Arch Biol Sci. 2024;76(4):477-490 https://doi.org/10.2298/ABS241025038I

Evaluation of the cytogenetic and genotoxic effects of an abamectin-based pesticide on *Allium cepa* roots

Pinar Ili[1] and Fikret Sari[2,*]

[1]*Department of Medical Services and Techniques, Denizli Vocational School of Health Services, Pamukkale University*, 20160 Denizli, Türkiye

[2]*Department of Plant and Animal Production, Tavas Vocational School, Pamukkale University*, 20500 Denizli, Türkiye

***Corresponding author**: fikretsari1@gmail.com; fsari@pau.edu.tr

Received: October 25, 2024; **Revised**: November 8, 2024; **Accepted**: November 10, 2024; **Published online**: November 25, 2024

Abstract: Abamectin, a widely used pesticide with insecticidal, anthelmintic, and acaricidal properties, has raised safety concerns due to its toxic effects on certain non-target organisms. The toxicity of abamectin, the active ingredient in the commercial pesticide formulation Alopec® EC, was evaluated using cytogenetic and comet assays on *Allium cepa* root tips. Mitotic index (MI) and phase index (PI) values were used for cytotoxicity assessment. Chromosomal aberration (CA) frequencies in the dividing cells and comet data were used for genotoxicity assessment. The root growth test showed a significant concentration-based decline in root growth after abamectin exposure, with a median effective concentration (EC_{50}) of 2.50 mg/L. Following 96-hour exposure to three concentrations of abamectin (1.25, 2.50, and 5.00 mg/L), cytogenetic and comet analyses indicated a significant concentration- and time-dependent decrease in the MI, alongside an increase in DNA damage. Additionally, there was a significant concentration-dependent rise in the total frequency of CAs. These findings show that abamectin is a pesticide with lethal effects on *A. cepa* root tip meristematic cells, even at lower concentrations, over prolonged exposure times, with CA-forming and DNA-damaging effects, and that it is highly cytotoxic and genotoxic.

Keywords: abamectin, toxicity, chromosomal aberration, DNA damage, *Allium cepa*

INTRODUCTION

Agrochemicals, which include pesticides and fertilizers, have become an important component of worldwide agricultural practices over the last century, enhancing crop yields and food production [1]. Pesticides are widely used in modern agriculture to eliminate the damage caused by pests, weeds, and plant diseases [2]. Even though pesticides aim to control target organisms, they usually exhibit adverse effects on non-target organisms [1,3]. As large amounts of pesticides reach the environment and contaminate the soil, water, and air [4,5], their extensive and indiscriminate use threatens public and environmental health [6,7], making pesticides of environmental and human health concern worldwide.

Avermectins, a class of macrocyclic lactones with nematocidal, acaricidal, and insecticidal activities, are derived from the soil-dwelling actinomycete *Streptomyces avermitilis* as a fermentation product [8]. As potential neurotoxins, avermectins such as doramectin, selamectin, abamectin, and ivermectin act through the glutamate-gated chloride channels in invertebrates and/or the gamma-aminobutyric acid-gated chloride channels in both invertebrates and vertebrates, resulting in membrane hyperpolarization in neurons, paralysis, and eventually death of the parasite [8,9]. Despite their short half-lives and different degradation pathways, avermectins can persist in water, sediment, and soil [8,10]. Abamectin, a key member of the avermectin family, is widely used as an insecticide due to its potent insecticidal, anthelmintic, and acaricidal properties [8]. Its versatility allows for applications not only in agriculture but also in pharmaceutical and veterinary fields, contributing to its extensive global use. The intensive use of abamectin

has recently raised concerns regarding its safety since it has various toxic effects on some non-target organisms [8,11,12]. Within this framework, environmental monitoring is important.

Biomonitoring is an effective tool to obtain a better understanding of the environmental effects of contaminants including pesticides, and the data obtained from biomonitoring studies are useful for making effective management decisions on the sustainability of environmental health [13,14]. As the use of living organisms in environmental monitoring has the advantage of revealing the complex effects of pollutants/toxicants [15,16], biomonitoring studies use various bioindicator species, including invertebrates [17,18] and vertebrates [19,20] as well as higher plants [13]. Among the higher plant species used to assess environmental contamination/pollution, *Allium cepa*, with a variety of advantages such as low cost, ease of handling, and good chromosome conditions for the study of chromosome damage or disturbance of cell division [21], is one of the most frequently employed species [22]. The *A. cepa* test is used for cytotoxicity and genotoxicity evaluations of different environmental contaminants [23]. It is more sensitive in detecting toxicity and genotoxicity than other tests [24]. *A. cepa* root tips are directly exposed to toxic materials dispersed in soil or water [24], making them highly sensitive to toxicants [25]. Furthermore, the strong correlation of the *A. cepa* test system with mammalian and non-mammalian test models enhances its significance in research [21,26].

The *A. cepa* test system has been employed to evaluate the toxicity of various pesticides, providing valuable information regarding their safety and environmental health. Considering the wide usage areas and intensive use of abamectin, it is important to understand all aspects of its toxic effects on non-target organisms for a comprehensive and accurate approach to environmental protection. The present study was conducted to investigate the cytotoxicity and genotoxicity potentials of abamectin as the commercial product Alopec® EC in *A. cepa* root tip meristematic cells after a 96-h exposure using the cytogenetic and comet assays. For cytotoxicity assessment, the mitotic index (MI) and phase index (PI) were used, while genotoxicity was evaluated through chromosomal aberration (CA) frequencies in dividing cells and comet assay data. The data obtained were comprehensively analyzed to reveal the potential relationships of each parameter with concentration and exposure time.

MATERIALS AND METHODS

Test organism, model insecticide, and preparation of test solutions

A. cepa (2n=16) was the test organism in this study. Healthy onion bulbs of nearly equal size (untreated) were purchased from a market in Denizli, Türkiye, and kept dry until the experiments. The model insecticide abamectin was purchased as a commercial formulation with the trade name of Alopec® EC (18 g/L abamectin). Before the experiments, the commercial formulation was diluted with distilled water (dH_2O) to give a stock solution at a concentration of 1 g/L. This stock solution was protected from light and kept at 4°C until use. The stock solution was then diluted with tap water to prepare the final test solution concentrations used in the experiments.

Determination of EC_{50}

The *A. cepa* root growth inhibition test was implemented following [21], with some modifications. Equal-sized onion bulbs (about 20 mm in diameter) were placed in glass test tubes (15 mL) filled with tap water and allowed to generate roots for 24 h. After 24 h, the best-rooted bulbs were selected to be used in the root growth inhibition test and treated with tap water (control) and nine different concentrations of abamectin (0.1, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 mg/L) for 96 h at 25±1°C in the dark, with each treatment consisting of five onion bulbs (as replicates). All solutions in the test tubes were renewed daily. At the end of an exposure time of 96 h, the lengths of 10 randomly selected roots from each onion bulb were gauged with a digital caliper. Using the mean root lengths for each of 10 treatments, the effective concentration of abamectin that inhibited root growth by 50% compared with the control (EC_{50}) was calculated.

Test solutions and treatment experiments

Onion bulbs with a diameter of 20 mm were cleaned from their dried roots, placed in glass test tubes (15

mL) filled with tap water and allowed to generate roots in laboratory conditions (at 25±1°C in the dark) for 24 h. Following the 24-h period, the best-rooted bulbs were selected. Based on the calculated EC_{50}, three concentrations of abamectin ($1/2\times EC_{50}$, EC_{50}, and $2\times EC_{50}$) were selected to encompass the recommended field application dose of the tested commercial pesticide formulation. Three exposure groups with these concentrations (1.25, 2.50, and 5.00 mg/L) were established for use in the cytogenetic and comet assays. Abamectin-free tap water was used as the negative control group and methyl methane sulfonate (MMS; 10 mg/L) as the positive control group. The selected onion bulbs were treated under laboratory conditions with solutions representing the five experimental groups for 24, 48, 72, and 96 h per assay. Each group consisted of five onion bulbs (as replicates) per exposure time, with all solutions in the test tubes renewed daily. The main reason for determining these exposure times in the study is that the cell cycle in *A. cepa* meristematic cells is 24 h [27].

Cytogenetic assay

At the end of each exposure time (24, 48, 72, and 96 h) for the treatment experiments, 10 to 15 randomly selected root tips (8-10 mm in length) were cut from each of five onion bulbs using sterile razor blades. This process was performed between 07:00 and 08:00 in the morning since the highest mitotic rate in *A. cepa* root meristems is between 06:00 and 09:00 [28]. After washing with dH_2O, the root tips were fixed in freshly prepared Carnoy solution (3 ethanol:1 glacial acetic acid, v/v) for 24 h and preserved in 70% alcohol at 4°C. They were then hydrolyzed with 1 N HCl in a water bath at 60°C for 7 min and rinsed with dH_2O three times for 5 min each. Following staining with 2% aceto-orcein at 60°C for 5 min, the root tips were placed on glass slides, covered with coverslips, and squashed; the coverslips were sealed with transparent nail polish to obtain semi-permanent slides. For each onion bulb in each experimental group, 10 slides were prepared for use in the subsequent cytogenetic analyses.

