CHAPTER -I

INTRODUCTION

1.1 Background

Use of pesticides has been increasing with the advancement in the agricultural practices. It has become necessary to eliminate various diseases which greatly damage the crop and reduce the yield so control of pests is a great challenge today to hold the growing population. Although pesticides are the easy way to control pests, indiscriminate use of these pesticides may harm the plant, human and disturb the ecosystem as well. Use of such chemicals in the field for the better production of the crops needs testing the genotoxic effect of such chemical. The current plan of work is proposed to study the effect of above chemical on *Allium cepa* on the basis of cytological aberrations during root tip mitosis.

Use of toxic chemicals started from late 1800 in Western countries. Chemical pesticides were imported and used in Nepal since 1950's for Malaria eradication and then for agricultural purpose. The initiation of pesticide use in Agriculture sector began from 1956 when DOA, HMG/N imported DDT for pest control purposes. More than 250 types of pesticides have been registered for use in Nepal. Fungicide equivalent to 15.5 MT of active ingredients are consumed annually in Nepal (PPD., 2000).

Fungi are the major harmful pests damaging the crops and reducing the yield. So, use of fungicides is also being increased in modern agricultural system to reduce damage of the crop caused by fungal diseases. Fungicides are the chemicals that inhibit fungal metabolism.

1.2 Copper oxychloride

Copper oxychloride is the most commonly used fungicide in South African vineyards to control Downy mildew of Grape caused by the fungus *Plasmopara viticola*.(Internet a). Throughout 1870's Downy mildew, which was widespread in Europe, vine was protected with Copper oxychloride . It is also used to control various diseases like blister blight of tea and coffee, leaf spot and fruit rot of banana, leaf blight of wheat and slug control.

Copper oxychloride 50% WP, an inorganic compound is a contact fungicide. Copper ions inhibit the metabolism of fungus when they react with sulfur containing enzymes in the plant (Internet b) Copper oxychloride is known by its trade names Dhanucop, Anucop, Curex, Blitox, Nagcopper etc

Copper oxychloride is light green in colour with mild characteristic odour with specific gravity 3.7, P^H 6.5 and miscible in water.

IUPAC name : Dicopper chloride trihydroxide

Molecular formula: $ClCu_2H_3O_3$

Molecular weight : 232.01

REG. NO. : 1332-40-7

Copper oxychloride 50% WP packet

1.3 Objectives

The main objectives of the present study are as follows:

- To study the effect of Copper oxychloride in mitotic activity of dividing cells.
- To observe the effect of different concentrations of Copper oxychloride and time period of treatment in dividing cells.
- To observe the cytological abnormalities if present.

1.4 Justification of the study

Copper oxychloride is one of the most useful fungicides used to Control various fungal diseases. It is most commonly used fungicide in South African vineyards to control Downy mildew and its toxicity to earthworms has been studied but its cytological effect in plant cells is not much known yet. So the present study has been carried out to understand its effect in the dividing cells of the root meristem of *Allium cepa*.

CHAPTER -II

LITERATURE REVIEW

2.1 Cytological effects of Fungicides

Shrestha (1982) revealed that Bavistin and Diathane M-45 were two fungicides capable of inducing physiological as well as cytological abnormalities in *Allium cepa* L. These fungicides inhibited the mitotic activity and induced chromosomal abnormalities such as stickiness, breaks and bridges. These fungicides exerted a weak C-mitosis.

Bhunya and Behera, (1984) studied the cytogenetical effects of fungicide Ediphenphos on the bone marrow chromosomes of mice in vivo. Dose, route, and duration of exposure largely influenced the aberration frequency. The chemical had been found to be mutagenic in the present test system.

Badr (1988) studied the effect of two fungicides Dithane and Denmart on root meristem of *Allium cepa.* Inhibition of mitosis and accumulation of metaphase cells were observed. Dithane reduced the frequency of prophase cells while Denmart reduced the frequency of anaphase and telophase. These fungicides induced chromosomal stickiness, breaks and bridges. Chromosome lagging, binucleated, multinucleated cells and micronuclei were also observed.

Singh et al. (2001) studied the effect of fungicides and weedicides on the severity of rice collar rot in rice field. The effectiveness of five fungicides on the in- vitro growth and sporulation of colour rot fungus, *Pestalotiopsis vesicolor* and two weedicides on the high yielding rice cultivars *Leima phou* (KD, 2-6-3) was studied. Visible response on the severity of rice collar rot was observed with the application of fungicides.

Chauhan et al. (1999) studied the effects of some fungicide s Mancozeb, Bavistin, Sulphex on cytomorphological change in *Allium cepa*. The fungicide inhibited mitotic index and the abnormalities found were binucleated cells, scattered metaphase, chromosomal bridges, laggard and multipolar anaphase.

Ahmad and Tasmin (1992) studied the cytotoxic effects of fungicide Tri-miltox on root meristem of *Allium cepa*. Various abnormalities were observed like chromosome fragmentation, laggards, micronuclei, single and multiple bridge formation.

Chand et al. (1991) studied the effect of Carbendazim in growing seed of sunflower and pearl millet. Various chromosomal abnormalities were observed in root tip cells and reproductive cells. Somatic cells showed abnormalities like stickiness, laggard chromosomes and chromosomal bridges at anaphase.

Pandey et al. (1994) studied the cytogenetic effect of fungicides Dithane M-45 and two insecticides Aldrex-30 and Metacid-50. It showed lethality on cell division and the abnormalities recorded were clumping, bridges, fragments, cytomixis, disturbed polarity etc.

Somashekhar et al. (1984) studied the effect of fungicide Topsin on root meristem of *Allium cepa* L. Fungicide showed mitodepression, chromosome clumping and disturbed anaphase gave rise to tripolar and tetrapolar cells and also induced various abnormalities like C-metaphase, spindle abnormalities and inhibition of cell plate formation. Clastogenic effects like chromosome fragmentation, gaps, breaks and bridges were also observed in anaphase and telophase. Disorientation of spindle fibers, micronuclei and inhibition of cytokinesis, precocious chromosome, and unequal distribution of chromatin material also observed.

Prakash, Lakshmi and Harini (1988) studied the cytological effect of fungicides Bavistin and Deltan on chili (Capsicum annum L.). Higher concentration affected the seed germination and seedling survival. Mean chiasma frequency per cell was decreased with the increased concentration of treatments. Stickiness and non-orientation of chromosome at metaphase I and chromatin bridges and laggards were observed at anaphase. The cytotoxic effects resulted pollen sterility.

Al- Najjar et al. (1980) found that Fungicides Vitavax-200 and Diathane S-60 caused highly reduction in metaphase index, significant increase in duration of metaphase stage and high frequency of chromosomal aberration mainly chromosomal bridges during anaphase.

Somashekhar and Gowda (1984) studied the cytotoxic effect of fungicide Vitavax on root meristematic cells of *Allium cepa.* The result of showed some antimitotic and cytotoxic effects like spindle abnormalities and inhibition of cell plate formation. Clastogenic effects like fragments, bridges were also found.

Hardy et al. (2001) studied the future of phosphate as a fungicide to the soil borne plant pathogen in natural ecosystem. Research has shown significant protective effects. However, phosphate also induced phytotoxicity, growth, abnormalities reproductive capacity and large differences in level of *P. cinnamomum* control between plant species.

Yuzbasioglu (2003) studied the cytogenetic effect of fungicide Afugan on the meristematic cells of *Allium cepa*. The chemical induced various chromosomal abnormalities like stickiness, C-mitosis, Bridges, lagging chromosomes, fragments, multipolar cells and micronuclei formation.

Dane and Dalgic (2005) studied the effect of fungicide Benomyl (benlate) on growth and mitosis in *Allium cepa* root meristem. Several abnormalities were induced in cell division. Mitotic frequency was decreased as the benomyl solution concentration was increased. Benomyl had negative effect on mitotic divisions in the plant cells.

2.2 Effects of Different Agrochemicals and Others

Cytological effects of insecticides on dividing cells were studied by Amer and Farah, 1974, Ravindran, 1971, Amer and Ali, 1969, Pandita, 1986, Rao et al., 1987, Ahmad et al., 1992, George and Ghareeb, 2001, Kumar and Kumar, 2004, and Sinha et al., 1989 and similarly, organophosphorous pesticide by Grover and Malhi, 1998.

Cytological effect of herbicides studied by Adhikary, 1982, Badr and Ibrahim, 1987, Bakale and Hadke, 1981, Wuu and Grant, 1967, El-Khodary et al., 1989, Butt and Bahidya, 1994, El-Ghamery et al., 2000, and Shrestha, 2002.

Effects of industrial effluents studied by Pun and Sakya, 1994, Shakya et al., 1999, and Rangaswamy et al., 1981; and the effect of others chemicals were studied by Amer and Mikhael, 1972, Pathak, 1999, Bhalla et al., 1976, Prasad and Das, 1977, Mercykutty and Stephen, 1980, and Reddy and Subhramanyam, 1981. Raj and Rao, 1972, Shehab, 1980b, Mercykutty and Stephen, 1980, George and Geethama, 1990, Sagoo et al., 1991, Williams et al., 1996 and Hengtai et al., 1997. Cytological effects of different metals were studied by Giri et al 1984 and Jayaprakash et al 1994.

CHAPTER -III

MATERIALS AND METHODS

For the present work root meristem of onion (*Allium cepa* L. 2n=16) were used. The root meristem was treated with copper oxychloride.

3.1 Materials

Onion (*Allium cepa* L.) meristem as bioassay.

3.2 Onion bulbs rooting method

Healthy onion bulbs were collected. Onion bulb was selected because of easy handling and convenience in cytological study. The bulbs were washed with water and old roots on the bulbs were removed. The bulbs were placed on the coupling jar filled with water touching its basal part with the water. The water of the jar was replaced at 24 hours intervals so as to check the growth of microorganisms.

