



UNIVERSITI PUTRA MALAYSIA

***PHYSIOLOGY OF *Erwinia mallotivora*- INFECTED PAPAYA
SEEDLINGS (*Carica papaya* L.) TREATED WITH SILICON***

NOOR SHAHIDA BINTI YAMANLUDIN

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By

NOOR SHAHIDA BINTI YAMANLUDIN

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

April 2015

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

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April 2015

Chairman : Associate Professor Yahya bin Awang, PhD
Faculty : Agriculture

Papaya dieback disease caused by *Erwinia mallotivora* has been a threat to the Malaysia's papaya industry destroying more than one million plants and chemical control of the disease is almost impossible. Based on the available information on the possible beneficial effects of silicon (Si) in increasing crop resistant to bacterial diseases in plants, this study was designed. This study was conducted with the intention to characterize the development of dieback disease on papaya seedlings, to investigate the effects of varying concentrations of Si on dieback disease development, physiological and biochemical aspects of *E. mallotivora* infected papaya seedlings as well as to elucidate the possible mechanisms of Si in mediating the beneficial effects in reducing the occurrence of dieback disease in papaya seedlings. Two cultivars of papaya were used in the first and second experiments which were papaya cultivar Eksotika and papaya cultivar Eksotika II. Only papaya cultivar Eksotika II was used in the third experiment. In the first experiment, eight week old plants inoculated with *E. mallotivora* suspension (1×10^8 CFU/ml) showed a dieback disease symptoms started from day 3 after inoculation (DAI) with small water soaked lesion at point of inoculation (3 cm in length) and the size of lesion has increased with time. Dieback of shoot occurred at 9 DAI and the plant was fully wilted and dead at 11 DAI.

Besides visual symptoms appearance of dieback disease, infection with bacteria *E. mallotivora* had also caused biochemical changes in papaya Eksotika and Eksotika II in the second experiment. Leaf of Eksotika II had higher content of total sugar, total protein, peroxidase activity and polyphenol oxidase activity compared to papaya Eksotika. In stem, papaya Eksotika II had higher total phenol and total protein compared to papaya Eksotika. Higher total protein, peroxidase activity and polyphenol oxidase activity were found in roots of papaya Eksotika II compared to papaya Eksotika. Papaya Eksotika II had higher photosynthetic rate compared to papaya Eksotika. However, stomata conductance was found higher in papaya Eksotika compared to papaya Eksotika II. There was no significant different in transpiration rate for both papaya cultivars. Studies on photosynthetic activity of both papaya cultivars

showed that non-infected plant had higher photosynthetic rate, stomatal conductance and transpiration rate compared to inoculated plant.

To elucidate the effects of Si in regulating dieback disease in the third experiment, two-week old papaya seedlings were sprayed with 50 ml of sodium silicate (28.5 % SiO₂, 8.5% Na₂O) at four level of SiO₂ (0, 50, 100, 150 mg/L), at a weekly interval for 8 weeks. Results showed lesion length and disease symptom were reduced when treated with Si. At 100 mg/L Si level, highest content of total sugar, total phenol, total protein, peroxidase activity, polyphenol oxidase activity, photosynthesis, transpiration rate and stomatal conductance were recorded. Si content in plant tissues increased markedly with increasing Si level in the applied solution of 0 mg/L (control), 50 mg/L, 100 mg/L and 150 mg/L, with their respective concentrations (0.120 mg/g DW, 0.164 mg/g DW, 0.246 mg/g DW and 0.218 mg/g DW).

In conclusion, papaya dieback disease symptoms for papaya cultivar Eksotika and Eksotika II occurred as early as day 3 after inoculation with bacteria *E. mallotivora*. Infection with *E. mallotivora* caused certain biochemical and physiological changes in papaya plants. Si applied as sodium silicate had shown positive effects on papaya plant infected with dieback disease caused by bacteria *E. mallotivora*. Si at 100 mg/L showed positive effects in reducing dieback disease caused by the bacteria *E. mallotivora*. However, treatment with sodium silicate did not prevent plants from dying. Papaya seedlings were dead at 18 days after inoculation with bacteria *E. mallotivora*. Results showed that sodium silicate at concentration of 100 mg/L and 150 mg/L SiO₂ were able to slow down the outbreak of dieback disease development but failed to stop dieback disease development and results in death of papaya plants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**FISIOLOGI ANAKBENIH BETIK (*Carica papaya* L.) YANG DIJANGKITI
Erwinia mallotivora DIRAWAT DENGAN SILIKON**

