UNIVERSITI PUTRA MALAYSIA

EXPRESSION OF CADHERIN, AN INSECTICIDE RESISTANCE ASSOCIATED GENE IN OIL PALM PEST, Tirathaba mundella AND POLLINATOR, Elaeidobius kamerunicus INDUCED BY EXPOSURE OF Bacillus thuringiensis

CALVIN TAN ZHE KHAI

FSPM 2020 5

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By

CALVIN TAN ZHE KHAI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirement for the Degree of Master of Science

January 2020

DEDICATION

TO

To my parents, family members, lecturers and friends
who have always been with me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EXPRESSION OF *CADHERIN*, AN INSECTICIDE RESISTANCE ASSOCIATED GENE IN OIL PALM PEST, *Tirathaba mundella* AND POLLINATOR, *Elaeidobius kamerunicus* INDUCED BY EXPOSURE OF *Bacillus thuringiensis*.

By

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January 2020

Chairman : Patricia King Jie Hung, PhD
Faculty : Agriculture and Food Sciences, Bintulu Campus

Tirathaba mundella is a major pest that potentially reduce the oil palm yield in plantations established on peat soil while *Elaeidobius kamerunicus* is a pollinating weevil that promote oil palm pollination. Current pest management strategies targeted to control the infestation of *T. mundella* and maintain the population of *E. kamerunicus*. *Bacillus thuringiensis* (Bt) insecticide has since been used to control insect pest in oil palm plantation while ensuring the population of pollinating weevil will not be adversely affected. However, studies have shown the progression development of resistance against Bt toxins among many pest insects, left alone the BT resistance in *T. mundella* was not well studied. Prior to this study, limited molecular data were available for these species and this constraint the study of insecticide resistance at the molecular level. In this study, *cadherin* gene, which often associated with the resistance against Bt toxin was investigated its relative expression in both *T. mundella* and *E. kamerunicus*. Insect samples were collected from two plantation sites, one with high exposure and one with low exposure to Bt insecticide. *T. mundella* collected from plantation with higher exposure to Bt toxin shows significant lower *cadherin* expression level and lower mortality against Bt as compared to *T. mundella* with low exposure to Bt. Relatively lower *cadherin* gene expression was observed at the early development stages of *T. mundella* collected from the plantation with higher Bt exposure. Relatively lower *cadherin* gene expression may confer protection to the pest against Bt as observed in the bioassay. In *E. kamerunicus, cadherin* gene was not expressed and they were not affected by the application of Bt insecticide. The findings of this study lead to the inference that prolong exposure of Bt insecticide may induce the progression development of *Bt*-resistance strain. Further study is needed to confirm the claim and unveil the mechanism.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

EKSPRESI *CADHERIN*, GEN YANG BERKAIT DENGAN RESISTENSI TERHADAP RACUN SERANGGA PADA PEROSAK KELAPA SAWIT, *Tirathaba mundella* DAN PENDEBUNGA, *Elaeidobius kamerunicus* DISEBABKAN PENDEDAHAN *Bacillus thuringiensis*.

Oleh

CALVIN TAN ZHE KHAI

Januari 2020

Pengerusi : Patricia King Jie Hung, PhD
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Tirathaba mundella adalah perosak utama yang berpotensi mengurangkan hasil kelapa sawit di ladang yang ditubuhkan di kawasan tanah gambut manakala *Elaeidobius kamerunicus* adalah serangga berguna yang membantu dalam pendebungaan kelapa sawit. Strategi pengurusan perosak semasa disasarkan untuk mengawal infestasi *T. mundella* dan mengekalkan populasi *E. kamerunicus*. *Bacillus thuringiensis* (Bt) merupakan racun serangga yang telah lama digunakan untuk mengawal perosak di ladang kelapa sawit pada masa yang sama memastikan populasi serangga berguna tidak akan terjejas. Walau bagaimanapun, banyak kajian telah menunjukkan perkembangan resistensi terhadap toksin Bt di kalangan serangga perosak tetapi resistensi Bt pada *T. mundella* tidak dikaji dengan baik. Sebelum kajian ini dijalankan, data molekul yang sedia ada agak terhad untuk spesies yang dikaji dan ia telah menjadi kekangan untuk mengkaji resistensi racun serangga pada peringkat molekul. Dalam kajian ini, gen *cadherin* yang sering dikaitkan dengan resistensi terhadap toksin Bt telah dikaji atas ekspresi relatifnya pada *T. mundella* and *E. kamerunicus*. Spesimen serangga telah dikumpul dari dua ladang kelapa sawit, iaitu ladang yang mempunyai kadar pendedahan yang tinggi dan rendah terhadap Bt racun serangga. *T. mundella* yang dikumpul daripada ladang yang mempunyai pendedahan dengan toksin Bt yang lebih tinggi menunjukkan tahap ekspresi *cadherin* yang ketara lebih rendah dan kadar kematian yang lebih rendah berbanding dengan sampel yang kurang terdedah terhadap Bt. Ekspresi gen *cadherin* yang relatif lebih rendah diperhatikan pada tahap perkembangan awal *T. mundella* yang dikumpulkan dari ladang dengan pendedahan Bt yang lebih tinggi. Ekspresi gen *cadherin* yang ketara lebih rendah berkemungkinan dapat memberikan perosak perlindungan terhadap Bt seperti mana yang diamati dalam bioassay. Pada *E. kamerunicus*, gen *cadherin* tidak diekspres dan mereka tidak terjejas dengan penggunaan racun serangga Bt. Penemuan kajian ini berkesimpulan pendedahan racun serangga yang memanjangkan berkemungkinan dapat mendorong perkembangan baka Bt-resisten. Kajian lanjut diperlukan untuk mengesahkan tuntutan tersebut dan memperlihatkan mekanisme tersebut.

