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*Full Length Research Paper*

# Genotoxicity evaluation of the insecticide ethion in root of *Allium cepa* L.

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**In this study, the genotoxic effects of ethion were investigated in the mitotic cell division of *Allium cepa*. Primary roots of *A. cepa* were treated with various concentrations (25, 50, 75, and 100%) of ethion solutions for different duration of time. The result revealed that increase in the concentration and duration of treatment decreases the mitotic indices. 24 h treatment at 100% concentration of ethion induced lowest mitotic index (20.08%) than that of the control (36.37%). The percentage of chromosomal abnormalities in different mitotic stages was significantly generally higher than that of the control in all the treatment period and concentrations. These abnormalities appeared in various degrees depending on the treatment duration and concentrations of ethion. The abnormalities in dividing cell reached a maximum value of 11.30% after 12 h of treatment at 75% concentration. The type of abnormalities produced were scattered prophase, non-synchronized condensation of chromosome, disturbed prophase, equatorial plate shifting, sticky chromosomes, C-metaphase and sticky metaphase. Overall, it can be concluded that ethion has a potential genotoxic effects on mitotic divisions in *A. cepa* root tip cells. So, it will be necessary to test the mutagenic potential of ethion on a more intensive and extensive basis especially on non-target systems before it is recommended for wider use in agricultural field.**

**Key words:** *Allium cepa*, chromosome aberration, ethion, mitotic index, genotoxicity, root tip.

## INTRODUCTION

In modern agricultural systems, pesticides are extensively used for disease control. Many cytological studies have been carried out to detect the harmful effects of various pesticides on different plants (Inceer and Beyazoglu, 2000; Lerda, 1992; Nandi, 1985). Most of these chemicals have been reported to have detrimental effect on the natural ecosystem and their residues in plants may also affect human health (Fujii and Inoue, 1983). In addition, chronic exposure to low levels of pesticides can cause birth defects, carcinogenicity (Feretti et al., 2007) and genotoxic effect (Steven, 1971; Amer and Farah, 1974).

Ethion is an organothiophosphate member of the organophosphate pesticide family that was first registered for use in the United States in 1965 (EPA, 1989b, d). This pesticide was first developed as a non-systemic insecticide and acaricide for use on fruit trees (EPA, 1989b, d). It is also used for control of insects on a wide variety of food, fiber, and ornamental crops. Other uses include applications as a topically applied pesticide agent on livestock to control biting flies, insects or skin parasites, such as ticks (EPA, 1989b, d). It is highly toxic to freshwater as well as marine fish and freshwater invertebrates (PHS, 1995; EPA, 1989). According to Gallo and Lawryk (1991) ethion is

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highly to moderately toxic by the oral route and caused an influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise. Ethion was negative in tests for point mutations (Kada et al., 1974; Waters et al., 1980), DNA repair (Shirasu et al., 1976), recom-bination (Waters et al., 1980), unscheduled DNA synthesis (Waters et al., 1980) and is anti-carcinogenic in rats and mice (Timbrell, 1991).

Several investigators had studied the side effect of the pesticides on the heredity material of different plant cell (Soliman and Lawryk, 2004; Jackson, 1969). In particular, *Allium cepa* possess many advantages in the field of environmental mutagenesis for screening and monitoring of genotoxic agents according to the standard protocol for the plant assays established by the International Program on Chemical Safety (IPCS) and the World Health Organization (Soliman and Lawryk, 2004). The use of plant root tips, particularly those of *A. cepa* and *Vicia faba*, as a bioassay test system for the genotoxicity of pesticides has shown extremely good correlation with the bacterial and mammalian systems (Soliman and Lawryk, 2004).

There is no much information on the cytogenetic changes induced by ethion in the *A. cepa* plant. Therefore, the objective of the present study was to characterize the action of ethion and to determine any possible effect by using *A. cepa* as a biological system. To accomplish these, mitotic index, chromosomal aberrations in different mitotic phases were evaluated in mitotic cells.

## MATERIALS AND METHODS

For the present work, root meristems of *A. cepa* L., (2n = 16) were used. Approximately equal sized and healthy looking bulbs of *A. cepa* were collected from National Agricultural Research Council (NARC), Lalitpur, Nepal. The bulbs were thoroughly washed with water and placed with basal side facing downward over glass vials containing water for root germination.

