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EFFECT OF SALICYLIC ACID ON DEVELOPMENT OF BACTERIA WILT DISEASE (Ralstonia solanacearum) IN TOMATO

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EFFECT OF SALICYLIC ACID ON DEVELOPMENT OF BACTERIA WILT DISEASE (*Ralstonia solanacearum*) IN TOMATO

By

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Specially Dedicated To:

My beloved mother, Zaliha binti Ma'at

My father, Madiha bin Sarudi

My brothers,

Noor Azizul Rahim Madiha and

Noor Saiful Nizam Madiha

My bestfriends,

Latifah Musanif, Azmah Azhari and Suriya Ismail

ABSTRACT

The effect of salicylic acid (SA) on infection potential of bacterial wilt disease on development of tomatoes caused by *Ralstonia solanacearum* was studied. The effect of different concentrations of SA on development of bacteria colony was tested in vitro. The in-vitro experiment was conducted in a completely randomized design with 10 concentrations of SA in 7 replicates for each treatment over 2 weeks. Two selected concentrations of SA were subsequently included in a pot experiment. The inhibition of SA on bacteria colony development showed significant differences at $P < 0.05$. Two best concentrations were selected, which are 10 and 40 ppm of SA to be sprayed onto tomato plants. A pot experiment was carried out in randomized complete block design to study the effect of foliar application of SA on the development of bacterial wilt disease on tomato. Each block consisted of 3 treatments (0, 10 and 40 ppm) with 5 tomato plants per treatment in the netted greenhouse. Six parameters were measured namely plant height, fresh and dry weight of plants, chlorophyll content, average fruit weight and disease incidence. Tomato plants were harvested 11 weeks after pathogen infection. Results showed that % of disease incidence in infected plants gradually increased with increasing time of infection (100% at 11 weeks). Treatment with 40 ppm of SA significantly reduced percentage disease incidence. Plant height, fresh and dry weight, average fruit weight and chlorophyll content significantly increased after foliar application of SA as compared with control. Thus, SA has potential to be used for controlling *R. solanacearum* in tomatoes.

ABSTRAK

Kesan salisilik asid ke atas potensi jangkitan penyakit kelayuan bakteria pada pertumbuhan tomato disebabkan oleh *Ralstonia solanacearum* dikaji. Kesan pelbagai kepekatan salisilik asid ke atas pertumbuhan koloni bakteria diuji secara in vitro. Eksperimen in-vitro dijalankan dalam 'completely randomized design' dengan 10 kepekatan salisilik asid dengan 7 replikasi bagi setiap rawatan selama 2 minggu. Dua kepekatan salisilik asid pilihan akan dimasukkan ke dalam eksperimen pot. Perencatan salisilik asid ke atas pertumbuhan koloni bakteria adalah signifikan pada $P < 0.05$. Dua kepekatan terbaik dipilih iaitu 10 dan 40 ppm salisilik asid disembur ke atas pokok tomato. Eksperimen pot dijalankan dalam 'randomized complete block design' untuk mengkaji kesan aplikasi foliar salisilik asid ke atas pertumbuhan penyakit layu pada tomato. Setiap blok terdiri daripada 3 rawatan (0, 10 dan 40 ppm) dengan 5 pokok tomato untuk setiap rawatan dalam rumah hijau berjaring. Enam parameter diukur iaitu tinggi pokok, berat basah dan berat kering pokok, kandungan klorofil, purata berat buah dan kejadian penyakit. Pokok tomato dituai selepas 11 minggu dijangkiti patogen. Keputusan menunjukkan % kejadian penyakit dalam pokok yang dijangkiti adalah meningkat dengan peningkatan masa jangkitan (100% pada 11 minggu). Rawatan dengan 40 ppm salisilik asid secara signifikan menurunkan peratusan kejadian jangkitan. Tinggi pokok, berat basah dan berat kering, purata berat buah dan kandungan klorofil secara signifikan meningkat selepas aplikasi foliar salisilik asid apabila dibandingkan dengan kawalan. Oleh itu, salisilik asid mempunyai potensi untuk digunakan dalam mengawal *R. solanacearum* tomato.

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APPROVAL

I certify that this research project report entitled "Effect of Salicylic Acid on Development of Bacteria Wilt Disease (*Ralstonia solanacearum*) in Tomato" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

cfu	–	colony forming units
CMV	–	cucumber mosaic virus
g	–	grams
HR	–	hypersensitive response
lb	–	pound
mL	–	milliliter
NPK	–	nitrogen, phosphorus, potassium
SA	–	salicylic acid
SAR	–	systemic acquired resistance
s. e.	–	standard error
ppm	–	parts per millions
°C	–	degree celcius
µL	–	microliter
%	–	percentage

CHAPTER 1

INTRODUCTION

1.1 Introduction

Tomato is one of the most popular vegetable crops for both fresh market and processing in Malaysia. Tomatoes are popular among consumers not only because of its tastes good but also because it contains high levels of vitamin C, lycopene and beta-carotene which are antioxidants that promote good health (Wang and Lin, 2005). The high demand for tomato makes it a high value crop that can generate much income for farmers.

Tomatoes are adapted to diverse environments. They are best grown in well drained and fertile soils containing high organic matter. The plants require moderate temperatures of 21 to 28 ^{o}C. Suitable temperature is important for flowering and fruiting in tomatoes. They are highly self pollinated (Peter and Abraham, 2007). Tomatoes are deep rooted crops and require sufficient amount of water to grow well. They are also being threatened by diseases caused by various kinds of pathogens. Pathogens such as *Fusarium oxysporum* (Fusarium wilt), *Alternaria solani* (Early blight) and *Phytophthora infestans* (Late blight) infect tomato resulting in severe yield losses. The pathogen that causing bacterial wilt disease in tomato is *Ralstonia solanacearum*.