Oleh

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Penyakit mati rosot betik yang disebabkan oleh *Erwinia mallotivora* telah menjadi ancaman kepada industri betik Malaysia dengan memusnahkan lebih daripada satu juta pokok dan kawalan kimia penyakit ini adalah hampir mustahil. Berdasarkan maklumat sedia ada berkenaan kemungkinan kesan baik silikon (Si) dalam meningkatkan daya tahan tanaman kepada bakteria di dalam tumbuhan, maka kajian ini dijalankan. Kajian ini dijalankan dengan tujuan untuk mencirikan perkembangan penyakit mati rosot pada anak benih betik, dan juga menyiasat kesan perbezaan kepekatan Si kepada anak benih betik dari segi aspek perkembangan penyakit mati rosot, fisiologi dan biokimia apabila dijangkiti oleh *E. mallotivora* dan untuk menjelaskan mekanisma Si yang berkemungkinan memberi kesan yang baik dalam mengurangkan kejadian penyakit mati rosot bagi anak benih betik. Dua kultivar betik telah digunakan dalam eksperimen pertama dan eksperimen kedua iaitu kultivar betik Eksotika dan betik Eksotika II. Hanya kultivar betik Eksotika II telah digunakan dalam eksperimen ketiga. Dalam eksperimen pertama, pokok berusia lapan minggu yang diinokulasi dengan bakteria *E. mallotivora* (1×10^8 CFU/ml) menunjukkan gejala penyakit mati rosot bermula dari hari ke-3 selepas inokulasi (DAI) dengan lecu basah yang kecil pada bahagian inokulasi (3 cm panjang) dan saiz lecu meningkat dengan peningkatan masa. Lecu basah pada bahagian pucuk berlaku pada 9 DAI dan tumbuhan layu sepenuhnya dan mati pada 11 DAI.

Selain simptom kemunculan penyakit mati rosot, jangkitan bakteria *E. mallotivora* juga telah menyebabkan perubahan biokimia dalam pokok betik Eksotika dan Eksotika II di dalam eksperimen kedua. Betik kultivar Eksotika II mempunyai kandungan jumlah gula, jumlah protein, aktiviti peroksidase (PO) dan aktiviti polifenol oksidase (PPO) yang lebih tinggi berbanding betik Eksotika di dalam daun. Dalam batang, betik Eksotika II mempunyai jumlah fenol dan jumlah protein yang lebih tinggi berbanding betik Eksotika. Jumlah protein, aktiviti peroksidase dan aktiviti polifenol oksidase yang lebih tinggi juga ditemui di dalam akar betik Eksotika II berbanding betik Eksotika. Betik Eksotika II mempunyai kadar fotosintesis yang lebih tinggi berbanding dengan betik Eksotika. Walau bagaimanapun, kealiran stomata didapati lebih tinggi di dalam

betik Eksotika berbanding betik Eksotika II. Tiada hasil yang ketara dalam kadar transpirasi untuk kedua-dua kultivar betik. Kajian ke atas aktiviti fotosintesis bagi kedua-dua kultivar betik menunjukkan tumbuhan yang tidak dijangkiti mempunyai kadar fotosintesis, kealiran stomata dan kadar transpirasi yang lebih tinggi berbanding tumbuhan yang telah disuntik dengan *E. mallotivora*.

Untuk menjelaskan kesan Si dalam mengawal penyakit mati rosot di dalam eksperimen ketiga, anak benih betik berusia dua minggu telah disemur dengan 50 ml natrium silikat (28.5 % SiO₂, 8.5 % Na₂O) pada empat tahap SiO₂ (0, 50, 100, 150 mg/L), setiap minggu selama 8 minggu. Keputusan menunjukkan bahawa panjang lecut dan gejala penyakit berkurangan apabila dirawat dengan Si. Pada tahap Si 100 mg/L, kandungan tertinggi bagi jumlah gula, jumlah fenol, jumlah protein, aktiviti peroksidase, aktiviti polifenol oksidase, fotosintesis, kadar transpirasi dan kealiran stomata telah direkodkan. Kandungan silikon dalam tisu tumbuhan meningkat dengan ketara selari dengan peningkatan tahap Si yang digunakan iaitu 0 mg/L (kawalan), 50 mg/L, 100 mg/L dan 150 mg/L, dengan kepekatan masing-masing 0.120 mg/g berat kering, 0.164 mg/g berat kering, 0.246 mg/g berat kering dan 0.218 mg/g berat kering.

