

Full Length Research Paper

Leaching patterns of fipronil in 3 kinds of soil in Hainan province, China

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Accepted 3 May, 2013

Fipronil, an insecticide for crop protection with good selectivity between insects and mammals, are toxic to plants and animals in wild. In this paper, the decay patterns of Fipronil in 3 kinds of arable layer soil in Hainan province were studied by means of simulated rain water leaching use tap water on the earthworms and duckweeds, respectively. The results showed that decays of Fipronil in different soils were high related with organic matter content in soil. The death rates of earthworms were 10% in up layer of clay loam (rice land) which organic matter content was 14.84 g/kg. After leaching for 14 days, Fipronil flowed into lower layer, death rates of earthworms was increased to 24%. The decreasing of death rate of earthworms were found in lower layer of three soils suggested that little Fipronil flowed into lower soils. Leaching water collected at 1 h from three soils was poisoned to cultured duckweeds which reduce chlorophyll contents and the maximal efficiency of PSII photochemistry (Fv/Fm). These studies confirmed that Fipronil was combined with organic matter at surface soil. It could not decay easily in field and will influence the crop which planted in Hainan province for long periods.

Key words: Fipronil, leaching, decay, organic matter, earthworms, duckweeds.

INTRODUCTION

The general pathways of pesticide decay in environment are volatilization, surface runoff, leaching, biological degradation by microbe, photolysis and absorption (Beltran et al., 1995). Fipronil was first developed by Rhone-Poulenc in France and patented under the US Patent. Fipronil was a broad spectrum insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the gamma-aminobutyric acid (GABA) receptor and glutamate-gated chloride channels (GluCl), components of the central nervous system (Zhao et al., 2003, 2004, 2005; Islam and Lynch, 2012; Murillo et al., 2011; Gold 2003; Tomoko et al., 2001). As a slow acting poison, Fipronil impacts highly toxic to fish, aquatic invertebrates, bees and

upland game birds (Stehr et al., 2006; Li et al., 2010). Fipronil applied to foliage partially photodegrades to form fipronil-desulfinyl. Fipronil and fipronil-desulfinyl are less volatile than water and can concentrate under field conditions. Fipronil was considering as a possible carcinogen and a potential ground water contaminate (Gan et al., 2012; Ou et al., 2006).

Before the fully banned of Fipronil in 2009 in China, it had been applied widely in rice and corn field about 5 tons per year in Hainan province alone. Leaching is one of the important indices for pesticides decay and especially useful in Hainan because from April to November, the typhoon seasons brought plenty of rain.

The leaching liquid on indicator organism can be used

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Table 1. The physicochemical properties of soils.

Contents	Unit	Latosolic red soil	Pond soil	Clay loam
Full N	g/kg	0.40±0.028	0.06±0.007	0.76±0.083
Instant P	mg/kg	6.04±0.89	0.36±0.041	107.4±10.89
Instant K	mg/kg	65.62±8.34	98.08±10.28	44.57±5.34
Exchanged K	cmol/kg	0.18±0.02	0.28±0.02	0.16±0.02
Exchanged Na	cmol/kg	0.06±0.01	0.07±0.01	0.11±0.02
Exchanged Ca	cmol/kg	1.52±0.13	0.69±0.08	6.23±0.74
Exchanged Mg	cmol/kg	0.24±0.02	0.60±0.06	0.33±0.04
Exchanged standard	cmol/kg	3.18±0.24	6.69±0.93	5.55±0.62
Organic matter	g/kg	8.6±0.75	4.11±0.62	14.84±2.31
Water content	%	1.4±0.31	2±0.32	1.2±0.18
Saturation water capacity	%	14.55±2.01	20.06±3.15	12.58±1.62
Organic matter percentage	%	0.65±0.07	0.44±0.06	0.90±0.09
pH	%	5.4±0.35	5.9±0.67	5.6±0.75

