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Effects of Imidacloprid on the Biodiversity of Soil Microbes in Selected Soils of Malaysia

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Abstract— **Imidacloprid is a systemic nicotinic compound with potent insecticidal activity against a wide range of pests. Although this pesticide is considered as relatively low toxic, but still there is great concern about its influence on soil microbial community. The current study was conducted to evaluate the effects of imidacloprid on soil microbial diversity. Two different molecular markers (ERIC-PCR and RAPD-PCR) were used for evaluation of genetic diversity in different soil samples which collected from selected non-polluted, semi-polluted and highly contaminated areas. The results showed that the application of imidacloprid has different impacts on soil bacterial community and the numbers of viable gram negative bacteria in soil can be reduced due to long-term use of this pesticide and the residues of this chemical in soil could be deleterious to some groups of soil microbes. Also cluster analyzing clearly showed that imidacloprid has significant negative impact on soil bacterial diversity in highly polluted farms and soil microbial balance has been gradually upset by application of more pesticides.**

Keywords-imidacloprid; diversity; bacteria; ERIC-PCR; RAPD-PCR; Malaysia

I. INTRODUCTION

Imidacloprid, 1- (6 – chloronicotinyl) – 2 – nitroimino – imidazolidine, is the first member of the neonicotinoid class of insecticides to be commercialized in 1991 and is used extensively for both crop protection and animal health applications [9,13]. Although the application of imidacloprid has been gaining popularity in agricultural and residential settings of countries especially in Malaysia, its environmental effects on soil microbial community have not been fully evaluated [1,3,5]. Few studies on influence of imidacloprid on soil microbial communities have shown adverse effects of this pesticide on different groups of soil microbes [2,14]. While many in the industry consider imidacloprid to be a pesticide of relatively low toxicity, it has been found to be extremely toxic to non-target insects like bees, and recently has led to resistance in some pests [13].

The aim of this study was to determine effects of imidacloprid on microbial diversity during long-term applications of imidacloprid, and to investigate what kind of microbes are affected more. To fulfill the objectives, different fields in Cameron Highlands area (in central part of Malaysia) have been selected due to intensive application of imidacloprid in the agricultural farms.

II. METHERIAL AND METHODS

A. Soil samples

Soil samples were collected from three different sites in Cameron Highlands in central part of west Malaysia. Soil sample selection was based on the history of the application of imidacloprid within these sites. Sampling site No. 1 was highly contaminated by imidacloprid due to heavily application of imidacloprid during recent years. Site No.2 was non-polluted area which was an experimental organic farm in Cameron Highlands that was protected from use of any agricultural chemicals including imidacloprid. And site No.3 was the area which imidacloprid had occasionally been used by farmers and consequently was moderately polluted by imidacloprid.

For sampling, in each site, over 10 random place were chosen for soil collection, and 1kg soil were taken from 15-25cm dept of soil then after mixing all collected soil with together, 1kg of mixture, representing of soil sample of that site, was taken into a clean plastic bag and transferred to the lab inside coleman (+4°C).

B. Residue Analysis

10 gram of each soil sample was transferred to centrifuge tubes and was suspended within acetonitrile, methanol and water (3:3:2) mixture. The suspension was shaken for 2h followed by centrifugation and filtered using syringe filter and consequently concentrated under rotary evaporation vacuum and completely dried under a gentle nitrogen gas stream. The residue was redissolved in a mobile phase, and aliquots of 25 µl were injected to liquid chromatography (HPLC) [7,15].

C. Bacterial isolation

One gram of each soil sample was suspended in 9ml sterile water and shaken for 5 min. One ml of each soil suspension was serially diluted (till 10^{-7}). Each dilution was

plated onto standard Mineral Salt Medium (MSM), Nutrient Broth (NB) and Tryptic Soy Broth (TSB) media containing imidacloprid (80 mg L^{-1}) and incubated at 28°C for 72 h [4,6,11].

After few days total viable bacteria for each soil sample were measured and plates screened for colonies that visually appeared different from each other. The different colonies were randomly selected and purified in NA medium and were stored inside glycerol: water (1:4) mixture and kept in -20°C. For genetic diversity analyses, each of these bacterial isolates was gradually re-cultured in NA medium and was analyzed by ERIC-PCR and RAPD-PCR.

