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RESEARCH ARTICLE

Biological and physiological responses of *Perionyx excavatus* to abamectin

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Abstract

Biological and behavioral responses of the tropical earthworm *Perionyx excavatus* towards different concentrations of abamectin were evaluated. Abamectin significantly reduced the biomass and reproduction (cocoon production) of *P. excavatus* as well as inducing histopathological alterations in the cuticle. Biomass loss was recorded in *P. excavatus* exposed to abamectin at a concentration as low as 0.1 mg active ingredient (a.i.) kg^{-1}, while atrophy, another physiological response, was observed at an abamectin concentration of 0.21 μg cm^{-2} in a filter paper test. Cocoon production was significantly reduced in the presence of abamectin, and no cocoons were produced at doses of 20 mg a.i. kg^{-1} or higher, while abamectin at 50 mg a.i. kg^{-1} induced extreme pathology, characterized by the loss of the integrity of the whole body wall and intestine of *P. excavatus*. Histopathological alterations can be used as a biomarker to evaluate the toxicological impact of exposure to abamectin.

Keywords Abamectin · *Perionyx excavatus* · Physiological morphology · Cocoon production · Histopathology

Introduction

Agriculture remains an important sector of Malaysia's economy, contributing 12% to the national gross domestic product (GDP) and providing employment for 16% of the population. In 2016, about 4.06 million ha of land was used for agriculture, of which vegetable plantations accounted for about 44,000 ha (1.08%). In terms of the land area used for cultivation, the top five vegetable crops and fruit plants were leaf mustard, round cabbage, chili, long bean, and tomatoes (MAMPU open data 2016).

However, during growth, these top five vegetable crops and fruit plants are easily damaged by many insects and diseases, such as the diamondback moth (*Plutella xylostella*), various leafminers (mostly Agromyzidae flies and Lepidoptera), two-spotted spider mite (*Tetranychus urticae*), tomato russet mite (*Aculops lycopersici*), armyworms (*Spodoptera* sp.), and tomato pinworms (*Keiferia lycopersicella*). The first record of *P. xylostella* in Malaysia was reported in 1925 and it became a serious pest of crucifers in this region after the mid-1940s (Corbett and Pagden 1941). In order to intensify agriculture practices, typically as monocultures, and overcome the subsequent pest invasions, different pesticides have been applied to control the outbreaks, including chlorpyrifos, organophosphates, pyrethroid, and abamectin (also known as avermectin). Among all the pesticides used, abamectin has been the most effective pesticide at controlling these pest outbreaks (Dybas 1989).

Abamectin is a mixture of 80% avermectin B1a and less than 20% avermectin B1b that is produced by the soil bacterium *Streptomyces avermitilis*. Research has shown that abamectin and avermectin have very similar biological and toxicological properties (Campbell 1989; Halley et al. 1993). Prior to use as a pesticide in agriculture farms, abamectin was used for the treatment and control of gastrointestinal nematodes as well as to control endo- and ecto-parasite infections in small ruminants (McKeller 1997; Chandrawathani and Nurul Aini 2012; Epe and Kaminsky 2013). Currently, abamectin is registered (RI. 01010119921065) for use on vegetable crops at a recommended application dose of 5–27 g ha^{-1} as a foliar spray (Lasota and Dybas 1990). However, most farmers exceed the recommended dosage and/or spray more frequently

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(typically as calendar spraying without prior scouting) in an attempt to ensure its effectiveness. In the calendar spraying method, farmers will apply pesticides at set interval days to control the pest outbreak, and without prior scouting means even though there is no sign of any pest damage, farmers still followed the scheduled plan to apply the pesticide in order to avoid any perceived possibility of lowering the product's price due to pest damage (Amoako et al. 2012; Halimatunsadiah et al. 2016). These actions have indirectly caused potential harm to soil organisms, and predominantly to the earthworms, resulting in a loss of biodiversity (Hole et al. 2005).

Earthworms are important soil organisms and are frequently used as indicators of soil quality as well as contamination levels. Their biological changes (biomarker of effect) and physiological changes are relatively immediate responses when they are exposed to contaminant (Lam and Gray 2003). These biological and physiological changes can be used as biomarkers of adverse conditions, and their use has been reported as early warning tools for the measurement of biological effects. Lourenço et al. (2011) reported the biological changes (histological alterations in tissue) in the earthworm *Eisenia andrei* after exposure to metals and radionuclides. Likewise the histopathology of *Eisenia fetida* changed after exposure to imidacloprid (Dittbrenner et al. 2011) as did the tropical earthworm *Eudrilus eugeniae* when exposed to heavy metals (Fernando et al. 2015).

Perionyx excavatus, a widely cultured compost earthworm in tropical regions, has been shown to be suitable as a test species for tropical soils (Maboeta et al. 1999; An and Lee 2008; De Silva et al. 2010). It was recently reported that *P. excavatus* is more sensitive than the temperate species (*Eisenia* species) to three of the commonly used pesticides: chlorpyrifos, carbofuran, and mancozeb (De Silva et al. 2010). Although a few reports have examined the toxicity of abamectin, they have focused on temperate earthworm species (Diao et al. 2007; Jensen et al. 2007; Kolar et al. 2008). Keeping in view none of them reported the histopathology and physiological changes in *P. excavatus* associated with exposure to abamectin. Here, we aim to study the effect of abamectin towards cellular responses by evaluating the changes in the histopathology, biomass, behaviour response, and cocoon production in *P. excavatus* at different concentrations and exposure times. With respect to the responses of *P. excavatus* to abamectin, the responses can be a tool in monitoring field populations where the assessment of conventional endpoints is difficult to be applied.

