

UNIVERSITI PUTRA MALAYSIA

ANTIFUNGAL ACTIVITIES OF SELECTED MEDICINAL PLANT CRUDE EXTRACTS ON PATHOGENIC FUNGI, Colletotrichum capsici AND Colletotrichum gloeosporioides

> LUCY JOHNNY FS 2011 24

ANTIFUNGAL ACTIVITIES OF SELECTED MEDICINAL PLANT CRUDE EXTRACTS ON PATHOGENIC FUNGI, Colletotrichum capsici AND Colletotrichum gloeosporioides



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

March 2011

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

ANTIFUNGAL ACTIVITIES OF SELECTED MEDICINAL PLANT CRUDE EXTRACTS ON PATHOGENIC FUNGI, Colletotrichum capsici AND Colletotrichum gloeosporioides

By

LUCY JOHNNY

March 2011

Chair: Professor Umi Kalsom Yusuf, PhD

Faculty: Science

The antifungal activities of the leaves extract of 15 selected medicinal plants; Alpinia galanga (L.) Willd., Alstonia spatulata Blume., Annona muricata L., Blechnum orientale L., Blumea balsamifera L., Centella asiatica L., Dicranopteris linearis (Burm. f.) Underw., Dillenia suffruticosa (Griff ex Hook.f. & Thomson) Martelli, Litsea garciae Vidal., Melastoma malabathricum L., Momordica charantia L., Nephrolepis biserrata (Sw.)., Pangium edule Reinw., Piper betle L., and Polygonum minus Huds., were evaluated on plant pathogenic fungi; C. capsici and C. gloeosporioides. C. capsici was isolated from chili, and C. gloeosporioides was isolated from mango. Different antifungal assays were employed in this study viz Agar-disc dilution assay to determine the inhibition of radial growth, dry mycelial weight assay to determine the inhibition of assay. The antifungal assays were carried out in five different treatments; which were distilled water as negative control, crude extract of leaves in methanol, chloroform, acetone and Kocide 101 and Benomyl as positive control. Seven species namely P. betle, A.



galanga, C. asiatica, M. charantia, B. balsamifera, P. minus, and D. suffruticosa were effective in inhibiting the growth of C. capsici at various concentrations. The methanol, chloroform and acetone leaf crude extracts of P. betle in all concentration were found to be the most effective in inhibiting the radial growth, aerial growth, and sporulation of *C. capsici*. Overall, the methanol leaf crude extract of *P. betle* in 10 μ g/mL showed the highest percentage in inhibiting the radial growth (85.25%), aerial growth (82.21%), and sporulation (80.93%) of C. capsici. The exact concentrations of *P. betle* that fully inhibited the growth of *C. capsici* (MICs) were 12.50 mg/mL in methanol, 17.50 mg/mL in chloroform, and 15.00 mg/mL in acetone. On the other hand, 4 species namely A. galanga, P. betle, M. malabathricum, and B. balsamifera were effective in inhibiting the growth of *C. gloeosporioides* at various concentrations. The methanol, chloroform and acetone leaf crude extracts of A. galanga in all concentration (except for 0.01 µg/mL of chloroform and acetone extracts) were found to be the most effective in inhibiting the radial growth, aerial growth, and sporulation of C. gloeosporioides. Overall, the methanol leaf crude extract of A. galanga in 10 µg/mL showed the highest percentage in inhibiting the radial growth (66.39%), aerial growth (68.21%), and sporulation (68.89%) of C. gloeosporioides. The exact concentrations of A. galanga that fully inhibited the growth of C. gloeosporioides (MICs) were 15.00 mg/mL in methanol, 17.50 mg/mL in chloroform, and 17.50 mg/mL in acetone. As a conclusion, the leaf crude extracts that exhibited effectiveness by showing more than 50% inhibition against C. capsici and C. gloeosporioides should be considered for further evaluation; with P. betle and A. galanga leaf crude extracts being the most effective in inhibiting the fungi respectively and thus, exhibited highest potential as new leading biofungicides in agriculture.

