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1 **Effects of long-term chlorpyrifos exposure on moulting and growth of**
2 ***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 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 (40mg/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.

Keywords: Insecticide, chlorpyrifos, moulting, growth, terrestrial isopods, *Armadillo officinalis*

65 1. Introduction

66

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

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

82 One of the most efficient approaches for assessing the effects of pesticides in the
83 environment is bio-monitoring, which involves the monitoring of bio-indicators in treated
84 versus untreated environments (Salines, 2012; Ferrante et al., 2015). In this context, several
85 studies have investigated the toxic impacts of chlorpyrifos using bio-indicators such as
86 terrestrial invertebrates (Gatti et al., 2002; Venkateswara et al., 2003; Fountain et al., 2007;
87 Jagar et al., 2007; Wang et al., 2012; Pelosi et al., 2014; Muangphara, 2015; Dolar et al.,
88 2021) and numerous effects have been reported. For instance, chlorpyrifos impairs growth
89 (Booth and O'Halloran, 2001) and fecundity (Alshawish et al. 2004; Zhou et al., 2007) in
90 earthworms, and reduces springtail abundance (Endlweber et al., 2006; Fountain et al., 2007).
91 Furthermore, it induces lethality (Nair et al., 2022; Morgado et al., 2016; Benmouhoub et al.,
92 2019), oxydative stress (Morgado et al., 2018) and changes in immune function (Dolar et al.,
93 2021) in terrestrials isopods.

94

95 Terrestrial isopods are widely used as bioindicators of environmental pollution due to
96 their sensitivity to the effects of anthropogenic activities (Longo et al., 2013; Mazzei et al.,
97 2013; Agodi et al., 2015). They also represent an abundant and widespread component of the
98 soil fauna (Caruso et al., 1987; Messina et al., 2011; Messina et al., 2012). The species
99 *Armadillo officinalis* has been reported as a good test-organism to assess soil contamination or
100 other environmental changes in its habitat (Agodi et al., 2015; Montesanto and Cividini,
101 2017).

102 Terrestrial isopods moult frequently throughout their life cycle in order to grow. So,
103 they shed their old cuticle and develop a new exoskeleton (Lefebvre, 2002). Moulting in
104 Oniscidea is regulated by a variety of neuroendocrine signalling cascades which involve
105 various hormones such as the ecdysteroid moulting hormones, the moult-inhibiting hormone
106 (MIH), and the crustacean hyperglycemic hormone (CHH) (Leblanc, 2007; Zou, 2020).
107 Several authors have recently shown that these endocrine pathways are highly sensitive to
108 aberrant signalling by environmental chemicals (Leblanc, 2007; Jubeaux, 2012; Legrand,
109 2016; Hosamani et al., 2017; Zou, 2020).

110 Numerous studies have highlighted the effects of pesticides on moulting (Baldwin et al.,
111 2001; Lemos et al., 2009; Palma et al., 2009; Lemos et al., 2010) and growth (Montagna and
112 Collins, 2007; Lemos, 2010; Singh et al., 2021) in Oniscidea crustacean. However, although
113 there have been some researches on the consequences of chlorpyrifos exposure (Volz et al.,
114 2003; Montagna and Collins, 2007; Taylor, 2020), to our knowledge, there is no data about
115 the impacts of chlorpyrifos on moulting in terrestrial isopods.

116 In ecotoxicological risk assessment of pesticides, particularly in isopods bioassay, most
117 studies use acute toxicity tests (Benmouhoub et al., 2019; Santos et al., 2010; Loureiro et al.,
118 2009; Kolar et al., 2008). Although these assays are effective for survival parameters (Calh a
119 et al., 2012; Santos et al., 2010) or avoidance behaviour parameters (Loureiro et al., 2002),
120 they are limited when more detailed and comprehensive effects of the toxicant on life stage
121 such as moulting and growth of the animal are aimed. In this case, long term exposure tests
122 are advisable as they could cover all the life stage of the animal and track the impacts of
123 pesticides over the time (Guimar es et al., 2023).

