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## Effects of cartap hydrochloride - an insecticide on *Hordeum vulgare* L.

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### ABSTRACT

In this study, we have examined the impact of an insecticide cartap hydrochloride on seed germination, shoot-root growth, total carbohydrate, free amino acid, protein content, mitotic division, chromosomal aberrations and micronuclei in barley (*Hordeum vulgare* L.). The experiment data showed significant ( $P < 0.05$ ;  $P < 0.001$ ) inhibition in germination, shoot-root growth, decreases in carbohydrate, amino acid, protein content and mitotic division and increases in the chromosomal aberration and micronuclei. These remarkable findings suggest that the insecticide cartap hydrochloride possess potential toxic effects and may bring changes on the physio-morphology, cell division and genetic materials of barley. © 2015 Trade Science Inc. - INDIA

### KEYWORDS

Cartap hydrochloride;  
Seed germination;  
Amino acid;  
Protein;  
Chromosomal aberrations;  
*Hordeum vulgare*.

### INTRODUCTION

The development of human activities and industrialization has led to an increased accumulation of toxic substances in the environment. The major sources of pollution are combustion of fossil fuels, mining and smelting activities, release of wastes, sewage waters and the use of fertilizers and pesticides<sup>[1-2]</sup>. Pesticides are heterogeneous category of chemicals used to control weeds, insects, fungi, nematodes and other parasitic pathogenic organisms designed to act upon a limited or broad spectrum of organisms. Recent researches have proved that the use of pesticides in large scale has not only affected the target organisms but also cause various toxicity to other organisms. Pesticides are potent chemical mutagens and the experiment report revealed that the ingredients of various agrochemicals possess mu-

tagenic and carcinogenic properties. The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage<sup>[3-7]</sup>. Researches on the impacts of currently used pesticides on biological systems have been increased in recent years, though the effects of some pesticides are still largely unknown<sup>[8-10]</sup>.

Cartap hydrochloride belonging to an organonitrogen group of insecticide (Figure-1), a derivative of nereistoxin which is a naturally occurring insecticidal substance isolated from the marine segmented worms *Lumbrinereis heteropoda* and *L. brevicirra*. It is a broad spectrum contact insecticides and applied extensively against rice stem borer, brinjal shoot borer and other chewing and sucking pests in various crops like sugarcane, banana, tomato, potatoes, cabbage, soybeans, peanuts,

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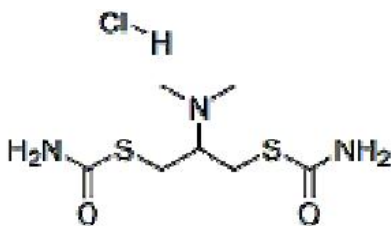


Figure 1 : Structure of cartap hydrochloride

sunflowers, maize, sugar beets, wheat, citrus, cotton, cauliflower etc.

Cartap is a moderately hazardous substance, according to its acute oral toxicity in rats and mice<sup>[11]</sup>. It causes no ocular irritations in rabbits<sup>[12, 13]</sup>. However, technical and soluble powder (50% SP) products of cartap have been found to cause acute lethality to rabbits in eye irritation tests<sup>[14, 15]</sup>.

Our recent review of literature revealed that there is lack of information on the impacts of cartap hydrochloride on higher plants. Hence, the present study was undertaken to examine the effect of cartap hydrochloride on seed germination, shoot-root growth, total protein, free amino acid, carbohydrate content, mitotic division and chromosomes of barley, *Hordeum vulgare* L.

## MATERIALS AND METHODS

### Chemicals

A commercial formulation of Cartap hydrochloride was purchased from local market as Bildan (Cartap hydrochloride – 50% SP). Other chemicals were purchased from E. Merck Co. India.

### Test system

Seeds of barley, *Hordeum vulgare* L. (2n = 14) cv PL172 were used for the present study.

