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JOURNAL OF ENVIRONMENTAL SCIENCES
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Environmental behaviors of phoxim with two formulations in bamboo forest under soil surface mulching

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ARTICLE INFO

Article history:
Received 27 November 2014
Revised 15 March 2015
Accepted 16 March 2015
Available online 17 June 2015

Keywords:
Phoxim
Formulation
Residue
Bamboo
Soil surface mulching

ABSTRACT

Phoxim (emulsifiable concentrate (EC) and granules (G)) has been widely used in bamboo forests. The persistence and magnitude of phoxim residues in the crop and soil must be investigated to ensure human and environmental safety. The environmental behaviors of the two formulations were investigated in a bamboo forest under soil surface mulching conditions (CP) and non-covered cultivation conditions (NCP). The half-lives of phoxim in soil under the two conditions in soil were 4.1–6.2 days (EC) and 31.5–49.5 days (G), respectively. Phoxim in EC could be leached from the topsoil into the subsoil. A minimized leaching effect was observed for G under NCP. Inversely, an enhanced leaching effect was observed for G under CP. The G formulation resulted in more parent compound (in bamboo shoots) and metabolite (in soil) residues of phoxim than in the case of EC, especially under CP conditions. In addition, the intensity and duration of the formulation effect on soil pH adjustment from G were more obvious than that from EC. Results showed that the environmental behaviors (distribution, degradation, residue) of phoxim in the bamboo forest were significantly influenced by the type of formulation. The prolongation effect from phoxim G might cause persistence and long-term environmental risk. However, bamboo shoot consumption could be considered relatively safe after applying the recommended dose of the two phoxim formulations.

Introduction

Bamboo shoot is one of the most popular types of non-timber forest product in Asian countries, and China is the world's largest producer and exporter. The bamboo shoots are exported to the USA, EU and Japan in large quantities every year. Although various practices are conducted to minimize the damage caused by insects during bamboo growth, the most effective strategy is still the application of insecticides. Phoxim, an organophosphorus pesticide with relatively high efficiency and low toxicity, is frequently employed in soil and foliage. In China, phoxim is applied to control underground pests in bamboo forests and for other plants, with application of up to 1000 tons per year (Huang et al., 2013). The emulsifiable concentrate (EC) of phoxim has been commonly used for many years. However, excessive application of insecticides will lead to great risk to the environment and human health (Wang et al., 2012). The use of a controlled-release formulation (CRF) is one of the best strategies to reduce the consumption of insecticide, and minimize its negative impact on the environment. CRFs produce a gradual and controllable release of insecticide over time, which allows a lower concentration of active ingredients to act effectively. In recent years, granules (G) of phoxim have also been applied in bamboo forests, especially for soil surface mulching cultivation.

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http://dx.doi.org/10.1016/j.jes.2015.03.019

During bamboo shoot production, soil surface mulching (bamboo leaf, straw and/or rice chaff are spread on the soil to increase the soil temperature and bring forward the harvest time about 1 month) emerged as an important industry practice in the 90s of the last century and has been becoming more and more popular. The food safety issue caused by contamination by pesticide residues arouses more concerns. Therefore, good knowledge of the pesticide fate in foodstuffs will benefit from properly assessing the human exposure and the environmental risk. However, the available studies on the environmental fate of phoxim are either too old to keep up with the times (Hohl and Barz, 1995; Mason and Meloan, 1976), or seldom focused on the influence of different formulations on its dissipation and residues in the field (Lin et al., 2011).

The different toxic effects induced by diverse pesticide formulations had been confirmed for bovine culture cells (Holeckova et al., 2013), microorganisms (Joly et al., 2013) and dermal exposure of pesticide operators (Berenstein et al., 2014). In addition, some previous works have focused on the influence of formulation on mobility (Wlodarczyk, 2014), leaching (Potter et al., 2010) and transport (Paradelo et al., 2014) of pesticides in soil under laboratory and field conditions. For example, the use of alginate CRF (alginate capsules) reduced the vertical mobility of metazachlor into the soil layer in comparison with the suspension concentrate (SC) formulation in soil column tests (Wlodarczyk, 2014). The influence of CRFs on pesticide loss by leaching has been studied using field-deployed lysimeters together with rainfall simulation (Potter et al., 2010); the authors reported that the use of a CRF with a clay-alginate polymer can decrease metolachor leaching. Furthermore, different pesticide formulations often result in the same active ingredients presenting different half-lives and terminal residues in plants and soil (Cao et al., 2005; Zhou et al., 2014). The degradation rate of chlorfenapyr nanoformulation was faster than that of SC, and the residue of the former was also less in soil (Cao et al., 2005). Many scientists concluded that the additives in the formulations were responsible for the differences (Paramasivam and Chandrasekaran, 2013; Sharma et al., 2011). In field and laboratory conditions, compared with the results from metazachlor applied alone, the addition of an adjuvant caused an increase of metazachlor residues at harvest time. Adjuvant addition caused a slowdown of leaching of metazachlor into the soil profile. Moreover, the addition of oil and surfactant adjuvants slowed down the degradation of metazachlor in soils (Kucharski and Sadowski, 2011).