### Preparation of suspension

Ethion was purchased from Enzymes, Pharmaceuticals and Industrial Chemicals (EPIC) Ltd. India. Four different concentrations of ethion (25, 50, 75 and 100%) were prepared just before the root treatment. When the roots of *A.cepa* L. were about 2 cm long, they were exposed to freshly prepared test solutions of different concentrations for 3, 6, 12 and 24 h at room temperature. Control roots were simultaneously treated with water in order to compare with that of ethion treated explants.

### Cytogenetic analysis

After treatment, the root tips were fixed immediately in aceto-alcohol (1:3) for 24 h and then transferred in 70% alcohol and stored in a refrigerator until use. Root tips were hydrolysed in 5 N HCl for 20 min at room temperature and then stained in 2% acetocarmine for 1 h. Root tips were then squashed in 2% aceto-orcein stain in 45% acetic acid as described by Savaskan and Toker (1991). Cell divisions and cytogenetical abnormalities were observed and photographed under a BH-2 Olympus research microscope. The various types of cells with normal and abnormal chromosomal behavior at various stages were observed and counted. The observations were recorded after studying around 4000 - 5000 cells from at least five different root tips treated with various concentrations of ethion. Mitotic indices of each phase and percentage chromosomal abnormalities in each mitotic phase were scored and analyzed by the method of Medeiros and Takahashi (1987).

### Statistical analysis

All experiments were repeated at least three times. The data shown represent the mean ± SD. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and significant differences between the means were assessed by Duncan's multiple comparison tests at $P \leq 0.05$.

## RESULTS

Table 1 represents the effect of ethion on *A. cepa* treated for different time duration and concentrations. The result showed that increase in the concentration and duration of treatment decreases the mitotic indices. Mitotic index (MI) value for control was the highest (36.37%). However, 24 h treatment at 100% concentration of ethion induced slightly lower mitotic index (20.08%) than that of the control. Thus, result revealed that MI is inversely proportional to the duration and concentration of ethion treated to the mitotic cells of *A. cepa.* There were great variations in the percentage of MI at different mitotic phases. The result showed significant variation in the frequency of prophase indices in treated roots than that of the control. It reached a maximum frequency of 92.49% after 12 h of treatment at 100% concentration compared with the control value of 86.67%. On the other hand, the metaphase indices showed nearly the same value of the control, with maximum value of 8.64% after 12 h of treatment at 25% concentration compared with the control value of 5.16%. However, the frequency of anaphase and telophase decreased in an irregular order, except at 3 h treatment at 25% concentration, which showed increased value (8.98%) compared with the control (8.15%).

Table 2 shows a total percentage of abnormal cells at each phase at different concentrations and duration of treatment. The observation showed that the mitotic abnormalities increased with increased concentration and the duration of insecticide treatment. All the concentrations were capable of inducing various types of chromosomal abnormalities in almost all the stages of mitosis. The abnormalities were observed mostly in the cells of treated roots. The non-treated roots (control) show very few abnormal cells (1.49%). However, treated roots exhibited various kinds of abnormalities with higher frequency. The percentages of chromosomal abnormalities in different mitotic stages were significantly higher than that of the control for all the treatment period and concentrations. The total percentage of abnormalities in dividing cells increased in irregular order and reached a maximum value (11.30%) after 12 h of treatment at 75% concentration.

