EVALUATION OF CYTOTOXIC EFFECT OF PHOSKILL ON THE ROOT TIPS OF ALLIUM CEPA L

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ABSTRACT

The present study reveals the hazardous effect of pesticide Phoskill on the root tips of Allium cepa L. The pesticides at different concentrations viz., 500, 1000, 1500, 2000 ppm concentration disturbed the chromosomes from normal behavior to abnormal. The Abnormalities such as endomitosis, diagonal anaphase, diagonal metaphase, binucleate condition, elongated nucleus, chromosomal bridges, clumping were observed. Initially mitodepressive effects and percentage of Abnormality was increase with the increase of concentration of phoskill.

KEYWORDS: Chromosomes abnormality, Phoskill, binucleate condition, endomitosis.

INTRODUCTION

The indiscriminate use of pesticides and herbicides in agriculture, as well as the increase of pollution in ecosystems due to industrial development, justifies the evaluation of the toxicity of these chemicals. They can be transformed into mutagenic or carcinogenic agents by vegetables, which are the first living beings in the food chain, absorbing the nutrients of polluted environments and acting as the toxic agents vectors to humans (Marcano et al., 2004). Pesticides causing environmental pollutions may be mutagenic or toxic to all living organisms. Use and constant exposure to these chemicals may result in change in the genetic constitution of an organism resulting in mutation in organism. Chromosomal aberrations can be accepted as indicators of genetic damage induced by pesticides (Reddi and Reddi, 1985).

Organophosphorus compounds are usually esters, amides or thiol derivatives of phosphoric acids. They form a large family of 50,000 chemical agents with biological properties that have important and sometimes unique implications for man (Kamanyire and Karalliedde, 2008). Monocrotophos is an organophosphate insecticide and it is acutely toxic to birds and...
humans and is usually esters, amides or thiol derivatives of phosphoric acids (Kanmanyire and Karalliedde, 2008). The present study is carried out to evaluate the genotoxicity and cytotoxicity of pesticide phoskill on chromosomes using the plant material *Allium cepa* L. (Onion) has been considered as a most efficient test material to indicate the presence of mutagenicity due to its kinetic characteristics of proliferation and possession of chromosomes suitable for cytotoxic study. Different parameters of *A. cepa* such as, root growth, mitotic index and chromosomal abnormalities was used to estimate the cytotoxicity caused by environmental pollutants. So, the present study was designed to investigate the mitotic index and frequencies of abnormalities in root tips cells of *A. cepa* L. to detect hazardous nature of organophosphorus pesticide phoskill.

**MATERIALS AND METHODS**

**Selection of Plant material:** The plant *Allium cepa* L. (2n=16) commonly called as Onion belongs to family Liliaceae was taken for the present study. Clean healthy bulbs were selected and allowed to culture in a suitable vial.

**Selection of pesticide:** The pesticide Phoskill 35 SL was taken to study the chromosomal abnormalities on the root tips of *Allium cepa* L. It is classified as organophosphate pesticide coming under Monocrotophos. It is commonly used to kill the pest like Shoot fly, Leaf Miner, Pod Borer, Mites, Aphids and Leaf Hoppers. It is also used to prevent insects and pests on Maize, Pea, Okra, Cabbage and all grams.

**Methodology:** Clean healthy onion bulbs (*Allium cepa* L.) was taken. The pesticide phoskill 36% SL was taken and prepared various concentrations viz., 500ppm, 1000ppm, 1500ppm and 2000ppm was prepared in three replications. The Onion bulbs are cultured on the pesticide solution and allowed to grow. The study was carried out on the root tips of well grown to 1-2 cm of *A. cepa* L. The roots are excised and stored in Carnoy’s fluid. The roots with root tips were cut carefully and placed at centre of the slide. Acetocarmine stain was added to the root tips and allowed for absorbtion of stain. The tips with stain were heated gently and allowed to cool. Carefully a cover slip was placed over the root tips and squashed gently without breaking the cover slips. Place thumb finger over cover slips and gently press until the cells were separated sufficiently. The slides were observed under microscope for various abnormalities and recorded. The percentage of mitotic index and frequency of abnormality was calculated as follows.
Determination of Mitotic Index

\[
\text{Mitotic Index} = \frac{\text{Total no. of cells divided}}{\text{Total no. of cells observed}} \times 100
\]

