GAZİOSMANPAŞA BİLİMSEL ARAŞTIRMA DERGİSİ (GBAD)
Gaziosmanpasa Journal of Scientific Research
ISSN: 2146-8168
http://dergipark.gov.tr/gbad
Araştırma Makalesi (Research Article)

Cilt/Volume : 8
Sayı/Number: 2
Yıl/Year: 2019
Sayı/Pages: 85-97

***Alınış tarihi (Received)*:** 29.03.2019
***Kabul tarihi (Accepted)*:** 11.10.2019

Toxicity Induced by Ridomil in *Allium cepa*: Physiological, Cytogenetic, Biochemical and Anatomical Approach

İlknur GÜÇ[a] , Kültiğin ÇAVUŞOĞLU[b] , Emine YALÇIN [b,*]

[a] *Giresun University, Science Institute, Department of Biology, 28200, Güre Campus, GİRESUN .*
[b] *Giresun University, Science and Art Faculty, Department of Biology, 28200, Güre Campus, GİRESUN .*
**Corresponding author: emine.yalcin@giresun.edu.tr*

ABSTRACT: In this study, the toxic effects of 125, 250 and 500 mg/L Ridomil fungicide were investigated in Allium cepa L. Toxic effects has been investigated in terms of physiological, cytogenetic, anatomical and biochemical aspects. The changes in germination percentage, weight gain and root length was investigated as physiological parameters; micronucleus (MN), mitotic index (MI) and chromosomal abnormality (CAs) frequency were as cytogenetic parameters. Oxidative stress indicators such as malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were analyzed for biochemical changes and also damages in root tip meristem cells were evaluated as anatomical parameters. As a result, a decrease in all selected physiological parameters and MI, and an increase in MN and CAs frequency were determined with a dose-dependent manner. Additionally, Ridomil caused fluctuations in SOD and CAT enzyme activities and a significant increase in MDA levels and these results indicated a oxidative stress formation. It has also been observed that Ridomil causes different types of anatomic damages in the root tip meristem cells. As a result, it was found that Ridomil can cause toxic effects in non-target organisms and A. cepa test material is a reliable biological indicator in observing these effects.

Keywords-*Allium cepa L., antioxidant enzymes, cytogenetic, oxidative stress, ridomil*

1. Introduction

Pesticides, which have been a part of our lives for many years, are defined as biocidal chemicals with toxic effects and their overuse leads to various environmental problems. In addition, pesticides may cause toxic effects by inhibiting various biochemical reactions in non-target organisms (Cavusoglu et al. 2011; Demirtas et al. 2015). An important group of pesticides are fungicides. Fungicides are chemical substances used in the fight against fungi which damage agricultural products. The use of fungicides is more common than other types of pesticides. Because fungicides are used as protective pesticides in many industries such as textiles, paints and wood. Ridomil is a fungicide commonly used to protect agricultural products such as tomatoes, cucumbers, onions and potatoes against fungal diseases. It has a permanent and systemic effect. Ridomil has an effect on prevention of Late Blight (Phytophthora infestans) disease in agricultural products and it is also used as an anti-infection agent in fruit, flowers and green tissues of plants (Tesserra and Giorgis 2007). Ridomil contains 64% mancozeb and 4% metalaxly-m and the main active ingredient is mancozeb.

Mancozeb forms a protective layer in the tissues of the plants and prevents the development of fungal spores, so that fungal growth is prevented and the plants are protected from the inside against fungi. Mancozeb is a water-soluble substance with a broad spectrum of protective properties having a molecular weight of 271.3 g/mol and contains Zinc and Manganese ethylene bis-dithiocarbamate (Osweiler et al. 1985). And also mancozeb has been reported to cause inhibition of lipid metabolism in non-target organisms, to cause allergic skin reactions, to have adverse effects on fertility and fetus. Carbon disulfide is released by chemical hydrolysis or metabolization of dithiocarbamates including mancozeb and this intermediate product causes abnormalities on nerve tissue. On the 11th day of pregnancy, developmental abnormalities were observed in the skin, eye, ear, muscle, skeleton and nervous systems of rats exposed to high doses (Preston and Hoffmann, 2015). In addition, DNA oxidation in peripheral blood cells, DNA single strand breaks and an increase in reactive oxygen species were detected in Wistar rats treated with mancozeb (Calviello et al. 2006). Mancozeb is also associated with decreased p53 expression and oxidative stress (Gullino et al. 2010).

In this study, toxic effects of Ridomil fungicide on A. cepa test material were investigated with the help of physiological, cytogenetic and anatomic parameters. Percentage of germination, root length and weight gain as physiological parameters; MN, CAs and the incidence of MI as cytogenetic parameters; SOD, CAT enzyme activities and MDA level were used as biochemical parameters. Also, cross sections taken from the root tips were used to determine the anatomical damages.

2. Material and Methods

2.1. Test Material and Application Doses

Ridomil (Ridomil Gold MZ 68 WG) was used as chemical agent. A. cepa bulbs of equal size obtained from a local convenience store in Giresun were used as test material. The bulbs were divided into 4 groups with 1 control and 3 applications. The bulbs in the control group were germinated in tap water, and the bulbs in application groups were germinated with 125 mg/L, 250 mg/L and 500 mg/L doses of Ridomil, respectively, at 24 °C for 72 h.

2.2. Measurement of Physiological Parameters

The root lengths of the bulbs were measured with the millimetric ruler on the basis of the radical formation, and the weight gains were measured with the precision scale. Weight changes were calculated by taking into consideration the weight differences measured before and after Ridomil application. Percentage of germination was determined with the help of Equation 1 (Atik et al. 2007).

