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Effects of long-term chlorpyrifos exposure on moulting and growth of *Armadillo officinalis* Dumèril, 1816 (Crustacea, Isopoda, Oniscidea)

Q1 Q2

 The corrections made in this section will be reviewed and approved by a journal production editor.

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Abstract

The aim of this study was to evaluate the long-term effects of the insecticide chlorpyrifos on the moulting and growth of the terrestrial isopods *Armadillo officinalis*. Adult woodlice were exposed to chlorpyrifos at concentrations of 5, 10, 20, 40 mg/kg dry soil and compared to a control. During 100 days of exposure, effects on moulting and growth were investigated. The duration of premoult, ecdysis and intermoult stages were estimated. Also, the cuticle width (CW) and length (CL) were measured and their size increase and specific growth were calculated. The results showed that exposure to chlorpyrifos induced adverse effects on moulting in the terrestrial isopod *Armadillo officinalis*, mainly at the higher concentrations (20 mg and 40 mg/kg dry soil). Indeed, it decreased the percentage of moulting animals, delayed moulting, decreased the number of completed moult, extended the premoult stage, prolonged ecdysis, and reduced intermoult duration. Furthermore, these findings showed that chlorpyrifos exposure impaired also growth of *Armadillo officinalis*, mainly at the highest concentration (40 mg/kg dry soil), by decreasing weight gain (0.94 ± 2.14 mg), cuticle length gain (0.26 ± 0.06 mm) and specific growth rate (SGR) (0.04 ± 0.01 mm/days) compared to the control (Weight gain = 16.5 ± 1.84 mg; CL gain = 0.65 ± 0.07 mm; SGR = 0.08 ± 0.01). The present study highlighted the adverse effects of chlorpyrifos on moulting and growth of *Armadillo officinalis*, which raises concern about consequences of chlorpyrifos exposure on population dynamic of this species.

Keywords:

Insecticide, Chlorpyrifos, Moulting, Growth, Terrestrial isopods, *Armadillo officinalis*

Introduction

Pesticides are intensively used in agriculture. However, these compounds raised concerns about their hazardous effects in soil organisms such as earthworms, springtails, snails and terrestrial isopods (Paoletti and Bressan, 1995; Hubbard et al., 1999; Robinson and Sutherland, 2002; Bengtsson et al., 2005; Bianchi et al., 2006; Sánchez-Bayo, 2011).

Among these pesticides, chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an organophosphate insecticide applied to control a wide range of insect pests in agricultural production as well as houseflies (EPA, 2011). In the Soummam valley, which is an important agricultural area in the north of Algeria, chlorpyrifos is one of the most commonly used insecticides (Benmouhoub et al., 2019). However, due to its broad-spectrum effectiveness, chlorpyrifos may have harmful consequences on non target organisms as inducing mortality, oxidative stress and metabolic disorders (Barron and Woodburnt, 1995; Zhang et al., 2017; Xinget al., 2019). The direct toxicity of chlorpyrifos is assumed to result from its specific inhibition of acetylcholinesterase (AChE), an enzyme that is primary involved in neurotransmission, and which also has many other physiological functions that are not related to the cholinergic system (Sepčić et al., 2019).

One of the most efficient approaches for assessing the effects of pesticides in the environment is bio-monitoring, which involves the monitoring of bio-indicators in treated versus untreated environments (Salines, 2012; Ferrante et al., 2015). In this context, several studies have investigated the toxic impacts of chlorpyrifos using bio-indicators such as terrestrial invertebrates (Gatti et al., 2002; Venkateswara Rao et al., 2003; Fountain et al., 2007; Jagar et al., 2007; Wang et al., 2012; Pelosi et al., 2014; Muangphra et al., 2015; Dolar et al., 2021) and numerous effects have been reported. For instance, chlorpyrifos impairs growth (Booth and O'Halloran, 2001) and fecundity (Alshawish et al., 2004; Zhou et al., 2007) in earthworms, and reduces springtail abundance (Endlweber et al., 2006; Fountain et al., 2007). Furthermore, it induces lethality (Nair et al., 2002; Morgado et al., 2016; Benmouhoub et al., 2019), oxydative stress (Morgado et al., 2018) and changes in immune function (Dolar et al., 2021) in terrestrials isopods.

Terrestrial isopods are widely used as bioindicators of environmental pollution due to their sensitivity to the effects of anthropogenic activities (Longo et al., 2013; Mazzei et al., 2013; Agodi et al., 2015). They also represent an abundant and widespread component of the soil fauna (Caruso et al., 1987; Messina et al., 2011; Messina et al., 2012). The species *Armadillo officinalis* has been reported as a good test-organism to assess soil contamination or other environmental changes in its habitat (Agodi et al., 2015; Montesanto and Cividini, 2017).

