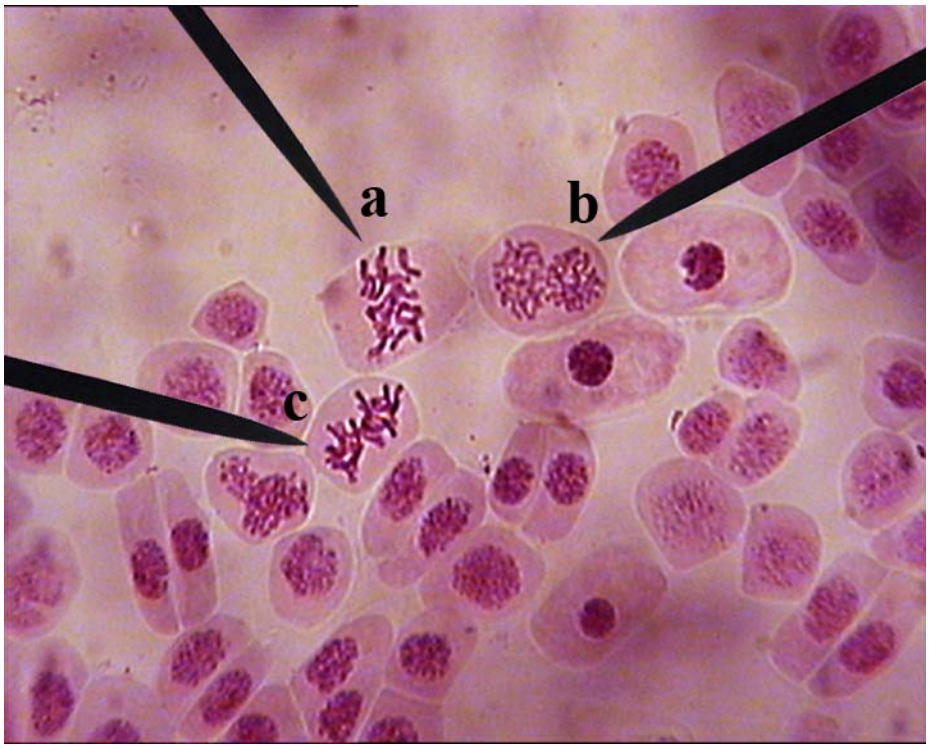
EFFECT OF FUNGICIDE METALAXYL ON DIVIDING ROOT CELLS OF *Allium cepa* L.



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ABSTRACTS

The study throws light on the cytological effects of a systemic fungicide Metalaxyl on the somatic cell division. The root meristems of healthy onion bulbs (*Allium cepa* L.) were used as bioassay under lab condition. The water culture roots were made as control measure to study the chromosomal behavior. Metalaxyl solution of different concentrations was used to observe its cyto-toxic effects in dividing cells.

In agriculture, the field recommended dose of Metalaxyl is 0.5 gm/1000 ml. Such concentration is recognized as 100% solution. The test solutions of different concentrations were prepared by dilution, *i.e.* 25%, 50% and 75%. The cytological effect was observed in different concentration of metalaxyl solution at different treatment periods *i.e.* 3, 6, 12 and 24 hours separately.

Metalaxyl showed mito-depressive effect on higher concentration and also affected on all phase with different concentration with respect to different time periods. It is observed that higher the period of treated time lower will be the value of mean mitotic index. The mitotic indices values were observed between 22 to 25% in treated meristem whereas control value was 31%.

The value of prophase index was increased with increase in time of treatment and concentration. The prophase index was highest due to prophase poisoning and lowest was metaphase index among all of the other phase indices. The fungicide accomplished to induce the various type of chromosomal aberrations. The untreated root cells showed relatively less abnormalities than that of treated. The commonly found abnormalities were plasmolized cells, bi-nucleated cells, stickiness of chromosomes at prophase and metaphase, diagonal metaphase, C-metaphase, equatorial plate shifting, non-synchronized arrangement of chromosomes, precocious chromosomes and arms, lagging chromosomes, inhibition of cell plate formation and unequal cytokinensis. These were major physiological aberrations encountered in root treated cell of *Allium cepa* L. The abnormalities included chromosome breaks, chromatin bridges and micronuclei were the major clastogenic aberrations found on Metalaxyl treatment. Although, abnormalities were also encountered at control, but frequency was very low. Frequencies of physiological effects were found to be higher than that of clastogenic abnormalities. The statistical analysis showed that the frequency of aberrations induced with the entire dose and treatment period differed significantly from that of control. So, Metalaxyl has potential to physiological as well as clastogenic effects on plant cells.

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ABBREVIATIONS AND ACRONYMS

$A_{Ana\text{-}Telo}$	=	Percentage of abnormalities at anaphase and telophase among the abnormal cells
A_{Meta}	=	Percentage of abnormalities at metaphase among the abnormal cells
Ana-Telo I	=	Anaphase and telophase Index
A_{Pro}	=	Percentage of abnormalities at prophase among the abnormal cells.
DNA	=	Deoxyribonucleicacid
Fig	=	Figure
Meta I	=	Metaphase index
MI	=	Mitotic index
Pro I	=	Prophase index
RNA	=	Ribonucleicacid
T_{Abn}	=	Total percentage of abnomal cells
$T_{Ana\text{-}Telo}$	=	Total percentage of abnormal cells at anaphase and telophase
T_{Meta}	=	Total percentage of abnormal cells at metaphase
T_{Pro}	=	Total percentage of abnormal cells at prophase
$TC_{Abn\ Ana\text{-}Telo}$	=	Total number of abnormal cells counted at anaphase and telophase
$TC_{Abn\text{-}Meta}$	=	Total number of abnormal cells counted at metaphase
$TC_{Abn\ -Pro}$	=	Total number of abnormal cells counted at prophase
TC_{Abn}	=	Total number of abnormal cells counted
$TC_{Ana\text{-}Telo}$	=	Total number of cells counted at anaphase and telophase
TC_{Meta}	=	Total number of cells counted at metaphase
TC_{Pro}	=	Total number of cells counted at prophase
TC	=	Total number of cells counted
TDC	=	Total dividing cells

1. INTRODUCTION

1.1 Background

No doubt, Nepal is an agricultural country. Amongst the agricultural countries, for the betterment of crops, pesticides are merely a tool in modern practice. Although, pesticides are the easiest way of counter measures to control pests, unsystematic use can yield adverse effects more than pests do. The haphazard use of pesticides may spoil plants, animals, soil, environment and ecosystem as well. Hence, testing the genotoxic effects of such chemicals is as important as considering them as a counter part.

The pesticide act 1991 and pesticide rule 1994 have already been made in our country, which measures manufacture, import, sell and use of pesticides. Environmental protection act was established in 1996 and rule framed thereby under recently been gazetted in 1997 (Bhatt,2006).

For the betterment of the crops, pesticides are of immense importance if used in a proper and scientific way. The use of pesticides should always be encouraged and practiced well. These chemicals when used properly can result in a good way and serve the purpose for what it is intended to. The health of the crops, increase in its yield, good quality of seeds for future preservation, increase in the quality of seed, no effect to the ecosystem and environment should always be intact. To the contrary, it's always the human error, negligence and maltreatment that lead the beauty of science and technology into a curse. However, no pesticides are safe but obviously there are safe methods.

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.

The current plan of work is proposed to study the effect of fungicide, Metalaxyl, on root tips of *Allium cepa* L. on the basis of cytological observation.

1.2 Metalaxyl

Metalaxyl is a systemic fungicide used in mixtures as a foliar spray for tropical and subtropical crops, as a soil for control of soil-borne pathogens and as a seed treatment to control downy mildrews. Matalaxyl may be invariably used on food crops, including tobacco, ornamentals, conifer, and turf applications (Anonymous, 1996).

Metalaxyl is a systemic, benzenoid fungicide. Trade names for products Metalaxyl include Apron, Delta-Coat AD, Ridomil and Subdue (Anonymous, 1996).

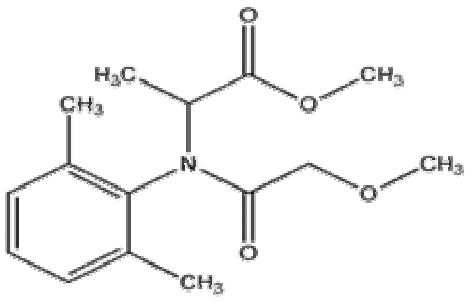
1.2.1 Molecular formula

$C_{15}H_{21}O_4N$

1.2.2 Chemical name

Methyl-N-(2,6-dimethylphenyl)-N-(2-xylyl)- DL-alaninate

1.2.3 Molecular model



1.2.4. Molecular weight

279.34

1.2.5. Physical and chemical properties

Metalaxyl is a colorless, odorless crystal. Its solubility in water is 7100 mg/L @ 20 C. Its melting point is 71.8 – 72.3 C. It is also soluble in methanol, benzene and hexane. Average half life of Metalaxyl is about 70 days.

1.3 Objectives

The main objectives of the present study are as follows:

- To study the mitotic effects of fungicide Metalaxyl in actively dividing root cells.
- To observe the nature and frequency of abnormalities in dividing cells.

1.4 Justification of the study

Metalaxyl is one of the most used fungicides to control various fungal diseases. It is most commonly used to control downy mildew in tropical and subtropical crops. It has various toxicological effects. It is slightly toxic when ingested and dermally applied and high dose intoxication manifests increased alkaline phosphatase. Studies including a dominant lethal assay in male mice indicate that Metalaxyl has no mutagenic potential. Available studies of the carcinogenicity of Metalaxyl are inconclusive. The liver is the primary target organ for Metalaxyl in animal systems. Metalaxyl is reported to be practically nontoxic to birds, fresh water fish and bees. The cytological effects of fungicide, Metalaxyl, is not yet studied on plant(Anonymous, 1996), so the present study is carried out in order to comprehend its effect on root meristem of onion cells.

2. LITERATURE REVIEW

2.1 Cytological effects of fungicides

Al-Najjar *et al.,* (1980) revealed the effects of two fungicides Diathane S-60 and Vitavax-200. These fungicides induced chromosomal anomaly like chromosomal bridge accompanied by highly significant reduction in mitotic index.

Shrestha (1982) observed the effects of Bavistin and Diathane M-45 in *Allium cepa* L. that both the fungicides induced physiological as well as cytological abnormalities. These fungicides inhibit mitotic activities and induced chromosomal aberrations such as stickiness, breaks and bridges. These fungicides exerted a weak C-mitosis.

Somashekhar *et al.,* (1984) studied cytological effects of fungicide Topsin in *Allium cepa* L. The fungicide showed mitodepression, C-metaphase, sticky metaphase, sticky anaphase, chromosomal clumping and disturbed anaphase giving rise to tripoar and tetrapolar cells. Clastogenic effects as fragmentation of chromosomes, gaps, breaks and bridges observed in anaphase and Telophase, disorientation of spindle fibers, micronuclei and inhibition of cytokinesis were also observed. Other abnormalities such as precocious chromosome, diagonal poles and unequal distribution of chromatin material were also noted.

Somashekhar *et al.,* (1984) studied mitodepressive effects, spindle disfunction, clastogenic effects, cytokinesis inhibition and mitostatic effects using the fungicide Vitavax in *Allium cepa* L.

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

Joshi *et al.,* (1982) observed the effects of two fungicides Bavistin and Diathane M-45 in *Allium cepa* L., *Eleusine corocana* Gaertn.and *Lycopersicum esculantum* L. The study revealed that the fungicides inhibited mitosis to a considerable extent and also induced chromosomal aberrations during mitotic division. The common chromosomal aberrations during mitosis were fragmentations of the chromosomes at late metaphase and anaphase, laggards and bridges at anaphase and micronuclei at telophase.

Badr (1988) used two fungicides Diathene and Denmart in *Allium cepa* L. These fungicides induced chromosomal stickiness, breaks and bridges, chromosomal lagging, binucleated and multinucleated cells and micronuclei formation.

Prakash *et al.,* (1988) studied the effects of fungicides.Bavistin and Diathane in Chilli (*Capsicum annum* L.). The fungicides inhibited seed germination and to survive the seedling the chromosomal abnormalities like univalent and multivalent stickiness and non-orientation of chromosomes at metaphase I, chromosomal bridge and laggards were also observed at anaphase.

Chand *et al.,* (1991) studied the effects of Carbendazin in growing seeds of sunflower and pearl millet. Carbendazine showed a variety of chromosomal aberrations in somatic (root tips) and reproductive (pollen mother) cells. The somatic cells of pearl millet showed aberrations at late prophase with stickiness and laggard chromosomes at anaphase. In somatic cells of sunflower chromosomal bridges and laggards were frequent during anaphase.

