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Acute toxicity of two pesticides (dursban, mancozeb) and their combined mixture on the terrestrial isopod *Armadillidium vulgare* (Oniscidea, Isopoda)

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Abstract: Our study focused on evaluating an acute toxicity test, where woodlice (*Armadillidium vulgare)* which is the most abandoned in our region , were exposed to pesticides (dursban and mancozeb) most used in Algeria.The concentrations of the pesticids tested are 720 mg/kg,360 mg/kg,90 mg/kg, 45 mg/kg for dursban; 800 mg/kg, 400 mg/kg, 200 mg/kg,100 mg/kg for mancozeb and 720/800 mg/kg, 360/400 mg/kg,90/200 mg/kg, 45/100 mg/kg for the mixture of dursban and mancozeb.Our results show that dursban was highly toxic (69% mortality after 28 days of the test) compared to mancozeb (19.5% mortality). On the other hand, in the case of the mixture (dursban ± mancozeb), the toxicity was lower (63% mortality) compared to the dursban tested alone. Contamination of woodlice with dursban and a mixture of dursban and mancozeb revealed morphological effects such as alteration of the marsupial cavity and expulsion of eggs from the outside of this cavity. Dursban and the mixture of dursban and mancozeb are toxic to *Armadillidium vulgare*, but mancozeb alone is not toxic. Dursban should be banned because it is bad for soil fauna and therefore probably for biodiversity in general and even human health.
Keywords: bio indicator, dursban, mancozeb, acute toxicity, *Armadillidium vulgare.*

INTRODUCTION

Contamination and then pollution of the soil, by various factors, directly causes serious consequences on living beings and indirectly on the environment. The development of organic chemistry marks the arrival (in 1960) of synthetic "pesticide" molecules that are widely used to improve agricultural production due to food needs that are constantly increasing with population growth.(Pretty, 2008). As a result, Algeria imported an average of 17159,648 tonnes of pesticides during 2017 at an estimated cost of US$ 855,38253.82 (ASISD, 2017).

The Soummam Valley, which is an agricultural area of 130,348 hectares, is known for its significant agricultural activity, particularly market gardening and arboriculture. A total of 1945, 077 tonnes of pesticides during 2017 was used for these activities (ASB, 2017). The survey of farmers in this regions showed that mancozeb and dursban are the most commonly used pesticides in agricultural practices due to their availability on the market and their proven efficiency agains pests. However,the systematic use of these products is currently being questioned, with growing awareness of the risks they can generate. One of the most effective approaches for assessing the effects of pesticides in the environment and there fore the risk associated with their contamination is bio-monitoring (Salines, 2012).

This bio monitoring principle has been used on various bio-indicators of soil pollution such as earthworms (Annelid) (Vermeulen et al., 2001), rats (Szepvolgyi et al., 1989), snails (Coline, 2011; Viard, 2004), and aquatic crustaceans (Palma et al, 2008). We add to this list of bio-indicators of soil quality, some Oniscidea species such as terrestrial isopods or woodlice (Grosset et al., 2005; Kammenga et al., 2000) which are abundant in various terrestrial ecosystems (Cortet et al., 1999; Camila et al., 2017) in which they constitute an essential link in the food web (Bureš et al., 2003).They contribute enormously in increasing soil fertility (Hassall et al., 1978, Curry, 1994; Camila, 2014). As such they can be used as bio-indicators for the eco-toxicological analysis of pesticide pollution (Van Gestel et al., 2018). It is in this context that the present work aims to evaluate the effect of the two most widely used pesticides i.e. mancozeb and dursban in the Soummam Valley in Algeria, on the survival and morphology of males and females *Armadillidium vulgare* ,the most abundant species in the study site.

Several studies have high lighted the effects of the two pesticides on the mortality of terrestrial isopod species (Morgado et al., 2016; Morgado et al., 2018; Jänschet al., 2005), but no acute pesticide toxicity studies have been carried out yet on the species *Armadillidium vulgare* Also, this is the first study investigating the effects of these pesticides on the morphology of thise species.

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MATERIALS AND METHODS

Study station

The Soummam Valley region extends for about 120 km from the southwestern slope of the Djurdjura mountain range (latitude 36°21'8 N; longitude 3°53'E) to the coast of the Mediterranean Sea. It is a socio-economic regional entity in the North East of Algeria. It is an agricultural area where the cultivated plots are located along side urban centers and scattered hamlets. It is covered by a fairly dense hydrographic network. It is also characterized by atypically. Mediterranean vegetation, composed mainly of bulges, cultivated plants and maquis of arborescent strata (cork oak, holm oak, Aleppo pine, poplar and olive tree). The climate is Mediterranean with hot, dry summers and cold, rainy winters.