Materials and methods

Test organism

Adult *P. excavatus* of 150–300 mg (wet weight) with a well-developed clitellum were obtained from an in-house culture at the Sakorn Nakhon Rajabhat University, Thailand. The earthworms were acclimatized in stock soil (see below) for at least a week and then a day in a wet paper towel without soil to void their guts before conducting the experiment.

Test soil and substance

Stock soils were prepared from 10% (*w*/*w*) fermented *Terminalia catappa* leaves, 3% (*w*/*w*) fermented cow dung (fermented for at least 2 weeks), 2% (*w*/*w*) peat moss, and 85% (*w*/*w*) soil with the stock soil moisture content adjusted to about 50% of the maximum water holding capacity. The moisture content was measured using a moisture meter (DM400, Guangdong, China). The soil preparations were mixed by hand and sieved following preparation before the water was added (mesh size, 2 mm). Standard toxicity test methods were only applied in the preparation of the test soil but not the test soil composition, which was mainly due to the relatively high costs of the standard test methods. Commercial grade abamectin [1.8% (*w*/*v*) EC] was purchased from Mitsomboon Chemical (Thailand) Co. Ltd.. Stock solutions of abamectin were diluted with distilled water to the required nominal concentrations.

Laboratory exposure

Filter paper test

Filter paper test (modified OECD Test 207) was conducted in a round, flat-bottomed (internal diameter 60 mm, height 30 mm, volume 65 mL) container lined with filter paper. Two milliliters of the prepared test concentration (0.0 (control), 0.3, 3, 30, 150, 300, 1500, and 3000 mg L^{-1}) of abamectin solution was added to the filter paper of each test unit to yield concentrations in the filter paper of 0 (control), 0.021, 0.21, 2.12, 10.6, 21.2, 106.1, and 212 μg cm^{-2}, respectively. Each treatment was performed with ten replicates, each consisting of one worm per container and maintained at ambient temperature (30 ± 2 °C). The morphology and behavior of *P. excavatus* were monitored over the first 10 min after exposure to abamectin, and then, the test containers were maintained at ambient temperature in the dark for 48 h.

Abamectin-treated soil test

All earthworms were exposed for 2, 7, and 14 days to soil pretreated with abamectin at different concentrations. Rectangular plastic containers (internal diameter 90 mm, length 145 mm) were filled with 500 g of the corresponding artificially abamectin-contaminated soil (0.1, 1.0, 10, 50, 100, 500, and 1000 mg active ingredient, (a.i.) kg^{-1} soil). Ten earthworms were released into each container with four replicates of each treatment. The tests were conducted at ambient

temperature (30 ± 2 °C) under a constant light intensity of 400–800 lx and a 16/8 h light/dark cycle.

A similar working protocol was applied to the reproduction test, where the earthworms were exposed to abamectin at a concentration of 0.0 (control), 0.1, 1.0, 10, 20, 30, 40, and 50 mg a.i. kg^{-1} soil and control soil (without abamectin) for 28 days, except finely ground cow dung (30 g) was added each week to each container as food.

Body mass measurements

Before and after exposure, the earthworms were rinsed with tap water, gently dried with a paper towel, and weighed to give the wet weight. Every 7 days up to 28 days, the biomass of all worms in each container was determined. The change in biomass of *P. excavatus* was expressed as the percentage loss according to formula: $100 - (Wx * 100)/Wo$, where *Wo* is the mean weight of the earthworms of each replicate at the beginning of the experiment and *Wx* is the mean weight of the earthworms of each replicate after x d of exposure. A similar experimental design was applied to the filter paper test, except the exposure time was 48 h.

Cocoon production

After 28 days of exposure, the living worms were counted, weighed, and then placed back in the same containers and observed for cocoon produced. After another 28 days, the produced cocoons were collected, counted, and placed on moist filter paper to determine the hatching rate. The newly hatched juveniles were carefully hand-sorted and counted.

Analysis of histological alterations

After exposure to abamectin at different concentrations, earthworms were anesthetized, killed, cut at the first 20 segments (segments 1–20, numbering from anterior), and then fixed in 10% (*w*/*v*) formalin and embedded into paraffin. The 20 segment block was then cut at 5-μm-thick longitudinal sections and stained with Harris Hematoxylin and Eosin stain (H&E) as well as a combined trichrome stain, Masson and fast green staining procedure (López-De León and Rojkind 1985). The stained tissue sections were then observed under a light microscope (Olympus CX41RF) equipped with Image Pro Express 4.0.1 imaging software.

Data analysis

Data from soil and filter paper tests were tested for normality and homoscedasticity and percentage data were arcsine transformed. The data were then analyzed by a one-way ANOVA and post hoc comparisons were conducted using Tukey's HSD. All statistical analyses were performed using the Minitab 16 Statistical software and significance was accepted at the $P < 0.05$ level.

Results and discussion

Filter paper test: behavioral monitoring and body mass measurements

The morphology and behavior of *P. excavatus* changed significantly within 48 h of exposure to abamectin (Fig. 1). When exposed to the two highest abamectin concentrations (106.1 and 212 μg cm^{-2}), *P. excavatus* reacted with intensive writhing and jumping, immediately exuded a pungent yellowish liquid (exuded coelomic fluid; ECF) posteriorly, and exhibited a spontaneous twitch-like movement of the whole body. Constrictions of the posterior end of the body were followed by sluggish movement within 6 min of exposure. By 8 min of exposure, the posterior end segments of the worms were nearly completely paralyzed and became rigid and brittle, with slow motion jerky movements only occurring at the anterior end. Finally, 100% mortality was observed within the first 10 min of exposure. These observations serve as an important criterion in deciding the optimum toxicity effect of this tropical earthworm for monitoring the use of abamectin as a pesticide.

