

**DEVELOPMENT OF A BIOPESTICIDE FOR THE CONTROL OF
SCHYZOPHYLLUM COMMUNE FR., A PATHOGEN OF OIL PALM SEEDS**

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**DEVELOPMENT OF A BIOPESTICIDE FOR THE CONTROL OF
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By

ANTARJO DIKIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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DEDICATION

I dedicate this humble effort, the fruit of my thoughts and study, to my affectionate wife Ir. Zakiah and son Nicky Rahmana Putra, daughter Nanda Marizky who have inspired me to the higher ideals of life.

I also dedicate to my father, H. Mohd. Sodikin, mother Almarhumah Mukiyem with her last words “ allow me ALLAH to pray for my son
in the rest-peace”

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

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Faculty : Agriculture

An effective and efficient biopesticide produced from antagonistic bacteria was developed for the control of *Schizophyllum commune* Fr., an important seedborne pathogen of oil palm causing brown germ and seed rot disease. Fifty two out of 265 bacterial isolates were found to inhibit the growth of *S. commune* in dual culture assay and 14 out of these 52 isolates produced antimicrobial substances and inhibited the growth of *S. commune*. Finally, 2 isolates, *Burkholderia multivorans* RU50 and *Microbacterium testaceum* RU7 were confirmed to produce a broad spectrum of antimicrobial substances in liquid media containing neopeptone plus lactose (1:1) and peptone plus maltose (1:1) respectively that were able to suppress *S. commune* and other species of fungi. The mixture of equal amount of supernatant containing antimicrobial substances from both bacterial species

resulted in the potential biopesticide. The potential biopesticide destroyed cell organs of the fungus. It contained pyrrolnitrin, phenylpyrrol, phenazine and 5 unknown compounds. It had a minimum inhibitory concentration (MIC) value of 1:65 in the liquid form and 1:310 in freeze dried powder form. It was also resistant to heat at 60°C for 2 hours, stable under irradiation of UV light at 312 nm for 5 hours, stable in sodium chloride solution up to 10%, stable on variable of pH 4-9 and had a shelf life of up to one year at 26±2°C and 5-10°C. Application of this biopesticide with a dilution of 1:4 with distilled water, as seed treatment to oil palm seeds was effective for the control of *S. commune* using vacuum infiltration at 400 mm Hg Vac. for 2 minutes or seed dipping for 30 minutes for non-germinating oil palm seeds. Oil palm seeds coated with the freeze dried talcum powder inhibited the colonization of *S. commune* on the seeds. Oil palm seeds treated with this biopesticide increased seed germination up to 77%.

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

**PEMBANGUNAN BIOPESTISID UNTUK SATU KAWALAN
SCHIZOPHYLLUM COMMUNE FR., SUATU PATOGEN
BIJI BENIH KELAPA SAWIT**

Oleh

ANTARJO DIKIN

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Biopestisid yang efektif dan efisien dihasilkan daripada bakteria antagonis untuk kawalan *Schizophyllum commune* Fr., sejenis patogen penting bijibenih kelapa sawit menyebabkan penyakit 'brown germ' dan reput bijibenih. Lima puluh dua daripada 265 asingan bakteria didapati mampu mengawal pertumbuhan *S. commune* pada kajian dwikultur dan 14 daripada 52 asingan menghasilkan bahan anti-mikrob dan merencat *S. commune*. Pada akhir kajian, 2 asingan, *Burkholderia multivorans* RU50 dan *Microbacterium testaceum* RU7 dipastikan menghasilkan bahan anti-mikrob yang berspektrum luas masing-masing pada medium cecair yang mengandungi neopepton dan laktosa (1:1), pepton dan maltosa (1:1) yang boleh merencatkan *S. commune* dan spesies kulat lain. Campuran supernatan anti-mikrob pada jumlah yang sama daripada kedua-dua

spesies bakteri ini menghasilkan biopestisid yang berpotensi. Biopestisid ini mempunyai ciri menghancurkan organ sel kulat. Biopestisid mengandung pirolnitrin, fenilpirol, fenazin dan 5 bahan yang yang belum dikenal pasti. Biopestisid mengandung konsentrasi penghambat terendah (MIC) dalam formulasi cecair 1:65, dalam formulasi tepung 1:310. Biopestisid tahan kepada suhu sehingga 60°C selama 2 jam, stabil apabila terdedah kepada sinar UV pada 312 nm selama 5 jam, stabil dalam larutan natrium klorida sehingga 10%, stabil pada pH 4 – 9, mempunyai tempoh penyimpanan sehingga 1 tahun pada suhu 26±2°C dan 5-10°C. Penggunaan biopestisid pada pencairan 1:4 dengan air suling, berkesan sebagai rawatan bijibenih kelapa sawit terhadap jangkitan *S. commune* dengan penggunaan vakum penyusupan 400 mm Hg Vac. selama 2 minit atau pencelupan bijibenih selama 30 minit untuk bijibenih belum cambah. Bijibenih kelapa sawit yang diselaputi campuran tepung talkum dan biopestisid menghalang jangkitan dari *S. commune*. Bijibenih kelapa sawit yang dirawat dengan biopestisid dapat meningkatkan pecambahan sehingga 77%.

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I certify that an Examination Committee met on 19 March 19 2007 to conduct the final examination of Antarjo Dikin on his degree in Doctor of Philosophy thesis entitled “Development of a Biopesticide for the Control of *Schizophyllum commune* Fr., a Pathogen of Oil Palm Seeds.” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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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 UPM or other institutions.

ANTARJO DIKIN

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
atm	Atmosphere
cfu	Colony form unit
cm	Centimeter
cm ²	Centimeter square
DAPG	2,4-diacetylphloroglucinol
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
FeSO ₄ .7H ₂ O	Ferrous sulphate heptahydrate
°C	Degree Celsius
g	gram
GN	Gram negative
GP	Gram positive
HCl	Hydrochloride acid
Hg	Mercury
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
I.D	Internal diameter
KH ₂ PO ₄	Potassium dihydrophosphate
K ₂ HPO ₄	Dipotassium hydrogen phosphate

kJ	Kilojoule
L	Liter
M	Molar
MgSO ₄ .7H ₂ O	Magnesium sulphate heptahydrate
MIC	Minimum inhibition concentration
mm	Millimeter
mL	Milliliter
mM	Millimolar
nm	Nanometer
NA	Nutrient agar
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
Na ₂ HPO ₄ ,	Disodium hydrophosphate
NaOH	Sodium hydroxide
NBY	Nutrient broth yeast
NH ₄ Mo	Ammonium molybdate
NH ₄ Cl	Ammonium chloride
NH ₄ NO ₃	Ammonium nitrate
NP	Neopeptone lactose
OD	Optical density
PDA	Potato dextrose agar

PM	Peptone maltose
Vac.	Vacuum
μM	Micrometer
μL	Microliter
RH	Relative humidity
RNA	Ribonucleic acid
rpm	Rotations per minute
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TLC	Thin layer chromatography
UV	Ultraviolet
WA	Water agar
Zn^{2+}	Zinc ion
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Zinc sulphate heptahydrate
λ	Lambda