Terrestrial isopods moult frequently throughout their life cycle in order to grow. So, they shed their old cuticle and develop a new exoskeleton (Lefebvre, 2002). Moulting in Oniscidea is regulated by a variety of neuroendocrine signalling cascades which involve various hormones such as the ecdysteroid moulting hormones, the moult-inhibiting hormone (MIH), and the crustacean hyperglycemic hormone (CHH) (LeBlanc, 2017; Zou, 2020). Several authors have recently shown that these endocrine pathways are highly sensitive to aberrant signalling by environmental chemicals (LeBlanc, 2017; Jubeaux et al., 2012; Legrand, 2016; Hosamani et al., 2017; Zou, 2020).

Numerous studies have highlighted the effects of pesticides on moulting (Baldwin et al., 2001; Lemos et al., 2009; Palma et al., 2009; Lemos et al., 2010) and growth (Montagna and Collins, 2007; Lemos et al., 2010; Singh et al., 2021) in Oniscidea crustacean. However, although there have been some researches on the consequences of chlorpyrifos exposure (Volz et al., 2003; Montagna and Collins, 2007; Taylor, 2020), to our knowledge, there is no data about the impacts of chlorpyrifos on moulting in terrestrial isopods.

In ecotoxicological risk assessment of pesticides, particularly in isopods bioassay, most studies use acute toxicity tests (Benmouhoub et al., 2019; Santos et al., 2010; Loureiro et al., 2009; Kolar et al., 2008). Although these assays are effective for survival parameters (Calh a et al., 2012; Santos et al., 2010) or avoidance behaviour parameters (Loureiro et al., 2002), they are limited when more detailed and comprehensive effects of the toxicant on life stage such as moulting and growth of the animal are aimed. In this case, long term exposure tests are advisable as they could cover all the life stage of the animal and track the impacts of pesticides over the time (Guimar es et al., 2023).

The aim of the present study was to evaluate the long-term effects of chlorpyrifos on the terrestrial isopod *Armadillo officinalis* using moulting parameters such as moulting frequency, the duration of premoult, ecdysis and intermoult stages and growth parameters such as weight gain, size gain and specific growth rate (SGR).

Materials and methods

Sampling and breeding

Adult males and females terrestrial isopods of the species *Armadillo officinalis* were collected in unpolluted natural area of the National Institute of Agricultural Research, Algeria (INRA) (latitude 36°21'8N; longitude 3°53'E). Then, they were bred in the laboratory of Applied Zoology and Animal Ecophysiology, Bejaia, Algeria, at room temperature (21°C±1°C), with a photoperiod of 13 h/11 h (light/dark). Twice a week, the colonies were sprayed with water and fed with potato tubers.

Chemical compound and soil

The pesticide tested in this experiment was the organophosphorus insecticide chlorpyrifos (CICLONER 48 EC with 480 g/L of chlorpyrifos).

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

Experimental set up

The toxicity test was conducted according to a modified version of the guideline for earthworm's sub-lethal test established by the Organization for Economic Co-operation and Development (OECD) (OECD, 2015). The selection of chlorpyrifos concentrations for this experiment was based on a preliminary assay where an acute toxicity test on *Armadillo officinalis* species was carried out in order to determine the lethal concentration 50 (LC₅₀) for chlorpyrifos. After 14 days of exposure, the result showed that the LC₅₀ of chlorpyrifos was 80.5 mg/kg. Thus, for the long-term exposure, chlorpyrifos was diluted in water to the following concentrations: 5, 10, 20 and 40 mg/kg soil (dry weight), which were about 1/16, 1/8, 1/4, 1/2 of LC_{50-14days} respectively. Pure Water was used as a control. Four replicates of each concentration and the control were prepared. Toxicity tests were performed in twenty transparent plastic boxes with perforated lids (30 cm × 20 cm × 10 cm), containing 250 g of moist soil. Each solution was sprayed on the soil of its respective box, before introducing 10 woodlice (5 males and 5 females) in intermoult phase with an average weight of 150 ± 7.6 mg per box. Woodlice were fed with potato tubers twice a week. Soil was kept moist during all the experiment by spraying water when necessary. Woodlice were exposed to the chemical for 100 days, during which moult monitoring and growth were also performed.

Moulting monitoring

To avoid the parturial moulting, and focus only on the growth moulting, the experiment was carried out outside the reproductive period of *Armadillo officinalis* that is from October to January. Moulting monitoring was launched along with the organisms exposure, and for 100 days, woodlice were systematically examined every 24hr. In fact, the observations were based on the appearance, size, and shape of white calcium sternal deposits on the first four pereon. This way, premoult phase (proecdysis) and its three main subphases (named PE1, PE2, PE3, as in Zidar et al., 1998) as well as the biphasic ecdysis and its subphases (Ea and Ep) were recorded and their duration calculated. In addition, intermoult period was calculated as the duration between the end of the first ecdysis to the end of the experiment for the woodlice that moult only once, and from the end of the first ecdysis to the beginning of the second premoult for the woodlice that moulted twice.

Growth monitoring

Tested woodlice were weighed every week. Then at the end of the experiment, the weight gain was calculated.

