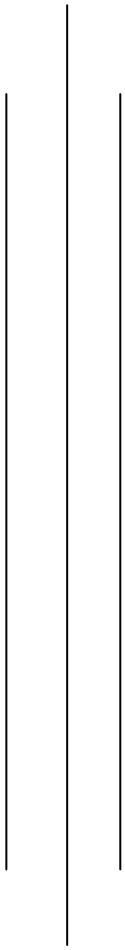
Cytological effects of the insecticide Penthoate in the somatic cell division of root meristem of *Allium cepa* L



Dissertation submitted as a partial fulfillment of M.Sc. in Botany

By

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CERTIFICATE

This is to certify that the dissertation work entitled " Cytological effects of the insecticide **Penthoate** in the somatic cell division of root meristem of *Allium cepa* L." has been carried out by Mr. Shailendra Karki under my supervision. It is based on the experiment performed by the student and the result has not been published or submitted for any other degree. I recommend this dissertation to be accepted as a partial fulfillment for M.Sc. Degree in Botany, Tribhuvan University of Nepal.

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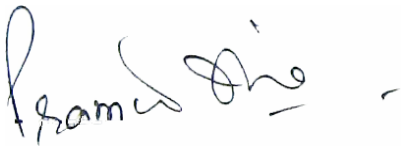
Letter of Approval

This dissertation entitled **"Cytological effects of the insecticide Penthoate in the somatic cell division of root meristem of *Allium cepa L*"** submitted by Mr. Shailendra Karki has been accepted as partial fulfillment of the requirement of M.Sc. Botany (Genetics and Plant Breeding).

Examination Committee



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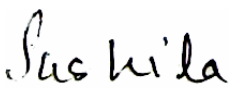

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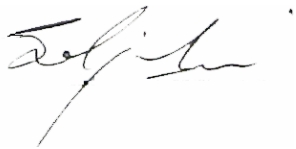

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Shailendra Karki

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ABBREVIATIONS AND ACRONYMS

$A_{ana\text{-}telo}$	**=**	**Percentage of abnormalities at Anaphase and telophase among the abnormal cells.**
A_{meta}	**=**	**Percentage of abnormalities at Metaphase among the abnormal cells.**
Ana-Telo I	**=**	**Ana-Telophase Index**
A_{pro}	**=**	**Percentage of Abnormalities at Prophase among the abnormal cells**
BSI	**=**	**British Standards Institution**
CAS	**=**	**Chemical abstracts service**
cf.	**=**	**compare**
DDT	**=**	**Dichlorodiphenyltrichloroethane**
DNA	**=**	**Deoxyribo Nucleic Acid**
Fig	**=**	**Figure**
Meta I	**=**	**Metaphase Index**
MI	**=**	**Mitotic Index**
PRO	**=**	**Pesticide registration office**
Pro I	**=**	**Prophase Index**
ISO	**=**	**International Organization for Standardization**
IUPAC	**=**	**International Union of Pure and Applied Chemistry**
T_{abn}	**=**	**Total percentage of abnormal cells**
$T_{ana\text{-}telo}$	**=**	**Total percentage of abnormal cells at Anaphase and Telophase**
T_{meta}	**=**	**Total percentage of abnormal cells at metaphase**
T_{pro}	**=**	**Total percentage of abnormal cells at prophase**
TC	**=**	**Total number of cells counted**
TC_{abn}	**=**	**Total number of abnormal cells counted**
$TC_{abn\ ana\text{-}telo}$	**=**	**Total number of abnormal cells counted at Anaphase and Telophase**
$TC_{abn\text{-}meta}$	**=**	**Total number of abnormal cells counted at Metaphase**
$TC_{abn\text{-}pro}$	**=**	**Total number of abnormal cells counted at Prophase**
$TC_{ana\text{-}telo}$	**=**	**Total number of cells counted at Anaphase and Telophase**
TC_{meta}	**=**	**Total number of cells counted at Metaphase**
TC_{pro}	**=**	**Total number of cells counted at Prophase**
TDC	**=**	**Total dividing cells**

CHAPTER I

1. INTRODUCTION

1.1 Background

Agriculture has undergone a drastic revolution from the pre-historic period to this modern era of science and technology. The adoption of the agriculture was made earlier during the Paleolithic age when early people felt inadequacy in their survival methods which involved hunting and gathering. Hence the process of sowing and harvesting commenced long before, in the Stone Age, the oldest record ever in the history of human civilization.
Since then several advances have been made gradually and continuously in the field of science and technology. These incredible innovations have accelerated the agriculture even more rapidly. The advent of modern techniques such as the use of pesticides has been widely adopted by the farmers all over the world including the developing countries like ours. The introduction of pesticide is proved to be a huge leap in the field of agriculture. It has helped us to overcome the immense loss of harvests due to the pests.
In Nepal 10 to 15 % of crop production damage is caused due to crop pests. The first use of pesticide was recorded in the 1940's to eradicate the Malaria outbreak by applying Dichlorodiphenyltrichloroethane (DDT). More than 250 different types of insecticides, 71 fungicides , 20 herbicides, 2 acaricides, 8 rhodenticides are redgistered for use (PRO 2001).

1.2 Insecticide

Insecticide is the chemical that is used to kill the harmful pests, specifically the insects that are most common pests of the economically important plants. Pesticide is a descriptive term, which includes all chemicals used to control animal or plant pests such as insecticide, fungicides, weedicides (herbicides) and rodenticides. Pesticides are chemical agents used to kill pests or inhibit their reproduction. They are intended to destroy organisms that are unwanted but should not harm those that are wanted i.e. a weedicide or weed killer will destroy weeds but leaves the crops we want to harvest for food unharmed. Ideally, pesticide should have no toxic effects when ingested by humans and animals but some may contain organic phosphorous compounds and fluorides which when ingested may cause damage to the nervous system or other organs of the body.

Pesticides are used not only to kill pests that attack crops but it also affects a wide range of organisms and pest predators. Pesticides differ based on their active ingredients which may include organochlorines, organophosphates and pyrethroids. Organophosphates are severe poisons which kill by inhibitory action of certain enzymes in the nervous system. After a pesticide is applied, it may meet a variety of fates. It may be lost to the atmosphere through volatilization, carried away to surface waters by run off and erosions or broken down by photolysis. In the soil, it may be taken up by plants, degraded into other chemical forms or leached downward. Pesticides that are insoluble or tightly bound to soil particles are most likely retained in the upper soil layers which may be lost to surface waters through leaching and erosion. The potential surface loss of pesticide depends on pesticide properties, soil type and the length of time after application.

Nevertheless, the use of pesticides may be noxious to the well being of lives and can be considered the health- hazard. Though the pesticide has become a necessity, the frequent and indiscriminate use of these chemicals has been proved to possess many undesirable secondary consequences on higher plants (Epstein and Legator, 1971, Amer and Farah 1974).

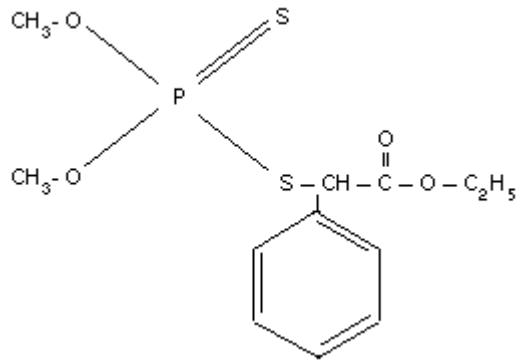
1.3 General Information on PENTHOATE

Penthoate, commonly known by its trade name Dhanuka Dhanusan 50, is an organophosphorus insecticide. Phenthoate is a broad spectrum, non-cumulative, organophosphorus pesticide; a cholinesterase inhibitor with contact and stomach action with no residual activity.Toxicity is increased after metabolism to the oxygen analogue. Phenthoate is phytotoxic to some plants. It has penetration and quick-knock down action. Organophosphorus compound such as Penthoate are popular insecticides because of their effectiveness and high degree of water solubility and rapid bio-degradation (Nelson et al 1990). However many of these compounds and their breakdown products are alkylating agents of DNA (Bedford and Robinson 1972). It is therefore important to continue to access potential genetic damage from limited exposure to organophosphorus insecticide (Degraeve et al 1984)The major crops, to which this insecticide is mainly applied, are cotton, paddy, groundnut, mustard, tobacco, potato, wheat, vegetables, grapevine, citrus, tea, coffee and ornamental plants. The pests that are controlled by this insecticide are aphids, jassids,fruit flies, leaf miners, steam borers, beetles, soft scales mosquito larvae and blowflies and various strains of houseflies and mites etc.

1.3.1 Application/Uses

Penthoate is recommended at dosages of 1000ml per hectare in 500 to 1000 liters water depending on the stage of crop growth.

1.3.2 Chemical Structure of Penthoate



1.3.3 Chemical Properties

Phenthoate (ISO, BSI, exception; JMAF-PAP)

IUPAC S-alpha-ethoxycarbonylbenzyl O,O-dimethyl phosphorodithioate

CAS No. 1 Acetic acid, mercaptophenyl-ethyl ester, S-ester with O, O-phosphorodithioate

CAS Reg. No. 2597-03-07

Molecular formula:	$C_{12}H_{17}O_4PS_2$
Molecular weight:	320.4
Colour:	reddish yellow
Melting point:	17.5 ± 0.5°C
Boiling point:	the compound decomposes at normal pressure before reaching the boiling point.
Vapour pressure:	4×10^{-5} torr at 40°C

Density d_4^{20}	1.226
Refractive index:	d_D^{20} /1.552 approx.
Flash point (/Cleveland):	165°C approx.
Partition coefficient:	Octanol//water log P = 3.69, 3.82 at two concentrations

Solubility of pure compound: in water at 24°C approx 10 mg/l.

1.3.4 Other Names for this Chemical (Chemical Synonyms)

Bay 33051, Bayer 18510, Cidemul[R], Cidial[R], dimephenthioate, dimephenthoate, dimethenthoate, Elsan[R], ENT 23438,ENT 27386, Erucin[R], Erusan[R], fenthoate, L-561, OMS 1075, PAP[R], Papthion[R], Phendal[R], Rogdial[R], S-2940, Tanone[R], TH 346-1, Tsidial[R].