Percentage of germination (%) = number of germinated bulb/total number of bulb x 100 (1)

2.3. Chromosomal Damage, MI and MN Test

The root tips were cut about 1 cm in length, fixed for 2 h in Clarke fixative (3: ethanol / 1: glacial acetic acid), washed in 96% ethanol for 15 min, and kept in 70% ethanol at 4°C due to experimental procedures. For the cytological preparation, the root tips were hydrolyzed at 1N HCl for 17 min at 60°C, incubated at 45% acetic acid for 30 min, and then stained in

acetocarmine for 24 h, squashed at 45% acetic acid and photographed at x500 under a research microscope (Irmeco IM-450 TI) (Staykova et al. 2005).

For the MN frequency, a total of 1.000 cells were observed in each application group and MN cells were counted at x500 under research microscope. The determination of MN is based on the criteria of Fenech et al. (2003). According to these criteria:

- The MN diameter should be 1/3 of the cell nucleus
- The shape of the MN should be oval or round
- The MN membrane should be distinguishable from the nucleus membrane.

MI was calculated according to Equation 2, counting 10,000 cells in each group.

Germination percentage (%) = number of germinated bulb/total number of bulb x 100 (2)

2.4. Observation of Anatomical Damages

Root tips were washed with distilled water for 2 min, cross-sectioned, stained with 5% methylene blue, and then photographed at x500 under research microscope.

2.5. Lipid Peroxidation

Lipid peroxidation was determined with the protocol proposed by Unyayar et al. (2006). The root tips (0.5 g) were homogenized in 5% trichloroacetic acid (TCA) with homogenizer (Ultraturrax T25-B). Then homogenates were centrifuged for 15 min at 12000 rpm at 24°C. In a 20% TCA solution, 0.5% thiobarbituric acid and supernatant were transferred to a new tube of equal volume and boiled at 96°C for 25 min. At the end of the period, the tubes were placed to ice bath and centrifuged at 10000 rpm for 5 min. The absorbance was measured at 532 nm and the MDA content was calculated using the 155 M-1cm-1 extinction coefficient and MDA levels was expressed in μM/g FW.

2.6. Determination of Antioxidant Enzyme

Enzyme extraction and preparation were carried out at 4 ° C. 0.5 g of fresh root material was collected, washed with distilled water and homogenized in 5 mL chilled sodium phosphate buffer (50 mM, pH 7.8).The homogenates were then centrifuged at 10,500 g for 20 minutes and stored at 4 ° C before the supernatant enzyme analysis (Zou et al. 2012).

2.6.1. Determination of SOD

SOD activity was measured according to the method proposed by Beauchamp and Fridovich (1971). The reaction mixture was prepared containing 1.5 mL 0.05 M sodium phosphate buffer (pH 7.8), 0.3 mL 130 mM methionine, 0.3 mL 750 μM nitroblue tetrazolium chloride (NBT), 0.3 mL 0.1 mM EDTA-Na2, 0.3 mL 20 μM riboflavin, 0.01 mL enzyme extract, 0.01 mL 4% (w / v) insoluble polyvinylpyrrolidone (PVPP) and 0.28 mL deionized water. The reaction was initiated by placing the tubes under 2 15 W fluorescent lamps for 10 minutes, and the tubes were terminated in the dark for 10 minutes. The absorbance was recorded at 560 nm. SOD activity was expressed as U/mg FW (Zou et al. 2012).

2.6.2. Determination of CAT

CAT activity was measured according to the method proposed by Beers and Sizer (1952). The CAT activity was measured using a UV-VIS Spectrophotometer at 25 ° C in a 2.8 mL reaction mixture containing 0.3 mL of 0.1M H2O2 just before use and 1.0 mL of distilled water and 1.5 mL of 200 mM sodium phosphate buffer (pH 7.8). The reaction was started by adding 0.2 mL of enzyme extract. CAT activity was measured by monitoring the reduction in absorbance at 240 nm as a result of H2O2 consumption. CAT activity OD240 nm min/g (Zou et al. 2012).

2.7. Statistical Analysis

Statistical analyzes were performed using the IBM SPSS Statistics 22 SP package program. MDA, SOD and CAT data were shown as mean ± SE (standard error); root length, weight, MN, CAs and MI data as mean ± SD (standard deviation). The statistical significance between the means was determined by One-way ANOVA and Duncan test, and p value was <0.05 when it was considered statistically significant.

3. Results and Discussion

3.1. Change in Physiological Parameters

The effects of Ridomil application on selected physiological parameters are shown in Table 1 and Figure 1. It was determined that the root length, weight gain and germination percentage decreased with the Ridomil concentration increased. In Group IV, germination rate and weight gain decreased by 2.1 and 8.7 times compared to the control group, respectively. And also Ridomil treatment significantly inhibited the root growth and decreased the root length by 26% in Group IV compared to control group. In addition, these decreases in root length and weight gain were found to be statistically significant (p <0.05).

Table 1. *Effect of Ridomil on physiological parameters*

Parameters	**Group I**	**Group II**	**Group III**	**Group IV**
Germination (%)	100	83	67	47
Weight gain (g)	$+8.18^{a}$	$+5.69^{b}$	$+3.69^{c}$	$+0.94^{d}$
Root length (cm)	11.05 ± 2.14^{a}	8.19 ± 2.28^{b}	5.94 ± 2.03^{c}	2.84 ± 1.31^{d}

*Group I: Control, Group II: 125 mg/L Ridomil, Group III: 250 mg/L Ridomil, Group IV: 500 mg/L Ridomil. Data were shown as mean ± SD (n=10). The statistical significance between the means was determined by using the "One-Way" ANOVA variance analysis following the Duncan test. The averages indicated by different letters (a-d) in the same line were statistically significant (p <0.05).

