TREATMENT OF ORGANOPHOSPHORUS INSECTICIDE MONOCROTOPHOS AND ITS EFFECT IN DIVIDING CELLS OF ALLIUM CEPA L.

A DISSERTATION SUBMITTED TO CENTRAL DEPARTMENT OF BOTANY FOR THE PARTIAL FULFILLMENT OF THE MASTER'S DEGREE OF SCIENCE

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ABSTRACT

The cytological effects of organophosphorus insecticide "Monocrotophos" has been studied on root meristem of *Allium cepaI L*. The out come of study is shown that Monocrotophos induced mitodepresive effect and chromotixic (cytological abnormalities). The effect included high lethality for cell division, c-metaphase, fragments, breaks, laggards, stickness and disturbed polarity.

The main abnormalities were plasmolysed cells, polar shifting (diagonally arranged chromosome in anaphase and telophase), c-metaphase, stickey metaphase, precocious arms, larggard, bridge, breaks in chromosome arms, fragmentation of chromosomes, delayed cytokinesis, diagonal cytokinesis, unequal cytokinesis and bi and tri nucleated in some cases.

ABBREVIATIONS

MI - Mitotic index

Prol - Prophase index

Meta I - Metaphase index

Ana-Telo I - Anaphase and Telophase index

TDC - Total dividing cells

TC - Total number of cells counted

TC_{pro} - Total number of cells counted at prophase

TC_{meta} - Total number of cells counted at Metaphase

$TC_{Ana+telo}$ - Total number of cells counted at Anaphase and Telophase

T_{Abn} - Total percentage of Abnormal cells

T_{pro} - Total percentage of Abnormal cells at Prophase

T_{Meta} - Total percentage of Abnormal cells at Metaphase

$T_{Ana+meta}$ - Total percentage of Abnormal cells at Anaphase

TC_{Abn} - Total number of Abnormal cells counted

TC_{AbnPro} - Total number of Abnormal cells counted at Prophase

$TC_{AbnMeta}$ - Total number of Abnormal cells counted at Metaphase

$TC_{Abn\ Ana\ +Telo}$ - Total number of Abnormal cells counted at Anaphase Ana TelophaseA$_{pro}$ - Percentage of Abnormalities at Prophase among the Abnormal cells.

A_{meta} - Percentage of Abnormalities at Metaphase among the Abnormal cells.

$A_{Ana+Telo}$- Percentage of Abnormalities at Anaphase and Telophase Among d the Among the Abnormal cells.

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CHAPTER – I

1. Introduction:-

The pests are those unwanted organisms which directly or, indirectly interfere with human activity. eg. Mites, weeds, aquatic plants, fungi, bacteria, virus, nematodes, snail, slug and birds. Since 1945, vast fields planted with one crop or, only a few crops have blanketed with variety chemicals to kill pests called pesticides.

Chemicals include in food additives, cosmetics, and drugs pesticides. 10,000 of them are produced annually in quantities between 500 and 1,00,000 kg (UNEP 1975). The use of pesticides has been increasing despite their potential hazardous nature in human environment. It is now obvious that use of pesticide has many secondary consequences (Reddy and Rao, Joshi and Bhuju, Joshi *et. al.* 1982) and their repeated use may even induce resistance in pest. They are intended to control. Pesticides can improve crop fields and help the control population of diseases. However, there is considerable evidence that the wide spread use of pesticide can have harmful effects on wild life, ecosystem structure and function and human health. The over use can lead to increase in crop losses and resurgence of the diseases. Pesticides produce the mutagenic and carcinogenic effect in plants and organisms and bring changes in genetic constitution. Mutagenic testing has been assumed increasing importance in content of society's responsibility not only to conserve the genetic material as a whole but also protect our own hereditary material from artificial increases in the frequencies of detrimental mutation (Bhunya and Behera 1988).

The U.S. environmental agency (EPA) has registered more then 2600 pesticide active ingradients, 567 herbicides, 610 insecticides, 670 fungicides and nematocides, 125 rodenticides and 630 disinfectants. According to their mode of action insecticides are classified as.

1. Stomach poison eg. Parathion, malathion.
2. Contact poison eg. DDT, BHC
3. Fumigants eg. Methyl bromide, HCN, CCl_4.
4. Systemic eg. Phosphoamidon, monocrotophos.

Their general toxicity.

Insectides > defoliants > dessicants> herbicides> fungicides.

The toxicity of insecticides.

Organophosphate > carbamates > cyclodienes > DDT relatives > botanicals > activators > inorganics.

In Nepal, the use of pesticide began in 1940's to eradicate Malaria out breaks throughout the country by using dichlorodiphenyl trichloroethane (DDT). More then 250 different types of insecticides and 40 herbicides are registered for their use in Nepal and their consumption is estimated to be approximately 55 metric tons par annum (MOPE 1998) and worth of Rs. 1.5 million is imported in every year 44% insecticides, 50% fungicides, 2% herbicides and 4% other (Uddin 2004). In 1977, Nepal pesticide and chemical industries Pvt. Ltd (NEPCIL) was established.

The danger of pesticides is not necessary result of direct application where some pesticides accumulate in the food to a toxic level and affect the public health. Although the use of insecticides has reduced significantly, the crop lands may still have some residual effect from persistent chemical

usage. Studies have indicated accumulation of DDT in rice, wheat and milk (Joshi, 1998).Out of 527 samples of rice analyzed, over 70% were found contaminated by DDT, in case of wheat DDT residues were higher then in rice. Similarly, out of 60 samples analyzed for DDT in milk in early 1980s, 55 samples contained high amount of DDT above MRL ie. 0.05 (E) mg/kg and the highest level of DDT detected was 1.2ppm (Joshi 1998). DDT residue in pulse, maize, oil, seed and fruits was also detected as being high. In this way, the frequency of cancer increases among people who have been exposed directly or, indirectly to pesticides.

Due to lack of education and ignorance, higher concentration of insecticides are being used then recommended dose in Nepal.

Monocrotophos:- Monocrotophos is used as systemic organophosphorus . Inclusion of Monocrotophos in PIC procedure because of it's high toxicity. It is likely to cause problem under condition of storage, transportation and use in developing countries. Acute oral LD_{50} S range from 0.9-6.7 mg /kg bw. It is used avian poison which kills birds. In Argentina 1995, 15,000 to 20,000 birds were killed. WHO recommends that the health and welfare of

workers and general population, the handling and application of monocrotophos should be entrusted only to supervised and well trained applicators, who follow the safety measures.

The recommended dose in 1ml per liter depending upon size and age of plant.

Common name :- Monocrotophos

Trade name :- Navacron, Monosil, Monohit, Monocil, Moncron, Red star Monocrotophos, Azodrin, Crotos, Bilobrin etc.

Chemical name (IUPAC):- Dimethyl (E) -1- methyl -2-(methyl carbamoyl) vinyl phosphate.

Chemical formula:- $C_7 H_{14} No_5 P$

Structural formula :- $(CH_30)_2$ P-O H

Properties (physical and chemical):- Monocrotophos is colourless, hygroscopic. Solubility is 1 kg /l (20^0C water), vapour pressure – 0.29 m pa (20^0C), melting point 54 – 55^0C, 50% loss occurs in 96 days at PH 5, 66 days at PH 7, 17 days at PH 9. It is a fast active insecticide with both systemic and contact action. Affect the nervous system. It persists for 7-14 days.

Manufacturer:- National organic industries limited, Bombay, India, Comlets chemical industrial Co. ltd. (R.O.C) Cyanomid (Brazil), Nantong pesticides factory (China), Sundat (S) Pte. Ltd. (Singapore) etc.

Objective: - The study is to find out the cytological effects of insecticides monocrotophos on root tip cells of Allium cepa.

Justification of study: - Pesticides are used generally in Terai to control the pest in Nepal. The large amount of pesticides are used annually in Nepal. Monocrotophos is used as trade name, Monohit Monocron, Monosil etc. The cytological effect of monocrotophos pesticide is undertaken to understand the genetic effects in root tip of Alliums cepa in lower or higher dose then the recommended dose.

CHAPTER II

2. Literature Review

By the effect of different pesticides, it has been found that the continuous and excessive use might be lead to mutagenic and carcinogenic effect (Kumar and sinha, 1989). Excessive and continuous use of pesticides, Herbicides, fungicides, laboratory agent, plant extracts of medicinal values, food additives and antibiotics are capable to induce breaking down of chromosome, clumping of chromosomes and inhibition of cell division.

Literature review revealed the studies of different chemicals, extracts and effluents effect on meristematic activity and growth of plants which are as follows.

Cytological Effect Of Pesticides In Plant Cell

Aberration of chromosomes by various insecticides like carbamate (N- methyl - Naphthyl carbamate) “Sevin” in roots of Alliums cepa were reported by Amer (1965).

Wuu and Grant (1966; a,b) observed chromosomal aberration including stickiness, extreme clumping, chromosome bridge, fragments and micronuclei etc caused by 15 different pesticides on root tip of barely and stated that the pesticides showed to be effective radiomimetic agents.

Phenol caused meiotic irregularities like stickiness, lagging chromosome and anaphase bridges in Vicia faba. Amer and Ali, (1968).

The insecticide DDT, Gammaxame, Folidol (diethylparanibophenly - thio-phosphate) have been known to produce mito-depressive effect and

cytological abnormalities like c-mitosis, stickiness, breakage, laggard, fragment, anaphase bridge, micronuclei. Amer and Ali, (1969).

Folidol should complete inhibition of anaphase at higher concentration and increased duration of treatment in root tip of Allium cepa Ravindra, (1971).

Kaul (1971) found 30% of irregular meiotic cells in Vicia faba plants treated with 2,2dichloropropionic acid. The abnormalities stickiness, clumping of chromosome, bridge and Laggards.

Disturbed prophase, meta-ana lagging chromosome, chromosome fragmentation, stickiness, anaphase bridge and multipolar anaphase were found deviation from normal root tip cell of vicia faba with treatment carbamate pesticides Rogar. Amer and Farah (1974).