1.3.5 ENVIRONMENTAL CONSIDERATIONS

Penthoate is highly toxic to fish, hence, suitable for only those areas where pisciculture is not practiced at all. It requires approximate effluent disposal system. It is also toxic to honey bees and hence recommended not to be sprayed during early hours of the morning. Poisoning symptoms in human after direct contamination include headache, dizziness, nausea, vomiting, diahhorea and even muscular twitching.

1.4 OBJECTIVE

The main objective of the present study is to figure out the cytological effects of the insecticide Penthoate in the root meristematic cells of *Allium cepa* L. in approximately recommended dose (100%) and those lower than that.

CHAPTER II

2. LITERATURE REVIEW

2.1 Cytological Effects of Insecticides

The cytological effects of insecticides (Dimecron-100 and Rogor-40) were studied on *Vicia faba*. The type of abnormalities found were: chromosome and chromatid breaks, dot delations, fragments, ring chromosomes, anaphase bridges and series of gaps in both meta- and anaphase stages. Star shaped metaphase was also noted (Reddy and Rao 1969).

Ravindran (1971) studied the effect of Folidol in *Allium cepa*. The insecticide Folidol produces many chromosome abnormalities such as anaphase inhibition, c-mitosis, chromatid and chromosome breakages, abnormal chromosome separation and micronuclei formation.

Amer and Farah (1974) reported that insecticide Rogor is capable of inducing mitotic abnormalities such as disturbed prophase, meta- and anaphases in both *Vicia faba* and *Gossypyum barbadense*, lagging chromosomes, chromosome contraction. Chromosome stickiness, chromosomes fragmentation, anaphase bridges and multipolar anaphases were observed only in *Vicia faba* L.

Amer et al. (1985) studied the effect of the insecticide Dichlorvos on root-mitosis of *Vicia faba*. Disturb meta- and anaphases where the chromosomes spread irregularly over the cell comprised the main type of abnormalities. Stickiness of the chromosomes, anaphase-bridges, micro- and binucleated interphase cells were also observed.

Amer et al. (1986) observed the effect of insecticide Rotenone on root-mitosis of *Vicia faba*. The type of abnormalities induced: disturbed meta- and anaphases (where, the chromosomes spread irregularly over the cell) in a high percentage, chromosome stickiness, lagging chromosomes and ana-telophase bridges.

Pandita (1986) observed the effect of insecticide Meta systox-R on the root meristem of *Allium cepa*. The results observed from root tips exposed to various insecticide concentrations. Chromosomal aberrations like bridges, fragments, laggards, chromosomal paling and micronuclei were found very often. Arrested ana telophases were also found.

The four organophosphorus insecticides viz. Dichlorovos, Monocrotophos, Phosalene and Oxydemetonmethyl induced clastogenic and turbogenic effects to different degree on root meristem of *Allium cepa*. These included chromosome breaks at metaphase, anaphase bridge, subchromatid connection at metaphase, heteromorphic diplochromosome at metaphase, ring chromosome and a fragment at metaphase, micronuclei, lagging chromosome at anaphase, forward chromosome at anaphase. Among four, Monocrotophos was observed to be more effective as a clastogenic agent followed by Dichlorovos, Phosalene and Oxydemetonmethyl repectively (Rao et al., 1987).

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

The four insecticides produced mitotic effects, cytogenetical effects and chromosomal effect. They are capable of producing a variety of mutations in *Capsicum* sp. Concerning the types of induced abnormalities disturbed meta- and anaphases comprised the most dominant type. Stickiness of chromosome, fragments, bridges, laggings, micronuclei were observed (Devadas et al., 1987).

Ahmed et al. (1992) studied the effects of Methyl Parathion and Tri-miltox on the mitosis of *Allium cepa*. The insecticide Methyl Parathion and Fungicide Tri-miltox both produced micronuclei, chromosomal fragments, laggard chromosomes, single and multiple bridge formation. But Methyl Parathion produced comparatively more chromosomal aberrations than Tri-miltox.

George and Ghareeb (2001) studied genotoxicity of the insecticide Cyolan on mitosis, meiosis and seed storage protein of *Vicia faba*. The used insecticide solution had

mitodepressive effect and induced a wide range of mitotic and meiotic abnormalities. The mitotic abnormalities include stickiness, laggards, bridges, fragments, disturbed phases, micronucleus and multipolar cells.

Cytotoxic effect of an insecticide Fenvalerate on root meristems of *Allium cepa* L was studied by Malla and Shakya (2003). The insecticide Fanvalerate significantly suppressed mitotic index value and showed variations in phase indices and chromosomal abnormalities. The abnormalities like c-metaphase, stickiness of chromosomes, equatorial plate shifting, precocious chromosomes, chromatin bridge, multipolar anaphase, binucleated, trinucleated and multinucleated cells were frequently produced. In addition lagging chromosomes, delayed, unequal and diagonal cytokinesis, micronuclei were other effects of this chemical.

The effects of 3 synthetic pyrethroid insecticides *viz.*, Cypermethrin, Alphamethrin and Fenvalerate on the mitotic activity and mitotic chromosomes in the root meristems of *Allium cepa* was studied by Bandaru V. Rao, Tanikella L. Narasimham and Muktinutalapati V. Subbarao. (2005). The test compounds elicited varying degrees of cytotoxic, turbagenic (toxicity to spindle) and clastogenic effects. In the ultimate analysis, Cypermethrin and Alphamethrin have more turbagenic and weak clastogenic activity whereas Fenvalerate has relatively strong clastogenic activity *in vitro*.

2.2 Cytological Effects of Pesticides

Amer (1965) observed the mitotic effect of pesticide N-methyl-1-napthyl carbamate "Sevin" on the root meristem of *Allium cepa*. It induced disturbed metaphases and anaphases, multipolar anaphase, multinucleated cells, star metaphase etc. The end-effect of pure and formulated Sevin depend mainly on the temperature of preparation of the agent solution. Continuous treatment with the insecticide for 24 hours nearly arrested mitosis. However, the root recovered after replacement in water for 48 hours and the rate of mitosis became normal again.

Amer and Ali (1969) observed the mitotic effects of pesticide IV, some phenols, on the root meristem of *Vicia faba*. The pesticide caused accumulation of the metaphase stage

on the expense of the ana-telophase stage. The induced types of anomalies were: disturbed type (disturbed metaphase, prophase-metaphase and disturbed ana-telophases), lagging chromosomes, stickiness, fragmentation and cytomixis.

Amer and Farah (1975) studied the mitotic effects of pesticide I, Isopropyl-N-phenyl carbamate and "Duphar" on *Vicia faba* and *Gossypium barbadense*. A clear decrease in the mitotic index, disturbed prophases, meta- and anaphases comprised the main type of induced abnormalities. Lagging chromosomes, multipolar anaphases, chromosome stickiness, anaphase bridges, chromosome fragmentation and multinucleated cells were also observed.

Some organophosphorus pesticide I induced the chromo/cytotoxic effects in *Barley* including fragments, ring chromosomes and c-mitotic configurations at metaphase. Chromatin bridges, laggards and multipolar cells were also observed at anaphase (Kaur and Grover, 1985).

Jain and Sarbhoy (1986) studied the effect of chlorinated pesticides I, on somatic chromosome of *Lens* and *Pisum*. The pesticides induced abnormalities like inactivation of spindle mechanism, condensation of chromosomes below their normal size, chromatin bridges at anaphase and telophase etc.

The immediate effect of all chlorinated pesticides III [Benzene Hexachloride (BHC), Lindane, Aldrin, Heptachor and Endrin] treatment was partial or entire inactivation of spindle mechanism followed by scattering of chromosomes. The spindle inactivation affected the chromosomes movement. The abnormalities include sticky chromosomes, multipolar spindle and unequal separation of chromosomes, polyploidy cells, multinucleate cells, chromatin bridge, multivalents etc. (Jain and Sarbhoy, 1987).

Grover and Malhi (1988) studied genotoxic effects of some organophosphorus pesticides III (Ekatin, Thiometon, Fenitrothion, Phorate, and Thimate) and MNNG (N-methyl-N-nitro-N-nitrosoguanidine) in root meristems of *Allium* and *Hordeum*. Both physiological type aberrations like c-mitosis, despiralization, lagging chromosomes, multipolar cells as well as clastogenic effects like chromosome breaks, ring chromosome, chromatin bridges and micronuclei were observed.

The cytological effects of the pesticide Phosalone were studied on root meristem of *Allium cepa*. The pesticide induced mitostatic effects. The mitotic index gradually decreases with the increase in concentration and time of treatment. Mutagenic effect was noticed with abnormalities like chromosome breakage and erosion, sticky bridge, laggard, multipolarity, clumping and pycnosis (Sinha et al, 1989).

Adam et al. (1990) studied the effect of two Organophosphorus insecticides namely Melathion and Tamaron on mitosis of *Vicia faba* root tips. They induced stickiness, abnormal prophase, spindle disturbance, lagging, bridge, despiralization, binucleate cell, contraction and asynchronization of chromosome in anaphase and telophase.

Sharma and Gautam (1991) studied the chromosomal aberrations induced by Phosphamidon and Penthoate in the bone marrow cells of mice. Both clastogenic as well as physiological types of aberrations were induced by these pesticides. The clastogenic type aberrations induced by both pesticides include chromosomal fragmentation, chromatid breaks, chromosomal and chromatid gaps; ring chromosomes, interchromatid exchanges, minute and dicentric chromosomes. The physiological type of aberrations caused by both of these pesticides include centromeric associations, exchange configurations, bizarre configurations, shrinkage of chromosomes, stickiness and end to end association.

Two pesticides Rogor and Bavistin and an antibiotic-streptomycin induced cytological effects on the pollen mother cells of *Lathyrus sativus* growth after seed treatment. All chemicals were capable of inducing cytological disturbances and genotoxic effect (Upendra et al, 1991).

Ahmad and Yasmin, (1992) studied the effects of Methyl parathion and Tri-miltox on the mitosis of *Allium cepa*. These chemicals produced an increased number of abnormalities such as bridge formation, laggard chromosome and micronuclei. Methyl parathion produced comparatively more chromosomal aberration that Tri-miltox.

Acharya (1999) studied the effect of Malathion on somatic cell division of *Allium cepa* L. The chemical showed various types of chromosomal abnormalities including abnormal

cellular behaviour or the cell. The common types of abnormalities were; non-synchronized movement of chromatids, spindle abnormalities, anaphase showing suspension of spindle fibres, anaphase with dilated (distorted) pole. Beside these precocious arms, laggards and chromosome fragments were also observed. The Melathion also showed its capability of inducing polyploidy. The effect of pesticide Metacid and water extract of *Taxus buccata* was studied by Manandhar (2000) on dividing cells of *Onion* root. Various types of cytological abnormalities like c-metaphase, chromosomal irregularities, retardation of chromosome movement, mitodepressive and clastogenic effect were noted.

