CHAPTER - I

INTRODUCTION

1.1 BACKGROUND

Nepal's population is increasing 2.27 per annum (2001). To support the increasing population His Majesty Government of Nepal (HMGN) has a goal to double food production. In order to meet the food requirements of the nation, a strong research system is vital. So prime needs is to boast up the production of vegetable or food by improved technique, culture practice to raise living standard of nation.

During second half of the 1960, the application of chemical pesticides for the crop improvement and protection was initiated by the department of Agriculture HMGN 1977, then the Nepal Pesticide and chemical Industries Pvt. Ltd (NEPCIL) was established.

Pesticides are also used by the ministry of health for the control of vector-borne disease. HMGN gazetted the pesticide Act, 2048 and pesticide regulation 2050 to regulate the import, distribution, formulation and use of chemical pesticide in the country.

In Nepal, the use of chemical pesticide began during fifties particularly to eradicate the malaria vector. Chemical are used for the indispensable requirements for the control of various disease. However, the use of chemicals has become necessity even though most of this agrochemical has become toxic and proved to have many undesirable consequences in plants. These chemical at higher concentration have been shown mutagenic as carcinogenic effect. In other hand in the positive view, the introduction of chemical is proved dependable, rapid, effective and economical means of controlling the majority of crop pest. (Srihari Reddy and Madhusudhana Rao, 1981).

By the use of pesticides, people are hopeful about tackling the world's major problem, i.e. the shortage of food due to over-growing population. Pesticides have proved to be boon to assist the agriculture. It is both cost effective and efficient way of controlling the undesired pests to gain a better yield harvest. However, some people think that the use of pesticides may be noxious to the well being of lives and can be considered the health- hazard. Still, the agriculturist or the farmers never pace back to use these products. They are promptly accepted in the market and regularly purchased by the farmers.

The quantity and quality of food and fibre production could not be maintained without substantial pesticide inputs (Pedigo, 1996). Frequent use the chemicals has been proved to prevent many undesirable secondary consequences on higher plants (Epstein and Legatar, 1971, Amer and Farah 1974) The chemical pesticide are categorized as insecticide 44%, herbicide 2%, and other 4% (Uddin, 2004).

Pesticide is a descriptive term, which includes all chemicals used to control animal or plant pest such as insecticides, fungicides, weedicides and rotendicides.

Pesticides are the chemical substances used for preventing, controlling destroying, repelling or mitigating pests. These chemicals are poisonous that kill organisms and in some circumstances harmful to crop.

Insecticides are the chemical that is used to kill the harmful pests, specially the insect that is most common pests of the economically important plants.

Effective and systematic insecticides are various formulation based on economic thresholds of insects pest that need to be applied judiciously.

Different author has studied the cytological effect of different pesticides, insecticides, fungicides, herbicides. (See literature review). It was proved from their experiential work to have the effects on chromosome might lead to genetic changes in the affected plants.

Many agronomists suppose that the situation would be far worse if no pesticides used at all, suggesting that if pesticides stopped by here, there would be tremendous increase in the cost of production. Pesticides are one of such chemicals, which protect the standing as well as stored crops and cereals. Each year some two million tons of pesticides products are scattered over the environment in an attempt to control pests and disease Two-Third of which is concentrated in the western industrialized countries 11 percent in the centrally planned economics, and the remaining 20 percent in the developing countries (UNEP 1985).

The present study deals with the cytological effect of the chemical or insecticide Imidacloprid in meristematic cells of *Allium cepa* L. according to the recommended dose.

1.2 General Information on Imidacloprid

Imidacloprid is a widely used insecticide with a relatively low human toxicity. Imidacloprid is a relatively new insecticide having first been registered for use in UK in 1931. In Nepal Imidacloprid has been registered on January 2006 (2062/10/5). Imidacloprid serves as non-repellent, rapid action, effective protection and convenient.

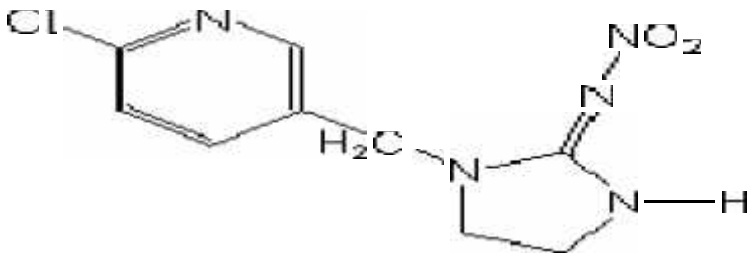
Imidacloprid is an insecticide manufactured by Bayer Crop science (part of the drug and chemical conglomerate Bayer AG). It is sold under several the trade names.

The chemical works by interfering with the transmission of stimuli in the insect nervous system. Specially, it causes a blockage in a type of

neuronal pathway that is; more abundant in insects than in warm-blooded animals (making the chemical selectively more toxic to insects than warm-blooded animals). This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventually death. It is effective on contact and via stomach action.

Imidacloprid is a systemic, choro-nicotinyl insecticide with soil, seed and foliage uses for the control of sucking insects including rice hoppers, aphids, thrips, whiteflies, termites, turf insects, soil insects and some beetles. This enables its use in applications as diverse as flea treatments for cats, control of beetle larvae in lawns and eradication or prevention of termite infestation in buildings and other areas where animals and people may be exposed to any residual chemicals.

1.2.1 Chemical structure of Imidacloprid



1.2.2 Molecular formula

$C_9H_{10}ClN_5O_2$

1.2.3 Molecular weight

255.7

1.2.4 Chemical Name

1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine

1.2.5 Formulation

Dustable powder, granular, seed dressing, soluble concentrate, suspension concentrate and wettable powder.

1.2.6 Trade Name

Admire, Condifor, Gaucho, Premier, Premise, Provado and Marathon all contain imidacloprid as the active ingredient.

1.2.7 Physical and chemical Properties

Imidacloprid is colourless crystals with a weak characteristic odour. Highly soluble in other solvents such as dichloromethane, isopropanol, toluene and fat. Its melting point is 136.4-143.8 C degrees. It can be phytotoxic if it is not used according to manufacture's specifications, and has been shown to be compatible with fungicides when used as a seed treatment to control insect pests.

1.3 Objective

The main objective of the present study is to find out the toxicity effect of the use of higher and lower level of recommended doses of insecticide chemical 'Imidacloprid' in terms of cytological experiment on the growing tissues of *Allium cepa* L.

1.4 Justification of the study

In Nepal, pesticides are used to control pest generally in terai. Large quantities of pesticides are used in Nepal annually. The information about the effect of these pesticides on plant life, environment as well as human life is sporadic, Imidacloprid is insecticide used in Nepal with different trade name like premise, premier, provado, Admire, Gaucho etc. The cytological effect of this insecticide is not yet studied so the present study

is carried out to find out its effect in cells of root meristems of *Allium cepa* L.

1.5 Limitations of the study

The study was carried out under following limitations:

- The work was absolutely lab based and no experiments were done in field.
- The cytological effects were directly included by various concentrations of the insecticide treatment in various time intervals.
- No recovery tests were done.

CHAPTER - II

LITERATURE REVIEW

The evidences accumulated in the past few decades have indicated that the countless number of insecticides, fungicides, laboratory agents, antibiotics, food additives and plant extracts of medicinal values are capable of inducing chromosomal behaviour, chromosomal aberrations and alters the rate of cell division in the treated organisms.

During the present observation on the topic cytological effect of insecticide Imidacloprid, the following literatures were reviewed.

2.1 Cytological effects of Insecticides

Insecticides represent a very large input of chemicals into our environment. The use of insecticides has assumed considerable significance in modern agricultural practices. However, screening of insecticides could have known or unknown mutagenic or carcinogenic effect on the biological system.

Ravindra (1971) studied the effect of Folidol in *Allium cepa*. The insecticide folidol produce many chromosome abnormalities such as anaphase inhibition chromatid and chromosome breakages, abnormal chromosome separation and micronuclei formation.

Amer et al. (1986) observed the effect of insecticide Rotenone or root mitosis of *Vicia faba*. The type of abnormalities induced- disturbed Meta- and anaphase (where chromosome spread irregularly over the cell) in a high percentage, chromosome stickiness, lagging chromosome.

The effects of insecticide Methamidophos is widely used to control pest, which attack different crops. The effect of pure insecticide

"Methamidophos" on the meiosis of *Vicia faba* has been studied. All the conducted treatment affected a significant percentage of abnormal in PMCs plant. Stickiness of the chromosome was shown and only Soheir et al (1986) observed few PMCs with chromosome fragments.

Rao et al. (1987) used the cytological effect of four-insecticide viz. Dhichorvos, monocrotopus, phosalane and oxydemetonmethyl on *Allium cepa* L. root tip. All the four insecticide induced clastogenic and turbogenic effects to different degree. Among the four insecticides, Monocrotophos was observed to be more effective as a clastogenic followed by Dichlorvos, Phosalane and Oxydemetonmethyl respectively.

Yonis et al. (1988) studied effect of insecticide Nuvacron on the mitotic behaviour of *Vicia faba*. The insecticide Nuvacron induced about 63% chromosome bridge 13% fragments 9% lagging chromosome and 1% ring of the total induced aberration.

Zakia et al. (1990) investigated the effect of insecticide Malathion and Tamaron on *Vicia faba* root tip cell. They suggested that the reduction in mitotic activity was due to the inhibition of the DNA synthesis, which considered one of the major prerequisites for a cell to divide. The most dominant types of abnormalities were stickiness and spindle disturbances. In addition, they induce other abnormalities like lagging, bridge, binucleate cells, contraction and synchronization of chromosome movement.

George and Ghareeb (2000) studied the effects of various treatment of Cylon insecticide on mitosis of *Vicia faba*. Prolonged treatment resulted in further inhibition of the mitotic index value. The most frequent types of abnormalities were stickiness, laggards, bridges, fragment, and disturbed phase.

Shrestha Shyam Sharan (2002) studied the cytological effect of insecticide Metasystox on *Allium cepa.* Induction of cytological abnormalities suggests highly chromotoxic effect. Abnormalities like fragmentation of chromosome, micronucleated cell. Highly stickiness and disturbed prophase suggested as the cytotoxic effect of the compound.

Malla and Shakya (2003) studied the cytotoxic effect of an insecticide Fenvalerate on root meristem of *Allium cepa*. It showed variation in phase indices and chromosomal abnormalities. The abnormalities like c-metaphase, stickiness of chromosome, equatorial plate shifting, precocious chromosome, Chromatin Bridge, multipolar anaphase were frequently observed due to effect of this chemical.

Shrestha Shristee (2004) studied the cytological effect of insecticides Quinalphos on *Allium cepa.* The over all result showed the abnormal characters of the dividing cells, the insecticide produced almost all major types of abnormalities. But the laggard and bridge were the rarest while c-metaphase, diagonal metaphase and diagonal anaphase were found to be the most frequent ones.

