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OPTIMIZATION OF SOIL POLLUTION MONITORING METHODS BY USE OF BIOLOGICAL TESTS

Elena BONCIU, Elena ROȘCULETE, Cătălin Aurelian ROȘCULETE, Aurel Liviu OLARU

University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania, Phone/Fax: +40251418475, E-mail: elena.agro@gmail.com, rosculeta2000@yahoo.com, catalin_rosculete@yahoo.com, liviu.olaru.dtas@gmail.com

Corresponding author: rosculeta2000@yahoo.com

Abstract

Among all the environmental factors, soil is the most stable component, due to its solid physical state. Soil is the basis of all food chains and terrestrial biodiversity. Therefore, a clean, unpolluted soil means reversing the decline of biodiversity, providing healthy agricultural raw materials, protecting human and animal health, etc. Pesticides are a broad group of chemicals commonly used in agriculture. Even though the impact on crop production is obviously profitable, pesticide residues are a common cause of soil pollution, especially in developing countries. Higher plants are recognized as excellent genetic models to detect environmental mutagens and are frequently used in environmental pollution monitoring studies. The objective of this paper was to determine the potential of the species Allium cepa to be used in biological tests for monitoring environmental pollution with herbicides. For this purpose, the biological material consisting of meristematic roots of A. cepa was exposed for 24 hours to the treatment with different doses of two types of herbicides, namely: 0.125, 0.200, 0.250 g/L for Pendimethalin and 0.225, 0.250 and 0,300 g/L for Aclonifen respectively. The obtained results show the sensitivity of the species A. cepa to the tested herbicides by the drastic reduction of the mitotic activity in the cell cycle and by the appearance of a large number of chromosomal aberrations in the mitotic cells. From this point of view, the use of the Allium biological test can contribute to optimization of the methods of monitoring the chemical pollution of soil with herbicides.

Key words**:** *pesticides, toxicity, soil, A. cepa, monitoring*

INTRODUCTION

The agri-food strategy of any country is determined by the need to establish guidelines for the sustainable biotechnological development of the agricultural system and the rural area, as a guarantee of achieving the objective of the population well-being [7, 8].

In the context of current climate changes, the increase in agricultural productivity, the sustainable protection of crops, but also the reduction of food waste represents important elements of ensuring sustainable food security [5, 6, 13, 14, 21].

In agriculture, pesticides are commonly used to obtain higher quality products and increase the production rate. Pesticides used in agriculture are organic compounds with low molecular weight and different solubility in water. The chemical character, shape and molecular configuration, solubility in water and polarity of the molecule can greatly influence the adsorption-desorption processes on soil colloids.

Apart from their beneficial effects, the pesticides are toxic substances. They residues remain in the atmosphere, being dangerous at the local and global level, for the health of ecosystems and the human population. Many studies using different biological tests have demonstrated the strong cytotoxic and genotoxic effects of herbicides, insecticides and fungicides [9, 17, 19, 20].

Pesticides lead to the generation of reactive oxygen species, such as hydrogen peroxide, superoxide and hydroxyl radicals. Since pesticides, which are widely used in agriculture are potentially carcinogenic, the need to expand the genotoxic evaluation of these chemicals by using different test systems becomes crucial.

The intensive use of pesticides has many side effects: environmental pollution, biological imbalances and even affecting the health of consumers, as a consequence of the pollution of soil, water and agricultural products. In genetics, genotoxicity describes the property of chemical agents (including pesticides) to produce various nuclear and chromosomal aberrations in cells and the production of mutations.

The pesticides are usually metabolically activated by plant peroxidases [11]. In soil, pesticides undergo some chemical transformations following the reactions with organo-mineral compounds of the soil. The physical and chemical properties of the soil are the most important factors that influence the chemical transformation of pesticides in soil. Numerous studies emphasize the role of soil microorganisms in the decomposition of pesticides, as well as the fact that there are few active substances that are not biologically degraded. Many pesticides are degraded in soil if a certain microbial culture medium or certain adjuvants, products that retain or degrade pesticides, are administered. There are also agricultural plants, such as sorghum and sugar cane which have the ability to decontamination the soil of pesticide residues through absorption and metabolic degradation. The EU has a complex legislation on chemicals, which has created the most advanced knowledge base in this field worldwide, and has established scientific organisms that carry out risk and hazard assessments of chemicals. The biomonitoring studies of the soils in EU indicate the presence of an increasing number of different dangerous chemical substances and therefore, the optimization of soil but also water and air monitoring methods is a very topical objective. One of these methods is the use of biological tests to determine the degree of soil pollution with chemical substances, such as the herbicides used to weeds control in agriculture. Plants are effective indicators for the detection of genotoxicity of chemical compounds and for in situ monitoring of genotoxic environmental contaminants.