Ralstania solanacearum or previously called *Pseudomonas solanacearum* is the causal agent of bacterial vascular wilt in solanaceous plants. The disease is present in the tropics and in the warmer climates throughout the world. It causes severe losses on tobacco, tomato, potato, and eggplant in some warm areas outside the tropics. However, many other hosts attacked by the disease. At least five races of the pathogen cause disease on the various hosts. One of the pathogen races attacks all solanaceous and many non-solanaceous crops as well as bananas. Two other races cause disease in plants of little importance (Agrios, 2005).

Ralstonia solanacearum is a soil borne pathogen that will remain in the soil for many years. The bacteria will propagate in the root vessels of tomato and block the function of xylem and phloem in water and nutrient transport. As the plants get insufficient water and nutrients, the leaves will start wilting. Finally, the infected plants may be stunted or completely wilted resulting in poor fruit quality such as small fruit size and significant yield loss (Ji *et al.*, 2005).

Bacterial wilt caused by *Ralstonia solanacearum* is among the most difficult diseases to control and there are no conventional pesticides known to provide effective control of this soil borne pathogen (Ji *et al.*, 2005). Today, farmers commonly use chemical pesticides such as zineb, maneb, or mancozeb mixed with copper compounds, Bordeaux mixture, fixed coppers and cupric hydroxide to control the spread of bacteria. Usually these pesticides are applied in the field as foliar spray. Although applications of pesticides have helped control plant disease but chemical control is very expensive and environmentally undesirable. Crop rotations and the use of resistant tomato cultivars are alternative ways to reduce bacterial wilt incidence (Agrios, 2005). However, characteristics such as high yield, quality and adaptation to regions are frequently taken into consideration without regard to resistance to disease. For this reason, the most viable method to protect plants against pathogens is through inducing resistance without changing any characteristics.

According to Ji *et al.* (2005), besides using chemical pesticides to control bacterial pathogens, recently abiotic compounds such as thymol have been used to control bacterial wilt disease in tomato. Abiotic compounds such as salicylic acid (SA) has potential in reducing bacterial wilt incidence. Salicylic acid plays an important role to induced resistance signal called Pathogen – Related Proteins (PR Proteins) to protect crops from pathogen infections (Vidhyasekaran, 2005). Salicylic acid is a phenolic compound, acts as plant regulator and confers plant resistance to some viral, bacterial and fungal diseases (Ozgonen *et al.*, 1999). The exogenous application of SA may influence physiological processes such as transpiration rate, stomatal closure, membrane permeability, growth and photosynthesis and antioxidant capacity of the plants (Radwan *et al.*, 2007).

According to Ozgonen *et al.* (2001), the application of SA had potential for the control of *Fusarium oxysporum* f.sp. *lycopersici* which is the pathogen (fungus) that cause Fusarium wilt disease in tomato. The effectiveness of application of salicylic acid (SA) to control *Ralstonia solanacearum* is unknown.

1.2 Objectives of Study

The objectives of this study were to:

(a) Determine the effect of different concentration of salicylic acid on development of tomato plants.

(b) Control bacterial wilt disease of tomatoes caused by *Ralstonia solanacearum*.

CHAPTER 2

LITERATURE REVIEW

2.1 Tomato

Tomato belongs to Solanaceae family (member of nightshade family) which is the same family as potatoes, peppers, and egg plants. Tomato is widely cultivated all over the world. Botanically, it also classified as a fruit, since it is developed from an ovary, although commercially it is recognized as a vegetable (Swiader and Ware, 2002).

Tomato originated from the Andean region of South America, in the area now covered by parts of Bolivia, Chile, Ecuador, Colombia and Peru. Archeological and circumstantial evidence all suggest that tomato was domesticated in Mexico, outside its centre of origin and the most likely ancestor is the primitive cherry tomato (*L. esculentum* var. *cerasiforme*). Tomato was introduced into Europe in an already fairly advanced stage of domestication soon after discovery of the new world. From there, it was taken to other parts of the world at various times; in the 17^{th} century to Chile, South and South-East Asia, and in the 18^{th} century to Japan and the United States (Siemonsna and Kasem, 1994).

2.1.1 Taxonomy of Tomato

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Solanales
Family	: Solanaceae
Genus	: Lycopersicon
Species	: *Lycopersicon esculentum* (Jones, 2008)

2.1.2 Cultivation of Tomato

2.1.2.1 Propagation of Tomato

Tomato is propagated by seed, through cuttings and grafting. Seed is normally planted in nurseries, and transplanted after four to six weeks old when the plant height is 15 to 20 cm tall (Winch, 2006).

2.1.2.2 Germination of Tomato Seeds

Germination of tomato seed normally takes about seven to ten days. Optimum pH is about six to seven and tomatoes need nitrogen as they grow (Winch, 2006). The best germination takes place at 18 to 30 °C. The seedlings were transplanted after four weeks (Singh *et al.*, 2004).

2.1.2.3 Flowering and Fruit Development

The flower clusters in tomatoes occur along the stem and contains both male and female parts. Tomatoes are a self – pollinated crop, mostly by wind. The number of flowers comprising an inflorescence may vary from four to twelve or more but not all of these will set fruits. The flowers must be adequately pollinated to avoid fruit defects (Swiader and Ware, 2002).

The tomato fruits will develop from the ovary and is composed of several irregularly shaped carpels. The mature fruits may be red, pink, orange, yellow or white (Swiader and Ware, 2002).

2.1.2.4 Growth Condition for Tomato

Tomato grows best in fertile soil which contains compost or manure. Most varieties of tomato need warm temperatures 21 to 23 ^{o}C for maximum pollen germination and fruit setting. The crop takes three to four months to produce yield. Irrigation and drainage is important especially during hot conditions. But fluctuations in soil moisture from dry to wet season may cause cracking of fruits. Excess watering can reduces tomato flowers from setting properly, and also causes fungus diseases (Singh, 2004). Tomatoes will grow best in light, warm, well drained and fertile soil and this is necessary for early and high fruit quality production. The suitable pH for tomato cultivation is in the range of 5.5 to 7.5 (Swiader and Ware, 2002).