Also, at the beginning of the experiment, tested woodlice were photographed under a stereo microscope Optika 4083.B5. Then, cuticle width (CW) and cuticle length (CL) were measured using ImageJ software (version 1.45 s). The same process was repeated at the end of the experiment and then the increase in size of (CW) and (CL) were calculated.

The specific growth rate (SGR) of size and weight were calculated using the following formula (as in Romano and Zeng, 2006): $SGR = (\ln W_f - \ln W_0) / IP \times 100$, where SGR is specific growth rate; W_f is the final size or weight; IP is

the intermolt period; W_0 is the initial size or weight.

Statistical analysis

Normality and equal variance tests were performed for all data. Statistical analysis was carried out using one-way analysis of variance (ANOVA) with Tukey post hoc test for multiple comparisons of means to determine significant differences relatively to control treatment. The level of significance was set at $p < 0.05$. All statistical analyses were performed using R software package (version 3.3.2).

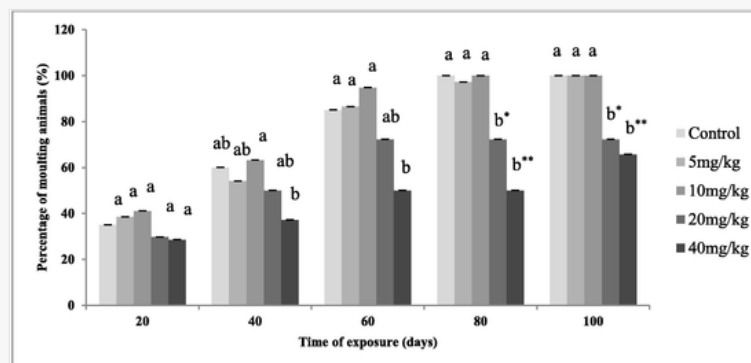
Results

Percentage of moulting animals during 100 days of exposure to chlorpyrifos

After 20 days of experiment, the results didn't show significant difference between the groups treated with the different concentrations of chlorpyrifos and the control (Fig. 1). However, a significant difference compared to the control was noted after 40 and 60 days of exposure to the highest concentration of chlorpyrifos (40 mg/kg soil) ($p < 0.05$). Also, a significant difference compared to the control was found after 80 days of exposure to the higher concentrations (20 and 40 mg/kg soil) ($p < 0.01$ and $p < 0.001$, respectively). After 100 days of experiment, 100 % of animals moulted in the control group. The same percentage of moulting animals was observed at the concentrations 5 and 10 mg/kg soil. However, a significant lower percentage of moulting animals was observed when they were exposed to 20 mg/kg soil (72 %) and 40 mg/kg soil (65.63 %) ($p < 0.01$ and $p < 0.001$, respectively).

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Fig. 1



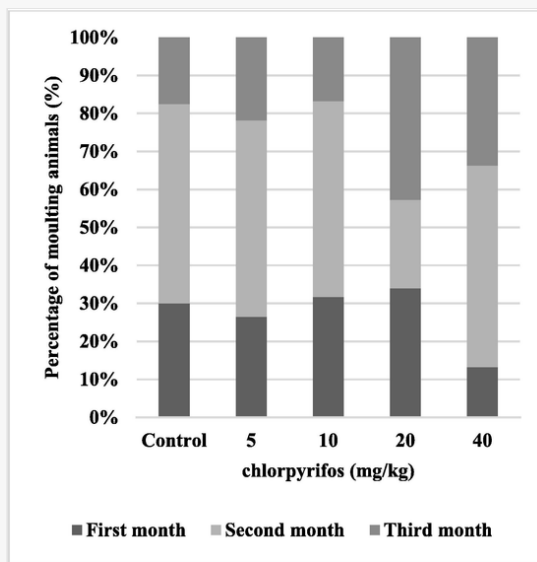
Percentage of moulting animals during 100 days of exposure to chlorpyrifos. Bars are means \pm SEM. Bars with different letters are significantly different ($p < 0.05$); one asterisk (*) indicates $p < 0.01$; two asterisks (**) indicate $p < 0.001$.

Distribution of the first moult per month of animals exposed to chlorpyrifos during 100 days

In the control group, 82.5 % of animals had moulted after two months of experiment, of which 30 % in the first month and 52.5 % in the second month (Fig. 2). Similar results were found in the groups exposed to the 5 and 10 mg/kg soil concentrations, with 77.67 % and 80.77 % of moulted animals after two months, respectively. In the group exposed to 20 mg/kg soil, only 41 % of animals had moulted after two months of exposure, of which 24.32 % the first month and 16.67 % the second month. In the group treated with the highest concentration (40 mg/kg soil), moult seemed to be drastically delayed, with only 8.57 % of animals that had moulted in the first month and 34.38 % in the second month.

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Fig. 2



Distribution of the first moult per month of animals exposed to chlorpyrifos. Data are presented as percentage of animals that moulted during each period.