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

AKTIVITI ANTIKULAT BAGI EKSTRAK TUMBUH-TUMBUHAN UBATAN TERPILIH KE ATAS KULAT PATOGEN, Colletotrichum capsici DAN Colletotrichum gloeosporioides

Oleh

LUCY JOHNNY

Mac 2011

Pengerusi: Profesor Umi Kalsom Yusuf, PhD

Fakulti: Sains

Aktiviti antifungal bagi ekstrak daun dari 15 spesies tumbuh-tumbuhan ubatan terpilih iaitu Alpinia galanga (L.) Willd., Alstonia spatulata Blume., Annona muricata L., Blechnum orientale L., Blumea balsamifera L., Centella asiatica L., Dicranopteris linearis (Burm. f.) Underw., Dillenia suffruticosa (Griff ex Hook.f. & Thomson) Martelli, Litsea garciae Vidal., Melastoma malabathricum L., Momordica charantia L., Nephrolepis biserrata (Sw.)., Pangium edule Reinw., Piper betle L., dan Polygonum minus Huds., diuji ke atas kulat patogenik terhadap tumbuhan; C. capsici yang dipencilkan daripada cili dan C. gloeosporioides daripada mangga. Ujian antikulat yang berlainan diaplikasikan di dalam kajian ini iaitu ujian 'agar-disc dilution' sebagai ujian untuk menentukan perencatan pertumbuhan jejari, diikuti dengan ujian 'dry mycelial weight' untuk menentukan perencatan pertumbuhan secara aerial, penentuan 'Minimum Inhibition Concentration (MIC)', dan ujian sprorulasi. Ujian antikulat dilakukan ke atas lima set rawatan yang berbeza iaitu air suling sebagai kawalan negatif, ekstrak asli daun dalam metanol, kloroform, aseton dan Kocide 101 dan Benomyl sebagai kawalan positif. Tujuh spesies iaitu *P. betle, A.*

galanga, C. asiatica, M. charantia, B. balsamifera, P. minus, dan D. suffruticosa didapati berkesan dalam merencat pertumbuhan C. capsici pada pelbagai kepekatan. Ekstrak daun P. betle dalam metanol, kloroform, dan aseton pada semua kepekatan didapati berkesan dalam merencat pertumbuhan jejari, pertumbuhan aerial, dan sporulasi C. capsici. Secara keseluruhan, ekstrak metanol daun P. betle pada kepekatan 10 µg/mL telah menunjukkan perencatan tertinggi bagi pertumbuhan jejari (85.25%), pertumbuhan aerial (82.21%), dan sporulasi (80.93%) C. capsici. Kepekatan spesifik bagi ekstrak daun P. betle yang merencat sepenuhnya pertumbuhan C. capsici (MICs) ialah 12.50 mg/mL dalam metanol, 17.50 mg/mL dalam kloroform, dan 15.00 mg/mL dalam aseton. Di samping itu, 4 spesies iaitu A. galanga, P. betle, M. malabathricum, dan B. balsamifera didapati berkesan dalam merencat pertumbuhan C. gloeosporioides pada pelbagai kepekatan. Ekstrak daun A. galanga dalam methanol, kloroform, dan aseton pada semua kepekatan (kecuali ekstrak kloroform dan acetone pada kepekatan 0.01 µg/mL) didapati berkesan dalam merencat pertumbuhan jejari, pertumbuhan aerial, dan sporulasi C. gloeosporioides. Secara keseluruhan, ekstrak metanol daun A. galanga pada kepekatan 10 µg/mL telah menunjukkan perencatan tertinggi bagi pertumbuhan jejari (66.39%), pertumbuhan aerial (68.21%), dan sporulasi (68.89%) C. gloeosporioides. Kepekatan spesifik bagi ekstrak daun A. galanga yang merencat sepenuhnya pertumbuhan C. gloeosporioides (MICs) ialah 15.00 mg/mL dalam metanol, 17.50 mg/mL dalam kloroform, dan 17.50 mg/mL dalam aseton. Sebagai kesimpulan, ekstrak daun yang menunjukkan keberkesanan lebih daripada 50% perencatan ke atas C. capsici dan C. gloeosporioides harus dipertimbangkan untuk ujian selanjutnya; dengan ekstrak daun P. betle dan A. galanga sebagai ekstrak yang paling berkesan dalam merencatkan

 \bigcirc

pertumbuhan kulat-kulat tersebut dan mempunyai potensi paling tinggi sebagai peneraju biofungisida dalam bidang pertanian.



