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Protective Role of *Olea europaea* L. Leaf Extract Against Deltamethrin Induced Genotoxic Effects: *Allium cepa* L. Model

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Abstract

In this research, the potential protective effect of *Olea europaea* L. (olive) leaf extract (OE) against the genotoxic effects of deltamethrin, a widely used insecticide, on *Allium cepa* L. root cells was revealed. *Allium* root tip cells assay were used to determine this effect. Deltamethrin (DM) treatments generally showed a significant decrease in mitotic index (MI) values (DM 2x group 48 hrs treatment about 96%) and a significant increase in chromosomal abnormality rates (DM 2x group 48 hrs treatment about 8 times) compared to the control group. OE treatments were observed to reduce abnormality rates (OE 2.0g/L 24 h treatment resulted in approximately 32% reduction) while keeping MI close to the control level (OE 0.5g 24 hrs treatment resulted in approximately 15% reduction). The most remarkable improvement was recorded in the DM 1x+OE 2.0g/L 48 hrs group. MI value was 7.33±0.4 and abnormality rate was 4.3±0.4, showing a significant improvement compared to the Deltamethrin-only groups. These results support the notion that OE extract as a herbal bioactive agent may have potential protective effect against insecticide-induced genotoxicity.

Keywords: *Allium cepa*; Deltamethrin; *Olea europaea*; Mitotic index; Chromosomal abnormality; Genotoxicity; Root tip test

1. Introduction

The widespread use of pesticides has significant toxic effects on both environmental and biological systems, resulting in serious health risks, especially in agricultural production. Genotoxicity is one of the most important of these effects, causing disruption of chromosomal structure and DNA damage during cell division. In determining such effects, the *Allium cepa L.* (onion) biotest system stands out as a reliable model for monitoring biomarkers such as mitotic index, chromosomal abnormalities and nuclear disorders [1]. Chauhan, Dikshith and Sundararaman showed that deltamethrin induced significant mitotic disturbances and chromosomal abnormalities in *Allium cepa* root meristem cells [2]. Contreras-Perera et al. reported that deltamethrin selection increased *kdr* mutations and altered the activity of insecticide detoxification enzymes in *Aedes aegypti*, suggesting that this compound may also affect genetic stability in different systems [3]. In this context, the search for natural antioxidants that can reduce the harmful effects of pesticides is of great importance. *Olea europaea L.* (olive) leaf is a powerful antioxidant and genoprotective agent thanks to its phenolic compounds, especially oleuropein. The extract of this plant has been found to reduce oxidative stress levels by reducing DNA damage and suppressing pesticide-induced genotoxicity in human lymphocytes [4].

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2. Material and methods

Certified *A. cepa* L. tubers were used as plant material in the study. *O. europaea* L. (OE) leaf extract was purchased in powder form from Gökçek Şifa Lab. Industry Trade Ltd. Co. was obtained. Deltamethrin was purchased from Ekin Agriculture Trade Ltd. Co.

In the research, certified *A. cepa* L. tubers were subjected to pre-rooting and divided into four groups for experimental treatments when 2-3 cm root length was reached. At 24 and 48 hours after the treatments, root tips were placed in Farmer fixative (3 Ethanol:1 Glacial Acetic Acid) and MI and chromosomal abnormality observations were performed for each group. The application groups are as follows.

2.1. Formation of Treatment Groups

A total of 23 different treatment groups were established using healthy *A. cepa* L. (shallot) roots. The groups were planned as control (distilled water), Deltamethrin-only groups (DM 1x, 2x and 4x; 24 and 48 h), *O. europaea* L. (OE) leaf extract-only groups (0.5 g/L and 2.0 g/L; 24 and 48 h) and Deltamethrin+OE combination groups (DM 1x, 2x, 4x + OE 0.5 g/L or 2.0 g/L; 24 and 48 h). For the deltamethrin, increasing concentrations (2x and 4x) were prepared based on the recommended dose (1x); OE extract powder were dissolved in distilled water in a magnetic stirrer at room temperature for 4 hours and then applied to rooting medium. For each group, root tips were treated with single and combined doses of DM (1x, 2x, 4x) and OE (0.5 g/L and 2 g/L). The effects of these treatments on mitotic index (MI) and chromosomal abnormality (CA) were evaluated comparatively.

