Ecotoxicology and Environmental Safety 81 (2012) 65–69

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Accumulation of chlorothalonil successively applied to soil and its effect on microbial activity in soil

Xiangwei Wu [a,b], Longyin Cheng [a], Zhengya Cao [a], Yunlong Yu [a,*]

[a] Department of Plant Protection, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, PR China
[b] College of Resources and Environment, Anhui Agricultural University, Hefei 230036, PR China

ARTICLE INFO

Article history:
Received 9 November 2011
Received in revised form
10 April 2012
Accepted 18 April 2012
Available online 8 May 2012

Keywords:
Chlorothalonil
Microbial activity
Accumulation
Repeated applications

ABSTRACT

The effect of successive chlorothalonil applications on the persistence of chlorothalonil, soil respiration activity, and dehydrogenase activity (DHA) in soil was investigated under laboratory conditions. The persistence of chlorothalonil in soil was prolonged significantly with the increase in the concentration applied. Repeated applications of chlorothalonil at 25 mg kg^{-1} led to its accumulation in soil. The effect of repeated chlorothalonil applications on soil respiration and DHA was found to be a concentration-dependent process. Soil respiration was permanently inhibited by the successive introductions of chlorothalonil at 25 mg kg^{-1}. DHA was reduced significantly on day 15 after four successive treatments of 10 mg kg^{-1} and 25 mg kg^{-1} of chlorothalonil, although a recovery trend could be found after the third and fourth treatments. Repeated chlorothalonil applications might increase the persistence of chlorothalonil in soil and thus alter soil microbial activity.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Fungicides are used extensively to protect crops against fungal pathogens in agricultural practice. Because fungicides can potentially exhibit toxicity to non-target organisms, there has been considerable interest in determining their impact on non-target organisms in soil, including soil beneficial microbes. Numerous studies have been carried out to evaluate the dissipation of fungicides in soil and the effects on soil-inhabiting microorganisms (Wang et al., 2009; Sukul et al., 2010; White et al., 2010; Yan et al., 2011). However, most of the studies were performed with a single application of a fungicide. Fungicides are usually applied repeatedly to the same crop over a growing season (Singh et al., 2002b). Repeated applications of fungicides might alter their dissipation behavior and their effect on soil microbial communities, thus posing a serious threat to soil health (Di Primo et al., 2003; Potter et al., 2005; Lancaster et al., 2010). There is therefore increasing concern about the effect of repeated fungicide applications on the persistence of fungicides and their effects on soil microbial activities.

Chlorothalonil (2,4,5,6-tetrachlorobenzene-1,3-dicarbonitrile, CAS No.: 1897-45-6) is a non-systemic and broad-spectrum foliar fungicide that is used in a wide range of fruits, vegetables and cash crops to control fungi imperfecti and ascomycetes. The dissipation behavior of chlorothalonil in soil and its effect on soil ecosystems have been investigated intensively (Sigler and Turco, 2002; Chu et al., 2008; Wang et al., 2011). The harmful effect of residual chlorothalonil on soil invertase, amylase, and dehydrogenase activities has been frequently observed (Tu, 1993; Chen and Edwards, 2001). Chen et al. (2001) reported that chlorothalonil has a significant stimulating effect on nitrification rates in soil. The application of both chlorothalonil and captan decreased fungal biomass and the Gram-positive to Gram-negative ratio (Podio et al., 2008). Bending et al. (2007) found that the soil eukaryote community structure was altered by chlorothalonil application, as assessed by denaturing gradient gel electrophoresis (DGGE) analysis. To the best of our knowledge, most of the reported results were obtained with a single application of chlorothalonil. Only a few studies were performed with repeated applications of chlorothalonil (Singh et al., 2002a, 2002b; Yu et al., 2006). The consequences of successive applications of chlorothalonil to soil differ from the effects of a single application. It is, therefore, necessary to assess the accumulation of chlorothalonil and its possible impact on soil microbial activity after successive applications because the fungicide is usually applied repeatedly at 1–5 times the recommended dosage per growing season or year (Potter et al., 2001; Chaves et al., 2007).

