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Cyto- and genotoxic effects of Actellic 50 EC and Rival pesticides on cells of Allium cepa L.

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Abstract. Pesticides are widely used synthetic compounds that can persist in the environment and pose significant risks to non-target organisms. Given their potential to induce genetic damage, their genotoxicity can be assessed through mutagenicity testing with suitable model systems. In the present study, the general toxic, cytotoxic, and mutagenic effects of two widely used pesticides, Actellic 50 EC (active substance pirimiphos-methyl) and Rival (active substance propamocarb hydrochloride), were evaluated using *Allium cepa* L. as a test model. Both formulations are used in agricultural practice to control insect pests, storage pests in cereals, and soilborne pathogens. The results demonstrated that Actellic 50 EC (at concentrations of 10 µg/L and 60 µg/L) and Rival (at concentrations of 40 µg/L and 80 µg/L) exerted a clear cytotoxic effect, as evidenced by a reduction in the mitotic index. Their genotoxic potential was confirmed by the induction of a wide spectrum of chromosomal aberrations, including anaphase and telophase bridges, chromosomal fragments, lagging and vagrant chromosomes. In addition, a significant increase in micronuclei frequency was observed, further supporting the genotoxic effects of the tested pesticides. Further studies using additional biological models and molecular genetic approaches are needed to better elucidate the mechanisms underlying the toxic action of these pesticides.

Key words: *Allium cepa*, pesticides, cytotoxicity, genotoxicity, mitotic index, chromosomal aberrations.

Introduction

The intensive use of pesticides in modern agriculture has led to their widespread distribution in the environment, raising serious concerns regarding their impact on non-target organisms and ecosystem stability (El-Aswad et al., 2026). Although pesticides are essential for crop protection and improved agricultural productivity, their persistence in soil and water systems increases the risk of chronic exposure and accumulation, which may result in adverse biological effects (Datta et al., 2018). Numerous studies have demonstrated that pesticide contamination can affect plant growth, induce oxidative stress, and disrupt key physiological and cellular processes. Beyond their intended pesticidal activity, many pesticides have been reported to exert cytotoxic and genotoxic effects on non-target organisms. These include inhibition of cell division, induction of chromosomal aberrations, and formation of micronuclei – widely accepted biomarkers of DNA damage (Datta et al., 2018; Cakmak-Arslan & Rasgele, 2025; Sarsar et al., 2025). Such genotoxic effects are of particular concern, as they may lead to mutations and long-term ecological consequences, affecting biodiversity and ecosystem functioning.

The pesticide formulations Actellic 50 EC and Rival are widely used in agricultural practice and differ in their biological activity and application. Actellic 50 EC contains pirimiphos-methyl, an organophosphorus insecticide commonly applied for the control of storage pests in cereals. Its pri-

mary mechanism of action involves inhibition of acetylcholinesterase; however, organophosphates are also associated with oxidative stress and DNA damage in non-target organisms. Rival contains propamocarb hydrochloride, a systemic carbamate fungicide used to control soilborne pathogens, particularly oomycetes. Despite their effectiveness, similar agrochemicals have been shown to induce cytotoxic and genotoxic effects depending on exposure conditions and concentration (El-Aswad et al., 2026).

The evaluation of pesticide-induced toxicity requires sensitive and reliable bioassays. Among plant-based systems, the *Allium cepa* assay is one of the most widely used and well-established methods for assessing cytotoxic and genotoxic effects of environmental pollutants (Venkataravanappa et al., 2025). This assay is based on the analysis of root meristem cells, which are particularly sensitive due to their high mitotic activity and direct contact with contaminants in the growth medium. The *Allium cepa* test offers several important advantages, including large and easily observable chromosomes, simplicity, cost-effectiveness, and high sensitivity to a wide range of chemical agents. Importantly, the results obtained with this model show strong correlation with those from other biological systems, including mammalian assays, supporting its reliability in environmental monitoring (Melo et al., 2024; Venkataravanappa et al., 2025). Recent experimental studies have confirmed its applicability in detecting pesticide-induced genotoxicity, demonstrating reductions in mitotic index and increased frequencies of chromosomal abnormalities following exposure to herbicides and other agrochemicals (Cakmak-Arslan & Rasgele, 2025; Sarsar et al., 2025; Jardim & Oliveira, 2025; El-Aswad et al., 2026). Given that plant roots are in direct contact with contaminated soils, they represent a primary target for pesticide exposure and an appropriate model for toxicity assessment (Venkataravanappa et al., 2025). Therefore, cytogenetic analysis of root meristem cells provides valuable insights into the mechanisms of pesticide action at the cellular and chromosomal levels.