2.1.3 Diseases of Tomato

2.1.3.1 Wilt Diseases

Wilt diseases are caused by bacteria and fungus. Bacterial wilt disease makes tomato plants wilt very rapidly, without yellowing and then die. These symptoms become worse in the wet tropics and in waterlogged soil. The causal agent of bacterial wilt is *Ralstonia solanacearum* which is a soil borne pathogen. Fusarium wilt disease makes the leaves of tomato become yellow. The other wilt disease is Verticillium wilt ("sleep disease") caused by a fungus which attacks the roots and the base of stems (Winch, 2006).

2.1.3.2 Viral Diseases

Virus diseases in tomato include tobacco mosaic, spotted wilt, cucumber mosaic, curly top and yellow top. All of the diseases are spread by aphids and originated in other Solanaceous plants (Winch, 2006). The symptoms are stunted growth of plants, with curled and malformed leaves and reduced yield (Swiader and Ware, 2002).

2.1.3.3 Fungal Diseases

There are many diseases caused by fungus infection and they show different symptoms on infected tomatoes. Early blight caused by *Alternaria solani* generally shows dark brown spots on stems, leaves and fruits. Late blight (*Phytophthora infestans*) affects both leaves and fruits (Swiader and Ware, 2002). Another disease is fruit rot caused by *Phytophthora lycopersici*. The infected plants produce less quantity of poor quality fruits which do not have marketable value (Singh *et al.*, 2004).

2.1.3.4 Bacterial Diseases

Bacterial spot is caused by *Xanthomonas vesicatoria* which is carried on the surface of seeds. Another bacterial disease is Bacterial canker (*Corynebacterium michiganense*), where the pathogen colonizes the water conducting tissues of plants. The bacteria can be found in seeds and in the soil (Swiader and Ware, 2002)

2.2 *Ralstonia solanacearum*

This is a member of the family Psedomonodaceae in the β-subdivision of the *Proteobacteria. Ralstonia* shares many similarities with *Burkholderia*, but can be distinguished on the assimilation of galactose, mannitol, mannose and sorbitol. Currently there is only one plant pathogen included in this genus, *Ralstonia solanacearum*, which is possibly the most significant bacterial phytopathogen worldwide; due to the wide host range and severity of the bacterial wilt diseases it causes (Waller *et al.*, 2002). Traditionally, the pathogen is classified into five biovars based on the utilization of certain sugars and sugar alcohols and into three races based on host range (Boer, 2001).

2.2.1 Taxonomy of *Ralstonia solanacearum*

Kingdom : Procaryotae

Division : Gracilicutes

Class : Proteobacteria

Family : Psedomonodaceae

Genus : *Rolstania*

Species : *Rolstania solanacearum* (Agrios, 2007)

2.2.2 Disease Cause by *R. solanacearum*

2.2.2.1 Southern Bacterial Wilt of Solanaceous Plants

Bacterial wilt on solanaceous crops appears as a sudden wilt. The pathogen is released into soil from infected plants, and neighbouring plants can be infected via root contact. It can also enter plants through pruning wounds. The pathogen can be disseminated into a clean field through contaminated water sources, symptomless yet contaminated seedlings, as well as humans or machinery carrying infested soils (Wang and Lin, 2005). Infected young plants die rapidly. Older plants first show wilting of the youngest leaves, or one sided wilting and stunting, finally the plants wilt permanently and die. In some plants, such as eggplant and tomato, they show similar disease symptomology for both races of *R. solanacearum*, characterized by severe rapid wilting of leaves and in the field symptoms may occur first in lower areas where moisture accumulates. Infected tomato plants may be chlorotic or stunted with adventitious roots on the stem. Other symptoms include vascular tissues of stems, roots, and tubers, turning brown, and in cross sections they ooze whitish bacterial exudates. Bacterial pockets develop around the vascular bundles in the pith and in the cortex, and roots and especially tubers often rot and disintegrate and by that time the plant wilts permanently (Agrios, 2007).

2.2.3 Control of Wilt Disease

Ralstonia solanacearum is difficult to eradicate once they are introduced into the soil but they can be suppressed. The usual methods being used are fumigation, rotation with non host crops, soil amendments, using resistant varieties of tomato and clean seedlings.

Growing Brassica manure like Indian mustard (*Brassica juncea*) and incorporating the plants into soil at flowering stage can also suppress the bacterial wilt pathogen. Commercial varieties with high and stable resistance as well as good fruit characteristics are not common. Examples of resistant or tolerant varieties include 'Arthaloka' in Indonesia, 'Delta' in Thailand, and 'Taichung AVRDC 4' in Taiwan (Wang and Lin, 2005). Bacterial wilt disease can be suppressed using thymol as biofumigant. Thymol is used for soil treatment before transplanting and it also prevents dramatic yield loss due to infection by *R. solanacearum* (Ji *et al.*, 2005).

There are several types of chemicals that can be used to control bacterial diseases especially wilt disease caused by *R. solanacearum*. The common chemical usually used is Bordeaux mixture. The mixture must be prepared freshly before application in the field. The chemical is also recommended as a spray at a concentration of 1 + 1 + 100 (copper sulfate in lb + calcium hydroxide in lb + water in gallons) and should be applied at five to seven day intervals. Chloropicrin, copper oxide and salicylic acid are other chemicals that can be used to control bacterial wilt disease (Vidhyasekaran, 2004).