used as pre-warning index for the groundwater pollution which caused by running off of applied pesticide through rain and irrigation. Besides, the migration of pesticides in soil can be obtained by simulation test in doors (Shan et al., 1994; Beltran et al., 1995). The fates of Fipronil in tropical climatic conditions were thoroughly studied (Chopra et al., 2011; Kumar et al., 2012; Lao et al., 2010). However, the retaining, migration and transformation of Fipronil and its effect on environmental security in Hainan agricultural field and freshwater is not estimated. Two species, namely earthworm and duckweeds were used as indices according to the National Standard of China for a long time. The major benefits of earthworm activities to soil fertility are converting large pieces of organic matter (e.g. dead leaves) into rich humus, and thus improving soil fertility. When the worm excretes this in the form of casts which are deposited on the surface or deeper in the soil, minerals and plant nutrients are made available in an accessible form. If pesticides killed earthworm, the soil structure will be condensed, resulting in a multitude of channels which allow the processes of both aeration and drainage malfunction, even hardly for plants to grow. Duckweed is so common that could be found in every aquatic habitat, but it is more addicted to still than running water. In this study, the leaching patterns of Fipronil in three main soils in Hainan province were simulated tested indoors. Our results showed that Fipronil is likely binding with organic matter and hard to leaching away, which in turns will do damage to plants in Hainan province.

MATERIALS AND METHODS

Agents

Five percent of Fipronil suspending agent (hereafter 5% agent) and

95% Fipronil active compound (hereafter 95% agent) were purchased from Shangdong A and Fine Agrochemicals Co., Ltd. Ultrapure water was made from Milli-Q integral Pure/Ultrapure water production unit, USA. Na₂HPO₄, Chlorination acetylcholine standard and other agents were purchased from Sigma Co, Ltd. Germany.

Soil type

The soil samples were collected from Latosolic red soil (corn land), pond soil (river side), and CLAY loam (rice land) from Practice Teaching Base of Environment and Plant Protection College, Hainan University, Danzhou, Hainan province, China. The soil samples were naturally dried, removal impurities, and past 2 mm sieves in diameter. The physicochemical properties of these soils showed in Table 1 were determined according to Bao (2005).

Leaching test of Fipronil in three soils

The transparent plastic bottle with 9 cm in diameter and 30 cm height were used as soil container. The base end fixed with gauze and adhesive tape, placed two layer of filter paper, put in tested soil, shake to make their density. The natural cultivated horizons were exactly simulated. Four soil layers were filled from bottom 20 to 15 cm, 15 to 10 cm and 10 to 5 cm, superficial layer was filled 5 to 0 cm. After column were ready, pure water were flooded to make it same as saturation moisture capacity in field. Both 5% Fipronil suspending agent and 95% Fipronil active compound were made 0.009 g/L standard solution. 10 ml and 2000 mg/L solution was mixed with 120 g wash sand, and then applied on the soil surface (Shan et al., 1994). Each column was added with 340 ml tap water in 3 h, which equal to 200 mm water precipitation.

Duckweeds cultivation and measurement of chlorophyll contents

The diluted solutions at 1, 2, and 3 h were collected to cultivated 200 duckweeds, the growth conditions and death number were recorded, respectively. Chlorophyll was extracted with 80% acetone from 0.1 g samples of duckweeds. The extract was measured spectrophotometrically at 475, 645, and 663 nm. Specific

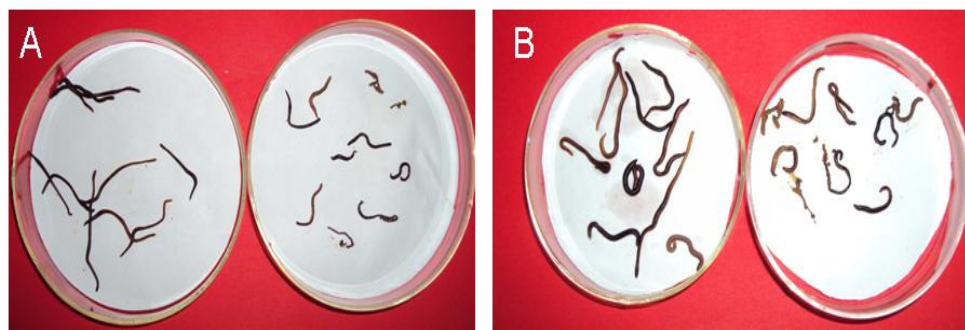


Figure 1. The death rate of earthworms in 3 soils leaching with Fipronil (A) Earthworms died in the first layer of clay soil treated with 5% Fipronil; (B) Earthworms died in the first layer of pond soil treated 95% Fipronil.

