ACUTE TOXICITY OF AN INSECTICIDE (ACETAMIPRID) ON *LUMBRICUS TERRESTRIS* (LINNAEUS, 1758)

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ABSTRACT

Earthworms are effective bio-indicators of biodiversity, soil quality and the environmental impact on cropping systems. Therefore, this study was conducted to assess the toxicological effect of Acetamiprid, a widely used insecticide by farmers in the region of Souk-Ahras (Algeria), on a biological model named Lumbricus terrestris using seven increasing concentrations 0, 5, 7, 9, 12.5, 25 and 50 mg/500 mg soil for four control periods; 24, 48, 72 and 96 hours. The physicochemical soil analysis indicated that the soil pH was strongly alkaline, and the pH water and pH KCl were respectively 8.52 and 7.72. The soil texture was sandy loam with a high level of organic matter (OM = 12.9%). In addition, the LC_{50} and LC_{90} values were remarkably decreased (LC_{50} = 12.4, 10, 7.30 and 6.69/500 mg soil; LC_{90} = 18.52, 14.21, 9.50, 9.15 mg/500 mg soil, corresponding respectively for each LC values to the four exposure periods 24, 48, 72 and 96 hours). Furthermore, no significant change was noticed in body weight and growth of earthworms treated with Acetamiprid at various concentrations. The mortality rate was concentration-dependent; increased with increasing concentration, and this was supported by the histopathological observations showing cellular alterations in the epidermis and muscle fibers. Additionally, treatment with Acetamiprid at high concentrations caused marked morphological abnormalities in the earthworms as evidenced by the winding of the body, loss of pigmentation, cut of the posterior part, lesions, or bloody wounds.

Keywords: *Lumbricus terrestris,* Acetamiprid, LC_{50}, LC_{90}, Growth, Mortality, Histological effects

INTRODUCTION

For several millennia, agriculture has relied on the resources of natural environments with humans fully integrated into the terrestrial ecosystem, however, a few years ago, agricultural activities started using external resources (fertilizers, pesticides, fossil fuels), and still up to now very dependent on them (Ojo and Zahid, 2022). Furthermore, pesticides are largely used to protect edible crops, economic plants, and lawns by controlling or killing target species, such as pests, insects, and weeds (Möhring *et al.,* 2020). The generalization of their use in most farms would testify to the quantitative improvement of the products, but also the fight against the pests of certain crops since they can cause many environmental risks and problems (Pretty and Pervez Bharucha, 2015). The misuse of pesticides can lead to serious problems, including the poisoning of farmers, destruction of non-target organisms, wide dispersal of pesticides in different environmental ecosystems, especially in food webs, and masses of freshwater and air, which can target human body through various routes of exposure (Martin *et al*., 2018). Several studies conducted in several countries around the world have shown the presence of pesticides in soil can threaten the soil environment and the life of the soil organisms, in particular, earthworms and terrestrial gastropods (Bhandari *et al.,* 2020). Earthworms are the most widely distributed soil invertebrate organism in Algeria, effectively contribute to the recycling of soil matter and are believed to be efficient environmental biomonitoring tools through measuring the effects of chemicals including pesticides in the laboratory or *in-situ* (Robidoux *et al.,* 2002). Earthworms are overall proven to be efficient bioindicators to vital monitoring, and hence their importance in the structure of ecosystems is explained by the fact that they constitute an ecologically dominant group of invertebrates, almost all soils contain at least one species of earthworms. Also, they can be exposed to contaminants via different routes (aqueous phase, vapor phase, and ingestion of the solid phase), thus organisms accumulating contaminants play an important role in the bio-monitoring of multiple pollutants, reflecting early responses to the deleterious effects of these pollutants on biological systems (Wang *et al*., 2017).

