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Studies on the toxic effect of Cypermethrin on the Meristem of *Allium Cepa* L.

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Abstract

Plants are direct recipients of agro-toxic and therefore important materials for assessing environmental chemicals for genotoxicity. Pesticides possess biological activity including genotoxic influence and can affect non-target organisms. The cyto and genotoxicological potency of cypermethrin pesticide was screened using *Allium cepa* L test. Onion bulbs were exposed to 96hrs for five concentrations (0.01, 0.02, 0.03, 0.04 and 0.05mg/l) of cypermethrin. A dose dependent reduction in *Allium cepa* root length was observed for the exposure of cypermethrin. Microscopic observations observed in the pesticides treated onions include sticky chromosomes, disturbed spindle and chromosome bridges. Dose dependent reduction was observed in the total mitotic dividing cells and mitotic index of the treated onions. The pesticides induced growth inhibition and caused cytogenotoxic effect on the meristamatic cells of *Allium cepa*. The data here in provide more information on the pesticide of which exposure to substantial concentration might constitute health risk to non-target organisms.

Keywords: Cypermethrin, *Allium cepa*, Genotoxicity, Chromosome

Introduction

The Indian economy's most significant sector is the agriculture industry, which makes a significant contribution to the nation's overall food output. A twofold increase in plant-based protein is required to meet the ever-growing human population's nutritional needs, which is relatively challenging with current farming methods. The use of pesticides is widely acknowledged to have a large positive impact on our ability to supply the world's demand for cheap, plentiful food and fibre; nonetheless, the intensive and widespread application of chemical insecticides has led to environmental risks in addition to control failures. There is a pressing need to explore for environmentally friendly substitutes when phytochemicals originating from various plants are significant. Plant responses to stress have been discovered to be significantly influenced by the use of plant growth regulators (Ananieva *et al*. 2004; El-Tayeb *et al*. 2006; Tsonev *et al.* 1998). The wide spectrum of protective effects that plant products like secondary metabolites and other related species have on genotoxicity has attracted a lot of attention in recent years. Higher plants are widely utilized in monitoring studies because they are considered as excellent genetic models for detecting environmental mutagens. According to Fisun and Rasgele (2009), environmental contaminants typically induce genetic harm to plant cells.

Researchers have examined induced chromosomal aberrations (CAs) and the presence of micronuclei (MNi) in the root tips of various plant species. The most often used experimental material is the root tips of various Allium species. *Allium cepa L.* is one of the best plants for identifying various xenobiotics. In 1981, Fiskesjo introduced the first alteration to the *Allium cepa* test for environmental monitoring. In the current study, cypermethrin's effects on various *Allium cepa* characteristics, including root shape, growth, mitotic index, and chromosomal abnormalities, were evaluated.

Materials and Methods

Test Chemical

The chemical which was used in this study was cypermethrin are expected to be potential genotoxicity on plant model. The chemical was obtained from the pioneer pesticide Pvt. Ltd, Industrial growth center, samba, J & K.

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Experimental Plant Organism

Allium cepa is the experimental organism employed. Genotoxicity have been assessed by treating *Allium cepa* root system with the cypermethrin. Equal sized and healthy onion bulbs were chosen. Disease and debride bulbs were not selected.

Test Procedure

Clean and healthy bulbs of onion were chosen for each treatment group. Before starting the experiments dry scales of bulbs were removed and then the onion bulbs were induced to root by placing them on culture tubes filled with distilled water as control and planted each bulb on culture tube filled separately with the freshly prepared concentration (0.01 to 0.05 mg/l) of the pesticide (Cypermethrin) for four different time periods 24, 48, 72 and 96 hrs. The contents of the culture tube were replaced with freshly prepared pesticide solution at every 24 hours. After the time exposure the root tips were collected and squash were prepared immediately.

