UNIVERSITI PUTRA MALAYSIA

IMIDACLOPRID BIODEGRADATION BY SOIL BACTERIA AND RELATIONSHIP TO SOIL BACTERIAL DIVERSITY

NASRIN SABOUR MOGHADDAM

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DOCTOR OF PHILOSOPHY

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By

NASRIN SABOUR MOGHADDAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2011

DEDICATION

I would like to dedicate this to my dear mother, father and my husband Reza Khakvar for their moral support, patient and confidence in me all the time. Their love and respect was my muse…

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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NASRIN SABOUR MOGHADDAM

February 2011

Chair: Associate Professor Mohamad Pauzi Zakaria, PhD

Faculty: Environmental Studies

Imidacloprid, [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin- 2-ylideneamine], is a second generation neonicotinoid pesticide (chloronicotinyl insecticide) that is used to treat plants against threatening pests. Application of imidacloprid as plant, seed and soil treatments has become a common practice to control pests on rice, maize, potatoes and vegetables. Besides controlling the pests in plants and soil, imidacloprid have come into direct or indirect contact to the nontargeted soil microorganisms and may affect their population and activity of microbial communities in soil.

Although the use of imidacloprid has been gaining popularity in agricultural and residential settings, the factors leading to its dissipation in soil are not clearly understood. Field dissipation rates of imidacloprid are widely variable, and it has been reported as a stable compound in the environment with half live exceeding 180 days in non-vegetated soil and more than three years in dry and aerobic conditions.

Whilst possible microbial metabolites of imidacloprid have been reported in soil metabolism studies, direct evidence of imidacloprid biodegradation and its effects on the genomic diversity of bacteria has not been fully evaluated especially in tropical area.

The objectives of this research were the isolation and identification of imidacloprid degrading bacteria from soil samples in laboratory condition, and to determine effects of imidacloprid on the diversity of soil bacteria using suitable molecular markers. Several extraction methods have been published for determination of imidacloprid residues in different matrixes, but there is no record of Soxhlet extraction for imidacloprid residue analysis and comparison of its efficiency with other extraction methods. Therefore we also optimized reliable extraction method with appropriate use of organic solvent, to quantify the concentration of imidacloprid present in the sandy clay loam soil, with applying Soxhlet and liquid extraction method using HPLC.

A comparison of the Soxhlet and liquid extraction methods for the sandy loamy clay soil indicated that, the two applied extraction methods performed equally well for the imidacloprid detection in terms of recovery percentage for lower concentration (1 mg kg^{-1}), but efficiency of liquid extraction over Soxhlet in higher concentrations (5 & 10 mg kg^{-1}) was confirmed.

Using standard Mineral Salt Medium (MSM), Nutrient Broth (NB) and Tryptic Soy Broth (TSB) media for different collected soils, a total of 120 different bacteria strains were isolated and biodegradative ability of all isolates were tested on N

limited and C limited media. As a result five bacteria were confirmed to be capable for degradation of imidacloprid in C limited media, which were identified as *Pseudomonas putida*, *Bacillus sp.*, *B. subtilis*, *Brevibacterium sp.* and *Rhizobium sp.* using 16S rRNA sequencing.

ERIC-PCR and RAPD-PCR were used to determine the influence of imidacloprid on soil bacterial diversity. The results showed that the long-term application of imidacloprid has different impacts on bacterial populations, and the numbers of viable Gram negative bacteria in soil can be reduced due to pesticides uses and Gram-positive bacteria gradually became dominant in the soils that had been treated by imidacloprid. The cluster analysis clearly showed that soil bacterial diversity has been changed in response to imidacloprid.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIODEGRADASI IMIDAKLOPRID OLEH BAKTERIA TANAH DAN HUBUNGANNYA TERHADAP KEPELBAGAIAN TANAH

Oleh

NASRIN SABOUR MOGHADDAM

Februari 2011

Pengerusi: Prof. Madya Mohamad Pauzi Zakaria, PhD

Fakulti: Pengajian Alam Sekitar

Imidakloprid, [1 - (6 – kloro-3-piridilmetil) -N- nitroimidazolidin -2- ylideneamine], adalah racun perosak generasi kedua neonicotinoid (racun serangga kloronicotinil) yang digunakan untuk merawat tanaman terhadap ancaman serangga. Aplikasi imidakloprid sebagai rawatan tanaman, benih dan tanah telah menjadi amalan umum untuk mengawal serangga pada padi, jagung, kentang dan sayur-sayuran. Selain mengawal serangga pada tanaman dan tanah, imidakloprid juga bertindak secara langsung atau tidak langsung terhadap mikroorganisma tanah bukan sasaran dan boleh menjejaskan populasi mereka dan aktiviti komuniti mikrob dalam tanah.