In this context, the present study aims to evaluate the cytotoxic and genotoxic effects of two widely used pesticides, Actellic 50 EC and Rival, on root meristem cells of *Allium cepa* L.

Materials and methods

Experimental setup

The *Allium cepa* test system, proposed by Fiskesjö (1985), with modifications by Ivanova et al. (2005) and Staykova et al. (2010), was used for the analysis of chromosomal aberrations. The model system has been adopted by the International Programme on Plant Bioassays (IPPB) for monitoring or testing contaminants in the environment. Annual seeds of *Allium cepa* (Density variety) were used for the present study, obtained from the certified producer "Sortovi Semena Bulgaria Ltd.", ensuring a well-defined origin and quality of the plant material. The onion seeds (50 each) were placed in petri dishes on three-ply filter paper under humid chamber conditions. The filter paper was moistened with the experimental solutions of different concentrations of Actellic 50 EC (10 μg/l and 60 μg/l) and Rival (40 μg/l and 80 μg/l). In the control, the filter paper was moistened with distilled water. The selected concentrations were based on literature data and preliminary testing, covering a range from environmentally relevant to higher doses capable of inducing measurable cytotoxic and genotoxic effects without completely inhibiting root growth (Leme & Marin-Morales, 2009; Rosculete et al., 2019). The exposure period of 72 h was chosen as a standard duration for the *Allium cepa* assay, ensuring sufficient root development and mitotic activity for reliable cytogenetic analysis (Fiskesjö, 1985; Grant, 1999; Jardim & Oliveira, 2025).

A minimum of 1000 cells, read from at least 5 microscopic preparations, from 5 different root tips were analysed from each experimental and control group. Microscopic preparations were observed using a light microscope (Leica DM 1000 LED; Leica Microsystems, Germany) at ×400 magnification and documented with a digital microphotography system (Flexacam i5; Leica Microsystems, Germany).

General toxicity, cytotoxicity, and genotoxicity assessment

In the present study, the total toxicity was calculated as the percentage of germinated seeds out of the total number of seeds set in the control and experimental samples. Mitotic index and phase indices were used as indices of cell proliferation for cytotoxicity analysis. The genotoxic effect was evaluated by determining the frequency of chro-

mosomal aberrations in *Allium cepa* root meristem cells. The methods used to report genotoxicity were the micronucleus assay and the anaphase aberration assay, which are well-established and widely accepted cytogenetic endpoints in plant bioassays (Fiskesjö, 1985; Grant, 1999; Leme & Marin-Morales, 2009; Nicuță et al., 2025).

Statistical analysis

Statistical comparisons between pesticide-treated samples and the control were performed using Student's t-test. Data are expressed as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$.

Results and Discussion

Total toxicity

Seed germination percentage is a commonly used indicator for assessing the overall phytotoxic effects of pesticides and other environmental contaminants, as it reflects early plant developmental sensitivity to toxic stress (Fiskesjö, 1985; Leme & Marin-Morales, 2009; Arık et al., 2025). In the present study, the highest germination was observed on the third day of exposure. A slight reduction in germination rate compared to the control was observed in samples treated with lower concentrations of the tested pesticides (10 µg/L Actellic 50 EC and 40 µg/L Rival), suggesting a weak inhibitory effect on early seed development. In contrast, exposure to higher concentrations (60 µg/L Actellic 50 EC and 80 µg/L Rival) did not result in a significant stimulatory or inhibitory effect on seed germination. The germination percentage remained comparable to the control, indicating the absence of pronounced acute phytotoxicity at these concentrations under the tested conditions.

