UNIVERSITI PUTRA MALAYSIA

FIELD EVALUATION OF PHAGE FERTILIZER ON CONTROLLING Ralstonia solanacearum IN TOMATO AND Erwinia mallotivora IN PAPAYA

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FP 2016 75

FIELD EVALUATION OF PHAGE FERTILIZER ON CONTROLLING *Ralstonia solanacearum* IN TOMATO AND *Erwinia mallotivora* IN PAPAYA

By

MOHD ZAFRUL ARIF RADHI

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

December 2016

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

FIELD EVALUATION OF PHAGE FERTILIZER ON CONTROLLING *Ralstonia solanacearum* IN TOMATO AND *Erwinia mallotivora* IN PAPAYA

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December 2016

Chairman : Tan Geok Hun, PhD
Faculty : Agriculture

The results of some previous studies have shown that bacteriophage, a virus that is 'eating' bacteria, by infecting and replicating in the bacterial cell-specific manner have potential to control plant pathogens. Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the major threats to the cultivation of tomatoes in Malaysia. In addition, papaya dieback caused by *Erwinia mallotivora* also brings huge losses to the papaya growers across the country since 2005 until now. In this study, field evaluation was conducted to determine the effectiveness of phage fertilizer for controlling bacterial wilt disease in tomato and papaya dieback in papaya. This fertilizer plus bacteriophage is the one with specific crop nutrient requirements for both crops that has been incorporated with bacteriophage. The attempts on tomato plants showed T1 (ACI (All Cosmos Industries) fertilizer + bacteriophage) recorded the lowest score of 0.5 compared to T2 (ACI fertilizer only) of 1.56 and T3 (control) of 3.02. There were significant differences between T1 and T3 at $p < 0.05$. Field study on papaya plants also recorded high percentage of plant mortality for all three treatments, T1, T2 and T3, three months after being inoculated with the pathogens *E. mallotivora*. T1 (ACI fertilizer + bacteriophage) recorded a percentage of 44.74% mortality, T2 (ACI fertilizer only) with 27.5% while T3 (control) with 69.77%. There was no significant difference between the three treatments recorded at $p < 0.05$. This proves the specificity of bacteriophage to the host (bacterial pathogens) plays an important role in determining the success of disease control using phage.

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

PENILAIAN LAPANGAN BAJA FAJ UNTUK KAWALAN *Ralstonia solanacearum* PADA POKOK TOMATO DAN *Erwinia mallotivora* PADA POKOK BETIK

Oleh

MOHD ZAFRUL ARIF RADHI

Disember 2016

Pengerusi : Tan Geok Hun, PhD
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Hasil daripada beberapa kajian sebelum ini mendapati bakteriofaj, iaitu sejenis virus 'pemakan' bakteria, berpotensi sebagai kawalan terhadap patogen tumbuhan dengan cara menginfeksi dan mereplikasi di dalam sel bakteria secara spesifik. Penyakit layu bakteria yang disebabkan oleh *Ralstonia solanacearum* merupakan salah satu ancaman utama terhadap penanaman tomato di negara ini. Selain itu, penyakit mati rosot betik yang berpunca daripada *Erwinia mallotivora* juga mendatangkan kerugian besar kepada penanam betik seluruh negara sejak tahun 2005 hingga kini. Dalam kajian ini, penilaian lapangan dilakukan untuk menentukan keberkesanan baja faj dalam mengawal penyakit layu bakteria pada tomato dan penyakit mati rosot pada betik. Baja faj ini merupakan kombinasi baja dengan formulasi khusus mengikut keperluan nutrien untuk kedua-dua tanaman tersebut yang telah dikombinasikan dengan bakteriofaj di dalamnya. Percubaan terhadap tanaman tomato menunjukkan T1 (baja ACI + bakteriofaj) mencatatkan skor penyakit terendah iaitu 0.5 berbanding T2 (baja ACI sahaja) iaitu 1.56 dan T3 (kawalan) iaitu 3.02. Terdapat perbezaan signifikan antara T1 dengan T3 pada $p<0.05$. Kajian lapangan terhadap tanaman betik pula mencatatkan hasil peratus kematian pokok yang tinggi untuk ketiga-tiga rawatan, T1, T2 dan T3 selepas tiga bulan diinokulasikan dengan patogen *E. mallotivora*. T1 (baja ACI + bakteriofaj) mencatatkan peratus kematian sebanyak 44.74%, T2 (baja ACI sahaja) sebanyak 27.5% manakala T3 (kawalan) sebanyak 69.77%. Tiada perbezaan signifikan antara ketiga-tiga rawatan dicatatkan pada $p<0.05$. Ini membuktikan kespesifikan bakteriofaj terhadap perumahnya (bakteria patogen) memainkan peranan penting dalam penentuan kejayaan kawalan penyakit menggunakan faj.

ACKNOWLEDGEMENTS

First of all, I am heartily thankful to my supervisor, Dr. Tan Geok Hun for her guidance, support and advice for my study. I appreciate her encouragement, efforts, scientific knowledge and understanding. With her excellent guidance and supervision, I am able to carry out the study smoothly. Again, million thanks to Dr. Tan Geok Hun.

Secondly, I wish to show my gratitude to my co-supervisors, Associate Professor Dr. Halimi Mohd Saud and Dr. Mohd Norowi Hamid. My study would not be complete without their crucial contribution, guidance and advice. I really appreciate their patience and encouragement to guide me throughout the study. Besides that, I would like to thanks the students and technical staff from Department of Agriculture Technology and Institute of Bioscience for their guidance, patience and assistance. Thank you for those who were willing to lend their hands to me when I carried out my work.

