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Research Article

CHROMOSOMAL ABERRATIONS INDUCED BY ACETAMIPRID IN *ALLIUM CEPA* L. ROOT MERISTEM CELLS

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ABSTRACT
The effects of acetamiprid (Insecticide) on the mitosis of *Allium cepa* L. was investigated with a view to ascertaining its mutagenic effects. Onion roots were treated with 0.1g /lt. water, 0.2g /lt. water and 0.3g /lt. water concentrations of acetamiprid at 6 hours, 12 hours and 18 hours duration and distilled water as control. There was a remarkable difference among the mitosis indices for 0.1g /lt., 0.2g /lt. and 0.3g /lt. of water concentrations of acetamiprid respectively compared with control. The result showed that acetamiprid induced cell mitotic abnormalities like stickiness, laggards, c-mitosis, bridges, multipolarity, fragmentations and picnosis at 0.1 g /lt., 0.2 g /lt., 0.3 g /lt. of water concentrations and different time of exposure. The result implicated acetamiprid as a mitotic depressor and confirmed acetamiprid as a mutagenic to plant cell, when absorbed in high dosage i.e. increasing concentrations increased the chromosomal aberrations.

Key Words: *Acetamiprid, Allium Cepa L., Chromosomal Aberrations, Insecticide, Mitotic Depressor, Mitotic Index, Mutagenesis*

INTRODUCTION
The mechanism leading to the formation of daughter cells and the retention of identical chromosome numbers and other hereditary factors in the newly formed cells, following treatment with various reagents have been studied by several workers (Panneerselvam *et al*., 2012; Nwangburuka and Oyelana, 2011; Yuzbasioglu *et al*., 2009; Fisun and Rasgele, 2009 and Umar, 2004). Due to their widespread use in agriculture, insecticides are some of the compounds most frequently released in to the environment. Despite the beneficial effects associated with the use of insecticides, many of these chemicals may pose potential hazards to humans and to nature. The current study is an attempt to investigate the effects of acetamiprid - a widely used insecticide, on mitotic activities in *Allium cepa* L. root tip cells. The justification for the use of acetamiprid is its popular use in control of sucking-type insects on leafy vegetables, fruiting vegetables, crops, fruits, ornamental plants and flowers, while *A. cepa* has been selected for its relatively low chromosome number (2n= 16). Its chromosomes are relatively large and the species is susceptible to cytological manipulations (Mercykutty and Stephen, 1980).
Acetamiprid is a member of a chemical family Neonicotinoid Insecticide. The IUPAC (International Union of Pure and Applied Chemistry) name of acetamiprid is (E) - N'- [(6- Chloro-3-Pyridyl) methyl] – N^2- Cyano - N'- methyl acetamidine. The molecular mass of this chemical is 222.67 g / mol. Acetamiprid is a systemic insecticide with translaminar activity. It is used to control hemiptera, lepidoptera, thysanoptera and coleoptera insects. It is an agonist of the nicotinic acetylcholine receptor, affecting the synapses in the insect central nervous system.
The present investigation was conducted using chromosome aberrations, detecting clastogenic activity qualitatively and quantitatively, in an *Allium cepa* L. test system in order to assess the mitotic depressions of the commercial insecticide acetamiprid. Farmers of this locality apply this insecticide as spray in different crops to control sucking insects @ 0.2 g / lt. of water randomly. There has been no study, to our knowledge on the genetic effects of acetamiprid in plant test systems.

MATERIALS AND METHODS

The insecticide used in this study is acetamiprid (CAS No. 135410-20-7), whose trade name is Pride. Its molecular formula is C_{10} H_{11}Cl N_4.

The plant used as test material was *Allium cepa* L. (2n= 16). Ten clean and healthy bulbs of *A. cepa* 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 with the base of the onion touching the surface of the water at room temperature. When the roots reached 1.5 - 2 cm in length, they were treated with different concentrations of insecticide acetamiprid dissolved with distilled water (0.1 g /lt., 0.2 g /lt. and 0.3 g/lt.), for 6, 12 and 18 hours. Controls were also treated with distilled water for the same time periods.

For mitotic studies, the root tips of *A. cepa* were fixed in 1:3 acetic acid-ethyl alcohol mixtures for overnight, followed by 5-7 minutes treatment in 45% acetic acid. Then root tips were hydrolyzed in 1 (N) HCl at 60^0C for 5 minutes, followed by staining with 2% aceto-orcein following the methods described by Sharma and Sharma (1980).