Pirimipphos-methyl a synthetic insecticide was incorporated into the study at dosage rates of 0.5, 1.0, 2.0g per 20g cowpea seeds for comparison. Result showed that all the seed powder had low insecticidal activity against the bruchid the toxicities of these seed powders increased with increase in dosage as well as increase in the period of exposure to the plant materials. Higher dosage rates (1 and 2g/ 20 cowpea seed) significantly kicked the adults of *Callosobruchus maculates* F. (Emeasor et al, 2005).

2.2 Cytological Effects of Different Agrochemicals and Others

Similar study on the cytological effects of different agrochemical like **Pesticides (**Amer and Ali, 1969; Kaur and Grover, 1985; Jain and Sarbhoy, 1986; Ajay et al., 1987; Lakshmi et al., 1988; Grover and Malhi, 1988; Jain and Sarbhouy, 1988; Singha et al., 1989; Upendra et al., 1991; Priya et al., 1995; Acharya, 1999; Manandhar, 2000 and Kumar and Kumar, 2004), **Herbicides (**Adhikary, 1982; Badr, 1983; Badr and Ibhrahim, 1987; El- Khodary et al., 1990; Chauhan et al,, 1990; Mehmet, Topaltos and Eyyup, 1991; Saeed, Ajmad and Robina, Yasmin, 1992; Currie et al., 1996; EI-Ghamery et al., 2000 and Shrestha, 2000), **Fungicide (**Rei B, J, 1974; Bhunya and Behera, 1984; Somashekhar et al., 1984; Prakash et al,, 1988; Badr, A., 1988; Chand et al., 1991; Pandey et al., 1994 and Shrestha, J, 2004) had been reported.

CHAPTER - III

MATERIALS AND METHODS

For the present work, the root meristem raised from the common onion (*Allium cepa* L. 2n=16) was used. The effect of systemic insecticide Imidacloprid was used as standard one.

3.1 Materials

Onion (*Allium cepa*) meristems as bioassay.

3.1.1 Onion Bulbs and Bulb Rooting Experiment

Healthy looking and approximately equal sized bulbs of *Allium cepa* L.was collected from the local market of the Kirtipur. The onion bulbs were selected because of easiness to handle, fast growth rate and convenient for cytological observations.

The bulbs were thoroughly washed with tap water and the old roots present in the bulbs were removed properly. To obtain the actively growing root tip, these bulbs were placed in the basal side facing downward placed over coupling jar filled with tap water with rooting bases touching the water level. The tap water in the jars was replaced at 24 hours intervals to check the growth the microorganisms and to prevent as injurious effect on the growth of the roots due to dissolution of yellow pigment from the outer scales of the bulbs by tap water.

3.1.2 Preparation of Suspension for Experiment

For the root treatment, four different concentration of Imidacloprid viz (25%), (50%), (75%), (100%) were prepared. When 1 ml of Imidacloprid was dissolved in 1000 ml of tap water, then it was recommended or

standard solution, which mean 100%. Similarly, the standard solution was made different concentration by adding tap water in that solution.

3.2 Methods

3.2.1 Treatment of the Rooting Bulbs

When the lateral roots of *Allium cepa* L. were about 2 cm long, they were exposed to the freshly prepared test solutions of different concentration for 3, 6, 12, 24 hours at room temperature, Control root were simultaneously treated with tap water in order to compare with that different concentration of Imidacloprid used. The schedules of treatment are given in Table.

Table no. 1: Schedule of Treatment

Time of transferring the materials to the suspensions (Imidacloprid)	Time period in hours	Fixing time
10:45 am (control)	0	10:45 am
7:45 am	3	10:45 am
4:45 am	6	10:45 am
10:45 pm (Previous night)	12	10:45 am
10:45 am (Previous day)	24	10:45 am

3.2.2 Preparation of Agent for Cytological Study

The following agents were used fro fixing and staining the tissues:

Fixing Agent - Acetic - Alcohol
Glacial Acetic acid 1 parts
Absolute alcohol 3 parts
Preserving Agent - Alcohol
70% Ethyl alcohol was used for short time preservation of tissue before staining.

Staining Agent - Aceto - Carmine 2%

Carmine	2gm
Glacial acetic acid	45ml
Distilled water	55ml

3.2.3 Cytological Fixation

The cellular and structural element all preserved in their natural condition by the process of fixation. In the present experiment, acetic alcohol is used for cytological fixation. The treated and control root tips of *Allium cepa* L. were thoroughly washed in tap water and excised and fixed in freshly prepared 1:3 Acetic alcohol for 24 hours. 70% Ethyl alcohol was used for short time preserving of tissues before staining. The fixing time was 10:45 am.

3.2.4 Aceto-Carmine Squash Technique

Cytological preparation were made by using the Aceto carmine squash technique, whereby the fixed roots (about 1 cm) were stained in 2% Acetocarmine mixed with 1 N HCL in ratio 9:1 for hydrolysis and squashed (about 2 mm root tips) were made in 45% Acetic Acid on a clean slide. More than 5 root tips were studied from each treated and non-treated onion bulbs.

3.2.5 Preparation of Permanent Slide and mounting Media

Celariers (1956) method was followed for the preparation of permanent slide. The composition of dehydration grade was as follows:

A)	Glacial Acetic acid	1part
	Butyl Alcohol	1 part
B)	Glacial Acetic acid	1 part
	Butyl Alcohol	3 part
C)	Butyl Alcohol	pure (100%)

After dehydration, the mounting of stained meristematic tissue was done in Euparol.

The microphotograph were taken from the permanent and freshly prepared temporary slides in Central Department of Botany, Tribhuvan University, Kirtipur.

3.2.6 Cytological Observation and Analysis

The observations of around 5000 cells from at least five different root tips treated with various concentration of solution (Imidacloprid) were recorded. The indices and abnormalities were scored and analyzed by using the following formula, according to Levan in 1949 (C.F.Kihlman 1971, Medeiros and Takahashi 1987). (See Appendix)

3.3 Statistical Analysis

"The Friedman's two-way analysis of variance by rank's method" (Siegal, 1956) was applied to calculate whether duration of treatment effect the mitotic index value of *Allium cepa* L. root tip cell or not. The data from table No. 7 and 8 were submitted for calculation as below:

$$X_r^2 = \frac{12}{Nk(k+1)} \sum_{j=1}^{k} (Rj)^2 - 3N(k+1)$$

Where,

N = 4 number of rows (concentration)

K = 4 numbers of columns (conditions, time of treatment)

Rj = sums of ranks in the j^{th} column

$\sum_{j=1}^{k}$ Directs one to sum of the squares of the sums of ranks over all k conditions

The degree of freedom (df) was determined by reference to the chi [x^2] square distribution with df = k - 1 and the significance value were matched at percentage (p) < 0.05.

CHAPTER - IV

RESULT

In the present study, the cytological effects of pesticide Imidacloprid on dividing cells in the root meristem of *Allium cepa* L. were analyzed. Comparative studies of control and Imidacloprid treated root meristems were done. The cytological effects have been done on the basis of variation in mitotic index and chromosomal abnormalities induced by the chemical. From statistical analysis, the obtained value for chi square (x^2) was found to be 3.9 where as tabulated value for chi square was 7.8 at percentage (pO <0.05. The results were tabulated as in Table no. 2 to 8 (See Appendix) and elaborated under separate sub-headings which are as follows.

4.1 Effects in Mitotic Index

The mitotic indices (MI) obtained for root tip cell treated with different concentrations of Imidacloprid in relation to duration of treatment has been shown in Table no. 3 (See Appendix) and Fig 1. Mitotic index did not show any regularity. The mitotic index value of treated meristems was higher than control at different concentration in all cases except some interruption in some periodic treatment. The mitotic index value is decreasing in 100% concentration at 3 hour, 25% at 6 hours and 75% at 12 hours of treatment than that of control. The mitotic index value is 50.8% at control. The highest mitotic index value is 57.93% in 100% concentration at 6 hours and the lowest value is 46.67% in 100% concentration at 3 hours of treatment.

4.2 Effects in Phase Index

4.2.1 Effects in Prophase Index

Prophase index (Pro I) is shown in Table no. 3. It is plotted against time and concentration in Fig 2. It shows that the prophase index is generally increased as the duration of treatment increase with respect to concentration. Prophase index is 88.32% at control and the maximum 92.7% in 100% concentration. This value decrease 87.57% in 25% concentration at 3 hours of treatment.

4.2.2 Effects in Metaphase Index

Metaphse index is shown in table no. 3. It is plotted against time and concentration in Fig. 3. Metaphse index did not show any regularity in its value. The metaphase index value is 7.17% at control. The maximum value of metaphase index is 8.5% at 6 hours of treatment of 25% concentration and minimum value is 4.1% at 3 hours of treatment of 100% concentration.

4.2.3 Effects in Anaphase and Telophase Index

The anaphase and telophase index (Ana-Telo I) has been shown in table no. 3 and is plotted against time and concentration in Fig 4. The anaphase and telophase index is 4.07% at control and it is maximum a 4.36% at 6 hours of treatment for 50% concentration. The least value is 2.11% at 3 hours of treatment for 100% concentration.

4.3 Relation between Mean Mitotic Indices and Mean Phase Indices

Mean Mitotic index and phase indices obtained from root tip cells of *Allium cepa* L. treated with Imidacloprid is shown in Table no. 4 and is plotted against time and concentration in Fig no. 9. The Mean Mitotic index increases with increase in treatment period than that of the control

except 6 hours of treatment. The value of mean mitotic index at control is 50.8% the Mean mitotic index is maximum at 12 hours of treatment where as it is lowest 47.07% at 6 hours of treatment.

Also increases with increases in treatment period than that of control. The mean Prophase value is lowest 88.32% at control whereas it is highest 91.14% at 24 hours of treatment. The Mean Metaphase index value is lowest 3.5% at 3 hours of treatment whereas it is highest 7.17% at control. The Mean Anaphase and Telophase index value decreases with increase in treatment period than that of control. The value of Mean Anaphase and Telophase index at control is 4.00% and it is lowest 3.80% of treatment

4.4 Percentage of Abnormal Cells

Table no. 5 shows total percentage of abnormal cells at each phase and different concentrations of Imidacloprid at different time of treatment.

4.4.1 Total Percentage of Abnormally Dividing Cells

The total percentage of abnormally dividing cells is shown in table no. 5 and is plotted against time and concentration in Fig 10. In normal untreated roots 3.96% abnormal cells were observed. The abnormalities in cells were observed mostly in treated roots. The percentage of abnormal cells did not show regularity with the time of treatment and concentration. Among all the treated concentrations and time period, the highest abnormal value is 7.71% at 100% concentration of 6 hours of treatment and lowest value is 2.4% at 25% concentration of 3 hours of treatment.

4.4.2 Total Percentage of Prophase Abnormalities

Table no. 5 and Fig 11 shows total percentage of Prophase abnormalities in different concentration of Imidacloprid and different time of treatment. In all groups percentage of prophase abnormalities decreases with increase in concentration and time of treatment than that of control. The prophase abnormalities at control is 0.7%. The highest abnormal value is 2.7% at 6 hours of treatment for 100% concentration.

