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Cytotoxic and genotoxic effects of the herbicide aclonifen on root meristem cells of Allium cepa L.

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Abstract

Aclonifen is a selective diphenyl ether herbicide widely used in agriculture for weed control; however, information regarding its cytotoxic and genotoxic effects on non-target plant cells remains limited. The present study aimed to evaluate the cytotoxic and genotoxic potential of aclonifen in root meristem cells of *Allium cepa* L. In a preliminary root growth inhibition assay, the concentration reducing root length to 50% of the control after 96 hours was determined as 100 mg L^{-1} and used as the median effective concentration. Accordingly, 50, 100, and 200 mg L^{-1} concentrations corresponding to one-half, one-fold, and two-fold this value were selected for cytogenetic analyses. Onion roots were exposed for 24, 48, and 72 hours, and root length, mitotic index, micronucleus frequency, and chromosomal aberrations were evaluated. Aclonifen significantly inhibited root growth in a concentration- and time-dependent manner. At 72 hours, root length decreased from 5.08 cm in the control group to 2.53 cm and 1.48 cm in the 100 and 200 mg L^{-1} groups, respectively. Similarly, the mitotic index declined from 55.28% in the control group to 24.86% and 15.38% at 100 and 200 mg/L, respectively. Genotoxicity endpoints increased markedly with increasing concentration and exposure duration. After 72 hours of treatment, micronucleus frequency increased from 0.12% in the control group to 2.86% at 200 mg/L, while total chromosomal aberrations rose from 1.72% to 23.64%. The most common abnormalities included chromosome laggards, stickiness, anaphase bridges, and c-mitosis. These findings demonstrate that aclonifen induces significant cytotoxic and genotoxic effects in *Allium cepa* root meristem cells by suppressing cell division and increasing chromosomal damage. The results provide new evidence regarding the biological effects of aclonifen on non-target plant systems and highlight the usefulness of plant-based bioassays in environmental risk assessment.

Keywords: *Aclonifen, Allium cepa, Cytotoxicity, Genotoxicity, Mitotic index, Chromosomal aberrations*

Introduction

Herbicides, which are widely used to achieve weed control in agricultural production, play an important role in enhancing crop productivity. However, intensive and indiscriminate herbicide use has become an increasing source of concern owing to environmental pollution and the potential adverse effects on non-target organisms. Herbicides applied to agricultural areas may persist for long periods in soil, water and plant systems and, upon being transported to environmental compartments, may exert various toxic effects on ecosystem components (Pérez-Lucas et al., 2019; Erguven et al., 2016).

Aclonifen [2-chloro-6-nitro-3-phenoxyaniline] is a selective herbicide belonging to the nitrophenyl ether (diphenyl ether) group and is widely used for the control of annual narrow- and broad-leaved weeds in sunflower, chickpea, lentil, carrot and various field crops (Erguven et al., 2016). Its commercial formulation, Challenge® 600 SC, contains 600 g/L aclonifen and has been licensed for use in Türkiye for many years. For many years, aclonifen was classified by the HRAC within Group F3 as a pigment-biosynthesis inhibitor whose mode of action was not precisely known. However, recent research has shown that the principal molecular target of this herbicide is solanesyl diphosphate synthase (SDPS), an enzyme involved in the plastoquinone biosynthetic pathway. In line with this discovery, aclonifen has been placed within Group 32 in the current HRAC classification (Kahlau et al., 2020). Inhibition of SDPS restricts plastoquinone production, disrupts carotenoid biosynthesis and, consequently, gives rise to phytotoxic effects characterised by the loss of photosynthetic pigments, chlorosis and plant death (Kahlau et al., 2020).

The widespread agricultural use of aclonifen has increased the importance of research concerning the environmental persistence of this compound and its effects on non-target organisms. In evaluations carried out by the European Food Safety Authority, aclonifen has been reported to exhibit moderate-to-high persistence in soil (EFSA, 2008; Erguven et al., 2016). Moreover, owing to its high adsorption capacity, aclonifen has been reported to remain in soil for long periods and to display low mobility (Pérez-Lucas et al., 2019). EFSA (2008) stated that aclonifen is moderately-to-highly persistent under aerobic conditions, with DT50 values ranging between 32.2 and 134 days. This situation suggests that aclonifen residues may accumulate in the environment and give rise to adverse effects on different groups of organisms.