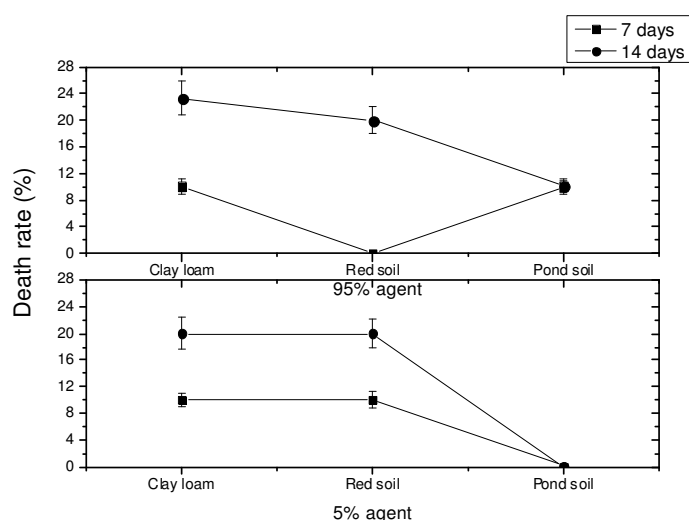


Figure 2. The death rate of earthworms in 3 soils leaching with Fipronil.

chlorophyll contents and total chlorophyll were determined according to the method of Wang et al. (2006).

Measurements of chlorophyll fluorescence

Modulated Chl fluorescence measurements were made in duckweed leaves at midday with a PAM-2000 portable fluorometer (Walz, Effeltrich, Germany) connected to a notebook computer with data acquisition software (DA-2000, Heinz, Walz). The experimental protocol of Demmig-Adams et al. (1996) was essentially followed.

Earthworms cultivation and measurement of acetylcholine

The leaching soil columns were separated into 4 equality parts with 5 cm depth each as mentioned above. Each soil cuts were put in 10 earthworms, observation the growth situation and death rate in 7 days intervals, the Chlorination acetylcholine in earthworms were measured in 14 days according to National Standard of China (GB/T 21809-2008[M]). Chlorination acetylcholine was measured by react with FeCl_3 in 1/15 mol/L PBS, pH7.2 with GE Ultrospec™ 2100 pro UV/Visible Spectrophotometer, USA at 540 nm according

to Mu (1994 and Kehr et al., 1998).

Determination of zymogen

The zymogen were measured by biurets agent, namely 1.5 g CuSO_4 and 6 g D-Potassium sodium tartrate dissolved in 300 ml and 10% NaOH, add water to 1000 ml. 0.2 ml samples was added 0.8 ml pure water and 4 ml biurets agent, heated in a water oven at 25°C for 30 min, then measured at 540 nm with GE Ultrospec™ 2100 pro UV/Visible Spectrophotometer, USA.

Statistical analysis

All experiments were repeated at least 3 times, results were means \pm SE. Figures were made by origin 8.0 software.

RESULTS

The death rate of earthworms in 3 soils leaching of Fipronil

As showed in Figures 1 and 2, the organic matter content in three kinds of soil had high relationship with death rates of earthworms. In the clay loam soil, which organic matter content was 14.84 g/kg, showed highest death rate at 7 day and 14 day. whilst, pond soil, which organic matter content was 4.11 g/kg, only 95% active compound Fipronil could led 10% death at 14 days. The 95% active compound Fipronil showed more toxic than 5% agent to earthworms. The analysis of death rates of earthworms showed that more earthworms died in the shallow soil in rice field and corn field, on the contrast, more death rates occurred in deep layer in soil from pond soil (river side). These results indicated that Fipronil was likely combined with organic matter, and hard to leaching by water. The contaminated Fipronil in field in Hainan province was harmful to animal in soil.

The death rates of earthworms in different layer at 14 days were showed in Figure 3. Similar decrease patterns were found in clay loam and red soil, which the organic