However, few reports were taken into account the probable effects of different cultivation processes (field, greenhouse, soil surface mulching, *etc.*) on the effects of pesticide formulations. In recent years, some scientists found that the cultivation process had a notable effect on pesticide dissipation. For example, the dissipation rate of cyprodinil under greenhouse conditions was much faster than for field conditions, either in strawberries or in soil (Liu et al., 2011). The half-lives of chlorpyrifos in greenhouse cucumbers (Liang et al., 2012) and rice (field conditions) (Zhang et al., 2012) were 1.60 days and 4.28 days, respectively. In our previous work (Liu et al., 2014), the degradation and metabolism of chlorpyrifos in a bamboo forest under two conditions (with and without soil surface mulching) were investigated. Results indicated that the soil surface mulching had a notable effect on the degradation, leaching, metabolism of chlorpyrifos granules. To the best of our knowledge, there is no information available about the behaviors (degradation, distribution, residues) of phoxim in bamboo forests. In particular, the influence of EC and G formulations on phoxim behaviors under soil surface mulching is not yet clear. The objective of this study was to evaluate the effect of formulation on phoxim residues in a bamboo forest under soil surface mulching conditions, by comparing the phoxim dissipation rates and terminal residues between EC and G formulations. This data would be helpful for the establishment of Maximum Residue Levels (MRLs) for phoxim EC and G products. The present work was also designed to investigate the residues of the two phoxim formulations in bamboo shoots and soil so as to determine the acceptable interval between spraying and harvest, which would be beneficial to the safe use of this plant and the reduction of any consumer health risks.

1. Materials and methods

1.1. Reagents and solutions

Pesticide analytical standards were purchased from the National Information Center for Certified Reference Materials (Beijing, China), with certified quality. Individual pesticide stock solutions (100 mg/L) were prepared in methanol and stored at −20°C. Then, a series of dilutions containing the mixture of standards were prepared (10 mg/L) in methanol. HPLC-grade acetonitrile and methanol were obtained from Merck (Merck, Darmstadt, Germany). A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain HPLC-grade water for the analyses. Other solvents were from Shanghai Sanying Chemical Reagents (Shanghai, China), with pesticide residue analysis quality.

1.2. HPLC-MS/MS

The LC system consists of a high performance liquid chromatograph (Waters, Milford, MA, USA) with a HSS T3 column (5 μm, 100 mm × 2.1 mm, *i.e.*, Waters). The mobile phase involving solvent A (0.05% formic acid, in water) and solvent B (acetonitrile) was eluted using a gradient program as follows: 80:20 of A:B (initial), 10%–90% A with 90%–10% B (0–5 min), 10:90 A:B (5–10 min), 10%–80% A with 90%–20% B (10–14 min),80:20 A:B (14–15 min). A subsequent re-equilibration time (3 min) was allowed between injections. The flow rate was 0.3 mL/min and the injection volume was 10 μL. The column and sample temperatures were maintained at 35°C.

MS/MS was performed on a Waters Quattro Premier triple-quadruple mass spectrometer equipped with an ESI source (Waters, Milford, MA, USA). MS/MS detection was performed in positive ion mode for phoxim and in positive mode for chlorphoxim separately. The monitoring conditions were optimized for the target compounds. Acquisition parameters were as follows: capillary voltage 3.5 kV, cone voltage 45 V, source block temperature 80°C, cone gas 50 L/hr, desolvation temperature 450°C, desolvation gas (nitrogen gas) 550 L/h, respectively. 298.9 (*m*/*z*) was selected as the precursor ion for phoxim, and its quantitative and qualitative product ions were 76.8 (m/z) and 129.0 (m/z), respectively, when the collision energies were both 22 V. As for chlorphoxim, 332.6/162.7 was selected as quantification ion transition, and 332.6/124.3 as confirmatory ion transition, with all the collision energies at 12 V. Multi-reaction monitoring mode (MRM) was selected as the scan mode. Under the described conditions, the retention times of phoxim and chlorphoxim were approximately 8.9 min and 9.0 min, respectively.

1.3. Pesticide analysis

A portion (25.0 g) of prehomogenized sample (bamboo shoot) was weighed in a 250 mL glass beaker. The sample was extracted with 50 mL of acetonitrile by homogenization with a high speed blender (Ultra-Turrax T18, IKA, Staufen, Germany) for 2 min. After the addition of 5 g NaCl, each mixture was shaken intensively for 1 min and centrifuged for 5 min at 8000 rpm. An aliquot of the organic phase (25.0 mL) was transferred and concentrated by a rotary evaporator at 40°C to near dryness, then dissolved in 1.0 mL methanol. Prior to analysis, the methanolic analyte was filtered through a 0.22 μm PTFE filter (Millipore, Milford, MA, USA).