2.3 Salicylic Acid

Many chemicals have been suggested as resistance inducers in plants. One of the chemicals is salicylic acid (SA). Salicylic acid is considered as one of the key components of defense signal transduction, which induces full set of systemic acquired resistance genes. Salicylic acid plays an important role in pathogen induced disease resistance in many plants. For example, SA is an effective inducer of resistance against Bean Yellow Mosaic Virus (BYMV) infection in *Vicia Faba* (Radwan *et al.*, 2007).

Salicylic acid and other abiotic compounds such as heavy metals, polyacrylic acid, mannitol, methyl salicylate and methyl jasmonate or jasmonic acid can induce synthesis of Pathogenesis – Related proteins (PR Proteins). PR proteins are encoded by the host plants when they are infected by pathogens (Vidhyasekaran, 2004).

Salicylic acid (SA) derived from cinnamic acid is a phenolic compound found in plants and acts as endogenous elicitors. Endogenous elicitors are plant constituents and released from the cell wall in response to pathogenic infection. Endogenous SA increases the synthesis of PR proteins in tobacco mosaic virus infected leaves. Exogenous application of SA also elicits PR protein accumulation and resistance. For example, spraying tobacco plants with SA induces the synthesis of PR – 1 proteins and resistance to alfalfa mosaic virus (Huang, 2001). According to Khan *et al.* (2003), the exogenous application of SA may influence a range of diverse processes in plants, including stomatal closure, ion uptake and transport, ethylene synthesis, seed germination, fruit yield, glycolysis and the growth-inhibiting effect of abscisic acid. Some essential oils were also confirmed to be effective in suppressing *R. solanacearum* in soil and reducing bacterial wilt disease in greenhouse pot experiments (Zhu and Yao, 2004).

The present study was conducted to determine the effectiveness of SA as exogenous elicitor against bacterial infection by *Ralstonia solanacearum* in tomato.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Design

Two experiments were conducted in this study. In the laboratory experiment, a completely randomized design was used while for the pot experiment, randomized complete block design was used.

3.2 Laboratory Experiment

3.2.1 Source of Inoculum

The pure *R. solanacearum* inoculum was obtained from Associate Professor Dr. Kamaruzzaman Sijam, from the Faculty of Agriculture in UPM Serdang.

3.2.2 Media Preparation

Nutrient Agar (NA) was prepared to grow the *R. solanacearum* and nutrient broth (NB) was prepared to make bacterial suspensions for the in-vitro experiment. Ten grams of NA powder and five grams of NB were weighed and 500 mL distilled water was poured into each the of Schott bottles. The bottles were shaken until the NA and NB dissolved. The bottles were closed and inserted into an autoclave at 121 ^{o}C for 20 minutes and cooled at room temperature for ten minutes. The NA was then poured into Petri dishes and left to coagulate in the laminar flow for one hour and 50 mL each of NB were poured into conical flasks.

3.2.3 In-vitro Experiment

The bacteria were cultured on the nutrient agar (NA). The culture was incubated for 24 hours. The bacteria were then transferred into conical flasks to propagate it in suspension. The conical flasks were shaken in an orbital shaker at 160 rpm for 24 hours. One µL of bacterial suspensions were transferred onto the NA plates using 1000 µL micropipette. The bacteria suspension was then spread onto the entire media surface using sterile 'hockey stick'. The plates were incubated for 24 hours before treating with salicylic acid.

3.2.4 Bacterial Inhibition by Salicylic Acid

The bacteria suspension firstly was spread on petri dishes and incubated for 24 hours to allow the colony to fully cover the surface of NA plates. Ten millimeter diameter pieces of filter paper were then dipped into different concentrations of salicylic acid (SA) and placed on the incubated petri dishes. The bacteria cultures were treated with SA concentrations of 0, 10, 20, 30, 40 and 50, 60, 70, 80, 90, or 100 ppm. Each treatment had seven replicates. The petri dishes were incubated for 24 hours to observe the effect of different concentrations of salicylic acid against the growth of *R. solanacearum*. After incubation period, the clear zones surrounding the bacterial colonies indicate positive effects of SA to suppress the bacterial growth. The growth of bacteria was observed and the diameters of inhibition zones were recorded. Finally, the best two treatments were chosen to be applied onto tomato plants in the field experiment.

3.2.5 Bacterial Suspension Preparation

Bacteria cultures were grown on nutrient agar (NA) plates at 28 °C for 24 hours. Bacterial suspension was adjusted to approximately 3 x 10^7 cfu/mL (Ji *et al.*, 2005) and 50 mL of the bacterial suspension was applied later to each polybag in the pot experiment.

3.3 Field Experiment

3.3.1 Sowing Tomato Seeds

Tomato seeds were sown in trays using jiffy media for three to four weeks old (Singh *et al.*, 2004) before transplanting into polybags. The tomato seeds (sterilized seeds) were provided by Taman Pertanian Universiti (TPU).

3.3.2 Soil Preparation and Tomato Transplanting

Polybags were filled with mixture 3:2:1 ratio of soil consisting of three parts of top soil and two parts of sand to one part of chicken dung. The tomato seedling where then transplanted into the polybags. After one week, the seedlings were fertilized with NPK blue fertilizer (2 g per polybag)

3.3.3 Application of Salicylic Acid

Two concentrations of salicylic acid i.e. 10 and 40 ppm of about 100 mL were sprayed onto tomato seedlings six days after transplanting. Each treatment consisted of 5 replications and each block consisted of 15 polybags. About 50 mL of bacterial suspension was drenched into the soil six days after SA was applied. Plants were observed for wilt disease for a period of 10 weeks.

3.3.4 Symptoms and Effects

The typical bacterial wilt symptoms were observed one week after bacterial inoculation. The symptoms were observed and recorded. Length of shoots was measured periodically (at 2 week intervals). Dry mass of each plant was determined at harvesting. Dry weight of shoots and roots were determined after the drying plant materials at 80 °C for 48 hours. The total of six parameters measured including weight of shoots and roots, plant height, chlorophyll content, average fruit weight per plant and disease incidence.