These findings suggest that while low-dose exposure may induce slight physiological stress, higher concentrations within the tested range do not significantly impair germination capacity (Table 1). The observed results are consistent with previous studies reporting that certain pesticides may not significantly affect germination rates, but can still induce cytogenetic and sub-lethal cellular effects that are detectable only at the microscopic or molecular level (Rosculete et al., 2019; Ili & Sari, 2024). This highlights the importance of using complementary endpoints, such as chromosomal aberration and micronucleus formation, for a comprehensive assessment of pesticide toxicity. Similar findings have been reported in studies using the *Allium cepa* model, where germination remained largely unaffected despite clear genotoxic responses at the cellular level (Leme & Marin-Morales, 2009; Arık et al., 2025).

Overall, the results indicate that the tested pesticides exhibit limited acute phytotoxic effects on seed germination under the experimental conditions, but this does not exclude the possibility of sub-lethal cytotoxic or genotoxic impacts, which are addressed in the subsequent analyses.

Table 1. Effect of tested pesticides on germination of *Allium cepa* seeds.

	Number of seeds	Number of germinated seeds	Number of ungerminated seeds	Germination %
Control	50	44	6	88
10 µg/l Actellic 50 EC	50	40	10	80
60 µg/l Actellic 50 EC	50	45	5	90
40 µg/l Rival	50	38	12	76
80 µg/l Rival	50	44	6	88

Cytotoxicity

The ability of the investigated pesticides to affect the proliferative activity of onion root meristem cells was studied. The level of cytotoxicity of an agent can be demonstrated by changes in the mitotic index, which may increase or decrease depending on the type and intensity of toxic exposure (Fernandes et al., 2007; Ili & Sari, 2024; Arık et al., 2025). A reduction in the mitotic index compared to the control is considered evidence that the growth and development of the organism are affected by the administered agent. In the present study, the mitotic index and phase index data obtained in control and experimental treatments revealed an inhibitory effect of Actellic 50 EC and Rival pesticides on cell division (Table 2).

Table 2. Mitotic index (IM) and phase indices (%) in *Allium cepa* treated for 72 h with different concentrations of Actellic 50 EC and Rival (X±SD) (marked with * are significant at p<0.05).

Sample	Control	10 µg/l Actellic 50 EC	60 µg/l Actellic 50 EC	40 µg/l Rival	80 µg/l Rival
Mitotic Index, IM	50.18±2.37	44.72±3.57*	40.75±7.25*	47.77±7.88	42.44±4.64*
Prophase index, IPph	85.54±6.23	88.51±2.25	84.72±5.62	88.57±3.16	86.09±3.96
Metaphase index, Imph	5.74±3.06	4.41±1.72	6.02±2.09	4.15±1.79	5.27±1.59
Anaphase index, Iaph	4.12±1.54	3.81±0.96	5.13±1.83	3.32±0.64	5.10±2.06
Telophase index, Itph	4.6±2.05	3.27±0.63	4.13±1.83	3.96±1.25	3.53±1.22

The highest number of dividing cells was recorded in the control, where the mitotic index value was higher than all experimental treatments (50.18±2.37; Table 2). The lowest level of cell proliferation was observed at the highest concentrations of the tested pesticides, 60 µg/L Actellic 50 EC (40.75±7.25) and 80 µg/L Rival (42.44±4.64). Lower concentrations (10 µg/L Actellic and 40 µg/L Rival) also induced a cytostatic effect, as evidenced by reduced mitotic activity compared to the control (Table 2). An inverse correlation between pesticide concentration and mitotic index was observed, indicating dose-dependent cytotoxicity. Such inhibition of mitotic activity in meristematic tissues is a well-documented response to pesticide exposure and is commonly associated with disturbances in cell cycle regulation and DNA integrity (Leme & Marin-Morales, 2009; Al-Ahmadi, 2013; Mesi & Kopliku, 2014; Bianchi et al., 2016; Popova et al., 2019; Arık et al., 2025; El-Aswad et al., 2026). Recent studies using the *Allium cepa model* have confirmed that reduced mitotic index is strongly linked to spindle apparatus disruption, oxidative stress, and activation of cell cycle checkpoints (Cakmak-Arslan & Rasgele, 2025; Venkataravanappa et al., 2025). These mechanisms collectively contribute to cytostatic effects and impaired cell proliferation.