Acetamiprid is used in crops against aphids, and acts directly on the receptors of the central nervous system causing consequently convulsions and paralysis of insects. Also, it has a systemic and translaminar action (penetrates the entire leaf and could be transported by the current of sap throughout the plant), and thus can reach insects on the opposite side of the leaf and new shoots. Acetamiprid acts on eggs, larvae, and adults. The persistence of organic matter in the active ingredient soil is 2 to 3 weeks (EPA, 2004). In this regard, this study assessed the toxicological effect of an Acetamiprid insecticide at various concentrations and exposure periods on *L. terrestris*.

MATERIALS AND METHODS

Sampling Site: The experimental study was conducted on earthworms obtained from the Forest of Mohamed El-Cherif Messaadia University, of Souk Ahras city (Northeast of Algeria), surrounded by El-Taref City from the east, Guelma City from the West, Tébessa City from the south, and Oum El-Bouaghi City from the southwest (Figure 1).

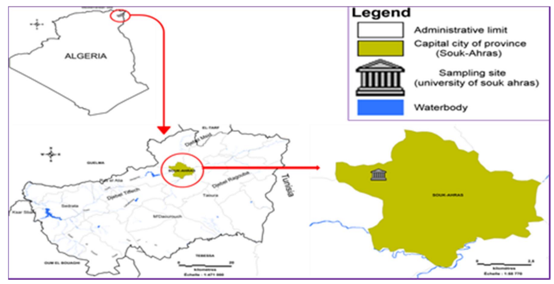

Figure 1: Collection site of *Lumbricus terrestris* – the Forest of the Mohamed El-Cherif Messaadia University, Souk Ahras City, Algeria

The Forest of Mohamed El-Chérif Messaadia University is a virgin area, rich in vegetation, earthworms, and terrestrial gastropods of which more than 15 species of wild plants, five species of earthworms, and two species of snail and slug, excluding the mush diversified insect species that have been identified.

Physicochemical Analysis of Soil: The soil samples collected randomly from the study site were physicochemically screened for common soil parameters including, pH, the pH-KCl, the hygroscopic water, and the soil texture according to the methods of Lag *et al*. (2008), whilst the electrical conductivity (EC) of soil was determined by the method of Okalebo *et al.* (2002). In addition, the capacities in the field and the saturation were measured according to the method of Balze (2000). The carbonates content of the soil was determined using the method ofHorvath *et al*. (2005), and the organic matter content in soil was determined by the method of Bonnefont *et al.* (1980).

Experimental Design

Animals: In this study, 105 adult earthworms were collected from soil at the depth of 20 to 30 cm at the beginning of the spring season (Bouche, 1972). Adults were separated from juveniles, as they are characterized by the proper development of clitellum, placed in plastic bins (17 cm wide and 45 cm long) containing native soil and plant residues obtained from the same sampling site, covered with mosquito nets, enabling worms breathing and preventing water evaporation, and then placed in a cool and air-conditioned room for 10 days of adaptation.

Chemical and determination of lethal concentration: In this study, Acetamiprid, a very active systemic insecticide was used (NCBI, 2023). The lethal concentrations were determined by using Finny's probit analysis, which is a method widely used in the field of ecotoxicology to explain the biological tests that use insecticides, fungicides, herbicides, and hence to calculate LD_{50} and LD_{90}. The principle of this method is based on the determination of the percentage of mortality which is then transformed into Probit, and the logarithm of the concentrations enables the determination of the regression line from which LC_{16}, LC_{50}, LC_{84} and LC_{90} can be estimated (Finny, 1971).

Treatments: A series of increasing concentrations (0, 5.7, 9, 12.5, 25 and 50 mg/g soil) were each diluted in one liter of distilled water and used for worm's treatment by spraying 300 ml of the solution on 500 g of soil, which then was well homogenized. The control earthworms were sprinkled with distilled water (Iordache and Borza, 2011). Experiments were carried out with three replicates, with each replicate containing five earthworms.

Body growth evaluation: The body growth rate was determined by daily weighing of the earthworms at 0, 24, 48, 72 and 96 hours using an electronic weighing balance.

Mortality test: The percentage of mortality was determined by counting dead individuals every 24 hours. The mortality was confirmed by the immobility of earthworms.

