UNIVERSITI PUTRA MALAYSIA

PREPARATION OF VERMICOMPOST FROM NEEM LEAF AND OIL PALM EMPTY FRUIT BUNCH AND EVALUATION OF ITS BIOACTIVITY ON FRUIT FLY Bactrocera dorsalis HENDEL

LOH KHYE ER

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By

LOH KHYE ER

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

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DEDICATION

To my family and friends, for their unconditional love, support and encouragement.

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

PREPARATION OF VERMICOMPOST FROM NEEM LEAF AND OIL PALM EMPTY FRUIT BUNCH AND EVALUATION OF ITS BIOACTIVITY ON FRUIT FLY *Bactrocera dorsalis* HENDEL

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January 2013

Chairman : Nor Azwady Abd. Aziz, PhD

Faculty : Science

Bactrocera dorsalis (Diptera: Tephritidae) is one of the major obstacles in the development of fruit industry in Malaysia. The pupation of *B. dorsalis* in the ground provides the possibility of controlling them using vermicompost, which is commonly applied as soil dressing around the base of plants. The palm oil industry generates empty fruit bunch (EFB) in large quantity as by-product, which need to be optimally exploited to obtain maximum benefits from their uses. Vermicomposting the mixture of EFB and neem (*Azadirachta indica*) leaves, with the latter known to have pesticidal value is of great interest. Therefore, vermicomposting of different ratio of EFB and neem leaves using the earthworm, *Eudrilus eugeniae* was conducted. The optimal mixture of neem leaf-empty fruit bunch for vermicomposting was determined. The earthworm performance, chemical and biological properties of different mixture composition were compared. This study found that vermicompost with the ratio of 10% neem leaves: 70% EFB: 20% cow dung (10%N:70%EFB:20%CD) showed the best earthworm performance and vermicompost quality. The study was then carried on by using

10%N:70%EFB:20%CD vermicompost and 10%N:90%EFB vermicompost, as cow dung control. The evaluation on the vermicompost in controlling *B. dorsalis* was conducted. The vermicomposts resulted in 10.67-12.80% reduction in adult emergence and 3.47-4.67% abnormal wing formation, significantly higher than the control. The dichloromethane fraction from vermicompost extract was shown to be the most bioactive fraction. Then, the characterization of *B. dorsalis* emergence inhibitor from vermicompost using metabolomics approach was conducted using the dichloromethane fraction. The classification of the 10%N:90%EFB vermicompost and its raw materials (neem leaf and EFB) were obtained by means of principal component analysis (PCA). The vermicompost, neem leaves and EFB were clustered into three different groups according to their proton nuclear magnetic resonance (^{1}H NMR) characteristics. The NMR profiles of the chemical constituents of the samples were correlated to the inhibition activity of the fruit flies using partial least square (PLS), wherein neem leaf was shown to be the major inhibitor. Based on NMR-metabolomics approach, the metabolites of the neem leaf responsible for the activity were identified as azadirachtin and salannin. In conclusion, neem leaf-empty fruit bunch-based vermicompost has the potential as biofertilizer-cum-biopesticide in controlling *B. dorsalis* population.

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

PENYEDIAAN VERMIKOMPOS BERASAL DARI DAUN SEMAMBU DAN TANDAN KELAPA SAWIT DAN PENILAIAN BIOAKTIVITINYA TERHADAP LALAT BUAH *Bactrocera dorsalis* HENDEL

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Bactrocera dorsalis (Diptera: Tephritidae) adalah salah satu daripada masalah utama dalam perkembangan industri buah-buahan di Malaysia. Pupasi *B. dorsalis* di dalam tanah memberikan kemungkinan untuk mengawal perosak ini dengan menggunakan vermikompos, yang biasanya ditaburkan di sekeliling pangkal tumbuhan. Industri minyak sawit menghasilkan tandan kelapa sawit (*empty fruit bunch*, EFB) sebagai sisa dalam kuantiti yang besar, yang perlu dieksploitasi secara optimum untuk mendapat faedah maksimum daripada penggunaannya. Pengkomposan-vermi (pengkomposan menggunakan cacing tanah) campuran EFB dan daun semambu (*Azadirachta indica*), dengan daun semambu diketahui mempunyai nilai racun makhluk perosak mempunyai nilai penyelidikan yang tinggi. Oleh itu, pengkomposan-vermi EFB dan daun semambu dalam nisbah yang berbeza telah dijalankan. Prestasi cacing tanah, ciri kimia dan biologi vermikompos telah dianalisa untuk menentukan campuran yang optimum untuk pengkomposan-vermi. Kajian ini mendapati bahawa vermikompos dengan nisbah 10% daun semambu: 70% EFB: 20% tahi lembu (10%N:70%EFB:20%CD) menunjukkan

