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EVALUATION OF CYTOTOXICITY OF THE HERBICIDE GALIGAN 240 EC TO PLANTS

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Abstract

Excessive use of pesticides in agriculture in the context of increased ecosystems pollution justifies assessing the cytotoxicity of these chemicals. The purpose of the research was to evaluate the cytotoxicity of the herbicide Galigan 240 EC to plants, using as a test plant the species Allium cepa (onion), which is commonly used in the cytotoxicity tests. Exposure to various herbicide concentrations (0.5, 1.0 and 1.5%) for 3, 8 and 24 hours had different effects on the mitotic index. Thus, a decrease in the percentage of the mitotic index was found, in direct correlation with the exposure time and herbicide concentration. The study also reveals a direct correlation between herbicide concentration, exposure time and chromosomal aberrations/nuclear alterations identified in meristematic cells of onion. The most common chromosomal aberrations identified were stickyness and laggards, while the nuclear alterations were represented by binucleated cells and cells with nuclear erosion.
These results suggest caution when applying the herbicide Galigan 240 EC to agricultural crops.

Key words*: herbicide, onion, mitodepresive, chromosomal aberrations.*

INTRODUCTION

In the modern agriculture, a large number of herbicides are being used to control a wide variety of weeds.
Herbicides are chemical means of plant protection against weeds, obtained by formulating and conditioning some biologically active ingredients of a toxic nature, which requires their use with great care. Herbicides used in modern farming practices can have dangerous effects. They can be transformed into mutagens by the crop plants that absorb polluted nutrients and act as toxic vectors for humans and animals.
Herbicides pollute soil and air, but also the aquatic environment. From this point of view, the herbicides are introduced into the aquatic environment both inadvertently through runoff events and intentionally through the use of those registered for use in waterways (Freeman, Rayburn, 2006). Also, intensive use of herbicides without adequate knowledge on its effects on soil enzymes may have adverse impact on soil biochemical processes and cycling of nutrients (Sireesha et al., 2012).
Soil pollution/contamination may affect or inhibit plant growth or may introduce toxic elements into the nutrient chain by absorbing them from plants and their accumulation in organic tissue. The agricultural plants are the raw material for many food products, and the pollution of the vegetable raw material has a direct influence on the quality and safety of food products (Bonea, 2013).
Galigan 240 EC is an herbicide of the diphenyl-ether group used for selective weed control in a wide range of fruit trees, vegetables, field crops, and non-crop areas. Galigan 240 EC contains oxyfluorfen (240 g/liter), an active substance in the diphenyl ether group, with long-lasting herbicidal contact and residual contact. Oxifluorfen is classified as potentially carcinogenic ingredient (Dragoeva et al., 2012).
The superior plants may serve as genetic tests for the screening and monitoring of various environmental pollutants, such as pesticides or heavy metals (Sărac et al., 2015; Petrescu et al., 2015). Two of the species best suited to citotoxicity testing of pesticides are *Allium cepa* (onion) and *Allium sativum* (garlic).
The effects of chemical substances on chromosomes are especially studied on meristematic tissues from root tips because they are easily obtained, experiences can be performed throughout the year and are less expensive.

MATERIALS AND METHODS

The biological material used was represented by dried and healthy onion bulbs, without any signs of disease or pest attack. First, the onion bulbs were immersed in glasses of water for 72 hours, when the meristematic roots reached the length of 15-20 mm, followed by immersed in dilutions of various concentrations of the herbicide Galigan 240 EC (0.5, 1.0 and 1.5%) for 3, 8 and 24 hours at room temperature. A number of 10 onion bulbs were used for each treatment variant as well as an untreated control that was immersed in tap water.

After expiration of the exposure time, the roots were harvested using a scalpel and processed according to the protocol of fixation, hydrolysis and staining to highlight the cytological activity and eventual presence of chromosomal aberrations.