Kesimpulannya, simptom penyakit mati rosot betik bagi betik Eksotika dan Eksotika II berlaku seawal 3 hari selepas inokulasi dengan bakteria *E. mallotivora*. Jangkitan *E. mallotivora* menyebabkan perubahan biokimia dan fisiologi tertentu dalam tanaman betik. Si yang digunakan iaitu natrium silikat telah menunjukkan kesan positif ke atas pokok betik yang dijangkiti penyakit mati rosot yang disebabkan oleh bakteria *E. mallotivora*. Si pada tahap 100 mg/L menunjukkan kesan positif dalam mengurangkan penyakit mati rosot pokok betik yang disebabkan oleh bakteria *E. mallotivora*. Walau bagaimanapun, rawatan dengan natrium silikat tidak menghalang tumbuhan daripada mati. Anak pokok betik mati pada 18 hari selepas inokulasi dengan bakteria *E. mallotivora*. Hasil kajian menunjukkan bahawa natrium silikat pada kepekatan 100 mg/L dan 150 mg/L SiO₂ dapat melambatkan simptom penyakit lecut basah tetapi gagal untuk menghentikan perkembangan penyakit mati rosot dan menyebabkan kematian pokok betik.

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I certify that a Thesis Examination Committee has met on 28 April 2015 to conduct the final examination of Noor Shahida Binti Yamanludin on her thesis entitled Physiology of *Erwinia Malloivora*- Infected Papaya Seedlings (*Carica Papaya L.*) Treated with Silicon 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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LIST OF ABBREVIATIONS

μmol	micromole
CFU	colony forming unit
DAI	days after inoculation
DW	dry weight
FW	fresh weight
h	hour
M	molar
min	minute
mM	milimolar
mmol	mili-mole
N	normality
OD	optical density
ppm	parts per million
YBGA	Yeast Bactopeptone Glucose Agar

CHAPTER 1

INTRODUCTION

1.1 Background Information

Papaya (*Carica papaya*), is a well-known commercial fruit consumed in most part of the world. Papaya plant is native to northern Mexico and Central America and it is widely grown in the subtropical and tropical regions including Malaysia. In Malaysia, papaya is mostly grown in state of Perak, Sarawak and Johor. The plant is a short-lived and fast-growing plant (Papaya Fruit Facts, 2011). According to Department of Agriculture Malaysia, papaya production in Malaysia had increased from 1993 to 2001 by an average rate of 5.21 % per annum. The market demand for papaya fruits was huge. Global papaya production in 2010 was estimated at 11.22 metric tonnes, growing at an annual rate of 4.35 % between 2002 and 2010. Global papaya production in 2010 was 7.26 % higher than 2009, and 34.8 2% higher than 2002 (FAOSTAT, 2012). Papaya fruit is important in Malaysian economy due to an export value of around RM100 – 120 million per year (Rabu and Mat Lin, 2005).

Papaya is sold in the market throughout the year in Malaysia. Papaya produced in Malaysia is exported to Singapore, Hong Kong, Middle East and Europe as well as for local consumption. The export market is expected to be extended to China, USA, Japan, Australia, New Zealand and Russia, when the government increase the activity promotion in the future (MOA, 2012). In year 2011, Malaysian Department of Agriculture reported that papaya planting area was 2,462 hectares (Ha) with a yield of 43,364 metric tonnes (t). These values increase from year 2011 to year 2013. In 2013, papaya planting areas were 2,869 hectares (Ha) with yield of 48,078 metric tonnes (t) (Figure 1.1 and Figure 1.2).