Ahamad and Yasmin (1992) studied the cytotoxic effects of fungicide tri-miltox on root meristem of *Allium cepa* L.Various abnormalities were observed like chromosomal fragmentation, laggards, micronuclei, and single and multiple bridge formation.

Pandey *et al.,* (1994) studied the cytological effects of fungicide Dithane M-45 and two insecticides Aldrex-30 and Metacid-50. It showed lethality on cell division and the abnormalities recorded were clumping, bridges, fragmentation, cytomixis, disturbed polarity, micronuclei, bi-nucleated nature and nuclear fusion.

Chauhan *et al.,* (1999) studied the effects of some fungicides Mancozeb, Bavistin, Sulphex and found cytomorphological changes in *Allium cepa* L. The fungicide inhibited mitotic index and the abnormalities found were bi-nucleated cells, scattered metaphase, chromosomal bridges, laggard and multipolar anaphase.
Singh *et al.,* (2001) studied the effects of fungicides on the weedicides on the severity of rice collar root in rice fields.The effectiveness of five fungicides on in-vitro growth and sporulation of color.

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

Shrestha (2004) studied the cytological effects of fungicide Carbendazin on meristematic cells of *Allium cepa* L. The abnormalities observed were plasmolysed cells, clumping of chromosomes, unequal condensation, disturbed prophase, equatorial pole shifting in metaphase and anaphase, precocious arms at anaphase, delay in cell plate formation and bi-nucleated cells.

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

Gurung (2005) studied the effects of fungicide Edifenphous on meristematic cells of *Allium cepa* L. The chemical was capable of inducing precoucious arms at metaphase and anaphase, stickiness, C-metaphase, fragmentation at metaphase, unequal cytokinesis and bi-nucleated and tri-nucleated cells. The mitodepressive, cytotoxic, and inhibition of spindle fiber mechanism were the accumulative effects of the fungicide.

2.2 Cytological effects of different agrochemicals and others

Cytological effects of different insecticides on dividing cells were studied by Kaur and Grover (1985), Pandita (1986), Rao *et al.,* (1987), Grover and Malhi (1988), Jain and Sarbhoy (1988), Sinha *et al.,* (1989), Upendra and Sinha (1991), Kara *et al.,* (1995), George and Ghareeb (2001), Thapa (2002), Malla and Sakya (2002), Kumar and Kumar (2004) had been studied.

Cytological effect of different herbicides studied by Bakale and Hadke (1981), Adhikary (1982), Badr (1983), Badr and Ibrahim (1987), Rao *et al.,* (1988), El-Khodary *et al.,* (1989), Chauhan and Sundaraman (1990), Topakatas and Rencuzogullari (1991), Butt and Vahidya (1994), El-Ghamery et al., (2000), Shrestha (2002), and Saxena *et al.*, (2004).

Effects of industrial effluents studied by Bhalla *et al.,* (1976) Rangaswamy *et al.,* (1981), Pun (1994), Shakya *et al.,* (1999), Abhram and Abhram (1991), Kaushik *et al.,* (1997), and Sudhakar *et al.,* (2001).

Effects of different plant extracts were studied by Shehab (1980b), Adam and Farah (1989), George and Geethamma (1990), Sagoo *et al.,* (1991), Banerjee (1992), and Williams *et al.,* (1996)

Effect of others chemicals were studied by Shehab (1980), Mercykutty and Stephen (1980), Sagoo *et al.,* (1991), Hengtai *et al.,* (1997), and Pathak (1999).

Cytological effects of different metals were studied by Giri *et al.,* (1984) and Jayaprakash *et al.,* (1994).

3. MATERIALS AND METHODS

To study the cytological effects of Metalaxyl on dividing root cells of onion (*Allium cepa* L., 2n = 16), root meristem of the same was used as bioassay system. The following major procedures are applied to carry out the experiment

3.1 Materials

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

3.2 Onion bulbs rooting method

Some healthy onion bulbs were collected. The bulbs were washed by water and old roots were removed. The bulbs were placed on the coupling jar filled with water and made sure that its basal part touches the water. The water of the jar was replaced at 24-hour interval so as to check growth of microorganisms.

3.3 Preparation of different concentration of Metalaxyl

As the recommended dose for Metalaxyl is 0.5 gm/1000 ml per hector, 0.5-gm Metalaxyl was added in 1000 ml of water to make the suspension (100%). Then, test solutions of different concentrations were prepared by dilution i.e. 25%, 50% and 75%.

3.4 Methods

3.4.1 Treatment of the rooting bulbs

When the lateral roots of the onion (*Allium cepa* L.) were about 2 cm long, they were exposed to freshly prepared test solutions of different concentrations for 3, 6, 12 and 24 hours respectively at room temperature. Those high concentrations of test solutions were not taken under consideration where they were proved to be highly fatal as the time period exposed was lengthened. The treatment schedules are as in table no. 1.

Table No.1: Schedules of treatment

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

3.4.2 Preparation of reagents for cytological study

The following agents were used for fixing and staining tissues.

Fixating 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.

Stainning agent (Iron-Aceto-Carmine 2%)

Belling's Iron-Aceto-Carmine 2% was used for the staining process. The stain was prepared by combination of the following agents.

Carmine	2 gm
Glacial Acetic acid	45 ml
Distilled water	55 ml
Ferric Chloride/Ferric Hydroxide	Few drops

3.4.3 Cytological fixation

The treated and controlled root tips of *Allium cepa* L. were excised, 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 tips, about 1 cm, were warmed with 2% Aceto-Carmine and about 2 mm of root tips were squashed on a clean slide. More than five root tips were used for each treated and non treated onion bulbs.

3.4.5 Preparation of permanent slide and mounting media

Celarier's (1956) method was used for the preparation of permanent slides. The compositions of different dehydration grades for permanent slide preparation were as follows:

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

After dehydration, the stained meristematic tissues were mounted in Euparol.

3.4.6 Microphotography

Microphotographs of different stages of cell division and chromosomal abnormalities were taken from either temporary slides or from the permanent slides prepared.

3.4.7 Cytological observation and calculation

The prepared slides were observed under compound microscope. Normal and abnormal cells were studied and counted. The observations were recorded approximately around 4,000 to 5,000 cells from at least five different root tips. The mitotic and phase indices along with abnormalities were scored and analyzed according to Levan formulae 1949 (cf. Kihlman 1971, Medeiros and Takahashi, 1987) as below.

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

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

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

Anaphase and Telophase Index (Ana-Telo I) = $\frac{TCAna-Telo \times 100}{TDC}$

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

Total Percentage of Abnormal cells at Prophase

$T_{Pro} = \frac{TCAbn\,\mathrm{Pr}o \times 100}{TDC}$

Total Percentage of Abnormal cells at Metaphase

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

Total Percentage of Abnormal cells at Anaphase and Telophase

$T_{Ana\text{-}Telo} = \frac{TCAbnAna-Telo \times 100}{TDC}$

Percentage of Abnormalities at Prophase among the abnormal cells

$A_{Pro} = \frac{TCAbn\,\mathrm{Pr}o \times 100}{TCAbn}$

Percentage of Abnormalities at Metaphase among the abnormal cells

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

Percentage of Abnormalities at Anaphase and Telophase among the abnormal cells

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

3.4.8 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* L. root tip cells or not. The data from table No.7 and No.8 were submitted for calculation as below.

$$\chi_r^2 = \frac{12}{NK(k+1)} \sum_{j=1}^{K} \left(R_j\right)^2 - 3N(k+1)$$

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

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

$$= \frac{12}{4 \times 4(4+1)}(16^2 + 9^2 + 10^2 + 5^2) - 3 \times 4(4+1)$$

$$= \frac{12}{80}(462) - 60$$

$$= 69.3 - 60$$

$$= 9.3$$

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

4. RESULTS

The cytological effects of fungicide Metalaxyl on root meristem of *Allium cepa* L. was analyzed from the experiment in the current study. A comparative study between the results of controlled and treated root meristem with respect to different time period of treatment and their variation in mitotic, phase indices and chromosomal abnormalities study was carried out. It had been observed that the chemical at different concentrations was capable of inducing different types of chromosomal abnormalities as shown in photo plate 1 to 5.

From statistical analysis, the calculated value for chi square (χ_r^2) was 9.3 whereas tabulated value was 7.8 at percentage (p) <0.05. The results are tabulated in Table 2 to Table 8 (see appendix).

The effects of the fungicide Metalaxyl on mitotic index and phase indices along with abnormalities at different mitotic stages are elaborated under separate headings as below.

4.1 Effect in mitotic index

The mitotic index of untreated root cells is higher than that of treated. Mitotic index decreased with increase in treatment of time and concentration of Metalaxyl as shown in table 3 and fig. 1. It was highest (26.86%) in 25% concentration at 3 hours and least (20.11%) in 75% concentration at 6 hours treatment and 31.05% at control.

4.2 Effect in phase indices

4.2.1 Effect in prophase index

Prophase index increased with increase in concentration and time of treatment as shown in table 3 and fig.2.It was highest (91.88%) in 100% concentration at 24 hours treatment period and least (82.06%) in 50% concentration at 6 hours treatment. The values didn't show much variation with varying period of treatment .The control value was 85.77%.

4.2.2 Effect in metaphase index

Metaphase index value showed no regularity with respect to concentration and treatment period as shown in table 3 and fig.3. It was highest (7.02%) in 50% concentration at 6 hours treatment and least (2.54%) in 50% concentration at 12 hours treatment. The value of control was 5.01%.

4.2.3 Effect in anaphase and telophase index

Anaphase and telophase index value didn't show any regularity with concentration and period of treatment as shown in table 3 and fig 4. It was highest (10.91%) at 50% concentration at 6 hours and least (4.82%) at 100% at 24 hours treatment and that of control was 9.02%.

(Mitotic index and phase indices of control as well as four different concentrations of Metalaxyl with respect to treatment time are shown in fig 6 to fig 9. Each figure reveals the comparison of control, mitotic and phase indices in same percentage with different period of treatment time.)

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. 5. The mean mitotic index decreased with increase in treatment period. Mean mitotic index was highest (25.01%) at 3 hours and least (21.04%) at 24 hours treatment and that of control was (31.05%). Mean prophase index increased with increase in treatment period. Mean prophase index value is greater than control for all treatment time period. It was highest (87.87%) at 24 hrs, least (86.20%) at 3 hours and 85.77% at control. Mean metaphase index was highest (5.63%) at 3 hours and least (4.67%) at 12 hours. Mean metaphase indices at different concentrations were less than that of control (5.01%) except 3 hours. Mean anaphase and telophase indices were less than that of control (9.20%) which is higher than that of maximum value (8.55%) at 6 hours of treated cells and least (7.23%) at 24 hours. Mean

metaphase index and mean anaphase and telophase index values didn't showed any regularity with treatment and 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 Metalaxyl at different time of treatment are given in table 5.

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 (2.58%). Abnormalities were found more in treated roots. Among treated cells, highest abnormality percentage was found (11.94%) in 25% concentration at 24 hours treatment and least i.e., 5.45% in 50% at 24 hours treatment.

4.4.2 Percentage of abnormalities among abnormal cells in prophase

Percentage of prophase abnormalities at different concentrations of Metalaxyl is shown in table 5 and fig. 11. The values did not show any regularity. The percentage of abnormality at control was 20.58%. Among all the treated concentrations of chemical and time period, the highest abnormal value was 51.28% at 100% concentration at 12 hours treatment period and least value was 11.25% at 50% concentration at 6 hours treatment which was lower than control value.

4.4.3 Percentage of abnormalities among the abnormal cells in metaphase

Percentage of metaphase abnormalities at different concentrations of Metalaxyl at different period of treatment are shown in table 5 and fig. 12. In normal untreated roots 38.23% of abnormal metaphase cells were found. Among treated roots, abnormality index was highest (33.33%) in 25% at 12 hours treatment and least (8.51%) in 50% at 3 hours treatment.

4.4.4 Percentage of abnormalities among abnormal cells in anaphase and telophase

Percentages of Anaphase and telophase abnormalities are shown in Table 5 and Fig. 13. Abnormal anaphase and telophase index found in untreated roots was 41.17% which was greater than abnormal prophase and metaphase indices in control. Among treated roots, highest abnormal anaphase and telophase index was 65.95% found at 50% at 3 hours treatment and least (31.50%) at 100% at 3 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 metalaxyl solution is shown in table 5. Abnormality indices of anaphase and telophase were higher than both of metaphase and prophase abnormality index except some 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 in untreated root was found 2.58% of total dividing cells.