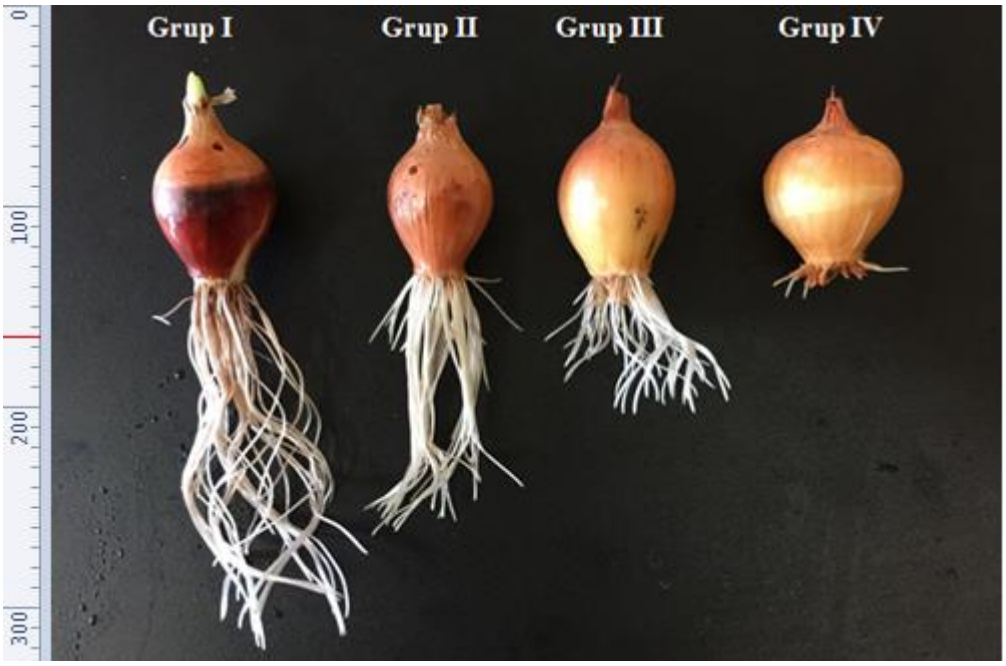

Figure 1. *The effect of Ridomil on root length*

3.2. Change in MN Frequency

The data on MN formation induced by the Ridomil application are shown in Table 2 and Figure 1. MN is an indicator of cytotoxicity that results from mitotic spindle damages or chromosome breaks. In this study, MN was not observed in the control group, but in Ridomil administration groups, an increase in MN frequency was detected depending on the increased Ridomil dose. The highest MN frequency was determined as 36.00 ± 3.97 in Group IV treated with 500 mg/L dose of Ridomil.

The data obtained for MI are shown in Table 2. MI is another parameter used to determine Ridomil cytotoxicity and is an indicator of cell proliferation. After the administration of Ridomil, the MI was decreased and the lowest MI was obtained in Group IV. In Group IV, MI was decreased by 62% compared to the control group.

In addition, it was determined that the observed increase in the MN frequency of the Ridomil application groups and the decrease in MI value were statistically significant (p <0.05) compared to the control group.

Table 2. *MN, MI and CAs frequencies induced by Ridomil*

Damage type	Group I	Group II	Group III	Group IV
MN	0.00±0.00[d]	6.10±2.02[c]	13.90±2.81[b]	36.00±3.97a
MI (%)	9.02[a]	8.33[b]	6.75[c]	5.61[d]
FRG	0.00±0.00[d]	19.20±3.16[c]	31.90±6.95[b]	41.40±5.23[a]
SC	0.20±0.42[d]	19.00±291[c]	25.50±4.09[b]	32.60±3.75[a]
B	0.00±0.00[d]	8.50±2.42[c]	15.30±3.23[b]	22.70±3.86[a]
UDC	0.00±0.00[d]	5.40±1.84[c]	10.70±3.30[b]	17.70±3.77[a]
CM	0.20±0.42[d]	4.40±1.96[c]	9.40±2.41[b]	14.80±2.62[a]
IP	0.00±0.00[d]	3.00±1.15[c]	5.40±1.65[b]	9.10±2.60[a]
BH	0.00±0.00[d]	1.60±1.17[c]	3.10±2.02[b]	7.40±1.90[a]

*Group I: Control, Group II: 125 mg/L Ridomil, Group III: 250 mg/L Ridomil, Group IV: 500 mg/L Ridomil. Data were shown as mean ± SD (n=10). For the frequency of MN 1.000 cells were counted in each group. For MI, 1.000 cells at each root tip in each group and 10.000 cells in total were analyzed. For CAs, 100 cells at each root tip in each group and 1.000 cells in total were analyzed. The statistical significance between the means was determined by using the "One-Way" ANOVA variance analysis following the Duncan test. The averages indicated by different letters (a-d) in the same line were statistically significant (p <0.05). MN: micronucleus, MI: mitotic index, FRG: fragment, SC: sticky chromosome, B: bridge, UDC: unequal distribution of chromatin, CM: c-mitosis, IP: inverse polarization, BC: binuclear cell.

3.3. Change in CAs Number

The CAs formation caused by Ridomil in *A. cepa* root tip cells is shown in Table 2 and Figure 2. Microscopic observations showed no other damage in the control group except a few sticky chromosomes and c-mitosis. The CAs observed in the groups treated with Ridomil and their number are in the form of the fragment> sticky chromosome> bridge> unequal distribution of chromatin> c-mitosis> inverse polarization> binuclear cell. The greatest effect of Ridomil on chromosomes has been upon the formation of fragment. In addition, it was determined that these increases in the number of CAs observed in the Ridomil treated groups according to the control group were statistically significant (p <0.05).