Cytogenetic analyses were performed by microscopically examining the prepared slides of the root tips through a B-600Ti Optika light microscope with a 4083. B5 OptikamB5 digital camera (Optika Microscopes, Italy). At least 200 cells in each of five microscopic regions per slide were observed, and the stages of these cells (interphase, prophase, metaphase, anaphase, or telophase) were detected. Based on the obtained data, the MI and PI values were used to assess the cytotoxic effect of abamectin.

The MI (%) was calculated per onion bulb using the following formula [29]:

$$MI = \left(\frac{NDC}{TNOC}\right) \times 100$$

where *MI* is the mitotic index, *NDC* is the number of the dividing cells, and *TNOC* is the total number of the observed cells.

The PI (%) for each mitotic phase was calculated per onion bulb through the following formula [30]:

$$PI = \left(\frac{NCP}{TNDC}\right) \times 100$$

where *PI* is the phase index, *NCP* is the number of cells in a specific phase, and *TNDC* is the total number of dividing cells.

At least 150 cells from each of five randomly selected slides per experimental group were observed, and CAs in the dividing cells were monitored. Based on the obtained aberrations data, the frequency of each CA was determined to be used for assessing the genotoxic effect of abamectin.

The frequencies of CAs (%) were calculated per onion bulb with the following formula [31]:

$$CAF = \left(\frac{NDCCA}{TNDC}\right) \times 100$$

where *CAF* is the chromosomal aberration frequency, *NDCCA* is the number of the dividing cells having a specific chromosomal aberration, and *TNDC* is the total number of the dividing cells.

Comet assay

To assess the genotoxic effect of abamectin by examining DNA damage levels in root tip meristematic cells at varying concentrations and exposure times, the alkaline comet assay was performed following a previously described protocol [32], with some modifications. At the end of each exposure time (24, 48, 72, and 96 h),

10-15 randomly selected root tips (8-10 mm in length) were cut from each of five onion bulbs as mentioned in the cytogenetic assay, and immediately chopped in a Petri dish containing 500 µL of ice-cold Tris-$MgCl_2$ buffer (0.2 M Tris, 4 mM $MgCl_2$ $6H_2O$, 0.5% Triton X-100, pH 7.5) using sterile razor blades to isolate the nuclei of the cells. Fifty µL of low-melting agarose in 0.75% phosphate-buffered saline (PBS) was mixed with 50 µL of the prepared suspension, then pipetted onto slides that had been pre-coated with 1% normal melting agarose in PBS the previous day. The slides were covered with coverslips and placed on an ice pack for 5 min to solidify the agarose. For each onion bulb in each experimental group, five slides were prepared for the subsequent comet analyses. The coverslips were removed, and the slides were lysed in a fresh lysing solution (2.5 M NaCl, 100 mM Na_2-EDTA, 200 mM NaOH, 1% Triton X-100, 10% DMSO, pH 10) for 60 min at 4°C. After the lysis step, the slides were kept in a horizontal electrophoresis chamber containing freshly prepared chilled buffer (1 mM Na_2-EDTA, 300 mM NaOH, pH>13) for 30 min at 4°C to allow the DNA to unwind, and then subjected to electrophoresis at 25 V and 300 mA for 30 min at 4°C. After maintaining in a neutralization buffer (400 mM Tris-HCl, pH 7.5) three times for 5 min each to remove excess alkali and detergent, the slides were rinsed with deionized water (diH_2O) two times for 2 min each and dried for 20 min. All steps described above were performed under dim light or in the dark to prevent artefactual DNA damage. The next step was the staining for which the silver staining method procedure [33] was followed with slight modifications. The slides were dried for 60 min at room temperature and then kept in a fixation solution comprising 15% trichloroacetic acid, 5% zinc sulfate, and 5% glycerol for 10 min at room temperature. After washing with diH_2O three times for 1 min, they were dried for 60 min at 37°C, rehydrated with diH_2O for 5 min, and then incubated with a staining solution comprising solution A (5% sodium carbonate) and solution B (0.2% silver nitrate, 0.2% ammonium nitrate, 0.5% tungstosilicic acid, 0.15% formaldehyde, and 5% sodium carbonate) for 20 min. After washing with diH_2O three times for 2 min each, they were kept in a stop solution of 1% acetic acid for 5 min to terminate the staining reaction, rinsed with diH_2O two times for 2 min each, and stored in light-protected boxes until analysis.

Each of two blind observers independently examined 100 randomly selected cells per slide under a light microscope, categorizing them into five classes ranging from 0 (no DNA damage) to 4 (maximal DNA damage), based on head size, tail length, and intensity. Based on averaged results of two independent classifications, DNA damage was expressed in arbitrary units (AU) and calculated using the following formula [34]:

$$Arbitrary\ Units\ (AU) = \sum N_i \times i$$

where N_i is the number of cells in a specific class, and i is the class number (0, 1, 2, 3, or 4).

Statistical analysis

The obtained experimental data were presented as the arithmetic mean±standard deviation (SD) of five onion bulbs. Statistical analyses were performed using Minitab 21 (Minitab Inc., State College, PA). Differences among the groups for each parameter measured per endpoint were statistically analyzed using one-way ANOVA, followed by Tukey's test. Pearson's correlation test was used to assess potential relationships between the parameter and both concentration and exposure time. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Determination of EC_{50}

In this study, the *A. cepa* root growth inhibition test was successfully performed, and different root growth rates after a 96-h exposure depending on the applied concentration were observed (Supplementary Fig. S1). As can be seen in Fig. 1, the mean root length was 4.63±0.65 cm in the tap water treatment (control) and ranged between 0.32±0.08 cm and 4.22±1.12 cm in the abamectin treatments. When the obtained root growth inhibition test results were statistically examined, different abamectin concentrations caused significant differences in root lengths ($F_{9, 490}$=276.28, P<0.001), with higher concentrations exerting a strong negative effect on root growth (r=-0.665, P<0.05). Based on the root length data in this study, the EC_{50} of abamectin was 2.50 mg/L (Fig. 1).

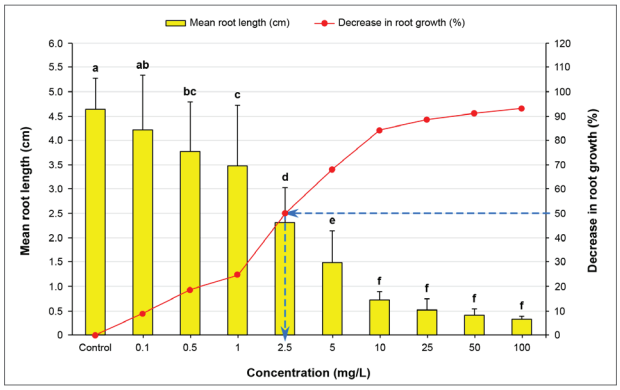


Fig. 1. Effects of different concentrations of abamectin ranging from 0.1 mg/L to 100 mg/L on *A. cepa* root length after a 96-h exposure. Root length values that do not share a superscript letter are statistically different (P<0.05). Note that the EC_{50} of abamectin is indicated by dashed blue line.

Cytogenetic assay

Based on the determined EC_{50} value, treatment experiments were carried out with the selected concentrations of abamectin (1.25, 2.50, and 5.00 mg/L) and negative and positive controls, and the MI values of the experimental groups at 24, 48, 72, and 96 h exposure times were calculated (Table 1). Analysis of the MI values for the experimental groups at identical exposure times revealed statistically significant differences among the groups at all exposure durations: $F_{4, 20}$=411.03, P<0.001 for 24 h; $F_{4, 20}$=221.83, P<0.001 for 48 h; $F_{4, 20}$=359.25, P<0.001 for 72 h; and $F_{4, 20}$=297.57, P<0.001 for 96 h. At each exposure time, the highest MI was observed in the control group and the lowest in the positive control group. The highest MI among the exposure groups was observed in the 1.25 mg/L group, and the lowest in the 5.00 mg/L group (Table 1). Examination of the MI values for the exposure times in identical experimental groups revealed that there were statistically significant differences among the exposure times in all groups ($F_{3, 16}$=114.42, P<0.001 for the 1.25 mg/L group; $F_{3, 16}$=101.97, P<0.001 for the 2.50 mg/L group; $F_{3, 16}$=22.89, P<0.001 for the 5.00 mg/L group; and $F_{3, 16}$=8.76, P<0.01 for the positive control group), except for the control group (P>0.05). In these four groups, the lowest MI was at 96-h and the highest at 24-h exposure time (Table 1). Abamectin caused inhibition in the MI in the exposure groups; however, the percentages of these reductions were lower than that in the positive control at all four exposure times. Moreover, correlation analyses revealed that the MI was negatively correlated with the concentration at all four exposure times (P<0.001) (Fig. 2A) and the exposure time at all four concentrations (P<0.001), except for 0.00 mg/L, where there was a positive correlation between the MI and the exposure time (P<0.05) (Fig. 2B), indicating a significant decrease in the MI with increasing concentrations and prolonged exposure time. The correlation of the MI with the concentration was strong at all four exposure times (r=-0.991 at 24 h, r=-0.948 at 48 h, r=-0.877 at 72 h, and r=-0.847 at 96-h exposure). The correlation of the MI with exposure time was moderate at 0.00 mg/L (r=0.468), and very strong at 1.25 mg/L (r=-0.937), 2.50 mg/L (r=-0.941), and 5.00 mg/L (r=-0.865).