3.3 Preparation of Suspension for the experiment

2 gm of Copper oxychloride was added in 1000ml of water to make the recommended dose suspension (100%). Similarly, different concentrations were prepared by diluting recommended dose suspension.

3.4 Methods

3.4.1 Treatment of the Rooting bulbs

When the lateral roots of the onion (*Allium cepa* L.) were about 2cm long they were exposed to freshly prepared test solutions of different concentrations for 3,6,12 and 24 hours at room temperature. As the period of treatment was prolonged and high concentrations were found to be lethal they were avoided. The schedules of treatment are given in table1.

Table No.1: Schedules of Treatment

Time of transferring the materials to the suspension (Copper oxychloride)	Time period (hour)	Fixing time
10:30a.m.(control)	0	10:30a.m.
07:30a.m.	3	10:30a.m.
04:30a.m.	6	10:30a.m.
10:30p.m.(previous night)	12	10:30a.m.
10:30a.m.(previous day)	24	10:30a.m.

3.4.2 Preparation of Reagents for Cytological Study

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

Fixing agent (Acetic alcohol)

Glacial acetic acid	1 part
Absolute alcohol	3 parts

Preserving agent

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

Stain: (Aceto-Carmine 2%)

Carmine	2gms
Glacial Acetic acid	45ml
Distilled water	55ml

3.4.3 Cytological Fixation

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

3.4.4 Aceto- Carmine Squash Technique

The fixed root tip (about 1cm) were stained in 2% aceto-carmin and squashed (about 2mm root tip) on a clean slide. More than 5 root tips were studied for each treated and non treated onion bulbs.

3.4.5 Preparation of permanent slide and Mounting Media

Celarier's (1956) method is used for the preparation of permanent slides. Different grades were prepared. The compositions of different grades were as follows:

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

After dehydration the stained meristematic tissues were mounted in Euparol. The permanent slide was observed under microscope and photomicrographs of different stages of cell division and chromosomal abnormalities were taken.

3.4.6 Cytological Observation and Calculation

The prepared slides were observed under compound microscope. Normal and abnormal cells were studied and counted. The observations were recorded on around 4000 cells from at least five different root tips treated with various concentration of Copper oxychloride. The mitotic and phase indices along with abnormalities were scored and analyzed by using Levan formulae 1949 (cf. Kihlman 1971, Medeiros and Takahashi, 1987) given in appendix.

3.4.6 Statistical Analysis

"The Friedman's two-way analysis of Variance by rank's method (Siegal 1956) was applied to calculate whether the time of treatment affects the mitotic index value of Allium cepa root tip cells or not. The data from table No. 6 and 7 was submitted for calculation as below:

$$\text{¡}_{r}^{2}\ \text{X}\frac{12}{NK\text{ƒ}k\ \Gamma 1\text{A}_{j\text{X}1}}\overset{K}{}\text{ƒ}R_{j}\ \text{Å}^{2}\ \text{Z}3N\text{ƒ}k\ \Gamma 1\text{A}$$

Where,

N = 4 number of rows (concentration)

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

R_j = sum of ranks in the j^{th} column

$\sum_{j}^{k} x_i$ = Directs one to sum of the squares of the sums of ranks overall 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

In the present study the effect of fungicide Copper oxychloride on root meristematic cells of *Allium cepa* L. was analyzed from the experiment. The comparative studies of results of controlled and treated root meristem with in relation to different time of treatment were done. The present study was carried out on the basis of variation of mitotic and phase indices and chromosomal abnormalities. The chemical at different concentrations was capable of inducing different types of chromosomal abnormalities as shown in photo plate 1 and 2. From statistical analysis, the calculated values for chi square (χ_r^2) was 9.3 whereas tabulated value (χ_r^2) was 7.8 at percentage (p) <0.05. The results are tabulated as in Table 2 to Table 8 (see appendix) and elaborated under separate sub headings are given below:

4.1 Effect in mitotic index

The Mitotic Index (MI) obtained from root tip cells treated with different concentrations of Copper oxychloride in relation to duration of treatment is shown in table 3 and fig. 1. On treatment mitotic index decreased than control. Mitotic index decreased with increase in concentration and time of treatment. It was highest (38.43%) at control and least (21.06%) in 0.1% concentration at 24 hours treatment.

4.2 Effect in phase index

4.2.1 Effect in prophase index

Prophase index values are shown in table 3. Variability in prophase index value in relation to duration of treatment is shown in fig.2. Prophase index increased with increase in concentration of chemical. It was highest (94.19%) in 0.075% concentration at 24 hours treatment period and least (87.41 %) in 0.05% concentration at 6 hours treatment. The values didn't show much variation with varying period of treatment.

4.2.2 Effect in metaphase index

Metaphase index values are shown in table 3 and fig.3 Metaphase index didn’t show any regularity. It was highest (6.17%) in 0.05% concentration at 6 hours treatment and least (2%) in 0.05% concentration at 12 hours treatment.

4.2.3 Effect in anaphase and telophase index

Anaphase and telophase index values are shown in table 3 and fig 4. Anaphase and telophase index decreased with increase in concentration and period of treatment except in some cases. It was 6.04% at control and was highest (6.56%) at 0.025% concentration at 3 hours and least (1.97%) at 0.1% at 3 hours treatment.

(Fig.5 to Fig. 8 also revealed the percentage of mitotic and phase indices in different period of treatment at four different concentrations of Copper oxychloride.)

4.3 Relation between Mean Mitotic Index and Mean Phase Indices

Mean mitotic index and mean phase indices are shown in Table 4 and fig. 9. The mean mitotic index decreased with increase in treatment period. Mean mitotic index was highest (38.43%) in control and least (22.18%) at 24 hours treatment. Mean prophase index increased with increase in treatment period except at 24hours. Mean prophase index value is greater than control for all treatment time period. It was highest (92.46%) at 12 hrs and least (89.06%) at control. Mean metaphase index was highest (4.9%) at control. Mean metaphase indices at different concentrations were less than that of control. Mean anaphase and telophase indices were also less than that of control (6.04%) which is the maximum value. Mean metaphase index and mean anaphase and telophase index values didn't show any regularity with treatment duration as mitotic index.

4.4 Percentage of Abnormal cells

Total percentage of abnormal cells and percentage of abnormal cells at each phase among the abnormalities with different concentration of Copper oxychloride at different time of treatment are given in table 5 and table 6.

4.4.1 Total Percentage of Abnormally Dividing Cells

Total percentage of abnormally dividing cells is shown in table 5 and fig. 10. In control, there were few abnormal cells (2.29%). Abnormalities were found more in treated roots. Among treated cells, highest abnormality percentage found was 6.51% in 0.025% concentration at 12 hours treatment and least i.e., 3.38% in 0.05% at 6 hours treatment which was lower than control value.

4.4.2 Total Percentage of Prophase Abnormalities

Total percentage of prophase abnormalities at different concentrations of Copper oxychloride is shown in table 5 and fig. 11. The values did not show any regularity.

The percentage of abnormality at control was 16.67%. Among all the treated concentrations of chemical and time period, the highest abnormal value is 39.66% at 0.1% concentration at 12 hours treatment period and least value is 5.17 % at 0.025% concentration at 6 hours treatment which is lower than control value.

4.4.3 Total percentage of Metaphase Abnormalities

Total percentage of metaphase abnormalities at different concentrations of Copper oxychloride at different period of treatment are shown in table 5 and fig. 12.In normal untreated roots 35.71% of abnormal metaphase cells were found. Among treated roots, abnormality index was highest (53.33%) in 0.05% at 24 hours treatment which is higher than that of control and least (10.34%) in 0.1% at 12 hours treatment.

4.4.4 Total Percentage of Anaphase and Telophase Abnormalities

Total percentages of Anaphase and telophase abnormalities are shown in Table 5 and Fig. 13. Abnormal anaphase and telophase index found in untreated roots was 47.62% which is greater than abnormal prophase and metaphase indices. Among treated roots, highest abnormal anaphase and telophase index was 61.40 % found at 0.05% at 12 hours treatment and least (25.00%) at 0.075% at 12 hours treatment.

4.5 Proportion of Abnormalities in Phases among Experimental groups

The proportion of abnormalities in dividing cells at different hours of treatment in different concentration of Copper oxychloride solution is shown in table 5. Abnormality indices of metaphase, anaphase and telophase were higher than prophase abnormality index for all concentrations and time periods. The highest percentage of abnormal cells was found in anaphase and telophase. Untreated root tip cells also showed abnormalities in prophase, metaphase, anaphase and telophase. Total percentage of abnormal cells was 2.29% of total dividing cells.

4.5.1 Abnormal mitotic phases in 0.025% Copper oxychloride treatment

Table 5 shows abnormal mitotic phase in the dividing cells of *Allium cepa* L. treated with 0.025% of Copper oxychloride . Prophase abnormality was not regular. It was highest at 12 hours treatment and low at 6 hours treatment. Metaphase

abnormality showed decrease with increase in treatment hour. It was highest at 12 hours treatment. Anaphase and telophase abnormality was highest at 3 hours treatment and least at 24 hours treatment.

4.5.2 Abnormal Mitotic Phases in 0.05% Coppers oxychloride treatment

Table 5 shows abnormal mitotic phases on 0.05% Copper oxychloride treatment. Prophase abnormality was highest at 3 hours treatment and least at 6 hours treatment. Prophase abnormality was not regular. Metaphase abnormality index also didn't show any regularity. It was highest at 6 hours treatment and least at 12 hours treatment. Anaphase and telophase abnormality was higher than prophase and metaphase in this treatment. It is highest at 12 hours treatment and least at 3 hours treatment.

