

Cytogenotoxic effects of alphamethrin and carbofuran based on mitotic index and chromosomal aberrations in *Allium cepa* root tip cells

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Abstract. Mili C, Kalita CK, Deka MJ, Das P, Tayung K. 2025. Cytogenotoxic effects of alphamethrin and carbofuran based on mitotic index and chromosomal aberrations in *Allium cepa* root tip cells. *Cell Biol Dev* 9: 71-77. Synthetic pesticides are widely used by farmers to protect crops from insect damage, which results in adverse effects on the environment. This study aimed to examine the cytogenotoxic potential of two pesticides, alphamethrin and carbofuran, using *Allium cepa* assay. The half maximum effective concentration (EC₅₀) was found to be 10 µg/mL for alphamethrin and 5 µg/mL for carbofuran. The toxicity was assessed with different concentrations of 1/10 × EC₅₀; 1/5 × EC₅₀; EC₅₀; 2 × EC₅₀; and 3 × EC₅₀ for both pesticides. Both pesticides demonstrated significant cytogenotoxic effects, evident from inhibited root growth, reduced mitotic index (MI), and increased chromosomal aberrations in onion (*Allium cepa* L.) root meristem cells. For alphamethrin, the lowest MI (12.24%) was observed at 20 µg/mL, while the highest chromosomal aberration frequency (CAF = 0.34) occurred at 30 µg/mL. Carbofuran exhibited a minimum MI of 14.90% at 10 µg/mL and the highest CAF (0.44) at 15 µg/mL. Moreover, the root growth inhibition demonstrated a dose-dependent pattern, while the MI showed neither a dose- nor a time-dependent pattern. The chromosomal aberrations observed were stickiness, vagrant, laggard chromosomes, C-metaphase, nuclear lesions, and spindle disturbance. These results underscore the urgent need for strong regulation and safer substitutes to minimize the ecological and health risks associated with widespread synthetic pesticide usage.

Keywords: *Allium cepa* assay, chromosomal aberration, EC₅₀, mitotic index, pesticides

Abbreviations: CAF: Chromosomal aberration frequency, DMSO: Dimethyl sulphoxide, EC₅₀: Half maximum effective concentration, EMS: Ethyl methanesulfonate, IPM: Integrated Pest Management, MI: Mitotic index, NAPCC: National Action Plan on Climate Change, NBAP: National Biodiversity Action Plan, ROS: Reactive oxygen species, WHO: World Health Organization

INTRODUCTION

The demand for crop production has increased significantly as a consequence of the growing global population. In recent times, to fulfill the food requirement, farmers have been using different synthetic chemicals, including pesticides, to increase food productivity. Zhang et al. (2011) reported that about 4.6 million tons of pesticides belonging to 500 different varieties are being used annually worldwide. The indiscriminate use of pesticides leads to environmental pollution and accumulation in crops, posing risks to non-target organisms. The majority of pesticides applied in agricultural practices are synthetic substances that act by obstructing an organism's crucial metabolic activity (Khan et al. 2023). Therefore, there is a need to investigate and determine the negative impact of these chemicals on living organisms at regular intervals.

According to Shahid et al. (2019), pesticides negatively affect plant photosystems, which impedes the process of photosynthesis. As an example, Shahid et al. (2023) revealed the overuse of hexaconazole pesticide reduces the length of plant organs (roots and shoots), fresh weight, chlorophyll pigments, and protein content in *Vigna radiata* (L.) R. Wilczek.

It has also been demonstrated that pesticides induce oxidative stress in plants by generating reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and superoxide anions (O⁻²) (Shahid et al. 2018; Peng et al. 2023). Additionally, pesticides alter phytosphere microbiota, affecting plant disease susceptibility, metabolism, fruit quality, and productivity (Sangiorgio et al. 2022). Likewise, pesticides can harm bees and cause the extinction of species that pollinate plants, which may result in pollinator decline (Hashimi et al. 2020). Moreover, it can cause direct harm to plants, such as retardation of plant growth, poor root hair formation, and yellowing of the shoots (Sheikh et al. 2020).

Concern has long been raised about the cytotoxic and genotoxic consequences of environmental contaminants due to the indiscriminate application of pesticides in agricultural practices. Onion (*Allium cepa* L.) assay is an affordable and reliable technique for assessing the deleterious impacts of these clastogenic and aneugenic substances (Konuk et al. 2007; Tütüncü et al. 2019). The clastogenicity of a chemical can be accurately determined by chromosomal aberration tests, while the mitotic index (MI) can be used to track cell division disruptions (Akgündüz

et al. 2020; Barman and Ray 2023). Indeed, data obtained from *A. cepa* assay were validated with the results of other eukaryotic and prokaryotic test systems (Fiskesjö 1988). When conducting *A. cepa* assay, physiological and cytological characteristics are taken into account to verify whether chemicals have any harmful effects that could prevent mitosis or result in aberrant mitotic and meiotic chromosomes (Sheikh et al. 2020).

FAO (2022) reported that more than 61,000 tons of pesticides were used in India during the year 2020. In fact, although banned in many countries, carbofuran is still illegally produced and used, particularly in rural areas of India (Nayak and Solanki 2021). Recently, a pyrethroid pesticide called cypermethrin has also been restricted in India due to its highly hazardous nature. Nevertheless, alphamethrin, which belongs to the same group usually believed to exhibit a faster knockdown effect against a wide range of pests, and is used extensively. However, the extensive use of such pesticides in agriculture raises concerns about crop genetics, environmental, and human health. To determine the degree of harm done to plants and other systems, it is crucial to assess the cytogenotoxic potency of regularly used pesticides. Therefore, this study aimed to assess the toxicity of two pesticides, alphamethrin and carbofuran, in a non-target bioindicator organism, *A. cepa*. The toxicity was assessed based on root growth inhibition, mitotic index, and chromosomal aberrations in *A. cepa* root tip cells.

