



UNIVERSITI PUTRA MALAYSIA

***RESISTANCE AGAINST SPINOSAD IN *Plutella xylostella* Linnaeus
(DIAMONDBACK MOTH) AND ITS CROSS RESISTANCE TO OTHER
INSECTICIDES***

NUR ADIBAH BINTI MOHD ISHADI

FP 2020 40



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By

NUR ADIBAH BINTI MOHD ISHADI

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

February 2020

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

RESISTANCE AGAINST SPINOSAD IN *Plutella xylostella* Linnaeus (DIAMONDBACK MOTH) AND ITS CROSS RESISTANCE TO OTHER INSECTICIDES

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February 2020

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Plutella xylostella (Diamondback moth, DBM) has developed resistance to different classes of insecticides over the years in Malaysia. This study aimed at establishing the resistance development and mechanism of DBM towards spinosad resistance. The parameters assessed were the role of specific enzymes (P450, GST and esterases), the fitness cost in resistant strain, mode of resistance inheritance, resistance stability and cross resistance potential with other selected insecticides. DBM was obtained from Ladybird Organic Farm, Selangor. Bioassay conducted on spinosad-selected (Spi-Sel) from parent (P) generation to the 15th generation (G15) showed approximately 42.81-fold increase in the resistance. P450 had significantly 4-fold higher activity ($p < 0.05$) in the Spi-Sel strain than in susceptible (SS) strain. Spi-Sel strain experienced high fitness cost during resistance development (relative fitness: 0.25). The larva of SS strain had significantly higher survival rate while larva of Spi-Sel strain required significantly shorter time to complete the stage. Spinosad resistance in Spi-Sel strain was governed by a single major autosomal factor that was inherited recessively. The recessiveness of the resistance was represented by the value of degree of dominance (D_{LC}) of F1 and F1', -0.6062 and 0.0311 respectively. The overlapping fiducial limit between the F1 and F1' concluding the autosomal inheritance. No significant deviation ($P > 0.05$) between the expected and observed mortality of the backcross (BC2) population implied monofactorial resistance. Spinosad-decaying (Spi-Dec) strain was produced by terminating the selection at G27 to study the resistance stability. The LC_{50} ranging from 547.33 to 132.57 ppm from G1 until G5 implying the unstable resistance. Moderate cross resistance was recorded towards emamectin and deltamethrin (RR = 3.8 and RR = 3.49, respectively) and low cross resistance recorded towards chlorantraniliprole (RR = 1.16). Unstable resistance accompanied by moderate to low cross resistance to other insecticides in the present finding suggested that resistance to spinosad in DBM can be reversed to susceptible status again and that resistance can be delayed by alternating the spinosad with insecticides of different classes and mode of action.

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

KERINTANGAN TERHADAP SPINOSAD DALAM *Plutella xylostella* Linnaeus (KUPU-KUPU INTAN) DAN RINTANG SILANGNYA KEPADA RACUN SERANGGA PEROSAK LAIN

Oleh

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Plutella xylostella (Kupu-kupu intan; DBM) telah menunjukkan kerintangan kepada kelas racun serangga perosak yang berbeza setelah sekian lama di Malaysia. Kajian ini bertujuan untuk membina perkembangan kerintangan dan mekanisma DBM terhadap spinosad. Parameter yang dinilai termasuklah fungsi enzim spesifik (P450, GST and esterases), 'fitness cost' dalam 'strain' rintang, mod pewarisan kerintangan, kestabilan kerintangan dan potensi silang rintang dengan racun serangga perosak yang lain. DBM telah diperolehi daripada Ladang Organik Ladybird di Selangor. Bioesei telah dijalankan ke atas generasi P sehingga generasi ke-15 (G15) menunjukkan nilai kerintangan sebanyak 42.81 kali ganda. Enzim P450 mempunyai 4 kali ganda aktiviti yang signifikan pada 'strain Spi-Sel' berbanding 'strain SS'. Kadar penerusan hidup bagi larva 'strain SS' lebih tinggi secara signifikan berbanding dengan 'strain Spi-Sel' namun larva 'Spi-Sel' mengambil masa yang signifikan lebih singkat untuk melengkapkan kitaran tersebut. Kerintangan spinosad dalam kajian ini melibatkan satu faktor autosomal yang diwarisi secara resesif. Tahap kedominanan (D_{LC}) melambangkan keresesifan tersebut dengan nilai -0.6062 dan 0.0311 bagi setiap satu F1 dan F1'. Had fidusial yang bertindih antara F1 dan F1' melambangkan pewarisan secara autosomal yang tidak melibatkan pengaruh induk dalam kes ini. Tiada diviasi yang signifikan ($P > 0.05$) antara kematian dijangka dan kematian direkod bagi populasi silang balik (BC2), menunjukkan bahawa kerintangan spinosad melibatkan hanya satu faktor (major). 'Strain spinosad-decaying' (Spi-Dec) telah dihasilkan melalui pemberhentian proses pemilihan pada G27 bagi tujuan kajian kestabilan kerintangan. Nilai LC_{50} mempunyai julat antara 547.33 ke 132.57 ppm daripada generasi pertama (G1) sehingga generasi kelima (G5), menunjukkan kerintangan yang tidak stabil. Silang rintang sederhana telah direkodkan terhadap emamectin dan deltamethrin ($RR = 3.8$ dan $RR = 3.49$, masing-masing) serta silang rendah rendah direkodkan terhadap chlorantraniliprole ($RR = 1.16$). Penurunan yang cepat dalam kerintangan spinosad berserta silang rintang sederhana dan rendah terhadap serangga perosak lain mencadangkan bahawa kerintangan spinosad dalam

