Genotoxic effect of ethephon on the root meristems of *Allium cepa* L.

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ABSTRACT

Genotoxic effect of ethephon [(2-chloroethyl) phosphonic acid], a PGR that has replaced calcium carbide as an agent for fruit ripening, was studied by using a root chromosome assay. Onion root meristems were cultured and analyzed after exposure with ethephon at concentrations of 200 ppm and 500 ppm for 2, 24, and 48 h. There was a time dependent decrease and an immediate toxic effect on dividing cells in mitotic index when compared to control (the mitotic indices of root meristems treated with 200 ppm ethephon for 2, 24, and 48 h were 11.1%, 10.7%, and 8.9%, respectively; and with 500 ppm ethephon for 2, 24, and 48 h the mitotic indices were 10.8%, 10.2%, and 9.0% respectively). The total chromosomal aberrations increased with prolonged exposures indicating duration dependent effect compared to control (the chromosomal aberrations of root meristems treated with 200 ppm ethephon for 2, 24, and 48 h were 14.5%, 15.6%, and 34.0% respectively; and with 500 ppm ethephon for 2, 24, and 48 h the chromosomal aberrations were 20.8%, 26.1%, and 39.8%, respectively). Chromosomal anomalies such as clumps and breaks were of frequent occurrence. This study proves the genotoxic effect of ethephon and emphasizes the judicial use of this compound as a PGR on edible plants and plant parts.

Keywords: Cytotoxicity, Chromosomal anomalies, Onion root meristems
INTRODUCTION

Ethephon [(2-chloroethyl) phosphonic acid] was discovered in 1965 and introduced commercially in 1973 by the AmChem/Union Carbide company as a plant growth regulator (PGR) (United States Environmental Agency 1995). The PGR promotes fruit ripening, abscission, flower induction, and other responses by releasing ethylene gas, a natural plant hormone. Ethephon has recently replaced calcium carbide as an agent for fruit ripening (Mursalat et al. 2013). In nature ripening is the final stage in the development of a fruit which involves series of physiological and biochemical events leading to changes in color, flavor, aroma, and texture that make the fruits both attractive and tasty (Suman and Seema 2011). The fruits soften as it ripens due to the conversion of chlorophyll to anthocyanin, starch to sugar, and the production of cell wall degrading enzymes respectively. Other specific changes include increased respiration and ethylene production, chlorophyll degradation, carotenoid synthesis, and production of essential oils (Rhodes 1980).

However, in the recent past this natural process of fruit ripening is hastened up by gregarious use of artificial fruit ripening agents. Fruits are dipped in solutions of ethephon to quicken ripening process (Contassot et al. 1987, Vincenti 1987, Kusch et al. 1990, Kulling et al. 1992). In plants, ethephon rapidly degrades to phosphate, ethylene, and chloride (U.S. Environmental Protection Agency 1988, Kidd and James 1991) and interferes in the growth process (Kidd and James 1991). The ethylene released promotes fruit ripening whereas residues of monochloroacetic acid (MCA) may be found in ethephon-treated commodities. MCA poisoning is known to produce symptoms such as vomiting, diarrhea, and Central Nervous System-excitability as early signs (Contassot et al. 1987, Vincenti 1987, Kusch et al. 1990, Kulling et al. 1992) and similar symptoms have been reported in many cases after the consumption of artificially ripened mangoes.

Ethephon has caused depression in the brain, plasma and red blood cells. Cholinesterase activity was depressed in both male and female dogs after 13 weeks of oral administration (US Environmental Protection Agency 1992, OHS Database 1993). According to El-Okazy et al. (2008), ethephon caused several signs of toxicity other than affecting cholinesterase, such as variations in organ weights where higher relative organs (liver, kidney, and spleen) weight were recorded. A developmental toxicity study on New Zealand white rabbits showed decreased body weight, food consumption and increased mortality at a maternal LEL 250 mg/kg. The fetotoxic LEL was 100 mg/kg/day, at which decreased fetal viability was reported (U.S. Environmental Protection Agency 1992). The growing awareness that chemicals present in the edible products causes deleterious, heritable change in the humans without immediate toxic effect (Auerbach and Robson 1946) has prompted the study on the effect of ethephon on root meristems of Allium cepa by determining the mitotic index and chromosomal anomalies.

MATERIAL AND METHODS

Ethephon, marketed by Bayer Crop Science, India, was used as the test chemical and commercially available onion was used as the test material. The outer dry scale leaves were removed. The stem were scraped slightly in order to expose the root primordial and placed in beaker containing distilled water. After the emergence of roots of about 3 cm, the root meristems of A. cepa were exposed to freshly prepared solutions of ethephon at concentrations of 200 and 500 ppm for a period of 2, 24, 48 h respectively. Similarly distilled water controls were maintained.

The bioassay, to monitor the potential genotoxic effect of ethephon, was carried out according to the classical A. cepa test by Levan (1938). The root tips were fixed in acetic ethanol and stained as per Haematoxylin squash method (Marimuthu and Subramaniam 1960). Mitotic indices were recorded (Grant 1982) from treated and control samples, examining a minimum of 2,000 nuclei per root involving 6 root tips from 3 onion bulbs (2 root tips from each bulb). The frequency of division was scored following Wilner and Soares (1980) method. The mitotic irregularities like break, anaphasic bridge, lagging chromosome, abortive anaphase, multipolar anaphase, star anaphase, ring metaphase, prophase, metaphasic, anaphasic, and telophasic clumps in the treated and control root tips were determined and classified on the basis of Buckton and Evans (1973).

