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EFFECTS OF LONG-TERM EXPOSURE TO ACETAMIPRID ON PARTURIAL MOULTING AND REPRODUCTION OF *ARMADILLO OFFICINALIS* DUMÈRIL, 1816 (CRUSTACEA, ISOPODA, ONISCIDEA)

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Abstract. The aim of this study was to evaluate the long-term effects of the insecticide acetamiprid on the parturial moulting and reproduction of the terrestrial isopod *Armadillo officinalis*. Adult woodlice were exposed to acetamiprid at 0.05, 0.1, 0.2, 0.4 mg/kg of dry soil. During 100 days of exposure, the effects on parturial moult and reproduction were investigated. The preparturial intermoult, the parturial premoult and the ecdysis stages were followed, and their duration was calculated. Also, an assessment of reproductive impairment was carried out using the following parameters: percentage of gravid females, incubation period length and the number of mancas released. The results showed that the exposure to acetamiprid significantly impaired the parturial moult in *Armadillo officinalis*. Indeed, it prolonged preparturial intermoult duration at the concentrations > 0.1mg/kg soil and prolonged parturial premoult and ecdysis duration at all tested concentrations. Furthermore, the results demonstrated that acetamiprid significantly impaired the reproduction of *Armadillo officinalis*. It decreased the percentage of gravid females at the highest concentration (0.4 mg/kg) and reduced the number of mancas released at all tested concentrations. These results raise concern about long-term effects of acetamiprid on terrestrial isopods' populations and suggest that acetamiprid could also pose a potential risk to other beneficial soil organisms and consequently reduce soil fertility.

INTRODUCTION

In recent decades, the overuse of pesticides and fertilizers in agriculture has contaminated soil at an alarming rate (van Gestel et al. 2009; Naaz 2013). This raised concern about the adverse effects of these compounds on soil fertility (Savadogo et al. 2009). Indeed, a significant amount of pesticides remains in the soil after their application, which could affect non-target soil organisms living in agroecosystems (Carvalho 2006).

Acetamiprid is a neonicotinoid insecticide displaying high efficiency against insects, such as aphids and white flies (Renaud et al. 2018; Saha et al. 2017). It is one of the most commonly used insecticides in Algerian agriculture (Gacem et al. 2023). It is not directly applied to the soil; however, it could be transferred to the terrestrial environment by rain, irrigation or other transport routes and therefore affect soil organisms (Renaud et al. 2018; Saggioro et al. 2019; de Lima e Silva et al. 2020). Recent studies demonstrated that acetamiprid was highly toxic to earthworms. It damages the epidermal and midgut cells and inhibits fecundity and cellulase activity of the earthworm *Eisenia fetida* (Wang et al. 2015). It was also reported that it affects behaviour, immune and antioxidant systems in *Eiseni aandrei* (Saggioro et al. 2019). Moreover, other studies showed that acetamiprid has potential toxicity concerning springtails and could affect their survival and reproduction (Oluseyi 2014; Renaud et al. 2018).

In order to evaluate the effects of pesticides on the environment, biomonitoring is one of the most effective approaches that involves bioindicators which contribute to the ecosystem functioning (Saline, 2012; Ferrante et al. 2015, 2018). Among the key-organisms, terrestrial isopods are a relevant model for soil ecotoxicology, in laboratory toxicity tests (Moumene et al. 2024; Benmouhoub et al., 2019), field surveillance (Longo et al. 2013; Hopkin et al. 1986) and biomarker studies (Morgado et al. 2018; Ferreira et al. 2015; Santos et al. 2010). They play an important role in decomposing leaf litter and, thus, contribute significantly to nutriment cycling and soil ecosystem services (van Gestel et al., 2018). Also, they represent an abundant and widespread component of the soil fauna and are sensitive to the effects of anthropogenic activities (Agodi et al. 2015; Longo et al. 2013; Mazzei et al. 2013). In this context, the species *Armadillo officinalis* Dumeril, 1816 has been described as an excellent test organism for assessing soil contamination and other environmental changes in its habitat (Agodi et al. 2015; Moumene et al. 2024).