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I certify that a Thesis Examination Committee has met on 30 January 2020 to conduct the final examination of Calvin Tan Zhe Khai on his Master of Science thesis entitled "Expression of Cadherin, an Insecticide Resistance Associated Gene in Oil Palm Pest, *Tirathaba mundella* and Pollinator, *Elaeidobius kamerunicus* Induced by Exposure of *Bacillus thuringiensis*" 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 Master of Science.

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This is to confirm that:

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LIST OF ABBREVIAITONS

Bt	*Bacillus thuringiensis*
ICP	Insecticidal crystal protein
CPO	Crude palm oil
FFB	Fresh fruit bunch
bp	Base pair
RPM	Rotation per minute
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
gDNA	Genomic deoxyribonucleic acid
cDNA	Complementary deoxyribonucleic acid
RNA	Ribonucleic acid
One-way ANOVA	One-way analysis of variance
SAS	Analytics software & solutions

CHAPTER 1

INTRODUCTION

Elaeidobius kamerunicus (Coleoptera: Curculionidae) is a beneficial insect that helps in the pollination of oil palm by visiting the male and female inflorescence (Dhileepan 1994). It has been introduced from Cameroon, Africa to Malaysia since 1981. The introduction of *E. kamerunicus* has increased the production of fresh fruit bunch (FFB) (Syed et al. 1982). However, the production of FFB is remarkably affected by pests that infesting oil palm. *Tirathaba mundella* (Lepidoptera: Pyralidae) is an important pest that causes serious damage to oil palm especially those planted on peat soil area (Yaakop and Manaf 2015; Masijan et al. 2015). The larvae of *T. mundella* are commonly found on immature fruitlets and male inflorescence. It feeds and makes holes on immature fruitlets, causing serious impact on the growth of fruit bunches (Ming et al. 2016). The infestation of *T. mundella* is widely spread in Malaysia and Indonesia (Hosang 2010). High infestation rate of *T. mundella* was reported in Sarawak particularly in areas such as Sibu, Miri and Mukah (Masijan et al. 2015).

Over the past decade, *Bacillus thuringiensis* (Bt) which produce species-specify *Cry* toxin has been broadly used to develop transgenic crops and play an important role in agricultural pest management (Jouzani et al. 2017). In Malaysia, Bt insecticide is widely applied in oil palm plantation to control the infestation of Lepidoptera pest as it is harmless to humans and non-target animals (Masijan et al. 2015). However, the prevalence of *Cry* toxins have raised concerns that the efficacy may be short-lived due to the possibility of the emergence of Bt resistant pests. Bt resistant strain has been reported from a broad range of crop pests from the order of Lepidoptera and Coleoptera (Ferré and Van 1995). The development of insecticide resistant strain had greatly reduced the efficacy of pest management strategies, commonly results in higher insecticide application frequencies and dosage (Bravo and Soberón 2008).

The development of molecular biotechnology has allowed scientists to study on the insecticide resistance at molecular level. Genes associated with the resistance mechanism against Bt insecticide have been reported in Lepidopteran and Coleopteran (Melo et al. 2014). *Cadherin* is one of the genes associated with the resistance against *Cry* toxin. *Cadherin* receptor in insect midgut was found to bind with the *Cry* toxin produced by Bt, the binding leads to the pore formation in insect midgut and kill the insect. Reduction of *cadherin* gene expression has been tightly associated with resistance capability against Bt insecticide in insects (Yang et al. 2012).

Nevertheless, there is lack of assessment on the effectiveness of the Bt insecticide against *T. mundella* after long term exposure despite the application is widely practiced to control the infestation. Besides, the study of the oil palm insect pest resistance gene was scarce. Limited of gene coding sequence was deposited in the NCBI database for both insects prior to this study. The lack of molecular data for the study of pest insecticide resistance constraint the study of insect behavior for the upgrowth of effective solution on the controlling of *T. mundella* and maintaining the population of *E. kamerunicus.*

The overall objectives of the current study are (1) to identify the gene fragment that associated with the Bt insecticide resistance in *T. mundella* and *E. kamerunicus*, (2) to study on the development of resistant strains in *T. mundella* and *E. kamerunicus*, (3) to identify the relationship between the gene expression and the Bt insecticide resistance. Several approaches were employed to amplify the DNA sequence of the *cadherin* gene that is associated with the *Cry* toxin resistance in *T. mundella* and *E. kamerunicus*. In addition, the development of insecticide resistance in both insects was assessed on the specimens collected from two estates with different pest management practices: Sabaju Estate (Bintulu, Sarawak) and Tinbarap Estate (Miri, Sarawak). Occasional application of Bt insecticide was practiced in Sabaju Estate while Tinbarap Estate was free from the insecticide over a year when the samples were collected. The *cadherin* gene expression was studied together with the insect mortality to determine its linkage with the insecticide resistance.

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