ACKNOWLEDGEMENTS

(In the name of God)

I am indebted to all generous individuals for their efforts, encouragement and kindness. I acknowledge with gratitude the assistance received from the following:

First and foremost, I would like to express my heartfelt and deepest gratitude to my supervisor, Professor Dr. Umi Kalsom Yusuf for her encouragement, advice, guidance, and supports throughout completing this study. Without her encouragement and valuable guidance, I could not have finished this dissertation.

I express my deepest thanks to my co-supervisor, Associate Professor Dr. Rosimah Nulit for her guidance and generous help to assist me whenever I needed help. She guided me step by step in order to write and finish my dissertation.

I would like to dedicate my appreciation to Dr. Hishamuddin Omar, Dr. Shamarina Shohaimi, and Dr. Latifah Zakaria for their valuable ideas, suggestions and guidance throughout the final steps in completing my dissertation.

My sincere appreciation is extended to the Laboratory Assistant, Madam Norida for all the suggestions, advice, help and cooperation in the proceedings of my laboratory works.

To my laboratory mates, thank you for yours advices and cooperation throughout this study.

I would like to express my deepest love and appreciation to my beloved family, for every second of supports and encouragement that companies every step of my journey not only in this project, but of my life. Without all of you, I will not be able to get this strength. I want to thank all of you, especially my father, Johnny Changai Lasa for his continuous prayers, my mother, Tang King Hua for unconditional love and supports on me, and to my sister, Landsay Johnny for always being there no matter what we are going through.

I also acknowledge with gratitude the scholarship, Graduate Research Fellowship (GRF) received from Universiti Putra Malaysia.

Finally, but not least, I would like to dedicate my thesis to all those who formally and informally gave me all that I required in order to finish my thesis. Without your guidance, knowledge, help and never ending supports, I would not be able to finish up my thesis.

Thank you and with love,

Lucy Johnny.

I certify that a Thesis Examination Committee has met on 10 March 2011 to conduct the final examination of Lucy Johnny on her thesis entitled "Antifungal Activities of Selected Medicinal Plant Crude Extracts on Pathogenic Fungi, *Colletotrichum capsici* and *Colletotrichum gloeosporioides*" in accordance with the Universities and University College 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 Master of Science.

Members of the Examination Committee were as follows:

Rusea Go, PhD Associate Professor

Faculty of Science Universiti Putra Malaysia (Chairman)

Hishamuddin bin Omar, PhD

Lecturer Faculty of Science Universiti Putra Malaysia (Internal Examiner)

Shamarina bin<mark>ti Shohaimi, PhD</mark>

Senior Lecturer Faculty of Science Universiti Putra Malaysia (Internal Examiner)

Latifah binti Zakaria, PhD

Lecturer School of Biological Science Universiti Sains Malaysia (External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date: 24 May 2011

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

Umi Kalsom Yusuf, PhD Professor Faculty of Science Universiti Putra Malaysia (Chairman)

Rosimah Nulit, PhD Senior Lecturer Faculty of Science Universiti Putra Malaysia (Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date:

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.

LUCY JOHNNY

Date: 10 March 2011

TABLE OF CONTENT

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	ix
DECLARATION	xi
LIST OF TABLES	XV
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	XX
CHAPTERUP M	
1 INTRODUCTION	1

2

3

LIT	ERATURE REVIEW	_			
2.1	Status of fungal infestation in crop				
	2.1.1 Pepper anthracnose caused by <i>Colletotrichum capsici</i>	12			
	2.1.2 Mango anthracnose caused by <i>Colletotrichum</i>				
	gloeosporioides	15			
2.2	Common fungal infestation	17			
2.3	Common practice to deal with fungal infestation				
2.4	Deleterious effect of fungicide				
2.5	Alternative to chemical fungicide				
2.6	The use of natural fungicide				
2.7	Some antifungal compounds isolated from plants				
2.8	Screening of antifungal activities of plants				
	2.8.1 Agar-disc dilution assay	34			
	2.8.2 Minimum inhibition concentration (MIC)	35			
	2.8.3 Dry mycelial weight assay	36			
	2.8.4 Spore germination assay	36			
2.9	List of plants with potential fungicide properties	37			
MA	TERIALS AND METHODS				
3.1	Plant materials	46			
3.2	Plant extraction for crude extract				
3.3	Culture media and source of fungi	47			
3.4	Antifungal assay	51			
	3.4.1 Agar-Disc Dilution assay	51			
	3.4.2 Dry Weight Mycelial assay	53			

