Evaluation mutagenic potential of pesticides through bioassays with *Allium cepa*

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The evaluation mutagenic potential of pesticides most used in southeastern Brazil, through bioassays with *Allium cepa*, it was an important study to understand harmful action of two classes of pesticides widely used in Brazil. The effects significant of cytotoxic and genotoxic in *Allium cepa* were evaluated in the following concentrations: 10 μL/mL, 25 μL/mL and 50 μL/mL, for Pyraclostrobin and Iminoctadine being in all cases compared to the negative and positive controls. With the results obtained it was possible to verify that the two pesticides have the ability to promote genetic changes. This study is a consequence of numerous complaints about harmful effects these substance, since small changes in DNA can cause irreversible problems the human health.


**INTRODUCTION**

The pesticides has being used in large scale in pest control in modern agriculture, the reason of this fact is the increase in productivity obtained with these substances. But indiscriminate use of these agents may bring harm to the environment and human health. In 2010 the Brazilian National Agency for Sanitary Surveillance conducted study in 26 states, noting high indices of pesticides in various types of foods marketed (Freire et al. 2013; Faria et al. 2014). As an example the state of *Espírito Santo*, southeastern Brazil, showed dissatisfaction in 26.47% of the samples on the level of pesticide. Organophosphate pesticides appear as the main chemical group responsible for this unsatisfactoriness (Chrisman et al. 2009; Boccolini et al. 2013).

It is believed that with the growth of agriculture in the state, the need for higher yields, fewer losses for pests and good results with positive balance motivates the irregular use of these substances. Thus a farmer resorts to using products and techniques to improve their production. Therefore makes use of pesticides in order to reduce costs, not checking the risks and operational security required for this type of work (Chrisman et al. 2009).

Organophosphates are chemicals that having the element phosphorus in its formation. Being widely used in agriculture as insecticides, pesticides and herbicides, with highlight between the pesticides that have the ability to react with Acetylcholinesterase. Causing decomposition of acetylcholine after transmission of nerve impulses from one neuron to another, causing the accumulation of acetylcholine in the synaptic receptors blocking the nerve transmissions (Tušarová et al. 1999; Smulders et al. 2003). In 1989 was enacted law nº 7802 which provides for research, experimentation and monitoring of pesticides, their components and related products. Law that guides and classifies all the harmful actions of these substances to human life, this being a major reference used in Brazil (Recena et al. 2006; Soares and Porto, 2009).

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Due to various problems caused by incorrect use of pesticides, several tests being conducted with the goal of check structural DNA damage (Burrack and Chapman, 2013; Sparks, 2013), among them is the test of micronucleus (Mn) (Rodríguez et al. 2015). This test consists in the investigation of cells previously exposed to chemical agents, aiming to detect possible chromosomal aberrations, micronuclei seen that are formed by the extrusion of whole chromosomes or from fragments during cell division (Ginzkey et al. 2014; Sanada et al. 2014; Rodriguez et al. 2015). An important feature of the micronucleus test is the ability to express chromosome damage when exposed to chemical agents indicating loss, breakage or presence of micronuclei. In several studies the test proved great effectiveness and reliability, besides presenting some advantages over other tests such as low cost and speed of analysis (Ayed-Boussema et al. 2011). Thus, the aim of this study was to evaluate the mutagenic potential of pesticides in use in southeastern Brazil through bioassay with Allium cepa to check the mutagenic effect of different concentrations of pesticides on the cells and what the possible relationship of concentration of the pesticide to abnormalities in cell division.

### MATERIALS AND METHODS

For the tests were selected two types of very pesticides currently marketed. Table 1 shows the main features of these substances used. The bulbs of Allium cepa were obtained commercially and in all bioassays biotypes of the same species and origin were used. The Iminocadine (Albesilate) is a contact fungicide chemical group of the guanidines which operates mainly in the biosynthesis of cell membrane lipids. Composed by: 1,1-imino(octamethylene) diguanidinium tris (alkylbenzenesulfonate). The Pyraclostrobin acts as an inhibitor of electron transport in the mitochondria of the cells of fungi by inhibiting the formation of ATP. Is essentially to metabolic processes in fungi. Composed by: Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl}phenyl)N-methoxycarbamate.