In terrestrial isopods, females' moulting and reproduction are closely related and synchronized (Lefebre 2002). In fact, females could experience two types of moulting: normal when not breeding and parturial during the reproduction period (Subramoniam 2000). In Oniscidea, moulting is regulated by a variety of endocrine hormones such as ecdysteroid moulting hormones produced by the moulting gland Y organ, the moult-inhibiting hormone (MIH), which is involved in the regulation of ecdysteroids during intermoult stages, the crustacean hyperglycemic hormone (CHH) involved in moulting regulation, and ecdysis-triggering hormone (ETH), which is a neuropeptide involved in the regulation of ecdysis (LeBlanc 2007; Nagaraju 2011; Pamuru 2019). The close relationship between reproduction and moulting could reveal common influencing endocrine factors in Oniscidea. For instance, ecdysteroids, the major hormonal factors controlling moulting, have been suggested to be involved in controlling female reproductive activity (Subramoniam 2000). Previous studies show that they play an important role in regulating vitellogenesis, ovarian maturation and protein synthesis (Wongsawang et al. 2005; Brown et al. 2009; Nagaraju 2011). However, current research demonstrated that chemical compounds could disturb some endocrine signalling pathways and consequently impact moulting and reproduction (LeBlanc 2007).

In particular, acetamiprid significantly impacts moulting (Hano 2017) and reproduction in different soil fauna species (Cheng et al. 2019; Saggioro et al. 2019; Cossi et al. 2020). However, to our knowledge, there is no literature concerning the effects of acetamiprid on parturial moult and reproduction of terrestrial isopods. Thus, the aim of this study was to evaluate the long-term effects of acetamiprid on the terrestrial isopod *Armadillo officinalis*, using parturial moult parameters such as preparturial intermoult length, parturial premoult ecdysis duration, and reproductive parameters such as percentage of gravid females, incubation period length and the number of mancas released.

MATERIALS AND METHODS

Isopod collection and breeding (sampling)

Adult males and females' terrestrial isopods of the species *Armadillo officinalis* were collected from an uncontaminated environment at the Algerian National Agricultural Research Institute (INRAA – 36°21'8"N, 3°53'E). They were then bred in the Laboratory of Applied Zoology and Animal Ecophysiology (Algeria) at room temperature (22°C ± 1°C) and a photoperiod of 13 h / 11 h (light/dark). The cultures were sprayed with water and fed with potato tubers twice a week.

Chemical compound and soil

The pesticide tested in this experiment was the neonicotinoid insecticide acetamiprid (Aceplan with 20% acetamiprid) purchased from RIVALE.

The soil used in this experiment was natural soil collected from the National Institute of Agricultural Research, Algeria (INRAA). The main properties of this soil were: pH = 8.56; total limestone = 3.6%; texture: 24% clay; 24% silt; and 52% sand.

Experimental set-up

The toxicity test was performed according to a modified version of the international OECD test for earthworms (OECD 2015). According to preliminary tests, acetamiprid was diluted in pure water to the following concentrations: 0.05, 0.1, 0.2 and 0.4 mg of active ingredient/kg of soil (dry weight). Pure water was used as a control. Eight replicates of each concentration and the control were prepared. Toxicity tests were performed in forty transparent plastic boxes with perforated lids (30 cm × 20 cm × 10 cm) containing 250 g of moist soil. Each concentration was sprayed on the soil of its respective box. Then, ten terrestrial isopods (five males and five females) in the intermoult phase with an average mass of 150 ± 2 mg were introduced in each box and fed with potato tubers twice a week. Soil was maintained moist during the experiment by spraying water when necessary. Terrestrial isopods were exposed to acetamiprid during 100 days on which parturial moult and reproduction were monitored.

Parturial moulting monitoring

The assay was carried out during the reproductive period of *Armadillo officinalis,* which is from March to June. In this experiment, only females were assessed. Parturial moult monitoring started along with animals' exposure, and during 100 days, the examination of animals was routinely carried out every 24 h. The observations were based on the appearance, shape and size of white calcium sternal deposits on the first four pereons, as described by Zidar et al. (1998). This way, parturial premoult and ecdysis stages were recorded and their duration calculated. In addition, the prepartu-rial intermoult period was calculated as the duration between the end of the last ecdysis and the beginning of the parturial premoult.

