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# Assessment of cytotoxicity and genotoxicity of Acetamiprid (CETAM®) and Fluazifop-P-butyl (Fusilade Max®) pesticides using the *Allium cepa* test

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**ABSTRACT**

The intensive and uninformed use of pesticides has become always a serious environmental threat worldwide. Synthetic and natural pesticides are expected to possess toxicities including genotoxicity. The present study was conducted to investigate the cytotoxicity and genotoxicity induced by commercial formulations of fluazifop-P-butyl (FPB) herbicide and acetamiprid (ACM) insecticide using *Allium cepa* assay. The results of root growth analysis indicated that FPB and ACM significantly inhibited the root growth by 70.5 and 51.6%, respectively at concentration of 0.1 ppm. The $EC_{50}$ values determined after 96-h exposure were 0.024 and 0.072 ppm for FPB and ACM, respectively. A concentration-dependent inhibition in the total mitotic index (M In %) ranged from 44.4 to 84.7 % was observed at all evaluated concentrations of FPB, representing ⅒, ½ and $EC_{50}$, compared to that of the control. The higher concentration ($EC_{50}$) of ACM also induced significant inhibition in MI by 67.6 %. All concentrations of tested FPB induced severe damage during the mitotic division. Different forms of chromosome aberrations were also detected at all concentrations tested of ACM. The present data revealed the potential risk of both pesticides, at tested concentrations, inducing genotoxicity on non-target organism. Also, it is concluded that *A. cepa* test can be used as a sensitive indicator to detect possible genotoxicity of pesticides.

**Keywords:** herbicide, neonicotinoid, *Allium cepa* assay, cytogenotoxic activity.

## INTRODUCTION

Pesticides are a broad category of chemicals that are commonly used in agriculture. They are utilized in agricultural areas to prevent pest-related losses (Karaismailoglu, 2013; Karaismailoglu, 2015). Excessive use of persistent pesticides, on the other hand, is dangerous due to their proclivity for accumulating in natural environments such as soil, water, and air. They persist in the environment, posing a dangerous effect to ecosystems and human health on a local and global scale. Pesticide residues are inadvertently transported to non-targeted species through food chains, causing damage to normal cell function (He et al., 2020). Some pesticides have been found to be toxic and/or genotoxic to various organisms, influencing population survival, fertility, and genetics (Wandscheer et al., 2017).

Various plant and animal bioassays have been used to evaluate the genotoxic potential of different pesticides and environmental contaminants (Kuchy et al., 2015). Some plant assays, such as mitotic index and phase, chromosomal aberrations, and micronucleus percentage in somatic cells, can be used to investigate the genotoxic effects of chemicals in living systems (Singh et al. 2008). Because this plant is very susceptible and trustworthy in comparison to other plant systems, *Allium cepa* is one of the most widely used plant materials in genotoxicity investigations (Karaismailoglu, 2015). The *Allium cepa* test is a low-cost, simple-to-use test that offers advantages over other tests that require sample preparation and the addition of an exogenous metabolic system (Leme and Marin-Morales, 2009).The use of the Allium test (AT) to assess the potential cytotoxic effects of a variety of chemical, physical, and biological agents have a long history in scientific literature, dating back to Levan's first inquiry in 1938 to Fiskesjö's (1985) and later Rank's (1989) more standardised approach (2003).

Acetamiprid is a broad-spectrum, highly effective, safety, insecticide belongs to neonicotinoid group, acts as a nicotinic agonist that reacts with nicotinic acetylcholine receptors (nACh-R). Acetamiprid is a systemic insecticide used to control and eliminate many insects (whitefly, aphids, thrips, beetles, cutworms, and bugs) affecting agricultural crops, which acquired resistance against other pesticides. It is active principally by ingestion although some contact action is also observed.

Fluazifop-P-butyl belongs to the arylophenoxypropionate group of graminicides. It is a post-emergence herbicide that acts by inhibiting acetyl-

CoA carboxylase enzyme activity, involved in the catalysis of the formation of malonyl-CoA during metabolism of lipids. Fluazifop-P-butyl herbicide offers superior post-emergence weed management for cotton and soybeans, among other crops.

No relevant previous studies on the genotoxicity and cytotoxicity of acetamiprid insecticide using *A. cepa* test. However, some cytogenetic studies have been investigated on another insecticide which belongs to the same group neonicotinoids, i.e., imidacloprid (IMI). Similar Studies have been observed in other herbicides belongs to the same group of fluazifop-P-butyl (arylophenoxypropionate), like quizalofop-p-ethyl and cycloxdim, which use *Allium cepa* test to assess cytogenotoxicty.

The objective of this study was to investigate the cytotoxic and genotoxic potency of a commercial formulation of acetamiprid insecticide and fluazifop-P-butyl herbicide by using *Allium cepa* test.

## MATERIALS AND METHODS

### 1. Chemicals used

Commercial formulations of acetamiprid (CETAM® 20% SL) and Fluazifop-P-butyl (Fusilade Max® 12.5% EC) were purchased from local pesticides market. Other chemicals used for staining and fixation were purchased from Sigma (USA), and Merk. Stock solutions of the test materials were prepared in distilled water and stored at room temperature in the dark.

### 2. Root growth inhibition test and determination of effective concentration ($EC_{50}$)

Almost equal-sized bulbs (92-94 g) of the common onion, *Allium cepa* untreated with plant growth regulators, were obtained from local farms. The *Allium* test was performed according to (Fiskesjö ,1985; Rank ,2003). Onions were kept cool and dry until use. Just before use, the yellow-brownish outer scales were removed carefully, leaving the ring of root primordia intact. The test procedure was performed in the laboratory at room temperature (25±1°C) and protected from direct sun light. All experiments were set up in completely randomized design.

Root growth inhibition was used to determinate the corresponding $EC_{50}$ values (effective concentration of pesticides, permitting 50% growth of the sample under study in relation to control) of both pesticides. Preliminary range of toxic concentrations finding was conducted with concentration ranges between five times higher and lower the manufacturers recommended

dose (7-10 concentrations for each pesticide with seven bulbs for each concentration). Concentrations between the highest concentration that inhibited root growth and the lowest concentration that rarely inhibited the root growth were assessed. The estimated $EC_{50}$ value for each pesticide was statistically determined from the regression curve of percentage root growth relative to control (inhibition) against pesticide concentrations after 96hrs 95% Fiducial limits, the $Ec_{50}$ and slope values were determined based on Finney' analysis (Finney, 1971). The toxicity assay is performed as 96hrs semi-static exposure test according to (Fiskesjö ,1985; Rank ,2003). First, the bulbs were placed onto small glass bottles filled with pesticide solution and distillated water for control treatment, which was renewed every 24hrs by fresh solution. Once the application process was ended, 6 bulbs were randomly selected from each group to determine the average root elongation of the groups. The lengths (cm) of 6 roots randomly selected from each bulb were measured using a ruler from the root basis to the root apex. First, the mean root elongation of each bulb was determined by calculating the average of these 6 roots. Then, the average root elongation of 6 bulbs were taken into consideration to calculate the mean root length per group. Root Inhibition is calculated using Equation No. 1.

$$RI(\%) = MRLC - MRLT \div MRLC \times 100$$

Where RI = root inhibition; MRLC = mean root length control; MRLT = mean root length treatment

The bulbs were weighted before and after the treatments. The first weight was subtracted from the last weight to determine the total weight change (g).