Determination of frequency of abnormality:

\[
\text{Frequency of abnormality} = \frac{\text{No. of abnormal cells dividing}}{\text{No. of cells dividing}} \times 100
\]

RESULTS AND DISCUSSION

The pesticide Phoskill belongs to Monocrotrophos (Organophosphorus) was taken for the present study on the root tips of *A. cepa* L. A variety of abnormalities was observed at chromosomal level by the treatment of pesticide. The concentration ranging from 500-2000ppm resulted in drastic change in the behavior of chromosomes. Significantly the mitotic index was reduced when compared with control. The maximum concentration 2000ppm was found to be highly toxic to the cells and becomes lethal to the roots, so the root initiation was poorly observed beyond 2000ppm. Most of the chemicals have been reported to have detrimental effect on the plants (Fuji and Inoue, 1983; Feretti *et al.*, 2007). The table 1 shows that the percentage index mitotic index in 2000ppm concentration was obtained as 23.84 whereas the control has represented as 76.00. Similarly the chromosomal abnormalities were increased with increase of pesticide concentration. The percentage of frequency of abnormal cells among the observed cells was higher (83.87) at 2000ppm concentration whereas the control showed only 1.47 of frequency of abnormalities. The abnormalities are supported by many workers and suggested that lowering of Mitotic index in treated root meristem could be due to inhibiton of DNA synthesis (Sudhakar *et al.*, 2001.) one or more mitotic phases arrested (Kabarity and Mallalah 1980). The inhibition of mitotic index due to chemicals or pesticides with the interference of normal process of mitotic cell division by reducing the normal cell division (Ghareeb and George, 1997; Badr 1983) Similar depressed activity of mitosis due to interruption of protein synthesis (Kim and Bendixen, 1987).

The chromosomal abnormalities induced by the pesticide Phoskill in the meristematic cells of *Allium cepa* L. has been represented in table. The different types of chromosomal abnormalities, *viz.*, clumping, laggards, elongated nucleus, DNA vacuolation, diagonal anaphase and metaphase, endomitosis, chromosomal bridges, precocious movement, binucleate cells and disorientation of chromosomes were observed. All the above disturbances is due to change in nucleic acid metabolism of the cell or dissolution of protein
covering the DNA in chromosomes (Mercykutty and Stephen, 1980). Appearance of bridges at various stages of cell division is due to general thickness of chromosomes (Nahla and Soliman, 1980).

As an extension of abnormality stickiness the Lagging chromosome was observed at higher concentration. It showed that abnormality is strictly oriented with chemical interference on the cell cycle. The lagging behavior also might be due to the hindrance of prometaphase movement of chromosomes accompanied by adhesion of centrometre to the nuclear membrane Nagpal and Grover, 1994 and Ajay and Sharboy, 1988. Diagonal orientation was due to a slight tilt in th spindle apparatus (Renjana et al., 2013) In Prophase, three abnormalities were predominantly observed such as endomitosis, elongated nucleus and DNA vacuolation was observed. In Metaphase, the abnormalities such as clumping, diagonal metaphase and disorientation of chromosome was observed. In Anaphase the abnormalities such as laggards, bridges, diagonal anaphase and precocious movement were observed. During telophase the abnormalities such as binuclete condition and disorientation of chromosomes was found to be more frequent. Disorientation of chromosome was not observed in control and 500ppm. It may be formed by improper functioning of spindle fibre formation and failure of cell plate formation during the process of cell division reported by Fenech and Crott 2002. The disorientation of and irregular arrangement of chromosomes is due to failure of spindle apparatus to organize and function in a normal way (Grant, 1978; Mansar 1984).

