EVALUATION OF FERMENTED PLANT EXTRACTS AND WAX DEGRADING BACTERIA ON *Phenacoccus solenopsis* Tinsley OF *Hibiscus* sp.

By

SULTAN AHMMED

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

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DEDICATION

I dedicated my thesis to beloved my mother, my wife and my son for their prayer, patient and support

During my study to achieve my goal

Grateful to Almighty Allah

and

Thanks to my family members as well as my friends who appreciated me about my study

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

EVALUATION OF FERMENTED PLANT EXTRACTS AND WAX DEGRADING BACTERIA ON *Phenacoccus solenopsis* Tinsley OF *Hibiscus* sp.

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Chairperson : Associate Professor Lau Wei Hong, PhD
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The mealybugs collected from hibiscus plants were confirmed as *Phenacoccus solenopsis*, *Maconellicoccus hirsurtus,* and *Paracoccus marginatus* based on their morphological characteristics and genes analysis such as *18S* and *28S ribosomal*, and *COI mitochondrial* genes. The percentage infestation of *P. solenopsis* was 40%, followed by *P. marginatus* (30%) and *M. hirsurtus* (15%) on *Hibiscus rosa sinensis* plants during specimen collection. *Phenacoccus solenopsis* was selected for further study based on the infestation level. It is one of the noxious sucking polyphagous pests causing severe losses and its control has been an issue of significance in pest management. Management of mealybug is challenging because of its wide host range, presence of a waxy coating on the body, and high reproductive potentiality. Fermented plant extracts (FPEs) and their wax degrading bacteria were screened for their relative toxicity against *P. solenopsis*. A total of 13 plant materials namely peppermint, Mexican mint, onion, garlic, turmeric, lemongrass, variegated mint, kaffir lime, lime, neem, galangal, mahogany, and ficus were selected for FPEs study based on their insecticidal properties. The FPEs of kaffir lime, turmeric, and ficus were effective in controlling *P. solenopsis* and scored the lowest LC_{50} value among the tested FPEs. All tested FPEs did not induce phytotoxicity to hibiscus leaves below 10% concentration (w/v) after 120 h postexposure, however, FPE of variegated mint and ficus had induced severe phytotoxic effect at >20% concentration to hibiscus leaves after 72 h post exposures. All FPEs were also evaluated for their repellency potential. Among the tested FPEs, fermented peppermint extract demonstrated the best repellent action against *P. solenopsis* after 1 h post exposure and it contained hydroxy-alpha-terpenyl acetate, 2-hydroxy methyl ester tetradecanoic acid, nonanol, and 2-heptanol attributed to repellent property while dropped after 24 h post exposure. The FPE of kaffir lime, turmeric, and ficus had less repellent action after 1 h and 3 h post exposure, however, fermented ficus extract could repel more than 50% *P. solenopsis* after 24 h post exposure. Based on repellent activity FPEs of pepper mint, Mexican mint, variegated mint, kaffir lime, galangal, lemongrass, and mahogany belonged to Class II (low repellent) after 24 h post exposure. FPEs of onion, turmeric, garlic and neem belonged to Class III (moderate repellent) and only fermented ficus extract belonged to Class IV

(high repellent) after 24 h post exposure. On contrary, FPEs of pepper mint, Mexican mint, variegated mint, lemongrass, lime, and mahogany exhibited decreased repellent activity while FPEs of ficus, onion, turmeric, garlic, neem, kaffir lime, and galangal showed increasing repellent activity with exposure period. All the FPEs contained phenolic and alcoholic compounds in very trace amounts while butanoic acid was the highest abundant in the FPEs of ficus attributed to the highest repellent activity (Class IV) for *P. solenopsis*. Based on all the test results, fermented ficus extract was selected for waxdegrading bacteria screening. A total of 10 bacteria were isolated from the fermented ficus extract and confirmed by morphological and molecular characterization. Among the tested bacteria isolates, *Klebsiella pneumoniae* 0F1B1 was highly disruptive (83 % mortality) and inhibited the growth of *P. solenopsis* compared to other isolates. *Bacillus* sp. 42F1B3 exhibited the best mealybug wax degrading ability. In the glasshouse trial, fermented ficus extract performed the best in terms of the percentage reduction of *P. solenopsis* without any remarkable phytotoxicity on *H. rosa sinensis*, followed by kaffir lime and turmeric. It was noticed that *H. cannabinas* was more prone to phytotoxicity damage by FPEs than *H. rosasinensis*. Plant growth and photosynthesis were more inhibited in the FPE of turmeric-treated plants than the FPEs of kaffir lime and ficus-treated plants during the glasshouse trial, but all affected plants recovered after 15 days post treatment. The findings of the current study suggest the fermented kaffir lime extract may have beneficial effects on the growth of hibiscus plants along with mealybug control.In conclusion, FPE of ficus and *Klebsiella pneumoniae* 0F1B1 as wax degrading bacterium are good candidates to control *P. solenopsis* either as an insecticide or repellent. Different plants may have different responses to the phytotoxicity effect of FPEs. Therefore, the FPE of kaffir lime is suggested as a good candidate for the management of *P. solenopsis* in hibiscus plants.

