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Effects of Repeated Applications of Chlorpyrifos on its Persistence and Soil Microbial Functional Diversity and Development of its Degradation Capability

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Abstract Effects of repeated applications of chlorpyrifos on its persistence and soil microbial functional diversity were studied under laboratory conditions. The results showed that the degradation rate of chlorpyrifos increased whereas its inhibitory effect on soil microbial communities gradually decreased with application frequency of chlorpyrifos. A bacterial strain DSP capable of utilizing chlorpyrifos as a sole source of carbon and energy was isolated 21 days after the third chlorpyrifos application, which indicated that the capability of soil microorganism for degrading chlorpyrifos was formed during the experiment. It could be concluded that repeated applications of chlorpyrifos had no lasting impact on soil health.

Keywords Chlorpyrifos · Degradation · Microbial diversity · Repeated application

Studies on the fate and ecotoxicology of pesticides are presently carried out mostly under a single application, but in practice, pesticides are usually applied repeatedly over a growing season. Repeated applications of pesticides may have an adverse effect on soil microbial functional diversity and subsequently influence soil fertility and plant growth, which pose serious threats to the sustainability of agricultural soils (Johnsen et al. 2001). Therefore, there has an interesting concern about the impacts of repeated pesticide applications on their persistence and soil microbial communities. Chlorpyrifos is a broad-spectrum organophosphate insecticide and acaricide widely used for insect pest control on grain, cotton, fruit, nut, and vegetable crops, as well as lawns and ornamental plants, which has caused a wide range of soil contamination (EPA 1997). The dissipation of chlorpyrifos after a single application in soil and its effect on soil microbial biomass C and N, microbial population, microbial respiration, and enzyme activities have been well investigated (Singh et al. 2002; Pandey and Singh 2004; Shan et al. 2006). However, so far little information is available on the effects of repeated applications of chlorpyrifos on its persistence and soil microbial functional diversity. The objectives of this study were: (1) to reveal the differences of chlorpyrifos degradation after repeated applications in soil; (2) to examine the effect of repeated chlorpyrifos applications on soil microbial functional diversity; and (3) to evaluate the effect of repeated chlorpyrifos applications on soil health.

Materials and Methods

Analytical grade chlorpyrifos (≥99.5%) was purchased from the Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Commercial formulation of chlorpyrifos (Dursban, 48% a.i.) was provided by Dow AgroSciences LLC. Soil used in this study was collected from a farm located at Zhejiang University, China. Its major properties were as follows: sand, 21.5%; silt, 71.1%; clay, 7.4%; organic matter content, 3.05%; water holding capacity, 39.4%; cationic exchange capacity, 10.6 cmol/kg; total nitrogen, 0.14% and a pH of 6.8.

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Soil samples (2 kg) were treated with chlorpyrifos formulation coupled with an appropriate amount of sterile distilled water to give a final concentration of 4 mg/kg, corresponding to the recommended dose. Soil samples received the same amount of sterilized water without chlorpyrifos were used as the controls. Soil water content was adjusted to 60% of water holding content. Soil samples were transferred to 3-L polypropylene flowerpots and incubated at 25 ± 1°C in the dark. The soils were retreated with chlorpyrifos at the same dose 42 and 84 days after the first treatment, respectively. All treatments were in triplicate. Two hours and 1, 3, 7, 14, 21, 35, and 42 days after

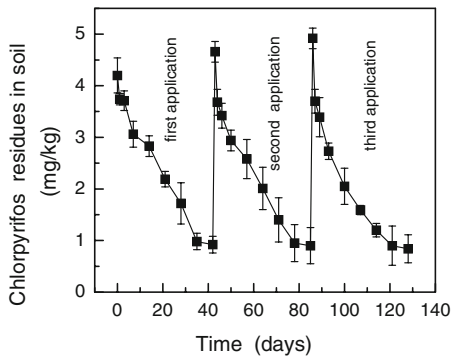

Fig. 1 Degradation of chlorpyrifos in soil after three repeated applications

Table 1 Kinetics data of chlorpyrifos degradation in soil

Application frequency	Dynamic function	DT_{50} (days)	r^2
1st	$C = 4.19e^{-0.0361t}$	19.2a	0.9674
2nd	$C = 4.10e^{-0.0379t}$	18.3ab	0.9768
3rd	$C = 4.14e^{-0.0402t}$	17.2b	0.9820

