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# Assessment of Cytotoxic and Genotoxic Effect of Carbamate Insecticide:Bendiocarb in *Allium cepa* Roots

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**Abstract:** *Bendiocarb is a chemical in the carbamate insecticide class that is widely used to protect foodstuffs consumed daily against pests and to increase their yield. In this study, genotoxic and cytotoxic effects of Bendiocarb on cells evaluated by Allium cepa L. test. Mitotic Index (MI), RAPD-PCR Test, Antioxidant activity determination and Apoptosis Necrosis cell death parameters were used as genotoxic and cytotoxic parameters. A. cepa seeds were divided into six (6) groups as one (1) control and five (5) treatment doses. All seeds were germinated with tap water for 24 hours and then treated with tap water in the control group and with 10 µM, 25 µM, 50 µM, 100 µM, 200 µM doses of Bendiocarb in the treatment group for 72 hours. At the end of the period, root tips were subjected to routine preparation procedures. As a result; Bendiocarb treatment revealed statistically significant differences in mitotic index values depending on the dose ($p<0.05$). In RAPD-PCR test, appearing of band formation were observed. In the determination of antioxidant activity, a significant decrease in SOD, CAT and GPx tests and an increase in MDA ratio were observed. In the apoptosis necrosis test, Ethidium bromide/Acridine orange dyes were mixed and the changes in the cells were evaluated with increasing doses. Bendiocarb has been found to cause serious genotoxic and cytotoxic toxic effects, which are dose-dependent.*

**Keywords:** *Pesticide, Insecticide Bendiocarb, RAPD-PCR, Antioxidant, Apoptosis.*

## 1. INTRODUCTION

Agricultural crops and other plants are treated in a controlled manner with pesticides and other chemicals under modern agricultural practices. Chemical pesticides are widely preferred for crop and grain protection because of their high biological activity against plant pests [1]. These pollutants pose a serious risk to plants, as well as to the health of humans and animals that use these plants as a source of food or feed. In countries where the economy is based on agriculture, using pesticides is a serious concern. The use of living organisms (bioindicators) is an important method for detecting stress factors caused by environmental pollutants and monitoring their effects [2]. Detecting cytotoxic and genotoxic damage in plants, the Allium test can be used. The Allium test was first used by Levan in 1983 and since then it has been considered as a standard material in studies to examine the effects of various chemical pollutants on chromosomes [3].

Bendiocarb (2,2-dimethylbenzo-1,3-dioxol-4-yl methylcarbamate) is a broad-spectrum carbamate insecticide developed specifically for the control of insects and other arthropod pests in industry and public health [4]. Bendiocarb was registered for use in the United States in 1980 for a variety of indoor, outdoor and also greenhouse uses. For oral exposure, bendiocarb is in Acute Toxicity Category I, the highest of the four categories.

In recent years, molecular tools such as cytogenetic tests and RAPD-PCR tests have been used to investigate the damage caused to the organism by agents with genotoxic and mutagenic effects on plants also. The basic principle of RAPD-PCR is that randomly selected oligonucleotides on the genomic DNA of the species of interest are randomly bound at low binding temperature and copies are made by PCR [5]. In the continuation of the technique, the product obtained is run on gel electrophoresis and stained and analyzed as band alterations for better observation. The results are evaluated according to whether the

bands are visible or not [6]. The biggest advantage of the RAPD-PCR method is that it can be used in studies on plant and animal genomes without the need for any prior knowledge of the gene sequences to be studied [7].

Molecular oxygen is an essential molecule in the oxidative metabolic processes of aerobic organisms, but harmful reactive oxygen species (ROS) such as superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^-$) can also be formed during these processes. Due to their high reactivity, these species can damage macromolecules. If the strength of the antioxidant defense system decreases or pro-oxidant processes increase, oxidative stress occurs [8,9,10].

Malondialdehyde (MDA) is the most abundant aldehyde in the cell in active form among the lipid peroxidation products formed as a result of the breakdown of lipid peroxides. It is formed as a result of peroxidation of fatty acids containing more than two bonds and causes cross-linking of membrane components. Therefore, deformation can alter membrane properties such as inactivation of membrane-bound enzymes and receptors. It is a parameter used as an indicator of lipid peroxidation [11].

Antioxidants play an important role in preventing oxidative stress caused by free radicals. Antioxidants neutralize free radicals by donating electrons and reduce oxidized molecules. Thus, they protect the structure and function of cells [12]. Common enzymes involved in the detoxification of free radicals include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). These enzymes reduce oxidative stress by interacting with different reactive oxygen species in biological systems [13]. These enzymes act on the level of oxidative stress by sweeping free radicals [14].

In this study, toxic effects of Bendiocarb, an insecticide, on *A. cepa* root tip meristem cells were investigated. For this purpose, cytotoxic and genotoxic effects on onions due to increasing doses were determined by using various parameters such as Mitotic Index, RAPD-PCR, Antioxidant Activity levels and Apoptosis Necrosis Test.