Walaupun penggunaan imidakloprid telah menjadi popular dalam amalan pertanian dan perumahan, faktor lesapan di tanah itu masih belum jelas difahami. Kodar lesapan lapangan imidakloprid adalah pelbagai, dan imidakloprid telah dilaporkan sebagai sebatian yang stabil dalam persekitaran dengan jangka hayat melebihi 180 hari dalam tanah tanpa-tanaman dan lebih daripada tiga tahun dalam keadaan kering

dan aerobik. Sesekali, terdapat kemungkinan metabolit mikrob imidakloprid wujud dalam kajian metabolisme tanah, namun bukti langsung biodegradasi imidakloprid dan kesannya pada kepelbagaian genom bakteria belum dinilai sepenuhnya terutama di kawasan tropika.

Objektif kajian ini adalah untuk memencilkan dan mengenalpasti degradasi bakteria imidakloprid dalam keadaan makmal dan menentukan kesan dari imidakloprid pada kepelbagaian genom bakteria tanah dengan menggunakan penanda molekul yang sesuai. Beberapa kaedah pengekstrakan telah diterbitkan untuk penentuan sisa baki imidakloprid dalam matriks yang berbeza, tetapi tidak ada catatan dari pengekstrakan Soxhlet untuk analisis sisa baki imidakloprid dan perbandingan kecekapannya dengan kaedah pengekstrakan yang lain. Oleh itu kajian ini juga termasuk mengoptimumkan kaedah pengekstrakan dengan penggunaan pelarut organik yang bersesuaian untuk mengukur kepekatan imidakloprid yang terdapat dalam tanah lempung liat berpasir dengan menggunakan kaedah pengekstrakan Soxhlet dan cecair HPLC.

Perbandingan antara pengekstrakan “Soxhlet” dan pengekstrakan cecair untuk tanah lempung liat berpasir menunjukkan bahawa kedua-dua kaedah pengekstrakan menghasilkan pengesanan imidakloprid yang sama dari segi peratusan pemulihan untuk kepekatan yang rendah (1 mg kg^{-1}), tetapi pengekstrakan cecair disahkan lebih cekap berbanding “Soxhlet” pada kepekatan yang lebih tinggi (5 & 10 mg kg^{-1}).

Dengan menggunakan standard “Mineral Salt Medium” (MSM), “Kaldu Gizi” (NB) dan “Tryptic Soy Broth” (TSB) pada media tanah terkumpul yang berbeza, sejumlah

120 jenis bakteria yang berbeza telah diasingkan dan kemampuan biodegradasi dari semua isolat pada media N terhad dan C terhad telah diuji. Keputusan menunjukkan, lima bakteria telah dikenalpasti mampu untuk menguraikan imidakloprid di media terhad C. Ia dikenalpasti sebagai *Pseudomonas putida, Bacillus sp, Bacillus subtilis., Brevibacterium sp. dan Rhizobium sp.* dengan menggunakan kaedah jujukan 16S rRNA.

ERIC-PCR dan RAPD-PCR telah digunakan untuk menentukan pengaruh imidakloprid terhadap kepelbagaian bakteria tanah. Keputusan kajian menunjukkan bahawa aplikasi imidaklorapid untuk suatu jangka masa panjang mempunyai kesan yang berbeza pada populasi bacteria. Jumlah Gram bakteria negatif yang terdapat didalam tanah dikurangkan oleh penggunaan racun perosak dan bakteria Gram positif secara berperingkat menjadi dominan di tanah yang telah dirawat oleh imidakloprid. Analisis kluster jelas menunjukkan bahawa kepelbagaian bakteria tanah berubah responsnya terhadap imidakloprid.

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The completion of this work was possible only by the kind support, advice and encouragement of many people and institutions.

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I certify that a Thesis Examination Committee has met on first of February 2011 to conduct the final examination of Nasrin Sabour Moghaddam on her thesis entitled "Imidacloprid Biodegradation by Soil Bacteria and its Relationship to Soil Bacterial Diversity" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NASRIN SABOUR MOGHADDAM

Date: 1 February 2011

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