DT_{50} values followed by different letters within a column are significantly different ($p \leq 0.05$)

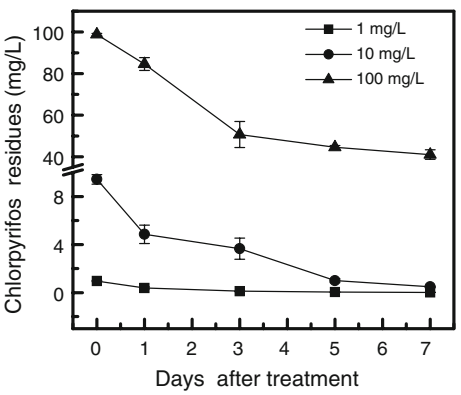

Fig. 2 Degradation of chlorpyrifos by the strain DSP in pure cultures

Table 2 Kinetics data of chlorpyrifos degradation by the strain DSP in pure culture

Chlorpyrifos concentration (mg/L)	Dynamic function	Degradation rate (mg/L · day)	r^2
1	$C = 0.73e^{-0.5475t}$	0.13	0.9770
10	$C = 9.15e^{-0.4183t}$	1.28	0.9695
100	$C = 91.17e^{-0.1310t}$	8.25	0.8968

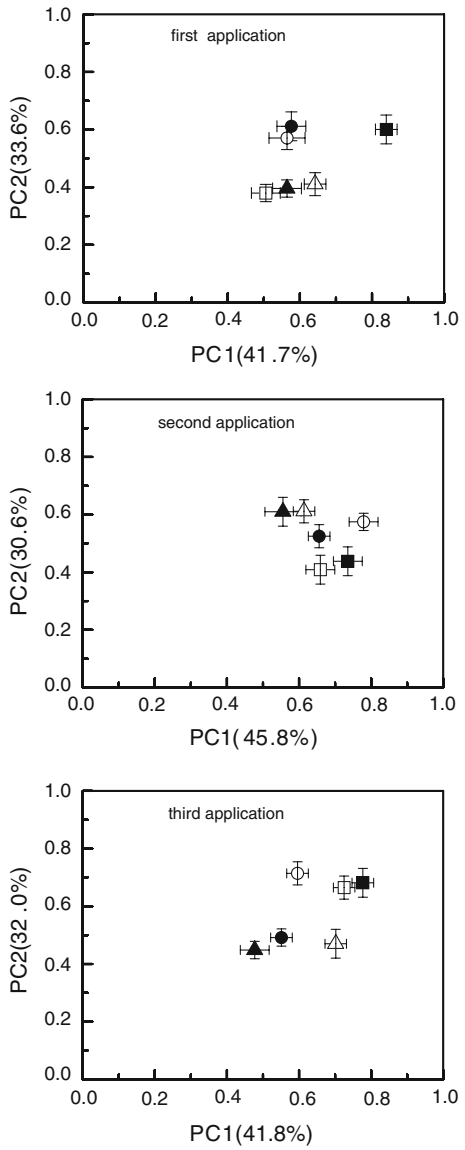

Fig. 3 Principle component analysis of substrate utilization patterns for each treatment at different sampling times. Control/3 days (■), Chlorpyrifos/3 days (□); Control/7 days (●), Chlorpyrifos/7 days (○); Control/21 days (▲), Chlorpyrifos/21 days (△). PC1 and PC2 represent the first and second principal components in the PCA profiles, respectively. The value in parentheses represents explained percentage of the variance

every chlorpyrifos treatment, soil samples (20 g) from each treatment were taken for determination of chlorpyrifos residues. Meanwhile, 10 g of soils were sampled for Biolog assay at 3, 7, and 21 days, respectively.

Biolog EcoPlate™ experiment was performed according to the procedures of Garland and Mill (1991). The Simpson index (*1/D*), Shannon–Weaver index (H'), and McIntosh index (*U*) were analyzed according to the methods of Zak et al. (1994). Principal component analysis (PCA) was used to characterize community level profiles.

Soil samples before and after each chlorpyrifos treatment were used to isolate the potential strain(s) capable of degrading chlorpyrifos with the methods described by Yu et al. (2006a). The isolated strain was identified by the 16S rRNA gene sequences.