## 2. Materials and Methods

### 2.1. Plant Material

For germination, *A. cepa* roots were treated with tap water at different times in 15 mL test tubes. After the roots germinated, the test tubes were changed and placed on test tubes containing the chemical and exposed to the chemical. Studies were conducted 24, 48 and 72 hours after *A. cepa*'s were exposed to Bendiocarb. In the experiments, different concentrations of Bendiocarb (10 µM- 25 µM- 50 µM- 100 µM- 200 µM) were applied to *A. cepa*.

### 2.2. Cytogenetic Impact and Mitotic Index (MI) Determination

*A. cepa* (2n=16) was used as test material. *A. cepa* test material was germinated with tap water for 24 hours. After 24 hours, tap water was exposed to the chemical by replacing it with different concentrations of Bendiocarb (10 µM-200 µM). The treatments were performed for 24, 48 and 72 hours under the same laboratory conditions. After 72 hours, the roots were cut into 1 cm lengths and incubated in a solution containing potassium metabisulphite and potassium nitrate for 24 hours. For cytogenetic evaluation, they were kept in acetocarmine for 4 hours and stained. After this step, the root tips were hydrolyzed in 1 N HCl at 60 °C for 10 minutes and made into a permanent preparation with the help of entellan. Damaged and total cells, one control and five dose preparations, were examined under a light microscope (Olympus BX53 DP72) with a 40X objective [15].

% MI = Number of dividing cells / Total number of cells X 100

### 2.3. DNA Isolation

Total genomic DNA was extracted from *A. cepa* root tip meristem cells using the Qiagen DNeasy plant mini kit (Qiagen, Hilden, Germany) after 72 h exposure to bendiocarb. The extraction method was applied following the instructions for use. Spectrophotometric measurements, optical density (OD) measurements were performed at 260 and 280 nm (ACTGene MicroSpectrophotometer) for the sampled DNA and OD260/OD280 was used for DNA purity. A260/A280 ratio indicates DNA purity and values of 1.8-2.0 indicate the amount of 'pure DNA' [16].

### 2.4. RAPD-PCR Method

RAPD-PCR was used to amplify a randomly selected regions of DNA. First, RAPD-PCR mix mixture was prepared and then transferred into eppendorf tubes in the same amounts. The prepared DNA isolates were added to the mix [17]. RAPD-PCR mix mixture; 2.5 µl PCR Buffer, 3 µl $M_gCl_2$, 2 µl dNTP, 2 µl DMSO, 1 µl randomly selected primer (OpC5), 0.5 µl Taq polymerase, 1 µl Template 1 µl and $ddH_2O$ was prepared as a total volume of 13.5 µl. 1X TAE buffer was used for running in the electrophoresis tank. For the gel preperation, 60 ml of 1X TAE buffer was taken and mixed with 0.9 g Agarose and heated in the microwave. 16 µL Ethidium bromide was added to the boiling homogenized gel. The gel was poured onto the tray and a comb was used to create wells. RAPD bands of the control and treatment groups were scored according to new band appearing and dissappearing band formations [18].

### 2.5. Preparation of Samples for Antioxidant Activities

"Bioassay Technology Laboratory ELISA Kit" was used to determine the amount of MDA in *A. cepa* test material. *A. cepa* roots were incubated in 1% (w/v) sodium hypochlorite for 10 minutes. This was followed by incubation in 75% (w/v) ethanol for 5 minutes and then surface sterilised three times with sterile water. After surface sterilization, seeds were placed in petri plates containing different concentrations of Bendiocarb (10 µM-200 µM). At the end of 72 h, the root tips were removed from the Bendiocarb, cut into 1 cm lengths and homogenized in 3.5 mL potassium phosphate buffer (0.1 M, pH 7.8) using a mortar and pestle. The homogenate was centrifuged at 15,000 g for 15 min at 4 °C. The supernatant obtained after centrifugation was separated for the measurement of malondial-dehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities [19].

Standards were prepared according to the method included in the kit. Standards were added 50 µl to the wells in a 96-well plate. Moving to the other wells, 40 µl each of the samples to be studied were taken and 10 µl each of anti-MDA, anti-SOD, anti-CAT and anti-GPx were added.

50 µL streptavidin-HRP was added to the standard and sample wells in the 96-well plate and the plate was incubated at 37 ˚C for 60 minutes. After incubation, the wells were washed 5 times for 30-60 seconds with 0.35 mL of wash buffer. 50 µL of standard solution A and then 50 µL of standard solution B were added to each well and incubated at 37 ˚C for 10 minutes.Then 50 µL of stop solution was added to each well and measured by setting the microplate reader to 450 nm [20].

### 2.6. Determination of Apoptosis-Necrosis Findings

*A. cepa* roots were germinated in tap water for 48 hours. After germination, they were treated with increasing doses (10µM-200µM) of Bendiocarb for 48 hours. At the end of 48 hours, *A. cepa* roots were cut into 1 cm lengths and stirred on a magnetic stirrer at 250 rpm for 5 min. Then incubated at +4 C for 20-25 min. Lysates were separated from *A. cepa* roots by filtration and transferred to an eppendorf tube. Ethidium bromide and Acridine orange dyes were mixed 1:1 ration in another eppendorf tube. The lysates and dye mixture were mixed at a ratio of 1:5, spread on the slide and covered with coverslip. After drying for 15 minutes, they were visualized by fluorescence microscopy.

