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*Article*

# The effects of lambda-cyhalothrin on juvenile forms of *Eisenia fetida* (Oligochaeta, Lumbricidae)

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**Abstract:** The aim of the study was to obtain the data on mortality and average weight changes in juvenile forms of earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae) exposed to the different dosages of lambda-cyhalothrin in laboratory conditions. Fourteen days after insecticide application, the decrease in average mass was noticed in earthworm groups treated with the highest dosage (20.45%). The highest weight increase was detected in the group treated with the recommended dosage in agricultural practice. The highest mortality rate was observed 72 hours and seven days after the treatment in groups treated with the highest dosage when almost one third of the initial populations were lost. The obtained results are important to protect the health of the environment and are of increasing interest in the context of protecting human health.

**Keywords:** lambda-cyhalothrin; pyrethroids; *Eisenia fetida*; mortality; earthworms

## 1. Introduction

Approximately 1.8 billion people worldwide are engage in agriculture practice and the majority use a broad range of plant protection products (PPPs) to protect the food and commercial products that they produce Alavanja [1]. Furthermore, pesticides are frequently used in public health programs and commercial applications, while many others use pesticides for the garden, lawn and cemetery maintenance, as well as in and around the home. The UN's Food and Agricultural Organization (FAO) defines the pesticides as any substance or mixture of substances proposed for preventing, destroying or controlling any pest, including vectors of human and animal diseases, unwanted species of plants, animals, fungi, bacteria or viruses causing harm, during or otherwise interfering with the production, processing, storage, food transport or marketing, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The definition also includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, as well as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Pesticides are one of the most potentially harmful chemicals introduced intentionally or accidentally into the environment and their inappropriate use is on increase [2,3]. According to FAOSTAT database, in 2017, the total pesticide use in agricultural practice worldwide was 4.113.591,25 tonnes, and only in Europe, 476.138,24 tonnes were used, which is 11.57% of the total worldwide amount. Regarding the rapid increase of the human population on the planet that affected the demand for sufficient food supplies around the world, pesticides are nowadays practically considered as the benefits chemicals. However, their adverse impacts on the non-target organisms are significant, as well as on the environment [4].

In the last few years, the use of pyrethroids has been growing since restrictions and interdictions have been placed on many of the organophosphorus insecticides [2,5]. According to Madkour [2], pyrethroids are the structural derivatives of naturally occurring pyrethrins and have greater potency. Since the beginning of the century, they have been recommended as an economically and environment friendly group of insecticides as they possess low mammalian toxicity, rapid decomposition in soil, leave no residue in the biosphere and are stable in sunlight [2,6]. Synthetic pyrethroids account for more than 30% of insecticides used worldwide in agricultural, domestic and veterinary applications [2,7]. During 2017, eighteen European Union countries have been used approximately 995.02 tonnes of pyrethroids in agricultural practice, where 70.12% of the total use were recorded only in four countries: France (270.1 tonnes), Spain (159 tonnes), Italy (143 tonnes) and Germany (125.6 tonnes).

One of the frequently used synthetic pyrethroids in agriculture and public health is lambda-cyhalothrin (IUPAC name: 1:1 mixture of (S)-$\alpha$-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propanecarboxylate and (R)-$\alpha$-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate) (Figure 1). This is a non-systemic pyrethroid insecticide and acaricide with contact and digestive action and repellent ability. Gives rapid knockdown with long residual activity. It is highly active against a broad spectrum of pests in agriculture: aphids, Colorado beetles, thrips, Lepidoptera larvae, Coleoptera larvae and adults in cereals, hops, ornamentals, potatoes, vegetables, cotton, and other crops. It is also frequently applied for insect and tick control in public health.

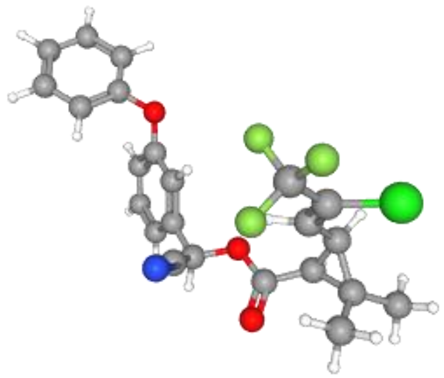

**Figure 1.** Chemical structure of lambda-cyhalothrin

Pyrethroids, including lambda-cyhalothrin, disrupt the normal functioning of the nervous system in an organism, causing paralysis or death [8]. Lambda-cyhalothrin is highly toxic to *Daphnia* sp., fish and bees, with moderate toxicity to algae. It is nontoxic to birds and earthworms.