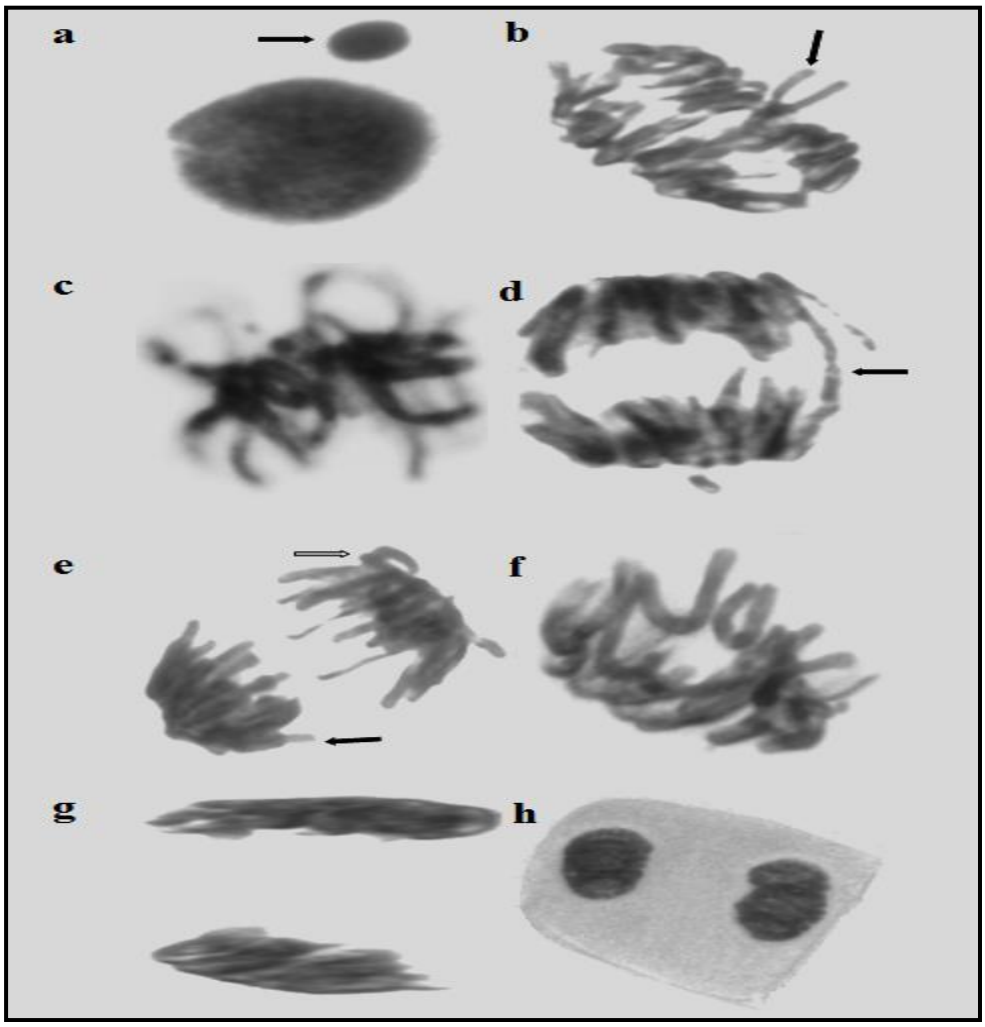

Figure 2. *MN and CAs induced by Ridomil in A. cepa root tip cells*
Arrow indicates MN **(a)**, fragment **(b)**, sticky chromosome **(c)**, bridge **(d)**, unequal distribution of chromatin (black arrow) and fragment (white arrow) **(e)**, c-mitosis **(f)**, inverse polarization **(g)**, binuclear cell **(h)**.

3.4. Change in Biochemical Parameters

The effect of Ridomil on oxidative stress parameters as MDA levels, CAT and SOD activities are shown in Figure 3-5. MDA levels were significantly increased after Ridomil application in *A. cepa* root tip cells. This increase is even more evident in the group of Ridomil administered at a dose of 500 mg/L. The highest MDA level was measured as 15 µM/g in Group IV, and the MDA level of this group was found to be approximately 3.0 times higher than the control group. When antioxidant enzyme activities were examined, it was determined that Ridomil application caused a dose dependent change in SOD and CAT activities. CAT activity was found to be increased 2.7 times in Group III compared to the control group and reached to 2.0 OD_{240nm}min/g. However, CAT activity decreased in Group IV and regressed to 1.65 OD_{240nm}min/g. A similar effect was observed in the SOD activity and the highest SOD activity was measured to be 185 U/mg FW in Group III and it was found to decrease to 150 U/mg FW in Group IV. In summary, it was determined that CAT and SOD enzyme activities increased due to the stress caused by 125 mg/L and 250 mg/L doses of Ridomil. However, due to the inhibition caused by the dose of Ridomil 500

mg/L, the enzyme activities decreased. In addition, according to the control group, MDA levels of the Ridomil treated groups and the changes in SOD and CAT enzyme activities were found to be statistically significant (p <0.05).