4.5.1 Abnormal mitotic phases in 25% Metalaxyl treatment

Table 5 shows abnormal mitotic phases in the dividing cells of *Allium cepa* L. treated with 25% of metalaxyl. Prophase abnormality increases from 3 to 6 hours and then decrease from 6 to 24 hours. It was highest (33.75%) at 6 hours treatment and lowest (14.16%) at 24 hours treatment. Metaphase abnormality did not show any regularity with treatment hours. It was highest (33.33%) at 12 hours treatment and lowest (14.16%) at 24 hours treatment. Anaphase and telophase abnormality decreases from 3 hours to 12 hours and then slightly increases at 24 hours. It was highest (64.19%) at 3 hours treatment and least (43.13%) at 12 hours treatment.

4.5.2 Abnormal mitotic phases in 50% Metalaxyl treatment

Table 5 shows abnormal mitotic phases on 50% Metalaxyl treatment. Prophase abnormality was highest (43.75%) at 12 hours treatment and least (11.25%) at 6 hours treatment. Prophase abnormality did not show any regularity with time period. Metaphase abnormality increases from 3 hours to 6 hours and decreases from 6 to 24 hours. It was highest (31.25%) at 6 hours treatment and least (8.51%) at 3 hours treatment. Anaphase and telophase abnormality was higher than prophase and metaphase in this treatment. The abnormalities of anaphase and telophase decrease from 3 to 12 hours and slightly increase at 24 hours. It was highest (65.95%) at 3 hours treatment and least (35.93%) at 12 hours treatment.

4.5.3 Abnormal mitotic phases in 75% Metalaxyl treatment

Table 5 shows abnormal mitotic phases on 75% Metalaxyl. Abnormality of prophase showed less variation in this concentration with respect to different time periods. Prophase abnormality was highest (30.55%) at 12 hours treatment and least (26.43%) at 24 hours treatment. Abnormality of metaphase did not show any regularity. Metaphase abnormality was highest (32.18%) at 24 hours treatment and least (12.03%) at 12 hours treatment. Anaphase and telophase abnormality didn't show any regularity. Anaphase and telophase abnormality was highest (59.09%) at 3 hours treatment and least (41.37%) at 24 hours treatment

4.5.4 Abnormal mitotic phases in 100% Metalaxyl treatment

Table 5 shows abnormal mitotic phases on 100% Metalaxyl treatment. Prophase abnormality was not so vary with time of treatment. It was highest (51.28%) at 12 hours treatment and least (46.80%) at 6 hours treatment. Metaphase abnormality decreases from 3 to 12 hours and increases at 24 hours. It was highest (20.54%) at 3 hours treatment and least (11.53%) at 12 hours treatment. Anaphase and Telophase abnormality increases from 3 to 6 hours and then slightly decreases up to 24 hours. It was highest (39.36%) at 6 hours treatment and least (31.50%) at 3 hours treatment.

4.6 Chromosomal behavior

Fungicide Metalaxyl induced 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 1-5.

4.6.1 Nature of abnormal cells in non-dividing cells

The abnormalities found in non-dividing cells were plasmolysed cells, 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, disturbed metaphases, non synchronized condensation of chromosomes were found. Diagonal metaphase, Fragmented Chromosomes were also found.

4.6.4 Nature of abnormal cells in anaphase and telophase

Diagonal anaphase, precocious arms and Laggards, unequal movement of chromosomes, precocious chromosome, bridges, fragmentation, and sticky anaphase were seen. Pole shift was also seen. In Telophase, unequal cytokinesis, delay in cell plate formation, bi-nucleated cells, pole shift, unequal condensation of daughter chromosomes, 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.

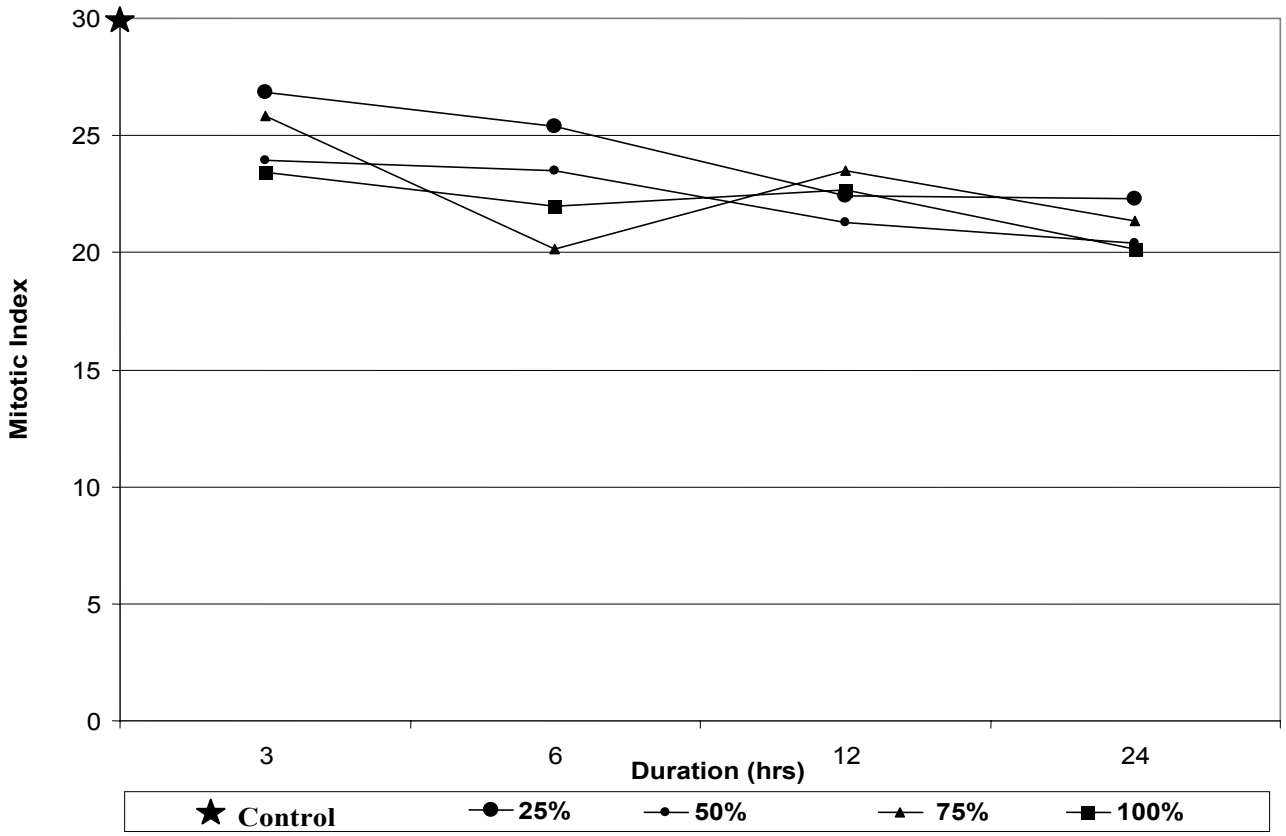


Fig. 1 Graph of Mitotic Index of *Allium cepa* L. root tip cells Vs treatment time with given concentrations of Metalaxyl.

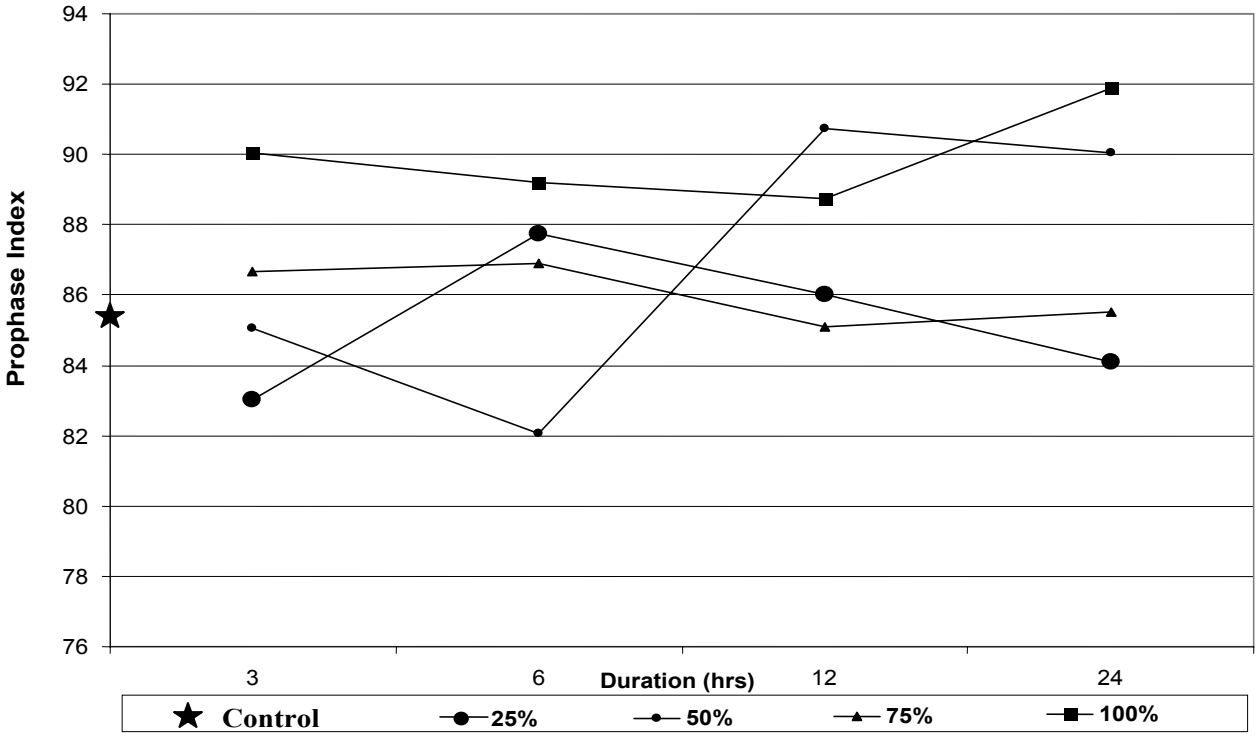


Fig. 2 Graph of Prophase Index of *Allium cepa* L. root tip cells Vs treatment time with given concentrations of Metalaxyl.

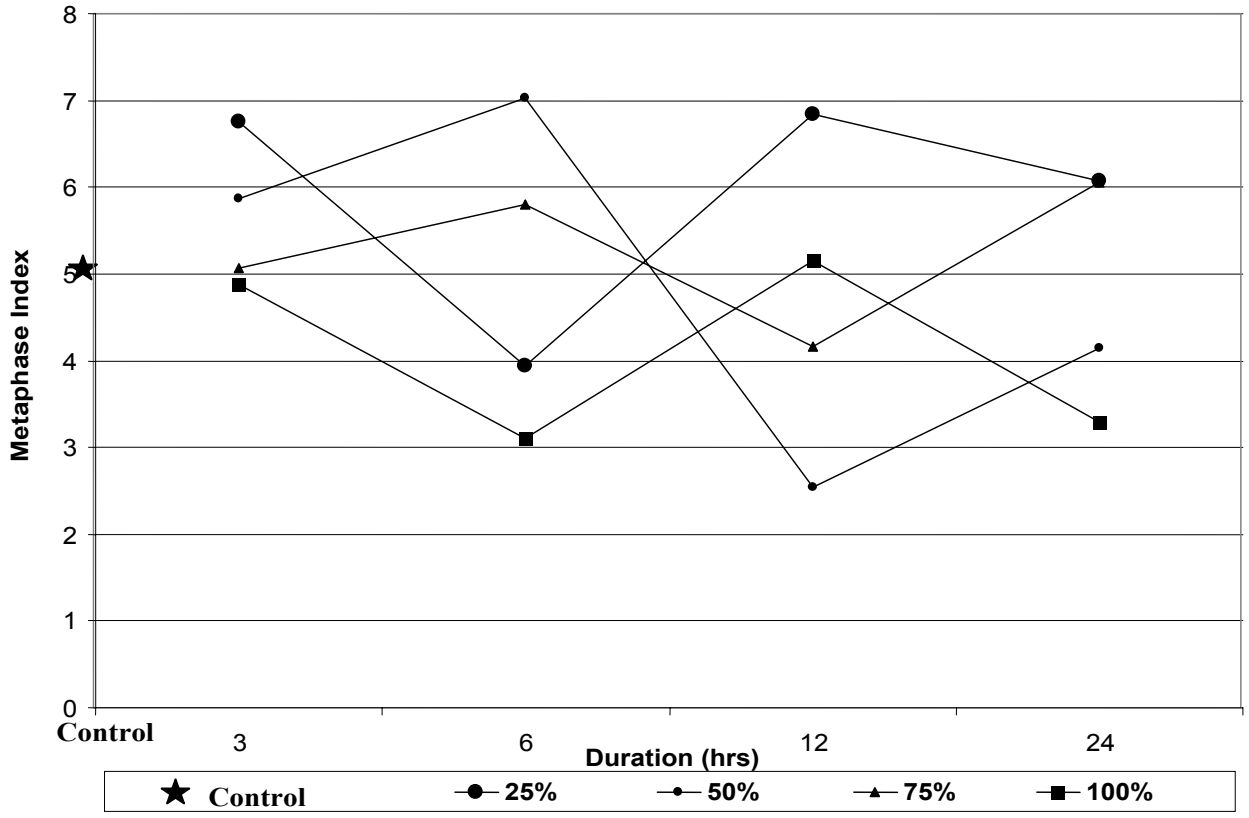


Fig. 3 Graph of Metaphase Index of *Allium cepa* L. root tip cells Vs treatment time with given concentrations of Metalaxyl.