Joshi and Bhaju (1982) found that maleic hydrazide induced both external morphology and chromosomal behavior meiotic division in Triticum aestivum (wheat).

Kaur and Grover (1985 a, b) investigated mitotic and meiotic effects of eight organophosphate eg. Dursban, parathion etc. in barely. They found ring chromosome, C-metophase, laggard, multipolar cells at anaphase, chromatin bridge.

The four Op insecticides viz. dichlorovos, monocrotophos, phosalene and oxydemetonmethyl induced clastogenic and turbogenic effect in root meristem of Allium cepa (Rao et al 1987). Monocrotophos was more effective as clastogenic followed by dichlorovos, phosalene and oxydemetonmethyl respectively.

BHC, Lindane, Aldrin, Endrin, (four) chlorinated pesticides induced genetic recombination, stickiness, fragmat, bridge, binucleated cell, cytomixis, multipolar, scatter chromosomes (Jain and Sarbhoy 1988).

The cytological effects organophosphate phosalone were studied on root meristem of Allium cepa. Concentration (0.25%, 0.5%, 0.75% and 1.0%) of pesticides were taken and subsequent knop's recovery was done for 96 hrs after treatment of up to 24 hours and 48 hours. Mitotic index gradually decreasing with increasing in concentration and time or treatment. Mutagernic effect like chromosome breakage and erosion, sticky, bridge, laggard, multiplicity, polyploidy, clumping. The slow rised the mitotic index after recovery for 48 hours and 96 hours of 24 hours and 48 hours and 48 hours respectively. Total percentage of abnormalities was increased concentration 0.25% and 0.75% and slightly to decreased in concentration 0.50% and 1.0% respectively. Material treated with 0.75% and 1.0% phosalone for 48 hours failed to show marked recovery with death of roots. Sinha et al, (1989).

The effect of two organophosphors insecticides malathion and tamaron on mitosis and some metabolic process of vicia faba root tips was studied by Adam et al (1990). It induced stickiness, abnormal prophase, spindle disturbance, lagging, bridge, binucleated cell, contraction and synchronisation of chromosome in anaphase and telophase.

Upendra and Sinha (1991) studies Rogar and Bavistin and on antibiotic (streptomycin) induced. Mutagenic effect in pollen mother cells of lathyrus sativus. All chemical were induced cytological disturbances and genotoxic effect.

Methyl parathion produced more chromosomal, aberration then tri miltox. Increased number of abnormal mitotic cells such as bridge formation, laggard chromosome and micronuclei. (Ahmad and yasmin 1992).

The root tip Allium cepa were exposed to malathion and post treated with the phyllanthus sp. extract and distilled water. This could neither restore the normal mitotic index nor bring about reduction in chromosomal irregularities. But residual analysis of treated and post treated cells showed the absence of pesticidal residues (Priya et.al, 1995).

Metacid showed severe cytological effect like C-metaphase, chromosomal irregulaties, retardation of chromosome movement, clastogenic effect etc then Taxus baccata extract on meristem of Allium cepa (Manandhar 2000).

Insecticides chloropyrifos treated on Allium sativum root showed mito depressive, chromosomal aberration like scattering chromosome, breaks, chromatid separations bridge, polarity, laggard, bincleated, multinucleated etc.it not only inhibits mitotic activity but also show impact on chromosomal behavior of cell (Yadav et.al. 2000).

Shrestha (2002) studied the cytological effects organophosphorus (metasytox) in meristematic cell of Allium cepa. It induced mitodepressive as well as positive chromatoxic effects. The abnormalities like plasmolysed cell, non synchronised movement of chromosome, precocious, laggard, bridge, diagonal and delay cytokinesis, bi and trinucleated cells were seen.

Kumar and kumar (2004) studied genotoxic effect of pesticides, Aldrin, Malathion and monocrotophos in root tip cell of Allium cepa and treated with 0.1% , 0.2% , 0.3% , 0.4% , 0.5% and 1% room temp. For 6 hours induced depression of mitotic index. Chromosomal abnormalites such as

stickiness, fragmentation, bridge, laggard and micronuclei have been found.

Bom (2005) studied the cytological effect of pesticide Dimethoate on mitotic activity and chromosomal behaviour in root meristematic cells of Allium cepa L. Chromosomal behaviour at higher concentration and larger time shows toxic effect. The abnormalities like c-metaphase, breaks and fragment chromosome (clastogenic effect), stickiness, disturbed meta and anaphase (cytotoxic effect), polyploidy, bi and tri nucleated cell were observed.

Cytological effect of fungicides in plant cells.

Chromosomal abnormalities like stickiness, breaks and bridges and inhibit mitotic activity was found but exerted a weak c-mitotic activity in Allium cepa, Eleusine corocana and lycopersicum esculentum root meristems. Among which Allium cepa was found to be the most sensitive when treated with Bavistin and Diathane M -45 (Joshi et.al, 1982).

Epiphenphos fungicides have been treated on bone marrow chromosome of mice in vivo influence the aberration frequency. It has been found to mutagenic in present test system (Bhunya and Behera 1984).

Various types of spindle abnormalities inhibits cell plate formation and antimitotic activity by Vitavax, a systematic fungicide was tested for it's antimitotic and clastogenic effect on Allium cepa (Somashekhar et.al1984).

Chromosomal aberration like univalent, multivalent stickiness, non orientation of chromosome at meta 1, bridges, laggard etc. was observed at anaphase on treatment Bavistin and Diathane to inhibit seed germination and seedling survival (Prakash et.al1988).

Carbendazin was treated with seeds of sunflower and pearlmillet, variety of chromosomal aberration in somatic (root tip) and reproductive (Pollen cell) cells observed more chromosomal aberration was more in pearl millet then sunflower . At late prophase leading to fragment and stickiness and laggard at meta and anaphase, while no aberration at telophase in somatic cell of pearl millet and abnormal bridge at anaphase and laggard at metaphase in sunflower somatic cells were observed. Abnormalities of reproductive cell at daikinesis and metaphase in pearl millet and laggard at metaphase in sunflower reproductive cells were observed (Chand et.al1991)

In Allium cepa root meristem, treatment of different concentration of Dithane M-45, Aldrex–30 and metacid–50 showed positive chromotoxic effect included high lethality for cell division , clumping, bridges, fragment, cytomixis, disturbed polarity etc. such genotoxic effects warrants frequent use of these chemicals (Pandey et.al1994)

Shrestha (2004) studied two cytological effects of fungicide Carbendazin on meristematic cell of Allium cepa. The percentage of abnormalities increases by increasing concentration and time period. Various abnormalities like plasmolysed cells, clumping, unequal and condensation of chromosomes, distrubed prophase, precious, arm unequal and diagonal cytokinesis , delay cell plate formation and binucleated cells etc were observed.

2.3 Cytological Effect of Herbicides In Plants Cells.

Various chromosomal abnormalities such as stickiness, condensation, breakage of chromosomes and delay in spindle formation was induced on treatment of 2,4 dichlorophenoxyacetic acetic acid and (2,4,5-D) and 2,4,5

trichlorophenoxy acetic acid (2,4,5-T) on root meristem cells of Allium cepa (Crocker 1952).

The seventeen different types of herbicides in different parts of different plant like staminal hairs of Tradescentia reflex, stipular leaf cells of Allium fistulosum was studied by Sawamura (1964 , 1965)

He used hormonal herbicides in which he studied metabolic and mitotic state, mitotic abnormalities like bridges, stickiness, retardation of chromosome movement in anaphase incomplete cell wall, binucleated cells with multicepta etc and non hormonal herbicide were found to suppress the spindle development.

Amer and farah (1975) studied IPC chemicals 0-iso- propyl –N- Phenyl carbonate (ipc) and 0-iso- propyl-N- (3-cloro) phenyl carbonate (CIPC) (weed control). A mixture IPC + CIPC (Duphar) was used in Egypt as inhibitor of potato sprouting. Decrease of mitotic index along with mitotic abnormalities on vicia faba treatment with IPC and Duphar had been observed.

2, 4 dichlorophenoxy acetic acid induced more effective morphological and cytological abnormalities. The common abnormality was formation of bridge and fragmentation (Adhikari 1982).

The turbutyrn treated in vicia faba produced mitotic abnormalities such as stickiness, chromosomal aberration, breakage and effect on spindle apparatus, mitodepresive action in which reduce the movement of DNA and Ribonuclic acid, it’s capacity to induce chromosomes abnormalities. (Bdr, 1983).

Herbicide i.e. Glean in Allium cepa and vicia faba in root meristem, it inhibited the mitotic division and depressed the DNA and RNA in root meristems of both plants, chromosomes damage included mitotic abnormalities, chromosomal aberration and stickiness (Badr and Ibrahim 1987).

Clastogenic effect likes laggards, bridge and fragments, chromosomal aberrationm, stickiness, C-metaphase, abnormal prophase, and polyploid was observed in treatment herbicide Tribunil on root meristem Allium cepa. (EL-Khodary et.al1990).

Herbicides like, Gasgard and Igran in barley seed with 10^{-12} 1% g/100cm^3 aqueous solution of these herbicides for 12 and 24 hours caused the decrease in mitotic index of root tips which was studied by (Topakatas et.al1991).

By treating and Atrazine and propazine on grain sorghum, multiple nucleoli in diplotene, early daikinesis and a synchronization of chromosome movement on metaphase, telophase metaphase II, and telophase II cells with lagging chromosome were detected. propazine cause less injury then Atrazine. (Currie et.al, 1996).

The percentage of aberration like sticking lagging, C-metaphase, bridges, irregular prophase, unequal distribution, fragment and few cell with micronuclei at interphase was found in higher in Allium cepa then vicia faba treated with Atrazine herbicide. EL-Ghamery et.al, (2000).

Shrestha (2002) investigated the cytological effects of herbicide Isoproturon on roots meristematic cell of Allium cepa. It induced various abnormalities like C-metaphase, precious arm at anaphase, stickiness,

unequal cells, unequal cytokinesis, shifting pole , equatorial plate shifting at metaphase , diagonal cytokinesis.

