J. BIOL. ENVIRON. SCI.,
2020, 14(42), 115-126

Original Research Article

Combined Effect of Two Agrochemicals on *Aporrectodea caliginosa* in a Semi-arid Land

Karim Bouazdia[1*]
[1]Department of Living Beings, Faculty of Exact Sciences and Natural and Life Sciences, Larbi Tebessi University –Tébessa, ALGERIA

Received: 11.09.2020; Accepted: 09.12.2020; Published Online: 25.12.2020

ABSTRACT

Glyphosate and deltamethrin are used worldwide to control pests in agriculture. However, information about their toxic effects on soil fauna is still insufficient. Therefore, we focused on the individual and combined effects of these pesticides, in artificial soil tests on the earthworm species *Aporrectodea caliginosa*. Endpoints were behavior, mortality, growth inhibition and morphology. The insecticide had a significant effect on the behavior. The percentage of migrant earthworms to the control soils increased with the increase of concentrations reaching 75%. At the opposite, 76.19% of earthworms preferred glyphon-treated soils. The insecticide showed toxicity with dose-response relationship, which leaded to values of LC_{50}-14days = 2215.71µg/kg and LC_{90}-14days = 51869.24µg/kg. However, Glyphon caused negligible mortality of *A.caliginosa*. The growth rate was inhibited by the insecticide much more than the herbicide. However, the mixture had a repulsive effect, an antagonistic interaction on the mortality and no effect on the growth rate of *A.caliginosa*. The insecticide altered morphology of exposed earthworms to different concentrations. Symptoms of this alteration ranged from color change of the tegument to lysis of the body wall. While, *A.caliginosa* treated with Glyphon and mixture showed intact bodies. Our data show that Glyphosate and Deltamethrin could be used safely if recommended doses are respected.

Keywords: Décis EC25, Glyphon SL, Behavior, Toxicity, Growth, Morphology

INTRODUCTION

The global pesticides market currently represents 40.475 billion dollars. Europe is the biggest consumer of pesticides in the world (with 31.7% of the market) followed by Asia (23.1%), then Americas (South: 20.8%; North: 20.6%) and Africa (3.8%). Algeria is ranked among the countries that use large quantities of pesticides where 30,000 tons of pesticides are applied each year (Chiali et *al*., 2013). In our country, with the development of agriculture, pesticides are increasingly used in crops. Thus, more than 400 pesticides are registered in Algeria, of which 40 varieties are more used in agriculture (Bordjiba & Ketif, 2009).

If these pesticides seemed to be beneficial, their harmful secondary effects were gradually highlighted. Pesticides have an impact on non-target soil organisms (Elodie, 2006). Some researchers estimate that only 0.3% of the applied amounts of pesticides reach their target. The remainder quantity affects all other living species with multiple consequences such as weakened immune system, reduced fertility and behavioral changes (Magdelaine, 2013).

The herbicide glyphosate is an effective tool for controlling all kind of weeds. Its global consumption reached 825 804 tons in 2014. Due to its non-selective nature, its agricultural use has long been limited to certain moments of the year in order to avoid damaging crops (Benbrook, 2016). Since 2015, debates around glyphosate illustrate the dependence on it, and underline the resistance to its ban in the agricultural sector (Barili et *al*., 2017) following its classification as "probable carcinogen" by the international cancer research center. Pyrethroids, synthetic analogues of pyrethrins, are a new range of pesticides with high efficacy and low environmental toxicity (Masoumi, 2009; Grojean, 2002). The impact of these pesticides on non-target invertebrates in agroecosystems is not well known.

Earthworms are among the best-known bioindicators, which have undeniable advantages for the evaluation of soil quality (Jansirani et *al*., 2012). The current earthworm species for standardized tests is *Eisenia fetida* or *Eisenia andrei*. However, these species are not representative of cultivated fields and less sensitive to pesticides than *A. caliginosa* (Savigny, 1826). This one can be considered as a relevant model test species (Pelosi et *al*., 2013; Bart et *al*., 2018). Similarly, Van Capelle et *al*., (2016) proposed *A.Caliginosa* as a non-target soil organism for the environmental risk assessment of genetically modified plants. Furthermore, a lot of

[*] Corresponding author: bouazdiak@yahoo.fr

contradictions and knowledge gaps highlight the need for further research into long-term earthworm exposure to mixtures of commercial formulations of pesticides (Pelosi et *al*., 2014).

Thus, the main purpose of the current study was to evaluate the responses of the earthworm species *A.Caliginosa*, in laboratory conditions, to the impact of two pesticides, commonly used in agriculture, which are Décis EC25 (pyrethroid insecticide) and Glyphon SL (glyphosate based herbicide).

MATERIALS AND METHODS

Biological material

The earthworms used in this study were collected from Youkous. This site is a water source in North of Tebessa, which is located in the Northeast of Algeria. Thus, we focused an unpolluted area to gather earthworms (Figure 1). Collected earthworms were identified, in laboratory, using the keys of Bouché (1972), Sim & Gerard (1985) and Reynolds (2018). The species used in this study is *A. caliginosa*. This species is dominant in the region of Tebessa (Bouazdia & Habes, 2017). The worms were maintained at 20.29±2.28°C for one month before the experiments.

