Original article

Individual and combined effects of tebuconazole and carbendazim on soil microbial activity

CrossMark

Caixia Wang [a], Feifei Wang [b], Qingming Zhang [b, *], Wenxing Liang [a]

[a] College of Agronomy and Plant Protection, Key Lab of Integrated Crop Pest Management of Shandong Province, Qingdao Agricultural University, Qingdao 266109, China
[b] College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China

ARTICLE INFO

ABSTRACT

Tebuconazole and carbendazim are the main fungicides in agricultural practice, and their potential toxicological effects have received considerable attention. However, very little is known about the combined effect of both the fungicides on soil microbial activity. Therefore, a mesocosm experiment was performed to ascertain the dissipation and effects of tebuconazole and carbendazim individual and combined applications on microbial properties (i.e., basal respiration, urease, alkaline phosphatase, invertase, and dehydrogenase) in soil. The results indicated that the dissipation of tebuconazole and carbendazim was affected by concentration applied when the two fungicides were applied individually. However, the degradation of both fungicides accelerated at low concentrations (1 mg kg^{-1}) and slowed down at moderate (10 mg kg^{-1}) to high (100 mg kg^{-1}) concentrations. Whether applied individually or in combination, low doses of both fungicides did not impart negative effects on soil respiration and enzymatic activities after seven days. However, increasing concentrations of moderate and high doses of tebuconazole significantly inhibited soil respiration and enzymatic activities. Apart from moderate doses of carbendazim which stimulated urease and invertase activities, other soil microbial activities were significantly inhibited by the moderate and high doses of carbendazim after seven days. The combined effects of the two fungicides at moderate and high concentrations were additive throughout the entire incubation time.

© 2015 Elsevier Masson SAS. All rights reserved.

1. Introduction

Currently, it is a common practice in crop protection to apply multiple pesticide mixtures instead of individual pesticides. This form of pesticide application likely results in the combined contamination of pesticide residues in the soil environment. The literature shows that various studies have investigated the degradation differences and effects of individual and combined pesticide use on the soil environment. For example, Singh et al. [1] found that the presence of fungicide chlorothalonil could retard the dissipation of the insecticides chlorpyrifos and fenamiphos in soil. The study by Swarcewicz and Gregorczyk [2] revealed that mancozeb significantly influence the degradation of pendimethalin but metribuzin does not affect the behavior of pendimethalin in soil. Tejada [3] observed that herbicidal glyphosate and diflufenican mixtures

contribute more toxicity to the soil biological activity (microbial C biomass and enzyme activities) than the individual herbicide. Therefore, it was deemed necessary to investigate and evaluate the effects of pesticide mixtures on soil environment for soil health.

Tebuconazole [methyl benzimidazol-2-yl carbamate] and carbendazim [(RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol] are two important fungicides used extensively to control various vegetable, fruit and other crop diseases worldwide [4,5]. The effects of the two fungicides on the soil environment have raised much concern. Previous studies have reported that higher concentrations (above 5 mg kg^{-1}) of tebuconazole decrease the soil microbial biomass and activity and alter the soil microbial communities in the short term [6–8]. Similarly, it has been reported in previous studies that higher concentrations (above 10 mg kg^{-1}) of carbendazim also have negative effects on soil respiration, dehydrogenase and phosphatase activities, soil nutrient cycling, and soil bacterial communities in the short term (within 30 days) [5,9–11]. However, these studies mainly focused on the individual effects of tebuconazole and carbendazim on the

soil environment, and little is known about the combined effects of these two fungicides on the soil environment. Due to the gradual application of tebuconazole and carbendazim mixtures in agriculture to control relevant diseases in recent years [12,13], further research is needed to achieve a better understanding of the effects of these two fungicides and their combined contamination effect on the soil environment.

It is well known that soil respiration and soil enzyme activities are two kinds of soil biological and biochemical properties, and they usually been used as integrative indicators of soil health [14,15]. In the present study, soil basal respiration and several important soil enzymes (urease, alkaline phosphatase, and invertase, dehydrogenase) were selected as indicators to assess and compare the differential influence of tebuconazole and carbendazim, individually and combined, upon application to the soil environment. At the same time, dissipation of the two fungicides when applied in soil, individually and combined, was also determined in the present study.

2. Materials and methods

2.1. Soil and pesticides

Soil was collected from the surface (0–20 cm) of a riparian zone located in the Yellow River Delta (118°48′ E and 37° 52′ N, Dongying, China), an area with no history of pesticide application. The collected soil was classified as fluvo-aquic soil according to the USDA Soil Taxonomy System. The physical and chemical properties of the soil were as follows: pH 7.8 (1:2.5 w/v in water in water), electrical conductivity 4.32 mS cm^{-1}, organic matter 12.3 mg kg^{-1}, organic nitrogen 82.5 mg kg^{-1}, available phosphorus 12.4 mg kg^{-1}, and available potassium 115.2 mg kg^{-1}. The soil was air-dried at room temperature and sifted through a 2 mm sieve prior to use.

The fungicide tebuconazole (97.5%) and carbendazim (98.5%) were obtained from Qingdao Hansen Biologic Science Co., Ltd., Qingdao, China.

