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Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*

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A B S T R A C T

In this study, we used two different types of bioassay, a contact filter paper toxicity bioassay and a soil toxicity bioassay, to compare the acute toxicity of twenty-four insecticides belonging to six chemical categories on earthworm species, *Eisenia fetida*. Results of the contact filter paper toxicity bioassay indicated that neonicotinoids were super toxic to *E. fetida* (48 h-LC_{50} value ranged from 0.0088 to 0.45 μg cm^{-2}), pyrethroids were very toxic (48 h-LC_{50} values ranged from 10.55 to 25.7 μg cm^{-2}) and insect growth regulators (IGRs) were moderately toxic (48 h-LC_{50} values ranged from 117.6 to 564.6 μg cm^{-2}) to the worms. However, antibiotics, carbamates and organophosphates induced variable toxicity responses in *E. fetida*, and were very to extremely toxic (48 h-LC_{50} values ranged from 3.64 to 75.75 μg cm^{-2}). Results of the soil toxicity bioassays showed a different pattern of toxicity except that neonicotinoids were the most toxic even under the soil toxicity bioassay system. The acute toxicity of neonicotinoids was higher than those of antibiotics, carbamates, IGRs and organophosphates. In contrast, pyrethroids were the least toxic to the worms under the soil toxicity bioassay system. It was concluded that irrespective of bioassay systems, earthworms were more susceptible to neonicotinoids than other modern synthetic insecticides.

1. Introduction

Earthworms are common soil organisms in most environments, and play an important role in improving structure and fertility of soil ecosystems (Bartlett et al., 2010). They modify soil organic matter both chemically and physically, mix leaf litter with the soil, facilitate the formation and stabilization of soil aggregates and improve soil porosity (Lavelle and Spain, 2001). It has been indicated that earthworms may represent up to 60–80% of the total animal biomass in soil (Ouellet et al., 2008; Jouquet et al., 2010). Unlike many other soil organisms that are protected by thick cuticle on the exterior of their bodies, earthworms are particularly susceptible to soil chemicals (Lanno et al., 2004; Nahmani et al., 2007). The bioaccumulation of insecticides in earthworms may not lead to significant effects to the animal itself, but may produce serious damages to higher trophic levels (Darling and Thomas, 2005; Hobbelen et al., 2006; van Gestel et al., 2011). Therefore, earthworms are suitable bioindicators of soil contamination, and can be used to provide safety thresholds for insecticide applications (Suthar et al., 2008; Lourenço et al., 2011).

The use of insecticides in agricultural systems is one of the most important factors contributing to the massive increase in food production worldwide (Kranthi et al., 2002). However, extensive use of insecticides causes an adaptation or resistance evolution in target pests and thereby a diminishing of the effectiveness of these chemicals (Wang et al., 2008). Due to structural and physiological similarities between pest and non-pest species, most insecticides are toxic not only to the target species, but also to a range of non-target organisms (Santos et al., 2010; Walker et al., 2010). Although numerous ecotoxicity studies have been carried out in recent years using earthworms, they are mostly focused on heavy metals, polychlorinated biphenyls (PCBs) and conventional insecticide categories such as organochlorines, organophosphates and carbamates (Diercxsens et al., 1985; Kamitani and Kaneko, 2007; Reinecke and Reinecke, 2007; Hackenberger et al., 2008). Neonicotinoids, insect growth regulators (IGRs) and antibiotics with novel modes of action are extensively used within cropping systems throughout the world, but few studies have examined their ecotoxicological impacts on earthworms.

In agricultural areas worldwide, there is an increasing concern about soil contamination due to the widespread use of insecticides (Zhou et al., 2006; Reinecke and Reinecke, 2007; De Silva and van Gestel, 2009; De Silva et al., 2010; Garcia et al., 2011; Santos et al., 2011). A number of soil animals have been proposed

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as indicator organisms of soil pollution with both advantages and drawbacks (Zhu et al., 2008). However, earthworms have long been used as a key index of ecotoxicology diagnosis although they are becoming extinct in many agriculture soils (Xiao et al., 2004; Zhu et al., 2008). Furthermore, the security of soil ecosystems is threatened by insecticides, which reduce earthworm population and lead to the build-up of waste materials (Edwards and Bohlen, 1992). The relationship between death and diminution of earthworms and the application of insecticides are still elusive, and little is known about the impact of novel insecticides on earthworms using the standard test method as described in the guidelines of the OECD (1984, 2004). The main aims of this ecotoxicology study are to get a more comprehensive understanding on the toxic effects of insecticides on earthworms, and to provide informative data for use in ecological risk assessment on soil ecosystem.