Studies conducted in recent years have revealed that aclonifen and some structurally related herbicides belonging to the same diphenyl ether family may produce various toxic effects in non-target organisms. Lee et al. (2021) reported that aclonifen caused developmental toxicity in zebrafish embryos and adversely affected mitochondrial functions. Similarly, Park et al. (2022) demonstrated that aclonifen could give rise to cellular stress and cytotoxic effects in mammalian cells. In studies carried out with bifenox, another herbicide possessing a

diphenyl ether structure, suppression of cell proliferation, disruption of the cell cycle and the triggering of apoptotic processes were reported (Park et al., 2023). Although these herbicides may exert their effects through different biological targets, the findings obtained indicate that diphenyl ether–derived herbicides may elicit various toxic responses in non-target organisms.

Among the biological test systems used to determine the cytotoxic and genotoxic effects of environmental pollutants, the *Allium cepa* test holds an important place owing to its high sensitivity, low cost, ease of application and the high correlation it shows with mammalian test systems (Leme & Marin-Morales, 2009; Tedesco & Laughinghouse, 2012; Torres-Bugarín et al., 2026). Owing to its large and readily observable chromosomes, rapid root growth and sensitivity to various chemicals, *A. cepa* is widely used in the determination of cytogenetic damage caused by pesticides. Parameters such as the mitotic index, micronucleus formation and chromosomal aberrations are regarded as reliable biomarkers in the evaluation of adverse effects on cell division and genetic material (Leme & Marin-Morales, 2009; Torres-Bugarín et al., 2026).

Previous studies conducted with different pesticides have shown that pesticide exposure suppresses mitotic activity, increases chromosomal aberrations and promotes micronucleus formation in *A. cepa* root meristem cells. In a recent study carried out with omethoate, a significant decrease in the mitotic index and an increase in the frequencies of micronuclei and chromosomal aberrations were reported (Karagöl et al., 2025). Similarly, applications of oxyfluorfen, a member of the diphenyl ether group, have been reported to reduce mitotic activity and to cause various chromosomal aberrations (Dragoeva et al., 2012). In addition, environmental samples containing pesticide residues have also been shown to cause genotoxic effects in the *A. cepa* system (Feretti et al., 2007).

Despite the widespread use of aclonifen, the cytotoxic and genotoxic effects produced by this herbicide in plant cells have not been sufficiently investigated. In particular, studies evaluating aclonifen-induced chromosomal damage and effects on cell division in the *Allium cepa* model are rather limited. Therefore, the present study aimed to evaluate the effects of Challenge® 600 SC, a commercial aclonifen formulation, on *Allium cepa* root meristem cells. Within this scope, root growth parameters, the mitotic index, micronucleus frequency and chromosomal aberrations were examined in order to reveal the biological responses induced by aclonifen exposure.

Materials and Methods

Experimental Design

In this study, aclonifen, a selective herbicide belonging to the diphenyl ether class, was used as the test substance. *Allium cepa* L. root meristem cells were chosen as the biological test system. Challenge® 600 SC (600 g/L aclonifen) was used as the commercial formulation. In order to ensure the homogeneous distribution of aclonifen in the test medium, 1% dimethyl sulfoxide (DMSO) was used as a co-solvent, and the DMSO proportion was kept constant in all treatment groups, including the negative control.

Healthy onions of similar size were selected, and their outer scales were removed without damaging the root primordia. The onions were germinated in the dark at room temperature and were separated into the experimental groups once the root lengths reached approximately 1–2 cm.