The present study, a bioassay with Allium cepa, in which 16 examples were placed in distilled water for 24 hours at room temperature, to stimulate the development of root meristem was performed. After this time, the bulbs were placed in test solutions for a period of 48 hours. It is noteworthy that all the experiments were done in duplicates and the blades of analysis were examined in blind test. For each slide 2000 cells were counted by identifying changes mitotic and micronuclei according to the criteria proposed by Titenko Holland et al. (1997) (Titenko-Holland et al. 1997). For positive control, Allium cepa biotypes were exposed to the copper sulphate (CuSO₄), since it has a mutagenic potential proven in the literature and as negative control, biotypes were exposed to distilled water. The roots length was used as an assessment of mitotic index as the reference standard, these case more precisely three roots were used. For each bulb, the length of the three longest roots was estimated with a ruler. In each case treatment was compared with negative control. Moreover the toxicity (growth inhibition) was defined as the difference between treatment and negative control was statistically significant. Data were statistically analyzed using the software ANOVA with significance level of 5%. Was used for perform linear regression the software BioEstat. ver. 5.0, being possible to confirm the results obtained from the ANOVA.

Methodology used to assess the formation of micronucleus is described below, being similar the proposed in another papers (Garriott et al. 2002; Kato et al. 2011):

1. An onion was placed in contact with distilled water so that the region where there was the formation of onion roots of ringtone the solution;

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### Table 1. Pesticides used for assessment of cytotoxicity and genotoxicity in Allium cepa.

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Trade name</th>
<th>Chemical Group</th>
<th>Class</th>
<th>Toxicological class</th>
<th>chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iminocadine (Albesilate)</td>
<td>Bellkute</td>
<td>Guanidines</td>
<td>Contact fungicide</td>
<td>Class I</td>
<td>Inert: Albesilate: 315g/L (31.5%m/v)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inert ingredients: 735g/L (73.5%m/v)</td>
</tr>
<tr>
<td>Piraclostrobin</td>
<td>Comet</td>
<td>Estrobutelines</td>
<td>Systemic fungicide</td>
<td>Class II</td>
<td>Piraclostrobin: 250 g/L ou 25.0% m/v</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inert ingredients: 802 g/L ou 80.2% m/v</td>
</tr>
</tbody>
</table>
Figure 1. Chromosomal abnormalities observed in meristematic cells of *Allium cepa* exposed to pesticides, photos taken at the time of analysis of biotypes (Viewed in 40x). A) Prophase, Anaphase and Telophase, normal processes. B) Chromosomes Laggards. C) Chromatid loose. D) Accessory Chromosome (micronucleus). E) Chromosome bridge. F) Chromatid and loose nuclear Defragmentation

Table 2. Analysis of root length Pyraclostrobin treated.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C-</th>
<th>C+1</th>
<th>C+2</th>
<th>10 μL/ml</th>
<th>25 μL/ml</th>
<th>50 μL/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>root length (cm)</td>
<td>4.0 ± 0.7</td>
<td>1.8 ± 0.4*</td>
<td>1.6 ± 1.3*</td>
<td>2.0 ± 0.7*</td>
<td>1.1 ± 0.6*</td>
<td>1.8 ± 0.4*</td>
</tr>
<tr>
<td>% growth</td>
<td>100</td>
<td>45*</td>
<td>40*</td>
<td>50*</td>
<td>28*</td>
<td>45*</td>
</tr>
</tbody>
</table>

*Significantly different value of the negative control. (p<0.05)

Legend: C-: Negative Control; C+1: Positive Control 1; C+2, Negative Control 2.

RESULTS AND DISCUSSION

The tests of cytotoxicity and genotoxicity were performed by the *Allium cepa* test based on some parameters of analysis, such as atypical chromosomal patterns, which consist of fragmentation, retardation and chromosomal bridge. Another aspect studied was the appearance of micronuclei formed by result of chromosomal breakage, clearly indicating the disturbances in the mitotic process. We also evaluated the appearance of the root length, biotypes established as control parameter, thus demonstrating the action of pesticides on cell mitosis.