A portion of soil sample (sieved through a 2 mm mesh, 2.0 g) was mixed with 1 mL distilled water for 5 min. Then methanol (5 mL) was added and vortexed for 2 min, and the sample was centrifuged for 5 min (4000 r/min). The superstratum (2.0 mL) was transferred and filtered through a 0.22 μm PTFE filter. The limit of detection (LOD) of analysis was 0.10 mg/kg for soil and 0.005 mg/kg for bamboo shoot, respectively.

1.4. Field experiments and sampling procedure

Soil pH was measured in water with the soil/water ratio of 1:5 (w/V) (Liu et al., 2010). The trial field was located in Linan, Zhejiang Province, China. The recommended dosages for the two formulations are 120 kg/ha (3% phoxim G, Tianyi Agriculture Chemistry Company, Zhejiang, China) and 1.13 kg/ha (40% phoxim EC, Xianlong Chemistry Company, Hubei, China), respectively. The field experiment was started on Nov 26, 2013. After the pesticide application, the surface soil was dug up to mix uniformly. On the next day, compound fertilizer was applied by hand (1000 kg/ha), and the soil surface was covered by bamboo leaf (20 cm in thickness). Representative soil samples were collected by random sampling (10 samples were mixed to obtain one representative sample for each sampling time). Phoxim and its metabolic residues were measured using the method described above. The soil (0–20 cm) was divided into three layers (0–5 cm, 5–10 cm and 10–20 cm) and collected (1 kg). Samples were collected randomly from each plot. The bamboo shoot sample (1 kg) was chopped and divided into two samples.

1.5. Data analysis

To determine the kinetics of the degradation, plots of concentration against time were made, and an exponential regression analysis (first-order rate equation) was then performed on each set of data.

$$C = C_0 e^{-kt}$$

where, C_0 and C_t represent the concentration of pesticide at initial and time t, and k is the rate constant.

2. Results and discussion

2.1. Effect of formulation on the behaviors of phoxim in soil under non-surface mulching condition (NCP)

The aims of CRFs are to diminish the active ingredient costs, to allow the release of the agent to the target at a controlled rate, and to maintain its concentration in the system within an optimum limit, over a specified period of time. These properties provide great specificity, minimizing the adverse effects and optimizing effectiveness (Dubey et al., 2011). In order to investigate the behaviors of phoxim in soil, the soil layer (0–20 cm, the cultivation soil layer for bamboo root growth) was divided into three parts: top layer (Tl, 0–5 cm), middle layer (Ml, 5–10 cm) and bottom layer (Bl, 10–20 cm). The results are shown in Fig. 1. Clearly, the dissipation rate of the CRF formulation (G) was slower than that of EC. After 29 days, phoxim EC in Tl was degraded to a level below the method quantification limit (0.10 mg/kg). However, phoxim G in Tl was even detected at 0.53 mg/kg in 121 days. The degradation dynamics of EC in Tl could be fitted to a first-order exponential decay model. The equation was:

$$C_t = 5.788e^{-0.112t} \quad \left(R^2 = 0.919\right),$$

the half-life was 6.2 days. Nevertheless, the degradation dynamics of G in Tl could not be fitted to any decay model. Before 22 days, phoxim was slowly released from the formulation and concentrated in Tl; then the dissipation curve pattern in Tl showed a subsequent descent. The slow-release effect was also found in our previous work for chlorpyrifos granules (Liu et al., 2014). After 22 days, the degradation equation of phoxim G in Tl was:

$$C_t = 5.604e^{-0.022t}\left(R^2 = 0.939\right),$$

the half-life was 31.5 days. Compared with the obtained half-lives, on one hand, the G formulation was notably more persistent in soil than EC, which also meant that the former had a longer period for pest control (bioefficacy). On the other hand, in a well-planned management system, pesticide should effectively control pests with little or no adverse environmental effects. Otherwise, the high amounts of pesticides used in agronomic practices would lead to the existence of polluted groundwater sources, mainly through leaching (Fenoll et al., 2014). In this study, after a rainfall at 20 days, phoxim was transported from the top soil to the subsoil. For the EC test, the phoxim concentration in Ml ranged from 0.16 mg/kg to 0.59 mg/kg during 15–89 days, and the residues in Bl were lower, with the highest residue of 0.36 mg/kg in 38 days. For the G test, the effect of rainfall on the pesticide leaching was more pronounced, and phoxim concentrations in Ml ranged from 0.41 mg/kg to 1.12 mg/kg during 22–89 days. However, there was no phoxim residue found in Bl, which suggested the leaching of phoxim decreased in the G formulation. The results proved that the CRF could reduce the pesticide leaching in soil, which was also reported by

