

THE EFFECT OF INSECTICIDE, MONOCROTOPHOS IN DIVIDING CELLS OF ALLIUM CEPAL.AND STUDY OF ITS MITOTIC INDEX

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Abstract

The effect of monocrotophos, insecticide has been studied in root tip of Allium cepa. The study shows that monocrotophos induced mitodespersive effect and cytological abnormalities. The abnormalities like plasmolysed cells, c-metaphase, stickymetaphase, precociousarms, laggard, bridge, fragmentation of chromosomes, delayed cytokinesis and bi and tri nucleated were observed. The mitotic index, prophase index, metaphase index and anaphase and telo phase index were obtained.

Key Words: *Monocrotophos, insecticides, pesticides, mitoticindex, Alliumcepa.*

Introduction

micals includes food additives, cosmetics, and drug pesticides. 10,000 of them are produced annually in quantities 500 and 1,00,000 kg (UNEP 1975). The use of pesticides has increased in spite of hazardous nature in human environment. It is now obvious that use of pesticides has many secondary consequences (Duggan et al. 1966, Reddy and Rao, Joshi and Bhuju, Joshi et al. 1986) and their repeated use may even induce resistant in pest. They are intended to control. The over use can lead to increase in crop losses and resurgence of the diseases.

In Nepal, the use of pesticides began in 1940's to eradicate Malaria outbreak throughout the country by using Dichlorodiphenyltrichloroethane (DDT). More than 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 ton per 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 necessarily result of direct application where some pesticides accumulate in the food to 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

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usage. Studies have indicated accumulation of DDT in rice and wheat (Joshi,1998) .Out of 527 samples of rice analysed ,Over 70% were found contaminated by DDT. In case of wheat DDT residues were higher than in rice.

Monocrotophos:

Common name :Monocrotophos

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

Trade name:Monohit,Monosil

Manufacturer: National organic industries limited .Bombay,India,Comlets chemical industries CO.Ltd (R.C.O)Cyanomid(Brazil)

Objectives: The cytological effect of insecticides,monocrotophos on root tip of *Allium cepa* and its mitotic index is to be studied.

Justification of Study: The large amount of pesticides are used ,generally in Terai region of Nepal.Monohit or MONOSIL is used as trade name for monocrotophos.The genetic effect in root tip of *Allium cepa* and its mitotic index in lower or higher doses than recommended doses is to understand by carrying out cytological effect of monocrotophos.

Literature Review

It has been found that effect of different pesticides leads to mutagenic and carcinogenic effect in long run(Kumar and Sinha 1989) .Excessive and continuous use of pesticides,herbicides ,fungicides,laboratory agent,plant extract of medical values ,food additives and antibiotics are induced in breaking down of chromosomes,clumping of chromosome and inhibition of cell division. Cytological Effect of pesticides in plant cell

The cytological effect of organophosphate phosalone were studied on root meristem of *Allium cepa*. Concentration (0.25%,0.5%,0.75%,and 1%) of pesticides were taken and subsequent Knops recovery was done 96 hours after treatment of up to 24 hours and 48 hours .Mitotic index gradually decreasing with increasing in concentration and time. Mutagenic effects like chromosome breakage ,sticky,bridge,laggard,polyploidy and clumping.The slow risedmitotic index after recovery for 48 hours and 96 hours of 24 hours and 48 hours respectively.The total percentage of abnormalities was increased concentration 0.25%, and 0.75% and slightly to decreased in concentration 0.50% and 1.0% repectively.Material treated with 0.75% and 1.0% phosalone for 48 hours failed to show recovery with death of roots.Sinha.et.al (1989).

Bom(2005) studied the cytological effect of pesticides Dimethoate on mitotic activity and

chromosomal behavior in root tip cells of *Allium cepa*. Chromosomal behavior at high concentration and large time shows toxic effect. The abnormalities such as c-metaphase, breaks and fragments chromosome, stickiness and polyploidy were observed.