**Table 1**. Mitotic index of *A. cepa* root tips exposed to different period and concentration of ethion.

| Duration of treatment | Treated dose (%) | No. of dividing cells | Prophase index (%) | Metaphase index (%) | Anaphase and telophase index (%) | Mitotic index (% ± SD) * |
|---|---|---|---|---|---|---|
| Control | 0 | 1741 | 86.67 | 5.16 | 8.15 | 36.37 ± 0.90$^{i}$ |
| 3 h | 25 | 1681 | 84.59 | 6.42 | 8.98 | 34.86 ± 1.60$^{h}$ |
| | 50 | 1617 | 89.17 | 6.67 | 4.14 | 32.93 ± 1.59$^{f}$ |
| | 75 | 1457 | 89.43 | 6.24 | 4.32 | 31.85 ± 1.66$^{f}$ |
| | 100 | 1378 | 88.60 | 4.28 | 7.11 | 29.32 ± 2.65$^{e}$ |
| 6 h | 25 | 1702 | 89.83 | 3.76 | 6.40 | 34.25 ± 1.25$^{gh}$ |
| | 50 | 1599 | 89.49 | 6.19 | 4.31 | 33.13 ± 0.87$^{fg}$ |
| | 75 | 1202 | 88.85 | 4.07 | 7.07 | 27.99 ± 1.39$^{d}$ |
| | 100 | 1175 | 88.25 | 4.34 | 7.40 | 24.38 ± 1.22$^{c}$ |
| 12 h | 25 | 1515 | 86.66 | 8.64 | 4.68 | 32.75 ± 1.18$^{f}$ |
| | 50 | 1192 | 88.84 | 5.36 | 5.78 | 27.48 ± 1.26$^{d}$ |
| | 75 | 1088 | 86.58 | 5.60 | 7.81 | 22.92 ± 1.64$^{b}$ |
| | 100 | 1066 | 92.49 | 4.03 | 3.47 | 22.12 ± 1.32$^{b}$ |
| 24 h | 25 | 1281 | 90.24 | 4.21 | 5.54 | 27.61 ± 0.94$^{d}$ |
| | 50 | 1012 | 89.62 | 4.54 | 5.83 | 24.68 ± 1.74$^{c}$ |
| | 75 | 917 | 90.51 | 5.99 | 3.48 | 20.45 ± 1.07$^{a}$ |
| | 100 | 931 | 89.58 | 4.08 | 6.33 | 20.08 ± 0.50$^{a}$ |

*Each value represents the mean ± standard devaition (SD) of three independent experiments per treatment. Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($P \leq 0.05$).

**Table 2.** Frequencies of abnormal cells induced in the chromosomes of *A. cepa* after different period of exposure to different concentrations of ethion.

| Duration of treatment | Treated dose (%) | Percentages of abnormal cells | | | Total abnormal cells (% ± SD)* |
|---|---|---|---|---|---|
| | | Prophase | Metaphase and telophase | Anaphase | |
| Control | 0 | 0.45 | 0.57 | 0.45 | 1.49 ± 0.05$^{a}$ |
| 3 h | 25 | 2.49 | 2.85 | 5.05 | 10.41 ± 0.72$^{fgh}$ |
| | 50 | 3.09 | 3.46 | 2.78 | 9.33 ± 1.19$^{defg}$ |
| | 75 | 1.78 | 2.40 | 2.95 | 7.13 ± 2.45$^{bc}$ |
| | 100 | 2.90 | 2.97 | 5.00 | 10.88 ± 0.84$^{gh}$ |
| 6 h | 25 | 1.70 | 1.99 | 3.32 | 6.93 ± 0.75$^{bc}$ |
| | 50 | 1.56 | 2.37 | 3.06 | 7.00 ± 0.87$^{bc}$ |
| | 75 | 2.41 | 2.16 | 3.91 | 8.48 ± 0.49$^{def}$ |
| | 100 | 2.12 | 2.12 | 4.51 | 8.76 ± 0.82$^{defg}$ |
| 12 h | 25 | 1.98 | 2.77 | 2.44 | 7.19 ± 0.58$^{bc}$ |
| | 50 | 2.51 | 3.02 | 4.19 | 9.73 ± 0.46$^{efgh}$ |
| | 75 | 2.38 | 3.49 | 5.42 | 11.30 ± 0.49$^{h}$ |
| | 100 | 2.34 | 2.62 | 1.31 | 6.28 ± 0.44$^{b}$ |
| 24 h | 25 | 1.79 | 2.57 | 3.35 | 7.72 ± 0.97$^{bcd}$ |
| | 50 | 2.86 | 3.35 | 4.94 | 11.16 ± 0.77$^{gh}$ |
| | 75 | 3.59 | 4.58 | 2.39 | 10.57 ± 0.82$^{fgh}$ |
| | 100 | 3.32 | 3.11 | 1.93 | 8.80 ± 0.72$^{defg}$ |

*Each value represents the mean ± standard deviation (SD) of three independent experiments per treatment. Data having the same letter in a column were not significantly different by Duncan's multiple comparison test ($P \leq 0.05$).

