INSECTICIDAL AND REPELLANT ACTIVITIES OF SELECTED BOTANICAL EXTRACTS AGAINST RICE WEEVIL (SITOPHILUS ORYZAE L.)

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DEDICATION

The thesis is first and foremost dedicated to Almighty Allah for seeing me through. Then to my parents for their unwavering support, advice, encouragement and prayers which guided me towards this achievement, I am very proud of them and may Almighty Allah reward them abundantly. The thesis is also dedicated to my brothers and sisters for their prayers and support.



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May Almighty Allah in his infinite mercy reward everyone abundantly.



ABSTRACT

Stored rice is infected primarily by adult rice weevil (Sitophilus oryzae L.). Botanical pesticides could be alternatives to synthetic insecticides due to their effectiveness and lower toxicity. This study investigated repellence activity, insecticidal activity and phytochemical composition of acetone and ethanol extracts of basil leaves (Ocimum basilicum), turmeric leaves (Curcuma longa) and akar mempelas stem (Tetracera macrophylla) against adult rice weevil (Sitophilus oryzae L.) on stored rice. Methodology involved preparing 0%, 10%, 20% and 30% concentrations of either ethanol or acetone extract of each plant to be examined for their repellence activity, contact toxicity and phytochemical composition using gas chromatography-mass spectroscopy (GC-MS). Results showed that acetone and ethanol extracts of the three plants exerted dose-dependent repellence and time-dependent contact toxicities. Ethanol extracts of the three plants showed superior repellence activity and contact toxicity were superior to those of their acetone extracts, while the repellence activity and contact toxicities of the ethanol and acetone extracts of basil were superior to those of turmeric and Akar mempelas. GC-MS indicated that basil acetone extract contained anethole, while the ethanol extract contained bis (2-Ethylhexyl) phthalate. Turmeric acetone extract contained Ar-turmerone, while the ethanol extract contained bis(2-Ethylhexyl) phthalate. Akar mempelas stem acetone extract contained 1-tetradecene, while the ethanol extract contained 1-octadecene and bis(2-Ethylhexyl) phthalate. In conclusion, acetone and ethanol extracts of basil leaves, turmeric leaves and akar mempelas stem exhibited effective dose-dependent repellant activities and time-dependent insecticidal activities against the adult rice weevil. However, the repellant and contact toxicity of ethanol extracts of the basil, turmeric and akar mempelas were superior to those of their acetone extracts, while the repellence and contact toxicities of the ethanol and acetone extracts of basil were superior to those of turmeric and akar mempelas.



ABSTRAK

Beras yang disimpan boleh dijangkiti terutamanya oleh kumbang padi dewasa (Sitophilus oryzae L.). Racun perosak botani mampu menjadi alternatif kepada racun serangga sintetik kerana keberkesanannya dan ketoksikan yang lebih rendah. Kajian ini menyiasat aktiviti penghalauan, aktiviti racun serangga dan komposisi fitokimia ekstrak aseton dan etanol daun selasih (Ocimum basilicum), daun kunyit (Curcuma longa) dan batang akar mempelas (Tetracera macrophylla) terhadap kumbang padi dewasa (Sitophilus oryzae L.) terhadap beras yang disimpan. Metodologi ini melibatkan penyediaan kepekatan 0%, 10%, 20% dan 30% sama ada ekstrak etanol atau aseton setiap tumbuhan yang akan diperiksa untuk aktiviti penghalauan, ketoksikan kontak dan komposisi fitokimia menggunakan kromatografi gas spektroskopi jisim (GC-MS). Hasil kajian menunjukkan bahawa ekstrak aseton dan etanol dari ketiga-tiga tumbuhan tersebut menunjukkan peningkatan bergantung kepada dos dan ketoksikan yang bergantung pada masa. Ekstrak etanol ketiga-tiga tumbuhan menunjukkan aktiviti unggul dan toksisiti sentuhan lebih tinggi daripada ekstrak asetonnya, manakala aktiviti penghalauan dan ketoksikan sentuhan ekstrak etanol dan aseton selasih lebih tinggi daripada ekstrak kunyit dan akar mempelas. GC-MS menunjukkan bahawa ekstrak aseton selasih mengandungi anethole dan ekstrak etanol mengandungi bis (2-Ethylhexyl) phthalate. Ekstrak aseton kunyit mengandungi Ar-turmerone, sementara ekstrak etanol mengandungi bis (2-Ethylhexyl) phthalate. Ekstrak aseton batang akar mempelas mengandungi 1tetradecene dan ekstrak etanol mengandungi 1-octadecene dan bis (2-Ethylhexyl) phthalate. Kesimpulannya, ekstrak aseton dan etanol daun selasih, daun kunyit dan batang akar mempelas menunjukkan aktiviti penghalau yang berkesan dan aktiviti racun serangga yang dosnya bergantung kepada masa terhadap kumbang padi dewasa. Walaubagaimanapun, ketoksikan penghalau dan sentuhan ekstrak etanol daun selasih, kunyit dan akar mempelas lebih tinggi daripada ekstrak aseton mereka, sementara ketahanan dan ketoksikan sentuhan ekstrak etanol dan aseton daun selasih lebih tinggi daripada kunyit dan *akar mempelas*.