2.2. *Allium cepa* Root Tip Harvesting

The 2-3 cm long root tips of the control group and each treatment group were placed in farmer's fixative early in the morning. After hydrolysis in 1 N HCl acid in a 60^0C water bath for 11 min, they were transferred to falcon tubes which containing freshly prepared acetocarmine and kept in the refrigerator until experiment.

2.3. *Allium cepa cepa* Root Tip Cells Assay (MI, CA)

For microscopy, mitotic stage distributions and chromosomal abnormalities were determined under light microscope. Mitotic index was calculated as the ratio of the total number of dividing cells to the total number of cells. During the determination of chromosomal abnormalities, the total number of cells undergoing normal mitosis and the number of cells with abnormalities were determined. As a result of this evaluation, abnormalities such as anaphase bridge, chromosome fragmentation, aberrant chromosome, sticky chromosome, lagging chromosome, unaligned chromosomes were observed. In each group, 10 onions were used and approximately 1500 cells were analyzed in total. All experiments were carried out with 3 replications. The data obtained were statistically evaluated by one-way ANOVA and Tukey tests ($p<0.05$).

3. Result and discussion

In this research, mitotic index (MI) and chromosomal abnormality (CA) rates of different treatment groups in *Allium cepa* L. root tip cells were evaluated by comparing with control group (MI: 12.59**,** CA: 2.2 ± 0.3).

3.1. *A. cepa* Mitotic Index Results

When the mitotic index (MI) values of all treatment groups are compared, it is shown in Table 1 that Deltamethrin treatments caused a significant decrease compared to the control group, while this effect was partially balanced in OE and combination groups.

Table 1 Mitotic Index (MI) of All Groups

Group	MI ± SE
Control	12.59 ± 0.3
DM 1x 24h	2.24 ± 0.25
DM 1x 48h	2.57 ± 0.30
DM 2x 24h	2.21 ± 0.25

DM 2x 48h	2.15 ± 0.35
DM 4x 24h	0.90 ± 0.25
DM 4x 48h	0.35 ± 0.20
OE 0.5g 24h	10.64 ± 0.3
OE 0.5g 48h	9.67 ± 0.35
OE 2.0g 24h	8.21 ± 0.4
OE 2.0g 48h	8.55 ± 0.3
DM 1x + OE 0.5g 24h	5.12 ± 0.25
DM 1x + OE 0.5g 48h	5.35 ± 0.30
DM 1x + OE 2.0g 24h	6.12 ± 0.25
DM 1x + OE 2.0g 48h	7.33 ± 0.4
DM 2x + OE 0.5g 24h	3.12 ± 0.25
DM 2x + OE 0.5g 48h	2.85 ± 0.25
DM 2x + OE 2.0g 24h	4.18 ± 0.3
DM 2x + OE 2.0g 48h	4.55 ± 0.25
DM 4x + OE 0.5g 24h	1.0 ± 0.20
DM 4x + OE 0.5g 48h	0.7 ± 0.20
DM 4x + OE 2.0g 24h	2.1 ± 0.20
DM 4x + OE 2.0g 48h	2.65 ± 0.20

Deltamethrin significantly decreased the MI values at all concentrations and exposure times; the highest decrease was observed in the DM 4x 48 hrs group. While OE treatments largely preserved mitotic activity, OE significantly reduced Deltamethrin-induced suppression in the combination groups (Figure 1).