At abamectin concentrations of 106.1 and 212 μg cm^{-2}, *P. excavatus* showed almost complete paralysis and then died within 10 min of exposure (Fig. 1g and h). Total mortality was observed in *P. excavatus* after a 24-h exposure to 21.2 and 10.6 μg cm^{-2} (Fig. 1e and f) and 48-h exposure to 2.12 and 0.21 μg cm^{-2} (Fig. 1c and d), respectively. At concentrations higher than 0.21 μg cm^{-2}, *P. excavatus* released excessive amounts of ECF, bleeding, and erosion leading to total body wall damage. After a 48-h exposure to abamectin at 0.21 μg cm^{-2}, some of the *P. excavatus* started to coil themselves. No mortality was observed after 48 h of exposure to abamectin at a concentration of 0.021 μg cm^{-2} (Fig. 1b). At abamectin concentrations below 21.2 μg cm^{-2}, the motile *P. excavatus* were observed trying to escape from the container including the control group (Fig. 1a).

Besides the behavioral changes, the presence of abamectin also induced a body mass reduction over the 48-h exposure period (Table 1). Compared to the control worms, the two lowest concentrations of abamectin (< 0.21 μg cm^{-2}) resulted in numerically lighter worms, but these were not significantly different to that in the control group ($P > 0.05$). However, the highest body mass loss in living worms was observed at an abamectin concentration of 2.12 μg cm^{-2}, which was a significantly larger loss compared to the control group. It was likely that even higher and significant body mass losses occurred at abamectin concentrations of 21.2 μg cm^{-2} and higher, but they could not be measured due to the extremely large volume of excluded ECF from the earthworms that stuck them tightly to the filter paper.

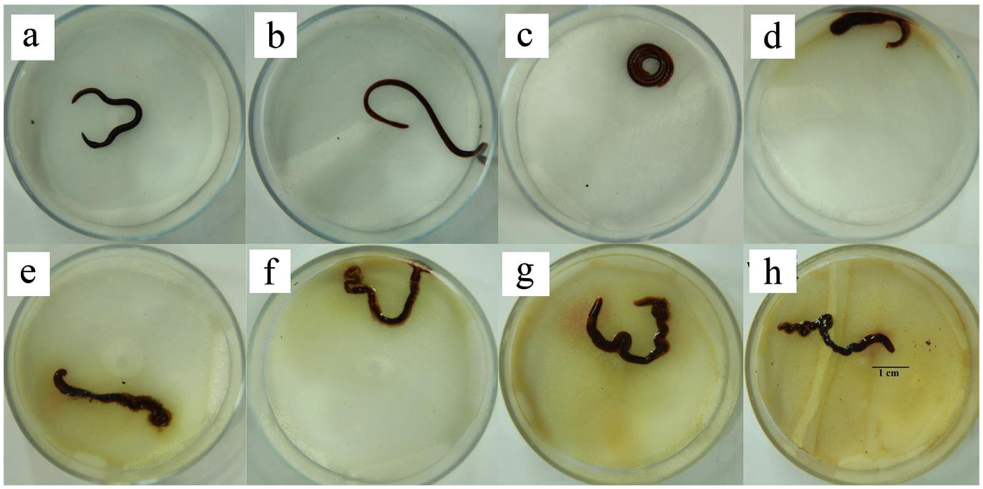

Fig. 1 Representative morphological changes shown by *P. excavatus* after a 48-h exposure to abamectin on filter paper at concentrations of **a** 0 (control), **b** 0.021, **c** 0.21, **d** 2.12, **e** 10.6, **f** 21.2, **g** 106.1, and **h** 212 μg cm^{-2}

Physiological adaptations are life history traits that are linked to how earthworms regulate their metabolism and behavior upon exposure to toxins, in this case to abamectin. In this study, on skin contact with abamectin, *P. excavatus* exuded ECF, which then acted as a protective layer and reduced the direct contact surface area to abamectin. In addition to the excretion of ECF, aestivation (lack of movement) and coiling were other responses observed in *P. excavatus* upon exposure to abamectin. Morphological changes, body mass reduction, and ECF secretion in earthworms are the responses most frequently observed and reported as acute signs of a toxicological effect (Dittbrenner et al. 2010; Katagi and Ose 2015).

Abamectin-treated soil test: behavioral responses, body mass changes, and mortality levels

A similar behavior by *P. excavatus* was observed in the 48-h soil exposure test. At the highest tested abamectin concentrations (500 and 1000 mg a.i. kg^{-1}), *P. excavatus* reacted with intensive writhing and jumping followed by 100% mortality within the first 30 min of exposure. Although no obvious behavioral response was observed in *P. excavatus* when exposed to 100 mg a.i. kg^{-1} and lower concentrations of abamectin, 100% and 90% mortality was observed after exposure for 24 h at 100 mg a.i. kg^{-1} and 48 h at 50 mg a.i. kg^{-1}, respectively. No mortality was observed with lower abamectin concentrations (0.1–10 mg a.i. kg^{-1}) or the control group in the first 48 h of exposure.

