STUDY OF THE ACUTE TOXICITY OF SOME PESTICIDES ON EARTHWORMS *EISENIA FOETIDA* (SAVIGNY, 1826)

Mădălina IORDACHE, Iacob BORZA

Banat's University of Agricultural Science and Veterinary Medicine Timişoara Calea Aradului no.119, 300645 Timişoara, Romania
E-mail: mada_iordache@yahoo.com

Abstract: *Earthworms represent a great part of the soil biomass, and due to this fact they are suitable bioindicators of the chemical contamination, expressing the soil health and quality. The effect of the chemicals on earthworms depends on the used substance, but regardless the substance nature, it must be considered in the ecological risk assessment both the direct and the indirect effects of it on soil life. This paper shows the results of a laboratory study through which was tested the acute toxicity of two types of pesticides (herbicides), homologated in Romania, on adult earthworms: GARDOPRIM PLUS GOLD 500 SC (classified in the risk category R50/53 - very toxic for aquatic organisms, able to cause long term adverse effects on environment, and belonging to the IVth group of toxicity) and HELMSTAR 75 WG (classified as N - dangerous for environment and belonging as well to the IVth group of toxicity),. The active substances of these herbicides are S-metolachlor and terbuthylazine and respectively tribenuron-methyl. The tested earthworm species was Eisenia foetida (Savigny, 1826). The research aimed to establish the effect of these two herbicides on earthworm mortality using the methodology in force enounced by OECD guidelines (Organisation for Economic Co-operation and Development). There were tested five concentration levels of each herbicide in laboratory conditions. The mortality assessment was realized at intervals by 24 and respectively 72 hours from exposure. Results showed that the herbicide GARDOPRIM PLUS GOLD 500 SC exerts an extremely toxic action on earthworms, and the second tested herbicide, HELMSTAR 75 WG was tolerated by earthworms. These findings demonstrated once again the sensitivity of these organisms such important for the soil health and fertility and highlight the necessity of precaution in using chemical substances that can get into the soil, under aspects of pedofauna abundance and activity, in order to maintain the biological equilibrium of soils.*

Key words: *earthworms, Eisenia foetida, toxicity, S-metolachlor, terbuthylazine, tribenuron-methyl*

INTRODUCTION

Earthworms represent a great proportion of biomass of terrestrial invertebrates (80%) and therefore they are suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality [BUSTOS-OBREGÓN AND GOICOCHEA, 2002; CULY AND BERRY, 1995; SHAHLA AND D'SOUZA, 2010; SOROUR AND LARINK, 2001]. Studies have shown that earthworm skin is a significant route of contaminant uptake [SHAHLA AND D'SOUZA, 2010; LORD ET AL., 1980] and thus investigation of earthworm biomarkers in the ecological risk assessment can be helpful [SHAHLA AND D'SOUZA, 2010; SANCHEZ-HERNANDEZ, 2006].

The pesticide effect on earthworms depends on the used chemical substance. Generally, herbicides manifest low toxicity on earthworms, but indirectly can produce the reduction of the populations by decreasing the organic matter input and weed coverage. The fungicides and fumigants are very toxic substances for earthworms [BOOTH AND O'HALLORAN, 2001; BOOTH ET AL., 2001; ABDUL RIDA AND BOUCHÉ, 2007]. The methods used for pesticide application could have various effects on the ecological groups of

organisms which populate the soil [RAO ET AL., 2003]. For example, the fungicide benomyl determines size reduction of the earthworm populations. The anecic species like *Lumbricus terrestris* are the most exposed to the surface applications and less affected by pesticide incorporation into the soil. Because *Lumbricus terrestris* borrows permanent galleries through the soil, it does not come in contact with the surface soil, located above the galleries. But, the endogeic species, like *Aporrectodea caliginosa,* which permanently extend their galleries as result of the feeding way, are prone to the chemical hazard [EDWARDS AND BROWN, 1982].

MATERIAL AND METHOD

The study concerning the influence of some pesticides (herbicides) on earthworms consisted of laboratory experiments. The herbicides were selected considering their wide utilisation in agriculture. Two herbicides were tested on earthworms: GARDOPRIM PLUS GOLD 500 SC and HELMSTAR 75 WG.