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

128 2. Materials and methods

129 2.1. Sampling and breeding

130 Adult males and females terrestrial isopods of the species *Armadillo officinalis* were
131 collected in unpolluted natural area of the National Institute of Agricultural Research, Algeria
132 (INRA) (latitude 36°21'8 N; longitude 3°53'E). Then, they were bred in the laboratory of
133 Applied Zoology and Animal Ecophysiology, Bejaia, Algeria, at room temperature
134 (21C°±1C°), with a photoperiod of 13h/11h (light/dark). Twice a week, the colonies were
135 sprayed with water and fed with potato tubers.

136

137 2.2. Chemical compound and soil

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

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

143

144 2.3. Experimental set up

145 The toxicity test was conducted according to a modified version of the guideline for
146 earthworm's sub-lethal test established by the Organization for Economic Co-operation and
147 Development (OECD) (OECD, 2015). The selection of chlorpyrifos concentrations for this
148 experiment was based on a preliminary assay where an acute toxicity test on *Armadillo*
149 *officinalis* species was carried out in order to determine the lethal concentration 50 (LC₅₀) for

150 chlorpyrifos. After 14 days of exposure, the result showed that the LC_{50} of chlorpyrifos was
151 80.5 mg/kg. Thus, for the long-term exposure, chlorpyrifos was diluted in water to the
152 following concentrations: 5, 10, 20 and 40 mg/kg soil (dry weight), which were about 1/16,
153 1/8, 1/4, 1/2 of $LC_{50-14days}$ respectively. Pure Water was used as a control. Four replicates of
154 each concentration and the control were prepared. Toxicity tests were performed in twenty
155 transparent plastic boxes with perforated lids (30 cm x 20 cm x 10 cm), containing 250 g of
156 moist soil. Each solution was sprayed on the soil of its respective box, before introducing 10
157 woodlice (5 males and 5 females) in intermoult phase with an average weight of 150 ± 7.6 mg
158 per box. Woodlice were fed with potato tubers twice a week. Soil was kept moist during all
159 the experiment by spraying water when necessary. Woodlice were exposed to the chemical
160 for 100 days, during which moult monitoring and growth were also performed.

161

162 2.4. Moulting monitoring

163

164 To avoid the parturial moulting, and focus only on the growth moulting, the experiment
165 was carried out outside the reproductive period of *Armadillo officinalis* that is from October
166 to January. Moulting monitoring was launched along with the organisms exposure, and for
167 100 days, woodlice were systematically examined every 24hr. In fact, the observations were
168 based on the appearance, size, and shape of white calcium sternal deposits on the first four
169 pereon. This way, premoult phase (proecdysis) and its three main subphases (named PE1,
170 PE2, PE3, as in Zidar et al., 1998) as well as the biphasic ecdysis and its subphases (Ea and
171 Ep) were recorded and their duration calculated. In addition, intermoult period was calculated
172 as a the duration between the end of the first ecdysis to the end of the experiment for the
173 woodlice that moult only once, and from the end of the first ecdysis to the beginning of the
174 second premoult for the woodlice that moulted twice.

175

176 2.5. Growth monitoring

177

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

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

184 The specific growth rate (SGR) of size and weight were calculated using the following
185 formula (as in Romano and Zeng, 2016): $SGR = (\ln W_f - \ln W_0) / IP \times 100$, where SGR is
186 specific growth rate; W_f is the final size or weight; IP is the intermoult period; W_0 is the initial
187 size or weight.

