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PRELIMINARY RESULTS REGARDING THE SENSITIVITY OF *TULIPA GESNERIANA* L. MERISTEMATIC ROOT CELLS TO FUNGICIDE FOLPAN

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Abstract
The genotoxicity of the pesticide Folpan was evaluated in meristematic root cells of Tulipa gesneriana cv. 'Leen van der Mark'. The statistical analyses of the results showed that the pesticide studied has a concentration-dependent toxicity and induces chromosomal aberrations. The lowest mitotic index (2,69%) was related with the highest tested concentration of Folpan (900 ppm). Various types of chromosomal and mitotic abnormalities such as binucleated cells, laggards, and disturbed ana-telophase with multiple chromosomal bridges revealed the clastogenic potential of pesticide tested.

Keywords: mitotic index, chromosomal aberrations, cytotoxicity, Folpet, Tulipa.

1. INTRODUCTION

After the initial use of sulfur compounds as fungicides, of arsenic compounds and chlorinated hydrocarbons for insect's control, the synthetic pesticides became integral part of agricultural practices. Pesticides were credited with the ability to reduce natural threats and thus increase productivity. Undoubtedly, intensive application of insecticides has been directly linked to increased productivity and sometimes significant health benefits (such as eradicating malaria), but also have been raised and scientifically proven the excessive risks of their use on the environment and human health.

In 2007, Marvin J. Levine was appreciating the pesticides as "a toxic bomb in our midst". By their nature, pesticide are toxic for some forms of life. In this context we initiated a study for evaluation of genotoxic effects of pesticide Folpan in meristematic root cells of *Tulipa gesneriana* L. cv. 'Leen van der Mark'.

2. MATERIALS AND METHODS

The chemicals used in this study were provided commercially. Folpan 80 WDG is classified as a Group M contact fungicide, with multi-site activity. The active ingredient of the contact fungicide Folpan 80 WDG was folpet N-(trichloromethylthio) phthalimide, included in the dicarboximide class of fungicides, in the class III of toxicity.

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The plant used as test material was *Tulipa gesneriana* L. (2n= 16). Three clean and healthy bulbs of *Tulipa gesneriana* L. cv. 'Leen van der Mark' were chosen for each treatment group. After the dry scales of bulbs were removed, tulips were grown in clean tap water, at room temperature.
When the roots reached 1.5-2 cm in length, they were treated with different concentrations of aqueous solution of Folpan containing 300, 600 and 900 ppm of active ingredients, for 6 hours. The concentrations were chosen to be lower than those doses used in agricultural field to control different diseases. The control was prepared by exposing the bulbs to water only. After time went by, the roots were collected and fixed in Carnoy 1:3 acetic acid-ethyl alcohol mixture for overnight, and then preserved in 70% alcohol at 4ºC for cytological studies. The root tips were hydrolyzed in 1N HCl at 60 ºC for 12 minutes, followed by staining with 2% aceto-orcein at 60ºC for 12 minutes. After proper fixation and staining, appropriate squash preparations were made for each of the treatments and control. Effects of chemical treatment and control on different chromosome plates were observed under light microscope. All observations were made from temporarily prepared slides. To determine the effects of this fungicide on mitotic index, 3000 cells were scored in the control, and in each treated sample. Mitotic index was computed by determining the mitotic cell frequency at the root tip cells as:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

Cytological abnormalities were also observed and scored. Photomicrographs of cells showing chromosomal aberrations, as well as showing normal mitosis, were taken using an Olympus CX31 microscope.
Percentage of cells showing chromosomal abnormalities, such as chromosomal fragments, anaphase and telophase bridges, vagrant chromosomes, as well as aberrant interphases (binucleated cells), were recorded at the appropriate mitotic stages (Șuțan *et al*., 2014).