2. Materials and methods

2.1. Earthworms

The Oligochaete *Eisenia fetida* is a currently used invertebrate species for ecotoxicological assessment of substances in soil, which is the OECD and International Standardization Organization (ISO) recommended earthworms test species (OECD, 1984, 2004; ISO, 1993). Adult earthworms (weighing between 350 and 500 mg) with well-developed clitella were purchased from the College of Animal Sciences, Zhejiang University, China, and cultured in the laboratory in artificial soil according to OECD guidelines (OECD, 1984, 2004). Soils were mixed with decayed leaves and decomposed pig manure, and kept at room temperature (20 ± 1 °C). Soil water content was measured every week and moisture was adjusted to 35% of the maximum water-holding capacity by adding distilled water whenever necessary.

2.2. Insecticides

Twenty-four insecticides from six different chemical classes were tested in this study (Table 1). The selected insecticides are widely used in agriculture around the world. Active ingredients were used instead of commercial formulations, aiming to document the acute effects of the active substances on the mortality rather than the effects of adjuvants added in the commercial products.

2.3. Toxicity test methods

2.3.1. Contact filter paper test

A modified contact filter paper test was performed (OECD, 1984). A piece of filter paper was placed in a 9-cm Petri dish and treated with the test substance dissolved in 2 ml of acetone. After the solvent was evaporated, the piece of filter paper was remoistened with 2 ml distilled water, and one earthworm was placed on it. The dish was incubated in the dark at 20 ± 1 °C for 48 h and mortality was recorded. An earthworm was considered dead if it failed to respond to a gentle mechanical touch on the front end. Earthworms were held on wet filter paper for 24 h at 20 ± 1 °C in the dark to have the gut contents purged before the dose response test.

A preliminary test was conducted to determine a concentration range for each chemical in which a 0–100% mortality of the earthworms was obtained. At least five concentrations and a control were included for each chemical. Ten replicates were used for each concentration. Acetone was used as the control. Treated earthworms were maintained at 20 ± 1 °C under 80–85% relative humidity in the dark.

2.3.2. Artificial soil test

Artificial soil consisted of 10% ground sphagnum peat (< 0.5 mm), 20% kaolinite clay (> 50% kaolinite) and 70% fine sand was used for artificial soil tests (OECD, 1984). A small amount of calcium carbonate was added to adjust the pH to 6.0 ± 0.5. In toxicity tests, the water content was adjusted to 35% of the dry weight. For each tested concentration, the desired amount of insecticide was dissolved in 10 ml acetone and mixed into a small quantity of fine quartz sand. The sand was mixed for at least 1 h to evaporate the acetone and then mixed thoroughly with the premoistened artificial soil in a household mixer. The final moisture contents of artificial soil were adjusted to the descried level by the addition of distilled water. A total of 0.65 kg soil (equivalent to 0.5 kg dry artificial soil) was placed in a 500-ml glass jar (surface area 63.6 cm^2) and 10 adult earthworms were added to each jar. Controls were prepared similarly but only with 10 ml acetone and no insecticide. The jars were loosely covered with polypropylene lids, allowing exchange of air, and stored at 20 ± 1 °C with 80–85% relative humidity under 400–800 lx of constant light. Mortality was assessed at 7 and 14 day after treatment.

Table 1
Detailed information of insecticides used in this study: name, manufacturer and stock.