The use of plants as test systems to assess the effects of pesticide soil pollution has many advantages related to: reproductive nature, the possibility of being applied in vivo, in vitro and in situ; standardization of the controlled method under laboratory conditions, which does not require large sample volume, extraction procedure or previous isolation, ethically suitable compared to animal tests and low cost, especially valuable in developing countries [4, 12]. From this point of view, the *Allium cepa* species is one of the most used in cytogenotoxicity tests of various pesticides or heavy metals in plant and animal systems [16, 18].

MATERIALS AND METHODS

For this experiment, we used onion bulbs as biological material, which were processed in according of the cytogenetic protocol, to obtain meristematic roots. Also, two herbicides were used for testing: Pendimethalin and Aclonifen respectively (Figure 1 and Figure 2).

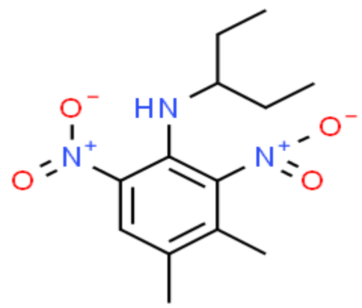

Fig. 1. Pendimethalin chemical structure
Source: [15].

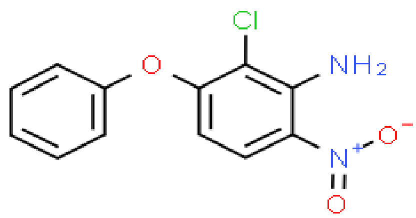

Fig. 2. Aclonifen chemical structure
Source: [1].

Pendimethalin is an herbicide of the dinitroaniline class used in premergence and postemergence applications to control annual grasses and certain broadleaf weeds. Its cytotoxicity has been demonstrated in many studies, both in plants and in animals [2, 3].

Aclonifen is a diphenyl ether herbicide which has been used in agriculture since the 1980s. Its mode of action has been uncertain, with evidence suggesting it might interfere with carotenoid biosynthesis or inhibit the enzyme protoporphyrinogen oxidase. This herbicide causes a bleaching phenotype to plants [10].

For this experiment, 10 healthy medium-size white onion bulbs were germinated in tap water for 72 hours, until the meristematic roots reached a length of 1-1.5 cm. The vegetal material was then transferred for 24 hours postincubation in the herbicides solutions, consisting of 3 experimental doses for each of the two herbicides, together with an untreated control, namely: 0.125, 0.200, 0.250 g/L for Pendimethalin and 0.225, 0.250 and 0.300 g/L for Aclonifen respectively.

After expiration of the treatment time, the meristematic roots were measured for the growth inhibition test and then, the biological material went through the stages of fixation, hydrolysis and staining with Schiff's reagent, after which the temporary microscopic preparations were prepared (according to the squash method) for microscopical analysis. The cytogenetic determinations concerned the mitotic index (MI) and the percentage of chromosomal aberrations (CA). The MI, characterized by the total number of cells in mitotic division in the cell cycle, has been used as a parameter to evaluate the cytotoxicity of different chemicals. Cytotoxicity levels of chemical stressors (such as pesticides) can be determined by increasing or decreasing MI. The percentage of chromosomal aberrations results from the total number of cells with aberrant chromosomes compared to the total number of cells in division. During the experiment, 500 cells were counted for each variant. The used microscope was Optika B-383 PL, equipped with photo camera. For the statistical comparison of the results, ANOVA analysis of variance and Duncan test were used ($P < 0.05$).

RESULTS AND DISCUSSIONS

The treatment of the biological material with three doses of the herbicides for 24 hours had different effects on meristematic growth as well as on the mitotic index to *A. cepa* (Table 1).