In the present study, chlorothalonil at initial rates of 5–25 mg kg^{-1} was successively applied to soil at 15 day intervals, because these concentrations are frequently found in soil after the application of chlorothalonil. The persistence of chlorothalonil and its effect on soil respiration and dehydrogenase activity were

* Corresponding author. Fax: +86 571 88982433.
E-mail address: ylyu@zju.edu.cn (Y. Yu).

0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2012.04.017

determined after each treatment. The aim of this study was to assess the accumulation of chlorothalonil and its potential effect on soil microbial activity after repeated applications. The results will be useful for predicting the environmental fate of this widely used fungicide and for understanding the ecotoxicological impacts of intensive treatments with chlorothalonil.

2. Materials and methods

2.1. Chemicals

Chlorothalonil (99.5% purity) was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. The commercial formulation of chlorothalonil (75% active ingredient, Syngenta Crop Protection, Inc.) was used for soil treatment. *n*-Hexane (chromatographic grade) was obtained from Siyou Chemical Co., Tianjin, China. Other reagents were all of analytical grade.

2.2. Soils

The soil sample used in this study was collected from a mulberry field located at Huajiachi Campus, Zhejiang University, Hangzhou, China, and contained no detectable amount of chlorothalonil residue. Surface soil taken from the top layer (0–15 cm) was air-dried at room temperature, mixed thoroughly, sieved (2 mm mesh) to remove stones and debris, and stored at room temperature for 48 h before use. Physical and chemical properties of the soil were measured with the methods described by Liu (1996) in the Institute of Soil Science, Zhejiang Academy of Agricultural Sciences, Zhejiang, China. The soil was classified as light loam, and its major properties were as follows: sand, 78.0%; clay, 22.0%; organic matter content, 1.45%; water holding capacity, 39.6%; cationic exchange capacity, 10.5 cmol kg^{-1}; total nitrogen, 0.09%; and pH 6.43.

2.3. Soil treatment and sampling

Soil samples (equivalent to 2.0 kg dw) were treated with the commercial chlorothalonil formulation coupled with the appropriate amount of sterile distilled water to give final concentrations of 5, 10, and 25 mg kg^{-1} of dry soil, corresponding to the recommended dose (3000 g of active ingredient per ha), twice the recommended dose, and five times the recommended dose, respectively. Soil samples were mixed thoroughly with a plastic spoon and passed through a sieve (2 mm) to distribute the chlorothalonil evenly. Soil samples receiving the same amount of sterilized water without chlorothalonil were used as the controls. Soil water content was adjusted to 60% of water holding content (WHC). Soil samples were transferred to 3 L polypropylene flower pots and incubated at 30 ± 1 °C in the dark. The soils were re-treated with chlorothalonil at the same dose 15, 30, and 45 days after the first treatment, respectively. All treatments were performed in triplicate. Two hours and 1, 3, 7, and 15 days after every chlorothalonil treatment, soil samples (25 g) from each treatment were taken for analysis of residual chlorothalonil and soil respiration. In addition, 15 g of soil was sampled for soil dehydrogenase activity at 15 days after each treatment.

2.4. Determination of chlorothalonil in soil

The soil sample (10 g) was weighed into a 100 mL Erlenmeyer flask and then 40 mL of *n*-hexane–dichloromethane (1:1, v/v) was added. Subsequently, the soil mixture was ultrasonicated in an ultrasonic bath for 20 min. The supernatant was decanted and collected in a Büchner flask through a filter paper on a Büchner funnel under vacuum. The left soil mixture was extracted again with 40 mL of *n*-hexane–dichloromethane (1:1, v/v), and the filter cake was rinsed twice with 10 mL of *n*-hexane. All filtrates were filtered through anhydrous sodium sulfate, collected in a 250 mL flat-bottom flask, concentrated to approximately 1 mL on a rotary evaporator, concentrated to dryness under a gentle nitrogen flow, and finally dissolved in 10 mL of *n*-hexane for gas chromatographic analysis.