Yuzbasioglu et.al(2003) on treatment Racer Flurochloride (herbicide) root meristem of Allium cepa. It produced various abnormalities like c-metaphase, laggards, polyploidy, breaks and fragments of chromosomes, micronucleated cell at interphase and reduced the mitotic division.

Saxena et.al, (2004) studied genotpxic effect of herbicides Diuron on root meristem cell of Alum sativa; it induced micro and binucleated cells and reduction of mitotic Index.

2.4 Cytological Effect of Industrial Effluents

Abraham (1974) investigated that frequency of mutant cells and events of mutation on staminal hair of plant when decay organic manure like cowdung (cowdung and sand in 1:3, 1:2 and 1:1 ratio) was supplied to Tradescantia clone. The frequency of somatic mutation like chromosome breakage, multipolar anaphase, bridge and laggard induced in staminal hair had been found to increase with increase in concentration of cow-dung insand. As water pollutants, the effect of mercuric chloride ($Hgcl_2$) and mercurous chloride (Hg_2cl_2) on Hydrilla verticillata showed much lower mitotie index and higher cytological aberration that control. Growth parameter like root length, shoot length, fresh weight, dry weight, biomass to control showed time and dose responsive of both $Hgcl_2$ and Hg_2cl_2 which acted inhibitory effects (Pal and Nandi, 1989).

In crotolaria the plant growing near Titanium factory from which geotaxis potential of pollutants effect was investigated. Growth retardation, iuhibition of cell division, chromosome clumping abnormalities which

were lacking in control plant in same environment (Abraham and Abraham 1991).

Equatorial plate shifting at metaphase, non synchronized movement of chromosome, precocious arm, polar displacement, delay cytokinesis ,clumping in prophase and stickiness, C-metaphase, bridges, laggards, micronuclei, polyploidy, binucleated cells abnormalities were obtained on effect of effluents of carpet dyeing industry in Allium cepa (Pun, shakya 1994).

Rank et.al(1998) studied genotoxicity testing of waste water sludge using in Allium cepa. The chromosomal aberration at anaphase and telophase were seen. The chromosome aberration increased with increase in concentration of heavy metals like Pb, Ni, Zn, Cd, in water.

Pathak (1999) studied the mitotic activity and chromosomal behavior carmosine food stuff dye treated root meristem of Allium cape. Various chromosomal abnormalities like c-metaphase , bridge formation, equatorial plate shifting, non-synchronized, chromosome, binucleated cells, fragmentation, inhibition of cell plate formation and unequal cytokinesis.

Sapkota (2000) studied the cytological effects of food stuff Tartrazine on mitotic activity and chromosome behaviour in root meristem of Allium cepa. Tartazine induced metabolic disorders and abnormalities like binucleated cells, equatorial plate shifting, c-metaphase , precocious arm at anaphase , stickiness at prophase, non synchronized arrangement of chromosome, bridge formation, unequal cytokinesis, inhibition of cell plate formation.

The silk dyeing industry effluents were examined for the induction of mitotic abnormalities in Allium cepa meristem. Abnormalities like

stickiness of chromosome fragment, bridge, laggards, binucleated cells and vacuolated nucleic were observed. (Sudhakar et.al2001).

2.5. **Cytological Effect of Plant Extract And Others.**

Raj and Rao (1972) observed that lathyrogen (free, non protein forming amino acid in lathyrus and vicia sativus) induce mitotic abnormalities like binucleated, multinucleated cells, stickiness, bridge, laggard and breaking chromosome. The extracts (ginger and turmeric rhizomes) were found toxic above 4% to root tip of Allium cepa and below this concentration induce abnormalities like chromosomal break, C-meta phase, binucleated cell and multipolar anaphase (Abraham et.al1976)

Bhalla et.al(1976) studied cytological response abnormalities like disturbed spindle apparatus, irregular separation of chromosome and lagging chromosome in smoke of various types cigarettes in root tip of Allium sativum.

Harmones (Gribberellicacid-GA, indolebutyric acid, 2,4 dichlorophenoxy acetic acid (2,4-D) produces abnormalities like bridge formation, C-mitosis, micro nuclear formation, stickiness, condensation chromosome in root tip mitosis of vicia faba (Prasad and Das 1977).

Abraham and cherjan found chumping chromosome, bridge metaphase on root meristem of Allium cepa treated to leaves extract of the betel vine.

Abnormalities like stickiness, disturbed metaphase, multipolar spindle, lagging chromosome, multinucleated cells observed by aliphatic alcohol, fatty alcohol from Euphorbia granulata and pulicaria (Shehab 1980). According to Shehab water extract from Teucrium pilosum (use against constipation by people of Quatar) had anti mitotic effect and

induced spindle disturbance, stickiness, C-mitosis, laggards, bridges polyploidy, chromosomal breaks etc in Allium cepa root.

Inorganic met.allic salts like zinc, Sodium , Rubidium, Copper, Lead, Cobalt Nickel induced abnormalities increased with increasing molecular weight as well as produce inhibition of mitosis treated in root meristem of Allium, cepa (Giri et al , 1984).

Shanker et al, (1987) found depressive action and abnormalities in Allium cepa by Acrylamide like chromatid breaks, fragmentation, bridge micro nuclei in anaphase.

Allium cepa root treated with 0.05%, 0.25%, o.50%, o.75% and 1.0% aqueous solution of sodium salicylate for 2, 4, 6, 8 hrs and 24 hrs. The increase in nuclear and chromosomal aberrations was depend on concentration and duration of treatment (Briand and Kapoor 1989).

The extract of cymbopogen proximus (gramineae induced chromosomal irregularities stickiness, laggard, bridge and mito depressive on cells of vicia faba (Adam and Farah 1989).

Leaf extract (Ricans communis) damaged the root meristem in Allium cepa. The percentage of abnormalities (Psychosis, clumping chromosome, fragmentation, lagging, bridge, scattering, multinucleated chromosomes) Increased with increasing concentration of extract 0.5% to 3% with arrest of mitotic at 4%. Abnormalities were detailly discussed by George and Geethamma 1990.

Zeerak (1991) studied effect gamma rays and ethyl methane sulphonate (EMS) on meiosis and on pollen and seed fertility in M_1 generation of a

local Brinjal of Kashmir valley found that treatments were more efficient in including meiotic abnormalities in radiation and combined then EMS.

C-metaphase scattering chromosomes randomly all over the cell, C-metaphase with chromosome grouped in two phase chromosome grouped in two groups, C-metaphase all chromosome clumped in single compact group studied by Morais et al (1991) by treatment of colchicine in root of Allium cepa.

Micronuclei, fragmentation, laggard, and single multiple bridge formation maximum cells with micronuclei with some bridge and minimum laggard chromosome abnormalities were observed by effect of methyl parathion and tri-miltox on mitosis of Allium cepa studied by Saeed and Yasmin (1992).

By bone marrow micro nucleus analysis, Mishra (1992) tested clastogenic potential of some hair dyes high toxicity of nitro phenol, nitrobenzene and phenyldiamine. All were clastogenic.

The abnormalities like stickiness, bridge, clumping chromosome etc were recorded by extract of different tobacco on Allium cepa. Banerjee (1992).

Clastogenic effects of two detergents, Roma and Triton-100 (potassium dichromate) on various concentration of root tip of vicia faba was observed. The chromium compound induced chromosomal aberration as well as C-metaphase on similar frequency as mixture chromium surfactants. Neither detergent produces clastogenic disturbances nor faciliates clastogenic effects of chromosome. Pietrini et al (1993).

Effect of extract from medicinal plant Alpinia nutans Rose and pogostemos heyneanus Benth on wister rat (invivo) was not effective inducing

structural and numerical aberration in bone marrow cells of rats but highly toxic in root tip cells of Allium cepa (Francisca and Takahashi 1994).

Meiotic abnormalities (univalent, laggard, bridge, stickiness, nondisjunction, cytomixis, precocious chromosome with stimulatory effect on plant height, no of branches pods and it's length) was observed. Caffeine induced Murphy-Cytological-Variability in Fenugreek, Trigonela foenum graecum studied by Anis and wanin (1997).

Several abnormalities of mitosis like laggard, bridges, acenctric fragment and polypolar distribution in Anaphase and polynuclear bodies in telophase were observed by the effect of Chinese lacquer on cell division of root tip of Allium cepa studied by Xing and lian in 1997.

Chapter – III

3. Materials and methods: - The root tip (meristem) coming out from the common lighted onion (Allium cepa. Var 2n = 16) was taken for the study cytological effect of Monocrotophos. The cytological effect of Monocrotophos was taken as standard one. The root tip of Allium cepa cells were studied to observe the effect of insecticide for mutagenic effect. The following procedures and materials were used in the experiment. The experiment was performed during July/August 2006.

3.1. Materials: - Root tip (meristem) of Allium cepa.

3.1.1. Selection of Rooting Bulbs: - Equal sizes and red light bulbs of Allium cepa were selected for the rooting. They were collected from the Kalimati, Kathmandu. The bulbs selected were devoid of any damage and healthy. The onion bulbs were selected because of fast growth rate, easiness to handle and convenience of cytological observation.

The bulbs were washed thoroughly with clean water and old root present in the bulbs were scraped out. The bulbs were placed with basal side facing downward over coupling jars filled with water in order to obtain the activity growing root tips. The water in the jars was refilled at 24-hours intervals so as to check the growth of micro-organisms and to present an injurious effect on growth of the roots due to dissolution of a yellow pigment from the outer scales of the bulbs by water. The amount of dissolution was minimum in distilled water.

3.1.2. Preparation of suspension for experiment: - Suspensions of monocrotophos was prepared in separate glass jar at the concentration of 25%, 50%, 75% and 100%. The glass jars were labeled accordingly.

3.1.3. Monocrotophos:- One ml of monocrotophos mixed in one liter of water takes as the standard solution and made different concentration by adding water in that solution.