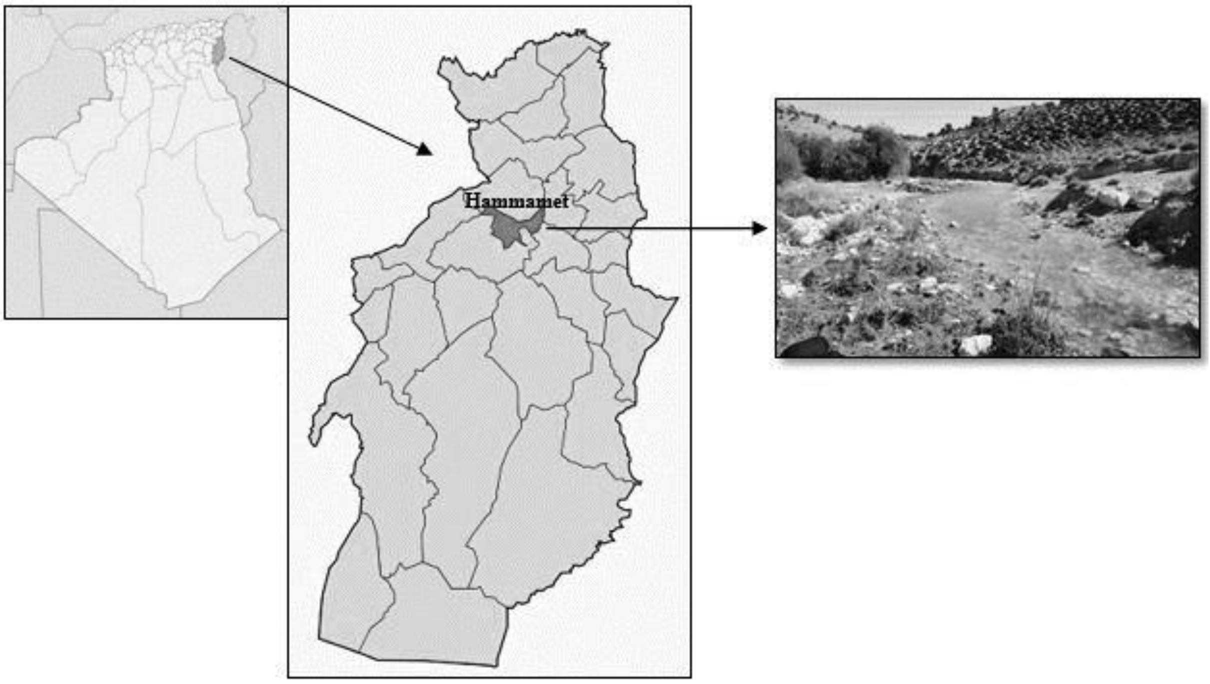

Figure 1. Geographical situation of the collecting site (Youkous-Hammamet) of earthworms.

Test chemicals

The pesticides being tested in these experiments, largely commercialized in Algeria, were an herbicide (Glyphon) and an insecticide (Decis). The stock solutions of each compound were prepared in water on the day of the experiments and were used immediately. Characteristics of the used pesticides are shown in Table 1.

Table 1. Characteristics of the herbicide Glyphon and the insecticide Décis.

Pesticides	**Herbicide**	**Insecticide**
Name	Glyphon	Décis
Active ingredient	N-phosphonométhyl-glycine	Deltaméthrin
RAD (µg/kg dry soil)	480	200

Table 2. Individual and combined concentrations (µg/kg soil dry weight) of Decis and Glyphon.

Decis	Glyphon	Mixture (Décis+ Glyphon)
50	120	C1=50+120
100	240	C2=100+240
200	480	C3=200+480
400	960	C4=400+960
500	1920	C5=500+1920

Behavior test

Behavior test was performed in terrariums and adapted for the species *A. caliginosa*. Each terrarium was divided into two equal sections with a vertical plastic separator. Then, we put 335g of control soil in a section and 335g of treated soil, with chosen concentration, in the other section. After removing the separator, 8 adults of *A. caliginosa* were placed in the separation line between the two sections. Terrariums were closed with perforated cover to prevent earthworms from escaping and allow air exchange. At the end of the test, the separator was reintroduced and the number of earthworms per section was counted. Damaged earthworms by the separator are considered belonging to the section where their heads are directed (Loureiro et *al*., 2005). To validate this test we should have no mortality of the earthworms (HundRinke & Wiechering, 2001).

Acute toxicity assay

Natural soils from particular source are not available all the year, and presence of micropollutants and native organisms can affect the experiment; so it is recommended to use artificial soil. The artificial soil consisted of 10% ground sphagnum peat (<0.5 mm), 20% kaolinite clay (>50% kaolinite), and 70% fine sand. A small amount of calcium carbonate was added to adjust the pH to 6.0 ± 0.5. The moisture content was adjusted to 35% of the final weight (OECD, 2004).

In the experiment, five concentrations of the pesticides, based on the recommended agriculture dose (RAD), were used (tab.2). For the control treatment, we used distilled water. Four replicates were performed for each concentration.

Mature individuals (with well-developed clitellum) weighting between 0.8 and 1.1 g were stored for 24 hours before use in Petri dishes on damp filter paper (in the dark) to void gut contents. After washing earthworms, ten individuals were introduced into each container and placed in an incubation chamber at a temperature of 20.29±2.28°C with a 12:12 photoperiod. The moisture content was kept using distilled water.
Mortality was counted every week by washing away the artificial soil, and different lethal concentrations were calculated for the tested pesticides. Earthworms were considered as dead when they did not react to a mechanical stimulus.

To assess tested mixture toxicity to earthworms, we opted to include independent-action prediction as follow (Rider et *al*., 2018):

$$M_{[a+b]} = M_{[a]}+M_{[b]} – (M_{[a]} \text{ x } M_{[b]})$$

Where:

$M_{[a+b]}$: is the mortality for the mixture.
$M_{[a]}$: is the mortality for the pesticide a.
$M_{[b]}$: is the mortality for the pesticide b.

Sub-lethal toxicity assay

During 28 days and every week, surviving earthworms were counted after being rinsed and dried. Then, growth rate was determined as the following formula (Martin, 1986):

$$C_n = \ln\frac{Pt}{P0} x100$$

Where:

C_n : is the growth rate for the concentration n.
P_t : is the weight after t days of exposure.
P_0 : is the weight in day 0.

Effect on the morphology

We followed morphological changes of the treated and untreated earthworm's bodies during exposure time. Stereomicroscope was used to confirm some symptoms.