Biological model

In this experiment, the terrestrial isopod *Armadillidium vulgare* was used as a test species.These organisms were collected by the National Institute of Agricultural Research ,Algeria (INRA) and then maintained in the laboratory of Applied Zoology and Animal Ecophysiology at room temperature (22C°±1C°),and a photoperiod of 13h/11h (ligh/dark). Only adult isopods (7pairs of periopods), a body mass of 15-25 mg were used in this experiment.The individuals selected in this test were healthy.

Chemical compound and soils

Two pesticides were used for this experiment which commercial formulations are: 1/the organophosphorus insecticide dursban that contains 480g/l of chlorpyriphos (DOW AGRO SCIENCE) and is used to treat many pests such as caterpillars and sucking insects (mealy bugs, aphids) and 2/ the dithiocarbamate fungicide, mancozeb with 80% of mancozeb (SHANGHA, DEZENTELU International TRADE CO): used for fruit tree treatments for example against apple scab.

The soil used for this biotest was taken from the *Armadillidium vulgare* sampling station and then subjected to physico-chemical soil analyses. The main properties of this soil are: pH = 8.56 ±0.05 (pHmeter), total limestone = 3.6 ± 0.2 (%) (Bernard's Calcimeter); texture (Robinson's pipette grain size):24 ± 1.08 (%) clay; 24 ± 1.53 (%) silt; 52 ± 2 (%) sand.Thus the soil is sandy-clay in nature according to the Atterberg triangle textures. The soil was heated to 80°C for several hours to destroy predators before isopods were introduced.

The experimental protocol

In these bioassays, the concentrations of both pesticides (dursban and mancozeb) were used in mg of active ingredient per kg of soil as used previously on earthworms (OECD, 1984; ISO, 2008). For this acute toxicity test, we took four different concentrations for each of the pesticides (dursban and mancozeb). The highest concentrations were those used by the farmer in the field (720 mg/kg for dursban and 800mg/kg for mancozeb). The three other concentrations were below the maximum concentrations (720 mg/kg ,800 mg/kg) in decreasing logarithmic order. The doses of pesticides used were: & dursban: 0 mg/kg (control) ,720 mg/kg, 360 mg/kg, 90 mg/kg, 45 mg/kg. & mancozeb: 0 mg/kg (control) ,800 mg/kg, 400 mg/kg, 200 mg/kg, 100 mg/kg. & Binary mixture (dursban ± mancozeb):0/0 mg/kg (control), 720/800 mg/kg, 360/400 mg/kg, 90/200 mg/ kg, 45/100 mg/kg.The test were repeted 4 times for each of these concentrations. Thus the experimental protocol adopted for our bioassay required the preparation of 60 plastic boxes of the same dimensions (30cm x 20cm x10cm), with perforated lids for ventilation.Contamination of the environment was carried out by spraying dilutions of prepared pesticides over the entire surface of the soil placed in the boxes. After impregnation with the pesticide for 2 hours, ten adulte (7 pairs of periopods) woodlice of each sex (5 males and 5 females) were introduced into each box. Woodlice were fed daily on litter collected from the sampling site composed of cork oaks, holm oaks, Aleppo pines, dry poplars, olive and carrot , potato peels and water. Mortality control was performed once a week. Dead woodlice were counted after 7, 14, 21 and 28 days.

Morphological effects of pesticides on *Armadillidium Vulgare*

During this experiment we looked for other morphological effects caused by the pesticides tested, by identifying all the morphological anomalies observed on dead or alive woodlice.

Statistical analysis

The statistical analysis was performed with R software package version 3.5.1 (2018) in GLMS. A post Hoc test (Tuckey method) was performed to compare between doses using the emmeans function in emmeans library.

Lethal concentration was determined using R soft ware by Koissi Saviet al (2017).

RESULTS

Acute toxicity test on the species *Armadillidium vulgare*

Effect of dursban on the survival of terrestrial isopods *Armadillidium Vulgare*

After 28 days ,the effect of dursban doses on *Armadillidium Vulgare* was significant ($p < 0.05$). A proportional relationship was observed between the doses used and the mortality obtained. (Fig.1). After already 7 days of exposure, the highest doses of dursban (720 mg/kg) caused 100% mortality in the population.The same result was observed after 14 days at a concentrations of 360 mg/kg.