Number of moults completed by animals during 100 days of exposure to chlorpyrifos

The results showed that the number of moults were not significantly different compared to the control when exposing animals to the lower concentrations (5 and 10 mg/kg soil) of chlorpyrifos (Fig. 3). However, a significant decrease in the number of moults was noted at the higher concentrations 20 mg/kg soil ($p < 0.05$) and 40 mg/kg soil ($p < 0.001$).


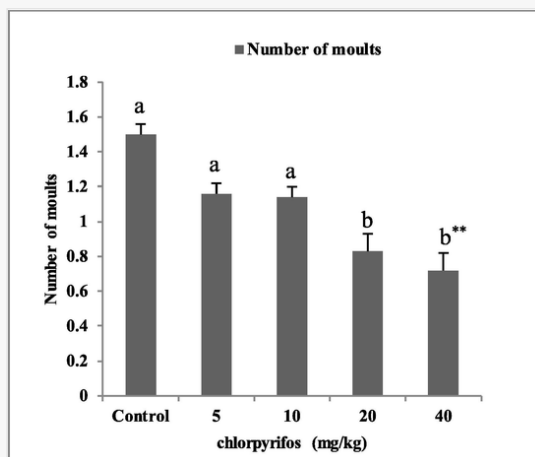
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Fig. 3



Mean number of moults completed by animals during 100 days of exposure to chlorpyrifos. Bars are means \pm SEM. Bars with different letters are significantly different ($p < 0.05$); two asterisks (**) indicate $p < 0.001$.

Effects of chlorpyrifos on the premoult duration (in days) and its substage duration (in days)

The results showed no significant difference between the groups treated with the lower concentrations (5 and 10 mg/kg soil) of chlorpyrifos and the control (Table 1). Concerning the groups treated with the higher concentrations (20 and 40 mg/kg soil) a significant increase in the premoult duration compared to the control was observed ($p < 0.01$ and $p < 0.001$, respectively).

Table 1

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Effects of chlorpyrifos on the duration (in days) of the first premoult substages (mean \pm SEM). Within a same column, values with different letters are significantly different ($P < 0.05$); one asterisk (*) indicates $p < 0.01$; two asterisks () indicate $p < 0.001$.**

	Whole premoult Duration (days)	Premoult substage duration (days)		
		PE1	PE2	PE3
Control	11.48 \pm 0.42 ^a	4.98 \pm 0.33 ^a	4.13 \pm 0.16 ^a	2.38 \pm 0.1 ^a
Min – Max	8–18	3–11	3–6	1–4
5 mg/kg	12.95 \pm 0.39 ^a	5.92 \pm 0.28 ^a	4.76 \pm 0.21 ^a	2.27 \pm 0.11 ^a
Min – Max	9–19	3–10	3–8	1–4
10 mg/kg	13.78 \pm 0.72 ^a	6.05 \pm 0.45 ^a	5.32 \pm 0.34 ^{b*}	2.41 \pm 0.13 ^a
Min – Max	9–34	3–17	3–15	2–5
20 mg/kg	14.88 \pm 0.96 ^{b*}	6.96 \pm 0.7 ^b	5.23 \pm 0.31 ^b	2.69 \pm 0.17 ^a
Min – Max	9–33	4–21	3–9	2–5


40 mg/kg	15.52 ± 1.32 ^{b**}	7.05 ± 0.98 ^b	5.90 ± 0.43 ^{b**}	2.57 ± 0.13 ^a
Min – Max	9–35	4–23	3–10	2–4

Regarding the effects of chlorpyrifos on the duration of the premoult substages, the results showed no significant difference on PE1 duration at the lower concentrations (5 and 10 mg/kg soil) compared to the control (Table 1). However, a significant prolongation in PE1 length at the higher concentrations (20 and 40 mg/kg soil) was noticed ($p < 0.05$ respectively). Concerning the effects on PE2 duration, except the lowest concentration (5 mg/kg soil), results demonstrated a significant difference to the control in all treatments and mainly at the highest concentration 40 mg/kg soil ($p < 0.001$). Finally, no significant difference in PE3 duration was found between the groups treated with the various concentrations of chlorpyrifos and the control.

Effects of chlorpyrifos on the duration of ecdysis and its substages

Ecdysis duration was not significantly affected by the chlorpyrifos treatments (Table 2). Concerning the substages, the only significant difference observed was an increase in Ep duration at a chlorpyrifos concentration of 20 mg/kg soil ($p < 0.05$).

Table 2

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Effects of chlorpyrifos on the mean duration (in days) of ecdysis and its substages Ea and Ep (mean ± SEM). Within a same column, values with different letters are significantly different ($p < 0.05$).