Determination of Inhibitory Concentration (IC<sub>50</sub>)

Various concentrations (based on the active ingredient) of test solution ranging from 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 ppm were prepared from the stock solution by diluting with tap water. Root elongation test was carried out to determine the IC<sub>50</sub> according to Environmental Protection Agency (EPA) guidelines<sup>[16]</sup>. Healthy and uniform sized seeds of barley were selected and surface sterilized with 5 % Tween-20 and 10 % Sodium hy-

pochlorite solution were treated for 10 min and washed thoroughly with distilled water. For each treatment triplicate of 50 seeds were used. From each concentration 7 ml of the freshly prepared test solutions were added to the Petri plates containing filter paper and the seeds were placed on the filter paper with adequate space. The Petri plates were kept in the BOD incubator for 120 hr at 25±1°C temperature in dark to facilitate the linear growth. After 120 hr the root growth was measured.

Root elongations were measured by following formula

$$\% \text{ of Relative root elongation} = \frac{\text{Average root elongation in treatment}}{\text{Average root elongation in control}} \times 100$$

### Test concentrations

Based on the IC<sub>50</sub> concentration determined in the preliminary root elongation test, four concentrations were selected viz. double the IC<sub>50</sub> value (12.5 ppm), the IC<sub>50</sub> concentration (6.25 ppm) and two two-fold dilutions of the IC<sub>50</sub> concentrations (1.56 and 3.125 ppm) were selected in order to provide a reasonable range of toxic and non-toxic concentrations.

### Measurement of seed germination and shoot-root growth

Seed germination and shoot-root growth assays were carried out similar to root elongation test using above selected four test concentrations. After 120 hr seed germination and shoot-root growth were measured according to EPA<sup>[16]</sup>. A seed was considered germinated when radicles had attained a length of not less than 5 mm.

$$\% \text{ of Relative seed germination} = \frac{\text{No. of seeds germinated in treatment}}{\text{No. of seeds germinated in control}} \times 100$$

$$\% \text{ of Relative shoot-root growth} = \frac{\text{Average shoot-root growth in treatment}}{\text{Average shoot-root growth in control}} \times 100$$

### Biochemical and cytogenetic assay

Presoaked (12 hr) seeds were treated with above four test concentrations of cartap hydrochloride for 6, 12 and 24 hrs. After the treatment, the seeds were thoroughly washed with running tap water for 1 hr and allowed to germinate on moist filter paper placed in Petri dishes at 25 ± 1°C in dark. Ethyl methane sulfonate (EMS 10 ppm) and tap water were also

maintained simultaneously as positive and negative controls. After germination some of the roots (when the root reached 1-1.5 cm length) were excised and fixed in acetic-ethanol (1:3) for cytogenetic assay and others were left for 120 hr after that shoots and roots were harvested to analyze the total protein<sup>[17]</sup>, carbohydrate and free amino acid content<sup>[18]</sup>. Cytogenetic assay was performed from the fixed root-tips by haematoxylin squash technique reported as earlier<sup>[19]</sup>. The frequencies of mitotic index (MI), chromosomal aberrations (CA), such as metaphase and anaphasic abnormalities and interphase cells with micronuclei (MN) were determined as described earlier<sup>[19]</sup>. For the analysis, a minimum of 5000 cells from 10 root tips were scored for each treatment.

**Statistical analysis**

All the data values are expressed as mean ± SD and the level of significance between the control and treated groups were evaluated by one-way analysis of variance (ANOVA) and multiple comparisons were

performed by Tukey’s HSD test.

**RESULTS**

In the preliminary root elongation test in *H. vulgare*, treatment with various concentrations (1.56 – 400 ppm) of cartap hydrochloride showed a concentration-dependent inhibition in root growth, along with morphological changes such as discoloration and stiffness of the roots at higher concentrations (i.e., 12.5 to 400 ppm). The dose response curve for the percent of growth as a function of the log concentration of cartap hydrochloride is presented in Figure-2. The concentration of cartap hydrochloride causing a 50% inhibition of root growth was estimated to be 6.0013 ppm (log10 concentration – 0.7782). Hence, we used 6.25 ppm as the IC<sub>50</sub> of Cartap hydrochloride for the subsequent experimentation.