Keywords: Fermentation, plant extract, *Phenococcus solenopsis*, wax degrading bacteria

SDG: Responsible consumption and production, zero hunger, no poverty

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

PENILAIAN EKSTRAK FERMENTASI TUMBUHAN DAN BAKTERIA PENGURAI LILIN TERHADAP *Phenacoccus solenopsis* Tinsley *Hibiscus* sp.

Oleh

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Fakulti : Pertanian

Koya yang dikumpul daripada pokok bunga raya telah disahkan sebagai *Phenacoccus solenopsis*, *Maconellicoccus hirsurtus* dan *Paracoccus marginatus* berdasarkan ciri-ciri morfologi dan analisis gen seperti *18S* dan *28S* ribosom, dan gen mitokondria *COI*. Peratusan serangan *P. solenopsis* ialah 40%, diikuti dengan *P. marginatus* (30%) dan *M. hirsurtus* (15%) pada pokok *Hibiscus rosa sinensis* semasa pengumpulan spesimen. *Phenacoccus solenopsis* telah dipilih untuk kajian selanjutnya berdasarkan kepada kadar serangan. *Phenacoccus solenopsis* merupakan salah satu jenis perosak penghisap polifagus yang berbahaya yang boleh menyebabkan kerugian teruk dan kawalannya telah menjadi satu isu penting dalam pengurusan perosak. Pengurusan koya adalah mencabar kerana julat perumahnya yang luas, kehadiran salutan berlilin pada badan dan pembiakan yang tinggi. Ekstrak fermentasi tumbuhan (FPE) dan bakteria yang mengurangkan lilinnya telah disaring untuk kajian ketoksikan relatifnya terhadap *P. solenopsis*. Sebanyak 13 bahan tumbuhan iaitu pudina, pudina Mexico, bawang merah, bawang putih, kunyit, serai, pudina bervariasi, limau purut, limau, mambu, lengkuas, mahogani dan ficus telah dipilih untuk kajian FPE berdasarkan kepada sifat keracunan terhadap serangga. FPE limau purut, kunyit dan ficus didapati berkesan dalam pengawalan *P. solenopsis* dan mencapai nilai LC_{50} terendah berbanding dengan FPE lain yang diuji. Semua FPE yang diuji tidak menyebabkan fitotoksisiti terhadap daun bunga raya di bawah kepekatan 10% (w/v) selepas 120 jam pasca pendedahan. Walau bagaimanapun, FPE pudina bervariasi dan ficus telah menyebabkan kesan fitotoksik yang teruk pada kepekatan >20% pada daun bunga raya selepas 72 jam pasca pendedahan. Semua FPE telah dinilai untuk potensi pencegahanya. Antara FPE yang diuji, ekstrak fermentasi pudina telah menunjukkan tindakan pencegahan serangga yang terbaik terhadap *P. solenopsis* selepas pendedahan 1 jam pasca pendedahan dan ia mengandungi hidroksi-alfa-terpenil asetat, asid tetradekanoik 2-hidroksi metil ester, nonanol dan 2-heptanol yang bersifat pencegah serangga. Walau bagaimanapun, aktiviti pencegahan serangga oleh ekstrak fermentasi pudina menurun selepas 24 jam pasca pendedahan. FPE limau purut, kunyit dan ficus mempunyai tindakan pencegahan serangga yang menurun selepas 1 jam dan 3 jam pasca pendedahan manakala ekstrak fermentasi ficus boleh mencegah lebih daripada 50% *P. solenopsis* selepas 24 jam