### 3. Genotoxicity assay

The genotoxicity assay is carried out with three sub-effective concentrations of each pesticide, based on calculated $EC_{50}$ values. The $EC_{50}$ of acetamiprid and fluazifop-P-butyl came to be 0.072 and 0.024 ppm, respectively. The onion roots were then exposed for 48hrs to three concentrations, representing ⅒ $EC_{50}$, ½ $EC_{50}$ and $EC_{50}$ concentrations of both pesticides and distilled water was used as negative control (Table 1.). Seven *A. cepa* bulbs were placed in small glass bottles to each concentration under the same laboratory conditions described above for 48hrs. The test solutions are changed after 24 hrs. At the end of the exposure period, roots were cut and immediately treated in a chilled Carnoy's fixative (ethanol: acetic acid = 3:1) for 24hrs and kept at 4°C overnight. The

roots were transferred to 70 % alcohol and kept in refrigerator at 4°C until use (Fiskesjö, 1994; Fachinetto et al.,2007).

**Table 1.** Experimental groups and tested concentrations of each pesticide

| Groups | Treatments* | |
|---|---|---|
| | Acetamiprid | Fluazifop-P-butyl |
| Group I | Distillated water | Distillated water |
| Group II | 0.007 | 0.001 |
| Group III | 0.036 | 0.012 |
| Group IV | 0.072 | 0.024 |

*Concentrations expressed in ppm for each pesticide.

### 3.1. Slides preparation and microscopic parameters

Chromosome and mitotic aberrations were assessed in *Allium cepa* root cells by using light microscope. After 48hrs exposure the roots were fixed in fixative solution (ethanol 70%: acetic acid glacial 45 %, 3:1) for 24hrs. Five root tips are cut off from every onion and placed in a test tube with about 2ml acetic acid 45% and boiling for 5 min. The roots were placed on a slide and the terminal root tip was cut off and used for further preparation. The rest of the root was removed from the slide. Afterwards the root tip was squashed in fresh filtered 1% acetocarmine stain and allowed to absorb stain for 5-10 min. The slides were examined for mitotic index, chromosomal aberrations, and cells with micronuclei. The categories of aberrations in mitotic cells included vagrant chromosomes, c-mitosis, stickiness in metaphase, multipolar anaphase, disoriented spindle, and binuclear cell Fiskesjö (1985).

### 3.2. Mitotic index (MI), mitotic phase (MP) and chromosomal aberrations (CA) analysis

For MI and MP, the different stages of mitosis were counted in one thousand (1,000) cells per slide per concentration. There were scored for the frequency and occurrence of different types of chromosomal aberrations in the dividing cells (Bakare et al., 2000). These are calculated as follows:

$$Frequency\ of\ Aberration\ (FOA) = No.of\ abbrent\ cells\ \div No.of\ cells\ scored\ \times 100$$

$$MI = NCT\ \div Total\ number\ of\ cells\ \times 100$$

Where MI= mitotic index; NCT= number of dividing cells in the treatment.

$$M\ In\ = MIC - MIT \div MIC \times 100$$

Where M In = mitotic inhibition; MIC = mitotic index of control; MIT = mitotic index of treatment.

### 4. Statistical analysis

Statistical analysis of root growth inhibition performed using Polo Plus (Probit and Logit Analysis Ver. 20, LEOA Software). Data are expressed as mean± SD. Statical analysis was performed by using one-way analysis of variance (ANOVA) to test for significant differences of all evaluated parameters in *A. cepa* roots exposed to corresponding pesticide concentrations. Means separation was determine with Duncan's multiple range test at $P < 0.05$ level (CoStat-statistic software, CoHort software).

## RESULTS

### 1. Root Growth Inhibition

The result presented in Table 2. show that the effective concentrations which retard 50% ($EC_{50}$) of the growth of *A. cepa* roots, after 96hrs exposure, are 0.072 and 0.024 ppm for acetamiprid and fluazifop-P-butyl (FPB) pesticides, respectively. As expected, the inhibitory effect of herbicide fluazifop-P-butyl was more effective (~ 3-fold) than the tested insecticide, acetamiprid.

**Table 2.** Effective concentration values (EC) of acetamiprid and fluazifop-P-butyl after 96hrs *Allium cepa* exposure.

| Pesticide | Slope ±SE | $EC_{50}$ (ppm) | 95% Fiducial Limits* | $X^2$ |
|---|---|---|---|---|
| **Acetamiprid** | 1.167±0.120 | 0.072 | (0.036 – 0.305) | 21.142 |
| **Fluazifop-p-butyl** | 0.886±0.081 | 0.024 | (0.010 – 0.041) | 10.948 |

*Calculated using Polo Plus (Probit and Logit Analysis Ver. 20, LEOA Software)

The data in Table (3). show the inhibitory effect of the selected concentrations of each pesticide on the growth of *A. cepa* roots. The analysis of the results showed that the root growth decreased with increasing concentrations. The average length of roots was 6.87 cm after 96hrs of growth in the control whereas the root length of acetamiprid was 5.93 and 1.29 in the lower and higher concentrations, respectively. Weight gains of the Acetamiprid treatments were showed a remarkable decrease like the other

growth parameters. Maximum weight gain came to be 9.76 g for control, while the higher concentration of Acetamiprid showed growth inhibition and came to be 2.13 g.

**Table 3.** Inhibitory effect of acetamiprid on the growth of *Allium cepa* roots after 96hrs of exposure.

| Conc. (PPM) | Mean Root Length (cm) | Percentage Root Inhibition (%) | Initial weight (g) | End weight (g) | Weight gain (g) |
|---|---|---|---|---|---|
| **control** | 6.87±0.75 | 0 | 93.20±1.36 | 102.96±1.99* | 9.76$^{a**}$ |
| **0.002** | 5.93±0.34 | 13.7 | 93.20±2.01 | 102.3±2.28 | 9.1$^{b}$ |
| **0.01** | 5.05±0.62 | 26.5 | 92.83±2.51 | 100.46±2.74 | 7.63$^{c}$ |
| **0.04** | 4.97±0.44 | 27.7 | 93.48±2.77 | 99.84±2.41 | 6.36$^{d}$ |
| **0.05**¥ | 4.39±0.47 | 36.1 | 93.38±2.65 | 99.36±1.92 | 5.98$^{d}$ |
| **0.1** | 3.32±0.68 | 51.6 | 94.06±1.41 | 97.86±1.44 | 3.81$^{e}$ |
| **0.2** | 1.29±0.51 | 81.2 | 91.92±1.47 | 94.05±1.69 | 2.13$^{f}$ |

* Data are expressed as Means ±SD. **Mean values followed by the same letter(s) in same column are not significantly different at the $p < 0.05$ level (Duncan's multiple range test). ¥ Concentration represents field rate.