The half-life of lambda-cyhalothrin on plant surfaces is 5 days. A representative soil half-life is 30 days with values ranging from 23-82 days [8]. In a field study, lambda-cyhalothrin degraded with a half-life of approximately 9 days by National Pesticide Information Centre, or 6-40 days according to MacBean [8]. Lambda-cyhalothrin has a low potential to contaminate groundwater due to its low water solubility and high potential to bind to soil [6]. According to MacBean [8], lambda-cyhalothrin is strongly adsorbed to soil and sediment organic matter.

The soil biota includes a remarkable species diversity that facilitates the maximum exploitation of the resources available in the different microhabitats at various levels of ecological niches [9]. Earthworms, as a part of soil biota, play a significant role in the integrated variety of ecosystem services and functions, such as nutrient supply for plant growth, water regulation, carbon sequestration, nutrient cycling [10], support for biodiversity through food webs, aeration and detoxification of harmful elements [11]. According to Yasmin and D'Souza [12], over 80% of terrestrial invertebrates biomass is represented with earthworms, which along with their important role in soil structuring and nutrient increasing, make them suitable and precise bioindicators of soil chemical contamination providing an early warning of deterioration in soil quality. Additionally, the earthworms ingest daily a large quantity of the manure, decomposed litter or other organic matter deposited on or in soil, serving as a mechanism for transformation and conversion of different organic matter into the rich humus [13].

Organization for economic co-operation and development [14] recommended red wiggler worm, *Eisenia fetida* (Savigny, 1826), as a standard test organism used in terrestrial ecotoxicology tests. It can be easily reproduced on a variety of organic matter, where its short generation time and susceptibility to chemicals resemble an adequate bioindicator soil organism.

The aim of the study was to obtain the data on mortality and average mass changes in juvenile forms of *E. fetida* exposed to the different concentrations/dosage of lambda-cyhalothrin in laboratory conditions.

## 2. Materials and Methods

Mixed age populations of *E. fetida* were purchased from a local manufacturer. After acclimatization for 48 h on the room conditions (temperature: 20±1 °C, humidity below 50%, natural dark/light ratio) with sufficient food, water and air ventilation, juvenile earthworms (specimens without developed clitellum) were selected. The tested specimens were deprived of food for 48 h before the pesticide application.

Plastic containers (11×9.5×4 cm) with lid were prepared for the following test. The bottom and the lid were perforated with holes (diameter less than 3 mm) in order to obtain sufficient ventilation and water drainage. Each container was supplied with 100 g of fresh humus purchased from a local manufacturer. The humus specification is shown in Table 1.

**Table 1.** Humus specification.

| Parameter | Unit | Value |
|---|---|---|
| Readily available nitrogen | mg/L | 121.2 |
| Nitrogen nitrate | mg/L | 120.6 |
| Phosphorus ($P_2O_5$) | mg/L | 108.7 |
| Potassium ($K_2O$) | mg/L | 73.5 |
| Air capacity | % | 20 |
| Field capacity | % | 352 |

Ten earthworms were placed into each container and covered with a lid. During the experiment, sufficient soil humidity in containers was maintained with distilled water. Through a 14 day study period, containers with earthworms were kept under room climate conditions (temperature: 20±1 °C, below 50%, natural dark/light ratio).

Lambda-cyhalothrin was mixed up with tap water in five dosages (Table 2) and applied by spraying once, at the beginning of the test on the substrate surface. The dosage was selected starting from the recommended dosage in agriculture for pest control on wheat. For the control treatment distilled water was used instead of insecticide. Three replicates of each treatment were performed. Test parameters were mortality (corrected %) and average earthworm mass (grams). The duration of the test was 14 days and the parameters were measured and calculated 48 h before the treatment, 24, 48, 72 h, 7 and 14 days after the treatment. Through the testing period, manure was provided as food. The earthworms were removed from the containers on the 15th day of the experiment.

**Table 2.** Lambda-cyhalothrin dosage (LCD) applied to the substrate surface in the container.

| Number | LCD in 80 ml of tap water (ml/80ml) | LCD per 100 $m^2$ ($ml/100m^2$) |
|---|---|---|
| Dosage 1 (D1) | 0.05 | 1.25 |
| Dosage 2 (D2)[1] | 0.08 | 2 |
| Dosage 3 (D3) | 0.1 | 2.5 |
| Dosage 4 (D4) | 0.5 | 12.5 |
| Dosage 5 (D5) | 1 | 25 |

[1] Recommended dosage in agriculture practice for pest control on wheat.