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LIST OF SYMBOLS AND ABBREVIATIONS

 \leq equal to or less than

greater thanpercentage

± - plus or minus

ANOVA - Analysis of variances

BCE - Before the Christian Era

C° - degrees Celsius

CAS - assigned number of registered compounds

GC - gas chromatography

GC-MS - gas chromatography-mass spectroscopy

cm - centimeter

CRD - Completely Randomized Design

FRIM - Forest Research Institute Malaysia

g/g - gram per gram

m - meter

Mc - control mortality

mg - milligram ml - milliliter

ml/min - milliliter per minute

mm - millimeter

Mo - observed mortality

Nc - insect number on the untreated half of filter paper

Nt - insect number on the treated half of the filter paper.

PR - Percent repellency

RT - Retention Time

μl - microliter

μm - micrometer

v/v - volume per volume



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LIST OF PUBLICATIONS

Journals:

Albariman, N.H., Sabran, S.F., Othman, N.W., Ishak, N., Dheyab, A.S., Anjur, N. (2020) Insecticidal and Repellant Activities of Southeast Asia Plants towards Insect Pests: A Review Asian Journal of Chemistry, 32(5), 1026-1032.

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CHAPTER 1

INTRODUCTION

1.1 Background

Plants pests embrace wide organisms including insects, mites, nematodes, rodents (animal pests), viruses, bacteria, fungi, chromista (microbes) and weeds (Kang, 2019). However, insects are still the most devastating pests to agriculture production (Kortbeek *et al.*, 2019). In addition, plant pests infest either wild or cultivated plants (Agrios, 2005) causing damage to those plant through damaging foliage (Dukes *et al.*, 2009) or even direct feeding (Edwards & Singh, 2006). The damage of plants not only restricted to pre-harvest crops but also extends to involve stored crops (Bebber *et al.*, 2019). Stored grains are attacked by 600 species of beetle, 70 moths and 355 mites (Nenaah, 2013). which increases the need for developing effective strategies to minimize the infestation of stored grains (Nenaah, 2013) and produce adequate and safe food to feed the population worldwide (Kang, 2019).

Plants developed strategies to defend themselves against those invasive pests (Wilkinson *et al.*, 2019), which could be physical through accumulating minerals (e.g. calcium oxalate crystals, amorphous calcium carbonates and silica) which make the plant tougher, mechanically through capturing the pest or chemically through the production of secondary metabolites (e.g. iridoids) (Carper *et al.*, 2019; Villard *et al.*, 2019). However, such auto-defense of plants against invasive pests is lost in case stored grains. Therefore; human has developed several controlling strategies for preventing and/or killing plant pests, such as chemical pesticides (Abhilash & Singh, 2009). However, the use of synthetic pesticides is frequently associated with unsafe application because of the serious health risk to the environment, local workers and the people live near the treated areas (Abhilash & Singh, 2009).