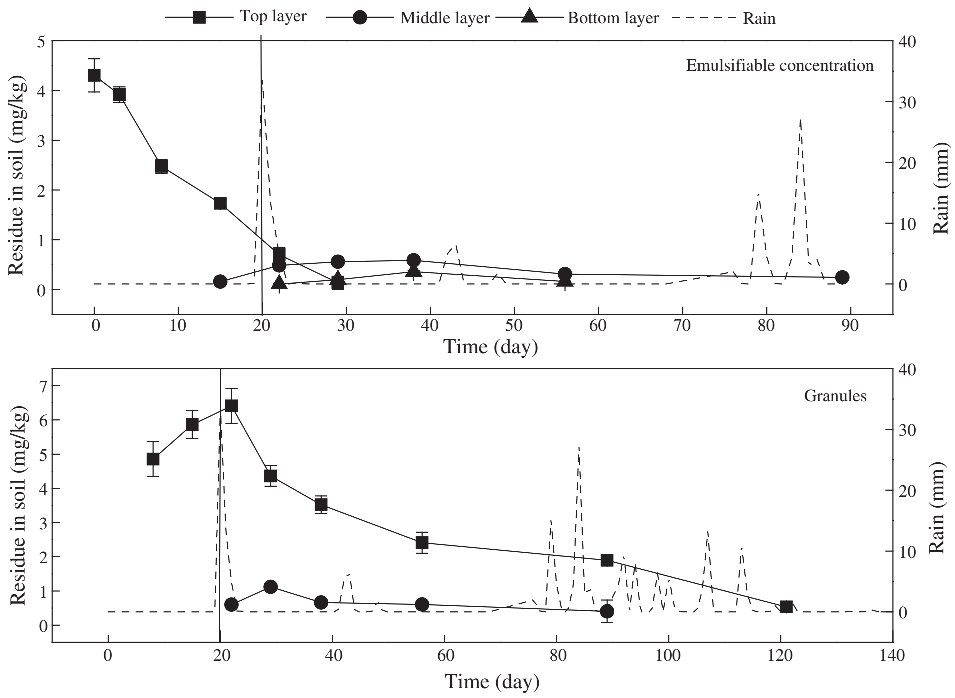

Fig. 1 – Distributions of phoxim formulations in different layers of soil under non-surface mulching condition.

Undabeytia et al. (2010). A field experiment was performed to determine alachlor leaching and bioefficacy in a commercial formulation (SC) and two CRFs (microencapsulation (MC), phosphatidylcholine–montmorillonite complexes (PCs)). Compared with SC, leaching to the 20- to 30-cm depth was reduced by 33% for MC and 25% for PC-clay formulations, respectively.

2.2. Effect of formulation on the behaviors of phoxim in soil under surface mulching conditions (CP)

The most important impact of soil surface mulching is to increase or maintain the soil temperature, and temperature is one of the important factors controlling pesticide behaviors in

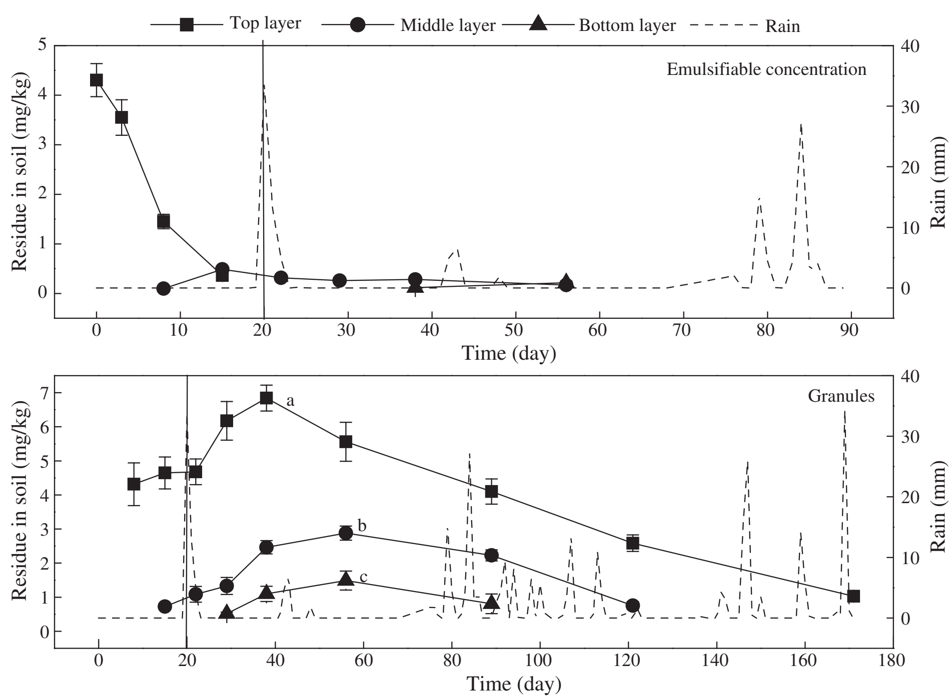

Fig. 2 – Distributions of phoxim formulations in different layers of soil under soil surface mulching condition.