Determination of the EC50

The EC50 value, on which the selection of the treatment concentrations was based, was determined on the basis of root growth inhibition as a result of preliminary trials. For this purpose, healthy *Allium cepa* onions of similar size were exposed for 96 h to aclonifen solutions of 25, 50, 100, 200 and 400 mg L^{-1} containing 1% DMSO. At the end of the exposure period, root lengths were measured, and the percentage growth inhibition relative to the control group was calculated for each concentration. Linear regression analysis was applied to the concentration–response data, and the concentration inhibiting root growth by 50% (EC50) was determined to be approximately 100 mg L^{-1}. Accordingly, the aclonifen concentrations of 50, 100 and 200 mg L^{-1} used in the main experiments represent ½EC50, EC50 and 2×EC50, respectively.

Experimental Groups and Exposure Conditions

Distilled water containing 1% DMSO was used in the negative control group, whereas 10 mg L^{-1} methyl methanesulfonate (MMS) was applied as the positive control group. Aclonifen treatments were carried out at concentrations of 50, 100 and 200 mg L^{-1}. The exposure periods were set as 24, 48 and 72 h. All concentrations are expressed as active ingredient equivalents (mg L^{-1} aclonifen)

Root Growth Analysis

In order to determine the effects of the aclonifen treatments on root development, root lengths were measured at the end of each treatment. The results obtained were expressed as mean ± standard deviation (SD) and, by comparison with the control group, growth inhibition was evaluated. Five onions were used in each treatment group, and the lengths of 10 randomly selected roots from each onion were measured, giving a total of 50 roots

evaluated. Root lengths were measured in centimetres and expressed as mean ± standard deviation. Root growth inhibition was calculated using the following formula:

Root Growth Inhibition (%) = [(mean root length of the control group − mean root length of the treatment group) / mean root length of the control group] × 100

Cytogenetic Analyses

At the end of the exposure period, root tips were excised and fixed in Carnoy's solution (ethanol–acetic acid, 3:1, v/v) for 24 h. Following fixation, the samples were hydrolysed in 1 N HCl at 60 °C for 5 min (Aksu Kalmuk, 2025). Staining was then carried out with Schiff's reagent for the cytogenetic examinations. The stained root tips were prepared as squash preparations in 45% (v/v) acetic acid. The preparations thus obtained were examined under a light microscope at ×400 magnification.

The mitotic index (MI), micronucleus (MN) frequency and chromosomal aberrations (CA) were evaluated (Fiskesjö, 1985; Leme & Marin-Morales, 2009). Five onions were used for each treatment group, and a total of 5000 cells were analysed by counting 1000 cells from each onion. The mitotic index was calculated by expressing the ratio of the number of dividing cells to the total number of cells as a percentage. The micronucleus frequency was evaluated in interphase cells, and the results were given as a percentage of the total number of cells examined. Chromosomal aberrations, on the other hand, were evaluated only in dividing cells, and the ratio of abnormal mitoses to the total number of dividing cells was calculated as a percentage (Grant, 1982).

Detailed evaluations of the chromosomal aberration profile were carried out only for the 72 h exposure groups. The reason for this is that, although the chromosomal aberrations occurred as the same types in the preliminary evaluations, their frequencies increased with exposure time, and the most pronounced genotoxic effect was observed in the 72 h treatments. Therefore, in order to avoid data repetition and to ensure a clearer presentation of the results, the chromosomal aberration profile was reported only for the 72 h treatment groups.

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analyses were performed using the SPSS 22.0 software package. Root length, mitotic index, micronucleus frequency and chromosomal aberration ratios were evaluated separately within each exposure period. Within this framework, the 24, 48 and 72 h treatment groups were analysed by one-way analysis of variance (ANOVA) in order to be compared with the corresponding control group. Dunnett's multiple comparison test was used to determine significant differences. Prior to analysis, the normality assumption of the data was assessed using the Shapiro–Wilk test and the homogeneity of variances using Levene's test. The level of statistical significance was accepted as $p < 0.05$.

Results

Effects on Root Growth

The aclonifen treatments were found to suppress the root development of *Allium cepa* in a dose- and time-dependent manner. In the control group, root lengths increased with increasing exposure time and were measured as 3.12 ± 0.21 cm, 4.36 ± 0.28 cm and 5.08 ± 0.34 cm at 24, 48 and 72 h, respectively. In contrast, root lengths in all aclonifen treatment groups were found to be significantly reduced compared with the control group ($p < 0.05$) (Table 1).

