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TOXICOLOGICAL AND HISTOPATHOLOGICAL IMPACT OF INSECTICIDE ACETAMIPRID AND AGRICULTURAL FERTILIZER ACTIVEG ON EARTHWORM (*Aporrectodea giardi*)

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ABSTRACT

Earthworms provide important information about the environmental risks so serve as useful biological indicators of soil contamination. The present study was aimed to assess the impact of insecticide 'Acetamiprid' and fertilizer 'Activeg' (widely used fertilizer in Algeria), on earthworm *Aporrectodea giardi*. The earthworms were subjected to four different doses of Acetamiprid *viz*., 25, 50, 100 and 200 mg 500 mg^{-1} soil for four periods *viz*., 24, 48, 72 and 96 h. The toxicological and histopathological evaluations were also conducted. Based on physiochemical analysis, soil proved to be a convenient environment for earthworms. The soil was sandy loam in texture, neutral in pH and rich in organic matter (8.93%) with a field capacity of 19.37. Earthworm mortality showed increase with increase in Acetamiprid dose with LD_{50} value varying from 89.03 to 240.23 mg kg^{-1}. Histological studies revealed marked alterations in epidermal and intestinal cells. Earthworm exposed to fertilizer Activeg showed neither earthworm mortality nor any toxicological effects on their tissue structure. The study confirmed the deleterious effect of Actamiprid on *A. giardi*, so it should be used with prudence in order to save the soil animal fauna.

Keywords: Acetamiprid, Activeg, *Aporrectodea giardi***,** earthworm, histopathology, LD_{50}, mortality

INTRODUCTION

The use of agrochemicals is effective in protecting agricultural crops against many biological pests; thereby these not only contributes in crop yield stabilization but also improve crop quality. The excessive use of these products may negatively impact the surrounding ecosystem including human health, because some organisms are vulnerable to the variations in biotic and abiotic conditions and may respond to them via measurable changes at morphological, physiological, cellular and molecular levels (Bekkar and Bouzned, 2014). The survival and reproductive capacity of endogenous organisms including earthworms are negatively affected by soil pollution (Leveque *et al.*, 2015). The presence/abundance of such organisms in an ecosystem serves as efficient bio-indicator of several ecosystem properties.

Earthworms act as good sentinel organisms of soil pollution because they come in direct contact with soil (Atanu et *al*., 2020). Earthworm play key role in soil ecological functions mainly in the recycling of soil nutrients, decomposition of organic matter and maintenance of soil structure (Onja, 2018). The methods of environmental quality evaluation, based on chemical properties of pesticides, have long been used but not found effective in assessing the impact of contaminants on different living organisms. However, new methods involving the use of biological, physical and biochemical parameters of living organisms at individual and ecosystems levels have overcome the deficiencies inherent in chemical analysis (Mathias *et al.*, 2011; Gountain 2013). The earthworm, *Aporrectodea giardi,* is mainly used as tropical biological animal model in soil related studies through targeting its bioindicators sensitivity to environmental conditions based on histopathology. The present study was aimed to assess the toxic effects of insecticide 'Acetamiprid' and agricultural fertilizer 'Activeg' (widely used in Algeria, especially in Souk-Ahras city) on earthworm (*A. giardi*).

MATERIALS AND METHODS

Sampling site properties

The representative samples were obtained from a house garden located within the municipal limits of Ouled-Driss, located 40 km away from Souk-Ahras city (northeast Algeria) on the border of Tunisia. This site is characterized by a continental climate with the Mediterranean and desert influence and rainfall varying between 300 and 1000 mm year^1 (Khoualdia and Hammar, 2017).