The PI values of the experimental groups calculated for each mitotic phase at 24-, 48-, 72-, and 96-h exposure times are presented in Table 1. After the PI values for the experimental groups at identical exposure times were examined, there were no statistically significant differences among the groups for the prophase PI at any of the exposure times (P>0.05); there were statistically significant differences only for the metaphase PI at 24-h exposure ($F_{4, 20}$=4.40, P<0.05), the anaphase PI at 48-, 72-, and 96-h exposures ($F_{4, 20}$=5.63, P<0.01; $F_{4, 20}$=5.41, P<0.01; and $F_{4, 20}$=3.19, P<0.05; respectively), and the telophase PI at 24-h exposure ($F_{4, 20}$=5.31, P<0.01). Analysis of the PI values for the exposure times in identical experimental groups revealed that there were no statistically significant differences among the exposure times for the metaphase PI in any of the groups (P>0.05) but there were statistically significant differences among the exposure times for the prophase PI in the 1.25 mg/L group ($F_{3, 16}$=22.63, P<0.001), the anaphase PI in the 1.25 mg/L group ($F_{3, 16}$=7.90, P<0.01) and the positive control group ($F_{3, 16}$=3.83, P<0.05), and the telophase PI in the 1.25 mg/L group ($F_{3, 16}$=13.26, P<0.001) and the positive control group ($F_{3, 16}$=3.83, P<0.05). In addition, correlation analyses revealed that the anaphase PI at all exposure times and the telophase PI values at 24 h and 96 h were negatively correlated with the concentration (P<0.05) and that the metaphase PI values at 24 h and 72 h were positively correlated with the concentration (P<0.05). The analyses also showed that the PI for prophase at 1.25 mg/L and for metaphase at 2.50 mg/L were positively correlated with exposure time (P<0.05) and that the PI for anaphase at 2.50 mg/L and for telophase at 1.25

Table 1. Effects of exposure to three different concentrations of abamectin (1.25, 2.50, and 5.00 mg/L) on mitotic cell division in *A. cepa* root tips at different exposure times

Exposure time	Concentration	TNOC	TNDC	MI±SD (%)	% inhibition in MI	PI±SD (%)			
						Prophase	Metaphase	Anaphase	Telophase
24 h	Negative control	11953	2758	$23.04\pm0.93^{a,A}$	–	$33.30\pm3.73^{a,A}$	$26.06\pm3.33^{b,A}$	$22.80\pm3.42^{a,A}$	$17.84\pm3.31^{a,A}$
	1.25 mg/L	11942	2345	$19.59\pm0.71^{b,A}$	14.97	$36.71\pm4.19^{a,A}$	$25.10\pm3.53^{b,A}$	$21.39\pm1.35^{a,B}$	$16.79\pm3.18^{a,B}$
	2.50 mg/L	12289	2020	$16.44\pm0.63^{c,A}$	28.63	$36.17\pm2.69^{a,A}$	$28.54\pm1.35^{ab,A}$	$19.61\pm1.56^{a,A}$	$15.67\pm2.37^{a,A}$
	5.00 mg/L	12081	948	$7.82\pm0.64^{d,A}$	66.07	$44.12\pm21.01^{a,A}$	$36.39\pm7.70^{a,A}$	$12.09\pm12.67^{a,A}$	$7.40\pm6.28^{b,A}$
	Positive control	11950	708	$5.95\pm1.09^{e,A}$	74.18	$40.54\pm9.28^{a,A}$	$33.62\pm7.16^{ab,A}$	$14.72\pm3.47^{a,A}$	$11.12\pm4.93^{ab,A}$
48 h	Negative control	11910	2698	$22.63\pm1.11^{a,A}$	–	$36.89\pm3.41^{a,A}$	$26.80\pm1.78^{a,A}$	$21.09\pm1.32^{a,A}$	$15.22\pm2.43^{a,A}$
	1.25 mg/L	12054	2217	$18.43\pm1.06^{b,A}$	18.56	$27.47\pm0.98^{a,B}$	$25.87\pm1.33^{a,A}$	$25.36\pm0.70^{a,A}$	$21.31\pm1.27^{a,A}$
	2.50 mg/L	11931	1238	$10.42\pm0.87^{c,B}$	53.96	$41.04\pm11.55^{a,A}$	$31.61\pm5.67^{a,A}$	$18.15\pm2.64^{ab,A}$	$9.20\pm7.00^{a,A}$
	5.00 mg/L	12284	834	$6.74\pm0.96^{d,AB}$	70.23	$43.96\pm28.68^{a,A}$	$35.32\pm14.69^{a,A}$	$9.92\pm11.41^{b,A}$	$10.80\pm11.10^{a,A}$
	Positive control	12102	720	$5.99\pm1.45^{d,A}$	73.55	$40.26\pm9.49^{a,A}$	$33.18\pm6.83^{a,A}$	$15.08\pm3.63^{ab,A}$	$11.48\pm5.01^{a,A}$
72 h	Negative control	12042	2858	$23.71\pm0.87^{a,A}$	–	$34.22\pm3.09^{a,A}$	$25.60\pm3.45^{a,A}$	$22.49\pm3.00^{a,A}$	$17.70\pm2.97^{a,A}$
	1.25 mg/L	12188	1475	$12.08\pm0.56^{b,B}$	49.05	$38.93\pm1.51^{a,A}$	$25.99\pm3.66^{a,A}$	$20.88\pm1.59^{ab,B}$	$14.20\pm2.26^{a,BC}$
	2.50 mg/L	12114	1149	$9.51\pm0.65^{c,B}$	59.89	$36.08\pm12.34^{a,A}$	$30.91\pm2.74^{a,A}$	$16.18\pm2.25^{abc,A}$	$16.82\pm10.38^{a,A}$
	5.00 mg/L	12197	737	$6.02\pm1.02^{d,B}$	74.62	$33.12\pm24.56^{a,A}$	$42.17\pm19.00^{a,A}$	$11.22\pm10.08^{bc,A}$	$13.49\pm18.63^{a,A}$
	Positive control	12354	571	$4.66\pm1.21^{d,AB}$	80.36	$50.23\pm11.85^{a,A}$	$34.69\pm15.19^{a,A}$	$8.81\pm6.62^{c,AB}$	$6.27\pm4.33^{a,AB}$
96 h	Negative control	12124	2940	$24.23\pm1.18^{a,A}$	–	$37.08\pm4.33^{a,A}$	$27.97\pm6.76^{a,A}$	$20.28\pm6.27^{a,A}$	$14.66\pm5.04^{a,A}$
	1.25 mg/L	12147	1281	$10.46\pm1.29^{b,B}$	56.81	$41.74\pm3.59^{a,A}$	$27.04\pm3.18^{a,A}$	$19.00\pm3.64^{a,B}$	$12.22\pm2.53^{a,C}$
	2.50 mg/L	12406	655	$5.26\pm1.61^{c,C}$	78.31	$41.85\pm16.82^{a,A}$	$34.58\pm4.73^{a,A}$	$14.39\pm5.88^{ab,A}$	$9.17\pm13.14^{a,A}$
	5.00 mg/L	12381	420	$3.44\pm0.81^{cd,C}$	85.80	$50.52\pm26.56^{a,A}$	$36.78\pm20.60^{a,A}$	$7.60\pm14.53^{b,A}$	$5.09\pm5.02^{a,A}$
	Positive control	12430	349	$2.82\pm0.56^{d,B}$	88.35	$60.84\pm22.39^{a,A}$	$30.08\pm15.63^{a,A}$	$5.59\pm6.57^{b,B}$	$3.50\pm3.21^{a,B}$

Data are presented as the mean±SD of five replicates (onions). Index values for concentrations at each exposure time with different lowercase superscripts in the same column are statistically different from each other ($P<0.05$). Index values for exposure times at each concentration with different uppercase superscripts in the same column are statistically different from each other ($P<0.05$). MI – mitotic index; PI – phase index; SD – standard deviation; TNDC – total number of the dividing cells; TNOC – total number of observed cells.

mg/L were negatively correlated with exposure time ($P<0.05$ and $P<0.01$, respectively). The general pattern showed a slight increase in PI values for prophase and metaphase, while PI values for anaphase and telophase decreased with increasing abamectin concentration at all exposure times.