DBM boleh dikembalikan kepada status lemah semula dan dilambatkan dengan mengilirkan spinosad dengan racun perosak yang berlainan kelas dan mod tindakan.



ACKNOWLEDGEMENTS

Alhamdulillah, all praises goes to Allah the Almighty that with His blessings, guides me throughout this doctorate journey.

My special thanks goes to my supervisor, Associate Professor Dr Norida Mazlan. Her consistent moral support, trust and patience she has given me since the early days knowing her always being an encouragement for me to give my best in this doctorate journey and in my future undertaking. Thank you very much Dr, may Allah reward you and your family abundantly in this life and hereafter. I also would like to express my humble gratitude to my committee member, Professor Dr. Dzolkhifli Omar and Associate Professor Lau Wei Hong for their invaluable help of constructive comments, advice and guidance which were a great help for the success of this study.

Sincere thanks to Pn Zaayah, Dr Farrah Melissa and all my friends of Postgraduate Room 2- Halimatun, Shu, Engku, Fatimah, Husna, Ain, Iman, Diyana, Aisyah, Hilmi and JJ, thank you very much for making my doctorate journey a fun and memorable one. I pray that all of us will succeed in paths we embark in the future.

Finally, my deepest gratitude goes to my beloved parents, Mohd Ishadi bin Sarbini and Rohani binti Awang, my sisters Anisah, Amirah, Atiqah and Aqilah and also to our big family. Thank you for always being the place I want to return to. I pray that the endless love, support and prayers will always be overflowing among us, insya Allah.

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

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LIST OF ABBREVIATIONS

P450	Cytochrome P450
GST	Glutathione-S-Transferase
ppm	Part per million
mm	millimetre
nAChRs	Nicotinic acetylcholine receptors
GABA	Gamma-aminobutyric acid
IRAC	Insecticide Resistance Action Committee
DCNB	2-dichloro-4-nitrobenzene
ld-pm	Log dose-probit mortality
LC	Lethal concentration
RR	Resistance ratio
FL	Fiducial limit
SE	Standard Error
mM	micromol
EDTA	Editic acid
PMSF	Phenylmethyl sulfonylfluoride
DTT	1,4-dithiothreitol
PTU	Phenylthiourea
BSA	Bovine serum albumin
D _{ML}	Effective dominance
SS	Susceptible
Spi-Sel	Spinosad-selected
Spi-Dec	Spinosad-decaying

CHAPTER 1

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae) is a well-known pest of Brassicacea. It is an oligophagous species, feed exclusively on the *Brassica* crops, primarily because of glucosinolate, a secondary metabolite that presence in the crops to enhance the feeding and oviposition in DBM. Zalucki et al. (2012) presented a detailed basis for estimating the worldwide control cost of diamondback moth, valued approximately US\$4 billion-US\$5 billion.

DBM multivoltine nature allows it to reproduce year-round; it can complete four generations in temperate areas per year while in tropical countries, it is capable to reach up to 20 generations (Lima-Neto et al., 2016; Knodel & Ganeshiarachchi, 2008). Practically, when more generations are produced and all life stages are available throughout year, it has resulted an increase in frequency of insecticide application. Therefore, DBM in tropical countries are subjected to more insecticides than the temperate countries.