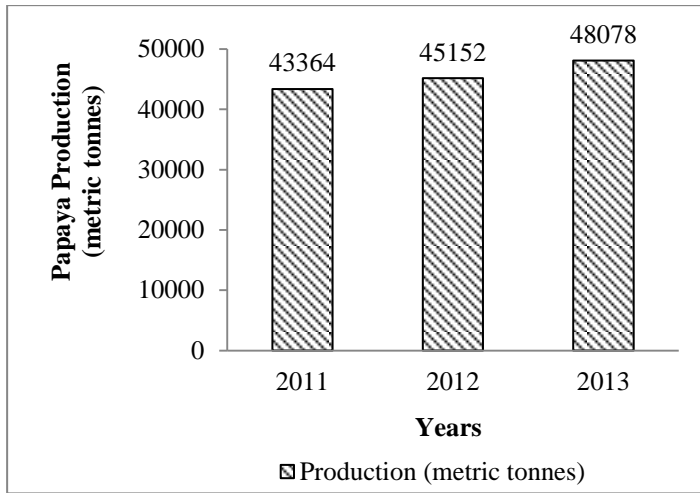


Figure 1.1. Papaya Production in Malaysia from Year 2011 to 2013.

(DOA, 2013)

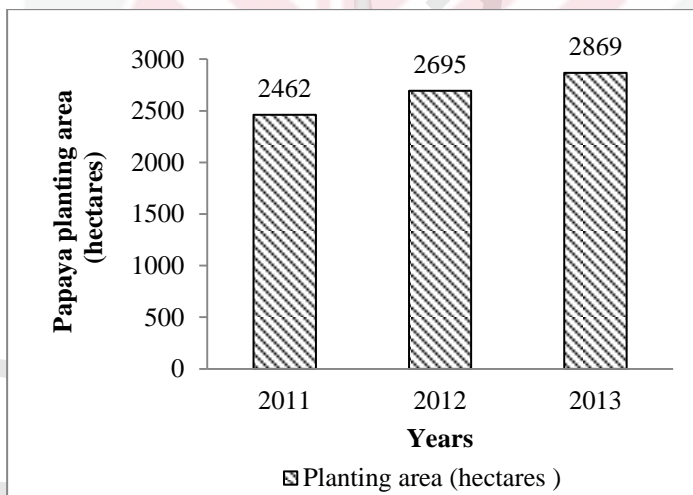


Figure 1.2. Papaya Planting Area in Malaysia from Year 2011 to 2013.

(DOA, 2013)

In expanding its cultivation in Malaysia, papaya has faced many problems. One of them is dieback disease. Papaya dieback disease caused by *Erwinia mallotivora* is a threat to papaya industry in Malaysia and around the world (Noriha *et al.*, 2011). There is no solution to counteract the disease once the plant has been infected with *E. mallotivora*. Infected papaya plant must be destroyed in order to prevent the spread and this has caused lots of losses to the papaya industry. Papaya cultivars infected by papaya dieback disease were Eksotika, Sekaki, Solo and Hong Kong. Infected areas was 806 hectares and estimated losses was RM30 millions (DOA, 2012).

One way to reduce the incidence and the spread of the bacteria dieback disease is by increasing its resistance. Among the technique is by increasing the content of silicon (Si) in plant tissues. The beneficial effect of silicon in increasing the crop resistance has been reported for both fungal and bacterial diseases in both Si-accumulators plant and Si non-accumulators plant (Wydra *et al.*, 2005). Apart from increasing disease resistance, Si have been reported to play a role in increasing photosynthetic activity, increase insect resistance, reduced mineral toxicity, improvement of nutrient imbalance, and enhanced drought and frost tolerance (Ma, 2004). Increasing level of Si in culture solution lead to increase in Si content of the leaves and thus, reduced disease incidence (Kanto, 2002). Menzies *et al.* (1991) found that infection efficiency, colony size, and germination of conidia were reduced when cucumbers were grown in nutrient solutions with high concentration of Si.

In the view of the effect of Si on disease resistance and the successful Si application in reducing disease incidence and severity and also enhanced host defence mechanisms, it is suggested that application of Si might help in inducing papaya defence against dieback disease caused by *E. mallotivora*. This would then help to reduce the overall occurrence and destruction of this disease on papaya. There were no investigation on Si application in papaya plant recorded and the possibility of using Si in reducing dieback diseases caused by *E. mallotivora* is unknown.

1.2 Objectives of the Study

The objectives of this study were:

- i. to characterise the development of dieback disease on papaya seedlings;
- ii. to investigate the effects of varying concentrations of silicon on dieback disease development, physiological and biochemical aspects of *E. mallotivora* infected papaya seedlings; and
- iii. to elucidate the possible mechanisms of silicon in mediating the beneficial effects in reducing the occurrence of dieback disease in papaya seedlings.

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