At the highest concentration, 200 mg L^{-1} aclonifen, root lengths were determined to be 1.36 ± 0.14 cm, 1.72 ± 0.17 cm and 1.48 ± 0.15 cm at 24, 48 and 72 h, respectively. The positive control group (MMS), on the other hand, showed the lowest root length values at all treatment times. In particular, at the 72 h treatment, the root length, which was 5.08 ± 0.34 cm in the control group, decreased to 2.53 ± 0.21 cm in the 100 mg L^{-1} aclonifen group, and this result was found to be consistent with the EC50 value determined in the preliminary trials (Table 1).

Table 1. Effects of different aclonifen concentrations and exposure times on root length (cm) in *Allium cepa*.

Concentration (mg/ L)	*24 h (cm)*	*48 h (cm)*	*72 h (cm)*
Control (%1 DMSO)	3.12 ± 0.21	4.36 ± 0.28	5.08 ± 0.34
50	2.61 ± 0.18*	3.28 ± 0.24*	3.74 ± 0.27*
100	1.98 ± 0.16*	2.41 ± 0.19*	2.53 ± 0.21*
200	1.36 ± 0.14*	1.72 ± 0.17*	1.48 ± 0.15*
Positive control (MMS)	1.12 ± 0.11*	1.08 ± 0.10*	0.96 ± 0.09*

Values are expressed as mean ± SD. *Statistically significant difference from the control group ($p < 0.05$).

Effects on the Mitotic Index

The aclonifen treatments were found to significantly reduce mitotic activity in the root meristem cells. In the control group, the mitotic index values were calculated as 68.42 ± 4.12%, 61.37 ± 3.86% and 55.28 ± 3.41% at 24, 48 and 72 h, respectively (Table 2).

A marked decrease in the mitotic index values occurred as a result of the aclonifen treatments. At the highest treatment dose, 200 mg L^{-1}, the mitotic index values were determined to be 27.53 ± 2.41%, 20.74 ± 1.96% and 15.38 ± 1.82% at 24, 48 and 72 h, respectively. In the positive control group, these values were determined to be 24.16 ± 2.08%, 18.63 ± 1.74% and 12.42 ± 1.56%, respectively. At the 72 h treatment, the mitotic index in the 200 mg L^{-1} aclonifen group was found to have decreased by approximately 72% compared with the control group (Table 2).

Table 2. Effects of aclonifen treatments on the mitotic index (MI) in *Allium cepa* root meristem cells.

Concentration (mg/ L)	***24 h MI (%)***	***48 h MI (%)***	***72 h MI (%)***
Control (%1 DMSO)	68.42 ± 4.12	61.37 ± 3.86	55.28 ± 3.41
50	52.16 ± 3.48*	43.85 ± 3.11*	36.42 ± 2.94*
100	39.74 ± 2.96*	31.28 ± 2.54*	24.86 ± 2.11*
200	27.53 ± 2.41*	20.74 ± 1.96*	15.38 ± 1.82*
Positive control (MMS)	24.16 ± 2.08*	18.63 ± 1.74*	12.42 ± 1.56*

Values are expressed as mean ± SD. *Statistically significant difference from the control group ($p < 0.05$).

Effects on Micronucleus Frequency

The aclonifen treatments were found to increase micronucleus formation in a dose- and time-dependent manner. In the control group, the micronucleus frequency remained at low levels at all treatment times and was determined to be 0.08 ± 0.03%, 0.10 ± 0.04% and 0.12 ± 0.05% at 24, 48 and 72 h, respectively.

In the aclonifen-treated groups, significant increases in micronucleus frequency were observed ($p < 0.05$). The highest values were detected in the 200 mg L^{-1} group, in which the micronucleus frequency was determined to be 1.18 ± 0.17%, 2.04 ± 0.26% and 2.86 ± 0.31% at 24, 48 and 72 h, respectively. In the positive control group, values of 1.42 ± 0.20%, 2.35 ± 0.29% and 3.18 ± 0.36% were obtained at the same times, respectively. At the 72 h treatment, the 200 mg L^{-1} aclonifen treatment caused an approximately 23.8-fold higher micronucleus formation compared with the control group (Table 3).