MATERIALS AND METHODS

Plant materials and chemicals

Immature and healthy onion (*A. cepa*) bulbs of uniform size were used in the present study. The onion bulbs were purchased from a local market. The root primordia of the onion bulbs were kept in touch with distilled water to promote root formation. The experiment was conducted in a laboratory condition with restricted direct sunlight exposure to maintain a steady rate of cell division. Two widely used insecticides, namely STOP 10% EC for alphamethrin and FURY® 3% CG for carbofuran, were considered for this study. The chemical profile of the alphamethrin and carbofuran is presented in Table 1. Test solutions were prepared using dimethyl sulphoxide (DMSO) (Himedia,

India), a biological cell penetrant. The stock solution of the test chemicals was prepared by dissolving in 1% DMSO (extrapure AR Grade, 99% purity).

Procedures

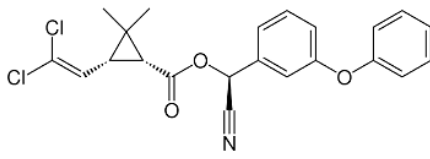
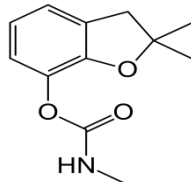
Determination of EC_{50}

The half maximum effective concentration (EC_{50}) for the test chemicals, alphamethrin and carbofuran, was determined by the method described by Özkara et al. (2015). Healthy and uniform-sized onion bulbs of about 4 cm diameter each were placed on separate conical flasks of water at room temperature for 48 h to produce roots. Thereafter, the best-growing six bulbs were selected and treated with different concentrations of the pesticides (1, 2.5, 5, 10, 15, and 20 $\mu\text{g}/\text{mL}$) at room temperature ($\sim 25^\circ\text{C}$) for 96 h. Additionally, a set of bulbs was treated with 1% DMSO as the negative control (to validate normal root growth), while ethyl methanesulfonate (EMS) (Himedia, $\geq 99\%$) was used as the positive control (to validate root inhibition). On the following day (i.e., 5th day), the lengths of the root bundles from the control and experimental sets (length of ten roots from each bulb) were measured in centimeters (cm) with a scale. Using effective control, root lengths plotted against test concentrations, the EC_{50} was calculated at 50% root growth inhibition.

Cytogenetic analysis

The cytogenotoxic efficacy of alphamethrin and carbofuran on cells of *A. cepa* root apex was determined using doses of $1/10 \times EC_{50}$, $1/5 \times EC_{50}$, EC_{50} , $2 \times EC_{50}$, and $3 \times EC_{50}$ as described by Sheikh et al. (2020). Initially, onion bulbs were placed on top of the Erlenmeyer flasks filled with distilled water at room temperature for 48 h. Five sets ($n = 3$) of bulbs with almost equal root lengths were placed in each test concentration of alphamethrin and carbofuran for 24 h and 48 h under laboratory conditions. One set of bulbs was placed in distilled water as a control. In the morning of the following day (6:00 - 7:00 a.m.), roots were cut off, and immediately the roots were fixed in Carnoy's solution (ethanol: acetic acid = 3:1) at 4°C for 24 h. Following fixation, the root tips were thoroughly washed with distilled water, stored in 70% ethanol, and refrigerated for further cytological analysis.

Table 1. Profile of two pesticides (alphamethrin and carbofuran) used in the present study

Chemical name	Alphamethrin	Carbofuran
Molecular Weight (MW)	$\text{C}_{22}\text{H}_{19}\text{Cl}_2\text{NO}_3$	$\text{C}_{12}\text{H}_{15}\text{NO}_3$
Chemical structure		
IUPAC Name	[(R)-cyano-(3-phenoxyphenyl) methyl] (1S,3S)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate
WHO classification	Class II	Class I and Class II

Staining and microscopic observations

The cytological slides were prepared by adopting the squashing technique (Sharma and Sharma 1965). The collected root tips were first hydrolyzed in a solution of 1N HCl and 2% acetocarmine (1:9), which was gently warmed for 5 min without boiling. Following that, the roots were gently washed with distilled water 3 times. The roots were then placed in 1% acetocarmine and heated intermittently until the acetocarmine started to boil. Thereafter, the root caps of healthy hydrolyzed roots were cut off with a sharp blade and placed on clean slides. A drop of 45% acetic acid was added to the root tips and covered with a coverslip. Finally, without disturbing the coverslip, the slide was swiftly heated and gently compressed by placing firm, even pressure between two folded pieces of Whatman No. 1 filter paper. The edges were sealed with nail polish and observed under a compound microscope (LABOMED®) at 400X magnification. At least 190 cells were scored collectively from three microscopic regions of a slide for each treatment.

Data analysis

For the cytotoxicity test, the mitotic index (MI) was computed for each dose and exposure time using the following formula (Balog 1982):

$$\text{MI (\%)} = (\text{Total number of divided cells} / \text{Total number of cells observed}) \times 100$$

Similarly, for the genotoxicity test, chromosomal aberration frequency (CAF) was calculated by the following formula (Sheikh et al. 2020):

$$\text{CAF} = \text{Total number of cells showing aberration} / \text{Total number of divided cells}$$

A comparative analysis of the calculated CAF values from the treatments and control groups was conducted to determine the genotoxic impact of the pesticides on the onion root tip cell genome. The mean and standard deviation (SD) were calculated using Microsoft Office Excel. Dunnett *t*-test (two-tailed) was performed to determine the significant differences ($p < 0.05$) between each treatment group and control using SPSS (Version 16.0).