For fluazifop-P-butyl (FPB), the data revealed that, the mean root length of the control was 6.87 cm whereas the mean root lengths of tested concentrations ranged from 0.65 cm (at conc. 0.63 ppm) to 4.87 cm (at conc. 0.007 ppm) Table (4). Similarly, weight gain showed less upon FPB treatments compared to that in the control. Maximum weight gain came to be 9.76 g in control, while the higher concentration of fluazifop-P-butyl cause growth inhibition with only 2.58 g increase. In fact, data in table (4) showed a linear growth inhibitor to all studied parameters with increasing pesticide concentration.

**Table 4.** Inhibitory effect of fluazifop-P-butyl on growth of *Allium cepa* roots after 96hrs of exposure.

| Conc. (PPM) | Mean Root Length (cm) | Percentage Root growth Inhibition (%) | Initial weight (g) | final weight (g) | Weight gain (g) |
|---|---|---|---|---|---|
| **Control** | 6.87±0.75 | 0 | 93.20±1.36 | 102.96±1.99* | $9.76^{a**}$ |
| **0.007** | 4.87±0.05 | 29.1 | 93.22±1.38 | 99.47±1.76 | $6.25^{b}$ |
| **0.015** | 3.69±0.05 | 46.3 | 93.52±0.63 | 98.57±1.55 | $5.05^{bc}$ |
| **0.03** | 2.44±0.14 | 64.5 | 93.55±1.68 | 98.23±1.65 | $4.68^{cd}$ |
| **0.12** | 2.03±0.25 | 70.5 | 93.45±1.78 | 97.03±1.87 | $3.58^{de}$ |
| **0.26** | 1.65±0.09 | 75.9 | 93.60±1.24 | 96.80±1.41 | $3.74^{e}$ |
| **0.38** | 0.85±0.08 | 87.6 | 93.23±1.59 | 96.20±1.74 | $2.97^{e}$ |
| **0.63**¥ | 0.65±0.09 | 90.5 | 93.82±1.61 | 96.4±1.85 | $2.58^{e}$ |

* Data are expressed as Means ±SD. **Mean values followed by the same letter(s) in same column are not significantly different at the $p < 0.05$ level (Duncan's multiple range test).
¥ Concentration represents field rate.

## 2. Analysis of genotoxicity

### 2.1. Mitotic index and mitotic phase

Data in Table 5. Showed that the analyzed number of dividing cells and mitotic index values of *Allium cepa* root meristematic cells after 48hrs exposure to three low concentrations, representing ⅒, ½ and $EC_{50}$ of fluazifop-P-butyl and acetamiprid pesticides. It is obvious that fluazifop-P-butyl herbicide significantly decreased mitotic index (MI) with all evaluated concentrations. The inhibitory effects were concentration-dependent and significant from the negative control, at which ranged from 44.4 to 84.7 %. For the acetamiprid insecticide, the data showed a significant inhibition in MI at the higher concentrations, 0.036 and 0.072 ppm, by 34.1 and 67.6 % inhibition, respectively. The frequency of chromosome aberrations ranged from 1.5-2.5 % for acetamiprid (Table 5).

The mitotic index is really a good cytotoxicity biomarker (Fiskesjö, 1985). The frequency of cell division is reflected in the mitotic index. The rise or reduction in MI can be used to measure an agent's cytotoxicity levels (Fernandes et al., 2007). MIs that are lower than the negative control could suggest that test substances have harmed the growth and development of

exposed organisms. Increased MIs, on the other hand, are the outcome of cell division induction and can be considered a harmful event to cells because it leads to uncontrolled proliferation and tumor formation (Hoshina, 2002). According to Mesi and Koplikua (2013) a decrease of less than 50% has a sub-lethal effect on the test organism, while a decrease of less than 22% has a deadly effect Mesi and Koplikua (2012); Singh (2000).

In our study, fluazifop-P-butyl significantly decreased the mitotic index (MI) in *A. cepa* root tip cells showing lethal effect at concentrations of 0.012 ppm (½ $EC_{50}$), whereas acetamiprid caused sub-lethal effect only at the higher concentration, 0.072 ppm ($EC_{50}$).

**Table 5.** Number of dividing cells and mitotic index of *Allium cepa* root meristematic cells exposed to ⅒ $EC_{50}$, ½ $EC_{50}$ and $EC_{50}$ of acetamiprid and fluazifop-P-butyl after 48hrs treatment application (n = 1000 cells).

| Treatment | Conc. (ppm) | No. of Dividing cells | Cells in Mitosis | (% MI)* | Cells With aberrations | (% FOA) | (M in)* |
|---|---|---|---|---|---|---|---|
| **control** | - | 178 | 50 | 28.1 [a] | - | - | - |
| **Acetamiprid** | **0.007** | 119 | 30 | 25.2 [b] | 27 | 2.7 | 10.25 [f] |
| | **0.036** | 135 | 25 | 18.5 [c] | 33 | 3.3 | 34.1 [e] |
| | **0.072** | 110 | 10 | 9.09 [e] | 11 | 1.1 | 67.6 [b] |
| **Fluazifop-P-butyl** | **0.002** | 95 | 15 | 15.7 [d] | 16 | 1.6 | 44.4 [d] |
| | **0.012** | 87 | 5 | 5.74 [f] | 15 | 1.5 | 77.6 [a] |
| | **0.024** | 93 | 4 | 4.30 [f] | 25 | 2.5 | 48.7 [c] |

*Mean values followed by the same letter(s) in same column are not significantly different at the $p < 0.05$ level (Duncan's multiple range test). Conc.: pesticide concentration. MI: Mitotic index. FOA: Frequency of Aberrations. M in: Mitotic inhibition.