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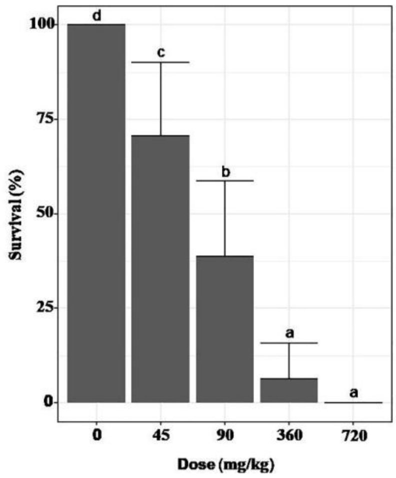

Fig. 1 Survival at 28 days(mean ± sd) of *Armadillidium vulgare* after exposure to single treatment of dursban. Different letters (a,b,c,d) indicate significant differences between concentrations (P < 0.05).

Effect of mancozeb on the survival of terrestrial isopods *Armadillidium Vulgare*

Mancozeb induced a lower mortality in woodlice,compared to dursban.However a significant decrease in survival ($p < 0.05$) was observed at 28 days at the concentrations of 200-800 mg/kg compared to the control condition.(Fig.2).

During the first week, the mortality rate of woodlice subjected to mancozeb was 5.12% for all concentrations (800,400, 200,100 mg/kg). At 28 days, the mortality was 18.5 % at the highest concentration (800 mg/kg) and 12.5 % at the lowest concentration (100 mg/kg).

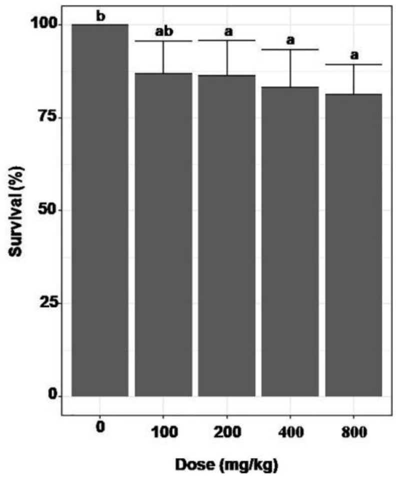

Fig. 2 Survival (mean ± sd) of *Armadillidium vulgare* after a 28days exposure to single treatment of mancozeb. Different letters (a,b) indicate significant differences between concentrations (P < 0.05).

Effect of the mixture (dursban ± mancozeb) on the survival of terrestrial isopods *Armadillidium Vulgare*

The effect of all doses was significant compared to the control.The concentration 45/100 and 90/200 mg/kg had the same effect ($p<0.05$) but beyond these doses, mortality became more and more significant.The dose 720/800 mg/kg caused the highest mortality.(Fig.3.) After already one week of exposure (7 days), the highest doses of the mixture (720/800 mg/kg) caused 100% of mortality in the population.The same result was observed for the concentrations 360/400 mg/kg after 14 days. After 28 days of exposure the lowest dose (45/100 mg/kg) revealed 30% of mortality.

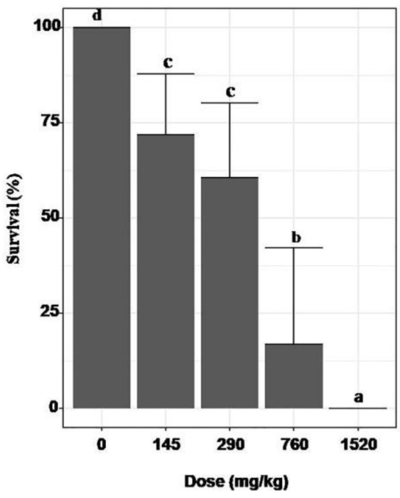

Fig. 3. Survival (mean ± sd) of *Armadillidium vulgare* after a 28 day exposure to a mixture of mancozeb and dursban. Different letters (a, b, c,d) indicate significant differences between concentrations ($P < 0.05$).

Determination of the LD50

The LD50 (the lethal dose of 50% of the population) of dursban after 14 days of experiment was 72.77 mg /kg. (Fig.4). Determination of the LD50 was not possible in an acute mancozeb toxicity test because mortality was not greater than or equal to 50% (25% mortality in the experimental population).

The LD50 of the binary mixture after 14 days of experiment was 330 mg/kg (Fig.5).