Prior to every weight measurement, the earthworms were extracted from the substrate, rinsed with distilled water, and dried for a few seconds on a filter paper. If any, the dead specimens were removed. The total number of individuals was measured on an analytical scale. The obtained measurement was divided with the number of alive individuals in order to obtain average mass per individual. The percentage of mortality was calculated according to Schneider-Orelli's formula [15].

Statistical analyses of obtained results were performed using TIBCO Statistica® 13.3.0. (University license).

## 3. Results and Discussion

The changes in average mass per individual during the experiment are shown in Figure 1. A significant increase in average mass per individual was observed in the control group, 72 h after treatment (52.25%). Fourteen days after insecticide application, the decrease in average mass was noticed in earthworm groups treated with dosage 4 and 5 (16.29% and 20.45% respectively). The highest mass increase was detected in the group treated with the recommended dosage in agricultural practice (D2). The obtained results are in accordance with Kula and Kokta [16], who observed weight losses at low concentrations of the tested pesticide, where mortality did not yet occur. They emphasized that changes in live earthworm weight are a sensitive parameter that could be used in the determination of sub-lethal effects when pesticides are tested.

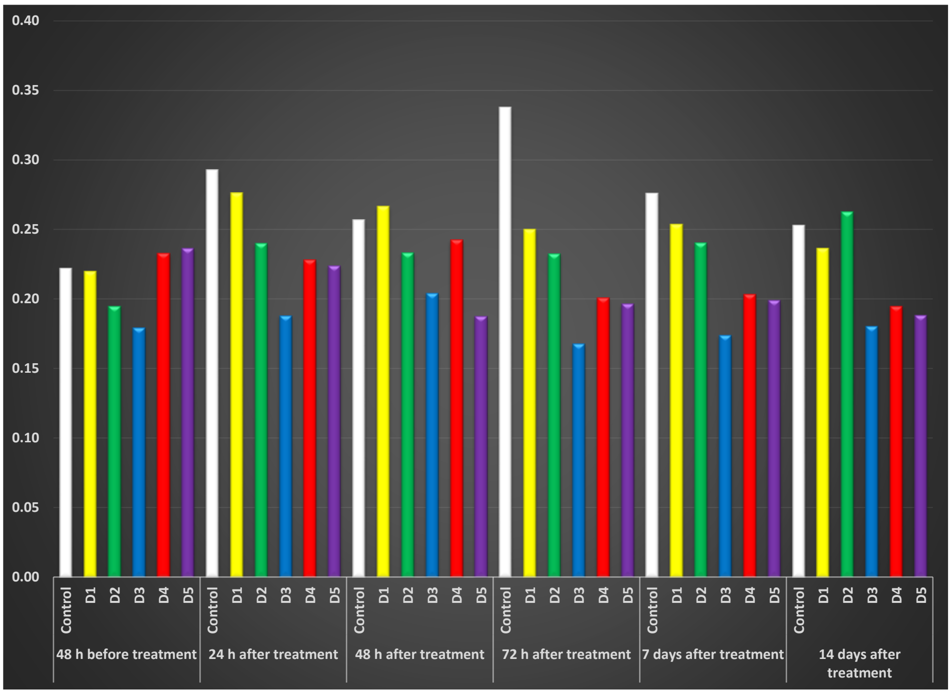


**Figure 1.** Changes in average mass per individual during the experiment (g).

The highest mortality rate was observed 72 hours and seven days after the treatment in groups treated with the highest dosage (D4 and D5) when almost one third of the initial populations were lost (30.00% and 33.33%, respectively) (Figure 2). Similarly, results were obtained by Pereira et al. [17] who investigated the avoidance behavior of the earthworm *Eisenia andrei* under exposure to the carbamate insecticide methomyl. Based on the results of the avoidance toxicity tests and significant adverse effects found at concentrations that are not environmentally relevant, they concluded that methomyl is not likely to be toxic for earthworms if properly used in the field. Furthermore, according to Yasmin and D'Souza (2010), sensitivity tests on different earthworm species have reviled that *E. fetida* is less sensitive compared to the other species.

One-factorial analysis of variance (ANOVA) revealed statistically highly significant differences when comparing different dosages of applied pesticide (pd=0.000000, for p<0.01) as the independent variable and average mass of individuals as dependent variable size. Comparisons of average masses of individuals between replicates and time (pr=0.875214 and pt=0.650103, for p<0.05) did not reveal statistically significant differences.