### 2.7. Statistical Analysis

All obtained data were calculated by one-way analysis of variance (ANOVA) and Tukey test to determine statistical significance. Standard deviation (SD) was used for mean values. $P<0.05$ was selected for significance level. All measurements were performed three times and the results are given as mean ± SD.

## 3. RESULTS

Allium test system was used to determine the genotoxic and cytotoxic activity of Bendiocarb. After *A. cepa* roots were exposed to Bendiocarb solution, the toxic effect of Bendiocarb was determined by analyzing morphological and cytogenetic changes in root tip meristem cells.

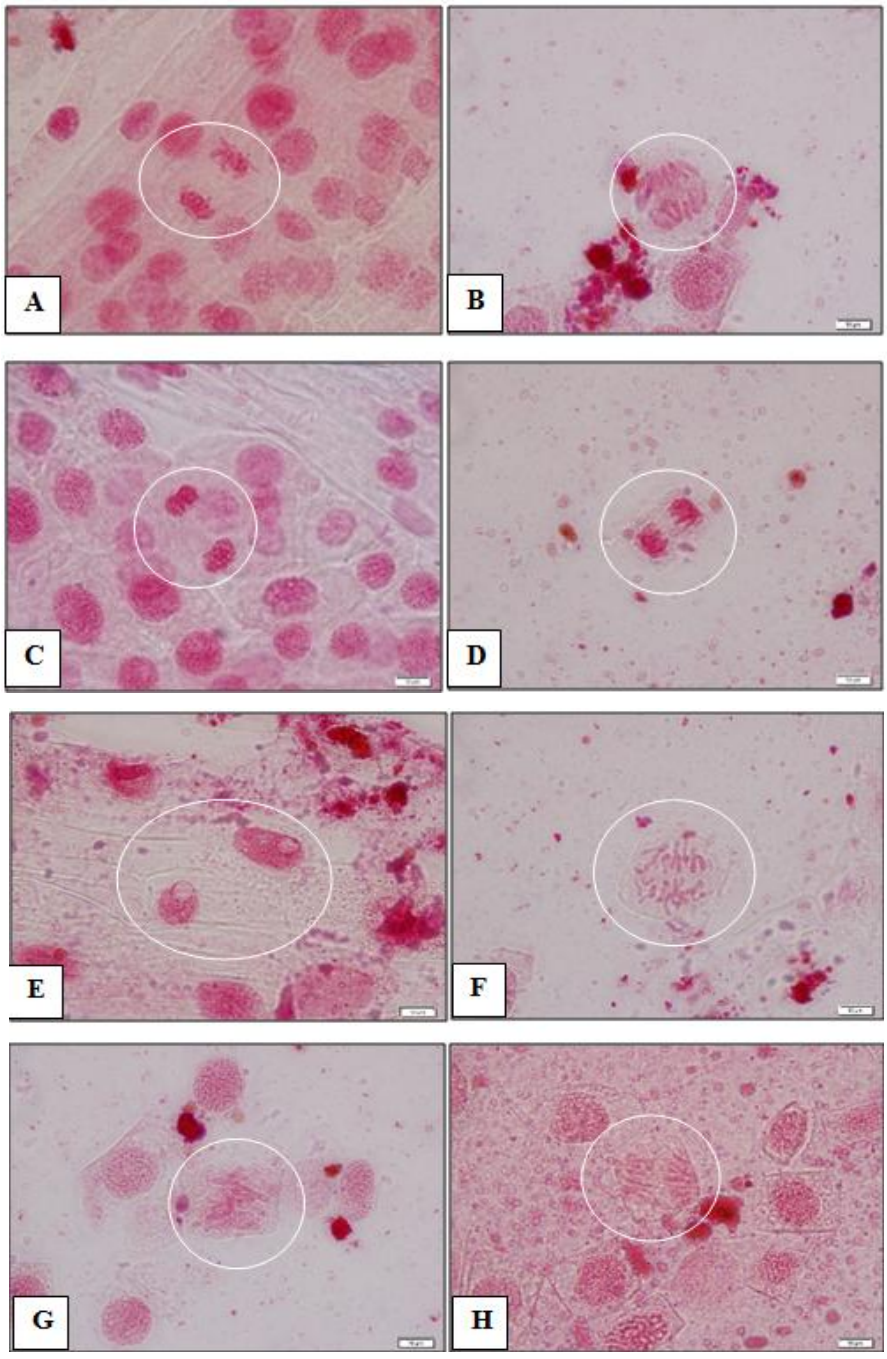
### 3.1. Determination of Mitotic Index (MI) and Chromosome Abnormalities

Mitotic indices (%) of *A. cepa* root tip meristem cells exposed to control and different concentrations of Bendiocarb solution were calculated. Depending on the duration of exposure of roots to Bendiocarb, MI increased significantly at different concentrations in the Bendiocarb treatment groups compared to the control group (Table 3.1.).

Different types of chromosomal abnormalities were detected at the end of 72 hours of exposure of Bendiocarb treatment groups to Bendiocarb compared to the control group. These anomalies were determined as adhesion in anaphase, C-mitosis, multiple nuclear lesions, normal metaphase, diagonal anaphase, irregular metaphase (Figure 3.1.).