3.2. Methods

3.2.1. Treatment of the rooting bulbs:- When the lateral roots of Allium cepa were about 2 cm long, they were exposed to the freshly prepared test solution of different concentration for 3, 6, 12 and 24 hours at room temperature. As the period of treatment was prolonged, however, high concentrations were found to be lethal and thus they were avoided. The control roots were treated with water to compare with different concentration of Monocrotophos solution. The methods of treatment were shown in following table.

Time of inserting the materials to suspension (monocrotophos)	Time periods in hours	Fixing
10:00 am control	0	10:00 am
7:00 am	3	10:00 am
4:00 am	6	10:00 am
10:00 pm (previous night)	12	10:00 am
10:00 am (previous day)	24	10:00 am

3.2.2. Preparation of Agent for cytological study: - The following chemicals were used for fixing and staining the tissues.

Fixing agent – Acetic alcohol

Glacial acetic acid	1 part
Absolute alcohol	3 parts

Preserving agent – alcohol

70% ethyl alcohol was used for short time preservation of tissues before staining. Staining procedure was done with 2% aceto caramine.

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

Two percent aceto carmine was used for staining . It was prepared by adding 2gm of carmine to a mixture of 55cc distilled water and 45cc glacial acetic acid and heated to boiling until the stain was dissolved. Then filter and stored in reagent bottle.

3.2.3. Fixation period:- The treated and control root tips of Allium cepa were excised and washed thoroughly in water and fixed in freshly prepared 1:3 acetic alcohol for 2 hours. The obtained root tips were preserved in 70% alcohol. The time of fixing was between 10:00 am and 10:30am.

3.2.4 Aceto carmine squash technique:- Cytological preparation was made by using Aceto carmine squash-technique where by the fixed root tips (about 1 cm) were stained in 2% Aceto carmine and squashed (about 2mm root tips) were made in 45% acetic acid on clean slide. More then five root tips were studied from each treated or non treated onion bulb.

3.2.5. Cytological slide preparation and Mounting media: - The slides were prepared from the stained roots having dark colour. The darkly stained roots were selected and placed on the clean slide. About 2mm root tips were required to squash. A clean cover slips was gently placed and squashed. To spread the cells properly, a cytological hammer was used to squash. The prepared slides were observed under the compound microscope. The given below dehydration grades were used in preparing permanent slides.

A. (i) Glacial acetic acid – 1 part

(ii) Butyl alcohol – 1 part

B. (i) Glacial acetic acid – 1 part

(ii) Butyl alcohol – 3 part

C. Butyl alcohol – pure (100%)

Some of the important slides were made permanent by dehydration in acetic acid butanol series and mounting in euparol. From freshly prepared temporary slides and permanent slides, microphotographs were taken in. The photographs were taken in central department of Botany, T.U. Kirtipur, Kathmandu.

3.2.6. Cytological observation and calculation.

The slides prepared by the above process were observed under the compound microscope. The observations were taken on around 3100 cells from at least five different root tips treated with various concentration of suspension of Monocrotophus (Control, 25%, 50%, 75% and 100%). The indices and abnormalities were scored and analysed by using the following

formulas according to Leven, 1949 (cf. Kihlman 1971, Medeivas and Takahashi 1987). The abbreviation is given in separate page.

Mitotic index (MI) $= \frac{TDC}{TC} X 100$

Prophase index (Pro I) $= \frac{TC\ pro}{TDC} X 100$

Metaphase index (Meta I) $= \frac{TC\ meta}{TDC} X 100$

Anaphase and telophase index (Ana – Telo I) $= \frac{TC\ Ana - telo}{TDC} X 100$

Total percentage of abnormal cells (T_{abn}) $= \frac{TC\ abn}{TDC} X 100$

Total percentage of abnormal cells at prophase (Tpro) $= \frac{TAbn\ pro}{TDC} X 100$

Total percentage of abnormal cells at metaphase T meta $= \frac{Tabn - meta}{TDC} X 100$

Total percentage of abnormal cells at Ana and telophase $T_{ana\text{-}telo}$ $= \frac{Tabn / Ana - telo}{TDC} X 100$

Percentage of abnormalities of prophase among the abnormal cells Apro $= \frac{Tabn\ pro}{T\ C\ Abn} X 100$

Percentage of abnormalities at metaphase among the abnormal cells Ameta $= \frac{Tabn\ meta}{TC\ Abn} X 100$

Percentage of abnormalities at anaphase and telophase among the abnormal cells $A_{ana\text{-}telo} = \frac{TAbn/Ana-telo}{T\,Cabn} X100$

3.3 Statistical Analysis:-

The data obtained were subjected to statistical analysis by the Friedman test (Siegel 1956) to determine whether the time of treatment affects the results (Mitotic index value).

The data from Table no 6 and 7 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 number of columns (Condition, time of treatment)

Rj = Sums of ranks in j^{the} column.

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

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.

CHAPTER – IV

4. Result

The cytological effect of insecticides monocrotophos in root meristem of Allium cepa was analysed. The main observations were focused on the variation of mitotic index and chromosomal abnormalities .The abnormalities include clastogenic like bridge, laggard, breakage of chromosome and hyperploids cells. The chemical was found to be lethal on cell division at higher concentration. Chromosomal abnormalities during treatment was proportional to concentration and duration of treatment. Dividing cells were completely absent in treatment with higher concentration.

The results are tabulated as in table no. 1 to table no. 7 (Appendix) and elaborated under separate sub-heading as given below.

Table no.3 and Table no. 4 Show the mitotic and other phase indices of Allium cepa with different concentration of Monocrotophus at different time of treatment respectively.

4.1 Effect in Mitotic index.

From figure no.1 shows the mitotic index at different concentration and duration of treatment of Monocrotophos. Mitotic index (MI) value decrease gradually with increase in time and concentration. The control value was 32.98 where as 24 hours of treatment in 100% concentration, the mitotic index was inversely proportional to concentration and time.

Statistical analysis, the calculated value for $X^2r = 12$ where as table value for $X^2r = 7.81$ at percentage $(p) < 0.05$.

4.2. Effect in prophase index.

Fig. no. 2. showed the prophase indices. The control value was 91.47 and it was maximum 97.80 at 24 hours treatment of 100% concentration. The values were increasing irregularly upto 24 hours treatment of 100% concentration. At control, the minimum value was obtained.

4.3. Effect in Metaphase index:-

Fig.3 showed the metaphase indices. The graph was plotted against time and concentration. The control value for metaphase index was 4.8 and minimum value of metaphase index was 5.42 at 50% concentration of 3 hours treatment. Minimum value or nil values were 6 hrs treatment of 100% concentration, 12hrs of 100% concentration, 24hrs of 25% and 100% concentration.

4.4. Effect in Anaphase and telophase index:- The Anaphase and telophase indices were in fig no. 4. The control index was 3.66 the nil values were at 12 hrs of 75% concentration, and 24hrs of 75% concentration. The percentage of mitotic and phase indices in different periods at different concentration of Monocrotophos were given in fig no. 5 to 8.

4.5. Relation between mean mitotic index and mean phase indices:- The mean mitotic index and phase indices obtained from root meristem cells of Allium cepa treated with monocrotophos in relation to time should in table no.3.

Mitotic index decreased with increased in treatment period from that of control value, 32.98 to 19.59. Prophase index value increased with

increased in treatment period from that of control value 91.47 to 97.80. Metaphase index value increased from control 4.85 to 4.83 of 3 hours treatment. After then index value decreased with increased in treatment time period from 3 hours, 4.83 to 0.75 of 24 hours. Anaphase and telophase value decreased gradually from control, 3.66 to 1.16 of 12 hours, then slightly increased by 1.47 in 24 hours treatment.

4.6. Total percentage of abnormal cells.

Total percentage of abnormal at each phase and different concentration of Monocrotophos at different time of treatment shown in table no.4.Total percentage of abnormal cells of dividing cells in given concentration of Monocrotophos shown in fig no. 10. There are 7.14% abnormal cells in control. All the treatment concentrations, the highest abnormal value were 16.52% in 50% concentration of 24 hours treatment. The percentage of the abnormal cells did not show any regularity with the time of treatment and concentration.

4.7. Total percentage of prophase abnormality.

The total percentage of prophase abnormalities in given concentration of Monocrotophos are shown in fig no. 11. The largest percentage abnormal value of prophase was 3.94 in control and highest abnormal value of prophase was 16.19 in 50% concentration of 24 hours treatment.

4.8. Total percentage of Metaphase Abnormally.

Total percentage of metaphase abnormalities in given concentration of monocrotophos are shown in fig no.12. The control value was 1.83% and maximum value obtained was 9.72% in 25% concentration of 12 hours treatment. The abnormality values were nil at 100% of 6 hours, 100% of 12 hours, 25%, 50%, 75%, and 100% concentrations of 24 hours treatment. The abnormalities value were decreasing or, increasing irregularly.

4.9. Total percentage of Ana-Telophase Abnormality.

Total percentage of Ana-Telophase abnormalities in given concentration of Monocrotophos are shown in fig no.13. The percentage of abnormalities values were nil at 75% concentration of 12 hours, 75% concentration of 24 hours treatment. Minimum percentage abnormality value was 0.33 at 50% concentration of 24 hours treatment and maximum was 1.87 at 50% of 3 hours treatment. The control value was 1.37%. In this way, abnormalities percentage were increasing or, decreasing irregularly.

4.10. Proportion of Abnormalities in phases among experimental groups.

In table no.4, the abnormal proportion in the dividing cells after different duration of treatment and different concentration of Monocrotophos solution are given. Abnormalities were higher in prophase then metaphase, Aana-telophase at different hours of treatments for all the concentration. The abnormalities were also found in control or, untreated root.