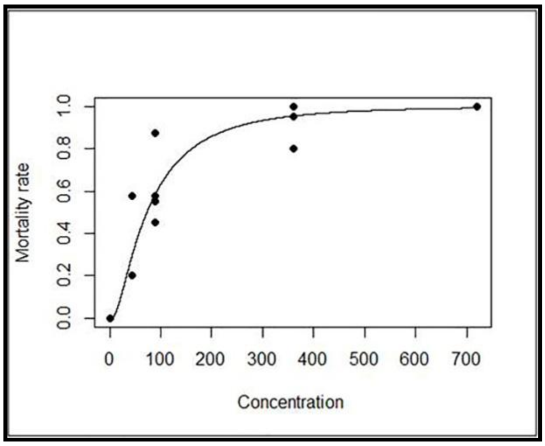

Fig. 4 Dose response curves for the acute toxicity test with *Armadillidium vulgare* in dursban (LD50).

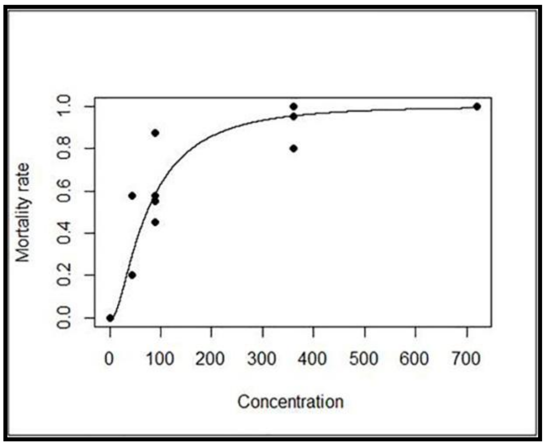

Fig. 5 Dose response curves for the acute toxicity test with *Armadillidium vulgare* submitted to a mixture of dursban ± mancozeb (LD50).

Morphological effects of pesticides on *Armadillidium Vulgare*

In the acute toxicity test conducted on woodlice, some morphological anomalies were observed in the individuals tested with dursban and the mixture of dursban ± mancozeb: *Paralysis of the anterior part in some individuals (Fig.6a).*Marsupium destruction and mucus release (Fig.6b).*Egg expulsion outside the marsupium (Fig.6c).

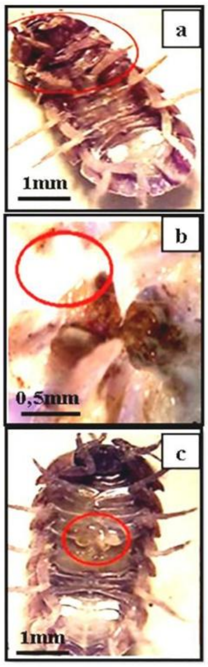

Fig. 6. Anomalies observed in woodlice contaminated by dursban, and the mixture dursban ± mancozeb: (a) paralysis of anterior part, (b) destruction of marsupium and release of mucus, (c) expulsion of eggs from marsupium.

DISCUSSION

After one month of acute toxicity testing on the Armadillidium vulgare population, a low toxicity of mancozeb was recorded (mortality of 19.5%).The same concentrations of this pesticide were tested by Yesguer (2015) on Aporrectodea caliginosa (Annelid species). These results corroborate those of our study. Also, Vermeulen et al (2001) and Ioriatti et al (1992) confirmed this low mancozeb toxicity respectively in the species Eisenia Andrei (Annelid) and the predatory mite Amblyseius andersoni.

Mancozeb had even no effect on adult Amblyseius fallacies (Acarid species) (Bostanian et al.,1998).These results are explained by the low solubility of this fungicide in lipids. According to Fabre et al (1954) this product accumulates poorly in animal tissues and therefore is easily diluted in water and dispersed after rain or irrigation. The LD50 for mancozeb was not determined because mortality was less than 50% (19.5%).Similar values were obtained by Yesguer 2015 on Aporrectodea caliginosa. In contrast, the LD50 of the same product was determined on other earthworm species (Annelid) ;1262mg/kg on Eisenia andrei by Vermeulen et al (2001), 500 mg/kg on Perionyx exacavatus by De Silva et al (2010).

By contrast, a 100% mortality rate could be observed after only 7 days exposure to dursban at a dose of 720 mg/kg or to the mixture of dursban ± mancozeb at 720/800 mg/kg.These results were similar to those obtained by Yesguer (2015) on Annelids submitted to a 720 mg/kg of dursban alone, and Zaidi (2017) on Octodrilus complanatus (Annelid) exposed to the mixture dursban ± mancozeb at a concentration of 720/800 mg/kg.Thises high toxicity of Chlorpyrifos was also reported by Morgado et al.(2016) on the terrestrial isopod Porcellionides pruinosus (75% mortality) and Palma et al. (2008) on three aquatic species Daphnia magna, Vibrio fischeri and Thamnocephalus platyurus.