Insecticide	Technical grade (a.i.)	Manufacturer
Neonicotinoid insecticides		
Acetamiprid	97%	Jiangsu Yangnong Chemical Co., Ltd.
Clothianidin	96.5%	Hunan Bide Biochemical Co., Ltd.
Imidacloprid	95.3%	Jiangsu Changlong Chemical Co., Ltd.
Nitenpyram	95%	Jiangsu Nantong Agro-chemical Co., Ltd.
Thiacloprid	97.75%	Tianjing Xingguang Chemical Co., Ltd.
Antibiotic insecticides		
Abamectin	93% B1a	Hebei Weiyuan Biochemical Co., Ltd.
Emaectin benzoate	89% B1a	Hebei Weiyuan Biochemical Co., Ltd.
Ivermectin	90.73% B1	Zhejiang Haizheng Chemical Co., Ltd.
Insect growth regulator insecticides		
Buprofezin	98.2%	Jiangsu Changlong Chemical Co., Ltd.
Chlorfluazuron	85.7%	Jiangsu Yangnong Chemical Co., Ltd.
Hexaflumuron	97.1%	Daliang Ruize Agro-chemical Co., Ltd.
Tebufenozide	92%	Jiangsu Baoling Chemical Co., Ltd.
Pyrethroid insecticides		
Cyhalothrin	95%	Jiangsu Yangnong Chemical Co., Ltd.
Cypermethrin	93.2%	Nanjing Red-sun Chemical Co., Ltd.
Fenpropathrin	94%	Nanjing Red-sun Chemical Co., Ltd.
Lambda-cyhalthrin	98%	Jiangsu Changlong Chemical Co., Ltd.
Carbamate insecticides		
Carbosulfan	88.89%	Suzhou Fumeishi Chemical Co., Ltd.
Isoprocarb	99.7%	Jiangsu Changlong Chemical Co., Ltd.
Metolcarb	96%	Nanjing Red-sun Chemical Co., Ltd.
Promecarb	98%	Jiangsu Yangnong Chemical Co., Ltd.
Organophosphate insecticides		
Chlorpyrifos	97%	Jiangsu Nantong Agro-chemical Co., Ltd.
Fenitrothion	95%	Huangyan Yongning Chemical Co., Ltd.
Phoxim	89%	Jiangsu Baoling Agro-chemical Co., Ltd.
Triazophos	80.5%	Hubei Xianlong Agro-chemical Co., Ltd.

A range of concentrations, 0, 0.1, 1.0, 10, 100 and 1000 mg kg^{-1} dry soil, were used in pre-trials to determine a concentration range that resulted in a 0–100% mortality. To obtain LC_{50}, six test concentrations and a control were used. Three jars, each containing 10 adult earthworms, were used for each concentration. The earthworms were preconditioned for 24 h under the same conditions described above in the untreated soil before the dose response test.

2.4. Statistical analysis

A probit analysis was conducted to assess the acute toxicity of insecticides to *E. fetida* using a program developed by Chi (1997). Significant level of mean separation ($P < 0.05$) was based on non-overlap between the 95% confidence limits of two LC_{50} values. For the contact filter paper test method, based on the resulting LC_{50} values, the insecticides were classified as supertoxic (< 1.0 μg cm^{-2}), extremely toxic (1–10 μg cm^{-2}), very toxic (10–100 μg cm^{-2}), moderately toxic (100–1000 μg cm^{-2}) or relatively nontoxic (> 1000 μg cm^{-2}) (Roberts and Dorough, 1984).

3. Results

3.1. Contact toxicity

The results of filter paper contact test are presented in Table 2. The results demonstrated that different insecticides varied widely

Table 2
Summary of parameter estimates for the acute toxicity with contact filter paper test of 24 insecticides to *Eisenia fetida*.