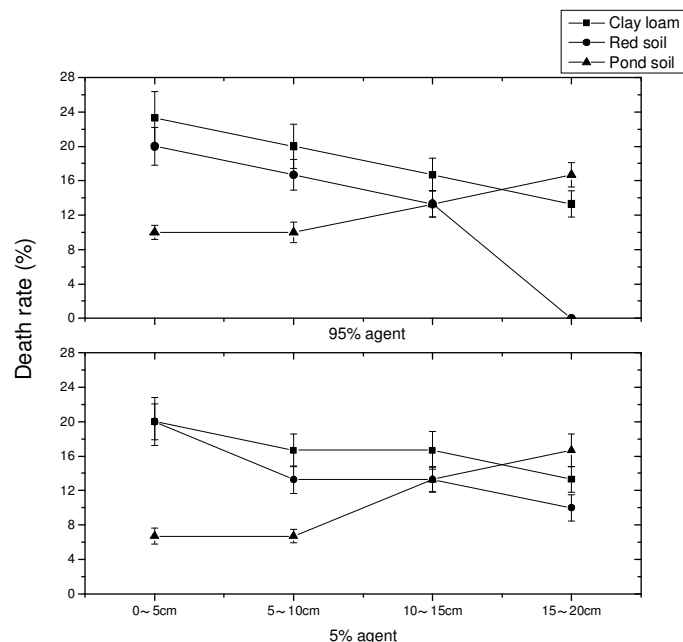


Figure 3. The death rate of earthworms at 14-day in 4 depths in 3 soils leaching with Fipronil.

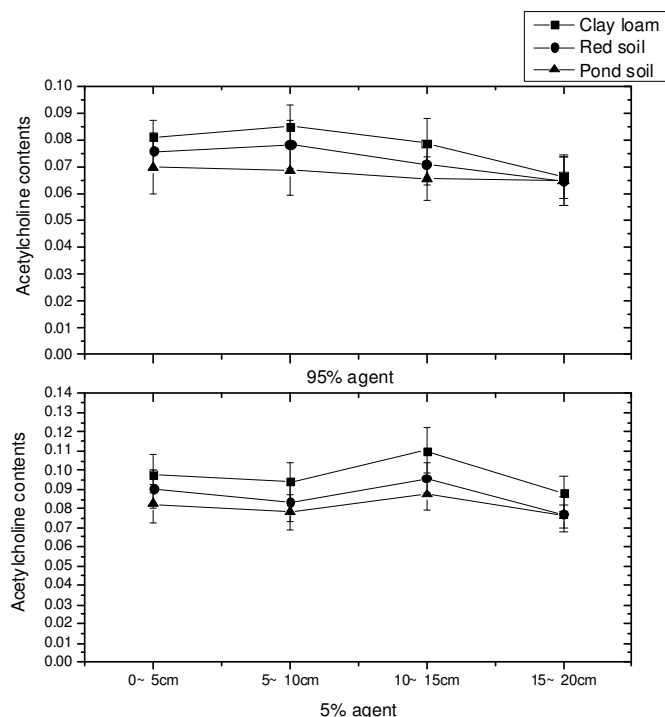


Figure 4. Acetylcholine contents in earthworms treated with Fipronil 5% agent and 95% agent in 3 soils.

matter contents were high than pond soil. The reduction death rates of earthworms suggested that the Fipronil contents were decreased along with the depth of soil.

Leaching by tap water only diluted little Fipronil from surface to deeper soil in clay loam and red soil. Pond soil, with lowest organic matter content, the Fipronil was diluted by water easily and more and more earthworms died in deep soils.

Figure 4 were the cetylcholine contents of earthworms at different layer in three kinds of soil. Similar patterns were found in 3 soils. After 14 days of water leaching, soils treated with 5% agent had highest cetylcholine contents of earthworms at 10~15 cm layer soil. Soils treated with 95% agent had highest cetylcholine contents of earthworms at 5~10 cm layer soil. These results could not consist with death rates of earthworms. The chemical compound acetylcholine (ACh) is a neurotransmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans. High contents of cetylcholine contents in earthworms suggested that Fipronil was toxic to earthworms at shallow soil not more than 15 cm.

Figure 5 were the zymogen contents of earthworms at different layer in three kinds of soil. Same patterns were found in three soils. After 14 days of water leaching, soils treated with 5% agent and 95% agent both had highest zymogen contents of earthworms at 0 to 5 cm layer soil. A zymogen (or proenzyme) is an inactive enzyme precursor. A zymogen requires a biochemical change (such as a hydrolysis reaction revealing the active site, or changing the configuration to reveal the active site) for it to become an active enzyme. The biochemical change usually occurs in a lysosome where a specific part of the precursor enzyme is cleaved in order to activate it. The amino acid chain that is released upon activation is called the activation peptide. The high-low-high-low patterns of zymogen were same as those of death rates of earthworms. The decrease of contents of zymogen in earthworms suggested that Fipronil was toxic to earthworms.