Squash Preparation

The root tip used for microscopic evaluation were harvested from three onion bulbs per concentration at 96 hrs. Root tips 1-2cm long were cut and placed in a watch glass and fixed in acetic acid (ethanol: glacial acetic acid in 3:1 ratio) for 10 – 15 minutes. The root tips were then hydrolyzed in 1N HCL at 60^{o}C for 5 minutes. Three root tips were squashed on slides and then stained with acetocarmine for 15 minutes (Sharma and Sharma, 1980). The cover slip was sealed on the slides with clean finger nail polish as suggested by Grant, 1982. After proper fixation and staining appropriate squash preparation were made for each of the treatment and control.

Scoring of Slides

Effects of chemical treatment and control on different slides were observed under light microscope (COSLAB) with camera attached (MDCE-5C) microscopy. Mitotic index and Phase index was calculated and different types of chromosomal aberrations were also observed in pesticide exposed root tip cells and control.

Mitotic Index = TDC x 100 / TC

Phase Index = TC x 100 / TDC

Where,

TDC – Total dividing cell

TC –Total dividing and non-dividing cells

Results

Effect of cypermethrin on root growth and root growth inhibition in *Allium cepa:*

Root number and Root length (cm)

The results given in Figure 1 revealed that the root numbers and root lengths were reduced in exposed group as compared with the control. The normal root number for the *Allium cepa* was 9.8±1.48 and the normal root length was 1.31±0.78. The root number was recorded in all the exposure groups of *Allium cepa* to be lower than the control group. The root number was 6.8±1.30 in 0.01mg/l, 6.2±1.30 in 0.02 mg/l, 5.0±1.58 in 0.03 mg/l, 2.8±1.30 in 0.04 mg/l and 2.4±1.51 in 0.05 mg/l respectively. The root length was recorded in all the exposure groups of *Allium cepa* to be lower than the control group. The root length was 1.03±0.56 in 0.01 mg/l, 1.02±0.53 in 0.02 mg/l, 0.82±0.62 in 0. 03 mg/l, 0.73±0.30 in 0.04 mg/l and 0.37±0.30 in 0.05mg/l respectively (Plate 1).

Growth Inhibition

The root growth inhibition (Figure 2) assay indicated that there was an increase in the inhibition of the growth of the onion roots as the concentrations of the test materials increased. There was no growth inhibition in the control which had the highest root length of 9.8 ± 1.48 cm. Maximum (-71.75 %) increase was recorded at 0.05mg/l of cypermethrin while all the remaining values were -21. 37% on 0.01mg/l exposure, -22.13% on 0.02mg/l exposure, -37.40% in 0.03mg/l exposure, -44.27% in 0.04mg/l concentration.

Effect of cypermethrin on mitotic index and mitotic depression in root tip cell of *Allium cepa* L.

Mitotic index

Figure 3 shows the result that mitotic index has decreased in exposed group as compared with the control group. The mitotic index is the reliable predictor of cell proliferation in the tissue. The treatment of cypermethrin on *Allium cepa* caused dose dependent decline in mitotic index. Mitotic index of control set was 39.54. From it declined 24.27 on 0.01mg/l exposure, 26.30 in 0.02mg/l, 19.78 in 0.03mg/l, 21.19 in 0.04mg/l and 18.58 in 0.05 mg/l of cypermethrin exposure.

Mitotic depression

The mitotic depression was observed (Figure 4) to be inversely proportional to the mitotic index. The mitotic depression values recorded varies from -33. 48% to -53.00% on the exposure of

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different concentrations of cypermethrin. The value of the mitotic depression recorded increase with increase in the concentration of the test chemical. The least mitotic depression (-33.48%) were observed in the *Allium cepa* treated with 0.02 mg/l concentration of cypermethrin. The highest mitotic depression (-53.00%) were observed in the *A. cepa* with 0.05mg/l concentration of cypermethrin.