prestasi cacing tanah dan kualiti vermikompos terbaik. Kajian ini kemudiannya diteruskan dengan menggunakan vermikompos 10%N:70%EFB:20%CD dan 10%N:90%EFB, sebagai kawalan tahi lembu. Penilaian vermikompos dalam mengawal *B. dorsalis* telah dijalankan. Vermikompos tersebut menunjukkan pengurangan 10.67-12.80% terhadap penghasilan lalat dewasa dan 3.47-4.67% pembentukan sayap yang tidak normal, kesan yang signifikan berbanding kawalan. Bioassai menunjukkan pencerakinan diklorometana ekstrak vermikompos adalah paling aktif. Seterusnya, pencerakinan diklorometana tersebut digunakan dalam perincian vermikompos menggunakan pendekatan metabolomik. Pengelasan vermikompos 10%N:90%EFB dan bahan asasnya (daun semambu dan EFB) telah diperolehi melalui *Principal Component Analysis* (PCA). Vermikompos, daun semambu dan EFB mengelompok kepada tiga kumpulan yang berbeza mengikut ciri *proton nuclear magnetic resonance* (^{1}H NMR). Profil NMR juzuk kimia sampel telah dikorelasikan dengan aktiviti perencatan lalat buah menggunakan *Partial Least Square* (PLS), dimana daun semambu adalah perencat utama. Berdasarkan pendekatan metabolomik NMR, metabolit daun semambu yang berperanan terhadap aktiviti tersebut dikenal pasti sebagai azadirachtin dan salannin. Kesimpulannya, vermikompos berasaskan daun semambu dan EFB mempunyai potensi sebagai baja-bio dan biopestisida dalam mengawal populasi *B. dorsalis*.

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I certify that a Thesis Examination Committee has met on 8th January 2013 to conduct the final examination of Loh Khye Er on her thesis entitled "**Preparation of vermicompost from neem leaf and oil palm empty fruit bunch and evaluation of its bioactivity on fruit fly *Bactrocera dorsalis* (Hendel)**" 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 degree of Doctor of Philosophy.

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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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LOH KHYE ER

Date: 8 January 2013

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon Nuclear Magnetic Resonance
1D	One-dimensional
^{1}H NMR	Proton Nuclear Magnetic Resonance
AA	Actinomycete agar
ANOVA	Analysis of Variance
C/N	Carbon-to-Nitrogen ratio
Ca	Calcium
$CDCl_3$	Deuterated chloroform
cfu	colony forming unit
EDTA	Ethylenediaminetetraacetic acid
EFB	Empty fruit bunch
Ex	Extract
F	F-ratio
FID	Free induction decay
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
H_f	Moisture content
HNO_3	Nitric acid
HPLC	High Performance Liquid Chromatography
IBS	Institute of Bioscience
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
K	Potassium

KCl	Potassium chloride
LHS	Laboratory of Natural Products
LV	Latent variable
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
Mg	Magnesium
MPOB	Malaysian Palm Oil Board
MUB	Modified universal buffer
N	Nitrogen
NA	Nutrient agar
$Na_2S_2O_3.5H_2O$	Sodium thiosulphate
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
P	Phosphorus
PC	Principal Component
PCA	Principal Component Analysis
PLS	Partial Least Square
*p*NP	Para-nitrophenol
*p*NPP	Para-nitropenyl phosphate
POME	Palm oil mill effluent
Ppm	Part per million
Q^2	Predictability
R	Ratio
R^2	Goodness of fit

RBA	Rose Bengal agar
rpm	Revolutions per minute
SIT	Sterile insect technique
SPSS	Statistical Package for Social Student
TFM	Tetrafluoroethylene
TMS	Tetramethylsilane
UPM	Universiti Putra Malaysia
V	Volume
VC	Vermicompost
VIP	Variable Importance in Projection
w	Weight vector
Wt	Weight
δ	Delta

CHAPTER 1

INTRODUCTION

Fruit flies are known as the major insect pest to fruit and vegetable crops worldwide. *Bactrocera dorsalis* (Diptera: Tephritidae) (Plate 1.1) is a species of fruit fly that is endemic to Southeast Asia, and is known as not a distinct species from *B. papayae*. This species is commonly known as the oriental fruit fly. *B. dorsalis* is one of the most destructive insect fruit pests in Malaysia, infests more than 300 host plants and commercial fruits (Chen *et al*., 2006). Many countries in Southeast Asia suffer major economic losses on commercial fruit cultivation from the infestations of tropical fruit flies.