Gas chromatographic analysis of chlorothalonil was performed with an Agilent 6890N gas chromatograph equipped with an electron capture detector (ECD) and an automatic injector (model 7683 B, Agilent Technologies, USA). A non-polar fused-silica HP-1 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 μm, Agilent Technologies, USA) was employed. Operating conditions were as follows: injector temperature, 250 °C; detector temperature, 300 °C; and nitrogen as carrier gas at a constant flow rate of 1.5 mL min^{-1}. The oven temperature was maintained at 80 °C for 1 min, increased to 180 °C at 25 °C min^{-1}, ramped to 250 °C at a rate of 15 °C min^{-1} and held for 3 min. A splitless volume of 1 μL was injected.

2.5. Determination of soil respiration activity

The respiration activity in the soil was measured as CO_2 production. To determine the evolution of CO_2, 5 g of fresh soil was weighed into a 70 mL narrow-mouthed glass bottle, and 2 mL of 0.1 mol L^{-1} glucose solution was subsequently introduced. The bottle was covered with a gas-tight butyl rubber stopper and incubated at 30 °C. The gas in the headspace was sampled with a gas-tight syringe 7 h after incubation, and 500 μL of the gas was injected into a special GC (Fuli 9790, Hangzhou Fuli Analytical Instruments Co. Ltd, China) equipped with a converter for the transformation of CO_2 into CH_4 and an FID for the detection of CH_4. A stainless-steel column filled with TDX-01 (1.5 m × 2 mm i.d., Hangzhou Fuli Analytical Instruments Co. Ltd) was used for separation. The operating conditions were as follows: injector, 120 °C; detector, 180 °C; oven, 100 °C; and converter, 300 °C. Nitrogen was used as carrier gas at a flow rate of 30 mL min^{-1}. The ignition gases used were hydrogen (30 mL min^{-1}) and air (300 mL min^{-1}). The amount of CO_2 in the sampled gas was quantified using standard curves obtained by injecting known amounts of CO_2. The respiration activity of soil was expressed as mg CO_2 g^{-1} dry soil 7 h^{-1}.

2.6. Measurement of soil dehydrogenase activity

Determination of dehydrogenase activity in the soil is based on the use of soluble 2,3,5-triphenyltetrazolium chloride (TTC) as an artificial electron acceptor, which is reduced to the red-colored compound triphenylformazan (Casida et al., 1964). Briefly, 5 g of fresh soil was mixed with 5 mL of 0.5% TTC in 0.5 M Tris–HCl buffer solution (pH 7.6) in a test tube and incubated at 37 °C for 24 h. The triphenylformazan (TPF) that was produced in the mixture was extracted with 10 mL of methanol on a mechanical shaker at 150 rpm for 1 h. The extract was filtered through filter paper. The concentration of TPF was colorimetrically determined at 485 nm by a spectrophotometer (Shanghai Spectrum Instruments Co., Ltd.) using a calibration curve.

2.7. Recovery assay

A recovery assay was conducted to confirm the validity of the method described above. Known amounts of chlorothalonil were added to 10 g soil samples to give final spiked concentrations of 0.1, 1, 10, and 100 mg kg^{-1} of dry soil, respectively. Extraction and analysis were performed in triplicate as described previously.

2.8. Statistical analysis

Mean and standard error of the data were calculated using Excel 2003 (Microsoft, USA). Significance of differences between treatments was determined by one-way analysis of variance (ANOVA) using the software SPSS 12.0 for Windows (SPSS Inc., USA).

3. Results

3.1. Evaluation of recovery

The average recoveries of chlorothalonil from the soil are shown in Table 1. The recoveries of chlorothalonil from soil ranged from 82.86% to 95.49% with a relative standard deviation (RSD) of less than 3.33%. The limits of detection and quantification were found to be 0.001 mg kg^{-1} and 0.01 mg kg^{-1} of dry soil, respectively. These data indicated that the extraction method is satisfactory for the analysis of residual chlorothalonil in soil.

Table 1
Recoveries of chlorothalonil from spiked soils.

Fortification level (mg kg^{-1})	Mean recovery ± standard deviation (%)	Relative standard deviation (%)
0.1	82.86 ± 2.52	3.04
1	95.39 ± 3.18	3.33
10	95.49 ± 2.86	2.99
100	93.71 ± 2.22	2.37

Download English Version:

https://daneshyari.com/en/article/4420641

Download Persian Version:

https://daneshyari.com/article/4420641

Daneshyari.com