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Microencapsulated chlorpyrifos: Degradation in soil and influence on soil microbial community structures

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ABSTRACT

Degradation kinetics of microencapsulated chlorpyrifos (CPF-MC) in soil and its influence on soil microbial community structures were investigated by comparing with emulsifiable concentration of chlorpyrifos (CPF-EC) in laboratory. The residual periods of CPF-MC with fortification levels of 5 and 20 mg/kg reached 120 days in soil, both of the degradation curves did not fit the first-order model, and out-capsule residues of chlorpyrifos in soil were maintained at 1.76 (±0.33) and 5.92 (±1.20) mg/kg in the period between 15 and 60 days, respectively. The degradation kinetics of CPF-EC fit the first-order model, and the residual periods of 5 and 20 mg/kg treatments were 60 days. Bacterial community structures in soil treated with two concentrations of CPF-MC showed similarity to those of the control during the test period, as seen in the band number and relative intensities of the individual band on DGGE gels ($p > 0.05$). Fungal community structures were slightly affected in the 5 mg/kg treatments and returned to the control levels after 30 days, but initially differed significantly from control in the 20 mg/kg treatments ($p < 0.05$) and did not recover to control levels until 90 days later. The CPF-EC significantly altered microbial community structures ($p < 0.05$) and effects did not disappear until 240 days later. The results indicated that the microcapsule technology prolonged the residue periods of chlorpyrifos in soil whereas it decreased its side-effects on soil microbes as compared with the emulsifiable concentration formulation.

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Introduction

The use of macromolecular substances as semi-permeable or contact-breakable membranes to encapsulate pesticides by chemical, physical or physico-chemical mechanisms is one approach to achieve microcapsule (MC) formulations with controlled-release properties (Heller, 1980; Dailey and Dowler, 1998; Tsuji, 2001). MC formulation is an advanced formulation that has several advantages over traditional formulations, including increased stability in the environment, reduced leaching from soil, and improved activity (Mogul et al., 1996; Frederiksen and Hansen, 2002; Bagle et al., 2012; Hack et al., 2012; Alonso et al., 2013). Since the Pennwalt Company first developed Parathion-methyl MC in 1974, over 200 agro-chemical companies have participated in the development and application of MCs. About 60 pesticides are commercially available in MC formulation today, including chlorpyrifos, avermectin and alachlor (Hua, 2010; Hack et al., 2012; Wang et al., 2013a).

Chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate, CPF) is a broad-spectrum organophosphorous insecticide that is widely used to control agricultural pests. Banning of the use of carbofuran has promoted CPF as an important substitute for controlling the underground pests in China (Jiang, 2008). However, the extensive use of CPF has led to contamination of the environmental and food matrix in some regions (Álvarez et al.,

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2013; Romeh and Hendawi, 2013; Wang et al., 2013b). CPF MC was recently developed as an alternative to conventional formulations to control pests with reduced application amounts and environmental effects (Frederiksen and Hansen, 2002; Zhu et al., 2010). Over 50 MC products containing CPF as an active substance have been registered and used in China (The data cited from Ministry of Agriculture of the People's Republic of China). However, while many studies on the product development and efficacy evaluation of CPF MC have been reported (Frederiksen and Hansen, 2002; Montemurro et al., 2002; Lláce et al., 2010; Zhu et al., 2010; Guo et al., 2011), as yet the environmental safety has not been assessed.

Microbes play important roles in soil fertility through their functions in nutrient cycling and organic matter decomposition (Wainwright, 1978). Most pesticides will eventually reach the soil following application, even foliar application, to affect the stability of the soil microbial community and ultimately influence soil fertility and plant productivity (Omar and Abdel-Sater, 2001; Singh and Singh, 2005). Intensive studies have been conducted to investigate CPF distribution, absorption, transfer and degradation in soil (Redondo et al., 1997; Li et al., 2005; Singh et al., 2006; Van-Emmerik et al., 2007; Gebremariam et al., 2012). The influences of CPF on soil microbes, including microbial biomass, microbial diversity, microbial populations, microbial respiration, and enzymatic activities, have also been studied frequently (Singh et al., 2002a, 2000b; Menon et al., 2004, 2005; Adesodun et al., 2005; Shan et al., 2006; Fang et al., 2009; Dutta et al., 2010; Wang et al., 2010). While certainly informative, most of these studies, however, do not focus on formulation, which is an important factor influencing the side effects of CPF. Organic solvent or emulsifiable concentration (EC) formulations of CPF could be classified as disposable release formulations. After introduction into the soil, CPF exerts maximum stress on soil microbes. This stress gradually weakens with CPF degradation. As the MC form of the pesticide provides a membrane-like obstruction, CPF is never in direct contact with the soils. In this case, soil microbes may be challenged by a progressive stress and then stabilize to certain levels for a longer period of time. One could hypothesize that the response of the soil microbes to the CPF MC may be distinct from their responses to non-controlled-release formulations. To the best of our knowledge, few studies have focused on evaluation of the effects of controlled-release CPF on the soil microbial communities. Therefore, the present study investigates the response of soil microbial communities to the stress from CPF MC and compares findings with CPF EC results, along with determination of the degradation kinetics of the two formulations. Denaturing gradient gel electrophoresis (DGGE) was used to analyze the polymerase chain reaction (PCR) products of region 3(V3) of the bacterial 16S rDNA and region ITS1 of the fungal deoxyribonucleic acid (DNA), to elucidate the DGGE patterns of microbial community structures among the different treatments.