4.4.3 Total percentage of abnormal Metaphase

Table no. 5 and is plotted against time and concentration in Fig 12. In all groups, percentage of metaphase abnormalities decreases with the increase in concentration and time of treatments than that of control. The metaphase abnormalities at control are 2.5%. The lowest abnormal value is 0.7% at 12 hours of treatment for 25% concentration.

4.4.4 Total Percentage of Ana-Telophase Abnormalities

Table no. 5 and Fig 13 shows total percentage of ana-telphase abnormalities in different concentration of Imidacloprid and different time of treatment. In normal untreated roots 0.46%, ana-telphase abnormalities were noted. Among treated roots, the maximum value is 1.86% at 6 hours of treatment for 100% concentration and minimum value is 0.76% at 6 hours of treatment for 25% concentration.

4.5 Proportion of Abnormalities in Phases among Experimental Groups

The proportions of abnormalities in dividing cells at different hours of treatments in different concentration of Imidacloprid solution are given in Table no. 5. Abnormalities in metaphase and ana-telophase were higher in comparison to prophase at different hours of treatments for all concentrations and time period. Among the recorded abnormalities, the

highest percentage of abnormal cells are found in metaphase which is 3.17% at 6 hour treatment in 100% imidacloprid whereas the lowest percentage are found in control which is 0.3% at 6 hours of treatment. The untreated roots also showed the abnormalities in all phase at higher rate similar to other phases.

4.5.1 Abnormal Mitotic Phases on 25% Imidacloprid Treatment

Table no. 5 and Fig 6 shows the abnormal mitotic phases in *Allium cepa* L. treated with 25% concentration of Imidacloprid. In this case, prophase abnormality was more than that of metaphase, Ana-telophase. Prophase abnormally was highest in 6 hours of treatment and it was lowest in 3 hours of treatment. Metaphase abnormality was highest in 6 hour of treatment and it was lowest in 24 hours of treatment which shows the decrease in abnormalities with increase of time. Ana-telophase abnormality was highest in 6 hours treatment and it was lowest in 6 hours of treatment.

4.5.2 Abnormal Mitotic Phases on 50% Imidacloprid Treatment

Table no. 5 and Fig 7 shows the abnormal mitotic phases in *Allium cepa* L. treated with 50% Imidacloprid. In this treatment, prophase abnormality was less than that of metaphase and ana-telophase. Prophase abnormality was not regular; it decreases in 24 hours of treatment. Metaphase abnormality was going on increasing from 3 hours upto 24 hours treatment and it attained highest value in 6 hours of treatment while ana-telophase abnormality was highest at 6 hours of treatment and lowest at 3 hours of treatment.

4.5.3 Abnormal Mitotic Phases on 75% Imidacloprid Treatment

Table no. 5 and Fig 8 shows the abnormal mitotic phase in *Allium cepa* L. treated with 75% of Imidacloprid.In this case, prophase abnormality was

less than metaphase and ana-telophase. Prophase abnormality was maximum at 6 hours of treatment and low at 3 hours of treatment, although it does not show any regularity. Similarly, metaphase abnormality does not show any regularity. It decreases at 3 hours of treatment then increase in 6 hours than decreases in 12 hours and again increases at 24 hours of treatment. Ana-telophase abnormality was maximum at 6 hours of treatment and was minimum at 3 hours of treatment.

4.5.4 Abnormal Mitotic Phase on 100% Imidacloprid Treatment

Table no. 5 and Fig 9 shows the abnormal mitotic phase in *Allium cepa* L. treated with 100% of Imidacloprid. Prophase abnormality does not show regularity, it decreases at 3 hours of treatment than increases at 6 hours than decreases at 12 hours and again decreases at 24 hours of treatment. Metaphase abnormality is maximum at 6 hours of treatment and was minimum at 12 hours of treatment. Metaphase abnormality does not show any regularity. Ana-telphase abnormality was going on increasing with time and concentration. Ana-telophase abnormality is maximum at 6 hours of treatment and was minimum at 3 hours of treatment.

4.6 Effects in Chromosomal Behaviour

The pesticide Imidacloprid is capable of inducing various types of chromosomal abnormalities in the meristematic cell of *Allium cepa* L. during mitotic cell division. Abnormal phases from treated groups are given in plate no... ... to .. ..

4.7 Nature of Abnormal Cells in Non-Dividing Cells

The abnormalities found in non-dividing phase were plasmolysed cell or shifting of nucleus to the polar position.

4.8 Nature of Abnormal Cells in Dividing Cells

The abnormalities found in dividing cells are as below:

4.8.1 Nature of Abnormal Cells in Prophase

Disturbed prophase, clumping of chromosomes and unequal condensation of chromatin thread.

4.8.2 Nature of Abnormal Cells in Metaphase

C-metaphase with shorting of chromosome at metaphase was the most common type of abnormality. C-metaphase, fragments, sticky metaphase were the dominant types of abnormalities found in metaphase. Star-shaped metaphase and breaks in chromosome were also noted.

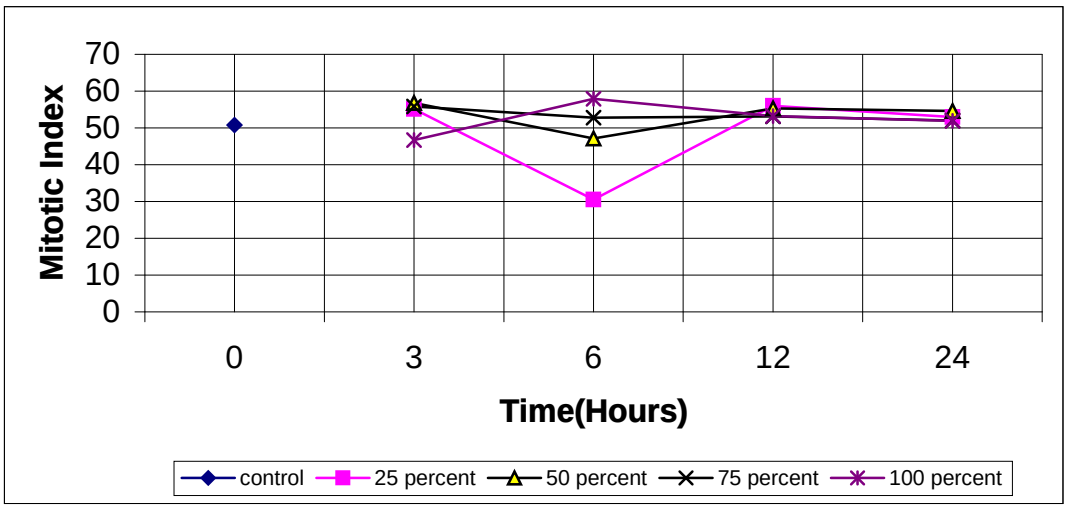
4.8.3 Nature of Abnormal Cells in Anaphase

Precocious arms and shifting of poles (diagonally arranged chromosome group is most common) Disturbed anaphase, laggard, bridges, fragmentation of chromosome were also observed.

4.8.4 Nature of Abnormal Cells in Telophase

The dominant abnormalities found in telophase were of (diagonal shifting is common type) Unequal cytokinesis, diagonal cytokinesis delay in cell plate formation and binucleated cells. Unequal condensation of daughter chromosomes and trinucleated cells were also noted in less frequency.

Figure 1: Graph Mitotic Index of *Allium cepa* L. root tip cells Vs Treatment Time with Given Concentration of Imidacloprid.



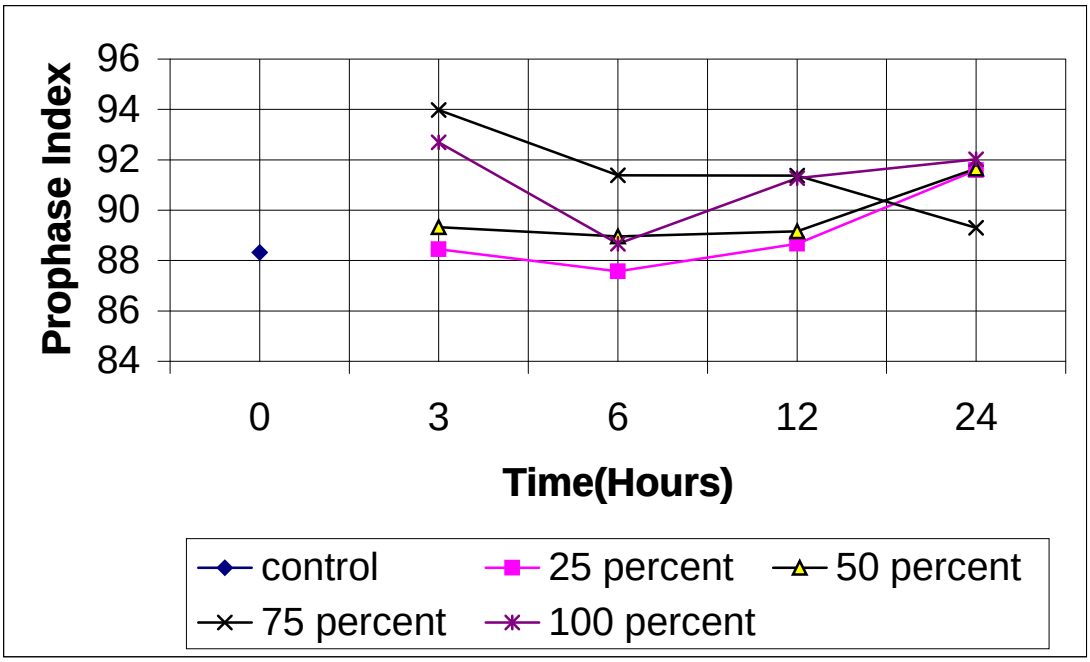


Figure 2: Graph Prophase Index of *Allium cepa* L. root tip cells Vs Treatment Time with Given Concentration of Imidacloprid.

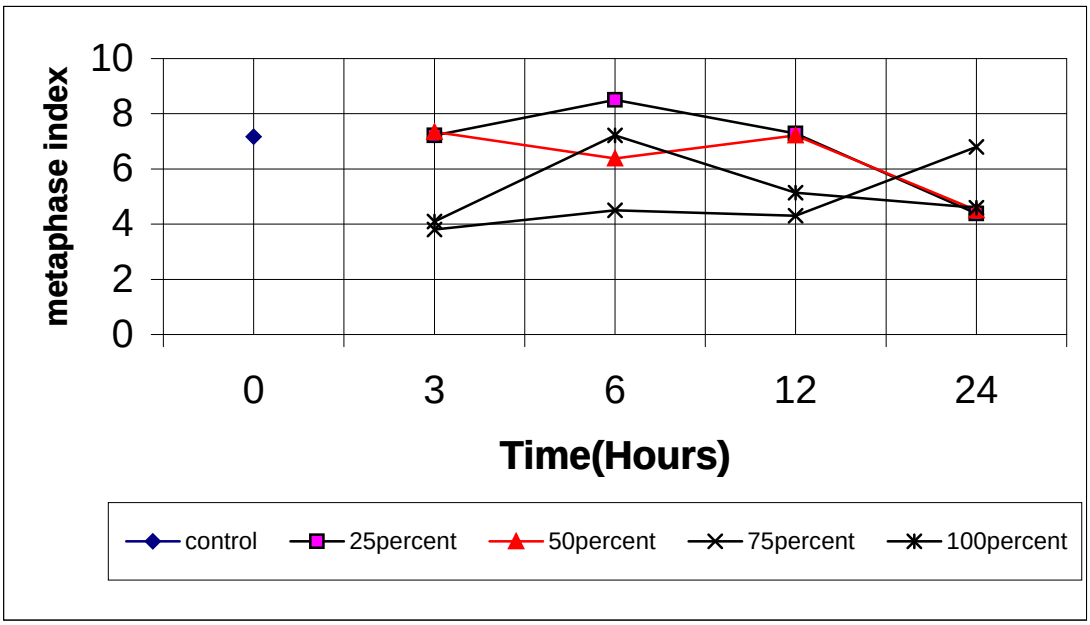


Figure 3: Graph Metaphase Index of *Allium cepa* L. root tips Vs Treatment Time with Given Concentration of Imidacloprid.

