The impacts of vinclozolin on soil microbial population

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Introduction

A fungicide of the dicarboximide type vinclozolin [3-(3,5-dichloro -phenyl)-5-methyl-5-vinyl- 1,3-oxazolidine-2,4-dione] was introduced to control various vegetable and orchard diseases. Vinclozolin has been shown to act as an environmental antiandrogen and influence gonad development and fertility.Moreover, vinclozolin is an endocrine disrupter chemical (EDC). Vinclozolin induced transcriptional alterations during gonadal sex determination and early testis development was examined. In this study, effects the diversity of the soil vinclozolin microbial community using soil samples collected from Taoyuan District Agricultural Research and Extension Station in Taiwan were examined. The diversity of the microbial community in soil is an important issue in modern soil microbiology and is one of the main indicators of toxic effects of pesticides in agriculture. 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 impact of vinclozolin on soil microbial community was visualized by PCR-DGGE and similarity between treatments was analyzed by unweighted pair-group method using arithmetic averages (UPGMA). Because of the unevenness of pesticides during application and to magnify the change of microbial communities, 5 and 50 mg kg^{-1} vinclozolin was used in this study.

Materials and methods

1.Materials

Vinclozolin used in the experiments were purchased from Fluka PESTANAL® Co., US. Purity of vinclozolin is 99.5%.

The soil sample was collected from Taoyuan District Agricultural Research and Extension Station (Pu soil). The soil texture was sandy loam with 2.72% organic matter content, 36.1% sand, 16.0% clay, 47.9% silt, and pH 5.06.

2. Sample Preparation

Keep the water holding capacity (45.07%) for each treatment. The incubation temperature was 30℃ to simulate room temperatures.

3. Extraction and Analysis of Chemicals

To determine the residual content of vinclozolin, 5 mL *n*-hexane was added to 10 g soil samples, which were then vortex for 1 min. Then, the samples were centrifuged at 5000 rpm for 10 min. The supernatants were collected and passed through 0.45-μm filters. The filtrate was analyzed by use of GC-ECD.

4. Extraction and Analysis of Microorganism

Total genomic DNA was extracted by use of an UltraClean™ 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 Vinclozolin in Sterile and Non-sterile Soils

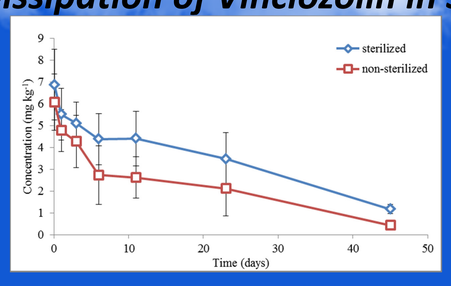

Fig.1 The degradation of 5mg kg^{-1} vinclozolin.□ represent nonsterilized Pu soil ◇represent sterilized Pu soil

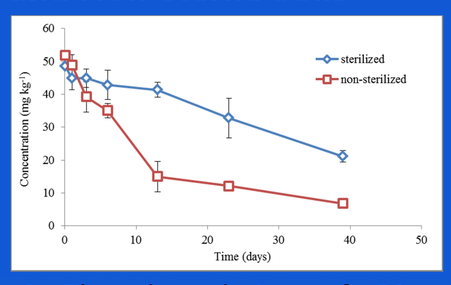

Fig.2 The degradation of 50mg kg^{-1} vinclozolin. □ represent non-sterilized Pu soil ◇represent sterilized Pu soil

2. Soil Bacterial Community with Vinclozolin Treatment

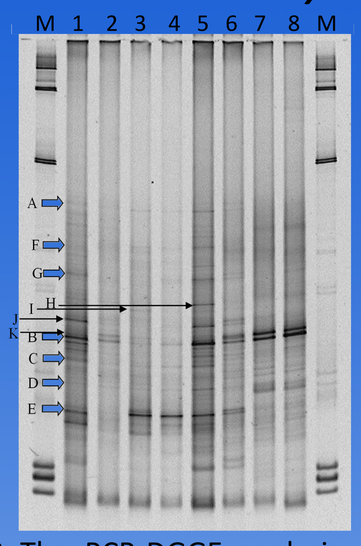

Fig.3 The PCR-DGGE analysis of 16S rDNA sequence fragments obtained from non-sterilized Pu soil of 5 mg kg^{-1} vinclozolin treatment. Letter M represents marker, and the number 1 is control in day 0. Number 2 to 8 represent the days 0, 1, 3, 6, 11, 23 and 45 day in this study, respectively.

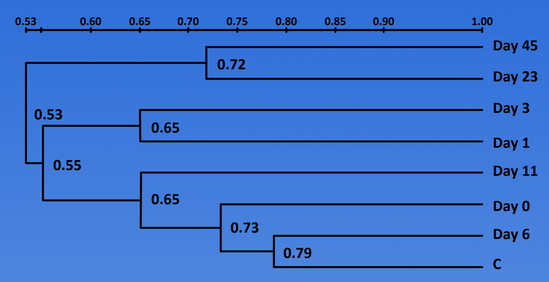

Fig. 4 Cluster analysis of bacterial community structures with different time of vinclozolin 5 mg kg^{-1} treated Pu soil by UPGMA. Abbreviation C means control.

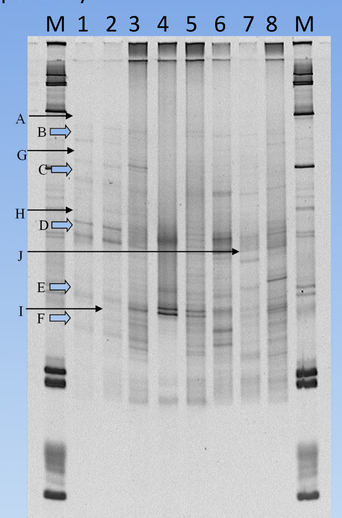

Fig. 5 The PCR-DGGE analysis of 16S rDNA sequence fragments obtained from non-sterilized Pu soil of 50 mg kg^{-1} vinclozolin treatment. Letter M represents marker, and the number 1 is control in day 0. Number 2 to 8 represent the days 0, 1, 3, 6, 13, 23, and 39 day in this study, respectively.

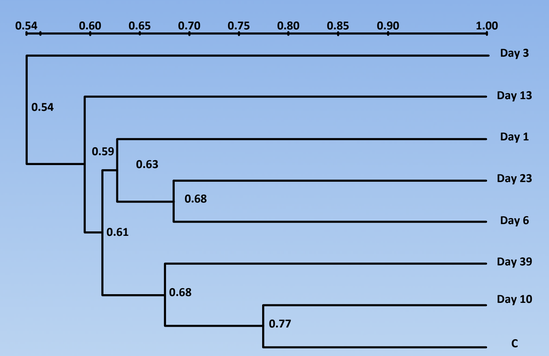

Fig. 6 Cluster analysis of bacterial community structures with different time of vinclozolin 50 mg kg-1 treated Pu soil by UPGMA. Abbreviation C means control.

Conclusions

In our study the result shows that when vinclozolin were completely degraded the soil microbial community cloud is partially recovered. Different treated time the soil microbial community was changed. The application of vinclozolin to soil will affect the soil microbial community, and it is important to notice the residual in our environment and to re-evaluate whether the regulation of vinclozolin has been updated.