Insecticide	Slope (SE)	χ^2 (d.f.)	LC_{50} (95% CI) μg cm^{-2}	Toxicity grade
Neonicotinoid insecticides				
Acetamiprid	4.33 (0.62)	1.41 (4)	0.0088 (0.0066–0.011)	Supertoxic
Clothianidin	4.12 (0.57)	0.86 (4)	0.28 (0.24–0.35)	Supertoxic
Imidacloprid	3.86 (0.57)	0.84 (4)	0.027 (0.018–0.036)	Supertoxic
Nitenpyram	5.85 (0.95)	1.32 (4)	0.22 (0.19–0.29)	Supertoxic
Thiacloprid	1.80 (0.24)	2.42 (4)	0.45 (0.36–0.61)	Supertoxic
Antibiotic insecticides				
Abamectin	2.95 (0.40)	0.60 (4)	23.08 (18.17–32.64)	Very toxic
Emamectin benzoate	3.62 (0.47)	4.04 (4)	30.2 (25.3–36.6)	Very toxic
Ivermectin	2.81 (0.40)	2.02 (4)	4.40 (2.86–5.82)	Extremely toxic
Insect growth regulator insecticides				
Buprofezin	5.28 (0.83)	2.98 (4)	564.6 (437.4–886.8)	Moderately toxic
Chlorfluazuron	4.26 (0.63)	4.78 (4)	536.2 (419.3–804.2)	Moderately toxic
Hexaflumuron	3.58 (0.49)	5.36 (4)	117.6 (93.57–166.3)	Moderately toxic
Tebufenozide	5.81 (0.95)	0.73 (4)	508.1 (401.7–775.8)	Moderately toxic
Pyrethroid insecticides				
Cyhalothrin	3.03 (0.41)	0.70 (4)	24.30 (19.02–34.99)	Very toxic
Cypermethrin	4.06 (0.55)	1.39 (4)	10.63 (6.89–15.41)	Very toxic
Fenpropathrin	3.01 (0.41)	1.94 (4)	10.55 (7.42–13.41)	Very toxic
Lambda-cyhalthrin	4.09 (0.57)	1.49 (4)	25.7 (21.0–34.7)	Very toxic
Carbamate insecticides				
Carbosulfan	2.82 (0.43)	0.29 (4)	75.75 (55.03–129.4)	Very toxic
Isoprocarb	1.97 (0.25)	4.96 (4)	3.64 (2.82–4.86)	Extremely toxic
Metolcarb	5.19 (0.80)	4.58 (4)	9.18 (7.17–10.8)	Extremely toxic
Promecarb	3.79 (0.50)	0.32 (4)	10.58 (8.81–12.62)	Very toxic
Organophosphate insecticides				
Chlorpyrifos	2.80 (0.34)	2.77 (4)	14.19 (11.58–17.92)	Very toxic
Phoxim	2.12 (0.30)	3.03 (4)	54.65 (40.40–85.79)	Very toxic
Pyridaphenthion	3.10 (0.46)	0.16 (4)	3.84 (2.87–6.18)	Extremely toxic
Triazophos	3.40 (0.43)	4.18 (4)	14.21 (11.83–17.62)	Very toxic

in their contact toxicities, and the different insecticides within the same chemical classes had different toxicities to *E. fetida*. Among the tested chemical classes, neonicotinoids showed the highest toxicity, followed by pyrethroids, while IGRs exhibited the lowest toxicity. The antibiotics, carbamates and organophosphates induced intermediate toxicity response to the animals. The descending order of toxicity for twenty-four insecticides was ranked as follows: acetamiprid > imidacloprid > nitenpyram, clothianidin > thiacloprid > isoprocarb, pyridaphenthion, ivermectin > metolcarb, fenpropathrin, promecarb, cypermethrin ≥ chlorpyrifos, triazophos > abamectin, cyhalthrin, lambda-cyhalthrin, emamectin benzoate > phoxim, carbosulfan ≥ hexaflumuron > tebufenozide, chlorfluazuron and buprofezin. The toxicity of acetamiprid was 64,159-fold higher than that of buprofezin at 48-h interval.

All the tested neonicotinoids were supertoxic to *E. fetida* (Table 2). Among the five neonicotinoids tested, acetamiprid exhibited the highest toxicity, while thiacloprid was the least toxic to *E. fetida*. Based on the LC_{50} values, acetamiprid was 51.1 and 31.8 times more toxic than thiacloprid and clothianidin, respectively. The order of toxicity to *E. fetida* based on LC_{50} values was as follows: acetamiprid > imidacloprid > nitenpyram, clothianidin > thiacloprid.