**Table3.1.** *Chromosomal abnormalities and mitotic index induced by bendiocarb at different doses and times*

| **Concentration (µM)** | **Number of Cells** | **Mitotic indeks %±SD 24 h** | **Mitotic indeks %±SD 48h** | **Mitotic indeks %±SD 72h** |
|---|---|---|---|---|
| Control | 705 | - | - | - |
| 10 µM | 601 | 12.36 ± 3.74 | 9.95 ± 3.05 | 7.14 ± 2.11 |
| 25 µM | 566 | 8.22 ± 2.89 | 7.07 ± 2.46 | 5.75 ± 1.65 |
| 50 µM | 500 | 5.39 ± 2.33 | 5.11 ± 2.22 | 3.61 ± 0.24 |
| 100 µM | 468 | 3.78 ± 1.25 | 3.20 ± 1.07 | 1.79 ± 0.15 |
| 200 µM | 374 | 3.52 ± 1.12 | 1.63 ± 0.04 | 0.92 ± 0.01 |



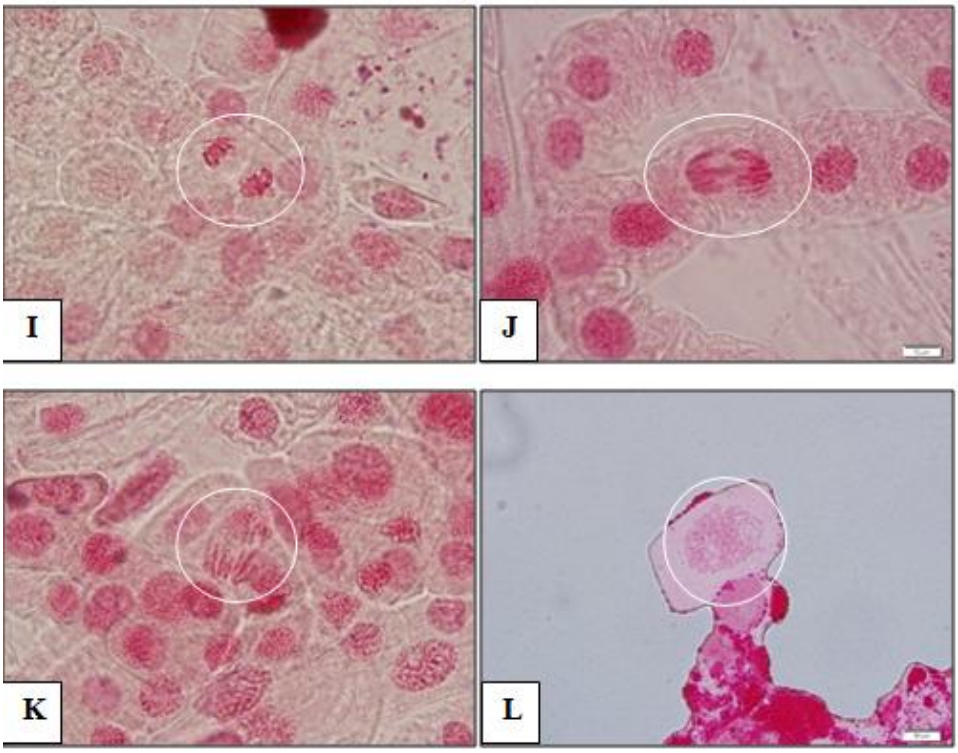



**Figure 3.1.** *Chromosome abnormalities induced by Bendiocarb in root tip meristem cells of A. cepa. (A) Anaphase, (B) Normal Metaphase, (C) Anaphase lagging behind, (D) Chromosome Shift, (E) Multiple Nuclear Lesions, (F) Diapausal Anaphase, (G) Irregular Metaphase, (H) Chromosome Fragments, (I) Chromosome Shift, (J) Chromosome Bridge, (K) Arctic Dry, (L) C Mitosis.*

### 3.2. Evaluation of RAPD-PCR Findings

RAPD-PCR results according to increasing concentrations of bendiocarb are shown in Figure 3.2. When the bendiocarb treatment groups were interpreted by comparing to the control group, it was seen that the normal bands disappeared and new bands were appeared.

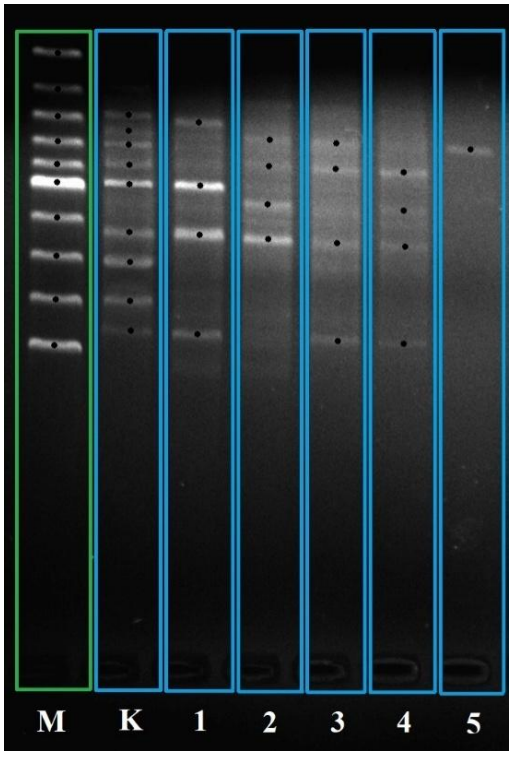



**Figure 3.2.** *RAPD bands generated with OpC5 primer in root tip meristem cells of A. cepa exposed to increasing concentrations of Bendiocarb. (C) Control, (M) Marker, (1) 10 µM, (2) 25 µM, (3) 50 µM, (4) 100 µM, (5) 200 µM.*