Disease incidence was calculated using the formula below:

Disease Incidence (DI):

$$\% \text{ of DI} = \frac{\text{Total Tomato Plants Infected}}{\text{Total Number of Plants Assessed}} \times 100$$

(Vidhyasekaran, 2004)

3.4 Data Analysis

All the data collected in both experiments were subjected to analysis of variance (ANOVA) using SAS (version 9.1) software and means were compared using Duncan's Multiple Range Test, $P < 0.05$.

CHAPTER 4

RESULTS

4.1 In-vitro Experiment

Results of the in vitro experiment showed suppression of *Ralstonia solanacearum* by salicylic acid (Table 1).

The inhibitory effect of SA on bacteria colony development was significant at $P < 0.05$. The SA at 40 ppm concentration showed significant inhibition in bacteria colony development compared to the control. The much reduced inhibition zones observed with 50, 60, 70, 90 and 100 ppm SA is not clear. A repeat of the in vitro study may be necessary. However, 10 and 40 ppm concentrations of SA were subsequently included in the pot experiment. The inhibition zones observed with 0, 10 and 40 ppm SA are illustrated in plates 1 and 2.

Table 1: Effect of different concentrations of salicylic acid on growth of *R. solanacearum* in-vitro

Concentration of salicylic acid (ppm)	Mean bacterial inhibition zone (cm)
0	0.0000[b]
10	0.03886[b]
20	0.07686[ab]
30	0.07614[ab]
40	0.18300[a]
50	0.04857[ab]
60	0.05057[ab]
70	0.04314[ab]
80	0.10471[a]
90	0.06043[ab]
100	0.07471[ab]
s.e.	± 0.0134

Means with same superscripts are not significantly different at $P < 0.05$ (DMRT)

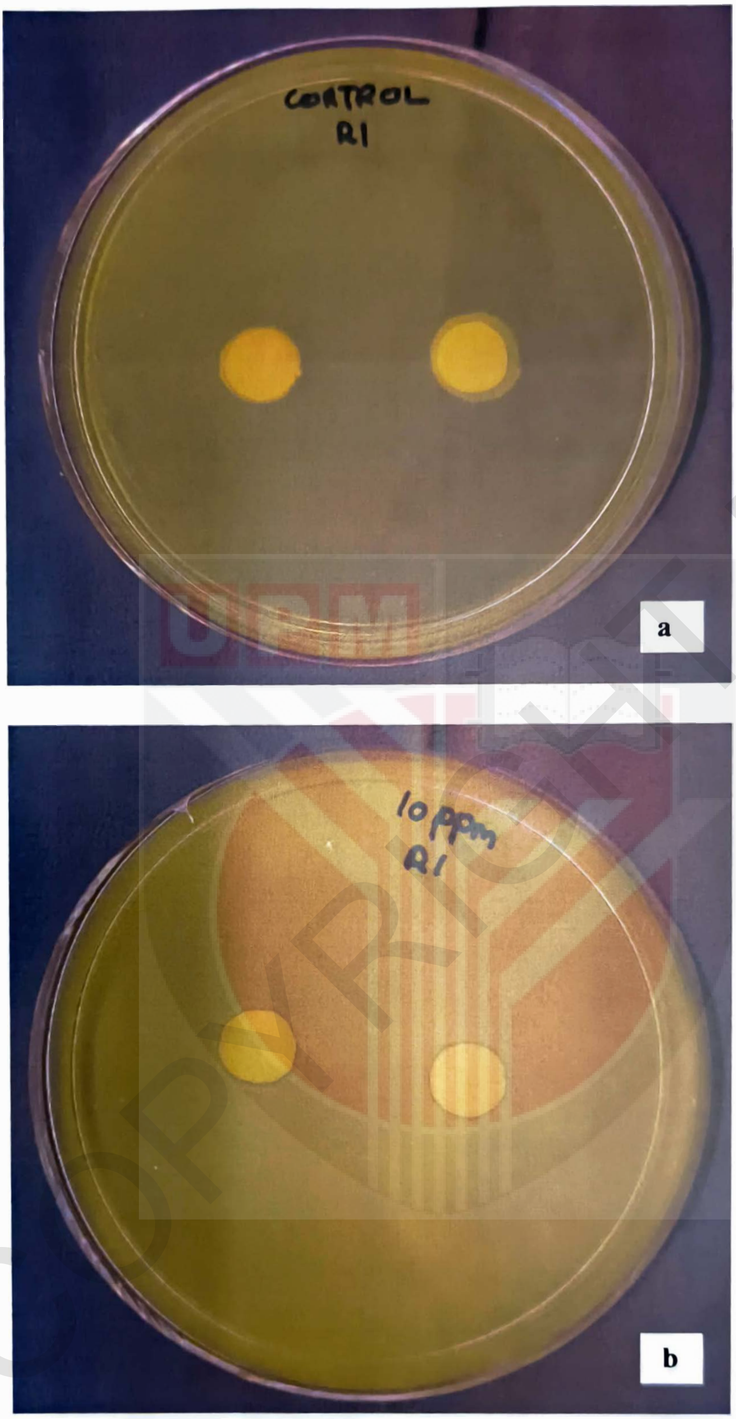

Plate 1: Inhibition of bacteria colony development treated with 10 ppm SA; (a) Control plate (0 ppm SA); (b) 10 ppm SA

Plate 2: Inhibition of bacteria colony development treated with 40 ppm SA;
(a) Control plate (0 ppm SA); (b) 40 ppm SA

4.2 Field Experiment

In the polybag experiment was conducted in netted greenhouse and data was collected for 10 weeks after incubation with *Ralstonia solanacearum*. Six parameters measured were height, fresh and dry weight, leaf chlorophyll content, average fruit weight and disease incidence.

4.2.1 Plant Height

The differences in height of tomato plants after foliar application of SA and inoculation with *R. solanacearum* were not significant at two weeks after inoculation (Table 2). All the treatments had the same height.