The types of CAs observed in the mitotic phases of root tip cells through microscopic examinations included polyploidy, C-mitosis, chromosome bridges, vagrant chromosomes, laggard chromosomes, ring chromosomes, and chromosome stickiness (Supplementary Fig. S2). The frequencies of CAs caused by the treatments in the study are presented in Table 2. Among the exposure groups, the most common CA was C-mitosis in the 5.00 mg/L group at 96 h (13.14±3.97%), whereas the least common CA was laggard chromosomes in the 1.25 mg/L group at 24 h (0.17±0.24%). Examination of the CA frequencies of the experimental groups at identical exposure times revealed statistically significant differences among the groups for most of the CA types at several exposure times ($P<0.05$) (Table 2). Significant differences in the total frequency of CAs were observed among the groups at all four exposure times ($F_{4, 20}=24.63$, $P<0.001$ for a 24-h exposure; $F_{4, 20}=46.79$, $P<0.001$ at 48 h; $F_{4, 20}=65.35$, $P<0.001$ at 72 h; and $F_{4, 20}=69.95$, $P<0.001$ at 96 h). At each exposure time, the lowest frequency of the total CAs was in the control group, and the highest was in the positive control group; the lowest frequency among the exposure groups was observed in the 1.25 mg/L group, and the highest in the 5.00 mg/L group (Table 2). Analysis of CA frequencies for the exposure times in identical experimental groups revealed statistically significant differences among the

Table 2. Effects of exposure to three different concentrations of abamectin (1.25, 2.50, and 5.00 mg/L) on the CAs in *A. cepa* root tips at different exposure times

Exposure time	Concentration	TNOC	TNDC	Frequency of CA (%)							
				PP	CM	CB	VC	LC	RC	ST	Total
24 h	Negative control	6053	1020	0.71±0.85^{b,A}	0.48±0.69^{b,A}	0.27±0.43^{c,A}	0.93±0.48^{b,A}	0.35±0.32^{bc,A}	0.15±0.33^{a,A}	1.02±0.61^{b,A}	3.91±1.64^{c,A}
	1.25 mg/L	5576	1023	1.67±0.83^{ab,A}	1.94±1.27^{ab,A}	0.80±0.90^{bc,A}	1.70±1.70^{b,A}	0.17±0.24^{c,B}	0.40±0.46^{a,A}	3.54±1.79^{ab,A}	10.23±2.24^{c,A}
	2.50 mg/L	5599	390	2.39±2.26^{ab,A}	2.55±1.44^{ab,B}	1.97±1.60^{abc,A}	5.87±1.18^{ab,A}	2.10±1.88^{abc,A}	1.57±1.10^{a,A}	4.63±1.79^{ab,A}	21.07±3.98^{b,A}
	5.00 mg/L	5082	270	2.98±2.62^{ab,A}	2.97±3.07^{ab,B}	3.01±0.95^{ab,A}	8.45±4.84^{a,A}	2.63±0.97^{ab,A}	1.93±0.47^{a,A}	6.15±3.18^{a,A}	28.12±8.67^{ab,A}
	Positive control	5487	278	4.84±2.69^{a,A}	5.48±2.39^{a,B}	3.87±2.05^{a,A}	7.63±3.50^{a,A}	3.57±1.72^{a,A}	1.88±2.64^{a,A}	5.66±2.32^{a,A}	32.94±7.07^{a,B}
48 h	Negative control	6236	1103	0.63±0.90^{a,A}	0.71±0.82^{c,A}	0.14±0.30^{c,A}	0.71±0.45^{b,A}	0.61±0.51^{b,A}	0.00±0.00^{a,A}	0.48±0.29^{b,A}	3.28±1.53^{d,A}
	1.25 mg/L	6203	1104	1.58±0.86^{a,A}	2.24±1.49^{bc,A}	0.74±0.82^{bc,A}	2.35±1.26^{b,A}	0.74±0.39^{b,A}	0.75±0.44^{a,A}	2.97±1.71^{ab,A}	11.38±3.38^{c,A}
	2.50 mg/L	6012	438	2.11±2.05^{a,A}	4.43±0.93^{ab,AB}	0.62±0.89^{bc,A}	5.24±1.03^{a,A}	3.73±2.37^{ab,A}	0.94±0.56^{a,A}	4.17±1.72^{a,AB}	21.24±2.57^{b,A}
	5.00 mg/L	6553	307	2.65±2.43^{a,A}	6.46±2.43^{a,B}	2.60±0.81^{ab,AB}	6.31±1.28^{a,A}	5.09±2.79^{a,A}	2.24±0.61^{a,A}	5.32±2.54^{a,A}	30.68±6.53^{a,A}
	Positive control	6459	317	4.06±1.89^{a,A}	7.47±2.32^{a,B}	3.41±1.86^{a,A}	6.53±2.52^{a,A}	3.06±1.27^{ab,A}	1.62±2.46^{a,A}	5.13±2.53^{a,A}	31.29±4.07^{a,B}
72 h	Negative control	6103	1080	0.26±0.36^{a,A}	0.40±0.40^{d,A}	0.51±0.33^{b,A}	0.87±0.76^{c,A}	0.87±0.76^{b,A}	0.36±0.52^{a,A}	0.26±0.36^{c,A}	3.51±2.28^{d,A}
	1.25 mg/L	5988	994	1.87±1.18^{a,A}	2.71±1.98^{cd,A}	0.81±0.86^{b,A}	2.21±1.54^{bc,A}	0.85±0.43^{b,A}	0.88±0.60^{a,A}	2.32±2.39^{abc,A}	11.66±4.87^{c,A}
	2.50 mg/L	5864	387	2.25±2.05^{a,A}	5.37±0.84^{bc,A}	0.71±1.01^{b,A}	5.03±1.78^{b,A}	5.58±2.88^{a,A}	1.02±0.58^{a,A}	1.52±1.40^{bc,B}	21.48±2.94^{b,A}
	5.00 mg/L	6262	329	2.48±2.20^{a,A}	8.48±2.75^{ab,AB}	1.35±1.29^{b,B}	8.64±1.24^{a,A}	3.69±2.85^{ab,A}	2.08±0.48^{a,A}	4.93±2.26^{ab,A}	31.66±5.36^{a,A}
	Positive control	6141	286	3.80±2.00^{a,A}	9.48±2.81^{a,B}	3.76±2.05^{a,A}	8.81±2.70^{a,A}	3.44±1.48^{ab,A}	1.69±2.59^{a,A}	5.65±2.75^{a,A}	36.63±2.36^{a,AB}
96 h	Negative control	5851	984	0.16±0.36^{b,A}	0.25±0.37^{c,A}	0.25±0.23^{b,A}	0.96±0.85^{c,A}	0.33±0.73^{b,A}	0.00±0.00^{b,A}	0.85±0.59^{c,A}	2.80±2.54^{d,A}
	1.25 mg/L	6129	1016	1.83±1.19^{ab,A}	3.08±1.76^{bc,A}	1.45±0.99^{ab,A}	2.16±1.53^{c,A}	0.82±0.41^{b,A}	0.85±0.55^{ab,A}	2.24±2.23^{abc,A}	12.44±4.97^{c,A}
	2.50 mg/L	6206	359	2.42±2.22^{ab,A}	6.26±1.56^{b,A}	0.75±1.05^{ab,A}	5.36±1.55^{b,A}	6.18±3.46^{a,A}	1.11±0.66^{ab,A}	1.69±1.60^{bc,AB}	23.77±3.65^{b,A}
	5.00 mg/L	6454	294	2.81±2.63^{ab,A}	13.14±3.97^{a,A}	1.24±1.20^{ab,A}	9.61±1.44^{a,A}	2.99±3.00^{ab,A}	2.34±0.67^{a,A}	5.57±2.62^{a,A}	37.69±6.24^{a,A}
	Positive control	6562	313	4.21±2.24^{a,A}	14.27±2.95^{a,A}	2.58±1.40^{a,A}	9.90±1.72^{a,A}	3.13±1.34^{ab,A}	2.19±2.41^{ab,A}	5.08±2.35^{ab,A}	41.37±3.51^{a,A}

Data are presented as the mean±SD of five replicates (onions). Frequency values for concentrations at each exposure time with different lowercase superscripts in the same column are statistically different (P<0.05). Frequency values for exposure times at each concentration with different uppercase superscripts in the same column are statistically different (P<0.05). CAs – chromosomal aberrations; CB – chromosome bridge; CM – C-mitosis; LC – laggard chromosome; PP – polyploidy; RC – ring chromosome; SD – standard deviation; ST – stickiness; TNDC – total number of the dividing cells; TNOC – total number of the observed cells; VC – vagrant chromosome.

exposure times for laggard chromosomes in the 1.25 mg/L group ($F_{3,16}$=3.35, P<0.05), for C-mitosis and stickiness in the 2.50 mg/L group ($F_{3,16}$=8.27, P<0.01 and $F_{3,16}$=4.94, P<0.05, respectively), for C-mitosis and chromosome bridges in the 5.00 mg/L group ($F_{3,16}$=9.34, P<0.01 and $F_{3,16}$=3.37, P<0.05, respectively), and for C-mitosis in the positive control group ($F_{3,16}$=10.29, P<0.01) (Table 2). As for the frequency of the total CAs, it was determined that there were no significant differences in the total CAs among the exposure times in any of the groups (P>0.05) except for the positive control group, where the frequencies of the total CAs for the exposure times significantly differed from each other ($F_{3,16}$=4.74, P<0.05). Moreover, correlation analyses revealed that the total CA frequency was positively correlated with the concentration at all four exposure times (P<0.001) (Fig. 2C), indicating a significant increase in the total CAs with ascending concentrations, and with the exposure time at 5.00 mg/L (P<0.05) (Fig. 2D), indicating a significant increase in the total CAs with prolonged exposure time at this concentration. The correlation of the total CA frequency with concentration was strong at all four exposure times (r=0.880 at 24 h, r=0.932 at 48 h, r=0.934 at 72 h, and r=0.948 at 96 h). However, the correlation of the total CA frequency with exposure time was moderate at 5.00 mg/L (r=0.472).