In order to highlight chromosomes and chromosomal aberrations was used the Feulgen-Rossenbeck method. The microscopic (temporary) preparations were performed according to the squash method, on the principle of tissue tightening between the microscopic blade and the microscopic lamella.

The experiments took place in the Genetics Laboratory of the Faculty of Agronomy Craiova.

RESULTS AND DISCUSSIONS

The exposure to various herbicide concentrations for 3, 8 and 24 hours had different effects on the mitotic index.

The analysis of the results demonstrates a decrease in the percentage of the mitotic index, in direct correlation with the exposure time and herbicide concentration (Figure 1).

Thus, at a concentration of 0.5%, the mitotic index recorded values from 36.8% (at 3 h exposure time), 33.7 (at 8 h exposure time) and 24.7% (at 24 h exposure time).

Also, at the 1.0% herbicide concentration, the mitotic index decreased to 36.6% (3 h), 31.1% (8 h) and 23.0% (24 h), respectively. But the mitotic index had the lowest values at the 1.5% herbicide concentration: 34% (3 h), 30.8% (8 h) and 20.1% at 24 h exposure time.

The mitotic index is a parameter that allows estimation of the frequency of cell division, and inhibition of mitotic activity is often an indicator of the effect of plant cytotoxicity (Marcano et al., 1998).

The decrease in mitotic index level shows the mitodepresive effect of the herbicide Galigan on cytological activity to onion, or the disturbance to a certain extent of the mitotic division. Also, the higher herbicide concentration, correlated with the higher exposure time, resulted in an increase in the frequency of prophases and, at the same time, a decrease in telophase frequency (Figure 2). Thus, at a concentration of 1.5% herbicide, prophase recorded a values from 66.1% (3 h) to 69.3% (8 h) and 71.1% (24 h). In the same vein, telophases values were between 17.5% (3 h), 13.0% (8 h) and 11.6% (24 h).

In terms of chromosomal aberrations, have been observed changes in organization and morphology of the chromosomes in the meristematic cells of onion exposed to treatment with the herbicide Galigan 240 EC. Thus, two categories of mitotic abnormalities have been identified: stickiness chromosomes and laggards chromosomes. In terms of nuclear anomalies, these were binucleated cells and cells with nuclear erosion.

The frequency of chromosomal aberrations and the frequency of nuclear abnormalities increased with the increase of the herbicide concentration and exposure time (Table 1). Thus, at a 1.5% herbicide concentration, the frequency of stickiness chromosomes was 2.7% (3 h), 4.1% (8 h) and 7.5% (24 h), while at the same concentration (1.5%), the frequency of laggards chromosomes was 3.1% (3 h), 3.9% (8 h) and 5.3% (24 h).

Nuclear abnormalities have been identified especially at the herbicide concentration of 1.5% and 24 hours (11% binucleated cells and 5% cells with nuclear erosion).

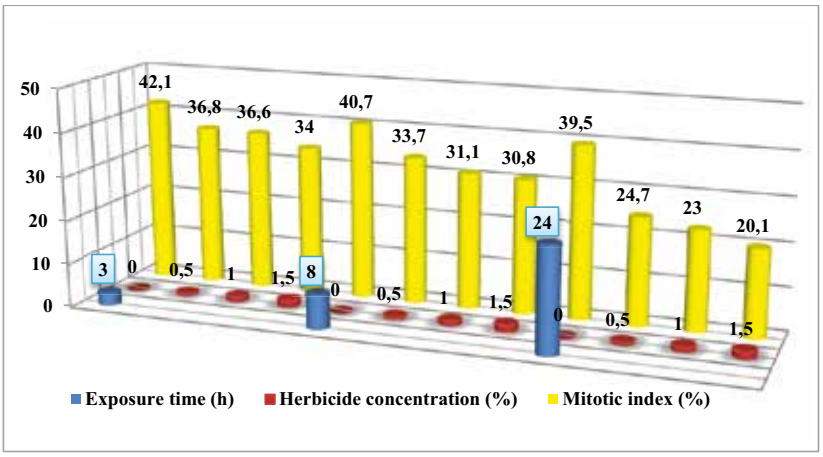

Figure 1. The decrease of mitotic index directly proportional to increase of herbicide concentration and increase of exposure time