Phase mitotic indices allow the determination of the relative duration of individual stages of cell division. In this aspect, a different distribution of cells in mitosis was observed among treatments. Meristematic cells exposed to 10 µg/L Actellic 50 EC, 40 µg/L Rival, and 80 µg/L Rival showed an accumulation of cells at prophase compared to the control (Table 2). The increase in the prophase index, accompanied by a decrease in metaphase and anaphase indices, suggests activation of a mitotic checkpoint between prophase and metaphase, likely triggered by cellular stress and DNA damage responses (Venkataravanappa et al., 2025; El-Aswad et al., 2026). At the same time, treatment with 60 µg/L Actellic 50 EC resulted in a lower proportion of prophase cells and increased metaphase and anaphase indices compared to the control. This may indicate an altered progression through mitosis, possibly reflecting disturbance in checkpoint regulation and premature transition between mitotic stages. Similar pesticide-induced alterations in mitotic phase distribution have been reported in recent *Allium cepa* studies, further supporting the sensitivity of this system for detecting cytotoxic effects (Venkataravanappa et al., 2025; El-Aswad et al., 2026).

Genotoxicity

Genotoxicity refers to the ability of physical, chemical, or biological agents to cause damage to genetic material or alter cellular structures, thereby affecting the function and behavior of chromosomes in the cell (Yan et al., 2013). Table 3 presents the incidence (% relative to the total number of cells analyzed and relative to dividing cells) with which chromosomal aberrations occur in *Allium cepa* cells after treatment with different concentrations of Actellic 50 EC and Rival. As can be seen from the data presented, after a 72-hour exposure period, the Actellic 50 EC and Rival pesticides exhibited genotoxic effects, inducing disorders at high frequency. The experimental treatments showed a significant increase in the number of aberrant cells for all pesticide concentrations tested relative to the control. A dose-dependent nature of the genotoxic effect was observed, with increasing pesticide concentration increasing the percentage of chromosomal disorders occurring in *Allium cepa* meristem cells (Table 3). The analysis of the spectrum of cytogenetic disorders gives us additional information on the mutagenic action of the Actellic 50 EC and Rival pesticides. They induce different types of mutations, from structural chromosomal disorders to abnormalities in the course of mitosis, which is a

prerequisite for incorrect divergence of genetic material. A characteristic correlation was found between the type of aberrations occurring and the different xenobiotic concentrations tested.

Table 3. Incidence of chromosomal aberrations (%) (X±SD) in *Allium cepa* treated for 72 h with different concentrations of Actellic 50 EC and Rival) (marked with * are significant at p<0.05).

Sample	Frequency of aberrations relative to the total number of cells analyzed	Frequency of aberrations relative to the number of dividing cells
Control	0.58±0.29	1.16±0.54
10 µg/l Actellic 50 EC	1.04±0.51	2.32±1.17
60 µg/l Actellic 50 EC	1.26±0.73	2.96±1.37*
40 µg/l Rival	1.23±0.31	2.75±0.31
80 µg/l Rival	1.32±0.31	2.94±0.84*

The values obtained for the frequency of clastogenic-type aberrations and the frequency of aneugenic-type disturbances for all pesticide doses tested lead to the conclusion that the higher tested concentrations of Actellic 50 EC and Rival exhibit a greater ability to induce impairment-related disturbances of the spindle apparatus. Conversely, the lower concentrations of pesticides tested induced, to a greater extent, disorders associated with damage and destruction of chromosomal structures (Tables 4 and 5). These differences in the spectrum of chromosomal aberrations and in the type of damage prevailing indicate the different mechanisms of their occurrence and the specificity in the mutagenic action of the different doses of pesticides tested.