Nevertheless, I convey my gratitude to my father, mother, beloved wife (Khairunnisa Izzati) and daughter (Aufa Humaira), family members and friends for their understanding and support during the completion of my study. Without their encouragement and support, this study would not have been completed. This study was funded by Ministry of Science, Technology and Innovation Grant Scheme (TechnoFund) (TF0512B074).

I certify that a Thesis Examination Committee has met on 23 December 2016 to conduct the final examination of Mohd Zafrul Arif bin Radhi on his thesis entitled "Field Evaluation of Phage Fertilizer on Controlling *Ralstonia solanacearum* in Tomato and *Erwinia mallotivora* in Papaya" in accordance with the Universities and University Colleges 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.

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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:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACI	All Cosmos Industries Sdn. Bhd.
ATCC	American Type Culture Collection
CRD	Complete Randomized Design
DNA	Deoxyribonucleic acid
dsRNA	Double stranded ribonucleic acid
NA	Nutrient agar
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
TBS	Tris-buffered saline

CHAPTER 1

INTRODUCTION

Agriculture remains an important sector of Malaysia's economy. In the year of 2014, Malaysia has a total of 7,839,000 ha of agricultural land from its total land area of 32,855,000 ha. Malaysia's Gross Domestic Product (GDP) was RM 1062.8 billion in 2015, which RM 94.1 billion (8.9%) from total GDP contributed by the agriculture sector. Total vegetable export value in 2013 was RM887.59 million (4.02% from total Malaysia food product export). The export value of tomato in 2013 was RM100.7 million (0.46% from total Malaysia food product export). Total fruit export value in 2013 was RM675.73 million (3.06% from total Malaysia food product export). The export value of pineapple (fresh and processed) in 2013 was RM38.61 million (0.17% from total Malaysia food product export) (Department of Agriculture, 2014).

Tomato production in Malaysia is commonly for domestic consumption. Tomato growing areas are usually in the highlands such as Cameron Highlands and Kundasang whereas lowland cultivation can be found in Kelantan, Johor and Pahang. Although there is no published data on the exact losses in Malaysia, bacterial wilt which brought about by a bacterial pathogen which is *Ralstonia solanacearum* dependably be the most wrecking causing agent in tomato and yield misfortunes up to 90 percent. Bacterial wilt is a critical disease, particularly in the lowlands. It was initially written about tomato and potato in 1910 (Bancroft, 1910). The pathogen had been accounted for to taint more than 200 plant species from 53 diverse plant families (Álvarez *et al*., 2010). The most widely recognized hosts are tomato, banana, eggplant, potato, groundnut and ginger. In fresh market tomato in Taiwan, malady occurrences of 15 to 55% have been accounted for, bringing about misfortunes surpassing 12 million U.S. dollars yearly (Lin *et al*., 2014).

Papaya or *Carica papaya* is another monetarily noteworthy fruit crop grown in Malaysia with an export value of USD 8.42 million in 2011 (FAOSTAT, 2015). In any case, papaya dieback disease has turned out to be one of the significant threats of this industry in Malaysia as of late. Symptoms commonly recognized include greasy, water soaked lesions and spots on leaves, as well as foliar and angular lesions. These lesions can prompt to optional contamination, which can in the end cause the demise of the papaya plant. This infection has been an issue for papaya producers for just about 10 years, devastating more than one million plants. The infection was initially distinguished in Malaysia close Batu Pahat, Johor in late 2003. Another occurrence was later announced in Bidor, Perak, in October 2004. All the more as of late, Maktar *et al*., (2008) detailed *E. papayae* as creating papaya dieback in Malaysia. Notwithstanding, the most recent review by Noriha *et al*., (2011) affirmed that the causal agent for papaya dieback in Peninsular Malaysia is *Erwinia mallotivora*.

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Mohd Zafrul Arif was born in Johor Bahru, Johor, Malaysia on 11th September 1984. He obtained his early education in Sekolah Kebangsaan Taman Tun Aminah, Skudai in 1991 and Sekolah Kebangsaan Sungai Tua Baru, Batu Caves from 1992 to 1996. He continued secondary education in Sekolah Alam Shah, Kuala Lumpur (SBP) from 1997 to 2001 and later enrolled in Kolej Matrikulasi Negeri Sembilan, Kuala Pilah for one year in 2002. He then pursued his tertiary education in Universiti Kebangsaan Malaysia, Bangi in year 2003 and did receive his Bachelor of Science (Hons.) in Microbiology in year 2006, under the Jabatan Perkhidmatan Awam (JPA) scholarship. After graduated, he joined Malaysian Agricultural Research and Development Institute (MARDI) as a research officer in the Strategic Resource Research Centre from 2007 until now. During his service with MARDI, he led few researches such as MARDI's Short Term Grant (indigenous microorganism research) and MARDI's RMKe-10 Mega Projects (development of biofertilizer standards and organic goat and chicken research) and MOSTI's Science Fund Project (vermicompost research). He was also an active collaborator in few projects such as the Development of Taman Tropika Kenyir in Terengganu, Integrated Organic Farming and Research on Climate Change. In 2014, he was offered to further his study by doing Master Degree in Agriculture Technology in Universiti Putra Malaysia under MARDI's scholarship.

LIST OF PUBLICATIONS

Mohd Zafrul Arif, R., Tan, G.H., Wan Azha, W.M. and Tony Peng, S.H. (2015). Field evaluation of Eksotika II (*Carica papaya* L.) against *Erwinia mallotivora* sp., causal agent for papaya dieback in Peninsular Malaysia. *Proceeding of International Congress of the Malaysian Society for Microbiology 2015* (ICMSM 2015), Bayview Beach Resort, Penang, Malaysia,7-10 December 2015, **P**:68-70

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