The ecotoxicity testing of the herbicides on earthworms was realised according to OECD methodology, on filter paper [12]. The tested earthworm species was *Eisenia foetida* (Savigny, 1826) (red worm, compost worm). Glass vials coated with filter paper were used, with appropriate sizes so that they don't overlap in the vial. Two types of vials were used: control vials and test vials. The herbicide in suspension was dissolved in distilled water, resulting five concentration levels in geometrical series, correspondent to those used in practice (or closed) in the cultures suitable for the treatment with such a product. One millilitre of each solution was introduced in each test vial, after what the vials were placed in horizontal position in front of a slow air stream ventilated until the filter paper from inside was dry. After drying, in each test vial was added 1 ml of distilled water to wet the filter paper. In the control vial, the filter paper was wetted only with 1 ml of distilled water. Immediately after distilled water was added, the vials were closed with plastic foil and then perforated to allow the earthworm access to oxygen (figure 1).

Figure 1. Vial for testing the toxicity of the herbicide GARDOPRIM PLUS GOLD 500 in species *Eisenia foetida* (Savigny, 1826) (red worm, compost worm)

In each vial was introduced a single adult earthworm, and every earthworm was previously maintained for 3 hours on wet filter paper, so that the content of their gut be eliminated, and then washed and dried. The vials were placed in horizontal position, the ambient temperature in the laboratory was approximately by 20°C ± 2°C. for 24 hours the vials was kept in dark, and ulterior exposed to the photoperiod conditions of May month, when the experiment was carried out. The test lasted 72 hours.

The five levels of the tested concentration for the herbicide GARDOPRIM PLUS GOLD 500 SC were as following: 6,25 $ml \cdot l^{-1}$, 12,5 $ml \cdot l^{-1}$, 25 $ml \cdot l^{-1}$, 50 $ml \cdot l^{-1}$, 100 $ml \cdot l^{-1}$, and for the herbicide HELMSTAR 75 WG were: 0,1 $ml \cdot l^{-1}$, 0,2 $ml \cdot l^{-1}$, 0,4 $ml \cdot l^{-1}$, 0,8 $ml \cdot l^{-1}$, 1,6 $ml \cdot l^{-1}$.

RESULTS AND DISCUSSION

The first tested herbicide was GARDOPRIM PLUS GOLD 500 SC, homologated in Romania by authorisation no. 215/11.10.2002, classified in the risk category R50/53 (very toxic for aquatic organisms, able to cause long term adverse effects on environment) and belonging to the IVth group of toxicity. This herbicide contains 312,5 g·l^{-1} S-metolachlor and 185.5 g·l^{-1} terbuthylazine. The action spectrum of the herbicide GARDOPRIM PLUS GOLD 500 SC includes the majority of the annual monocotyledonous and dicotyledonous weeds and it is applied both pre- and post-emergently (table 1).

Table 1

Ecotoxicity effects of the herbicide GARDOPRIM PLUS GOLD 500 SC on different life forms in statical conditions [12, 13]

Crrt. no.	Species	Dose	Toxicity level
1	*Oncorhynchus mykiss* (rainbow trout)	8.32 mg·l^{-1}, 96 h	LC50
2	*Daphnia magna* (water flea)	35.2 mg·l^{-1}, 48 h	EC50
3	*Desmodesmus subspicatus* (green alga)	0.211 mg·l^{-1}, 72 h	ERC50

Notes:
LC50 – represents the mean lethal concentration, the concentration of a substance which results in death of 50% of the tested organisms, during the whole testing period [12];
EC50 – represents the half maximal effective concentration of the tested substance which determines a response (reaction) to 50% of the tested organisms, during the whole testing period [14];
ERC50 – represents the concentration causing 50% inhibition of growth rate [14].

The S-metolachlor is mainly taken by weeds during the seed germination through coleoptile. So, the weeds are removed before plant emergence, during emergence or at short time after emergence. The root uptake is lower. The terbuthylazine is taken by weeds through roots and leaves. The herbicide GARDOPRIM PLUS GOLD 500 SC is applied in dose of 4-5 l · ha^{-1}, depending on infestation degree and weed dominance [13].

Exposing the earthworms to the action of herbicide GARDOPRIM PLUS GOLD 500 SC, for five concentrations (6,25 ml·l^{-1}, 12,5 ml·l^{-1}, 25 ml·l^{-1}, 50 ml·l^{-1}, 100 ml·l^{-1}), there was found after 24 test hours that all earthworms died in all test vials, for all tested concentration levels (figure 2).