## 2.2. Chromosomal and mitotic aberrations (CAs)

The normal mitotic stages and different types of chromosome aberrations which are caused in response to the cytotoxic effect of tested pesticides are shown in figures 1-6. Normal mitotic stages were shown in the *A. cepa* control (Figure 1): (A) Prophase, (B) metaphase, (C) anaphase, and telophase(D). The harmful effect of tested pesticides showed a wide range of chromosomal aberrations in dividing *Allium cepa* test. These abnormalities are shown in Figures 2-5. For example, two forms of chromosomal aberrations were observed in root tips of *A. cepa* during mitosis (Figure 2): A. stickiness prophase and B. pulverized chromatin. At metaphase stage, numerous forms

of chromosome aberrations were found (Figure 3): A. c-Metaphase, B. chromosome breakage, C. chromatin erosion, D-F. different types of stickiness, G. diagonal, H. vagrant chromosome, and I. scattered metaphase.

Moreover, different types of abnormalities appeared during anaphase stage in *A. cepa* roots exposed to acetamiprid, at all tested concentrations (Figure 4). Among these abnormalities: A. late separation of chromosomes, B. vagrant chromosomes, C. anaphase bridge, C. diagonal anaphase, D-F. different shapes of multipolarity, G. loss of chromosomes, H. c-mitosis in anaphase, I. delayed anaphase, and J. sticky anaphase. In addition, two forms of abnormalities were observed in the telophase stage (Figure 5): A. star shaped and B. stickiness telophase.

On the other hand, all concentrations used of fluazifop-P-butyl herbicide cased only four types of chromosome aberrations (Figure 6): A. ghost cell, B. binucleated/multi. Nucleated cells, C. nuclear budding and D. micronuclei.

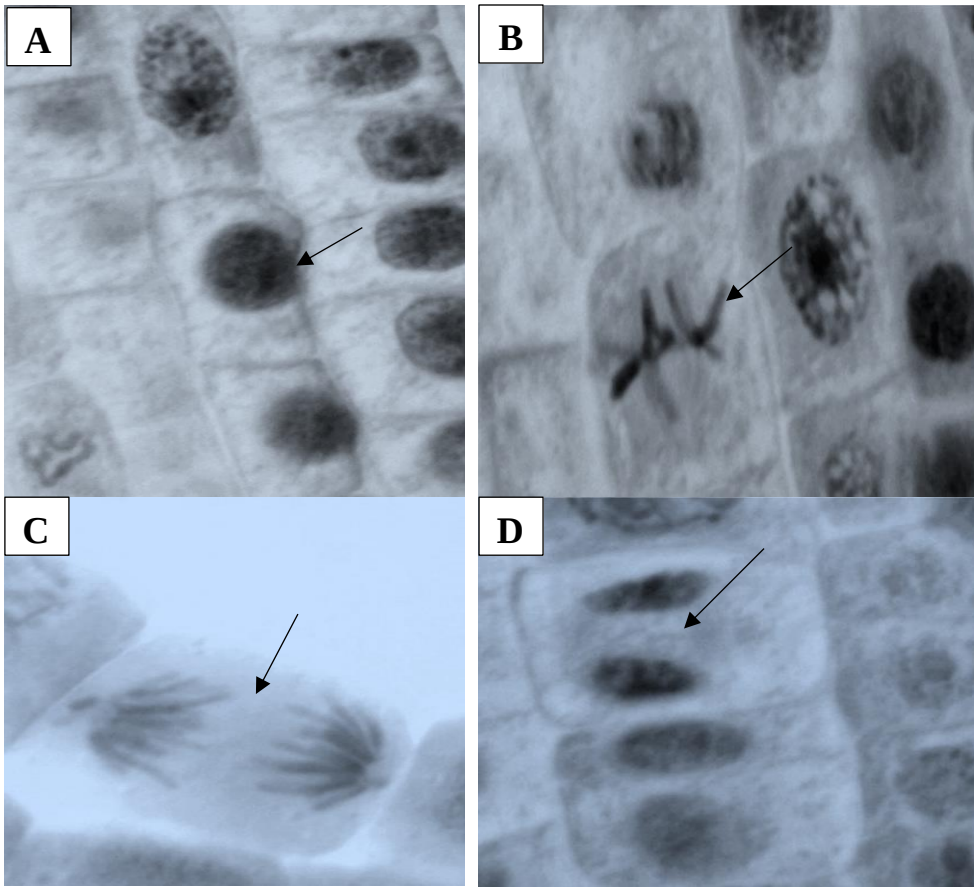



**Figure 1.** *Allium cepa* cells in normal mitotic division **A.** Prophase (the chromosomes are visible and tangled) **B.** metaphase (chromosomes are arranged in the equatorial plate) **C.** anaphase (the sister chromatids separate moving towards the spindle poles), **D.** telophase (each daughter chromosome has arrived at the spindle pole).

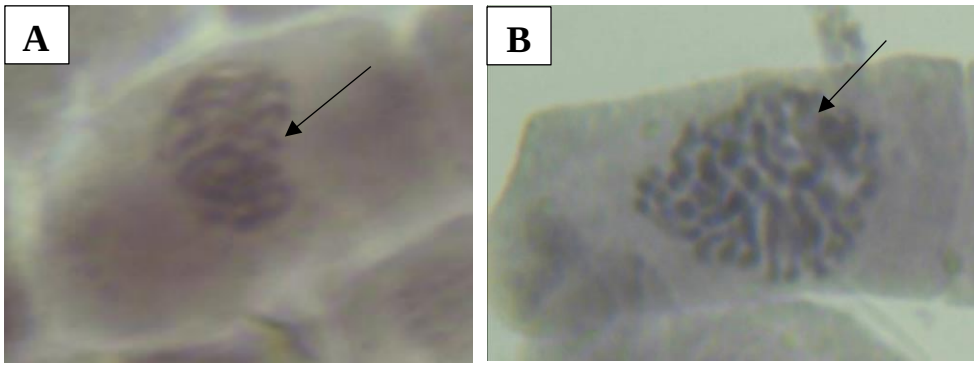
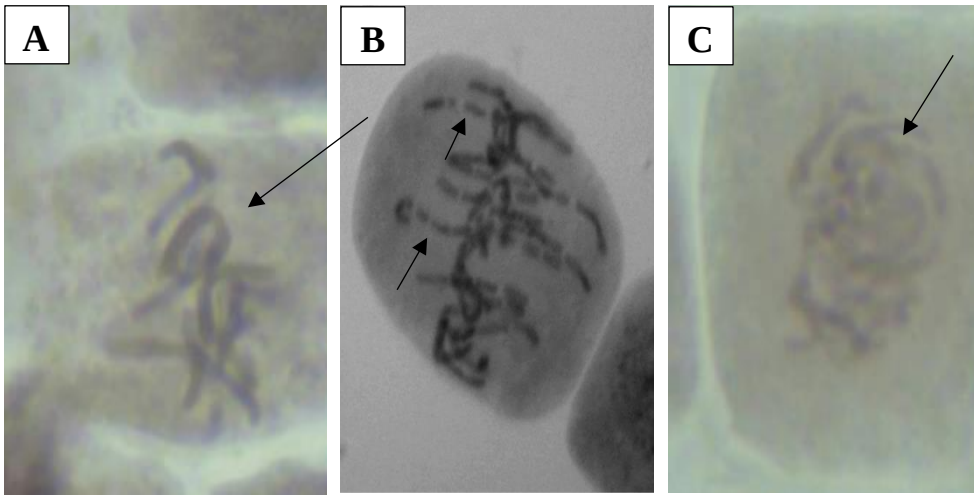



**Figure 2.** Chromosomal aberrations detected in prophase of *Allium cepa* root meristematic cells exposed to acetamiprid insecticide for 48hrs period, **A.** Stickiness prophase and **B.** Pulverized chromatin at prophase.