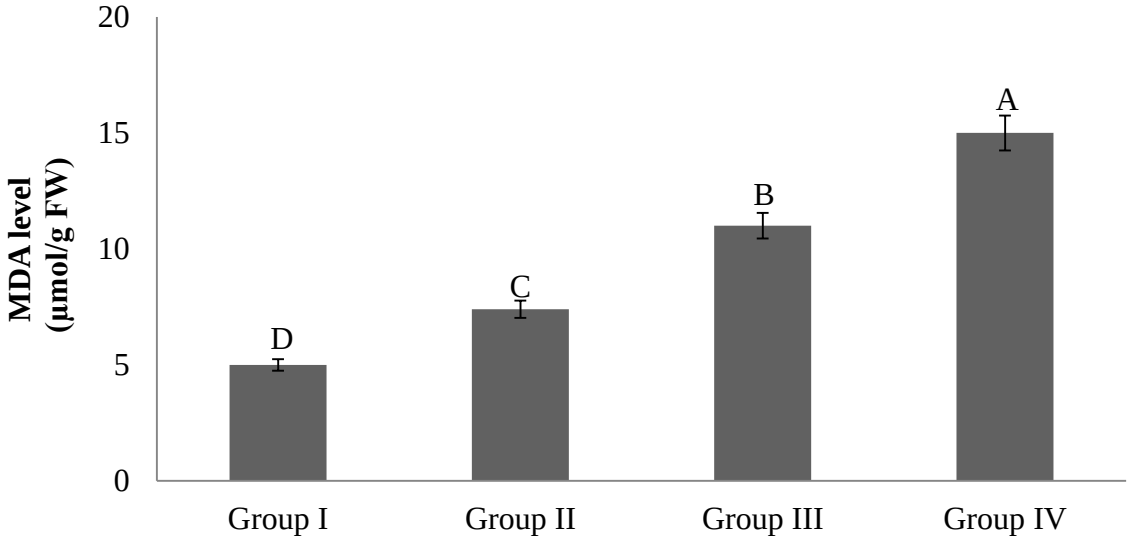

Figure 3. *Effect of Ridomil on MDA levels*

Group I: Control, Group II: 125 mg/L Ridomil, Group III: 250 mg/L Ridomil, Group IV: 500 mg/L Ridomil. Each histogram is a decimal mean; the vertical lines above the bars indicate standard error. The averages indicated by different letters (A-D) were statistically significant (p <0.05).

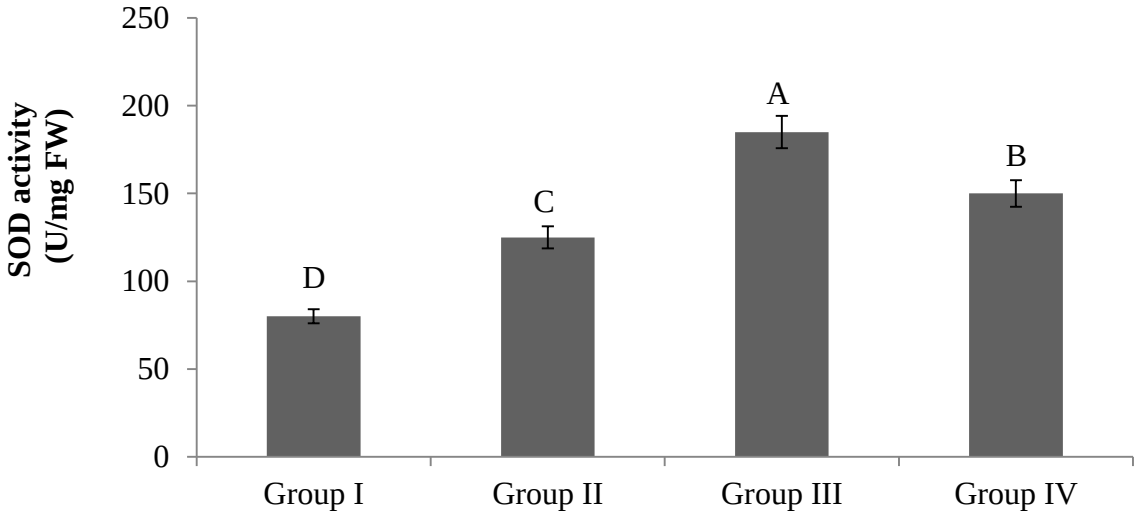

Figure 4. *Effect of Ridomil on SOD activity*

Group I: Control, Group II: 125 mg/L Ridomil, Group III: 250 mg/L Ridomil, Group IV: 500 mg/L Ridomil. Each histogram is a decimal mean; the vertical lines above the bars indicate standard error. The averages indicated by different letters (A-D) were statistically significant (p <0.05).

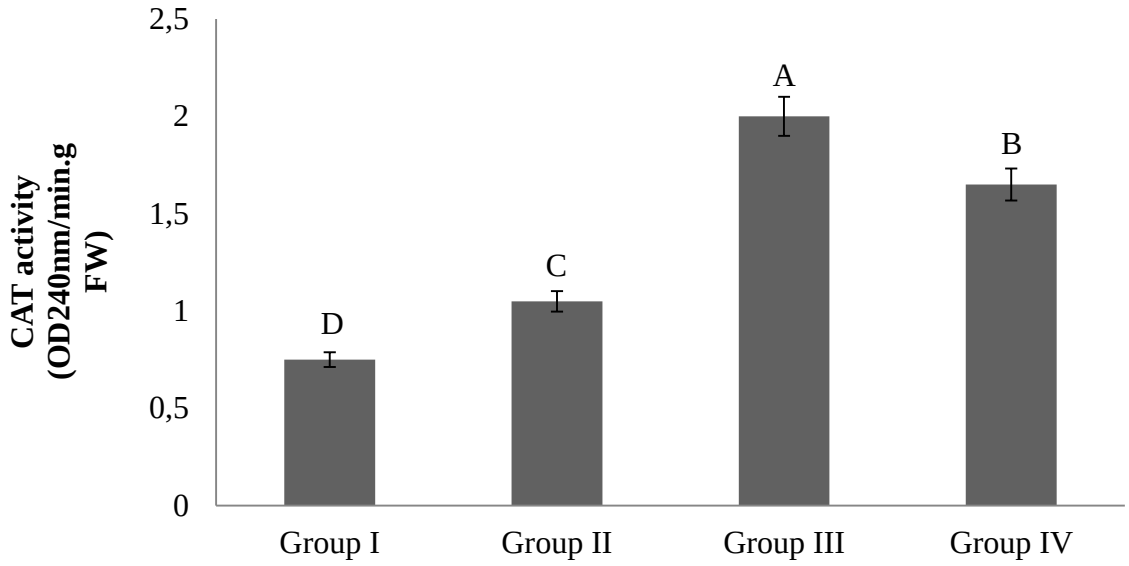

Figure 5. *Effect of Ridomil on CAT activity*

Group I: Control, Group II: 125 mg/L Ridomil, Group III: 250 mg/L Ridomil, Group IV: 500 mg/L Ridomil. Each histogram is a decimal mean; the vertical lines above the bars indicate standard error. The averages indicated by different letters (A-D) were statistically significant (p <0.05).