RESULTS AND DISCUSSION

Effect on root growth

The root growth inhibition assay at different concentrations (1, 2.5, 5, 10, 15, and 20 $\mu\text{g/mL}$) of both pesticides showed that the root length decreased significantly ($p < 0.05$) with the increasing concentrations of the chemicals (Table 2). The maximum root length of 5.58 ± 0.13 cm was observed in the negative control, while a minimum root length of 1.33 ± 0.17 cm was found in the positive control. However, the root growth inhibition demonstrated a clear dose-dependent pattern in the preliminary EC_{50} analysis. The decline in root growth with increasing concentrations was in line with the results of Fattah and Omer (2021), wherein glyphosate was studied on onion roots with the concentrations of 0.5, 1.0, 2.0, and 4.0 mL/L for 24 h. This kind of inhibition trend was also reported for the abamectin (Kalefetoğlu 2021) and profenofos (Kumar and Prasad 2024) pesticides on *A. cepa* roots. This result may be

attributed to the higher concentration of pesticides leading to the deactivation of proteins or enzymes, which may be involved in the mechanism of root growth (Lushchak et al. 2018). In plant genotoxicity investigations, the EC_{50} value turned out to be a crucial and trustworthy measure for determining the experimental concentrations and concentration-response correlations (Saxena et al. 2010). In our study, the EC_{50} value was found to be 10 $\mu\text{g/mL}$ for alphamethrin and 5 $\mu\text{g/mL}$ for carbofuran (Figure 1). Thereafter, doses of $1/10 \times \text{EC}_{50}$, $1/5 \times \text{EC}_{50}$, EC_{50} , $2 \times \text{EC}_{50}$, and $3 \times \text{EC}_{50}$, and distilled water (control) were used for treatments at 24 and 48 h duration.

Mitotic index (MI)

The total number of cells observed under the microscope at 400X is presented in Tables 3 and 4. The lowest MI (12.24%) was observed in the root tips treated with alphamethrin at a 20 $\mu\text{g/mL}$ concentration for 48 h, while in carbofuran treatment, the lowest MI (14.90%) was observed at a concentration of 10 $\mu\text{g/mL}$ for a 24 h duration. The present results partially align with the findings of Sheikh et al. (2020), who investigated the cytogenotoxic effects of malathion and cypermethrin using *A. cepa* assay. Furthermore, these findings also corroborate with several previous studies on the effect of various pesticides in different plant as well as animal models, such as Amaç and Liman (2021), Gogoi et al. (2021), and Saquib et al. (2021). However, the reduced MI may result from interference with biological processes like DNA or protein synthesis (Pathak et al. 2022). For instance, there are two possible reasons for a reduction in mitotic activity: either DNA synthesis is inhibited or the G2 phase of the cell cycle is blocked by the tested pesticides, preventing the cell from progressing to the M phase (Yekeen and Adeboye 2013). In addition, the MI exhibited neither a dose- nor a time-dependent pattern that agrees with the report of Couoh-Puga et al. (2025), who studied the effect of chlorpyrifos and lindane in root cells of *Allium fistulosum* L. Overall, the decrease in MI at different concentrations indicates the cytotoxic potency of the studied pesticides.

Table 2. Inhibition of root growth of *Allium cepa* at different concentrations of tested pesticides

Test substance	Concentration ($\mu\text{g/mL}$)	Root length (cm)
Negative control (1% DMSO)		5.58 ± 0.13^a
Positive control (1% EMS)		1.33 ± 0.17^e
Alphamethrin	1	4.76 ± 0.23^b
	2.5	4.71 ± 0.10^b
	5	4.13 ± 0.51^b
	10	3.25 ± 0.19^c
	15	3.22 ± 0.32^c
Carbofuran	20	2.09 ± 0.41^d
	1	4.58 ± 0.12^b
	2.5	4.13 ± 0.23^b
	5	3.18 ± 0.10^c
	10	2.23 ± 0.27^d
	15	2.10 ± 0.19^d
	20	2.07 ± 0.14^d

Note: Mean value \pm SD, $n = 3$; Different letters in the same column indicate statistically different values (Dunnett *t*-test $p < 0.05$, two-tailed)