### 3.3. Evaluation of MDA Level

When the effect of bendiocarb application on MDA enzyme activity was compared with the control group, it was determined that there was a significant dose-dependent increase in enzyme activity ($p<0.05$) (Figure 3.3.).

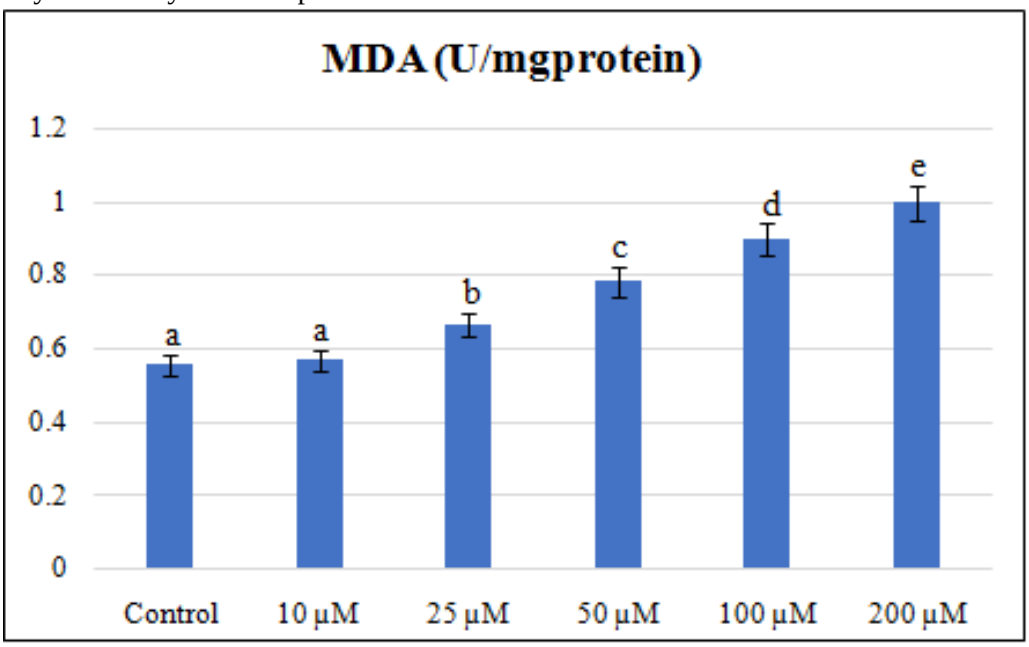



**Figure 3.3.** *Comparison of MDA enzyme activities in A. cepa root tip meristem cells of control and treatment groups. Groups with different letters in the columns indicate groups with a statistically significant difference ($p<0.05$).*

### 3.4. Evaluation of SOD Enzyme Activity

In terms of SOD enzyme activity, significant changes were observed when the control group was compared with the groups treated with increasing doses of Bendiocarb and a decrease in the enzyme activity was detected ($p<0.05$) (Figure 3.4.).

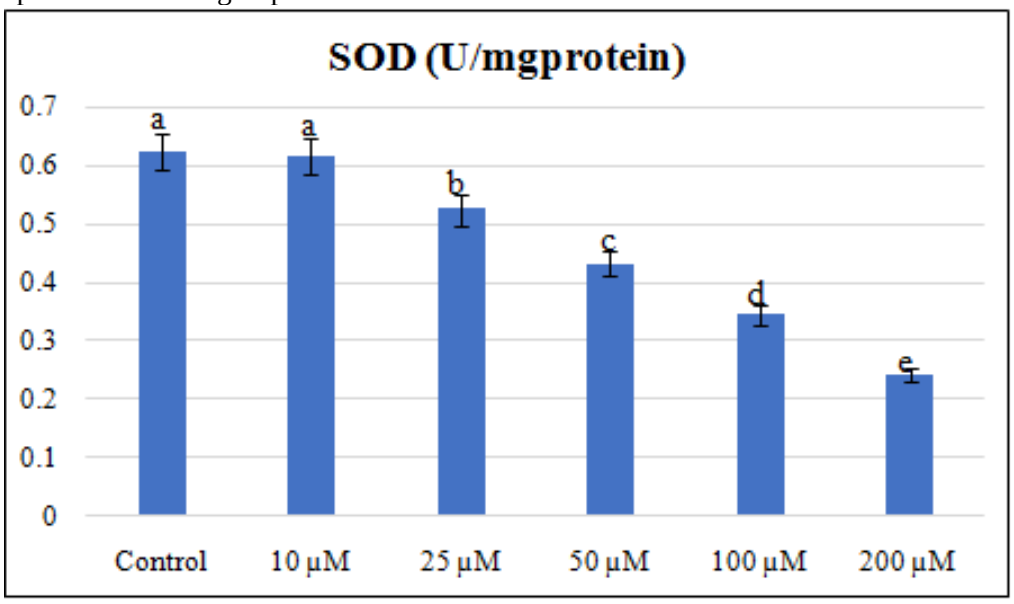



**Figure 3.4.** *Comparative analysis of SOD enzyme activities of control and treatment groups.Groups with different letters in the columns indicate groups with a statistically significant difference ($p<0.05$).*