4.5.3 Abnormal mitotic phases in 0.075% Copper oxychloride treatment

Table 5 shows abnormal mitotic phases on 0.075% Copper oxychloride. Prophase, metaphase, anaphase and telophase abnormality didn't show any regularity. At prophase, it was high at 6 hours and low at 24 hours treatment. Metaphase abnormality was least at 12 hours treatment and highest at 6 hours treatment. Anaphase and telophase abnormalities are highest at 3 hours and least at 12 hours of treatment.

4.5.4 Abnormal Mitotic Phases in 0.1% Copper oxychloride treatment

Table 5 shows abnormal mitotic phases on 0.1% Copper oxychloride treatment. Prophase abnormality was highest at 12 hours treatment and least at 24 hours treatment. Metaphase abnormality was highest at 3 hours treatment and least at 12 hours treatment. Anaphase and Telophase Index was highest at 6 hours treatment and least at 24 hours treatment.

4.6 Chromosomal Behaviour

Fungicide Copper oxychloride nduced various types of chromosomal abnormalities in the root meristematic cells of *Allium cepa* L. during mitotic cell division. Abnormal Phases from treated groups are given in photo plates 1-2.

4.6.1 Nature of abnormal cells in Non-dividing cells

The abnormalities found in non-dividing cells were plasmolysed cells (plate 2: N) and shrinkage of cells.

4.6.2 Nature of abnormal cells in Prophase

Unequal condensation of chromatin threads, stickiness of chromosomes, diluted cells and disturbed prophase were the abnormalities found in prophase.

4.6.3 Nature of Abnormal cells in Metaphase

Equatorial plate shifting, C-metaphase (Plate 1: A), Relaxed chromosome at equator (Plate 1:D), disturbed metaphase, non synchronized condensation of chromosomes were found. Diagonal metaphase (Plate 1: B), Fragmented Chromosomes were also found.

4.6.4 Nature of Abnormal cells in Anaphase and Telophase

Diagonal anaphase (Plate 1: C), precocious arms (Plate 1:G and H), Laggards, unequal movement of chromosomes, precocious chromosome, bridges, fragmentation, sticky anaphase were seen. Pole shift and Relaxed C-star anaphase (Plate 1: E) were also seen. In Telophase, unequal cytokinesis (Plate 2: K), delay in cell plate formation, binucleated cells (Plate 2: J and K), pole shift (Plate 1: F), unequal condensation of daughter chromosomes (Plate2: L), diagonal Telophase were found.

4.6.5 Nature of Abnormal cells in Control

Plasmolysed cells were seen in interphase. In prophase, disturbed cells, unequal condensation of chromatin threads were observed. Precocious arm, diagonal anaphase and Telophase were found frequently.

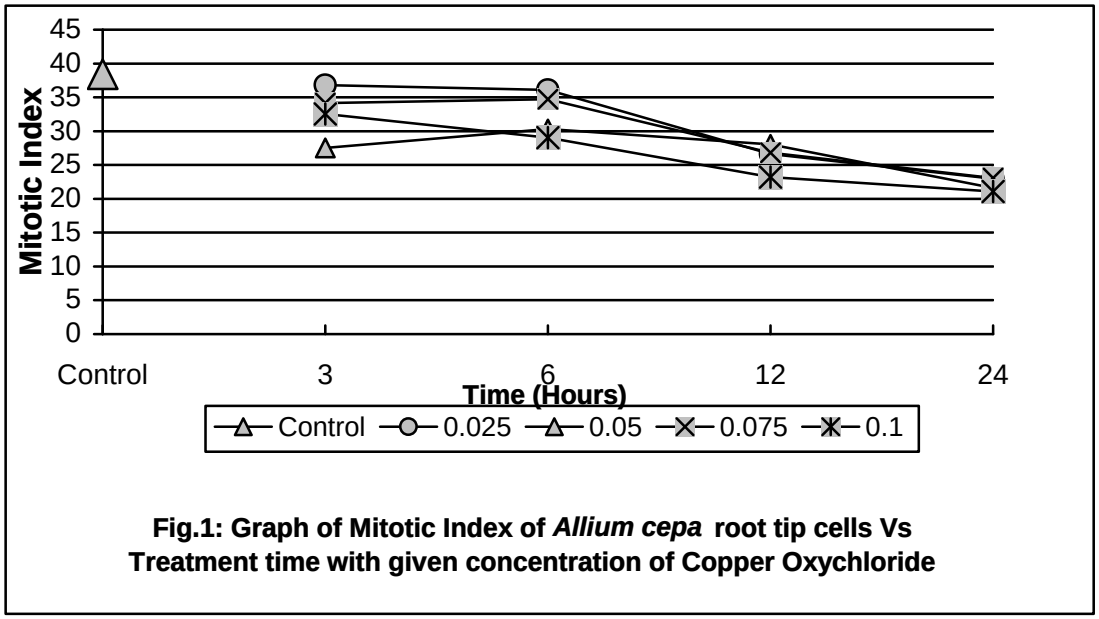


Fig.1: Graph of Mitotic Index of *Allium cepa* root tip cells Vs Treatment time with given concentration of Copper Oxychloride

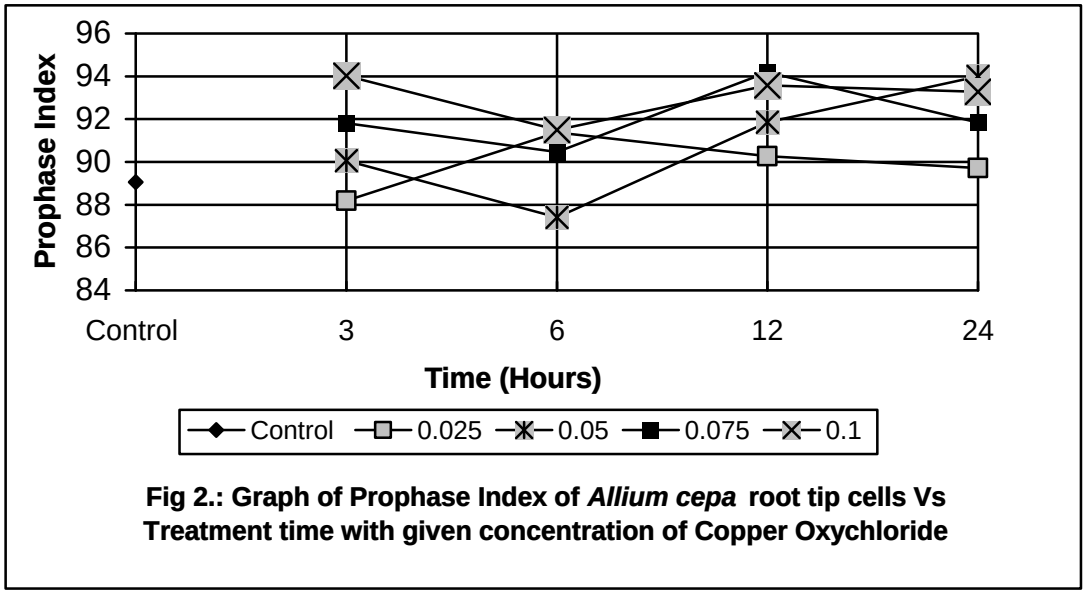


Fig 2.: Graph of Prophase Index of *Allium cepa* root tip cells Vs Treatment time with given concentration of Copper Oxychloride

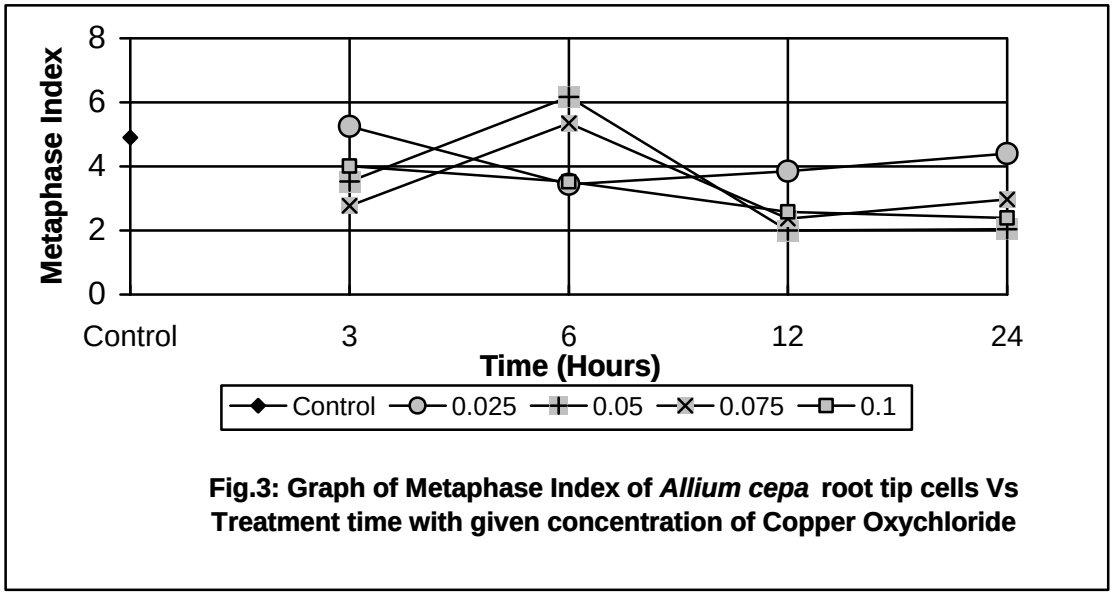


Fig.3: Graph of Metaphase Index of *Allium cepa* root tip cells Vs Treatment time with given concentration of Copper Oxychloride