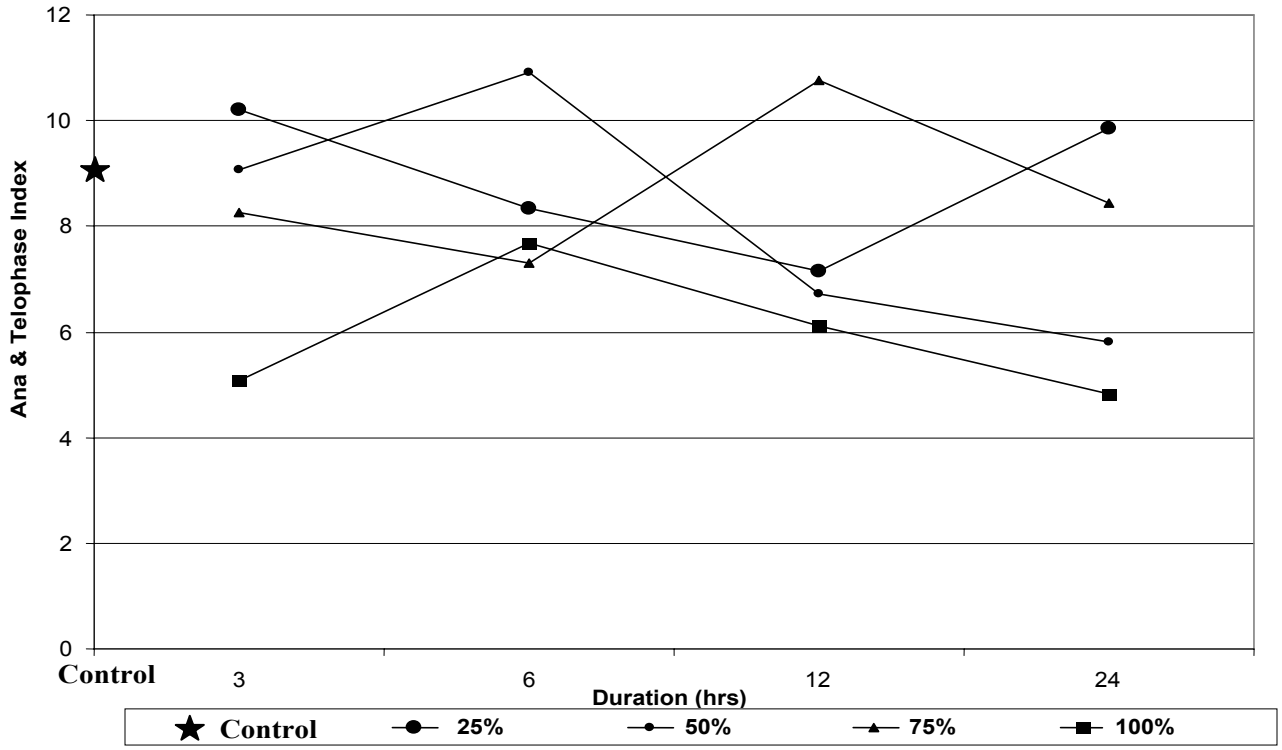


Fig. 4 Graph of Ana and Telophase Index of *Allium cepa* L. root tip cells Vs treatment time with given concentrations of Metalaxyl.

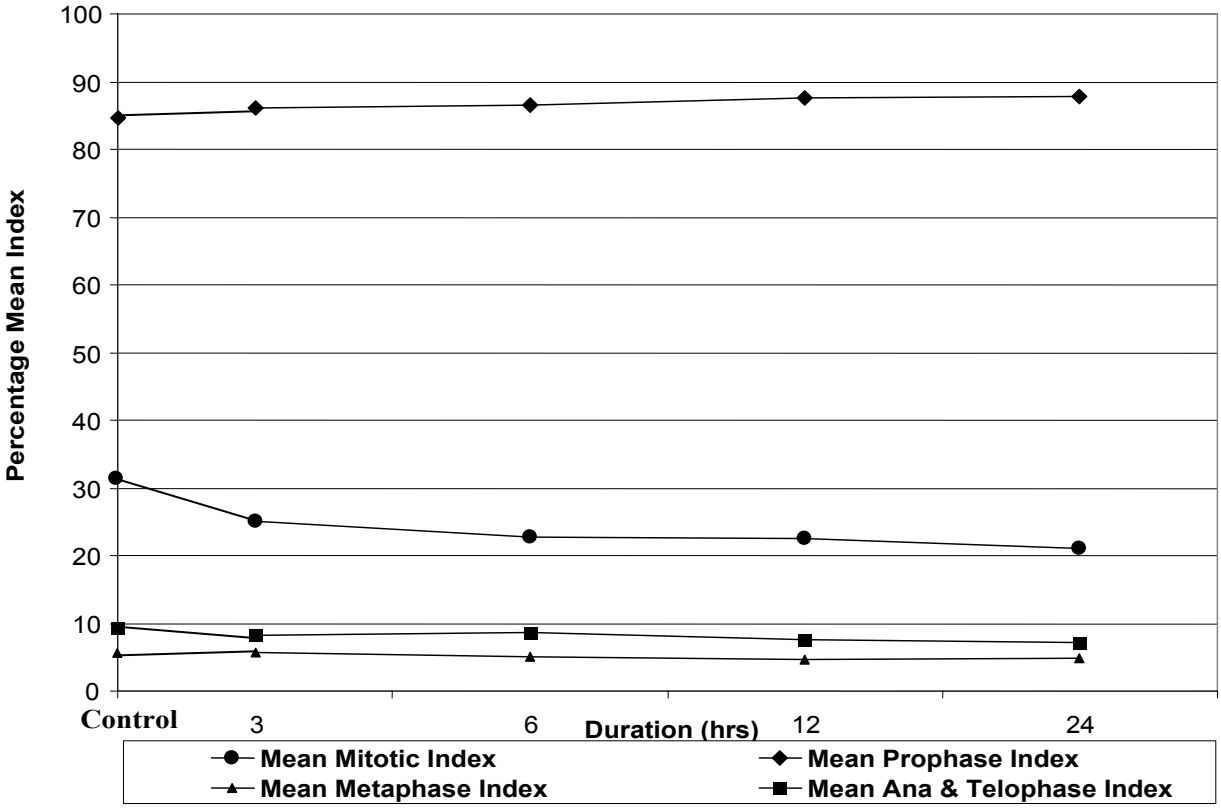


Fig. 5 Graph of Mean Indices of Mitotic, Prophase, Metaphase and Ana and Telophase Vs treatment time with mean percent concentration of Metalaxyl.

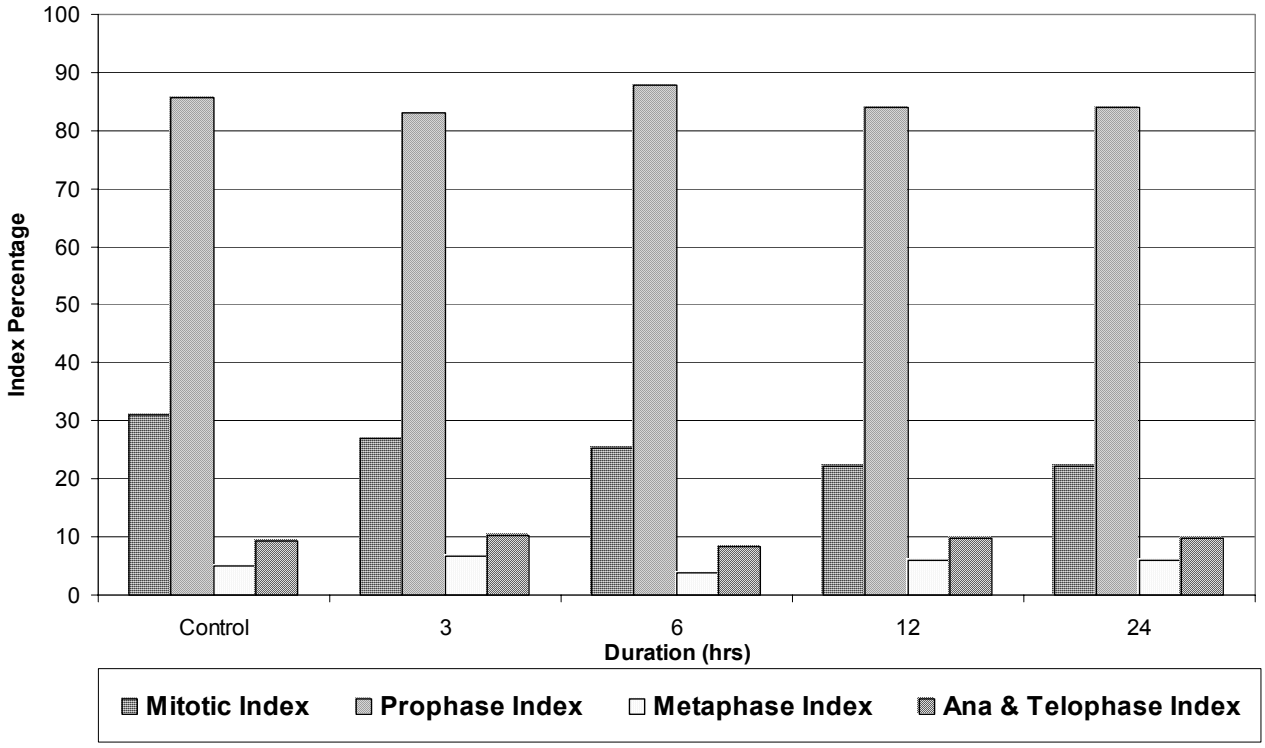


Fig. 6 Bardiagram of Mitotic, Prophase, Metaphase and Ana and Telophase indices Vs treatment time with 25% concentration of Metalaxyl.

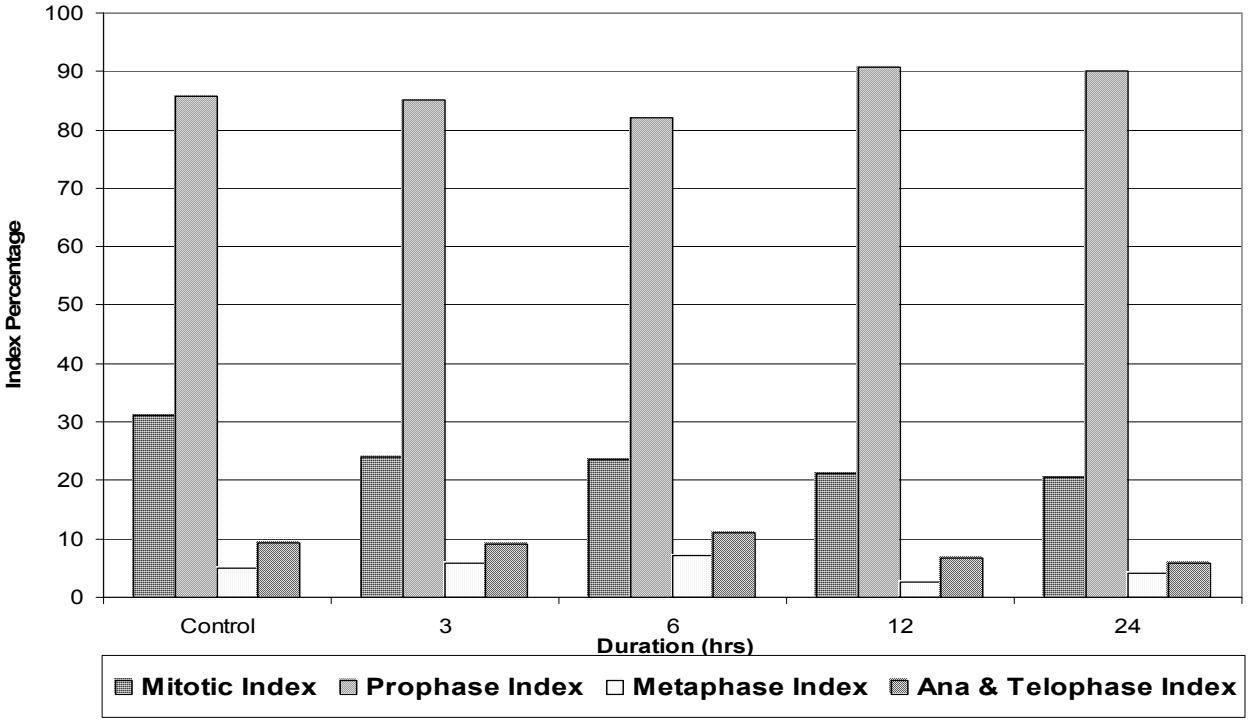


Fig. 7 Bardiagram of Mitotic, Prophase, Metaphase and Ana and Telophase indices Vs treatment time with 50% concentration of Metalaxyl.