The Fischer LSD test confirmed the existence of statistically significant differences in the average weight of individuals relative to the applied dosage and the untreated control group, but also to the recommended dosage (D2) and concentrations lower than the recommended (D1) compared to the higher applied dosages.

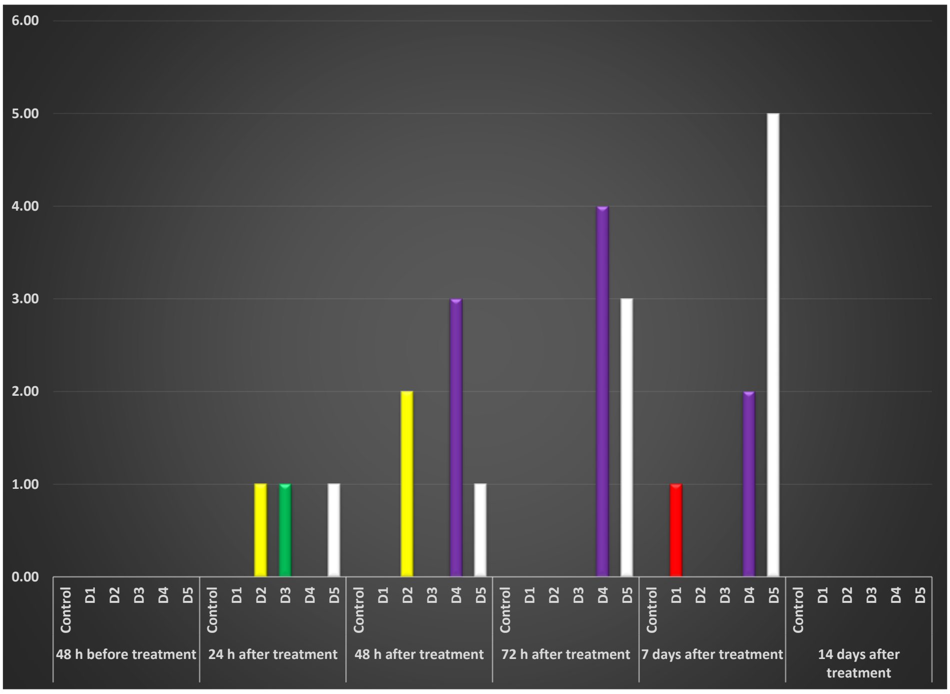


**Figure 2.** Mortality rate expressed according to Schneider-Orelli's formula (corrected %) [15].

The obtained results are in accordance with results obtained by Garcia et al. [18], who investigated the effects of lambda-cyhalothrin on *E. fetida* under tropical and temperate regions experimental conditions. They found that except at the highest concentrations, lambda-cyhalothrin had no effects on mortality and biomass of *E. fetida*. Oppositely to the results acquired from the experiments on the influence of pyrethroids and organophosphate pesticides on mortality rate and weight on *E. fetida*, which showed low or no observed significance, Wang et al. [19], proved that neonicotinoid insecticides, such as imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid have high acute toxicity to *E. fetida*. Especially, imidacloprid and thiacloprid, which within the levels found in soils caused epidermal and midgut cell damage in earthworms. Therefore, the application of neonicotinoid insecticides poses a serious threat to *E. fetida* and other earthworm species in soils. Moreover, according to the results of their study, it could be concluded that besides the standard ecotoxicological tests and parameters monitored at sub-lethal concentrations, the changes in reproduction, cellulase activity and earthworm histology should be included as more sensitive methods, which could have an important value for population and ecosystem protection, as well as for pollutant monitoring and early warning protocols.

Earthworms are in very close contact with any substances present in the soil. They have sensitive receptors on body surfaces with which sense chemicals in the soil, but they also ingest soil particles together with the microscopic organisms on which they feed. Therefore, they are very suitable as models for the chemical toxic effects testing on soil biota [20]. According to Kokta and Rothert [16], where pesticides are used on bare soil or with very little plant cover, as was the case here, the estimation is that nearly the total amount of applied pesticides will enter the soil. This is important for protecting the health of natural environments and is of increasing interest in the context of protecting human health, as well as other terrestrial vertebrates that prey upon earthworms [12].