Cytological effect in fungicides in plant cells.

Carbendazin was treated with seeds of sunflower and pearl millet, variety of chromosomal aberration in root tip (somatic cell) and reproductive cell (pollen grain). Cell observed more chromosomal aberration in pearl millet than 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 on sunflower somatic cells were observed. Abnormalities of reproductive cell at diakinesis and metaphase in pearl millet and laggard at metaphase in sunflower reproductive cells were observed (Chand et al 1994).

Cytological Effect of Herbicides in Plant Cells

Herbicides like, Gas and Igran in barley seed with 10^{-12} / 100% cm^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 al 1991.

Cytological Effect of Industrial Effluents

In crotalaria the plant growing near Titanium factory from which geotaxis potential of pollutants effect was investigated. Growth retardation, inhibition of cell division, chromosome clumping abnormalities which were lacking in control plant in same environment (Abraham and Abraham 1991).

Cytological Effect of Plant Extract and Other

Allium cepa root treated with 0.05%, 0.25%, 0.50%, 0.75%, and 1.0% aqueous solution of sodium salicylate for 2, 4, 6, 8 hrs and 24 hrs. The increase in nuclear and chromosomal aberration was depend on concentration and duration of treatment (Briand and Kapoor, 1989)

Material and Methods

The root tip (meristem) from onion bulb was taken for study cytological effect of Monocrotophos. The following procedures and materials were used in the experiment.

Material: Root tip of *Allium cepa*

Selection of Rooting bulb: Equal size and red light bulbs of *Allium cepa* were selected for rooting. The bulb selected were devoid of any damage and healthy.

The bulb was washed thoroughly with clean water and placed with basal side facing downward over jar filled with water for growing roots. The water in jar was refilled at 24 hours interval to check the growth of micro-organisms.

Preparation of suspension for experiment: suspension of monocrotophos was prepared in separate glass jar at concentration of 25%, 50%, 75% and 100% respectively.

Methods

Treatment of rooting bulbs: when the lateral roots of *Allium cepa* were about 2 cm long, they were exposed to freshly prepared test solution of different concentration for 3, 6, 12 and 24 hours at room temperature.

Preparation of Agent for cytological study: For fixing and staining the tissues, following chemicals were used.

Fixing agent: Acetic alcohol

Preserving agent: 70% ethyl alcohol

Staining : 2% acetocarmine

Fixing period: The time was between 10:00 am and 10:30 am

Cytological slide preparation and mounting media:

The darkly stained root was selected and placed on clean slide. About 2mm root tip was required to squash. The clean cover slip was gently placed and squashed. The slide was 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%)

Cytological Observation and Calculation

The slides were observed under compound microscope. The observation were taken on around 3100 cells from at least five different root tips treated with various concentration of suspensions of

Monocrotophos(control,25%,50%,75% and 100%) .The following formulae was used.

$$\text{Mitotic index (MI)} = \frac{\text{TDC}}{\text{TC}} \times 100 \quad (\text{TDC} = \text{Total diving cells, Tc} = \text{total cells counted})$$

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

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

$$\text{Anaphase and Telophase index (Ana-Telo)} = \frac{\text{TC ana-telo}}{\text{TDC}} \times 100$$

Result

The effect of insecticides, monocrotophos in root tip of *Allium cepa* was analysed .Variation of mitotic index and chromosomal abnormalities were observed. Bridge, laggard, breakage of chromosome, and hyperploidy cells were found. Chromosomal abnormalities during treatment was proportional to concentration and during of treatment. Dividing cells were completely absent in treatment with higher concentration.