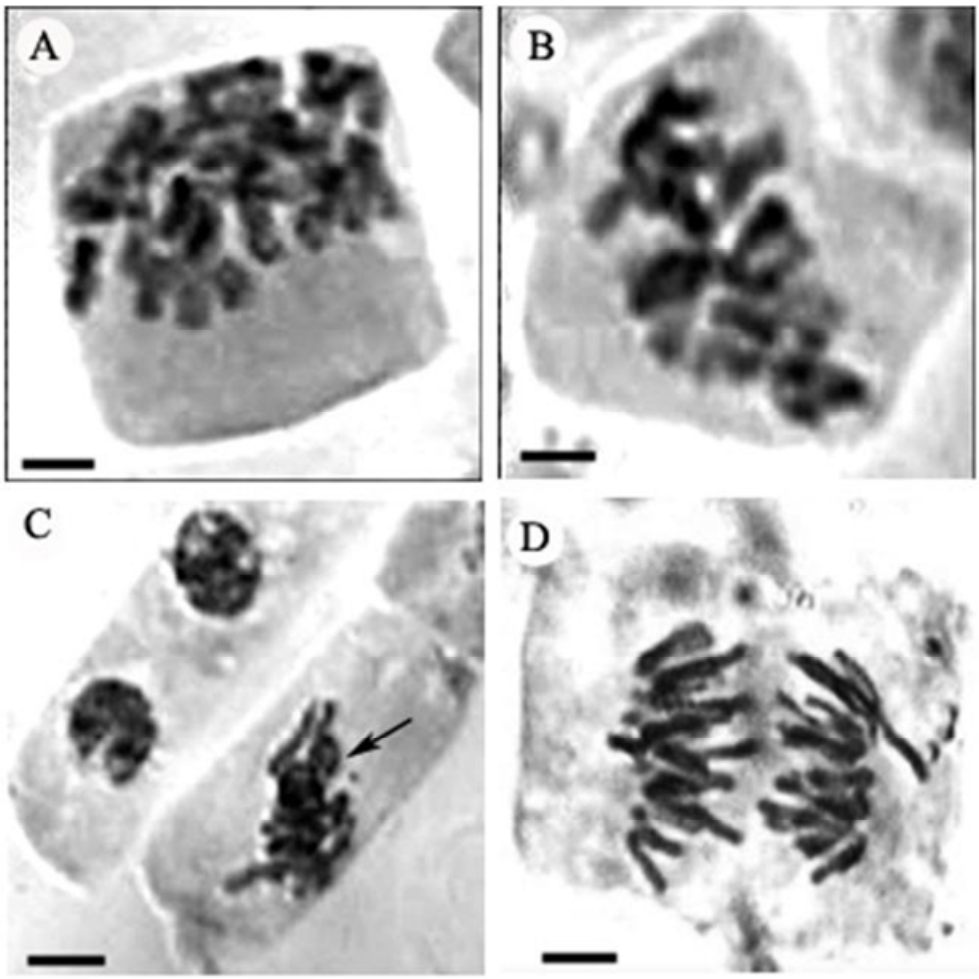


**Figure 1.** Different chromosomal behaviors found in *A. Cepa* roots cells treated with ethion. A: C-metaphase; B: diagonal metaphase; C: sticky metaphase; D: precocious arms in anaphase. Arrow head indicates the sticky chromosomes at metaphase. Bar = 10 µm.

### Nature of abnormal cells in different mitotic phases

Representative samples of the chromosomal aberration types are shown in Figures 1 and 2. Various types of abnormalities such as sticky chromosomes, unequal condensation of chromatin threads, disturb and dilution of cells, clumping of chromosomes and C-prophase were noticed in prophase. Among them, stickiness of chromosome, diagonal metaphase and C-metaphase with shortening of chromosome was the most common type of abnormality (Figures 1A - C). In some cases, metaphases with lags and equatorial plate shifting (diagonally placed) were also observed. During anaphase, polar shifting of chromosomes, precocious chromosomes (Figure 1D), bridges, non-synchronized movement of chromosomes (Figure 2A), stickiness and disturbed anaphase were frequently found. However, polar shifting, delayed cytokinesis leading to binucleated cells (Figure 2B), Plasmolysed cell (Fig. 2C), unequal cytokinesis (Figure 2D) were the common abnormalities found in telophase.