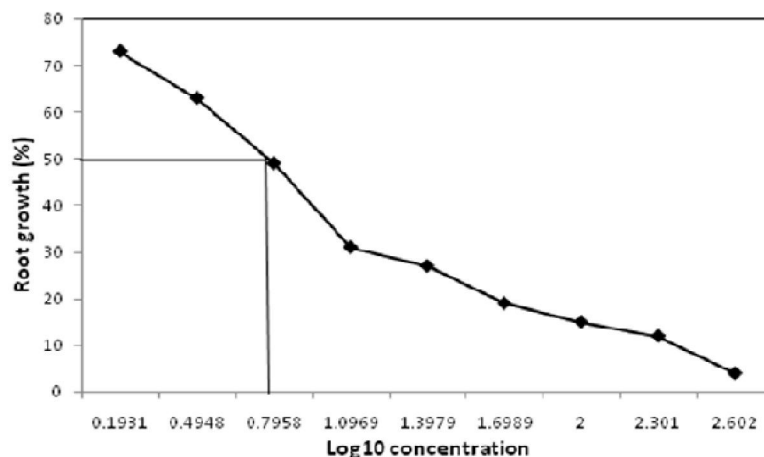


Figure 2 : IC<sub>50</sub> of cartap hydrochloride on *Hordeum vulgare* root growth

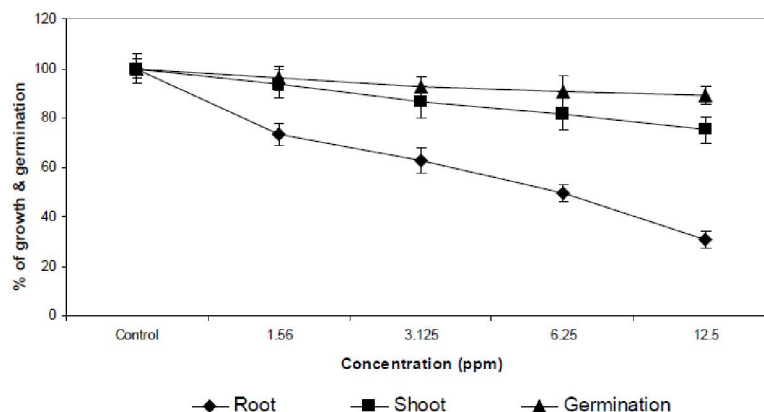


Figure 3 : Effects of cartap hydrochloride on seed germination and shoot-root growth

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### Effects of cartap hydrochloride on seed germination and shoot-root growth

The results of cartap hydrochloride on seed germination and shoot-root growth are presented Figure 3. Treatments with cartap hydrochloride at all concentrations (except 1.56 ppm) showed reduction in germination rates. A statistically significant ( $P < 0.05$ ) differences were observed in the rate of germination between treated and control group except 1.56 ppm. The inhibition of germination was observed in dose-dependent manner. However, there is no significant difference between treated groups. The influence of cartap hydrochloride on shoot-root growth were affected significantly ( $P < 0.05$ ;  $P < 0.001$ ) in all tested concentrations when compared to control. A significant difference was also observed between the treated groups and the highest growth retardation was observed in 12.5 ppm.

### Effects on total carbohydrate, free amino acid and protein content

The results of the impact of cartap hydrochloride on biochemical contents in *H. vulgare* are presented in

Figure- 4 to 6. It was observed that the total carbohydrate content was significantly ( $P < 0.05$ ) decreased at all concentrations and all duration of the treatments when compared to control. Dose and duration dependent decreases were observed in all treatments. Treatment for 24 hr exhibit more reduction than 6 hr and 12 hr. The effects of CH on free amino acid and protein content also showed similar trend as in total carbohydrate. All concentrations and all duration exhibited significant ( $P < 0.05$ ;  $P < 0.001$ ) decrease. Treatment with positive control (EMS 10 ppm) significantly ( $P < 0.05$ ) decreased the carbohydrate, free amino acid and protein content.