D. ERIC and RAPD PCR

Primers for ERIC-PCR and RAPD PCR were prepared from NHK Bioscience Solutions Co. (S. Korea). The sequences of forward and reverse ERIC primers which previously described by Nicholson and Hirsch (2005) were: Forward: 5'- CAC TTA GGG GTC CTC GAA TGT A-3' and Reverse: 5'- AAG TAA GTG ACT GGG GTG AGC G-3' and the random primers pack was bought from NHK Bioscience Solution Company.

Total bacterial DNA was extracted from selected bacteria using Medici *et al*., (2003) methods with few modifications. Final volume for both PCR was 50µL. The PCR reaction mix contained of 5ng of bacterial total DNA as template. Amplification was performed in a BioRAD (*i*-cycler) thermocycler (USA) with the following program: Initial denaturation step at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min (denaturation), 52°C for 1 min (annealing) for ERIC-PCR [10] and 30°C for RAPD-PCR [16], 72°C for 8 min (extension), and with final extension step at 72°C for 5 min.

PCR products were separated onto agarose gels and stained in ethidium bromide for and illuminated under UV light. The standard DNA marker (DNA ladder 100bp) was used as molecular size marker. After staining of electrophoresis gels of ERIC and RAPD-PCR products, the gels were photographically scanned and loaded into a computer and analyzed using GEL-Compar II software (Belgium).

III. Results and Discussion

A. Bacterial Isolation

High and lower volume of total viable bacteria was observed in organic and polluted sites respectively (Figure 1). Though, the differences in total viable bacteria among these three different soil samples was low but the results showed that the application of imidacloprid has negative impact on total bacterial populations inside soil and the numbers of viable bacteria biomass in soil can be reduced due to long-term use of this pesticide and the residues of this chemical in soil could be deleterious to most groups of soil microbes. Different pesticides have different effect on soil total viable bacteria amount. Some pesticides such as pentachlorophenol (PCP) have negative impact on total viable bacteria in treated soils while some other studies shown that total viable bacteria in the soils that have been contaminated with some pesticides such as cypermethrin were increased [12,17].

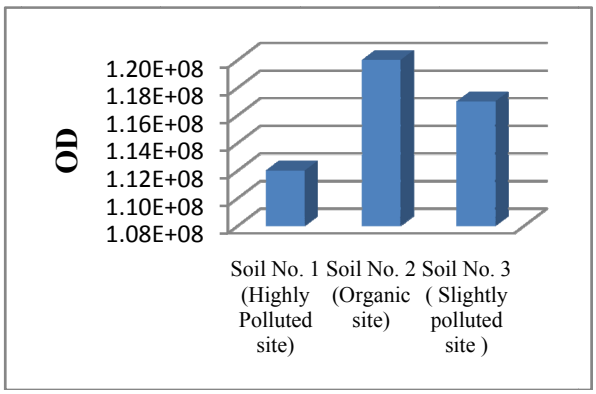

Figure 1. Total viable bacteria in soil samples

A total of 60 colonies from three soil sample were isolated, purified and stored. Majority of bacterial isolates from soil No.2 (organic farm, non-polluted) were gram negative bacteria (64.7%) while the numbers of gram negative bacteria were decreased in slightly polluted (60%) and highly polluted (50%) respectively. The current results indicate that imidacloprid has negative effects on some gram-negative bacteria in soil and gram-positive bacteria gradually became dominant in the soils that had been treated by imidacloprid. Previously similar effects of some pesticides on G- to G+ bacteria ratio have been reported [11,18].

Although still in highly polluted soil gram-negative bacteria consist 50% of soil bacterial population but still this reduction of gram-negative bacteria population in imidacloprid-polluted soils can increase anxiety about the negative effects of imidacloprid on soil biological fertility.