Several chemical classes of insecticides include organochlorine (e.g. dichlorodiphenyltrichloroethane), organophosphates (e.g. glyphosate), carbamate (e.g. carbofuran), triazines (e.g., simazine), pyrethroids (e.g. permethrin) and neonicotinoid (e.g. imidacloprid) (Nicolopoulou-Stamati et al., 2016). The health risks of insecticides that could affect human health could range from minor pain to severe paralysis and death because they can bind to different enzymes, receptors, and other proteins (Gupta et al., 2019). For example, short term exposure to insecticides lead to harmful effects on several human organs including lung, blood, liver, kidney, endocrine glands, and even immune system, while chronic exposure results in several hazards to nervous system, lung, skin, eyes, gastrointestinal tract and development of cancer, particularly in children (Organization, 2019). Additionally, the cost of chemical pesticides is becoming increasingly inaccessible to farmers, particularly in developing countries (Jahromi et al., 2012). Moreover, the flawed use of pesticides is also associated with a critical threat to non-target species, such as potential natural enemies of the pests. Therefore, finding effective, safe, and cost-effective alternatives pest control strategy is needed (Abhilash & Singh, 2009).

Nowadays, the utilization of pesticides of plant origin; known as botanical pesticides, becomes one of the possible techniques (Javed *et al.*, 2007). Fortunately, pesticides of a botanical origin have been reported to be effective with the advantages of lower toxicity and a shorter degradation period compared to the synthetic ones (Gregoire *et al.*, 2009; Pavoni *et al.*, 2019). In recent times, the use of environment-friendly and biodegradable botanicals to control pests and other insect vectors of diseases are promising (Pavoni *et al.*, 2019). In addition, investigating the pesticidal activity of botanical extracts should be associated with a phytochemical investigation for secondary metabolites that are responsible for the insecticidal activity of the plant extract since such secondary metabolites are produced by plants to protect themselves from pests and diseases or attract pollinators (Stevenson, 2019). For example, pyrethrin is a commercial insecticide which is derived from flowers of *Tanacetum cinerariifolium* (Xu *et al.*, 2019). Therefore, if these substances are identified and extracted from plants, they can be recruited in the production of commercial insecticides.

One of the most global concerns is the world food security which encounters a threat of invasive plant pests that destroy pre- or post-harvest of crops (Bebber *et al.*, 2019). For example, 60% of the global caloric need comes from wheat, maize,

rice (*Oryza sativa*) and soya (Almu *et al.*, 2019), and rice is one of the most important global nutritional crops constituting an essential cereal food for at least 3.5 billons people worldwide (Almu *et al.*, 2019). In Malaysia, at least 19 different pests attack, rest or live on rice plant in Malaysia alone (Ooi, 2015a). On the other hand, rice weevil (*Sitophilus oryzae* L.) is one of the primary insects that attack stored rice and this insect is resistant to most of the synthetic insecticides (Benzi *et al.*, 2009; Nenaah, 2013).

Essential oils from tea, cinnamon, cloves, lemongrass and thyme were reported to be dose-dependent repellant and insecticidal against adult (*Sitophilus oryzae* L.) (El-Salam, 2019). Similarly, essential oils of spearmint leaves (*Mentha spicata* L.) and clove showed insecticidal activity against rice weevil (*Sitophilus oryzae* L.) (Saad *et al.*, 2017). In addition, the seed extract of *Sesbania aegyptica* was effective insecticidal against weevil (*Sitophilus oryzae* L.) (Ahmed & Al-Moajel, 2019). Likewise, the leave extract of *Agave americana* leaf showed a repellant and insecticidal activity against the adult rice weevil (Maazoun *et al.*, 2019).