Table 1. Results regarding the mitotic index and root growth to *A. cepa* exposed to some herbicides

Herbicide/ Doses (g/L)		Mitotic index (%)±SD	Average length (cm) ± SD
Pendi-methalin	Ct	59.6±0.7a	2.3±0.05a
	0.125	43.2±0.5a	2.1±0.07b
	0.200	36.4±0.8b	1.9±0.09c
	0.250	21.5±0.4c	1.5±0.03d
Aclonifen	Ct	48.1±0.2a	2.1±0.05a
	0.225	33.2±0.5b	1.7±0.08b
	0.250	25.4±0.3c	1.5±0.04c
	0.300	11.3±0.1d	1.3±0.02d

Note: Means with the same letter do not differ statistically at the level of 0.05.
SD=Standard deviation
Source: Own calculation.

The sensitivity of *A. cepa* to Pendimethalin herbicide action can be observed through the effects of inhibiting meristematic growth, compared to the untreated control, respectively reduced of the mitotic activity, through the decrease of MI, in direct correlation with the Pendimethalin concentration.

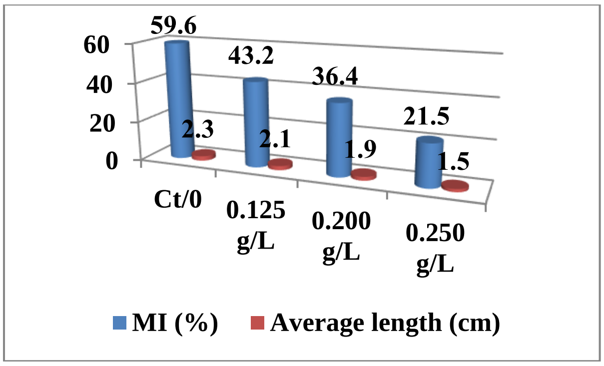

Fig. 3. The decrease of the mitotic index and the inhibition of meristematic growth in *A. cepa* roots exposed to different doses of Pendimethalin
Source: Own design and calculation

The MI value was between the limits of 59.6% (Ct) and 21.5% in the case of the variant exposed to dose of 0.250 g/L herbicide. Regarding the meristematic growth, the average length value was between the limits of 2.3 cm (Ct) and 1.5 cm in the case of the variant exposed to dose of 0.250 g/L herbicide (Figure 3).

The effect of Aclonifen on mitotic activity and meristematic growth in *A. cepa* was somewhat similar to that produced by Pendimethalin. It can be observed, however, that the effect of mitodepression as well as that of marked inhibition of meristematic growth, compared to the control variant, were more pronounced in the case of this herbicide, but the treatment doses were also higher.

As can be seen in Figure 4, the MI value was between the limits of 48.1% (Ct) and 11.3% in the case of the variant exposed to dose of 0.300 g/L Aclonifen.

Regarding the meristematic growth, the average length value was between 2.1 cm (Ct) and 1.3 cm in the case of the variant exposed to dose of 0.300 g/L herbicide.

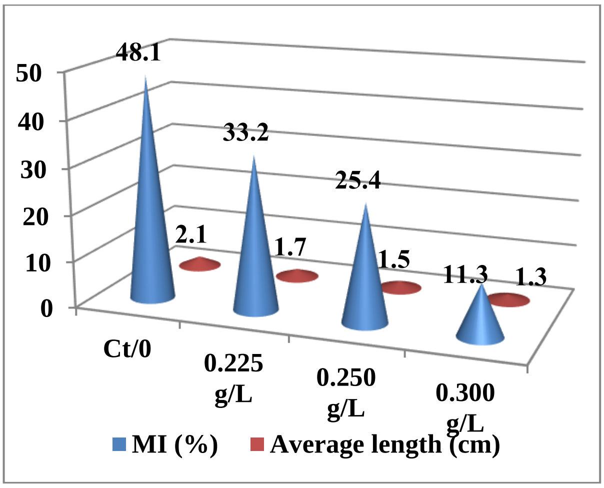

Fig. 4. The decrease of the mitotic index and the inhibition of meristematic growth in *A. cepa* roots exposed to different doses of Aclonifen

Source: Own design and calculation.

To quantify chromosomal aberrations, all phases of the mitotic division were evaluated: prophase, metaphase, anaphase and telophase. This analysis allows a much more precise assessment of cell damages, as a result of clastogenic or aneugenic effects on the tested biological material.

The cytogenetic results regarding the evaluation of the types and frequency of chromosomal aberrations identified in the meristematic cells of *A. cepa* exposed to the action of the Pendimethalin and Aclonifen herbicides are highlighted in Table 2.