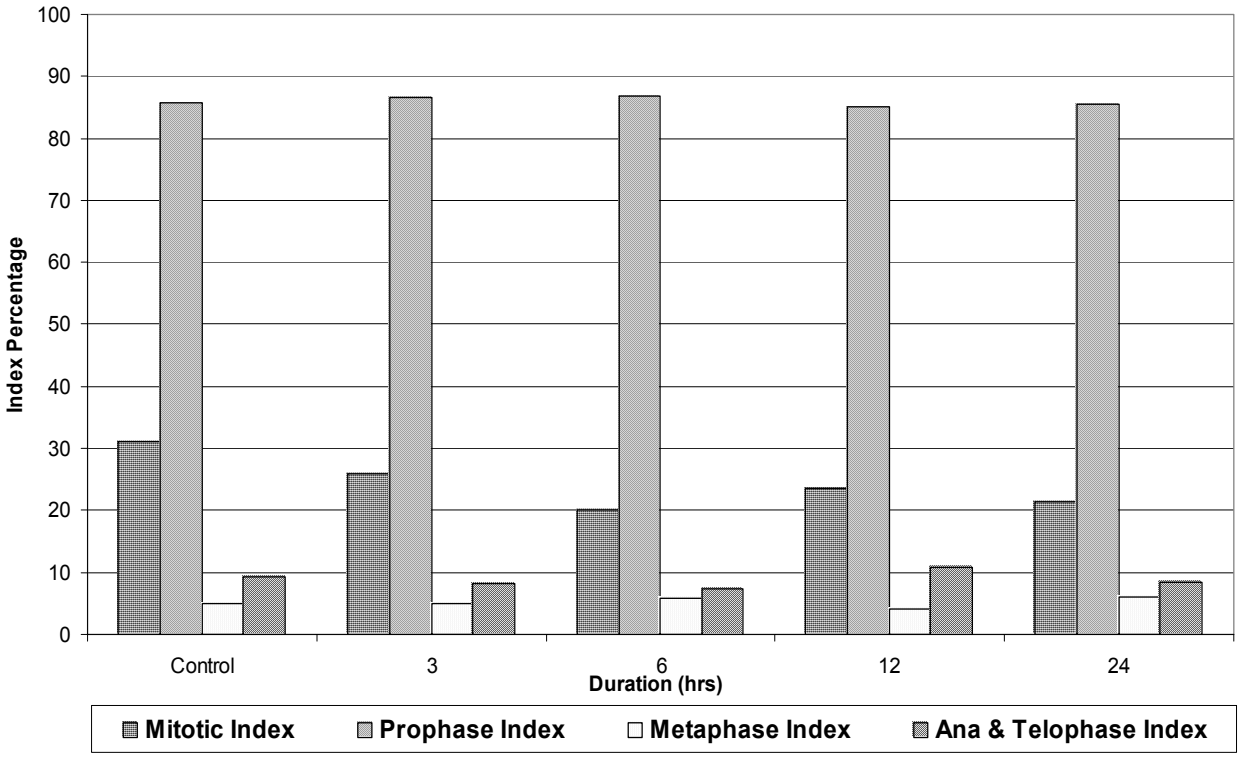


Fig. 8 Bardiagram of Mitotic, Prophase, Metaphase and Ana and Telophase indices Vs treatment time with 75% concentration of Metalaxyl.

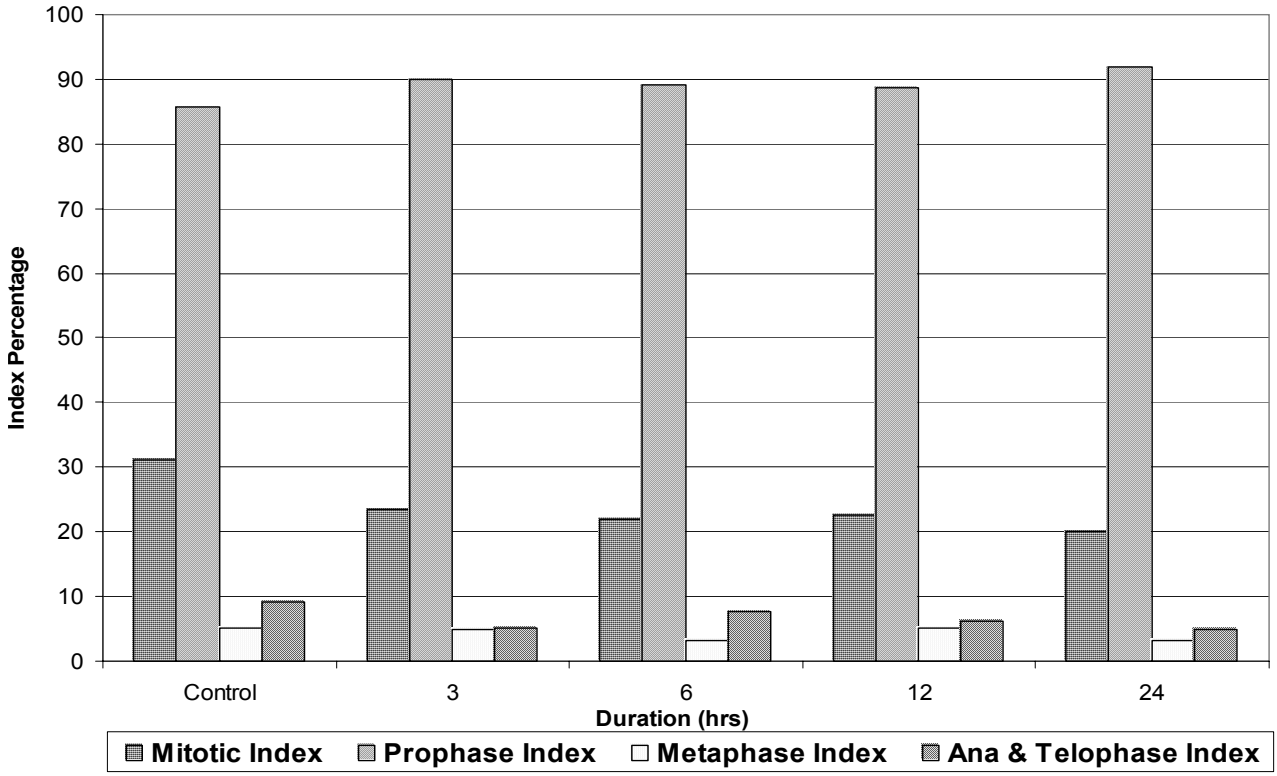


Fig. 9 Bardiagram of Mitotic, Prophase, Metaphase and Ana and Telophase indices Vs treatment time with 100% concentration of Metalaxyl.

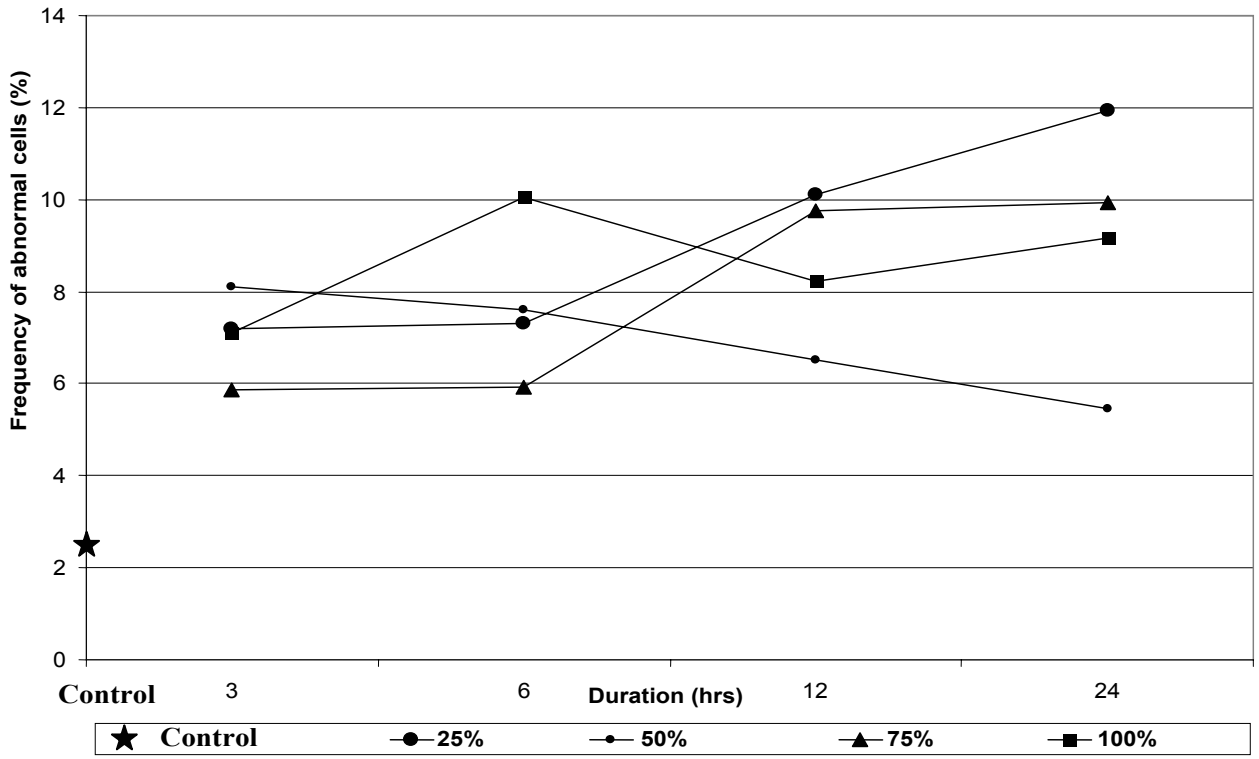


Fig. 10 Graph of Frequency of abnormal cells Vs treatment time with given concentration of Metalaxyl.