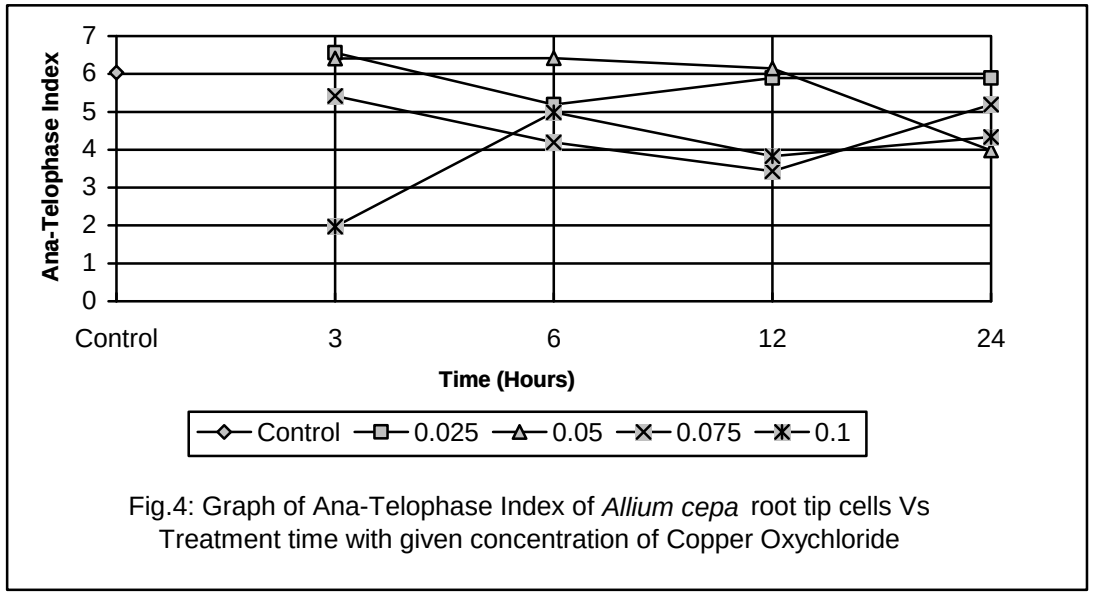


Fig.4: Graph of Ana-Telophase Index of *Allium cepa* root tip cells Vs Treatment time with given concentration of Copper Oxychloride

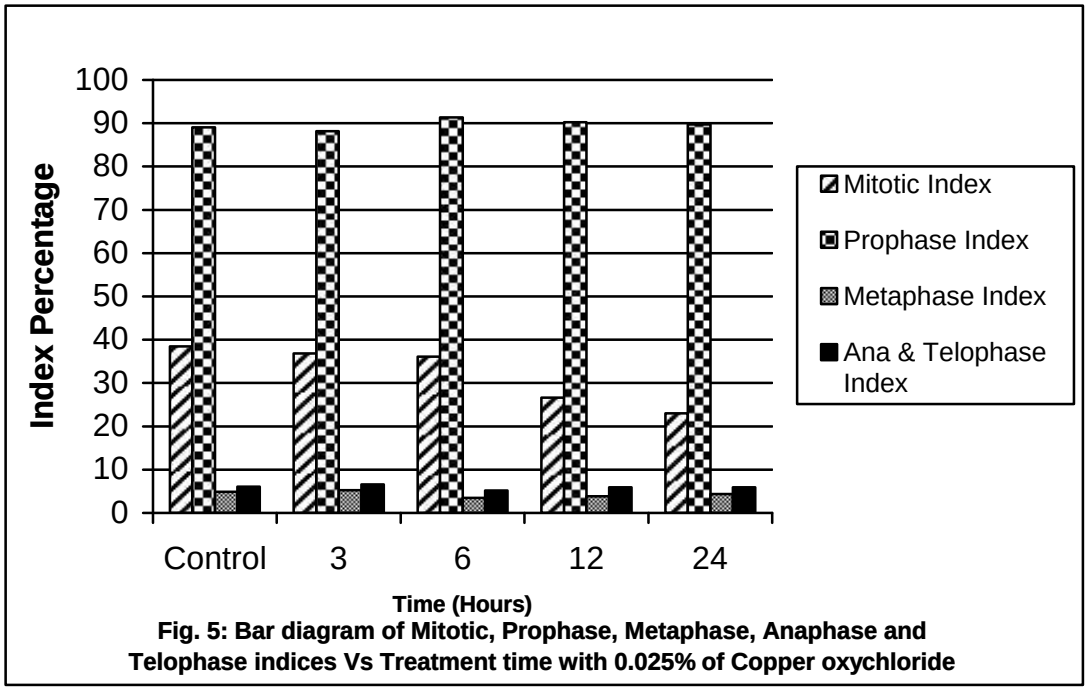


Fig. 5: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase indices Vs Treatment time with 0.025% of Copper oxychloride

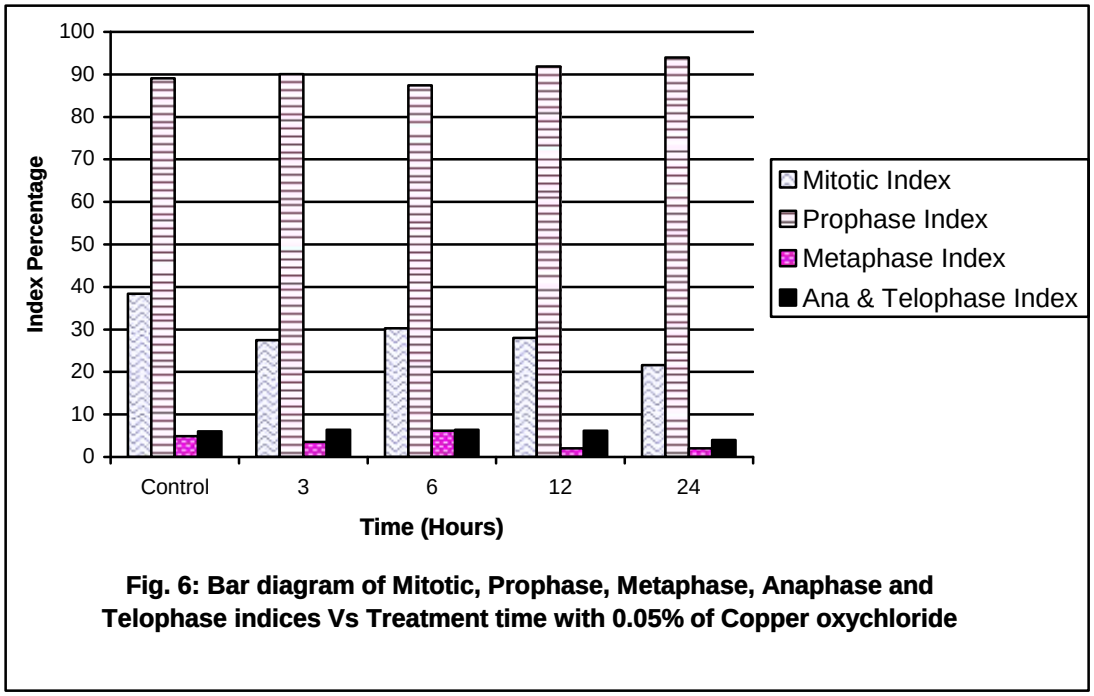


Fig. 6: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase indices Vs Treatment time with 0.05% of Copper oxychloride

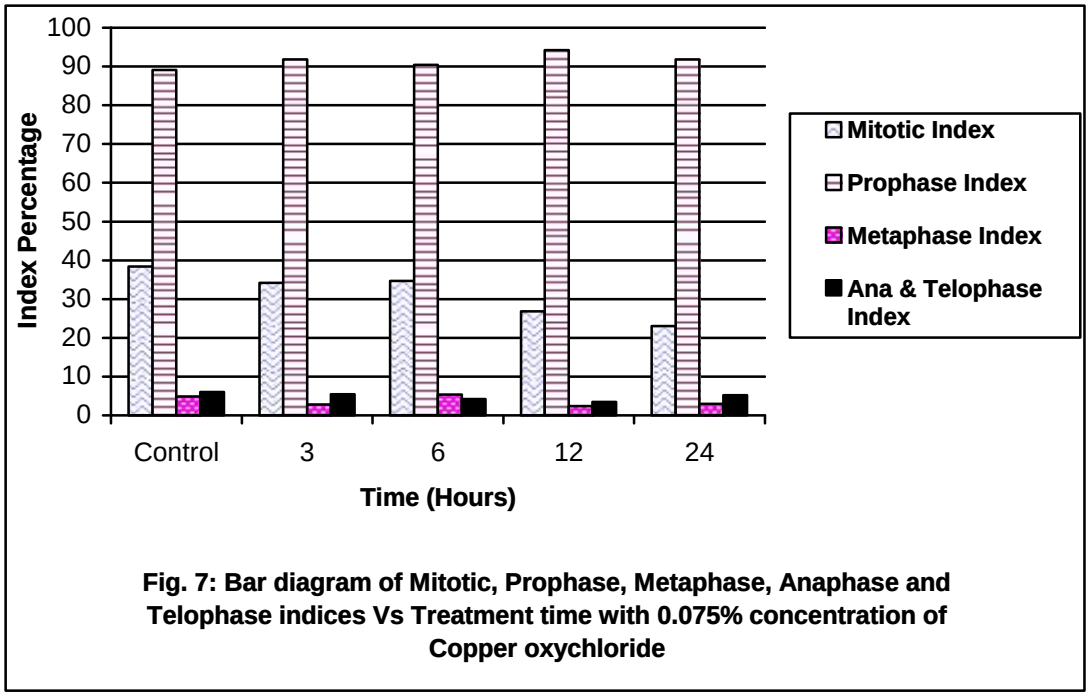


Fig. 7: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase indices Vs Treatment time with 0.075% concentration of Copper oxychloride