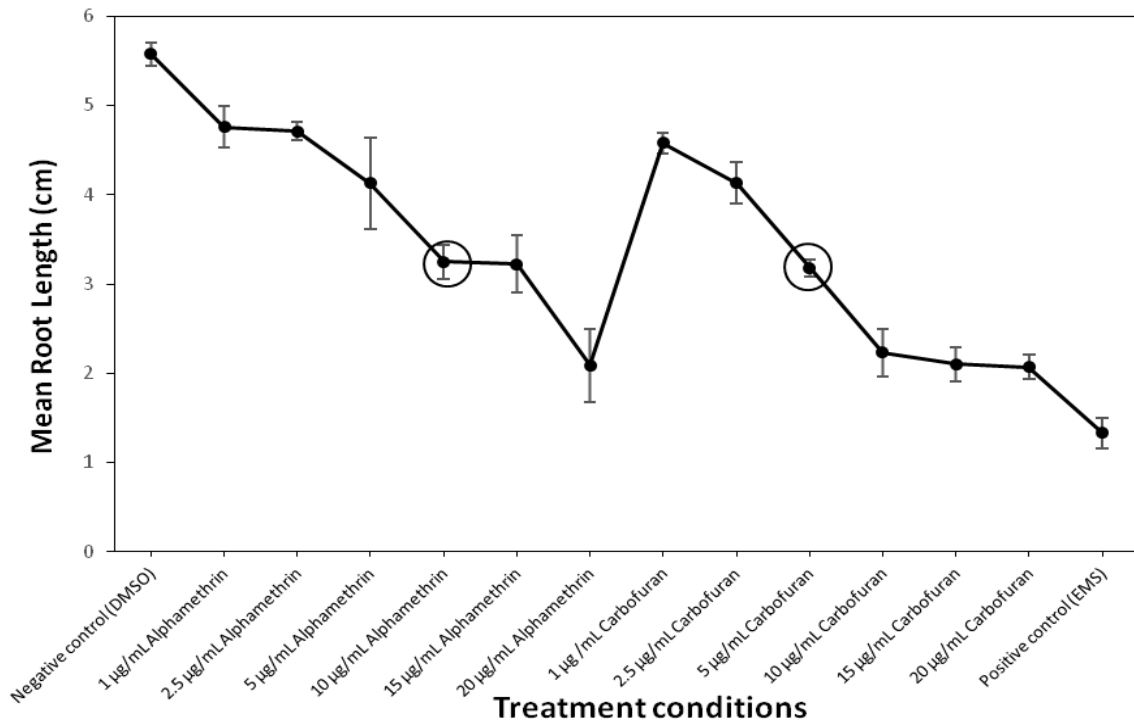


Figure 1. Dose-response curve of test concentrations with EC₅₀ values indicated by circles. Error bars indicate the standard deviation (SD); n = 3

Table 3. Mitotic indices and chromosomal aberrations observed in the root apex of *Allium cepa* treated with alaphamethrin

Treatment	Conc. (µg/mL)	Duration (h)	Total no. of cells analyzed	No. of dividing cells scored	No. of cells showing aberrations	Mitotic index (MI) (%)	Chromosomal aberration frequency (CAF)
Control	Dist. H ₂ O	24	197	115	-	58.37	-
		48	199	129	-	64.82	-
Alaphamethrin	1	24	276	88	20	31.88	0.22
			281	76	12	27.04	0.15
			307	62	17	20.19	0.27
			298	68	15	22.81	0.22
			311	70	13	22.51	0.18
	2	48	287	57	15	19.86	0.18
			266	49	15	18.42	0.30
			310	51	17	16.45	0.33
			294	36	11	12.24	0.30
			279	46	16	16.48	0.34

Table 4. Mitotic indices and chromosomal aberrations observed in the root apex of *Allium cepa* treated with carbofuran

Treatment	Conc. (µg/mL)	Duration (h)	Total no. of cells analyzed	No. of dividing cells scored	No. of cells showing aberrations	Mitotic index (MI) (%)	Chromosomal aberration frequency (CAF)
Control	Dist. H ₂ O	24	202	98	-	48.51	-
		48	196	112	-	57.14	-
Carbofuran	0.5	24	236	64	17	27.11	0.26
			228	67	11	29.38	0.16
			256	54	19	21.09	0.35
			275	41	17	14.90	0.41
			222	63	15	28.37	0.23
	1	48	291	59	23	20.27	0.38
			300	53	14	17.33	0.26
			275	62	21	22.54	0.41
			290	51	17	17.58	0.33
			227	37	16	16.29	0.44

Chromosomal aberration

The chromosomal aberration analysis revealed that all the tested concentrations of the pesticides caused chromosomal abnormalities, mostly during the metaphase and anaphase stages of the mitotic cell division (Figure 2). Most of the chromosomal abnormalities include laggard chromosomes, vagrancy, stickiness, nuclear lesions, C-metaphase, and spindle disturbance. Mechanically, a ‘vagrant chromosome’ is a chromosome that moves independently and prematurely towards a cell pole, possibly resulting in an uneven distribution of chromosomes during cell division (Elseehy 2015), while a ‘laggard chromosome’ fails to properly segregate and migrate to the opposite poles, lagging behind the other chromosomes (Mihajlović et al. 2021). The ‘sticky chromosomes’ appear clumped together or adhered to one another rather than properly separating during cell division (McGill et al. 1974). The ‘C-metaphase’ occurs when chromosomes align near the equator of the cell but are not correctly attached to the spindle fibres (Barman et al. 2020). The ‘nuclear lesion’ refers to damage to the nucleus (Udo et al. 2014).

The induction of laggard chromosomes has been ascribed to the spindle apparatus's inability to properly organize and function (Patil and Bhat 1992). Additionally, mitotic instability and breakage-fusion-bridge cycles involving broken chromatids that are started by chromosome arms broken during the primary event have been linked to chromosomal breaks that result in chromosome laggards (Fluminhan et al. 1996). It has been described that stickiness is a form of

physical attachment that mostly affects the proteinaceous matrix of chromosomes, causing them to lose their typical appearance and instead have a sticky surface that results in clustering (Okorie Asita et al. 2017; Hore and Tanti 2023; Karagol et al. 2025). Al Achkar et al. (1989) also suggested that chromosome stickiness might be caused by recombinations at non-random chromosome locations, such as the junction of hetero- and euchromatin and telomeric areas. C-metaphase could be caused by the spindle apparatus's inability to function normally, while chromosomes that are vagrants travel toward the poles ahead of their corresponding chromosomal group, causing the daughter cells' chromosomes to be unequally separated (Shakoori et al. 2017). Such vagrancy and C-metaphases can be attributed to the inhibition of ATPase by the pesticides that resulted in spindle disorganization (Rao et al. 2005; Topatan et al. 2024). A similar kind of chromosomal abnormalities has been reported in several previous investigations, such as Rosculete et al. (2019), Kalefetoğlu (2021), and Haq et al. (2023). Bıyüksüz et al. (2025) observed the presence of vagrant chromosomes in *A. cepa* root cells after exposure to triflumuron insecticide at concentrations of 1.6, 10.0, and 24.2 µg/L for durations ranging from 24 to 72 h. Similarly, Ili and Sari (2024) reported laggard chromosomes at a concentration of 1.25 mg/L and both C-mitosis and chromosomal stickiness at 2.50 mg/L in *A. cepa* roots treated with an abamectin-based pesticide for the duration of 24-96 h.

