UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

NG LEE CHUEN

ITA 2012 3

DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

By

Ng Lee Chuen

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

August 2012

DEDICATION

To my beloved parents, husband, daughter, sister and brothers for their inspiration, love and support

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

By

NG LEE CHUEN

August, 2012

Chairperson: Professor Sariah Meon, PhD

Institute : Institute of Tropical Agriculture

Rice production consumes lots of water. Due to increasing water scarcity, research on aerobic rice cultivation has been intensified. Poor root anchorage, unavailable phosphorus (P), low soil organic matter and blast disease caused by *Pyricularia oryzae* are the major constraints of aerobic rice cultivation systems. A study was carried out with the aim to inoculate commercially prepared rice straw compost (RSC) with a consortium of microbes in order to produce a microbial-fortified product, and evaluate its bio-efficacy on rice blast severity, growth, yield and soil health of rice variety M4 using an aerobic cultivation system. Four bacterial isolates [*Pseudomonas aeruginosa* (P1), *Corynebacterium agropyri* (P7), *Enterobacter gergoviae* (P9) and *Bacillus amyloliquefaciens* (S3)] and two *Trichoderma* spp. [(*Trichoderma harzianum* (T1) and *Trichoderma virens* (T2)] isolated from rice rhizospheres have been isolated and selected based on *in vitro* tests for the production of indole-acetic acid (IAA), siderophore, chitinase, volatile compounds, abilities to solubilize

phosphate and suppress mycelia growth of *P. oryzae*. These isolates were proven to be compatible as a mixed culture. *Enterobacter gergoviae, B. amyloliquefaciens, T. harzianum* and *T. virens* had significantly increased seed germination and seedling establishment, due to their ability to produce IAA and solubilize phosphate. Seedlings pre-inoculated with *P. aeruginosa*, *C. agropyri* and *T. harzianum* have higher production of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase corresponding to lower incidence of blast disease under greenhouse conditions. Total microbial activity based on fluorescein diacetate (FDA) hydrolysis measured in rhizosphere soil was positively correlated (r = 0.76, P = 0.04) to the seedling vigor index. These isolates were further inoculated into commercial rice straw compost (RSC) as carriers. The stability and the survival of the introduced microbes were evaluated based on total culturable plate count and FDA hydrolysis in storage up to 38 weeks. The viability of all the introduced bacteria in the microbial-fortified RSC in both sterilized and non-sterilized conditions decreased with time of storage. At week 38th, in sterilized RSC, all introduced bacteria was detected at 1.00 $\log_{10}$ cfu/g and none of the introduced bacteria was detected in unsterilized RSC. The viability of *T. harzianum* and *T. virens* remained constant (5.48–7.78 $\log_{10}$ cfu/g) throughout the storage period and was associated with the better colonization and proliferation due to no competition from the indigenous *Trichoderma* spp. A greenhouse experiment indicated that soil amended with the microbial-fortified RSC significantly improved yield (1768.42, 1052.00 and 2233.33%) and decreased rice blast severity based on AUDPC (area under disease progress curve) (74.52, 86.31 and 86.70%) compared to the control (mineral soil alone),

when *P. oryzae* inoculated at 14, 56 and 80 DAS (days after sowing), respectively. In soil amended with microbial-fortified RSC also improved soil total microbial activity (4.49 µg/g/0.5h in control; 7.32 µg/g/0.5h in soil amended with microbial-fortified RSC) at harvest, as well as physico-chemical properties included soil EC, bulk density and moisture content. Microbial-fortified RSC introduced during soil preparation was effective in reducing rice blast severity, promoting plant growth, yield and improving soil health for rice variety M4 under aerobic cultivation system.

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

PEMBANGUNAN KOMPOS JERAMI PADI YANG DIPERKAYAKAN DENGAN MIKROB UNTUK PENANAMAN PADI AEROBIK

Oleh

NG LEE CHUEN

Ogos, 2012

Pengerusi: Profesor Sariah Meon, PhD

Institut : Institut Pertanian Tropika

Tanaman padi adalah tanaman yang memerlukan air yang banyak. Seiring dengan peningkatan kebimbangan terhadap kekurangan air, penyelidikan ke atas penanaman padi aerobik telah pun dipergiatkan. Kelemahan cengkaman akar pokok, ketersediaan fosforus (P) dan bahan organik yang rendah dalam tanah bersamaan dengan masalah penyakit karah yang disebabkan oleh *Pyricularia oryzae* menjadi halangan utama dalam tanaman padi aerobik. Kajian ini bertujuan menghasilkan kompos jerami padi yang diperkayakan dengan mikrob melalui inokulasi konsortium mikrob ke dalam kompos komersial, dan menilai keberkesanan biologinya terhadap kawalan penyakit karah, pertumbuhan, hasil and kesihatan tanah pada padi varieti M4 yang ditanam dengan sistem aerobik. Empat bakteria [*Pseudomonas aeruginosa* (P1), *Corynebacterium agropyri* (P7), *Enterobacter gergoviae* (P9) dan *Bacillus amyloliquefaciens* (S3)] dan dua *Trichoderma* spp. [(*T. harzianum* (T1) dan *T. virens* (T2)] telah dipencilkan dan dipilih daripada rizosfera padi berdasarkan