CHAPTER III

3. MATERIALS AND METHODS

For the present work, root meristems of common onion (*Allium cepa* L 2n=16) were used. The effect of an insecticide Penthoate is used as standard one. Following are the major procedures applied to carry out the whole experiment.

3.1 Materials

Onion, *Allium cepa* L., meristems as bioassay.

3.1.1 Onion Bulbs and Bulb Rooting Experiment

Healthy looking and approximately equal sized bulbs of onion (*Allium cepa* L.) were collected from the local market of Kathmandu. The onion bulbs were selected because of easiness in handling, fast growth rate and convenience in cytological observations.

The bulbs were thoroughly washed with tap water and the old roots present in bulbs were removed properly. To obtain the actively growing root tips, these bulbs were placed with their basal side facing downward over coupling jars filled with tap water. The water in the jar was replaced at 24 hour intervals so as to check the growth of microorganism and to prevent any injurious effect on the growth of the roots due to dissolution of a yellow pigment from the outer scale of the bulbs by the tap water.

3.1.2 Preparation Of Suspension For Experiment

For the root treatment four different concentration of Penthoate viz. 25%, 50%, 75% and 100% were prepared. The 1 ml of Penthoate was dissolved in 1000 ml of tap water in order to prepare solution of concentration 100%. Similarly 0.5ml of Penthoate was dissolved in 1000ml of tap water to make solution of 50%. Then by dissolving 0.75ml in 1000ml of tap water, 75% concentration was prepared. And by dissolving 0.25ml in 1000ml of tap water the solution of 25% concentration was prepared. These four differently concentrated solutions were prepared just before the root treatment.

3.2 Methods

3.2.1 Treatment of The Rooting Bulbs

As the lateral roots of onion (*Allium cepa* L.) were about 2 cm long, they were exposed to the freshly prepared test solutions of different concentrations for 3, 6, 12 and 24 hours at root temperature. As the period of treatment was prolonged and the root tips were treated with higher concentrations, the effect was found to be lethal and thus they were avoided. Control roots were simultaneously treated with tap water in order to compare with the effect on the treated roots. The method of treatment is given in table 1.

Table 1: Method of treatment

Times of transferring the materials to the suspension (Penthoate)	Time period (hour)	Fixing time
10:30 am (control)	0	10:30 am
07:30 am	3	10:30 am
04:30 am	6	10:30 am
10:30 pm (previous night)	12	10:30 am
10:30 am (previous day)	24	10:30 am

The following agents were used for fixing and staining the tissues.

Fixing agent-Carnoy's Fluid I

Glacial acetic acid	1 part
Absolute alcohol	3 parts

Preserving Agent-70% Ethanol

70% ethyl alcohol was used for short time preservation of tissues before staining.

Stain-Belling's Iron- Aceto-Carmine (1926)

Carmine	0.5gm
Glacial acetic acid	45 ml
Distilled water	55 ml

3.2.2 Cytological Fixation

The treated and control root tips of Onion (*Allium cepa* L.) were excised, thoroughly washed in tap water and fixed in freshly prepared 1:3 acetic alcohol for 2 hours and preserved in 70% ethyl alcohol. The fixing time was around 10:30.

3.2.3 Aceto-Carmine Squash Technique

The fixed root (about 1 cm) were stained in aceto-carmine and squashed (about 2 mm root tip) on a clean slide. More than five root tips were studied from each treated or non-treated onion bulb.

3.2.4 Preparation of Permanent Slide And Mounting Media

Celarier (1956) was followed for the preparation of permanent slides. The composition of dehydration grades was as follows:

(A) Glacial acetic acid	1 part
Butyl alcohol	1 part
(B) Glacial acetic acid	1 part
Butyl alcohol	3 parts
(C) Butyl alcohol	pure (100%)

After dehydration the mounting was done in Euparol and necessary photomicrographs were taken from permanent slides.

3.2.5 Photography

With the help of digital camera, various stages of the mitotic division in the permanent slide were photographed.

3.2.6 Cytological Observation And Analysis

The observations were recorded between 4000-5000 cells from at least five different root tips treated with various concentrations of Penthoate. The indices and abnormalities were scored and analysed by using the following formulae, according to Levan 1949 (c.f. Kihlman 1971; Medeiros and Takahashi 1987). given in appendix.

3.3 Statistical Analysis

"The Friedman's two-way analysis of variance by ranks" method (Siegal 1956) was applied to calculate whether the time of treatment effects the mitotic index of *Allium cepa* root tip cells or not. The data from table 8 were submitted for calculation as below:

$$\chi^2 = \frac{12}{NK(K+1)} \sum_{j=1}^{K} (Rj)^2 - 3N(K+1)$$

where,

N= 5 number of rows (concentration)

K= 4 number of columns (duration of treatment)

Rj= sums of ranks in the f^{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 the reference to the chi (χ) square distribution with df= k-1 and the significance value were matched at percentage (p)<0.05.

CHAPTER IV

4. RESULTS

From the above experiment, the effects of insecticide Penthoate on the mitotic activity of meristematic cells of onion were analysed. The comparative studies of the results of controlled and root meristems treated with different concentration of Penthoate for different of time were done. The results are tabulated from Table no. 2 to Table no. 8 (see appendix) and with the help of statistical analysis, value for chi square (χ^2) was calculated which is equal to 4.8

4.1 Effect on Mitotic index

The mitotic indices obtained from dividing root meristematic cell, treated with different concentrations of Penthoate for different duration of time have been tabulated in the Table no.3 (see appendix). The graph of mitotic indices of different concentrations of insecticide was plotted against different duration of time in Figure no.1.

The result showed that the mitotic index increased slightly with the increase in the concentration of Penthoate. Also, it increased with the increase in duration of treatment except in some cases. MI values of the cells treated with various concentrations of Penthoate are lower than that of control except for those treated with 75% for 6, 12, & 24 hours. Mitotic index is least at 25%/3hrs which is equal to 20.83, whereas it is highest at 75%/6hrs which is equal to 83.16.

4.2 Effect on Phase index

4.2.1 Effect on Prophase Index

Figure 2 shows the graph of prophase index plotted against duration of treatment for different concentrations. The result showed that the Prophase Index increased with increase in concentration and also with the increase in duration of treatment. At control, prophase index is 90.12 and the maximum value is 96.85 at 75%/6hrs. Minimum value is 85.38at 50%/3hrs.

4.2.2 Metaphase Index

Figure 3 and Table no.3 reveals the values of Metaphase indices at different levels. It appears that the value decreases with the increase in duration of treatment except in 50% of Penthoate at 3hrs where it increased with the increase in hours and also in 100% at 6hrs The maximum value is 5.87at 50%/3hrs and the minimum value is 1.4 at 25%/3hrs. With respect to the value at control, other values seem to fluctuate in great range.

4.2.3 Anaphase and Telophase Index

According to figure 4 and Table 3, it seems that the value of Anaphase and Telophase index seems to decrease with the increase in time except for those treated with 75% Penthoate for 3hrs where the value increases abruptly. Also, the value decreases with the increase in concentration of the insecticide. The maximum value is 8.85at 75%/3hrs and the minimum value is 1.44 at 75%/6hrs. All the other values fluctuate highly in comparison to the value of control.

4.3 Relation between mean mitotic index and mean phase indices

The values of mean mitotic index and phase indices are tabulated in Table no.4 and the graph of mean indices was plotted against duration of treatment in figure 5. The value of mean mitotic index appears to remain approximately constant with the increase in the duration of treatment. The maximum value is 61.56 at 24-hour treatment and the minimum value is 43.31 at 3-hour treatment.

For prophase index and metaphase index, the value seems to be almost constant. In other words, the indices are not affected by the increase in duration of treatment. But in the case of Telophase-Anaphase index, the value fluctuates considerably. It increases from control to 3hrs and suddenly decreases at 6-hour duration and then is almost constant.

4.4 Percentage of Abnormal cells

The total percentage of abnormal cells at each phase treated with each concentration of Penthoate for particular duration of treatment was tabulated in Table no.5.

4.4.1 Total Percentage of Abnormality in dividing cells

Figure 10 reveals the percentage of abnormality at each level of concentration and duration. The data fluctuates randomly but every sample showed some degree of abnormalities. Maximum value is 6.06% obtained in the cells treated with 100% Penthoate for 6 hours and minimum value is 0.97% obtained from those treated with 25% Penthoate for 3 hours. Altogether, 62% (i.e. 10 out of 16) treated samples showed more abnormality than the control sample.

4.4.2 Total Percentage of Abnormality in Prophase

Figure 11 shows that the fluctuation of the values persists in the random manner and the maximum value is 44.44% at the concentration 25% and duration 3hrs. Some samples did not show any abnormality at all. The control treatment showed lowest abnormality, which is only2.89%.Fourteen different samples showed more abnormality than control.

4.4.3 Total Percentage of Abnormalities in Metaphase

Figure 12 shows the percentage of abnormally dividing cells in metaphase. The graph does not appear even, which gives the idea of irregular rise and fall in the values. The lowest value is 27.08% obtained in the cells treated with 25% of Penthoate for 12 hours whereas the highest one is 68.78 % obtained in the cells treated with 50% Penthoate for 3 hours. The value for control is 50.72%, which can be considered approximately average. Here also, eleven different treated samples showed more abnormality than in the case of control sample.

4.4.4 Total Percentage of Abnormalities in Anaphase and Telophase

The values for total percentage of abnormalities were shown in Table no.5 and figure 13, which reveals a usual case of randomness. The maximum value is 53.01% obtained from the cells treated with 100% Penthoate for 24 hours whereas the minimum value is 11.11% obtained from the cells treated with 25% Penthoate for 3 hours.

4.5 Chromosomal Behavior

The insecticide Penthoate was able to induce various types of chromosomal abnormalities in the meristematic cells of root of *Allium cepa* L. during mitotic cell division.

4.5.1 Nature of Abnormal Cells in Non-Dividing Cells

Few plasmolysed cells in almost all concentrations of all durations were found. Binucleate cells were also found in most of the concentrations. Tri-nucleolate cells were also found frequently in 75% /6hrs whereas they were few in 50% and 100%. In 25%, no such tri- and tetra-nucleolate stages were observed. Another peculiar abnormality was the occurrence of micronucleus which was frequently observed in the cells treated with 100% Penthoate for 12hrs. In other concentrations, no such abnormality was secured.