Effect of Acetamiprid on the morphology of *Lumbricus terrestris:* The morphological alterations in control earthworms and those treated with different concentrations of the test insecticide following the selected exposure periods were macroscopically observed.

Histological examination: Three earthworms of each concentration were taken, washed with distilled water, transferred to boxes containing 01% agar-agar for 96 hours to empty the soil in their digestive tract, cut into two parts, and then placed in cassettes containing normalized formalin (Oluah *et al.,* 2010). The fixed earthworms were subjected to histological procedures of serial alcohol dehydration, embedding in paraffin wax, mounting, sectioning and staining with Haematoxylin-Eosin for microscopic observation. The histological sections were observed using a light microscope (x40) and microphotographed using a digital camera (Fine pix 40i, Fuji, Japan) (Berrouk *et al.,* 2021).

Statistical Analysis: The significant differences between multiple groups were tested using analysis of variance (ANOVA). Significant means were separated using LSD and $p<0.05$ was considered significant. The correlation between the growth and the different chemical concentrations on one hand and the growth as a function of time was tested by the Pearson correlation coefficient. All data analyses were done using SPSS Version 22 (IBM SPSS, 2019),

RESULTS

Physicochemical Properties of the Soil: As indicated in Table 1, the results of the physicochemical analysis of the native soil indicated that earthworms can live in alkaline soil. The soil had pH water and pH KCl in the interval of 8.52 – 7.70, a sandy-loam soil texture with a saturation rate of 82%, and high level of organic matter (OM = 12.90%).

Effect of Treatment on Body Weight Growth: The body weight of treated earthworms compared with controls revealed no significant concentration-dependent increase ($p>0.05$) (Figure 2).

Table 1: Physicochemical properties of the Mohamed El-Cherif Messaadia University forest soil, the habitat of *Lumbricus terrestris*

Physicochemical parameters	Content
pH (H_2O)	8.52
pH (KCl)	7.70
Electrical conductivity	2.08 mS /cm
Organic matter level	12.90%
Carbonate level	2-10%
Saturation capacity	82%
Field capacity	41.47
Texture	Sandy-loam

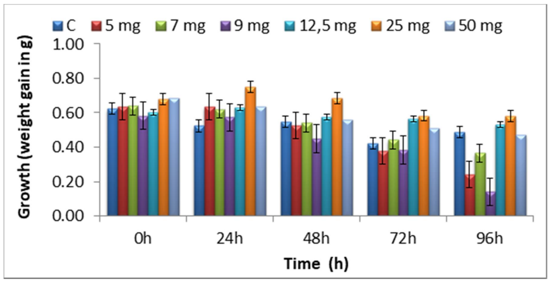

Figure 2: Effect of increasing concentrations of Acetamiprid on growth of *Lumbricus terrestris*

Similarly, there was no significant correlation ($p>0.05$) between growth and concentrations as evidenced by the Pearson correlation coefficient test.

Table 2 revealed a time-dependent decrease in the values of lethal concentrations LC_{16}, LC_{50}, LC_{84} and LC_{90}. Also, the values of the regression coefficients determinant were found to be close to 1, indicating a strong correlation between the concentrations of Acetamiprid and the mortality rate of earthworms.

Table 2: Various lethal concentrations values as a function of exposure periods (24, 48, 72 and 96 hours)

Lethal concentrations (mg)	Exposure period (hours)			
	24	48	72	96
LC_{16}	9.09	7.61	5.96	5.24
LC_{50}	12.40	10.00	7.30	6.69
LC_{84}	16.93	13.13	8.96	8.53
LC_{90}	18.52	14.21	9.50	9.15
Equation	$Y = 7.37x - 3.05$	$Y = 8.41x - 3.41$	$Y = 11.22x - 4.69$	$Y = 9.40x - 2.75$
R^2	0.98	0 .95	0.94	0.88

Percentage of Earthworm Mortality: As shown in Figure 3, the control earthworms showed no mortality (zero mortality rate), but the low concentrations of Acetamiprid-treated animals reveal a low mortality rate, which hence increases proportionally with the increase in the used concentrations.

