

INSYIRAH ISHAK

MASTER OF SCIENCE

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**OPTIMIZATION OF *Metarhizium anisopliae*
(MET-GRA4 STRAIN) MASS PRODUCTION: A
BIO CONTROL AGENT AGAINST RED PALM
WEEVIL, *Rhynchophorus ferrugineus***

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**Thesis Submitted in Fulfilment of the Requirement for the
Degree Master of Science
in the School of Marine and Environmental Sciences
Universiti Malaysia Terengganu**

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DEDICATION

I dedicated this thesis to my beloved parents,

Ishak Long and Rogayah Hasan.

Thanks for nursing me with patience, moral support and endless love.

You enlighten my life and this postgraduate journey till the end.

Love,

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

**OPTIMIZATION OF *Metarhizium anisopliae* (MET-GRA4 STRAIN) MASS
PRODUCTION: A BIO CONTROL AGENT AGAINST RED PALM
WEEVIL, *Rhynchophorus ferrugineus***

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Main supervisor : Associate Professor Wahizatul Afzan Azmi, PhD

Co-Supervisor : Ng Lee Chuen, PhD

School : School of Marine and Environmental Sciences

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* is one of the serious insect pests for major cultivated crops worldwide. The use of bio control agent such as entomopathogenic fungi is highly recommended as it is a host specific, environmental friendly and cost effective. A strain of *Metarhizium anisopliae* namely MET-GRA4 was isolated from Terengganu soil has the potential to be commercialized as bio-pesticide against RPW. Thus, study was conducted to screen and optimize the mass production of *M. anisopliae* using One Factor A Time (OFAT) and Response Surface Methodology (RSM); Central Composite Design (CCD) model. The OFAT and RSM models used to investigate the interaction of incubation time, water amount and additional supplement. OFAT model factors first screening showed that 15 ml of water, 4 weeks of incubation time yielded 1.01 g of spores. The second screening, yeast yielded 1.367 g higher than peptone, 0.982 g. The CCD models optimization found the optimum conditions to mass produce MET-GRA4 was at 4 weeks of incubation, yeast 2.5% - 3% and 15 ml water content, yielded 1.39 g with 77.14% to 86.43% spore viability. Then, the bioassay of adult RPW was tested to determine the pathogenicity of mass produced MET-GRA4 spore. The harvested mass produced spores were infected on adult RPW for 21 day with same spore concentration 5×10^8 conidia/ml but different viability. The LT_{50} of 80-99% viable spores recorded at 8.6 days with 92% of mycosis effect, while the lowest spore viability 30-49% took 21.37 days with 16.6% of mycosis effect. The growth rate of spore germ tube showed a gradual increment as the incubation time

significantly increased from 6 hours to 48 hours (Pearson correlation $p < 0.05$, $R = 0.77$). Spores from pure culture, mass production and emerged from RPW cadavers were then characterized by molecular technique. The DNA sequencings from samples obtained were BLAST in NCBI showed 99% similarity with *Metarhizium anisopliae*, (accession number: JNNZ01000133.1, JNNZ01000133.1 and APNC01000595.1). In summary, this study provides the most optimum condition using rice substrate to mass produced *M. anisopliae* strain MET-GRA4 which was infective and pathogenic on adult RPW.

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PENGOPTIMUMAN PENGELUARAN BESAR-BESARAN *Metarhizium anisopliae* (MET-GRA4): AGEN KAWALAN BIOLOGI MENENTANG KUMBANG PALMA MERAH, *Rhynchophorus ferrugineus*

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2018

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Pusat Pengajian : Pusat Pengajian Sains Marin dan Sekitaran

Kumbang Palma Merah, *Rhynchophorus ferrugineus* (RPW) adalah salah satu daripada serangga perosak yang utama bagi tanaman palma yang ditanam secara meluas di dunia. Penggunaan agen kawalan biologi seperti kulat entomopatogenik sangat disyorkan kerana kulat ini khusus untuk satu perumah (hos), mesra alam dan kos efektif. Strain *Metarhizium anisopliae* iaitu MET-GRA4 yang telah dipencilkan daripada tanah dari Terengganu mempunyai potensi untuk dikomersialkan sebagai agen kawalan bio terhadap RPW. Oleh itu, satu kajian telah dijalankan untuk menyaring dan mengoptimumkan penghasilan *M. anisopliae* dengan menggunakan analisis One Factor A Time (OFAT dan Response Surface Methodology (RSM); model Central Composite Design (CCD). Analisis OFAT dan RSM digunakan untuk mengkaji interaksi masa inkubasi, jumlah air dan penambahan suplemen. Keputusan saringan pertama dari faktor OFAT, menunjukkan bahawa 15 ml air dan 4 minggu masa inkubasi menghasilkan 1.01 g spora. Saringan kedua dengan penambahan yis menyarankan menghasilkan 1.369 g spora berbanding peptone. Pengoptimuman menggunakan Central Composite Design (CCD) mendapati keadaan optimum untuk menghasilkan 1.39 g spora MET-GRA4 memerlukan 4 minggu inkubasi, yis 2.5% - 3% dan 15 ml kandungan air dengan kadar pertumbuhan spora adalah 77.14% hingga 86.43%. Bioesei terhadap RPW dewasa diuji untuk menentukan kadar patogenik spora MET-GRA4. Spora yang dituai telah diuji terhadap RPW dewasa, menggunakan kepekatan spora yang sama iaitu 5×10^8 conidia / ml dengan kadar pertumbuhan spora yang berbeza. RPW yang diuji didapati mati dalam masa 21 hari

selepas dijangkiti dengan spora META-G4. Pertumbuhan spora 80-99% mencatatkan kematian LT_{50} 8.6 hari dengan 92% kesan jangkitan penyakit. Manakala pertumbuhan spora yang terendah 30-49% mengambil masa 21.37 hari dengan 16.6% kesan jangkitan. Kadar pertumbuhan spora menunjukkan kenaikan secara beransur-ansur apabila masa inkubasi meningkat dari 6 jam hingga 48 jam (korelasi Pearson $p < 0.05$, $R = 0.77$). Spora dari pencilan tulen, pengeluaran besar-besaran dan dari bangkai RPW kemudian dikenalpasti menggunakan teknik molekular. Jujukan DNA yang diperoleh di BLAST di NCBI telah menunjukkan kesemua sampel adalah *Metarhizium anisopliae* dengan kesamaan 99% dengan nombor rujukan (JNNZ01000133.1, JNNZ01000133.1 dan APNC01000595.1). Kesimpulannya, kajian ini telah menyediakan formulasi yang paling optimum dengan menggunakan substrat beras untuk menghasilkan MET-GRA4 dalam skala besar. Spora MET-GRA4 mampu menyebabkan penyakit dan membunuh RPW dewasa.