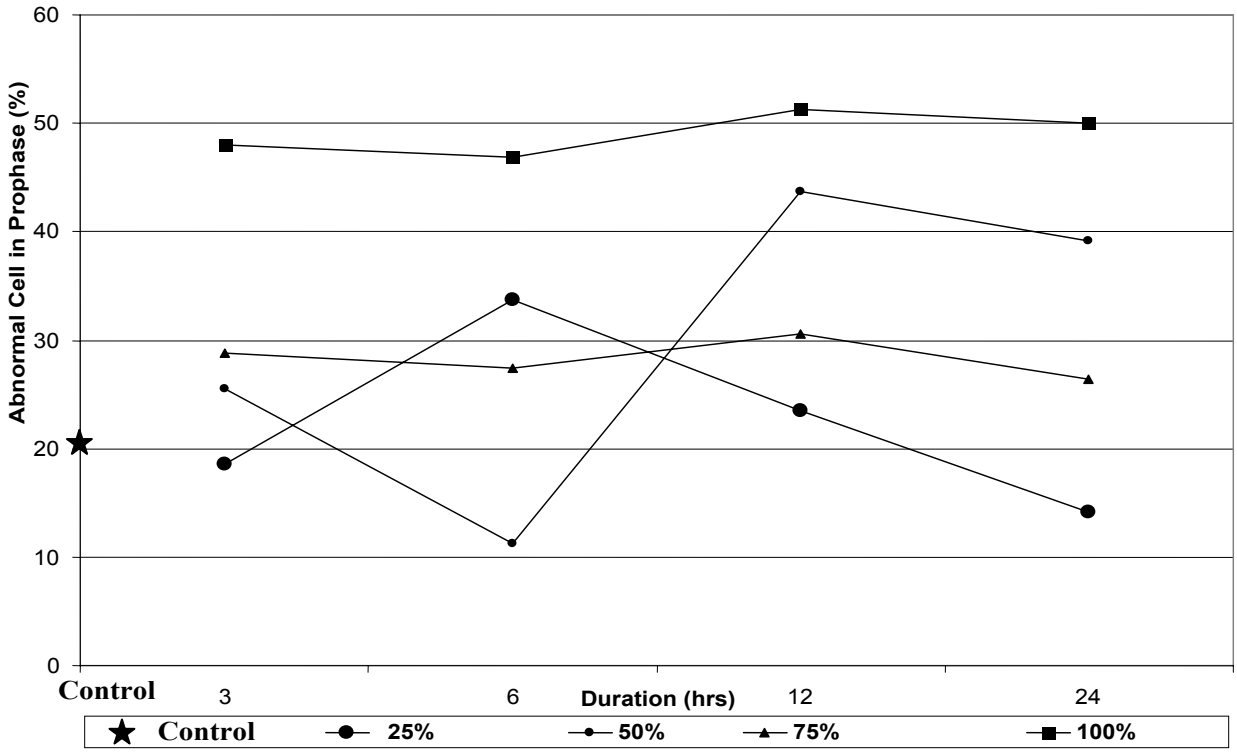


Fig. 11 Graph of percentage of abnormalities among the abnormal cells in Prophase Vs treatment time with given concentration of Metalaxyl.

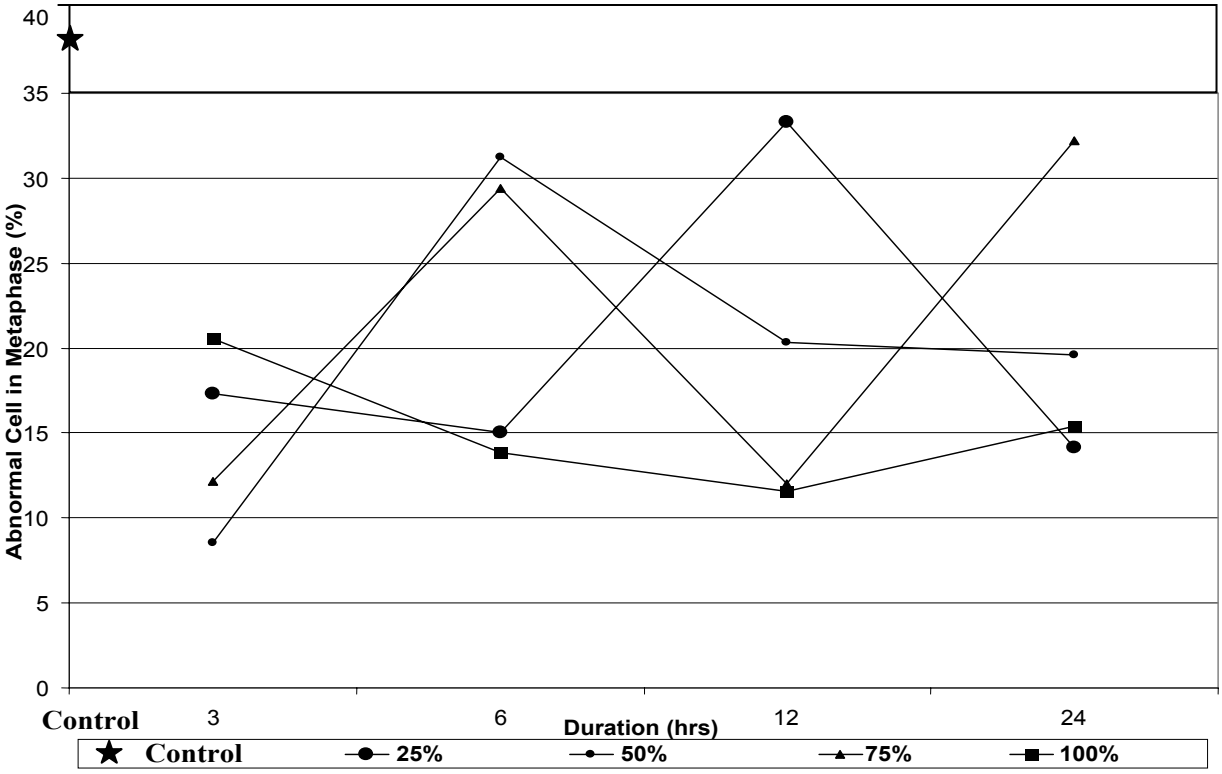


Fig. 12 Graph of percentage of abnormalities among the abnormal cells in Metaphase Vs treatment time with given concentration of Metalaxyl.

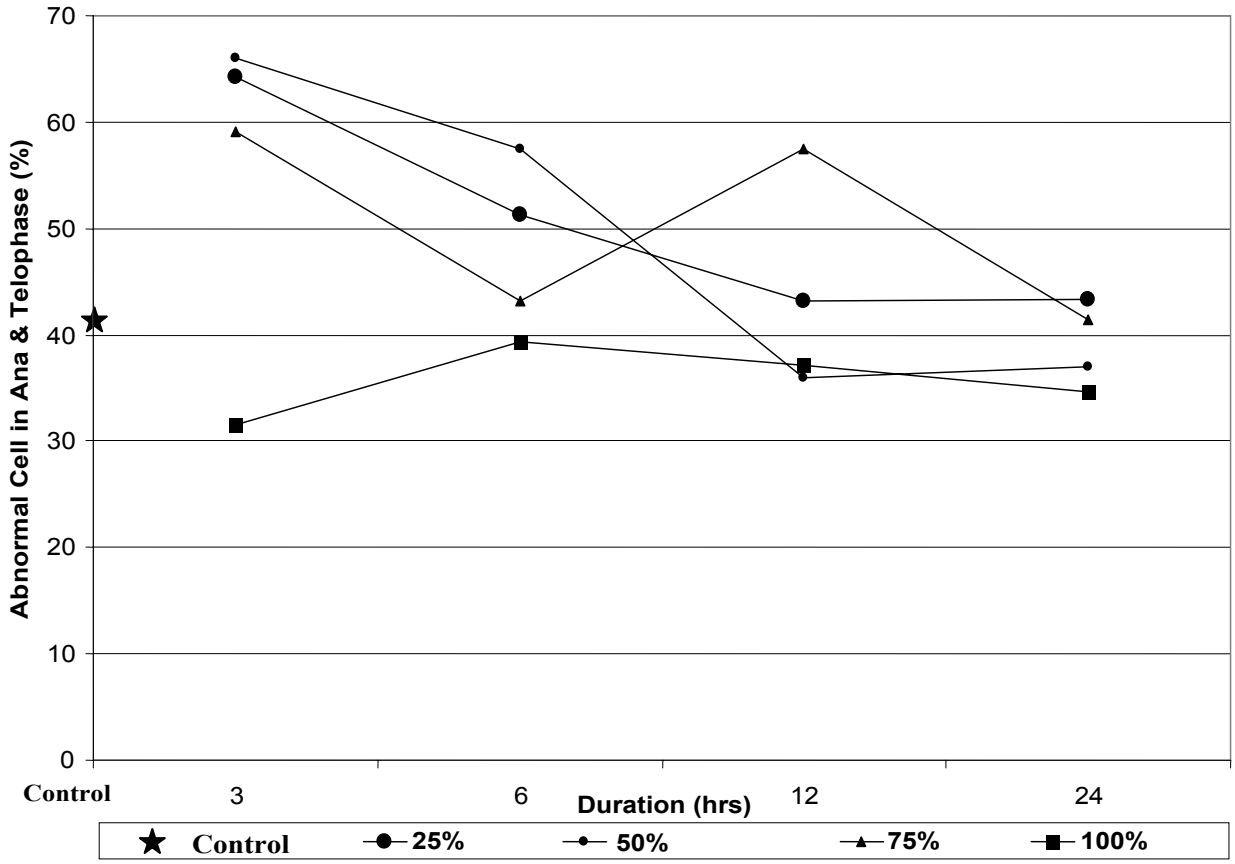
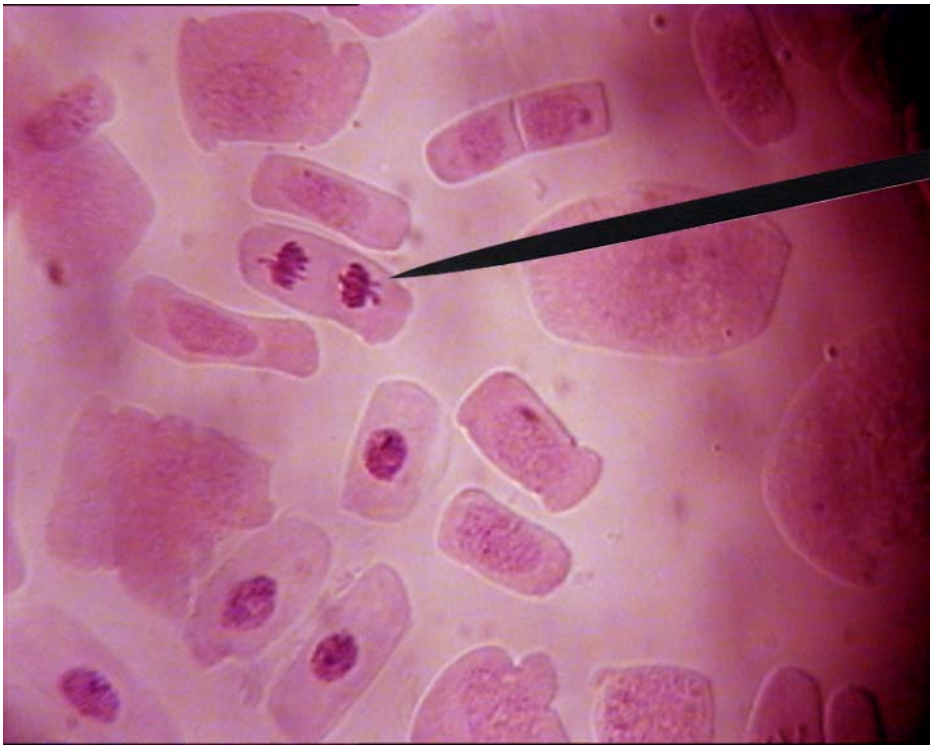
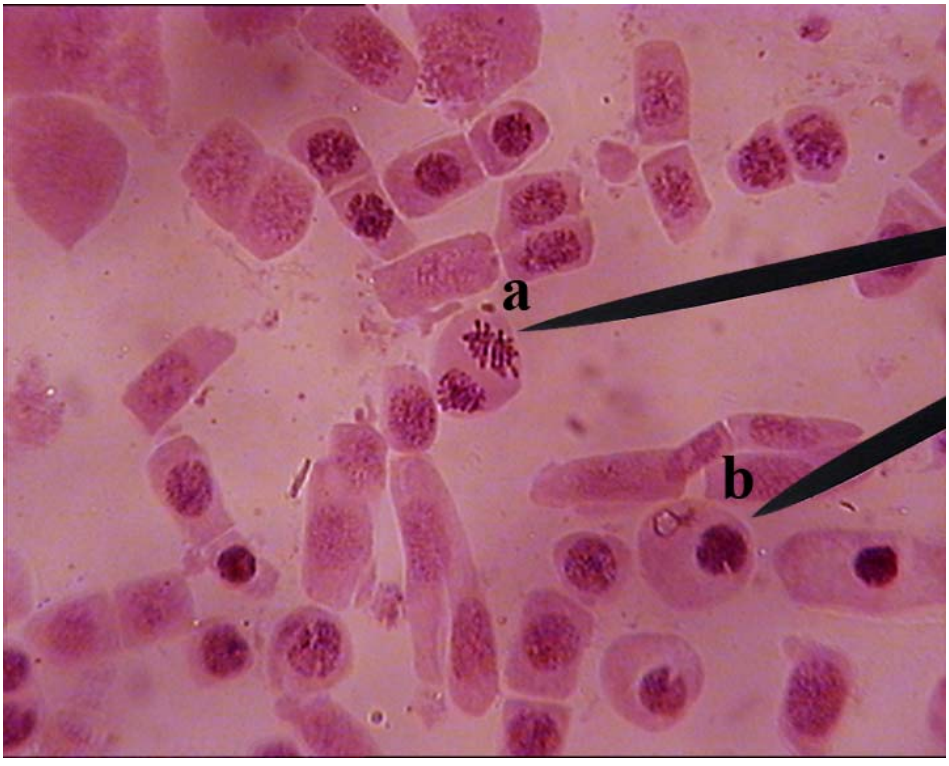


Fig. 13 Graph of percentage of abnormalities among the abnormal cells in Ana and Telophase Vs treatment time with given concentration of Metalaxyl.