Physicochemical analysis of soil

Compost soil samples collected from the study site were evaluated for some physicochemical properties. The soil samples were prepared by removing grit and gravels and then air-dried. The soil pH was determined as per the method of Baize and Jabiol (1995). For determination of pH_{water}, a solution mixture of 10 g fine soil and 50 mL distilled water was thoroughly agitated, then kept standing as such for 15-30 min, and pH value noted using a pH meter. For measuring $pH_{KCl,}$ 20 g fine soil was dissolved in 100 mL distilled water and then 50 mL KCl added to it, agitated for 5 min and allowed to stand for 30 min. The $pH_{KCl,}$ value read on a pH meter. The electrical conductivity of soil was determined as per the method of Bacha and Bechim (2005). Briefly, a soil solution was prepared by mixing 10 g fine soil with 50 mL distilled water, then thoroughly agitated and kept standing for 15-30 min. The EC values were read on a conductivity meter.

The hygroscopic water, including field capacity and saturation capacity, was determined as per the method of Baiz (1988). For measuring soil hygroscopic water or non-usable water, a sample of 30 g fine soil, taken on aluminium paper, was dried in an oven at 105°C for 24 h. The hygroscopic water was determined by using the formula (Laval, 2011; Elodie, 2013):

$$\text{Hydroscopic water (\%)} = \frac{\text{Weight of water}}{\text{Weight of dry soil}} \times 100$$

Where, weight of water was obtained by subtracting the weight of water before drying from the weight of water after drying.

For the determination of field- and saturation-capacity of soil, the saturated dough solution prepared by drop-wise adding water to soil until shiny and slippery dough was formed. Then, it was allowed to stand for 1 h and kept in an oven at 105°C for 24 h. The field and saturation capacity were determined as follow:

$$\text{Saturation rate (SR)} = \frac{X}{Y} \times 100$$

$$\text{Field capacity (FC)} = \frac{\text{Saturaation rate}}{2}$$

Where, Y = Dry soil weight; and X = Weight of non-dried soil

The soil texture and carbonate content were determined as per the method of Guesmia (2019). A mixture of 10 mL dilute HCl, 30 mL distilled water and 10 g fine soil was agitated and left as such to stand. The carbonate content (%) was determined as follows:

$$CaCO_3\ (\%) = \frac{\text{Weight of released carbon dioxide x 2.274}}{\text{Soil weight}} \times 100$$

Where, the weight of released CO_2 is determined by subtracting the initial weight of beaker + acid (W1) and 10 g soil (W1 + 10 g soil = W2) from the final weight of beaker, acid and soil (W3).

The soil organic matter content was determined as per the method of Laval (2011). In this method, 10 g oven-dried soil sample was calcined in a muffle furnace at 375°C for 16 h. The organic matter (OM) content was determined as follows:

$$\text{Organic matter (\%)} = \frac{\text{Dry soil weight (g)} - \text{Weight of incinerated soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

$$\text{Organic matter (\%)} = \frac{(W1 - W0) - (W2 - W0)}{W1 - W0} \times 100$$

Where W_0 is empty capsule weight, W_1 is final weight, and W_3 is weight of capsule containing ash.

Biological materials

Aporrectodea giardi abundantly omnipresent earthworm species in the eastern region of Algeria was used as laboratory animal model. The earthworms were collected by digging the superficial soil layer up to 10-15 cm depth. The earthworms were kept in plastic bags (45 individuals per bag). Each bag was filled with same garden soil up to 10 cm for better adaptation and acclimatization for 14-21 days. The earthworms were fed with leaf and plant debris throughout the experimental period (Mokatel and Touahria, 2015).

Chemical materials

The insecticide 'Acetamiprid' and agricultural fertilizer 'Activeg' were procured from Agricultural Cooperative, Souk-Ahras city, Tunisia. 'Acetamiprid' is an odourless blue, floury solid water-soluble insecticide of neonicotinoid family used against insects and cereal suckers (Fiche de Conseil, 2013). 'Activeg' power 20 is used to improve the quality of cereals by foliar spray in root application in wettable powder form.