	Whole Ecdysis	Ecdysis substages	
		Ep	Ea
Control	2.08 ± 0.04 ^a	1.08 ± 0.04 ^a	1.0 ± 0.0 ^a
Min – Max	2–3	1–2	1–1
5 mg/kg	2.35 ± 0.1 ^a	1.35 ± 0.1 ^a	1.0 ± 0.0 ^a
Min – Max	2–4	1–3	1–1
10 mg/kg	2.46 ± 0.28 ^a	1.27 ± 0.13 ^a	1.19 ± 0.16 ^a
Min – Max	2–12	1–5	1–7
20 mg/kg	2.58 ± 0.16 ^a	1.5 ± 0.13 ^b	1.08 ± 0.05 ^a
Min – Max	2–5	1–3	1–2
40 mg/kg	2.14 ± 0.08 ^a	1.14 ± 0.08 ^a	1.0 ± 0.0 ^a
Min – Max	2–3	1–2	1–1

Effects of chlorpyrifos on intermoult duration

The results showed that chlorpyrifos reduced significantly the intermoult duration of all exposed groups compared to the control. The effect was stronger at the concentrations of 5 mg/kg and 40 mg/kg soil ($p < 0.0001$ respectively) (Fig. 4).


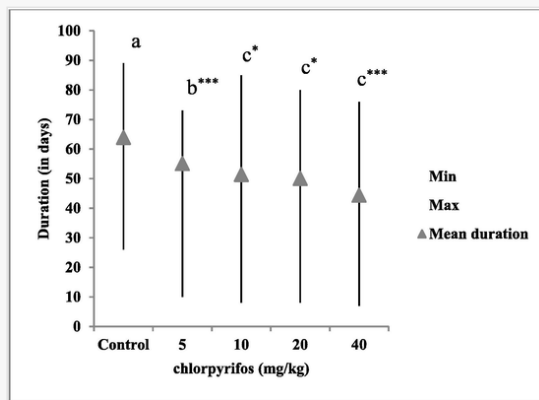
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Fig. 4



Mean duration (in days) of intermoult (mean \pm SEM) of all animals exposed to chlorpyrifos during 100 days. Values with different letters are significantly different ($p < 0.05$); one asterisk (*) indicates $p < 0.01$; three asterisks (***) indicate $p < 0.0001$.

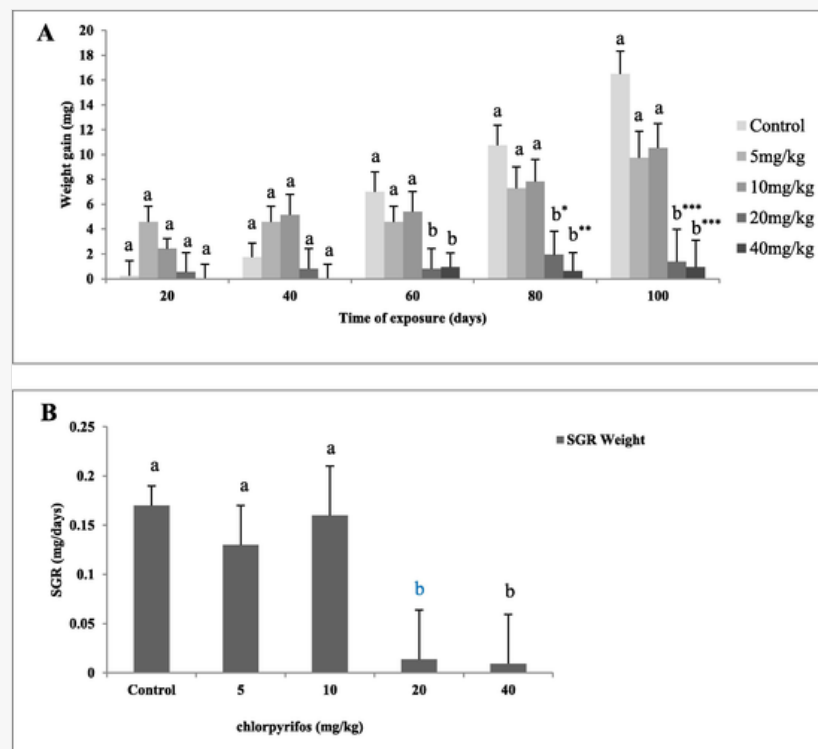
Effects of chlorpyrifos on growth

Effects of chlorpyrifos on weight gain

After 20 and 40 days of experiment, no significant difference was noted between the groups treated with different concentrations of chlorpyrifos and the control (Fig. 5). A significant difference was found at the higher concentrations of chlorpyrifos (20 and 40 mg/kg soil) after 60 days ($p < 0.05$ respectively) and 80 days of experiment ($p < 0.01$ and $p < 0.001$ respectively). After 100 days of exposure to chlorpyrifos, the results did not show any significant difference between the control and treatment with the lower concentrations (5 and 10 mg/kg soil). However, chlorpyrifos impaired significantly the weight gain of animals exposed to the higher concentrations of chlorpyrifos (20 and 40 mg/kg soil) ($p < 0.0001$ respectively).