PHOTO PLATE I

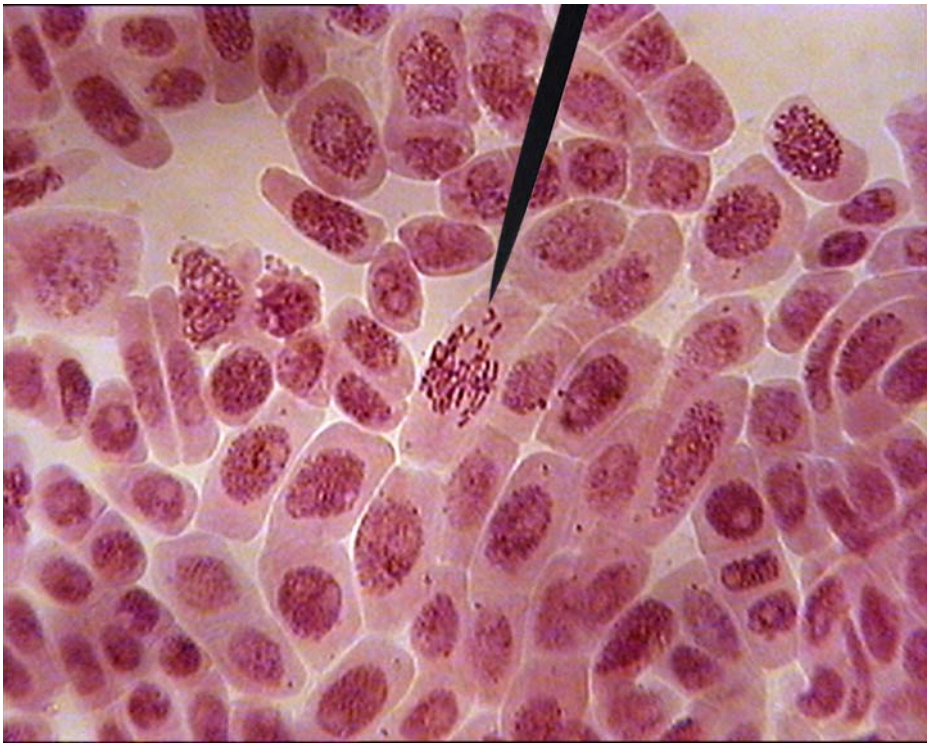


Anaphase with precoucious arm

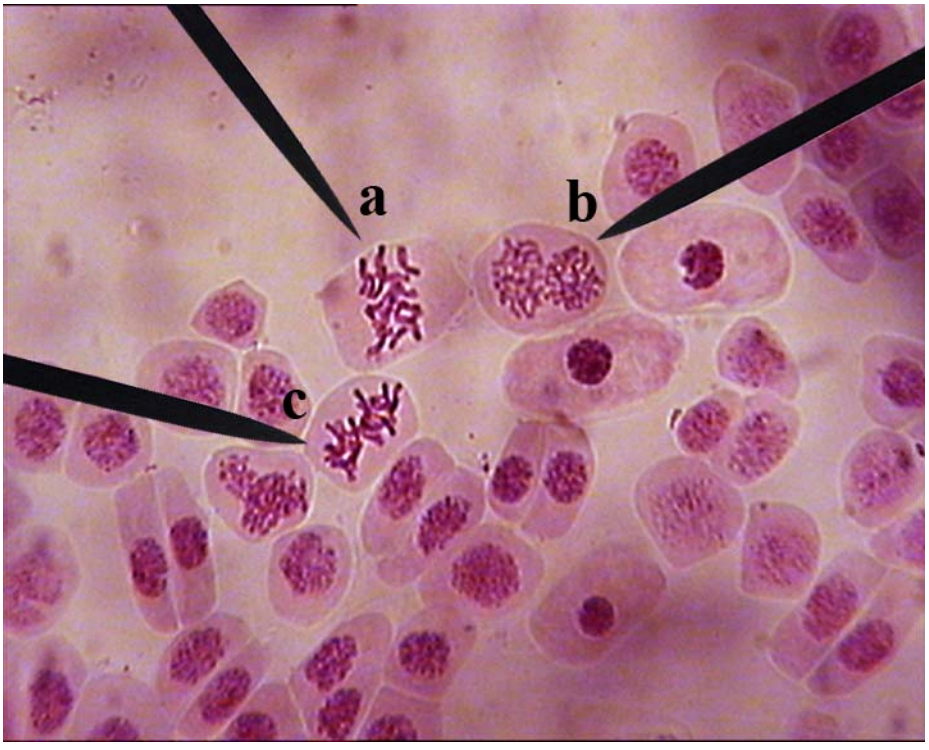


a) Non-synchronized condensation of chromosomes at telophase b) Plasmolysed cell

PHOTO PLATE II

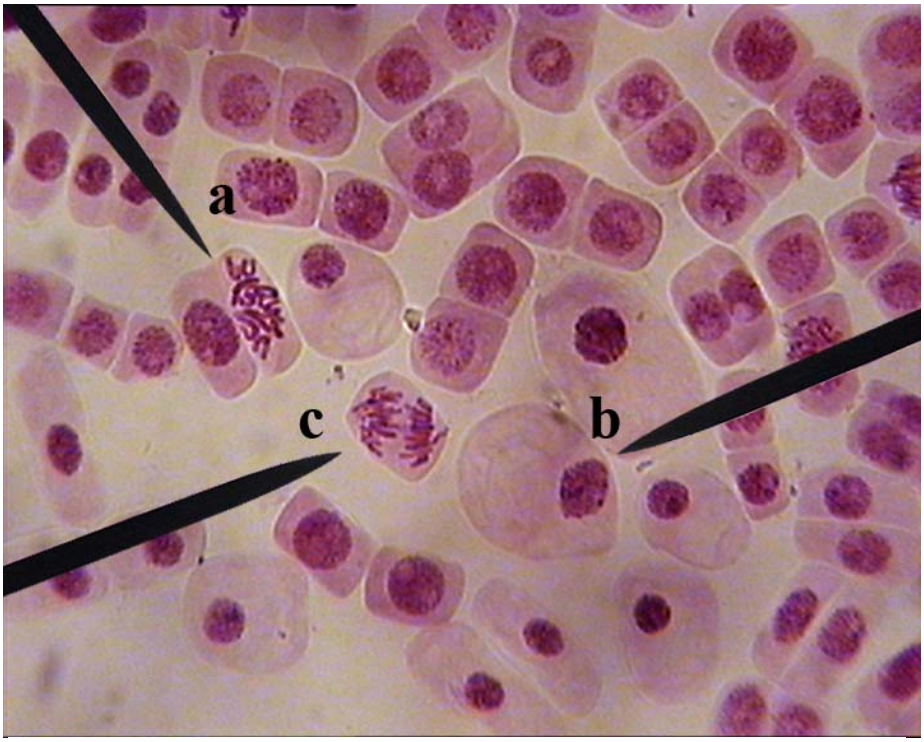


C-metaphase with excluded chromosomes to equatorial plate

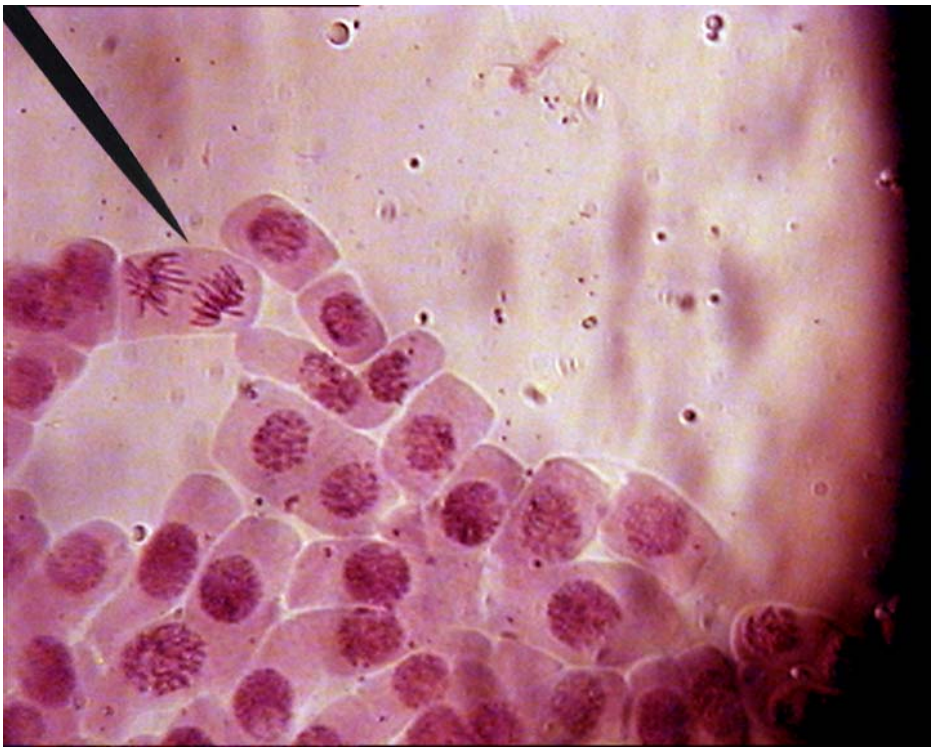


a) Early C-metaphase b) Polyploidy stage-secondary metaphase c) Late C-metaphase

PHOTO PLATE III

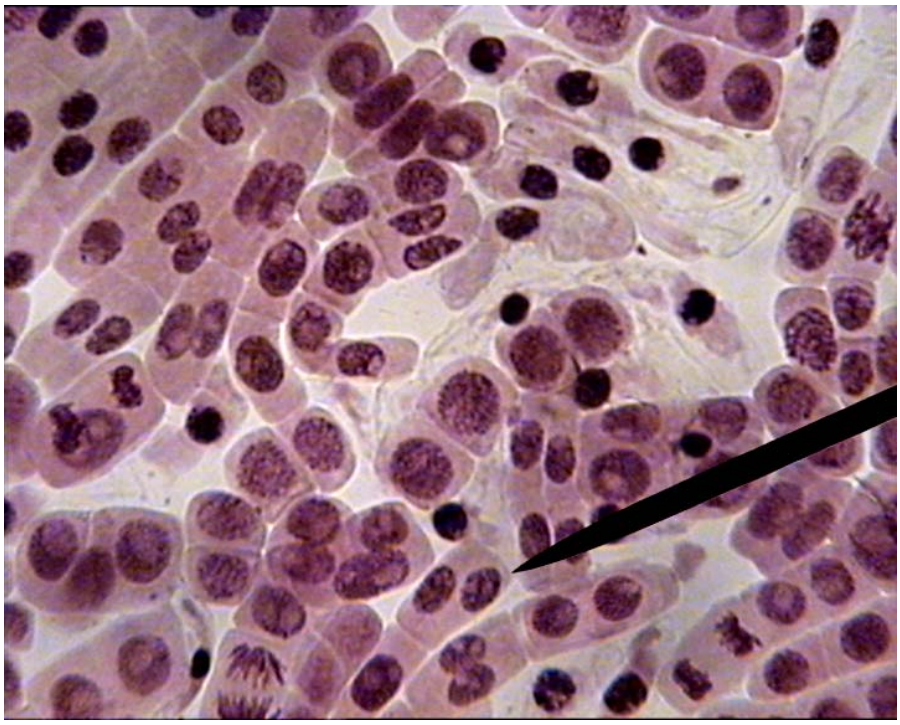


a) C-metaphase b) Plasmolysed c) Anaphase with precoucious arm

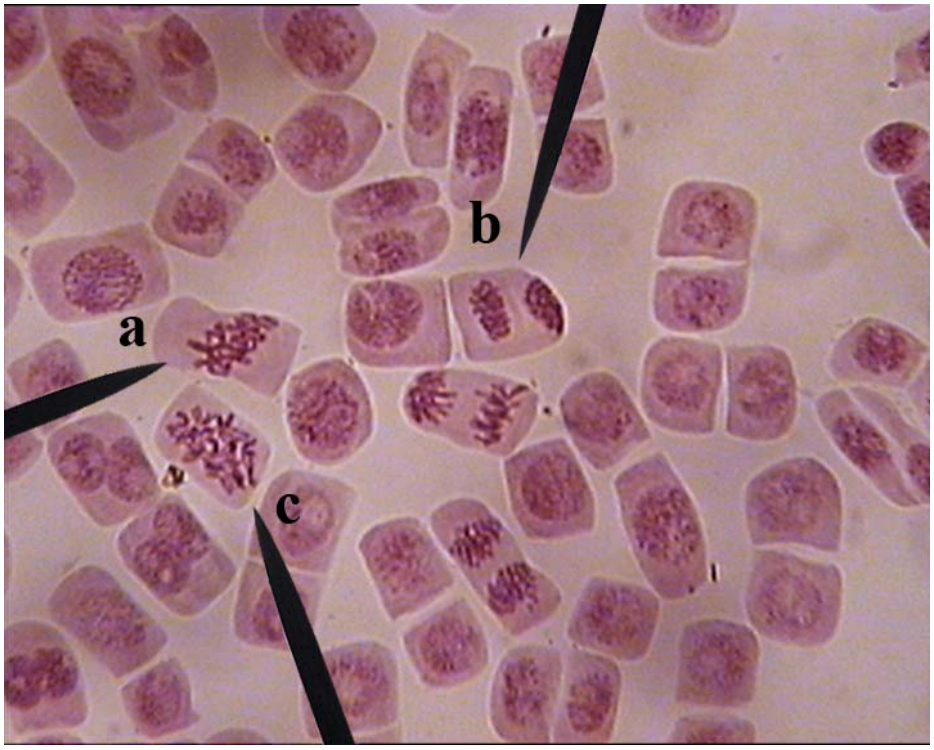


Late anaphase with precoucious arm and shifted pole

PHOTO PLATE IV

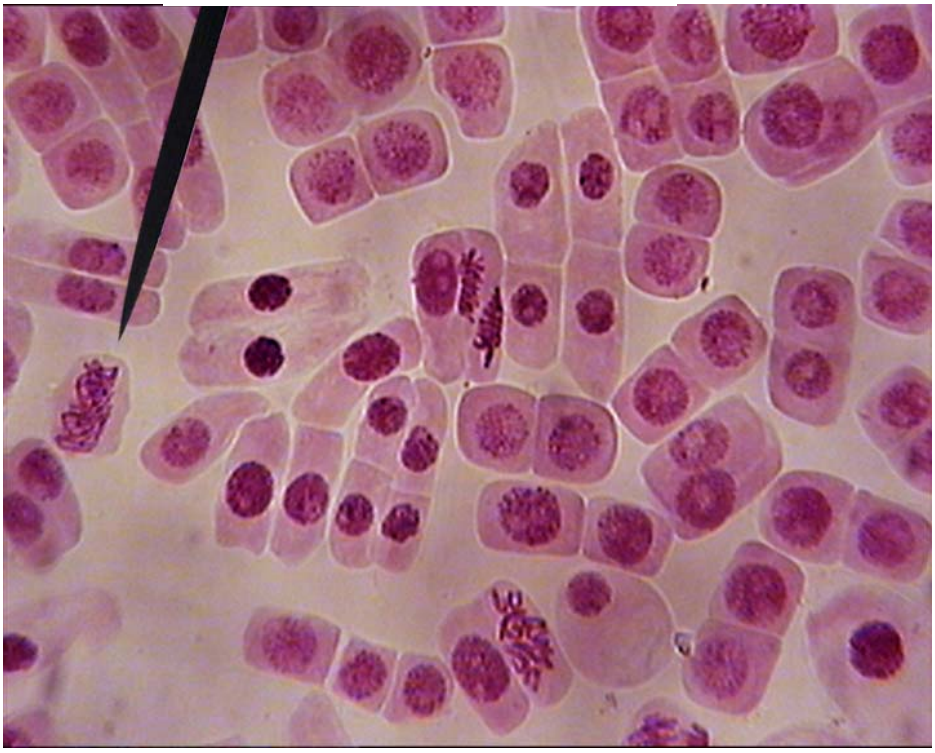


Bi-nucleated cell

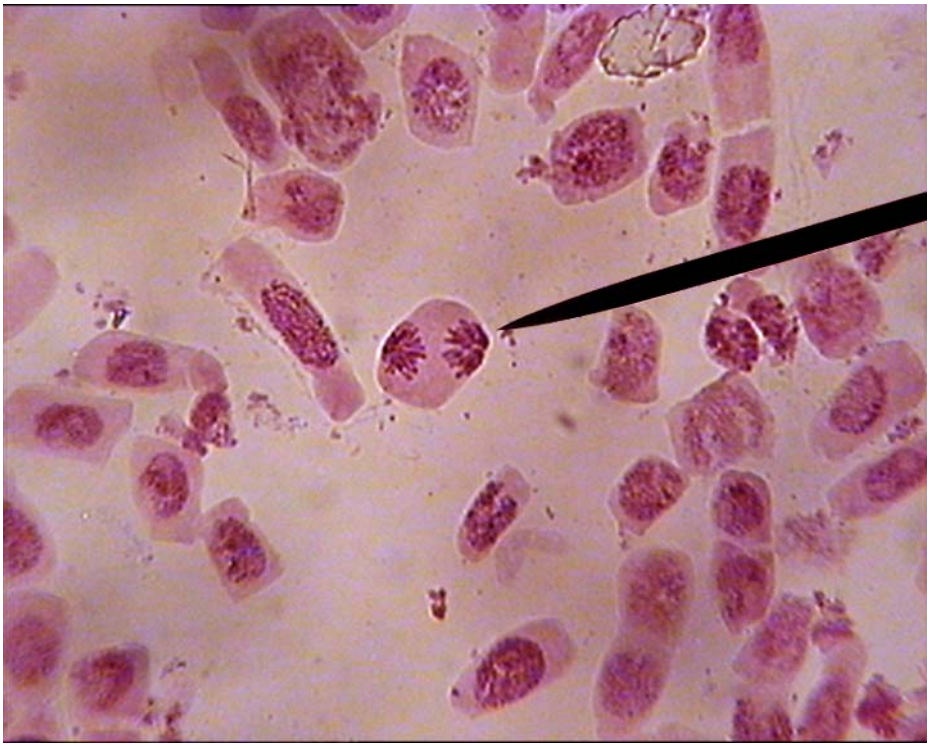


a) C-metaphase, side view b) Telophase c) Disturbed metaphase

PHOTO PLATE IV



Sticky metaphase



Diagonal telophase

5. DISCUSSION

The present study has been undertaken to determine the cytological effect of fungicide Metalaxyl on the meristematic cells of root of *Allium cepa* L. As it is quite apparent from the literature that fairly a large number of pesticides have been proved to be effective mutagenic agents and demonstrated to induce chromosomal aberrations in mitotic system (Wuu and Grant 1966, Amer and Ali 1983, Somashekhar *et a*l, 1984) of various plants.

The calculated chi-square (χ_r^2) value is 9.3 which is greater than tabulated value (7.8) shows the effectiveness of the chemical with respect to time of treatment in dividing cells. This value showed that increasing of time of treatment make lesser in cell division.

The value of mitotic index decreased with increase in time of treatment and concentrations with respect to value of control. Such drop in mitotic index indicates that chemical interfere the normal sequence of mitosis thus preventing a number of cells from entering the prophase, state at interphase. (El-Khodary *et al,*1989) Decrease in Mitotic index value might be caused by decreasing of the ATP level, enzyme production or enzyme function like induction, repression or feedback inhibitions and the pressure from the function of the energy producing centre. (Jain and Sarbhoy 1988, 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). Mitotic index value decreased may be 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).

With few exceptions, the prophase index value increased with increase in concentration of solutions and duration of treatment. 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 of metaphase index, no regularity was shown. Metaphase index was less than prophase index. This may be due to accumulation of dividing cells in prophase i.e. prophase poisoning and restrict on prophase.

Ana and telophase indices were higher than the metaphase index and lower than the prophase index. Anaphase and telophase indices did not show any regularity. The increasing anaphase and telophase indices might be due to delay in the completion of mitotic cycle. Similar result was also reported by Shehab (1980 b).

Few abnormalities were found in control roots whereas higher frequencies of abnormalities were observed in cells treated with Metalaxyl. Few abnormalities were observed in prophase and metaphase cells with respect to anaphase and telophase.

Unequal condensation of chromatin threads, non–synchronized condensation of chromosomes, stickiness and disturbed prophase were abnormalities seen in prophase. Similar results were found by Mallah and Kabarity (1982) in *Allium cepa* L. Stickiness may be due to the physiological effect of fungicide which effect on protein of the chromosomes. Stickiness is due to the improper folding of chromosome fibers which makes the chromatids connected by means of sub-chromatid bridges (Mc Gill *et al*., 1974). Equatorial plate shifting, non-synchronized chromosome, c-metaphase, fragmentation and stickiness were the abnormalities found in metaphase. Similar abnormalities were observed by Mallah and Kabarity (1982). Equatorial plate shifting during metaphase may be due to de-polymerization of spindle fibers (Medeiros and Takahashi, 1987). Non synchronized arrangement of chromosomes may be due to the spindle abnormalities produced by chemical.