Statistical analysis

LC_{50} (the concentration that is lethal to 50% of individuals), as following 95% confidence interval, was calculated using the program prism 6.01 (GraphPad software, La JollaCalifornia, USA). Statistical analysis was performed using the Minitab software (Minitab 16.0 for Windows). On the basis that they are used, one-way ANOVA ($p < 0.05$) test for assessing the effects of contaminants on growth. With post hoc, in comparison of means (growth), Tukey (HSD) test was applied. Data are presented as mean ± standard deviation (SD).

RESULTS

Behavior test

Figure 2 shows the distribution of earthworms at the end of behavior test. The distribution of individuals is equal in the terrariums containing control soil in the two sections. However, in the terrariums containing the control soil in one section and the treated soil with Decis in the other section, the percentage of migrating earthworms to the control soil increased with increasing concentrations reaching a maximum of 71,43%. However, earthworms exposed to the herbicide glyphon showed non-significant avoidance responses at different concentrations; preferring the treated section at the concentration 480 µg/kg where 76.19% of individuals had migrated. In the same way as the insecticide, the mixture had a repulsive effect on the earthworms. This behavior becomes more accentuated with increasing concentration.

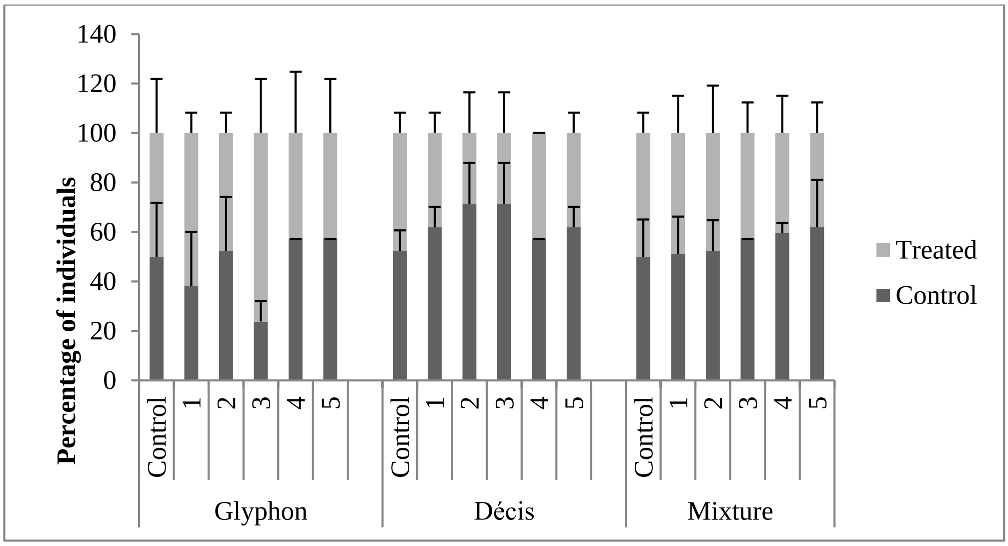

Figure 2. Distribution of adults of *A.caliginosa* between control and treated soil with different concentrations of the pesticides and their mixture. (m±SD; n=30).

Toxicity assay

After four weeks of exposure of the earthworms to different concentrations of glyphon and the mixture (glyphon + decis), the results revealed negligible mortality of the tested earthworms. Thus, lethal concentrations (LC_{50} and LC_{90}) could not be determined.

Whereas, the insecticide Décis caused higher mortality percentage of *A.caliginosa* compared to glyphon and mixture. The results showed that LC_{50} and LC_{90} values decreased during exposure time (Table 3; Figure 3) and no mortality recorded in control series. Thereby, the toxicity of Décis displayed a concentration and exposure time-response relationship.

Figure 3 presents the evolution of probits as a function of decimal logarithms of the concentrations.

Table 4 shows the real and predicted mortality under independent-action model for the earthworms exposed to the mixture. It is noted that there is no compatibility between results, suggesting an antagonistic effect between the pesticides.

Table 3. Median lethal concentration values of the insecticide Décis for the earthworm *A. caliginosa* and their confidential limits.

Exposition time (weeks)	LC50 [Lower limit ; Upper limit]	LC50 [Lower limit ; Upper limit]
1	2220,98[867,99;5682,98]	33724,77[13190,07;86296,96]
3	839,42[352,40; 1999,53]	16715,70[7017,41; 39817,32]
4	805,93[340,49; 1907,62]	15862,62[6701,65; 37546,38]

Table 4. Real and predicted mixture mortality under Independent Action (I.A) model.

	Glyphon		Decis		Predicted mixture mortality under I.A model	Real mixture mortality
	Concentration	Mortality	Concentration	Mortality		
7 days	Control	0	Control	0	0,00	0
	1	0,25	1	2,22	1,92	0
	2	0,5	2	10,28	5,64	0
	3	0	3	12,78	12,78	0,75
	4	0	4	17,78	17,78	0,75
	5	0	5	26,11	26,11	1
14 days	Control	0	Control	0	0,00	0
	1	0,75	1	4,72	1,93	0
	2	1	2	13,06	1,00	0
	3	0	3	15,28	15,28	1
	4	0,25	4	20,28	15,46	1
	5	0,25	5	31,11	23,58	1,5

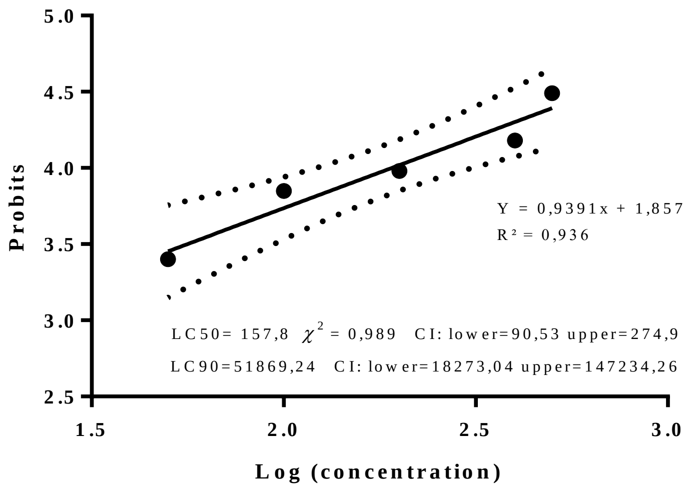

Figure 3. Fourteen days median lethal concentrations (µg/kg soil dry weight) of the insecticide Décis : probit Analysis.

