



UNIVERSITI PUTRA MALAYSIA

***ANTIFUNGAL ACTIVITY OF ALLAMANDA SPP. EXTRACTS AND THEIR
MICROEMULSION FORMULATIONS AGAINST ANTHRACNOSE
(COLLETOTRICHUM GLOEOSPORIOIDES) DISEASE OF PAPAYA***

FARAH FARHANAH BINTI HARON

FP 2012 61

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DOCTOR OF PHILOSOPHY

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By

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

October 2012

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

ANTIFUNGAL ACTIVITY OF *ALLAMANDA* SPP. EXTRACTS AND THEIR MICROEMULSION FORMULATIONS AGAINST ANTHRACNOSE (*COLLETOTRICHUM GLOESPORIODES*) DISEASE OF PAPAYA

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October 2012

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An effective and efficient microemulsion biofungicide derived from *Allamanda* spp. was developed for the control of *Colletotrichum gloeosporioides*, the most important pathogen of papaya anthracnose. Leaf extracts of *Allamanda blanchetti*, *Allamanda cathartica*, *Allamanda cathartica* ‘Alba’, *Allamanda cathartica* ‘Jamaican Sunset’ and *Allamanda oenotheraefolia* were screened for antifungal activities *in vitro* and extracts that showed strongest activity were screened *in vivo*. Petroleum ether, chloroform and methanol extracts of all species resulted in production of antifungal substances that significantly inhibited the growth of *C. gloeosporioides* ($P < 0.05$).

Antifungal activity of chloroform extract of *A. cathartica* ‘Alba’, *A. cathartica* ‘Jamaican Sunset’ and *A. blanchetti* gave the smallest mean of percentage inhibition of radial growth (PIRG) and number of spores as compared to *A. cathartica* and *A. oenotheraefolia* where maximum inhibition (100%) was observed with the 5 mg/ml and higher treatments. SEM observations showed that active crude extracts resulted

in hyphal degradation which the hyphae were retarded and agglutinated. Results proved that *Allamanda* spp. possessed fungistatic and fungicidal properties and the effect increased at higher extract concentrations. A significantly lower disease incidence, severity, and index were observed in *Allamanda* treated papaya fruits. Fruits coated with chloroform extract of *A. cathartica* 'Jamaican Sunset' at 5 and 7 mg/ml concentrations showed maximum reduction in anthracnose incidence (17% and 0%, respectively) and disease severity scores were always 1 (1-25%) and 0 (0%).

Antifungal effects of the most effective extracts of all *Allamanda* spp. were supported by the presence of chemical constituents identified by GC-MS. Campesterol, β -sitosterol, stigmasterol, plumericin, squalene, and α -Tocopherol were detected as major compounds in *Allamanda* species that were possibly responsible for the antifungal activity. Bioautography-guided isolation was performed on the chloroform extract of *A. cathartica* 'Jamaican Sunset' and based on the available spectral data from MS, IR, ^1H -, ^{13}C -, DEPT-135, HMQC, HMBC, COSY NMR spectra obtained on a Varian AS 400 spectrometers using CDCl_3 as solvent, the pure active antifungal sesquiterpene was identified as plumericin ($\text{C}_{15}\text{H}_{14}\text{O}_6$, MW: 290).

Microemulsion formulation was prepared by constructing ternary phase diagrams between alkyl polyglucosides (APG) surfactants (Agnique MBL 510 H or/and Agnique MBL 530 H), dimethylamide carrier oil (Agnique AMD 810), and water, in order to select the best points from the isotropic microemulsion regions. From the seven phase diagrams constructed, eight microemulsion solutions were derived and all solutions were stable after 4 weeks. Surface tension values of microemulsion solutions were in the range of 29 to 31 mN/m, while particle sizes were in the range of 51.79 to 1801.05 nm. *Allamanda* microemulsion was formulated with the active

ingredient (a.i), *Allamanda* concentrated liquid crude extract (ACLCE) with 9.75% plumericin.