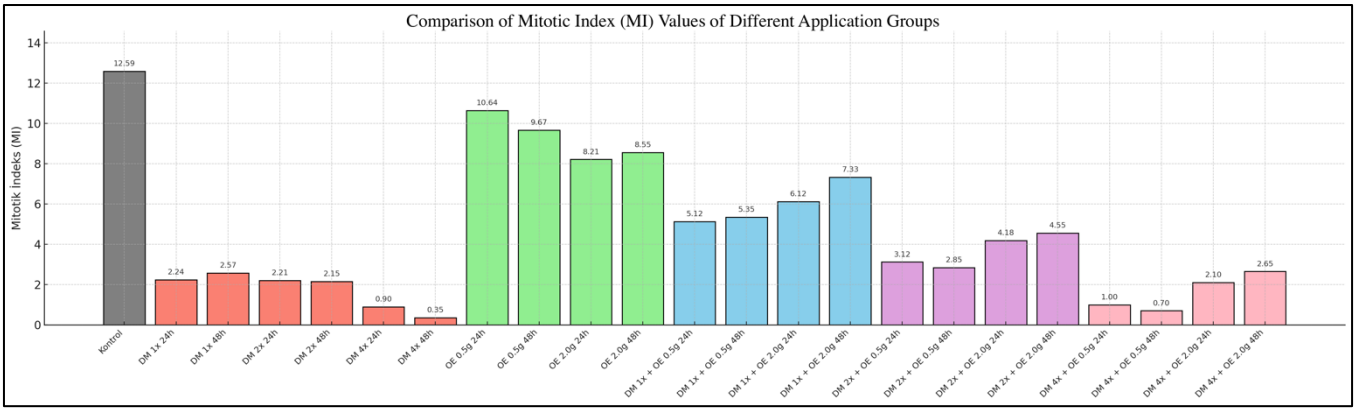

Figure 1 Comparison of Mitotic Index (MI) Values of Different Treatment Groups

In the figure, the gray bar represents the control group; red bars represent alone Deltamethrin treatments , green bars represent alone OE (olive leaf extract) treatments; blue, purple and pink bars represent Deltamethrin+OE combination groups. Values were calculated based on the mean ± standard error of the mean (SE) for each group.

- Gray bar → Control group
- Green bars → OE applications only (OE 0.5g/2.0g, 24-48h)
- Orange bars → Deltamethrin applications only (DM 1x/2x/4x, 24-48h)
- Blue, purple and pink bars → DM + OE combination groups (DM 1x/2x/4x + OE, 24-48h)

3.2 Effects of Treatments on *A. cepa* Chromosomal Abnormality (CA)

It was determined that chromosomal abnormality rates observed in all treatment groups increased significantly in Deltamethrin treatments compared to the control group. It was determined that OE and combination groups provided protective effects at varying levels. The findings are presented in Table 2.

Table 2 Chromosomal Abnormality Rate of All Groups

Group	Abnormality ± SE
Control	2.2 ± 0.3
DM 1x 24h	10.5 ± 0.8
DM 1x 48h	13.8 ± 0.8
DM 2x 24h	16.2 ± 0.9
DM 2x 48h	18.9 ± 1.0
DM 4x 24h	17.8 ± 1.4
DM 4x 48h	15.7 ± 1.3
OE 0.5g 24h	4.9 ± 0.5
OE 0.5g 48h	3.5 ± 0.5
OE 2.0g 24h	2.9 ± 0.4
OE 2.0g 48h	3.1 ± 0.4
DM 1x + OE 0.5g 24h	7.8 ± 0.6
DM 1x + OE 0.5g 48h	6.5 ± 0.5
DM 1x + OE 2.0g 24h	5.9 ± 0.5
DM 1x + OE 2.0g 48h	4.3 ± 0.4
DM 2x + OE 0.5g 24h	9.2 ± 0.7
DM 2x + OE 0.5g 48h	8.1 ± 0.6
DM 2x + OE 2.0g 24h	6.8 ± 0.6
DM 2x + OE 2.0g 48h	5.5 ± 0.5
DM 4x + OE 0.5g 24h	12.2 ± 0.8
DM 4x + OE 0.5g 48h	14.5 ± 0.9
DM 4x + OE 2.0g 24h	10.5 ± 0.8
DM 4x + OE 2.0g 48h	11.3 ± 0.8

While chromosomal abnormality rates increased significantly in the deltamethrin-treated groups, OE treatments showed values close to the control level; in the combination groups, it was observed that OE reduced the genotoxic effect especially under low and medium dose Deltamethrin conditions (Figure 2).