Comet assay

Five damage classes were observed in the slides of *A. cepa* root tip meristematic cells based on head size and tail length and intensity (Supplementary Fig. S3). The comet

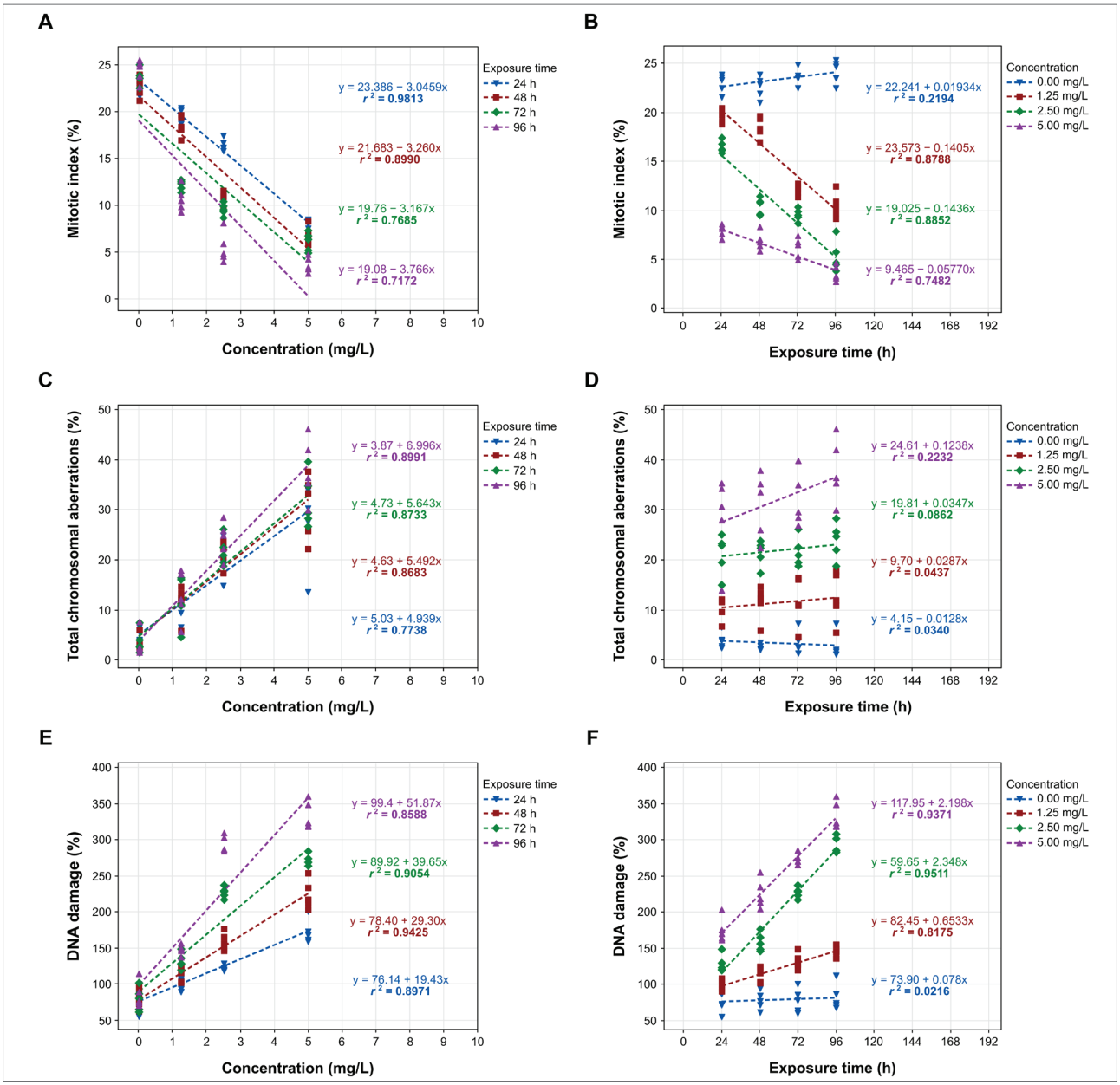


Fig. 2. Correlation graphs demonstrating concentration-response and time-response relationships. Correlation of the MI with the concentration (**A**) and the exposure time (**B**). Correlation of the total CA frequency with the concentration (**C**) and the exposure time (**D**). Correlation of the DNA damage with the concentration (**E**) and the exposure time (**F**).

assay results are given in Table 3. Increased DNA damage levels between 28.93% and 333.33% in the positive control and exposure groups compared to the negative control group were determined at four exposure times. After the DNA damage levels of the experimental groups at identical exposure times were examined, it was found that there were statistically significant differences among the groups at all four exposure times ($F_{4, 20}$=217.89, P<0.001 for a 24 h; $F_{4, 20}$=216.89, P<0.001 at 48 h; $F_{4, 20}$=414.01, P<0.001 at 72 h; and $F_{4, 20}$=369.94, P<0.001 at 96 h). At each exposure time, the lowest DNA damage level was determined in the negative control group and the highest in the positive control group. The lowest DNA damage level in the exposure groups was in the 1.25 mg/L group and the highest in the 5.00 mg/L group (Table 3). After the DNA damage levels for the exposure times in identical experimental groups were analyzed, it was determined that there were

Table 3. Effects of exposure to three different concentrations of abamectin (1.25, 2.50, and 5.00 mg/L) on the DNA damage in *A. cepa* root tips at different exposure times

Exposure time	DNA damage (AU)				
	Negative control	1.25 mg/L	2.50 mg/L	5.00 mg/L	Positive control
24 h	76.40±15.44^{d,A}	98.50±7.41^{d,B}	127.10±12.29^{c,D}	172.60±16.84^{b,D}	314.40±17.31^{a,B}
48 h	77.50±12.41^{e,A}	112.60±10.81^{d,B}	157.10±12.70^{c,C}	222.80±20.32^{b,C}	320.60±15.03^{a,B}
72 h	78.00±16.55^{e,A}	130.80±10.92^{d,A}	225.90±7.58^{c,B}	271.90±7.40^{b,B}	333.60±12.02^{a,AB}
96 h	82.50±18.18^{d,A}	144.70±6.91^{c,A}	292.00±11.66^{b,A}	332.10±19.38^{a,A}	357.50±10.23^{a,A}

Data are presented as the mean±SD of five replicates (onions). DNA damage values with different lowercase superscripts in the same row are statistically different (P<0.05). DNA damage values with different uppercase superscripts in the same column are statistically different (P<0.05).

statistically significant differences among the exposure times in all the groups ($F_{3, 16}$=24.28, P<0.001 for the 1.25 mg/L group; $F_{3, 16}$=214.81, P<0.001 for the 2.50 mg/L group; $F_{3, 16}$=82.53, P<0.001 for the 5.00 mg/L group; and $F_{3, 16}$=9.39, P<0.01 for the positive control group) except for the negative control group (P>0.05). In these four groups, the highest DNA damage level was at the 96-h exposure time and the lowest at the 24-h exposure time (Table 3). Additionally, correlation analyses showed that the DNA damage level was positively correlated with concentration at all four exposure times (P<0.001) (Fig. 2E) and with exposure time at all concentrations (P < 0.001) (Fig. 2F), indicating a statistically significant increase in DNA damage with higher concentrations and longer exposure durations. The correlation of DNA damage with concentration was strong at all four exposure times (r=0.947 at 24-h exposure, r=0.971 at 48 h, r=0.952 at 72 h, and r=0.927 at 96 h). The correlation of DNA damage with exposure time was strong at 1.25 mg/L (r=0.904), 2.50 mg/L (r=0.975), and 5.00 mg/L (r=0.968).

DISCUSSION

Abamectin is one of the most extensively utilized pesticides worldwide in agriculture but also pharmaceutic and veterinary medicine. It is known to persist in water, soil, sediment, and crops, posing a potential risk to non-target organisms. Therefore, it is important to investigate all aspects of its toxic effects on non-target organisms for reliable and robust environmental protection and management.