Effect of cypermethrin on Phase index

Prophase index (PI)

The frequency of prophase decreased with increase in different concentration of cypermethrin. The prophase index values were abruptly varied in all the concentrations. The prophase index value of control was 43.97. At 0.01mg/l exposure appeared 40.77 decreased and 0.02mg/l concentration 39.56 was recorded. But a sharp (50. 00 to 58.62) increased at 0.03, 0.04 and 0.05mg/l respectively (Figure 5).

Metaphase Index (MI)

The frequency of metaphase decreased with increase in different exposure of cypermethrin. The metaphase index value of control was 22.46 and the highest value of metaphase index 38.88 was obtained in 0.03mg/l of cypermethrin. The lowest value of metaphase index 6.52 was observed in 0.04mg/l exposure (Figure 6).

Anaphase Index (AI)

The frequency of anaphase decreased with increase concentration of the test chemical. The anaphase index decreases with increase in concentration. The anaphase index value of control was 18.32 and the lowest anaphase index (10.34) recorded at 0.05mg/l of cypermethrin and the maximum anaphase index (21.97) was obtained at 0.02mg/l concentration (Figure 7).

Telophase Index (TI)

The frequency of telophase decreased with increased concentrations of cypermethrin. The telophase index of control was 16.23 while the maximum telophase index (32.60) was observed at 0.04mg/l similarly the lowest telophase index (11.11) appeared at 0.03mg/l of cypermethrin (Figure 8).

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Chromosomal Aberrations

The genotoxic effects that were observed in the present study included different chromosomal aberrations. These include lagging chromosome, fragments, granular, sticky chromosomes abnormal anaphase and metaphase. Other chromosomal aberrations observed in this study are binucleate, micronuclei (Plates 1, 2 and 3).

Figure 1: Effect of cypermethrin (mg/l) on root number and root length in onion root tip cells of *Allium cepa* L.

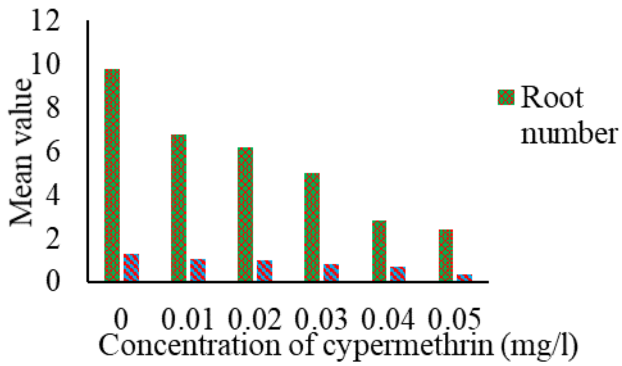

Figure 2: Percent changes of Growth Inhibition (%) in onion root tip cells of *Allium Cepa* L. exposed to different concentrations of cypermethrin (mg/l)

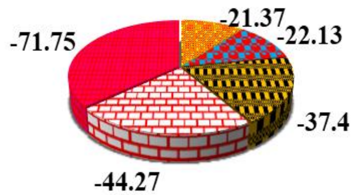

Figure 3: Effect of cypermethrin (mg/l) on Mitotic Indices in root tip cells of *Allium cepa* L.

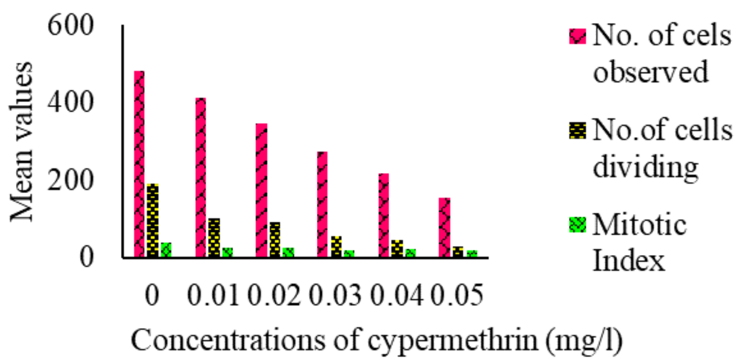

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Figure 4: Percent changes of Mitotic Depression (%) in onion root tip cells of *Allium Cepa* L. exposed to different concentrations of cypermethrin (mg/l)

