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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF CITRUS MEALYBUG (Planococcus citri) AND ITS RESPONSE TO SELECTED FERMENTED PLANT EXTRACTS

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By

KHADEM AHAD GUL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATION

I want to dedicate this small piece of effort to every individual who live and die for the cause which benefits other individuals, families, groups, society, nations or globe. When and where those great people are remembered my late father “Mula Soor Gul Khadem” will be remembered for his dedication, devotion, diligence and unflinching commitments towards his duties and responsibilities. The love and support for the people whom he helped in need are unforgettable. Let me admit that what if I reach the pinnacles or become the limelight even I would say I am nothing and would have been nothing without his constant help and support towards my appropriate nurturing, nourishment and prosperity. However, I have missed the presence of my father upon every step I have taken towards fulfilling his dream. I will be missing him onwards and dedicating my milestones with proud and by saying “Father I miss you”.

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

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Chairman : Associate Professor Lau Wei Hong, PhD
Faculty : Agriculture

This project was conducted to evaluate the effect of FPEs against *P. citri*. Prior to carry out research on the FPEs, the mealybugs collected from citrus plants were confirmed as *P. citri* based on their morphological characteristics and genes analysis such as *Internal transcribed spacer (ITS2), 18S ribosomal* and *Cytochrome c oxidase I* (*COI) mitochondrial* genes. A total of 10 plant materials were selected for FPEs study based on their toxicity and repellent efficacy, namely onion, garlic, turmeric, aromatic ginger, lemongrass, variegated mint, Mexican mint, peppermint, kaffir lime and lime. Among the tested FPEs, the fermented Mexican mint extract, fermented turmeric extract and fermented onion extract were highly disruptive on *P. citri* with more than 80% mealybugs died after 120 hours post treatment. The fermented Mexican mint extract tested in this study was the best FPE in causing mortality to *P. citri*, however it was the least effective repellent to *P. citri* among the FPEs. Fermented Mexican mint extract, fermented turmeric extract and fermented onion extract had scored less than 10% (w/v) LC_{50} value while other FPEs had required more than 10% (w/v) to induce 50% morality of *P. citri* after 72 hours post treatment. The fermented variegated mint was the best repellent to *P. citri*. When the FPE concentration was increased to 10% (w/v), fermented garlic extract, fermented kafir lime extract and fermented onion extract had caused higher repellency percentage to *P. citri* than fermented lime extract, fermented turmeric extract, fermented Mexican mint extract, fermented peppermint extract, fermented aromatic ginger extract and fermented lemongrass extract. The phytotoxic effect of these FPEs was recorded after 24, 48 and 72 hours post treatment. Concentration of FPEs at 5% (w/v) and 10% (w/v) did not induce phytotoxicity to citrus leaves after 24, 48 and 72 hours post treatment. Fermented Mexican mint extract had induced higher phytotoxic effect to citrus leaves compared to the fermented turmeric extract and fermented onion extract at different time intervals. The severity level of the phytotoxic effect caused by fermented Mexican mint extract increased with time and concentrations, and became severe after 72 hours post treatment. The mealybug wax was detached after treated with FPE concentrations above 10% (w/v). With increasing concentration and time of FPE

treatment, the yield of wax was also increased. The fermented onion extract showed the highest mealybug wax gained compared to other FPEs and also the chloroform after 30 seconds and 60 seconds post treatment. The fermented turmeric extract performed better than chloroform after 30 seconds post treatment, but the performance dropped after 60 seconds post treatment. The fermented Mexican mint extract had the least effect on the mealybug wax among the tested FPEs in this study. As a conclusion, the present research findings have revealed the potential use of FPEs in controlling *P. citri*. The fermented Mexican mint, turmeric and onion extracts are a good candidate for controlling *P citri* while the fermented variegated mint extract is a good repellent among the tested FPEs. These findings are new and novel for biopesticide development. With further investigation on the chemical composition and mode of action of the FPE will enable the selection of functional FPE in citrus mealybug control.

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

IDENTIFIKASI MORFOLOGI DAN MOLEKULAR KOYA SITRUS (*Planococcus citri*) DAN TINDAKBALASNYA TERHADAP EKSTRAK FERMENTASI TUMBUHAN YANG TERPILIH