3.5. Changes in Root Anatomy

The anatomical changes induced by Ridomil in *A. cepa* root meristem cells are shown in Figure 6. As a result of microscopic observations, no damage was observed in the anatomical structure of the control group cells. However, anatomical damage such as unspecific conduction tissue, flattened cell nuclei, thickening of the cortex cell wall, accumulation of some substances in the cortex cells, epidermis deformation and cortex cell deformation was observed in Ridomil treated groups.

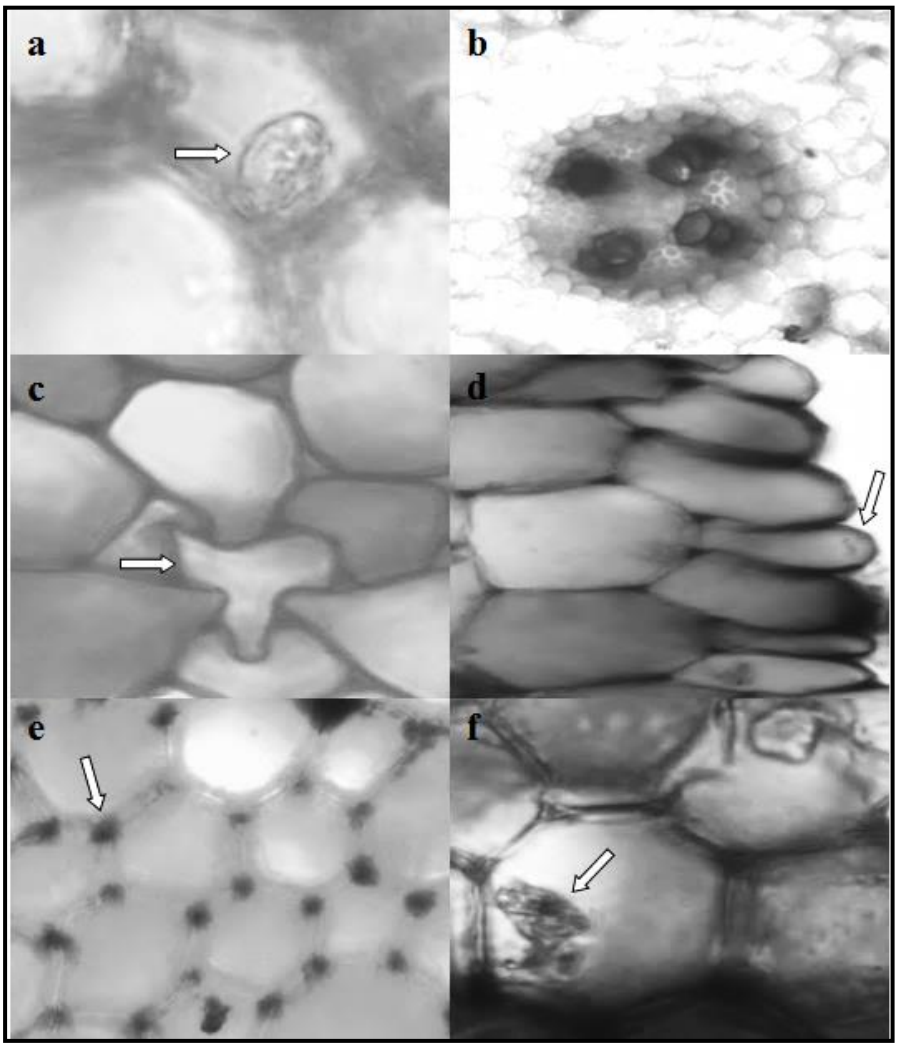

Figure 6. *Anatomical damages induced by Ridomil*
Arrow indicates flattened cell nuclei (a), unspecific conduction tissue (b), cortex cell deformation (c), epidermis cell deformation (d), thickening of the cortex cell wall (e), accumulation of some substances in the cortex cells (f).

Pesticides protect plants against the toxic effects of pests. However, they also cause serious harm to non-target organisms. In this study, the toxic effects of Ridomil were investigated with the help of *A. cepa* which is preferred as non-target organism. *A. cepa* is an indicator material for determining the effects of Ridomil on non-target organisms. The toxic effects of Ridomil were investigated by using physiological, cytogenetic, biochemical and anatomical parameters.

As a result of experimental studies, it was determined that Ridomil application decreased all the selected parameters such as germination percentage, root length and weight increase. In the literature, there are some studies conducted with fungicides and other pesticides whose active substance is Mancozeb and confirms our results. For example, Bicakci et al. (2017) reported that diazinone administration decreases the weight increase, root length and germination percentage in proportion to the dose increase in *A. cepa*. Tort et al. (2004) found that diniconazole fungicides reduced the wet-dry weight, chlorophyll a and total chlorophyll content in barley due to increased concentration, and also prevented

physiological events such as photosynthetic activity and chlorophyll synthesis. Soykan and Koca (2014) reported that increasing doses of dichlorvos (DDVP) insecticide decreased root length in *A. cepa*. The reason for this is that some of the various structural and numerical changes that occur in chromosomes cause the death of the cells that provide root elongation. In addition, it was emphasized that DDVP inhibited stem prolongation by preventing cell division.

