Full Length Research Paper

Effects of organic insecticides, Kingbo and Azdar 10 EC, on mitotic chromosomes in root tip cells of *Allium cepa*

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In this study, two organic insecticides (Kingbo and Azdar 10EC) extracted from plants and used as agricultural pesticides were investigated for cytotoxic and genotoxic effects using root tips of *Allium cepa* assay. Three different concentrations (0.625, 1.62 mL.L⁻¹ and 2.5 mL.L⁻¹) were used for different periods of time (8, 16 and 24 h). A single treatment of the effects of the two organic insecticides was used. The tested concentrations decreased the mitotic index compared to the control; 0.625 mL.L⁻¹ of Kingbo treatment was statistically significant, while that of 1.62 mL.L⁻¹ for 16 h and 2.5 mL.L⁻¹ for 8 and 16 h increase the mitotic index. This increase was significant. Single treatment of Azdar 10EC decreased the mitotic index and its effect was non-significant compared to the control, while 2.5 mL.L⁻¹ treatment for 24 h increased the mitotic index and was statistically significant. In addition, the different treatments caused diverse types of chromosome abnormalities during metaphase, anaphase and telophase stages, and they were statistically significant after treating with 0.625 and 1.62 mL.L⁻¹ Kingbo for 8 and 16 h and 0.625 mL.L⁻¹ Azdar 10 EC for 8 h. The chromosomes abnormalities were stickiness, disturbance, c-metaphase, c-anaphase, stare metaphase, chromosome bridges in anaphase and telophase, lagging chromosome and micronuclei appearing in interphase cells. The result indicates that both organic insecticides had cytotoxic and genotoxic activities on mitotic index and chromosomal aberration.

**Key words:** Organic insecticides, mitotic chromosomes, root tips, *Allium cepa*.

INTRODUCTION

The use of plant material or crude plant extracts for the protection of crops and stored products from insect pests is probably as old as crop protection itself (Thacker, 2002). Cytogenetic effects of synthetic chemical used for plant protection have been well documented and previously investigated by many authors (Qureshi et al., 1988; Vyuyan, 2002; Mekki, 2008). Almost all studies confirm the harmful effects of synthetic chemicals used in agriculture and increase in environmental pollution, which is a global problem (Soliman, 2010).

Some plants contain components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanical pesticides or botanicals (Malkhan et al., 2012). The secondary compounds of plants are a vast repository of compounds with wide range of biological activities. Unlike compounds synthesized in laboratories, secondary compounds from plants are virtually guaranteed to have biological activity and that activity functions highly in protecting the producing plant from a pathogen, herbivore or competitor (Duke, 1990).

Yet, many reports reveal that drugs of plant origin are...
genotoxicity to measure their harmful effects on chromosomes during the M-phases of cell cycle that may lead to death of cells. Root tips of A. cepa were used as an experimental material.

MATERIALS AND METHODS

Tested materials

Kingbo is a botanical insecticide, a product of Beijing Kingbo Biotech Company Limited., China. It is extracted from wild medical plant, *Sophora flavescens* Ait and natural oils. Its effective ingredient is 0.6% Matrine C_{15}H_{24}N_{2}O, with mol. wt. of 248.36 (Figure 1). It can be used on a wide variety of plants such as vegetables, fruit trees, shrubs, grapes, grasses and flowers, and its recommended dosage is 2 – 2.5 mL.L^{-1}. Azdar 10EC is also a botanical insecticide, a product of T.Stanes & Company Limited., India; it is a tetratorsterpinoid isolated from the seeds of the neem tree, *Azadirachta indica* A. juss. Its effective ingredient is Azadirachtin A (10 g/L) C_{27}H_{46}O_{16}, with mol. wt. of 720.73 (Figure 2). It is used for whitefly, thrips, cutworms, aphids, scale insects, bollworm, citrus and tomato leaf miner; its recommended dosage is 2 – 2.5 mL.L^{-1}.

Sample preparations

Plant test system is widely used for monitoring genotoxicity of chemicals due to many advantages, such as low cost, availability throughout the year, ease of handling, good chromosome condition for the study of chromosome damage and good correlation with other test systems (Sobita and Bhagirath, 2005). In this study, *A. cepa* (2N = 16) root tips were used as an experimental material. Healthy onion bulbs were obtained from local markets. The loose outer scales and old roots of the onion were scraped and suspended in small beaker with distilled water.