### Effects on mitotic index (MI), chromosomal aberrations (CA) and micronuclei (MN)

The results of cartap hydrochloride on the MI, CA and MN of *H. vulgare* root meristem cells are presented in Figure-7 to 10. Significant ( $P < 0.05$ ) inhibition of MI was observed in the exposure at all concentration and this is in a dose-dependent manner (Figure-7). However, there was no duration related inhibition observed with in the treated group. Figure - 8 & 9 shows

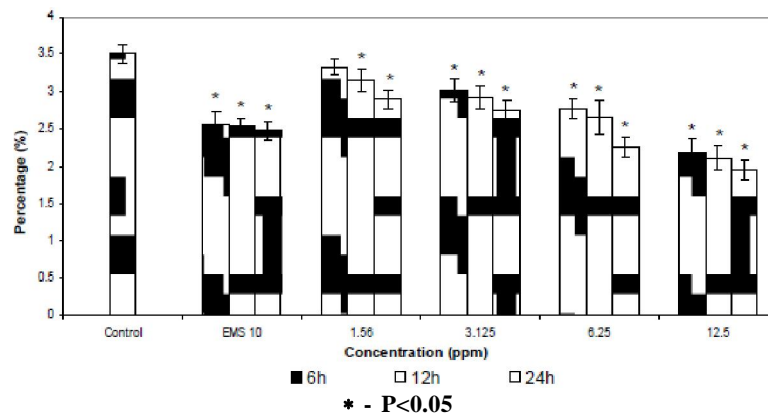


Figure 4 : Effects of cartap hydrochloride on total carbohydrate content

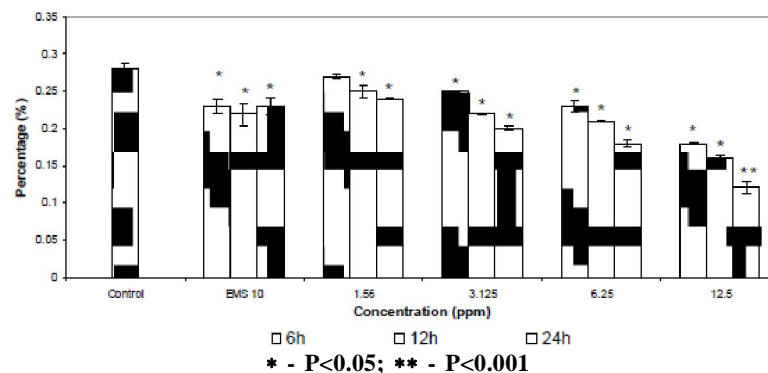


Figure 5 : Effects of cartap hydrochloride on total free amino acid content

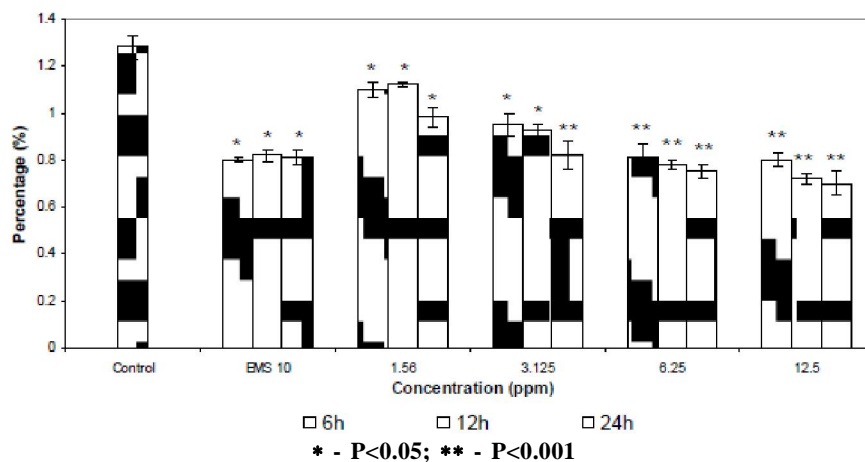


Figure 6 : Effects of cartap hydrochloride on total protein content

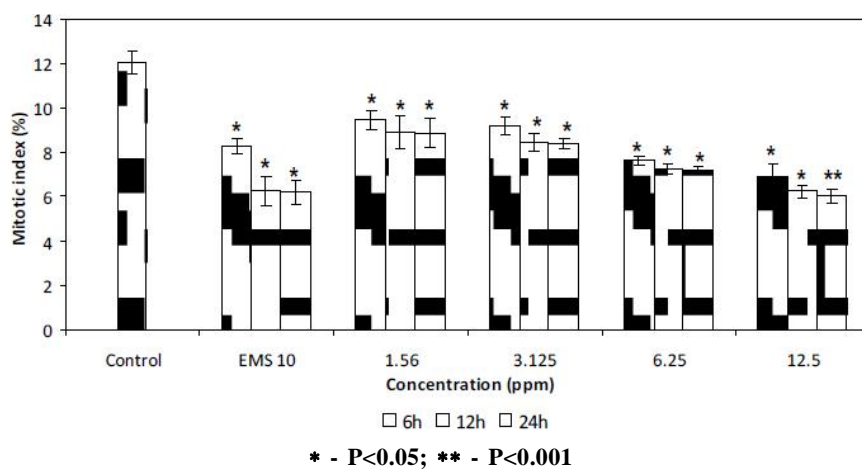


Figure 7 : Effects of cartap hydrochloride on mitotic index

the frequencies and various types of chromosomal and mitotic aberrations. A drastic increase of CA was observed at all concentration and durations and this is statistically highly significant ( $P < 0.001$ ). Also significant duration related increase was observed with in each concentration. The highest percent of aberrations were recorded in 12.5 ppm treatment. The frequencies of C-metaphase, stickiness, disturbed metaphase and anaphasic bridges were found in all concentrations. A gradual increase of fragments was observed when the concentration increased and maximum frequency observed in 24 hr treatment at 3.125, 6.25 and 12.5 ppm. The frequency of cells with micronucleus is shown in Figure-10. A gradual significant ( $P < 0.05$ ;  $P < 0.01$ ) increases in MN frequency were observed in all treatments. The increases of frequency were observed in dose-dependent manner but not duration. The highest value was recorded in 12.5 ppm. Positive control (EMS) exhibits significant ( $P < 0.05$ ) reduction in MI and in-

creases CA and MN.