Basil (selasih; Ocimum basilicum.; Family: Lamiaceae), on the other hand, is an aromatic plant that contains essential oils consisting from methyl chavicol, methyl cinnamate, eugenol, methyl eugenol, linalool, geraniol, geranial, camphor and neral. In addition, basil is a plant that is famous in many countries worldwide including Malaysia for its therapeutic benefits (Ali & Setzerb, 2013). In addition, the repellant and insecticidal activities of basil against adult and larvae of mosquito (Anopheles arabiensis) as well as a repellant activity against diamondback moth (Plutella xylostella) were reported (Ali & Setzerb, 2013; Chokechaijaroenporn et al., 1994; Elsiddig, 2019; Kianmatee & Ranamukhaarachchi, 2007; Kraikrathok et al., 2013; Nour et al., 2009). Similarly, turmeric (Curcuma longa; family: Zingiberaceae) is an aromatic spice plant, of which rhizome is well-known to contain curcumin and use in the traditional medicine for its therapeutic benefits (Chanda & Ramachandra, 2019; Govindarajan & Stahl, 1980). Interestingly, turmeric possesses insecticidal properties against insects and fungi. The isolated compounds of turmeric rhizome; ar-turmerone and curcumin, showed insecticidal activity against the larvae of Culex pipiens (Abdelgaleil et al., 2019). Most studies in the literature focused on the repellant and insecticidal activity of rhizome extracts against several plant pests due to the presence of ar-turmerone rhizome, which was reported to be insecticidal (De Souza Tavares et al., 2016). However, ar-turmerone was also isolated from the leaves of turmeric in a high concentration (De Souza Tavares *et al.*, 2016). In addition, essential oil and its principal bioactive (e.g. α-phellandrene) in the leave extract of turmeric exhibited insecticidal activity against the larvae of *Lucilia cuprina* (Diptera:Calliphoridae) (Chaaban *et al.*, 2019). In addition, one study reported that the essential oil extract of the leaves of turmeric exhibited contact and fumigant toxicity against adult rice weevil (*Sitophilus oryzae* L.) (Tripathi *et al.*, 2002), which needs to be further evaluated due to the scarcity of studies in the literature about the insecticidal or the repellant activities of the turmeric leaves against adult rice weevil.

The genus *Tetracera* embraces several species distributed in Asia, Malaysia, Australia and New Caledonia, and the species of the genus *Tetracera* are mostly aromatic (Hoogland, 1953). *Akar mempelas* (*Tetracera macrophylla*) Family: Dilleniaceae), is one species of such genus known with a local name; '*Akar mempelas*' and it is a wild plant distributed in Southeast Asia, Malaysia and Indonesia (Ong *et al.*, 2011). The decoction of the stem of *akar mempelas* is taken orally by local Malaysian for alleviating physical weakness (Ong *et al.*, 2011), while the collected sap of the stem of this plant is used orally by locals for relieving night fever (Sabran *et al.*, 2016). However, there is no data in the literature about the insecticidal or repellant activity of the stem extract of *akar mempelas* against adult rice weevil (*Sitophilus oryzae* L.) or even other insects. In addition, no data have been reported about the phytochemical composition of the stem of *akar mempelas*.

1.2 Problem statement

The use of synthetic pesticides in most of the developing countries, including Malaysia is, however, frequently associated with an unsafe application and incorrect training. This poses a serious health risk to the environment, local workers and the people living near the treated areas. The increasing public concern over pesticide safety and possible damage to the environment has resulted in increased attention being given to natural products for the control of pests. Therefore, the selected botanical pesticide of basil (*Ocimum basilicum*), turmeric (*Curcuma longa*) and *akar mempelas* (*Tetracera macrophylla*) to decrease the spreading of major pest that causing serious damage of stored rice are one of the solutions. Still, botanical pesticide control may become an alternative control for rice pests, as it is considered

as an environmentally safe form of pest control. Further development of botanical pesticides against pests of stored rice is targeted the intended pests without adversely affecting the human health or the environment. The future development and use of safer pesticides in Malaysia will need to address safety concerns using botanical pesticide as basil leaves, turmeric leaves and *akar mempelas* stem extracts against rice weevil insect (*Sitophilus oryzae* L.) on stored rice. This will impact positively on health by controlling disease-causing vectors and food security as well.