The MI, a biomarker of cell proliferation [35,36], is a parameter for assessing anthropogenic pollutants/toxicants [22,37-39]. Consistent with the results of previous studies regarding the effect of abamectin on the MI [12,38], a significant decrease in MI, dependent on both concentration and exposure time, was observed in the present study as a result of abamectin exposure, indicating that abamectin has a cytotoxic effect on *A. cepa* root tip meristematic cells. Based on the obtained MI results, the most cytotoxic abamectin concentration was 5.00 mg/L, followed by 2.50 and 1.25 mg/L. In previous studies, abamectin was reported to be a cytotoxic pesticide [12,38,40], in agreement with the detection of its cytotoxic effect in this study. As it decreases MI, abamectin may have a mitochondrial depressive effect. This implies a negative effect on the cell cycle in *A. cepa* root tip meristem, preventing cells from entering prophase and stopping the cycle in interphase. The proportion of dividing cells decreased while that of non-dividing cells in interphase increased with increasing abamectin concentrations at all exposure times. The blockage of the cell cycle stages (G1, S, and/or G2) prevents cell proliferation [41] and inhibits root growth. A 50% inhibition of MI compared with the control group is considered to be a toxicity limit value, with the evaluation of toxicity of a given chemical based on this value obtained in toxicity studies. Thus, decreases below 50% (i.e. inhibition above 50%) relative to the control group have a sublethal effect on the test organism, while decreases below 22% (i.e. inhibition above 78%) suggest lethal effects [7,42,43]. In the present study, inhibition of the MI ranging from 14.97% to 85.80% after exposure to abamectin suggested that the toxic limit of abamectin in *A. cepa* after exposure for 96 h is a concentration below 1.25 mg/L. Based on the inhibition rates, it can be concluded that the abamectin concentration of 5.00 mg/L at 24-h exposure induces sublethal effects on *A. cepa*, concentrations of 2.50 and 5.00 mg/L at 96-h exposure have a lethal effect and a 1.25 mg/L concentration at 96-h exposure is sublethal. The concentrations of 2.50 and 5.00 mg/L

at 48 h and 72 h induce sublethal effects in *A. cepa*. These toxicity evaluations emphasize the necessity of a detailed examination of the impacts of abamectin on non-target organisms for the maintenance of environmental health.

The PI is a parameter used for assessing cell division inhibition [43,44]. The high PI value in the cell division phase suggests that cells in this phase take longer to transition to the next phase and complete division [44,45]. After analyzing the PI values for different mitotic phases, statistically significant changes were detected for several cases. In general, the PI values for prophase and metaphase slightly increased and the PI values for anaphase and telophase decreased with increasing abamectin concentrations. These changes in PI values may be due to the cytotoxic effect of abamectin, which aligns with the observed decreases in MI values. The effect of abamectin on PI can be ascribed to obstacles at the beginning of cell division or to prolonged maintenance of prophase and metaphase due to mitotic stress in cells caused by abamectin.

Given that the toxic effects of various chemicals, including pesticides, may not be immediately evident, assessing their genotoxicity is essential in ecotoxicology studies [42]. Genotoxicity is a crucial toxicological endpoint for assessing the safety of chemicals [46]. One widely used method for genotoxicity testing involves estimating CAs in dividing cells, a reliable parameter for assessing pesticide-induced genotoxicity [22,36,40,43].

In this study, abamectin exposure led to the formation of multiple CA types, such as polyploidy, C-mitosis, chromosome bridges, vagrant chromosomes, laggard chromosomes, ring chromosomes, and stickiness, and significantly increased CA frequency compared to the control. A notable concentration-dependent rise in overall CA frequency induced by abamectin was observed. This increase in CA frequency, alongside a decrease in mitotic index (MI), aligns with findings from studies on *A. cepa* and *V. faba* exposed to other herbicides [47]. Previous research also reports that several pesticides are highly genotoxic, leading to increased CA frequencies [10,12,43]. Thus, our findings suggest that abamectin exerts genotoxic effects on *A. cepa* root tip meristematic cells, inducing CAs, which is consistent with studies identifying abamectin as highly genotoxic [12,38,40].

Furthermore, CA data in this study revealed that abamectin exposure significantly increased the frequencies of C-mitosis, vagrant chromosomes, laggard chromosomes, and stickiness compared to other CA types. These findings suggest that abamectin's CA-inducing genotoxicity is likely linked to mitotic spindle impairment [48]. The observed CA types indicate that abamectin acts as both a clastogenic and aneugenic agent in *A. cepa* root tip meristematic cells [22].

The comet assay, or single-cell gel electrophoresis, allows researchers to detect DNA damage, specifically strand breaks, at the cellular level without relying on cell division [49]. DNA damage often results from increased free radical and reactive oxygen species (ROS) activity following exposure to various chemicals. While organisms can repair DNA damage, the effectiveness of this repair depends on the concentration and duration of exposure, the characteristics of the DNA-damaging chemical, and the species involved [50].

In this study, a significant concentration- and time-dependent increase in DNA damage was observed following abamectin treatment, suggesting a DNA-damaging genotoxic effect on *A. cepa* root tip meristematic cells. This finding aligns with the observed increase in total CA frequency mentioned earlier. The DNA-damaging effects of abamectin observed here are consistent with previous studies that used *A. cepa* as a model organism to assess the genotoxicity of other pesticides, such as imazethapyr [51], flubendiamide [39], and fenaminosulf [52].

Pesticides are known to induce ROS production, leading to oxidative stress and DNA strand breaks [13,50]. Although biochemical changes in root tip meristematic cells in response to abamectin exposure were not assessed in this study, it is plausible that abamectin induced ROS production, resulting in oxidative stress within *A. cepa* root tip cells. Thus, the DNA-damaging genotoxic effects observed here may stem from the oxidative imbalance caused by abamectin in *A. cepa* root tip cells.

The literature is extensive regarding the toxicity of abamectin across various organisms and cell types; however, to our knowledge, only two studies have specifically investigated its toxic effects on *A. cepa* [12,38]. In the present study, we examined the cyto-genotoxicity of abamectin, using the commercial

formulation Alopec® EC, by exposing *A. cepa* root meristematic cells to three different concentrations and assessing effects through cytogenetic and comet assays. While this study shares some aspects with previous research, such as using *A. cepa* as the test organism and conducting root growth and cytogenetic analyses, it distinguishes itself by introducing a new commercial pesticide, selecting a high concentration range that includes the recommended application level, sampling root tips at 24-hour intervals to represent four different exposure times, and employing the alkaline comet assay.

Examining the toxic effects of different commercial pesticide formulations on non-target organisms is crucial, as commercial products often exhibit higher toxicity than the active ingredients alone, likely due to additional excipients [23,53]. Moreover, by including the recommended application concentration in the concentration range, this study offers insights into potential environmental effects of abamectin exposure at realistic levels. Sampling root tips across four different exposure times also allowed for the assessment of temporal variations in toxicity parameters, an important consideration given the persistence of pesticides like abamectin in the environment and their cumulative impact on non-target organisms.

The use of the alkaline comet assay provided a quantitative measure of DNA damage in individual cells, extending beyond cytogenetic analysis of CAs alone and offering a more comprehensive view of abamectin's genotoxicity. Collectively, the findings of this study contribute to the existing literature on abamectin toxicity and offer a foundation for future research. These insights also provide valuable guidance for researchers and policymakers in addressing global environmental challenges, particularly as pesticide use continues to rise.

CONCLUSIONS

Given the ongoing environmental and public health challenges worldwide, investigating the toxic effects of pesticides is essential for guiding environmental management and conservation strategies. In this study, we evaluated the toxic effects of abamectin, the active ingredient in the commercial pesticide formulation Alopec® EC, on *A. cepa* root tips and analyzed toxicity parameter changes based on concentration and exposure duration. The root growth assay demonstrated a significant concentration-dependent reduction in root growth following abamectin exposure, with an EC_{50} value of 2.50 mg/L. Cytogenetic and comet analyses revealed that abamectin exposure caused a concentration- and time-dependent decrease in the MI, a significant increase in DNA damage, and a concentration-dependent rise in total CA frequency.

These results indicate that abamectin exerts lethal effects on *A. cepa* root tip meristematic cells, even at lower concentrations with prolonged exposure, alongside its ability to induce CAs and DNA damage, highlighting its significant cytotoxicity and genotoxicity. To safeguard public health and ecosystems, careful regulation of abamectin concentrations, particularly in agroecosystems, is essential. Further research is recommended to fully elucidate the mechanisms underlying abamectin's toxic effects in *A. cepa* root tip meristematic cells and other organisms.

Funding: The authors received no specific funding for this work.