## DISCUSSION

The pesticides have broad spectrum of activity with long residual effects and wide use in agriculture and horticulture. The studies about the effect of different pesticides found out some of the genotoxic effects on plants (Tartar et al., 2006). This study was undertaken to evaluate the possible genotoxic and cytological changes induced by ethion on root meristem cells of *A. cepa*. The changes on mitotic activities like mitotic index, phase indices and induction of chromosomal abnormalities appeared in varying degrees depending on the dose and duration of treatment of ethion (Table 1). The changes of mitotic activity in plant have been attributed to many factors. The inhibition of mitotic index may be due to the interference of ethion in the normal process of mitosis by reducing the number of the dividing cell (Ghareeb and George, 1997; Badr, 1983). Many other investigations were attributed to

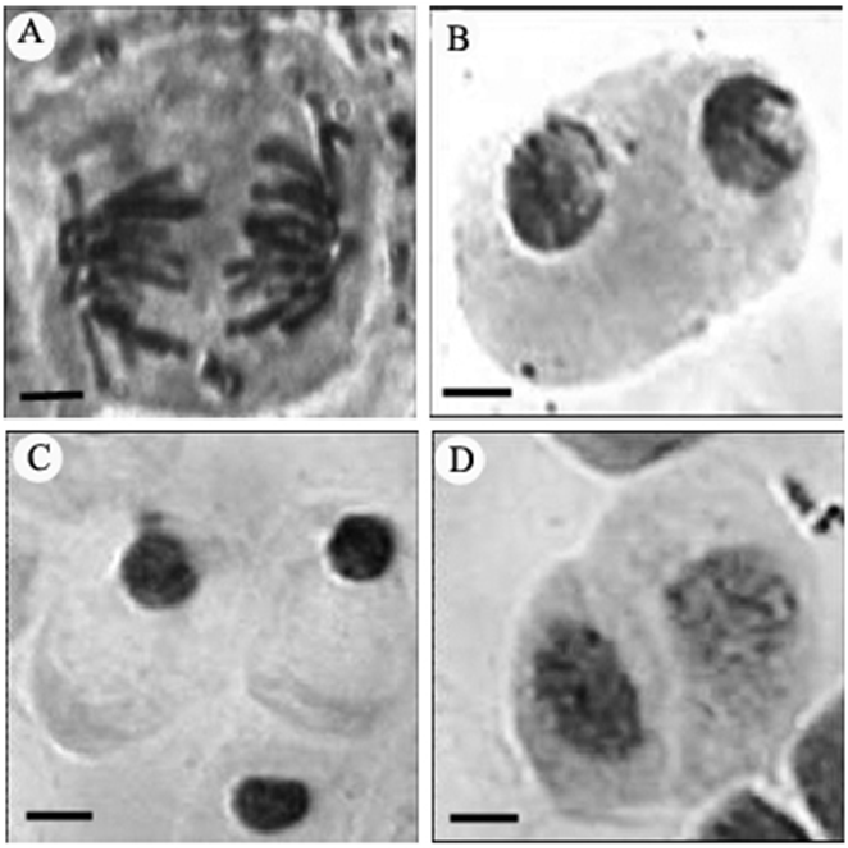


**Figure 2.** Different chromosomal behaviors found in *A. Cepa* roots cells treated with ethion. A: Non-synchronized movement of choromosomes at anaphase; B: telophase with deleating cytokinesis; C: plasmolysed cells; D: unequal cytokinesis. Bar = 10 µm.