4.11. Abnormal mitotic phases on 25% monocrotophos treatment:-

The abnormal mitotic phases in Allium cepa treated with 25% of monocrotophos are shown in table no.4. Mitotic abnormality increased with time and concentration of treatment. Prophase abnormality was high at 24 hours treatment and decreased simultaneously up to 3 hours. Metaphase abnormality was lowest or, nil at 24 hours and highest was 9.72 at 12 hours treatment. From 12 hours to 3 hours, abnormality was decreased. While at ana- telophase, abnormality was 1.45 at 24 hours treatment and lowest 0.53 at 12 hours treatment. Prophase abnormalities were the highest among other phases.

4.12. Abnormal mitotic phases on 50% monocrotophos treatment:-

The abnormal mitotic phases in Allium cepa treatment with 50% of monocrotophos are shown in table 4. Prophase abnormality was increasing from 3 hours to 24 hours treatments. Metaphase abnormalities decreased from 3 hours to 24 hours treatment. It was nil at 24 hours treatment. Anaphase and telophase abnormalities were also decreasing from 3 hours to 24 hours treatment. Prophase abnormalities were the highest among other phases.

4.13. Abnormal mitotic phases on 75% monocrotophos treatment:-

Abnormal mitotic phases in Allium cepa treated with 75% monocrotophos are shown table no.4. Prophase abnormality was increased from 3 hours to 24 hours treatment. Metaphase abnormality was going

decreased from 3 hours to 24 hours treatment where it was nil at 24 hours treatment. Ana and telophase abnormality was decreased from 3 hours to 6 hours and nil at 12 hours and 24 hours treatment.

4.14. Abnormal Mitotic phase on 100% Monocrotophos treatment.

Abnormal mitotic phases in Allium cepa treated with 100% of monocrotophos are shown in table 4. Prophase abnormalities were going on increasing from 3 to 24 hours treatment. Metaphase abnormality was only found 2.79 at 3 hours and nil at 6, 12 and 24 hours treatment.

Ana and telophase abnormality was seen irregular. The highest abnormality was 1.46 at 6 hours treatment and lowest was 0.56 at 12 hours treatment.

4.15. Effect in chromosomal Behaviour.

The monocrotophos was induced various kinds of chromosomal abnormalities in meristematic cells of Allium cepa during mitotic cell division. Abnormal phases from treated groups are given in plates in the next page.

4.16. Nature of Abnormal cells in Non dividing cells.

The abnormalities found in non dividing cells were plasmolysed cells, reduction in the size of nucleus and shrinkage of cells and unequal nucleated cells.

4.17. Nature of Abnormal cells in prophase.

Unequal condensation of chromosome, clumping chromosome, sticky, disturbed nature of chromosomes were observed.

4.18. Nature of Abnormal cells in Metaphase.

Equatorial plate of shifting, fragmentation of chromosome c-metaphase and sticky chromosomes were dominant types of abnormalities. In some concentration and time duration diagonal metaphase was also seen.

4.19. Nature of Abnormal cells in Anaphase and Telophase.

Shifting poles (diagonally arranged chromosomes) and unequal movement of chromosomes, Precocious arm, laggard were frequently found. Laggard, fragmented anaphase, disturbed anaphase, bridge formation and breaks of chromosomes, also reported. Unequal condensation of daughter chromosomes, shifting of poles, and delay in cell plate formation, unequal cytokinesis, diagonal cytokinesis, bi and tri nucleated cells were found in telophase.

4.20. Nature of Abnormal cells in Control.

Some abnormalities were seen in control cells. Plasmolysed cell tri-nucleus in interphase, diagonal, c-metaphase and stickiness in Metaphase, diagonal and disturbed anaphase and diagonal and unequal cytokinesis in telophase were observed.

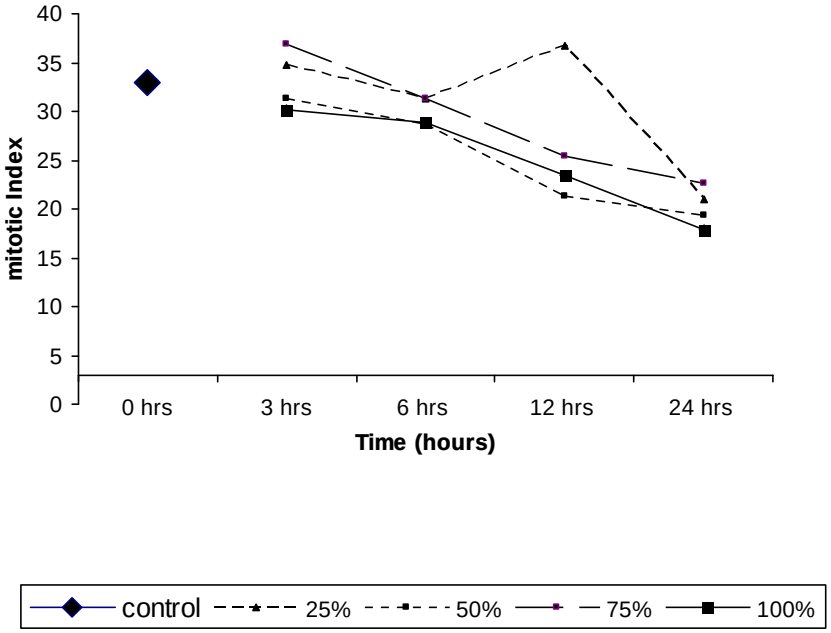


Fig No:-1

Graph of Mitotic Index of Allium cepa root tip cells Vs treatment time with given concentration of monocrotophos

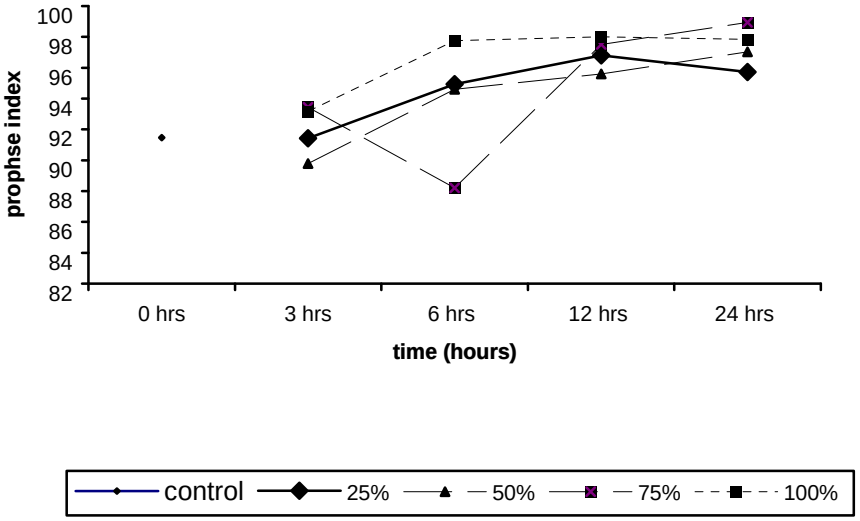


Fig No:- 2

Graph of prophase Index of Allium cepa root tip cells Vs treatment time with given concentration of monocrotophos

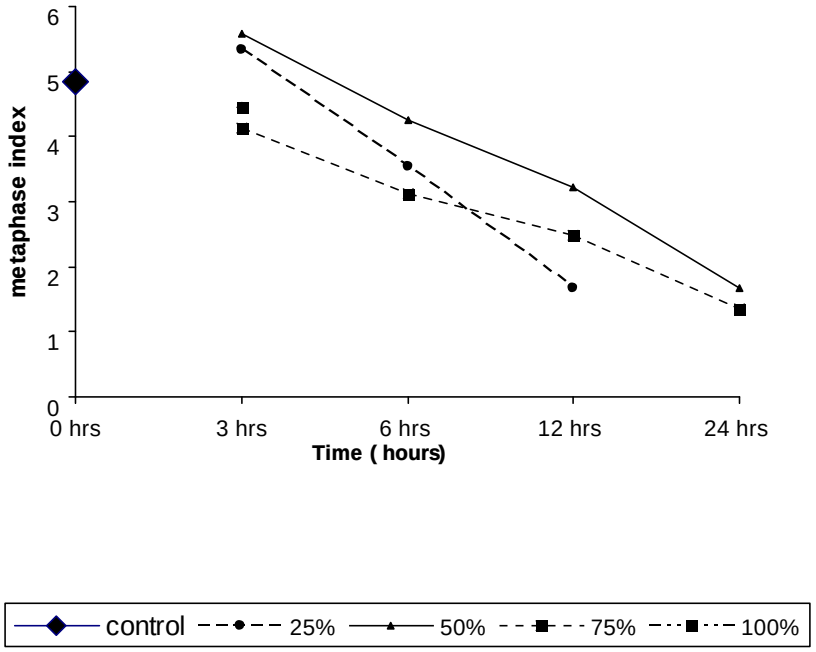


Fig No:- 3
Graph of metaphase Index of Allium cepa root tip cells Vs Treatment time with given concentration of monocrotophos

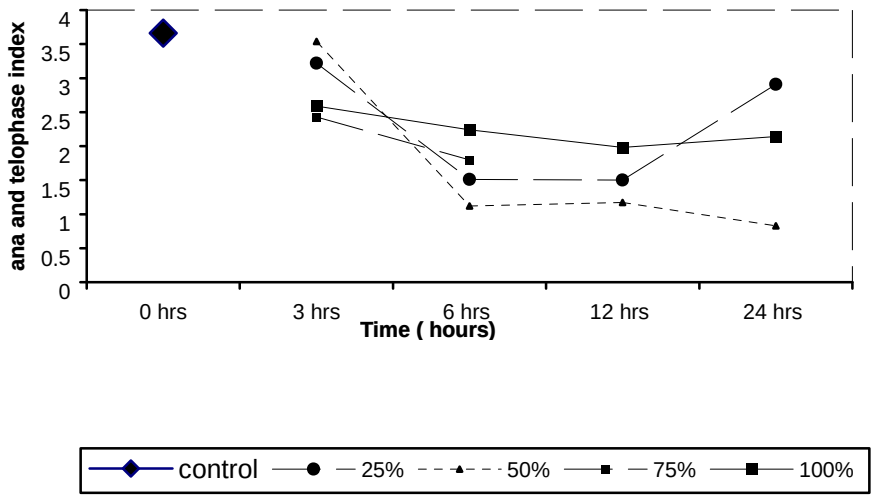


Fig No:-4
Graph of ana and telophase Index of Allium cepa root tip cells Vs Treatment time with given concentration of monocrotophos

Fig No:- 5

Bar diagram of mitotic prophase, metaphase and anaphase and telophase indices vs treatment time 25% concentration of monocrotophos.