4.5.2 Nature of abnormal cells in Prophase

Stickiness, disturbed-prophase and dumbbell-shaped nucleus (only one in 75%, 3hrs) were the abnormalities seen in the prophase. Sticky prophase was maximum in 50% for 6hrs. Few dumb-bell shaped prophases were observed in 75% concentration for 3 and 6 hours.

4.5.3 Nature of abnormal cells in Metaphase

Number of abnormalities were secured such as diagonally placed equatorial plate, C-metaphase, stickiness, polyploidy, fragmentation, precocious chromosome and disturbed metaphase. Among these, the most common abnormality is the diagonally placed equatorial plate. They were observed most frequently at 50% concentration for 3hrs. The polyploidy and c-metaphases where common at 100% for 3hrs.

4.5.4 Nature of abnormal cells in Anaphase

Distorted and disturbed, precocious chromosome diagonally placed equatorial plate and longitudinal arrangement, tri-polar anaphase, stickiness, non-synchronized were frequently observed abnormalities. Rare abnormalities are the formation of bridge, c-anaphase and lag- formation. Bridge-formation is almost negligible since only one cell was found to have it when treated with 75% concentration of Penthoate for 12 hours.

4.5.5 Nature of abnormal cells in Telophase

The main abnormalities are diagonal Telophase, shifted pole, early cytokinesis, longitudinal cleavage in normal anaphasic cell, precocious chromosome, presence of micronucleus and multi-nuclei among which the rare abnormalities are micro-nucleus and multi-nuclei which were observed only in 75% concentration for 12 hours.

4.5.6 Nature of abnormal cells in Control

No abnormal cells were recorded in either interphase or prophase of controlled cells of *Allium cepa L.* whereas in metaphase, several abnormalities such as diagonal plate, fragmentation, c-metaphase, stickiness and polyploidy were observed. In Telophase and Anaphase, early cytokinesis, unequal cytokinesis, non-synchronization, diagonal plate, fragmentation, disturbed phases and rarely bridge formation are observed.

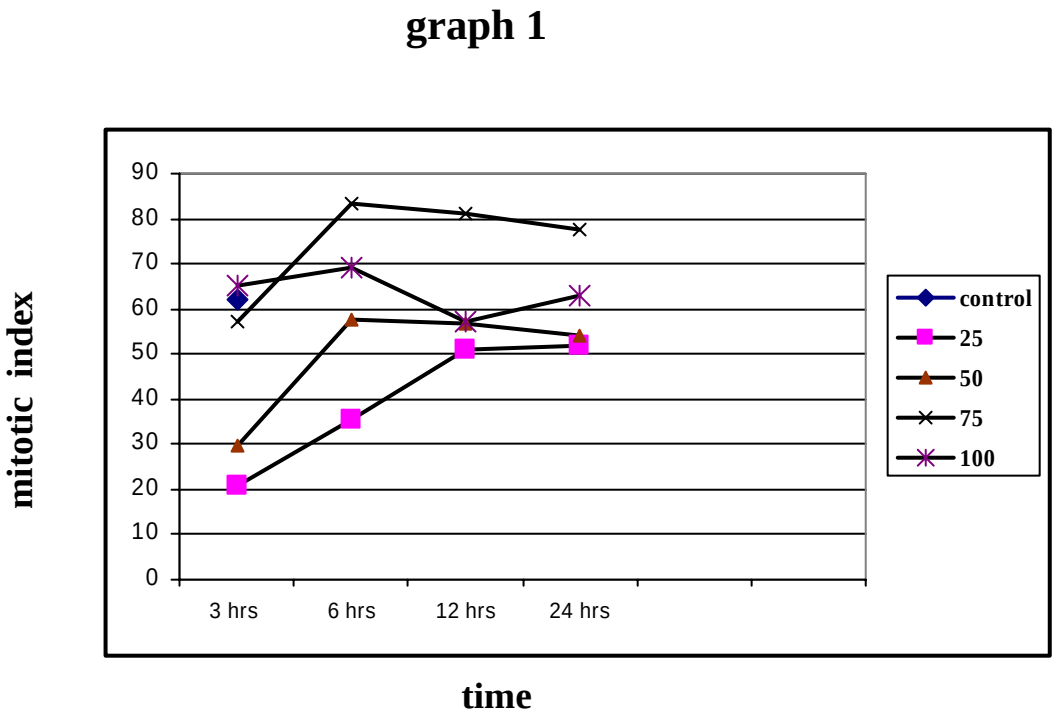


Figure 1: Graph of Mitotic Index of *Allium cepa* root tip cells vs Treatment time with given concentration of Penthoate

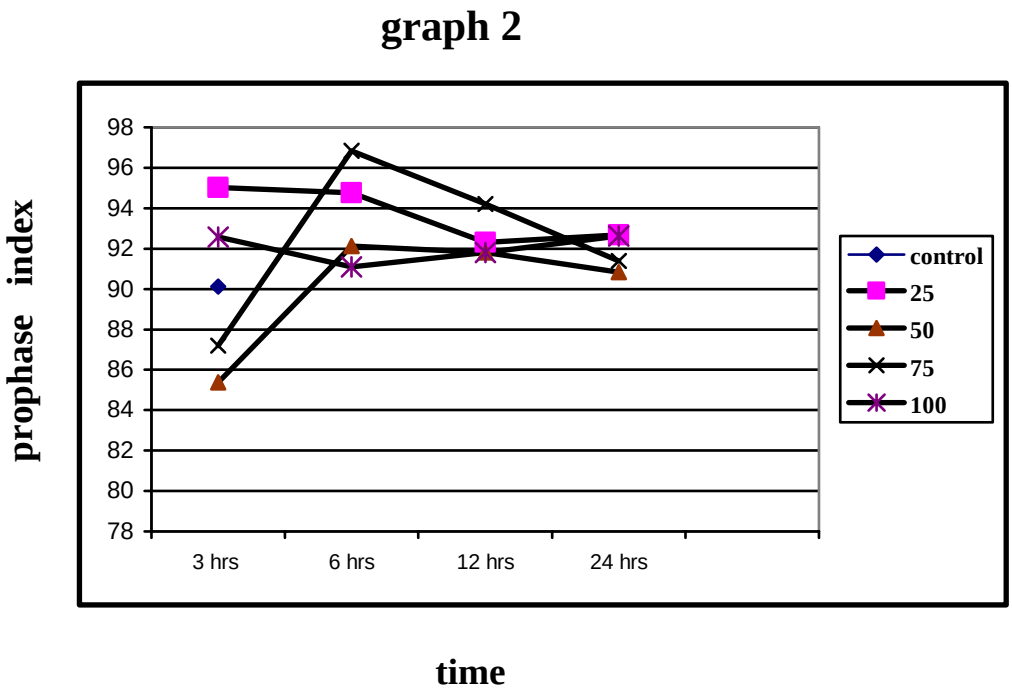


Figure 2: Graph of Prophase Index of *Allium cepa* root tip cells vs Treatment time with given concentration of Penthoate

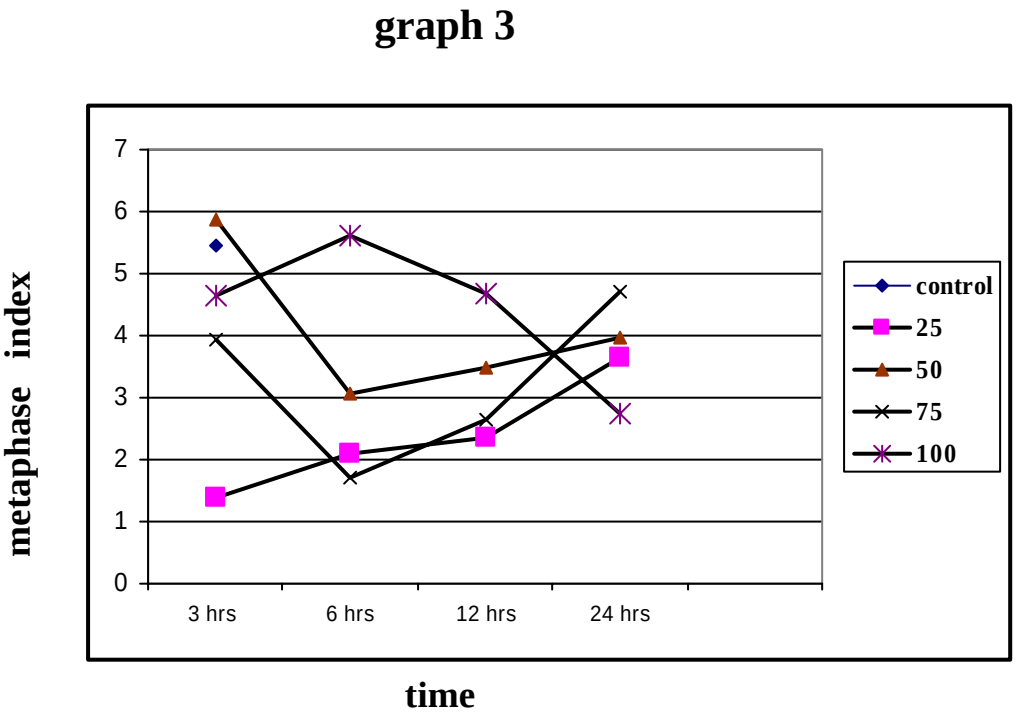


Figure 3: Graph of Metaphase Index of *Allium cepa* root tip cells vs Treatment time with given concentration of Penthoate