188

189 2.6. Statistical analysis

190

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

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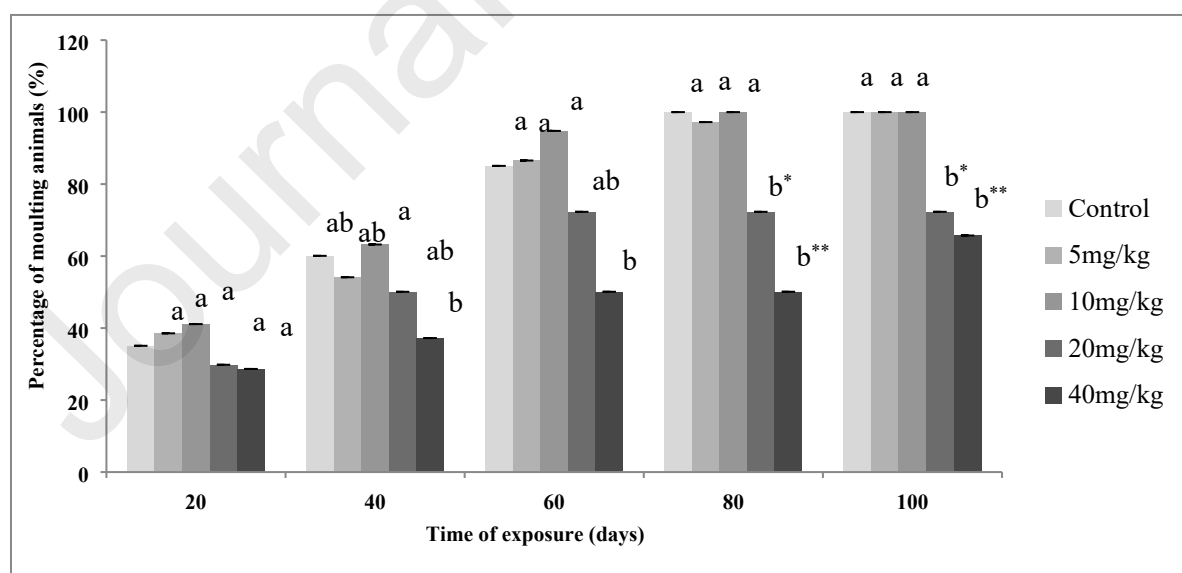
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3. Results

3.1. 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 (40mg/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 40mg/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 $p < 0.001$.

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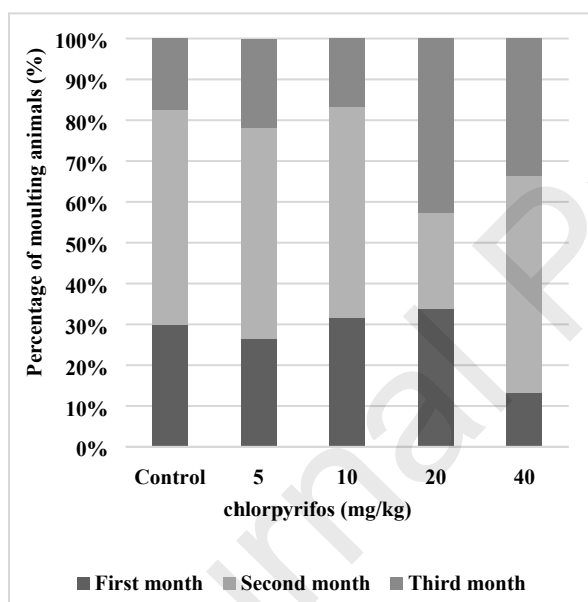
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247 3.2. Distribution of the first moult per month of animals exposed to chlorpyrifos during 248 100 days

249 In the control group, 82.5% of animals had moulted after two months of experiment,
250 of which 30% in the first month and 52.5% in the second month (Fig. 2). Similar results were
251 found in the groups exposed to the 5 and 10 mg/kg soil concentrations, with 77.67% and
252 80.77% of moulted animals after two months, respectively. In the group exposed to 20 mg/kg
253 soil, only 41% of animals had moulted after two months of exposure, of which 24.32% the
254 first month and 16.67% the second month. In the group treated with the highest concentration
255 (40 mg/kg soil), moult seemed to be drastically delayed, with only 8.57% of animals that had
256 moulted in the first month and 34.38% in the second month.