pasca pendedahan. Berdasarkan kepada aktiviti pencegahan serangga oleh FPE pudina, pudina Mexico, pudina bervariasi, limau purut, lengkuas, serai dan mahogani tergolong dalam Kelas II (pencegah serangga rendah) selepas 24 jam pasca pendedahan. FPE bawang merah, kunyit, bawang putih dan mambu tergolong dalam Kelas III (pencegah serangga sederhana) dan hanya ekstrak fermentasi ficus tergolong dalam Kelas IV (pencegah serangga tinggi) selepas 24 jam pasca pendedahan. Sebaliknya, FPE pudina, pudina Mexico, pudina bervariasi, serai, limau dan mahogani menunjukkan penurunan aktiviti mencegah serangga manakala FPE ficus, bawang, kunyit, bawang putih, mambu, limau purut dan lengkuas menunjukkan peningkatan aktiviti pencegahan serangga dengan tempoh pendedahan yang meningkat. Semua FPE mengandungi sebatian fenol dan alkohol dalam jumlah yang sangat sedikit manakala asid butanoik adalah sebatian yang paling banyak dalam FPE ficus yang memberi kesan pencegahan serangga yang tertinggi (Kelas IV) terhadap *P. solenopsis*. Berdasarkan kepada semua keputusan ujian yang dijalankan, ekstrak fermentasi ficus dipilih untuk menyaring bakteria yang boleh mengurai lilin. Sebanyak 10 bakteria telah dipencilkan daripada ekstrak fermentasi ficus dan disahkan dengan pencirian morfologi dan molekul. Antara bakteria yang diuji, *Klebsiella pneumoniae* 0F1B1 adalah sangat berkesan (83 % kematian) dan menghalang pertumbuhan *P. solenopsis* berbanding dengan bakteria yang lain. *Bacillus* sp. 42PB3 mempamerkan keupayaan mengurai lilin koya yang terbaik. Dalam kajian di rumah kaca, ekstrak fermentasi ficus menunjukkan prestasi terbaik dari segi pengurangan peratusan *P. solenopsis* tanpa sebarang fitotoksisiti pada *H. rosa sinensis*, diikuti dengan limau purut dan kunyit. *Hibiscus cannabinas* lebih cepat menunjukan kerosakan fitotoksisiti oleh FPE berbanding dengan *H. rosa sinensis*. Pertumbuhan tumbuhan dan fotosintesis boleh dihalang dengan rawatan FPE kunyit berbanding dengan FPE limau purut dan ficus semasa percubaan di rumah kaca, tetapi semua tumbuhan yang terjejas telah pulih selepas 15 hari pasca rawatan. Penemuan kajian ini mencadangkan ekstrak fermentasi limau purut memberi manfaat kepada pertumbuhan pokok bunga raya bersama dengan kawalan koya. Kesimpulannya, FPE ficus dan *K. pneumoniae* 0F1B1adalah calon yang baik untuk mengawal *P. solenopsis* sama ada sebagai racun atau pencegah serangga. Tumbuhan yang berbeza mungkin mempunyai tindak balas yang berbeza terhadap kesan fitotoksisiti FPE. Oleh itu, FPE limau purut dicadangkan sebagai calon yang baik untuk pengawalan *P. solenopsis* pada pokok bunga raya.

Kata kunci: Fermentasi, ekstrak tumbuhan, *Phenococcus solenopsis*, bakteria pelupus lilin

SDG: Penggunaan dan pengeluaran yang bertanggungjawab, sifar kelaparan, tiada kemiskinan

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CRD	Completely Randomized Design
DMRT	Ducan's Multiple Range Test
dH_2O	Distilled Water
FPE	Fermented Plant Extract
GCMS	Gas Chromatography-Mass Spectrometry
g	Gram
h	Hour
LC_{50}	Lethal Concentration 50
LDM	Leaf Dipping Method
LIP	Laboratory of Insect Pathology
mg/L	Milligram Per Litre
min.	Minute
mL	Milliliter
mm	Millimeter
nm	Nanometer
p. t	Post Treatment
SAS	Statistical Analyses System
SEM	Scanning Electron Microscopy
SPAD	Soil Plant Analysis Development
rpm	Rotation Per Minute
RT	Retention Time
RCBD	Randomized Complete Block Design
TAM	Top Application Method