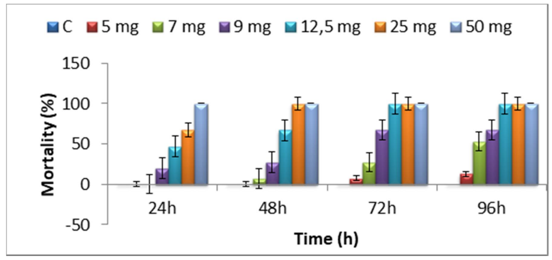

Figure 3: Changes in mortality of *Lumbricus terrestris* exposed to increasing concentrations of Acetamiprid after 96 hours

Acetamiprid-Induced Behavioral and Morphological Alterations in *Lumbricus terrestris*: Earthworms exposed to Acetamiprid had behavioral disturbances, evidenced by their emerging on the soil surface and rolling up on themselves. Similarly, several morphological abnormalities were observed such as bloody lesions, loss of skin pigmentation as well as, the strangulation of the posterior part of the body up to the total disintegration, especially with the strong concentrations

Histological Observations: The muscle tissue sections from control earthworms revealed a normal organization of the circular and longitudinal muscles (Figure 4A) since Acetamiprid at 12.5 mg/g soil-treated earthworms caused a slight alteration in the muscle tissue, which overall kept its structural integrity (Figure 4B). Marked histological alterations in the muscle tissue were found in 25 mg /g soil of Acetamiprid, showing the formation of vacuoles, and alterations in muscle fibers (Figure 4C), and similarly, treatment with Acetamiprid at 50 mg/g soil showed alteration in epithelial tissue and muscle fibers, and loss of internal organization (Figure 4D).

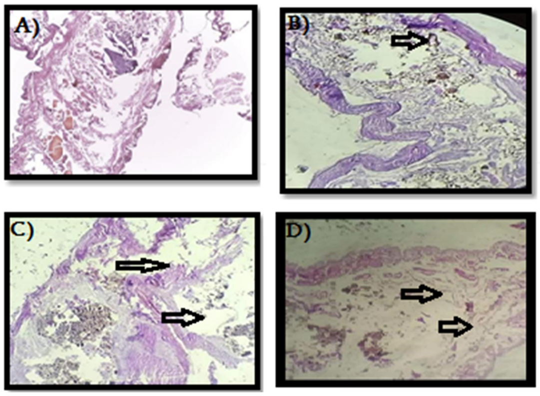

Figure 4 (A, B, C and D): Histological sections of *Lumbricus terrestris* and treated with different concentrations of Acetamiprid after 96 hours (Light microscope, Gr x 40). *Key:* *A - Control, B - Acetamiprid at 12.5 mg/g soil, C - Acetamiprid at 25 mg/g soil, D - Acetamiprid at 50 mg/g soil*

DISCUSSION

Physicochemical Properties of the Soil: In this study, the soil pH was found to be strongly alkaline, meanwhile, the pH water and pH KCl of the soil ranged between 8.52 and 7.72, and the soil texture was sandy-loam with a high level of organic matter (MO = 12.9 %), promoted the survival, growth, reproduction and distribution of *L. terrestris*. In this context, Curry (1998) had mentioned that soil texture can affect the distribution and abundance of earthworms. Edwards and Bohlen (1996) had explained that soils with low organic matter level generally do not support a high density and diversity of earthworms. According to Crumsey *et al.* (2013), acidic soils influence earthworm dynamics and dispersal. It was reported that the physicochemical analysis of soil earthworm sampling sites in Tébessa City showed that the pH of all soils is alkaline, and the texture of the soil was clayey-sandy-loamy with low or high organic matter level (Bouazdia, 2019).