Author contributions: Conceptualization, PI and FS; methodology, PI and FS; experiments; PI and FS; investigation, PI and FS; writing – original draft preparation, FS; writing – review and editing, PI. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: Data underlying the reported findings have been provided as a raw dataset available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Ili%20and%20Sari_Dataset.pdf

REFERENCES

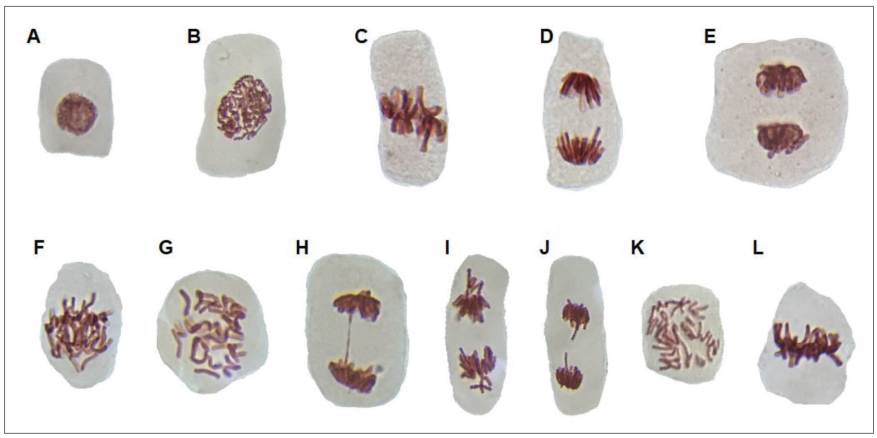
1. Carvalho FP. Pesticides, environment, and food safety. Food Energy Secur. 2017;6(2):48–60. https://doi.org/10.1002/fes3.108
2. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD, Dar OI, Singh K, Jasrotia S, Bakshi P, Ramakrishnan M, Kumar S, Bhardwaj R, Thukral AK. Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci. 2019;1(11):1–16. https://doi.org/10.1007/s42452-019-1485-1
3. Sari F. Lethal and sublethal effects of the pyrethroid insecticide tau-fluvalinate on the non-target organism Gammarus roeseli: A study of acute toxicity, genotoxicity and locomotor activity. Arch Biol Sci. 2022;74(4):347–58. https://doi.org/10.1007/s42452-019-1485-1

4. Pimentel D, Levitan L. Pesticides: Amounts applied and amounts reaching pests. Bioscience. 1986;36(2):86–91. https://doi.org/10.2307/1310108
5. Tudi M, Ruan HD, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT. Agriculture development, pesticide application and its impact on the environment. Int J Environ Res Public Health. 2021;18(3):1112. https://doi.org/10.3390/ijerph18031112
6. Akashe MM, Pawade UV, Nikam AV. Classification of pesticides: A review. Int J Res Ayurveda Pharm. 2018;9(4):144–50. https://doi.org/10.7897/2277-4343.094131
7. İli P. Tau-fluvalinat içerikli bir insektisitin sitotoksik ve genotoksik etkilerinin Allium testi kullanılarak incelenmesi. Iğdır Üniv Fen Bil Enst Der. 2024;14(1):75–86. https://doi.org/10.21597/jist.1326695
8. Bai SH, Ogbourne SM. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. Chemosphere. 2016;154:204–14. https://doi.org/10.1016/j.chemosphere.2016.03.113
9. Salman M, Abbas RZ, Mehmood K, Hussain R, Shah S, Faheem M, Zaheer T, Abbas A, Morales B, Aneva I, Martínez JL. Assessment of avermectin-induced toxicity in animals. Pharmaceuticals. 2022;15(3):332. https://doi.org/10.3390/ph15030332
10. Sheikh N, Patowary H, Laskar RA. Screening of cytotoxic and genotoxic potency of two pesticides (malathion and cypermethrin) on *Allium cepa* L. Mol Cell Toxicol. 2020;16(3):291–9. https://doi.org/10.1007/s13273-020-00077-7
11. Liang Y, Dong B, Pang N, Hu J. ROS generation and DNA damage contribute to abamectin-induced cytotoxicity in mouse macrophage cells. Chemosphere. 2019;234:328–37. https://doi.org/10.1007/s13273-020-00077-7
12. Kalefetoğlu Macar T. Investigation of cytotoxicity and genotoxicity of abamectin pesticide in *Allium cepa* L. Environ Sci Pollut Res. 2021;28(2):2391–9. https://doi.org/10.1007/s11356-020-10708-0
13. Camilo-Cotrim CF, Bailão EFLC, Ondei LS, Carneiro FM, Almeida LM. What can the *Allium cepa* test say about pesticide safety? A review. Environ Sci Pollut Res. 2022;29(32):48088–104. https://doi.org/10.1007/s11356-020-10708-0
14. Dhananjayan V, Jayanthi P, Ravichandran B, Jayakumar R. Biomonitoring and biomarkers of pesticide exposure and human health risk assessment. In: Singh P, Singh S, Sillanpää M, editors. Pesticides in the Natural Environment. Elsevier; 2022. p. 563–84. https://doi.org/10.1016/B978-0-323-90489-6.00021-5
15. Azizullah A, Häder DP. A comparison of commonly used and commercially available bioassays for aquatic ecosystems. In: Häder D-P, Erzinger GS, editors. Bioassays: Advanced Methods and Applications. Amsterdam: Elsevier; 2018. p. 347–68. https://doi.org/10.1016/B978-0-12-811861-0.00017-6
16. Sari A, Sari F. A comparative examination of acute toxicities of three disazo dyes to freshwater macroinvertebrates Gammarus roeseli (Crustacea: Amphipoda) and Chironomus riparius (Insecta: Diptera). Chem Ecol. 2021;37(8):683–703. https://doi.org/10.1080/02757540.2021.1974008
17. Ozdemir A, Duran M, Akyildiz GK, Sen A. EROD and metallothionein in Limnodrilus profundicola (Oligochaeta: Tubificidae) as an indicator of pollution exposure in the Curuksu stream of Menderes river, Denizli–Turkey. Desalination Water Treat. 2011;26(1–3):98–103. https://doi.org/10.5004/dwt.2011.2116
18. Sari A. Assessment of pollution variability across the central part of the Büyük Menderes River (Turkey) using water physicochemical parameters and biomarker responses in the non-biting midge Chironomus riparius (Diptera: Chironomidae). Chem Ecol. 2023;39(1):59–77. https://doi.org/10.1080/02757540.2022.2147515
19. Vasconcelos AM, Daam MA, dos Santos LRA, Sanches ALM, Araújo CVM, Espíndola ELG. Acute and chronic sensitivity, avoidance behavior and sensitive life stages of bullfrog tadpoles exposed to the biopesticide abamectin. Ecotoxicology. 2016;25(3):500–9. https://doi.org/10.1007/s10646-015-1608-4
20. Wang Y, Zhu YC, Li W. Interaction patterns and combined toxic effects of acetamiprid in combination with seven pesticides on honey bee (Apis mellifera L.). Ecotoxicol Environ Saf. 2020;190:110100. https://doi.org/10.1016/j.ecoenv.2019.110100
21. Fiskesjö G. The Allium test as a standard in environmental monitoring. Hereditas. 1985;102(1):99–112. https://doi.org/10.1111/j.1601-5223.1985.tb00471.x
22. Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: A review on its application. Mutat Res Rev Mutat Res. 2009;682(1):71–81. https://doi.org/10.1016/j.mrrev.2009.06.002
23. Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV., Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia. 2018;71(3):191–209. https://doi.org/10.1080/00087114.2018.1503496
24. Fiskesjö G. The Allium test — an alternative in environmental studies: the relative toxicity of metal ions. Mutat Res Fundam Mol Mech Mutagen. 1988;197(2):243–60. https://doi.org/10.1016/0027-5107(88)90096-6
25. Cabuga CC, Joy J, Abelada Z, Rose R, Apostado Q, Joy B, Hernando H, Erick J, Lador C, Lloyd O, Obenza P, James C, Presilda R, Havana HC. *Allium cepa* test: An evaluation of genotoxicity. Proc Int Acad Ecol Environ Sci. 2017;7(1):12–9.
26. Constantin MJ, Owens ET. Introduction and perspectives of plant genetic and cytogenetic assays a report of the U.S. environmental protection agency Gene-Tox program. Mutat Res Rev Genet Toxicol. 1982;99(1):1–12. https://doi.org/10.1016/0165-1110(82)90027-6
27. Kihlman BA. Root tips for studying the effects of chemicals on chromosomes. In: Hollaender A, editor. Chemical Mutagens. Boston, MA: Springer; 1971. p. 489–514. https://doi.org/10.1007/978-1-4615-8969-3_8
28. Sharma CBSR. Plant meristems as monitors of genetic toxicity of environmental chemicals. Curr Sci. 1983;52(21):1000–2.
29. Rashid F, Singh D, Attri S, Kaur P, Kaur H, Mohana P, Quadar J, Vig AP, Bhatia A, Singh B, Walia H, Arora S. Modulation of atrazine-induced chromosomal aberrations and cyclin-dependent kinases by aqueous extract of Roylea cinerea (D.Don) Baillon leaves in *Allium cepa*. Sci Rep. 2022;12(1):12570. https://doi.org/10.1038/s41598-022-16813-z
30. Liman R, Ali MM, Ciğerci İH, İstifli ES, Sarıkurkcu C. Cytotoxic and genotoxic evaluation of copper oxychloride through