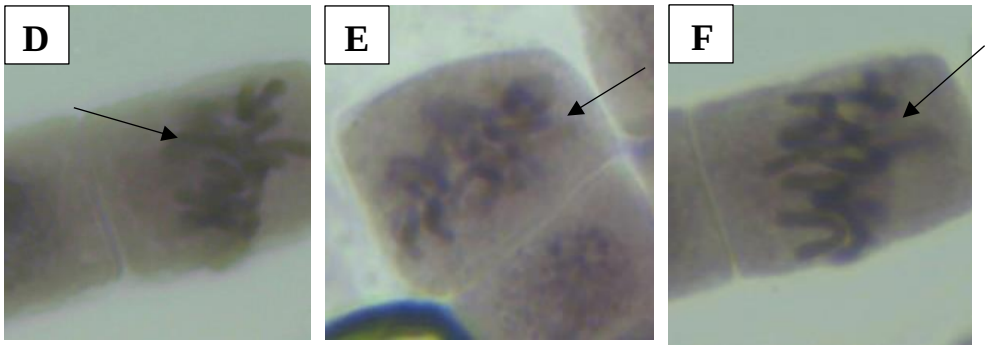
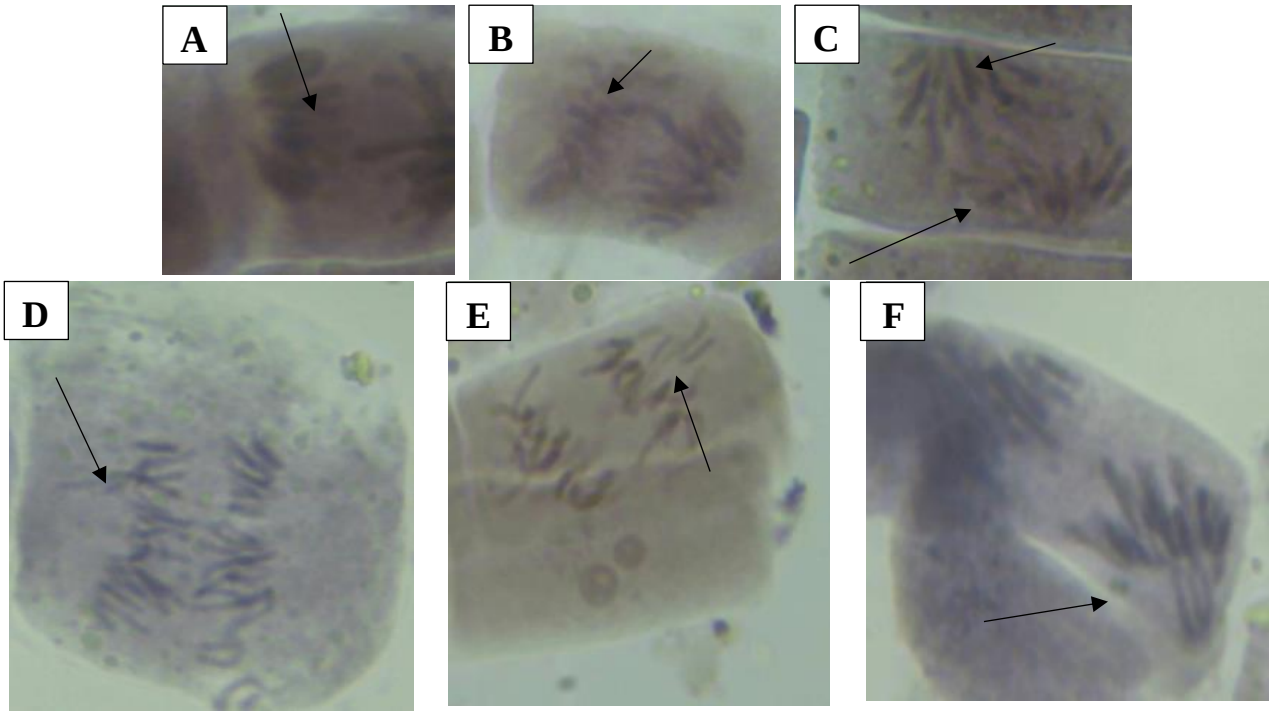



**Figure 3.** Chromosomal aberrations detected in metaphase of *Allium cepa* root meristematic cells exposed to acetamiprid insecticide for 48hrs period **A.** c-Metaphase, **B.** Chromosome breakage in metaphase, **C.** Chromatin erosion in ball metaphase **D.E., and F,** Different shapes of Stickiness in metaphase, **G.** Diagonal metaphase, **H.** Vagrant chromosome, **I.** Scattered metaphase.