Table 4. Frequency of occurrence (%) of different chromosomal aberrations of clastogenic type analyzed by the *Allium* test. First row - % relative to total cells (N), second row - % relative to dividing cells (N').

Sample	Cells with chromosomal bridges	Cells with chromosomal fragments	Cells with despiralized chromosomes	Cells with multipolar anaphase	Cells with micronuclei	Total
Control	0.06	0.07	0.00	0.00	0.15	0.28
	0.11	0.14	0.00	0.00	0.30	0.55
10 µg/l Actellic 50 EC	0.21	0.23	0.00	0.02	0.25	0.70
	0.48	0.50	0.00	0.04	0.54	1.56
60 µg/l Actellic 50 EC	0.15	0.15	0.00	0.00	0.04	0.34
	0.37	0.39	0.00	0.00	0.08	0.84
40 µg/l Rival	0.22	0.17	0.02	0.04	0.28	0.73
	0.52	0.41	0.04	0.08	0.70	1.75
80 µg/l Rival	0.07	0.23	0.07	0.00	0.10	0.47
	0.16	0.44	0.16	0.00	0.18	0.94

Table 5. Frequency of occurrence (%) of different chromosomal aberrations of aneugenic type analyzed by the *Allium* test.

Sample	C-mitosis	Cells with lagging chromosomes	Cells with wandering chromosomes	Cells in diagonal anaphase	Cells with asynchronous mitosis	Total
Control	0.07	0.06	0.15	0.04	0.00	0.31
	0.14	0.11	0.29	0.07	0.00	0.62
10 µg/l Actellic 50 EC	0.12	0.08	0.11	0.02	0.02	0.34
	0.27	0.16	0.25	0.04	0.05	0.76
60 µg/l Actellic 50 EC	0.48	0.22	0.23	0.00	0.00	0.92
	1.08	0.47	0.58	0.00	0.00	2.12
40 µg/l Rival	0.23	0.08	0.19	0.04	0.02	0.51
	0.54	0.08	0.43	0.08	0.05	1.19
80 µg/l Rival	0.33	0.13	0.32	0.02	0.06	0.85
	0.71	0.27	0.68	0.04	0.12	1.82

Micronuclei were the most common aberration in the samples treated with the lower concentrations of the Actellic 50 EC and Rival pesticides (Figs. 1 and 2). Micronuclei are observed in the daughter interphase cells, but are the result of genotoxic effects to which the parent cell has been subjected. They are formed during telophase of mitosis when the nuclear envelope is repaired around the daughter chromosome complexes (Camilo-Cotrim et al., 2022; Cakmak-Arslan & Rasgele, 2025). Micronuclei result from the lagging of chromosomes or their fragments in the preceding phases of mitosis, and consequently, they are not incorporated into the daughter nuclei. Cells with micronuclei are predominantly seen in samples with a high frequency of disruption. The high number of micronuclei in *Allium cepa* cells treated with 10 µg/l Actellic 50 EC and 40 µg/l Rival is evidence of the genotoxic action of these pesticides. A large number of cells with chromosome bridges and fragments were also found in these experimental treatments (Figs. 3-6).

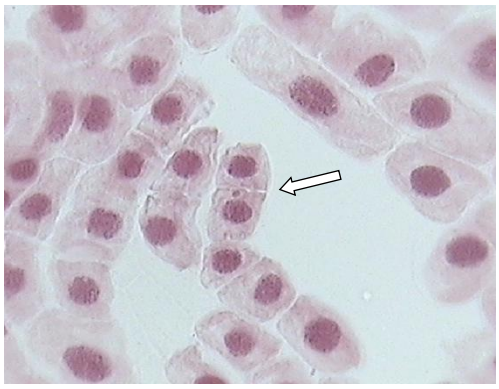

Fig. 1. A micronucleus in a sample treated with 10 µg/l Actellic 50 EC, magnification 400x.