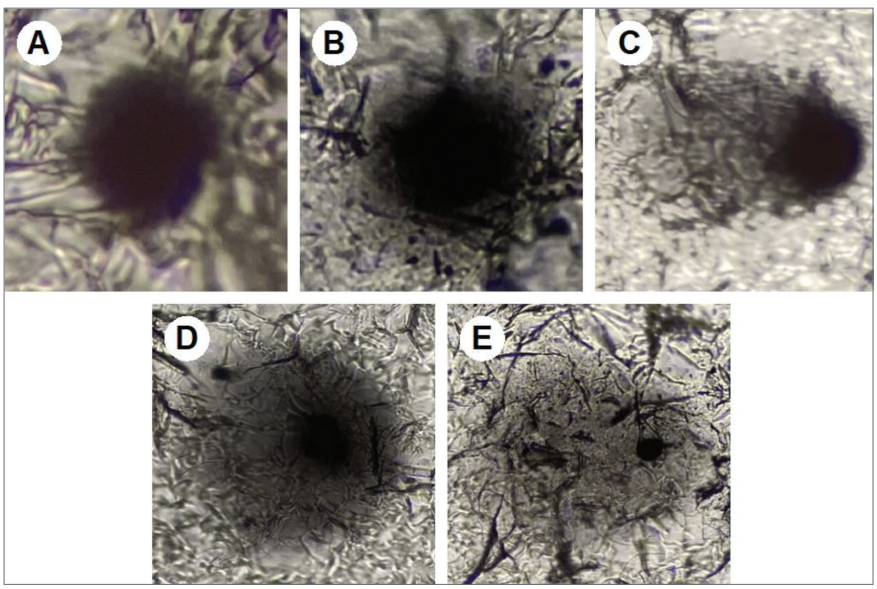
Allium test and molecular docking studies. Environ Sci Pollut Res. 2021;28(33):44998–5008. https://doi.org/10.1007/s11356-021-13897-4
31. Akbaba GB. Toxicity assessment of zinc sulfate: A commonly used compound. Toxicol Ind Health. 2020;36(10):779–87. https://doi.org/10.1177/0748233720944771
32. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu J-C, Sasaki YF. Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen. 2000;35(3):206–21. https://doi.org/10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J
33. Nadin SB, Vargas-Roig LM, Ciocca DR. A silver staining method for single-cell gel assay. J Histochem Cytochem. 2001;49(9):1183–6. https://doi.org/10.1177/002215540104900912
34. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol. 2004;26(3):249–61. https://doi.org/10.1385/MB:26:3:249
35. Ping KY, Darah I, Yusuf UK, Yeng C, Sasidharan S. Genotoxicity of Euphorbia hirta: An *Allium cepa* assay. Molecules. 2012;17(7):7782–91. https://doi.org/10.3390/molecules17077782
36. Karaismailoglu MC. Investigation of the potential toxic effects of prometryne herbicide on *Allium cepa* root tip cells with mitotic activity, chromosome aberration, micronucleus frequency, nuclear DNA amount and comet assay. Caryologia. 2015;68(4):323–9. https://doi.org/10.1080/00087114.2015.1109927
37. Smaka-Kincl V, Stegnar P, Lovka M, Toman MJ. The evaluation of waste, surface and ground water quality using the Allium test procedure. Mutat Res Genet Toxicol. 1996;368(3–4):171–9. https://doi.org/10.1016/S0165-1218(96)90059-2
38. Ahmed FAW. Cytotoxic and genotoxic potency screening of WIDE-SPEC pesticide on *Allium cepa* L. root meristem cells. J Nat Sci Res. 2014;4(24):100–8.
39. Ciğerci İH, Liman R, İstifli ES, Akyıl D, Özkara A, Bonciu E, Colă F. Cyto-genotoxic and behavioral effects of flubendiamide in *Allium cepa* root cells, Drosophila melanogaster and molecular docking studies. Int J Mol Sci. 2023;24(2):1565. https://doi.org/10.3390/ijms24021565
40. Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHOK1 cells. Environ Sci Pollut Res. 2015;22(21):17041–52. https://doi.org/10.1007/s11356-015-4927-3
41. Zhu S, Zhou J, Zhou Z, Zhu Q. Abamectin induces apoptosis and autophagy by inhibiting reactive oxygen species-mediated PI3K/AKT signaling in MGC803 cells. J Biochem Mol Toxicol. 2019;33(7):e22336. https://doi.org/10.1002/jbt.22336
42. Mesi A, Kopliku D. Cytotoxic and genotoxic potency screening of two pesticides on *Allium cepa* L. Procedia Technol. 2013;8:19–26. https://doi.org/10.1016/j.protcy.2013.11.005
43. Karaismailoglu MC. Assessments on the potential genotoxic effects of fipronil insecticide on *Allium cepa* somatic cells. Caryologia. 2017;70(4):378–84. https://doi.org/10.1080/00087114.2017.1371992
44. Wijeyaratne WMDN, Wickramasinghe PGMU. Chromosomal abnormalities in *Allium cepa* induced by treated textile effluents: Spatial and temporal variations. J Toxicol. 2020;2020:8814196. https://doi.org/10.1155/2020/8814196
45. Kwankua W, Sengsai S, Kuleung C, Euawong N. Sunlight decreased genotoxicity of azadirachtin on root tip cells of *Allium cepa* and Eucrosia bicolor. Ecotoxicol Environ Saf. 2010;73(5):949–54. https://doi.org/10.1016/j.ecoenv.2010.04.001
46. Turkez H, Arslan ME, Ozdemir O. Genotoxicity testing: progress and prospects for the next decade. Expert Opin Drug Metab Toxicol. 2017;13(10):1089–98. https://doi.org/10.1080/17425255.2017.1375097
47. de Souza CP, Guedes T de A, Fontanetti CS. Evaluation of herbicides action on plant bioindicators by genetic biomarkers: a review. Environ Monit Assess. 2016;188(12):694. https://doi.org/10.1007/s10661-016-5702-8
48. Seth CS, Misra V, Chauhan LKS, Singh RR. Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and Comet assay approach. Ecotoxicol Environ Saf. 2008;71(3):711–6. https://doi.org/10.1016/j.ecoenv.2008.02.003
49. Mandal A, Giri S, Giri A. Assessment of toxicity, genotoxicity and oxidative stress in Fejervarya limnocharis exposed to tributyltin. Environ Sci Pollut Res. 2024;31(10):14938–48. https://doi.org/10.1007/s11356-024-32220-5
50. Prathiksha J, Narasimhamurthy RK, Dsouza HS, Mumbrekar KD. Organophosphate pesticide-induced toxicity through DNA damage and DNA repair mechanisms. Mol Biol Rep. 2023;50(6):5465–79. https://doi.org/10.1007/s11033-023-08424-2
51. Liman R, Ciğerci IH, Öztürk NS. Determination of genotoxic effects of Imazethapyr herbicide in *Allium cepa* root cells by mitotic activity, chromosome aberration, and comet assay. Pestic Biochem Physiol. 2015;118:38–42. https://doi.org/10.1016/j.pestbp.2014.11.007
52. Liman R, Ciğerci IH, Akyıl D, Eren Y, Konuk M. Determination of genotoxicity of Fenaminosulf by Allium and Comet tests. Pestic Biochem Physiol. 2011;99(1):61–4. https://doi.org/10.1016/j.pestbp.2010.10.006
53. Cossi PF, Herbert LT, Yusseppone MS, Pérez AF, Kristoff G. Toxicity evaluation of the active ingredient acetamiprid and a commercial formulation (Assail® 70) on the non-target gastropod Biomphalaria straminea (Mollusca: Planorbidae). Ecotoxicol Environ Saf. 2020;192:110248. https://doi.org/10.1016/j.ecoenv.2020.110248

SUPPLEMENTARY MATERIAL

Supplementary Fig. 1S. Root growth of *A. cepa* exposed to tap water (control) and different concentrations of abamectin ranging from 0.1 mg/L to 100 mg/L for 96 h.



Supplementary Fig. 2S. Typical mitotic stages in *A. cepa* root tip meristematic cells (**A–E**) and the CAs detected in the cells after treatment experiments (**F–L**). **A**) interphase; **B**) prophase; **C**) metaphase; **D**) anaphase; **E**) telophase; **F**) polyploidy; **G**) C-mitosis; **H**) chromosome bridge; **I**) vagrant chromosome; **J**) laggard chromosome; **K**) ring chromosome; **L**) stickiness.



Supplementary Fig. 3S. Classification of *A. cepa* root tip meristematic cells following the comet assay according to the head (nucleus) size and the tail length and intensity. **A** – Class 0; **B** – class 1; **C** – class 2; **D** – class 3; **E** – class 4. Stain is silver nitrate. Original magnification: 400 ×.