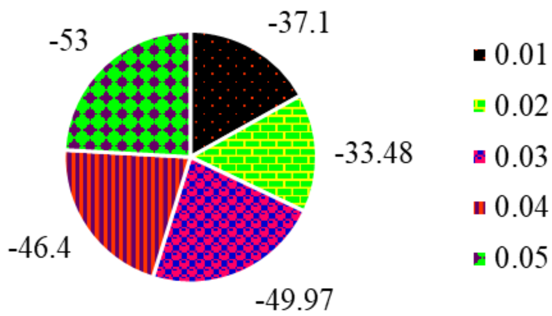

Figure 5: Effect of cypermethrin (mg/l) on Prophase Index in root tip cells of *Allium cepa* L.

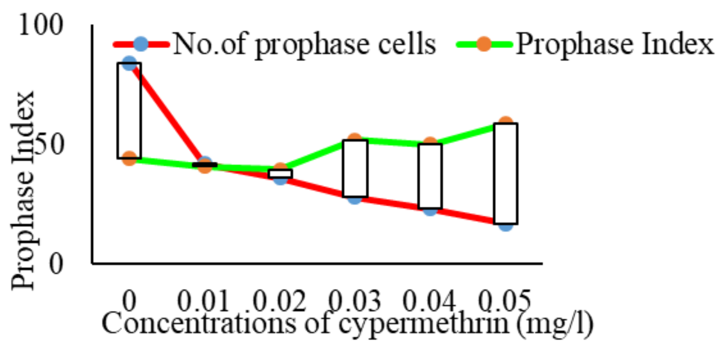

Figure 6: Effect of cypermethrin (mg/l) on Metaphase Index in root tip cells of *Allium cepa* L.

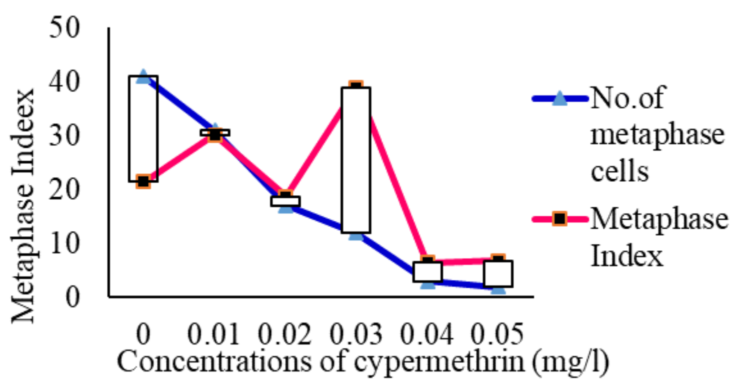

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Figure 7: Effect of cypermethrin (mg/l) on Anaphase Index in root tip cells of *Allium cepa* L.

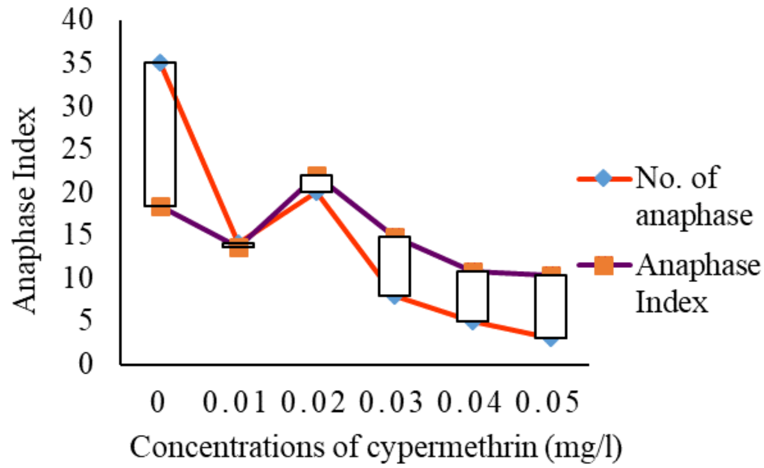


Figure 8: Effect of cypermethrin (mg/l) on Telophase Index of root tip cells in *Allium cepa* L.