Growth inhibition

Significant decrease of the growth rate of earthworms treated with 240, 480 and 1920 µg/kg of the herbicide is noted after the first and the last week compared to the control series (Figure 4A). On the other hand, after the first week, a non-significant decrease of the growth rate is noted in the series treated with the concentrations 50, 100, 400 µg/kg compared to the control. However, the earthworms treated with 200 and 500 µg/kg of the insecticide showed a significantly reduced growth rate. This growth inhibition is observed in all the series after the remaining exposure period (Figure 4B). Conversely, mixture had no effect on the growth rate of earthworms as it is shown in Figure 4C.

Effect on morphology

Morphological alterations such as shrinking, swelling, color change, lysis of the wall and taper appear in different regions of the body of earthworms treated with different concentrations of Decis after 4 weeks of exposure. These anomalies are accentuated with the increase of the concentration (Figure 5). In contrary, earthworms exposed to different concentrations of the herbicide glyphon and mixture showed intact bodies.

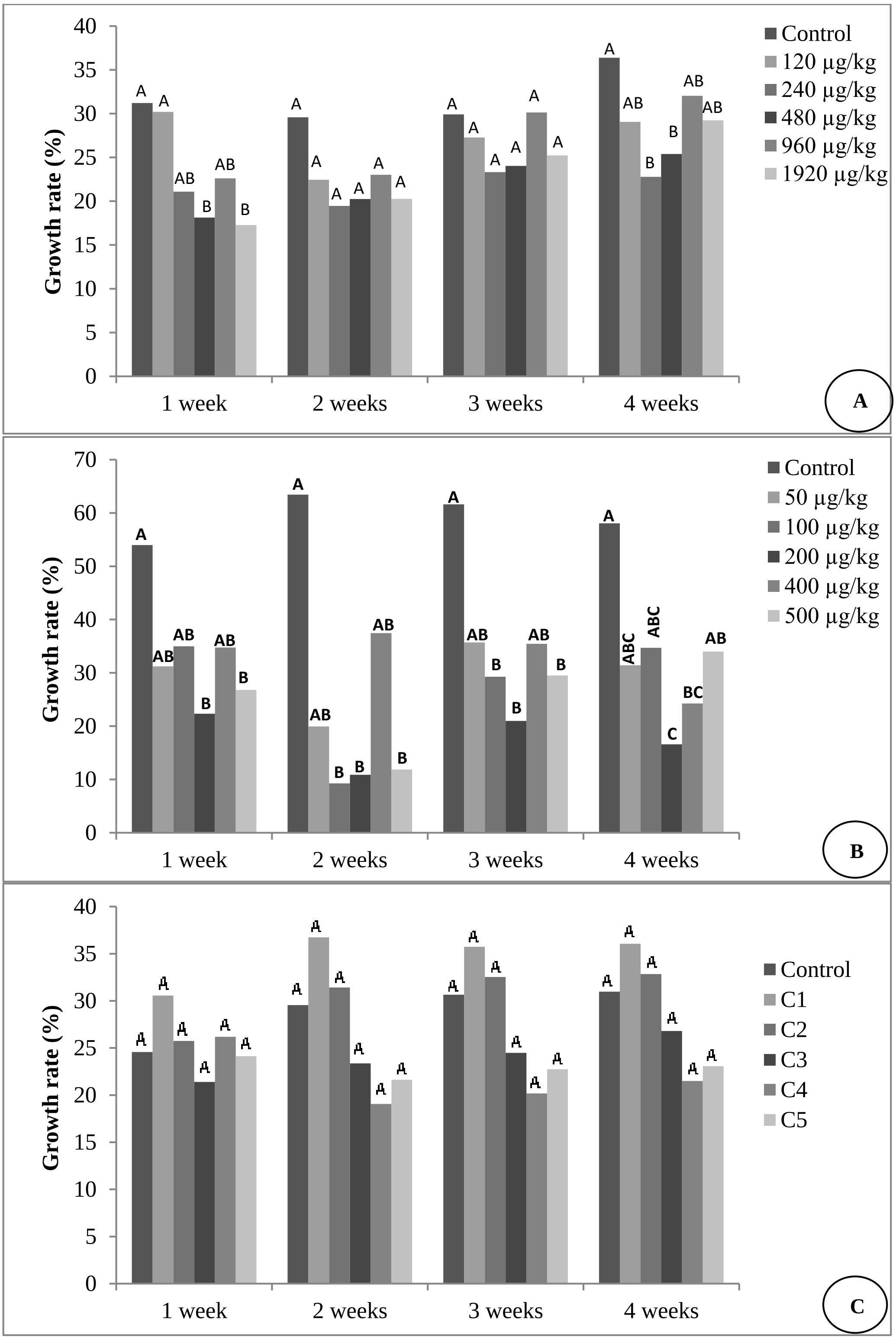

Figure 4. Effect of the pesticides at different concentrations on the weight growth rate of *A. caliginosa* during exposure time: A: Glyphon, B: Décis and C: Mixture. (Different letters indicate significant difference between different concentrations amongst the same period (Tukey's HSD Test)).

Figure 5. Morphological anomalies of the earthworms exposed to the insecticide Décis in artificial soil at different concentrations; A: Control; B: 50µg/kg ; C : 100µg/kg ; D : 200µg/kg ; E : 400µg/kg and F : 500µg/kg.