After proper fixation and staining, appropriate squash preparations were made for each of treatment and control. Effect of chemical treatment and control on different chromosome plates were observed under light microscope. To determine the effects of this chemical on mitotic index, 2000 cells were scored in control group and in each treated group. The mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells.

In this study a statistical analysis was done to estimate standard error (SE) of the results. Photomicrographs of cells showing chromosomal aberrations as well as showing normal mitosis were taken using Olympus microscope.

RESULTS AND DISCUSSION

The acetamiprid induced chromosomal aberrations such as stickiness, laggards, c-mitosis, bridges, multipolarity, fragmentations, picnosis etc. (Fig. 3) are present in *Alliun cepa* L. root tip cells. Acetamiprid induced chromosomal aberrations at 0.1, 0.2 and 0.3 g /lt. was statistically significant when compared with untreated control (Table 1). The result also showed that acetamiprid decreased mitotic index (MI) with increasing concentration in treated plants with different concentration and treatment periods (Table 1; Fig. 1). The results also revealed that most of the aberrations observed in chromosomes were at metaphase and anaphase and very few at prophase and telophase compared with untreated control, which explains its cytotoxicity in plant test system. In this study it is clearly observed that rate of chromosomal aberration is proportional to the increment of acetamiprid concentration and duration of treatment (Fig. 2).

Numerous potentially mutagenic chemicals have been studied mainly because they can cause damaging and inheritable changes in the genetic material (Panneerselvam *et al*., 2012 and Nwangburuka and Oyelana, 2011). The commercial form of the insecticide was tested because this is the form that is utilized in agriculture and introduced into the environment.

In this study, acetamiprid decreased the MI in *A. cepa* root tip cells. MI is considered as a parameter that allows one to estimate the frequency of cellular division (Marcano *et al*., 2004). The decrease of MI in *A. cepa* was significant in all concentrations when compared to control, which was dose and duration dependent. Similar type of results were also observed by many authors in the treatment with antimalarial drug (Nwangburuka and Oyelana, 2011), herbicide illoxan (Yuzbasioglu *et al*., 2009) and glycidol (Panneerselvam *et al*., 2012). There are some possible mechanisms for chemically decreased mitotic index in plant cells. The first is that a decrease in MI could be due to blocking of G_1 suppressing DNA synthesis (Shcneiderman *et al*., 1971). The second possible mechanism is a blocking of G_2 preventing the cell from entering mitosis (Van't, 1968). The lowering of the mitotic index might have been achieved by the inhibition of DNA synthesis at S- phase (Sudhakar *et al*., 2001). Another possible mechanism is explained by Chand and Roy (1981).

Table 1: Mitotic Index (mi), Type and Percentage of Mitotic Abnormalities in the Root Tip Cells of *Allium Cepa* l. Exposed to Acetamiprid

Time of Treatment (hrs)	Conc. (g/lt.)	Mitotic Index (Mean ± SE)	Mitotic abnormilities % S	L	C-M	B	M	F	P	% Total Abnormalities
6	Control	18.17 ± 2.4	0	0	2.45	0	0	0	0	2.45 ± 1.1
	0.1	11.87 ± 1.7	2.19	5.9	4.38	0.88	0.88	0	0.25	14.48 ± 6.5
	0.2	11.17 ± 1.4	9.55	2.82	3.17	0.71	0	1.05	2.11	19.41 ± 8.68
	0.3	9.25 ± 1.5	8.14	7.55	4.05	1.57	0.58	0.58	5.2	27.67 ± 9.17
12	Control	15.37 ± 1.6	0	0	0	0	0	3.5	0	3.5 ± 1.52
	0.1	10.32 ± 1.3	6.11	5.12	2.04	0.51	1.53	0	1.51	6.81 ± 7.52
	0.2	10.20 ± 1.8	9.5	6.71	0	3.65	0	1.82	1.5	23.18 ± 7.42
	0.3	8.25 ± 1.5	8.43	6.78	7.31	1.69	1.11	1.69	2.2	29.21 ± 8.14
18	Control	15.42 ± 1.3	0	0	4.5	0	0	0	0	4.5 ± 2.03
	0.1	9.45 ± 1.1	9.2	5.78	2.55	1.28	1.28	0	1.07	21.16 ± 7.96
	0.2	8.77 ± 1.3	5.43	0	11.27	4.07	0	0	5.03	25.80 ± 8.74
	0.3	7.5 ± 1.1	9.42	5.36	5.96	3.87	0.59	0	6.68	31.88 ± 9.71