### 3.5. Evaluation of CAT Enzyme Activity

In terms of CAT enzyme activity, it was determined that there was no statistically significant change between the control group and 10 µM application group, but there was a significant decrease in increasing doses ($p<0.05$) (Figure 3.5.).

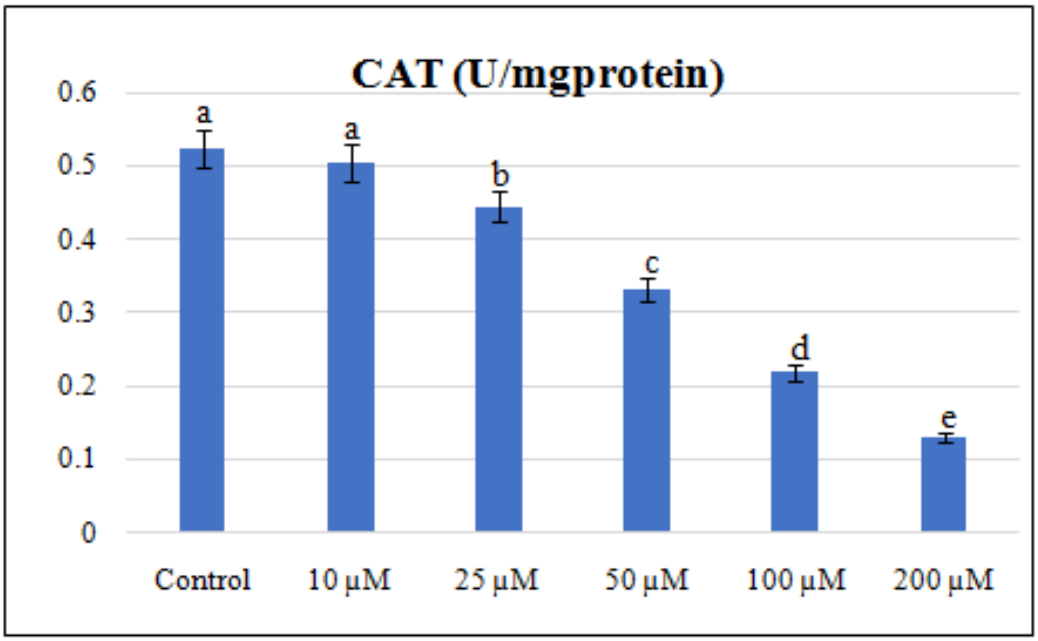



**Figure 3.5.** *Comparison of CAT enzyme activities in A. cepa root tip meristem cells of control and treatment groups.Groups with different letters in the columns indicate groups with a statistically significant difference (p<0.05).*

### 3.6. Evaluation of GPx Enzyme Activity

In terms of GPx enzyme activity, it was determined that there was no statistically significant change between the control group and the 10 µM application group, but there was a significant decrease in increasing doses (p<0.05) (Figure 3.6.).

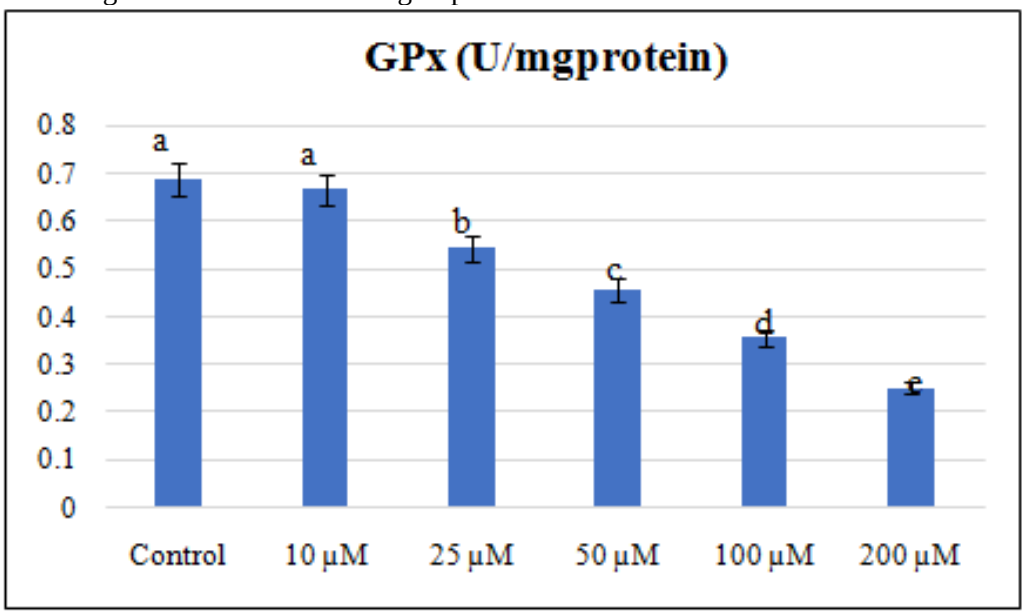



**Figure 3.6.** *Comparison of GPx enzyme activities in A. cepa root tip meristem cells of control and treatment groups.Groups with different letters in the columns indicate groups with a statistically significant difference (p<0.05).*

