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

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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*.

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 soi), 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.

Graphical abstract

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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, Xing et 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 (Saline, 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 Jager 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, particulary 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 (Calhôa 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 (Guimarães 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).

Section snippets

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 (21C°±1C°), 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

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)

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,

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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