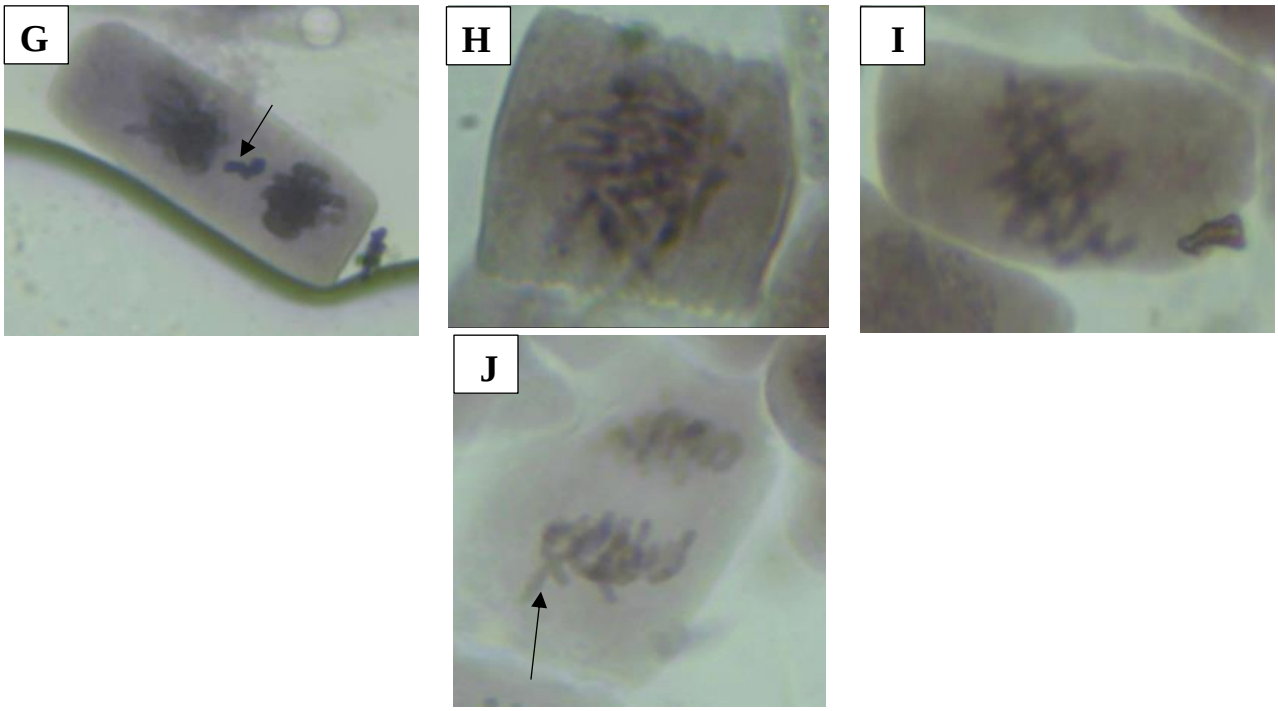



**Figure 4**. Chromosome aberration detected in Anaphase of *Allium cepa* root meristematic cells exposed to acetamiprid insecticide for 48hrs period. **A.** Late separation of chromosomes at anaphase and vagrant chromosomes, **B.** Single bridge of anaphase and vagrant chromosomes, **C.** Diagonal anaphase orientation fault of equatorial plate, **D.E.** and **F.** different shape of multipolarity of anaphase, **G.** Loss of chromosome, **H.** c-Mitosis in anaphase, **I.** Delayed anaphase and **J**. Sticky anaphase showing vagrant chromosomes.

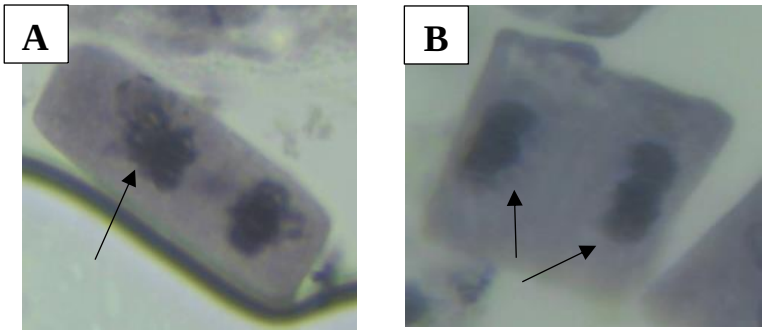


**Figure 5.** chromosomal aberrations detected in telophase of *Allium cepa* root meristematic cells exposed to acetamiprid insecticide for 48hrs period; **A.** Star shaped in telophase and **B.** Stickiness in telophase.

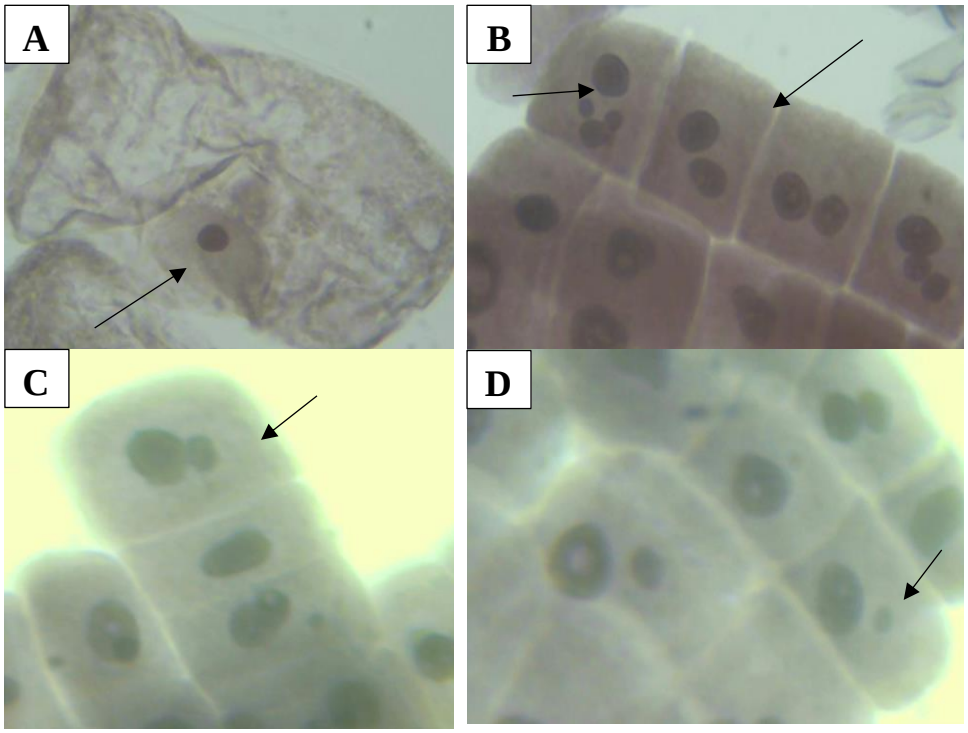



**Figure 6.** Different types of chromosome aberrations detected in division stages of *Allium cepa* root meristematic cells exposed to fluazifop-P-butyl herbicide. **A.** Ghost cell, **B.** Binucleated/Multinucleated cells, **C.** Nuclear budding and, **D.** Micronuclei.

## DISCUSSION

Higher plants, such as *A. cepa*, are frequently used as genetic models to investigate cytotoxic and genotoxic effects such as chromosome abnormalities and mitotic cycle disturbances. The cytotoxic effects of pesticides on plants can cause chromosome aberrations in dividing cells, which reflects the harmful effect of tested pesticides on plant genome (Adesuyi et al. 2018). Because of its sensitivity, *A. cepa* has shown to be an excellent model for studying pesticide genotoxicity. Using the *Allium cepa* assay, the genotoxic effects of several pesticides, including herbicides (Amaç

and Liman 2021), fungicides (Aragăo et al., 2021), and insecticides (Macar, 2020).