Sticky chromosomes may be due to delay in chromosome movement to the poles (Ajay and Sarbhoy, 1988). According to Darlington (1942) stickiness is due to the disturbances in the nucleic acid metabolism in the cell. Similar result was obtained in herbicide treatment by El-Khodary *et al.,* (1989). C-metaphase may be due to inhibition of transcription of spindle protein messengers (Mercykutty and Stephen, 1980). Occurrence of C-mitosis configuration may be explained by disturbances in synthesis of proteins, nucleic acids and antagonism

between these substances (Dewey and Miller, 1969). Similar type of abnormality was also observed in *Allium cepa* treated 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.

The chromosomal fragmentation at metaphase might be due to the action on DNA molecules responsible for the linear continuity of the chromosomes (Grant, 1978). The chemical may be stimulating some of the lysis 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)

According to Amer and Farah (1974), Fragments might arise due to stickiness of chromosomes and consequent failure of separation of chromosomes.

Shifting of poles, precocious arms, unequal movement of chromosomes, precocious chromosomes, laggards, bridge, fragments and breaks in chromosomes were abnormalities found in anaphase. Shifting of pole in anaphase may be due to the effect in spindle mechanism. De-polymerization of the spindle fibers caused the shifting of poles (Pathak,1999 and shrestha,2002). 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).The chromosomal stickiness leading to sticky metaphase and anaphase bridges and nuclear pulverization are possible due to the effect of the chemical in breaking the protein moiety of the nucleo-protein backbone (Patnaik *et al.,* 1984). Anaphasic bridge may be formed due to unequal exchange or dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the

formation of dicentric chromosomes. The dicentric chromosome is pulled equally to both the poles at anaphase and a bridge is formed. (Sax,1940). Similar type 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 effluence 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 bi-nucleated and tri-nucleated cells (Grant, 1978; Badr and Ibrahim, 1987).C-metaphase and bi- nucleated cells are the consequences of inhibited cell cycle in which chromosome DNA is replicated but not distributed in the usual way (Brown and Dyer,1972*)* Telophase bridge was also observed. Bridges in anaphase and telophase may be due to stickiness of chromosomes at metaphase stage (El-Khodary *et al.*, 1989).

In interphase, micronuclei, bi-nucleated and multi-nucleated cells were observed. Micronuclei may originate from lagging chromosomes (El-Khodary *et al.,* 1989). Micronuclei are true mutagenic effects (Auerbach,1962).The lagging chromosomes were observed in the mitotic stages. The bi-nucleated cells are the consequence of inhibition in cell plate formation. Butt and Vahidya *et al.,* (1994) also obtained similar result. Pundhir *et al.,*

(1996) also observed bi-nucleated and tri-nucleated cells. Studying the Nundecane induced cytological aberration in root tips of *Allium cepa* L.

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

Metalaxyl, a fungicide, can also cause adverse effects on plant cell, including mitodepressive, turbogenic and clastogenic effects. The study shows that the chemical Metalaxyl on its higher concentration causes toxic effect to the dividing cells. The recommended dose of the chemical is not suitable for plant as it affects in proper cellular functioning. It has been found to be lethal at this commercial recommended dose. From this study it can be concluded that only low concentration of the chemical seems to be appropriate in order to prevent mutational threats in the plants.

RECOMMENDATION

The current conclusion has been come out from the observation carried under lab conditions. It may not be match with that of the field experiments due to different condition from lab condition. There are a number of physical factors like solubility, PH, buffer, temperature etc, which can influence on the cell division and may vary from that of lab condition. So, it is recommended that the experiment should be carried out under natural condition with different physical as well as biological factors.

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