Reproduction monitoring

All females were monitored daily, and after the first parturial moult, gravid females were separated and maintained individually in plastic boxes containing moist soil contaminated with the same concentration of acetamiprid (0.05; 0.1; 0.2 and 0.4 mg a.i/ kg of soil d.w.). This way, the following reproductive parameters were studied: percentage of gravid females, gestation period length and the number of mancas released.

Statistical analysis

Normality test (Kolmogorov-Smirnov (K-S) was performed on all data. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey test for multiple comparisons of means to determine significant differences relative to control treatments. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using R version 3.3.2.

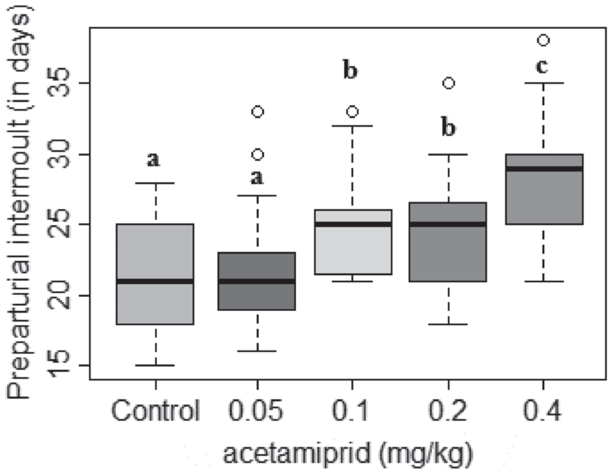

Figure 1. Mean duration (in days) of preparturial intermoult of all females exposed to acetamiprid for 100 days. Different letters indicate different groups ($p < 0.05$).

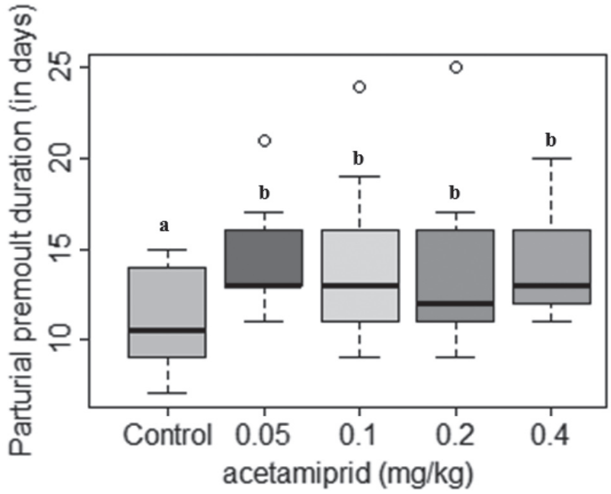

Figure 2. Mean duration (in days) of parturial premoult of all females exposed to acetamiprid during 100 days. Different letters indicate different groups ($p < 0.05$).

RESULTS

Effects of acetamiprid on preparturial intermoult duration

The results showed no significant difference between the groups treated with the lowest concentration (0.05 mg/kg dry soil) of acetamiprid and the control (Figure 1). However, a significant increase in pre-parturial intermoult duration was observed in groups treated with higher concentrations of acetamiprid: 0.1 mg/kg (p = 0.001), 0.2 mg/kg (p = 0.0003) and 0.4 mg/kg (p = 0.0001).

Effects of acetamiprid on parturial premoult duration

The results showed that acetamiprid significantly prolonged the parturial premoult duration of all exposed groups compared to the control (0.05 mg/kg, p = 0.0001; 0.1 mg/kg, p = 0.01; 0.2 mg/kg, p = 0.005, and 0.4mg/kg, p = 0.003; Figure 2).

Effects of acetamiprid on parturial ecdysis duration

The results showed a significant increase in ecdysis duration of all groups treated with acetamiprid compared to the control (p = 0.0001; Figure 3).