1.3 Objectives

The goal of this work is to reduce the rice weevil (*Sitophilus oryzae* L.) on stored rice through achieving the following objectives:

- (i) To determine the repellant activities of basil dry leaves (*Ocimum basilicum*), turmeric dry leaves (*Curcuma longa*) and *akar mempelas* dry stem (*Tetracera macrophylla*) against adult rice weevil insect (*Sitophilus oryzae* L.) on stored rice.
- (ii) To evaluate the insecticidal activity of basil dry leaves (*Ocimum basilicum*), turmeric dry leaves (*Curcuma longa*) and *akar mempelas* dry stem (*Tetracera macrophylla*) against adult rice weevil insect (*Sitophilus oryzae* L.) on stored rice.
- (iii) To profile phytochemical content of basil dry leaves (*Ocimum basilicum*), turmeric dry leaves (*Curcuma longa*) and *akar mempelas* dry stem (*Tetracera macrophylla*) by using gas chromatography-mass spectroscopy.

1.4 Scope of the study

Plants pests embrace wide organisms including insects, mites, nematodes, rodents (animal pests), viruses, bacteria, fungi, chromista (microbes) and weeds. However, insects are still the most devastating pests to agriculture production. One of the most global concerns is the world food security which encounters a threat of invasive plant pests that destroy pre- or post-harvest of crops. For example, 60% of the global caloric need comes from wheat, maize, rice (*Oryza sativa*) and soya, and rice is one of the most important global nutritional crops constituting an essential cereal food for

at least 3.5 billons people worldwide. In Malaysia, at least 19 different pests attack, rest or live on rice plant in Malaysia alone. On the other hand, rice weevil (*Sitophilus oryzae* L.) is one of the primary insects that attack stored rice and this insect is resistant to most of the synthetic insecticides.

The human has developed several controlling strategies for preventing and/or killing plant pests, such as chemical pesticides. However, the use of synthetic pesticides is frequently associated with unsafe application because of the serious health risk to the environment, local workers and the people live near the treated areas. There are several chemical classes of insecticides including organochlorine dichlorodiphenyltrichloroethane), organophosphates (e.g. (e.g. glyphosate), carbamate (e.g. carbofuran), triazines (e.g., simazine), pyrethroids (e.g. permethrin) and neonicotinoid (e.g. imidacloprid). The health risks of insecticides that could affect human health could range from minor pain to severe paralysis and death because they can bind to different enzymes, receptors, and other proteins. Many herbal plants which known as botanical pesticides act as natural insecticides and could be provided with an alternative tool for control of stored rice pests also preventive of many pest's diseases in several developing countries. The current study aims to achieve effectiveness and potential of pesticides of plant origin for application in stored rice in Malaysia. The selected botanical pesticides are to decrease the spreading of rice weevil that causing serious damage to stored rice.

1.5 Significance of the study

This study differs from previous studies since it is searching for major pests on stored rice and applied an extract of basil leaves (*Ocimum basilicum*), turmeric leaves (*Curcuma longa*) and *akar mempelas* stem (*Tetracera macrophylla*) to control the infestation of adult rice weevil insect (*Sitophilus oryzae* L.) on stored rice.

The three selected plants were studied for their repellant and contact toxicities against several plant pests, adult rice weevil and their phytochemical composition. Interestingly, these plants are available in Malaysia and their repellant and insecticidal activities as well as their phytochemical composition should be studied to compare those activities and phytochemical composition to those studied in the other countries.

The results of this study may provide insight for the discovery and development of promising botanical effective repellants and insecticides from the extracts of basil leaves, turmeric leaves and *akar mempelas* stem against rice weevil insect (*Sitophilus oryzae* L.) on stored rice. In addition, this study would contribute to the production of newly defined botanical formulation that could provide an environmentally friendly alternative for controlling rice weevil insect (*Sitophilus oryzae* L.) on stored rice.



