Cytological effects induced by Agil herbicide to onion

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Abstract The purpose of this study was to investigate in laboratory the cytological effects of Agil herbicide on the onion (Allium cepa) root meristematic cells, in particular the mitotic index and mitotic abnormalities. Cytological experiments were conducted using herbicide concentrations of 0.5 ppm, 1.0 ppm and 1.5 ppm for 8 h, 24 h and 48 hours in the presence of a control for each combination of them. Mitotic index decreased with increasing the herbicide concentration at each exposure time. Also, the higher concentration and the higher exposure time of the Agil herbicide caused an increase of prophase frequency and decrease of telophase frequency. In the same vein, the total chromosomal aberration increased with an increasing the Agil herbicide concentration (the frequency of the chromosomal aberration was markedly higher at 1.5 ppm compared to other test concentrations). The obtained results indicate that Agil herbicide had the ability to cause production of some mitotic abnormalities. These abnormalities appeared in varying degrees depending on the herbicide concentration. Our study reveals a direct correlation between herbicide concentration, exposure time and mutagenic effects observed in exposed Allium cepa cells. We conclude that when applied in high doses, the Agil herbicide shows cytotoxic effects to Allium cepa plants.

Key words

Allium cepa, herbicide, mitotic index, chromosomes

Modern agronomy, plant breeding, pesticides and fertilizers, and technological improvements have sharply increased yields from cultivation, but at the same time have caused widespread ecological damage and negative human health effects. For years, farmers and horticulturists have used weed killers to kill undesirable plants, but weeds have a strong will to survive so they become resistant to many types of industrial weed killer. Making the most of herbicides in today’s environment means taking the time to find the exact agricultural weed killer and using the minimum amount to accomplish the task. Herbicides available to the general public are less likely to do damage to the environment. Pesticides which are used in the modern agricultural practices for disease control have some dangerous effects (4). Genetic changes induced by herbicides, their metabolites and residues are expressed by various endpoints, which include: structural changes in chromosomes and chromatids, called chromosomal aberrations (breaks, deletions, inversions, gaps, translocations, rings) and other disturbances (stickiness, clumping, erosion). Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. For this purpose, the Allium cepa is one of the most frequently used higher plant species. The Allium test for genotoxicity has been used on pesticides in other studies (2). The cytogenetic effects of the herbicide Avenoxan, active substance 2,4-D, were investigated in both Allium cepa and Allium sativum (5).

The herbicide Agil (active substance propaquizafop 100 g/l) has a molecular weight 443.88 g mol⁻¹ and the molecular formula is C₂₂H₂₅ClN₂O₅. Treatments with Agil herbicide is used especially for weed control (during vegetation) in onion (Allium cepa) and garlic (Allium sativum) farms. To avoid undesirable effects, when you choose a herbicide, must by law be considered genotoxic and cytotoxic effects on plants. The repeated and indiscriminate uses in addition to the extreme stability of such pesticides have led to their accumulation in plants, animals, soils and sediments, thus effecting prevailing contamination of the environment.

Material and Methods

The objective of this study was to determine any possible affects of Agil herbicide by using Allium cepa root meristematic cell test, which is one of the reliable genotoxic test used in laboratories. Allium cepa (2n=16) was used as test organism. The experiment was maintained in laboratory conditions at 20±2 °C. First, 12 bulbs (30-35 cm in diameter) of a commercial variety of onion were chosen. Clean and healthy onion bulbs were set up and allowed to produce roots in distilled water for 48 h. After the homogeneously
rooted. 3 onion bulbs remaining in distilled water (the control) and 9 bulbs transferred to the different Agil herbicide solutions (0.5, 1.0 and 1.5 ppm) for 8, 24 and 48 h. The root tips were fixed in 3:1 mixture of ethanol and glacial acetic acid. Next day, the roots were transferred to 70% alcohol and stored in refrigerator until use. Before coloring, the root tips were macerated in a solution of 1N HCl for 5 min and HCl 50% for 16 min; after that, the roots were washed with distilled water. To carry out the chromosomes view using the optic microscope, these were colorized with the Schiff reactive, prepared in the laboratory. The effective colorized operation was the introduction to 3-4 cc colorant solution from onion roots placed in glass ampoules, at room temperature. After max 30 minutes, the meristematic tissues were colored in purple-red. To increase the chromosomes-cytoplasm contrast and the optimal microscopic preparation view, before the study under the microscope the colored roots were kept in a 45% acid acetic solution, for 15 minutes, to remove colorant excess.

Microscopic slides were done by pressing. From this point of view, the slides were prepared by putting a drop of 45% acetic acid on the root tip, placing the cover slip over the material and tapping with a pencil to disperse the cell. Cells division and cytogenetical abnormalities were observed under a MBL-2000 Kruss research microscope, by considering 15 areas per slide. The mitotic index and chromosomal aberrations were investigated in cytogenetic analysis for each concentration and exposure time. The number of abnormal cells was counted in each phase of mitosis. The mitotic index was determined by scoring more than 100 cells per slide and was calculated as the percent ratio of dividing cells and total numbers of cells scored. In chromosomal aberrations test, 50 cells in anaphase were examined for aberrations per slide. The mitotic abnormalities a scored were anaphase bridges and binucleated cells.

The analysis of variance was used to assess the significant differences between control and each treatment. The data obtained from this experiment was analysed by statistically meaningful value was considered as p<0.05.

