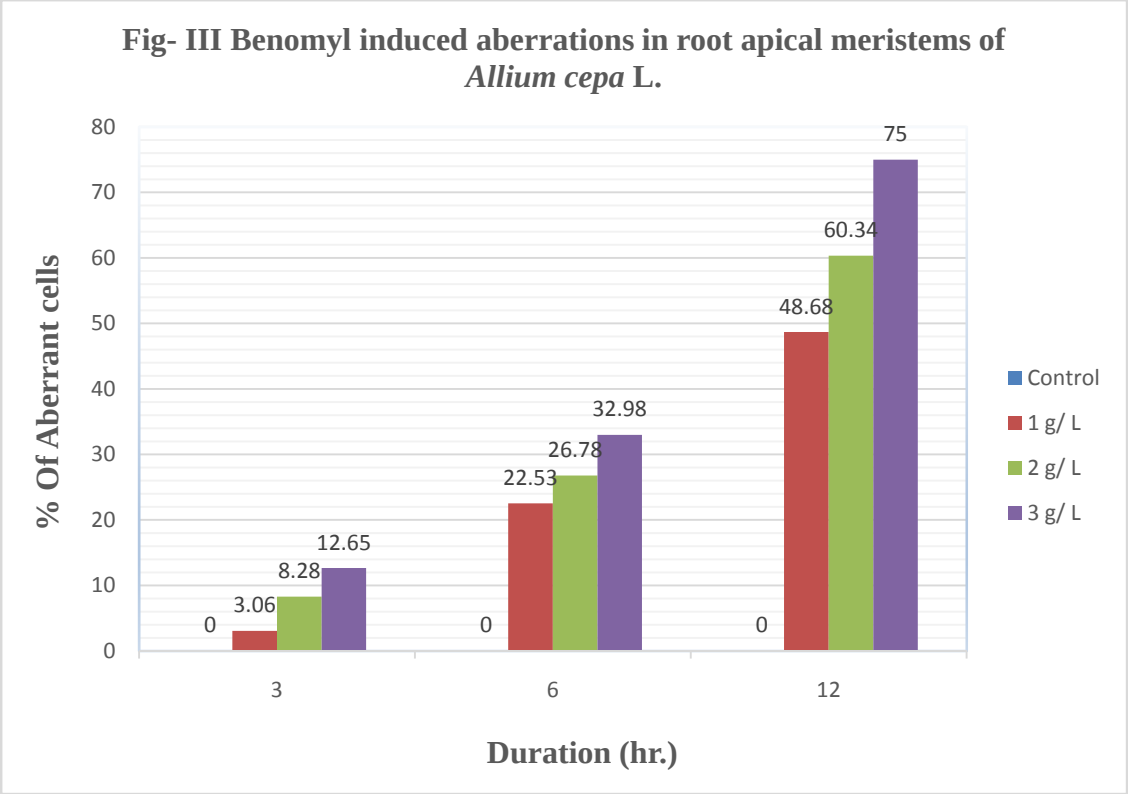


Table-II:Benomyl induced chromosomal aberrations in root apical meristems of *Allium cepa L.*

	Concentration (g/ L)	Total No. of divided cells (N)	Total No. of aberrant cells (n)	% of aberrant cells = n X 100 / N
3	Control	215	0	0
	1	196	06	3.06
	2	181	15	8.28
	3	166	21	12.65
6	Control	224	0	0
	1	142	32	22.53
	2	112	30	26.78
	3	97	32	32.98
12	Control	260	0	0
	1	76	37	48.68
	2	58	35	60.34
	3	32	24	75.00



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