EVALUATION AND CHARACTERIZATION OF SPIDER VENOM FOR CONTROLLING BLOW FLY, *Chrysomya megacephala* FABRICIUS (DIPTERA: CALLIPHORIDAE)

By

SAW SIEW FEN

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Chairperson: Assoc. Prof. Lau Wei Hong, PhD
Faculty: Agriculture

Blow fly (*Chrysomya megacephala*) is a well-known cosmopolitan pest in most poultry farms. In Malaysia, an unpublished data of spider species *Leucauge* sp. and *Pardosa* sp. have shown insecticidal activity against the larvae of *Spodoptera litura* and *Spodoptera exigua*. However, their potential use in controlling *C. megacephala* is unknown. Hence, the objectives of this study were to evaluate the insecticidal activity of spider venom(s) against *C. megacephala* as well as to characterize and evaluate the spider toxin(s) with potential insecticidal activity. The spider venoms were extracted from the field collected spiders, precipitated and fractionated by HPLC. The crude venom of *Pardosa* sp. did not induce mortality in maggots but the crude venom of *Leucauge* sp. had caused 100% mortality towards the maggots. The lethal dosage (LD_{50}) value of both crude venom and partial purified venom of *Leucauge* sp. was 38.92 and 33.19 µg/g, respectively. The SDS-PAGE results had revealed that protein bands of supernatant of partial purified venom contained low molecular weight proteins (less than 11 kDa). Three HPLC purified fractions namely F7, F8 and F9 showed positive response to maggots with LD_{50} values of 1.61 µg/g, 2.33µg/g and 2.12 µg/g, respectively. The Q-TOF mass spectrometry analysis had confirmed these fractions fall into the class of disulfide-rich neurotoxins and linear cytolytic peptides (1-10 kDa), within the toxicity range of the reported Ctenitoxin from *Phoneutria nigriventer* on *Musca domestica*. The potency test had confirmed negative response of *Leucauge* sp. venom towards *Artemia salina* which is a common organism used for toxicity test.

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

PENILAIAN DAN PENGENALPASTIAN BISA LABAH-LABAH DALAM KAWALAN LANGAU HIJAU, *Chrysomya megacephala* FABRICIUS (DIPTERA: CALLIPHORIDAE)

Oleh

SAW SIEW FEN

Mei 2018

Pengerusi: Prof. Madya Lau Wei Hong, PhD
Fakulti: Pertanian

Chrysomya megacephala adalah serangga perosak kosmopolitan yang terkenal di ladang ternakan ayam. Di Malaysia, data *Leucauge* sp. dan *Pardosa* sp. yang belum diterbitkan telah terbukti mempunyai aktiviti pengawalan serangga perosak yang boleh membunuh *Spodoptera litura* dan *Spodoptera exigua.* Walau bagaimanapun, kegunaan bisa labah-labah dalam mengawal *C. megacephala* belum dapat dikenalpasti lagi. Maka, objektif kajian ini adalah untuk menilai aktiviti kawalan serangga perosak dalam bisa labah-labah serta mencirikan dan mengenalpasti toksin labah-labah yang mempunyai potensi dalam kawalan serangga perosak. Bisa labah-labah yang telah diekstrak dari sampel labah-labah digunakan untuk penilaian aktiviti kawalan serangga perosak. Bisa labah-labah yang mempunyai aktiviti kawalan serangga perosak diteruskan penilaian dengan pemendakan dan pemeringkatan bisa dengan menggunakan HPLC. Bisa mentah daripada *Pardosa* sp. tidak menyebabkan kematian kepada berengga tetapi bisa mentah *Leucauge* sp. menyebabkan 100% kadar kematian berengga. Nilai dos maut (LD_{50}) untuk bisa mentah dan bisa separa tulen daripada *Leucauge* sp. adalah 38.92 µg/g dan 33.91 µg/g. SDS-PAGE yang menunjukkan jalur protein bagi supernatan separa tulen telah dikesan mempunyai berat molekul yang rendah iaitu kurang daripada 11 kDa. Tiga pecahan tulen HPLC iaitu F7, F8 dan F9 telah menunjukkan keputusan positif terhadap berengga dengan nilai dos maut (LD_{50}) 1.61 µg/g, 2.33 µg/g serta 2.12 µg/g. Spektrometer jisim masa penerbangan (Q-TOF mass spectrometry) telah membuktikan ketiga-tiga pecahan ini daripada kelas neurotoksin disulfida dan peptide sitolisi linear (1-10 kDa), dalam julat ketoksikan yang dilaporkan sebagai Ctenitoxin daripada *Phoneutria nigriventer* pada *Musca domestica.* Ujian potensi telah membuktikan bahawa bisa *Leucauge* sp.

memberikan respons negatif terhadap *Artemia salina*, iaitu organisma yang biasa digunakan untuk ujian ketoksikan.