DISCUSSION

Effect on Behavior

Avoidance reaction of earthworms to contaminated soils was proposed as the simplest, sensitive, valid and feasible soil toxicity assessment test (Lukkari et *al*., 2004). Our results revealed that the percentage of earthworms migrating to control soil increased with the increase of the concentration of Décis. In the same way, Lukkari et *al*., (2004) reported that soil contaminated with discharges of copper-Nickel had evident effect on the avoidance behavior of *Aporrectodea Tuberculata, Lumbricus rubellus* and *Dendrobaena octaedra.* At the opposite, in six chambers test the species *E.fetida* was attracted to median and higher concentrations of deltamethrin. This result shows partially that deltamethrin is not dangerous for *E. fetida* (Gherhardt & Bolcu, 2011) which is less sensitive to pesticides than *A. caliginosa* (Pelosi et *al*., 2013).

Unlike insecticide, the herbicide glyphon had no significant effect on the avoidance behavior of *A. caliginosa.* Similarly, *Eisenia andrei* did not avoid treated soil with high concentrations of glyphosate (Santos et *al*, 2012). However, Glyphosate induced significant avoidance effect on *Pontoscolex corethrurus* (Buch et *al*, 2013) *Octolasion cyaneum* (Salvio et *al*, 2016) and *E.andrei* (Casabé et *al*, 2007).

Earthworms, having chemoreceptors and sensory tubers, have a high sensitivity to chemicals in soils (Reinecke et *al*, 2002), However some products may not be detected by earthworms which may die in the soil test without trying to escape (Garcia et *al*, 2004).

However, mixture had synergistic effect on treated earthworms. Another study found that two mixtures of chlorpyrifos (CPF)+clothianidin (CLO) and heavy metal chromium [Cr(VI)]+CPF+CLO+acetochlor (ACE) exhibited synergistic effects on *E. fetida*. The other two quaternary mixtures of CLO+fenobucarb (FEN)+ACE+Cr(VI) and Cr(VI)+FEN+CPF+ACE at low concentrations also displayed synergistic effects on the earthworms. In contrast, the mixture of Cr(VI)+FEN had the strongest antagonistic effects on *E. fetida*. Besides, the quinquenary mixture of Cr(VI)+FEN+CPF+CLO+ACE also exerted antagonistic effects (Yang *et al.*, 2018).

Toxicity test

Artificial soil test indicated an important mortality of *A.caliginosa* exposed to the insecticide Décis. This mortality depends on the concentration and exposure time. Our results are similar to those of Benetti et *al*., (2017) where deltamethrin caused high mortality of the earthworms in contaminated soils. Also, Song et *al*., (2015) found that survival rate of *E.fetida,* exposed to deltamethrin in artificial soil, decreased with the increase of concentration (100-125 mg/kg lead to a maximal mortality of 90%). No survival was found at 602.5μg/cm^2 after 72 hours in filter paper test. In the same way, Singh et *al*., (2019) have evaluated the toxicity of different concentrations of triazophos, deltamethrin and their mixture (triazophos + deltamethrin) to *Eudrilus eugeniae* in filter paper test. The mortality of earthworms reached 100% at the concentration of 0.0447μg/cm^2. Wang et *al*., (2012) reported that four types of pyrethroids (cyhalothrin, lambda cyhalothrin, fenpropathrin, cypermethrin) were very toxic to *E.fetida*.

Otherwise, many studies showed that the insecticide Deltamethrin had no toxic effect in different species of earthworms. Thus, Blattner et *al*., (2012) indicated that deltamethrin is not toxic to *E.fetida* with LC_{50} higher than 1290 mg/kg of dry soil. Zhang et *al*. (1985) reported that 14 days LC_{50} was 70.85 mg/kg in earthworms *E.fetida* exposed to deltamethrin. We noticed that CL_{50} and CL_{90} values are much higher than concentrations used in toxicity test, which confirm that deltamethrin is not toxic to *A. caliginosa* at the recommended agriculture dose.

The herbicide glyphon had no toxic effect on the earthworms *A. caliginosa*. Similarly, Correia & Moreira, (2010) showed, in many tests, that glyphosate had no mortality effect on *E.fetida*. This herbicide was not toxic to earthworms *Eudrilus Eugeniae* with LC_{50} of 3578 ± 14.22 mg of glyphosate/kg dry soil (Kpan et *al.*, 2018).

Few works were dedicated to study the toxic effect of glyphosate amongst terrestrial invertebrates, because this compound is rapidly adsorbed to soil particles and is not biologically available anymore (Bonnard et *al*, 2019). In fact, Kpan et *al*, (2018) showed that the weak toxicity of glyphosate toward *Eudrilus Eugeniae* could be explained by the non-affinity of the molecules of this compound to the biological systems of animals.

Combined effect of the tested pesticides on the earthworm's mortality was negligible which concluded to an antagonistic effect. Our results were confirmed by Chen et *al*., (2018) who noticed an antagonistic effect between the herbicide Tribenuron-methyl and the fungicide Tebuconazole on *Eisenia fetida*. Same effect was reported with Triazophos and Deltamethrin on mortality of *Eudrilus eugeniae* (Singh et *al*., 2019), with chrome and cadmium on *E.fetida* (Aebeed & Amer, 2018), with 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Glyphosate in *E.fetida* (Lazurick et *al*., 2017) and with Glyphosate and captain in *A. caliginosa* (Springett & Gray, 1992)

However, Yang et *al*., (2017) found that the binary, ternary and quaternary mixtures of chlorpyrifos, fenobucarb, clothianidin and acetochlor exhibited a clear synergistic effect on the mortality of *E. fetida*.

The variable sensitivity of earthworms to pollutants may be due to (1) ecological differences of the different species and their food habitats (ecological groups) and (2) specific physiological characteristics of each species (Nahmani et *al.,* 2003).