Toxicological studies

The four concentrations of 'Acetamiprid' and 'Activeg' evaluated were 25, 50, 100 and 200 mg 500 mg^{-1} soil, and each treatment was replicated three times with each replication comprising of 9 individual earthworms. The concentration of each chemical was prepared in distilled water. The 'Acetamipride' or 'Activeg' solutions were sprayed at 24 h interval for 4 consecutive days. Control earthworms were sprayed with distilled water only. The mortality rate of earthworm was determined by counting the number of dead earthworms in each treatment during four periods. The mortality was confirmed by immobility of earthworms while touching them with an object (Oluah et *al*., 2010). The LD_{50} was determined by the method of Bliss (1938) using Probit analysis statistical method based on the transformation of sigmoid curve of Trevan (1927) to a straight curve. The mortality data were represented as mean ± standard error. The significance of variation due to the changes in concentrations and exposure time was tested by two-way ANOVA with unpaired Student's t test.

Histopathological examination

The histological studies of earthworm tissues were performed at the end of experiment in Anatomo-Pathology Laboratory of Souk-Ahras Hospital (Northeast Algeria) as per the method of Madjri and Hachad (2014). Three earthworms from each treatment were randomly selected, washed with distilled water and put in jars containing 1% agar jelly. These jars were kept as such for 96 h to facilitate the removal of soil digestive system and dehydrated with upgrading ethanol (70-100%) for 15 min before cutting them into 2 pieces (Oluah et *al.*, 2010). The samples were embedded in formalin wax for 18 h and sectioned into small slides using a semi-automated rotary microtome. The sections were spread on the slides containing a drop of water and passed on hot plate. The remaining paraffin was dissolved by keeping the slide rack in an oven at 150°C. The processed samples were stained successively with xylene, ethanol and hemalin for 10, 3 and 2 min, respectively. The stained sections were washed with tap water and stained with eosin for 5 min and again washed with tap water before staining with ethanol and acetone + xylene for 2 min each. The stained sections were mounted in xylene. The tissue sections were observed at 40X under a binocular microscope and photographed with the help of a microscope-mounted camera.

RESULTS AND DISCUSSION

The soil was sandy loam in texture, neutral in reaction (pH 7.14 and 6.88 in water and KCl, respectively) and electrical conductivity was 0.151 m mhos cm^{-1}), rich in organic matter (8.93%) and strongly calcareous with $CaCO_3$ content of 52.98% and field capacity of 19.374. The study revealed that the earthworms survived in organic matter rich neutral sandy loam soil. Madjri and Hachad (2014) reported that *Lumbricus terresteris* survive in basic, loamy clay textured soils rich in organic matters with mean rate of total limestone content. Gountan (2013) evidenced the effects of pedoclimatic conditions on the density and biomass of soil macrofauna including earthworms. Bazri (2015) found that *Aporrectodea caliginosa* prefers alkaline soil (pH, 8.21), but *Octolasion lacteum* prefers acid soil (pH, 6.22), indicating that every species of earthworm has its optimal pH for growth. The soil type, depth and texture affect the earthworm population abundance. Moore *et al.* (2015) reported that soil acidity influences the survival of *L. terresteris* and life cycle (reproduction) of *Amynthas hawayanus*. Earthworms are sensitive to soil-texture, because of their size and roughness of soil physicochemical properties created by soil texture, particularly, the soil-water retention capacity. Bazri (2015) reported that *Octolasion lacteum* and *Octodrilus maghrebinus* survive in organic matter rich soil.

Effect of acetamiprid on earthworm mortality and LD values

The mortality of earthworms increased with increase in ‘Acetamiprid’ concentration and exposure time (Table 1; Fig. 1). Anthropogenic activities often lead to the accumulation of inorganic and organics in soil and the earthworms living in close proximity with contaminated soil may experience stress at different levels. Blouin *et al.* (2013) reported that earthworms are among the soil animals exposed to pollution, because they feed on soil mixed with organic matter and the products of pollution. In present study, mortality rate increased with increase in acetamiprid concentration or exposure periods.