## DISCUSSION

Cartap, an organonitrogen insecticide, widely used for agricultural pest has long been recognized as an analogue of nereistoxin and categorized as a safe compound. Its basic chemical structure is *S,S*-(2-dimethylaminotrimethylene)bis(thiocarbamate) and it is normally used as its hydrochloride. The commercial names of cartap include Padan<sup>®</sup>, Thiobel<sup>®</sup> and Vegetox<sup>®</sup>. The oral  $LD_{50}$  of cartap in rats, mice and monkey are 325-392, 150-225 and 100-200 mg/kg of body weight, respectively<sup>[12,13,15,20,21]</sup>. However, a technical and soluble powder (50% SP) product of cartap has been found to cause acute lethality to rabbits in eye irritation tests<sup>[14,15]</sup>. Cartap exerted a dose- and time-dependent cytotoxic effect in  $C_2C_{12}$  cells by inducing ROS gen-



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eration via a  $\text{Ca}^{2+}$ -dependent mechanism<sup>[22]</sup>. Recently Boorugu and Chrispal<sup>[23]</sup> reported a case of intentional ingestion of cartap hydrochloride as a suicidal attempt in a farmer which subsequently resulted in severe respiratory failure. However, there is lack of information of the cartap hydrochloride influence on higher plants. Hence, the present investigation was carried out.

In the present study, treatments with different concentrations of cartap hydrochloride significantly decreased the seed germination and shoot-root growth. The growth inhibition of plants can result from several possible mechanisms such as cell cycle delay, cell death, and photosynthesis damage<sup>[24-26]</sup>. Siddiqui et al.,<sup>[27]</sup> reported the inhibition of seed germination and seedling growth in *Penesetum americanum* L. due to the application of organophosphate insecticides. Siddiqui et al.,<sup>[28]</sup> and Jabee et al.,<sup>[29]</sup> also reported the reduction in germination and shoot-root growth by the treatments of pesticides and herbicides.