Table 3. Effects of different aclonifen concentrations and exposure times on micronucleus (MN) frequency in *Allium cepa* root meristem cells.

Concentration (mg/ L)	***24 h MN (%)***	***48 h MN (%)***	***72 h MN (%)***
Control (%1 DMSO)	0.08 ± 0.03	0.10 ± 0.04	0.12 ± 0.05
50	0.31 ± 0.08*	0.56 ± 0.11*	0.82 ± 0.14*
100	0.64 ± 0.12*	1.12 ± 0.18*	1.68 ± 0.22*
200	1.18 ± 0.17*	2.04 ± 0.26*	2.86 ± 0.31*
Positive control (MMS)	1.42 ± 0.20*	2.35 ± 0.29*	3.18 ± 0.36*

Values are expressed as mean ± SD. *Statistically significant difference from the control group ($p < 0.05$).

Effects on Total Chromosomal Aberrations

The aclonifen treatments were found to increase the total chromosomal aberration frequency with increasing dose and exposure time. In the control group, the total chromosomal aberration rates were determined to be 1.24 ± 0.32%, 1.48 ± 0.36% and 1.72 ± 0.41% at 24, 48 and 72 h, respectively.

In the aclonifen-treated groups, marked increases in chromosomal aberration frequency were observed. The highest values were obtained in the 200 mg L^{-1} group, in which the total chromosomal aberration rates were calculated as 12.48 ± 1.05%, 18.72 ± 1.44% and 23.64 ± 1.86% at 24, 48 and 72 h, respectively. In the positive control group, these values were determined to be 15.26 ± 1.21%, 22.84 ± 1.73% and 27.18 ± 2.04%, respectively. At the 72 h treatment, the total chromosomal aberration frequency in the 200 mg L^{-1} aclonifen group was found to have increased approximately 13.7-fold compared with the control group (Table 4).

Table 4. Effects of aclonifen treatments on total chromosomal aberration (CA) frequency in *Allium cepa* root meristem cells.

Concentration (mg/ L)	***24 h CA (%)***	***48 h CA (%)***	***72 h CA (%)***
Control (%1 DMSO)	1.24 ± 0.32	1.48 ± 0.36	1.72 ± 0.41
50	4.86 ± 0.58*	6.94 ± 0.72*	8.42 ± 0.83*
100	8.16 ± 0.77*	11.84 ± 1.02*	14.26 ± 1.18*
200	12.48 ± 1.05*	18.72 ± 1.44*	23.64 ± 1.86*
Positive control (MMS)	15.26 ± 1.21*	22.84 ± 1.73*	27.18 ± 2.04*

Values are expressed as mean ± SD. *Statistically significant difference from the control group ($p < 0.05$).

Chromosomal Aberration Profile

The main chromosomal aberrations observed in the 72 h treatments were chromosome laggards, chromosome stickiness, anaphase bridges, c-metaphase, irregular anaphase–telophase, binucleate cells and nuclear deformations.

Chromosome stickiness was found to be the most common type of chromosomal aberration in all treatment groups. The stickiness rate, which was determined to be 0.42 ± 0.07% in the control group, rose to 5.62 ± 0.44% in the 200 mg L^{-1} aclonifen treatment. Similarly, c-metaphase, anaphase bridge and irregular anaphase–telophase aberrations were also observed to increase in parallel with the increasing dose.

While the chromosome laggards were 0.21 ± 0.05% in the control group, they were determined to be 2.54 ± 0.21% in the 200 mg L^{-1} aclonifen group. The nuclear deformations, on the other hand, rose from 0.17 ± 0.04% in the control group to 3.48 ± 0.28% in the same treatment group. In the positive control group, all types of chromosomal aberrations were found to be observed at the highest levels (Table 5).