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Fig. 5



Effects of chlorpyrifos on weight. (A) weight gain (mg); (B) SGR of weight (mg/days). Bars are means \pm SEM. Bars with different letters are significantly different ($p < 0.05$); three asterisks indicate $p < 0.0001$.

Effects of chlorpyrifos on weight's specific growth rate

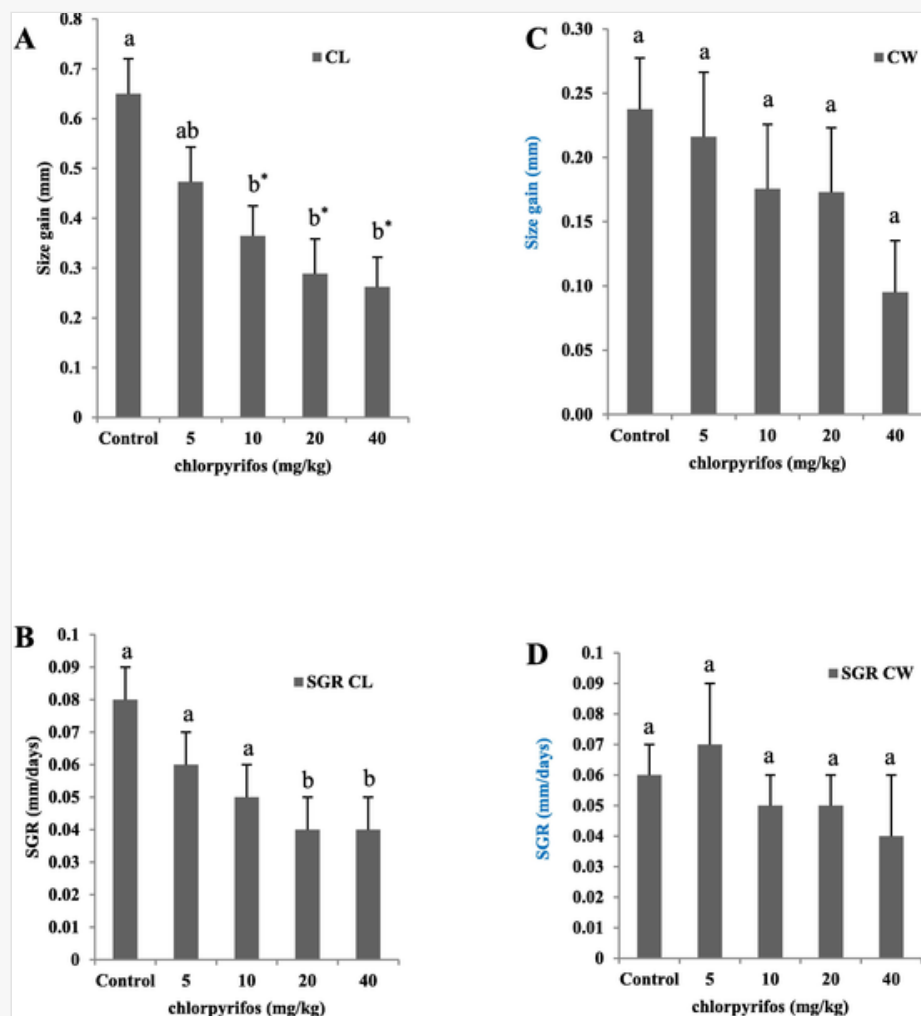
The results showed no significant difference between the groups treated with the lower concentrations (5 and 10 mg/kg soil) of chlorpyrifos and the control. However, a significant decrease in weight's specific growth rate (SGR) was noted at the higher concentrations of chlorpyrifos ((20 and 40 mg/kg soil) compared to the control ($p < 0.05$ respectively) (Fig. 5).

Effects of chlorpyrifos on size gain

The results showed that chlorpyrifos exposure impaired significantly size gain of the treated groups (Fig. 6). Cuticle length (CL) gain decreased with increasing concentrations of chlorpyrifos and was significantly different from the control at the concentrations of 10, 20 and 40 mg/kg soil ($p < 0.01$ respectively).

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Fig. 6



Effects of chlorpyrifos on size. (A) size gain of CL (mm); (B) SGR of CL (mm/ days); (C) size gain of CW (mm); (D) SGR of CW (mm/days). Bars are means \pm SEM. Bars with different letters are significantly different ($p < 0.05$); One asterisks (*) indicates $p < 0.01$.

Concerning the effects of chlorpyrifos on the cuticle width (CW) gain, the results did not show any significant difference between the treated and control groups (Fig. 6).

Effects of chlorpyrifos on specific growth rate of cuticle length and cuticle width

The results showed that the specific growth rate of cuticle length was significantly lower in the groups treated with the higher chlorpyrifos concentrations (20 mg and 40 mg/kg soil) compared to the control ($p < 0.05$ respectively) (Fig. 6).

On the other hand, no significant difference in specific growth rate of cuticle width was found between the groups treated with the different concentrations of chlorpyrifos and the control (Fig. 6).