Percentage of gravid females during a 100-day exposure to acetamiprid

The results did not show a significant difference between groups treated with different concentrations of acetamiprid except at the highest concentration of 0.4 mg/kg ($p < 0.05$; Figure 4).

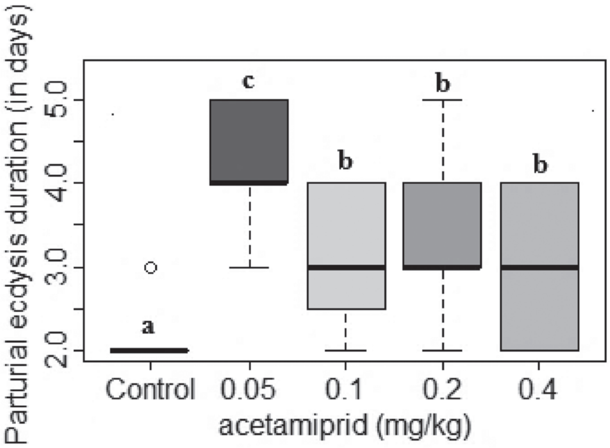

Figure 3. Mean duration (in days) of parturial ecdysis of all females exposed to acetamiprid during 100 days. Different letters indicate different groups ($p < 0.05$).

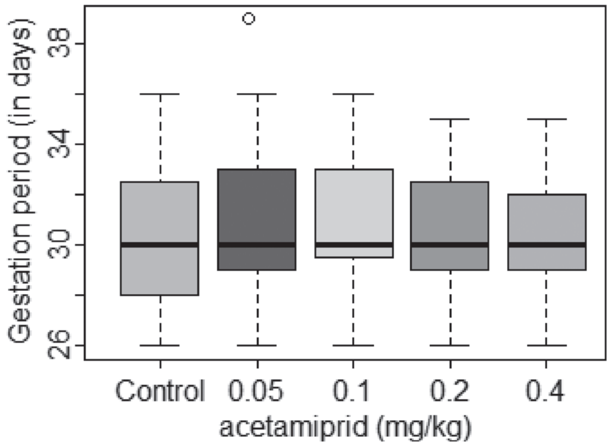

Figure 5. Mean duration (in days) of gestation period of all gravid females exposed to acetamiprid during 100 days.

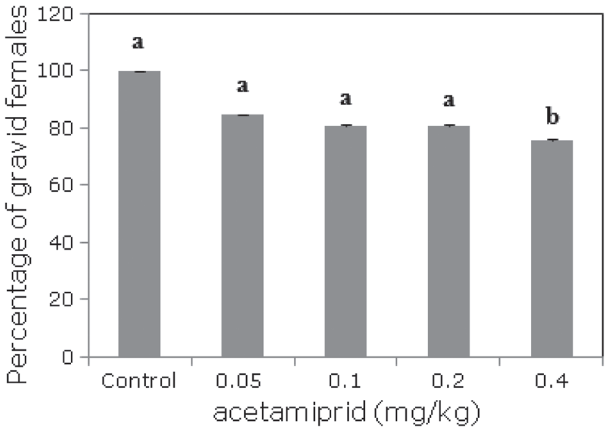

Figure 4. Percentage of gravid females during a 100-day exposure to acetamiprid. Bars are means ± SE. Different letters indicate different groups ($p < 0.05$).

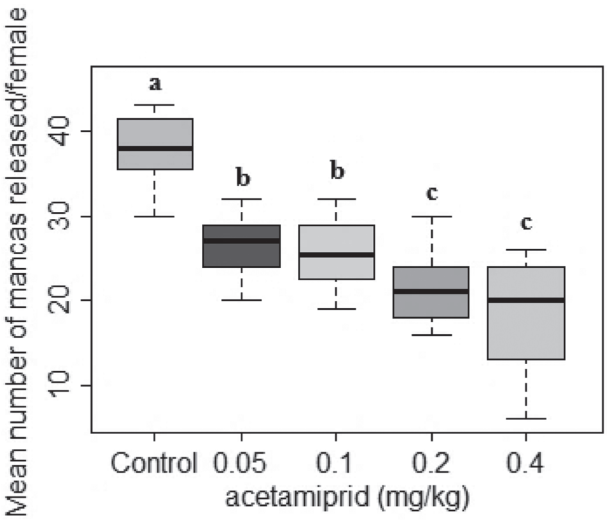

Figure 6. Mean number of mancas released per female during 100 days of exposure to acetamiprid. Different letters indicate different groups ($p < 0.05$).