The selected pyrethroids were very toxic to *E. fetida*. The toxicities of fenpropathrin and cypermethrin were similar, and were significantly higher than those of cyhalothrin and lambda-cyhalthrin. The high to low ranking of toxicities of pyrethroids were as follows: fenpropathrin, cypermethrin > cyhalothrin, lambda-cyhalthrin. IGRs were moderately toxic to *E. fetida*. These four insecticides were the least toxic among the chemicals tested in the present study. Among the four IGRs tested, hexaflumuron had the highest toxicity, which was 4.8, 4.6 and 4.3 times more toxic than buprofezin, chlorfluazuron and tebufenozide, respectively.

The evaluated antibiotics were either very or extremely toxic to *E. fetida*. Among the three antibiotics selected, ivermectin had higher toxicity to *E. fetida* than emamectin benzoate and abamectin. Based on the LC_{50} values, ivermectin was 6.9 and 5.2 times more toxic than emamectin benzoate and abamectin, respectively. Therefore, the relative toxicity (LC_{50}) of antibiotics to *E. fetida* in decreasing order was ivermectin, abamectin and emamectin benzoate. Similar to antibiotics, carbamates were either very or extremely toxic to *E. fetida*. Among the selected carbamates, isoprocarb and metolcarb showed relative higher toxicity, while carbosulfan exhibited the lowest toxicity to *E. fetida*. The relatively toxicity (LC_{50}) of carbamates to *E. fetida* in decreasing order was as follows: isoprocarb > metolcarb, promecarb > carbosulfan. Similar to antibiotics and carbamates, organophosphates were also either very or extremely toxic to *E. fetida*. Among the four organophosphates, pyridaphenthion showed the highest toxicity, followed by chlorpyrifos and triazophos, while phoxim showed the lowest toxicity to the animals. The toxicity order of organophosphates from high to low was as follows: pyridaphenthion > chlorpyrifos, triazophos > phoxim.

3.2. Soil toxicity

The acute toxicities to *E. fetida* of the 24 insecticides from artificial soil test are shown in Table 3. The data exhibited a clear concentration-dependent relationship, and the mortality increased when exposure period increased for all insecticides tested. Similar to the results of contact toxicity, each of the insecticides evaluated using artificial soil test showed different degree of toxicity to *E. fetida*.

At 7-day interval, neonicotinoids showed the highest toxicity, followed by antibiotics and carbamates. The toxicity of IGRs

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Table 3
Summary of parameter estimates for the acute toxicity with artificial soil test of 24 insecticides to *Eisenia fetida*.