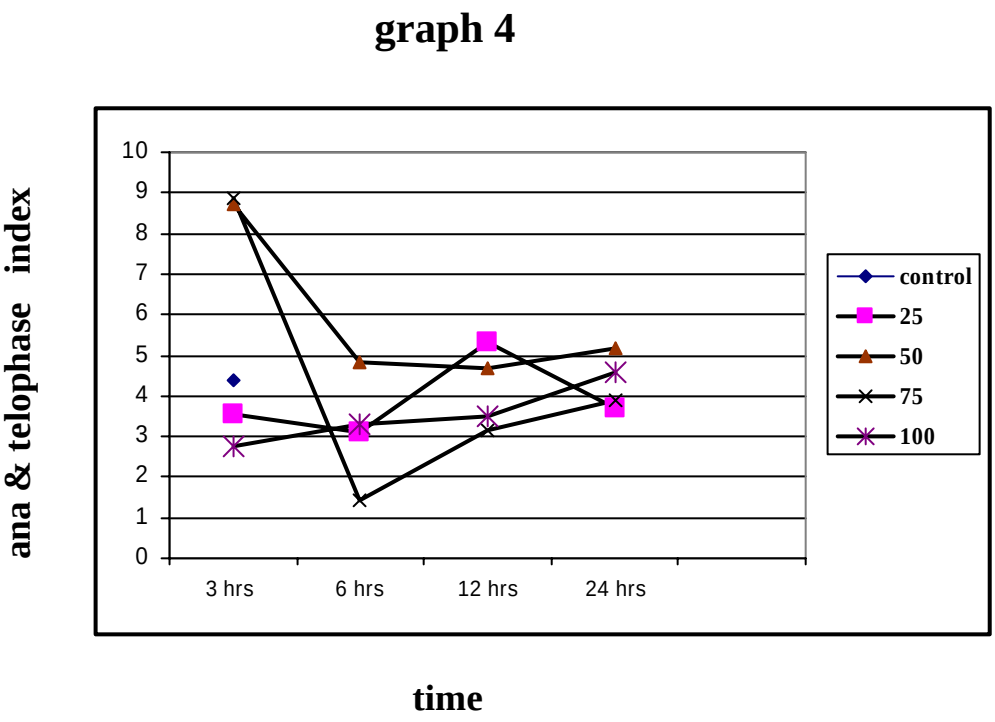


Figure 4: Graph of Anaphase and Telophase Indices of *Allium cepa* root tip cells vs Treatment time with given concentration of Penthoate

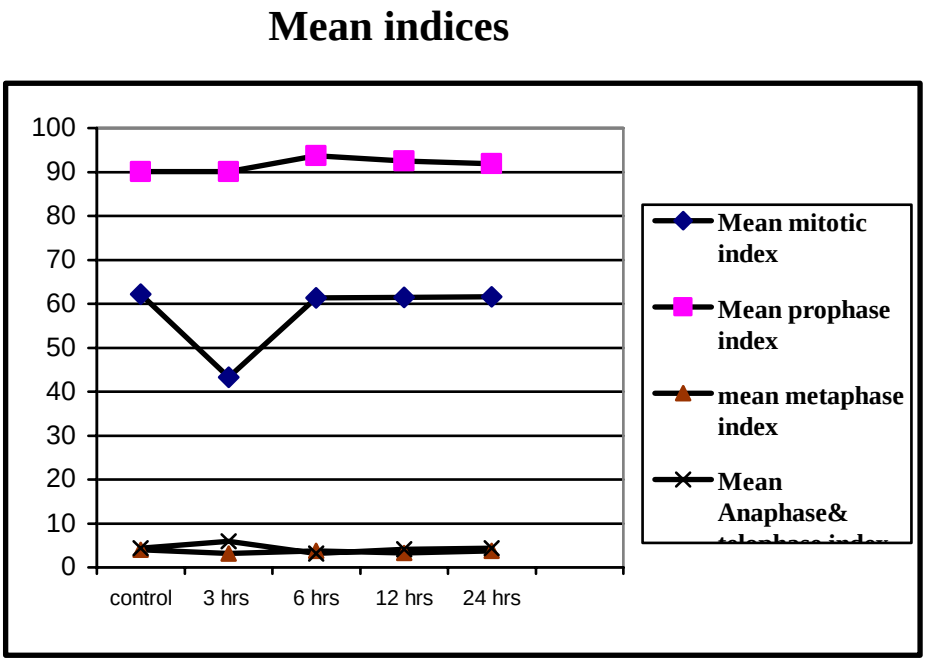


Figure 5: Graph of Mean-Mitotic, -Prophase, -Metaphase, -Anaphase and Telophase vs Treatment time with given concentration of Penthoate

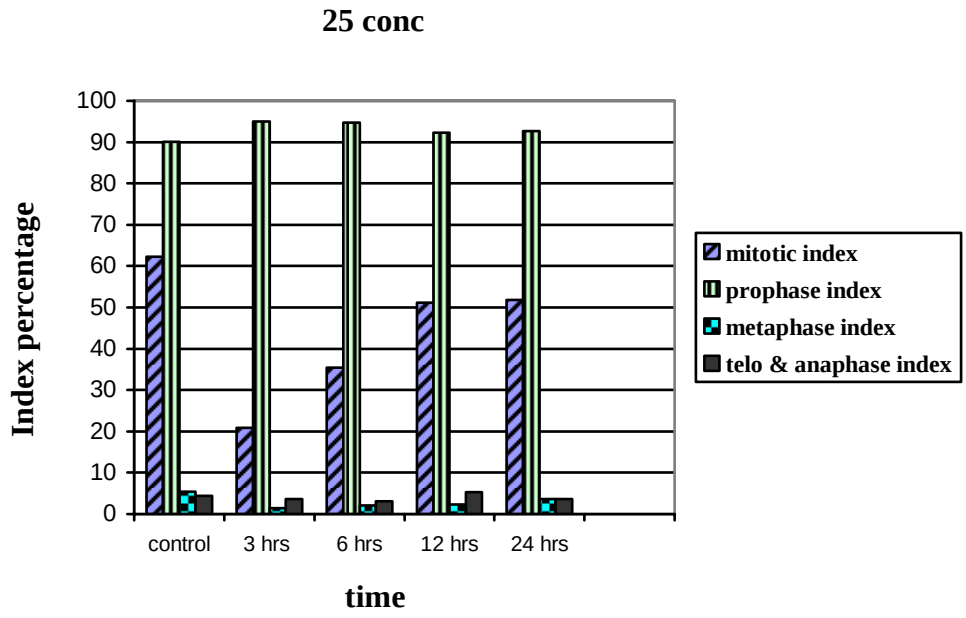


Figure 6: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices vs Treatment time with 25% concentration of Penthoate

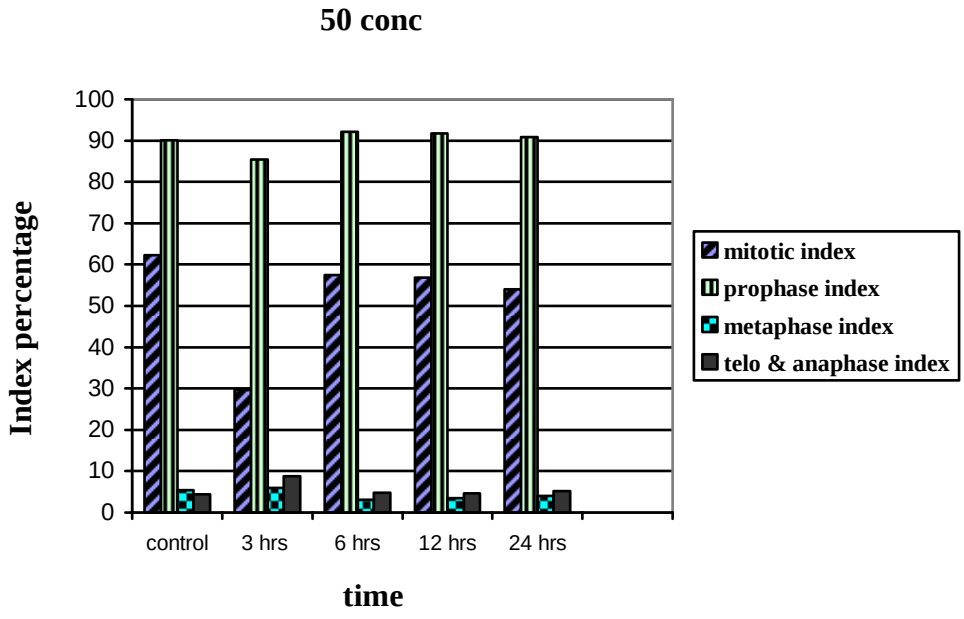


Figure 7: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices vs Treatment time with 50% concentration of Penthoate

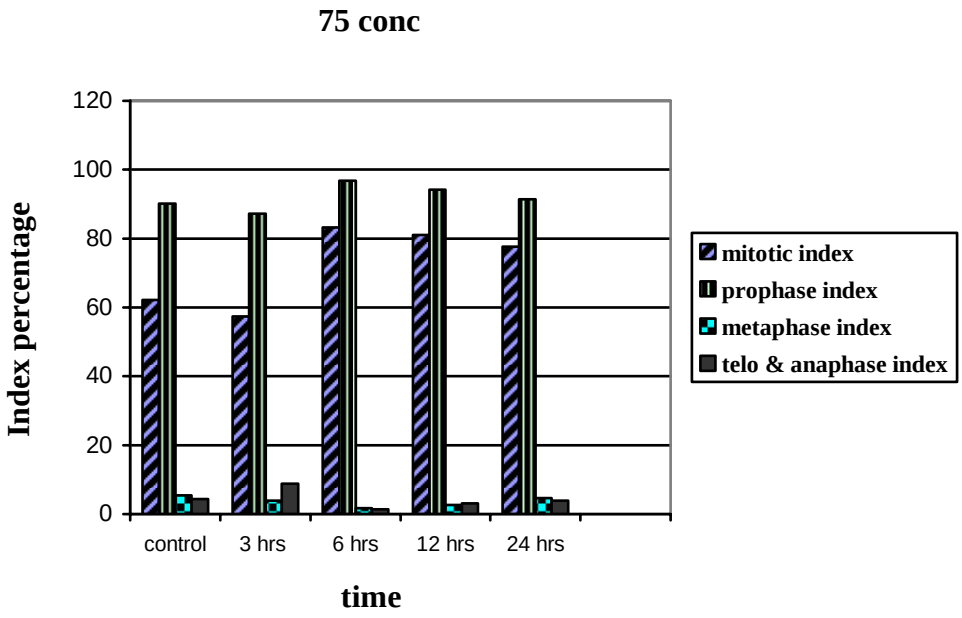


Figure 8: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices Vs Treatment time with 75% concentration of Penthoate