Table 1 Biomass loss and mortality rate of *P. excavatus* after a 48-h exposure to abamectin

Abamectin concentration (mg L^{-1})	Abamectin concentration (μg cm^{-2})	Biomass loss (%)	Mortality rate
0.0 (control)	0.0 (control)	10.77 ± 5.86^{b}	0.00
0.3	0.021	12.11 ± 4.33^{b}	10.00
3.0	0.21	15.22 ± 5.70^{b}	30.00
30	2.12	21.39 ± 11.73^{a}	97.50
150	10.6	nil*	100.00
300	21.2	nil*	100.00
1500	106.1	nil*	100.00
3000	212	nil*	100.00

Derived from the actual concentration (x mg L^{-1}) by $2x/9\pi$ μg cm^{-2}

*nil = at abamectin concentrations higher than 2.12 μg cm^{-2}, the biomass loss could not be reliably measured due to the deformed shape of *P. excavatus* and their strong attachment to the filter paper

Comparison of the soil test with the filter paper test revealed that the earthworms had broadly similar reactions when exposed to higher concentrations of abamectin, but that the soil test was less sensitive. The presence of the soil likely reduced the exposed surface area of the worm and so led to a lower bioavailability of abamectin to *P. excavatus* in the soil, as well as adding a different toxic mechanism. This is mainly because in the soil, abamectin is bound firmly and easily to the organic carbon in soil particles (Virginia et al. 1990) and is then ingested by the worms through feeding or burrowing and so absorbed through the gut (Zang et al. 2000). Without soil, the body surface of the earthworm is in direct contact with abamectin and hence shows the increased toxicity. Similar results were reported in *E. fetida*, where the estimated toxicity to abamectin was higher in the filter paper test compared to that in the artificial soil test (Sun et al. 2005), and in another similar study, this toxicity was reported to differ between the two tests by about 200-fold (Zang et al. 2000).

Prolonged exposure to abamectin for up to 7 days revealed no significant differences in mortality between the control group and those exposed to abamectin at less than 10 mg a.i. kg^{-1}. However, a longer exposure period (14 days) significantly ($P < 0.05$) increased the mortality of *P. excavatus* by approximately 2.5 to 7.5% for abamectin concentrations of less than 10 mg a.i. kg^{-1}. A similar trend was observed in *P. excavatus* when exposed to abamectin at 50 mg a.i. kg^{-1} (Fig. 2). The surviving worms (≤ 50 mg a.i. kg^{-1}) had a

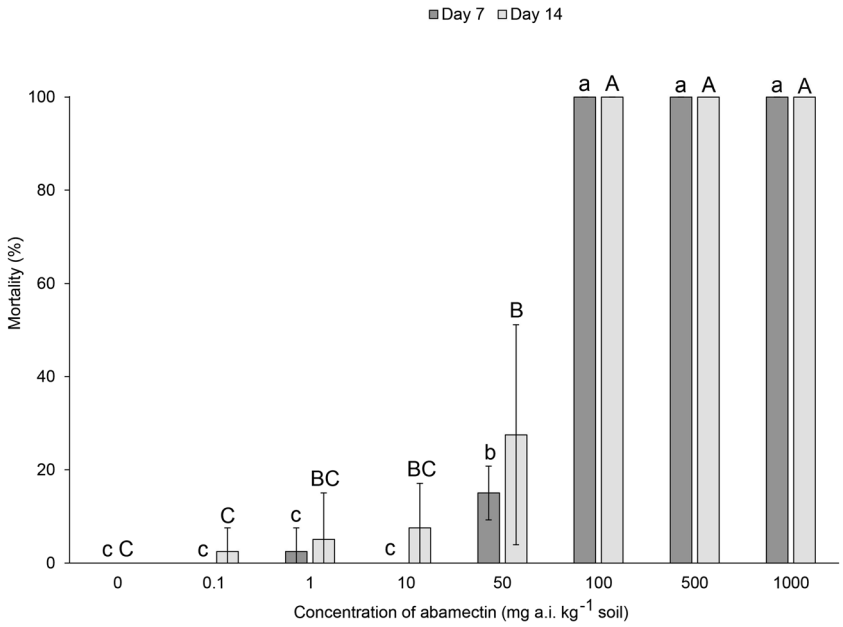

Fig. 2 Mortality rate of adult *P. excavatus* after a 7- and 14-day exposure to different doses of abamectin. Data are shown as the mean ± 1SD, derived from four replications. Means with a different letter are significantly different (ANOVA: Tukey's test; $P < 0.05$)

tendency to show a decreased biomass (Fig. 3) in the presence of abamectin in the test soil. The only increment in biomass was exhibited by those in the uncontaminated soil (control group), as even the presence of abamectin at the very low concentration of 0.1 mg a.i. kg^{-1} resulted in a significant reduction in average biomass ($P < 0.05$). The smallest biomass loss at day 7 (5.1%) was found with the lowest abamectin concentrations (0.1 mg a.i. kg^{-1}), some four-fold less than that at an abamectin concentration of 50 mg a.i. kg^{-1} (20.7%), although the highest biomass loss (approximately 30%) was observed with abamectin at 0.1 and 10 mg a.i. kg^{-1}. A broadly similar trend was observed after a longer 14-day exposure period, with significantly higher biomass losses seen at abamectin concentrations above 1.0 mg a.i. kg^{-1} (35.2–48%) compared to that (13.0%) at the lowest abamectin concentration (0.1 mg a.i. kg^{-1}).

Asides compromised metabolism and loss of ECF, biomass loss in earthworms caused by contaminants can result from the use of energy reserves for toxin elimination (Johnston et al. 2014) as well as for diverting the reserved energy for reproduction. Under these conditions, tissue is catabolized to support reproduction, resulting in a net weight loss (Maboeta et al. 2004; Johnston et al. 2014). Biomass loss in earthworms can also be due to reduced feeding and increased movement by trying to avoid contaminants, resulting in a reduced energy input and increased energy output shifting the balance to a negative energy gain. However, the relevance of this notion to the effects of abamectin on *P. excavatus* has yet to be confirmed.