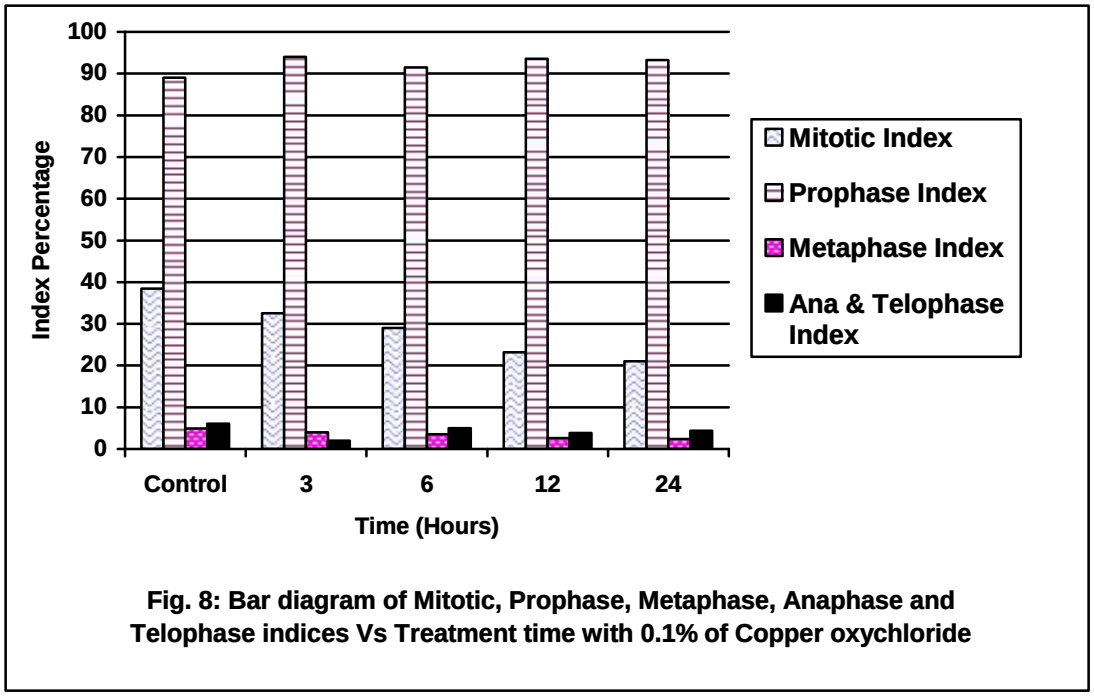


Fig. 8: Bar diagram of Mitotic, Prophase, Metaphase, Anaphase and Telophase indices Vs Treatment time with 0.1% of Copper oxychloride

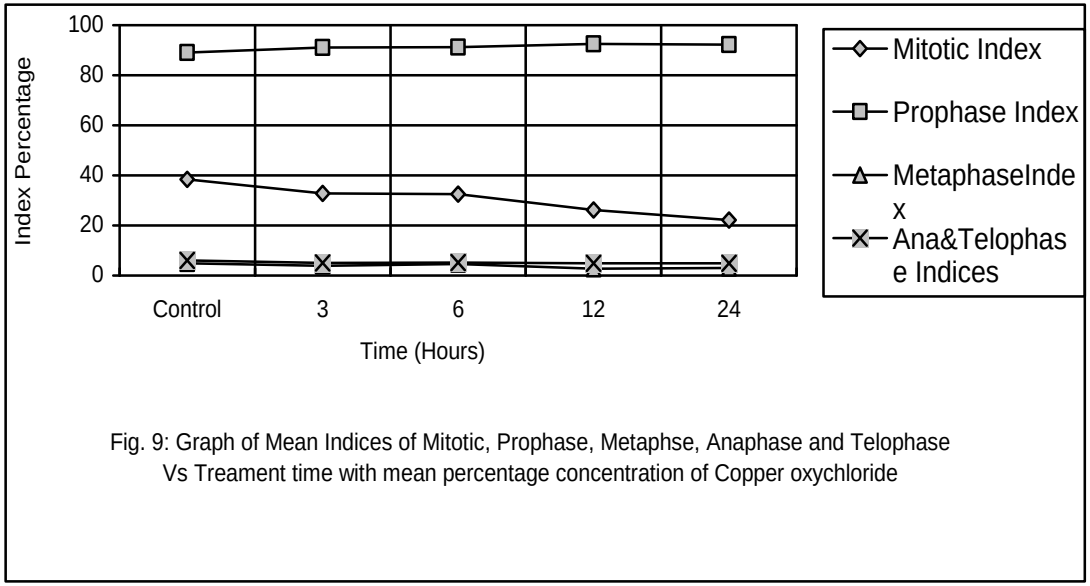


Fig. 9: Graph of Mean Indices of Mitotic, Prophase, Metaphse, Anaphase and Telophase Vs Treament time with mean percentage concentration of Copper oxychloride

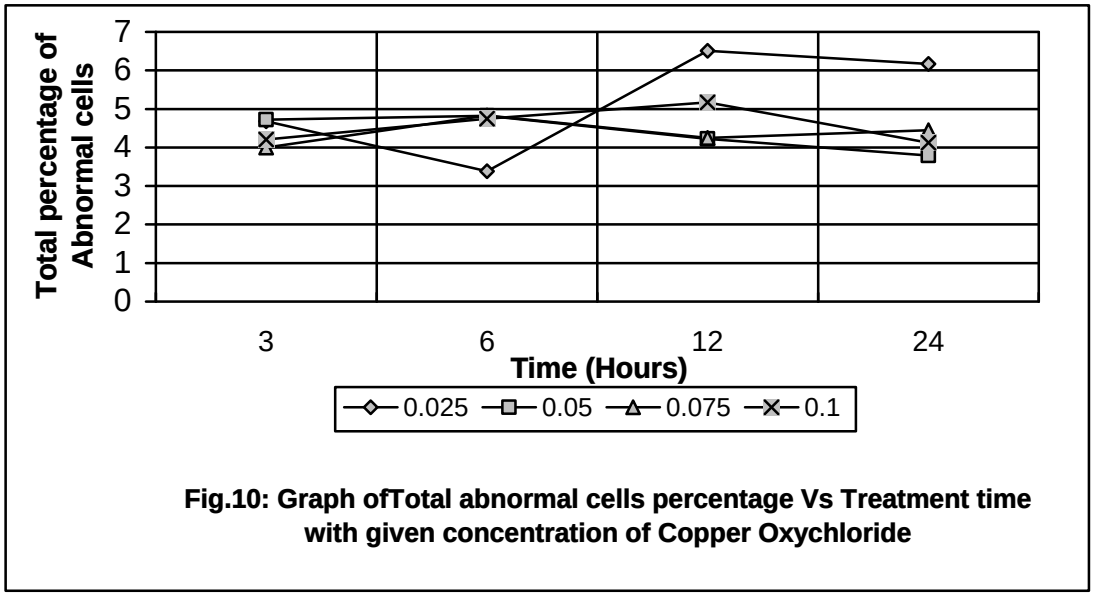


Fig.10: Graph ofTotal abnormal cells percentage Vs Treatment time with given concentration of Copper Oxychloride

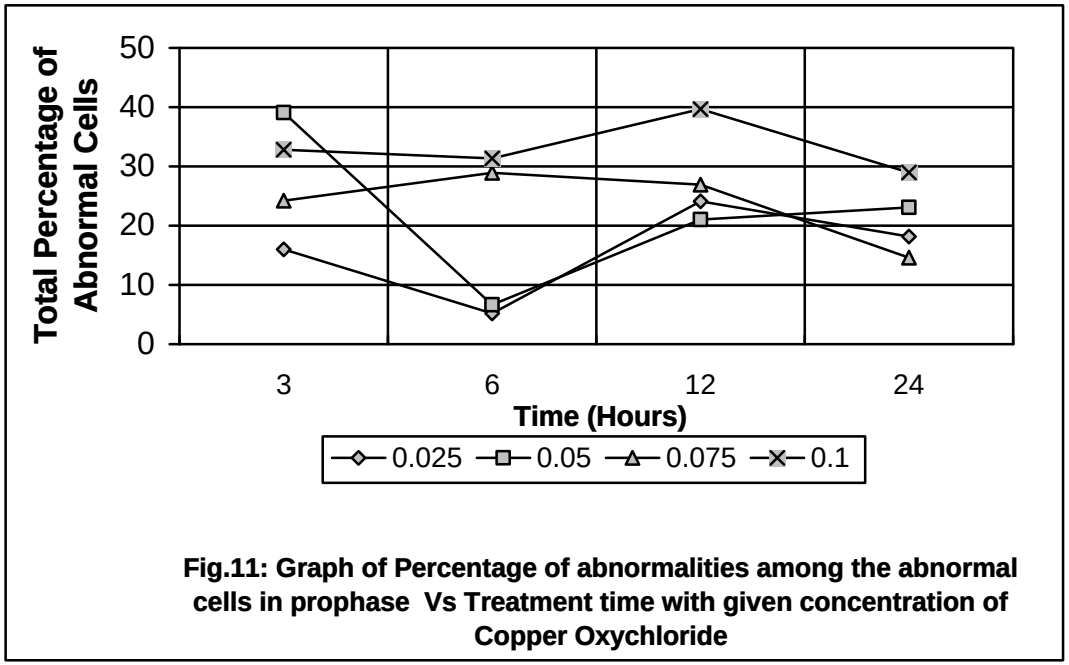


Fig.11: Graph of Percentage of abnormalities among the abnormal cells in prophase Vs Treatment time with given concentration of Copper Oxychloride

