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THE EFFECTS OF FUNGICIDE BENOMYL (BENLATE) ON GROWTH AND MITOSIS IN ONION (*ALLIUM CEPA* L.) ROOT APICAL MERISTEM

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In this study, the effects of benomyl, a systemic fungicide were investigated in the mitotic cell division in onion *(Allium cepa)* root tip cells during germination. For this aim, different concentrations (1, 2, 5, 10 and 20 mM) of benomyl solutions were used. All the concentrations used caused several abnormalities in mitotic cell divisions and the mitotic frequency in the onion root tip cells decreased as the concentration of benomyl solution increased. Based on our findings, it is reported that benomyl has some negative effects on mitotic divisions in onion root tip cells.

Keywords: Benomyl – toxicity – *Allium cepa* – root tip – mitosis

INTRODUCTION

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 [22].

Benomyl was first reported as a fungicide in 1968 and introduced onto the market in 1971 by the US company Du Pont [17]. 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 turf. Formulations include wettable powder, dry flowable powder and dispersible granules [22]. In Turkey, benomyl is used especially in the treatment of *Pyricularia oryzae* Cav. in rice. Although the field use of pesticides has now become a common practice in rice cultivation, there is not much information on their effects on different plants.

During our research we found studies about benomyl effectiveness, but little information is available about its cellular effects on different plants. An *in vitro* bioassay was conducted and a logistic dose-response model was developed for the fungicide benomyl in coneflower (*Echinacea* sp.). Benomyl was very effective in controlling

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Sclerotinia sclerotiorum in both bioassay and greenhouse evaluations [20]. Benomyl has been found to lessen the adverse impact of ozone on plants. Comparing plants treated with benomyl to untreated plants it is found that, on exposure to ozone, a greater fraction of light absorption energy was cycled through the photosynthetic system in benomyl-treated plants, as shown by the higher PSII-mediated electron flow and the higher fraction of open PSII reaction centers [5].

The factors influencing the uptake of pesticides by the shoots applied via seed treatment have been widely studied. From the two- or three-leaf stage up to the heading phase, systemic fungicides or insecticides applied as seed treatment to wheat, barley or rice were taken up mainly by the roots [12]. Since many of the pesticides have high pest-management rating, it was considered desirable to find out some alternative safer toxicant. More experiments are needed to demonstrate precisely the effects of such chemicals and establish their *in situ* levels of toxicity. The aim of this study is to determine the influence of benomyl in onion *(Allium cepa)* root tip cells during mitosis.

MATERIAL AND METHODS

A simple, fast and easy-to-perform method was carried out for the quantification of the inhibitory effects of benomyl on *Allium cepa.* The method uses germination, root elongation and growth as parameters in the presence of varying concentrations of benomyl. All experiments were performed on adventitious roots of the onion *Allium cepa.* The onions that are used in the experiment had been prepared as described by Wierzbicka [21]. They were grown in a plastic cylinder (8 cm in length, 6 cm in diameter) in opaque containers at 22 °C in distilled water.

At the beginning of the experiment 10 onions were set up and allowed to produce roots in distilled water. On the second day the bulbs with the poorest growth were discarded and other 7 were used. When the roots reached a length of 2 cm, 5 onions were transferred to different concentrations of benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) and the others were left in the previous solution and treated as controls. Benomyl (Benlate WP (wettable powder) 50%) was obtained from Du Pont Company. Five concentrations of benomyl were used 1, 2, 5, 10 and 20 mM. After 1 week treatment, the root tips of onions were fixed in Carnoy fixative (3 alcohol : 1 acetic acid) and hydrolysed in 1 N HCl at 50 °C for 5 minutes followed by squashing in a 2% aceto orcein stain. The experiments were repeated 3 times and the statistical analysis was performed acoording to these results. Slides were kept in a freezer and examined within a month. The Allium test was conducted according to Rank and Nielson with slight modification [15]. Slides were coded and scored for mitotic index (at least 500 cells/slide) and mitotic aberrations. The evaluation of chromosomal structural changes is performed during the first cell cycle after benomyl application. The aberrations were characterized and classified into the following categories: fragments, bridges, lagging chromosome, *c*-mitosis, multipolarity and lack of cytokinesis. The photographs were taken by an Olympus photomicroscope.

RESULTS

The results of this study performed with different concentrations of benomyl were evaluated in three steps; the effect of benomyl concentrations on the development of the onion roots, the ratio of abnormalities during mitosis and the kind of abnormalities caused by benomyl concentrations.

