



UNIVERSITI PUTRA MALAYSIA

**ANTIBACTERIAL ACTIVITY OF SALICYLIC ACID AND COPPER-BASED
FUNGICIDES AGAINST *Pseudomonas fuscovaginae* AND
*Xanthomonas oryzae***

MUNIRAH MOHD RADZI

FP 2016 32

**ANTIBACTERIAL ACTIVITY OF SALICYLIC ACID AND COPPER-BASED
FUNGICIDES AGAINST *Pseudomonas fuscovaginae* AND *Xanthomonas oryzae*.**

MUNIRAH BINTI MOHD RADZI

**A project submitted to Faculty of Agriculture, Universiti Putra Malaysia, in
fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of
the degree of Bachelor of Horticultural Science.**

**Faculty of Agriculture
Universiti Putra Malaysia**

2015

CERTIFICATION

This project entitled “ANTIBACTERIAL ACTIVITY OF SALICYLIC ACID AND COPPER-BASED FUNGICIDES AGAINST *Pseudomonas fuscovaginae* AND *Xanthomonas oryzae*” is prepared by Munirah Binti Mohd Radzi and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

Student's name:

Student's signature

Munirah Binti Mohd Radzi.

Certified by:

.....

(PROF. MADYA DR. KAMARUZAMAN BIN SIJAM)

Project Supervisor,

Department of Plant Protection,

Faculty of Agriculture,

Date :

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah S.W.T for the strength and His blessings that enable me to complete my final year project successfully.

I would like to dedicate special appreciation to my project supervisor, Prof. Madya Dr. Kamaruzaman Bin Sijam for his endless supervision and constant support. His constructive comments and suggestions really help me a lot throughout the project.

Also, a special thanks to the all staffs of Bacteriology Laboratory, Department of Plant Protection especially Ms. Junaina, Laboratory Assistant and others staff for their help and assistance during the laboratory works.

I would also like to express deepest love to my beloved family for their continuous encouragements, moral and financial supports during the completing of this study. Last but not least, a special appreciation to all of my supportive and helpful friends for their kindness, encouragement and contributions in giving ideas as well help during the conduct of this project.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS	ii
LIST OF PLATES	iv
LIST OF TABLE	v
LIST OF FIGURES	vi
LIST OF APPENDICES	vii
LIST OF SYMBOL AND ABBREVIATIONS	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER	
1 INTRODUCTION	1
1.1 Background of <i>Oryzae sativa L</i>	1-2
1.2 Objective	3
1.3 The Hypothesis of the study	3
2 LITERATURE REVIEW	4
2.1 Rice (<i>Oryzae sativa. L</i>)	4 -5
2.2 Plant Pathogenic	6
2.3 Bacterial Leaf Blight	7-10
2.3.1 <i>Xanthomonas oryza pv. oryzae</i>	11
2.4 Bacterial Sheath Brown Rot	12 -14
2.4.1 <i>Pseudomonas fuscovaginae</i>	14
2.5 Salicylic Acid as control agents	15
2.6 Copper-based fungicide as control agents.....	15

3	METHOD AND MATERIALS	16
3.1	Isolation of bacteria <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> and <i>Pseudomonas fuscovaginae</i> .	16
3.2	Preparation concentration of copper-based Fungicide used.	16
3.3	Preparation concentration of Salicylic acids (SA).....	16
3.4	In vitro antibacterial activity SA and fungicide; plate agar diffusion method.....	17
3.5	Statistical Analysis and Statistical Analysis	18
4	RESULT AND DISCUSSIONS	19
4.1	The Inhibition Zone of Plant Pathogenic Bacteria towards Copper-based Fungicide.	19-20
4.2	In Vitro Antibacterial activity of Copper-based Fungicide against Plant Pathogenic Bacteria	21-22
4.3	The Inhibition Zone of Plant Pathogenic Bacteria towards Salicylic Acid	23-24
4.4	In Vitro Antibacterial activity of Salicylic Acid against Plant Pathogenic Bacteria	25-26
5	CONCLUSION	26
	REFERENCES	27-29
	APPENDICES	30-31

LIST OF PLATES

Plate	Title	Page
1	The lesion on the older leaves show the water-soaked to yellow-orange stripes on the leaf blades or leaf tips.	9
2	The lesion on young leaves. The bacterial ooze on the leaf (A) and then the bacterial ooze becomes dry up (B).	10
3	<i>Xanthomonas Oryza pv oryzae</i> .	11
4	The discoloration of the leaves (A) and the infected grains (B).	13
5	Zone of inhibition produced by copper-based fungicide against <i>Xanthomonas oryzae pv. oryzae</i> (A) and <i>Pseudomonas fuscovaginae</i> (B).	19
6	Zone of inhibition produced by Salicylic Acid (SA) against <i>Xanthomonas oryzae pv. oryzae</i> (A) and <i>Pseudomonas fuscovaginae</i> (B).	23