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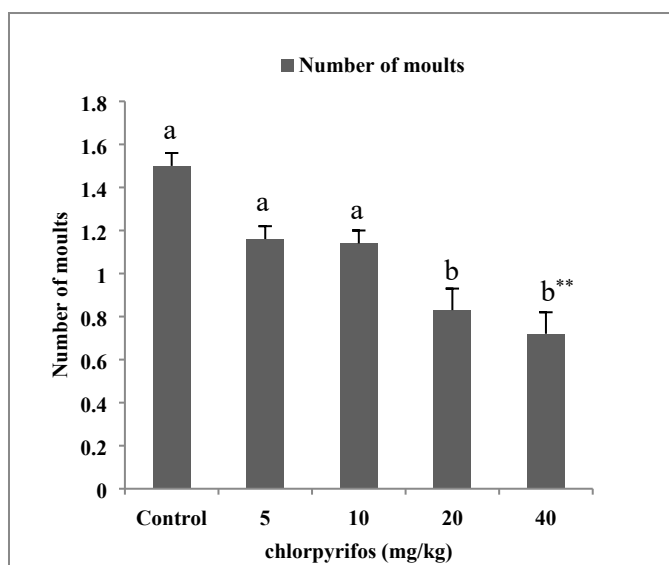
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259 **Fig.2.** Distribution of the first moult per month of animals exposed to chlorpyrifos. Data are presented as
260 percentage of animals that moulted during each period.

261

262 3.3. Number of moults completed by animals during 100 days of exposure to 263 chlorpyrifos

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



268

269 **Fig.3.** Mean number of moults completed by animals during 100 days of exposure to chlorpyrifos. Bars are
 270 means \pm SEM. Bars with different letters are significantly different ($p < 0.05$); two asterisks (**) indicate $p <$
 271 0.001.

272

273 3. 4. Effects of chlorpyrifos on the premoult duration (in days) and its substage duration 274 (in days)

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

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

289

290

291

292 Table 1

293 Effects of chlorpyrifos on the duration (in days) of the first premoult substages (mean \pm SEM). Within a same
 294 column, values with different letters are significantly different ($P < 0.05$); one asterisk (*) indicates $p <$
 295 0.01; two asterisks (**) indicate $p < 0.001$.

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

3.5. Effects of chlorpyrifos on the duration of ecdysis and its substages

E

cdysis duration was not significantly affected by the chlorpyrifos treatments (Tab. 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

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 ($p < 0.05$).

331

	Ecdysis		
		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

3.6. Effects of chlorpyrifos on intermolt duration

The results showed that chlorpyrifos reduced significantly the intermolt 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).

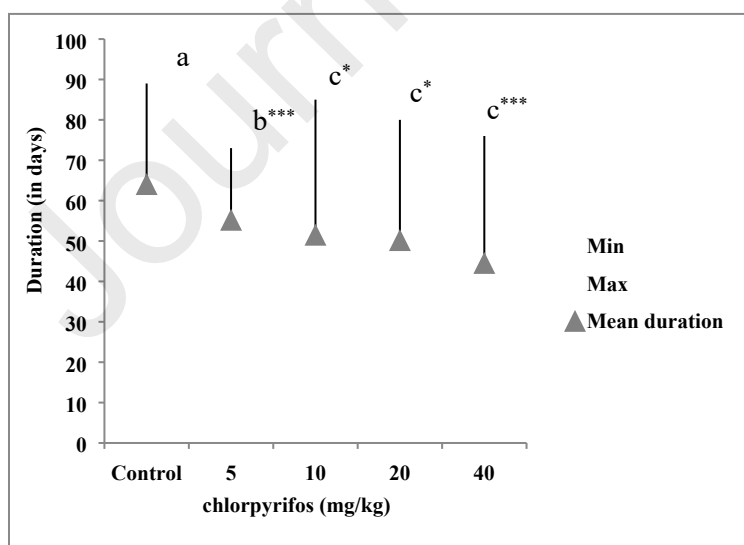


Fig. 4. Mean duration (in days) of intermolt (mean ± SEM) of all animals exposed to chlorpyrifos during 100 days. Values with different letters are significantly different ($p < 0.05$); one asterisk (*) indicates $p < 0.01$; three asterisks (***) indicate $p < 0.0001$.