Table 1 shows the negative effect of benomyl concentrations on the development of the onion roots. From the table general toxicity can be determined. We can see that after the 3rd day, the development of the onion roots in 20 mM concentration of benomyl remained constant and after the 6th day, there was approximately no change in the length of the treated roots compared to the control (Table 1). Variance analysis was used to determine the significance of the values. As the variance of groups was proved to be homogeneous, Tukey HSD test was performed with SPSS.

As a result of binary comparison, there was significant difference ($p < 0.05$) between 1 and 2 mM ($p = 0.009$), 1 and 5 mM ($p = 0.004$), 1 and 10 mM ($p = 0.003$), 1 and 20 mM ($p = 0.000$), 2 and 20 mM ($p = 0.002$), 2 mM and control ($p = 0.000$), 5 and 20 mM ($p = 0.005$), 5 mM and control ($p = 0.000$), 10 and 20 mM ($p = 0.008$), 10 mM and control ($p = 0.000$), 20 mM and control ($p = 0.000$).

Table 1
The effects of benomyl concentrations on the growth of the onion root tips

Benomyl concentration	Root length (cm)							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Mean value ± SD
1 mM	3	4	5	5.5	6	6	6	5.071 ± 1.170
2 mM	3	3.5	3.9	4	4	4.2	4.2	3.828 ± 0.434
5 mM	3	3.5	3.8	3.9	3.9	4	4	3.728 ± 0.363
10 mM	3	3.5	3.6	3.8	3.9	3.9	4	3.671 ± 0.345
20 mM	2	2.3	2.5	2.5	2.5	2.5	2.5	2.400 ± 0.191
Control	3	4.5	5.5	6.3	6.5	7.5	8	5.900 ± 1.733

The effect of benomyl concentrations on mitotic frequency

The effect of benomyl concentrations on mitotic frequency is shown in Figure 1. All the concentrations have negative effects on mitosis. The frequency of mitosis decreases as the concentration of benomyl increases (Fig. 1).

The ratio of abnormalities during mitosis

The ratio of abnormalities of onion root tip cells during mitosis is shown in Figure 2. It can be seen that the ratio of abnormalities increases parallel to the increase in concentration of benomyl (Fig. 2).

The kind of abnormalities caused by benomyl concentrations

In prophase, instead of normal chromatin condensation chromatin granulation was observed (1 and 2 mM concentrations). Chromosomes were bent down outside of the equatorial plane and polarity was seen in metaphase. In anaphase chromosomal bridges and change in the plane of the cell division were observed (2, 5 and 10 mM concentrations) (Fig. 3b, c). In telophase lack of cytokinesis (two nuclei in one cell)