However, the treatments showed significant differences after 4, 6, 8 and 10 weeks of inoculation. Plant height for both SA treated plants was significantly higher compared to the infected control plants. The height of tomato plants treated with two concentration of SA showed significant increases in height, while the height of infected control plants decreased with increasing time of infection.

Table 2: Effect of salicylic acid on height of tomato plants infected with *R. solanacearum*

Treatment	Plant Height (cm)				
	Weeks after inoculation				
	2	4	6	8	10
T1 - *R. solanacearum* + distilled water (control)	18.207^{a}	28.633^{b}	24.173^{b}	13.227^{b}	6.81^{b}
T2 - *R. solanacearum* + 10 ppm SA	18.527^{a}	48.887^{a}	51.060^{a}	85.693^{a}	104.25^{a}
T3 - *R. solanacearum* + 40 ppm SA	18.327^{a}	56.267^{a}	64.720^{a}	93.640^{a}	118.88^{a}
s. e.	0.570	4.737	6.329	13.229	18.070

Note: Means within columns with same superscripts are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

4.2.2 Fresh and Dry Weight

The fresh and dry weight of tomato plants 10 weeks after being infected with bacteria showed significant differences between the SA treatments and the untreated control (Table 3).

The dry weight of tomato plants showed significant differences between the SA treatments. Treatment with 40 ppm SA showed the highest dry weight which was 90.81 g. The lowest dry weight of plants was in the infected control which was 2.27 g.

Table 3: Effect of foliar applied salicylic acid on fresh and dry weight of tomato plants after 10 weeks of inoculation with *R. solanacearum*

Treatment	Fresh Weight (g)	Dry Weight (g)
T1 - *R. solanacearum* + distilled water (control)	32.00[b]	2.27[a]
T2 - *R. solanacearum* + 10 ppm SA	312.00[a]	68.02[b]
T3 - *R. solanacearum* + 40 ppm SA	409.33[a]	90.81[c]
s. e.	69.264	13.762

Note: Means within columns with same superscripts are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

4.2.3 Chlorophyll Content

The total chlorophyll content significantly increased in leaves of tomato plants treated with SA compared to infected control plants (Table 4).

The total chlorophyll content in infected plants was decreased with increased time of infection which was 45.847, 33.33 and 22.893 at 14, 28 and 48 days, respectively. The plants were observed to be stunted in growth with strongly wilt leaves due to infection by *R. solanacearum*. The tomato plants that were treated with SA increased in chlorophyll content over the 10 weeks of infection. Treatment 3 showed the highest chlorophyll content.

Table 4: Effect of salicylic acid on total chlorophyll content in leaves of tomato plants inoculated with *R. solanacearum*

Treatment	**Total chlorophyll (SPAD readings)**		
	Days after pathogen inoculation		
	14	28	42
T1 - *R. solanacearum* + distilled water (control)	45.847^{a}	33.333^{b}	22.893^{b}
T2 - *R. solanacearum* + 10 ppm SA	36.840^{b}	38.860^{a}	41.207^{a}
T3 - *R. solanacearum* + 40 ppm SA	36.467^{b}	40.220^{a}	43.567^{a}
s. e.	1.635	1.111	3.309

Note: Means within columns with same superscripts are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

4.2.4 Fruit Weight

Total fruit weights of tomatoes were harvested after 10 weeks of inoculation period showed significant differences between the SA treated plants and the control (Table 5). There were no significant differences between the SA-treatments plants.

The infected control plants did not produce any fruits during the experimental period. Most of the plants were infected by the pathogen and with severe wilting leaves. The plants did not produce any flowers. The highest average fruit weight recorded was 28.833 g. The result was obtained is due to the high fruit number produce by the Treatment 3 of tomato plants.

Table 5: Effect of foliar applied salicylic acid on yield of tomato plants after 10 weeks inoculation with *R. solanacearum*

Treatment	Fruit Weight (g)
T1 - *R. solanacearum* + distilled water (control)	0.000[b]
T2 - *R. solanacearum* + 10 ppm SA	23.250[a]
T3 - *R. solanacearum* + 40 ppm SA	28.833[a]
s. e.	4.860

Note: Means within columns with same superscripts are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

4.2.5 Disease Incidence

All treatments were affected by foliar application of SA and inoculation with *R. solanacearum*. Table 6 shows the disease incidence in tomato plants after 10 weeks of inoculation with *R. solanacearum*. There were no significant differences in disease incidence on day 14. All tomato plants showed healthy growth with no wilting symptoms.

Disease incidence observed at day 28 and 42 showed significant differences between treatments. The highest value of disease incidence was observed in Treatment 1 which is 1.571 (100%). Infected plants with highest value of disease incidence were severely wilted and stunted in growth. However, disease incidences with the SA treatments were the same for day 28 and day 42.

Table 6: Disease incidence of bacteria wilt diseased in tomato plants treated with salicylic acid

Treatment	Days After Inoculation		
	14	28	42
T1 – *R. solanacearum* + distilled water (control)	0	100.00 (1.571[a])	100.00 (1.571[a])
T2 – *R. solanacearum* + 10 ppm SA	0	20.00 (0.201[b])	20.00 (0.201[b])
T3 – *R. solanacearum* + 40 ppm SA	0	13.33 (0.134[b])	13.33 (0.134[b])
s. e.	0	14.055	14.055

Note: Values in parenthesis are data transformed to Arc sine values prior to statistical analysis. Means with the same superscript are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