In tropical countries, intensive planting of host plants has provided a convenient environment for DBM to sustain, however the production of the host plants on the other hand are largely threatened by the pest (Regupathy, 1996). In the Southeast Asia, the countries depended primarily on the agriculture and as they are seeking to enter the global market by providing fresh fruits and vegetables, it is crucial to produce high cosmetic standards fresh produce (Thuy et al., 2012). In order to meet this demand, insecticides are used intensively to control the pests. However, frequent and malpractice of insecticides application leads to resistance development of DBM to a wider range of insecticides.

Earlier, studies have proven DBM resistance to the early generations of insecticides such as organophosphates, pyrethroids and organochlorines as well as newer generations with novel mode of actions including spinosad, neonicotinoids and juvenile hormone disruptor (Meghana et al., 2018; Zhang et al., 2017; Park et al., 2004) Acknowledged as one the few insect species resistant to majority of the insecticide classes (Furlong et al., 2012), DBM is ranked second in the insecticide resistance list by Arthropod Pesticide Resistance Database (APRD,2018). Entailing resistance, DBM has also confers cross resistance to several groups of insecticides, either the insecticides are within the same or different groups. Studies have shown the DBM ability to confer cross resistance towards insecticides via different mechanisms such as by sharing of similar target sites, augmentation of detoxification enzymes and sharing of the midgut binding site (Chen et al., 2011; Gong et al., 2014; Wang et al., 2013).

Spinosad is an insecticide derived from soil actinomycete *Saccharopolyspora spinosa* that was firstly commercialized in 1997. Primarily it activates the nicotinic acetylcholine receptor and have gamma amino butyric acid (GABA) receptor as the secondary target site (Salgado, 1998). In Asia, it was initially introduced to control the DBM and was firstly introduced in Malaysia in 1998 (Samsuddin et al., 2004). However, in 2002, the first resistance case towards spinosad was recorded in DBM field population in the USA (Zhao et al., 2002) and subsequently, several other cases were recorded (Shono & Scott, 2003; Ferguson, 2004; Sayyed et al., 2004).

In Malaysia, up until today, DBM is the major pest to the crucifers' production since 1941 (Syed et al., 1997). Although chemical control is the most common mean of controlling DBM in crucifers' production areas in Malaysia, up until now, there are a dearth of information on this topic in the country, specifically ones that emphasize on the effects of insecticides on DBM resistance. The latest study focusing on DBM resistance in Malaysia were conducted in 2016 (Nabihah, 2016). Realizing the importance of recent knowledge in this aspects, this study was conducted to fill the gap. In addition to that, it is crucial to understand current DBM resistance status in Malaysia to be shared with farmers and suggest appropriate solution to them.

Therefore, the objectives of this study were:

1. To study the spinosad resistance in laboratory selected (Spi-Sel) DBM strain
2. To investigate the resistance development of spinosad in Spi-Sel strain.
3. To study the activity of detoxification enzymes involved in the development of resistance.
4. To examine the effect of the resistance on the fitness of Spi-Sel strain.
5. To characterized the mode of inheritance of the spinosad resistance in the Spi-Sel strain.
6. To investigate the stability of spinosad resistance and cross resistance potential to other insecticides.

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LIST OF PUBLICATIONS

Journal

Nur Adibah Mohd Ishadi, Norida Mazlan. Insecticide Use Impacts on Pest Resistance: An Evidence from Diamondback Moth. *International Journal of Sciences: Basic and Applied Research* (2015) 22(1): 131-150

Conference Proceedings

Nur Adibah, M.I., Norida, M., Dzolkhifli, O., Lau, W.H. 2019. High Resistance *Plutella xylostella* to Spinosad Exhibited Low Cross Resistance to Other Insecticides. 8th International Conference on Management of the Diamondback Moth and Other Crucifer Insect Pests. March 4-8, 2019, World Vegetable Centre, Shanhua, Taiwan. Oral Presenter.

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Ishadi, N.A.M., Norida, M., Omar, D. and Lau, W.H. 2014. A Preliminary Study: Toxicity Level of Spinosad and Emamectin Benzoate on *Plutella xylostella* Population From Ulu Yam, Selangor. International Symposium on Insect: Harnessing the Power, Unlocking its Potential. December, 1 – 3, 2014, Bayview Hotel Melaka, Malaysia. Poster presenter.



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