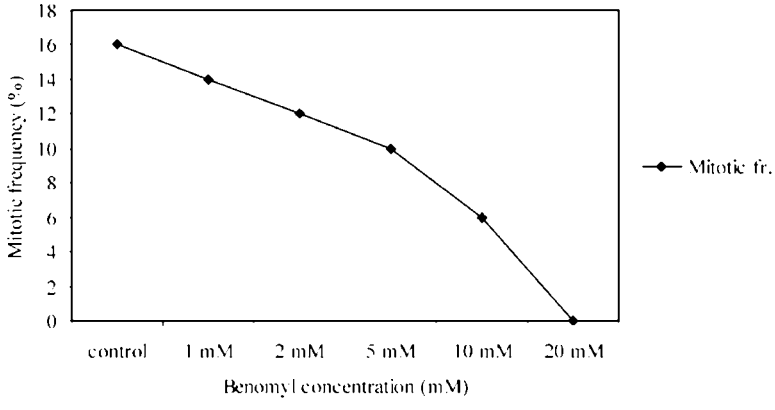


Fig. 1. The effect of benomyl concentration on mitotic frequency

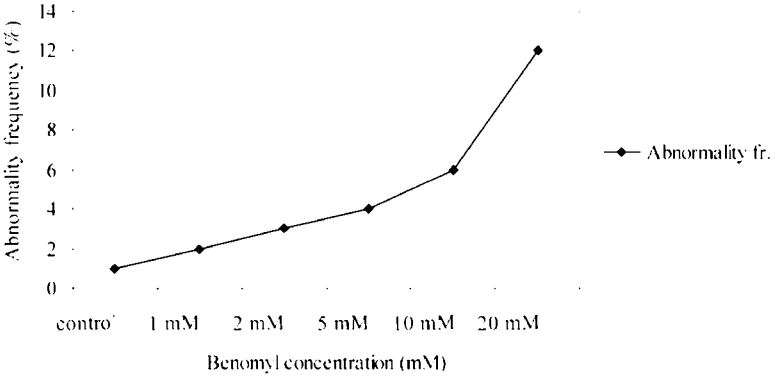
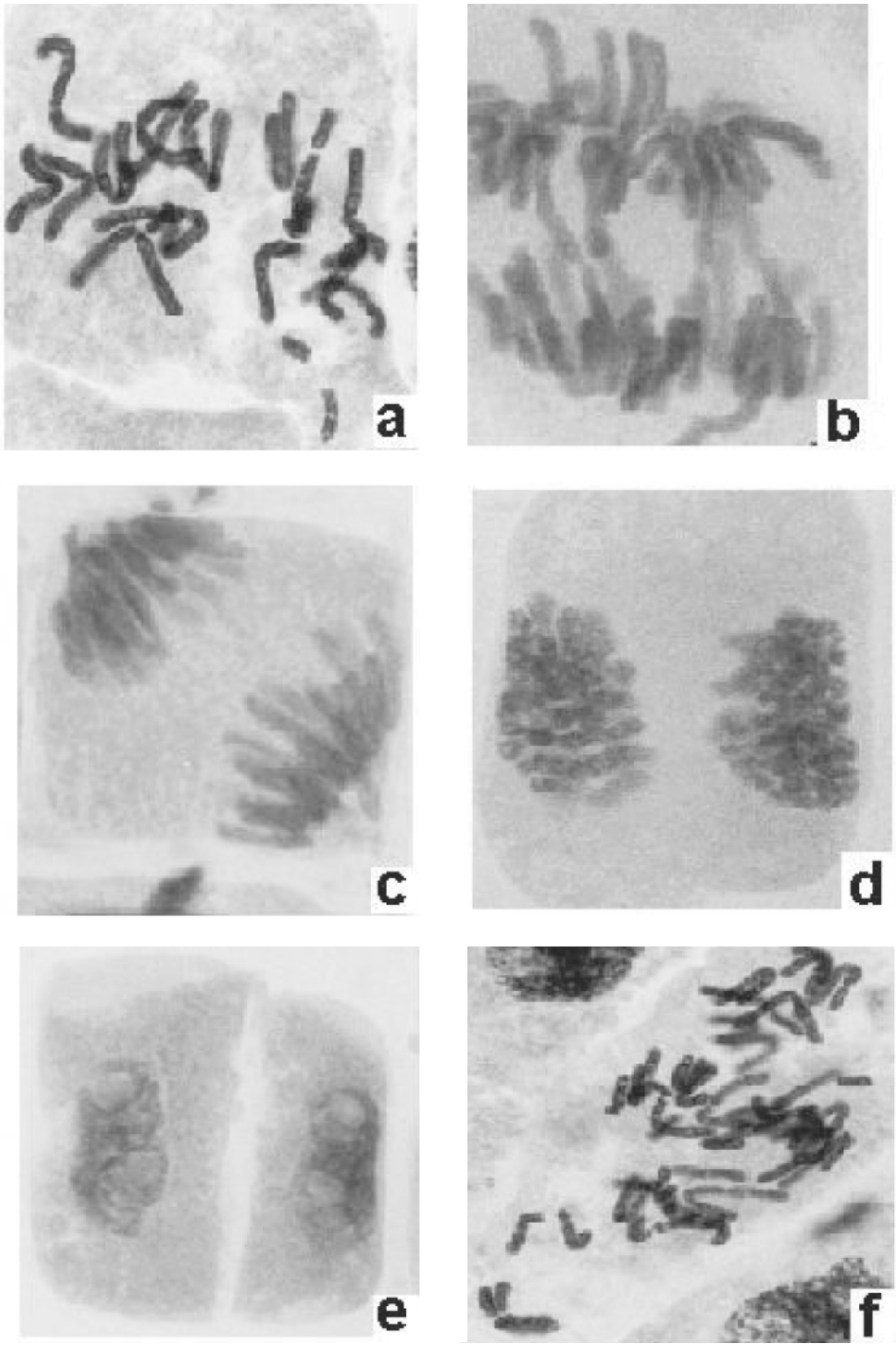


Fig. 2. The effect of benomyl concentrations on abnormal cell frequency

Fig. 3. The mitotic aberrations in onion root tip meristematic cells: a) *c*-mitosis, b) chromosomal bridges, c) diagonally spindle, d) perpendicular spindle and chromatin granulation, e) asymmetrical cells, f) poliploidy


a
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was observed and also instead of chromatin restitution, chromosomes become swollen (2 and 5 mM concentration) (Fig. 3d). In cytokinesis because of unequal cytoplasmic division asymmetrical cells were observed (2 and 5 mM concentration) (Fig. 3e). Vacuolization and chromatin deformation was observed in interphase (10 and 20 mM concentrations) (Fig. 4a, d). C-mitosis, diploidy and polyploidy were seen in metaphase (5 and 10 mM concentrations) (Fig. 3a, f).