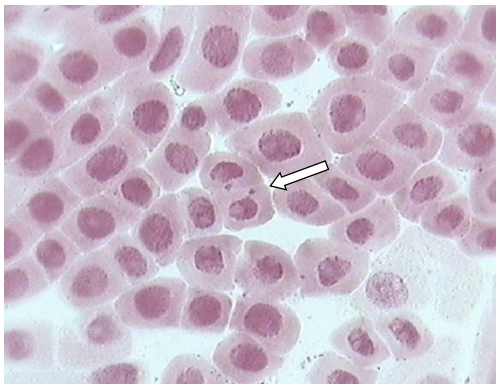

Fig. 2. A micronucleus in a sample treated with 40 µg/l Rival, magnification 400x.

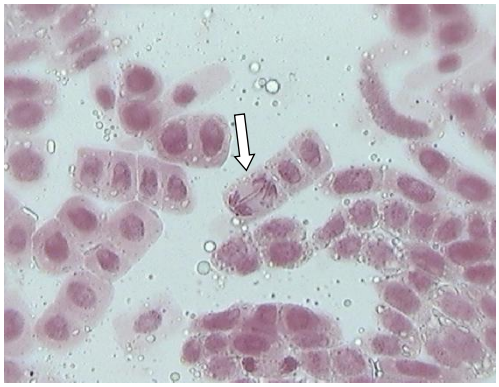

Fig. 3. Anaphase bridges in a sample treated with 10 µg/l Actellic 50 EC, magnification 400x.

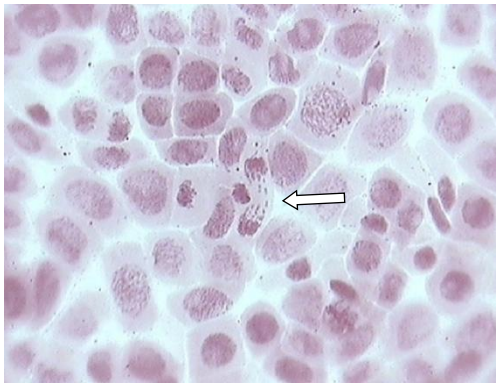

Fig. 4. Fragments in a sample treated with 10 µg/l Actellic 50 EC, magnification 400x.

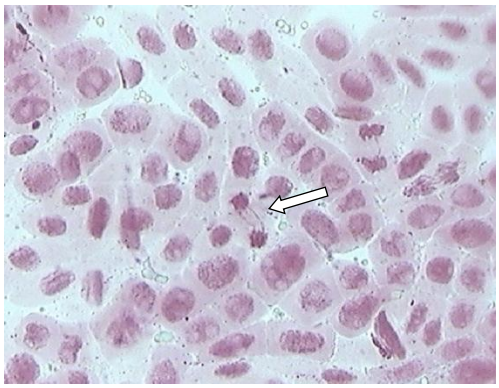

Fig. 5. Telophase bridges in a sample treated with 40 µg/l Rival, magnification 400x.

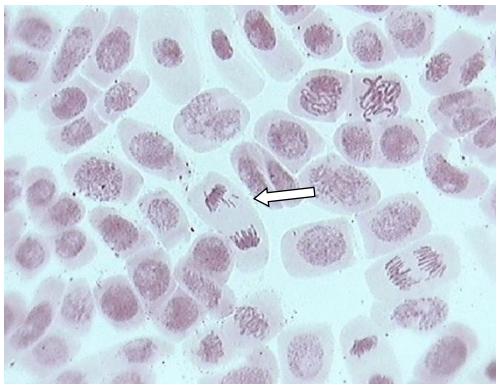

Fig. 6. Fragments in a sample treated with 40 µg/l Rival, magnification 400x.

The presence of chromosome bridges across anaphase and telophase demonstrates the ability of the pesticide to cause DNA breaks leading to aneucentric translocations (Kalaev & Popova, 2014). Fragmentation of chromosomes is an indicator of the destruction of their structure associated with the lysis of DNA molecules and is an indicator of genomic instability.