UPM	Universiti Putra Malaysia
µl	Microliter
w/v	Weight to Volume

CHAPTER 1

INTRODUCTION

The genus *Hibiscus* belongs to Malvaceae family which comprises of several hundred species woody shrubs, small trees, and both annual and perennial herbaceous plants (Fatima et al., 2016; Taylo and Magdalita, 2021). Hibiscus are used for landscaping, construction, rope, fibre and paper production, food and beverage, folk medicine, symbolism and culture, national and state symbol, etc. Several species notably *H. syriacus* L. and *H. rosa-sinensis* L. are widely grown as ornamental plants (El Mokni and Iamonico, 2020). *Hibiscus rosa-sinensis* L. is one of the most widely cultivated flowering plants in the tropical and sub-tropical places including Asia (Magdalita et al., 2010). It is the national flower of Malaysia and commonly grown in Malaysia (Bhaskar, 2011).

Several researchers (Fatima et al., 2016; Taylo and Magdalita, 2021) reported a high percent incidence of thrips, flea beetle, mealybugs, and soft scale insects on *Hibiscus* spp. Till date, five mealybug species namely *Ferrisia sylirii* (Cockerell), *Maconellicoccus hirsutus* (Green), *Paracoccus marginatus* (Williams and Granara de Willink), *Phenacoccus solenopsis* (Tinsley), and *Pseudococcus jackbeardsleyi* (Gimpel and Miller) have been reported infesting the hibiscus plant such as *Hibiscus rosa-sinensis* L. (national flower), *H. liliiflorus* L. and hibiscus hybrids in Malaysia (Williams, 2004; Sartiami et al., 2016). *Phenacoccus solenopsis* (Tinsley) is one of the most destructive mealybug species commonly found on *Hibiscus* sp. It has been reported as a serious and invasive pest of *H. rosa-sinensis* L. in Pakistan, India (Hodgson et al., 2008), Nigeria (Akintola and Ande, 2008) and China (Wang et al., 2009; Wu and Zhang, 2009). Beside *H. rosa-sinensis* L., *P. solenopsis* (Tinsley) also attacked other *Hibiscus* species such as *H. cannabinas* L. (Kenaf) which is cultivated as a cash crop in Malaysia (Roslan et al., 2011; Hanim, 2012; Kamal et al., 2012). This crop has been cultivated as an alternative material to produce bio-composite and used for production of furniture, pulp, paper, fibre board, animal feed and materials for construction and automotive. Kyoto Protocol also recognized it as an eco-friendly industrial organic material to be effective in reducing global warming (Mohd Rifaat, 2008). Among the kenaf insect pests, mealybug is one of the most destructive pests on kenaf in Indian subcontinent (Vennila et al., 2011). The highest level of mealybug infestation (47.58%) was reported in BJRI Kenaf-3 (Bot kenaf) causing 47.82% fibre yield loss in Bangladesh (BJRI Annual report, 2017). In Malaysia, *P. solenopsis* (Tinsley) was observed on kenaf (Malisa et al., 2011; Roslan et al., 2011). Infestation of mealybug could cause yellowing of leaves, defoliation, flower buds that failed to open, ceasing the natural process of photosynthesis, ultimately reduced growth, wrinkled and curling younger leaves, bunchy top and death of plants malformation of shoots, leaves, flowers and fruits (Dhawan,1980; Culik and Gulan, 2005; Chong et al., 2008; Nagrare et al., 2011; Sartiami et al.,2016), toxin injection into plant tissue and virus transmission (Ben-Dov, 2005; Hoffman et al., 2011).