### 3.7. Evaluation of Apoptosis Necrosis Findings

In the control group, the density of viable cells appearing in green color was observed. At increasing doses, the density of cells appearing green at 10 µM was close to that of the control group. At 25 µM, it was observed that yellow cells started to form next to green cells. At 50 µM, green, yellow and orange cell formation was observed. At 100 µM, orange and red cell formation was observed. When increased to 200 µM, there was a significant increase in red cell density (Figure 3.7).

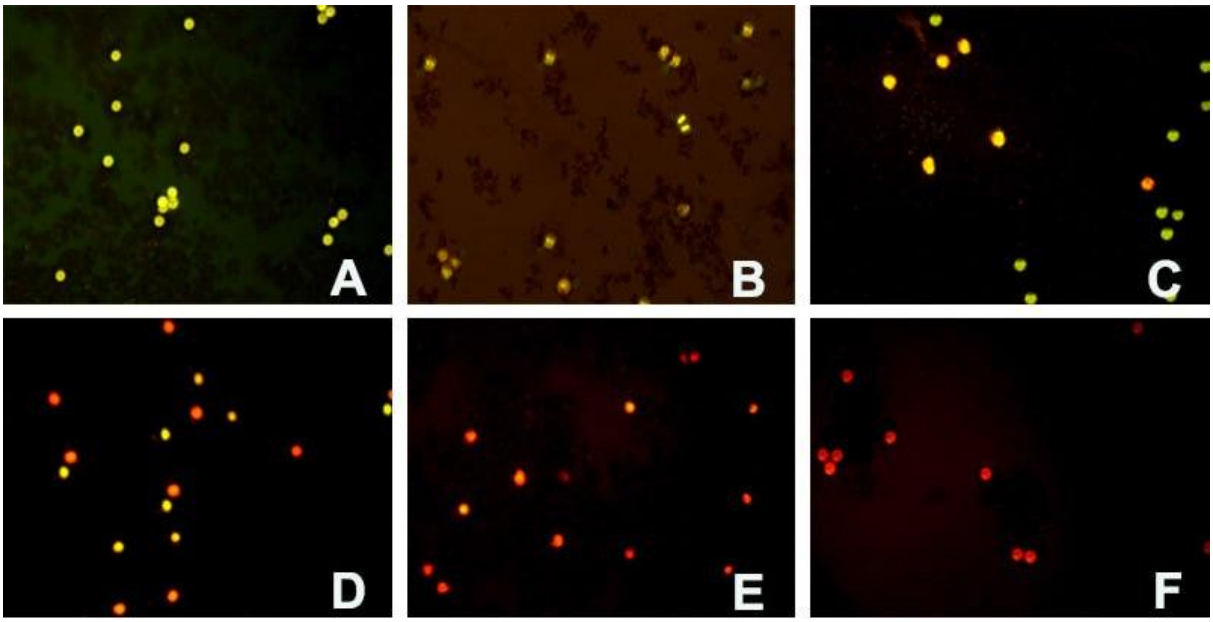



**Figure3.7.** *Images of necrosis and apoptosis observed in root tip meristem cells of A. cepa exposed to increasing concentrations of bendiocarb (A) Control, (B) 10 µM, (C) 25 µM, (D) 50 µM, (E) 100 µM, (F) 200 µM.*

## 4. DISCUSSION

Pesticides, used to control agricultural and domestic pests, are chemicals that can harm plants and animals. But using improperly, many of them pose potential hazards from contamination of food, water and air. This causes serious health problems for humans as well as the entire ecosystem [21,22,23]. There are several reports of genetic damage and mutagenic effects of pesticides in plants and animal [24,25].

Plant species such as *A. cepa, Hordeum vulgare, Pisum sativum, Tradescantia sp., Vicia faba, Zea mays, Lycopersicum esculentum* are mostly preferred to determine the cytotoxic effects of chemicals. *A. cepa* is a frequently used test material due to its physiological structure, fixed number of chromosomes, rapid response to genotoxic materials, easy accessibility and low cost [26,27,28]. Andrioli N.B. et al. 2012, a fungicide widely used in agricultural and industrial applications, was evaluated using the *A. cepa* test [29]. In this study, toxic effects of Bendiocarb on *A. cepa* root tip meristem cells were determined with many of parameters for genotoxic and cytotoxic asessment.