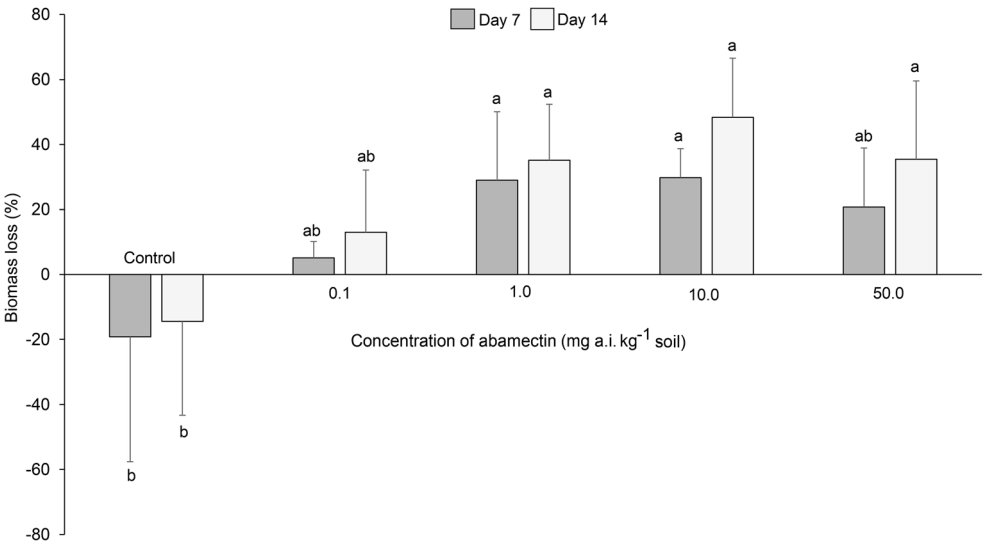

Fig. 3 Biomass loss (%) of adult *P. excavatus* after a 7- and 14-day exposure to different doses of abamectin. Data are shown as the mean ± 1SD, derived from four replications. Means with a different letter are significantly different (ANOVA: Tukey's test; $P < 0.05$)

After 28 days of exposure to abamectin at concentrations higher than 10 mg a.i. kg^{-1}, the surviving *P. excavatus* showed fragmentation of their segments, amputation at the posterior end, and they became very thin and shorter. A significantly higher mortality rate (83–98%; $P < 0.05$) and biomass loss (90.3–97.3%) was found with abamectin concentrations of 10–50 mg a.i. kg^{-1} compared to those at 1.0 (20%, 57.3%) and 0.1 (2.5%, 24.8%) mg a.i. kg^{-1}, although the control group showed an increased biomass (67.7%) without any mortality (Fig. 4).

With respect to the very low abamectin concentrations (0.1 and 1.0 mg a.i. kg^{-1}), the biomass loss of *P. excavatus* was more sensitive than the mortality level in indicating the toxic effects of abamectin. Biomass loss in earthworms has previously been reported for fungicides (Helling et al. 2000) and pesticides (De Silva et al. 2010; Domínguez et al. 2015; Nunes et al. 2016), where it appeared to be a good indicator of physiological stress.

Cocoon production

In addition to reduced biomass and increased mortality, reproduction in *P. excavatus* was also significantly ($P < 0.05$) reduced when exposed to sublethal concentrations of abamectin. No juveniles or cocoons were found in the soil treated with abamectin at 20 mg a.i. kg^{-1} or higher concentrations after 28 days.

An average of 44.3 juveniles was produced per worm in the control soil. The number of offspring was found to be equal to the number of cocoons produced, consistent with reports that this species generally has just one offspring per cocoon (Edward et al. 1998; Bhattacharjee and Chaudhuri 2002), and so giving a hatchling rate of almost 100%. A decreased number of juveniles were produced as the concentration of abamectin increased (Table 2), with significantly lower ($P < 0.05$) juvenile numbers being produced at abamectin concentrations of 10 mg a.i. kg^{-1} and higher. Among all the juveniles produced (375 individuals), two (0.53%) were found to have an abnormal morphology, including having two heads (Fig. 5) at an abamectin concentration of 10 mg. a.i. kg^{-1}. However, the effect of this treatment was not significant compared to the control and the lower concentration of abamectin ($P = 0.073$).

Table 2 Effects of a 28-day exposure to different concentrations of abamectin (0 (control), 0.1, 1.0, 10, 20, 30, 40, and 50 mg a.i. kg^{-1}) on *P. excavatus* reproduction

Abamectin concentration (mg a.i. kg^{-1} soil)	No of juveniles
Control	44.25 ± 16.19^{a}
0.1	35.00 ± 9.76^{a}
1.0	13.00 ± 7.62^{b}
10.0	1.50 ± 3.00^{b}
20.0–50.0	No cocoon or offspring was observed

Data are shown as the mean ± SD, derived from four replications. Means with a different letter are significantly different (ANOVA: Tukey's test; $P < 0.05$)

The average reproduction rate observed in this study (1.4 cocoons $worm^{-1}$ day^{-1}) is very similar to the number obtained by Reinecke and Hallatt (1989) in an environmental controlled chamber, but is just over 3.3-fold higher than that reported by Karmegam and Daniel (2009), at 0.42 cocoons $worm^{-1}$ day^{-1}. However, this reproduction rate is still very low as compared to the 80–92 juveniles (56 days) reported by De Silva et al. (2010). Regardless, there was a similar trend of an increasing dosage of toxin significantly reducing the number of juveniles produced. Jensen et al. (2007) evaluated the toxic effect of abamectin using *Eisenia fetida* and found that no cocoons were produced by *E. fetida* at an abamectin soil concentration of 5 mg a.i kg^{-1}, while cocoon production