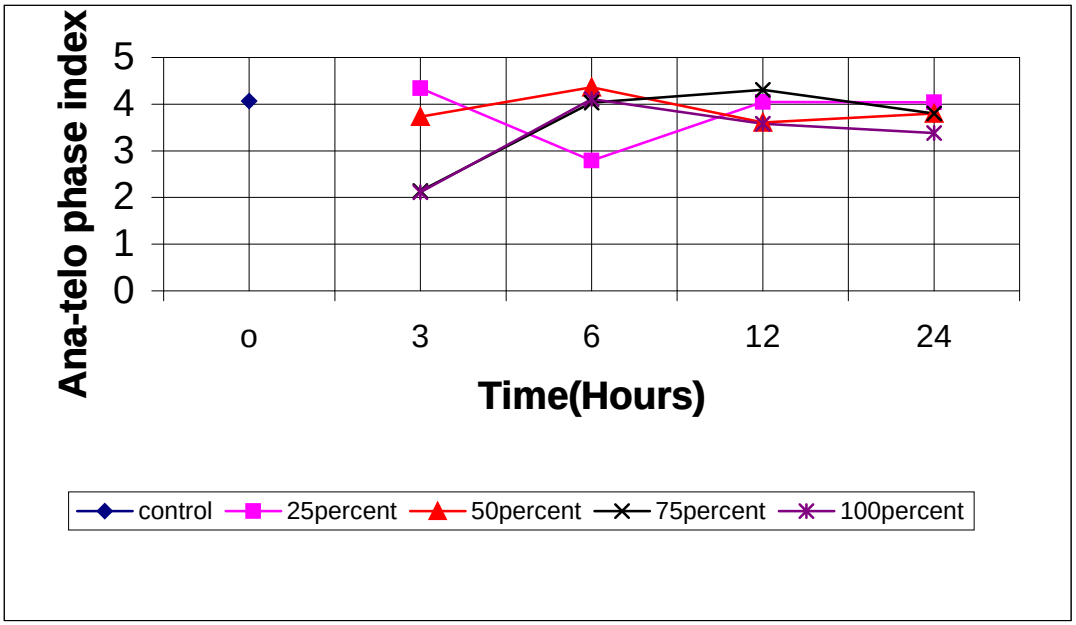


Figure 4: Graph Anaphase and Telophase Index of *Allium cepa* L. root tip cells Vs Treatment Time with Given Concentration of Imidacloprid.

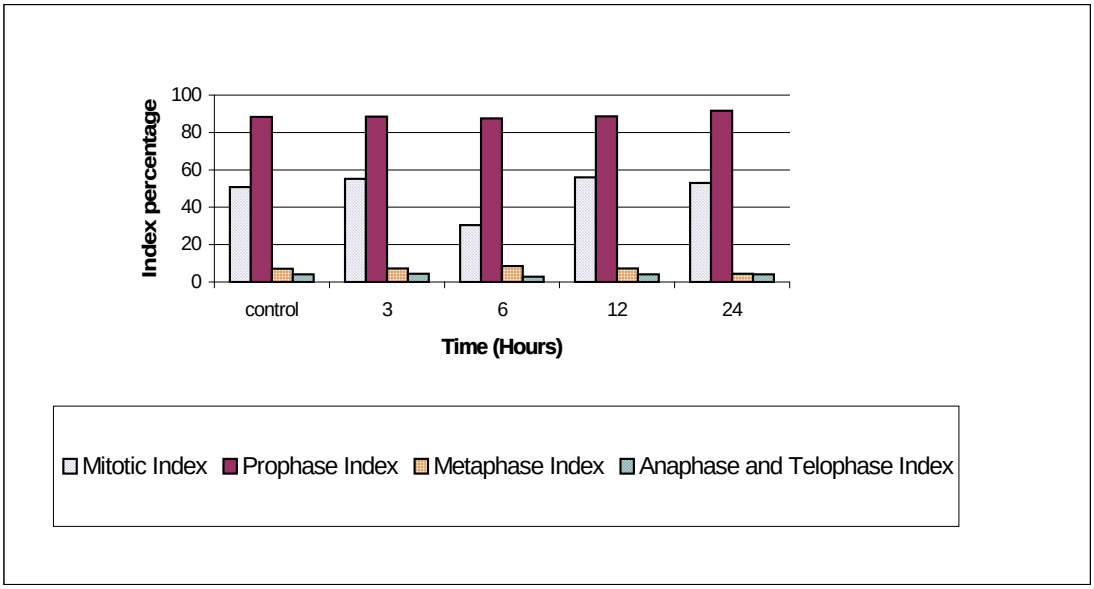


Figure 5: Bar Diagram if Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices Vs Treatment Time with 25% Concentration of Imidacloprid.

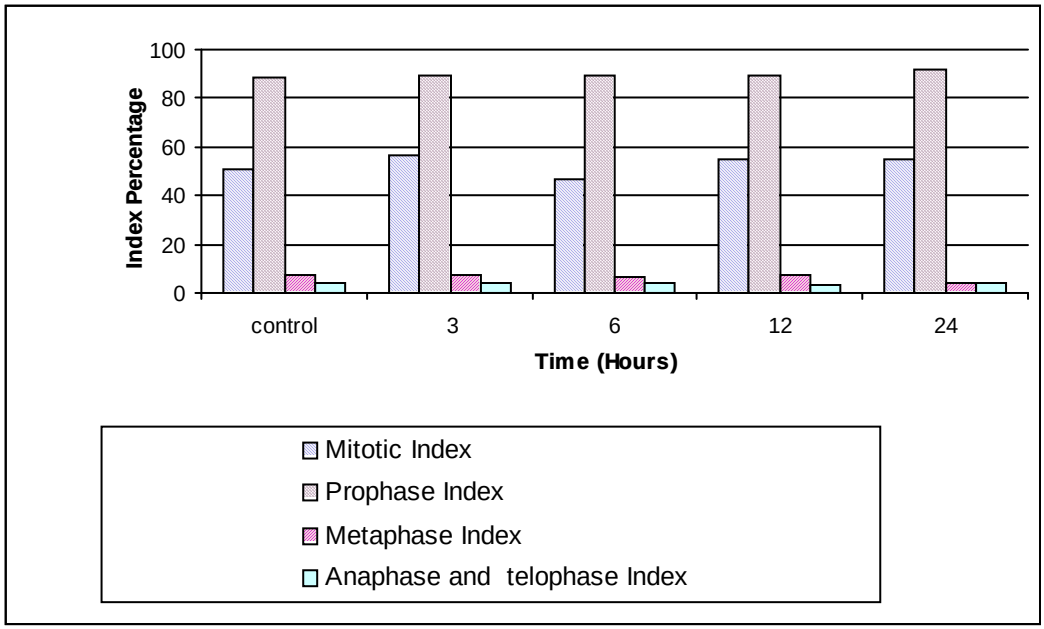


Figure 6: Diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices Vs Treatment Time with 50% Concentration of Imidacloprid.

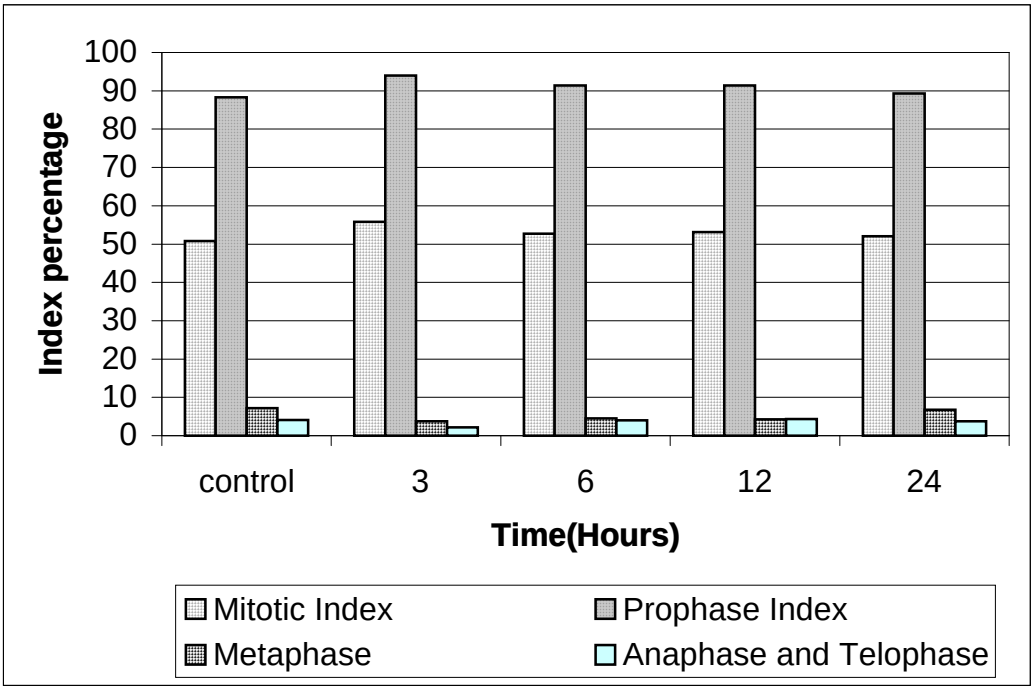


Figure 7: Bar Diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices Vs Treatment Time with 75% Concentration of Imidacloprid.