Figure 2. Acute toxicity test of the herbicide GARDOPRIM PLUS GOLD 500 SC, different levels of concentrations (ml·l^{-1}) on species *Eisenia foetida* (Savigny, 1826) (red worm, compost worm), 24 h

Exception was the control vial, where the earthworms survived even after 72 hours of test, manifesting at the test end features similar to those before their introduction into the vials. The ecotoxicity test was repeated by 10 times, in the same earthworm species, with the same result.

The second herbicide that was tested was HELMSTAR 75 WG, homologated in Romania by authorisation no. 2596/14.12.2005, classified as N (dangerous for environment) and belonging to the IVth group of toxicity. This herbicide contains 75% tribenuron-methyl and is a selective herbicide, with post-emergent application, to fight against the dicotyledonous weeds from wheat and barley cultures. It is mainly absorbed at leaf level, but also through roots, when weeds are in the first stages of development (10-15 cm height) and the straw cereals are in the phase of shoot formation and until the first internode was formed. HELMSTAR 75 WG is applied in dose of 15-20 g·ha^{-1}, depending on the soil type, weed spectrum and their height [13].

Data concerning the ecotoxicology of the active substance (tribenuron-methyl) contained by HELMSTAR 75 WG are given in the table 2 [15].

Table 2

Ecotoxicity effects of the herbicide HELMSTAR 75 WG asupra on different life forms in statical conditions [15]

Crrt. no.	Specification	Toxicity value	Tested species	Toxicity degree
1	Bio-concentration factor: BCF (days)	0.08	-	Reduced
2	Bioaccumulation potential	-	-	Reduced
3	Mammals – acute oral dose: DL50 (mg·kg^{-1})	5000	*Rat*	Reduced
4	Birds – acute oral dose: DL50 (mg·kg^{-1})	2250	*Colinus virginianus*	Reduced
5	Fishes – acute oral dose: DL50 (mg·l^{-1})	738	*Oncorhynchus mykiss*	Reduced
6	Fishes – chronic dose at 21 days (mg·l^{-1})	560	*Oncorhynchus mykiss*	-
7	Aquatic invertebrates – acute dose: EC50 (mg·l^{-1})	894	*Daphnia magna*	High
8	Aquatic invertebrates: chronic dose at 21 days (mg·l^{-1})	120	*Daphnia magna*	-
9	Aquatic plants: EC50 (mg·l^{-1})	0.0099	*Lemna minor*	High
10	Algae –acute dose: EC50 (mg·l^{-1})	0.11	*Raphidocelis subcapitata*, 120 h	Moderate
11	Bees –oral dose: DL50 (µg·bee^{-1})	9.1	-	Moderate
12	Earthworms –acute dose : DL50 (mg·kg^{-1})	1000	*Eisenia foetida*	Moderate
13	Arthropods (1): dose 0.03 kg·ha^{-1} Effect in % on: - mortality - reproduction	 14 10	*Typhlodromus pyri*	Strong
14	Microorganisms in soil: - nitrogen mineralization - carbon mineralization	Insignificant effect	-	-

The granulated herbicide HELMSTAR 75 WG was dissolved in distilled water. The five concentration levels tested on earthworms were: 0,1 $ml \cdot l^{-1}$, 0,2 $ml \cdot l^{-1}$, 0,4 $ml \cdot l^{-1}$, 0,8 $ml \cdot l^{-1}$, 1,6 $ml \cdot l^{-1}$. The concentration levels have been established similar to the first tested herbicide: in ascending order, in geometric series, with correspondence in practice. According to the work protocol, the test lasted 72 hours. After this time period, at the test end, there was found that all earthworms survived, both in control and in test vials, for all concentrations (figure 3).

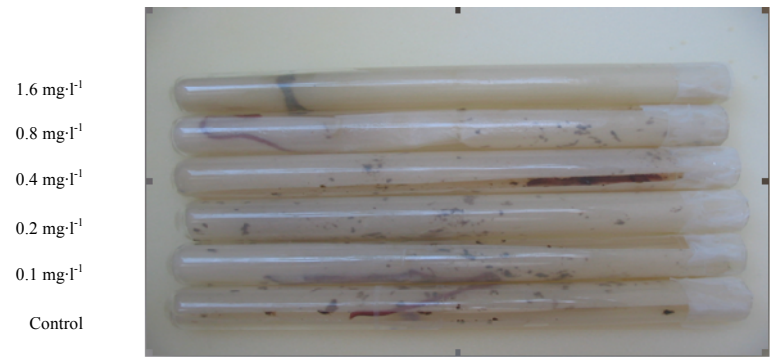

Figure 3. Acute toxicity test of the herbicide HELMSTAR 75 WG, different levels of concentrations ($mg \cdot l^{-1}$) on earthworm species *Eisenia foetida* (Savigny, 1826) (red worm, compost worm), 72 h