B. ERIC and RAPD-PCR Fingerprinting

After determination of band weights and their scoring, using Gel-Compar software, Jaccard's and Dice's indices were determined for comparison of profiles and UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) was used to draw similarity dendrogram for isolates (Figure 2). In ERIC-PCR profiling gram positive and gram negative isolates were divided into separate clusters while in RAPD-PCR no distinct clusters were drawn for gram negative and gram positive isolates.

The similarity of the patterns in both methods varied from 20 to 99% and. The highest similarity was observed among the bacteria isolated from soil No. 1 (highly polluted) while the lowest similarity was among Organic-farm's bacteria. In polluted farm, most gram negative and positive isolates were over genetically similar over 80%. Except those 4 isolates that were common in all 3 farms, the isolates that were dominant in polluted farm were different from non polluted farm (Organic farm).

Cluster analysis clearly shows that genomic distance between organic farm's (No.2) and highly polluted farm (No.1) bacteria was higher than genomic distance between

organic farm's bacteria and slightly polluted farm's (No.3) bacteria demonstrating that imidacloprid is significantly capable to change soil dominate bacteria in highly polluted farms and soil microbial balance has been gradually upset by application of more pesticide.

REFERENCES

[1] Atlas, M. R., Hortowitz, A., Krichevsky, M., and Bei, A. K. 1991. Response of microbial population to environmental disturbance. Microbial Ecology. 22: 249-256.

[2] Ahmed S., and Ahmad, M. S. 2006. Effect of insecticides on the total number of soil bacteria under laboratory and field conditions. Pakistan Entomology. 28: 63-68.

[3] Bromilow, R. H., Evans, A. A., Nicholls, P. H. Todd, A. D. and Briggs, G. G. 1996. The effect on soil fertility of repeated applications of pesticides over 20 years. Pesticide Science. 48: 63-72.

[4] Fantroussi, S., Verschuere, L., Verschuere, W. and Top, E. M. 1999. Effects of phenylurea herbicides on soil microbial communities estimated by analysis of 16s RRNA gene fingerprints and community-level physiological profiles. Applied and Environmental Microbiology. 65 (3): 982-988.

[5] Johnsen, K., Jacobsen, C. S., Torsvik, V. and Sorensen, J. 2001. Pesticide affects on bacterial diversity in agricultural soil- a review. Boil. Fertil. Soils. 33: 443-453.

[6] Li, X., Jiang, J., Gu, L., Ali, S., He, J. and Li, S. 2008. Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples. International Biodeterioration and Biodegradation. 62: 331–335.

[7] Liu, H., Liu, H., Song, J., Zhang, S., Qu, L., Zhao, Y., Wu, Y., and Liu, H. 2005. Analysis of residues of imidacloprid in tobacco by high-performance liquid chromatography with liquid-liquid partition cleanup. Journal of Pest Management Science. 61: 511-514.

[8] Medici, D.D., et al., 2003. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect Salmonella enterica serotype Enteritidis in poultry. Applied and Environmental mMicrobiology. 69: 3456-3461.

[9] Moriya, K., Shibuya, K., Hattori, Y., Tsuboi, S. and Shiokawa, K. 1992. 1-(6-Chloronicotinyl)-2- nitroimino-imidazolidines and related compounds as potential new insecticides. Bioscience, Biotechnology, and Biochemistry. 56 (2): 364-365.

[10] Nicholson, P.S. and Hirsch, P.R. 1998.The effects on the diversity of the culturable soil bacterial. Journal of Applied Microbiology. 84: 551-558.

[11] Rath, A. K., Ramakrishnan, B., Kumarawamy, S., Bharati, K., Singla, P. and Sethunathan, N. 1998. Effect of pesticides on microbial biomass of flooded soil. Chemosphere. 37: 661-671.

[12] Sato, K. 1993. Effect of a pesticide, pentachlorophenol (PCP) on soil microflora I. Effect of PCP on microbiological processes in soil percolated with glycine. Plant and Soil. 75: 417-426.

[13] Sheets, Larry, P. 2001. Imidacloprid: A neonicotinoid insecticide. In: Krieger R., ed. Handbook of Pesticide Toxicology. Vol. 2: 1123-1130.