Table 5. Profile of chromosomal aberrations induced by aclonifen in *Allium cepa* root meristem cells after 72 h of exposure.

Group	*LC*	*SC*	*AB*	*CM*	*IAT*	*BN*	*ND*	*Total CA*
Control (%1 DMSO)	0.21 ± 0.05	0.42 ± 0.07	0.26 ± 0.06	0.28 ± 0.06	0.24 ± 0.05	0.14 ± 0.04	0.17 ± 0.04	1.72 ± 0.41
50 mg/ L	0.92 ± 0.10	2.04 ± 0.18	1.16 ± 0.12	1.34 ± 0.13	1.12 ± 0.11	0.84 ± 0.09	1.00 ± 0.10	8.42 ± 0.83
100 mg /L	1.54 ± 0.14	3.48 ± 0.29	1.96 ± 0.18	2.24 ± 0.21	1.92 ± 0.17	1.28 ± 0.13	1.84 ± 0.16	14.26 ± 1.18
200 mg/ L	2.54 ± 0.21	5.62 ± 0.44	3.08 ± 0.26	3.84 ± 0.31	3.12 ± 0.25	1.96 ± 0.18	3.48 ± 0.28	23.64 ± 1.86
MMS (10 mg/L)	2.96 ± 0.24	6.48 ± 0.51	3.64 ± 0.29	4.42 ± 0.35	3.58 ± 0.28	2.26 ± 0.21	3.84 ± 0.31	27.18 ± 2.04

LC = laggard chromosome; SC = chromosome stickiness; AB = anaphase bridge; CM = c-metaphase; IAT = irregular anaphase-telophase; BN = binucleate cell; ND = nuclear deformation.

Discussion

In this study, the commercial aclonifen formulation Challenge® 600 SC was shown to produce marked cytotoxic and genotoxic effects on the root meristem cells of *Allium cepa*. The responses obtained were found to increase with dose and exposure time, manifesting as reductions in root growth and mitotic activity and as increases in the frequencies of micronuclei and chromosomal aberrations.

The suppression of root growth is regarded as one of the most sensitive indicators of phytotoxicity in the *Allium* test system, since root elongation directly reflects the processes of cell division and cell expansion in meristematic tissues (Leme & Marin-Morales, 2009; Torres-Bugarín et al., 2026). In this study, aclonifen treatments significantly reduced root length at all concentrations, and the strongest inhibition was observed in the 200 mg L^{-1} group at the end of 72 h of exposure. The results obtained indicate that aclonifen adversely affects root development and cellular proliferation. Similarly, *Allium* studies carried out with various pesticides have also reported marked reductions in root growth (Feretti et al., 2007; Karagöl et al., 2025). The root elongation inhibition observed in this study suggests that aclonifen disrupts the physiological processes required for normal root development.

The mitotic index, reflecting the proportion of actively dividing cells within a cell population, is regarded as one of the reliable indicators of cytotoxicity (Leme & Marin-Morales, 2009). In our study, significant decreases in mitotic index values were determined following aclonifen treatments. The decreases occurring particularly at concentrations of 100 and 200 mg L^{-1} became more pronounced as the exposure time increased. These findings show that aclonifen suppresses cell division in root meristem tissues. Significant decreases in mitotic index values have similarly been reported in studies carried out with the insecticide omethoate and with the herbicide oxyfluorfen, which belongs to the same diphenyl ether class (Dragoeva et al., 2012; Karagöl et al., 2025). It is considered that this decrease in mitotic activity may be associated with disturbances in chromosome organisation and in the functioning of the spindle fibres during cell division. Moreover, considering that aclonifen inhibits solanesyl diphosphate synthase (SDPS), a key enzyme in plastoquinone biosynthesis, the observed cytotoxic and genotoxic effects may also be associated with oxidative stress and cellular disturbances resulting from impaired plastoquinone metabolism.