Effects of acetamiprid on the duration of the gestation period

The results showed that the duration of the gestation period was not significantly affected by the treatments regardless of the concentration of acetamiprid (Figure 5).

Effects of acetamiprid on the mean number of mancas released per female

The results showed that the exposure to acetamiprid significantly decreased the mean number of mancas released per female ($p = 0.0001$; Figure 6).

DISCUSSION

Our findings first highlighted that the acetamiprid exposure disturbed the parturial moulting in *Armadillo officinalis*. Recent studies revealed that neonicotinoid insecticides could disrupt moulting in several animal species (Li et al. 2024; Krishnan et al. 2021; Macaulay et al. 2019). For instance, Barmentlo et al. (2019) found that the neonicotinoid thiacloprid delayed moulting in the damselfly *Ischnura elegans* at a concentration of 9.86 µg/L after three weeks of exposure. Furthermore, Macaulay et al. (2019) reported that exposing the mayfly *Deleatidium* spp. to the neonicotinoid imidacloprid and clothianidin at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, and 4.0 µg/L) decreased significantly the moulting propensity with increasing pesticide concentrations during the second and third weeks of exposure. Also, Hano et al. (2017) exposed the marine crustacean kuruma prawn (*Marsupenaeus japonicas*) to 6, 13, 25, 50, 100, 200 µg/L of acetamiprid and observed that after 96 h of exposure, moulting frequency showed a tendency to decrease with increasing concentrations of acetamiprid mainly at the highest concentrations (100 µg/L and 200 µg/L).

Our results showed that the preparturial intermoult stage was significantly extended in the isopods exposed to acetamiprid. Similar results were also reported by Al-Badran et al. (2019) in brown shrimps (*Farfantepenaeus aztecus)* after 36 days of exposure to 15 µg/L of the neonicotinoid insecticide imidacloprid. Neonicotinoid insecticides act on the central nervous system as agonists of the nicotinic acetylcholine receptors (nAChRs), and thus disrupt synaptic transmission (Matsuda et al. 2009). This suggests that they could interrupt some endocrine signalling pathways and thus impact moulting. According to LeBlanc (2007), the disruption of the moulting cycle by toxic exposure could result from an alteration in the levels of the ecdysteroid moulting hormone. In our study, acetamiprid probably inhibited the ecdysteroids hormone synthesis, which delayed the next moult and extending the intermoult period. Another hypothesis is that acetamiprid interfered with the moulting inhibiting hormone (MIH) (Hosamani et al. 2017).

Moreover, our results showed that the duration of the parturial premoult stage was significantly prolonged when isopods were exposed to acetamiprid. According to Zou (2020), the slowdown of moulting could be due to the inhibition of epidermal chitobiase (NAG), which is one of the chitinolytic enzymes required for the degradation of the chitinous exoskeleton that will be shed. The same results were also documented by Lemos et al. (2009) where the premoult stage of the terrestrial isopods *Porcellio scaber* was significantly extended after 5 weeks of exposure to 100 mg a.i./kg of the fungicide vinclozolin. These authors suggested that it is probably due to the alteration of the exuviation factor, which is essential for animals to complete their premoult. According to Zou (2020), the impairment of chitinolytic enzymatic activity by a moulting-disrupting chemical could be through alteration in ecdysteroids titers via perturbation in ecdysteroidogenesis and/or changes in inhibitory peptide hormones such as moulting inhibitory hormone (MIH) or crustacean hyper glycemic hormone (CHH). In addition, it could also be caused by a direct disruption of epidermal ecdysteroid signalling through interfering with the ecdysteroid receptor (EcR) (LeBlanc 2007).