## 4. Conclusions

The effects of PPPs on soil biota depend on many abiotic, biotic and anthropogenic factors such as characteristics and fate of the compound, its application method, frequency and rate, as well as the site properties: climate, soil texture, pH, organic matter content and the soil organism species diversity and richness. Because of the complexity of the potential interactions between some or all of these factors, it is not surprising that the PPPs can cause different toxicity effects on certain soil species. More studies, both laboratory and field, using different chemicals, soil types, organisms and test conditions are needed in order to improve the environmental risk assessment of PPPs and their fate in soil.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Alavanja, M.C.R. Introduction: pesticides use and exposure extensive worldwide. *Rev Environ Health* **2009**, *24*, 303–309.
2. Naglaa K. Madkour Protective effect of curcumin on oxidative stress and DNA fragmentation against lambda cyhalothrin-induced liver damage in rats. *J App Pharm Sci* **2012**.
3. Bursić, V.; Vuković, G.; Gvozdenac, S.; Petrović, A.; Popović, A.; Marinković, D.; Petrović, M. Abuse of plant protection products. *Journal on Processing and Energy in Agriculture* **2016**, *20*, 189–192.
4. Mezei, M.; Bursić, V.; Vuković, G.; Petrović, A.; Beuković, D.; Marinković, D.; Beuković, M. Pesticides and the loss of biodiversity: European Hare.; Bor, Serbia, 2018; Vol. 26, pp. 220–225.
5. Soderlund, D.M.; Clark, J.M.; Sheets, L.P.; Mullin, L.S.; Piccirillo, V.J.; Sargent, D.; Stevens, J.T.; Weiner, M.L. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* **2002**, *171*, 3–59.
6. Kale, M.; Rathore, N.; John, S.; Bhatnagar, D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol Lett* **1999**, *105*, 197–205.
7. Prasamthi, K.; Muralidhara,; Rajini, P.S. Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. *Food Chem Toxicol* **2005**, *43*, 299–306.

8. *The pesticide manual: a world compendium*; MacBean, C., British Crop Protection Council, Eds.; Sixteenth edition.; British Crop Protection Council: Alton, Hampshire, 2012; ISBN 978-1-901396-86-7.
9. Datta, S.; Singh, J.; Singh, S.; Singh, J. Earthworms, pesticides and sustainable agriculture: a review. *Environ Sci Pollut Res* **2016**, *23*, 8227–8243.
10. Putten, W.H. van der; Mudgal, S.; Turbé, A.; Toni, A. de; Lavelle, P.; Benito, P.; Ruiz, N. *Soil biodiversity: functions, threats and tools for policy makers*; Bio Intelligence Service: Parijs, 2010; pp. 1–250;.
11. Altieri, M.A. The ecological role of biodiversity in agroecosystems. *Agric Ecosyst Environ* **1999**, *74*, 19–31.
12. Yasmin, S.; D'Souza, D. Effects of Pesticides on the Growth and Reproduction of Earthworm: A Review. *Appl Environ Soil Sci* **2010**, *2010*, 1–9.
13. Sandoval, M.C.; Veiga, M.; Hinton, J.; Klein, B. Review of biological indicators for metal mining effluents: a proposed protocol using earthworms.; Columbia, 2001; Vol. 25, pp. 67–79.
14. Test No. 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) Available online: https://www.oecd-ilibrary.org/environment/test-no-222-earthworm-reproduction-test-eisenia-fetida-eisenia-andrei_9789264070325-en (accessed on Mar 28, 2020).
15. Püntener, W. *Manual for field trials in plant protection*; Agricultural Division; 2nd ed.; Ciba-Geigy Limited, 1981.
16. Kula, H.; Kokta, C. Side effects of selected pesticides on earthworms under laboratory and field conditions. *Soil Biol Biochem* **1992**, *24*, 1711–1714.
17. Pereira, J.L.; Antunes, S.C.; Ferreira, A.C.; Goncalves, F.; Pereira, R. Avoidance behavior of earthworms under exposure to pesticides: is it always chemosensorial? *J Environ Sci Health B* **2010**, *45*, 229–232.
18. Garcia, M.; Scheffczyk, A.; Garcia, T.; Römbke, J. The effects of the insecticide lambda-Cyhalothrin on the earthworm Eisenia fetida under experimental conditions of tropical and temperate regions. *Environmental Pollution* **2011**, *159*, 398–400.
19. Wang, K.; Pang, S.; Mu, X.; Qi, S.; Li, D.; Cui, F.; Wang, C. Biological response of earthworm, Eisenia fetida, to five neonicotinoid insecticides. *Chemosphere* **2015**, *132*, 120–126.
20. Reinecke, S.A.; Reinecke, A.J. The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. *Ecotox Environ Safe* **2007**, *66*, 244–251.