3. RESULTS AND DISCUSSIONS

The overall interpretation of the results obtained in experiments organized to highlight the cytogenetic effects of fungicide Folpan showed a concentration dependent decrease in the mitotic index, which explain its cytotoxicity in plant test system, as it is shown in Figure 1. The lowest value of MI (2.69%) was corresponding for the highest concentration of Folpan (900 ppm), showing a decrease of 65%. This inhibition of mitotic division can be attributed to the effect of toxic substances contained by the tested fungicide on DNA/protein synthesis of the biological system (Chauhan *et al*., 1998). Decrease of mitotic index could be related with alterations in the growth and development of the exposed organism (Hoshina, 2002, cited by González *et al*., 2011). Moreover, Ping *et al*. (2012) evaluating the genotoxicity of *Euphorbia hirta* extract on mitotic cells in *Allium cepa* root tips, appreciated that "the decrease of mitotic index could be interpreted as cellular death". However, similar results have been reported for other fungicidesusing *Allium cepa* assay. Liman *et al*. (2011) reported a lower mitotic index in *Allium cepa* meristematic root cells exposed to Fenilaminosulf than in the negative control. Bernardes *et al*. (2015) reported that Tebuconazole was more cytotoxic than Difenoconazole, causing a significant reduction in the mitotic index relative to

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the controls. The similarity of our results reflects the utility of higher plants for monitoring the cytotoxic and genotoxic effects of some chemicals.

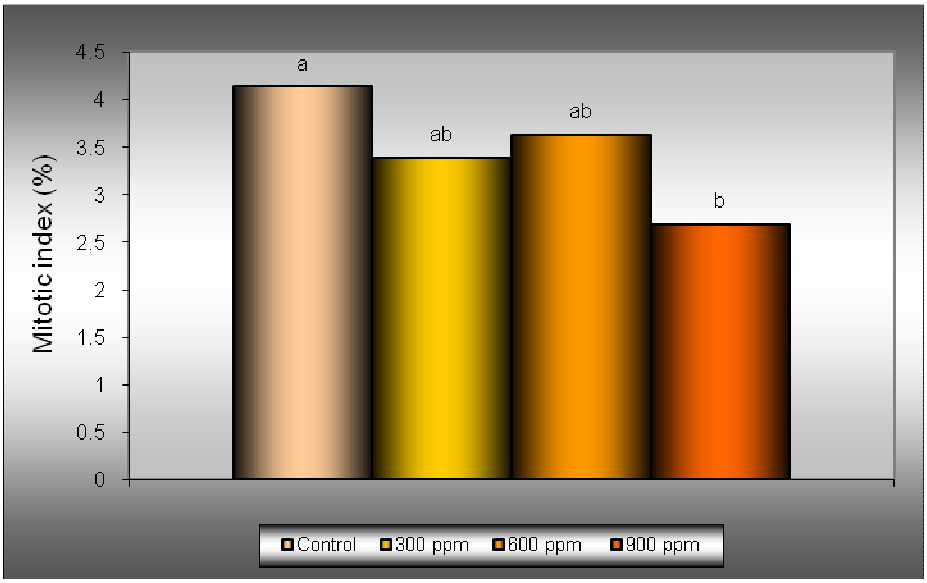

Figure 1. Mitotic index of Tulipagesneriana cv. 'Leen van der Mark' root tip cell exposed to different concentrations of Folpan(a, b: the significance ofdifferencesbetweenexperimental variantsusingDuncantest, p<0,05).

Fungicide Folpan induced chromosomal aberrations such as chromosomal fragments, anaphase and telophase bridges, vagrant chromosomes, and binucleated interphase cells. The frequencies of induced chromosomal aberrations were statistically significant at 600 and 900 ppm Folpan when compared with untreated control. The number of chromosomal aberrations increased with increasing concentrations of Folpan solutions, suggesting its mutagenic potential in a dose dependent manner. The most common chromosomal aberration in metaphase and anaphase cells was chromosome fragments. Occurrence of anaphase and telophase bridges was also noticed. The consequences of chromosomal breakage could be deletion, restitution, or joining of the resulted fragments with other fragments or chromosomes (Popescu*et al*. 2013).The merge of broken ends during metaphase can lead to the formation of acentric fragments, which are frequently lost, and dicentric chromosomes which may form bridges at anaphase. Anaphase bridges are the background for other chromosomal breaks. According with Bushberg *et al*. (1994), this process is self-generating, leading to serious disturbancesof genetic information of the daughter cells. These kind of chromosome aberrations indicate the clastogenic effect of Folpan in meristematic root cells of *Tulipa gesneriana* cv. 'Leen van der Mark'.

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Table1. Percentage of chromosomal aberrations induced of Folpan at different concentrations in meristematic root cells of Tulipa gesneriana cv. 'Leen van der Mark'.