The formulations were evaluated for their efficacy in controlling papaya anthracnose and their effects on the postharvest quality of treated fruits during storage. Overall, the eight formulations showed very good activity in reducing the incidence and index of anthracnose to as low as 0 to 28% at concentration of 7% w/w while severity scores were always 0 and 1 (0-25%), in comparison with Benocide[®]. *Allamanda* microemulsion coatings delayed ripening process (10 °C, 80% RH) in commercial packaging observed by the minimum weight loss, slower change in external peel colour, greater firmness which were good in maintaining the storage quality of papaya up to 30 days without affecting their soluble solids concentration (SSC) values as compared to control.

A fungicide composition of nano-emulsified homogeneous mixture coded as AM8, comprising 35% w/w of *Allamanda* concentrated liquid crude extract (ACLCE), 26% w/w aqueous medium (water), 13% w/w of alkylpolyglucoside surfactant and an oil phase of dimethylamide of 26% w/w (AM8), was chosen as the best *Allamanda* microemulsion formulation (Patent No.: PI2011004439). It was designed and optimized to achieve maximum fungicide performance with EC₅₀ and EC₉₅ values of 7.067 and 18.390% (w/w) at 95% confidence limit, had a shelf life up to six months, and was found to be more effective and efficient in comparison with the common commercial fungicide formulation, Benocide[®] (benomyl 50% WP).

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

AKTIVITI ANTIKULAT EKSTRAK *ALLAMANDA* SPP. DAN FORMULASI MIKROEMULSINYA TERHADAP PENYAKIT ANTHRAXOS (*COLLETOTRICHUM GLOESPORIOIDES*) PADA BETIK

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Mikroemulsi biofungisid berkesan dan efisien daripada *Allamanda* spp. telah dicipta untuk kawalan *Colletotrichum gloeosporioides*, patogen penting penyebab anthraknos pada betik. Ekstrak daun *Allamanda blanchetti*, *Allamanda cathartica*, *Allamanda cathartica* 'Alba', *Allamanda cathartica* 'Jamaican Sunset' dan *Allamanda oenotheraefolia* telah disaring secara *in vitro* dan ekstrak yang menunjukkan aktiviti terkuat telah disaring secara *in vivo*. Ekstrak petroleum eter, kloroform dan metanol daripada kesemua spesies menghasilkan pengeluaran bahan antikulat yang ketara menghalang pertumbuhan *C. gloeosporioides* ($P < 0.05$). Aktiviti antikulat ekstrak kloroform daripada *A. cathartica* 'Alba', *A. cathartica* 'Jamaican Sunset', dan *A. blanchetti* memberi min peratus rencatan pertumbuhan jejarian (PIRG) dan bilangan spora terkecil berbanding *A. cathartica* dan *A. oenotheraefolia* di mana rencatan maksimum (100%) telah diperhatikan dengan rawatan 5 mg/ml ke atas. Pemerhatian SEM menunjukkan bahawa ekstrak

aktif menyebabkan degradasi hifa yang mana hifa terbantut dan berkepul. Keputusan membuktikan bahawa spesies *Allamanda* memiliki sifat merencat dan membunuh kulat dan kesannya meningkat dengan kenaikan kepekatan ekstrak. Insiden penyakit, tahap jangkitan, dan indeks yang lebih rendah secara ketara telah diperhatikan pada buah betik yang dirawat dengan *Allamanda*. Buah yang disalut dengan ekstrak kloroform daripada *A. cathartica* 'Jamaican Sunset' pada kepekatan 5 dan 7 mg/ml menunjukkan pengurangan maksimum pada insiden anthraknos (17% dan 0%, masing-masing) dan skor keterukan penyakit adalah pada tahap 1 (1-25%) dan 0 (0%).