LIST OF TABLE

Table	Title	Page
1	Treatments for <i>Xanthomonas oryza pv. oryzae</i>	17
2	Treatments for <i>Pseudomonas fuscovaginae</i>	17



LIST OF FIGURES

Figure	Title	Page
1	Mean of inhibition zone diameter of copper-based fungicide against plant pathogenic bacteria	21
2	Mean of inhibition zone diameter of Salicylic Acid against plant pathogenic bacteria	25

LIST OF APPENDICES

Appendix	Title	Page
1	Result of ANOVA for the interaction between different concentration of copper-based fungicide against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> .	31
2	Result of ANOVA for the interaction between different concentration of Salicylic Acid against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> .	31
3	Result of ANOVA for the interaction between different concentration copper-based fungicide against <i>Pseudomonas fuscovaginae</i> .	32
4	Result of ANOVA for the interaction between different concentration of Salicylic Acid against <i>Pseudomonas fuscovaginae</i> .	32

LIST OF SYMBOL AND ABBREVIATIONS

Xoo	=	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>
Pf	=	<i>Pseudomonas fuscovaginae</i>
Ppm	=	part per million
%	=	percentage
SA	=	Salicylic Acid
µm	=	micromilimeter
cm	=	centimeter
g/L	=	Gram per liter
g/mL	=	Gram per milliliter
RR	=	Recommendation Rate
CRD	=	Completely Randomized Design
ANOVA	=	analysis of variance
SAS	=	Statistical Analysis System
LSD	=	Least Significant Different
MHA	=	Muller-Hilton Agar

ABSTRACT

Rice plant (*Oryzae sativa L.*) is the main food crop planted in Malaysia. Rice is the main source of carbohydrates consumed by the people. However, rice plants can be infected by bacterial blight disease and brown sheath rot which are the main diseases that attack rice plants. These diseases were reported to be attacked on rice paddy in Peninsular Malaysia. The symptoms of bacterial blight disease included wilting of seedlings and yellowing and drying of leaves. While brown sheath rot shows rotting in sheaths and grains of seedlings and mature plants. No chemical has been recommended for both diseases. However, salicylic acid and copper-based fungicide have been reported. This study is conducted to: 1) To determine the efficiency of salicylic acid and copper-based fungicides on different concentrations to inhibit bacterial pathogen. The sample of bacteria will be obtained from the Bacteriology Lab, Department of Plant Protection of Agriculture Faculty. The bacteria are already isolated from the infected rice plants. The experiment will be conducted using *in vitro* antibacterial activities of salicylic acid and copper-based fungicides; plate agar diffusion method. The parameter is an inhibition zone of growth of causal agent. The copper-based fungicide that will be used is copper hydroxide fungicide with different concentrations in ppm. Salicylic acid is expected to be most effective at 1.5 ppm, which caused the largest inhibition zone. Antibacterial activity of copper-based fungicide against bacteria recorded 4.25 g/L gives the largest inhibition zone.

ABSTRAK

Tanaman padi (*Oryza sativa L.*) adalah sumber tanaman makanan utama di Malaysia. Beras adalah sumber utama karbohidrat yang dimakan oleh semua orang. Walaubagaimanapun, tanaman padi boleh dijangkiti penyakit hawar daun dan 'brown sheath rot' yang merupakan penyakit utama yang menyerang tanaman padi. Penyakit-penyakit ini dilaporkan menyerang tanaman padi di semenanjung Malaysia. Tanda-tanda penyakit hawar daun adalah anak benih layu dan kering daun. Penyakit 'brown sheath rot' pula menunjukkan reput pada pelepah, biji benih dan pokok matang. Tiada kimia disarankan untuk kedua-dua penyakit ini, bagaimanapun, acid salisilik dan racun kulat berasaskan kuprum dilaporkan dapat merencatkan penyakit ini. Kajian ini dijalankan untuk : 1) untuk menentukan keberkesanan asid salisilik dan racun kulat berasaskan kuprum pada kepekatan yang berbeza untuk merencatkan bakteria. Sampel bacteria diperolehi dari Makmal Bakteriologi, Jabatan Perlindungan Tumbuhan, Fakulti Pertanian. Bacteria dipisahkan daripada pokok padi yang dijangkiti. . Eksperimen akan dijalankan menggunakan dalam aktiviti anti-bakteria vitro asid salisilik dan racun kulat berasaskan tembaga; plat agar kaedah penyebaran. Parameter adalah zon perencatan pertumbuhan agen penyebab penyakit. Racun kulat berasaskan tembaga yang akan digunakan adalah tembaga racun kulat hidroksida dengan kepekatan yang berbeza dalam ppm. Asid salisilik dijangka paling berkesan pada 1.5 ppm yang menyebabkan zon perencatan terbesar. Aktiviti anti-bakteria racun kulat berasaskan kuprum melawan bakteria dilaporkan pada kepekatan 4.25 g/L member zon perencatan yang luas.