CncentrationFolpan (ppm)	**Chromosomal aberrations (%)**			**Percentage of chromosomal aberrations (%** ± SE)
	Metaphase	**Anaphase**	**Telophase**	
0	0	0	0	0^{b}
300 ppm	0.23	0.17	0.31	0.23 ± 0.07^{ab}
600 ppm	0.16	0.47	0.43	0.35 ± 0.16^{a}
900 ppm	0.21	0.44	0.62	0.42 ± 0.15^{a}
Percentage of chromosomal aberrations (% ± SE)	0.20 ± 0.036^{bc}	0.36 ± 0.16^{ab}	0.45 ± 0.15^{a}	0.33 ± 0.15 / 0.25± 0.20

*each value represents the mean ± standard error (ES) for each experimental variant. Means with the same latter exponent do not differ significantly at the level of $p<0.05$ by the Duncan test.

Figure 2 shows chromosomal aberrations at different phases of cell cycle that were observed in root tip cells of *Tulipa gesneriana* cv. 'Leen van der Mark'.

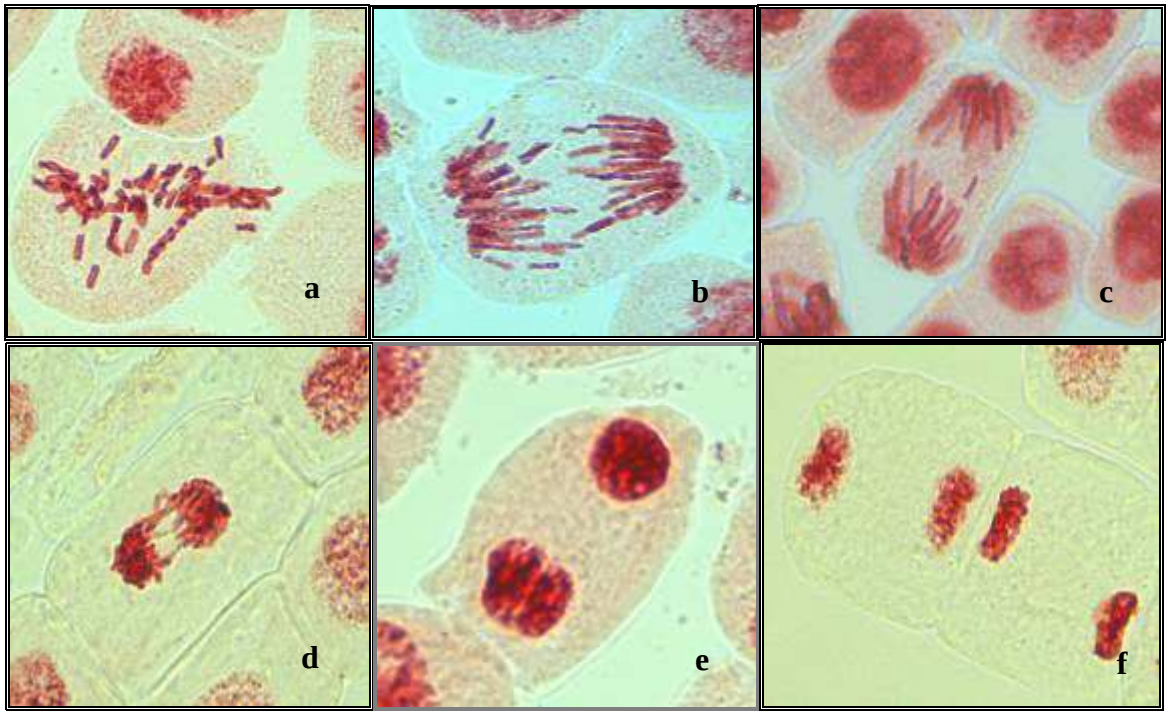

Figure 2. Photomicrographs of cytogenetic aberrations in Tulipa gesneriana cv. 'Leen van der Mark' meristem cells exposed to Folpan; (a-c) cells with chromosomes fragments; (d) anaphase bridge; (e-f) binucleated cells.

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4. CONCLUSIONS

These preliminary results suggest that the fungicide Folpan has a mitodepressive effect on meristematic root cells of *Tulipa gesneriana* cv. 'Leen van der Mark'. Induced chromosome aberrations such as chromosome fragments, anaphase and telophase bridges, vagrant chromosomes, indicate the clastogenic effect of Folpan in root tip cells of *Tulipa gesneriana* cv. 'Leen van der Mark'. Further investigations should be performed to confirm these effects, by using more specific methods.

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