357

358 **3.7. Effects of chlorpyrifos on growth**359 **3.7.1. Effects of chlorpyrifos on weight gain**

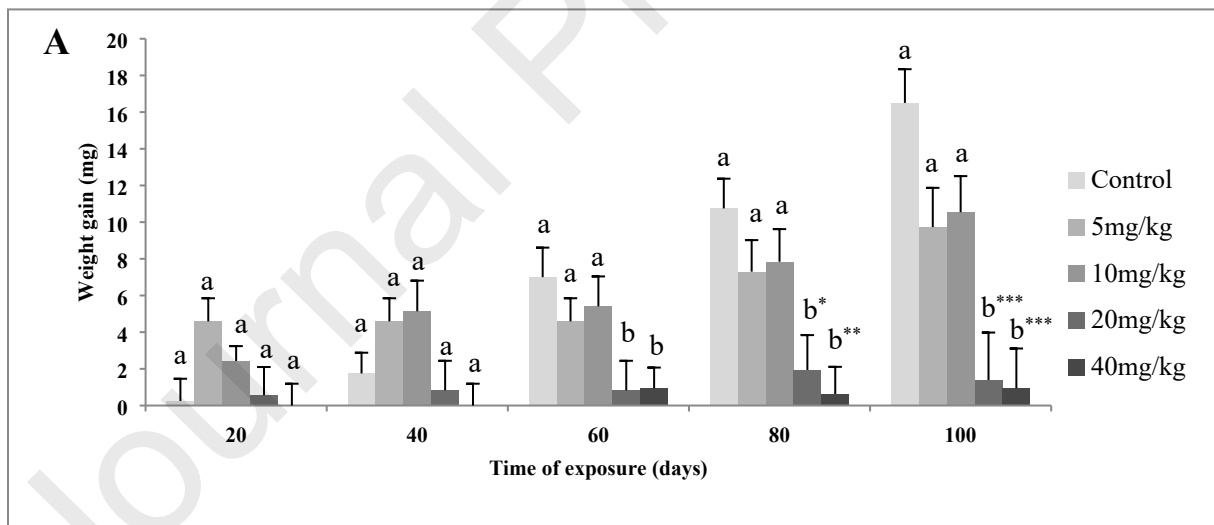
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361 After 20 and 40 days of experiment, no significant difference was noted between the
 362 groups treated with different concentrations of chlorpyrifos and the control (Fig.5). A
 363 significant difference was found at the higher concentrations of chlorpyrifos (20 and 40 mg/kg
 364 soil) after 60 days ($p < 0.05$ respectively) and 80 days of experiment ($p < 0.01$ and $p < 0.001$
 365 respectively). After 100 days of exposure to chlorpyrifos, the results did not show any
 366 significant difference between the control and treatment with the lower concentrations (5 and
 367 10 mg/kg soil). However, chlorpyrifos impaired significantly the weight gain of animals
 368 exposed to the higher concentrations of chlorpyrifos (20 and 40 mg/kg soil) ($p < 0.0001$
 369 respectively).