Effect on growth

Our results revealed that the insecticide Decis induced a decrease of the weight of all exposed earthworms during the study period. Similarly, Shi et *al*., (2007) had compared the effect of different concentrations of lindane and deltamethrin on the growth of *E.fetida* in artificial soil test during 14 days. Deltamethrin had inhibited the growth rate of earthworms in comparison to control series. Also, Zhang et *al*., (1985) reported that deltamethrin had toxic effect on development, growth and reproduction of earthworms. Thus, the weight of the treated earthworms decreased along the test time. Many studies had observed the negative effect of pesticides on the growth of earthworms as Cyfluthrine, Carbaryl, Chlorpyrifos, Fipronil and Imidaclopride (Mostert et *al.*, 2007), Buprofezin, Lufenuron and Triflumuron (Badawy et *al*., 2013).

Depta et *al*. (1999) indicated that one of the potential adaptation strategies of earthworms to pollution is avoidance. The earthworms, placed in polluted soils, would thus be able to differentiate organic matter according to its level of contamination.

On the other hand, glyphon induced significant inhibition of the growth rate of treated earthworms compared to control. Correia and Moreira (2010) reported the same results with the species *E.fetida*. Thus, treated earthworms showed weight loss of about 50%. In the same way, Springett and Gray (1992) confirmed the toxic effect of glyphosate, with concentrations lower than RAD, on the growth of *A.caliginosa*. At the opposite, no effect of glyphosate on the growth of *A.Caliginosa* was reported in artificial soil (Martin, 1982) and in native soil from New Zealand (Doliner, 1991). Therefore, treated *E.fetida* with glyphosate showed no significant difference of the growth rate with control during 28 days (Zhou et *al.*, 2013). Thus, Kpan et *al*, (2018) have shown no effect of this herbicide on the earthworm biomass. These differences amongst results are perhaps due to the diversity, origin and history of used species.

At the opposite of the individual effect of the pesticides, the mixture of Glyphon and Decis had no effect on the growth rate of *A.caliginosa*. Similarly, Santos et *al*., (2011) had noticed that the majority of the mixtures of Glyphosate and Dimethoate had effects smaller than the prediction based on the individual responses on the growth of *Eisenia andrei*.

Effect on morphology

Morphological alterations as swelling, shrinking, lysis of the wall, color change, narrowing, fragmentation and taper were observed in earthworms exposed to different concentrations of the insecticide Décis (50, 100, 200, 400 et 500 µg/kg) along 4 weeks. Similarly, *Perionyx excavatus* and *Eisenia andrei* displayed abnormal swelling, secretion of mucous, bleeding and fragmentation when exposed to different xenobiotic as lead acetate, tetra ethyl lead (TEL) and méthyl-tert-butyl éther (MTBE) (Venkateswara et *al*., 2004, 2003) and Aldrine, Endosulfan, Heptachlor and Lindane (Rajendra et *al*.,1990). Velki & Cimovic (2015) have evaluated the toxicity of a battery of pesticides at different temperatures in *E. fetida* and noticed that lambda-cyhalothrine, imidaclopride, the mixture of chlorpyrifos with cyperméthrine, azoxystrobin, cyproconazole, the mixture of difenoconazole with propiconazole, fluazifop-p-butyl, diquat and tembotrione caused morphological alterations to treated earthworms. In addition, *E.fetida* exposed to phorate suffered of winding, wall disruption, appearance of internal glandular cell mass and disintegration of circular and longitudinal muscles that lead to fragmentation of the body (Kumar & Singh, 2017).

REFERENCES

Aebeed, A. S., & Amer, A. H. (2018). Combined toxicity of cypermethrin, a pyerthroid insecticide and metal to earthworms *Eisenia fetida. Med Tox Curr Res*, 1(1), 4-10.

Badawy, M. E., Kenawy, A., & El-Aswad, A. F. (2013). Toxicity assessment of buprofezin, lufenuron, and triflumuron to the earthworm *Aporrectodea caliginosa*. *International Journal of Zoology*, *2013*.

Barilli E., Jeuffroy M.H., Gall J., de Tourdonnet S., Médiène S. 2017. Weed response and crop growth in winter wheat–lucerne intercropping: a comparison of conventional and reduced soil-tillage conditions in northern France. *Crop and Pasture Science* 68(11): 1070-1079.

Bart, S., Amossé, J., Lowe, C. N., Mougin, C., Péry, A. R., & Pelosi, C. (2018). *Aporrectodea caliginosa*, a relevant earthworm species for a posteriori pesticide risk assessment: current knowledge and recommendations for culture and experimental design. *Environmental Science and Pollution Research*, *25*(34), 33867-33881.

Benbrook, C. M. 2016. « Trends in glyphosate herbicide use in the United States and globally ». *Environmental Sciences Europe*, 28(3), doi : 10.1186/s12302-016-0070-0.

Benetti, F., Pigatin, L. B. F., Kanashiro, M. M., Rodrigues, R. N., & Rezende, M. O. D. O. (2017). Vermicompost Incorporation into Soil Contaminated with Deltamethrin as an Attempt to Reduce the Toxicity Caused by this Pyrethroid: A Structural Study of Soil. *American Journal of Environmental Sciences*, *13*(4), 297-306.

Blattner, J. R., Safety, P., & Murray, F. J. (2012). Deltamethrin Has Not Been Clearly Shown to Cause Reproductive Toxicity.

Bonnard. N, Jargot. D, Falcy. M, Pillière. F, Robert. S, Fastier. A, Oudar. S, and Rambourg. M.-O. (2019). Base de données fiches toxicologiques. Glyphosate - Edition : Septembre 2019.

Bordjiba, O. & Ketif, A. 2009. Effet de trois pesticides (Hexaconazole, bromiuconazole et Fluazifop-p-butyl) sur quelques Métabolites physico-biochimiques du blé dur : *Tritucum durum* Desf. *European Journal of Scientific Research*, 6 (2): 260-268.

Bouché M.B., 1972. Lombriciens de France. Ecologie et Systématique. Ann. *Zool.Ecol Anima*. Hors-sér., 671pp.