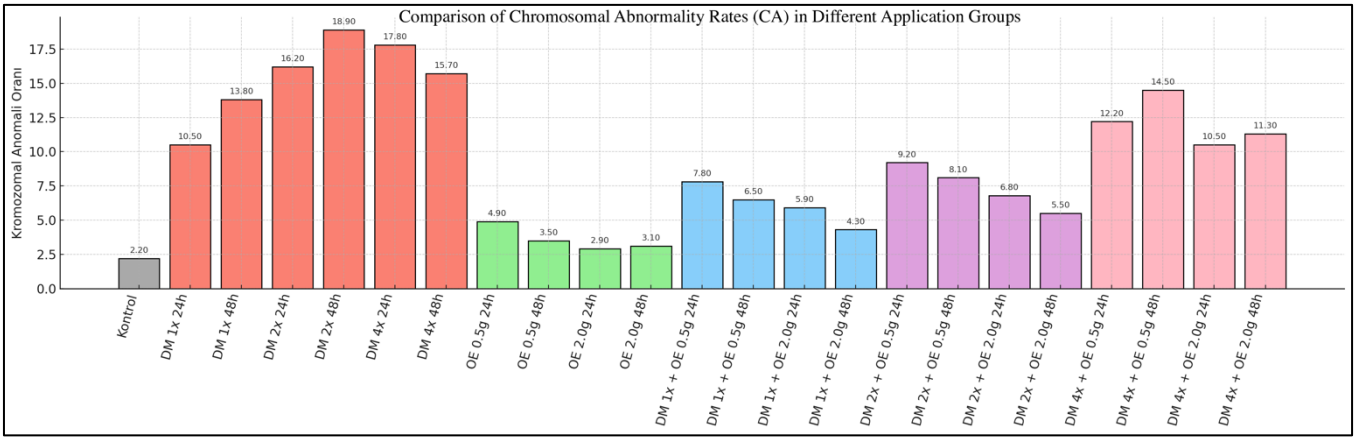

Figure 2 Comparison of Total Chromosomal Abnormality (CA) Rates of Different Treatment Groups

Gray bar indicates control group; green bars indicate alone OE, red bars indicate alone Deltamethrin; blue, purple and pink bars indicate Deltamethrin+OE combinations.

- Gray bar → Control group
- Green bars → OE applications only *(OE 0.5g/2.0g, 24-48h)*
- Orange bars → Deltamethrin applications only *(DM 1x/2x/4x, 24-48h)*
- Blue, purple and pink bars → DM + OE combination groups *(DM 1x/2x/4x + OE, 24-48h)*

In the research, mitotic index (MI) and chromosomal abnormality (CA) ratios of different treatment groups on *Allium cepa* L. root meristem cells were evaluated with reference to control group values (MI: 12.59 ± 0.3; CA: 2.2 ± 0.3). The findings clearly demonstrated the cell division-suppressive and genotoxic effects of Deltamethrin, and showed that *Olea europaea* L. leaf extract possesses protective potential against these toxic effects.

In the Deltamethrin (DM) treated groups, increasing concentration and exposure times had a significant suppressive effect on MI and a dose-dependent increasing effect on CA rates. Notably, in the DM 4x 48 h group, the MI value decreased to 0.35 ± 0.20, and the CA rate increased to 17.8 ± 1.3. These results indicate that Deltamethrin exerts cell cycle-inhibiting and clastogenic effects. The observed decrease in MI and increase in CA support the hypothesis that Deltamethrin may disrupt chromosomal structure by directly interacting with DNA at the cellular level. In a study supporting this conclusion, Saxena et al. reported that Deltamethrin exposure in *A. cepa* and *Allium sativum* root meristem cells suppressed mitotic activity and induced structural alterations in DNA as revealed by FTIR analysis [5]. Similarly, El-Gerbed demonstrated that Deltamethrin treatment caused severe histological and ultrastructural damage in mouse kidney tissue, and that this damage could be significantly mitigated by the antioxidant lycopene [6].