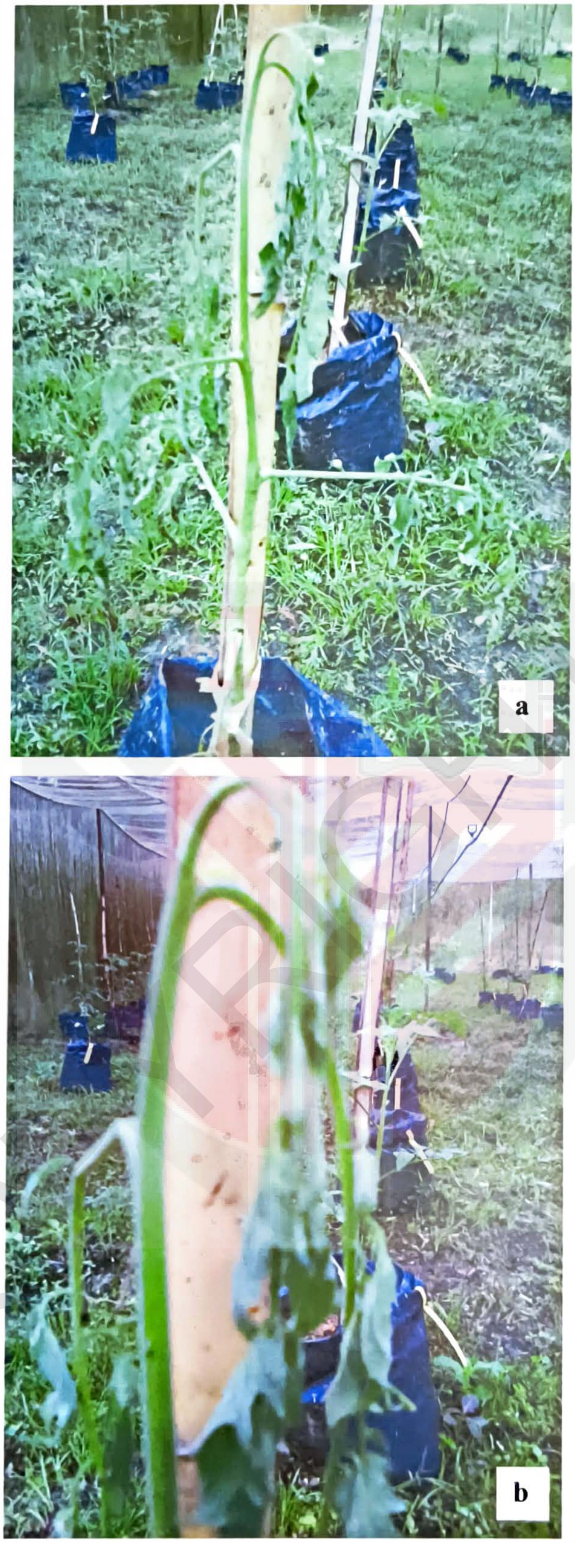

Plate 3: Wilting symptoms in tomato plants after infection by *R. solanacearum*
(a) General view; (b) Close-up

CHAPTER 5

DISCUSSION

5.1 In vitro Experiment

The inhibitory effect of salicylic acid (SA) on the development of *R. solanacearum* did not increase linearly with increasing concentration. After incubation for 24 hours, colony development of bacteria was not inhibited in the control plate. There was no inhibition zone surrounding the disc. The 40 ppm of SA showed strong inhibitory activity with highest inhibitory zone. According to Avilez *et al.* (2008), phenol compounds or phenols can act as antimicrobials.

Ralstonia solanacearum is a gram-negative bacterium which has cell walls that consists of lipopolysaccharides. This type of membrane makes the bacteria resistant to certain types of antibiotics. The growth of the bacteria colony can be suppressed with compounds applied has the potential to disrupt cell wall integrity by altering protein reaction (Campbell and Reece, 2005; Ji *et al.*, 2005) and salicylic acid is also one of the phenol elicitors and has been known to induce PR proteins and offer disease resistance in plants (Huang, 2001). The SA may potentially act as an antimicrobial that can cause the destruction of cell walls and death of microbes (Candela *et al.*, 1995; Ozgonen *et al.*, 2001).

Momol *et al.* (2000) indicated that thymol and palmarosa oil (essential oil had significant efficacy against *R. solanacearum*. Results of the present stud shov s that SA treatments can suppress the growth of the pathogenic bacteria, *R. solan icearum*, the causal agent of bacterial wilt disease in tomato.

5.2 Field Experiment

5.2.1 Effect of Salicylic Acid on Plant Height of Tomato

Tomato plants grown in *R. solanacearum* pathogenized soil sprayed with SA, markedly increased plant heights when compared to the infected control. The plant height of tomato increased over time. It was also observed that with SA treatments growth of tomato increased rapidly. The results were similar to that reported by Hegazi and El-Shraiy (2007). In their study, the exogenous application of SA affected the plant size and number of leaves and increased height of common bean plant. The similar results were observed by El-Shraiy (2004), who reported that acetyl acetic acid treatments on potato plants promoted growth in plant height and increased number of leaves per plant.

Hutchenson and Buchanan (1983) were reported that the increases in photosynthetic rate of plants not only can induce resistance but also promote the growth of plants. Rapid photosynthesis rate will promote metabolism activity and generating rapid new cell production that can contribute to increases in plant height.

5.2.2 Effect of Salicylic Acid Application on Fresh and Dry Weight of Tomato

The fresh and dry weights of tomato plants that was inoculated with *R. solanacearum* and sprayed with SA were much higher than untreated infected control plants (Table 3). This showed that exogenous application of SA reduced wilting in tomatoes caused by the pathogen (Ozgonen *et al.*, 2001). Wilting can contributed to lower fresh weight of plants. The salicylic acid potentially inhibits colonization of bacteria in the tomato plants. Salicylic acid can also promote induction of PR proteins in plants and thus inhibit growth of bacteria. Similar results was obtained by Hooft van Huijsduijnen *et al.* (1986), who reported that spraying tobacco plants with salicylic acid induced both the synthesis of PR proteins and resistance of plants to virus infection. Exogenously applied SA was first shown to induce PRs and protect against pathogen infection locally and systematically, making SA an endogenous signal for SAR.