Insecticide	7-days			14-days		
	Slope (SE)	χ^2 (d.f.)	LC_{50} (95% CI) mg kg^{-1}	Slope (SE)	χ^2 (d.f.)	LC_{50} (95% CI) mg kg^{-1}
Neonicotinoid insecticides						
Acetamiprid	9.01 (1.21)	3.10 (4)	1.72 (1.58–1.97)	8.93 (1.15)	2.42 (4)	1.52 (1.41–1.67)
Clothianidin	12.30 (1.93)	5.53 (4)	7.44 (6.65–9.06)	9.25 (1.25)	1.44 (4)	6.06 (5.60–6.77)
Imidacloprid	12.04 (1.85)	2.10 (4)	3.15 (2.86–3.71)	10.0 (1.37)	1.25 (4)	2.82 (2.61–3.17)
Nitenpyram	9.72 (1.33)	2.75 (4)	4.42 (4.11–4.76)	9.14 (1.21)	1.62 (4)	3.91 (3.57–4.20)
Thiacloprid	10.62 (1.65)	0.15 (4)	12.13 (10.78–14.90)	8.55 (1.20)	2.32 (4)	10.96 (9.93–12.77)
Antibiotic insecticides						
Abamectin	14.59 (2.42)	0.74 (4)	31.50 (28.44–38.00)	10.67 (1.53)	1.11 (4)	27.86 (25.72–31.45)
Emamectin benzoate	9.97 (1.41)	2.54 (4)	196.2 (179.5–225.3)	9.10 (1.21)	1.41 (4)	175.3 (162.3–195.4)
Ivermectin	8.04 (1.08)	1.78 (4)	68.11 (62.07–78.01)	7.24 (0.89)	2.84 (4)	56.34 (51.95–61.83)
Insect growth regulator insecticides						
Buprofezin	10.14 (1.50)	5.62 (4)	425.1 (383.1–505.4)	8.03 (1.05)	3.96 (4)	363.3 (333.9–407.4)
Chlorfluazuron	10.99 (1.62)	2.64 (4)	406.5 (369.9–474.5)	10.72 (1.55)	0.99 (4)	381.8 (351.0–434.8)
Hexaflumuron	14.64 (2.44)	0.71 (4)	420.1 (379.3–507.2)	9.73 (1.35)	1.39 (4)	374.2 (344.5–422.8)
Tebufenozide	9.67 (1.44)	0.53 (4)	434.8 (389.7–522.3)	9.72 (1.36)	0.09 (4)	386.7 (354.3–441.8)
Pyrethroid insecticides						
Cyhalothrin	9.63 (1.70)	0.33 (4)	1530 (1308–2082)	9.92 (1.54)	0.31 (4)	1369 (1213–1691)
Cypermethrin	9.62 (1.60)	0.12 (4)	1467 (1273–1910)	10.58 (1.57)	1.91 (4)	1272 (1148–1512)
Fenpropathrin	7.80 (1.35)	0.43 (4)	1532 (1306–2079)	9.13 (1.30)	2.01 (4)	1246 (1126–1464)
lambda-cyhalthrin	7.97 (1.50)	1.32 (4)	1623 (1353–2367)	9.92 (1.53)	0.31 (4)	1370 (1214–1692)
Carbamate insecticides						
Carbosulfan	9.15 (1.31)	1.29 (4)	146.8 (132.5 –173.1)	10.6 (1.52)	1.15 (4)	130.1 (120.2–146.8)
Isoprocarb	9.02 (1.20)	1.46 (4)	69.4 (64.3–76.9)	8.53 (1.09)	0.69 (4)	60.8 (56.4–65.9)
Metolcarb	8.91 (1.19)	2.19 (4)	108.1 (99.7–120.8)	8.03 (1.01)	1.04 (4)	93.8 (86.8–102.1)
Promecarb	10.78 (1.58)	3.00 (4)	31.23 (28.36–36.55)	9.24 (1.26)	2.36 (4)	28.43 (26.15–32.08)
Organophosphorus insecticides						
Chlorpyrifos	12.09 (1.87)	0.15 (4)	421.3 (380.7–501.9)	109.5 (1.60)	0.93 (4)	384.9 (353.5–440.3)
Phoxim	6.18 (0.88)	1.77 (4)	1083 (960.4–1305)	6.31 (0.80)	4.48 (4)	901.5 (821.3–1017)
Pyridaphenthion	9.31(1.27)	1.48 (4)	273.3 (254.1–294.0)	9.46 (1.27)	1.23 (4)	243.7 (226.9–265.8)
Triazophos	9.62 (1.34)	1.84 (4)	381.4 (350.2–433.3)	9.40 (1.27)	1.14 (4)	347.5 (322.3–384.3)

and organophosphates were similar, and they were higher than that of pyrethroids. The decreasing order of the average acute toxicity of these six chemical classes was as follows: neonicotinoids > antibiotics, carbamates > organophosphates, IGRs > pyrethroids.

At 14-day interval, neonicotinoids still exhibited the highest toxicities, and the decreasing order of the average acute toxicity to *E. fetida* of the six chemical classes was similar to that at the 7-day interval. The order of toxicity to *E. fetida* based on LC_{50} values was as follows: acetamiprid > imidacloprid > nitenpyram > clothianidin > thiacloprid > abamectin, promecarb > ivermectin, isoprocarb > metolcarb > carbosulfan > emamectin benzoate > pyridaphenthion > triazophos, buprofezin, hexaflumuron, chlorfluazuron, chlorpyrifos, tebufenozide > phoxim > fenpropathrin, cypermethrin, cyhalthrin and lambda-cyhalthrin. The toxicity of acetamiprid was 901-fold higher than that of lambda-cyhalthrin to *E. fetida* at 14-day interval. Among the five neonicotinoids tested, acetamiprid showed the highest toxicity, followed by imidacloprid, nitenpyram and clothianidin. In contrast, thiacloprid had the least toxicity to *E. fetida*. The toxicities of antibiotics and carbamates were similar to *E. fetida*. Among the antibiotics evaluated, the toxicity of abamectin to *E. fetida* was 6.3- and 2.0-fold higher than those of emamectin benzoate and ivermectin, respectively. Among the four carbamates selected, promecarb showed the highest toxicity to *E. fetida*, and was 4.6-, 3.3- and 2.1-fold more toxic than carbosulfan, metolcarb and isoprocarb, respectively. Among the organophosphates tested, pyridaphenthion showed the highest toxicity, followed by triazophos and chlorpyrifos, while phoxim was the least toxic to *E. fetida*. The toxicity of the four IGRs was similar, and their LC_{50} values ranged from 363.3 (333.9–407.4) to 386.7 (354.3–441.8) mg kg^{-1}. Similar to IGRs, the toxicity of the four pyrethroids was also similar with LC_{50} values ranging from 1246 (1126–1464) to 1370 (1214–1692) mg kg^{-1}.