Treatments

*A. cepa* was suspended in a small beaker (50 ml) with distilled water to enhance the growth of the root tips until they reach 0.5 - 1 cm in length; then it was transferred to another beaker containing freshly prepared solution of botanical insecticides and left for different periods of time. Three different concentrations for three different periods of time were used, and one bulb of onion was used for each treatment.

Negative control for each time was used; the root tips of *A. cepa* were treated with distilled water only, and used as a comparative sample for the effects of tested organic insecticides.

Three different concentrations (0.625, 1.62 and 2.5 mL.L^{-1}) were prepared to investigate the effect of the recommended dosage, half and quarter of the recommended dosage for both insecticides. Single effect of the insecticides was examined.

Slides preparation

The roots were treated with different concentrations (0.625, 1.62 and 2.5 mL.L^{-1}) for different periods of time (8, 16, 24 h), then the roots were detached, fixed in freshly prepared 3:1 (v/v) ethanol alcohol and glacial acetic acid for 24 h. Root tips of *A. cepa* were hydrolyzed in 1 N HCL at 60 degrees centigrade for 8 min; roots tips were then washed with distilled water several times and stained with 1% aceticarmin. Five temporary slides were prepared using the squash technique. Two root tips on each slide were examined for the effects of botanical insecticides on mitotic index (MI). The

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**Figure 1.** Chemical structure of matrine *merck index, eleventh Edition*.  
**Figure 2.** Chemical structure of Azadirachtin* merck index, eleventh Edition*.  

not free from toxic effects (Sousa et al., 2009). A number of plant extracts have been reported to have antimitotic and chromosome damaging properties. Yadav and Rathore (1984) investigate the aqueous fruit extracts of *Emblica officinalis*; Sobita and Bhagirath (2005) investigate the effect of extracts of *Nerium odorum, Andrographis paniculata, Nytanthes arbortis-lis, phlogacanthus thrysiflorus, Solanum indicum* and *Kaempferia galangal*; Mondal et al. (2006) investigated the effect of different *Ipomosea* species; Adebibe and Sanyaolu (2009) investigated the effect of *Vernonia amygdalina* Del. Leaf; Surendrajit and Bhagirath (2011) investigated the aqueous effect of *Croton caudatus* Geiseler leaves; Tülay (2012) investigates the effect of plant extracts; Al-Ahmadi (2013) investigated the effects of organic insecticides (Baicao No.2 and Baicao No.3) extracted from plants.

Regarding plant bioassay, *Allium cepa, Lactus sativa, Zea mays* and *Vicia faba* have been the most common species used for cytogenotoxicity evaluation. These species also show high sensibility to toxic compounds and they do not have small chromosomes (Singh and Das, 2002; Lubini et al., 2008; Sousa et al., 2009).

In this study, two botanical insecticides (Kingbo and Azdar 10 EC) were investigated for their cytotoxicity and
Table 1. Total number of examined cells, mitotic index and aberration frequency after treatment with different concentrations of organic insecticides for different periods of time on cells of root tip of Allium cepa.

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (ML/L)</th>
<th>Treatment/hour</th>
<th>Number total cells</th>
<th>Divided cell</th>
<th>Total number of abnormal cell</th>
<th>Mitotic index</th>
<th>Chromosomal Aberration frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>d.water</td>
<td>8</td>
<td>2062</td>
<td>218</td>
<td>13</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>d.water</td>
<td>16</td>
<td>2316</td>
<td>215</td>
<td>4</td>
<td>9.3</td>
<td>0.04</td>
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<tr>
<td></td>
<td>d.water</td>
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<td>2217</td>
<td>302</td>
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<td>13.6</td>
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</tr>
<tr>
<td></td>
<td>0.625</td>
<td>8</td>
<td>2019</td>
<td>139</td>
<td>25</td>
<td>6.9*</td>
<td>0.18*</td>
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<tr>
<td></td>
<td>0.625</td>
<td>16</td>
<td>2156</td>
<td>157</td>
<td>13</td>
<td>7.3</td>
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<td></td>
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<td>2153</td>
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<td>8.6</td>
<td>0.13</td>
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<td></td>
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<td>16</td>
<td>2151</td>
<td>233</td>
<td>24</td>
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<td>1.62</td>
<td>24</td>
<td>2234</td>
<td>171</td>
<td>30</td>
<td>7.7</td>
<td>0.18</td>
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<td></td>
<td>2.5</td>
<td>8</td>
<td>2501</td>
<td>266</td>
<td>17</td>
<td>10.6*</td>
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<td>2.5</td>
<td>16</td>
<td>2151</td>
<td>230</td>
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<td>10.7*</td>
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<td>176</td>
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<td>13</td>
<td>6</td>
<td>0.09</td>
</tr>
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<tr>
<td></td>
<td>2.5</td>
<td>24</td>
<td>2203</td>
<td>326</td>
<td>26</td>
<td>14.8*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Significant at 0.05.