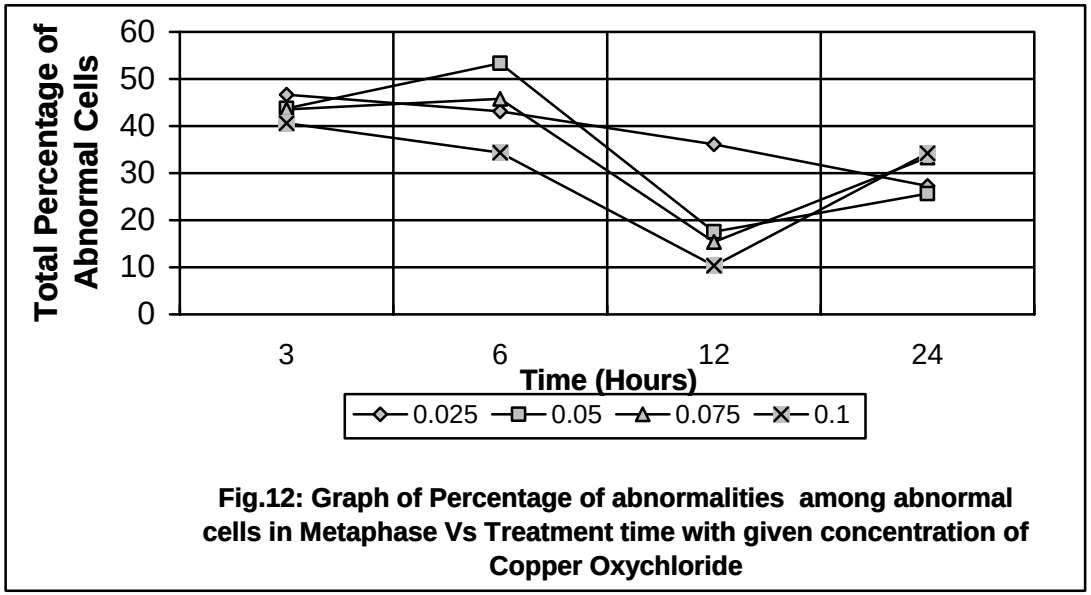


Fig.12: Graph of Percentage of abnormalities among abnormal cells in Metaphase Vs Treatment time with given concentration of Copper Oxychloride

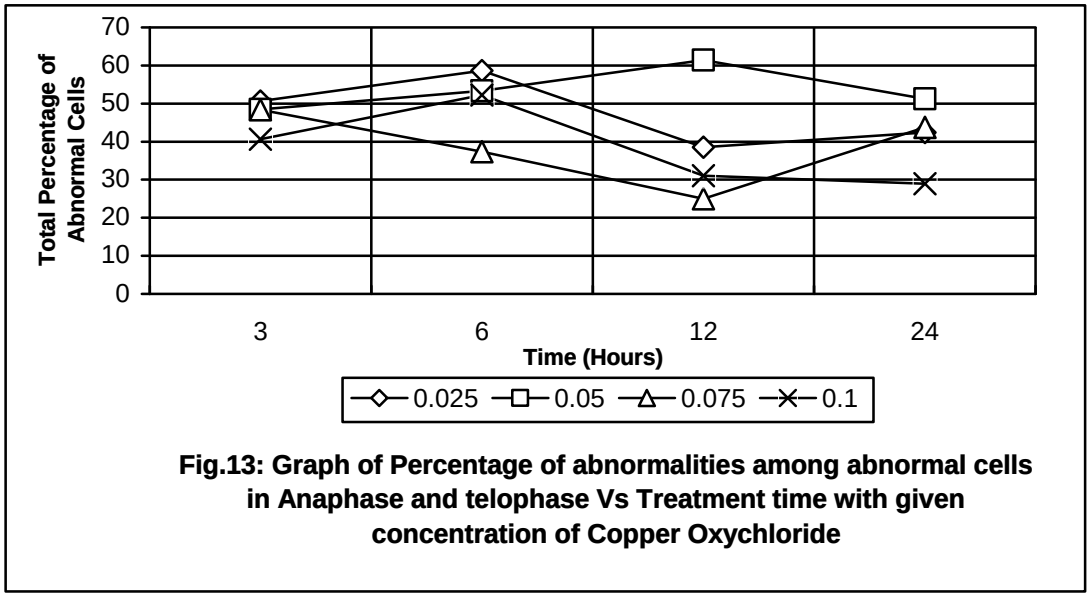
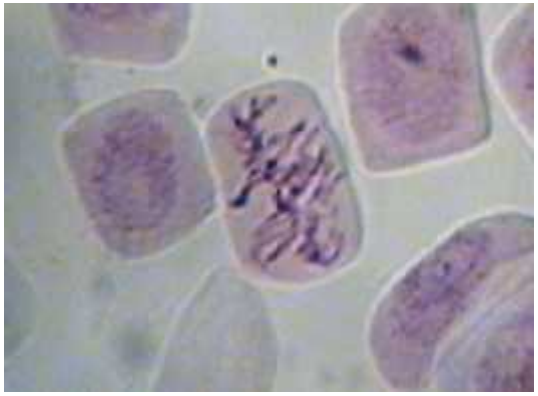


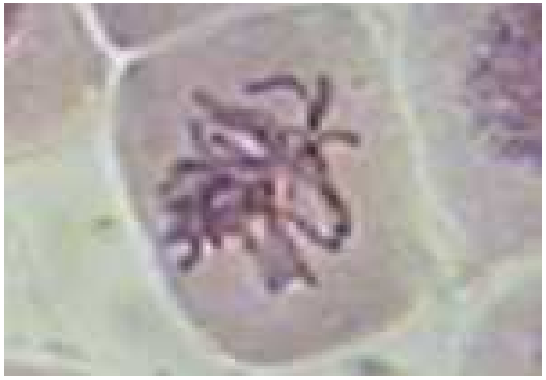
Fig.13: Graph of Percentage of abnormalities among abnormal cells in Anaphase and telophase Vs Treatment time with given concentration of Copper Oxychloride

A C-metaphase

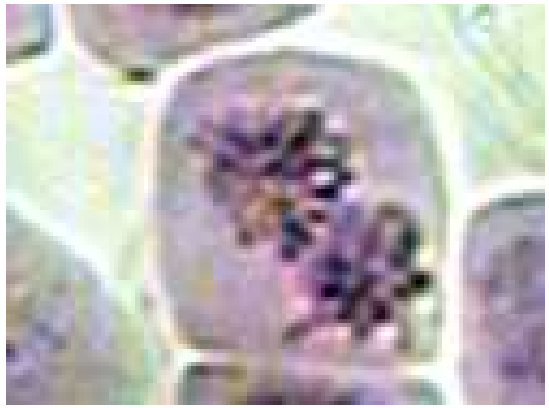


B. Diagonal Metaphase

C. Diagonal Anaphase



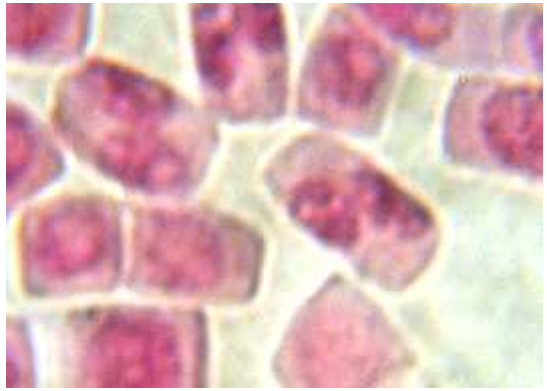
D. relaxed chromosomes at equator



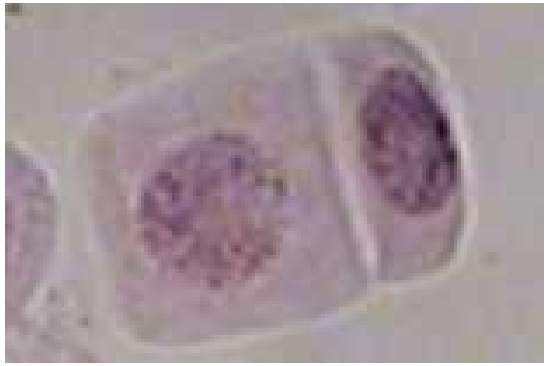
E. Star shaped relaxed C-Anaphase

F. Shifting of pole in C-Anaphase

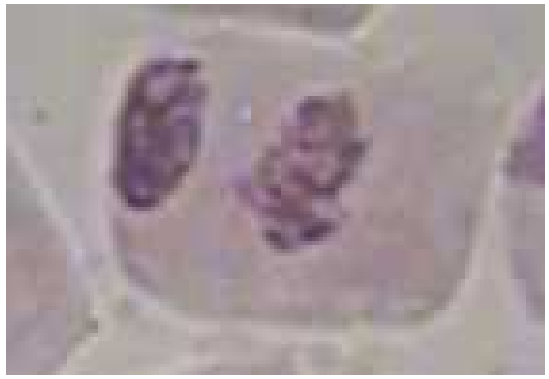
I. Unequal Condensation of Daughter Chromosomes



J. Binucleated cells (central)



K. Unequal Cytokinesis



L. Pole displacement and unequal condensation

CHAPTER -V

5. Discussion

Present work has been carried out to know the effect of fungicide Copper oxychloride on the mitotic activities and chromosome behaviour during the somatic cell division in root meristem of *Allium cepa* L. Copper oxychloride was capable of inducing various types of cytological abnormalities during somatic cell division in root meristem of Allium cepa L.