Of the 16 analyzed biotypes, abnormalities were found in 94.4%. Root length showed significant differences compared to the negative control. The fact proves effective action of the pesticide in the phase of cell proliferation. Each sample to be analyzed was prepared in two blades each having a root. Thus it was possible to estimate the number of micronuclei and abnormalities (laggard chromosomes, chromosome bridges and fragments). This experiment was conducted in 2,000 cells, in Figure 1 it is possible to verify some of obtained results, a fact that demonstrates the feasibility of the study.

Bioassays with Pyraclostrobin concentrations of 10 μL/mL, 25 μL/mL and 50 μL/mL were used there was a significant reduction in root growth (Table 2) (p <0.05).
Table 3. Analysis of abnormalities visualized in anaphases / telophases roots treated with Pyraclostrobin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C-</th>
<th>C+1</th>
<th>C+2</th>
<th>10 µ/mL</th>
<th>25 µ/mL</th>
<th>50 µ/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Fragment</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Latecomer</td>
<td>0</td>
<td>46</td>
<td>27</td>
<td>1</td>
<td>86</td>
<td>27</td>
</tr>
<tr>
<td>% Bridge</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Micronuclei</td>
<td>0</td>
<td>10*</td>
<td>12*</td>
<td>0.5</td>
<td>0.4</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Significantly different value of the negative control. (p<0.05)
Legend: C-: Negative Control; C+1: Positive Control 1; C+2, Negative Control 2.

Table 4 - Analysis of root length treated with Iminoctadine Tris (Albesilato).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C-</th>
<th>C+1</th>
<th>C+2</th>
<th>10 µ/mL</th>
<th>25 µ/mL</th>
<th>50 µ/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length (cm)</td>
<td>4.0 ± 0.7</td>
<td>1.8 ± 0.4*</td>
<td>1.6 ± 1.3*</td>
<td>1 ± 0.6*</td>
<td>0.6 ± 0.1*</td>
<td>0.4*</td>
</tr>
<tr>
<td>% Growth</td>
<td>100</td>
<td>45*</td>
<td>40*</td>
<td>25*</td>
<td>15*</td>
<td>10*</td>
</tr>
</tbody>
</table>

*Significantly different value of the negative control. (p<0.05)
Legend: C-: Negative Control; C+1: Positive Control 1; C+2, Negative Control 2.

Table 5. Analysis of abnormalities seen on the roots telophases anaphases and treated with Iminoctadine tris (Albesilato).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C-</th>
<th>C+1</th>
<th>C+2</th>
<th>10 µ/mL</th>
<th>25 µ/mL</th>
<th>50 µ/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Fragment</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>% Latecomer</td>
<td>0</td>
<td>46</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Bridge</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>% Micronuclei</td>
<td>0</td>
<td>10*</td>
<td>12*</td>
<td>0.6</td>
<td>1.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*Significantly different value of the negative control. (p<0.05)
Legend: C-: Negative Control; C+1: Positive Control 1; C+2, Negative Control 2.

The analysis of root length showed a reduction of 50%, 72.5% and 55% at concentrations exposed 10 µL/mL, 25 µL/mL and 50 µL/mL respectively compared to the negative control. The statistical test showed significant differences at all concentrations tested.

The analysis of the abnormalities observed in dividing cells revealed a high percentage of laggard chromosomes (Table 3), more precisely in anaphase/telophase in the 25 µL/mL. The presence of forty-four micronuclei in the concentration of 10 µL/mL, thirty-one micronucleus concentration of 25 µL/mL and center and forty-three in the concentration of 50 µL/mL were quantified.

In tests conducted with Iminoctadine Tris (Albesilate) concentrations of 10 µL/mL, 25 µL/mL and 50 µL/mL were used. All concentrations tested had reduced root growth (Table 4) in the rates of 75%, 85% and 90% lower than the negative control.