same slides were analysed for the types and frequencies of chromosomal abnormalities (CF) produced by the examined insecticides.

Scoring of slides and data analysis
The slides were viewed under light microscope (Phenix P H 50 DB047VU) using the 40X objective lens immersion. The most representative ones for each structural aberration were photographed using Phenix micro Image analyzer Software 2008 En V2, 2.

Mitotic index
On one slide for each treatment, a total of 2000 cells were scored. The mitotic index (MI) was expressed as the number of dividing cells per total cells scored, as seen in the following equation:

\[
\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cell}}{\text{Total number of cell examined}} \times 100
\]

Cytotoxicity
The mitotic index of the treated cells at each dose of each insecticide was compared with that of the negative control using the SPSS 16.0 for Windows statistical package. Two-way Analysis of Variance was the statistical method used for determining the significance of difference at \( P = 0.05 \).

\[
\text{Chromosomal aberration frequency (CF)} = \frac{\text{Total number of abnormal cell}}{\text{Total number of divided cell}}
\]

RESULT AND DISCUSSION
Mitotic index (MI)
Table 1 and Figures 3 and 4 show the data of total cells counted in microscopic observations. Mitotic index (MI) which measures the proportion of cells in the M-phase of cell cycle inhibition could be considered as cellular death or delay in the cell proliferation kinetics (Rojas et al., 1993; chromosomal aberration frequency (CF) that reveals chromosomes aberrations was observed after treatment with tested material.

The mitotic index of root tip cells that were treated with different concentrations of Kingbo and Azdar 10EC (0.625, 1.62 and 2.5 mL.L\(^{-1}\)) for different periods of time (8, 16 and 24 h) decreased compared to that of the negative control (root tips treated with distilled water only). Root tips treated with Kingbo show increase in (MI) after
A toxic cycle and toxicity of the tested extracts might interact with DNA subsequent mitotic accumulation of interphase cells may be due to the increased number of interphase or dead cells and the decreased mitotic index of root tip cells of A. cepa. Inhibition of cytokinesis was also reported by Njagi and Gopalan (1980) and Sobita and Bhagirath (2005).

These effects on mitotic index indicate a potential mito-depressive that leads to cytotoxic effects. Similar inhibition of cytokinesis was also reported by Borah and Talukdar (2002).

The increase of mitotic index may result from shortening the duration of mitotic cycle and allowing the interphase cells enter the subsequent division stages. Haroun and Al Shehri (2001), Haroun (2010) and Abderrahman et al. (1997) found out that treatment with *Peganum harmala* extraction increased the mitotic index of root tip cells of *A. cepa*.

**Chromosomal aberration (CA)**

*Allium cepa* assay is a sensitive test, and it has been shown to have correlation with tests in other living systems and serves as an indicator of toxicity of the tested material (Fiskesjo, 1985). Chromosomal aberrations (CA) are changes in chromosome structure that results from a break or exchange of chromosomal material (Swierenga et al., 1991).

Sobita and Bhagirath (2005) reported that chromosomal aberrations that resulted from different treatments indicate a clastogenic effect of the tested materials. Table 2 and Figures 5 and 6 show the result of single effect of the two organic insecticides on chromosomes of root tip cells of *A. cepa*.

Even though the control treatment had no tested concentrations of the insecticides, the apical meristem showed cytological abnormalities with low frequency. This might be due to the auto- mutagenic substance (Dubinin and Scerbako, 1962; Kaushik, 1996). Teas et al. (1965) suggested that as seedlings roots increase in length, aberrations are less likely to continue in mitosis and when root becomes 2 to 3 cm in length, the aberration caused in control condition becomes insignificant, which means increase in mitotic index of control.