The calculated chi-square value (9.3) which is greater than tabulated value (7.8) shows the effectiveness of the chemical to the dividing cells. The value of the mitotic index decreased with increase in time of treatment and concentrations compared to the value of control. Decrease in Mitotic index value may be due to some effects of the chemical on enzyme production or enzyme function like induction, repression or feedback inhibition (Wuu and Grant, 1967). Decreased mitotic index value is due to the inhibition of cell division which is due to the effect on the synthesis of DNA, RNA, Protein and energy (Hess, 1983). It may be suggested that MI value decreased due to blockage of G1 phase suppressing DNA synthesis or prolonged G2 phase preventing the cell from entering mitosis (Badr and Ibrahim, 1987; Van't Hof, 1968).

The prophase index value increased with increase in concentration of solutions and duration of treatment except in some cases. The increased Prophase index shows prophase poisoning where cells entered into mitosis but they were arrested in

the prophase resulting in high frequency of prophase cells (Prasad and Das, 1977). Higher prophase index at higher concentrations of chemical could be due to effect of fungicide on spindle formation that prolonged the prophase stage. Similar results were found by Kaur and Grover (1985). In the present study metaphase index didn't show any regularity. Metaphase index was less than prophase index. This may be accumulation of dividing cells in prophase and did not enter another phase. Mean metaphase index values were less than that of control.

The mean Ana and Telophase indices were higher than the metaphase index and lower than the prophase index. It may be due to prolonged prophase or accumulation of abnormal anaphase and telophase cells. The increasing anaphase and telophase indices might be due to delay in the completion of mitotic cycle. Such result was also reported by Shehab (1980 b).

In control roots few abnormalities were found whereas higher frequencies of abnormalities were observed in cells treated with Copper oxychloride. In prophase cells, few abnormalities were observed in comparison to metaphase, anaphase ands telophase.

Abnormalities found in prophase were unequal condensation of chromatin threads, disturbed prophase, stickiness and non–synchronized condensation of chromosomes. Similar results were found by Mallah and Kabarity (1982) and Shrestha (2002) in *Allium cepa* L. In prophase unequal condensation of chromatin threads may be attributed to the agglutination of the chromosomes.

The abnormalities found in metaphase were equatorial plate shifting. Diagonal metaphase was more frequent. Stickiness of chromosomes, non-synchronized arrangement of chromosomes, c-metaphase was frequently observed. Similar results were observed by Pathak (1999); Sapkota (2000) and Shrestha (2002). Equatorial plate shifting during metaphase may be due to depolarization of spindle fibers (Medeiros and Takahashi, 1987). Non synchronized arrangement of chromosomes may be because of spindle abnormalities produced by chemical.

Stickiness has been attributed to the improper folding of chromosome fibers which make the chromatids connected by means of sub-chromatin bridges (Mc Gill et al. 1974). According to Ajay and Sarbhoy (1988), sticky chromosomes may be due to delay in chromosome movement to the poles. C-metaphase was also frequently observed. It may be due to inhibition of transcription of spindle protein messengers (Mercykutty and Stephen, 1980). Similar type of abnormality was also observed in the mitosis of *Allium cepa* after treatment with herbicide Garlon-4 (El-Khodary et al. 1989) and Methyl parathion and Tri-miltox (Ahmad and Yashmin, 1992). The cause behind abnormalities like C-metaphase, equatorial plate shifting seems to be due to disturbance in spindle mechanism.

Relaxed chromosomes and relaxed star-shaped C-anaphase were found. This may be due to the disturbance in metabolism process which resulted in long and uncondensed chromosomes. The chromosomal fragmentation at metaphase might be due to the effect of Copper oxychloride on DNA molecules responsible for the linear continuity of the chromosomes (Grant,

1978). The chemical may be stimulating some of the lyases involved in protein removal in DNA so that nucleases act upon it. Endonucleases cause internal cuts in DNA, thus leading to the formation of fragments (Pandita, 1986)

Fragments might arise due to stickiness of chromosomes and consequent failure of separation of chromosomes. Similar results were observed in other insecticides Rogor on *Vicia faba* and *Gossipium barbadense* (Amer and Farah, 1974).

Abnormalities found in Anaphase were shifting of poles, precocious arms, unequal movement of chromosomes; precocious chromosomes, laggards, star anaphase, bridge, fragments and breaks in chromosomes. Copper oxychloride has induced shifting of poles in anaphase. It may be due to the effect in spindle mechanism. Diagonal anaphase was frequent. Similar abnormality was also reported by Pathak (1999) on somatic cells of *Allium cepa* treated with Carmoisine; Shrestha (2002) on root meristem of *Allium cepa* L, with Isoproturon. According to them, depolymerisation of the spindle fibers caused the shifting of poles. The precocious arms and precocious chromosomes may be the result of unequal spindle movement in which some chromosome arms are pulled towards the extremity of the pole. Unequal separation of the chromosomes at anaphase stage may be due to disruption in the spindle mechanism during anaphase.

Bridge formation may be due to general stickiness of chromosomes at metaphase stage and failure of anaphasic separation (Tomkins and Grant, 1972) or because of breakage and reunion of chromosomes (Abraham and Koshy, 1979) Similar types of abnormality was also observed in the mitosis of *Vicia faba* after treatment with the organophosphorous insecticides (Amer and Farah, 1985). Lagging chromosomes may be due to delay in movement towards the pole. The lagging of

chromosome is due to interruption of protein metabolism caused by disturbance in RNA synthesis (Shrestha and Sakya, 2005)

The abnormalities found in telophase were shifting of poles including diagonal and longitudinal types, delay in cell plate formation, unequal cytokinesis and unequal condensation of daughter chromosomes.

The unequal condensation of daughter chromosomes and unequal cytokinesis were also reported by Rangaswamy et al (1981) in onion root tip cells treated with effluent from Lac and Paint. According to him, the unequal condensation may be due to the mitostatic property of the effluent and unequal cytokinesis probably due to the disturbance in cell metabolism. Similar types of abnormalities were reported by Pathak (1999) on *Allium cepa* root treated with Carmoisine. Shifting of pole in telophase may be because of continuity of such abnormality from metaphase and anaphase.

Delay in cell plate formation caused a delay in the completion of mitotic cycle. Delay or failure of cytokinesis resulted binucleated and tri-nucleated cells (Grant, 1978; Badr and Ibrahim, 1987). Telophase bridge was also observed. Bridges in anaphase and telophase may be due to stickiness of chromosomes (Abraham and Koshy, 1979).

The shifting of nucleus to the polar position may be due to the imbalance in the osmoregulation of the cells that caused cells to be Plasmolysed and nucleus was shifted to the pole (Rangaswamy et al., 1981)

6. CONCLUSION

The study shows that the effect of Copper oxychloride fungicide chemical exhibits mito-inhibitory and clastogenic effect in the dividing cells. The use of highly concentrated chemical is toxic to the dividing cells. Present study indicates that the commercially recommended suspension dose was found to be lethal to the dividing cells. In other to prevent mutational hazard in the plants one should use lowly concentrated fungicides.

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SUMMARY

The present study describes a cytological experiment to determine the effect of fungicide Copper oxychloride on the root meristematic cells of *Allium cepa*. The root meristems were treated with different concentrations of Copper oxychloride i.e., 0.025%, 0.05%, 0.075% and 0.1% for different duration of time i.e., 3, 6, 12 and 24 hours for each concentration.

In the study, mitotic index, phase indices and abnormality indices were calculated and abnormal phases were studied. The present study shows that Copper oxychloride has mito-depressive effect and causes various types of abnormalities. Mitotic index decreased with increasing concentration and period of treatment. Mitotic index was least in 0.1% concentration at 24 hours treatment. It shows that treatment with higher concentrations and longer period of treatment is toxic. Phase indices were also affected by different concentration of chemical in relation to different periods of treatment. Prophase index increased with increase in concentration and period of treatment. Metaphase and Ana-telophase indices showed decreasing tendency with increase in concentration and period of treatment.

Highest MI value was 36.81% at 0.025% concentration at 3 hours treatment and least i.e. 21.06% at 0.1% at 24 hours treatment. Highest prophase index was 94.19% at 0.075% at 12 hours treatment and least i.e. 90.06% at 0.05% at 3 hours treatment.

In control 2.29% of abnormal cells were found. Among treated cells, highest abnormality percentage was found (6.51%) in 0.025% concentration at 12 hours treatment and least (3.38%) in 0.05% at 6 hours treatment.

Among different phases highest frequency of abnormal cells were found in anaphase and telophase i.e. 61.40% at 0.05% at 12 hours treatment. Least abnormal cells were found in prophase i.e. 39.66% at 0.1% concentration prophase at 12 hours treatment.

Copper oxychloride induced various types of cellular abnormalities. The abnormalities were diluted cells, unequal condensation of chromatin threads in prophase, equatorial plate shifting, C-metaphase, stickiness, disturbed metaphase, diagonal anaphase, precocious chromosomes, precocious arms, laggards, bridges, fragmentation, sticky anaphase, pole shift in anaphase and telophase, unequal cytokinesis, delay in cell plate formation, binucleated cells, unequal movement of chromosomes, diagonal telophase and unequal condensation of daughter chromosomes. The abnormalities may be attributed to the disturbance in the spindle mechanism and metabolic disturbances caused by the chemical.

The results obtained from the study shows that fungicide Copper oxychloride is cytologically effective, mito-depressive, clastogenic and is lethal at higher concentrations. From the study, it can be suggested that higher concentration of Copper oxychloride is toxic to the plant so recommended dose of chemical should be lowered so as to prevent the mutation in plant.