2.2. Experimental design

A three-month microcosm experiment was carried out, following a modified method by Yan et al. [5]. For each treatment, sieved soils of 1.5 kg dry weight (DW) were placed into enamel trays and artificially contaminated by spraying the relevant amount (20 mL) of acetone solution of tebuconazole and N,N-dimethylformamide solution of carbendazim to give the following final concentrations: 1, 10, and 100 mg kg^{-1} of DW soil for tebuconazole (TEB); 1, 10, and 100 mg kg^{-1} of DW soil for carbendazim (CAB); 1 mg kg^{-1} + 1 mg kg^{-1}, 10 mg kg^{-1} + 10 mg kg^{-1}, and 100 mg kg^{-1} + 100 mg kg^{-1} for the combination of TEB and CAB, respectively. Concentrations were set according to the design described by Muñoz-Leoz et al. [8] and Wang et al. [11], with slight modification. Soil samples were thoroughly mixed with a plastic spoon to assure uniform fungicide distribution and maintained 1 h in a fume hood to allow evaporation of the solvent. Subsequently, soil samples were transferred to a 3-L brown flowerpot and covered with perforated polypropylene sheets. Untreated soil samples were reserved as the control. Each treatment for each sampling time was replicated three times. Soil moisture content was adjusted to a 60% water holding capacity and maintained by periodic addition of deionized water. Soil samples were incubated in the dark at 25 °C. Samples were taken from each of the treatments at 0, 7, 30, 60, and 90 days of incubation to determine the TEB and CAB dissipation, soil basal respiration, and enzyme activities.

2.3. Determination of tebuconazole and carbendazim

TEB and CAB residue in soil was extracted and determined using the previously reported procedure described by Chen et al. [16] and Yu et al. [17], respectively. In brief, a 10 g DW soil sample was placed into 250 mL Erlenmeyer flask and extracted with 50 mL methanol: distilled water (70:30, v/v) for TEB and methanol for CAB by ultrasonication for 30 min, respectively. The extracts were filtered through a Buchner funnel using 20 mL methanol as washing solvent. For TEB, the filtrates were pooled and transferred to a 500 mL separatory funnel and 100 mL of 5% saturated NaCl solution was added. Then, the solution was extracted three times with 40, 40, and 20 mL dichloromethane. The dichloromethane layer was collected in a 250 mL round bottom flask and concentrated to near dryness with a rotary evaporator at 40 °C. For CAB, the filtrates were only collected in a flask and concentrated to approximately 2 mL at 60 °C. The concentrated product was transferred a 10 mL volumetric flask and adjusted to 10 mL with methanol, then filtered through a 0.22 μm pore membrane filter prior to analysis.

TEB and CAB were measured using an Agilent 1100 high performance liquid chromatography system equipped with a UV detector and an Agilent HC-C18 (2) reversed-phase column (4.6 × 250 mm, 5 μm). The mobile phase was methanol:water (80:20, v/v) for TEB and methanol:water (50:50, v/v) for CAB at a flow rate of 0.8 mL min^{-1}. The detection wavelength was 223 nm and 270 nm for TEB and CAB, respectively. The injection volume was 10 μL. For each fungicide, the standard curve was established at the concentrations of 1, 5, 10, 25, 50, and 100 mg L^{-1}. Each of them was conducted in three times with R^2 value above 0.99. The method's limit of quantification (LOQ) for TEB and CAB based on the instrument's response to the lowest calibration standard was 0.03 and 0.05 mg kg^{-1}, respectively. The average recoveries of TEB and CAB were 92.3% and 97.7%, respectively.

2.4. Measurement of soil basal respiration and enzyme activities

Basal respiration was determined according to ISO 16072 Norm [18], CO_2 released by soil samples from airtight jars at 30 °C for 3 days was captured in beakers containing 0.2 M NaOH and titrated with 0.1 M HCl. The activities of urease and invertase, two important enzymes in the nitrogen and carbon cycle in soils [19], were determined according to Guan et al. [20], as described in Yan et al. [5]. Alkaline phosphatase plays a critical role in the phosphorus cycle of soils as a catalyzer for the hydrolysis of esters and anhydrides of phosphoric acid. Its activity was determined according to Tabatabai [21]. A 0.1 p-nitrophenol phosphatase solution (pH 11.0) was used as the substrate for methods described by Yan et al. [5]. Dehydrogenase, linked with microbial respiratory processes, is an important indicator of overall microbial activity of soils. Its activity was assayed by the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC) to the red compound triphenyl formazan (TF), using detailed procedures previously performed by Yao et al. [22].

2.5. Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) using the SPSS 18.0 software. Each parametric test was preceded by an evaluation of the homogeneity of variance. Post-hoc comparisons (LSD tests) were employed to identify notable difference, considered significant at $P < 0.05$ and $P < 0.01$ levels. The degradation rate constant and half-life of TEB and CAB were calculated using first-order kinetic equations $C_t = C_0 e^{-kt}$ and $t_{1/2} = \ln 2/k$, where C_t denotes the concentration of the pesticide residue at time t, C_0 denotes the initial concentration, k denotes the dissipation degradation rate constant, and $t_{1/2}$ represents the half-life.