Effect of Acetamiprid on the Growth of *Lumbricus terrestris:* The growth and reproduction of earthworms are among the parameters sensitive to the toxic effects of different pesticides used because the drop in body weight of earthworms and the decrease in their reproduction rate positively influence their abundance in natural ecosystems. Xiao *et al.* (2006) have mentioned that the herbicide acetochlor affects the growth and reproduction of *Eisenia fetida.* Furthermore, Curry and Good (1992) and Wang *et al.* (2015) suggested that organophosphate and organochlorine insecticides can inhibit earthworm fecundity and decrease their biomass. The body weight decrease was previously reported in earthworms treated with three herbicides (Propyzamide, Benfluraline and Metribuzine) (Bilalis *et al.,* 2013) and Isoproturon (Lackmann *et al.,* 2018). Similarly, the growth rate of *Aporrectodea caliginosa* decreased after 28 days of treatment with the herbicide (Sekator OD), in addition to a marked decrease in the body weight gain in 32.7 mg/kg Karate Zeon exposed *A. caliginosa* (Bouazdia, 2019).

Acetamiprid-induced Mortality in *Lumbricus terrestris*: Earthworm mortality is a very sensitive index of the toxic effects of pesticides, and earthworm survival remains the first parameter addressed by eco-toxicological tests. In this regard, Xiao *et al.* (2006) showed that the herbicide acetochlor at concentrations of 20 – 80 mg/L caused sublethal toxicity in *E. fetida* species. Yesguer (2015) has reported very high mortalities of *A. caliginosa* reaching up to 100% after one week of exposure to chlorpyrifos, and similarly, Berrouk *et al.* (2021) have showed significant mortalities in *Aporrectodea giardia* exposed to the insecticide Acetamiprid. Contrarily, Lackmann *et al.* (2018) have reported no mortality in *L. terrestris* exposed to Isoproturon even at the highest concentrations.

Effect of Acetamiprid on the Morphology of *Lumbricus terrestris*: According to Bouazdia (2019), *A. caliginosa* exposed to the insecticide Karate Zeon at a high concentration (32.7 mg/kg) for 4 weeks had swelling of the anterior region, accentuated degeneration of the posterior part resulting in body fragmentation, and besides, Sekator OD herbicide exposed *A. caliginosa* did not cause morphological abnormalities even at the highest concentrations. Moreover, Rathore and Nollet (2012) have reported contraction of the longitudinal muscles, coiling of the body, rigidity of the body, swellings on the body, and bursting of swellings creating wounds and bleeding in agrochemical exposed worms. Many authors have also reported that the use of some pesticides induced the rupture of the cuticle and the exit of cœlomic fluid in treated earthworms (Miglani and Bisht, 2019; Zhang *et al*., 2020).

Histological Effect: Histological observations of tissues and cells are valuable tools for assessing the toxic effects of contaminants on earthworms. Herein, previous studies have reported various histological alterations in pesticides exposed worms, including tissue weakening, hypertrophy of glandular cells with the formation of vacuoles in Butachlor-exposed earthworms (Muthukaruppan *et al.,* 2005), and damage in the epidermal cells and the intestinal cells in insecticides exposed worms (Wang *et al.,* 2015). As earlier reported by Bouchachia and Aissani (2016), the insecticide Durban and the fungicide propicol-70 can cause serious alterations in the testicles and ovaries of *E. fetida*. In addition, epidermal and muscular tissue alterations were previously reported in earthworms exposed to Acetamiprid (Berrouk *et al*., 2021).

Conclusion: The abundance and biodiversity of earthworms are very good indicators of soil health, and they play an important role in agriculture and natural environments as an environmental quality monitoring tool. Several species of earthworms have become model organisms for toxicological and ecological research, particularly in the assessment of soil contamination by different pesticides. As part of the biomonitoring of natural ecosystems, this study investigated the effect of an insecticide, Acetamiprid, on the earthworm *L. terrestris* and revealed that: (i) adverse concentration-dependent effects on the morphology, growth, survival, and histology of *L. terrestris,* and (ii) effective contribution for the regulatory authorities to understand the effects of insecticides (Acetamiprid) on non-target organisms (*L. terrestris*), which could provide useful information on the expected dangers of different pesticides detected in the natural environment.

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