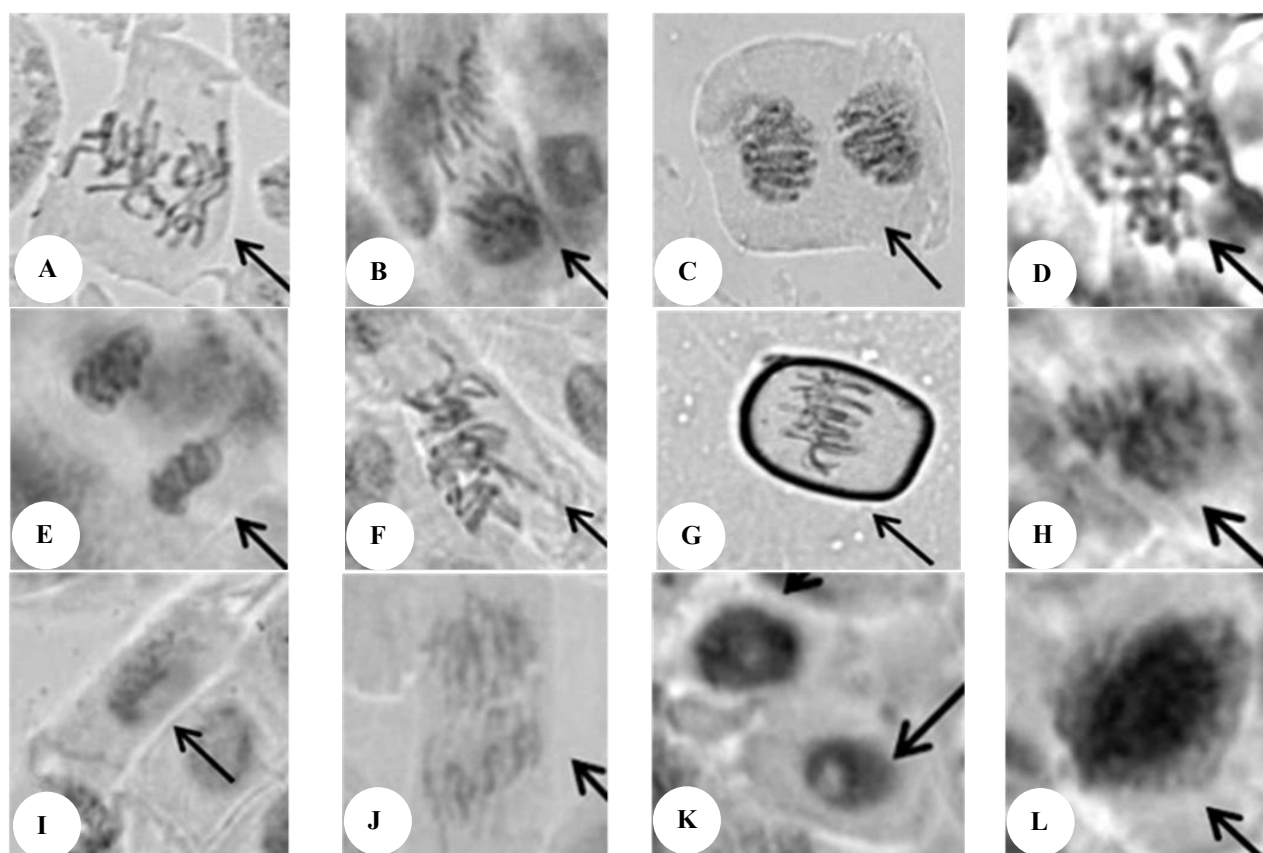


Figure 2. Possible chromosomal aberration observed during 24 and 48 h treatment in different concentrations of alphamethrin and carbofuran at 400X magnification: A. Vagrant metaphase, B. Spindle disturbance, C. Sticky anaphase, D. Laggard chromosomes, E. Sticky telophase, F. C-metaphase, G. Laggard chromosomes, H. Sticky metaphase spindle disturbance, I. Morphological change (elongated) in cell shape, J. Spindle disturbance, K. Nuclear lesion, L. Sticky prophase

In the present study, alphamethrin showed both cytotoxic and genotoxic potential, which is in partial agreement with the findings of Asita and Makhalemele (2008), who reported exhibiting the cytotoxic potential only, rather than genotoxicity. A similar pesticide, cypermethrin, belonging to the same chemical class, has already been reported to exhibit both cytotoxic and genotoxic potential (Sheikh et al. 2020), which also supports our findings. Nonetheless, carbofuran has been demonstrated to exert cytotoxicity through DNA strand breakage, reactive oxygen species (ROS) generation, and mitochondrial dysfunction in human umbilical vein endothelial cells (Saquib et al. 2021). Likewise, Soloneski et al. (2008) also elucidated the entanglement of carbofuran in the sister chromatid exchange, micronuclei frequencies, and cell-cycle progression in Chinese hamster ovary (CHOK1) cells. Collectively, these findings provide evidence and demonstrate the toxic effects of the respective pesticides.