The infected plants showed wilting symptoms with reductions in fresh and dry weight after 10 weeks of inoculation. As indicated by Agrios (2005), the fresh weight reduction of infected tomato plants was probably caused by colonization of bacteria in both primary and secondary xylem tissue. The colonies will then block the xylem and prevent water transfer to the entire plant. The plant suffers from water stress and result in severe wilting and stunted growth. The reduction in plant height of infected tomato plants was related to inhibition in chlorophyll biosynthesis. The situation can cause decreases in biomass and might result from high levels of lipid peroxidation mediated cell damage in tomato tissues (El-Khallal, 2007).

5.2.3 Effect of Salicylic Acid on Chlorophyll Content of Tomato

One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. Photosynthesis is the main physiological process important for plant growth. The present study showed that the total chlorophyll content gradually decreased in leaves of infected tomato plants with increasing time of infection. Similar results were obtained by El-Khallal (2007). It was reported that the reduction in total chlorophyll in tomato leaves as a result of Fusarium wilt disease may be a consequence of fungal infection which lead to inhibition of chlorophyll biosynthesis.

The chlorophyll content significantly increased in leaves of tomato plants treated with SA as compared to the control. It has been suggested that treatments with hormonal elicitors markedly affected the efficiency of photosynthetic apparatus in infected tomato leaves with a better potential for resistance. According to Hutchenson and Buchanan (1983), the SA stimulated pigment formation and in turn the efficiency of photosynthetic apparatus with a better potential for resistance. The photosynthesis apparatus of resistant plants can be more efficient than that of susceptible ones, and a decrease in photophosphorylation rate usually occurring after an infection can be compensated by an increase in efficiency of the photosynthetic apparatus (Chandra and Bhatt, 1998). Also, high chlorophyll content in SA-treated plants could be attributed to its stimulatory effect on rubisco activity (El-Khallal, 2007).

Khan *et al.* (2003) had indicated that the exogenous application of SA increased net photosynthetic rates for corn and soybean. Their findings suggest that the increase in photosynthetic rates following spray application of phenolic compounds were the result of increased carbon dioxide uptake activity at the chloroplast level, rather than simple increases in stomata opening. In the present study, there was no attempt to elucidate the mechanism of action.

5.2.4 Effect of Salicylic Acid on Fruit Weight of Tomato

The average fruits weight for the two SA treatments increased with increasing time of infection. The results also showed that the infected plants did not produce any fruits due to severe wilting and stunted growth caused by the bacterial wilt disease. Similar results were also obtained by Ji *et al.* (2005). They reported that the use of thymol as soil fumigant prevented the dramatic yield loss due to bacterial wilt observed in untreated tomatoes. They also reported that palmarosa oil provided significant disease reduction and increased tomato yield.

Singh and Kaur (1980) had also reported that exogenous application of SA to different crops species have been shown to elicit beneficial effects on yield of the plants. An increase in the number of pods and yield has reported in mung bean. Foliar application of SA can increase chlorophyll content and help promote the growth of tomato plants by enhancing photosynthesis activity.

5.2.5 Disease Incidence

The phenomenon of systemic resistance has attracted attention as a new strategy for controlling plant disease. The reduction was evident with the reduction in disease incidence in treated plants compared to the infected control. Percentage of disease incidence in infected tomato plants was higher as compared to SA-treated plants. Almost all control plants were infected by *R. solanacearum* as shown by wilting symptoms. Ji *et al.*, 2005 reported about 92.5% wilting in treatment without application of essential oil (thymol), indicating high inoculum pressure. Disease incidence indicated that plants treated by thymol were less susceptible to bacterial wilt.

Phenolic compounds such as SA can induce a range of defence genes for encoding pathogenesis-related (PR) proteins, several of which have been shown to posses antifungal or antibacterial properties (Bowles, 1990). SA-induced effects slow down the progress of the disease and symptoms expression. Dassi *et al.* (1998) indicated that increased PRs were a defensive response of plants to pathogen invasion. According to Naylor *et al.* (1998), SA inhibited PVX (potato virus X) RNA accumulation in SA-treated tissue. Tobacco plants that were treated with SA 3 days prior to cucumber mosaic virus (CMV) inoculation consistently showed a delay of 5 to 6 days in the onset of systemic symptoms, compared with untreated plants. SA was able to delay development of systemic disease symptoms caused by CMV. The increased concentration of phenols in plant tissue following pathogen attack is an important defence mechanism by which pathogen activity may be limited or pathogen populations are decreased (Zhu and Yao, 2004).

CHAPTER 6

CONCLUSION

Many types of phenolic compounds such as thymol, palmarosa oil and salicylic acid are being tested to be applied to crops to control pathogenic bacteria and fungi. Results of the in-vitro experiment showed that salicylic acid at 10 ppm or higher concentrations can suppress colony growth of *Ralstonia solanacearum*.

In the field experiments exogenous application of salicylic acid was shown to promote plant height, increased fresh and dry weights, increased chlorophyll content, gave higher fruit weight and reduced disease incidence in tomato plants. The result of this study showed that salicylic acid had potential for use in the control of *R. solanacearum* in tomatoes. However, dosage and methods of application in the field will need to be optimized to achieve effective and efficient disease control.

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APPENDIX A1

Plate 4: General view of tomato plants in field experiment

Plate 5: (a) and (b) Healthy tomato plants treated with salicylic acid and *R. solanacearum*

APPENDIX A2

Plate 6: Wilting symptoms in untreated control plant

Plate 7: Ripened tomato fruits from healthy plants

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Effect of Salicylic Acid on Development of Bacteria Wilt Disease (*Ralstonia solanacearum*) in Tomato" by the supervisor in joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

Norhayati binti Madiha

Date: 08/05/2009