Table 1: Effect of Acetamiprid on mortality (%) of earthworm, *Aporrectodea giardi* [The values are mean ± standard error (S)]

Exposure time to acetamiprid (h)	Control	Doses of Acetamiprid (mg)			
		25	50	100	200
24	0 ± 0	0 ± 0	0 ± 0	2.66 ± 0.58	2.66 ± 1.15
48	0 ± 0	0.33 ± 0.58	0.33 ± 0.58	4.66 ± 2.08	5.66 ± 1.58
72	0 ± 0	0.66 ± 0.58	0.66 ± 0.58	6.00 ± 2.08	6.66 ± 1.00
96	0 ± 0	0.66 ± 0.58	1.00 ± 0	6.66 ± 1.53	7.33 ± 1.15

The ‘Acetamiprid’ concentrations leading to 16, 50, 84 and 90% killing of *A. giardi* population (i.e. LC_{16}, LC_{50}, LC_{50} LC_{84} and LC_{90}, respectively) were 41.21, 89.03, 192.33 and 240.23 mg kg^{-1} $soil^{-1}$. LC_{50} of acetamiprid in case of *A. giardi* was 89.03 mg kg^{-1} soil, and CL_{90} was 240 mg kg^{-1} soil. Neither mortality nor toxicological effects were observed in *A. giardi* exposed to ‘Activeg’, even at very high concentrations. Bakkar and Bouzned (2015) reported that the Etalon (herbicide) disturbs the enzymatic activity of catalase in *Lumbricus terresteris*, Madjri and Hachad (2015) found that Mikal-Flash (fungicide) increases the MDA content (lipid peroxidation marker) in *L. terresteris*. Moussavou (2010) reported that pesticides, heavy metals and other chemical pollutants

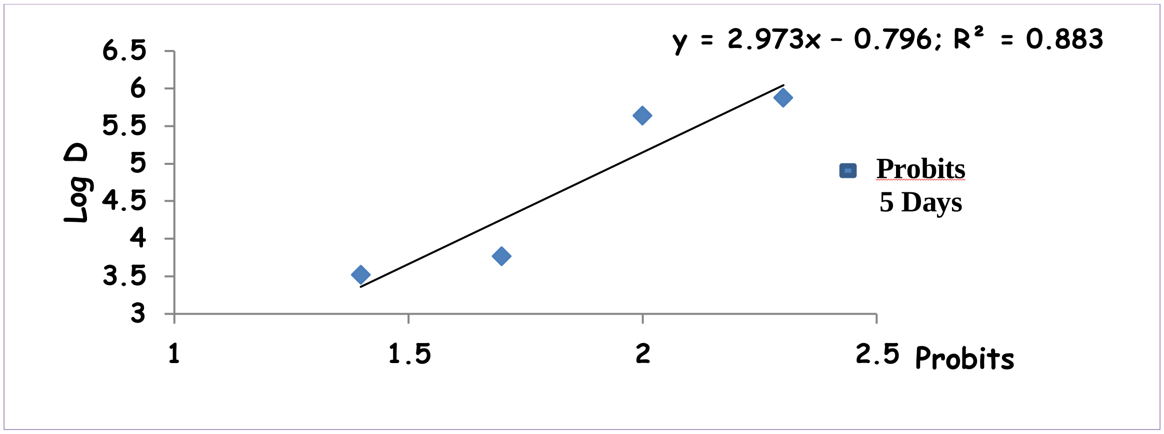

Fig. 1: A curve showing the mortality of *A. giardi* in the function of used Acetamiprid concentrations (R^2 = Determination coefficient)

poison and disturb the natural habitat of animals; and lead to ecological imbalance. Pelosi *et al.* (2014) reported that insecticides show negative effect on earthworms than herbicides and fungicides; further reducing the pesticides use increases the density of earthworm population. Yesguer (2015) found the LD_{50} of 73.6 and 60 mg kg^{-1} soil for Manebe and dursbam on *Aporrectodea calginosa*. Mekahlia *et al.* (2016) showed that the application of Sekator mixed with fertilizer could have nefast effect on *L. terresteris* and cause significant changes in the measured biomarkers.