Several types of chromosomal aberrations were identified through microscopic analysis, the most common being stickiness, vagrant, laggard and ring chromosomes. Sticky type aberrant chromosomes had the highest frequency and ring type chromosomes had the lowest frequency (Fig. 5).

Table 2. Results regarding the chromosomal aberrations to *A. cepa* exposed to some herbicides

Herbicide/ Doses (g/L)		**CA (%)**				
		S	**V**	**L**	**R**	Total CA± **SD**
Pendi-methalin	Ct	1.4	0.3	0.3	0.1	2.1±0.55a
	0.125	3.2	1.8	3.9	0.9	9.8±0.25b
	0.200	4.8	2.1	3.3	2.5	12.7±0.11c
	0.250	6.1	3.2	3.4	3.6	16.3±0.31d
Aclonifen	Ct	1.7	0.5	0.4	0.3	2.9±0.55a
	0.225	4.1	2.1	3.4	1.2	10.8±0.35b
	0.250	5.8	3.2	3.3	1.9	14.2±0.54c
	0.300	7.1	2,6	4.8	4.1	18.6±0.26d

Note: Means with the same letter do not differ statistically at the level of 0.05.

CA=Chromosomal aberrations; S=Sticky chromosomes; V=Vagrant chromosomes; L=Laggards chromosomes; R=Ring chromosomes; SD=Standard deviation

Source: Own calculation.

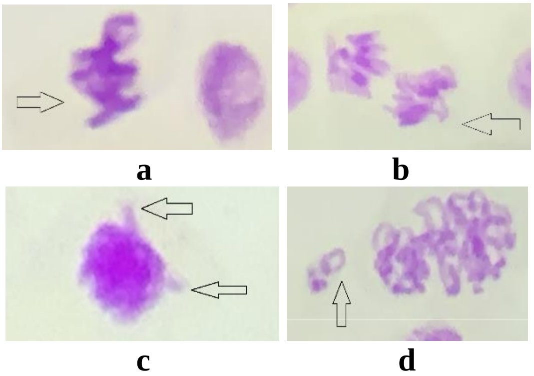

Fig. 5. Some chromosomal aberrations induced by Pendimethalin and Aclonifen herbicides in *A. cepa* cells: sticky chromosomes (a); vagrant chromosomes (b, c); ring chromosome (c)

Source: Own cytogenetic pictures

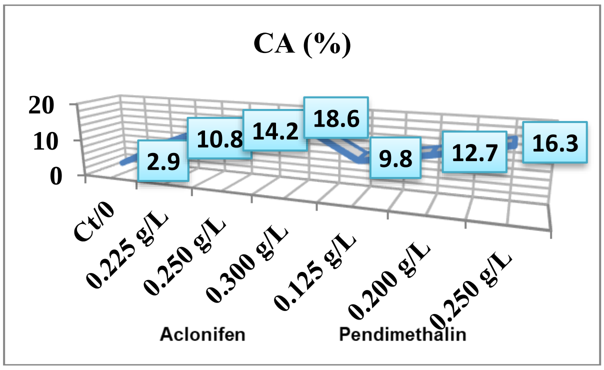

Fig. 6. Total chromosomal aberrations (CA %) induced in *A. cepa* roots after exposure to different doses of Aclonifen and Pendimethalin herbicides

Source: Own design and calculation.

The frequency of total chromosomal aberrations (CA%) recorded values between 2.1 and 2.9% in case of the control variants and respectively 16.3% and 18.6% in case of the highest doses of Pendimethalin and Aclonifen herbicides (Figure 6).

CONCLUSIONS

Higher plants are recognized as excellent genetic models to detect environmental mutagens and are frequently used in environmental pollution monitoring studies. In this context, *A. cepa* can be used to evaluate chromosomal aberrations and mitotic cycle disorders but also to evaluate the toxicity of many chemical agents.

The sensitivity of *A. cepa* to tested herbicides action was suggested through the inhibition of the meristematic growth, reduction of the mitotic activity by the decrease of mitotic index and appearance of several chromosomal aberrations respectively.

Reduction of the mitotic index and chromosomal aberrations appearance in the meristematic cells is an important indicator in environmental pollution monitoring, especially for the assessment of contaminants with toxic and cytotoxic potential. Therefore, the biological *Allium* test has potential for estimating, to a certain degree, the chemical pollution level of the soil.

The aneugenic and clastogenic effects of the tested herbicides can be much more significant, but not noticeable with the means of study in this case.

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