The mixture dursban ± mancozeb causes a lower (63 %) mortality than that observed for the dursban tested alone (69%).Similar observations were reported by Zaidi (2017) on earthworms (Octodrilus compla - natus). On the other hand, some studies have shown that the effect of a mixture does not necessarily reflect the effects of individual substances (Padhi et al., 2008).

The possible interactions between the components of a mixture of pollutant are quite complex and make it very difficult to predict the overall effect (Lodivici et al., 1994). The toxic effects and significant mortality induced by dursban are explained by the high liposolubility of this insecticide; this property allows it to penetrate easily into the tissucs of animals (Rice et al., 1997). After penetration inside the body, thise products inhibits the functioning of the enzyme acetylcholine esterase (neurotransmitter of the nervous system) (Mileson et al.,1998; Venkateswara et al., 2004 ; Sanchez et al.,2014 ; Morgado et al.,2018) and thus prevent acetylcholine degradation (Regnault, 2005). This neurotransmitter accumulates in the synapses and causes hyperexcitation leading to death.

The acute toxicity test of dursban on Armadillidium vulgare revealed a lethal dose of 50% of the population after 14 days (LD50 = 72.77 mg/kg), this dose is close to that found by Hu et al. (2004) (83.626 mg/kg) and Zhou et al.(2007) (91.78 mg/kg) on earthworms (Aporrectodea caliginosa). Yesguer (2015) determined an LD50 of 60mg/kg with the same pesticide on Aporrectodea caliginosa (Annelid),whereas Lister et al.(2011) reported an LD50 between 26.6 and 38.9 mg/kg on Lumbricus rebellus. Ma et al.(1993) and Chen et al.(2014) reported that dursban has a high toxicity on Eisenia foetida (Annelid) with respective values of 1077 mg/kg and 384.9 mg/kg. According to Kula et al. (1997) ,the variation in the LD50 value of the pesticide is due to the difference in substrate composition, environmental conditions and species tolerance. According to Belfroid et al. (1993); Kula et al. (1997); De silva et al. (2009) it varies with organic matter, texture, pH, temperature and exposure time. The difference observed between the LD50 of dursban in Aporrectodea caliginosa earthworms by Yesguer (2015) and that obtained on a terrestrial crustacean (Armadillidium vulgare), with the same environmental conditions and concentrations of this insecticide, is mainly due to the biological characteristics of the species studied. Like all crustaceans, Armadillidium vulgare is protected by a chitinous cuticle, while earthworms (Annelid) have a thin epidermis. Further -more, the diet of earthworms is composed of soil particles (Eggleton, 2006), making them highly vulnera -ble to contaminants compared to woodlice.

The modification of the structure of the cuticle observed in woodlice under the effect of pesticides made this cuticle fragile causing the destruction of the marsupium, which induces the release of mucus and the expulsion of eggs. The paralysis noticed in wood -lice treated with the dursban is not a consequence of moulting since these individuals were not moulting during this period referring to Montesanto (2017). This paralysis has already been reported by Louat (2013) on the insect Drosophila melanogaster with the same insecticide (Chlorpyrifos). It is due to the inhibition of the enzyme Acetylecholinestrase which causes an accumulation of Acetylcholine in the cholinergic synapse leading to hyperexcitation of neurons. Paralysis is finally leading to death in insect (Louat, 2013).

CONCLUSION

The acute toxicity test carried out showed that dursban caused 100% mortality in the population at the dose used by farmers (720 mg/kg).The second pesticide mancozeb,caused less toxocity (19.5% morta-lity).When a mixture of the two pesticides was tested, the toxicity was lower (63% mortality) compared to the dursban alone. The contamination of woodlices by dursban also reveals an alteration in the marsupial cavity. The latter causes the eggs to be expelled outside the cavity hindering the embryonic development of woodlice. Dursban, an insecticide widely used in agriculture,definitely represents a danger for all soil fauna. In the long term, the frequent use of dursban would be responsible for the decrease in the abundance of soil biodiversity reducing its biological activity. Consequently, this could lead to the loss of soil fertility, and agricultural yields.

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AUTHORS CONTRIBUTION

Metodology: Moumene M., Yesguer S., Mahdeb M., Charfi F.; Data collection: Salhi A. M.; Data processing: Mouloud M. N., Kadji H. D.; Data validation: Sayah C. M.; Writing, original draft preparation: Benmouhoub K. H.; Writing- review and editing: Montesanto G., Habould C.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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