Micronucleus formation is regarded as one of the most important biomarkers of genotoxic damage, as it originates from chromosomes or chromosome fragments that fail to be incorporated into the daughter nuclei during division (Leme & Marin-Morales, 2009; Torres-Bugarín et al., 2026). In this study, the micronucleus frequency was found to increase significantly in a manner dependent on both concentration and exposure time. Marked increases in micronucleus formation were observed particularly at 48 and 72 h of exposure. The highest micronucleus frequency was determined in the 72 h, 200 mg L^{-1} aclonifen treatment group and approached the values obtained in the positive control group. In this group, the micronucleus frequency showed an approximately 23.8-fold increase compared with the control. These results clearly demonstrate that aclonifen causes genetic damage in meristematic cells. Similarly, significant increases in micronucleus frequency have also been reported in studies carried out with environmental samples containing pesticide residues and with various agrochemicals (Feretti et al., 2007; Karagöl et al., 2025).

Chromosomal aberrations are among the important indicators in the evaluation of the genotoxic effects of environmental pollutants. In this study, the total frequency of chromosomal aberrations was found to rise markedly as the dose and exposure time increased in response to aclonifen treatments. The highest chromosomal aberration frequencies were observed in the 72 h treatments. The total chromosomal aberration frequency reached its highest level of 23.64% in the 72 h, 200 mg L^{-1} group (control: 1.72%). Among the aberrations observed, chromosome stickiness was determined to be the most common. Chromosome stickiness is generally regarded as an indicator of severe damage occurring in the chromatin structure and is associated with disturbances in chromosomal proteins or in DNA organisation (Leme & Marin-Morales, 2009). In addition, chromosome laggards, anaphase bridges, c-metaphase, irregular anaphase–telophase cells, binucleate cells and nuclear deformations were also observed at high rates in the treatment groups. Similar chromosomal aberrations have also been reported in *Allium* studies carried out with oxyfluorfen and other pesticides (Dragoeva et al., 2012; Karagöl et al., 2025).

In particular, the predominant occurrence of chromosome stickiness, together with the accompanying increases in the frequencies of c-metaphase and chromosome laggards, suggests that aclonifen disrupts normal chromosome segregation during mitosis. Aberrations such as c-metaphase and chromosome laggards reflect an aneugenic effect associated with the impairment of spindle function, whereas chromosome stickiness, anaphase bridges and nuclear deformations reflect a clastogenic effect associated with damage to the chromosome/chromatin structure. These findings suggest that aclonifen acts through both aneugenic and clastogenic mechanisms. Such disturbances may lead to chromosome loss, structural chromosome damage and, consequently, micronucleus formation. The parallel increases observed in chromosomal aberrations and micronucleus frequency in our study also support this assessment and indicate that aclonifen gives rise to genomic instability in root meristem cells.

Previous studies carried out with aclonifen on non-target organisms have revealed that this herbicide produces developmental toxicity in zebrafish embryos and may cause cytotoxic effects in mammalian cells (Lee et al., 2021; Park et al., 2022). Furthermore, studies carried out with bifenox, which belongs to the same diphenyl ether family in structural terms, have reported the suppression of cell proliferation and an increase in cellular damage (Park et al., 2023). The findings obtained in this study are consistent with these investigations and show that aclonifen may produce adverse effects not only on target weeds but also on non-target biological systems.

Conclusion

Taken together, the findings of the present study demonstrate that aclonifen induces significant cytotoxic and genotoxic effects in the root meristem cells of *Allium cepa*. The present study provides new evidence regarding the cytotoxic and genotoxic potential of aclonifen in non-target plant systems and confirms the suitability of the *Allium cepa* assay as a sensitive and reliable tool for the assessment of herbicide-induced environmental risks. Future studies integrating molecular biomarkers and oxidative stress parameters would be valuable for clarifying the mechanisms underlying aclonifen-induced genotoxicity.

Declarations

Ethical Approval Certificate

The present study was conducted using the Allium cepa test system and did not involve experimental animals or human participants. Therefore, ethical committee approval was not required.

Author Contribution Statement

Nurşen Aksu Kalmuk: Conceptualization, methodology, investigation, data collection, formal analysis, visualization, writing – original draft preparation, writing – review and editing.

Fund Statement

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Conflict of Interest

The author declares no conflict of interest.

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