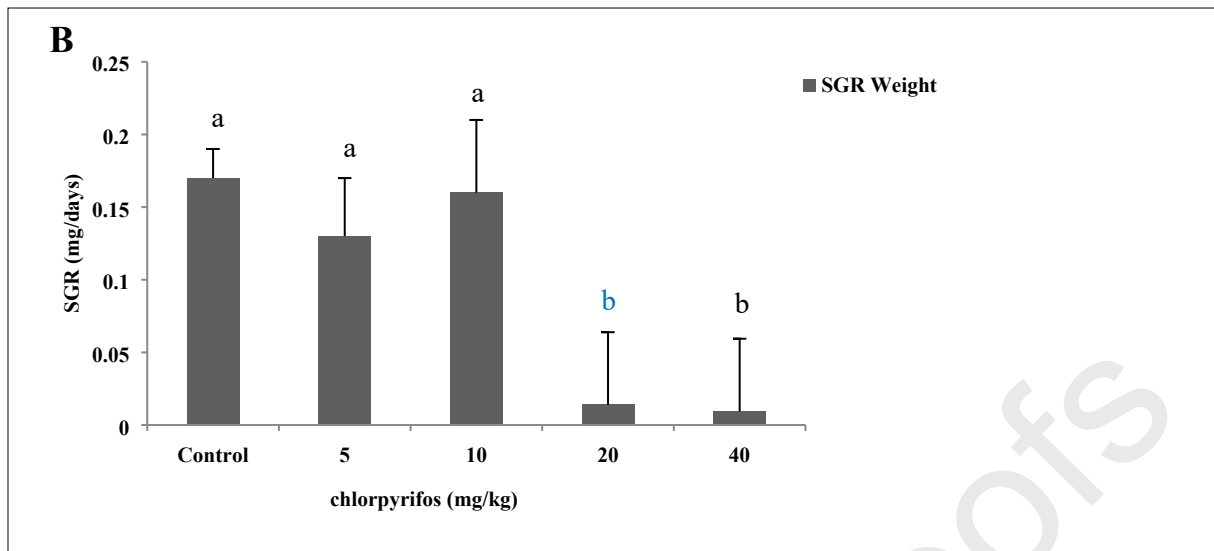
370 **3.7.2. Effects of chlorpyrifos on weight's specific growth rate**

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

375



376



377

378 **Fig. 5.** Effects of chlorpyrifos on weight. (A) weight gain (mg); (B) SGR of weight (mg/days). Bars are means \pm
 379 SEM. Bars with different letters are significantly different ($p < 0.05$); three asterisks indicate $p < 0.0001$.

380

381 3.7.3. Effects of chlorpyrifos on size gain

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

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

388

389 3.7.4. Effects of chlorpyrifos on specific growth rate of cuticle length and cuticle width

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

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

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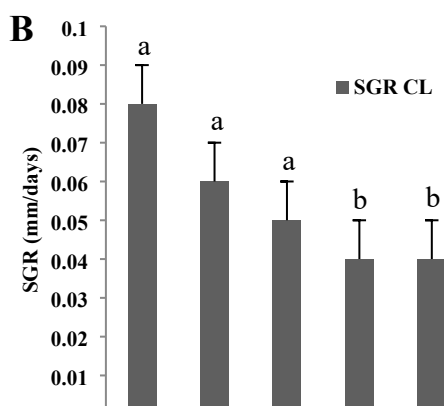
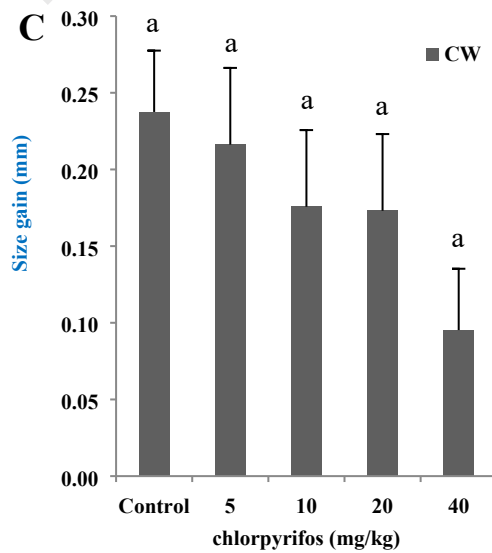
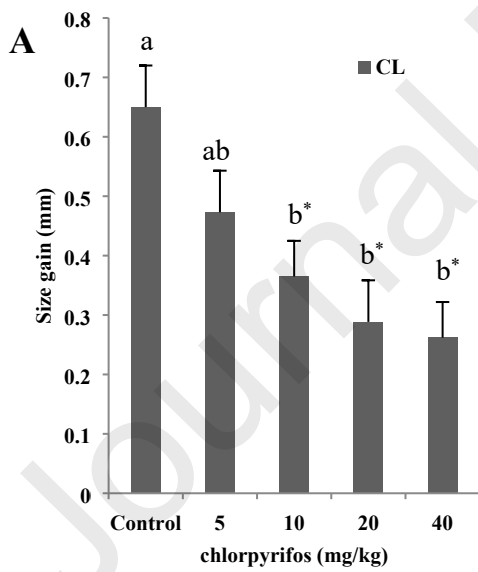
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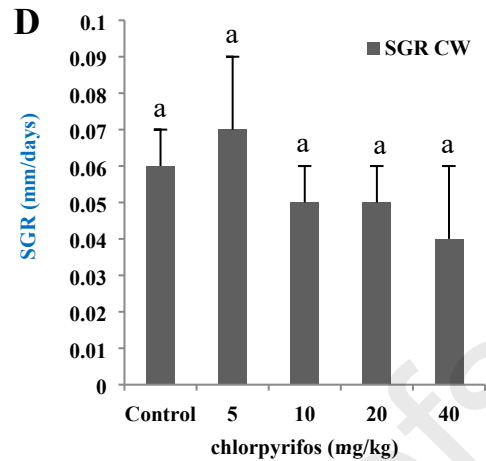
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412 **Fig. 6.** Effects of chlorpyrifos on size. (A) size gain of CL (mm) ; (B) SGR of CL (mm/ days) ; (C) size gain of
 413 CW (mm) ; (D) SGR of CW (mm/days). Bars are means \pm SEM. Bars with different letters are significantly
 414 different ($p < 0.05$); One asterisks (*) indicates $p < 0.01$.