CHAPTER 1

INTRODUCTION

1.1 Background of *Oryzae sativa L.*

Rice is the second largest produced cereal in the world. Rice belongs to the genus *Oryza*. The scientific name for rice is *Oryzae sativa L.* . There are two species of cultivated rice, which are the common rice, *Oryzae sativa L.* and the African rice, *Oryzae glaberrima* Steud (Tsunoda and Takahashi) . Rice plant is the staple food for most people in the world. Rice production has become a main crop production all around the world, mostly in Asian countries. Rice has primarily become a main source of carbohydrates for the Asian peoples.

However, bacterial leaf blight has been the most serious disease ever attacked on rice plants. It caused losses both in the quantity and quality of rice. The control of the disease should be done before the farmers happen to loss a large yield on yield. There is some method that can be used and has been used to control the disease. Plants need nitrogen to grow, but somehow excess used of nitrogen can lead to some problem and disease. Therefore, a balanced amount of nitrogen should be used. Used a resistant varieties also has been proven to be effective in control the disease.

Other than that, ensure the drainage and field clean to prevent the from being attacked by the disease. The other disease is brown sheath rot. The disease also caused a high loss of yield production. According to Razak et al., 2013, the highest disease incidence was recorded in the state of Pahang (62%) and Selangor (62%), while the most severe infection was recorded in Pahang (55%) and Terengganu (61%). To control the disease, used a healthy and clean seed since the disease has been a seed-borne disease. Used a foliar fungicide, for example benomyl and copper oxychloride to control the brown sheath rot disease.

1.2 The Objectives

The specific objectives the study be conducted is;

- Determining the efficiency of salicylic acid and copper-based fungicide on different concentration to inhibit growth of the causal agent.

1.3 The Hypothesis of the study

The hypothesis of the study was;

- Ho; SA and copper-based fungicides will not inhibit the growth of bacteria.
- Ha; SA and copper-based fungicides will inhibit the growth of bacteria.

REFERENCES

Abeygunawardena, D.V.W (1968). Studies on Fungicidal Control of Rice Disease.

Central agricultural Research Institute, Ceylon.

M. A. AL-Saleh (2010). Pathogenic variability among five bacterial isolates of *Xanthomonas campestris pv. vesicatoria*, causing spot disease on tomato and their response to salicylic acid. *Journal of the Saudi Society of Agricultural Sciences* (2011). 10,47-51.

Zeigler, R., & Alvarez, E, (1987). Bacterial Sheath Brown Rot of Rice Caused by *Pseudomonas fuscovaginae* in Latin America. Retrieved May 20, 2015 from http://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1987Articles/PlantDisease71n07_59

RUDGARD S. A. , PETTITT T. R. AND HADLEY P. (1990). Tenacity, biological activity and redistribution on copper fungicides on cocoa in controlled environments. *Crop Protection Vol.9.*

Silverman P. , Seskar M. , Kanter D. ,Schweizer P. , Metraux J. P. , and Raskin I.,
(1995). *Salicylic Acid in Rice. Biosynthesis, Conjugation, and Possible Role.* Plant
Physiol. (1995) 108; 633-639

Global Rice Science Partnership. (n.d). *Cultivated Rice Species.*, Retrieve Novembar
7, 2015 from <http://ricepedia.org/rice-as-a-plant/rice-species/cultivated-rice-species>

Razak, A., Zainudin, N., Sidiqe, S., Ismail, N., Mohamad, N., & Salleh, B. (2009).
Sheath Brown Rot Disease of Rice Caused by *Pseudomonas Fuscovaginae* in the
Peninsular Malaysia. *Journal of Plant Protection Research.* Volume 49, Issue 3,
Pages 244–249, ISSN (Online) 1899-007X, ISSN (Print) 1427-
4345, DOI: [10.2478/v10045-009-0037-x](https://doi.org/10.2478/v10045-009-0037-x), September 2009

OU. S. H., (1985). *Rice Disease, Second Edition* (pp.61-80) Great Britian : Cambrian
News (Aberystwyth) Ltd.

**Anand A. , Uppalapati S. R. , Ryu C. M., Allen S. N. , Kang L. , Tang Y. , Mysore
K. S., (2008).** Salicylic Acid and Systemic Acquired Resistance Play a Role in
Attenuating Crown Gall Disease Caused by *Agrobacterium tumefaciens.* *Plant
Physiol.* 146(2): 703–715.

Corne M.J. Pieterse and Leendert C. van Loon. (1999). Salicylic Acid- independent Plant Defense Pathways. *Elsevier Science*. S1360-1385(98)01364-8

Robert F. Chandler. Jr. (1979). *Rice in The Tropics; A Guide to the Development of National Programs*. United States of America ; Westview Press. Inc. Pages 1-21

T.W. Mew & J.K. Misra,(1994). *A Manual of Rice Seed Health Testing*. International Rice Research Institute (IRRI) : Manila Phillippines. Pages 92.