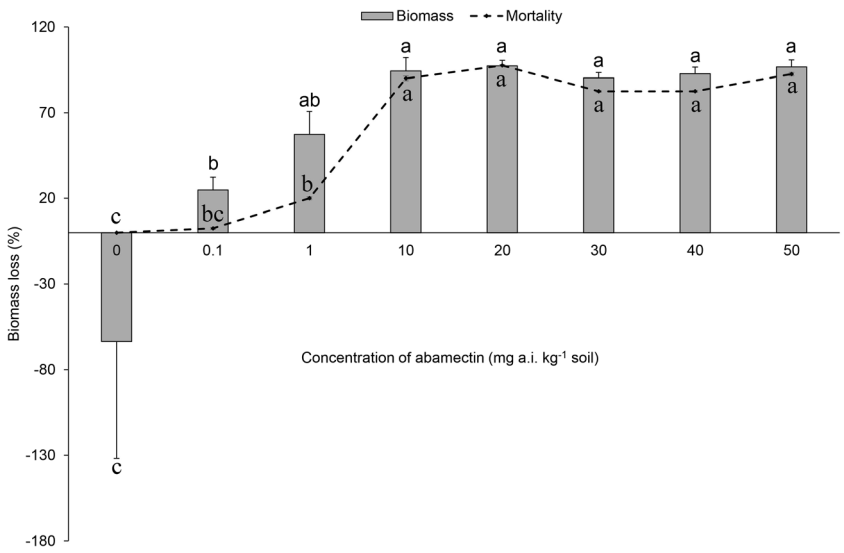

Fig. 4 Biomass loss and mortality rate of adult *P. excavatus* after 28-day exposure to different doses of abamectin. Data are shown as the mean ± SD, derived from four replications. Means with a different letter are significantly different (ANOVA: Tukey's test; $P < 0.05$)

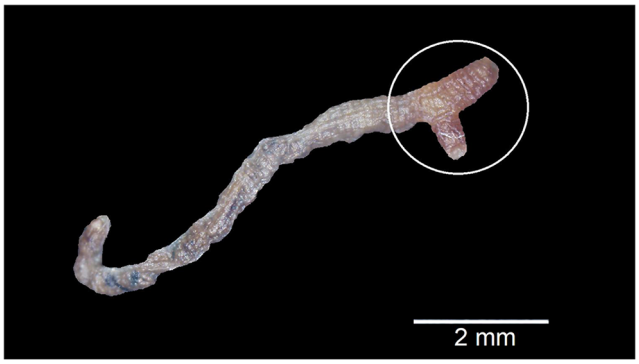

Fig. 5 Abnormal morphology (white circled) shown by a newly hatched *P. excavatus* offspring produced in an abamectin concentration of 10 mg a.i. kg^{-1}

was reduced by more than 50% at 1 mg a.i. kg^{-1}. Gaupp-Berghausen et al. (2015) also reported a decreased reproduction of *Lumbricus terrestris* and *Aporrectodea caliginosa* with increasing glyphosate dosages.

Histological alterations of the earthworm's body wall and intestines

Generally, the body wall of *P. excavatus* has three layers, namely the epidermis (E), circular muscle (CM), and longitudinal muscle (LM), as shown in Fig. 6a. The presence of abamectin caused histological alterations in these three layers. Abamectin at a very low concentration (0.021 μg cm^{-2}) did not cause any gross morphological change in the body wall, although an infarction in the body wall prior to the subtle loss of Masson's trichrome staining in body wall was evident (Fig. 6b). With increasing abamectin concentrations, the morphology of the body wall started to show changes, where at 0.21 μg cm^{-2}, the mucocytes of the E showed an irregular surface and cellular compartmentation (Fig. 6c). Further increasing the concentration of abamectin to 2.12 μg cm^{-2} induced an extreme pathology of the body wall of *P. excavatus*, where the whole body wall lost its intact nature (Fig. 6d). Besides the body wall, histological observation also showed the disintegration of the E, CM, and LM of the clitellum (Fig. 7a–d).

The intestine of *P. excavatus* from the control treatment consisted of the epithelium (EP), CM, and chloragogenous cells (ChC), as shown in Fig. 8a. Histopathological changes were observed in the transverse section of the intestine (EP and ChC) during exposure to abamectin at 50 mg a.i. kg^{-1}. As shown in Fig. 8b, the arrangement of the villi and feathering of the epithelium surface were damaged with a clear