Chlorpyrifos residues from the soils were extracted and determined according to the methods of Yu et al. (2006a). The recovery experiment of chlorpyrifos in the soil was conducted to confirm the validity of the extraction method. Known concentrations of chlorpyrifos in 20 g of dry soil (0.1, 1, and 10 mg/kg) were spiked. All treatments were in triplicate.

Results and Discussion

The average recoveries of chlorpyrifos from the soil at levels of 0.1, 1, and 10 mg/kg were 104.8 ± 2.9%, 95.0 ± 1.5%, and 92.0 ± 0.3%, respectively. The limits of detection and quantification were measured to be 0.001 and 0.01 mg/kg of dry soil, respectively. These data indicated that the extraction method was considered to be satisfactory for requirements of chlorpyrifos residues analysis.

The degradation of chlorpyrifos after repeated applications in soil is shown in Fig. 1. The half-life of chlorpyrifos, calculated by first-order function, was 19.2, 18.3, and 17.2 days for the first, second, and third applications, respectively (Table 1). The results indicated that repeated applications of chlorpyrifos significantly ($p \leq 0.05$) accelerated its degradation. Similar to this result, Robertson et al. (1998) found enhanced degradation of chlorpyrifos in previously chlorpyrifos-treated soil. Contrast results had also been reported by Singh et al. (2002) that the half-life of chlorpyrifos was obviously extended with its application times.

To explore the possible factors contributing to enhanced degradation of chlorpyrifos, isolation of indigenous microorganisms capable of degrading chlorpyrifos was conducted before and after each chlorpyrifos addition. The result showed that no chlorpyrifos-degrading strain was isolated from the tested soil before chlorpyrifos treatment. However, a bacterial strain DSP utilizing chlorpyrifos as the sole carbon and energy sources was isolated from the soil sample 21 days after the third chlorpyrifos treatment. The strain DSP was identified as an unknown species of *Escherichia* (accession number EU770569). Figure 2 shows the degradation patterns of chlorpyrifos at levels of 1, 10, and 100 mg/L in pure culture. The degradation rates of chlorpyrifos at concentrations of 1, 10, and 100 mg/L by the strain DSP in pure culture were calculated to be 0.13, 1.28, and 8.25 mg/L · day, respectively (Table 2). These results indicated that some indigenous microorganisms

Table 3 Diversity indices of soil microbial community after repeated applications of chlorpyrifos

Application frequency	Concentration (mg/kg)	Days after treatment	Simpson index (*1/D*)	Shannon index (H')	McIntosh index (*U*)
1st	Control	3	25.67 ± 0.61a	3.29 ± 0.05a	7.03 ± 0.14a
1st	4	3	20.71 ± 0.26b	3.25 ± 0.02a	5.73 ± 0.31b
1st	Control	7	21.18 ± 0.32a	3.17 ± 0.07a	5.55 ± 0.16a
1st	4	7	20.35 ± 0.46a	3.24 ± 0.01a	5.07 ± 0.42a
1st	Control	21	18.21 ± 0.31a	3.02 ± 0.10a	4.84 ± 0.25a
1st	4	21	18.37 ± 0.30a	3.14 ± 0.04a	4.85 ± 0.24a
2nd	Control	3	18.77 ± 0.63a	3.17 ± 0.06a	5.52 ± 0.21a
2nd	4	3	15.18 ± 0.10b	2.93 ± 0.02a	4.03 ± 0.07b
2nd	Control	7	16.08 ± 0.38a	2.92 ± 0.08a	4.34 ± 0.63a
2nd	4	7	16.34 ± 0.59a	2.97 ± 0.05a	4.52 ± 0.21a
2nd	Control	21	13.87 ± 0.11a	2.81 ± 0.12a	3.55 ± 0.24a
2nd	4	21	15.06 ± 0.49b	2.94 ± 0.16a	3.93 ± 0.32a
3rd	Control	3	17.21 ± 0.27a	3.03 ± 0.07a	4.99 ± 0.14a
3rd	4	3	15.40 ± 0.12a	2.97 ± 0.06a	4.10 ± 0.30a
3rd	Control	7	10.30 ± 0.38a	2.87 ± 0.02a	2.84 ± 0.37a
3rd	4	7	12.19 ± 0.25b	2.89 ± 0.12a	2.90 ± 0.04a
3rd	Control	21	12.47 ± 0.59a	2.87 ± 0.13a	3.74 ± 0.42a
3rd	4	21	15.00 ± 0.15b	2.89 ± 0.07a	4.46 ± 0.26a