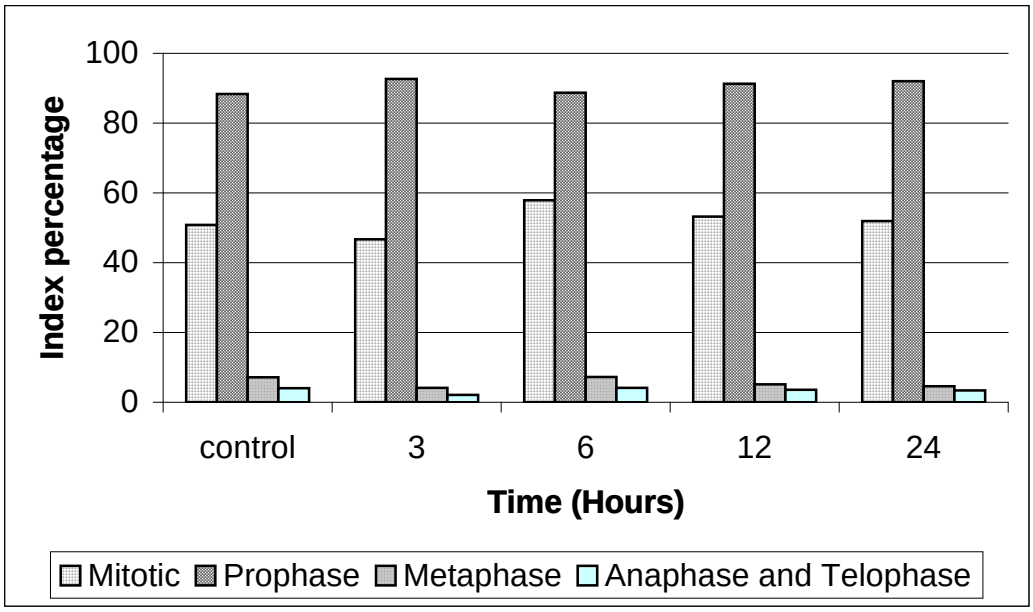


Figure 8: Bar Diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices Vs Treatment Time with 100% concentration of Imidacloprid.

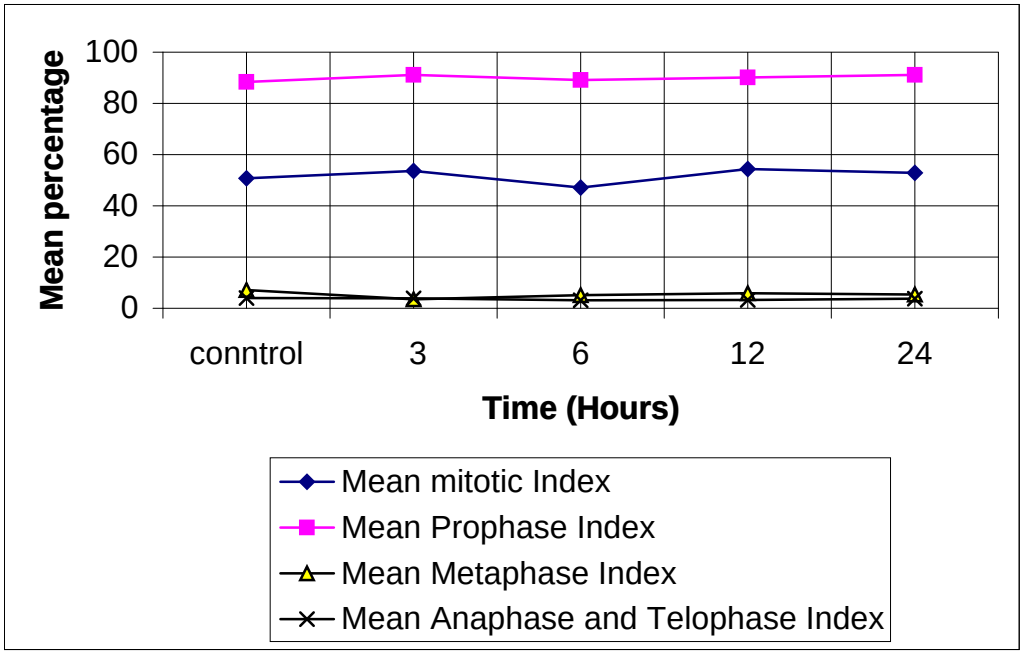


Figure 9: Graph of Mean Indices of Mitotic, Prophase, Metaphase, Anaphase and Telophase Vs Treatment Time with Mean percentage Concentration of Imidacloprid.

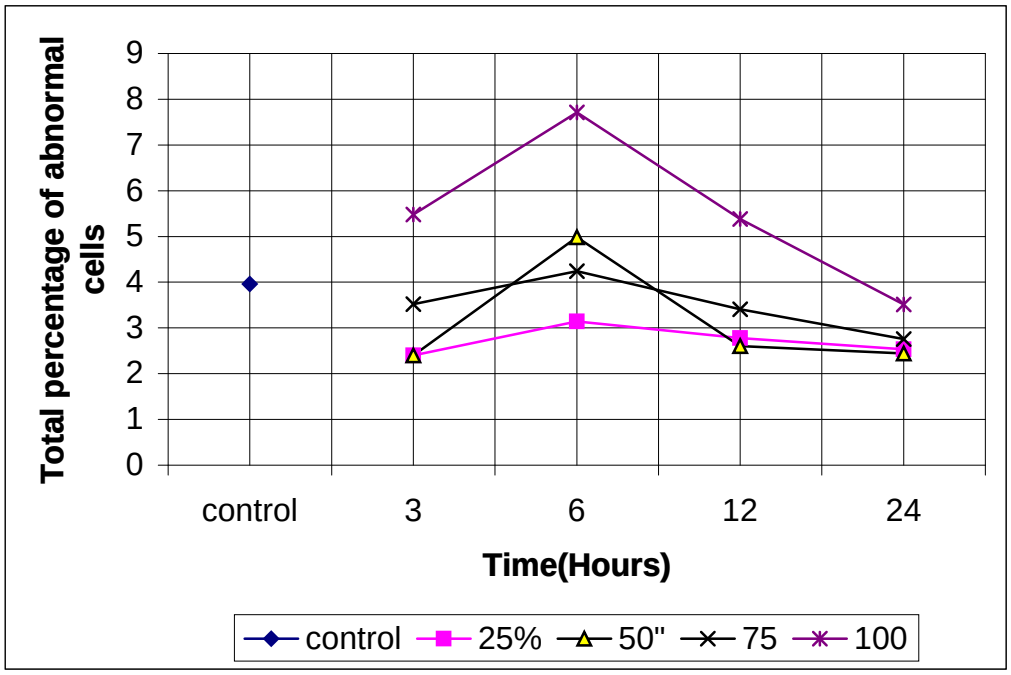


Figure 10: Graph of Total Percentage Abnormal cells Vs Treatment time with Given Concentration of Imidacloprid.

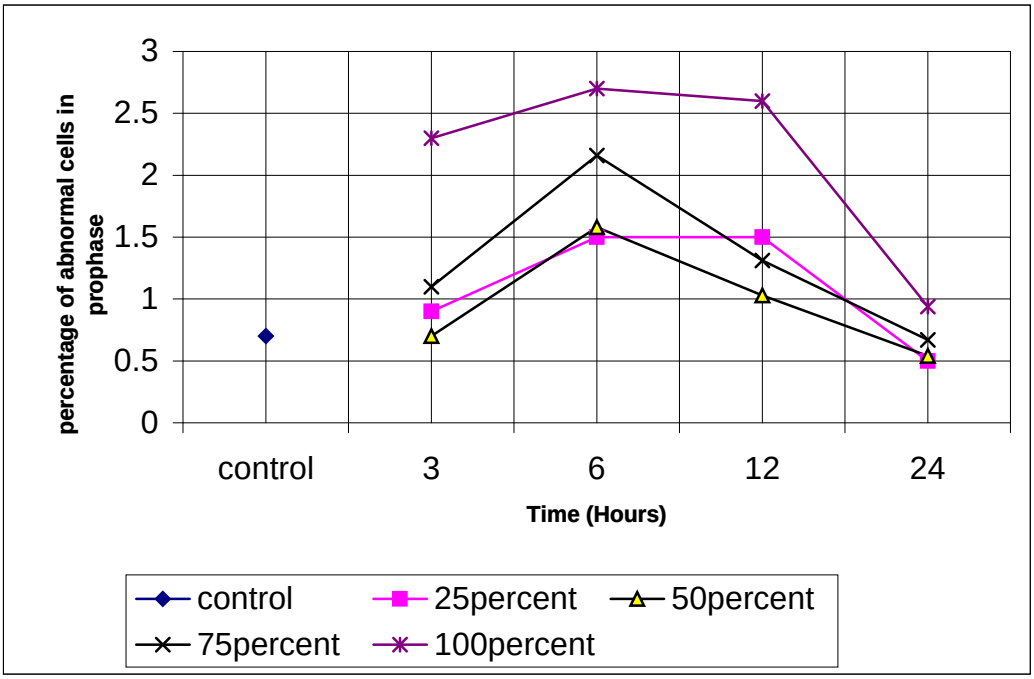


Figure 11: Graph of Total Percentages Abnormal Cells in Prophase Vs Treatment time with Given Concentration of Imidacloprid.

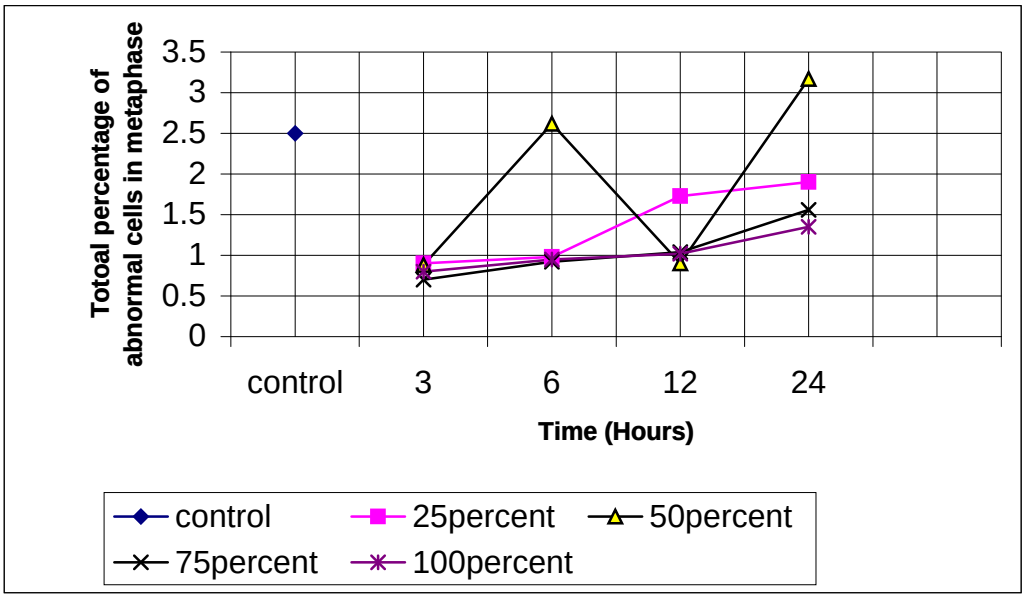


Figure 12: Graph of Total percentage of abnormal cells in Metaphase Vs Treatment Time with Given Concentration of Imidacloprid.