the environment. Normally, high temperature could promote the hydrolysis, adsorption and degradation of pesticide (Liu et al., 2010). In the EC test under CP conditions, the degradation of phoxim followed this rule (Fig. 2). The degradation dynamic equation of EC in Tl was:

$$C_t = 5.103e^{-0.171t}\left(R^2 = 0.982\right),$$

the half-life was 4.1 days. The results showed that phoxim EC degraded faster in CP than that in NCP. This demonstrated that the cultivation process could influence the degradation of pesticide (non-CRF), which was also reported for phoxim (EC) in soil under greenhouse conditions, with its half-life measured at 3.5 days (Wang et al., 2011). However, phoxim G still presented a slow-release effect first, and then a subsequent decline. The release of phoxim reached the peak in 38 days, then the subsequent degradation equation of phoxim G in Tl was:

$$C_t = 7.438e^{-0.014t}\left(R^2 = 0.979\right),$$

with the half-life of 49.5 days. Compared with the half-life of phoxim G in NCP (31.5 days), the degradation of phoxim was prolonged by the surface mulching (CP) process. In addition, the period of slow release in CP was longer, and the released quantity of phoxim was higher (the peak was 6.84 mg/kg at 38 days). For NCP, the release of phoxim reached the peak in 22 days, with the concentration of 6.41 mg/kg. The prolongation of phoxim degradation in CP might result from the mulching material (bamboo leaf). The leaves could intercept the rainfall, which resulted in a gentle release process. Moreover, the leaching of phoxim in CP soil was also influenced by the interception by mulching material. The leaching effect was delayed after rainfall at 20 dac. The peak concentrations of phoxim from leaching in Ml and Bl were found at 56 days (b, c point). The corresponding value from the NCP test of Ml was 29 days, and none of the pesticide could be transported into Bl. The comparison showed that the leaching effect of phoxim G under CP conditions could not only be delayed, but also enhanced. A previous study (Goldreich et al., 2011) tested the effect of soil wetting and drying cycles (WDCs) on metolachlor fate (desorption, leaching, and weed control) applied as the CRF and as the commercial formulation (EC). The tests were conducted in batch experiments using Teflon centrifuge tubes. The results indicated that WDCs increased metolachor release from soil, which was similar to our results. However, enhanced metolachlor leaching under WDCs was observed for EC. In contrast to our results, when metolachlor was applied as a CRF, leaching was suppressed and not affected by WDCs. In this study, the enhanced phoxim leaching under CP was observed for G. The different performance might be induced by the mulching material, which kept the moisture and temperature of the soil at a relatively stable level. So the leaching effect took place slowly over a long period, and more pesticide had sufficient time to slowly leach from the topsoil to the subsoil. This prolonged leaching process could not take place with the drastic changes of soil resulting from WDCs.

2.3. *Effect of formulation on the terminal residue of phoxim in bamboo shoots under NCP/CP conditions*

One of the major disadvantages of pesticide use is that the residues may remain in foods and exceed the MRLs. This problem is being viewed seriously by international organizations, such as the United States Environmental Protection Agency (USEPA), the Codex Alimentarius Commission, WHO and FAO of the United Nations. Therefore, the terminal residue of pesticide in the edible part is considered the most important assessment for its risk. In this work, the bamboo shoot samples (edible part, with average length 15–20 cm) were divided into two parts: upper part (10–15 cm length) and bottom part (5 cm). The residues of phoxim in different samples under the two conditions are presented in Table 1. Phoxim could be detected in the bamboo shoots of 89–91 days for the treatment of G + CP, with the concentrations of 6.3–11.2 μg/kg. However, for the treatment of EC + CP, only small amounts of phoxim could be detected in the bottom part of bamboo shoots. After 97 days, all the concentrations of phoxim in the bamboo shoots were below the LOD of the analytical method (5 μg/kg). Compared with the EC formulation, the G formulation might result in more pesticide residues in bamboo shoots. Some previous works on formulation effects under field conditions indicated that the different adjuvants in the formulations could be the reason for the formulation effect on pesticide residue. The study of rotenone in suspension concentrate (SC) and water dispersible granule (WDG) forms under field conditions showed that formulation had a significant effect on the terminal residue in cabbage (Zhou et al., 2014). The formulation effect on pesticide residue was due to the different adjuvants present. Adjuvants used in SC and WDG formulations were very different in that there was more surfactant in SC. Formulation type affected the initial concentrations and finally led to different terminal residues in cabbage. All the previous works focused on the formulation effect under field conditions, and the pesticides were directly applied onto the edible agricultural products. However, the pesticide was directly applied onto the soil surface in the bamboo forest to control underground insects. The only pathway for pesticide entering into the bamboo shoots was through absorption. So the possible reason for the formulation effect in this study might be from the long-lasting presence of phoxim in soil, so that phoxim could have a long time to be absorbed or permeated into the bamboo shoots. Moreover, there was no remarkable difference between the two formulations under NCP conditions (all below the LOD). This performance might have resulted from the harvest time for NCP, which is almost 30 days behind that for CP, and hence the total quantity of phoxim residue in soil was relatively less in that period.