	3.4.3	Minimum inhibitory concentration (MIC) assay	55				
	3.4.4 Sporulation assay						
3.5	Statist	Statistical analysis					
RF	ESULT						
4.1	Confir	Confirmation of C. capsici					
4.2	Confir	Confirmation and characterization of <i>C. gloeosporioides</i>					
4.3	The ef	The effect of plant leaf crude extracts on the radial growth of					
	C. cap	osici and C. gloeosporioides – Agar-disc dilution assay	64				
	4.3.1	Percentage inhibition of radial growth of <i>C. capsici</i>					
		by leaf crude extracts in methanol, chloroform, and					
		acetone	64				
	4.3.2	Percentage inhibition of radial growth of <i>C</i> .					
		gloeosporioides by leaf crude extracts in methanol,					
		chloroform, and acetone	72				
4.4	The ef	fect of plant leaf crude extracts on the aerial growth of					
	C. cap	osici and C. gloeosporioides – Dry mycelial weight					
	assay		80				
	4.4.1	Percentage inhibition of dry mycelial weight of <i>C</i> .					
		<i>capsici</i> by leaf crude extracts in methanol,					
		chloroform, and acetone	80				
	4.4.2	Percentage inhibition of dry mycelial weight of <i>C</i> .					
		<i>gloeosporioides</i> by leaf crude extracts in methanol,					
		chloroform, and acetone	88				
4.5	Minim	num inhibition concentration (MIC)	96				
	4.5.1	Determination of minimum inhibition concentration.					
		(MIC) of <i>C. capsici</i> by leaf crude extracts in					
		methanol, chloroform, and acetone	96				
	4.5.2	Determination of minimum inhibition concentration					
		(MIC) of <i>C. gloeosporioides</i> by leaf crude extracts in					
		methanol, chloroform, and acetone	99				
4.6	The ef	The effect of plant leaf crude extracts on the sporulation rate					
	of C . c	of C. capsici and C. gloeosporioides –Sporulation assay					
	4.6.1	Percentage inhibition of sporulation of <i>C. capsici</i> by					
		leaf crude extracts in methanol, chloroform, and	100				
	1.5.0	acetone	102				
	4.6.2	Percentage inhibition of sporulation of C.					
		gioeosporiolaes by leaf crude extracts in methanol,	110				
4 -	/ T1-	chioronorm, and acetone	110				
4.7	The co	biliparison of the effectiveness of plants leaves crude					
	extrac	extracts in inhibiting the radial growth (fungistatic) versus					
	aerial	growin (lungicidal)	118				

		4.7.1	The comparison of the effectiveness of plants leaves	
			crude extracts in inhibiting the radial growth	
			(fungistatic) versus aerial growth (fungicidal) of C.	
			capsici	118
		4.7.2	The comparison of the effectiveness of plants leaves	
			crude extracts in inhibiting the radial growth	
			(fungistatic) versus aerial growth (fungicidal) of <i>C</i> .	
			gloeosporioides	122
	4.8	The co	mparison of the percentage of inhibition radial growth,	
		dry my	celial weight and sporulation by <i>P. betle</i> and <i>A</i> .	
		galang	ra	124
5	DIS	CUSSI	ON	127
	CT II			
6	SUN	AMARY	Y, CONCLUSION AND RECOMMENDATIONS	
	FOF	R FUTU	JRE RESEARCH	148
REFERI	ENCES/B	IBLIO	GRAPHY	151
BIODAT	BIODATA OF STUDENT			162
LIST OF	F PUBLIC	CATION	NS	163

 \bigcirc