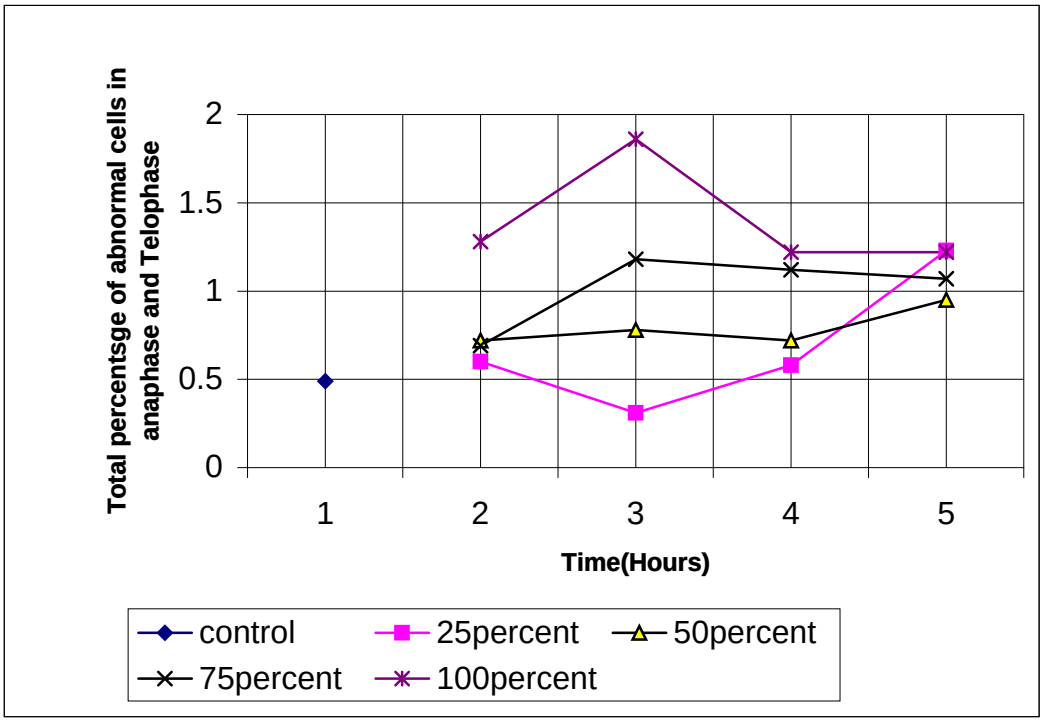


Figure 13: Graph of Total percentages Abnormal Cells in Anaphase and Telophase Vs Treatment Time with Given Concentration of Imidacloprid.

Fig.: Precocious Chromosome

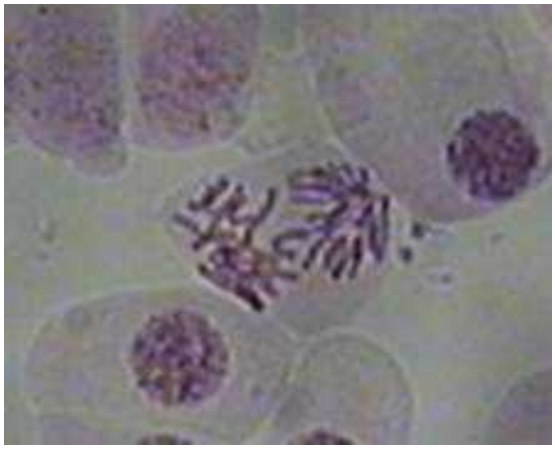


Fig.: Lagging Chromosomes

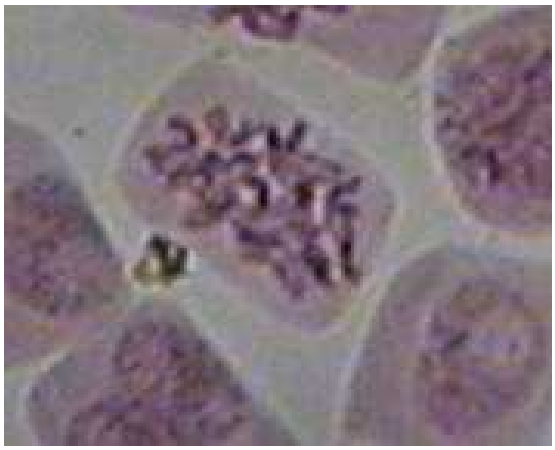


Fig.: C-Metaphase Chromosomes Synchronisation

Fig.: Non- condensation at Telophase

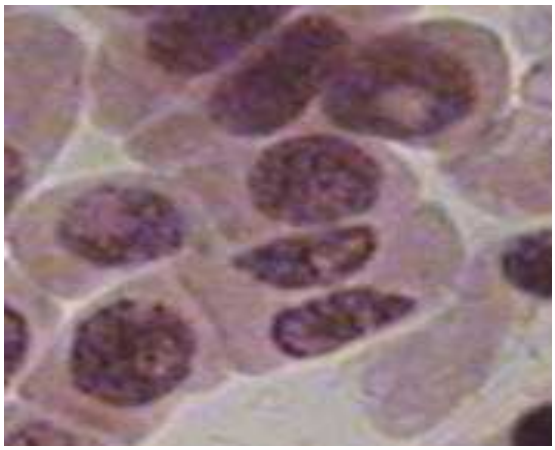


Fig.: Binucleated cell (Lower)

Fig.: some cells with early Prophase

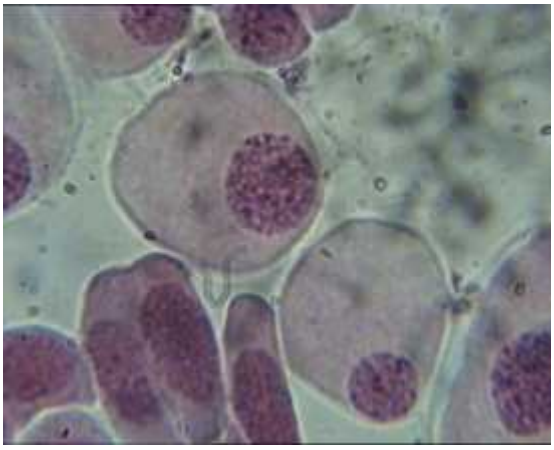


Fig.: Plasmolysed cells

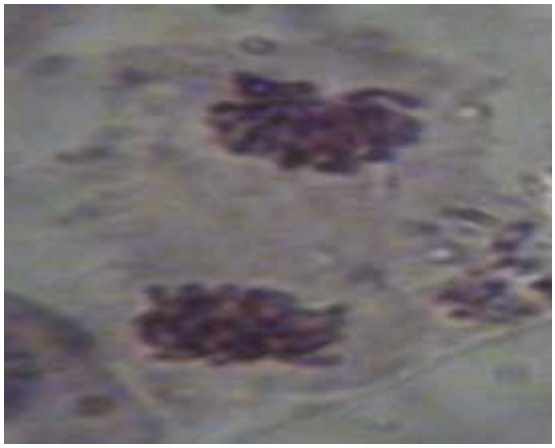


Fig.: Mid Telophase

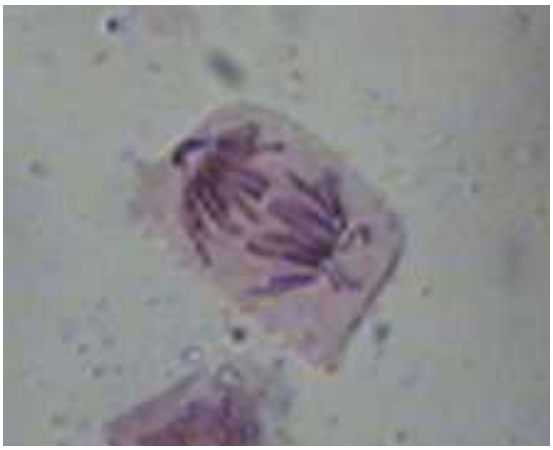


Fig.: Anaphase with Precocious arms

Fig.: Anaphase with diagonal Pole formation

Fig.: C- Metaphase

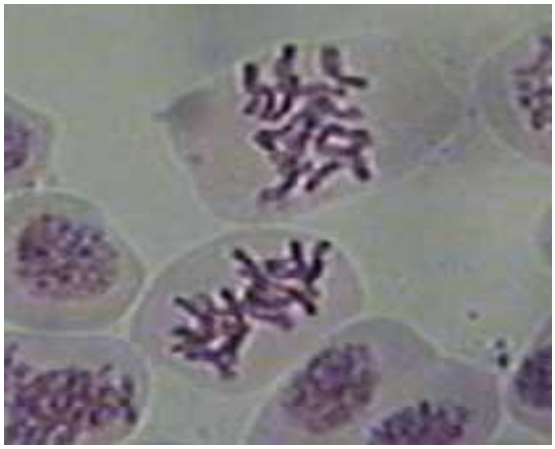


Fig.: C-Chromosome orientation at equatorial region

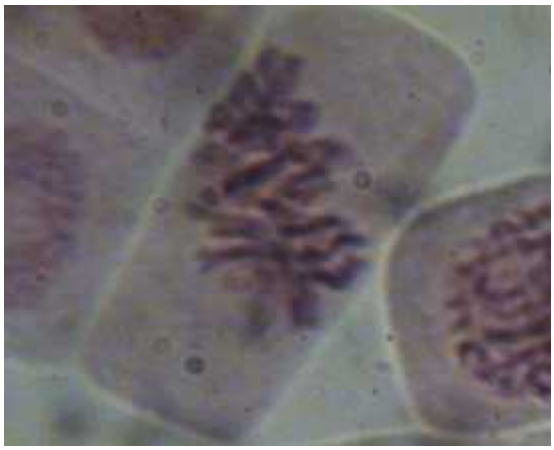


Fig.: Diagonal Metaphase

Fig.: Mid Prophase Chromosomes

CHAPTER - V

5.1 Discussion and Conclusion

The present work has been performed with the objective to acquire the result of the cytological effects of insecticides Imidacloprid on somatic cell division in root meristems of *Allium cepa*. It has been observed that Imidacloprid is capable to induce various types of abnormalities in root meristem of *Allium cepa*. Chromosomal aberrations in somatic and reproduction cells by pesticide Rogar and Bavistin have been reported by [Upendra and Sinha 1991].

In present observation, the mitotic index value increased as concentration and time of treatment increases corresponding in relation to control. The average mitotic index value was greater in 24 hour (52.88%). It suggests that it has stimulatory effect on mitotic cell division. Similar result was obtained by Shrestha (2004) in *Allium cepa* L. treated with preservative Potassium Metabisulphate. Rao *et al.* (1988) also clearly shown the increase in mitotic indices value root meristems treated with Endosulfan. Such increase in the mitotic indices is the result of accumulation of c-metaphase configuration (Badr, 1983).But at the same time when the dose is increased, the MI value has found to be increased. The reduction in mitotic activity with respect to control samples may be described as due to the inhibition of DNA synthesis which is one of the major prerequisites for a cell to divide. (Zakia et al 1989).

By the observed data, the calculated statistical chi-square value is 3.9 but the tabulated chi-square value is 7.8.A data becomes significant when the chi-square value is greater than this value. Since the chi-square value of

the present experiment is less than the given tabular value, it can be concluded that the chemical is less significant. In other words, it has substantially less significant mutagenic effect on the growing root tip cells of *Allium cepa* L.