2.4. *Effect of formulation on the metabolism of phoxim in the bamboo forest under NCP/CP conditions*

Organophosphorus pesticides (OPs) are of great environmental concern due to their widespread use over the past several decades and potential toxic effects to non-target organisms, primarily on the nervous system of animals. Moreover, some metabolites of OPs in the environment also have the same toxic effects. Therefore, to fully understand OPs' impact on the environment, we need to investigate the impacts caused by both the parent pesticides and their metabolites. The investigation of phoxim's metabolism in crops began 20 years ago. Hohl and Barz (1995) reported four metabolites (oxime, primary amine, N-malonate, and N-malonic

Table 1 – Residue levels of phoxim in bamboo shoots after treatment with different formulations under two conditions (μg/kg).

Application time (day)	Treatment with G + CP		Treatment with G + NCP		Treatment with EC + CP		Treatment with EC + NCP	
	Upper	Bottom	Upper	Bottom	Upper	Bottom	Upper	Bottom
89	8.5 ± 1.3	11.2 ± 1.5			<LOD	6.5 ± 0.7		
91	6.3 ± 0.6	9.1 ± 1.2			<LOD	5.2 ± 0.9		
93	<LOD	5.5 ± 0.9			<LOD	<LOD		
97–112	<LOD	<LOD			<LOD	<LOD		
121–142			<LOD	<LOD			<LOD	<LOD

G: granules; EC: emulsifiable concentrate.
CP: soil surface mulching conditions; NCP: non-covered cultivation conditions.
Data are presented as mean ± SD. The limit of detection (LOD) of analysis was 0.10 mg/kg for soil and 0.005 mg/kg for bamboo shoot, respectively.

acid conjugate of phenylacetonitrileamine) in plants and the cell suspension cultures of soybeans. Five intermediates were identified for the degradation of phoxim in river water by the aid of HPLC–MS/MS (Lin et al., 2011). In this work, HPLC–MS/MS was also used for the investigation of phoxim metabolism in the bamboo forest. Chlorphoxim was detected in the soil samples (Fig. 3), and the results can be seen in Table 2. The residues of chlorphoxim ranged from 132.2 to 536.7 μg/kg in the topsoil (Tl) during 38–121 days in the treatment of G + CP. For formulation G, more chlorphoxim could be detected in the soil of Tl under CP conditions. However, the comparison was reversed for the EC formulation, where more chlorphoxim was found in the soil samples under NCP conditions. The difference might be caused by the notable slow-release effect from G under CP conditions. Phoxim was slowly released from G, and the metabolism process correspondingly lasted for an extended period. However, phoxim could be degraded rapidly from EC under CP/NCP conditions, and the metabolite (chlorphoxim) also appeared quickly. Because chlorphoxim (logP = 5.03) is more hydrophobic than its parent (logP = 3.38), its leaching is more difficult. As a result, no metabolites could be found in almost all of the subsoil samples (Ml and Bl, except for G + CP at 89 days). For bamboo shoots, there were no chlorphoxim residues detected. Chlorphoxim was fixed in the topsoil, which made it difficult to absorb by the bamboo stem in the subsoil. Besides, there were no other intermediates detected in the soil and bamboo shoot samples. Compared with the other results for phoxim metabolism mentioned above, various metabolites could be found in different plants or the environment. The structure of the metabolite for phoxim was determined by the matrix (plant, soil, water, *etc.*), but the quantity of metabolite residue was influenced by the formulation and cultivation style.

2.5. *Effect of formulation on the pH of soil under NCP/CP conditions*

The pH of soil is known to play a key role in pesticide behaviors (degradation, adsorption, leaching, *etc.*). The degradation rate of several pesticides has been found to be related to pH (Rodrigues et al., 2013). In a study investigating the effect of two different formulations on the residue of rotenone in cabbage and soil, the adjuvants in SC or WDG formulations were postulated as the key factor in the changes of soil properties, such as pH and structure of soil particles. All these factors eventually lead to different terminal residues in soil (Zhou et al., 2014). In our work, the hypothesis was confirmed by experimental data (Fig. 4). The pH values of the two formulations were 2.80 (EC) and 6.94 (G), respectively. The changes of soil pH could be expected to be variable due to the different acid–base properties of the two formulations. The small fluctuation of pH in the subsoil (Ml and Bl, data not shown) meant the formulation effect on soil pH mainly took place in the topsoil. For EC, the pH of soil was decreased by the acid formulation. After 15 days, the formulation effect on soil pH was mitigated and an increase in pH appeared. The maximum pH decrements under the two conditions were 0.17 (NCP) and 0.30 (CP), respectively. The intensity and duration of the formulation effect on pH from G were more obvious than that from EC. The pH of soil was increased quickly by the alkaline formulation. After 38 days, the effect disappeared. The maximum of pH increment under the two conditions were 0.57 (NCP) and 0.82 (CP), respectively. Compared with the maximum values of pH decrement/increment, the formulation effect was significant under CP conditions. In our previous work (Liu et al., 2014), the mulching material (straw) was found to have a notable effect on the soil pH, due to the leachate of straw (pH: 6.13–7.61). Then the straw could indirectly affect the adsorption and degradation behavior of chlorpyrifos and its metabolites in soil. However, in this work, the bamboo leaves were hard to dissolve or leach by the rain to form leachate for soil pH adjustment. In addition, there was little rain during the experiment, so the contribution from acid rain to pH adjustment of the soil was insignificant. Moreover, the pH values of soil samples during the whole period of experiment were all below 4.05. The fluctuation of pH in the acid range had little effect on pesticide behaviors.