The analysis of abnormalities showed a significant increase in micronuclei concentration of 50 µL/mL (Table 5). It was also noted the presence of chromosomal fragmentation proportionally increased concentration: 17%, 20% and 30% for the concentration of 10 µL/mL, 25 µL/mL and 50 µL/mL.

The result of the micronucleus test samples were made
from the bioassays with Pyraclostrobin and Iminoctadine Tris (Albesilato). These tests were significantly different from the negative control regarding the amount of micronuclei. In addition, the other changes in the genetic material can be seen in the Figure 2.

The morphological changes occurred in chromosomal level, as well as degradation of the nucleus. This study showed that different dosage in exposure periods miscellaneous can promote great changes in the DNA structure, therefore can lead to mutagenic effects in live organisms. Similar study were conducted with rodents and obtained similar results (Kligerman et al. 1993; Taneja, 2000; Oliveira et al. 2012). With morphological changes in chromosomes due to action of the pesticides it is can say that incorrect exposure promote DNA mutations. Issues that may affect the person who comes into direct contact with this product and your successors. The root irregular growth was altered with exposure dose of the pesticide being inversely proportional, that is, the higher the dose, the lower were growth. Thus, it is believed that exposure to pesticides also generates impractical economic effects, because the root growth of cells demonstrates the difficulty in reproducing biotype and also alter your growth.

The analyzes of changes in the mitotic index proved the formation of micronuclei becoming evident that the results are according to dosage applied. However, we note that in the second addition the process grows cell defragmentation until it enters the third phase dosage (higher concentration). In the higher concentration there is a decrease in defragmentation process, perhaps by a possible attempt to repair. Higher doses showed large amounts of micronuclei formed, as well as chromosomal fragments, compared as negative controls. There by it was possible to check the action of pesticides on mitotic rate, formation of nuclei, as well as the size of the root. Thereby significant differences were observed between the systems studied relative the control group, thus indicating that workers exposed to pesticides are subject to the actions these chemical agents. Such behavior have been highlighted by other studies, showing the harmful effects of pesticides.

The Pyraclostrobin (N-methoxy carbamate) is an effective systemic fungicide which acts as an inhibitor of electron transport in the mitochondria of the cells of fungi inhibiting the formation of ATP. In general, the known data show a low acute and chronic toxicity of pyraclostrobin and your concentration normally it is within the standards set by Brazilian law. According to Ministry of Agriculture, the systemic fungicide, which is the active ingredient pyraclostrobin, it has genotoxic effects in some fish species. In this research the pyraclostrobin significantly different compared to the negative control (p) <0.05, and present an increased mitotic index and laggard chromosomes that were not observed in the positive controls (Table 2).

The Iminoctadine Tris (Albesilato) \((C_{72}H_{131}N_{7}O_{9}S_{3})\) belonging to the chemical group of guanidine, is a fungicide used in crops of coffee, peach and potato. The Iminoctadine Tris (Albesilato) was significantly different compared to the negative control p<0.05, therefore, higher potential for formation of micronuclei present in 67% of analyzed slides (Table 4), in addition to causing a decrease in mitotic index, which was observed in 33% of the blades.

The increased use of agrochemicals has caused environmental and health impacts of the population, mainly professionals working directly with these products (Recena et al. 2006; Soares and Porto 2009). How much of the agriculture in the state comes from family culture, where children still undergoing training assist parents, this problem becomes even more serious because children are more sensitive to genetic changes. This problem becomes more serious considering that treats irreversible problem and which may be passed to the descendants (Fujii and Inoue, 1983; Giri et al. 2002; Benedetti et al. 2013).
CONCLUSION

After adapting and improving of techniques already published, the bioassays revealed that the *Allium cepa* was affected by the action of pesticides containing pyraclostrobin and iminoctadine. The effects significant of cytotoxic and genotoxic were evaluated in the following concentrations: 10 μL/mL, 25 μL/mL and 50 μL/mL, for Pyraclostrobin and Iminoctadine being in all cases compared to the negative control. With the results obtained it was possible to verify that the two pesticides have the ability to promote genetic changes. Thus any manipulation of these substances must be performed with adequate safety equipment. This study besides proving the harmful potential of these substances warn the serious problems that these substances can generate.

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