As a result of cytogenetic analyzes, it was found that Ridomil application caused increase in MN and CAs numbers in root tip cells and a decrease in MI. In addition, these alterations were directly related to the administered Ridomil dose. All these cytogenetic damages can be explained by Ridomil toxicity. It is known that mancozeb, the active ingredient of Ridomil, attacks the macromolecules such as DNA, causing mitotic abnormalities, DNA fragmentation and mutation. In addition, all these damages are thought to be triggered by inducing intracellular oxidation and free radical formation (Cooke et al. 2003; Srivastava et al. 2012; Dias et al. 2014). There are some studies conducted in the literature using different pesticides in a manner that confirms our results on cytogenetic effects. For example, Kuchy et al. (2016) reported that endosulfan, dichlorvos and carbendazim pesticides cause damage in the form of bridges, fragments, sticky chromosomes, c-mitosis, multiple polarity and ring chromosomes in root tip cells of *A. cepa.* They also found that MI decreased. Pandey et al. (1994) found that dithane M-45, aldrex-30 and metacid-50 pesticides promote anaphase bridge, fragment and sticky chromosome formation in *A. cepa.* Liman et al. (2015) observed that imazethapyr herbicide reduces MI in *A. cepa* root cells. Ozkara et al. (2015) reported that 25, 50 and 100 ppm doses of pyracarbolid fungicide increase the frequency of MN in the root tip cell of *A. cepa* in parallel with the dose increase. Karaismailoglu (2016) found that the increased dose of Pyriproxyfen insecticide increases the frequency of MN in *Allium cepa* root tip cells.

Ridomil administration resulted in a continuous increase in MDA levels which are indicative of lipid peroxidation and an increase in antioxidant enzymes SOD and CAT activities followed by a decrease. This decrease in SOD and CAT enzyme activities is thought to be due to the denaturation of protein structures caused by Ridomil. Because Mancozeb, the active ingredient of Ridomil, is bound to sulfhydryl groups, it causes inhibition of enzyme activity and degradation of important pathways such as lipid metabolism, respiration, ATP production and cell death (Thind and Hollomon, 2018). The changes observed in SOD and CAT enzyme activities as a result of Ridomil application can be explained by these effects of mancozeb. The cell is also in a balance of antioxidant and oxidant compounds. Disruption of this balance causes oxidative damage in the cells. And also inhibition of antioxidant enzymes such as SOD and CAT can lead to oxidative stress and increased MDA levels. This hypothesis confirms the increase in MDA level as a result of Ridomil administration. Dias et al. (2014) reported that MDA content increased after mancozeb application in lettuce leaves. They also suggested that this increase was due to oxidative damage caused by mancozeb's indirect ROS production or inhibition of antioxidant enzymes. Cavusoglu et al. (2014) reported that application of lambda cyhalotrin caused an increase in SOD activity and MDA levels in *A. cepa* root cells depending on dose and duration. They also reported that CAT activity increased due to dose increase, but decreased with increasing application time.

Microscopic examination of *A. cepa* root tip cells showed that Ridomil application caused anatomic damage such as non-prominent transmission tissue, cortex cell deformation, cell deformation of epidermis, accumulation of substance in cortex cells, cortex cell wall

thickening and flattened cell nucleus. In order to tolerate the toxic effects of chemicals, the plants have developed mechanisms such as activation of the detoxification system, reduction of transport to other tissues, thickening of the cortex cells, increasing the epidermis cells and deposition of suberin in the cell wall (Baker, 1981). As a result of these mechanisms, it is inevitable that some anatomical changes occur in the plant and thus the effects of chemicals can be reduced. For example, Liu et al. (2004) reported that toxic substance exposure caused a thickened cell wall in *Vicia faba* L. and that this anatomical change acted as a barrier to restrict the transport of toxic substances to other cells. Ozturk et al. (2006) found that the application of metalaxly, tomato seedlings reduce leaf mesophilus, lower epidermis and total leaf thickness, as well as palisade parenchyma cells show an intermittent structure, and sponge parenchyma cells have deteriorated internal structures. Demirtas et al. (2015) reported that 25, 50 and 100 ppm doses of Dinicanazole fungicides promote anatomic damage such as cell deformation, nonspecific transmission tissue, flattened cell nucleus and necrosis in *A. cepa* root tip cells.

4. Conclusion

As a result, it was determined that Ridomil caused many negative effects on non-target organism as A. cepa. For this reason, the use of Ridomil should be limited or dose levels which do not have toxic effects for non-target organisms should be preferred. In addition, people should be made aware of pesticide toxicity and natural agriculture. However, it should be kept in mind that if all the people do their duty and responsibility, all these negativities can be eliminated.

5. References

Atik, M., Karaguzel, O., Ersoy, S., 2007. Effect of Temperature on Germination Characteristics of *Dalbergia sissoo* Seeds. Mediterranean Agricultural Sciences, 20, 203-210.

Baker, A.J.M., 1981. Accumulators and Excluders-Strategies in the Response of Plants to Heavy Metals. Journal of Plant Nutrition, 3, 643-654.

Beauchamp, C., Fridovich, I., 1971. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. Analytical Biochemistry, 44, 276-287.

Beers, R.F., Sizer, I.W., 1952. Colorimetric Method for Estimation of Catalase. Journal of Biological Chemistry, 195, 133-139.

Bicakci, U., Cavusoglu, K., Yapar, K., Acar, A., Yalcin, E., 2017. The Investigation of Diazinon Toxicity in Root Tip Cells of *Allium cepa* (Onion) L. Iğdır University Journal of the Institue of Science and Technology, 7, 49-56.

Calviello, G., Piccioni, E., Boninsegna, A., Tedesco, B., Maggiano, N., Serini, S., Wolf F.I., Palozza, P., 2006. DNA Damage and Apoptosis İnduction by the Pesticide Mancozeb in Rat Cells: İnvolvement of the Oxidative Mechanism. Toxicology and Applied Pharmacology , 211, 87-96.

Cavusoglu, K., Gur, B., Yalcin, E., Demirtas, G., Cicek, F., 2014. The Effect of Lambda-Cyhalothrin on Root Tip Cytology, Pigment Contents and Antioxidant Defense System of *Allium cepa*. Cytologia, 79, 95-101.

