Effect of three fungicides on degradation of diuron in tea field soil and impacts on soil bacterial community

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Introduction

In practice, pesticides are usually applied simultaneously or one after another for crop protection, and this type of pesticide application often leads to a combined contamination of pesticide residues in the soil environment.The effect of mix application of three fungicides, benomyl, tebuconazole and triadimefon, with herbicide, diuron, on persistent of these pesticides on soil was investigated. Meanwhile, the impact of mixed application on soil bacterial communities was also monitored. Here PCR amplification of 16S rDNA followed by denaturing gradient gel electrophoresis (PCR-DGGE) was used to study complex microbial populations.

Objective

In this study, the effect of mix application of three fungicides, benomyl, tebuconazole and triadimefon, with herbicide, diuron, on persistent of these pesticides on soil was visualized by PCR-DGGE and similarity between treatments was analyzed by unweighted pair-group method using arithmetic averages (UPGMA).

Materials and methods

1.Materials

Chemicals used in the experiments were all purchased from Dr. Ehrenstorfer, Augsburg, Germany. Purity of diuron is 97.5%. Purity of benomyl is 98.0%. Purity of tebuaconazole is 98.8%. Purity of triadimefin is 99.0%.

The soil sample was collected from The Experimental Forest College of Bioresources and Agriculture,National Taiwan University No.12-44. The soil texture was sandy clay loam with organic matter content, sand, clay, % silt, pH , and cation exchange capacity (CEC).

2.Sample Preparation

Soil samples after air dry were meshed through a 2-mm sieve and treated with stock solution to final concentrations of diuron 50 mg kg^{-1}, benomyl 25 mg kg^{-1}, tebuaconazole 10 mg kg^{-1} and triadimefin 10 mg kg^{-1} for each. Keep the water content with field capacity (22.63%) for each treatment. The incubation temperature was 28℃ to simulate room temperatures.

3.Extraction and Analysis of Chemicals

To determine the residual content of pesticide in this study, 5 mL acetonitrile was added to 10 g soil samples, which were then vortex for 3 min. Then, the samples were centrifuged at 5000 rpm for 10 min. The supernatants were collected and passed through 0.45-μm filters. Then repeat it again. The filtrate was analyzed by use of HPLC-DAD.

4.Extraction and Analysis of Microorganism

Total genomic DNA was extracted by use of an UltraCleanTM Soil DNA Isolation kit (MO BIO Laboratories, West Carlsbad, CA, USA). The 16S rDNA was amplified at 94 ℃ for 2 min, followed by 35 cycles of 94 ℃ for 30 sec, 60 ℃ for 1 min, 72 ℃ for 1 min, and a final extension at 72 ℃ for 10 min. Samples of 20 *μL of PCR products were loaded onto an 8% (w/v)* polyacrylamide gel that contained 40%-60% denaturing gradient of formamide and urea. Electrophoresis was run at 60 ℃ in 1X TAE for 12 hr at a constant voltage of 75 V. The DGGE profiles were analyzed by an unweighted pair-group method with arithmetic averages (UPGMA), and the similarity was calculated by the coefficient of DICE with Quantity One® software (Bio-Rad, USA). The UPGMA method was used to examine the change in the bacterial community under different temperatures and incubation days.

Results and Discussion

1.Dissipation of diruon in sterile and unsterile soils

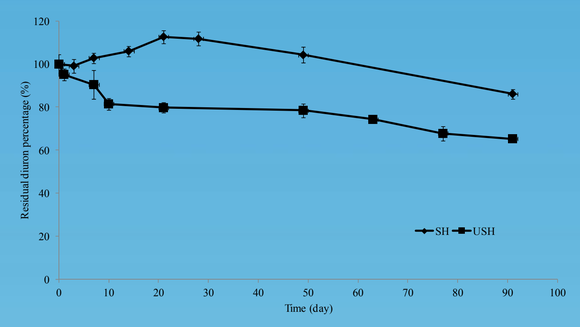

Fig. 1 The diuron degradation rate in sterilized and unsterilized soil.

2.Soil bacterial community with diuron treatment

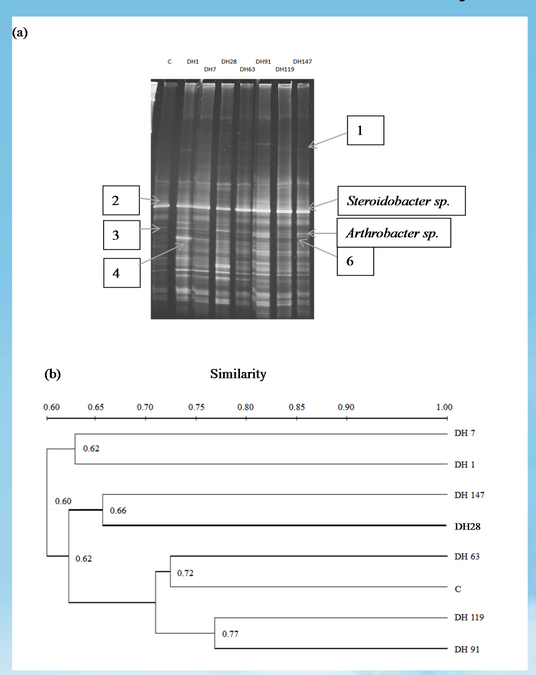

Fig. 2 (a) PCR-DGGE analysis of 16S rDNA sequence fragments and (b) UPGMA cluster analysis of bacterial community structures obtained from diuron treatment soil with difference incubation time.