The collected leaching water at 1, 2, and 3 h was used to culture duckweeds in order to confirm Fipronil leaching effects. The death rates, chlorophyll contents and chlorophyll fluorescence kinetics parameters were analyzed at 7 days after duckweeds cultured. Results showed that the 95% agent showed more toxic than 5% agent to duckweeds. The Fipronil contents in leaching water were decreased along with leaching time, and negative relationship with organic matter contents. The Fipronil in pond soil leached faster than those of clay loam and red soil, which lead to higher death rate and sharper decrease of death rates. On the other hand, the chlorophyll contents which showed the photosynthetic activities of duckweeds, showed reversed data. These results indicated that the Fipronil was not likely leaching form clay loam as from pond soil (Figure 6 and 7).

As seen from Table 2, the maximal efficiency of PSII photochemistry (Fv/Fm) of duckweeds cultured in 2 and 3 h leaching water were increase 7.6 and 18.4% comparing to duckweeds cultured in 1 h leaching water. The electron

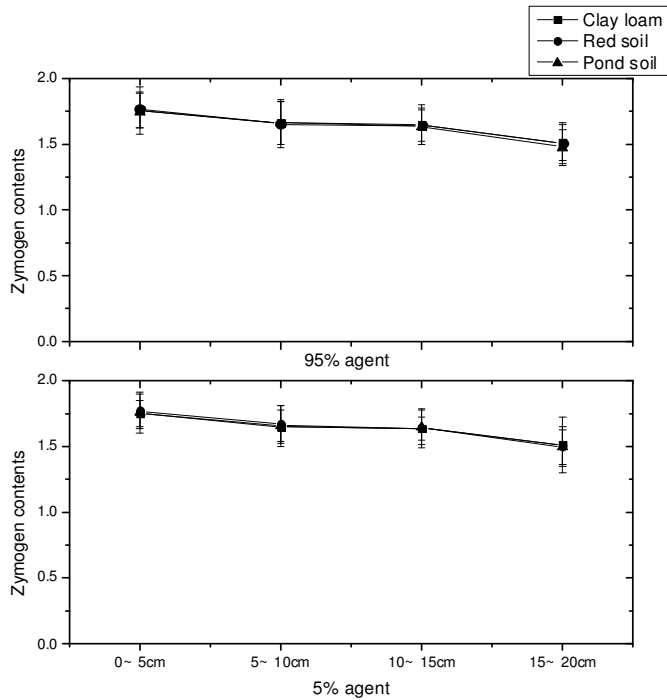


Figure 5. Zymogen contents in earthworms treated with Fipronil 5% agent and 95% agent in 3 soils.

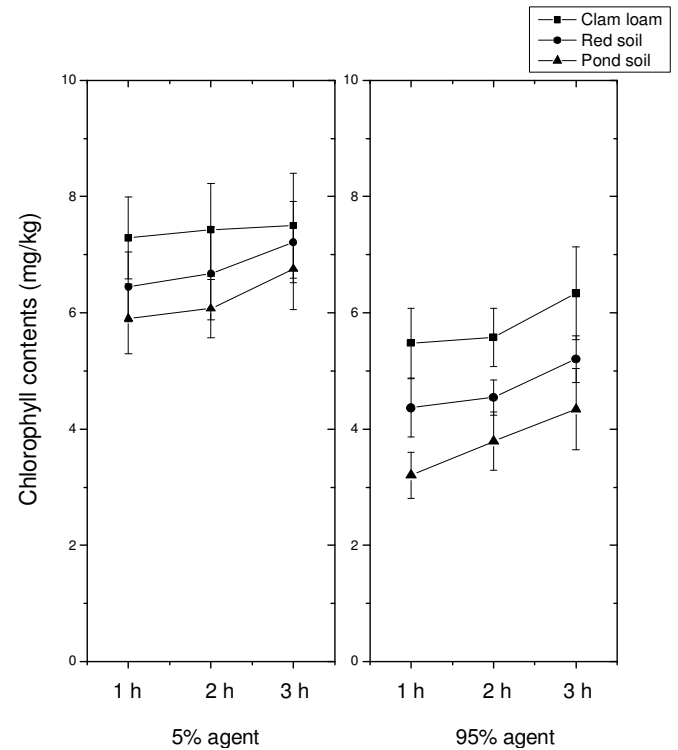


Figure 7. Chlorophyll contents of Duckweeds treated with leaching water from Fipronil 5% agent and 95% agent in 3 soils.