ANOVA 2-tailed t-test (Addin- StatistiXL, MS Excel, 2007) was carried out to bring out the probability of significant difference (level of significance P<0.001) among the treated and control root tips.

RESULTS AND DISCUSSION

The present study was directed to determine the genotoxic effect of ethephon on biological systems. Treatment of A. cepa root meristematic cells with two different concentrations of ethephon resulted in the decrease of mitotic index (Table 1) during all durations as compared to control. Ethephon had an immediate toxic effect on the dividing cells as it reduced the
mitotic index after 2 h exposure. The mitotic index scored also showed a time-dependent reduction in the frequency of division.

The present investigations showed that the test chemical brought about both duration as well as concentration dependent significant reduction in the mitotic index when compared to the control samples (Table 1).

Ethephon also induced structural aberrations of chromosomes at both doses and durations tested (Table 2 and 3). The types of chromosomal aberrations observed were clumps, breaks, c-metaphase, aborted anaphase, and tripolar anaphase. The percentage of chromosomal anomalies during treatment with 200 ppm ethephon for 2, 24, and 48 h are 14, 15, and 34%, respectively, and during treatment with 500 ppm ethephon for 2, 24, and 48 h were 20, 26, and 39% respectively, showing a time-dependent and dose-dependent increase. Among the chromosomal anomalies observed the clumps and breaks were of frequent occurrence. Fragmented chromosomes were seen only in cells treated in 500 ppm ethephon and was absent in cells treated with 200 ppm ethephon. However, the decrease in mitotic index and increase in chromosomal anomalies was not significant.

Table 2. Quantification of mitotic anomalies induced by ethephon in the root meristem of Allium cepa. 

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Duration of Treatment (hours)</th>
<th>Total number of Cells in division</th>
<th>No of aberrant cells</th>
<th>% of cells showing MAs ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>2</td>
<td>1,337</td>
<td>194</td>
<td>14.51 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1,292</td>
<td>205</td>
<td>15.86 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1,085</td>
<td>369</td>
<td>28.60 ± 0.34</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>1,318</td>
<td>272</td>
<td>20.60 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1,237</td>
<td>324</td>
<td>26.10 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1,093</td>
<td>436</td>
<td>39.80 ± 0.55</td>
</tr>
<tr>
<td>Control*</td>
<td></td>
<td>1,574</td>
<td>25</td>
<td>1.60 ± 0.21</td>
</tr>
</tbody>
</table>

Similar observations were made in bone marrow cells of three groups of pregnant female mice administered separately with 50 mg kg⁻¹, 100 mg kg⁻¹, 150 mg kg⁻¹ bw day⁻¹, of ethephon in water (El-Raouf and Girgis 2011). Their study revealed an increase in all types of structural chromosomal aberrations (chromatid breaks and gaps, centrometric attenuations, endomitosis, deletions and fragments) and a decrease in mitotic index as compared to control. The increase in all types of structural chromosomal aberrations was highly significant at high dose treatment. An increase in all types of structural chromosomal aberrations and a decrease in mitotic index as compared to the control were also observed in fetal liver cells (Raouf and Girgis 2011). Ethephon also brought about a reduction in the DNA, RNA, and protein content of dam’s brain, liver and kidney, and fetal liver cells compared to control. A significant decrease in the cholinesterase content was detected in blood plasma of dams and in brain tissues of the dams and fetuses in ethephon treated mice. Ethephon increased cholesterol (mg Dl⁻¹) and LDH (U L⁻¹) levels and reduced the levels of triglyceride and hemoglobin. However glucose level was not affected.

Treatment with combinations of gibberellic acid and ethephon (2-chloroethylphosphonic acid) showed a reduction in weight gain and low dry matter and a significant decrease in mean liver, kidney and spleen weights. The activity of liver AST showed significant dose dependent decrease in groups treated with the combination of gibberellic acid and ethephon. Determination of transaminases such as (ALT) and (AST) activities may reflect the performance and/or damage of liver tissue. They are important and critical enzymes in the biological processes. They are considered as specific indicator of liver function and/or damage. Thus, this study indicated that ethephon and gibberellic acid in combination caused several signs of toxicity which includes variations in organ weights where higher relative organs (liver, kidney, and spleen) weights were recorded in groups treated with ethephon together with gibberellic acid. This effect might be due to acidic effect of gibberellic acid which might together with stomach acidity keep ethephon from rapid degradation because ethephon is stable at pH less than or equal to 3 and decomposing to liberate ethylene and phosphonic acid at higher pH values (El-Okazy 2008).
Similarly ethephon treated mice showed greatest inhibition in brain ACh E (El-Okazy 2008). Ileum segment of smooth Old Wister Albino male rats incubated with $10^{-7}$ M ethephon caused a statistically significant decrease in $E_{\text{max}}$ value of ACh which is related to the down regulation of the number of receptors that ACh can be bound (Cetinkaya and Baydan 2010).

**CONCLUSION**

The present study showed that ethephon is a mitotoxic and a genotoxic agent that reduced the mitotic index in a dose- and time-dependent manner and induced chromosomal aberration respectively in onion chromosomes. The results agree with many published studies in other organisms. No previous research work on the effects of ethephon on plants was found. This study emphasizes the need for judicious use of chemicals on edible plants and plant parts.

**References**


Vincenti M. 1987. A propos d'un cas d'intoxication percutanee par l'acide monochloracétique. Thèse médécine (Direction J. Jouglard), 26 mai, Marseille, France.