Precocious chromosome and arms formation could be caused by stickiness of chromosomes reported by (Kaur and Grover, 1985). The other related abnormality chromosomal bridges was reported by Tomkins and Grant 1972. Hence the pesticide Phoskill was considered as highly toxic.
Table 1: showing the percentage of chromosome abnormality due to effect of pesticide Phoskill on the root tips of *A. cepa* L.

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Conc. of pesticide Phoskill (PPM)</th>
<th>Mitotic index (%)</th>
<th>Frequency of abnormality (%)</th>
<th>Endo-mitosis (%)</th>
<th>Elongated nucleus (%)</th>
<th>DNA vacuolation (%)</th>
<th>Clumping (%)</th>
<th>Diagonal metaphase (%)</th>
<th>Disorientation (%)</th>
<th>Laggards (%)</th>
<th>Bridges (%)</th>
<th>Diagonal anaphase (%)</th>
<th>Precocious movement (%)</th>
<th>Binucleate condition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>76.00 ± 2.5</td>
<td>1.47 ± 0.28</td>
<td>0.31 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.52 ± 0.02</td>
<td>_</td>
<td>0.57 ± 0.04</td>
<td>0.22 ± 0.06</td>
<td>_</td>
<td></td>
<td>0.21 ± 0.06</td>
<td>0.21 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>59.58 ± 1.00</td>
<td>10.57 ± 0.19</td>
<td>1.95 ± 0.02</td>
<td>2.29 ± 0.42</td>
<td>4.02 ± 0.02</td>
<td>_</td>
<td>1.08 ± 0.55</td>
<td>0.48 ± 0.10</td>
<td>0.12 ± 0.07</td>
<td>0.22 ± 0.07</td>
<td>0.57 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>42.13 ± 2.00</td>
<td>22.63 ± 0.24</td>
<td>3.01 ± 0.03</td>
<td>6.98 ± 0.02</td>
<td>8.43 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>1.08 ± 0.55</td>
<td>0.48 ± 0.10</td>
<td>0.12 ± 0.07</td>
<td>0.84 ± 0.08</td>
<td>1.20 ± 0.14</td>
<td>0.60 ± 1.87</td>
<td>0.84 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>1500</td>
<td>35.90 ± 2.5</td>
<td>26.58 ± 0.20</td>
<td>3.79 ± 0.02</td>
<td>13.92 ± 0.06</td>
<td>11.39 ± 0.02</td>
<td>0.50 ± 0.06</td>
<td>1.89 ± 0.26</td>
<td>0.88 ± 0.06</td>
<td>0.37 ± 0.06</td>
<td>1.51 ± 0.09</td>
<td>1.64 ± 0.06</td>
<td>1.13 ± 0.05</td>
<td>1.89 ± 0.16</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>23.84 ± 1.5</td>
<td>83.87 ± 1.11</td>
<td>8.06 ± 0.03</td>
<td>32.25 ± 0.15</td>
<td>24.19 ± 0.03</td>
<td>0.80 ± 0.05</td>
<td>3.22 ± 0.34</td>
<td>1.45 ± 0.36</td>
<td>0.64 ± 0.05</td>
<td>3.87 ± 0.10</td>
<td>3.22 ± 0.55</td>
<td>2.25 ± 0.21</td>
<td>3.22 ± 0.45</td>
</tr>
</tbody>
</table>

a. Chromosomal bridges  
d. Precocious movement  
b. Diagonal metaphase  
e. Disorientation  
c. Clumping  
f. DNA Vacuolation
The chromosomal abnormalities observed at different concentration of Pesticide Phoskill.

a. Diagonal anaphase d. DNA Vacuolation
b. Laggards e. Clumping
c. Diagonal metaphase f. C-Mitosis

CONCLUSION
The present investigation revealed that the insecticide Phoskill exerts mitodepressive effect and related abnormalities found to be more predominantly occurring on the root tips of Allium cepa L. Hence the pesticide application must be strictly dose oriented in unavoidable situations. Further avoiding the chemical pesticides in the field of agriculture leads to creating a pollution free generation. The continous use of these hazardous chemicals will create a irreversible cytogenic effects not only in plants but also in higher organisms. Finally the work insists on importance of organic or natural farming should be followed in agriculture.

REFERENCE