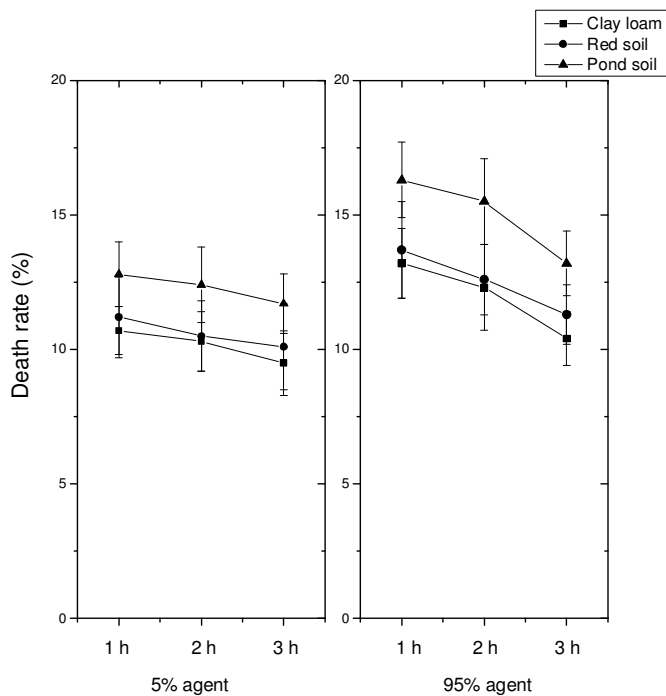


Figure 6. Death rates of Duckweeds treated with leaching water from Fipronil 5% agent and 95% agent in 3 soils.

transport rate (ETR), photochemical quenching (qP), and the actual photochemical efficiency of PS II (Φ PS II) of

duckweeds cultured in 2 and 3 h leaching water were also increased compared to duckweeds cultured in 1 h leaching water, respectively. However, the non-photochemical quenching (qN) which reflects the process competing with PS II photochemistry for absorbed excitation energy (Campbell et al., 1998) decreased 24.7 and 23.2% ($P < 0.01$) in duckweeds cultured in 2 and 3 h leaching water, respectively. These indicated that after the photosystem structures breakdown, the light energy absorbed by chloroplast could not transport to PSI but dissipated as heat through Non-photochemical quenching (NPQ) in duckweeds when damaged by Fipronil in 1 h leaching water. The decrease of Fipronil in leaching water in 2 and 3 h helped duckweeds resume its normal photosynthesis ability.

DISCUSSION

The biodegrading of fipronil and its major metabolites fipronil sulfide (MB 45950), fipronil-desulfinyl (MB 46513) and fipronil sulfone (MB 46136) were studied in grape leaves and soil (Mohapatra et al., 2010). Fipronil was easier degraded than its metabolites in soils (Shuai et al., 2012; Spomer and Kamble, 2010). Our results found that Fipronil as an effective pesticides would not cause earthworms death resulting in the reduce densities of earthworms in deep soil. However, it is likely binding to

Table 2. PSII photochemistry of duckweeds treated with leaching water form Fipronil 5% agent and 95% agent in 3 soils.

Leaching water time (h)	Fv/Fm	ETR	qP	NPQ	ΦPSII
1	0.65±0.02	12.9±0.02	0.61±0.02	0.81±0.01	0.33±0.02
2	0.70±0.01	14.0±0.64	0.74±0.04	0.61±0.05	0.41±0.01
3	0.77±0.02	20.1±0.82	0.76±0.05	0.63±0.02	0.46±0.02

organic matter. The more organic contents in soil, the more risk of Fipronil contamination. The different death rates along with the changes in cetylcholine and zymogen contents improved that Fipronil could do continuous toxic to earthworms, which in turn, harmful to oil. The same results of earthworms and duckweeds suggested that they could be used as bio-indicator for Fipronil in field of Hainan province. The leaching test showed that Fipronil is chronic pesticide which binds with organic matter. It was harmful to plants that grow in soil surface and earthworms in shallow soil. Leaching does not reduce the Fipronil contents in soil surface but need long time for them to decay. Earthworms and duckweeds could be use as indices for potential toxic of Fipronil in soils of Hainan province.

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