Oleh

KHADEM AHAD GUL

Ogos 2021

Pengerusi : Profesor Madya Lau Wei Hong, PhD
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Projek ini dijalankan untuk menilai kesan ektrak fermentasi tumbuhan (FPEs) terhadap *P. citri*. Sebelum memulakan kajian ke atas FPE, koya telah dikutip dari pokok sitrus dan telah disahkan sebagai *P. citri* berdasarkan kepada ciri-ciri morfologi dan juga analisa molekular gen seperti *Internal transcribed spacer (ITS2), 18S ribosomal* dan *Cytochrome c oxidase I* (*COI) mitochondrial*. Sebanyak 10 bahan-bahan tumbuhan telah dipilih berdasarkan kepada ketoksikan dan kebolehan menghalau serangga untuk kajian FPE, iaitu bawang merah, bawang putih, kunyit, daun cekur, serai, daun pudina bervariasi, daun bangun-bangun, daun pudina, limau purut dan juga limau nipis. Antara kesemua FPE yang telah diuji, didapati ekstrak fermentasi daun bangun-bangun, ekstrak fermentasi kunyit dan juga ekstrak fermentasi bawang menyebabkan kerosakkan yang tinggi ke atas *P. citri* dengan lebih dari 80% kematian koya selepas 120 hari pasca rawatan. Ekstrak fermentasi daun bangung-bangun terbukti FPE yang terbaik dalam menyebabkan kematian *P. citri*, tetapi, ianya kurang berkesan dalam menghalau *P. citri* berbanding dengan FPE yang lain. Ektrak fermentasi daun bangun-bangung, ekstrak fermentasi kunyit dan ekstrak fermentasi bawang telah mendapat nilai LC_{50} yang kurang daripada 10% (w/v) manakala FPE yang lain memerlukan kepekatan lebih dari 10% (w/v) untuk membunuh 50% *P. citri* selepas 72 jam pasca rawatan. Ekstrak fermentasi daun pudina bervariasi merupakan penghalau *P. citri* yang terbaik. Apabila kepekatan FPE dinaikkan ke 10% (w/v), ekstrak fermentasi bawang putih, ekstrak fermentasi limau purut dan ekstrak fermentasi bawang telah menunjukkan peratusan yang lebih tinggi dalam menghalau *P. citri* berbanding dengan ekstrak fermentasi limau, ekstrak fermentasi kunyit, esktrak fermentasi daun bangung-bangung, ekstrack fermentasi daun pudina, ekstrak fermenasi daun cekur, dan tapaian ekstrak serai. Kesan fitotoksik FPE telah dicatatkan selepas 24, 48 dan 72 jam pasca rawatan. Kepekatan FPE pada 5% (w/v) dan 10% (w/v) tidak menyebabkan fitotoksik ke atas daun sitrus selepas 24, 48 dan 72 jam pasca rawatan. Esktrak fermentasi daun bangun-bangun telah mencetus kesan fitotoksik yang lebih tinggi terhadap daun sitrus berbanding dengan esktrak fermentasi kunyit dan ekstrak fermentasi bawang pada tempoh masa yang berlainan. Tahap kesan

fitotoksik yang disebabkan oleh esktrak fermentasi daun bangun-bangun meningkat dengan masa dan kepekatan, dan semakin teruk selepas 72 jam pasca rawatan. Lapisan lilin pada koya telah tertanggal selepas dirawat dengan kepekatan FPE lebih daripada 10% (w/v). Peningkatan kepekatan dan masa rawatan dengan FPE telah meningkatkan hasil nyah lilin. Ekstrak fermentasi bawang telah menunjukkan penyahlilin koya yang paling tinggi berbanding dengan FPE yang lain dan juga kloroform selepas 30 saat dan 60 saat pasca rawatan. Ekstrak fermentasi kunyit telah menunjukkan kebolehan yang lebih baik berbanding klorofom selepas 30 saat pasca rawatan, tetapi kebolahannya telah menunjukkan kadar penurunan selepas 60 saat pasca rawatan. Ekstrak fermentasi daun bangun-bangun kurang berkesan terhadap penyahlilin koya berbanding dengan PFE lain yang diuji dalam penyelidikan ini. Kesimpulannya, penemuan dalam penyelidikan ini telah mendedahkan kebolehan FPE dalam kawalan *P. citri*. Esktak fermentasi daun bangun-bangun, kunyit dan bawang adalah pilihan yang terbaik dalam kawalan *P. citri* manakala ektrak fermentasi daun pudina bervariasi merupakan sebagai penghalau serangga terbaik di kalangan FPE yang telah diuji. Kesemua penemuan ini adalah baru dan idea asli untuk pembangunan biopestisida. Penyelidikan secara mendalam terhadap komposisi kimia dan cara tindak balas FPE akan dapat memilih FPE yang berfungsi dalam kawalan koya sitrus.