Kesan antikulat ekstrak yang paling berkesan daripada kesemua *Allamanda* spp. dosokong oleh kehadiran bahan kimia yang dikenalpasti oleh GC-MS. Campesterol, β -Sitosterol, stigmasterol, plumericin, squalene, dan α -tokoferol telah dikesan sebagai sebatian utama dalam spesies *Allamanda* yang mungkin bertanggungjawab ke atas aktiviti antikulat. Pengasingan-berpandu bioautografi telah dijalankan ke atas ekstrak kloroform daripada *A. cathartica* 'Jamaican Sunset' dan berdasarkan data spektrum daripada MS, IR, ^1H , ^{13}C , DEPT-135, HMQC, HMBC, COSY NMR yang diperolehi melalui varian AS 400 spektrometer yang menggunakan CDCl_3 sebagai pelarut, bahan tulen aktif antikulat yang dikenal pasti adalah plumericin ($\text{C}_{15}\text{H}_{14}\text{O}_6$, MW: 290).

Formulasi mikroemulsi telah disediakan dengan membina gambar rajah fasa pertigaan antara surfaktan poliglukosida alkil (APG) (Agnique MBL 510 H atau / dan Agnique MBL 530 H), minyak pembawa dimetilamida (Agnique AMD 810), dan air, untuk memilih titik terbaik dari kawasan isotropik mikroemulsi. Daripada tujuh rajah fasa yang dibina, lapan formulasi mikroemulsi telah diperolehi dan kesemua formulasi adalah stabil selepas tempoh 4 minggu. Nilai ketegangan

permukaan cecair mikroemulsi adalah dalam lingkungan 29 hingga 31 mN/m, manakala saiz zarah berada dalam julat antara 51.79 kepada 1801.05 nm. Semua komposisi mikroemulsi terpilih telah digunakan untuk menyediakan formulasi mikroemulsi *Allamanda* dengan bahan aktif (a.i.), cecair ekstrak pekat *Allamanda* (ACLCE) dengan 9.75% plumericin.

Formulasi kemudiannya dinilai untuk keberkesanan mereka dalam mengawal anthraknos betik dan kesannya terhadap kualiti lepas tuai buah yang dirawat semasa penyimpanan. Secara keseluruhan, lapan formulasi menunjukkan aktiviti yang sangat baik dalam mengurangkan insiden dan indeks anthraknos serendah 0 hingga 28% pada kepekatan sebanyak 7% w/w manakala skor keterukan adalah pada tahap 0 dan 1, berbanding Benocide[®]. Salutan mikroemulsi *Allamanda* melambatkan proses masak (10°C, 80% RH) dalam pembungkusan komersial yang diperhatikan melalui penurunan berat badan minimum, perubahan perlahan dalam warna kulit buah, kekerasan yang lebih tinggi yang mana lebih baik dalam mengekalkan kualiti penyimpanan betik sehingga 30 hari tanpa menjejaskan nilai kepekatan pepejal larut (SSC) berbanding kawalan.

Komposisi racun kulat nano-emulsi yang homogenus berkod AM8 terdiri daripada 35% w/w daripada cecair pekat ekstrak mentah *Allamanda* (ACLCE), 26% w/w medium akues (air), 13% w/w surfaktan poliglukosida alkil, dan 26% w/w minyak pembawa dimetilamida, telah dipilih sebagai mikroemulsi terbaik *Allamanda* (No. Paten: PI2011004439). Ia telah direka secara optimum untuk mencapai prestasi racun kulat maksimum dengan nilai EC₅₀ dan EC₉₅ 7.067 dan 1.839% (w/w) pada had keyakinan 95%, mempunyai jangka hayat sehingga enam bulan, dan didapati lebih berkesan dan efisien berbanding formulasi racun kulat komersial, Benocide[®] (benomyl WP 50%).