4. Discussion

With uniform area of contact exposure but various exposure period, the comparative acute toxicity of the tested insecticides to *E. fetida* in the two different test systems were almost all toxic in filter paper substrate medium. On the contrary, these insecticides were comparatively less toxic in soil substrate medium except for neonicotinoids. These results indicated that a toxicity estimation of an insecticide apparently differed with different test methods, and probably also differed with different types of chemical molecules, which were in confirmation with the results obtained by Heimbach (1984). Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms in that the insecticides are absorbed mainly by the skin; however, it fails to represent the situations in the soil ecosystem (Miyazaki et al., 2002; Grumiaux et al., 2010; Tripathi et al., 2010). Artificial soil test is more representative of the natural environment of earthworms, and the insecticides are absorbed mainly by gut in this method (De Silva and van Gestel, 2009; Udovic and Lestan, 2010). Therefore, artificial soil test is more adequate when toxicity of insecticides to earthworms are evaluated.

Neonicotinoids are among the most effective insecticides for the control of sucking and chewing pests. They act as competitive inhibitors on nicotinic acetylcholine receptors (nAChR) in the central nervous system (Elbert et al., 2008). Neonicotinoids may potentially endanger soil organisms including earthworms because they are system compound (Ishaaya and Degheele, 1998). Imidacloprid, in comparison with carbaryl, cyfluthrin, chlorpyrifos and fipronil, had a more negative effect on earthworm *Aporrectodea trapezoide* Duges (Mostert et al., 2002).

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Results from this study also show that neonicotinoids are the most toxic to *E. fetida* among the six chemical classes tested. Considering the high efficacy of neonicotinoids against target organisms, environmental managers should carefully evaluate the use of them in integrated pest management (IPM) programs to avoid serious damage to earthworms.

Pyrethroids bind to a distinct receptor site on sodium channel and prolong the open state by inhibiting channel deactivation and inactivation, and these compounds have high insecticidal activities through contact and stomach actions (Bloomquist, 1996). However, pyrethroids are relatively less toxic to mammalian because they are rapidly metabolized in mammals and ecosystems (Narahashi, 2000). It has been demonstrated that most of the pyrethroids are non-toxic to earthworms in soil toxicity tests (Inglesfield, 1984; Roberts and Dorough, 1984). However, some exceptions do occur, for example, cypermethrin is highly toxic to *Perionyx excavatus* (Gupta et al., 2010). Our results show that the toxicity of pyrethroids to *E. fetida* is the lowest in artificial soil test, while they are very toxic to *E. fetida* in contact toxicity tests, which indicate that pyrethroids are easier to be absorbed by skin than by gut.