Picnotic and degenerated nuclei were seen in interphase. Also degeneration of some of the cells and lagging chromosome fragments were observed in 10 and 20

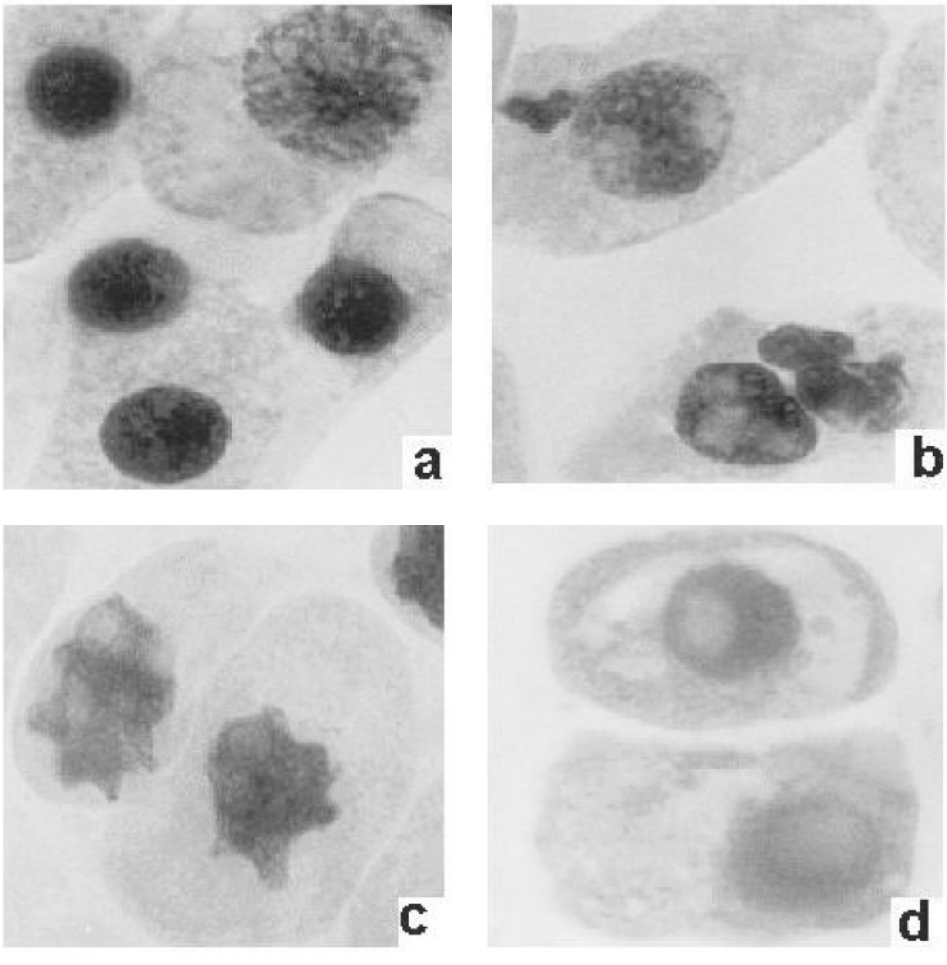

Fig. 4. The abnormalities in interphase on onion root tip meristematic cells: a) picnotic nucleus and chromatin deformation, b) swollen lagging chromosomes and multinucleated cells, c) degenerated nuclei, d) vacuolated meristematic cells

mM concentrations (Fig. 4a–c). Two and 5 mM concentrations caused genotoxic effect (chromosomal bridges, lagging chromosomes, chromosomal fragments, etc.) (Figs 3a, 4b).

DISCUSSION

Benomyl is a colorless crystalline solid (pure compound). It is mildly irritating to skin but without dermatitis hazard. Dermal: LD_{50} = >10,000 mg/kg (rabbit), oral: LD_{50} = >10,000 mg/kg (rat), inhalation: LC_{50} = >2 mg/l (dry product, 4-hour exposure, rat), eyes: 10 mg of dry 50% powder or 0.1 ml of 10% suspension in mineral oil caused only temporary mild conjuctival irritation (rabbit) (Benomyl Chemical Profile 8/89, Du Pont Company.).