Fig No:- 6

Bar diagram of mitotic prophase, metaphase and anaphase and telophase indices vs treatment time 50% concentration of monocrotophos.

Fig No:-8

Bar diagram of mitotic prophase, metaphase and anaphase and telophase indices vs treatment time 100% concentration of monocrotophos

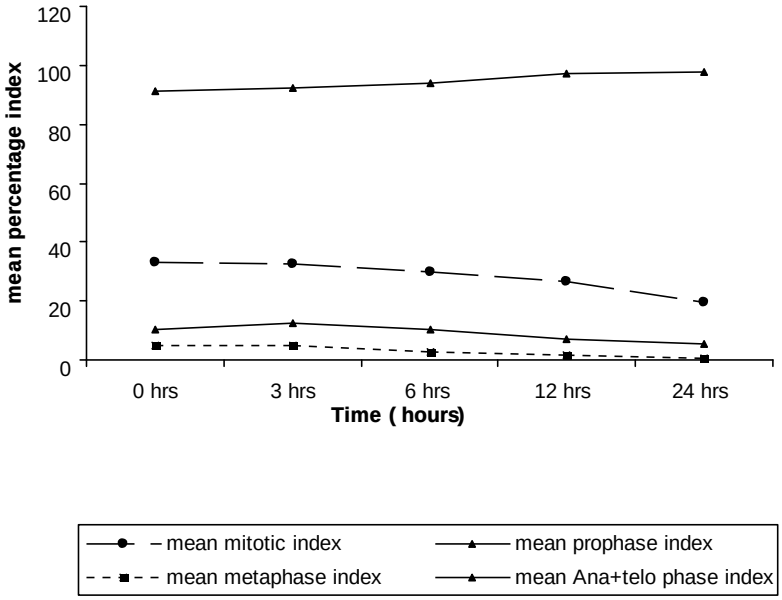


Fig :- 9

Graph of mean indices of mitotic, prophase, metaphase and anaphase and telophase Vs treatment time with mean percentage concentration of monocrotophos.

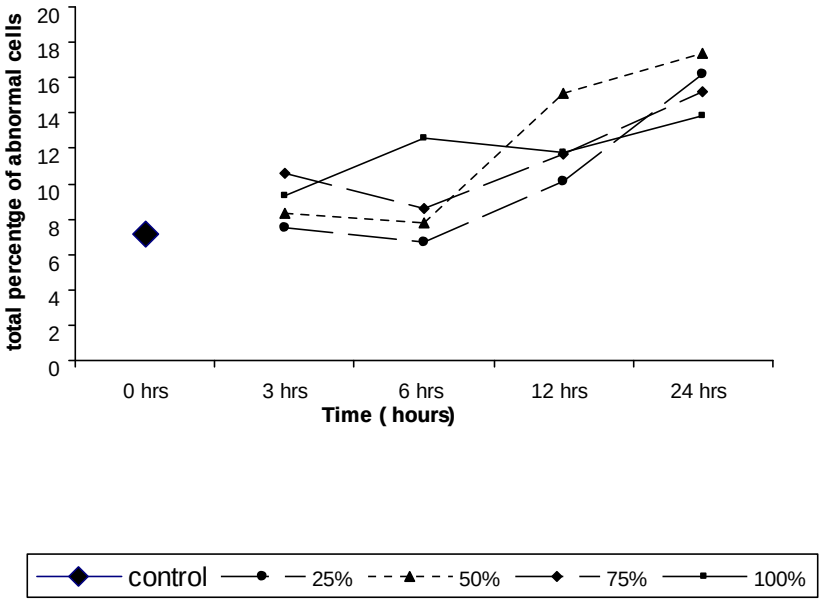


Fig :- 10
Graph of total abnormal cells percentage Vs treatment time with given concentration of monocrotophos.

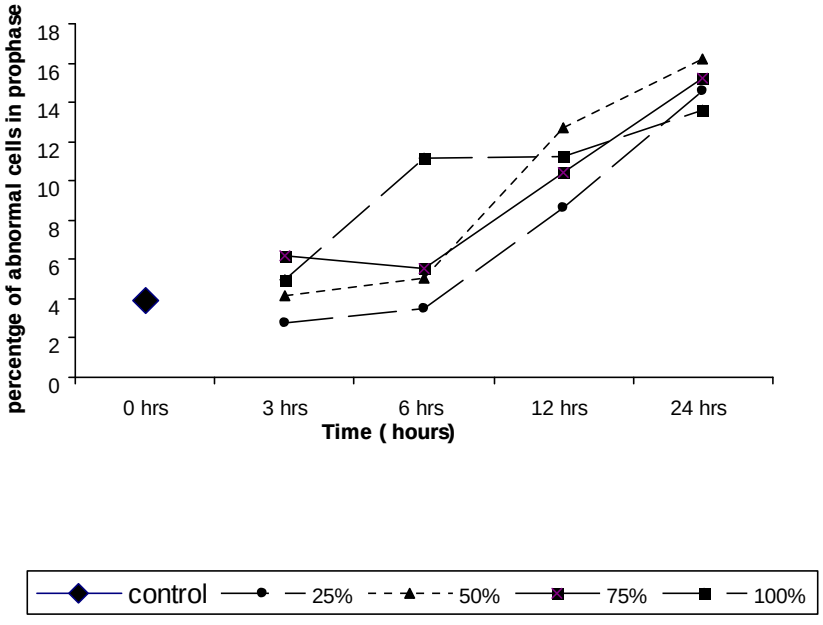


Fig :- 11
Graph of percentage of abnormalities among the abnormal cells in prophase Vs treatment time with given concentration of monocrotophos

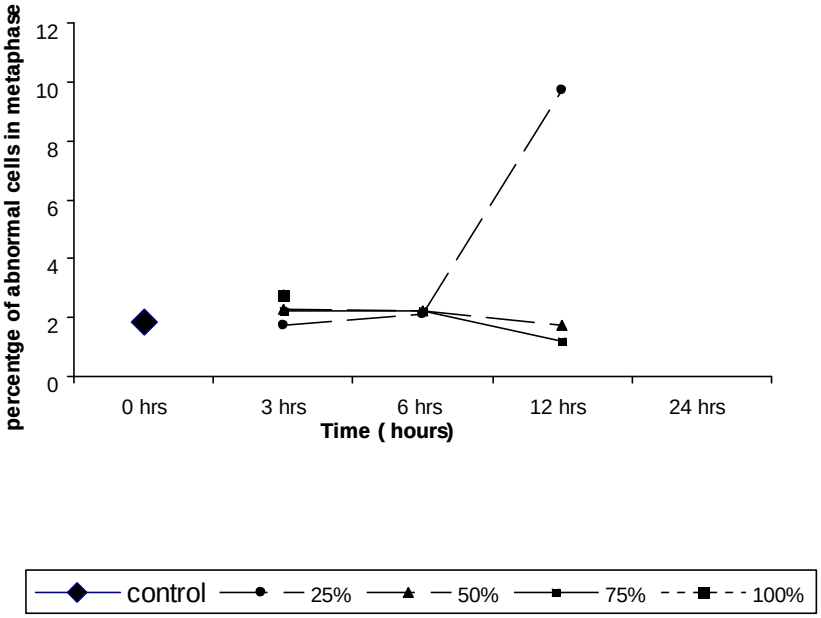


Fig :- 12

Graph of percentage of abnormalities among the abnormal cells in metaphase Vs treatment time with given concentration of monocrotophos