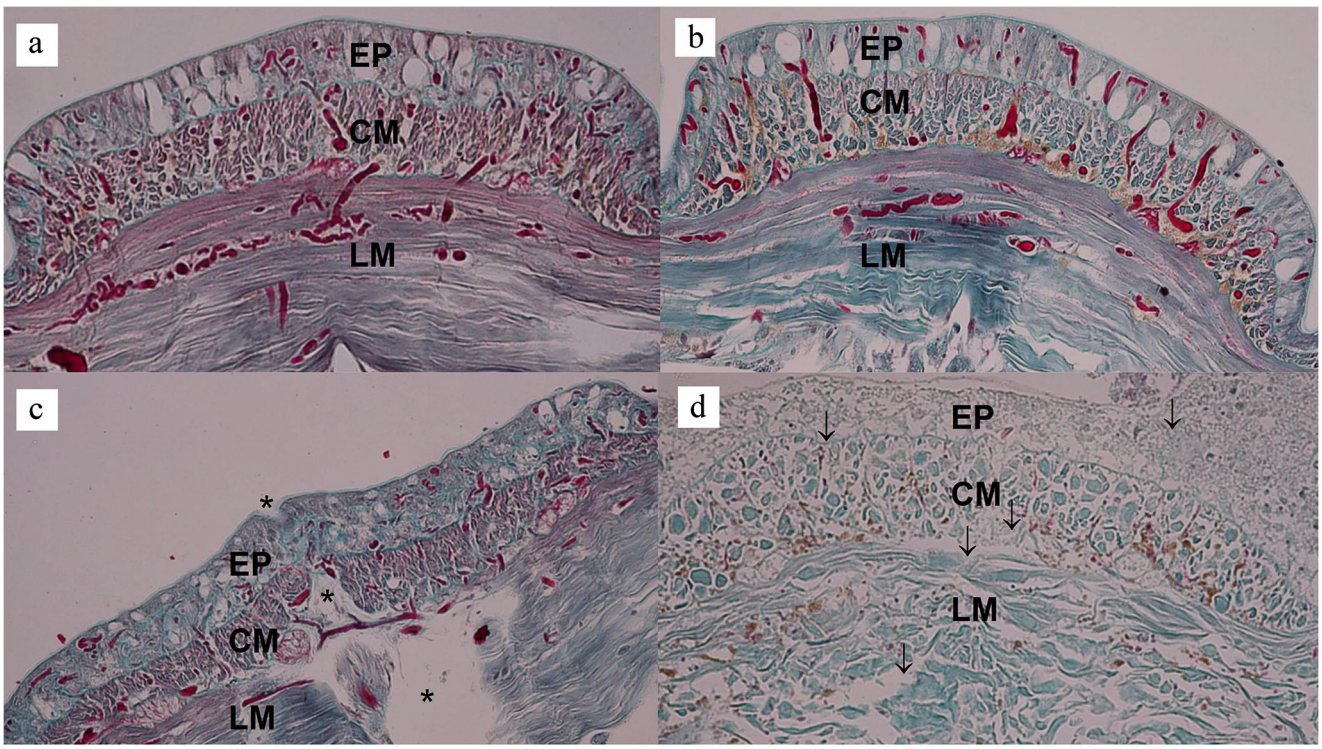

Fig. 6 Histological changes observed in longitudinal sections of the epidermal surface in *P. excavatus* after exposure to abamectin on filter paper at **a** 0 (control), **b** 0.021, **c** 0.21, and **d** 2.12 μg cm^{-2}. **a** Epidermis and muscles layer in control earthworms, **b** an infarction in body wall prior to the loss or subtle of Masson's trichrome stain in the epidermis and muscle layers, **c** atrophy response with mucocytes of the epidermis showing an irregular surface and cellular compartmentation, and **d** an extreme pathology characterized by a loss of the integrity of the whole body wall. Key: *EP* epidermis, *CM* circular muscle layer, *LM* longitudinal muscle layer, *disrupted cellular compartmentation and an increased occurrence of intercellular spaces, ↓ the thinning and dispersion of epidermis, circular, and longitudinal muscle layer