The exposure of earthworm *A. giardi* to various test doses of agricultural fertilizer 'Activeg' neither affected the earthworm mortality nor showed any toxic effects on their survival and growth during the study period.

Histopathological changes in earthworms exposed to chemicals

The histological study of 'Acetamiprid'- and 'Activeg'-treated and untreated earthworms revealed that the tissues in untreated control had normal structure of epithelium, circular muscles and chloragogen cells (Fig. 2a). However, the tissue sections of earthworms treated with 25 and 50 mg 'Acetamiprid' showed some histological modifications in the form of epidermis alterations along with the formation of some vacuoles (Fig. 2b,c). The histological sections of earthworms treated with 100 mg 'Acetamiprid' depicted degradation of epithelial cells and muscle fibers and formation of vacuoles (Fig. 2d). The high concentration of 'Acetamiprid' (200 mg) caused marked histological alterations, manifested by destruction of circular muscle and dispersion of chloragogen cells (Fig. 2e). On the other hand, the treatment of earthworms with 'Activeg' did not induce any histological alteration, even at highest test dose (200 mg), showing similar tissue structures as found in control earthworm (Fig. 3a).

Pesticides and heavy metals accumulate in the digestive tissues of earthworms after oral soil ingestion, or through their skin which lack protective cuticle tissues. Exposure to pesticides causes cell damage. Oluah *et al.* (2010) reported that histological study of tissues and cells serve as a valuable tool for assessing the toxic effects of contaminants on soil animal. The examination of histological sections of earthworm exposed to various concentrations of 'Acetamipirid' significant tissue damages, as manifested in the form loss of epidermal cell integrity, the appearance of vacuoles, dispersion of chloragogen cells and separation of muscle fibres, in addition to serious tissue alterations which often lead to the destruction of body tissues. The histological sections of earthworms exposed to 'Activeg' did not show any alterations histology. Oluah *et al.* (2010) found serious histological alterations in *Nukkadrillus mbae* exposed to atrazine herbicide. While Kilic (2011) reported damage and accumulations in circular muscles of earthworms exposed to xenobiotics. Gao *et al.* (2013) found that triazole fungicide induces tissue necrosis in *Eisenia foetida*, while Zeriri (2014) showed histological effects in *Octadrilus complanatus* exposed to methomyl insecticide. Nayak *et al.* (2018) found that phosphogypsin at 10% concentration caused