Buch, A. C., Brown, G. G., Niva, C. C., Sautter, K. D., & Sousa, J. P. (2013). Toxicity of three pesticides commonly used in Brazil to *Pontoscolex corethrurus* (Müller, 1857) and *Eisenia andrei* (Bouché, 1972). *Applied soil ecology*, *69*, 32-38.

Casabé, N., Piola, L., Fuchs, J., Oneto, M. L., Pamparato, L., Basack, S., ... & Kesten, E. (2007). Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments*, *7*(4), 232-239.

Celine Pelosi, Joimel S, Makowski D. (2013). Searching for a more sensitive earthworm species to be used in pesticide homologation tests — a meta-analysis. *Chemosphere*. 90: 895–900.

Chen, J., Saleem, M., Wang, C., Liang, W., & Zhang, Q. (2018). Individual and combined effects of herbicide tribenuron-methyl and fungicide tebuconazole on soil earthworm *Eisenia fetida*. *Scientific reports*, 8(1), 1-9.

Chiali F. Z. (2013). Effets métaboliques d'un régime à base de purée de pomme de terre contaminée par les pesticides chez le rat wistar. Thèse doctorat Physiologie et Biochimie de la Nutrition. Tlemcen. Université Abou Bekr Belkaid Tlemcen. 205p.

Correia, F. V., & Moreira, J. C. (2010). Effects of glyphosate and 2, 4-D on earthworms (*Eisenia fetida*) in laboratory tests. *Bulletin of environmental contamination and toxicology*, *85*(3), 264-268.

Depta, B., Koscielniak, A., Rozen, A. (1999). Food selection as a mechanism of heavy metal resistance in earthworms. *Pedobiologia* 43, 608–614.

Doliner, L.H. 1991. *Discussion document: Preharvest use of glyphosate herbicide.* Canada D91-01. Pesticide Directorate, Agriculture Canada, Ottawa.

Elodie Guigon-Moreau (2006). Transfert des pesticides vers les eaux superficielles et l'atmosphère : caractérisation et modélisation sur le bassin versant de la Vesle. Thèse de Doctorat Université de Pierre et Marie Curie. Spécialité : Géosciences et Ressources naturelles.

Garcia, M. (2004). Effects of pesticides on soil fauna: development of ecotoxicological test methods for tropical regions (Vol. 19). Cuvillier Verlag.

Gherhardt, T., & Bolcu, C. (2011). Avoidance Behavior of *Eisenia Fetida* to Acetone, Deltamethrin and Glyphosate. *New Frontiers in Chemistry*, *20*(2), 1.

Grojean S (2002). Etude de la réponse neuronale dans un modèle d'asphyxie cérébrale chez le rat nouveau-né. Influence d'une hyperbilirubinémie associée. These doctorat. 318pp.

Hund-Rinke, K., & Wiechering, H. (2001). Earthworm avoidance test for soil assessments. *Journal of Soils and Sediments*, *1*(1), 15-20.

Jansirani D., Nivethitha S., Singh M. 2012. Production and utilization of vermicast using organic wastes and its impact on *Trigonella foenum* and *Phaseolus aureus*. *Int J Res Biol Sci* 2(4): 187-189.

Bouazdia Karim & Habes Dahbia. (2017). Earthworm Species Identified in the Region of Tebessa (Eastern Algeria). *International Journal of Zoological Research*, 13, 38-44.

Kpan, G. K. K., Ehouman, M. N. G., Toure, M., Brou, L. Y., Tiho, S., Traore, S. K., & Dembele, A. (2018). Toxicité d'un Herbicide à base de Glyphosate sur le ver de Terre *Eudrilus Eugeniae* KINBERG, 1867 (Oligochaeta, Eudrilidae).

Kumar, S., & Singh, S. M. Toxicological effects of organo phosphate pesticide, phorate on the prostomial region of earthworm, *metaphire posthuma*.

Lazurick, C., Lidzbarski, N., Owings, R., Brotherton, J., & Steele, E. (2017). Investigating the toxicity and accumulation of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and glyphosate in *Eisenia fetida*. *Journal of the South Carolina Academy of Science*, *15*(2), 6.

Loureiro, S., Soares, A. M., & Nogueira, A. J. (2005). Terrestrial avoidance behavior tests as screening tool to assess soil contamination. *Environmental pollution*, *138*(1), 121-131.

Lukkari, T., Taavitsainen, M., Väisänen, A., & Haimi, J. (2004). Effects of heavy metals on earthworms along contamination gradients in organic rich soils. *Ecotoxicology and environmental safety*, *59*(3), 340-348.

Magdelaine C (2013). Les pesticides ou produits phytosanitaires. En ligne : http://www.notre-planete.info/ecologie/alimentation/pesticides.php#.

Martin, N. A. (1982). The effect of herbicides used on asparagus on the growth rate of the earthworm *Allolobophora caliginosa*. *New Zealand Plant Protection*, *35*, 328-331.

Martin, N.A.(1986). Toxicity of pesticides to *Allolobophora caliginosa* (Oligochaeta: lumbricidae).*New Zealand journal of agricultural research*, 29 (4), 699–706.

Masoumi A, Goldenson B, Ghirmai S et al. (2009). 1 alpha, 25-dihydroxyvitamin D3 interacts with curcuminoids to stimulate amyloid-beta clearance by macrophages of Alzheimer's disease patients. *J Alzheimers Dis*, 17(3):703–17. doi:10.3233/JAD-2009-1080

Mostert, M. A. (2007). The ecotoxicity of five insecticides to the Pheretima group (Oligochaeta) occurring on golf courses in the Pretoria region (South Africa) (Doctoral dissertation, University of Pretoria).

Nahmani, J., Lavelle, P., Lapied, E., & van Oort, F. (2003). Effects of heavy metal soil pollution on earthworm communities in the north of France: The 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, *47*(5-6), 663-669.