However, studies into the genotoxic effects of neonicotinoid insecticides, especially acetamiprid, in A. cepa are still very rare (Fioresi et al., 2020). Despite Acetamipride is widely used insecticide against agricultural insects, still there are less research on its harmful effects on treated plants. so, using the *Allium cepa* assay to examine the genotoxic effects of lower concentrations of this substance is critical for providing information on this extensively used insecticide.

Our present study revealed that concentration-dependent and statistically significant of root growth of *A. cepa* by both tested pesticides at all tested concentrations compared to the control group. As expective, fluazifop-P-butyl herbicide was more effective in inhibition of *A. cepa* root growth ($EC_{50}$= 0.024 ppm) than acetamiprid insecticide ($EC_{50}$= 0.072 ppm). Fluazifop-P-butyl (FPB) is a selective herbicide belongs to aryloxyphenoxypropionate group. n sensitive plants, it causes oxidative stress by inhibiting lipid production, free radical formation, and oxidative stress (Ore and Olayinka, 2017).

In comparison to the control, root growth inhibition was related with a considerable decrease in mitotic index (MI). The MI is a parameter that allows for the estimation of cellular division frequency and is used to consistently identify the presence of cytotoxic pollutants in the environment (Fiskesjö, 1985). A 50% reduction in the mitotic index compared to control is considered a limit value; decreases below 50% have a sublethal effect on the test organism, while decreases below 22% have a lethal effect (Mesi et al., 2012; Singh, 2000). In our current study, the reduction in Mi values for both pesticides were in concentrations-dependent manner. The herbicide fluazifop-P-butyl resulted more cytotoxic than acetamiprid insecticide, which caused highly significant cytotoxicity at concentrations of 0.012 (½ $EC_{50}$) and 0.024 ($EC_{50}$) ppm, by 77.6 and 84.7 % MI inhibition, relative to the control, respectively. These data are agreement with the quizalop-p-ethyl herbicide cytotoxic effects on *A. cepa* roots (Rosculete et al., 2019).

Similarly, at a concentration of 1.5 percent, cycloxydim, an herbicide belonging to the same group aryloxyphenoxypropionate, caused sublethal cytotoxicity in *Allium cepa* (Rosculete et al., 2019). In *A. cepa* meristematic roots, the mitodepressive effect of these herbicide described its cytotoxic and polluting potential. Other authors reported comparable results about the cytotoxic and genotoxic potential of numerous herbicides using the Allium

test, which were agreement with our current findings, e.g., Gul et al., (Liman et al., 2015).

Our present study indicted also that the acetamiprid insecticide belongs to the neonicotinoids revealed a significant reduction in the MI at the higher tested concentration tested causing sublethal cytotoxic effect (MI value was below 50% relative to the control). However, some cytogenetic studies have been investigated on another insecticide which belongs to the same group. Neonicotinoids, i.e., imidacloprid (IMI). The neonicotinoid IMI is one of the most frequent used pesticides worldwide. Fioresi et al., (2020) found that mitotic index in the of *A. cepa* root cells, treated with IMI, at concentrations of 175 and 1750 µg/ml, was significantly lower than the control. The induction of chromosomal adhesion and micronuclei was linked to IMI's genotoxic effect (Fioresi et al. 2020). These data are in line with our present study. After IMI exposure, Bianchi et al. (2016) detected high levels of chromosomal adherence in *A. cepa*. Our findings support those of Karabay and Oguz (2005), Demsia et al. (2007), and Bianchi et al. (2016), who identified IMI as a clastogen.

The present results regarding the type and frequency of abnormalities in root meristematic cells of *Allium cepa* reported that fluazifop-P-butyl herbicide, at all evaluated concentrations, resulted in high frequencies and severity in chromosome and mitotic aberrations. Other herbicides, such as quizalofop-p-ethyl and cycloxdim, were found to cause a high number of mitotic aberrations and nuclear anomalies in *Allium cepa* cells, yielding similar results (Rosculete et al. 2019). Our present study also revealed that acetamiprid insecticide induced different types of chromosomal aberrations at all evaluated concentrations including breaks, bridge, c-mitosis, stickiness, vagrant, spindle, and fragments.

The frequency of cells with sticky chromosomes was the most frequent chromosome abnormality observed in root tips of *A. cepa* treated with both quizalofop-p-ethyl and cycloxdim herbicides (Rosculete et al. 2019). Such sticky chromosomes are suggested to reflect highly toxic effects and probably cause cell death (Donghua et al., 1996). The suppression of mitotic activity is often used to assess cytotoxicity (Bianchi et al., 2016). The genotoxicity is considered one of the most serious side effects of pesticide exposure (Boumaza et al., 2016). C-mitosis in the result of damaged mitotic apparatus due to genotoxic substances in the cells, and is stimulated by many chemicals (Fiskesjö, 1985; Firbas and Amon, 2014).

The most common chromosome aberration detected in root tips of *A. cepa* treated with both quizalofop-p-ethyl and cycloxdim herbicides was the frequency of cells with sticky chromosomes (Rosculete et al. 2019). The suppression of mitotic activity is a common cytotoxicity measurement (Bianchi et al., 2016). One of the most dangerous side effects of pesticide exposure is genotoxicity. C-mitosis occurs when the mitotic apparatus is destroyed because of genotoxic compounds in the cells, and it is induced by a variety of pesticides (Fiskesjö, 1985; Firbas and Amon, 2014).

## CONCLUSION

The present study demonstrated that both fluazifop-P-butyl (FPB) herbicide and acetamiprid (ACM) insecticide have cytotoxic and genotoxic effects when tested on *Allium cepa*. The FPB was much more toxic than ACM. Both pesticides decreased mitotic index which was always correlated by the higher concentrations for ACM pesticide. These findings implied that the reduction of MI might be because of inhibition of the cell cycle, thus slowing down the progression through mitosis. Our results indicate that caution should be taken applied when using higher concentrations of the neonicotinoid acetamiprid insecticide and Fluazifop-P-butyl herbicide that induce cytological changes on non-targeted organism.

## REFERENCES

Adesuyi, AA; Njoku, KL; Ogunyebi, AL; Dada, EO; Adedokun, AH; Jolaoso, AO; Akinola, MO. (2018). Evaluation of the Cytogenotoxic Effects of Emulsifiable Concentrate form of Amitraz Pesticide on *Allium cepa* L. J. Appl. Sci. Environ. Manage. 22 (11): 1837-1843.

Amaç E. and Liman R. (2021). Cytotoxic and Genotoxic Effects of Clopyralid Herbicide on Allium Cepa Roots. Environ Sci Pollut Res Int. 28(35):48450-48458.

Aragão F.B., Duarte I.D., Fantinato D.E., Galter I.N., Silveira G.L., Reis G.B., Andrade-Vieira L.F. and Matsumoto S.T. (2021). Toxicogenetic of tebuconazole based fungicide through Lactuca sativa bioassays. Ecotoxicol. Environ. Saf. 15 (213):111985.