Results

The effect of contaminant on plants was reported to be depending on the concentration and the kind of contamination. In the current research study, the effect of Agil herbicide on the onion (Allium cepa) root meristematic cells was assessed. Among the broad rage of organic pollutants contaminating soil-water environment, pesticides are of great environmental concern. The use of plants for the evaluation of environmental pollutants such as pesticides is becoming common practice because plants are direct recipients of agrotoxics, so they are important material for genetic test and for environmental monitoring of places affected by such pollutants. Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division (4); inhibition of mitotic activities is often used for tracing cytotoxic substances. The effect of Agil herbicide on the mitotic index (MI%) of Allium cepa root meristematic cells are reported in table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Herb conc. (ppm)</th>
<th>Mitotic index (MI%±SE)</th>
<th>Mitotic phases (%)</th>
<th>Abnormalities (%)</th>
<th>Anaphase bridges (%)</th>
<th>Binucleated cells (%)</th>
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<tbody>
<tr>
<td></td>
<td>Pt</td>
<td>Mt</td>
<td>At</td>
<td>Tt</td>
<td>(%)</td>
<td>(%)</td>
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<td>8</td>
<td>Ct</td>
<td>42.1±0.02</td>
<td>50.3</td>
<td>19.1</td>
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<tr>
<td></td>
<td>0.5</td>
<td>36.8±0.02</td>
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<td>16.5</td>
<td>11.6</td>
<td>22.0</td>
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<td>53.8</td>
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<tr>
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<td>1.5</td>
<td>34.0±0.02</td>
<td>66.1</td>
<td>6.3</td>
<td>10.1</td>
<td>17.5</td>
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<tr>
<td>24</td>
<td>Ct</td>
<td>40.7±0.02</td>
<td>49.9</td>
<td>20.1</td>
<td>8.5</td>
<td>21.5</td>
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<td>59.1</td>
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<td>6.1</td>
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<tr>
<td>48</td>
<td>Ct</td>
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<td>50.1</td>
<td>19.4</td>
<td>9.7</td>
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<td>71.1</td>
<td>13.2</td>
<td>4.1</td>
<td>11.6</td>
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*No of cells examined/slide = 150; MI = Mitotic index; SE = Standard error; P = Prophase; M = Metaphase; A = Anaphase; T = Telophase.
When the same concentrations of Agil herbicide has been introduced at 8, 24 and 48 hours, the obtained results showed different effects on mitotic index. Analysis of the results from the effect of the concentration and the time of exposure on the MI showed a decrease in % MI of exposed roots it was dependent on the concentration and time of treatment. There were significant differences between different herbicide concentrations and the control. From these observations, the mitotic index decreased proportionally with increasing herbicide concentration and with increasing exposure time (fig. 1). Thus, at concentration of 0.5 ppm, MI had values down to 36.8% (8 h) to 33.7 (24 h) and 24.7% (48 h). Also, at 1.0 ppm Agil concentration, the mitotic index decreased to 36.6% (8 h), 31.1% (24 h) and 23.0% respectively (48 h). But the mitotic index had the lowest values at 1.5 ppm concentration of the herbicide Agil: 34.0% (8 h), 30.8% (24 h) and only 20.1% of 48 hours exposure time.

Fig. 1. The mitotic index (MI) decreased proportionally with increasing herbicide concentration and with increasing exposure time.
Fig. 2. The higher concentration and the higher exposure time of the Agil herbicide caused an increase of prophase frequency and decrease of telophase frequency.

According to other authors (1), decrease of mitotic index level shows that experimental material had mitodepressive effect resulting in the inhibition of cells access to mitosis. Also, the higher concentration and the higher exposure time of the Agil herbicide caused an increase of prophase frequency and decrease of telophase frequency (fig. 2). Thus, at concentration of 1.5 ppm, prophase had values down to 66.1% (8 h) to 69.3% (24 h) and 71.1% (48 h). In the same vein, the telophase values were 17.5% (8 h), 13.0% (24 h) and 11.6% (48 h).

The changes in the organization and morphology of the chromosomes in the root tips exposed to the herbicide were observed (fig. 3). They identified some mitotic abnormalities, as follows anaphase bridges and binucleated cells. Anaphase bridges were observed mainly on monobridges, but di- and tribridges were also observed though in less number. This could happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence. This bridges cause structural chromosome mutations (3). When increase the herbicide concentration and exposure time, increased the frequency of cells with mitotic abnormalities. Thus, at herbicide concentration of 1.5 ppm, frequency of the anaphase bridges was 1.7% (8 h), 2.3% (24 h) and 6.5% at 48 hours, while at the same concentration (1.5 ppm), the frequency of binucleated cells was 2.1% (8 h), 2.4% (24 h) and 3.1% at 48 hours exposure time.

According to our findings we can say that Agil herbicide can produce negative effects on mitotic divisions in onion root tip cells. The concentration and exposure time-dependent inhibition of mitotic index and the appearance of some chromosomal aberrations illustrate the cytotoxic potential of herbicide Agil in Allium cepa. In conclusion, our study reveals a direct correlation between herbicide concentration, exposure time and mutagenic effects observed in exposed Allium cepa cells. The results of our study explain the genotoxic effect of Agil herbicide, it would be better if it is examined by other eucaryotic test systems.

Fig. 3. The correlation between herbicide concentration, exposure time and some abnormalities observed in exposed Allium cepa cells

Conclusions

The nature and concentrations of the herbicides can contribute in setting up the toxicity of the compounds to the plant. The onion mitotic index decreased proportionally with increasing herbicide Agil concentration and with increasing exposure time. Higher concentration of the Agil herbicide caused an increase of prophase and decrease of telophase frequency.

The concentration and exposure time-dependent inhibition of mitotic index and the appearance of some mitotic abnormalities illustrate the cytotoxic potential of herbicide Agil in Allium cepa.

To avoid undesirable effects, when you choose a herbicide, must by law be considered genotoxic and
cytotoxic effects on plants. The results of our study explain the genotoxic effect of Agil herbicide, it would be better if it is examined by other eucaryotic test systems.

References