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I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Mst. Motmainna on her PhD thesis entitled "Determination of Allelopathic Noxious Weed Species as Potential Bioherbicide for Weed Control in Malaysia" in accordance with the universities and university college act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15th March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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INTRODUCTION

The tropical climate in Malaysia is suitable for producing numerous fruits. Fruit crops in Malaysia cover approximately 375,000 ha (5.4 percent) of land (Zakaria and Rahim, 2014). Fruit production is 1.8 million metric tons and export value is USD 127.8 million, mostly to Singapore, Hong Kong and the Middle East. Pineapple, papaya, watermelon, starfruit, banana, orange, mangosteen and durian are the major fruits for local markets as well as export markets (Salleh and Yusof, 2006). The per capita fruit intake currently stands at 44.88 kg (Zakaria and Rahim, 2014).

In Malaysia, citrus crops have occupied 2,836.32 hectares of agricultural land with 25,421.59 metric tonnes of fruit production recorded in 2018 (DOA, 2018). There have been reports of many insect pests on citrus. Among them, mealybug *Planococcus citri* is the most important pests of citrus (Ahmadi et al., 2012). *P. citri* is a polyphagous pest which attacks many important cash crops, ornamental plants, and fruit plants. These mealybugs are soft-bodied, small plant-sucking insects that form the second largest scale insect's family. In various temperate and tropical regions, the new shoots and leaves of plants in greenhouses and orchards are attacked by *P. citri*. They prefer to hide in sheltered areas and form thick colonies. *P. citri* have been reported to transmit pathogens such as Grape Vein Leaf Roll Virus and Grape Vein Virus in grapes (Sforza et al., 2003). Apart from *P. citri*, the oleander mealybug, *Paracoccus burnerae* is also found as a pest in the citrus plants (Johnson and Giliomee, 2013).

Several methods such as cultural, biological, botanical, chemical, and integrated pest management have been to control mealybugs (Culik and Gullan, 2005). Cultural method such as pruning is believed could alter the microclimate of the mealybug (Franco et al., 2004). For cleaning nursery stock, hot water (47-58 °C) dipping was carried out at 2, 5, 10 and 20 min where it provided 99- 100% killing of vine mealybug, *Planococcus ficus* (Haviland et al., 2005). Controlled atmosphere treatments with ultralow oxygen were created on the bench grafts of dormant grape. It attained complete control of *P. ficus* and did not show any harm to vine growth (Liu et al., 2010).

Predators and parasites including *Cryptolaemus montrouzieri, L. dactylopii, Leptomastix epona, Anagyrus pseudococci*, etc have contributed to the regulation of different mealybugs (Daane et al., 2004). Mixture of mineral oil with some insecticides such as parathion, methidathion, fenoxycarb and imidacloprid are effective on citrus mealybug (Draga, 2005). However, they could be harmful to human health and the environment. They can cause resistance to chemical insecticides in target species and also destroy benificial insects (Franco et al., 2004; Ahmadi et al., 2012). Citrus production in Malaysia will be affected if there is no appropriate management for mealybugs. Due to their wide host range, waxy coating on the body, and high reproduction, it is not easy to control mealybugs in the field.

Garbage enzymes or fermented plant extracts are produced by fermented food of kitchen waste/ fruits peel/ garden waste, water, and molasses/brown sugar. They are multiuse solutions for housing and farming usages (Tang, and Tong, 2011). High concentration of acetic acid and low pH could be the key factor of the fermented enzyme to be effective in removing odor, cleaning and avoiding blockage of drainage, etc. The byproduct such as acetic acid, "vinegar", alcohol and propionic acid can resolve severe insecticide resistance situations and suitable to provide alternative to the chemical management (Prakash, 2011). Drecampbell. (2021) reported the successful use of acidic solutions such as apple cider vinegar to kill mealybugs. Since acidic solutions have been proven to show positive control of mealybugs in the field, the byproduct of fermented food or plant waste could provide a cheaper source of acidic solutions for mealybug control. A preliminary study was conducted to test whether fermented plant waste could remove the waxy layer of mealybugs at the Laboratory of Insect Pathology, Department of Plant Protection in Universiti Putra Malaysia in 2019 (unpublished data). The preliminary test using unfiltered FPEs has shown removal of waxy layer from the body of test mealybugs and the mealybugs died within three days. There is also necessary to confirm the mealybug identity for this study since there are different species of mealybugs found in the field. Therefore, this study was carried out with the following objectives:

1. To study the morphological and molecular characteristics of mealybugs;
2. To determine the efficacy, repellence, wax removing effect and phytotoxicity of fermented plant extracts against mealybugs and citrus leaves.

1.1 Scope of the study

Citrus plants are from the important fruits grown in Malaysia and are severely damaged by insect pests. However, there is a significant lack of research on the incidence of insects especially, mealybugs, and control methods of *Planococcus citri* in Malaysia. Therefore, it is expected that this research will be the only one of insect associated with citrus, the identification study of *P. citri* and its management through FPEs.

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