depression of mitotic activity due to the inhibition of protein synthesis (Kim and Bendixen, 1987). Mitotic inhibition could also be due to the inhibition of DNA synthesis which is considered as one of the major prerequisites for cell to divide (Badr, 1983). However, Chand and Ray (1981) reported that the reduction of oxidative phosphorylation resulting in lowered ATP level in the cell could be the other factor of inhibition of DNA and RNA synthesis in herbicide treated plants. Furthermore, Wuu and Grant (1967) reported that pesticides in a cell may exert some fundamental effects on enzyme production or enzyme function, induction, repression or feedback inhibition could be a possible reason for the decreasing mitotic index. In this report, the mitotic index decreased with increasing concentrations and duration of treatment of ethion (Table 1). This result suggests that ethion caused cytological changes and induced a wide range of mitotic abnormalities in the root tip cells of *A. cepa*. Similar results were obtained after treating *A. cepa* root cells with insecticides and pesticides (Ajay and Sarbhoy, 1987; El-Khodary et al., 1989). However, the value of prophase index increased with increase in the concentration of solution as well as duration of treatment except for few groups (Table 1). This may be attributed to the prolongation of prophase stage affecting the spindle formation by the pesticides solution. This prophase accumulation was similar to that observed by El-khodary et al. (1989) and Prasad and Das (1977). According to them, prophase poisoning is the stage in which the cells entered into mitosis but were arrested in the prophase resulting in high frequency of prophase cells. However, the result shows that the metaphase, ana-telophase indices decreased with increase in the duration of treatment and concentration of insecticide. The decrease in metaphase and anatelophase indices with concentration and duration of treatment may be due to prolonged prophase or the dividing cells blocked in prophase and do not enter for further phase at all.

Abnormalities were observed in all the mitotic phases of treated cells. The percentage of abnormalities gradually increases with the duration of treatment and concentration of ethion (Table 2). Stickiness, abnormal disturbed prophase, disturbed metaphase and anaphase were the main types of anomalies observed. Beside these bridges, laggards, delayed cytokinesis, metaphase with lag chromosome, diagonal phase, binucleated interphase cells

cells and unequal cytokinesis were also observed. Similar abnormalities recorded with treatment of fluorochloridone on *A. cepa* root tips (Yuzbasioglu et al., 2003). The most common types of irregularities found in prophase were scattered prophase, non-synchronized condensation of chromosome and disturbed prophase as indicated by the abnormal arrangement of chromatin threads**.** Similar type of abnormal prophase with irregular chromosomes was reported by El-Khodary et al. (1990; 1989) in *A. cepa* roots treated with herbicide Tribunil and Garlon-4. The percentage of the disturbed metaphase was higher than the disturbed anaphase (data not shown). These abnormalities could also be explained by the failure of the spindle apparatus to organize and function in a normal way and caused an irregular orientation of chromosomes (Grant, 1978; Mansour, 1984). Another prominent anomaly observed was the sticky nature of chromosomes which could be due to delay in chromosomal movement. As a result, the chromosomes could not reach the poles and remained scattered in the cytoplasm and appeared condensed and sticky (Ajay and Sarbhoy, 1988). However, Klasterska et al. (1976) suggested that chromosomal stickiness arose due to improper folding of chromosome fibers into single chromatid and thus there is an intermingling of fibers, making chromosomes to become attached to each other by means of subchromatid bridges. The most common type of aberration in the present study was C-metaphase induced by high dose of ethion. According to Nagl (1970), treatment of insecticides to the roots caused blockage of the cell cycle at metaphase which subsequently results into C-metaphase. The most observed type of aberrations in anaphase cells was precocious anaphase. Precocious arms and precocious chromosomes formation could be caused by stickiness of chromosomes (Kaur and Grover, 1985). Other interesting abnormalities were bridges, sticky bridges and chromosomal bridges which were observed frequently in the different treatments. This could be due to general stickiness of chromosome breakage and reunion (Tomkins and Grant, 1972; Badr, 1983).

The present observation revealed that the insecticide ethion exerts a mitodepressive effect upon the root tip cells of *A. cepa*. It has capability of producing variety of mutants, chromosomal aberrations and toxic effects in the long run, even below the recommended dose. Such chromosomal abnormalities can affect the vigor, fertility, yield and resistance to the pathogens. Therefore, it is suggested to test the mutagenic potential of ethion on a more intensive and extensive basis especially on non-target systems before it is recommended for wider use in agricultural field.

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