"MI" (Mitotic Index) is a parameter that provides an estimate of the frequency of division of cells. This parameter is used to reliably determine the presence of cytotoxic pollutants in the environment [30, 31]. In this study, it was found that MI values decreased and cytological anomalies increased on *A. cepa* depending on the application time (72 hours) and dose increase. Kanev, M., et.al. (2017) investigated the genotoxic and cytotoxic effects of Ergene river water on *A.cepa* root tip cells [32]. As a result of the study, it was determined that water samples taken from the area where domestic wastes were discharged caused a significant decrease in the mitotic index in the root tips of *A. cepa* test compared to the control group. On the other hand, water samples taken from the area where industrial wastes were discharged intensively increased the mitotic index, but showed cytotoxic effects by causing abnormalities such as asynchronization and C-mitosis.

RAPD assay has been proposed as a genotoxicity method that can be used to detect DNA damage (e.g. DNA insertion, DNA breakage) and mutations (point mutations and large rearrangements) when appropriate optimization conditions are provided. Therefore, the RAPD assay is considered a reliable, sensitive and reproducible test [7,33]. In this study, the effect of increasing doses of Bendiocarb was examined using RAPD-PCR method to show its toxic effect on *A. cepa* root tip meristem cells. Increasing doses of bendiocarb caused the disappearance of some DNA bands and changes in band intensities depending on DNA traces. In groups exposed to 200 µM Bendiocarb for 72 hours, changes in genetic structure were observed. In the

study using RAPD method, the disappearance of bands and alterations in band intensities compared to the control and treatment groups reduced the possibility of genomic template instability of DNA repair mechanisms and indicated that Bendiocarb may cause genotoxicity in *A. cepa* root tip cells.

Antioxidant enzymes having a increased great importance in maintaining the oxidative balance in cells [34]. GPx, CAT and SOD enzyme activities are the most important defense mechanisms against oxidative stress caused by free radicals. SOD enzyme enables the conversion of superoxide radicals into hydrogen peroxide molecules. CAT enzyme is one of the most important mechanisms involved in the conversion of these hydrogen peroxide molecules into water add oxygen. With the contribution of these enzymes in the cell, oxidative balance is kept under control. If the oxidative balance is shifted, increasing free radicals cause severe damage to macromolecules and cell membranes. The MDA molecule is the final product of lipid peroxidation in the cell membrane. The MDA molecule is considered both a precursor and a consequence of oxidative stress. MDA increase is an important indicator of cell membrane damage. Bicakci,U., is., et al,. (2017) examined the antioxidant activity of ginger against diazinon toxicity in *A. cepa* meristem cells [35]. It was found that increasing doses of diazinon caused an increase in MDA levels and thus caused damage to the cell membrane. Likewise, other studies using the *A. cepa* test have shown that diazinon causes severe oxidative stress [36]. Cavusoglu, K., et al. (2021) used CAT, SOD and MDA assays to observe the oxidative stress caused by tetraconazole administration. The effect of the insecticide tetraconazole on antioxidant enzyme activities in *A. cepa* roots was investigated as a dose-response relationship [38]. As a result, it was found that the activities of antioxidant enzymes such as SOD and CAT increased significantly ($p<0.05$) with increasing tetraconazole concentration. The activities of these enzymes were more than doubled compared to the control group. Similarly, MDA levels were significantly increased in a dose-dependent manner as a result of tetraconazole treatment ($p<0.05$). MDA level was found to be more than 3 times higher than the control group. Gupta, M., et al. (2004), triazole fungicides triggered the formation of reactive oxygen species and increased the activities of antioxidant enzymes such as SOD and CAT in sweet potato (*Ipomoea batatas* L.) plants[37]. It has been reported that tetraconazole administration increased membrane lipid peroxidation despite activation of the antioxidant defense system, as indicated by increased MDA levels.In this study, it was observed that Bendiocarb doses caused a statistically significant difference in terms of SOD and CAT activities and MDA formation compared to the control group ($p<0.05$). Bendiocarb has been shown to cause cell membrane damage.

There are very few studies in the literature determining the toxic effects of Bendiocarb on *A. cepa* root tip meristem cells. In our study, it was observed that root tip meristem cells exposed to Bendiocarb solution were subjected to toxic effects depending on concentration and time. Increased exposure to Bendiocarb solution caused chromosome abnormalities and DNA damage in root tip meristem cells. As a result of the study, Bendiocarb was found to have a genotoxic effect on human health. It caused significant changes in enzyme activities. Mitotic Index Determination, RAPD-PCR, Antioxidant Activity Determination (SOD, CAT and GPx) and Apoptosis Negros parameters were determined [38].

İlçe, Z. (2019) demonstrated that LPS can induce apoptosis in blood cells with cell counting results. However, VE+SS combination was found to be more effective against apoptosis than individual application. In this study, apoptosis necrosis was determined using *A. cepa* material. Bendiocarb was found to increase the amount of apoptosis following increasing doses [39].

## 5. Conclusion

The genotoxic and cytotoxic effects of Bendiocarb treatment in *A. cepa* root tip meristem cells have not yet been investigated. The severity of genotoxic and cytotoxic effects in root tip meristem cells increased as the concentration and exposing time of Bendiocarb increased. Root tip cells are more sensitive cell type for exposing to pesticides, showing increased cellular and chromosomal damage as well as DNA damage. The longer the exposure period, increases the degree of these damages.To determine the potential toxicity

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