Cytological observation (Figure 7) indicates that all the tested concentrations of the two organic insecticides (0.625, 1.62 and 2.5 mL.L⁻¹) cause chromosome abnormalities mostly during metaphase, anaphase and telophase stages. They were statistically significant after treating with 0.625 and 1.62 mL.L⁻¹ Kingbo for 8 and 16 h and 0.625 mL.L⁻¹ Azdar 10 EC for 8 h. Most types of chromosome aberrations observed in high percentage were stickiness, disturbance, c-metaphase, chromosome bridges in anaphase and telophase, lagging chromosome and micronuclei appearing in interphase cells; while s-metaphase, s-anaphase and fragments were observed in low percentage.

Treatment with Kingbo (0.625 and 1.62 mL.L⁻¹) caused high chromosome frequency after 8 and 16 h of exposure; also treatment with Azdar 10 EC (0.625 and 2.5 mL.L⁻¹) for 8 and for 16 h caused high chromosome aberration frequency compared to the control.

Chromosome stickiness means loss of normal appea-
Table 2. Type of chromosomal aberrations on root tip cells of *A. cepa* after treatment with different concentrations of the tested organic insecticides for different periods of time.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Concentration (mg/ml)</th>
<th>Duration of time (h)</th>
<th>AF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Distilled water</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>4</td>
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<td></td>
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<td>24</td>
<td>2</td>
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<tr>
<td></td>
<td>0.625</td>
<td>16</td>
<td>18</td>
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<td></td>
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<tr>
<td>Kingbo</td>
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<tr>
<td>Azdar 10 EC</td>
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<td>16</td>
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<td></td>
<td></td>
<td>24</td>
<td>12</td>
</tr>
</tbody>
</table>

Sticky, and it is seen with sticky surface, causing chromosomes agglomeration (Babich et al., 1997). It might be due to the effect of pollutants and chemical compounds on the physical-chemical properties of DNA, protein or both, the formation of complexes with phosphate group in DNA, DNA condensation or formation of inter and intra chromatid cross links (Shahin and El-Amoodi, 1991; Turkogul, 2007; Tülay and Özlem, 2010). Also stickiness might be due to the dimerization and partial dissolution of nucleoproteins, breakage and exchange of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes (Onyenwe, 1983). Disturbance during metaphase and anaphase, star metaphase, star anaphase arises because of the effect of the treatment on the spindle that leads to failure of spindle mechanism (Yadav, 1986). Khakdan and Piri (2012) suggested that plant extracts act as a toxic agent on formation of the mitotic spindle, with the chemical action...
Boerhaavia diffusa had a nucleotoxic action.

Chromosomes bridge during anaphase and telophase raises when the chromosomes fail to separate because of chromosomes stickiness Yadav (1986). Chromosome fragment is an indication of chromosome break, and can be a consequence of anaphase/telophase bridges (Singh, 2003). Also, Darlingin (1942) reported that stickiness may result in fragmentation of chromosomes from the stress of anaphase movement or in the bridge formation when the chromosomes fail to separate.

C-metaphase (colchicine metaphase) appeared because of the inactivation of the spindle followed by a random scattering of the chromosomes over the cell (Levan, 1938; Auti et al., 2010). Micronuclei (MN) often result from the acentric fragments or lagging chromosomes that fail to incorporate into daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes (Albertini et al., 2000; Krishna and Hayashi, 2000; Tülüay and Ozlem, 2010).

**Conclusion**

The result of this study indicates that the single treatment of these two organic insecticides (Kingbo and Azdar 10 EC) decreased the mitotic index and was statistically significant. Still, some examined concentrations of the investigated insecticides caused an increase in the mitotic index. Furthermore, different treatments of the organic insecticides caused chromosomes abnormalities such as stickiness, disturbance, chromosome bridges on anaphase and telophase stage, lagging and fragments. Decrease in the mitotic index and increase in the chromosomal aberration frequency indicates that they had a cytotoxic effect on cells division; chromosomes abnormalities indicate that the two insecticides have a clastogenic property that leads to genotoxic effects. In addition, Kingbo was more effective in mitotic index and chromosomes abnormalities than Azdar 10EC.

**REFERENCES**