Bakare A.A., Mosuro A.A. Osibanjo O. (2000). Effects of simulated Leachates on chromosomes and mitosis of Allium cepa (L). J. Environ. Biol. 21: 263-271.

Bianchi J., Fernandes T.C. and Marin-Morales M.A. (2016). Induction of mitotic and chromosomal abnormalities on *Allium cepa* cells by pesticides imidacloprid and sulfentrazone and the mixture of them. Chemosphere 144: 475-483.

De Souza R.P., de Souza C.P., Bueno O.C. and Fontanetti C.S. (2017). Genotoxicity evaluation of two metallic-insecticides using Allium cepa and Tradescantia pallida: a new alternative against leaf-cutting ants. Chemosphere. 168:1093-1099.

Demsia G., Vlastos D., Goumenou M., and Matthopoulos D.P. (2007). Assessment of the genotoxicity of imidacloprid and metalaxyl in cultured human lymphocytes and rat bone-marrow. Mutat. Res. 634: 32-39.

Donghua L., Wusheng J. and Chunli W. (1996). Effects of Zn2+ on root growth, cell division and nucleolus of Allium cepa L. J. Environ. Sci. 8: 21-25.

Fachinetto J.M., Bagatini M.D., Durigon J., Silva A.C.F. and Tedesco S.B. (2007). Efeito anti-proliferativo das infusões de Achyrocline satureioides DC (Asteraceae) sobre o ciclo celular de Allium cepa. Rev. Bras. Farmacogn. 17: 49-54.

Fernandes T.C.C., Mazzeo D.E.C. and Marin-Morales M.A. (2007). Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifluralin herbicide. Pestic. Biochem. Physiol. 88: 252-259.

Finney J. (1971). Probit Analysis. 3rd Edition. Cambridge University Press. London.

Fioresi V.S., de Cássia Ribeiro Vieira B., de Campos J.M.S. and de Silva Souza T. (2020). Cytogenotoxic activity of the pesticides imidacloprid and iprodione on Allium cepa root meristem. Environ. Sci. Pollut Res. 27: 28066-28076.

Firbas P. and Amon T. (2014). Chromosome damage studies in the onion plant Allium cepa L. Caryologia. 67(1):25-35.

Fiskesjö G. (1985). The Allium test as a standard in environmental monitoring. Hereditas. 102: 99-112.

Fiskesjö G. (1994). The Allium Test II: Assessment of chemical's genotoxic potential by recording aberrations in chromosomes and cell divisions in root tips of Allium cepa L. Environ. Toxicol. Water Qual. 9: 234-241 .

He B., Wang X., Yang C., Zhu J., Jin Y., and Fu Z. (2020). The regulation of autophagy in the pesticide-induced toxicity: angel or demon. Chemosphere. 242: 125-138.

Hoshina M.M. (2002). Induction of chromosome aberrations in the Allium cepa test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere. 72 (5):722-725

Karabay N.U. and Oguz M.G. (2005). Cytogenetic and genotoxic effects of the insecticides imidacloprid and methamidophos. Genet. Mol. Res. 4: 653-662.

Karaismailoglu M.C. (2013). Deltamethrin ve quizalofop-petil pestisitlerinin Helianthus annuus L. (Ayçiçeği) kök ucu hücreleri üzerine mutajenik etkilerinin araştırılması [Investigation of mutagenic effects of the pesticides Deltamethrin and Quizalofop-p-ethyl on root tip cells of Helianthus annuus L. (Sunflower)] [MSc thesis]. Trabzon: Karadeniz Technical University.

Karaismailoglu M.C. (2015). 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. 68(4): 323-329.

Kuchy A.H., Wani A.A. and Kamili A.N. (2015). Cytogenetic effects of three commercially formulated pesticides on somatic and germ cells of Allium cepa. Environ. Sci. Pollut. Res. 23: 895-906.

Leme D.M. and Marin-Morales M.A. (2009). Allium cepa test in environmental monitoring: a review on its application. Mutat. Res. 682: 71-81.

Levan A. (1938). The effect of colchicine on root mitosis in Allium. Hereditas. 24: 471-486.

Liman R., Ciğerci I.H. and Öztürk N.S. (2015). Determination of genotoxic effects of Imazethapyr herbicide in Allium cepa root cells by mitotic activity, chromosome aberration, and comet assay. Pestic. Biochem. Physiol. 118: 38-42.

Macar T.K. (2021). Investigation of cytotoxicity and genotoxicity of abamectin pesticide in Allium cepa L. Environ. Sci. Pollu Res. 28: 2391-2399.

Mesi A., and Kopliku D. (2013). Cytotoxic and Genotoxic Potency Screening of Two Pesticides on Allium cepa L. Food Environ. Procedia. Technol. 8: 19-26.

Mesi A., Kopliku D. and Golemi S. (2012). The use of higher plants as bio-indicators of environmental pollution - a new approach for toxicity screening in Albania. MJSS 2012. 3(8): 237-248.

Ore A. and Olayinka E.T. (2017). Fluazifop-p-butyl, an aryloxyphenoxypropionate herbicide, diminishes renal and hepatic functions and triggers testicular oxidative stress in orally exposed rats. Toxicology and Industrial Health. 33(5): 406-415.

Plewa M.J., Wagner E.D., Gentile G.J. and Gentile J.M. (1984). An evaluation of the genotoxic properties of herbicides following plant and animal activation. Mutation Research. 136: 233-245.

Rank J. (2003). The method of Allium anaphase-telophase chromosome aberration assay. Ekologija. 1: 38-42.

Rosculete C.A., Bonciu E., Rosculete E. and Olaru L.A. (2019). Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on Allium cepa. Int. J. Environ. Res. Public Health. 16: 75.

Singh P., Srivastava A.K. and Singh A.K. (2008). Cell cycle stage specific application of cypermethrin and carbendazim to assess the genotoxicity in somatic cells of Hordeum vulgare L. Bull Environ. Contam. Toxicol. 81(3): 258-261.

Singh R.J. (2000). Plant Cytogenetics. 2nd ed. New York: CRC Press. p.12.

Turkoglu S. (2009). Genotoxic effects of mono-, di-, and trisodium phosphate on mitotic activity, DNA content, and nuclear volume in Allium cepa L. Caryologia. 62(3): 171-179.

Verma S., Arora K., and Srivastava A. (2016). Monitoring of genotoxic risks of nitrogen fertilizers by Allium cepa L. mitosis bioassay. Caryologia. 69: 343-343.

Wandscheer A.C.D., Marchesan E., Tededco S.B., (2017). Cytogenotoxicity of rice crop water after application of the tricyclazole fungicide. Anais da Academia Brasileira de Ciências. 89(2): 1251-1258.