[14] Singh, J. and Singh, D.K. 2005. Bacterial, Azotobacter, Actinomycetes, and Fungal Population in Soil after Diazinon, Imidacloprid, and lindane treatments in groundnut (Arachis hypogaea L.) fields. Journal of Environmental Science and Health Part B. 40: 785-800.

[15] Singh, B. K., and Walker, A. 2006. Microbial degradation of organophosphorus compounds. FEMS Microbiological Review. 30: 428–471.

[16] Yang, Y. H., Yao, S. and Qi, Y. 2000. Effects of agricultural chemicals on sequence analysis diversity of soil microbial community: A study with RAPD marker. Microbial Ecology. 39: 72-79.

[17] Zhang, B., Zhang, H., Jin, B., Tang, J., Li, B., Zhuang, G., Baiz, H. 2008. Effect of cypermethrin insecticide on the microbial community in cucumber phyllosphere. Journal of Environmental Sciences, 20: 1356–1362.

[18] Zhang, C., Liu, X., Dong, F., Xu, J., Zheng, Y., and Li, L. 2010. Soil microbial communities' response to herbicide 2, 4-dichlorophenoxyacetic acid butylester. European Journal of Soil Biology, 1: 1-6.

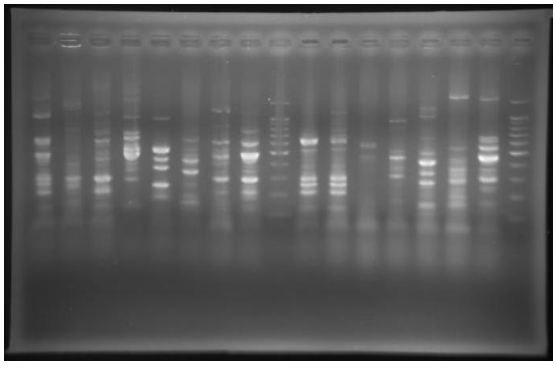

Figure 2. The results of ERIC-PCR experiment on some selected bacterial isolates

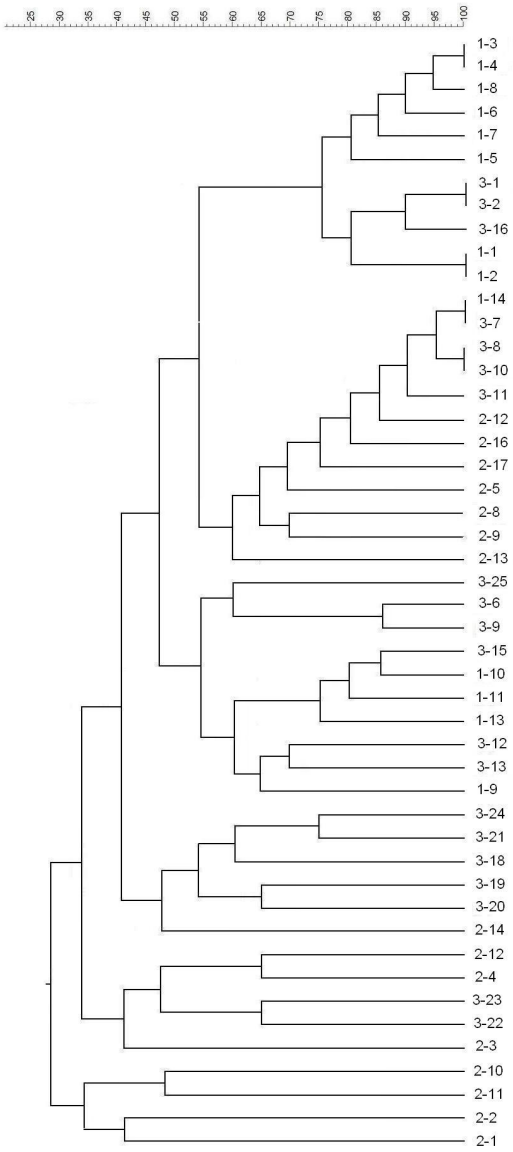

Figure 3. Dendrogram drawn from UPGMA comparison of bacterial isolated by ERIC-PCR