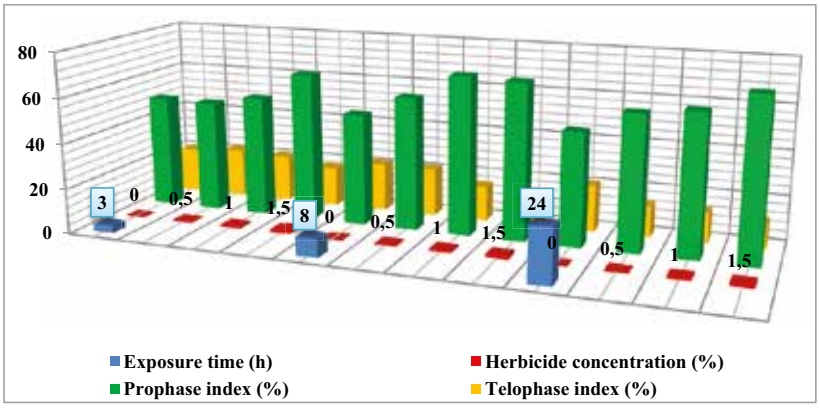

Figure 2. Graphical representation of increase of prophases frequency and decrease of telophases frequency with increasing concentration of herbicide Galigan 240 EC and increasing exposure time

Table 1. Quantification of chromosomal aberrations and nuclear abnormalities identified in meristematic cells of onion treated with the herbicide Galigan 240 EC

Exposure time (h)	Herbicide conc. (%)	Chromosomal aberrations		Nuclear abnormalities	
		Stickiness (%)	Laggards (%)	Binucleated cells (%)	Cells with nuclear erosion (%)
3	0 (Ct)	0	0	0	0
	0.5	1	0	0	0
	1.0	0	1	0	0
	1.5	2.7	3.1	2	1
8	0 (Ct)	0	0	0	0
	0.5	0	1.9	1	0
	1.0	0	2.0	1	1
	1.5	4.1	3.9	3	2
24	0 (Ct)	0	0	0	0
	0.5	0	2.3	3	2
	1.0	0	4.1	8	2
	1.5	7.5	5.3	11	5

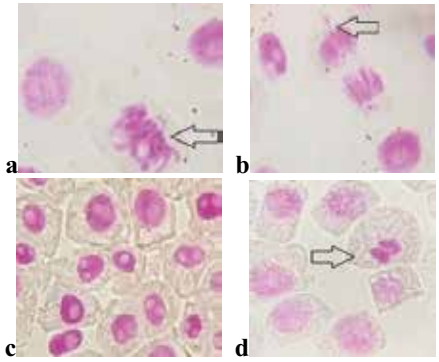

Figure 3. The chromosomal aberrations and nuclear abnormalities identified in meristematic cells of the onion exposed to treatment with herbicide Galigan 240 EC: stickiness chromosomes in methaphase (a); laggards chromosomes in anaphase (b); binucleated cells (c); cell with nuclear erosion (d)

CONCLUSIONS

The study reveals the mitodepresive effect of treatment with the Galigan 240 EC herbicide to onion, and shows the direct correlation between herbicide concentration, exposure time and cytological mutagenic effects identified in meristematic cells. The results suggest caution when applying the herbicide Galigan 240 EC to agricultural crops.

Repeated and without discernment use of herbicides invariably leads to the accumulation of phytotoxic substances in plants and soil and contaminates the environment.

The optimal solution for avoiding cytotoxic effects can be the application of minimum concentrations of herbicides and the combination of these chemical methods of weed control with biological methods, in the context of sustainable agriculture, for the protection of the environment and the increase of the quality of life.

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