Cavusoglu, K., Yapar, K., Oruc, E., Yalcin, E., 2011. Protective Effect of *Ginkgo biloba* L. Leaf Extract Against Glyphosate Toxicity in Swiss Albino Mice. Journal of Medicinal Food, 14, 1263-1272.

Cooke, M.S., Evans, M.D., Dizdaroglu, M., Lunec, J., 2003. Oxidative DNA Damage: Mechanisms, Mutation and Disease. The FASEB Journal, 17, 1195-1214.

Demirtas, G., Cavusoglu, K., Yalcin, E., 2015. Anatomic, Physiologic and Cytogenetic Changes in *Allium cepa* L. Induced by Diniconazole. Cytologia, 80, 51-57.

Dias, M.C., Figueiredo, P., Duarte, I.F., Gil, A.M., Santos, C., 2014. Different Responses of Young and Expanded Lettuce Leaves to Fungicide Mancozeb: Chlorophyll Fluorescence, Lipid Peroxidation, Pigments and Proline Content. Photosynthetica, 52, 148-151.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E., 2003. Human Micronucleus Project. HUMN Project: Detailed Description of the Scoring Criteria for the

Cytokinesis-Block Micronucleus Assay Using Isolated Human Lymphocyte Cultures. Mutation Research, 534, 65-75.

Gullino, M.L., Tinivella, F., Garibaldi, A., Kemmitt, G.M., Bacci, L., Sheppard, B., 2010. Mancozeb: Past, Present and Future. Plant Disease, 94, 1076-1087.

Karaismailoglu, M.C., 2016. The Evaluation of The Genotoxic and Cytotoxic Effects of Pyriproxyfen İnsecticide on *Allium Cepa* Somatic Chromosomes with Mitotic Activity, Chromosome Abnormality and Micronucleus Frequency. Turkish Journal of Life Sciences, 1, 65-69.

Kuchy, A.H., Wani, A.A., Kamili, A.N., 2016. Cytogenetic Effects of Three Commercially Formulated Pesticides on Somatic and Germ Cells of *Allium cepa*. Environmental Science and Pollution Research, 23, 6895-6906.

Liman, R., Cigerci, I.H., Ozturk, N.S., 2015. Determination of Genotoxic Effects of Imazethapyr Herbicide in *Allium cepa* Root Cells by Mitotic Activity, Chromosome Aberration, and Comet Assay. Pesticide Biochemistry and Physiology, 118, 38-42.

Liu, H.Y., Liao, B., Lu, S.Q., 2004. Toxicity of Surfactant, Acid Rain and Cd^{2+} Combined Pollution to the Nucleus of *Vicia faba* Root Tip Cells. Chinese Journal of Applied Ecology, 15, 493-496.

Osweiler, G.D., Carlson, T.L., Buck, W.B., Von Gelder, G.A., 1985. Organic Synthetic Fungicides. In Clinical and Diagnostic Veterinary Toxicology, Kendal/Hunt Publishing Company, Dubuque 231-242.

Ozkara, A., Akyil, D., Eren, Y., Erdogmuş, S.F., Konuk, M., Saglam. E., 2015. Assessment of Cytotoxic and Genotoxic Potential of Pyracarbolid by *Allium test* and Micronucleus Assay. Drug and Chemical Toxicology, 38, 337-341.

Ozturk, I., Tort, N., Tosun, N., 2006. The Effect of Metalaxyl Application on Anatomical Structure of Tomato (*Lycopersicon esculentum* Mill.). The Journal of Agricultural Science, 12, 14-22.

Pandey, R.K., Shukla, R., Datta, S.K., 1994. Chromotoxic Effect of One Fungucide (Dithane M-45) and Two Insecticide (Aldrex-30 and Metacid-50). Cytologia, 59, 419-422.

Preston, R.J., Hoffmann, G.R., 2015. Genetic Toxicology. In Klaassen, C.D., Watkins, J.B., III. eds, Casarett and Doull's Essentials of Toxicology, Third Edition, McGraw-Hill, New York 135-147.

Soykan, H., Koca, S., 2014. Effects of Dichlorvos (DDVP) on Mitosis and Chromosomes in *Allium cepa* L. Root Tip Meristem Cells. Cumhuriyet Science Journal, 35, 36-45.

Srivastava, A.K., Ali, W., Singh, R., Bhui, K., Tyagi, S., Al-Khedhairy, A.A., Srivastava, P.K., Musarrat, J., Shukla, Y., 2012. Mancozeb-Induced Genotoxicity and Apoptosis in Cultured Human Lymphocytes. Life Sciences, 90, 815-824.

Staykova, T.A., Ivanova, E.N., Velcheva, I.G., 2005. Cytogenetic Effect of Heavy Metal and Cyanide In Contamined Waters from the Region of Southwest Bulgaria. Journal of Cell and Molecular Research, 4, 41-46.

Tesserra, M., Giorgis, G.W., 2007. Impact of Farmers' Selected IDM Options on Potato Late Blight Control and Yield. African Crop Science Society, 8, 2091-2094.

Thind, T.S., Hollomon, D.W., 2018. Thiocarbamate Fungicides: Reliable Tools in Resistance Management and Future Outlook. Pest Management Science, 74, 1547-1551.

Tort, N., Turkyilmaz, B., Dereboylu, A.E., Tosun, N., 2004. Morphological and Physiological Effects of a Fungicide with a Diniconazole agent on Some Barley Culture Forms. Ege Journal of Agricultural Research, 41, 169-179.

Unyayar, S., Celik, A., Cekic, F.O., Gozel, A., 2006. Cadmium-Induced Genotoxicity, Cytotoxicity and Lipid Peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis 21, 77-81.

Zou, J., Yue, J., Jiang, W., Liu, D., 2012. Effects of Cadmium Stress on Root Tip Cells and Some Physiological Indexes in *Allium cepa* var. *agrogarum* L. Acta Biologica Cracoviensia Series Botanica, 54, 129-141.