There are several control measures available, but none is absolute. If there is no proper control method on this insect pest, the crop production in Malaysia will be affected as

well. Although the insecticides have provided high efficacy in insect pest control in general, but it may induce resistance in mealybugs due to their high reproductive potentiality and multiple generations each year (Mark and Gullan, 2005). Sometimes, the efficacy of conventional insecticides against mealybugs has been proven unsatisfactory and ineffective because of the waxy covering on body that hinders penetration of the active ingredients of insecticides (Sparks et al., 1996; Joshi et al., 2010; Nagrare et al., 2011). Besides, indiscriminate insecticide application will disrupt the potential natural enemies and pest resurgence of new insect leading to additional insecticides application to control these pests. An increased in field resistance instances to insecticides have been documented due to detrimental effects on the environment rendering insecticide-based control techniques ineffective (Depickère et al., 2012). These drawbacks led to explore new eco-friendly alternatives as a part of integrated control strategy for mealybugs.

Fermented plant extracts (FPEs) are produced by fermenting food of kitchen waste/ fruits peel/ garden waste, water, and molasses/brown sugar. The microorganisms present in the waste metabolize the food waste into acetic acid or "vinegar", ethanol and propionic acid. High concentration of acetic acid at low pH is an important factor of the FPEs to be effective in reducing odor, cleaning, and avoiding blockage of drainage, controlling insect etc. Besides the acetic acid, alcohol and propionic acid can resolve severe insecticidal resistance situations and suitable to offer alternative to the chemical control of insect pests (Prakash, 2011). According to Drecampbell (2021), the acidic solutions such as apple cider vinegar could kill mealybugs successfully. The byproduct of FPEs could offer a cheaper source of acidic solutions for mealybug management. Dar et al. (2019) stated that FPEs seem to appear as a promising alternative to replace conventional chemical control and could get around the worst situations of insecticide resistance.

During the fermentation process, the fermenting microbes such as bacteria, yeasts, and molds are involved in the decomposition of plant materials and wax degradation (Doyle and Meng, 2006; Arunkumar et al., 2017a; Mokoena, 2017). A number of bacteria such as *Acinetobacter, Actinomycetes, Alcaligenes, Arthrobacter, Bacillus, Corynebacteria, Flavobacterium, Micrococcus, Neisseria, Nocardia, Plesiomonas, Proteus, Pseudomonas, Rhodococcus, Xanthomonas, Zoogloea* are involved in the wax degradation (Ridgway et al., 1990; Roper 2004; Xu et al., 2013). They degrade wax by utilizing it as the sole carbon source and energy for their growth (Arunkumar et al., 2017a). Salunkhe et al. (2013) and Arunkumar et al. (2017a) demonstrated the potential promising wax degrading result for bacteria against mealybug. Ibrahim et al. (2020) had isolated *B. cereus, B. subtilisi, B. licehniformis* and *Pseudomonas putida* from *Plenococcus citri* (Risso). Krishnamoorthy et al. (2020) also reported *Bacillus* sp isolated from *Paracoccus marginatus* as efficient biocontrol agent for mealybugs. The application of FPE in crop protection program will reduce the usage of synthetic pesticides in agricultural insect pest.

Regarding the waxy coating body *P. solenopsis* (Tinsley) along with the drawbacks of traditional insecticides, it is challenging to explore eco-friendly management alternatives. However, several researchers have reported that FPEs could serve as an anti-microbial agent or insecticide, and they have a sour smell like vinegar that acts as

repellant to insects (Burnette, 2010; Cortesia et al., 2014; Rahmat et al., 2104; Nazim, 2017). A few number of researches on the efficacy of FPEs have been carried out to control aphids and white flies (Nzanza and Mashela, 2012), 28-spotted beetles (Baloc and Bulong, 2015), cutworm (Sahayaraj, 2011, Subash and Raju, 2014), and white grub (Ayub et al., 2021). Recently, Khadem et al. (2022) have reported the use of FPEs as a promising candidate to repel and to control *Planacoccus citri* (Risso). In fact, there was not any attempt to test the insecticidal activity of FPE and wax degrading bacteria associated with fermentation against *P. solenopsis* (Tinsley). Therefore, this study was undertaken to discover a safer and effective pest control approach to control the mealybugs infesting the *H. rosa sinensis* L. and *H. cannabinus* L. under laboratory and field conditions with the following objectives:

1. Identification of mealybugs collected from *Hibiscus rosa sinensis*.

2. Evaluation of fermented plant extracts against mealybugs under laboratory condition.

3. Evaluation of wax-degrading bacteria against mealybugs under laboratory condition.

4. Field evaluation of fermented plant extracts against mealybugs on *H. rosa sinensis* and *H. cannabinus*.

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