The roots treated with alphamethrin showed the highest chromosomal aberration frequency (CAF) (0.34) at 30 µg/mL for 48 h, and the lowest CAF (0.15) was at 2 µg/mL for a 24 h duration (Table 3). On the other hand, the roots treated with carbofuran showed the highest CAF with a value of 0.44 at 15 µg/mL concentration for 48 h duration, while the lowest CAF (0.16) was observed at 1 µg/mL for 24 h duration (Table 4). However, the data obtained in the present study showed that the treatment duration does not correspond to an increase in mitotic aberrations. This could be because different concentrations of alphamethrin and carbofuran have varied saturation levels (Sheikh et al. 2020).

The findings of this study hold important ramifications for pesticide regulation and public health policy. The World Health Organization (2005) classifies the synthetic pyrethroid alphamethrin as moderately dangerous. Despite this, alphamethrin is still extensively sold in markets under formulations like STOP 10% EC in India. In contrast, carbofuran is known to be extremely harmful and has been subject to strong restrictions in several nations, including the US, Canada, and EU members (Otieno et al. 2010). However, it is still utilized in India in granular form (e.g., FURY® 3% CG), particularly in rural areas of northeastern regions. This draws attention to a regulatory gap in risk communication, labeling standards, and training programs, all of which are mandated under India's Insecticides Act (1968). Moreover, the observed cytogenotoxic effects in *A. cepa* reinforce the necessity of regular environmental toxicity screening and the incorporation of non-target organism bioassays into regulatory frameworks. Aligning pesticide use with national environmental priorities, such as the National Action Plan on Climate Change (NAPCC) and the National Biodiversity Action Plan (NBAP), requires a shift toward Integrated Pest Management (IPM), stronger control on unsafe pesticide imports and formulations, and enhanced inter-agency collaborations among regulatory agencies, agricultural, and environmental departments.

In conclusion, the present study demonstrates that both alphamethrin and carbofuran exhibit cytogenotoxic effects on *A. cepa* root meristem cells. A dose-dependent inhibition of root growth was observed, with carbofuran showing a stronger inhibitory effect ($EC_{50} = 5 \mu\text{g/mL}$) compared to

alphamethrin ($EC_{50} = 10 \mu\text{g/mL}$). Furthermore, both pesticides caused a reduction in MI, reflecting disrupted cell division processes. The induction of various chromosomal aberrations, particularly at higher concentrations and longer exposure durations, indicates the genotoxic nature of these pesticides. Overall, the present study highlights the need for cautious management of pesticides due to their implications for biological and environmental safety, as well as the potentially harmful effects of these agrochemicals on plant cellular processes. While this study evaluates the cytotoxic potential of tested pesticides, further research employing cutting-edge molecular and genomic approaches is recommended to elucidate the underlying mechanisms of pesticide-induced genotoxicity. These insights will not only strengthen scientific knowledge but also enlighten policy reform and safer agricultural practices, contributing to both human health protection and environmental sustainability.

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REFERENCES

- Akgündüz MÇ, Çavuşoğlu K, Yalçın E. 2020. The potential risk assessment of phenoxyethanol with a versatile model system. *Sci Rep* 10 (1): 1209. DOI: 10.1038/s41598-020-58170-9.
- Al Achkar W, Sabatier L, Dutrillaux B. 1989. How are stickychromosomes formed? *Ann Genet* 32 (1): 10-15.
- Amaç E, Liman R. 2021. Cytotoxic and genotoxic effects of clopyralid herbicide on *Allium cepa* roots. *Environ Sci Pollut Res* 28 (35): 48450-48458. DOI: 10.1007/s11356-021-13994-4.
- Asita OA, Makhalemele R. 2008. Genotoxicity of chlorpyrifos, alphathrin, efekto virikop and springbok to onion root tip cells. *Afr J Biotechnol* 7 (23): 4244-4250. DOI: 10.5897/AJB08.550.
- Balog C. 1982. The mitotic index in diploid and triploid *Allium* roots. *Cytologia* 47 (3-4): 689-697. DOI: 10.1508/cytologia.47.689.
- Barman M, Ray S. 2023. Cytogenotoxic effects of 3-epicaryoptin in *Allium cepa* L. root apical meristem cells. *Protoplasma* 260 (4): 1163-1177. DOI: 10.1007/s00709-023-01838-6.
- Barman M, Roy S, Ray S. 2020. Colchicine-like metaphase and cell cycle delay inducing effects of leaf aqueous extract of *Clerodendrum inerme* (L.) Gaertn. in *Allium cepa* root apical meristem cells. *Cytologia* 85 (3): 197-201. DOI: 10.1508/cytologia.85.197.
- Bıyıkısız G, Kalefetoğlu Macar T, Çavuşoğlu K, Yalçın E. 2025. A study investigating the multifaceted toxicity induced by triflumuron insecticide in *Allium cepa* L. *Sci Rep* 15 (1): 24839. DOI: 10.1038/s41598-025-10777-6.
- Couoh-Puga ED, Gómez-Maldonado MC, Escalante-Herrera KS, Osorio-Mendoza EA, Moreno-Ortiz G, Noreña-Barroso E, Wissner JL, Rodríguez-Fuentes G. 2025. Effect of chlorpyrifos and lindane in root cells of *Allium fistulosum*: Genotoxicity, cytotoxicity, and oxidative stress. *Toxicol Environ Health Sci* 1-10. DOI: 10.1007/s13530-025-00267-7.
- Elsehy M. 2015. Evaluation of high fructose corn syrup (hfc) genotoxic effect on *Allium cepa* genome. *J Agric Chem Biotechnol* 6 (5): 91-103. DOI: 10.21608/jacb.2015.44195.
- FAO. 2022. Pesticides use, pesticides trade and pesticides indicators—Global, regional and country trends, 1990–2020. FAOSTAT Analytical Briefs, no. 46. Rome. DOI: 10.4060/cc0918en.