CHAPTER 2

LITERATURE REVIEW

2.1 Plants with insecticidal and repellant activities

Globally, utilization of botanicals is becoming a trend of important means to protect crop harvest and environment from pollution by pesticides (Prakash et al., 2008). The new agricultural trend is directed toward exploring and developing friendly insecticides (Matassini et al., 2020). For such reason, the botanical pesticides could be probable alternatives (Javed et al., 2007) since several botanical pesticides provide effectiveness against several plant pests, lower toxicity, multiple mechanisms of insecticidal activity and shorter degradation period (Gregoire et al., 2009; Pavoni et al., 2019; Campos et al., 2019). Interestingly, there are promising evidence indicated the effectiveness and efficacy of botanical pesticides from aromatic plants in controlling agriculture pests (Campos et al., 2019). In general, botanical pesticides are characterized by several advantages over synthetic ones including availability, abundance, ease to be obtained, low toxicity to mammals, least pollution to the environment, minimal hazards to health, lower pest resistance to their pesticide effects, minimal adverse effects on the growth of plants, no effect on viability of seeds, no effect on the quality of grains cooking, and low cost (Prakash et al., 2008). For such reasons, the European Union took steps to enhance the environmental awareness about the hazards of using synthetic pesticides and recommended several strategies for sustainable use of pesticides among them using botanical pesticides as a safe option (Stoytcheva, 2011).

Up to know, the pesticide efficacy of several plants has been approved including sweet flag, bel, neem, mahua, senwar, karanj pyrethrum and tobacco, while efforts of exploring new bio pesticides from plants are ongoing (Prakash *et al.*, 2008).

In Europe, it was reported that seed extract of black pepper (*Piper nigrum* L.) against third instars of European chafer (*Rhizotrogus majalis* larvae) has been reported to be effective (Scott *et al.*, 2005), while the standardized twig extract of *Aglaia odorata Lour*. (Meliaceae) was effective in controlling infestation of the European corn borer (*Ostrinia nubilalis* Hübner) (Ewete *et al.*, 1996). In Southeast Asia countries (Malaysia, Indonesia, Myanmar, Thailand, Laos, Vietnam, Cambodia (Perry & Metzger, 1980), a shortlist was adopted to demonstrate some plant extracts that reported to exert an insecticidal and/or repellant activities against some common plant pests, which are organisms that attack and infest plants causing diseases and damages (Kang, 2019), (Table 2.1).

Table 2.1: List of some insecticidal and repellant plants against insects infested crops in some Southeast Asia countries

Plant species	Activity	Insects	References
Piper retrofractum (Indonesia)	Insecticidal	(Crocidolomia pavonana F.) and (Plutella xylostella L.)	(Dadang & Prijono, 2009)
Piper nigrum (Malaysia)	Larvicidal	Larvae of Spodoptera litura	(Fan et al., 2011)
Piper nigrum (Malaysia)	Larvicidal	Larvae of rice weevil, (Sitophilus oryzae L.) and rice moth (Corcyra cephalonica)	(Khani <i>et al.</i> , 2012)
Piper longum (Malaysia)	Insecticidal	Housefly (Musca domestica L.)	(Prodhan <i>et al.</i> , 2012)
Piper retrofractum (Thailand)	Insecticidal	Diamondback moth (<i>Plutella</i> xylostella)	(Kraikrathok <i>et al.</i> , 2013)
Piper aduncum (Malaysia)	Adulticidal	Housefly (Musca domestica)	(Mee et al., 2009).
Azadirachta excelsa (Indonesia, Malaysia and the Philippines)	Insecticidal	Larvae of Crocidolomia binotalis	(Teik Ng <i>et al.</i> , 2003)
Azadirachta indica (Indonesia)	Insecticidal	Second instar larvae of Crocidolomia binotalis Zellar	(Prijono, 1998)
Azadirachta indica (Malaysia)	Insecticidal	Silver leaf whitefly (Bemisia tabaci)	(Islam et al., 2011)
Azadirachta indica A.Juss (Vietnam)	Repellant	Maize weevil bugs (Sitophilus zeamais)	(Thanh <i>et al.</i> , 2011).
Azadirachta indica L. (Malaysia)	Repellant	Gold dust beetle (<i>Hypomeces</i> squamosus L.)	(Singh et al., 2013).
Azadiracta indica (Indonesia)	Insecticidal	Nephotettix virescens D., Nilaparvata lugens S., S. incertulas, and Leptocorisa oratorius	(Abdullah <i>et al.</i> , 2015).

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