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of supervisory committee were as follows:

Lau Wei Hong, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ganesan Vadamalai, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision2012-2013) are adhered to.

Signature: _____
Name
Chairman of
Supervisory
Committee: Assoc. Prof. Dr. Lau Wei Hong

Signature: _____
Name
Member of
Supervisory
Committee: Assoc. Prof. Dr. Ganesan Vadamalai

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LIST OF ABBREVIATIONS

ºC	Degree Celsius
ANOVA	Analysis of variance
APS	Ammonium persulphate
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
h	Hour
HPLC	High performance liquid chromatography
HSD	Honest significant difference
KCl	Potassium Chloride
LC_{50}	Medium lethal concentration
LD_{50}	Lethal median dosage
mg	Milligram
$MgCl_2$	Magnesium chloride
min	Minute
ml	Milliliter
mM	Millimolar
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaH_2PO_4	Sodium dihydrogen phosphate
ng	Nanogram
Q-TOF	Quadrupole Time-of-Flight
SAS	Statistical Analysis System

SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulphate
s	Seconds
TEMED	N-N-N-N-Tetramethyl-Ethylenediamine
TFA	Trifluoracetic acid
Tris-HCl	Tris-hydrochloride
µg	Microgram
µl	Microliter
v/v	Volume/volume
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

Chrysomya megacephala is distributed worldwide and known as the most threatening stomachic dipteran pathogens vectors. It has caused losses to the animal industry and caused medical problems (Ghandour, 1988; Norris, 1965). According to Sulaiman *et al.* (1988 & 1989), *C. megacephala* is also the predominant helminth parasite eggs vector in Malaysia. Studies have been reported that *C. megacephala* is well recognized as a critical food contaminator (Illingworth, 1926; Patton, 1930). The adults have caused nuisance in market places such as on sweets, meat, fruits, fish and other foodstuffs as well as in slaughterhouses (Greenberg, 1973). Hence, *C. megacephala* has attracted the attention of researchers including epidemiologists and hygienists (Greenberg, 1971).

The flies breed in animal manure and are considered potential vectors of pathogenic bacteria and causing diseases to poultry animals. The most common treatment for fly control is chemical pesticides such as organophosphorus compounds and pyrethroids. However, using chemicals to control fly may affect the health of animals. Manure that has been sprayed with chemicals to remove the maggots in the poultry farms will not be considered as biofertilizer. Hence, it is important to introduce biopesticide which causes no harm to the environment and save to the animals.

The venom toxin of some spiders is unsafe to many organisms including humans. However, many researchers are currently studying the role of spider venom in pharmacological study as well as pest control. The components of spider venom have potential pharmaceutical and medical values which have led to researcher's attention progressively in new drugs development and journal publications (Vassilevski *et al.*, 2009; Windley *et al.*, 2012; Bonning *et al.*, 2014; Dutertre, 2014; Pineda *et al.*, 2014; Kalia *et al.*, 2015). King and Hardy (2013) claimed that spider venoms are new candidates for bioinsecticide industry due to their richness in novel toxin compounds. The venom peptide, disulfide-rich neurotoxins (1-10 kDa) can cause lethality to target insects. Unlike snake venom, spider venom has low presence of postsynaptic toxins which block the action of neurotransmitter (Herziq *et al.*, 2011).

Spider venom is also getting attention due to its functional compounds and potential insecticidal activity that can be used as a bioinsecticide. In Malaysia, spider venom has shown insecticidal activity against the larvae of *Spodoptera litura* and *Spodoptera exigua* (Chooi, 2016). However, their potential use in controlling *C. megacephala* is still unknown. Hence, the objectives of this study were:

1. to evaluate the insecticidal activity of spider venom against *C. megacephala*.
2. to characterize and evaluate the spider toxin(s) with potential insecticidal activity.

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