The IGRs inhibit chitin synthesis and kill target insect by disturbing exoskeleton formation after molting (Schneider et al., 2008), and the insecticides are generally able to specifically kill the target insect pests with minimal impact on non-target organisms (Carmo et al., 2010). Our studies demonstrate that the selected IGR insecticides have relatively low intrinsic toxicity to *E. fetida*, compared with the other insecticides tested, which was consistent with the results of previous studies (Luo et al., 1999). Antibiotics are a group of broad-spectrum macrocyclic lactone insecticides and have high efficiency against target organisms (Lasota and Dybas, 1991). It is well known that the toxicity mechanism of antibiotics is based on their specific action on γ-aminobutyric acid (GABA) receptor to block the nervous signal transmission at the neuromuscular junctions. In general, antibiotics block electrical activity in vertebrate and invertebrate nerve and muscle by increasing the membrane conductance to chloride ions (Bloomquist, 1993). Among the six classes of chemicals tested, antibiotics and carbamates induce intermediate toxicity responses to *E. fetida*. Toxic effects of antibiotics on earthworms have been demonstrated in other studies, confirming their potential risk to soil invertebrates (Wislocki et al., 1989; Gunn and Sadd, 1994; Diao et al., 2007).

Although organophosphates and carbamates have different structures, they both inhibit acetylcholinesterase (AChE) activity (Carmo et al., 2010). The toxicity of organophosphates to earthworms depends on the assessed parameter. Certainly these chemicals will show very high toxicity if one assesses AChE inhibition and the consequent physiological damage (Rao et al., 2003; Reddy and Rao, 2008). In general, most organophosphates are not very toxic to earthworms. In this study, the toxicities of organophosphates to *E. fetida* evaluated using artificial soil test method are similar to those of IGRs, but are relatively lower compared with those of neonicotinoids, antibiotics and carbamates. Similar results have also been obtained in other earthworm species (Stenersen et al., 1973; Stenersen, 1979). It has been demonstrated that many organophosphates such as azinphosmethyl, diazinon, fenitrothion and malathion are only slightly toxic or not toxic to earthworms (Hopkins and Kirk, 1957; Griffiths et al., 1967; Voronova, 1968). Since organophosphates and carbamates both inhibit AChE, the higher toxicity of carbamates to *E. fetida* than organophosphates has led to the reversibility of the inhibitory pathway that characterizes carbamates as compared to organophosphates.

This study provides important information on the ecological relevance of these types of toxicity data for use in ecological risk assessments or derivation of soil quality standards. Results reported herein show the acute toxic effects of a range of insecticides on earthworms, but the adverse effect of sub-chronic or chronic exposure is also important in ecological risk assessments (Jensen et al., 2007; Liu et al., 2011). To date, the growth and reproduction of earthworms have also been important endpoints used in environmental ecotoxicity (van Gestel et al., 1991,1992; An and Lee, 2008; Wu et al., 2011). In addition, many other chemicals and particularly insecticides have already been tested for their ability to induce avoidance on earthworms. (De Silva and van Gestel, 2009; Owojori and Reinecke, 2009; Santos et al., 2011). Moreover, sub-lethal endpoints involving various biomarkers are also used, which include lysosomal membrane stability measured by neutral red retention time, as well as genetic effects assessed by comet assay (Lock and Janssen, 2002; Xiao et al., 2006). Besides, numerous studies have been carried out using earthworms in recent years, but they mostly focus on single insecticide (Spurgeon et al., 2003). Mixed insecticides are becoming increasingly popular in agricultural use for their high efficiency, convenience and rapid actions. It is well known that mixed insecticides generally cause significant synergistic toxic effects on both target species and beneficial species (Clark et al., 2002; Ahmad et al., 2009). Single-insecticide experiments fail to reflect field situations where multiple insecticides or insecticide mixtures are used (Zhou et al., 2011). Therefore, more studies on the long-term effects of insecticides on earthworms are needed for adequate ecological risk assessment. Furthermore, juvenile earthworms may be more sensitive to pollutants than adults (Zhou et al., 2008). Estimating ecotoxicological risk using toxicity data from adults and single-insecticide experiment may lead to underestimation of the effects of pollutants on soil invertebrate populations (van Straalen and Denneman, 1989). Data from juvenile earthworms experiments should therefore also be considered when proposing a safe environmental concentration for a specific insecticide (Booth and O'Halloran, 2001).

5. Conclusions

Toxicity of the selected insecticides to earthworms varies with the category of chemicals, as well as with the bioassay procedures followed. Result of contact filter paper toxicity bioassay does not always reflect the susceptibility of earthworms exposed under natural soil conditions except for those of neonicotinoids, which are the most toxic of all insecticides under both bioassay systems.

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