Plate 1.1: Adult male *B. dorsalis*

In Malaysia, a total damage on fruit and vegetable crops was reported in unprotected situations and as much as 50% loss was resulted despite chemical control methods were employed (Vijaysegaran, 1996). The economic impact of fruit flies includes not only the direct losses of yield and increased cost of control, but also an export barrier due to strict quarantine regulations. The losses and the expenditure incurred on eradication are estimated to cause annual losses of over RM12.8 million in the year 1987 alone and expected to increase annually (Vijaysegaran, 1988).

Globally, certain species of fruit flies such as *B. dorsalis* are known as major proclaimed quarantine pests. However, the availability of effective insecticides is decreasing as old, broad-spectrum pesticides are being banned from use due to their negative ecological impact. Furthermore, the use of chemical insecticides that may remain on the fruit's skin will have negative impact on consumer's health upon consumption. Fruit bagging method was impractical in large field fruit cultivation and sterile insect technique does not achieved satisfactory control due to the polyphagous and polyandrous, as well as the long distance migratory ability of the flies (Singh and Singh, 1998). Therefore, an effective biological control of fruit flies could serve as an alternative way to control this pest.

The understanding of the life cycle of fruit flies enabled the attempts in the biological control of their population by targeting certain stage of their life cycle. The entire life cycle of *B. dorsalis* takes about 21-25 days, which begin with eggs, then hatch and become larvae; the larvae further develop into pupae and finally emerge into adults. There are three larval stages that are first, second and third instar. The third instar larva

detaches from the fruit and drops to the ground to transform into pupa when mature. Pupation usually occurs in the soil at the depth of 1-5 cm from the surface.

The pupation of the third instar larvae in the soil provides the possibility of controlling the pest fruit fly using vermicompost, as vermicompost is commonly applied as soil dressing around the base of plants. The fruit flies spend part of its life cycle, which is from third instar larvae until emerging into adult flies in the ground (Figure 1.2). The high possibility that the third instar larvae, pupae and adult flies come into contact with the vermicompost has granted its potential in affecting the metamorphosis of *B. dorsalis*. Hence, a research targeting the suppression of *B. dorsalis* adult emergence through vermicompost exposure is of great interest.

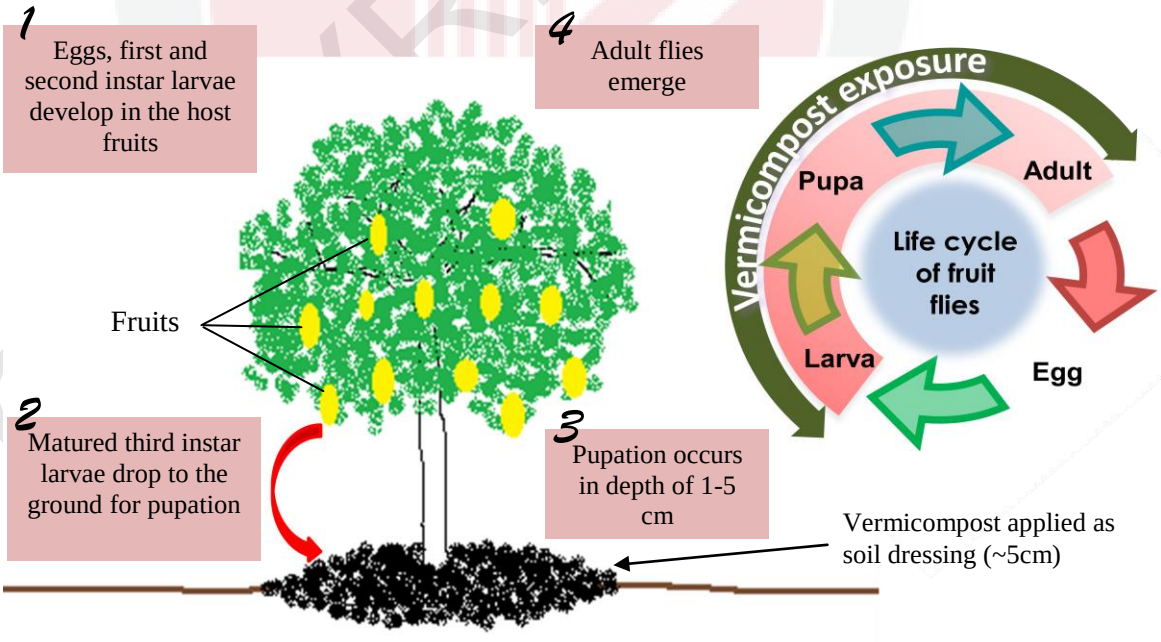

Figure 1.2. Illustration showing the possible stages of fruit flies' life cycle expose to vermicompost