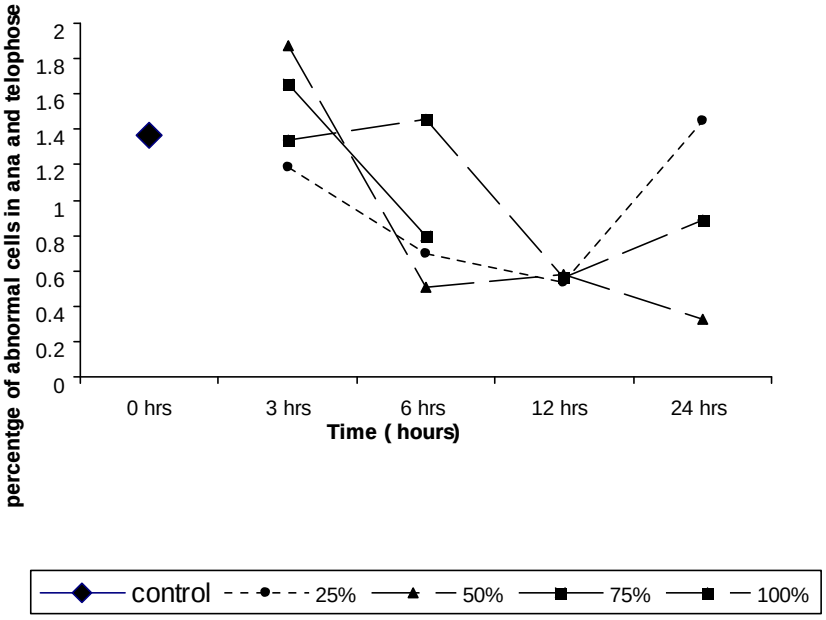
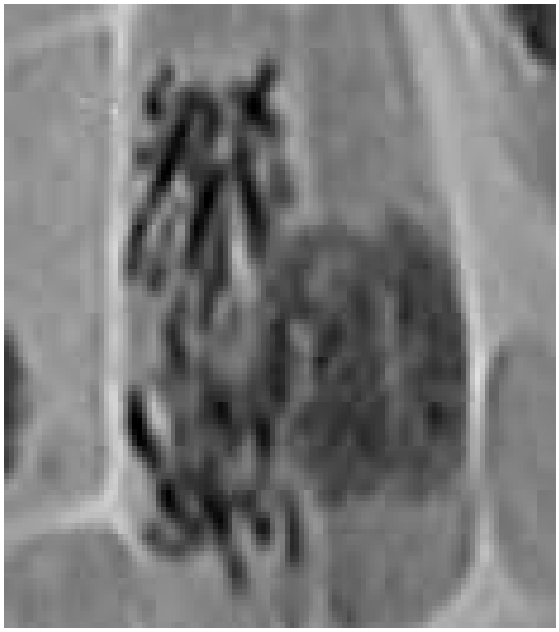
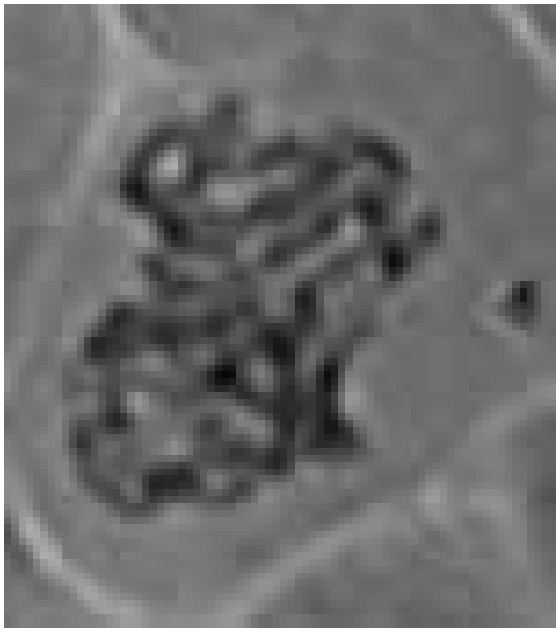
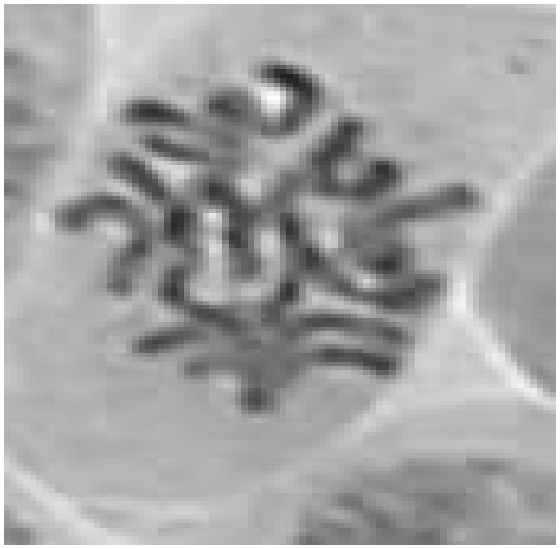


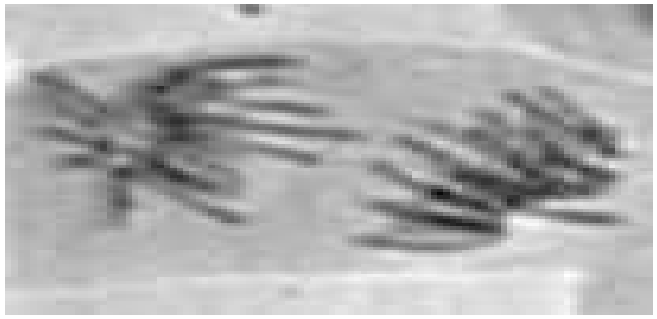
Fig :- 13

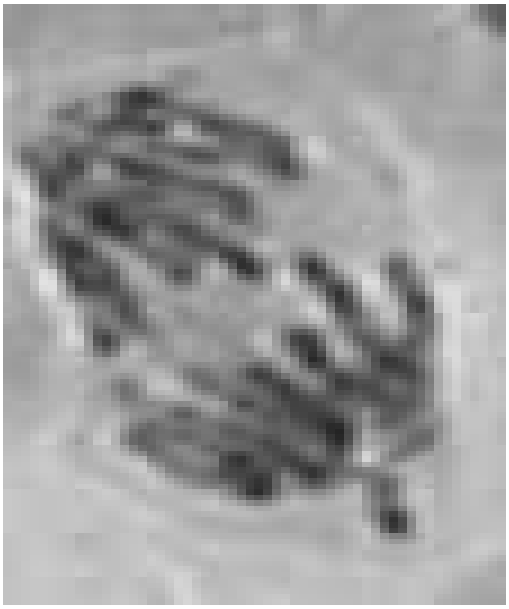
Graph of percentage of abnormalities among the abnormal cells in anaphase and telophase Vs treatment time with given concentration of monocrotophos

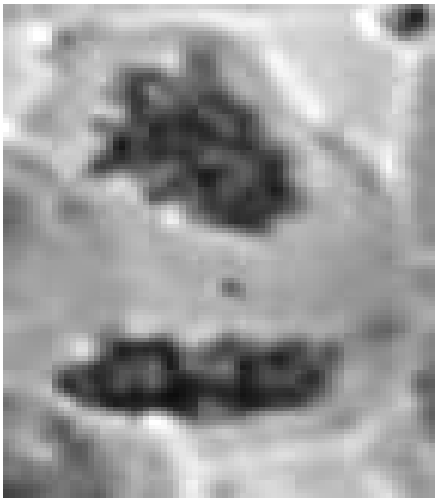


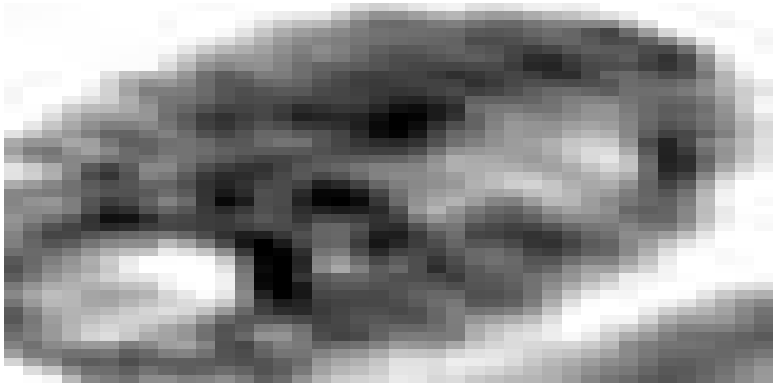












CHAPTER – V

5. Discussion and Conclusion.

Monocrotophos, widely used organophosphors insecticide, showed variation on different mitotic activities and induction of abnormal chromosomes during somatic cell division on different concentration on root tip (meristem) of Allium cepa L. It reflects that monocrotophos posesses poisonous characters in the present experiment.

It was observed that mitotic index value decreases as the concentration and time of treatment increases with relation to control. The average mitotic index value was 32.60 in 3 hours and 24 hours (19.59). The reduction of mitotic activity seems to be common effect of most pesticides tested for their action on mitosis.

Blocking of mitotic cycle during interphase which may result from prolonged G_2 period or, to the inhibition of DNA and RNA biosynthesis caused mitotic inhibition by pesticides. Increase concentration and prolonged period of treatment resulted in increased reduction in the amount of both nucleic acid (Badr and Ibrahim, 1987).

The mitodepressive effect of plant extracts of cymbopogon proximus, cuffa operculata, pteridium aquilimum and Achillea fragrantissima on bioassays materials have been reported by some authors (Adam and Farah 1989 and shehab *et al*, 1978).

By the observation data the calculated X^2 (chi square) value is 12 where as the tabular (X^2) chi square value is 7.81. This value shows the effectiveness of chemicals related with the time of treatment in cell division. These value shows that time of treatment of monocrotophos did

effects on mitotic value. Longer the treatment, less the division of the cell recorded.

The prophase index was increasing with the increasing in time and concentration. The mean prophase index was 92.26 at 3 hours and 97.80 at 24 hours. The increased prophase index shows prophase poisoning where cells entered into mitosis but they were arrested in the prophase in high frequency (Prasad and Das 1997), which result of effect of monocrotophos at prophase stage.

The increased prophase index in high concentration may be due to prophase stage effecting the spindle formation by chemical. Similar result was obtained by treating isoproturon (chemical) in Allium cepa root Shrestha (2004).

Metaphase index showed no regularity. Metaphase index was higher then ana- telophase index and lower then prophase index. Decreased metaphase index with increased the time of treatment. It shows high accumulation of abnormal metaphase cells (Pathak, 1999) and same result obtained by treating potassium metasulphite on root tip of Allium cepa Shrestha (2004).

Anaphase and telophase indices were higher then metaphase index in 24 hours treatment and lower the prophase index and in remaining 3, 6 and 12 hours treatment, metaphase index was greater then ana–telophase. In this way, no regularity was seen. In average, ana-telophase index decreased with increasing time and concentration due to prolonged prophase and not entered

into further division. Similar result was obtained by treating Carbendazin in Allium cepa root meristem (Shrestha 2004).

In control cells abnormities were observed. Most of abnormalities were seen in metaphase, anaphase and telophase. In prophase clumping chromosomes and distributed chromosome were found. Such observation was obtained in vicia faba after seed soaked treated with Roger (Amer and Farah 1974).

In prophase dilution and disintegration of chromatin material, unequal condensation of chromatin thread, non synchronised chromosomes were found.

The abnormally arranged chromatin threads just appeared like non synchronization in distribution chromosomes. Mallah and Kabarity (1982) reported similar abnormalities in Allium cepa root treated with cannabis sativa.

Shrestha R. (1991) reported that high Stickiness in metaphase–1 by treating monocrotophos and Phosphoamiden in pollen mother cells of vicia faba. It causes arrest at metaphase. Similar result by using Rotenone in treating vicia faba root (Amer and Michel 1986).

Jain and Sarbhoy (1988) suggested that sticky nature of chromosome at metaphase due to delay in chromosome movement by pesticides treatment.