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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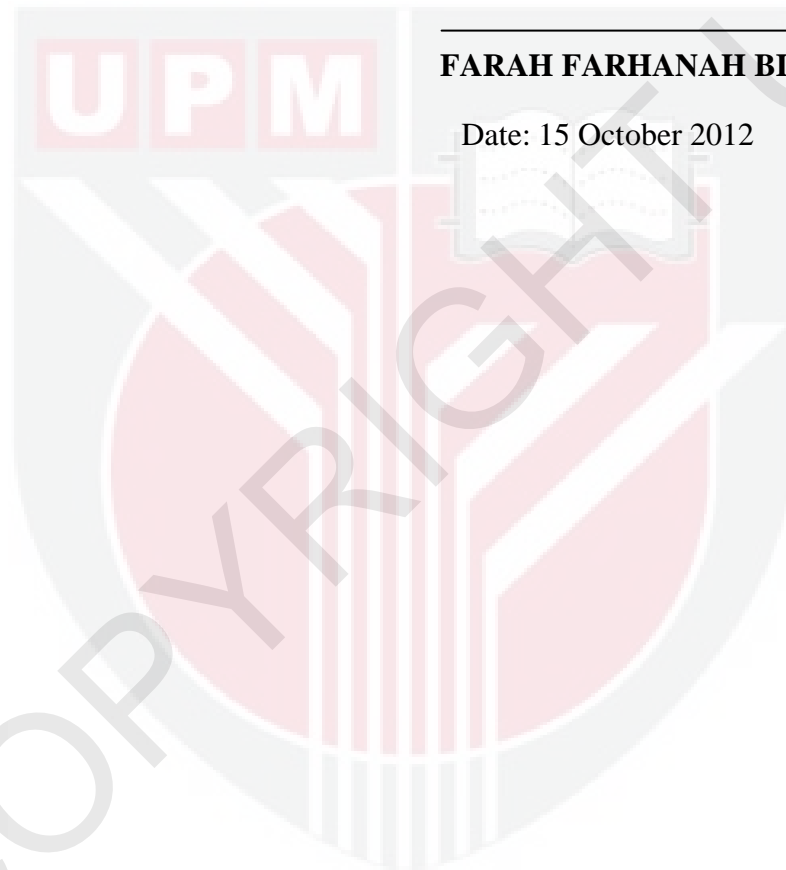
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



ACKNOWLEDGEMENTS

All praises and thanks are to Allah SWT, the magnificent and merciful. I beseech Allah's blessings of peace for the Holy Prophet Muhammad (peace and blessings be upon him), the messenger of Allah, who advised us that education is to be imbibed from cradle to grave.

First and foremost, I avail myself of this opportunity to record my sincerest thanks and appreciation to my supervisor, Associate Professor Dr. Kamaruzaman Sijam who has supported me throughout my study with his patience and knowledge whilst allowing me the room to work in my own way. I offer my sincerest gratitude to my supervisory committee members, Professor Dr. Dzolkhifli Omar for his guidance as well as for providing necessary information regarding this study, and Professor Dr. Mawardi Rahmani for his valuable advice and extensive discussions on my work.

I take this opportunity to sincerely acknowledge the Ministry of Science, Technology, and Innovation (MOSTI) Malaysia, for their sponsorship throughout the whole study in providing financial assistance in the form of National Science Fellowship which buttressed me to perform my work comfortably.

My grateful thanks extended especially to the staff of Microbiology Laboratory, Pathology Laboratory, Toxicology Laboratory and all the members of the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia for their valuable help and support.

Most of the results described in this thesis would not have been obtained without close collaboration with few laboratories. I am highly indebted to the United States Department of Agriculture-Agriculture Research Service-Natural Product Utilization Research Unit, (USDA-ARS-NPURU) in the conduct of my research attachment in the National Center for Natural Product Research (NCNPR), The University of Mississippi, USA for helping me carry out part of my research work at the institute. My warm appreciations are due to all colleagues, Ph.D students, and friends of that institute.

I owe a great deal of appreciation to the staff of Gas Chromatography-Mass Spectrometry Laboratory, Nano Particle Size Analyzer Laboratory at the Department of Chemistry, Faculty of Science, UPM and the staff of Postharvest Technology Laboratory, Department of Crop Science, Faculty of Agriculture, UPM, for the laboratory facilities and their sincere assistance.

I would like to pay my highest regards to my family for their sincere encouragement throughout my life and lifting me uphill this phase of life. This journey was much easier with them around and I owe everything to them. Last but not least, I would like to thank all those who contributed in many ways to the successful completion of this study and made this thesis possible as an unforgettable experience for me.

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