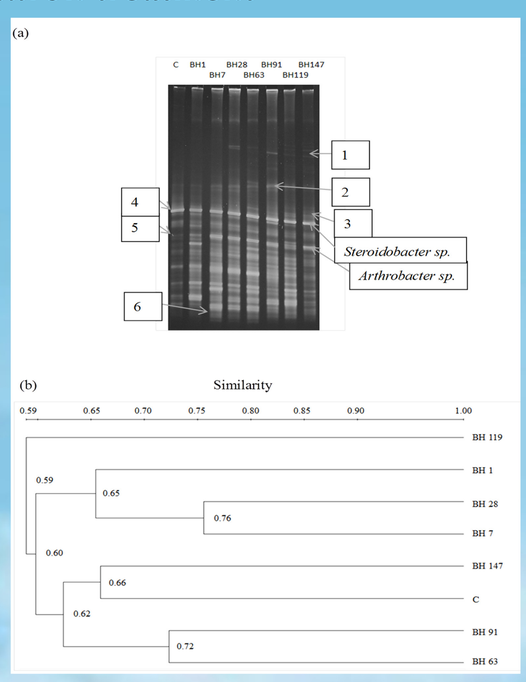

Fig. 3 (a) PCR-DGGE analysis of 16S rDNA sequence fragments and (b) UPGMA cluster analysis of bacterial community structures obtained from benomyl+diuron treatment soil with difference incubation time.

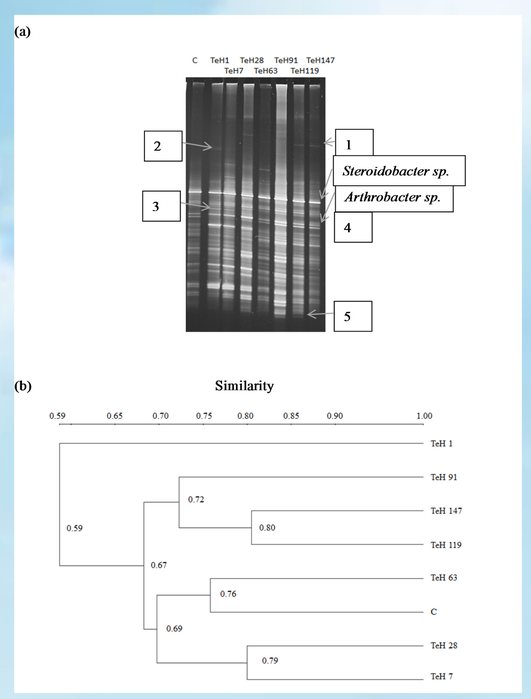

Fig. 4 (a) PCR-DGGE analysis of 16S rDNA sequence fragments and (b) UPGMA cluster analysis of bacterial community structures obtained from tebuconazole+diuron treatment soil with difference incubation time.

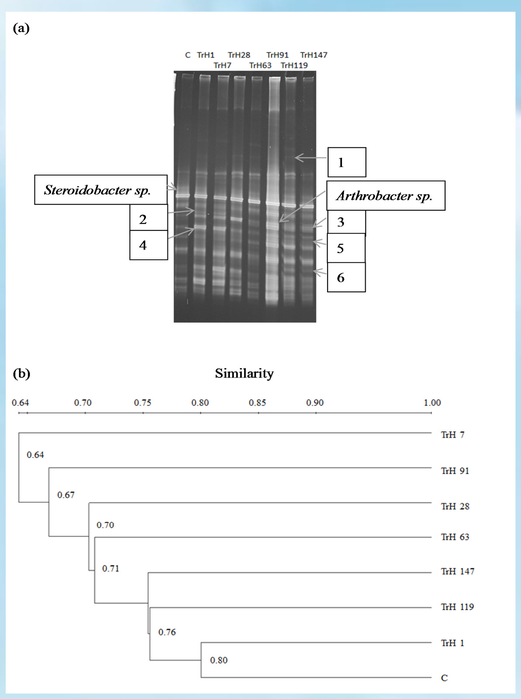

Fig. 5 (a) PCR-DGGE analysis of 16S rDNA sequence fragments and (b) UPGMA cluster analysis of bacterial community structures obtained from triadimefon+diuron treatment soil with difference incubation time.

Conclusions

In this study we found the treatments of triadimefon with diuron and benomyl with diuron would affect the soil bacterial communities. The impact on bacterial community may inhibit the degradation of diuron and therefore increase the persistence of diuron in soil.