All values are mean ± SD of three replicates. Data in the same column followed by different letters are significant difference ($p \leq 0.05$)

became adapted to chlorpyrifos after its repeated applications. Similar phenomenon had been revealed by Yu et al. (2006b) that three bacterial strains capable of utilizing chlorothalonil as a sole source of carbon and energy were isolated 21 days after the fourth treatment of chlorothalonil.

The diversity indices *1/D*, *H′*, and *U* are used to indicate the dominant population, the richness, and evenness of soil microorganisms (Simpson 1949). In the present study, the *1/D* and *U* obviously decreased during the initial 3 days after three repeated applications and thereafter gradually recovered to the similar level as the control. However, no significant effect on *H′* was observed after repeated chlorpyrifos applications, indicating that the richness of soil microorganisms was not apparently affected. In contrast, Singh et al. (2002) reported that a small but significant reduction in richness was observed following the second treatment with chlorpyrifos. A similar trend on soil microbial functional diversity was also observed in PCA plots (Fig. 3). As shown in Table 3 and Fig. 3, the inhibition effect of chlorpyrifos on soil microbial communities gradually decreased with its application frequency. This might be resulted from toleration and adaptation of soil microorganisms to chlorpyrifos under the selective pressure of the chemical, and these soil microorganisms may utilize chlorpyrifos as a source of carbon and energy and proliferate substantially. This phenomenon had been demonstrated that fungal populations and denitrifying bacteria can tolerate chlorpyrifos residues at a range of 10–300 mg/kg in an agricultural loam (Martinez-Toledo et al. 1992).

The results obtained in this study indicated that the dissipation of chlorpyrifos was accelerated with its application frequency. The inhibitory effect of chlorpyrifos on soil microbial functional diversity was observed during the initial 3 days after each application, which thereafter recovered to the control level. Furthermore, this inhibition gradually decreased with application times of chlorpyrifos.

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References

EPA (1997) Review of chlorpyrifos poisoning data. EPA, Washington, DC

Garland JL, Mill AL (1991) Classification and characterization of heterotrophic microbial community-level sole-carbon-source utilization. Appl Enviorn Microbiol 57:2351–2359

Johnsen K, Jacobsen CS, Torsvik V, Sorensen J (2001) Pesticide effects on bacterial diversity in agricultural soils-a review. Biol Fertil Soils 33:443–453

Martinez-Toledo MV, Salmeron V, Gonzalez-Lopez J (1992) Effect of the insecticides methylpyrimifos and chlorpyrifos on soil microflora in an agricultural loam. Plant Soil 147:1573–5036

Pandey S, Singh DK (2004) Total bacterial and fungal population after chlorpyrifos and quinalphos treatments in groundnut (*Arachis hypogaea* L.) soil. Chemosphere 55:197–205

Robertson LN, Chandler KJ, Stickley BDA, Cocco RF, Ahmetagic M (1998) Enhanced microbial degradation implicated in rapid loss of chlorpyrifos from the controlled-release formulation suSCon Blue in soil. Crop Prot 17:29–33

Shan M, Fang H, Wang X, Feng B, Chu XQ, Yu YL (2006) Effect of chlorpyrifos on soil microbial populations and enzyme activities. J Environ Sci 18:4–5

Simpson EH (1949) Measurement of diversity. Nature 163:688

Singh BK, Walker A, Wright DJ (2002) Persistence of chlorpyrifos, fenamiphos, chlorothalonil, and pendimethalin in soil and their effects on soil microbial characteristics. Bull Environ Contam Toxicol 69:181–188

Zak JC, Willing MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26:1101–1108

Yu YL, Fang H, Wang X, Wu XM, Shan M, Yu JQ (2006a) Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation 17:487–494

Yu YL, Shan M, Fang H, Wang X, Chu XQ (2006b) Responses of soil microorganisms and enzymes to repeated applications of chlorothalonil. J Agric Food Chem 54:10070–10075