Furthermore, our findings showed a significant increase in parturial ecdysis duration in all groups treated with acetamiprid compared to the control. Similarly, Lemos et al. (2009) noted changes in the ecdysis period of *Porcillio scaber* after 7 days and 14 days of exposure to vinclozin. According to Zou (2020), environmental toxicants could, in theory, affect the signalling of ecdysis-triggering hormone (ETH). This suggests that acetamiprid probably inhibited this neurohormone and caused ecdysis deficiencies in *Armadillo officinalis*.

Acetamiprid impaired the reproduction in *A. officinalis* females in the current study. The percentage of gravid females was significantly lower in the group treated with the highest concentration of acetamiprid (0.4 mg/kg). The same results were also found by Fischer et al. (1997) in the terrestrial isopod *Porcellio scaber* after eight weeks of exposure to 20, 30 and 40 mg/kg of dimethoate insecticide. Reproduction in the crustacean suborder Oniscidea is regulated by a variety of endocrine hormones (Nagaraju 2011; Pamuru 2019). However, some of these hormones could be highly sensitive to environmental chemicals (LeBlanc 2007). In this context, recent studies have reported that acetamiprid could act like an endocrine disruptor (Ma et al. 2022; Mosbah et al. 2018; Baines et al. 2017). This leads to the hypothesis that acetamiprid probably inhibited the ecdysone hormone and consequently induced reproductive disruptions in the females exposed to the highest concentration. Cheng et al. (2019) also reported reproductive disorders, such as abnormalities in the development of ovaries and fallopian tubes in silkworm *Bombyx mori* after 96 h of exposure to 0.01 mg L^{-1} of acetamiprid. According to these authors, these reproductive perturbations were related to the low ecdysteroid 20-hydroxyecdysone (20E) levels in the exposed animals. The levels of 20E and the expression of its related gene were down regulated at 0.01 mg L^{-1} acetamiprid exposure, indicating that acetamiprid can inhibit the increase of ecdysone and affect the synthesis of vitellin.

Concerning the effects of acetamiprid on the duration of the gestation period, the results showed no significant difference compared to the control. The same results were also documented by Negro et al. (2015) in the freshwater burrowing crab, *Zilchiopsis collastinensis* after 10 days of exposure to the insecticides endosulfan (100, 200 and 400 ng/L) and chlorpyrifos (48, 240 and 1200 ng/L).

In addition, the results showed that the mean number of mancas released in animals exposed to acetamiprid was significantly lower compared to the control group. It has been reported that some endocrine hormones such as ecdysteroids and methyl farnesoate are involved in egg production and maturation (Rodriguez et al. 2002; Mak et al. 2005; Nagaraju 2011). Thus, any interference of acetamiprid with these hormones could disrupt egg production. For instance, Picone et al. (2022) noted a decrease in egg production and an inhibition of hatching ratio in the copepod *Acartia tonsa* after 30 days of exposure to 10 ngL^{-1} of acetamiprid. Another hypothesis is that acetamiprid allocated the capacity of oviposition in *Armadillo officinalis* females. That what Cheng et al. (2018) reported in female mites *Amblyseius cucumeris* after 30 days of exposure to acetamiprid concentrations > 2.24 mg a.i. L^{-1}.

CONCLUSION

The findings of the present study showed that acetamiprid exposure induced adverse effects on the parturial moult of the terrestrial isopod *Armadillo officinalis*. It extended preparturial intermoult at 0.1, 0.2 and 0.4 mg/kg soil and prolonged parturial premoult and ecdysis duration at all tested concentrations. Furthermore, the results demonstrated that acetamiprid significantly impaired the reproduction of *Armadillo officinalis*. It decreased the percentage of gravid females at the highest concentration (0.4 mg/kg) and reduced the number of mancas released at all tested concentrations. These results raise concerns about long-term effects of acetamiprid on non-target animals such as terrestrial isopods and suggest that acetamiprid could pose a potential risk to other beneficial soil organisms and consequently reduce soil fertility.

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