This value shows the less effectiveness of the chemical related at the time of treatment in cell division. Pal and Bhunya (1992) also reported that at higher dose and lowest dose treated tip were not statistically significant.

Prophase index value did not show any regularity in all cases prophase index was greater than that of control value. This indicates that the chemical has some kind of mechanism for the prophase poisoning where cells are able to enter mitosis but be arrested in the prophase and the further division is ceased, thus, resulting high frequency of prophase cells (Prasad And Das, 1977). The increased prophase index in higher concentration may be due to the prolongation of prophase stage effecting the spindle formation by chemical. Shrestha (2002) observed similar result in Isoproturon treated root tips of onion. In average, the prophase index value was increased with increase in time and concentration. The mean prophase index was grater in 24 hours (91.14%).

Metaphase index showed no regularity. Metaphase indices are obtained in highly fluctuating manner. Metaphase index was higher than Ana-telophase indices and lower than prophase index. In average, metaphase index value was decrease with increase in duration of treatment. These decrease in metaphase index value may be due to prolonged prophase or the dividing blocked in prophase and do not enter in further phase. The increase in metaphase index may be due to the high accumulation of

metaphase cells. Similar result was obtained by Shrestha (2004) in onion root tip treated with Potassium Metasulphite.

In control samples, few abnormal cells were observed but the frequency is quite less as compared to those in the treated samples. Abnormalities were discovered often in metaphase, anaphase and telophase than in prophase. Ana-telophase indices value were lower than the prophase and metaphase. Ana-telophase indices also do not show any regularity. In average, Ana-telophase indices values decrease with increase in time and concentration. This decrease in indices may be due to prolonged prophase and do not enter into further division. Similar result was obtained by Shrestha (2004) in Carbendazin treated root tips of onion.

In control cells, very few abnormalities were obtained in comparison to the treated ones. Most of abnormalities were seen in metaphase, Ana-telophase in comparison to prophase. In prophase, clumping of chromosome and disturbed prophase are found. Disturbed prophase was indicated by the abnormal arrangements of chromatin thread. Similar observation was made in *Vica faba* and *Gossypium barbandense* after seed soak and root treatment with "Rogar" (Amer and Farah, 1974).

Abnormalities in interphase are the binucleate cells, presence of micronuclei, and tri-nucleolate cells. Formation of binucleate cells was one of the common abnormalities in the light microscopic studies (Chauhan & Sundarman, 1990).Mechanism of cytokinesis in plant cells involves the formation of new cell wall with the help of different organelles such as microtubules, golgi complex and possibly mitochondria (Molleuhar et al. 1980, Pickett-Heaps 1967). This possibly suggests that the chemical interferes in the process of cytokinesis by

affecting the functions of microtubules and golgi complex and leads to the formation of binucleate cells (Chauhan and Sundaram, 1990).

Various types of abnormalities found in metaphase were c-metaphase, stickiness in metaphase and disturbed metaphase. Polyploidy and star shaped metaphases were also observed.

High percentage of c-metaphase was found in metaphase treated with insecticides Imidacloprid. This type of abnormality was produced because of inhibition of spindle fibre formation. In this case, it causes an arrest mainly at metaphase (EL-Khodary et al, 1989). Similar type of abnormalities in metaphase cells were observed in root meristem of *Allium cepa* L. after treated with insecticides Rotenone (Amer and Mikhael, 1986).

Another abnormalities produced by insecticide Imidacloprid were stickiness in metaphase. The sticky nature of chromosome at metaphase may be due to the delay in chromosome movement. Thus the chromosome could not reach to the poles and remains scattered in the cytoplasm and appear condensed and sticky (Ajay and Sarbhoy, 1988). Similar type of abnormality in metaphase cells were observed in root meristem of *Allium cepa* L. after treated with Acrylad monomer (Shanker et al. 1987) also by Abdel-Rhem and Ragab (1989) after treatment with IKI 7899 and XRD 473 on root meristems of *Vicia faba.*

In metaphase, a few polyploidy cells were found when treated with insecticide Imidacloprid. Deysson (1968) suggested that c-metaphase may lead to polyploids cells and thus degenerated without further division. Similar result was obtained by Yuzbasioglu *et al.* (2003) after treatment with herbicide Racer Flurochloride on the meristem cells of

Allium cepa L. Briand and Kapoor (1988) reported the presence of polyploidy cell in the *Allium cepa* L. root treated with sodium salicylate.

Star shape metaphase was found in very few cells in metaphase treated with Imidacloprid. Similar type of abnormality was observed after treatment of *Vicia faba* roots with Rogar (Amer and Farah, 1974) and considered as being a fore step of complete disturbance of spindle (Amer, 1966).Diagonal orientation of the chromosomes was also frequently obtained in the metaphasic cells.

Disturbance in chromosome orientation, either in metaphase or anaphase was also a common abnormality which may be the result of the effect in the centromere activity or the spindle fibers (Zakia et al 1989) Selim et al (1981) referred this type of abnormality to be formed due to spindle interruptions caused by the treatment with synthetic organic insecticides.

The main types of abnormalities found in anaphase were precocious arm and shifting of poles (diagonal shifting). Besides them fragments and breaks in chromosome unequal movement of chromosome, laggards and bridges were also frequently found.

Precocious arm and precocious chromosome formation is the result of unequal spindle movement in which some chromosome arm are pulled towards the extremity of the pole (Shrestha, 2004). The precocious arm may be one or more. Chaurasia and Sinha (1987). In *Allium cepa* L. and mice treated with urea also reported such type of abnormalities. Such type of abnormality was reported by Adhikary (1982) in *Allium cepa* L. root meristems treated with 2, 4,-D and2, 4, 5-Trichloro-phenoxy acetic acid: by Sapkota (2000) in *Allium cepa* L. root meristems treated with Carmoisine: by Shrestha (2000) in *Allium cepa* L. root meristem treated

with Isoproturon. Precocious arm and precocious chromosomes were also induced by different fungicides (Bassicol, Ceresan wet, Thisal etc) in Barley root meristem (George et al, 1970).

The shifting of pole in anaphase is the effect of insecticide Imidacloprid in spindle mechanism and may be due to failure of spindle apparatus to organize and function in a normal way depolymerization of spindle fibres was reported by Medeiros and Takahashi (1987) on *Allium cepa* L. root tip treated with *Luffa operculata.* According to them, depolymerization of the spindle fibres caused the shifting of poles. Similar abnormality was also noticed by Bajracharya (1995) on Allium sativum treated with carpet dyeing effluent.

Fragment at anaphase is due to breakage at photosynthetic period either at G_2 or prophase (Kaul, 1972) exposure to higher concentration of the extract causing disturbance in RNA metabolism and consequent lack of DNA, protein synthesis leads to acute fragmentation of chromosome (Sharma and Sharma).

The unequal movement of daughter chromosome in anaphase may be due to disturbance and inhibition of spindle mechanism. Similar abnormalities were also reported by Rangaswamy *et al.* (1981) in onion root tips treated with effluent from lac and paint, plywood and sugar factory.

The formation of lagging chromosome may be attributed to hindrance of prometaphase movement accompanied by adhesive of centromere of one or more chromosome to outer layer of the plasma and movement of the other to toward equatorial plates (Borthelmess, 1977) such result was also reported by Kumar and Kumar (2004), on root meristem of *Allium cepa* L. treated with pesticide Aldrin. The lagging chromosome can be

attributed to the failure of chromosomal movement (Permjit and Grover, 1985).

Another abnormality, the tripolar anaphase is also frequent in the present study. Since the mitotic spindle fibres are composed of protein, it is apparent that Imidacloprid could be responsible for the disruption of microtubule, causing ‘y’ and ‘x’ type of configuration in metaphase. Such disorientation may produce tripolar anaphase and multipolar anaphase. Bale and Mathew (1987) reported tripolar anaphase by sodium fluoride.EI-Khodary at al. 1988 by herbicide Garlon-4 on root meristem of Allium cepa.

The non-synchronized cells in Anaphase are also observed which may be due to the disturbance and inhibition of spindle mechanism. Similar observations were reported by Rangaswamy et al. (1981) in root tip treated with effluent from lac and paint, plywood and sugar factory. (Sujata Shrestha, 2002).

The bridge at anaphase and telophase may be formed due to general stickiness of chromosome at metaphase stage (Abraham and Koshy, 1979). Similar type of result was obtained by Acharya (1999) on root meristem of *Allium cepa* L. treated with pesticide Malathion. Bridge are also formed due to breakage and reunion of the chromosomes (Permjit and Grover, 1985).

The abnormalities found in telophase were shifting of poles that has perhaps led to the formation of diagonal and longitudinal types. (diagonal and longitudinal), unequal cytokinesis, diagonal cytokinesis, delay in cell plate formation. Beside these, binucleate cells and trinucleated cells were also found. The unequal cytokinesis resulted in formation of unequal size of cells and may be due to disturbance in cell metabolism. Similar result

was reported by Bhandla (1992) in *Cyclotella meneghianiana f. unipunctata.*

Delay in cell plate formation cause delay in the completion of mitotic cycle. Inhibition of cytokinesis leads to the accumulation of binucleated and trinucleated cells. Binucleated cells are interpreted as consequences of inhibited cell cycle in which chromosome DNA is replicated but not distributed in usual way (Brown and Dyes, 1972).

Beside above mention abnormalities in dividing cells abnormality was also found in non-dividing cells (interphase cells). The abnormalities found in interphasic cells were shifting of nucleus to the polar position of plasmolysed cells and reduction in size of the nucleus of the cell.

The Conducted experiment showed the clastogenic activity of Imidacloprid and found out the clastogenic potentially of the compound and concluded this insecticide is mutagenic to the dividing cell of the onion root tip.

Hence, from the above observation it can be concluded that treatment of insecticide Imidacloprid on the mitotic indices of *Allum cepa* is non-significant in recommended does but chromosomal behaviour at higher concentration and longer time treatment shows toxic effect. Increase in mitoactive action of compound and increase in prophase index indicates that this pesticide acts as prohpase poisoning. The abnormalities like c-metaphase; precocious chromosomes, binucleated, trinculeated and polyploidy cells suggest that the chemical affects the mitotic spindle so it is turbogenic in nature. The abnormalities like breaks and fragmentation of chromosome suggests clastogenic effects of the compound. The abnormalities like stickiness, disturbed prophase, and disturbed metaphase suggest cytotoxic effect of the compound. Though it shows mutagenic effect but does not affect to the morphology.

5.2 Summary

Imidacloprid is an insecticide used for the control of sucking insects including rice hoppers, aphids, whiteflies, termites, soil insects and some beetles. The present experiment is meant to figure out the cytological effects of the insecticide on the root meristematic cells of *Allium cepa L. The concentration* of 25%, 50%, 75%, 100% were prepared and the root tips were treated for the different durations such as 3,6,12 and 24 hours. Hence, along with the control sample, altogether 17 different samples were prepared and for the every sample, the procedure of counting all the normal and abnormal cells, whether dividing or non-dividing were done.