The high overall frequency of mutations induced by the higher tested concentrations of the studied pesticides (60 µg/l Actellic 50 EC and 80 µg/l Rival) is largely due to the large number of cells with C-mitosis (Table 5). C-mitosis results from the disruption of the strands of the spindle, resulting in characteristic configurations of chaotically dispersed chromosomes in the cells (Figs. 7 and 8). This suggests a genotoxic effect of the pesticides related to direct or indirect effects on the achromatic apparatus.

In these samples (60 µg/l Actellic 50 EC and 80 µg/l Rival), a high frequency of cells with lagging and 'vagrant' chromosomes was observed (Figs. 9-12), confirming the leading role of disorders related to the formation and function of the mitotic spindle.

The comparative analysis of samples treated with different concentrations of Actellic 50 EC and Rival revealed the dose-dependent nature of the abnormalities caused by damage to the mitotic apparatus. Similar effects have been observed by other authors in studies of different pesticides and environmental toxicants using the *Allium cepa* test system (Andrioli et al., 2012; Ozakca & Silah, 2013; Stoyanov et al., 2018). In addition to these detected disorders, single cells with asynchronous mitosis, multipolar and diagonal anaphases, as well as those with despiralized chromosomes, were also registered by the anaphase method in the course of the present study. The analysis of the results obtained shows that the tested Actellic 50 EC and Rival pesticides have a pronounced genotoxic effect on the onion root meristem, which is evidenced by both the significantly higher frequency of chromosomal aberrations recorded in the experimental treatments compared to the control and their significant diversity found.

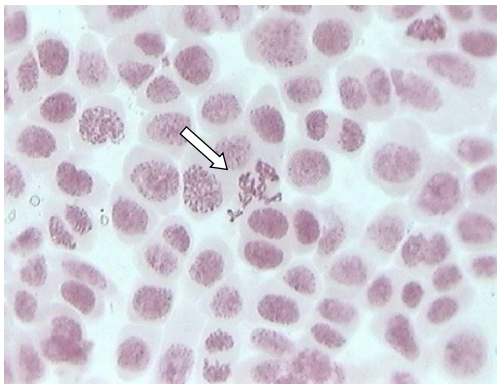

Fig. 7. C-mitosis in a sample treated with 60 µg/l Actellic 50 EC, magnification 400x.

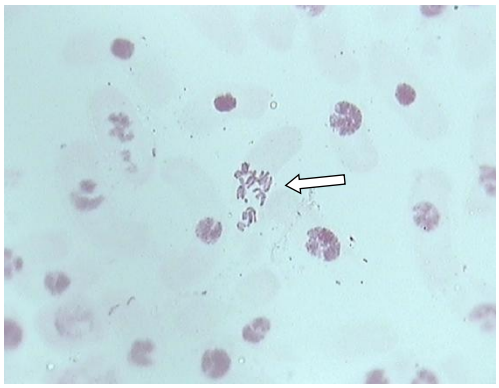

Fig. 8. C-mitosis in a sample treated with 80 µg/l Rival, magnification 400x.

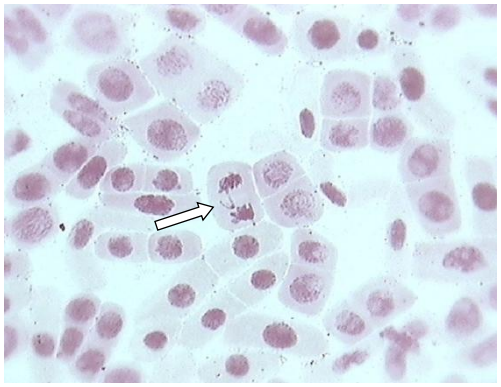

Fig. 9. Lagging chromosome in a sample treated with 60 µg/l Actellic 50 EC, magnification 400x.