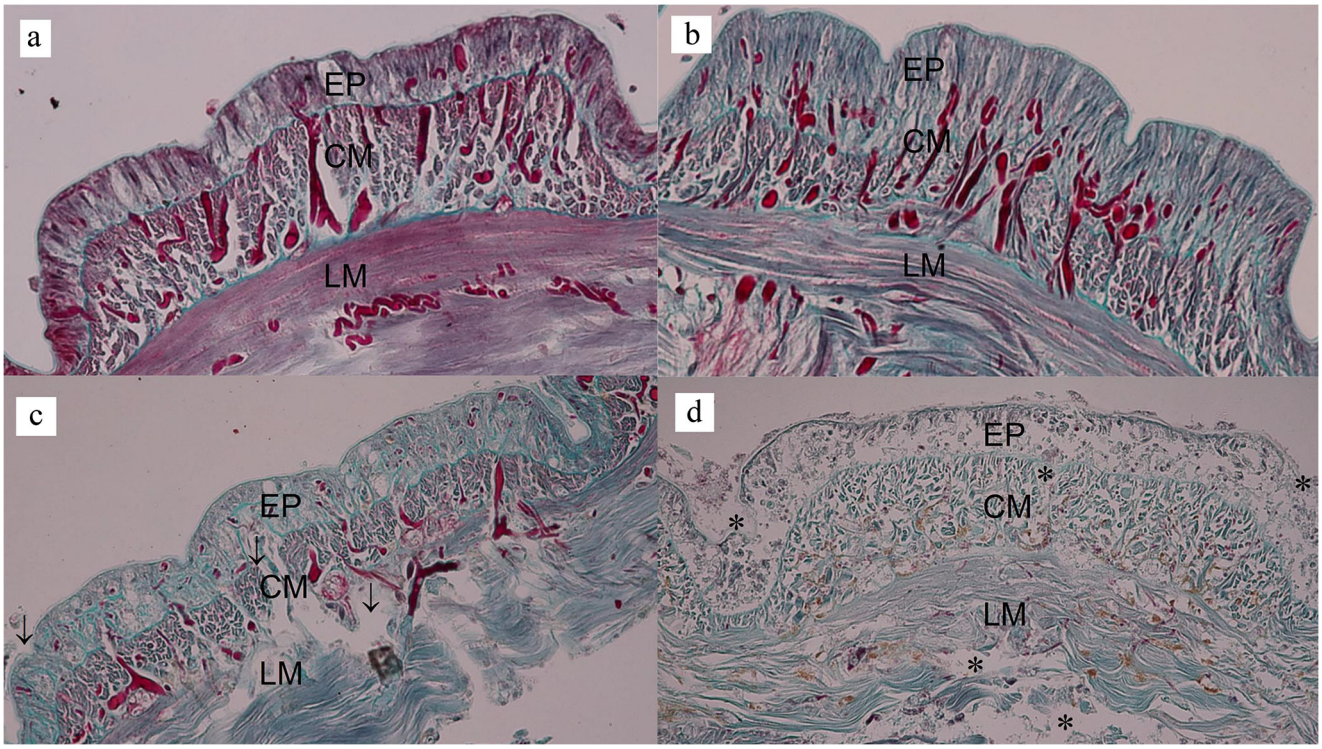

Fig. 7 Histological changes observed in longitudinal section of the clitellum in *P. excavatus* to abamectin after exposure to abamectin on filter paper at **a** 0 (control), **b** 0.021, **c** 0.21, and **d** 2.12 μg cm^{-2}. **a** Normal clitellum (control), **b** loss of or subtle Masson's trichrome staining in the clitellum, loss of cell shape and structural organization of the muscle layer and drastic damage in the clitellum (**c**, **d**); ↓ disrupted cellular compartmentation and an increased occurrence of intercellular spaces. Key: * cell disintegrated

evidence of necrosis being observed in the EP. In addition, the ChC were totally detached from the muscle layer.

A number of studies have evaluated the effect of abamectin on different earthworm species, namely *Eisenia fetida* (Jensen et al. 2007; Torkhani et al. 2011), *Eisenia andrei* (Kolar et al. 2008), and *Lumbricus terrestris* (Torkhani et al. 2011). However, no published data is available on the histopathology induced by abamectin. Nevertheless, histopathological changes in earthworms have frequently been reported after exposure to organic and heavy metal pollution (Reddy and Rao 2008; Kiliç 2011), insecticides (Wang et al. 2015), pesticides (Saxena et al. 2014; Rico et al. 2016), and herbicides (Morowati 2000). Disintegration of the body wall, damage to the muscles layers, and deformation of the ChC were the most common responses observed and reported in earthworms after exposure to pesticides. However, no hypertrophy was observed in this study when *P. excavatus* was exposed to abamectin. Atrophy is the general physiological process of tissue breakdown and, as a result, the body wall of the earthworm becomes thinner. This is most probably because of the mechanism of action of abamectin, which inhibits transmission between inhibitory motoneurons and muscles (Shoop et al. 1995; Ding et al. 2001) and so results in a slow-onset rigid paralysis. However, this hypothesis has yet to be confirmed.

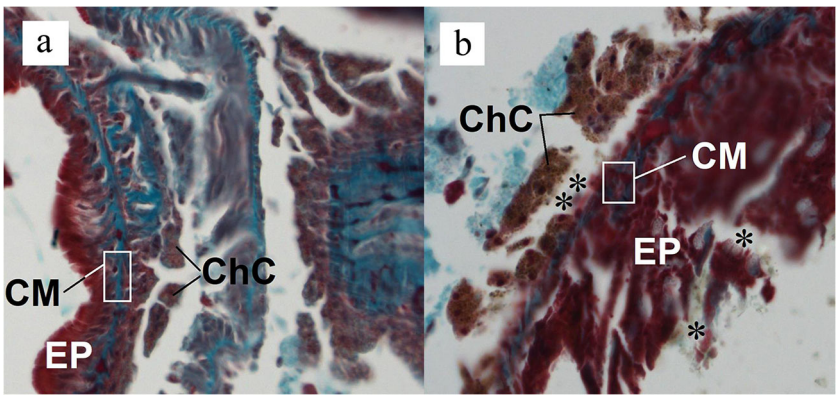

Fig. 8 Effect of abamectin on the intestine tissue of *P. excavatus* after a 48-h exposure to abamectin at **a** 0 (control) or **b** 50 mg kg^{-1} soil showing the irregular cell shape, cellular compartmentation with almost total damage to the epithellum (EP), and chloragogue cells (ChC). Key: * cytoplasm of sparse density, intercellular space enhanced, and ChC totally detached with the circular muscle layer (CM)

According to Markad et al. (2015), the histological changes in earthworms when exposed to contaminated soil or the contaminant itself are caused by a reinforced biochemical and cytogenotoxic response, where the membrane lipid in the exposed earthworms is seriously damaged, mainly because the antioxidant defense system of the organism is insufficient to handle the introduced substances. The results of this study showed a similar response profile, where the higher the concentration of abamectin, the more seriously damaged was the body wall and digestive system. Biomarkers, such as morphological and histological alterations, are helpful and can be an important element in the ecological risk assessment of soil conditions as well as a tool to assess the pesticide toxicity on earthworms.

Conclusion

In summary, we demonstrated that abamectin has significant effects on *P. excavatus*, including concentration-dependent distinct morphological changes, biomass loss, reduced cocoon production, and histological alterations. Abamectin is widely used in tropical countries to overcome different phytophagous pests in agricultural farms. Farmers may start applications at a recommended dosage (5–27 g ha^{-1}) but typically progress to higher effective dosages depending upon local conditions and pest resistance in an attempt to ensure its effectiveness. This can control the pest outbreak, but the effect of the abamectin on soil dwelling organisms, including earthworms (a soil engineer), remains largely unknown.

From the results of this study, the drawback of this high dosage application of abamectin is that the growth and reproduction of the tropical earthworm *P. excavatus* is detrimentally affected. Moreover, pesticides are often applied as cocktails, and the additive or synergistic effects of other pesticide residues in the soil with abamectin on soil organisms remain to be determined. Future and more detailed studies are necessary to evaluate the effect of abamectin on ecologically different earthworms that are found surrounding vegetable farms and their histopathological alterations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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