Discussion

In the present study, our results have firstly highlighted that the exposure to chlorpyrifos altered the moulting of *Armadillo officinalis*. Indeed the percentage of moulting animals during the 100 days of exposure was significantly lower at the higher chlorpyrifos concentrations (20 and 40 mg /kg) compared to the control. Several studies revealed that environmental chemicals could impair moulting in Oniscidea crustaceans (LeBlanc, 2017; Jubeaux et al., 2012; Legrand, 2016; Hosamani et al., 2017; Zou, 2020). For instance, Drobne and Strus (1996) also reported a decrease in rate of moulting in the terrestrial isopods *Porcellio scaber* exposed to zinc.

Our research showed also that chlorpyrifos delayed drastically the first moult, mainly at the highest concentration (40 mg/kg). Chlorpyrifos has been noted as the primary inhibitor of the enzyme acetylcholinesterase which leads to the accumulation of acetylcholine in the synaptic cleft, inducing the failure of translation of hormonal regulation and consequently the impairment of endocrine homeostasis in the organism (EPA, 1992; Ubaid ur Rahman et al., 2020). According to LeBlanc (2017), the disruption of the normal moult cycle by toxicant exposure can be attributed to an alteration in levels of ecdysteroid moulting hormone produced by the moulting gland Y organ. Hirano et al. (2009) documented that exposure of the mysid *Americamysis bahia* to nonylphenol delayed significantly moulting and this effect was linked to the suppression of ecdysteroid 20-hydroxyecdysone (20E) content. However, Lemos et al. (2009) who exposed the terrestrial isopods *Porcellio scaber* to the fungicide Vinclozolin noted a delay in moulting despite an increase in 20E concentration. According to the authors, this could be a result of side effects associated with hyperecdysionism.

Moreover, the number of moults completed by the terrestrial isopods exposed to the higher concentrations (20 and 40 mg/kg soil) of chlorpyrifos was significantly lower compared to the control group. The same results were also reported by Lemos et al. (2010) when exposing the terrestrial isopods *Porcellio scaber* to 100 mg/kg of Vinclozolin. Similarly, Ohmstead and Leblanc (2001) noted that the insecticide methoprene decreased moulting frequency in the crustacean *Daphnia magna* and associated this effect to the negative impact of methoprene on the terpenoid hormone Methyl farnesoate (MF) produced by the mandibular organs, which is involved in the modulation of ecdysteroids. In fact, it was demonstrated that some insecticides would mimic insect juvenile hormones and could act in the same manner as methyl farnesoate (MF) and impact crustacean moulting (Zou, 2020).

Furthermore, our findings showed a significant increase in the premoult stage and substage (PE1, PE2) durations in the groups treated with the higher chlorpyrifos concentrations (20 and 40 mg/kg). Lemos et al. (2009) reported the same results when exposing the terrestrial isopods *P.scaber* to the fungicide Vinclozolin and related the premoult prolongation to the too high level of 20E found in animals exposed to Vinclozolin. In fact, the required decrease in ecdysone concentration below the threshold value for which the exuviation factor is released, (and which is indispensable to complete the premoult), has not been attained. It seems that chlorpyrifos also can cause an elevation in the ecdysteroid titers. That was observed by Volz et al. (2003) in female Grass Shrimp *Palaemonetes pugio* exposed to 100 and 200 ng/L nominal chlorpyrifos. This enhancement in the 20E titre could be due to disruptions in ecdysteroidogenesis in Y-organs and/or changes in inhibitory peptide hormones, such as the moult-inhibiting hormone (MIH) or the crustacean hyperglycemic hormone (CHH) which are involved in the regulation of moulting (Zou, 2020). Also, this alteration could be caused by a direct disruption in epidermal ecdysteroid signaling by interfering with ecdysteroid receptor (EcR) (LeBlanc, 2017).

Regarding the effect of chlorpyrifos on ecdysis duration, a significant delay in the duration of posterior ecdysis (Ep) at the high concentration of 20 mg/kg was observed. This could be due to the adverse impact of chlorpyrifos on the ecdysis-triggering hormone (ETH) either by its direct inhibition or by interfering with its release. This neuropeptide from the central nervous system (CNS) is involved in ecdysis regulation and its lack causes ecdysis deficiencies (Mykles et al., 2010). Furthermore, according to Zou (2020), environmental toxicants could in theory impact the signalling of this neurohormone, and thereby, affect the moulting process. Lemos et al. (2009) also noted a disruption in ecdysis when exposing the terrestrial isopods *Porcellio Scaber* to vinclozolin, and explained it as being the result of the too high level of ecdysteroid and the lack of exuviation factor.