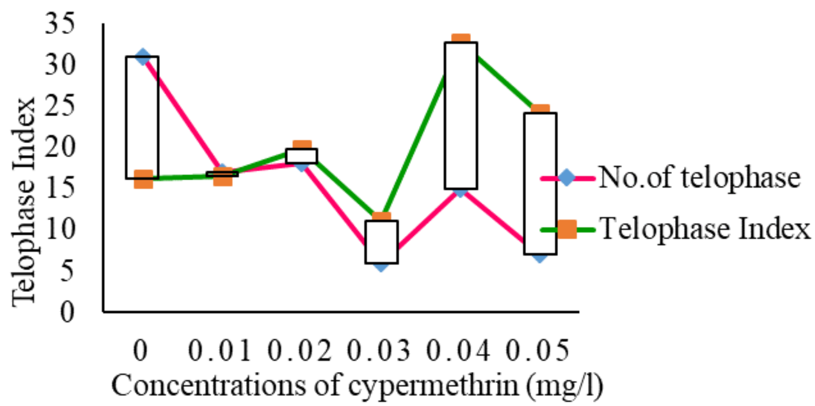


Plate 1: Effect of different concentrations of cypermethrin application on root growth of *Allium cepa* L.

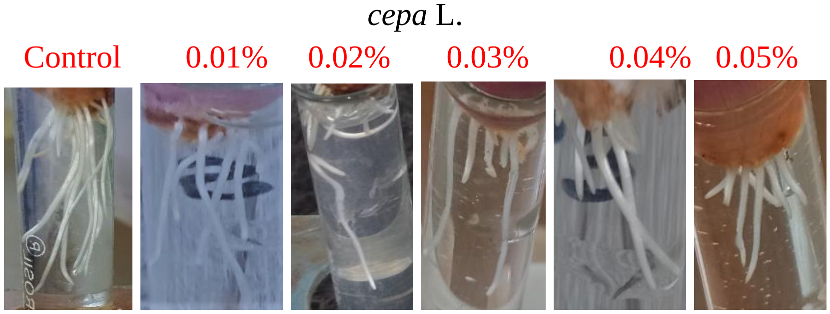
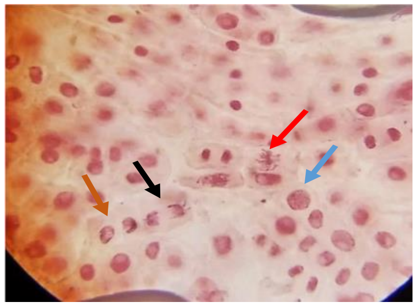


Plate 2 Pesticide-free *Allium cepa* root tip cells display typical cell division stages.



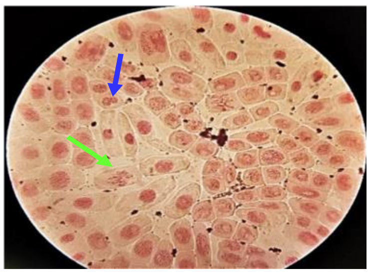
Prophase

Metaphase

Anaphase

Telophase

Plate 3 Pesticide-treated *Allium cepa* root tip cells have chromosomal abnormalities.