ujian *in vitro* terhadap penghasilan indole-asetik asid (IAA), siderofor, kitinase dan sebatian mudah meruap, keupayaan untuk melarutkan fosfat dan menyekat pertumbuhan miselia *P . oryzae*. Pencilan telah dibuktikan serasi untuk dijadikan kultur campuran. *Enterobacter gergoviae*, *B. amyloliquefaciens, T. harzianum* dan *T. virens* telah menunjukkan peningkatkan secara signifikan dalam percambahan biji benih dan pertumbuhan awal anak benih berdasarkan keupayaan menghasilkan IAA dan melarutkan fosfat. Anak benih pra-inokulasi dengan *P. aeruginosa, C. agropyri* dan *T. harzianum* pula menunjukkan pengeluaran peroksidase, polifenol oksidase dan fenilalanine ammonia-liase yang lebih tinggi sepadan dengan kejadian penyakit karah yang lebih rendah dalam keadaan rumah hijau. Jumlah aktiviti mikrob berasaskan hidrolisis diasetat pendarfluor (FDA) pada rizosfera berkorelasi secara positif (r = 0.76, P = 0.04) dengan indek kecergasan anak benih. Pencilan selanjutnya ditambahkan ke dalam kompos jerami padi (KJP) komersial yang berfungsi sebagai pembawa. Kestabilan KJP yang diperkayakan dengan mikrob dan kemandirian mikrob yang diperkenalkan telah dinilai berdasarkan jumlah kiraan koloni yang tumbuh dan hidrolisis FDA setelah menyimpan selama 38 minggu. Pada minggu ke-38, pada KJP yang telah disteril, kesemua bakteria yang diperkenalkan telah dikesan dengan 1.00 $\log_{10}$ cfu/g dan tiada bakteria yang diperkenalkan dikesan dalam KJP tanpa disteril. Kebernasan *T. harzianum* dan *T . virens* didapati berkekalan (5.48–7.78 $\log_{10}$ cfu/g) sepanjang tempoh simpanan adalah berkaitan dengan kolonisasi and proliferasi yang lebih baik akibat tanpa persaingan dengan spesies *Trichoderma* asli. Kajian di rumah hijau menunjukkan tanah yang ditambahkan dengan KJP yang diperkayakan dengan

mikrob menunjukkan peningkatan hasil (1768.42, 1052.00 dan 2233.33%) dan pengurangan penyakit berdasarkan KBLPP (kawasan di bawah lengkung perkembangan penyakit) (74.52, 86.31 dan 86.70%) secara signifikan berbanding dengan kawalan (tanah mineral sahaja) apabila inokulatan dengan *P. oryzae* dilakukan pada 14, 56 dan 80 HLT (hari lepas tabur). Penambahan KJP yang diperkayakan dengan mikrob juga meningkatkan jumlah aktiviti mikrob (4.49 µg/g/0.5h pada kawalan; 7.32 µg/g/0.5h pada tanah yang ditambah dengan KJP yang diperkayakan dengan mikrob) dan menambahbaik sifat-sifat fizik-kimia tanah termasuk kekonduksian elektrik, ketumpatan pukal dan kandungan air. Penyediaan tanah bersama dengan KJP yang diperkayakan dengan mikrob berkesan untuk mengurangkan penyakit karah, menggalakkan pertumbuhan, hasil (t/ha) dan kesihatan tanah pada tanaman padi varieti M4 yang ditanam dengan sistem aerobik.

ACKNOWLEDGEMENTS

I wish to express my deepest and sincere gratitude to Prof. Dr. Sariah Meon, the chairman of my supervisory committee for her dedicated effort, support, intellectual guidance throughout the entire period of research and preparation of this dissertation.

I would also thank my committee members Assoc. Prof. Dr. Zainal Abidin Mior Ahmad, Assoc. Prof. Dr. Radziah Othman and Dr. Sariam Othman for the constructive comments, advices and helps at various stages of my research. My sincere appreciation to the Universiti Malaysia Terengganu and the Ministry of Higher Education Malaysia through a doctorial fellowship, the Ministry of Science, Technology and Innovation (MOSTI) for the research grant administered through the science fund of MOA and also to the Institute of Tropical Agriculture and the Faculty of Agriculture, Universiti Putra Malaysia for providing the research facilities.

My grateful thanks to numerous colleagues, friends and laboratory officers of the Plant Pathology, Plant Physiology and Soil Science departments for their excellent assistance and co-operation.

Finally, my heartfelt thanks go to my beloved parents, husband and daughter for their love, understanding, patience and spiritual support during this research.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committe were as follow:

Sariah Meon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Radziah Othman, PhD
Associate Profesor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Zainal Abidin Mior Ahmad, PhD
Associate Profesor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Sariam Othman, PhD
Malaysian Agricultural Research and Development Institute
(External member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Univeristi Putra Malaysia

Date:

TABLE OF CONTENTS