Stickiness and clumping chromosome as sickly prophase, sticky bridge at ana-telophase, c-metaphase were found in metaphase. Similar result was reported by Shehab (1980) in Allium cepa treated water extract Teucrium pilosum.

The abnormalities found in anaphase were shifting of poles (diagonally arranged chromosome group) precocious arm and non synchronised movement chromosomes, fragment, breaks of chromosomes, bridge and lagging were found. Precocious arm may be one or, more Adhikari (1982) reported in Allium cepa root tips treating with 2, 4 dichlorophenoxy acetic acid and 2, 4, 5 trichlorophenoxy acetic acid.

Precocious arm and precocious chromatin is formed due to unequal movement in which some chromosome arms pulled extremity of pole (Pathak 1999 and Sapkota 2000).

Laggard at Anaphase due to chemical breaking protein moiety of nucleoprotein back bone of chromosome (Patnaik et al, 1984).

Lagging arm may be attributed hinderance of prometaphase movement. Such result was obtained by kumar (2004) using Aldrin in root of Allium cepa.

The bridge and telophase may be due to a stickiness of chromosomes at metaphase stage (Abraham and Koshy, 1979, Badr and Ibrahim 1987). Similarly such result was obtained by Acharya (1999) on root of Allium cepa using pesticides malathion. Shifting of pole including diagonal and longitudinal type, unequal daughter chromosome, unequal cytokinesis, delay cellplate formation, bi and tri nucleated cell and bridge formation were abnormalities in telophase.

Unequal condensation of daughter chromosome resulted in formation of unequal cell size or, unequal cytokinesis resulted unequal cell size due to disturbance in cell metabolism. Such result was obtained by Ranga swamy et al (1981) in root tip of onions treated with effluent from lack and paint.

The bi and tri nucleated cells were result by failure of cell plate formation according to sapkota (2000) in onion root tip cells treated with Tartrazine. Bi nucleated cells were interpreted as consequences of inhibited cell cycle in which chromosomal DNA is replicated but not distributed in usual ways Brown and Dyes (1972). The polar shifting in telophase was because of continuing such abnormalities from meta and anaphase.

Some abnormal interphase cells or, plasmolysed cells have been recorded following various treatment suggesting cell death. The shifting of nucleus to polar due to imbalance osmoregulation of cells caused plasmolysed and shifting aside Rangaswamy et al (1981). Abnormal prophase cell with decomposed nuclear material were observed in high frequency suggesting pre-prophasic effect of suspension.

From above observation, it is concluded that Monocrotophos is mitodepressive effect in meristem of Allium cepa.

1) Clastogenic effects:-The abnormalities like breaks, bridges, micro nucleated and fragmentation of chromosomes suggested as clastogenic effect of monocrotophos.

2) Cytotoxic effects:- Highly stickiness and disturbed prophase and metaphase suggested as cytotoxic effect of the monocrotophos.

3) Turbogenic effect:- C-metaphase, Laggard, bi and tri nucleated cells, precocious chromosomes suggest that monocrotophos affects the mitotic spindles so, it is turbogenic. In this way, Monocrotophos induces

chromosomal aberration. These aberrations help us to known the mutational changes in organisms.

SUMMARY

The cytological effects of organophosphorus insecticide “Monocrotophos” have been studied on root meristem of Allium cepa. It is widely used in agriculture to destroy the undesired insects-pest to produce high yield. The root tip of Allium cepa was treated in control (water) and different concentration of Monocrotophos (25%, 50%, 75% and 100%) in different time periods 3, 6, 12 and 24 hours respectively.

The out come of study is shown that Monocrotophos induced mitodepresive effect and chromotoxic (cytological abnormalities). The effect included high lethality for cell division, c-metaphase, fragments, breaks, laggards, stickiness, and disturbed polarity.

The main abnormalities were plasmolysed cells, polar shifting (diagonally arranged chromosome in anaphase and telophase), c-metaphase, sticky metaphase, precocious arms, laggard, bridge, breaks in chromosome arms, fragmentation of chromosomes, delayed cytokinesis, diagonal cytokinesis, unequal cytokinesis and bi and tri nucleated in some cases.

The observed results revealed the potentially severe mutagenic in nature of insecticide monocrotophos which not only showed cytological abnormalities but also interfered the mitotic activity by prophase poisoning.

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Table: 1 is in another file

Table:-2
Mitotic, prophase, metaphase, Anaphase and telophase indices of Allium cepa with different concentration of monocrotophos at different time of treatment.

Duration of treat mint in hours	Concentration of more colophons in percentage	Mitotic index	Prophase index	Metaphase index	Anaphase and telophase index
3 hrs	Control	32.98	91.47	4.85	3.66
	25%	34.75	91.42	5.34	3.22
	50%	31.25	91.03	5.42	3.54
	75%	34.21	93.43	4.12	2.43
	100%	30.22	93.16	4.45	2.38
6 hrs	25%	31.23	94.94	3.53	1.51
	50%	28.73	94.61	4.26	1.12
	75%	31.38	88.20	3.12	1.61
	100%	28.79	97.75	-	2.24
12 hrs	25%	36.75	96.81	1.67	1.50
	50%	21.29	95.60	3.22	1.17
	75%	25.47	97.51	2.48	-
	100%	23.52	98.01	-	1.98
24 hrs	25%	20.95	97.24	-	2.91
	50%	20.04	97.49	1.66	0.83
	75%	19.55	98.63	1.36	-
	100%	17.85	97.85	-	2.14

Table: - 3
Mean mitotic, prophase, metaphase and anaphase and telophase indices treated With mean percentage concentration of monocrotophos at different duration of treatment

Duration of treatment time	Mean mitotic index	Mean prophase index	Mean metaphase index	Mean anaphase and telophase index
Control	32.98	91.47	4.85	3.66
3 hours	32.60	92.26	4.83	2.89
6 hours	30.03	93.87	2.72	1.62
12 hours	26.75	96.98	1.84	1.16
24 hours	19.59	97.80	0.75	1.47

Table:-4
Total percentage of abnormal cells and parentage abnormal cells at each phase among the abnormalities with different concentration of monocrotophos at different time of treatment

Duration of treatment of in hours	Concentration of monocrotophos in percentage	Total percentage of abnormal cells	Total percentage of abnormal cells in prophase	Total percentage of cells in metaphase	Total percentage of abnormal cells in ana and telophase
3 hrs	Control	7.14	3.94	1.83	1.37
	25%	7.55	4.60	1.75	1.19
	50%	8.34	4.17	2.29	1.87
	75%	9.28	5.25	2.25	1.78
	100%	8.69	4.55	2.79	1.34
6 hrs	25%	7.37	4.54	2.12	0.70
	50%	7.95	5.04	2.24	0.67
	75%	8.56	5.54	2.21	0.80
	100%	12.59	11.13	-	1.46
12 hrs	25%	10.16	8.66	9.72	0.53
	50%	15.08	12.73	1.75	0.58
	75%	11.63	10.45	1.17	-
	100%	11.78	11.22	-	0.56
24 hrs	25%	13.18	14.72	-	1.45
	50%	16.52	16.19	-	0.33
	75%	15.21	15.21	-	-
	100%	15.53	14.64	-	0.89

Table :- 5

Percentage of abnormal and normal cells at each phase with different concentration of monocrotophos at different time of treatment.

Duration of treatment on hours	**Concentration of monocrotophos in percentage**	**Prophase percentage**		**Metaphase percentage**		**Anaphase and telophase percentage**	
3 hrs		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
	Control	94.27	55.12	3.25	25.64	2.46	19.23
	25%	93.91	60.97	3.88	23.17	2.19	15.85
	50%	94.76	50.00	3.41	27.50	1.82	22.50
	75%	97.20	56.56	2.06	24.24	0.72	19.19
	100%	97.05	52.38	1.81	32.14	1.13	15.47
6 hrs	25%	97.60	51.64	1.52	28.76	0.87	9.58
	50%	97.32	63.38	2.19	28.16	0.48	8.45
	75%	97.96	64.70	1.07	25.88	0.95	9.41
	100%	99.09	88.39	-	-	0.90	11.60
12 hrs	25%	98.12	85.21	0.78	11.22	1.08	6.12
	50%	97.58	84.46	1.72	11.65	0.68	3.88
	75%	98.52	89.88	1.47	11.25	-	-
	100%	98.38	95.18	-	-	1.61	4.81
24 hrs	25%	98.45	91.00	-	-	1.73	9.00
	50%	97.40	97.97	2.00	-	0.60	2.02
	75%	98.38	100	1.61	-	-	-
	100%	98.52	94.25	-	-	1.47	6.09

Table:-6

Mean mitotic index values of Allium cepa root tip cell treated with different concentration of monocrotophos at different time of treatment

Concentration of monocrotophos in percentage	Mitotic index (K) , treated indifferent time period			
	3 hours	6 hours	12 hours	24 hours
25%	34.75	31.23	36.75	20.95
50%	31.25	28.73	21.29	20.04
75%	34.21	31.38	25.47	19.55
100%	30.22	28.79	23.52	17.85

Table:-7

Ranks of four matched groups (concentration) of mitotic index value of Allium cepa under four condition (time)

Concentration of monocrotophos in percentage	Condition (K) , treated indifferent time period			
	3 hours	6 hours	12 hours	24 hours
25%	4	3	2	1
50%	4	3	2	1
75%	4	3	2	1
100%	4	3	2	1
Total (Rj)	16	12	8	4

Calculation

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

$$= \frac{12}{4X\,4(4+1)}\{(16)^2 + (12)^2 + (8)^2 + (4)^2\} - 3X\,4(4+1)$$

$$= \frac{12}{80}(256+144+64+16) - 3\,X\ 4\,X\ 5$$

$$= \frac{12}{80}(480) - 60$$

= 12 X 6 – 60

= 72-60

= 12

Where,

N= number of rows (concentration)

K= number of columns

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

Note: ranking was done assuming lowest score =1 and higher score is=4 for above four conditions.