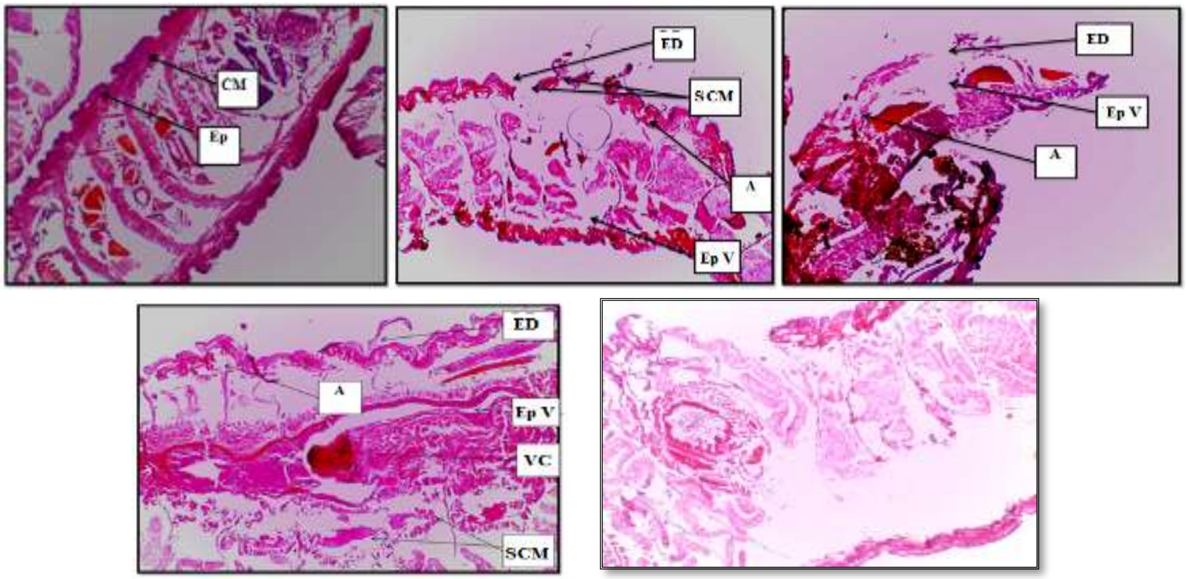

Fig. 2: Histological sections of earthworm *A. giardi*; Top left) untreated control; Top middle) 25 mg 'Acetamiprid' treated earthworm; Top right) 50 mg 'Acetamiprid' treated earthworm; Bottom left) 100 mg 'Acetamiprid' treated earthworm; Bottom right) 200 mg 'Acetamiprid' treated earthworm; Ep: epithelial; CM: Circular muscles; A: Alteration in tissues; ED: Epidermis detachment; SCM: Separation of circular muscles and Ep V: Epidermis vacuoles), CV: Vascular congestion (magnification 25X)

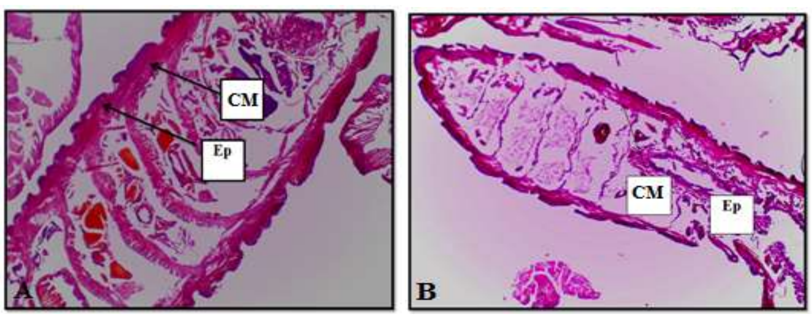

Fig. 3: Histological section of *A. giardi;* [A] Untreated control*;* [B] Treated with 200 mg Activeg (magnification x 25). The histological section shows normal organization of epithelial layers with no histological alterations and hence it is comparable to control histology; CM: Circular muscles; Ep: Epithelial tissue

damage to muscle fibres and caused alteration in enzyme activities in *Glyphidrillus tuberosus*, thus affecting the physiology and ecological functions of these worms. Pelosi (2021) reported that 92% earthworm sampled from agricultural fields applied with Imidaclopride insecticide, Diflufenican herbicide and Epoxiconazole fungicide had pesticide in their body. Bourabia (2013) found that thiamethoxam caused cell membrane alterations, breaks in basal membrane and partial degeneration of certain digestive cells in a *Helix aspersa* model.

Conclusion: Several species of earthworms have become model organisms for eco- logical and toxicological research. *A. giardi* along with other lumbricians are the engineers of ecosystems, because they play essential role in organic matter decomposition and soil structure formation and aeration. *A. giardi* are bio-indicators of contamination by different pollutants *viz*., insecticides, herbicides, fungicides and heavy metal. The physicochemical analysis of soil revealed that the soil has a sandy-loam texture, rich in organic matter and with neutral pH, which explains its suitability for the survival of *A. giardi*. Toxicological study showed that acetamiprid cause mortality of *A. giardi* which increases with dose and period of exposure. No toxicological mortality was observed in *A. giardi* exposed to 'Activeg'. The acetamiprid causes serious alterations in epidermal cells, muscle cells and vascular congestion.

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