- Fattah YM, Omer AH. 2021. Evaluation of genotoxic and cytotoxic effects of glyphosate on *Allium cepa*. *Technium BioChemMed* 2 (1): 131-140. DOI: 10.47577/biochemmed.v2i1.3332.
- Fiskesjo G. 1988. The *Allium* test—an alternative in environmental studies: the relative toxicity of metal ions. *Mutat Res/Fundam Mol Mech Mutagen* 197 (2): 243-260. DOI: 10.1016/0027-5107(88)90096-6.
- Fluminhan Jr A, de Aguiar-Perecin ML, Dos Santos JA. 1996. Evidence for heterochromatin involvement in chromosome breakage in maize callus culture. *Ann Bot* 78 (1): 73-81. DOI: 10.1006/anbo.1996.0098.
- Gogoi J, Das K, Dutta PA. 2021. Effect of preservatives and pesticides on mitotic index of *Allium cepa* roots-biological model experiment for genotoxicity. *Poll Res* 40 (3): 777-781.
- Haq FAN, Etikawati N, Solichatun. 2023. Effect of cypermethrin insecticide on root chromosome morphometry of scallion (*Allium fistulosum*). *Cell Biol Dev* 7 (1): 14-21. DOI: 10.13057/cellbioldev/v070101.
- Hashimi MH, Hashimi R, Ryan Q. 2020. Toxic effects of pesticides on humans, plants, animals, pollinators, and beneficial organisms. *Asian Plant Res J* 5 (4): 37-47. DOI: 10.9734/APRJ/2020/v5i430114.
- Hore P, Tanti B. 2023. Toxic effect of 2, 4-D on cytology of *Vigna radiata* (L.) Wilczek. *Intl J Exp Res Rev* 30: 276-281. DOI: 10.52756/ijerr.2023.v30.025.
- Ili P, Sari F. 2024. Evaluation of the cytogenetic and genotoxic effects of an abamectin-based pesticide on *Allium cepa* roots. *Arch Biol Sci* 76 (4): 477-490. DOI: 10.2298/ABS241025038I.
- Kalefetoğlu MT. 2021. Investigation of cytotoxicity and genotoxicity of abamectin pesticide in *Allium cepa* L. *Environ Sci Poll Res* 28 (2): 2391-2399. DOI: 10.1007/s11356-020-10708-0.
- Karagol S, Macar O, Çavuşoğlu K, Yalçın E. 2025. Toxicological assessment of omethoate insecticide in *Allium cepa* L. *Sci Rep* 15 (1): 18663. DOI: 10.1038/s41598-025-03923-7.
- Khan BA, Nadeem MA, Nawaz H, Amin MM, Abbasi GH, Nadeem M, Ali M, Ameen M, Javaid MM, Maqbool R, Ikram M, Ayub MA. 2023. Pesticides: Impacts on agriculture productivity, environment, and management strategies. In: Aftab T (eds.). *Emerging Contaminants and Plants: Interactions, Adaptations and Remediation Technologies* (pp. 109-134). Springer International Publishing, Cham. DOI: 10.1007/978-3-031-22269-65.
- Konuk M, Liman R, Cigerci IH. 2007. Determination of genotoxic effect of boron on *Allium cepa* root meristematic cells. *Pak J Bot* 39 (1): 73. DOI: 10.1007/s10616-013-9673-0.
- Kumar P, Prasad V. 2024. Cytotoxic and mitodepressive effect of insecticide profenofos on root meristem of *Allium cepa* L. *Environ Ecol* 42 (2): 420-426. DOI: 10.60151/envec/JQAI6367.
- Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. 2018. Pesticide toxicity: A mechanistic approach. *EXCLI J* 17: 1101-1136. DOI: 10.17179/excli2018-1710.
- McGill M, Pathak S, Hsu TC. 1974. Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. *Chromosoma* 47 (2): 157-166. DOI: 10.1007/BF00331803.
- Mihajlović AI, Haverfield J, FitzHarris G. 2021. Distinct classes of lagging chromosome underpin age-related oocyte aneuploidy in mouse. *Dev Cell* 56 (16): 2273-2283. DOI: 10.1016/j.devcel.2021.07.022.
- Nayak P, Solanki H. 2021. Pesticides and Indian agriculture - A review. *Intl J Res Granthaalayah* 9 (5): 250-263. DOI: 10.29121/granthaalayah.v9.i5.2021.3930.
- Okorie Asita A, Moramang S, Rants'o T, Magama S. 2017. Modulation of mutagen-induced genotoxicity by vitamin C and medicinal plants in *Allium cepa* L. *Caryologia* 70 (2): 151-165. DOI: 10.1080/00087114.2017.1311166.
- Otieno PO, Lalah JO, Virani M, Jondiko IO, Schramm KW. 2010. Carbofuran and its toxic metabolites provide forensic evidence for Furan exposure in vultures (*Gyps africanus*) in Kenya. *Bull Environ Contam Toxicol* 84 (5): 536-544. DOI: 10.1007/s00128-010-9956-5.
- Özkar A, Akyl D, Eren Y, Erdoğan SF. 2015. Potential cytotoxic effect of Anilofos by using *Allium cepa* assay. *Cytotechnology* 67 (5): 783-791. DOI: 10.1007/s10616-014-9716-1.
- Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S, Mohapatra A, Pandey V, Rana N, Cunill JM. 2022. Current status of pesticide effects on environment, human health and its eco-friendly management as bioremediation: A comprehensive review. *Front Microbiol* 13: 962619. DOI: 10.1007/s11033-023-08424-2.