Abbreviations: *S: Stickiness; L: Laggards; C-M: C-mitosis; B: Bridges; M: Multipolarity; F: Fragmentations; P: Picnosis*

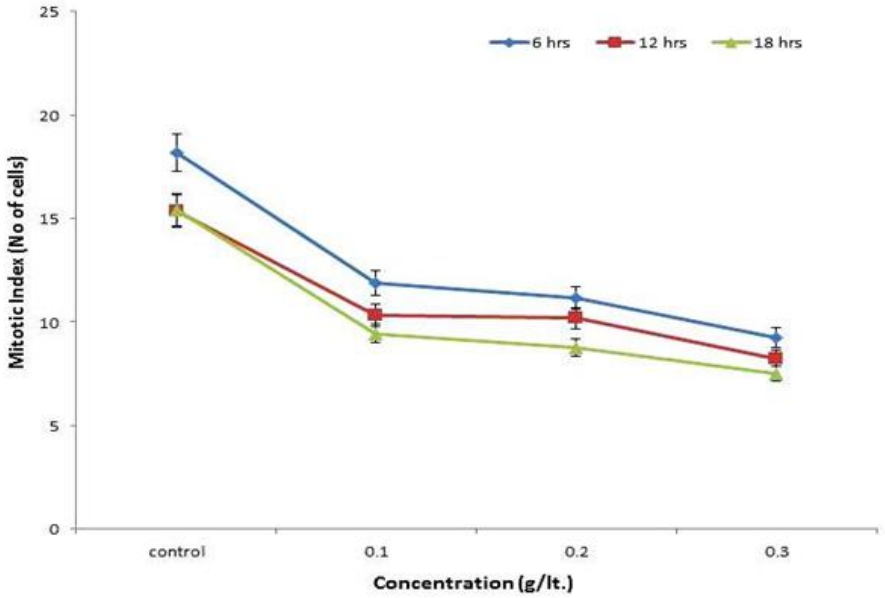

Figure 1: Mitotic Index of *Allium cepa* L. Root Meristem Cells Treated with Acetamiprid at Different Times and Concentrations

Acetamiprid increased the percentage of abnormal cells in *A. cepa*. This increase was significant in all concentrations applied when compared to the control and was also dose and duration dependent. Common abnormalities were stickiness, laggards and c-mitosis. Fiskesjo (1985) reported that sticky chromosomes indicate highly toxic chemical effects that are usually not reversible and will probably lead to cell death. These results were reported by many investigators following treatment with some pesticides (Yuzbasioglu *et al*., 2009 and Ateeq *et al*., 2002). Generally, a mitotic poison causes disturbance of the spindle apparatus, resulting in a c-mitosis effect, which means the complete absence of a spindle. A weak c-mitotic effect produces lagging chromosomes that do not attach to the spindle apparatus.