2.6. *Evaluation of food safety and suggestion of proper use of the two formulations*

China established 50 μg/kg as the MRL for phoxim in stalk and stem vegetables (bamboo shoots included). In EU and USA, the MRL for phoxim in bamboo shoots is 10 μg/kg. The corresponding MRL in Japan (Positive List System) is 20 μg/kg. According to the terminal residue results, the residue behavior of phoxim in bamboo shoots under different treatments followed a trend such that shorter harvest intervals led to more phoxim residues. Except for the results from 89 days

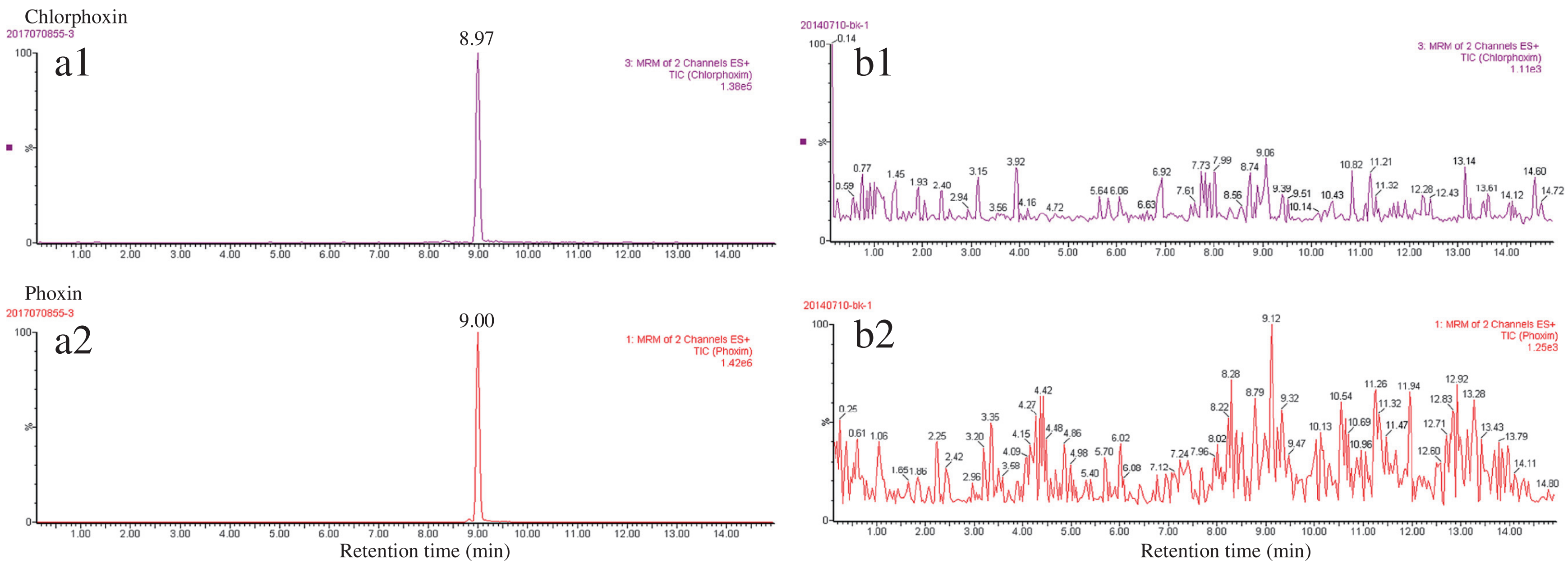

Fig. 3 – Representative chromatograms of phoxim and chlorphoxim in soil sample. (a) Phoxim and chlorphoxim detected in soil sample, (b) blank sample of soil.

Table 2 – Residue levels of chlorphoxim in soil after treatment with different formulations under two conditions (μg/kg).

Application time (day)	Treatment with G + CP		Treatment with G + NCP	
	Top layer	Middle layer	Top layer	Middle layer
0–29	<LOD	<LOD	<LOD	<LOD
38	132.2 ± 8.0	<LOD	180.3 ± 13.5	<LOD
56	361.7 ± 19.8	<LOD	457.3 ± 35.7	<LOD
89	536.7 ± 35.7	162.1 ± 17.3	<LOD	<LOD
121	202.7 ± 16.3	<LOD	<LOD	<LOD
171	<LOD	<LOD	<LOD	<LOD
Application time (day)	Treatment with EC + CP		Treatment with EC + NCP	
	Top layer	Middle layer	Top layer	Middle layer
0–3	<LOD	<LOD	<LOD	<LOD
8	126.4 ± 10.3	<LOD	154.5 ± 12.7	<LOD
15	176.2 ± 18.4	<LOD	258.1 ± 23.6	<LOD
22	<LOD	<LOD	294.3 ± 35.2	<LOD
29–89	<LOD	<LOD	<LOD	<LOD

G: granules; EC: emulsifiable concentrate.
CP: soil surface mulching conditions; NCP: non-covered cultivation conditions.
Data are presented as mean ± SD. The limit of detection (LOD) of analysis was 0.10 mg/kg for soil and 0.005 mg/kg for bamboo shoot, respectively

(11.2 μg/kg, which exceeded the limits of EU and USA), the other quantities of phoxim detected in bamboo shoots were below the MRLs mentioned above. This result suggested that the shoots would be relatively safe for consumption after applying the recommended dose of the two phoxim formulations.