1. Materials and methods

1.1. Chemicals

Commercial formulations of CPF, in 40% EC and 36% MC, were obtained from DOW Chemical Co., USA and Xten Chemical Co., Japan, respectively. CPF standard (99.5%) was purchased from Shanghai Pesticide Research Institute, China.

1.2. Soil

The soil sample used in this study was collected from a field (0–20 cm) in Zhejiang Academy of Agricultural Sciences, Hangzhou, China that was used for growing rice for cultivation of pesticide-susceptible strains of rice pests, and had not been treated with any pesticide for the previous 10 years. The soil was sieved (2 mm) to remove stones and debris, analyzed with standard protocols (Institute of Soil Science, Academia Sinica, 1979) and classified as silt loam with the following characteristics: sand 20.8%, silt 74.3%, clay 8.0%, organic matter content 3.23%, water holding capacity 41.2%, cationic exchange capacity 9.6 cmol/kg, total nitrogen 0.18% and pH 6.8.

1.3. Soil treatment

Soil samples were pre-incubated at 25°C in the dark for 6 days. Then, the samples were separately treated by a predetermined amount of CPF in MC and EC formulations following proper dilution with distilled water, to achieve a certain concentration of insecticide and obtain soil moisture of 60% of the soil water holding capacity. Five treatments, including a control, recommended dosages (MC and EC, 5 mg (active ingredient)/kg), and four times the recommended dosages (MC and EC, 20 mg (active ingredient)/kg), were used in this experiment. The control treatments received the same amount of sterilized distilled water without CPF. Each treatment was performed in triplicate. Dosed samples (8 kg per treatment) were mixed thoroughly and transferred to 30 cm × 20 cm × 15 cm polypropylene containers. Each container was covered with black fabric and incubated in a climate chamber at 25°C. Soil moisture was determined and maintained by regular addition of sterilized water every 2 days. At fixed intervals of 0 (2 hr), 7, 15, 30, 45, 60, 90, 120, and 240 days after treatment, aliquots of the soil sample (60 g) were collected to determine residues of CPF and soil microbial community structures.

1.4. Determination of residual CPF in soil

CPF residues in soil were determined by the method proposed by Chu et al. (2008), with the shaking time optimized to 4 hr. Soil samples were extracted by acetone–petroleum ether (1:1, V/V). The extracts were prepared for gas chromatography (GC) analysis after filtration, washing by 3% sodium sulfate, drying by anhydrous sodium sulfate, concentration on rotary evaporator, and dissolution in 10.0 mL of acetonitrile.

CPF residues were determined by a Shimadzu GC-2010 (Shimadzu Corp., Kyoto, Japan) equipped with a Ni^{63} electron capture detector and a fused silica capillary column (HP-5) (Supelco Corp., Bellefonte, Pennsylvania, USA) (30 m length, 0.32 mm internal diameter, and 0.33 μm film thickness). The operating conditions were as follows: injector port, 280°C; detector, 300°C; column, 240°C; carrier gas (N_2) flow rate, 50 mL/min; and injection volume, 2 μL.

Three replicate analyses were carried out at four different spiking levels to test the validity of the aforementioned method for extracting CPF from the soil. Soil samples without CPF were spiked with MC and EC at concentrations of 0.1, 1.0, 5.0, and 20.0 mg/kg.

1.5. Determination of out-capsule CPF in soil

Five soil samples without CPF were spiked separately with EC and MC mixtures and the last concentration of CPF in soil was 20 mg/kg, and the percentage of CPF from EC in each sample was 0%, 5%, 25%, 50% and 100%, respectively. The soil samples (20 g) were then transferred to a 2.5 cm × 25 cm glass column,

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