- Patil BC, Bhat GI. 1992. A comparative study of MH and EMS in the induction of chromosomal aberration on lateral root meristem in *Clitoria atematea* L. *Cytologia* 57 (2): 295-264. DOI: 10.1508/cytologia.57.259.
- Peng X, Wang N, Sun S, Geng L, Guo N, Liu A, Chen S, Ahammed GJ. 2023. Reactive oxygen species signaling is involved in melatonin-induced reduction of chlorothalonil residue in tomato leaves. *J Hazard Mater* 443 (Pt A): 130212. DOI: 10.1016/j.jhazmat.2022.130212.
- Rao BV, Narasimham TL, Subbarao MV. 2005. Relative genotoxic effects of cypermethrin, alphamethrin and fenvalerate on the root meristems of *Allium cepa*. *Cytologia* 70 (3): 225-231. DOI: 10.1508/cytologia.70.225.
- Rosculete CA, Bonciu E, Rosculete E, Olaru LA. 2019. Determination of the environmental pollution potential of some herbicides by the assessment of cytotoxic and genotoxic effects on *Allium cepa*. *Intl J Environ Res Public Health* 16 (1): 75. DOI: 10.3390/ijerph16010075.
- Sangiorgio D, Spinelli F, Vandelle E. 2022. The unseen effect of pesticides: The impact on phytobiota structure and functions. *Front Agron* 4: 936032. DOI: 10.3389/fagro.2022.936032.
- Saqub Q, Siddiqui MA, Ansari SM, Alwathnani HA, Al-Khedhairi AA. 2021. Carbofuran cytotoxicity, DNA damage, oxidative stress, and cell death in human umbilical vein endothelial cells: Evidence of vascular toxicity. *J Appl Toxicol* 14 (5): 847-860. DOI: 10.1002/jat.4150.
- Saxena PN, Gupta SK, Murthy RC. 2010. Carbofuran induced cytogenetic effects in root meristem cells of *Allium cepa* and *Allium sativum*: A spectroscopic approach for chromosome damage. *Pest Biochem Phys* 96 (2): 93-100. DOI: 10.1016/j.pestbp.2009.09.006.
- Shahid M, Ahmed B, Zaidi A, Khan MS. 2018. Toxicity of fungicides to *Pisum sativum*: A study of oxidative damage, growth suppression, cellular death, and morpho-anatomical changes. *RSC Adv* 8 (67): 38483-38498. DOI: 10.1039/c8ra03923b.
- Shahid M, Khan MS, Kumar M. 2019. Kitazin-pea interaction: Understanding the fungicide-induced nodule alteration, cytotoxicity, oxidative damage, and toxicity alleviation by *Rhizobium leguminosarum*. *RSC Adv* 9 (30): 16929-16947. DOI: 10.1039/c9ra01253b.
- Shahid M, Singh UB, Khan MS. 2023. Metabolomics-based mechanistic insights into revealing the adverse effects of pesticides on plants: An interactive review. *Metabolites* 13 (2): 246. DOI: 10.3390/metabo13020246.
- Shakoori AR, Aftab S, Al-Ghanim K. 2017. Structural Changes in Chromosomes. In: Bhat T, Wani A (eds.). *Chromosome Structure and Aberrations*. Springer, New Delhi. DOI: 10.1007/978-81-322-3673-3_12.
- Sharma AK, Sharma A. 1965. *Chromosome Techniques: Theory and Practice*. Butterworths, London.
- Sheikh N, Patowary H, Laskar RA. 2020. Screening of cytotoxic and genotoxic potency of two pesticides (malathion and cypermethrin) on *Allium cepa* L. *Mol Cell Toxicol* 16 (3): 291-299. DOI: 10.1007/s13273-020-00077-7.
- Soloneski S, Reigosa MA, Molinari G, González NV, Larramendy ML. 2008. Genotoxic and cytotoxic effects of carbofuran and furadan® on Chinese hamster ovary (CHOK1) cells. *Mutat Res* 656 (1-2): 68-73. DOI: 10.1016/j.mrgentox.2008.07.007.
- Topatan ZŞ, Kalefetoğlu Macar T, Macar O, Yalçın E, Çavuşoğlu K, Acar A, Kutluer F. 2024. Alleviatory efficacy of *Achillea millefolium* L. in etoxazole-mediated toxicity in *Allium cepa* L. *Sci Rep* 14 (1): 31674. DOI: 10.1038/s41598-024-81586-6.
- Tütüncü E, Yalçın E, Acar A, Yapar K, Çavuşoğlu K. 2019. Investigation of the toxic effects of a carbamate insecticide methiocarb in *Allium cepa* L. *Cytologia* 84 (2): 113-117. DOI: 10.1508/cytologia.84.113.
- Udo IJ, Akpan GA, Esenowo IK. 2014. Cytotoxic effects of (5) medicinal plants on mitosis in *Allium cepa* root tips. *Curr Res J Biol Sci* 6 (2): 71-75. DOI: 10.19026/crjbs.6.5501.
- World Health Organization. 2005. Safety of pyrethroids for public health use. Available at: <https://iris.who.int/bitstream/handle/10665/69008/?sequence=1> (Accessed on 19th July 2025).
- Yekeen TA, Adeboye MK. 2013. Cytogenotoxic effects of cypermethrin, deltamethrin, lambda-cyhalothrin, and endosulfan pesticides on *Allium cepa* root cells. *Afr J Biotechnol* 12 (41): 6000-6006. DOI: 10.5897/AJB2013.12802.
- Zhang WJ, Jiang FB, Ou JF. 2011. Global pesticide consumption and pollution: with China as a focus. *Proc Intl Acad Ecol Env Sci* 1 (2): 125. DOI: 10.0000/issn-2220-8860-piaees-2011-v1-0012.