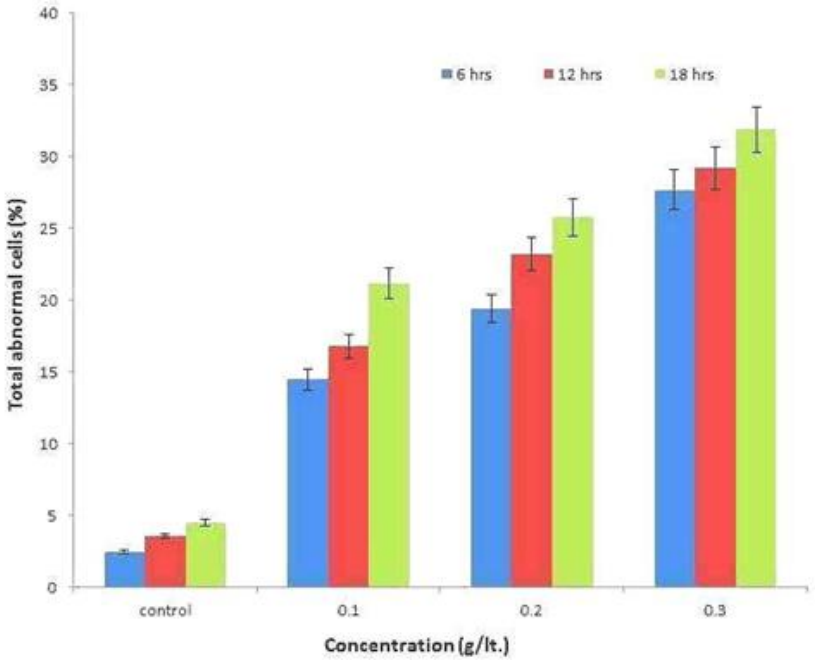

Figure 2: cytotoxic Effects of Acetamiprid at Different Times and Concentrations in A*llium Cepa* l. Root Tip Cells

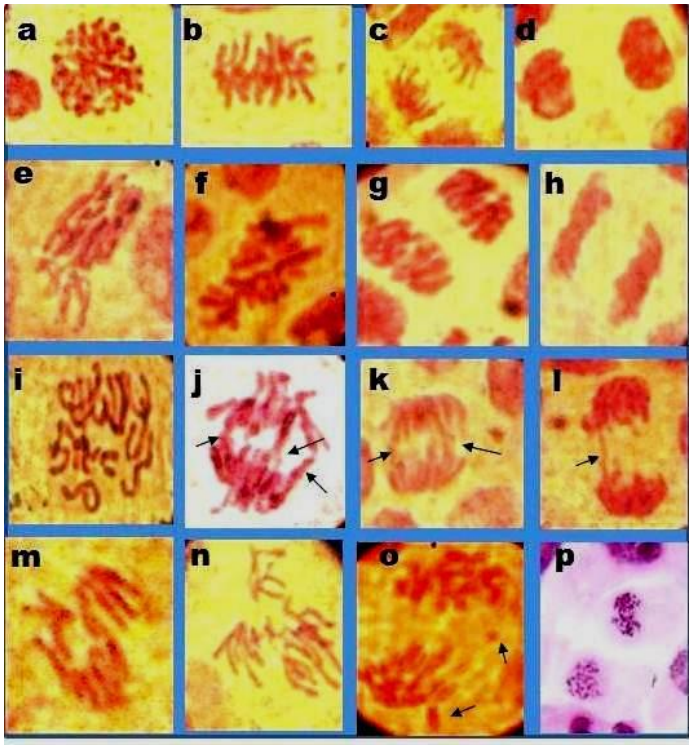

Figure 3: Normal and Abnormal Stages of Mitosis in the Root Tip Cells of *Allium Cepa* L. Treated With Acetamiprid a-p: a: Normal Prophase; **b:** Normal Metaphase; **c:** Normal Anaphase; **d:** Normal

Telophase; **e-f:** Stickiness; **g:** Anaphase laggard; **h:** Telophase laggard; **i:** c-mitosis; **j-k:** Anaphase bridge; **l:** Telophase bridge; **m-n:** Multipolarity; **o:** Fragmentations; **p:** Picnosis.

The formation of c-mitosis, lagging chromosomes and multipolarity may be due to the disturbance in the spindle formation which was effected by the herbicide (Haliem, 1990 and Badr *et al*., 1985).

According to Ateeq *et al*. (2002) pentachlorophenol (PCP), 2, 4- dichlorophenoxy acetic acid (2, 4- D) and 2- chloro-2, 6- diethyl- N- (butoxymethyl) acetanilide (butachlor) induced chromosome aberrations at a statistically significant level. These herbicides induce breaks, bridges, stickiness and laggards in *A. cepa* root tip cells.

Acetamiprid increased chromosomal aberrations significantly in all concentrations (0.1, 0.2 and 0.3 g /lt.) at each hours tested (6 hours, 12 hours and 18 hours). In conclusion, plant models such as chromosomal aberrations (stickiness, laggards, c-mitosis etc.) have been found to be highly sensitive in the detection of hazards arising from insecticides, pesticides, industrial contamination and heavy metals. The data obtained here support these findings and indicate that plant bioassays can be used as an important and integral part of test batteries for the detection of genotoxic effects of chemicals used in the environment.

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