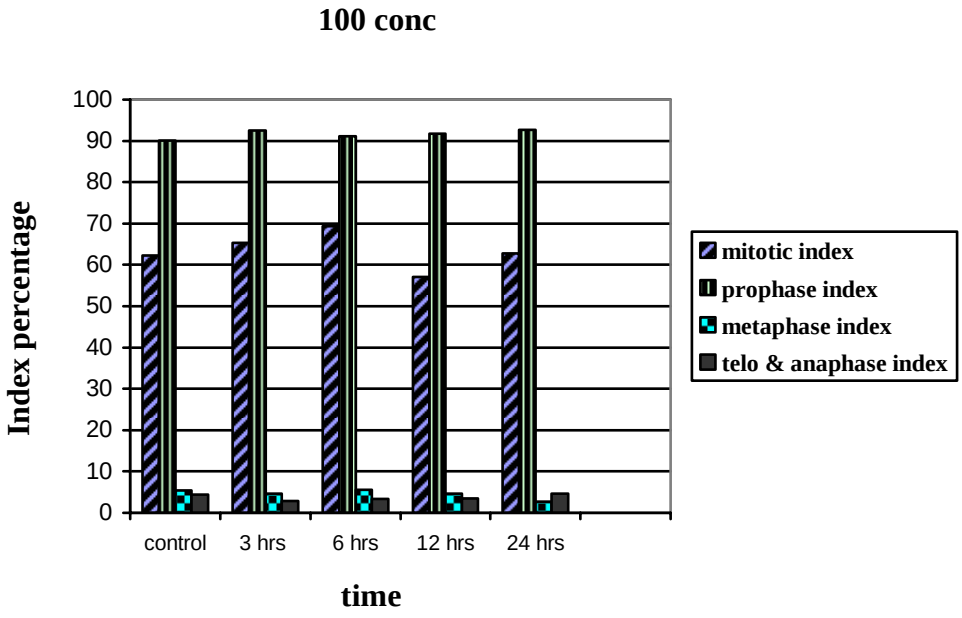


Figure 9: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices vs Treatment time with 100% concentration of Penthoate

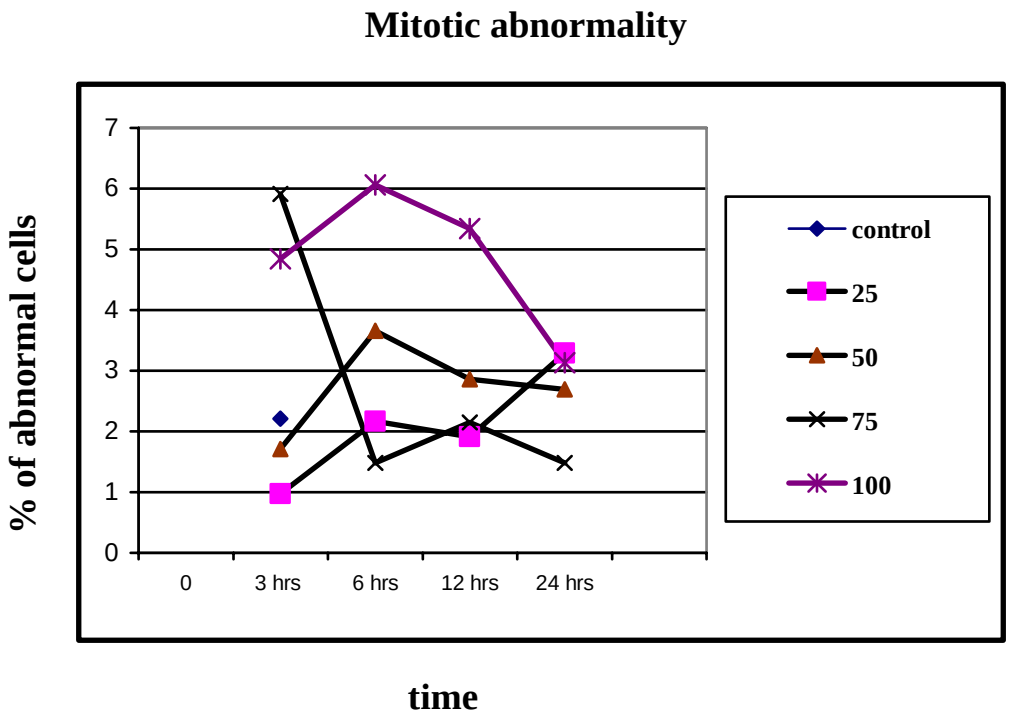


Figure 10: Graph of Total abnormal cells percentage vs Treatment time with given concentration of Penthoate

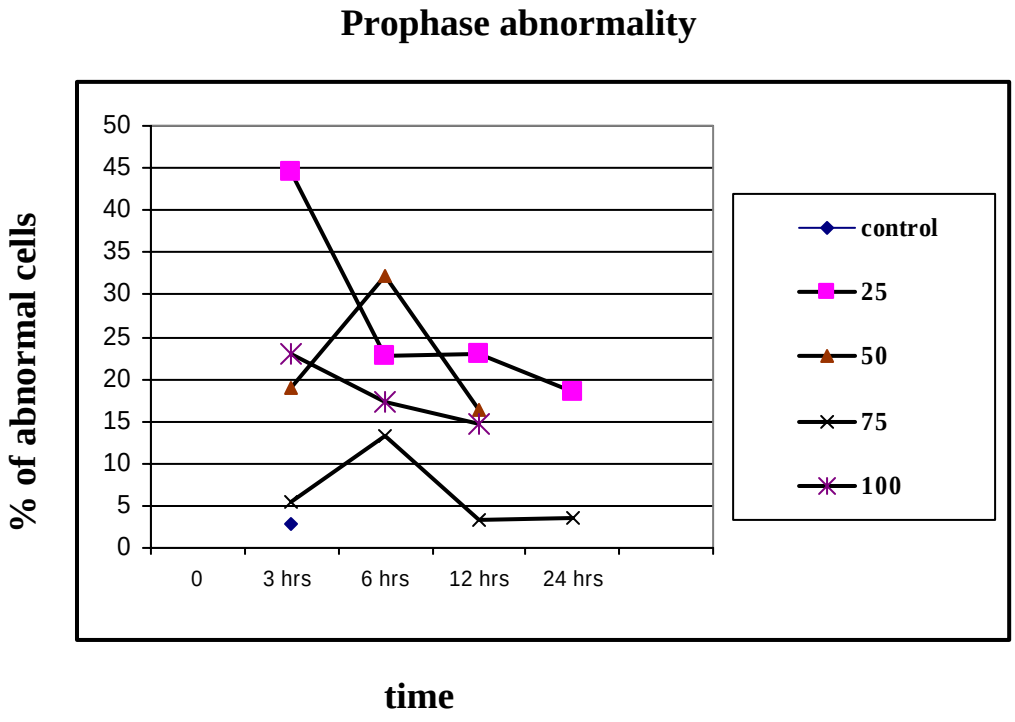


Figure 11: Graph of Percentage of Abnormalities among the abnormal cells in Prophase vs Treatment time with given concentration of Penthoate

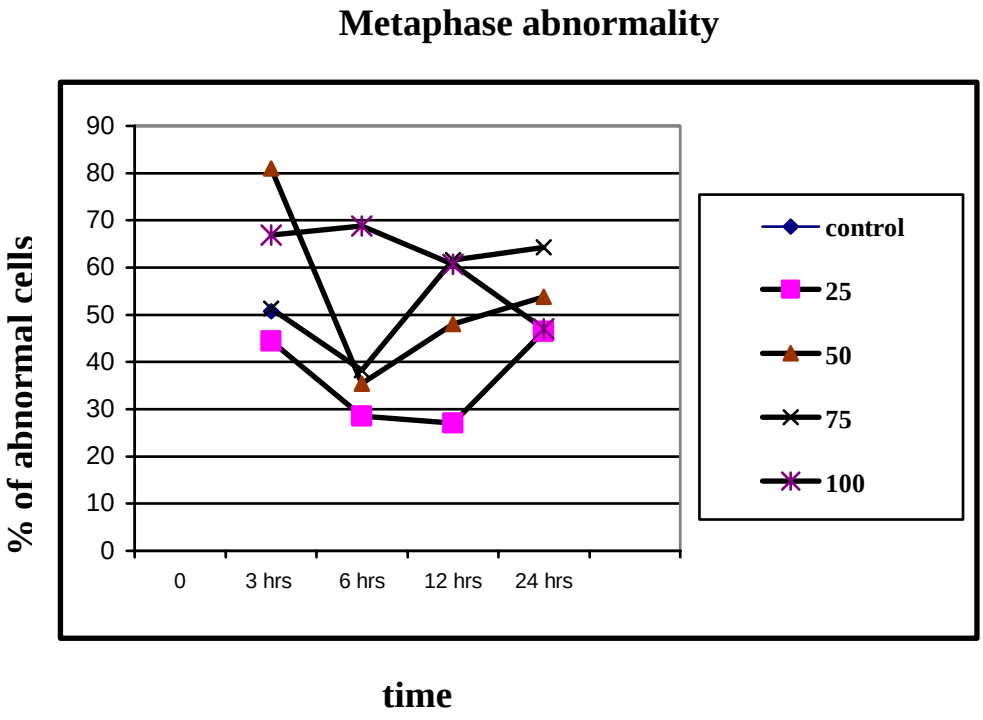


Figure 12: Graph of Percentage of Abnormalities among the abnormal cells in Metaphase vs Treatment time with given concentration of Penthoate

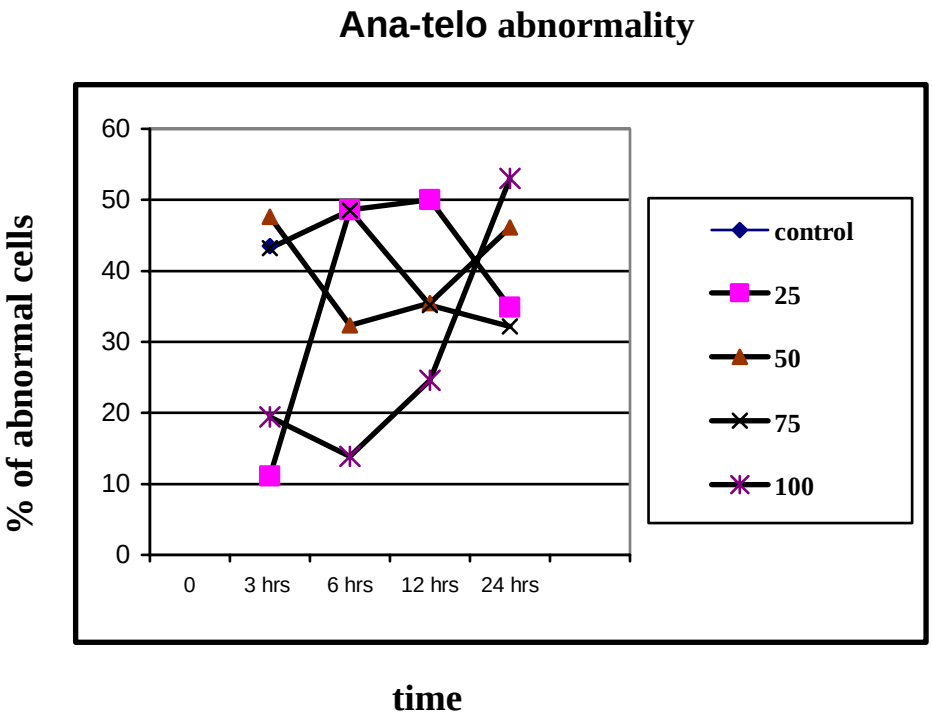


Figure 13: Graph of Percentage of Abnormalities among the abnormal cells in Anaphase and Telophase vs Treatment time with given concentration of Penthoate