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418 4. Discussion

419

420 In the present study, our results have firstly highlighted that the exposure to chlorpyrifos
 421 altered **the moulting** of *Armadillo officinalis*. Indeed the percentage of moulting animals
 422 during the 100 days of exposure was significantly lower at the higher chlorpyrifos
 423 concentrations (20 and 40 mg/kg) compared to the control. **Several studies revealed that**
 424 **environmental chemicals could impair moulting in Oniscidea crustaceans** (Leblanc, 2007;
 425 Jubeaux, 2012; Legrand, 2016; Hosamani et al., 2017; Zou, 2020). For instance, Drobne and
 426 Strus (1996) also reported a decrease in rate of moulting in the terrestrial isopods *Porcellio*
 427 *scaber* exposed to zinc.

428

429 Our research showed also that chlorpyrifos delayed drastically the first moult, mainly
 430 at the highest concentration (40 mg/kg). Chlorpyrifos has been noted as the primary inhibitor
 431 of the enzyme acetylcholinesterase which leads to the accumulation of acetylcholine in the
 432 synaptic cleft, inducing the failure of translation of hormonal regulation and consequently the
 433 impairment of endocrine homeostasis in the organism (EPA, 1992; Ubaidurrahman, 2020).
 434 According to Leblanc (2007), the disruption of the normal moult cycle by toxicant exposure
 435 can be attributed to an alteration in levels of ecdysteroid moulting hormone produced by the
 436 moulting gland Y organ. Hirano et al. (2009) documented that exposure of the mysid
 437 *Americamysis bahia* to nonylphenol delayed significantly moulting and this effect was linked

438 to the suppression of ecdysteroid 20-hydroxyecdysone (20E) content. However, Lemos et al.
439 (2009) who exposed the terrestrial isopods *Porcellio scaber* to the fungicide Vinclozolin
440 noted a delay in moulting despite an increase in 20E concentration. According to the authors,
441 this could be a result of side effects associated with hyperecdysonism.