Benomyl completely degrades to carbendazim within several hours in acidic or neutral water. The half-life of carbendazim is 2 months [10]. Since benomyl is a systemic fungicide, it is absorbed by plants. Once it is in the plant, it accumulates in veins and at the leaf margins. The metabolite carbendazim seems to be the fungicidally active agent. Benomyl residues are quite stable; with 48 to 97% remaining as the parent compound 21 to 23 days after application [18]. Benomyl and its main metabolite carbendazim bind to microtubules (an essential structure of all cells) and therefore interfere with cell functions such as cell division and intracellular transportation. [22].

The studies on the effects of different pesticides found out some of the genotoxic effects of pesticides on plants [1, 2, 7, 16]. Genetic changes induced by pesticides, their metabolites, and residues are expressed by various endpoints, which include; structural changes in chromosomes and chromatids, called chromosomal aberrations (breaks, deletions, inversions, gaps, translocations, rings) and other disturbances (stickiness, clumping, erosion). Disturbances in the mitotic or meiotic division, like spindle inactivation, causing so-called *c*-mitosis, non-disjunction, and other irregularities in the chromosome distribution during anaphase, resulting in polyploid or aneuploid cells. While we observed normal mitotic divisions in the control group, different kinds of abnormalities were seen in all of the benomyl concentrations varying to phases of mitosis. In our study we observed some abnormalities in both interphase and mitotic divisions in meristematic cells of *Allium cepa.* These abnormalities were; the defects in the miotic spindle, the lack of directioning of the mitotic spindle, karyokinesis without cytokinesis. Furthermore, the negative effects on chromatin condensation and decondensation and some abnormal vacuoles were observed in the interphase.

The effect of various fungicides used as seed treatment has been reported in some field crops and cereals [3, 12]. In different studies with different pesticides, some similar results were found. Insecticide endosulphan caused several chromosomal abnormalities in the mitotic cell divisions and endosulphan produced a decrease of about 75% in the mitotic cell division frequency, depending on the dose [1]. In Spring Wheat (*Triticum aestivum* L.) uptake and fate of fungicide triticonazole

applied as seed tretament was investigated and active ingredient concentration in the plant shoots was found [12]. In another study, the changes that could take place in the root meristem regions of onion with an application for different periods and at different concentrations of insecticide decis was investigated. Treatment with decis for 24 hours negatively affected the onion root meristematic cells. The shape of the nucleus of the onion meristematic cells was changed and bigger nuclei were seen [7]. *Herbicide monuron* caused abnormal development in some plant tissues, especially in leaf epidermis. Phasalon and Cycloheximide had effect on onion root tip cells, it is reported that *in vitro* and *in vivo* nuclear abnormalities took place after the treatment with these chemicals [2, 16].

The effect of benomyl on *Ascomycete ascochyta rabiei* (Pass.) was investigated. Microscopic analyses revealed that within the examined isolates five different combinations of cell number and number of nuclei in spores existed [4]. The fungicide benomyl was evaluated for effectiveness in controlling three common fungal contaminants, as well as their impact on the growth and development of Arabidopsis seedlings. Benomyl proved to be an effective inhibitor of all three contaminants in concentrations as low as 2 ppm within the agar medium, and no evidence of phytotoxicity was observed until concentrations exceeded 20 ppm [13].

Plants are the main recipients of pesticides, regardless of whether they themselves represent the target organism (e.g. weed) or whether the targets are pests, pathogenic fungi, etc. They are exposed to pesticides from direct application, through the uptake from soil and water, and from atmospheric drift. Pesticides tend to be very reactive, mostly electrophilic, compounds that can react with various nucleophilic centres of cellular biomolecules, including DNA [8], or form even more reactive electrophilic products that either modify cellular components or are metabolized to more or less stable products.

In our experiments the negative side-effects of benomyl on onion root tip cells during mitosis were investigated. Treatment of the onion tip cells with benomyl caused several negative effects in mitotic cell division. According to us, the reason of *c*-mitosis and the change in the plane of the cell division is the effect of benomyl on the polymerization of microtubules. Microtubules have directive function on transport of cellular elements to equatorial plane thus lack of cytokinesis in different benomyl concentrations can be explained by the inhibition of microtubules. Abnormal chromation condensation is related to the inhibition of enzymes and histon proteins. So benomyl concentration may have effect on inhibition of enzymes. And also in meristematic root tip cells of *Allium cepa* there was no vacuolization in the control group during interphase but in 20 mM concentration of benomyl big vacuoles were seen. Vacuolization of the meristematic cells shows that the division specificity of the cells are effected by the given benomyl concentrations.

In our opinion, more detailed studies should be done on different types of the chemicals, which are used as pesticides.

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