Plate 1: Cytological effects of Penthoate

fig.1 c metaphase

fig.2 sticky metaphase

fig.3 precocious arm

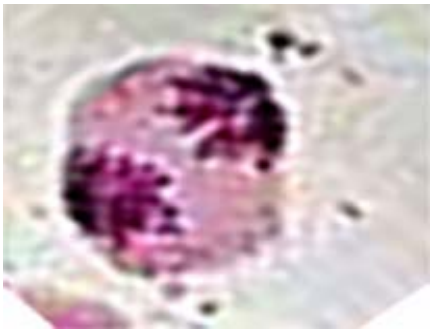


fig.4 diagonal anaphase

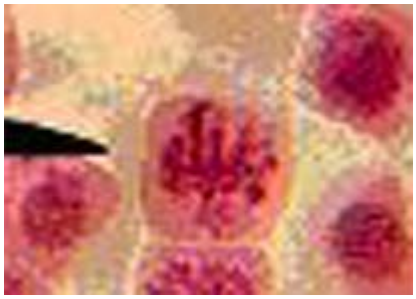


fig.5 star metaphase

fig.6 Normal Telophase

CHAPTER V

5. DISCUSSION AND CONCLUSION

The present work has been performed with the objective to acquire the results of the cytological effects of insecticide Penthoate on the mitotic activities and chromosome behaviour during the somatic cell division in the root meristem of *Allium cepa* L. It has been observed that the insecticide is capable of inducing various type of abnormalities in the dividing root meristem of *Allium cepa* L. the organophosphorus insecticide has considerable mitoinhibitory effect.

The mitotic index value seems to increase with the increase in the concentration in every duration of treatment whereas almost all treated samples show slightly less MI value than that in the control. This suggests a little mito-depressive action of compound. But at the same time when the dose is increased, the MI value has been 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 1990)

The statistical analysis reveals the value of chi-square as 4.8. On the contrary, the tabular value is 7.815. 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.

Prophase indices are found to be greater than that of control. This indicates that the chemical has some kind of mechanism for the prophase poisoning where cells are able to enter mitosis but get arrested in the prophase and the further division is ceased, thus, resulting high frequency of prophase cells (Prasad And Das, 1977).

Metaphase indices are obtained in highly fluctuating manner. The usual case is the increase in value in most of the samples except for the few ones such as in those of 75 % concentration for 6 hours and 25% for 3 hours. In case of Anaphase and Telophase indices, for the most of the samples, the value decreased with the increase in duration of treatment.

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.

Abnormalities in interphase are the binucleate cells, presence of micronuclei, dumbbell shaped nucleus and tri-nucleolate cells. Formation of binucleate cells was one of the common abnormalities in the light microscopic studies (Chauhan & Sundararaman, 1990). Mechanism of cytokinesis in plant cells involves the formation of new cell wall with the help of diffent organelles such as microtubules, golgi complex and possibly mitrocondria (Mollenhaur et al. 1980, Pickett-Heaps1967) 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 Sundararaman, 1990)

Very few dumb bell shaped nuclei were found. These types of nuclei were also reported by Salgo in 1983 and he described it as the consequence of the Telophase cells with persistent bridges which leads us to believe that dump bell shaped nuclei could have been formed as a result of the reconstitution of Telophase with such bridges.

Presence of micronuclei in the interphase cells was also prevalent. This was frequent in those samples treated with 100% Penthoate for 12 hours. Micronuclei have been reported by Clowes (1964) to be formed as a result of exclusion of acentric fragments of chromosomes out of the nuclear membrane during the completion of mitosis.

One of the major abnormalities found in the metaphasic cells is sticky metaphase (Plate 1). This stickiness of chromosomes may be attributed to the delay in the chromosome movement by insecticide treatment. Thus the chromosomes could not reach to the poles and remain scattered in the cytoplasm and appear condensed and sticky (Ajay, 1988).

However Mc Gill et al (1974) and Klastersaka et al (1974) proposed that the stickiness is due to the improper folding of chromosome fiber into single chromatid and chromosomes. . Another frequently occurring abnormality is c-metaphase (Plate 1). This occurs as a result of inhibition of spindle fibre formation and the further division is arrested at metaphase.(Dl-Khodarey et al, 1989)

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 (Farah 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.

Diagonal orientation of the chromosomes was also frequently obtained in the metaphasic cells. Very few star metaphases were found (Plate 1). Such type was observed also in Vicia faba roots with Rogor (Amer And Farah **)** and it is considered as being a fore-step of the complete disturbance of spindle (Amer 1966)

Fragmentation of the chromosomes was also observed frequently which can be attributed to the chromosomal breakages due to the effect of chemical (Ahmad and Yasmin, 1992). Penthoate is an organophosphorus insecticide and most organophosphorus compounds are radiomimetic and induce chromosomal breaks (Wuu and Grant 1966, Amer And Farah, 1980). According to Grant (1982),
the chromosomal fragmentation is multiple breaks resulting in the loss of chromosome integrity and this has been regarded to involve direct action on DNA (Grant 1978).

A considerable number of polyploid cells were observed in the treated samples. Deysson (1968) suggested that c-metaphase may lead to polyploid cells and thus formed degenerated without further division. Briand and Kapoor also reported the presence of polyploid cell in the *Allium cepa* root treated with sodium salicylate.

The abnormalities found in anaphase cells were shifting of poles, longitudinal arrangement of chromosomes, precocious arm, few bridges and one or two lag chromosomes. Besides these, tri-polar and tetra-polar are also occurred frequently

Bridges are formed due to breakage and reunion of the chromosomes (Permjit and Grover, 1985) while the lagging chromosome can be attributed to the delayed terminalization, stickiness of chromosome ends or because of the failure of chromosomal movement (Permjit and Grover, 1985).

Some unequal separation of the chromosomes at the anaphase stage was also obsereved and such cases are reported by Sinha et al, 1989 in the root tip of *Allium cepa* L. treated by Phosaline. This may be due to disruption in the spindle mechanism during anaphase (Raghuvanshi and Joshi, 1965). This unequal and irregular anaphasic separation might in the long run lead to multipolarity. Such multipolar spindles were noted in the onion after root treatments with Isopropyl-Phenyl-Carbamate and has been attributed to partial suppression of spindle action (Doxey, 1949)

The shifting of poles in anaphase may be the effect of Penthoate in spindle mechanism. Similar abnormality was reported by Pathak (1999) on somatic cells of *Allium cepa* treated with Carmoisine; Shrestha (2002) on root tips of *Allium cepa* with Isoproturon. According to them, depolymerisation of the spindle fibres causes the shifting of poles. Similar abnormality was also noticed by Bajracharya (1995) on *Allium sativum* treated with carpet dyeing effluent.

The precocious chromosomes were also noted during the observation. Precocious arms and precocious chromosomes formation may be the result of unequal spindle movement in which some chromosome arms are pulled towards the extremity of the pole. Such type of abnormality was reported by Adhikary (1982) in *Allium cepa* root meristems treated with 2,4-D and 2,4,5-Trichloro-phenoxy acetic acid; by Sapkota (2000) in *Allium cepa* root meristems treated with Carmoisine; by Shrestha (2002) in *Allium cepa* 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).

One 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 Penthoate could be responsible for the disruption of microtubules, causing ‘y’ or ‘x’ type of configurations in metaphase. Such disorientation may produce tripolar and multipolar

anaphase. Bale and Mathew (1987) reported tripolar anaphase by sodium fluoride; El-Khodary et al; (1988) by herbicide Garlon-4 on root mitosis 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 ovservations were reported by Rangaswamy et al. (1981) in onion root tips treated with effluent from lac and paint, plywood and sugar factory. (Sujata Shrestha, 2002)

The abnormalities found in Telophase were shifting of poles that has perhaps, led to the formation of diagonal and longitudinal types. Besides, unequal cytokinesis, unequal condensation of daughter chromosomes, delay in cell plate formation and non-synchronized movement of chromosomes were also ovserved. Polar shifting in telophase may be the continuation of the same in metaphase and anaphase.

The shifting of nucleus position may be due to the imbalance in the osmoregulation of the cells that caused cells to be plasmolysed and shifted the nucleus aside sometimes touching the cell wall. This phenomenon was described by Rangaswamy et al (1981). The unequal sizes of cells and nuclei were because of non-synchronized condensation of nucleus and unequal cytokinesis.

Bi-nucleate cells are also frequently observed. Delay in cell plate formation causes the delay in the completion of mitotic cycle. Delay or failure of cytokinesis would account for the occurrence of binucleated and trinucleated cells (Grant 1978; Badr and Ibrahim 1987). This statement was also supported by Sapkota (2000) and Shrestha (2002). Grant (1982a) cited a number of chemicals and pesticides which are capable of causing multinucleate condition.Hence it can be concluded that the Penthoate is also mutagenic to the dividing root tip cell of the onion.