Similarly, a research published in *Scientific Reports* demonstrated that *Melissa officinalis* leaf extract significantly reduced manganese-induced cytogenotoxicity and improved mitotic activity in *Allium cepa* [7]. In addition, medicinal plants such as *Viburnum opulus* and *Equisetum arvense* have also shown similar protective effects against pesticide and heavy metal exposure by increasing mitotic index and decreasing chromosomal abnormalities. Likewise, *Hypericum perforatum* has been confirmed to enhance mitotic index and reduce chromosomal abnormalities under heavy metal and pesticide exposure in *Allium cepa* root cells [8].

In the *Olea europaea* (OE)-only treated groups, MI values remained close to control levels (e.g., 10.64 ± 0.3), while CA rates were consistently low. Türkez and Toğar showed that OE treatment reduced permethrin-induced chromosomal aberrations and sister chromatid exchanges in human lymphocytes [4]. In addition, Aloriby et al. demonstrated that OE extract suppressed DNA damage through its strong antioxidant capacity in both in vitro and in vivo models [9].

In the DM + OE combination groups, the protective effect of OE was particularly evident under low and medium Deltamethrin doses. In the DM 1x + OE 2.0 g/48 h group, MI increased from 2.57 to 7.33 ± 0.4, while CA decreased from 13.8 to 4.3 ± 0.4. Mekircha et al. reported that OE oil alleviated Deltamethrin-induced oxidative stress and hormonal

imbalances [10]. In the high-dose combination group (DM 4x + OE), MI increased to 2.65 ± 0.2 and CA decreased to 11.3 ± 0.8, suggesting that OE was able to promote mitotic activity even under highly toxic conditions. Supporting this, Sheweita et al. found that OE and other natural antioxidants significantly reduced DNA damage even under high-dose pesticide exposure [11].

Overall, OE treatments, both alone and in combination with DM, significantly contributed to the preservation of mitotic activity and reduction of chromosomal aberration rates.

4. Conclusion

This research revealed that Deltamethrin, a widely used synthetic insecticide, caused marked mitotic depression and a high level of chromosomal abnormalities in *Allium cepa* L. root meristem cells. Cell division was severely suppressed, and clastogenic effects were observed in the genetic material, particularly under high-dose and long-term Deltamethrin exposure. In contrast, treatment with *Olea europaea* L. (olive) leaf extract alone supported cell division and did not induce genotoxic stress. The observation that mitotic index values remained close to control levels and chromosomal abnormality rates were low in OE-treated groups suggests that the protective effect may be linked to the antioxidant properties of the extract.

The most remarkable finding was observed in the combination groups where OE was administered together with Deltamethrin. In particular, in the group treated with the recommended concentration of Deltamethrin and high-concentration OE (2.0 g/L, 48 h), the mitotic index was significantly elevated and the chromosomal abnormality rate markedly reduced compared to the group treated with Deltamethrin alone. This suggests that OE extract can reduce pesticide-induced genotoxicity and promote cell division to a certain extent. However, the protective effect of OE was limited at the highest Deltamethrin doses (4x), indicating that phytochemicals may not provide full bioprotection at elevated pesticide concentrations.

In conclusion, *Olea europaea* L. leaf extract may be considered a promising plant-derived bioactive agent for reducing pesticide-induced genotoxic damage. This research highlights the potential of naturally occurring phytochemicals in mitigating environmental toxicity and reinforces the utility of the *Allium* root tip assay as a reliable biomarker for assessing the genoprotective effects of plant extracts. Future research should aim to elucidate the mechanisms underlying OE's protective action using various plant systems, cell lines, and molecular biomarkers.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that there is no conflict of interest in this manuscript.

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