Mitotic index is higher in couple of treated samples than in the control. However, most of dividing cells were in the prophase stage, which can be well explained by the prophase poisoning mechanism that ceased the further division. Hence, though the MI does not seem to be affected significantly by the treatment of chemicals, the division is ultimately ceased due to arrest of mitotic activity at the prophase stage.

The mitotic index value of treated meristems was higher than control at different concentration in all cases except some interruption in some periodic treatment. The mitotic index value is decreasing in 100% concentration at 3 hour, 50% at 6 hours and 75% at 12 hours of treatment than that of control. The mitotic index value is 50.8% at control. The highest mitotic index value is 57.93% in 100% concentration at 12 hours and the lowest value is 30.5% in 25% concentration at 6 hours of treatment.

Regarding the abnormal characters of the dividing cells, the insecticide produced almost major types of abnormalities. However, the Laggards and bridge were the rarest while c-metaphase, Diagonal-metaphase and diagonal-anaphase were found to be the most frequent ones.

Abnormalities in metaphase and ana-telophase were higher in comparison to prophase at different hours of treatments for all concentrations and time period. Among the recorded abnormalities, the highest percentage of abnormal cells are found in metaphase which is 3.17% at control whereas the lowest percentage are found in ana-telo which is 0.3% at 6 hours of treatment. The untreated roots also showed the abnormalities in all phase at higher rate similar to other phases.

In conclusion, the observed result revealed the potentially mutagenic nature of the insecticide Imidacloprid that not only showed the cytological abnormalities in the dividing cells, but also interfered with the mitotic activity by the mechanism of prophase poisoning

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APPENDICES

The statical analysis can be calculated by following calculation

Mitotic Index (MI) = $\frac{TDC \mid 100}{TC}$

Prophase Index (pro I) = $\frac{TC_{Pro} \mid 100}{TDC}$

Metaphase Index (Meta I) = $\frac{TC_{Meta} \mid 100}{TDC}$

Anaphase and telophase index = $\frac{TC_{AnaZTelo} \mid 100}{TDC}$

Total percentage of Abnormal cells (T Abn) = $\frac{TC_{Abn} \mid 100}{TDC}$

Total percentage of Abnormal cells at prophase

$$T_{pro} \text{ X} \frac{TC_{AbnPro} \mid 100}{TDC}$$

Total percentage of Abnormal cells at Metaphase

$$T_{meta} \text{ X} \frac{TC_{AbnMeta} \mid 100}{TDC}$$

Total percentage of Abnormal cells at Ana and Telophase

$$T_{Ana.Ztelo} \text{ X} \frac{TC_{AbnAnaZTelo} \mid 100}{TDC}$$

Percentage of Abnormalities at metaphase among the abnormal cells

$$T_{pro} \text{ X} \frac{TC_{AbnPro} \mid 100}{TC_{Abn}}$$

Percentage of Abnormalities at metaphase among abnormal cells

$$A_{Meta} \text{ X} \frac{TC_{AbnMeta} \mid 100}{TC_{Abn}}$$

Percentage of Abnormalities at Anaphase and Telophse among the abnormal cells

$$T_{AnaZTelo} \text{ X} \frac{TC_{AbnAnaZTelo} \mid 100}{TC_{Abn}}$$

Table no 2: Total number of counting cell of Allium cepa.L.at each phase with different concentration of Imidacloprid at different time treatment

Duration of Treat ment Hours	Imidacloprid percentage	Total cell counted	Total cells in interphase	Dividing cell			Prophase			Metaphase			Anaphase and telo phase		
				Total	Normal	Abnormal	Total	Normal	Abnormal	Total	Normal	Abnormal	Total	Normal	Abnormal
	Controlled	4635	2279	2356	2257	99	2081	2073	18	169	108	61	96	72	20
3	25	4475	2007	2468	2407	61	2183	2160	23	178	155	23	107	92	15
	50	4812	2175	2735	2656	81	2434	2400	34	201	174	27	102	82	20
	75	4443	1964	2479	2408	71	2330	2302	28	96	62	34	53	44	9
	100	4266	2275	1991	1882	109	1846	1800	46	82	44	38	42	38	25
6	25	4473	2211	2262	2189	73	1981	1947	34	193	173	20	63	69	19
	50	4286	2266	2020	1919	101	1797	1765	32	129	75	53	88	78	16
	75	4427	2082	2335	2241	94	2134	2089	45	107	85	22	94	67	27
	100	4619	1943	2676	2468	203	2373	2300	73	193	108	85	110	60	50
12	25	4578	2011	2567	2496	71	2276	2236	40	187	169	18	104	89	15
	50	4459	1994	2465	2379	86	2198	2152	46	178	156	22	89	71	18
	75	4319	2024	2295	2191	104	2097	2040	57	99	75	24	99	76	23
	100	4569	2139	2430	2297	133	2218	2153	65	125	87	38	87	57	30
24	25	4109	1931	2178	2119	59	1995	1974	21	95	77	18	88	68	20
	50	4384	1988	2396	2323	73	2196	2172	24	109	86	23	91	67	26
	75	4293	259	2234	2158	76	1995	1966	29	154	131	23	85	61	44
	100	4567	2197	2370	2266	104	2181	2138	43	109	77	32	80	61	29

Table no 3: Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices of Allium cepa L. with different concentration of Imidacloprid at different time of treatment.

Duration of treatment in hours	Concentration of Imidacloprid in percentage	Mitotic index	Prophase index	Metaphase index	Anaphase and telophase index
	control	50.83	88.33	7.17	4.07
3	25	55.15	88.45	7.21	4.34
	50	56.84	88.99	7.35	3.73
	75	55.80	93.99	3.87	2.14
	100	46.67	92.72	4.12	2.11
6	25	50.57	87.58	8.53	2.79
	50	47.13	88.96	6.39	4.36
	75	52.74	91.39	4.58	4.03
	100	57.93	88.68	7.21	4.11
12	25	56.07	88.66	7.28	4.05
	50	55.28	89.17	7.22	3.61
	75	53.14	91.37	4.31	4.31
	100	53.18	91.28	5.14	3.58
24	25	53.01	91.60	4.36	4.04
	50	54.65	91.65	4.55	3.80
	75	52.04	89.30	6.89	3.80
	100	51.89	92.03	4.60	3.38

Table no. 4: Mean Mitrotic, Prophase, Metaphase, Anaphase and Telophase Indices treated with mean percentage concentration of Imidacloprid at different time of treatment.

Duration of treatment in hours	Mean Mitotic Index	Mean Prophase Index	Mean Metaphase Index	Mean Anaphase & Telophase Index
Control	50.8	88.32	7.17	4
3	53.6	91.11	3.5	3.9
6	47.07	89.14	5.1	4.17
12	54.39	90.11	5.9	3.24
24	52.88	91.14	5.4	3.8

Table no. 5: Total percentage of abnormal cells and percentage of abnormal cells at each phase among the abnormalities with different concentration of Imidacloprid at different time of treatment.

Duration of treatment in hours	Concentration of Imidacloprid in percentage	Total percentage of abnormal cells	Total percentage of abnormal cells in Prophase	Total percentage of abnormal cells in Metaphase	Total Percentage of abnormal cells in Ana
	Control	3.96	0.7	2.5	0.76
3	25	2.4	0.9	0.9	0.6
	50	2.4	0.7	0.98	0.72
	75	3.52	1.1	1.73	0.69
	100	5.48	2.3	1.9	1.28
6	25	3.14	1.5	0.88	0.76
	50	4.98	1.58	2.62	0.78
	75	4.24	2.16	0.9	1.14
	100	7.71	2.7	3.17	1.86
12	25	2.78	1.5	0.7	0.58
	50	2.67	1.03	0.92	0.72
	75	3.41	1.31	1.04	1.12
	100	5.38	2.6	1.56	1.22
24	25	2.53	0.5	0.8	1.23
	50	2.44	0.54	0.95	0.95
	75	2.76	0.67	1.02	1.07
	100	3.51	0.94	1.35	1.22

Table no. 6: Percentage of abnormal and normal cells at each phase with different concentration of Imidacloprid at different time of concentration.

Duration of treatment in hours	Concentration of Imidacloprid in percentage	Prophase percentage		Metaphase percentage		Anaphase and Telophase percentage	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
	Control	91.8	18.8	4.78	61.6	3.28	20.11
3	25	89.7	37.70	6.43	37.70	3.81	25.51
	50	90.36	41.9	6.55	33.33	3.08	24.61
	75	93.98	39.43	2.57	47.88	1.82	12.66
	100	95.64	42.20	2.33	34.86	15.83	22.93
6	25	88.94	46.57	7.90	27.39	3.14	26.01
	50	91.97	31.68	3.96	52.4	4.05	15.84
	75	93.21	47.8	3.79	23.40	2.98	28.71
	100	93.19	35.09	4.37	40.86	2.42	24.01
12	25	89.58	56.33	6.77	25.35	3.84	29.85
	50	90.45	53.48	6.55	25.58	2.97	31.03
	75	93.10	54.80	3.42	23.07	3.46	22.11
	100	93.70	48.8	3.78	28.57	2.47	22.55
24	25	94.14	25.5	3.63	30.50	3.7	33.89
	50	93.49	32.8	3.70	31.50	2.79	35.5
	75	91.10	38.15	6.07	30.26	4.16	31.57
	100	94.35	41.34	3.39	37.5	2.24	27.88

Table no. 7: Mean Mitotic index values of *Allium cepa* root tip cell treated with different concentration of Imidacloprid at different time of treatment.

Duration of treatment in hours	Mitotic index (K) treated in different time period			
	3 hours	6 hours	12 hours	24 hours
25	55.15	30.5	56.0	53.00
50	56.8	47.13	55.28	54.65
75	55.79	52.74	53.13	52.03
100	46.67	57.93	53.18	51.89

Table no. 8: Ranks of four matched groups concentration of Mitotic index value of *Allium cepa* under four conditions (Times).

Concentration of Imidacloprid in percentage	Mitotic index (K) treated in different time period			
	3 hours	6 hours	12 hours	24 hours
25	3	1	4	2
50	4	1	3	2
75	4	2	3	1
100	1	4	3	2
Total (Rj)	12	8	13	7

$$X_r^2 = \frac{12}{Nk(k+1)} \sum_{j=1}^{k} (Rk)^2 - 3N(k+1)$$

$$= \frac{12}{4x4\,(4+1)} \{(12)^2+(8)^2 + (13)^2 + (7)^2 - 3 \times 4\,(4 + 1)\}$$

$$= \frac{12}{80} \{144 + 64 + 169 + 49 - 3 \times 4\,(4 + 1)\}$$

$$= \frac{12}{80} (426) - 60$$

$$= \frac{12}{80} \times 426 - 60$$

$$= 63.9 - 60$$

$$= 3.9$$

Where,

N = 4 number of rows (concentration)

K = 4 numbers of columns (conditions, time of treatment)

Rj = sums of ranks in the j^{th} column

Note: Ranking was done assuming lowest score = 1 and highest score is = 4 for above condition.