The analyses revealed that the total carbohydrate content was significantly decreased with the treatment of CH and the maximum decrease (44.28%) was found in 12.5 ppm at 24 hr treatments. Similar results also observed in wheat with fungicides benlate and calixin<sup>[30]</sup>. Faten<sup>[31]</sup> reported remarkable decrease of monosaccharide content in radish leaves after application with the insecticide cyanophos. In the case of amino acid content, treated with all the concentrations significantly reduced the amount. Faten<sup>[31]</sup> also observed treatment with high concentration of cyanophos insecticide reduced the amino acid content in the radish plant. Singh and Shaner<sup>[32]</sup> reported that

enzymes in different amino acids biosynthesis pathways identified as a target of several pesticides. These suggest that inhibition of the branched chain amino acids pathway causes a unique change in the level of free amino acids in plants. In addition Abd El-Mageed<sup>[33]</sup> reported that after 2 and 7 days from cyanophos application a significant decrease of amino acids was observed. Abdullah et al.,<sup>[34]</sup> found that different insecticides showed differences on total amino acids content of cotton leaves. Kerns and Goylor<sup>[35]</sup> observed that some pesticides caused a significant change in total amino acids pools which may have resulted from the effect of pesticides. It has been suggested that the toxicant produced by the application of pesticides inhibits protein synthesis by binding to the larger ribosomal subunits inducing change in the enzyme system<sup>[36]</sup>, ceasing ATP and NADP formation<sup>[37]</sup> thus reduced the protein content. In this study we also recorded significant decreases of protein content during all duration and all concentrations. This result is in agreement with earlier<sup>[33, 38]</sup> who showed that the total soluble protein decreased by insecticide treatment cyanophos and malathion. Application of systemic fungicides Benlate and Calixin also found to decrease the total protein content in *Triticum aestivum*<sup>[30]</sup>.

The effects of CH on MI, CA and MN along with positive (EMS) and negative control are presented in Figure 7-10. The results clearly indicate that the CH can induce genotoxic effects in plant. Different concentrations of CH influence the MI and induce the CA and MN in root tip cells of the treated samples. The inhibition of MI and induction of CA and MN in plant cells

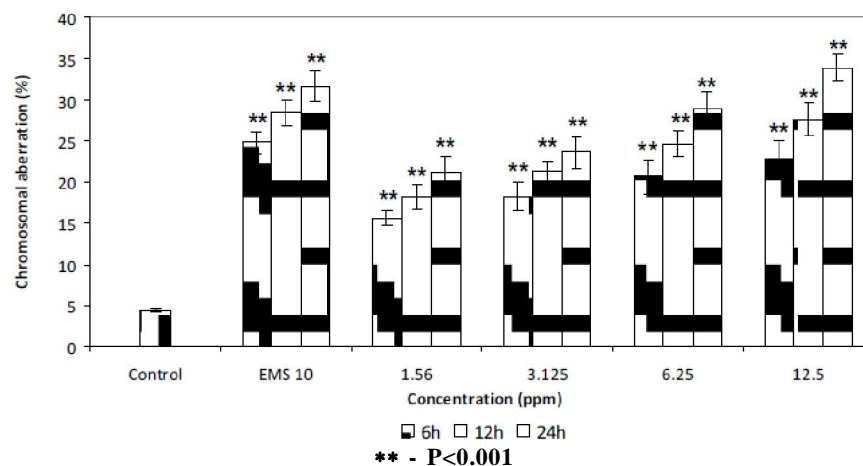


Figure 8 : Effects of cartap hydrochloride on chromosomal aberration

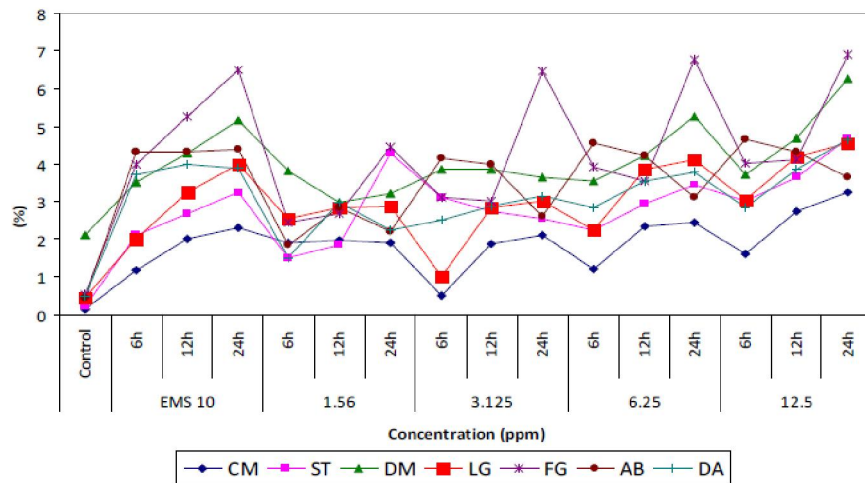


Figure 9 : Various types of chromosomal aberrations induced by cartap hydrochloride on root meristem cells of *Hordeum vulgare*.