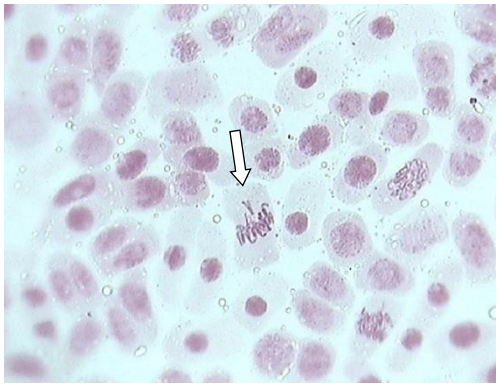

Fig. 10. A "wandering" chromosome in a sample treated with 60 µg/l Actellic 50 EC, magnification 400x.

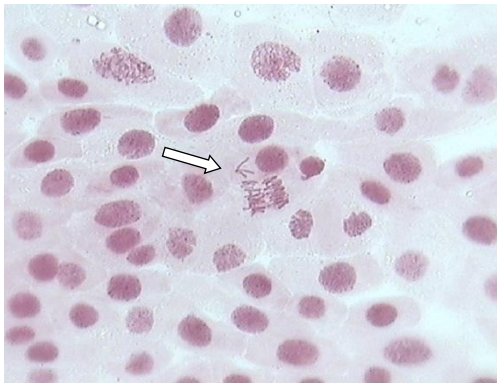

Fig. 11. A "wandering" chromosome in a sample treated with 80 µg/l Rival, magnification 400x.

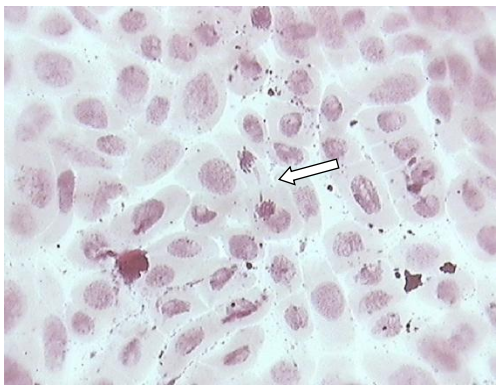

Fig. 12. Lagging chromosomes in a sample treated with 80 µg/l Rival, magnification 400x.

Conclusions

Based on the results obtained using *Allium cepa* as a model system, it can be concluded that both Actellic 50 EC and Rival exhibit clear cytotoxic and genotoxic effects on plant meristematic cells. These effects are manifested by reduced mitotic activity, disturbances in the mitotic apparatus, and induction of structural and numerical chromosomal aberrations. Both pesticides caused a significant decrease in the mitotic index across all experimental treatments compared to the control, indicating cytostatic activity. The strongest inhibition of cell division was observed at the highest concentrations (60 µg/L Actellic 50 EC and 80 µg/L Rival), while lower concentrations (10 µg/L and 40 µg/L, respectively) produced a milder but still evident suppressive effect on cell proliferation. In addition, alterations in the distribution of mitotic phases confirmed disruption of normal cell cycle progression. The genotoxic effects were evidenced by a significant increase in the frequency and diversity of chromosomal aberrations, including chromosome bridges, fragments, lagging and vagrant chromosomes, C-mitosis, and micronuclei formation. These abnormalities indicate that both clastogenic and aneugenic mechanisms are involved in the toxic action of the tested pesticides. A concentration-dependent pattern of genotoxicity was observed. Lower concentrations of both pesticides were predominantly associated with structural chromosomal damage (clastogenic effects), whereas higher concentrations induced more pronounced disturbances of the mitotic spindle apparatus, leading to aneugenic abnormalities. Despite the clear cytotoxic and genotoxic effects, no marked phytotoxic effect on seed germination was observed, as germination rates in treated groups remained comparable to the control. This suggests that early germination processes are less sensitive to the tested concentrations, while cellular and chromosomal endpoints provide a more sensitive measure of pesticide toxicity.

Overall, the results demonstrate that Actellic 50 EC and Rival exert dose-dependent cytotoxic and genotoxic effects in *Allium cepa* root meristem cells, highlighting their potential risk to non-target plant systems and supporting the usefulness of plant bioassays in environmental toxicity assessment. These findings are in agreement with previous reports on pesticide-induced cytogenetic damage in plant bioindicator systems.

Acknowledgments

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