Table No. 2: **Total no of counting cells of *Allium cepa* at each phase with different concentration of copper oxychloride at different time of treatment**

Duration of Treatment (Hours)	Concentration of copper oxychloride (%)	Total no. of cells counted	Interphase	Dividing cells			Prophase			Metaphase			Anaphase and Telophase		
				Total	Normal	Abnormal	Total	Normal	Abnormal	Total	Normal	Abnormal	Total	Normal	Abnormal
	Control	4780	2943	1837	1795	42	1636	1629	7	90	75	15	111	91	20
3 hrs	0.025%	4352	2750	1602	1527	75	1412	1400	12	84	49	35	105	67	38
	0.05%	4932	3575	1357	1293	64	1223	1198	25	48	20	28	87	56	31
	0.075%	4537	2987	1550	1488	62	1423	1408	15	43	16	27	84	54	30
	0.1%	4677	3156	1521	1457	64	1430	1409	21	61	35	26	30	4	26
6hrs	0.025%	4753	3037	1716	1658	58	1569	1566	3	59	34	25	89	55	34
	0.05%	5072	3535	1557	1462	75	1361	1356	5	96	56	40	100	60	40
	0.075%	4839	3160	1719	1596	83	1555	1531	24	92	54	38	72	41	31

	0.1%	4862	3451	1411	1344	67	1301	1280	21	50	27	23	71	36	35
12hrs	0.025%	4790	3515	1275	1192	83	1150	1130	20	49	19	30	75	43	32
	0.05%	4821	3470	1351	1294	57	1240	1228	12	27	17	10	83	48	35
	0.075%	4563	3340	1223	1171	52	1152	1138	14	29	21	8	42	29	13
	0.1%	4832	3711	1121	1063	58	1050	1027	23	29	23	6	43	25	18
24 hrs	0.025%	4650	3581	1069	1003	66	959	947	12	47	29	18	63	35	28
	0.05%	4765	3735	1030	991	39	969	960	9	21	11	10	41	21	20
	0.075%	4679	3600	1079	1031	48	990	983	7	32	16	16	56	35	21
	0.1%	4378	3456	922	884	38	860	849	11	22	9	13	40	29	11

Table No.3: Mitotic, Prophase, Metaphase, Anaphase and Telophase Indices of *Allium cepa* L. with different concentration of Copper oxychloride at different time of treatment

Duration of treatment (Hours)	Concentration of copper oxychloride (%)	Mitotic Index	Prophase Index	Metaphase Index	Anaphase and telophase Index
	Control	38.43	89.06	4.90	6.04
3	0.025	36.81	88.19	5.25	6.56
	0.05	27.51	90.06	3.53	6.41
	0.075	34.16	91.81	2.77	5.42
	0.1	32.52	94.02	4.01	1.97
6	0.025	36.10	91.38	3.44	5.18
	0.05	30.30	87.41	6.17	6.42
	0.075	34.70	90.46	5.35	4.19
	0.1	29.02	91.49	3.52	4.99
12	0.025	26.62	90.27	3.85	5.89
	0.05	28.02	91.85	2.00	6.15
	0.075	26.80	94.19	2.37	3.43
	0.1	23.20	93.58	2.58	3.83
24	0.025	22.99	89.71	4.40	5.89
	0.05	21.62	93.99	2.04	3.98

	0.075	23.06	91.84	2.97	5.19
	0.1	21.06	93.28	2.39	4.34

Table No. 4: Mean mitotic, prophase, metaphase, Anaphase and Telophase Indices treated with mean concentration of Copper oxychloride at different time of treatment

Duration of treatments (Hours)	Mean mitotic index	Mean prophase index	Mean metaphase Index	Mean Anaphase & telophase index
Control	38.43	89.06	4.9	6.04
3	32.75	91.02	3.89	5.04
6	32.53	91.19	4.62	5.2
12	26.16	92.46	2.7	4.83
24	22.18	92.20	2.95	4.85

Table No.5: Total Percentage of abnormal cells and percentage of abnormal cells at each phase among the abnormalities with different concentration of Copper oxychloride at different time of treatment

Duration of treatment (Hrs)	Concentration of Copper oxychloride (%)	Total Percentage of Abnormal cells	Total Percentage of abnormal cells in Prophase	Total Percentage of Abnormal cells in Metaphase	Total Percentage of Abnormal cells in Anaphase and Telophase
3	Control	2.29	16.67	35.71	47.62
	0.025	4.68	16.00	46.67	50.67
	0.05	4.72	39.06	43.75	48.44
	0.075	4.00	24.19	43.55	48.39
	0.1	4.21	32.81	40.63	40.63
6	0.025	3.38	5.17	43.10	58.62
	0.05	4.82	6.67	53.33	53.33
	0.075	4.83	28.92	45.78	37.35
	0.1	4.75	31.34	34.33	52.24
12	0.025	6.51	24.10	36.14	38.55
	0.05	4.22	21.05	17.54	61.40
	0.075	4.25	26.92	15.38	25.00
	0.1	5.17	39.66	10.34	31.03
	0.025	6.17	18.18	27.27	42.42

24	0.05	3.79	23.08	25.64	51.28
	0.075	4.45	14.58	33.33	43.75
	0.1	4.12	28.95	34.21	28.95

Table No. 6: Percentage of abnormal and normal cells at each phase with different concentration of Copper oxychloride at different time of treatment

Duration of treatment in hours	Concentration of Copper oxychloride in percentage	Prophase percentege		Metaphase percentage		Anaphase and Telophase percentage	
3		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
	Control	0.38	88.67	4.08	0.81	4.95	1.08
	0.025	0.74	87.39	3.05	2.18	4.18	2.37
	0.05	1.84	88.28	1.47	2.06	4.12	2.28
	0.075	0.96	90.83	1.03	1.74	3.44	1.93
	0.1	1.38	92.63	2.30	1.70	0.26	1.70
6	0.025	0.17	91.25	1.98	1.45	3.20	1.98
	0.05	0.32	87.09	3.59	2.56	3.85	2.56
	0.075	1.4	89.06	3.14	2.21	2.38	1.80
	0.1	1.48	90.71	1.91	1.63	2.55	2.48

12	0.025	1.56	88.62	1.49	2.35	3.37	2.50
	0.05	0.88	90.89	1.25	0.74	3.55	2.59
	0.075	1.144	93.5	1.71	0.65	2.37	1.06
	0.1	2.051	91.61	2.05	0.53	2.23	1.60
24	0.025	1.12	88.58	2.71	1.68	3.27	2.61
	0.05	0.87	93.20	1.06	0.97	2.03	1.94
	0.075	0.64	91.10	1.48	1.48	3.24	1.94
	0.1	1.19	92.08	0.97	1.40	3.14	1.19

Table No. 7: Mean mitotic index values of *Allium cepa* L. root tip cell treated with different concentration of copper oxychloride at different time of treatment

Concentration of Copper Oxychloride in %	**Mitotic index (k), treated in different time periods**			
	3 hrs.	**6 hrs.**	**12 hrs.**	**24 hrs.**
0.025	36.81	36.10	26.62	22.99
0.05	27.51	30.30	28.02	21.62
0.075	34.16	34.70	26.80	23.06
0.1	32.52	29.02	23.80	21.06

Table No. 8: Ranks of four matched groups (concentration) of Mitotic Index values of *Allium cepa* L. under four conditions (times)

Concentration of Copper Oxychloride in %	Mitotic index (k), treated in different time periods			
	3 hrs.	6 hrs.	12 hrs.	24 hrs.
0.025	4	3	2	1
0.05	2	4	3	1
0.075	3	4	2	1
0.1	4	3	2	1
R_j	13	14	9	4

Levan formulae 1949 (C.F. Kihlman 1971, Medeiros and Takahashi, 1987)

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

$$\text{Prophase Index (Pro I)} = \frac{TC\,\text{Pr}o \mid 100}{TDC}$$

$$\text{Metaphase index (Meta I)} = \frac{TCMeta \mid 100}{TDC}$$

$$\text{Anaphase and Telophase Index (Ana-Telo I)} = \frac{TCAna\,\text{Z}Telo \mid 100}{TDC}$$

$$\text{Total Percentage of Abnormal cells } (T_{Abn}) = \frac{TCAbn \mid 100}{TDC}$$

Total Percentage of Abnormal cells at Prophase

$$T_{Pro} = \frac{TCAbn\,\text{Pr}o \mid 100}{TDC}$$

Total Percentage of Abnormal cells at Metaphase

$$T_{Meta} = \frac{TCAbnMeta \mid 100}{TDC}$$

Total Percentage of Abnormal cells at Anaphase and Telophase

$$T_{Ana\text{-}Telo} = \frac{TCAbnAna\,\text{Z}Telo \mid 100}{TDC}$$

Percentage of Abnormalities at Prophase among the abnormal cells

$$A_{Pro} = \frac{TCAbn\,\text{Pr}o \mid 100}{TCAbn}$$

Percentage of Abnormalities at Metaphase among the abnormal cells

$$A_{Meta} = \frac{TCAbnMeta \mid 100}{TCAbn}$$

Percentage of Abnormalities at Anaphase and Telophase among the abnormal cells

$$A_{Ana\text{-}Telo} = \frac{TCAbnAna\ ZTelo \mid 100}{TCAbn}$$

Calculation:

$$\text{¡}\ {}_{r}^{2}\text{X}\frac{12}{NK\text{ƒ}k\ \Gamma 1\text{Å}_{j\text{X}1}}\ {}^{K}\text{ƒ}R_{j}\ \text{Å}^{2}\ \text{Z}3N\text{ƒ}k\ \Gamma 1\text{Å}$$

N = Number of rows (concentration) = 4

K = Number of columns (condition, time of treatment) = 4

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

$$\text{X}\frac{12}{4 \mid 4\text{ƒ}4\ \Gamma 1\text{Å}}\{(13)^{2}\ \Gamma (14)^{2}\ \Gamma (9)^{2}\ \Gamma (4)^{2}\}\ \text{Z}3 \mid 4(4\ \Gamma 1)$$

$$\text{X}\frac{12}{80}(462)\ \text{Z}60$$

X9.3