OECD (1984) Ligne directrice de l'OCDE pour les essais de produits chimiques no. 317. Bioaccumulation chez les oligochètes terrestres, OECD— Ligne directrice de l'OCDE pour les essais de produits chimiques. Paris, France

Rajendra, K., Rajesh, C.G., Mirza, U.B., 1990. Toxicity assesment of four insecticides to earthworm, *Pheretima postuma*. *Bull. Environ. Contam. Toxol*. 45, 358–364.

Reinecke, A. J., Maboeta, M. S., Vermeulen, L. A., & Reinecke, S. A. (2002). Assessment of lead nitrate and mancozeb toxicity in earthworms using the avoidance response. *Bulletin of Environmental Contamination and Toxicology*, *68*(6), 779-786.

Reynolds, J.W. 2018. Preliminary Key to Algerian Megadriles (Annelida, Clitellata, Oligochaeta), based on external characters, insofar as possible. *Megadrilogica*. 24(1): 1-16.

Rider, C. V., & Simmons, J. E. (Eds.). (2018). *Chemical Mixtures and Combined Chemical and Nonchemical Stressors: Exposure, Toxicity, Analysis, and Risk*. Springer.

Salvio, C., Menone, M. L., Rafael, S., Iturburu, F. G., & Manetti, P. L. (2016). Survival, reproduction, avoidance behavior and oxidative stress biomarkers in the earthworm *Octolasion cyaneum* exposed to glyphosate. *Bulletin of environmental contamination and toxicology*, *96*(3), 314-319.

Santos, M. J. G., Ferreira, M. F. L., Cachada, A., Duarte, A. C., & Sousa, J. P. (2012). Pesticide application to agricultural fields: effects on the reproduction and avoidance behavior of *Folsomia candida* and *Eisenia andrei*. *Ecotoxicology*, *21*(8), 2113-2122.

Santos, M. J., Morgado, R., Ferreira, N. G. C., Soares, A. M., & Loureiro, S. (2011). Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. *Ecotoxicology and environmental safety*, 74(7), 1994-2001.

Shi, Y., Shi, Y., Wang, X., Lu, Y., & Yan, S. (2007). Comparative effects of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (*Eisenia fetida*). *Pesticide biochemistry and physiology*, *89*(1), 31-38.

Sims, R. W. & Gerard, B. M. (1985). Earthworms: Keys and Notes for the Identification and Study of the Species. In: Barnes, R. S. K. & Crothers, J. H. (Eds.), *Synopses of the British Fauna.* (New Series) No. 31, London: E. J., 171 pp.

Singh, S., Tiwari, R. K., & Pandey, R. S. (2019). Acute toxicity evaluation of triazophos, deltamethrin and their combination on earthworm, *Eudrilus eugeniae* and its impact on AChE activity. *Chemistry and Ecology*, *35*(6), 563-575.

Singh, S., Tiwari, R. K., & Pandey, R. S. (2019). Acute toxicity evaluation of triazophos, deltamethrin and their combination on earthworm, *Eudrilus eugeniae* and its impact on AChE activity. *Chemistry and Ecology*, *35*(6), 563-575.

Song, Y., Kai, J., Song, X., Zhang, W., & Li, L. (2015). Long-term toxic effects of deltamethrin and fenvalerante in soil. *Journal of hazardous materials*, *289*, 158-164.

Springett, J. A., & Gray, R. A. J. (1992). Effect of repeated low doses of biocides on the earthworm *Aporrectodea caliginosa* in laboratory culture. *Soil Biology and Biochemistry*, *24*(12), 1739-1744.

Springett, J.A. and R.A.J. Gray. 1992. Effect of repeated low doses of biocides on the earthworm *Aporrectodea caliginosa* in laboratory culture. *Soil Biol. Biochem*. 24(12):1739-1744.

Van Capelle C, Schrader S, Arpaia S (2016). Selection of focal earthworm species as non-target soil organisms for environmental risk assessment of genetically modified plants. *Sci Total Environ* 548-549: 360–369.

Velki, M., & Ečimović, S. (2015). Changes in exposure temperature lead to changes in pesticide toxicity to earthworms: A preliminary study. *Environmental toxicology and pharmacology*, *40*(3), 774-784.

Venkateswara Rao J, Kavitha P (2004). Toxicity of azodrin on the morphology and acetylcholine esterase activity of the earthworm *Eisenia fetida. Environmental Research* 96, 323–327.

Venkateswara Rao, J., Surya Pavan, Y., Madhavendra, S.S., (2003). Toxic effects of chlorpyrifos on survival, morphology and acetylcholinesterase activity of the earthworm *Eisenia fetida*. *Ecotoxicol. Environ*. *Saf*, 54, 296–301.

Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., & Wang, Q. (2012). Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicology and environmental safety*, *79*, 122-128.

Yang, G., Chen, C., Wang, Y., Peng, Q., Zhao, H., Guo, D., ... & Qian, Y. (2017). Mixture toxicity of four commonly used pesticides at different effect levels to the epigeic earthworm, *Eisenia fetida. Ecotoxicology and environmental safety*, 142, 29-39.

Yang, G., Chen, C., Yu, Y., Zhao, H., Wang, W., Wang, Y., ... & Wang, X. (2018). Combined effects of four pesticides and heavy metal chromium (VI) on the earthworm using avoidance behavior as an endpoint. Ecotoxicology and environmental safety, 157, 191-200.

Zhang, R. W., Li, Z. X., Bai, Q. Y., & Cai, D. J. (1985). Detection of the toxicity of pesticides on earthworms by standard method. *Acta Scientiae Circumstantiae*, *5*(3), 327-333.

Zhou, C. F., Wang, Y. J., Li, C. C., Sun, R. J., Yu, Y. C., & Zhou, D. M. (2013). Subacute toxicity of copper and glyphosate and their interaction to earthworm (*Eisenia fetida*). *Environmental pollution*, *180*, 71-77.