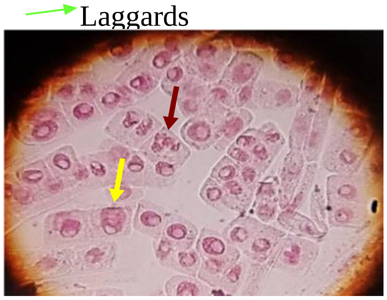


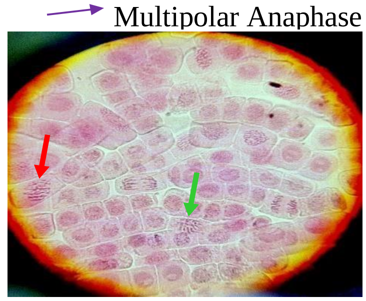
Micro nucleus

Laggards

Anaphase Bridge

Multipolar Anaphase





Polyploidy

Telophase Puffing

Disoriented Metaphase

Granules

Telophase with one end puffing

Strap nucleolus

High degree of clumping

C-metaphase

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Discussion

Plants are direct recipients of agrotoxics, making them important subjects for genetic testing and environmental monitoring of areas where such pollutants are present. As a result, the use of plants for the evaluation of environmental pollutants like pesticides is becoming more and more common.

According to Yekeen *et al*. (2013) and Patra and Sharma (2002), the higher plants Allium cepa (onion), Tradescantia paludosa, and Vicia faba are suitable to test organisms for the investigation of the cytogenotoxicity of environmental mutagenic substances and complex mixtures because they have relatively large monocentric chromosomes in reduced numbers.

The present study's findings indicate that varying quantities of the pesticide cypermethrin are harmful, inhibiting the continuous development of roots. It is well known that the meristematic zone, where cell division is most active, has a higher oxygen consumption rate associated with respiration; it is conceivable that the decrease in oxygen consumption takes place in the lengthening zone, where oxidation reactions are less advantageous than in the meristematic zone (Lerda *et al*. 2010).

Examining the root tips of Allium cepa for chromosomal abnormalities allowed researchers to assess the genotoxic effects of various cypermethrin doses. Higher doses prevent normal morphological characterization of the various stages of cell division and hinder cell division in general. When compared to the control, there was a substantial difference between the treatments. In root tips treated with petroleum compounds, the normal cell cycle was disturbed, and the results varied between treatments. Such findings are not dissimilar from those of other Allium test results.

By measuring cytological factors including the mitotic index and the amount of chromosome abnormalities, such as micronuclei, laggards, ghost cells, and vagrants, as well as sticky chromosomes (metaphase), it was possible to predict the potential cytotoxic and genotoxic consequences. Numerous writers have noted a decrease in the mitotic index after treating Allium cepa with a pesticide (Asita and Matebsi, 2010).

It has been suggested that chromosome abnormalities and chemical toxicity are related. For instance, Yuzbasioglu *et al*. (2009), Pulate and Tarar (2014), and Tripathy and Patel (2014) examined the cytotoxic effects of the herbicides Illaxan, Tilt, and Procion, respectively, and found that the identification of C-metaphase, sticky metaphase, multipolar anaphase, bridges, and laggards in the root tips of Allium cepa is an indicator of the cytotoxicity of these chemicals. With these findings, it may be inferred that the pyrethroid insecticide cypermethrin used indiscriminately in agricultural fields above the tolerance level can have negative impacts on biological systems, including humans.

The onion root tip cells, cell division, and chromosomal alterations all exhibit harmful effects. The issue of agrochemical pollution can be solved, however, by concerted efforts to minimize the usage of pesticides and use natural solutions for insect incursion through organic farming. Regulations that restrict the use of agrochemicals should be supported, along with safer, non-toxic alternatives for both humans and the environment. So-called "natural pesticides" like plant pesticides, microbiological and biological agents, and inorganic minerals are examples of alternatives to conventional pesticides that are not synthesized but rather obtained from nature. These treatments are typically thought to be less harmful to human health than synthetic pesticides and might be a good alternative.

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