Apart from the terminal residue in food, pesticide residue in soil is also worthy of attention. Significant risk of groundwater pollution has been observed as a result of rapid leaching of highly soluble pesticides when used in agronomic practices as conventional formulations. This risk can be minimized through the application of the pesticide at a set rate using CRFs. Mobility experiments showed that the use of CRFs markedly reduces the presence of isoproturon and imidacloprid in the leachate compared to technical products, and to a lesser extent for cyromacine due to its high water solubility (Fernández-Pérez et al., 2011). A continuous and intensive rainfall might result in translocation of pesticides into some deeper layers of the soil, which is of high importance due to the possible contamination of groundwater, and the application of a formulation with a controllable release of the active substance might considerably minimize this process (Wlodarczyk, 2014). In this work, the results from NCP conditions (the leaching of phoxim was reduced for G formulation) also supported the above viewpoint. However, the leaching minimization effect from CRFs was negated when the pesticide was applied onto the soil with surface mulching, and the leaching effect from phoxim G under

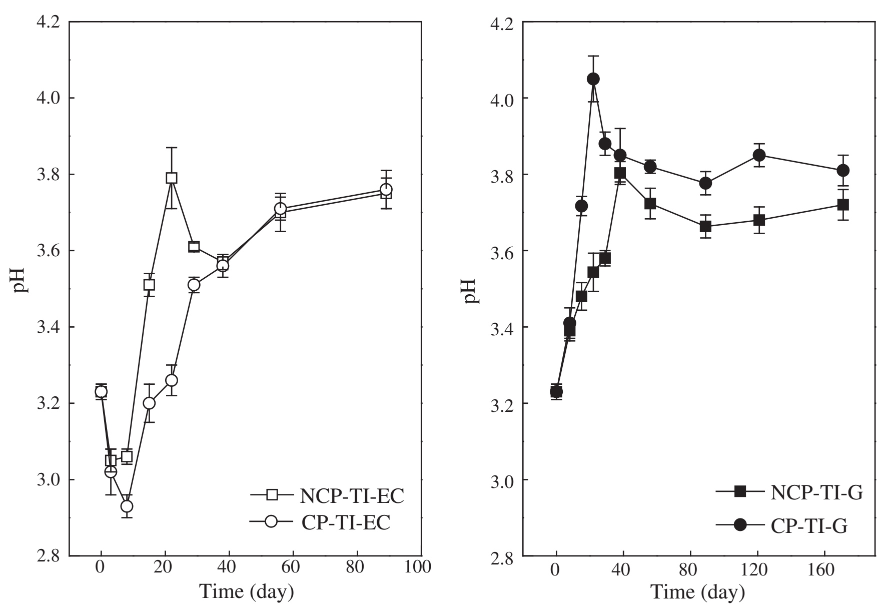

Fig. 4 – Formulation effect on pH of top layer of soil (Tl) under noncovered cultivation condition (NCP) and surface mulching condition (CP).

CP conditions was stronger than that from phoxim EC. Moreover, more residues of metabolite could be observed in soil from the CRF. Although the leaching effects for the metabolite from the two formulations were not obvious (owing to the high hydrophobicity of the metabolite), the application of CRF should be avoided or reduced in areas with high groundwater level.

3. Conclusions

The results of this study showed that the environmental behaviors (distribution, degradation, residue) of phoxim in a bamboo forest were significantly influenced by the types of samples and formulations. The degradation of phoxim was prolonged when it came from a CRF formulation, due to the slow-release effect of pesticide. The prolongation effect was particularly evident in the soil surface mulching cultivation, and might cause persistence and long-term environmental risk. Phoxim residues in the harvested bamboo shoot samples from two formulations under two conditions did not basically exceed the MRLs, which suggested that it bamboo shoot consumption could be considered relatively safe after applying the recommended dose of the two phoxim formulations. However, the high risk to groundwater from the enhanced leaching effect for CRF under soil surface mulching conditions also deserves our attention, especially for highly hydrophilic compounds (pesticides and their metabolites).

Acknowledgments

We gratefully acknowledge the financial support from the Applied Research Project in the Public Interest of Zhejiang Province (Nos: 2013C32106, 2015C32071), the Fundamental Research Funds for the Central Non-profit Research Institution of CAF (No. RISF61252), and the Special Fund for Forestry Scientific Research in the Public Interest (No. 201304705). The authors state that there are no conflicts of interest.

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