6. SUMMARY

Penthoate is an organophosphorus insecticide widely used in agriculture to destroy the undesired insect-pests in order to produce a substantially high yield. The present cytological study indicates the mitodepressive action of the insecticide on the root meristematic cells of *Allium cepa* L. when treated for longer time. The decrease in the mitotic value and the alterations of other phase index values were found related with concentration of the insecticide and duration of treatment.The mean Mitotic Indices were found 43.31%, 61.36%, 61.51%, and 61.56% in 3, 6, 12 and 24 hours of treatment respectively, while the value of mitotic index for control was 62.2%. The decrease in mitotic index values in all treated groups suggested inhibitory action of penthoate. The overall result is the decrease of Mitotic activity for the samples treated for 3 hours and the increase in MI with the further increase in the duration of treatment. But most of the 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 chemical, the division is ultimately ceased due to arrest of mitotic activity at the prophase stage.** Further, the prophase index also increased with the increase in duration of treatment which is again explained by the prophase-poisoning phenomenon. The insecticide produced almost all major types of abnormalities, Laggards and bridge were the rarest while c-metaphase, sticky metaphase, diagonal-metaphase and diagonal-anaphase were found to be the most frequent ones. Observations of such abnormalities are attributed to metabolic disturbance, disturbance in spindle formation and depolymerization of nucleoprotein. The calculated chi square value which is 4.8 and lesser than the tabulated value of 7.815 shows that the insecticide is less significant. In other words, it has substantially less significant mutagenic effect on the growing root tip cells of *Allium cepa* L. Therefore the recommended dosage is suitable for use.

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8. APPENDIX

The indices and abnormalities were scored and analyzed by using the following formulae, according to Levan 1949 (cf. Kihlman 1971; Medeiros and Takahashi 1987).

(I) Mitotic Index (MI) = $\frac{\text{TDC x 100}}{\text{TC}}$

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

(III) Metaphase Index (Meta I) = $\frac{TC_{meta} \text{ x } 100}{\text{TDC}}$

(IV) Ana-Telophase Index (Ana-Telo I) = $\frac{TC_{ana\text{-}telo} \text{ x } 100}{\text{TDC}}$

(V) Total percentage of abnormal cells (T_{abn}) = $\frac{TC_{abn} \text{ x } 100}{\text{TDC}}$

(VI) Total percentage of abnormal cells at prophase (T_{pro}) = $\frac{T_{abnPro} \text{ x } 100}{\text{TDC}}$

(VII) Total percentage of abnormal cells at metaphase (T_{meta}) = $\frac{T_{abnMeta} \times 100}{TDC}$

(VIII) Total percentage of abnormal cells at Anaphase and Telophase

$$T_{ana\text{-}telo} = \frac{T_{abnAna\text{-}Telo} \times 100}{TDC}$$

(VIII) Percentage of Abnormalities at Prophase among the abnormal cells

$$A_{pro} = \frac{T_{abnPro} \times 100}{TC_{abn}}$$

(IX) Percentage of Abnormalities at Metaphase among the abnormal cells

$$A_{meta} = \frac{T_{abnMeta} \times 100}{TC_{abn}}$$

(X) Percentage of Abnormalities at Ana-Telophase among the abnormal cells

$$A_{ana\text{-}telo} = \frac{T_{abnAna\text{-}Telo} \times 100}{TC_{abn}}$$

Table 2:Total No. of cells of *Allium cepa* L. at each phase with different concentration of Penthoate at different time of treatment

Duration in Hrs	Penthoate in %	Total Cells Counted	Interphase			Dividing Cells			Prophase			Metaphase			Anaphase & Telophase		
			Total	Nor	Abn	Total	Nor	Abn	Total	Nor	Abn	Total	Nor	Abn	Total	Nor	Abn
	Control	5000	1890	1890	-	3110	3041	69	2803	2801	2	170	135	35	137	107	30
3	25	4450	3523	3523	-	927	918	9	881	877	4	13	9	4	33	32	1
	50	4125	2900	2889	11	1225	1204	21	1046	1042	4	72	55	17	107	97	10
	75	5397	2301	2285	16	3096	2913	183	2700	2690	10	122	28	94	274	195	79
	100	4388	1519	1481	38	2869	2730	139	2656	2624	32	133	40	93	80	53	27
6	25	4528	2921	2919	2	1607	1572	35	1523	1515	8	34	24	10	50	23	17
	50	4550	1934	1933	1	2616	2520	96	2410	2379	31	80	46	34	126	95	31
	75	5500	926	894	32	4574	4506	68	4430	4421	9	78	52	26	66	33	33
	100	4118	1264	1254	10	2854	2681	173	2600	2570	30	160	41	119	94	70	24
12	25	4905	2398	2398	-	2507	2459	48	2314	2303	11	59	46	13	134	110	24
	50	4860	2099	2095	4	2761	2682	79	2535	2522	13	96	58	38	130	102	28
	75	5195	982	980	2	4213	4122	91	3969	3966	3	111	55	56	133	101	32
	100	4004	1720	1710	10	2284	2162	122	2097	2079	18	107	33	74	80	50	30
24	25	5039	2428	2426	2	2611	2525	86	2420	2404	16	95	55	40	96	66	30
	50	4466	2053	2050	3	2413	2348	65	2192	2192	-	96	61	35	125	95	30
	75	4860	1088	1088	-	3772	3716	56	3447	3445	2	178	142	36	147	129	18
	100	4213	1566	1565	1	2647	2546	83	2452	2452	-	73	34	39	122	78	44

Table 3:Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices of *Allium cepa* L. with Different concentration of Penthoate at different time of treatment					
Duration of treatment (hours)	Concentration of Penthoate (%)	Mitotic Index	Prophase Index	Metaphase Index	Anaphase & Telophase Index
	Control	62.2	90.12	5.46	4.40
3	25	20.83	95.03	1.4	3.55
	50	29.69	85.38	5.87	8.73
	75	57.36	87.20	3.94	8.85
	100	65.38	92.57	4.63	2.78
6	25	35.49	94.77	2.11	3.11
	50	57.49	92.12	3.05	4.81
	75	83.16	96.85	1.70	1.44
	100	69.30	91.10	5.60	3.29
12	25	51.11	92.30	2.35	5.34
	50	56.81	91.81	3.47	4.70
	75	81.09	94.20	2.63	3.15
	100	57.04	91.81	4.68	3.50
24	25	51.81	92.68	3.63	3.67
	50	54.03	90.84	3.97	5.18
	75	77.61	91.38	4.71	3.89
	100	62.82	92.63	2.75	4.60

Table 4: Mean Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices treated with mean concentration of Penthoate at different time of treatment				
Duration of treatments (hours)	Mean mitotic Index	Mean Prophase Index	Mean Metaphase Index	Mean Anaphase & Telophase Index
Control	62.2	90.12	3.96	4.40
3	43.31	90.04	3.11	5.97
6	61.36	93.71	3.71	3.16
12	61.51	92.53	3.28	4.17
24	61.56	91.88	3.76	4.33

Table 5: Total Percentage of abnormal cells and Percentage of abnormal cells at each phase among the abnormalities with different concentration of Penthoate at different time of treatment

Duration of treatment	Concentration of Penthoate (%)	Total % of Abnormal cells	Total % Abnormal cells in Prophase	Total % of Abnormal cells in Metaphase	Total % of Abnormal cells in Anaphase & Telophase
	Control	2.21	2.89	50.72	43.47
3 hrs	25	0.97	44.44	44.44	11.11
	50	1.71	19.04	80.95	47.61
	75	5.91	5.46	51.37	43.17
	100	4.84	23.02	66.90	19.42
6 hrs	25	2.17	22.85	28.57	48.57
	50	3.66	32.29	35.41	32.29
	75	1.48	13.23	38.23	48.53
	100	6.06	17.34	68.78	13.87
12 hrs	25	1.91	22.91	27.08	50.00
	50	2.86	16.45	48.10	35.44
	75	2.15	3.29	61.53	35.16
	100	5.34	14.75	60.65	24.59
24 hrs	25	3.29	18.60	46.51	34.88
	50	2.69	-	53.84	46.15
	75	1.48	3.57	64.28	32.14
	100	3.13	-	46.98	53.01

Table 6: Percentage of Abnormal and Normal cells at each phase with different concentration of Penthoate at different time of treatment

Duration of treatment	Concentration of Penthoate (%)	Prophase %		Metaphase %		Anaphase & Telophase %	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
	Control	90.06	0.06	4.34	1.12	3.44	0.96
3 hrs	25	94.60	0.43	0.97	0.43	3.45	0.10
	50	85.06	0.32	4.49	1.38	7.91	0.81
	75	86.89	0.32	0.90	3.03	6.29	2.55
	100	92.57	1.11	1.39	3.24	1.84	0.94
6 hrs	25	94.27	0.50	1.50	0.62	1.43	1.05
	50	90.94	1.18	1.75	1.60	3.63	1.18
	75	96.65	0.20	1.13	0.57	0.72	0.72
	100	60.04	1.05	1.43	4.16	2.45	0.84
12 hrs	25	91.86	0.44	1.83	0.51	4.38	0.95
	50	91.34	0.47	2.10	1.37	3.69	1.01
	75	94.13	0.07	1.30	1.32	2.39	0.76
	100	91.02	0.78	1.44	3.24	2.19	1.31
24 hrs	25	92.07	0.61	2.10	1.53	2.52	1.14
	50	90.84	-	2.52	1.45	3.93	1.24
	75	91.33	0.05	3.76	0.95	3.41	0.47
	100	92.63	-	1.28	1.43	2.94	1.66

Table 7: Mean Mitotic index of *Allium cepa* L. root tip cell treated with different concentration of Penthoate at different time of treatment

Percentage of Penthoate	Mitotic Index (k), treated in different time periods			
	3 hrs	6 hrs	12 hrs	24 hrs
25	20.83	35.49	51.11	51.81
50	29.69	57.49	56.81	54.03
75	57.36	83.16	81.09	77.61
100	65.38	69.30	57.04	62.82

Table 8: Rank of Five matched groups concentration of Mitotic Indices of value of *Allium cepa* L. under four conditions (times)				
Percentage of Penthoate	Mitotic Index (k), treated in different time periods			
	3hrs	6hrs	12hrs	24hrs
25	1	2	3	4
50	1	4	3	2
75	1	4	3	2
100	3	4	1	2
Rj	6	14	10	10

$$\chi^2 = \frac{12}{NK(K+1)} \sum_{j=1}^{K} (Rj)^2 - 3N(K+1)$$

$$= \frac{12}{4 \times 4(4+1)} (6^2 + 14^2 + 10^2 + 10^2) - 3 \times 4(4+1)$$

$$= \frac{12}{16 \times 5} \times 432 - 12 \times 5$$

$$= 4.8$$

where,

N= 4 number of rows (concentration)

K= 4 number of columns (duration 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 the reference to the chi (X) square distribution with df= k-1 and the significance value were matched at percentage $(p)<0.05$.