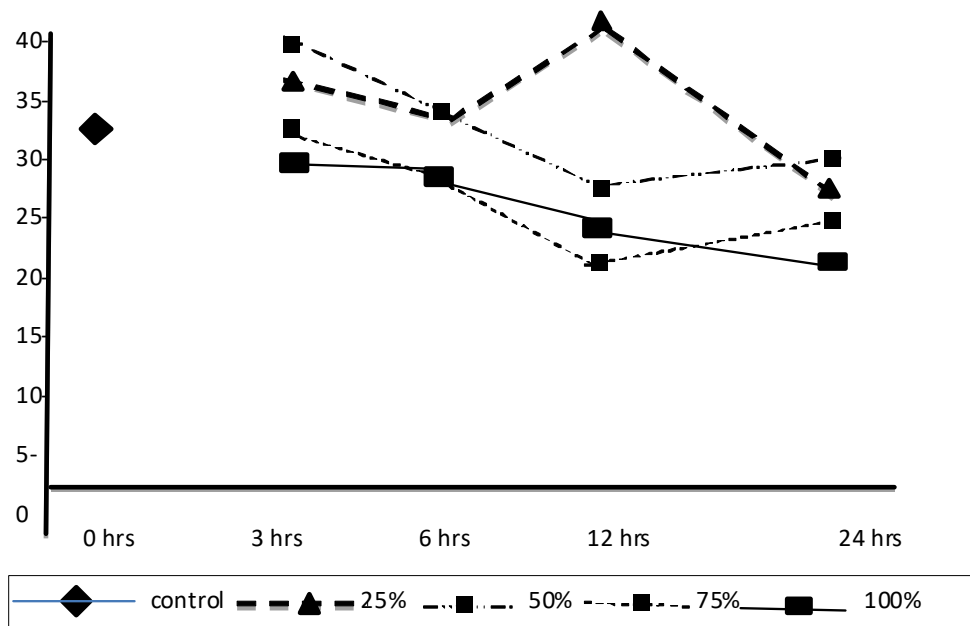


Fig: Graph of Mitotic Index of *Allium cepa* root tip cells Vs treatment time with given concentration of monocrotophos.

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.

Effect in prophase index:

The control value was 91.47 and maximum 97.80 at 24 hours treatment of 100% concentration. The value was increasing irregularly upto 24 hours treatment of 100% concentration.

Effect in Metaphase index:

The control value was 4.8 and minimum value was 5.42 at concentration of 3 hours treatment.

Effect in Anaphase and telophase index

The control index was 3.66, the nil value was at 12 hrs of 75% concentration, and 24hrs of 75% concentration.

Discussion and Conclusion:

Monocrotophos showed variation on different mitotic activities and induced chromosomal abnormalities during somatic cell division on different concentration on root tip of *Allium cepa*. Therefore, Monocrotophos has poisonous character 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 of pesticides tested for their action on mitosis.

The increase prophase index in high concentration 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 prophase in high frequency (Prasad and Das 1997), which result of effect of monocrotophos at prophase stage.

Metaphase index showed no regularity. Metaphase index was higher than anaphase-telophase index and lower than prophase index. Decreased metaphase index with increased the time treatment. It shows high accumulation of abnormal metaphase cells (Pathak, 1999).

Anaphase and telophase indices were higher than metaphase index in 24 hours treatment and lower than the prophase index and remaining 3, 6, and 12 hours treatment, metaphase index was greater than anaphase-telophase. In this way, no regularity was seen. In average, anaphase-telophase index decreased with increasing time and concentration due to prolonged prophase and not entered into further division. Similar result was obtained by treating Carbendazim in *Allium cepa* root (Shrestha 2004). Jain and Sarbhoy (1988) suggested that sticky nature of chromosome at metaphase due to delay in chromosome movement by pesticides treatment. Stickiness, and clumping chromosomes,

bridge formation, lagard, fragmentation of chromosome and c-metaphase were found. From above observation, it is concluded that monocrotohos shows clastogenic effect (breaks, bridge, micronucleated), cytotoxic effects and turbogenic effect (C-metaphase, lagard, precocious chromosomes and bi and tri nucleated cells).

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