Vermicompost is produced from the decomposition of organic materials by the action of earthworms through a non-thermophilic process (Atiyeh *et al*., 2000). It is a stabilized product having greater contents of most nutrients in forms that are more available to plants (Suthar, 2009). Vermicomposts have been shown to suppress populations of plant parasitic nematodes (Arancon *et al*., 2002) and plant pathogens (Chaoui *et al*., 2002b). Other researchers also reported that vermicomposts suppressed numbers of jassids, aphids and spider mites (Rao *et al*., 2001). In addition to that, field treatments with vermicomposts decreased the occurrence of leaf miner on groundnuts (Ramesh, 2000). Many works has been carried out on vermicomposting of wide varieties of organic materials such as animal dung, forestry wastes, agricultural wastes and food wastes (Logsdon, 1994; Singh and Sharma, 2002; Yan *et al*., 2012). Industrial waste such as paper pulp, guar gum and distillery wastes also have been used to turn into nutrient rich vermicompost (Suthar, 2006). These finding have opened up the possibility of turning different types of bio-wastes into valuable vermicompost.

Malaysia is known as the leading producer and exporter of palm oil in the world. In 2008, Malaysia produced 17.7 million tonnes of palm oil on 4,500,000 hectares of land (MPOB, 2008). In oil palm plantations, large quantity of by-products in the form of empty fruit bunch (EFB) is produced, with one tonne of fresh fruit bunches produces about 0.22 tonne of EFB. In recent years, much interest in the utilization of EFB as organic mulch and fertilizer supplement has been developed due to the positive results of agronomic research on EFB (Lim and Chan, 1987; Gurmit *et al*., 1989). Their application to land was found to be beneficial to soil physical and chemical properties. The EFB constitute of a large quantity of nutrient content, the general range of nutrients

offered are ranges from 1.20 to 2.40% potassium (K), followed by 0.34 to 0.66% nitrogen (N), 0.17 to 0.20% magnesium (Mg) and 0.03 to 0.10% phosphorus (P). The benefits of EFB on soil include improvement in soil structure due to better aeration, increased water holding capacity, and increase in soil pH (Menon *et al*., 2003). Therefore, EFB is a potentially good bedding material for vermicomposting and use as organic amendment to improve crop yield.

Besides, a material that serves as the source of bioactive ingredients for the vermicompost is needed. Neem leaf appeared to be the suitable candidate for this purpose. Neem (*Azadirachta indica*) is a large, evergreen, hardy tree which grows easily and survives even on dry, nutrient-lean soils. Its leaves and fruit are known to possess pesticidal properties, and recently have attracted global attention due to its potential as a source of environment-friendly pesticides (Kumar, 2002). Neem contain compounds that can control over 540 insect pest species through antifeedant, oviposition deterrence, repellent, growth regulating, ovicidal and sterilant activities (Schmutterer and Singh, 2002). The bioactivity of neem product has been attributed to various compounds contained in the seed and leaf, but azadirachtin appears to be the most important compound that contributes to its pesticidal activities against many species of insect pests (Nawrot and Harmatha, 1994). Therefore, the bioconversion of the mixture of neem leaves and EFB into vermicompost is highly significant to be studied seeing that a biofertilizer-cum-biopesticide may be resulted.

However, the composition of neem leaf in the vermicompost need to be compromised as its bioactive compounds may also harm the worms. The determination of the optimal

mixture composition has become an important element in this study prior to the evaluation of the vermicompost against fruit fly. A laboratory scale experiment evaluating the effect of vermicompost in affecting the life cycle of *B. dorsalis* was not reported by other researchers. Furthermore, there was no previous study done on the characterization of vermicompost using metabolomics approach. Nevertheless, the determination of the emergence inhibitor may only be answered rather conjecturally based on NMR interpretation and comparison with reported data. Therefore, the objectives of the present study are:

1. To determine the optimal mixture of neem leaf-empty fruit bunch for vermicomposting.
2. To evaluate the potential of neem leaves-empty fruit bunch-based vermicompost in controlling tephritid fruit fly, *B. dorsalis* (Diptera: Tephritidae).
3. To characterize the *B. dorsalis* (fruit fly) emergence inhibitor from neem leaf-empty fruit bunch-based vermicompost using metabolomics approach.

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