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

452

453 Furthermore, our findings showed a significant increase in the premoult stage and
454 substage (PE1, PE2) durations in the groups treated with the higher chlorpyrifos
455 concentrations (20 and 40 mg/kg). Lemos et al. (2009) reported the same results when
456 exposing the terrestrial isopods *P. scaber* to the fungicide Vinclozolin and related the premoult
457 prolongation to the too high level of 20E found in animals exposed to Vinclozolin. In fact, the
458 required decrease in ecdysone concentration below the threshold value for which the
459 exuviation factor is released, (and which is indispensable to complete the premoult), has not
460 been attained. It seems that chlorpyrifos also can cause an elevation in the ecdysteroid titers.
461 That was observed by Volz et al. (2003) in female Grass Shrimp *Palaemonetes pugio* exposed
462 to 100 and 200 ng/L nominal chlorpyrifos. This enhancement in the 20E titre could be due to
463 disruptions in ecdysteroidogenesis in Y-organs and/or changes in inhibitory peptide
464 hormones, such as the moult-inhibiting hormone (MIH) or the crustacean hyperglycemic
465 hormone (CHH) which are involved in the regulation of moulting (Zou, 2020). Also, this
466 alteration could be caused by a direct disruption in epidermal ecdysteroid signaling by
467 interfering with ecdysteroid receptor (EcR) (Leblanc, 2007).

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

478

479 Also, our results showed that chlorpyrifos exposure significantly reduced intermoult
480 duration compared to the control. On the opposite, Taylor (2020) noted a prolongation in
481 intermoult stage when exposing the American Lobster Larvae (*Homarus americanus*) to
482 different concentrations of chlorpyrifos. Nevertheless, our results corroborate the observations

483 of Montagna and Collins (2007) who found that chlorpyrifos significantly decreases
484 intermoult duration in *Palaemonetes argentinus*. During intermoult period, ecdysteroids are
485 under the negative regulatory control of the moult-inhibiting hormone (MIH), which
486 maintains low-circulating ecdysteroid levels until the next moult (Leblanc, 2007).
487 Chlorpyrifos exposure probably induced an inhibition of this hormone and consequently
488 enhanced ecdysteroid that led to stimulate the next moult and reduced the intermoult stage.

489

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

503

504 Furthermore, chlorpyrifos also impaired *Armadillo officinalis* growth by decreasing
505 significantly its CL gain and its CL specific growth rate. Similarly, a significant decrease in
506 CL specific growth rate was noted by Taylor (2020) when exposing American Lobster
507 (*Homarus americanus*) larvae to chlorpyrifos. On the opposite, chlorpyrifos had no effect on
508 size growth in glass shrimps (*Palaemonetes argentinus*) (Montagna and Collins, 2007).
509 However, various synthetic molecules are also known to retard growth in animal species. For
510 instance, Bisphenol A at a concentration of 10 and 100 mg/kg soil induced a significant
511 decrease in terrestrial isopods *P. Scaber* size gain (Lemos et al., 2010). Also, Singh et al.
512 (2021) showed that malathion exposure altered CL gain in freshwater crabs *Poppiana dentata*.
513 These authors suggested that exposure to toxicants triggers the detoxification processes,
514 resulting in a reallocation of energy to these processes at the expense of growth. In this
515 context, Verslycke et al. (2004) found that chlorpyrifos exposure significantly hinders the
516 allocation of energy to growth in the mysid *Neomysis integer*.

517 Besides, growth disruption may also be attributed to perturbations in moulting. In fact,
518 to grow, terrestrial isopods have to periodically shed their exoskeleton during the moulting
519 cycle, and any disturbance of this latter, can potentially lead to the alteration of growth
520 process. As mentioned above, chlorpyrifos exposure reduced significantly intermoult duration
521 thereby probably preventing the completion of the new exoskeleton. Indeed, intermoult stage
522 is the time of full activity, feeding, and muscle growth (Steel, 1982; Lemos and Weissman,
523 2020).

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

528

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

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553

554 **Declaration of interest**

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557

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841 **Highlights**

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- Exposure to chlorpyrifos delayed moulting in *Armadillo officinalis*.

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- 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*.