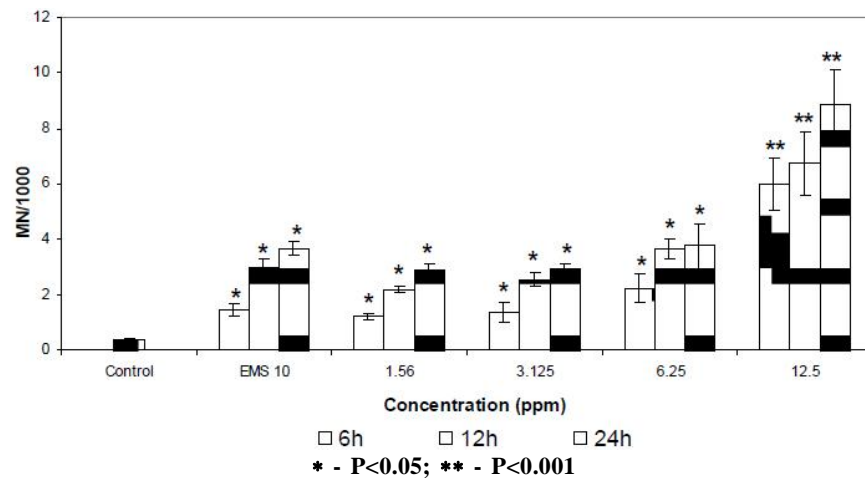


Figure 10 : Effect of cartap hydrochloride on induction of micronuclei

by several pesticides have been reported earlier by different workers<sup>[9,39-41]</sup>. In the present study, we also observed significant reduction of MI and found maximum achieved in 12.5 ppm at 24 hr treatments. The decrease in the MI could be either due to blocking of G<sub>1</sub> suppressing DNA synthesis or inhibition of DNA synthesis at S-phase<sup>[42]</sup> or blocking in G<sub>2</sub> preventing the cells from entering mitosis<sup>[43]</sup>. Parul Singh et al.,<sup>[9,40]</sup> studied the effects of insecticides (Profenophos & Cypermethrin) and fungicides (Mencozeb & Carbendazim) on different stages of cell cycle of barley and found S phase is more sensitive.

Various types of chromosomal abnormalities such as C-mitosis, stickiness, disturbed metaphase, lagging, fragments, bridges, disturbed anaphase and MN were observed after treatment with CH. Fragments, disturbed metaphase, bridges and stickiness

were the most predominant abnormalities. High frequency of chromosomal breaks and micronuclei induced by CH indicates clastogenic potential of the test compound. The induction c-mitosis and disturbed metaphase may be impairment of mitotic spindle function is probably due to the interaction of fenazaquin with tubulin-SH group<sup>[44]</sup>. The stickiness is presumably due to the intermingling of chromatin fibers which leads to subchromatid connections between chromosomes<sup>[45]</sup>. The presence of lagging chromosomes may be attributed to the delayed terminalization, stickiness of chromosome ends or failure of chromosome movements<sup>[46, 47]</sup>. Induction of chromosomal and chromatin bridges may result from stickiness and the separation of daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus remains connected by chro-

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matin bridges<sup>[48]</sup>. MN, which often results from the acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the cell cycle, can cause cell death or loss character(s) due to the deletion of primary genes<sup>[49]</sup>. Such type of observations already reported by several workers in various pesticides<sup>[9, 39-41, 50-52]</sup>.

### CONCLUSION

In summary, it may be concluded that the insecticide cartap hydrochloride may possess potential toxic effects on cell division, genetic materials and can bring physio-morphological changes in barley, *Hordeum vulgare* L.

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