CONCLUSIONS

Performing the ecotoxicity tests with the herbicides above mentioned, on the earthworm species *Eisenia foetida* (Savigny, 1826), in laboratory condition, there was found the following conclusions:

• The herbicide GARDOPRIM PLUS GOLD 500 SC manifests an extremely toxic action on earthworms, determining 100% mortality within earthworms after 24 and respectively 72 test hours. The single exception was in the control vials, where the earthworms survived for more than 72 hours.

• The herbicide HELMSTAR 75 WG, in the tested conditions, was tolerated by earthworms, unlike the other herbicide.

• The results obtained by ecotoxicity testing on this earthworm species represent another confirmation of the fact that these organisms living in soil, with major role in maintaining its fertility state, are sometimes extremely sensitive to pesticides that get into the soil, highlighting the idea that the use of the chemical substances must be made with maximal precautions and responsibility, to not affect on long time, or possible irreversibly, the earthworm life and, through this, their activity such important for the fertility of the agricultural lands.

ACKNOWLEDGEMENTS

Publishing of this paper was financially supported by the research grants UEFISCDI PN II CAPACITATI-MODUL III no. 432/16.06.2010 and UEFISCDI PN II-RU-PD, no. 109/02.08.2010, code PD-591.

BIBLIOGRAPHY

1. ABDUL RIDA, A.M., BOUCHÉ, M.B., 1997 – *Earthworm toxicology: from acute to chronic tests.* Soil Biology and Biochemistry, 29: 699-703;

2. BOOTH, L.H., HODGE, S, O'HALLORAN, K., 2001 – *Use of biomarkers in earthworms to detect use and abuse of field applications of a model organophosphate pesticide*. Bull. Environ. Contam. Toxicol. 67: 633–640;
3. BOOTH, L.H., O'HALLORAN, K., 2001 – *A comparison of biomarker responses in the earthworm Aporrectodea caliginosa to the organophosphorous insecticides Diazinon and Chlorpyrifos*. Environ. Toxicol. Chem., 20, 2494-2502;
4. BUSTOS-OBREGÓN, E., GOICOCHEA, R.I., 2002 – *Pesticide soil contamination mainly affects earthworm male reproductive parameters.* Asian Journal of Andrology 4(3) 195-199;
5. CULY, M.D., BERRY E.C., 1995 – "Toxicity of soil-applied granular insecticides to earthworm populations in cornfields". *Down to Earth,* vol. 50, 20-25;
6. EDWARDS, P.J., BROWN, S.M., 1982 – *Use of grassland plots to study the effects of pesticides on earthworms.* Pedobiologia 24:145-150;
7. LORD, K.A., BRIGGS, M.C., MANLOVE, R., 1980 – *Uptake of pesticides from water and soil by earthworms.* Pesticide Science, 11(4): 401–408;
8. RAO, J.V., PAVAN, Y.S., MADHAVENDRA, S.S., 2003 – *Toxic effects of chlorpyrifos on morphology and acethylcholinesterase activity in the earthworm Eisenia fetida*. Ecotoxicol. Environ. Saf. 54: 296-301;
9. Sanchez-Hernandez, J.C., 2006 - *Earthworm biomarkers in ecological risk assessment*. Reviews of Environmental Contamination and Toxicology. 188: 85–126
10. SHAHLA, Y., D'SOUZA, D., 2010 – *Effects of pesticides on the growth and reproduction of earthworm.* Applied and Environmental Soil Science. 2010(2010): 1-9;
11. SOROUR, J., LARINK, O., 2001 – *Toxic effects of benomyl on the ultrastructure during spermatogenesis of the earthworm Eisenia fetida*. Ecotoxicology and Environmental Safety, 50(3): 180-188;
12. ***OECD GUIDELINE FOR TESTING OF CHEMICALS - *Earthworm, Acute Toxicity Tests*, 1984, April 4 (*http://www.oecd.org*);
13. ***http://www.syngenta.ro;
14. ***http://sis.nlm.nih.gov/enviro/iupacglossary/glossarye.html;
15. ***http://sitem.herts.ac.uk/aeru/footprint/en/Reports/655.htm#2.