Also, our results showed that chlorpyrifos exposure significantly reduced intermoult duration compared to the control. On the opposite, [Taylor \(2020\)](#) noted a prolongation in intermoult stage when exposing the American Lobster Larvae (*Homarus americanus*) to different concentrations of chlorpyrifos. Nevertheless, our results corroborate the observations of [Montagna and Collins \(2007\)](#) who found that chlorpyrifos significantly decreases intermoult duration in *Palaemonetes argentinus*. During intermoult period, ecdysteroids are under the negative regulatory control of the moult-inhibiting hormone (MIH), which maintains low-circulating ecdysteroid levels until the next moult ([LeBlanc, 2017](#)). Chlorpyrifos exposure probably induced an inhibition of this hormone and consequently enhanced ecdysteroid that led to stimulate the next moult and reduced the intermoult stage.

Moreover, our results also highlighted that the exposure to chlorpyrifos affected significantly the growth of *Armadillo officinalis* by decreasing its weight gain and specific growth rate. These results corroborate previous studies, which reported weight loss in terrestrial isopods exposed to pesticides ([Drobne et al., 2008](#); [Kolar et al., 2010](#); [Lavtizar et al., 2016](#); [Morgado et al., 2016](#)). This leads to the hypothesis that growth retardation could be due to changes in the feeding behaviour of *Armadillo officinalis*. In fact, several previous studies highlighted a decrease in feeding behaviour in terrestrial isopods exposed to stressors ([Abdel-Lateif et al., 1998](#); [Donker et al., 1998](#); [Morgado et al., 2016](#)). Such an alteration in feeding behaviour could be due to neurotoxic effects of chlorpyrifos. Indeed, the fact that pesticides induced changes in feeding behaviour is often assimilated to acetylcholinesterase inhibition ([Bayley and Baatrup, 1996](#); [Morgado et al., 2016](#)). For instance, [Blažič et al. \(2005\)](#) noted that the pesticide-induced inhibition of feeding in *Porcellio scaber* coincides with acetylcholinesterase inhibition.

Furthermore, chlorpyrifos also impaired *Armadillo officinalis* growth by decreasing significantly its CL gain and its CL specific growth rate. Similarly, a significant decrease in CL specific growth rate was noted by [Taylor \(2020\)](#) when exposing American Lobster (*Homarus americanus*) larvae to chlorpyrifos. On the opposite, chlorpyrifos had no effect on size growth in glass shrimps (*Palaemonetes argentinus*) ([Montagna and Collins, 2007](#)). However, various synthetic molecules are also known to retard growth in animal species. For instance, Bisphenol A at a concentration of 10 and 100 mg/kg soil induced a significant decrease in terrestrial isopods *P. Scaber* size gain ([Lemos et al., 2010](#)). Also, [Singh et al. \(2021\)](#) showed that malathion exposure altered CL gain in freshwater crabs *Poppiana dentata*. These authors suggested that exposure to toxicants triggers the detoxification processes, resulting in a reallocation of energy to **Q3** these processes at the expense of growth. In this context, [Verslycke et al. \(2004\)](#) found that chlorpyrifos exposure significantly hinders the allocation of energy to growth in the mysid *Neomysis integer*.

Besides, growth disruption may also be attributed to perturbations in moulting. In fact, to grow, terrestrial isopods have to periodically shed their exoskeleton during the moulting cycle, and any disturbance of this latter, can potentially lead to the alteration of growth process. As mentioned above, chlorpyrifos exposure reduced significantly intermoult duration thereby probably preventing the completion of the new exoskeleton. Indeed, intermoult stage is the time of full activity, feeding, and muscle growth ([Steel, 1982](#); [Lemos and Weissman, 2020](#)).

In addition, growth alteration could also be related to the disruption by chlorpyrifos of some signalling pathways, like the mTOR pathway, which is involved in growth and developmental processes, and/or TGF β , which is implicated in the growth, development and innate immunity of invertebrates, including crustaceans ([Li et al., 2020](#)).

In summary, the present study demonstrated that the long-term exposure to chlorpyrifos induced harmful effects on moulting and growth of the terrestrial isopods *Armadillo officinalis*, and suggests that chlorpyrifos could also pose a potential risk to other beneficial soil organisms.

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Q4 Uncited references

[Jager et al. \(2007\)](#).


Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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 The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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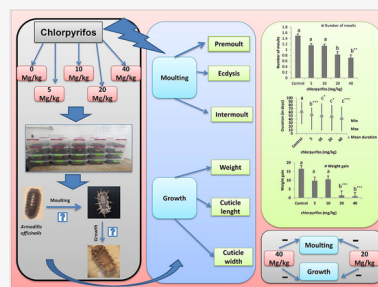
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Graphical abstract

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Highlights

- Exposure to chlorpyrifos delayed moulting in *Armadillo officinalis*.
 - Chlorpyrifos affected the moulting frequency in *Armadillo officinalis*.
 - Exposure to chlorpyrifos extended premoult and ecdysis stages in *Armadillo officinalis*.
 - Chlorpyrifos reduced intermoult duration in *Armadillo officinalis*.
 - Chlorpyrifos impaired growth by decreasing weight gain, cuticle length gain and the specific growth rate of *Armadillo officinalis*.
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Queries and Answers

Q1

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