

**DEVELOPMENT OF DISEASE SUPPRESSIVE COMPOST AND POTTING  
MIX FOR CONTROL OF BACTERIAL WILT OF TOMATO**

**MASYITAH**

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**DEVELOPMENT OF DISEASE SUPPRESSIVE COMPOST AND POTTING  
MIX FOR CONTROL OF BACTERIAL WILT OF TOMATO**

**By**

**MASYITAH**

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**DEVELOPMENT OF DISEASE SUPPRESSIVE COMPOST AND POTTING MIX FOR CONTROL OF BACTERIAL WILT OF TOMATO**

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**December 2004**

**Chairman : Associate Professor Datin Hiriyati Abdullah, PhD**

**Faculty : Agriculture**

In Malaysia, bacterial wilt caused by *Ralstonia solanacearum* is considered a major factor limiting tomato production in the lowlands. Compost usage had effectively suppressed diseases. Therefore, developing and evaluating composts for suppression of bacterial wilt of tomato were carried out. Three types of composts, made from corn stalk, rice straw and tree bark, each mixed with chicken dung, were composted using the forced aeration method. Their maturity, quality and disease suppression were evaluated in the laboratory, greenhouse and field experiments.

The phytotoxicity test showed that the corn stalk and rice straw compost extracts significantly ( $P < 0.01$ ) decreased germination, root length and germination index of tomato compared to control. However, the tree bark compost extract at both 10% and 30% concentrations did not affect germination, root length and germination index of tomato.

In plant bioassay, the corn stalk and rice straw composts significantly ( $P < 0.01$ ) decreased seed germination and fresh weight of tomato seedlings compared to the control. However, the tree bark compost up to 50% concentration did not affect seed germination and fresh weight of tomato.

An experiment on suppression of *Pythium* damping off disease showed that the tree bark compost gave the highest disease suppression of 80.58% and 71.96% in tomato and *Brassica* sp, respectively. Greenhouse trial on suppression of bacterial wilt of tomato caused by *R. solanacearum*, showed that all composts significantly reduced the disease but treatment with the tree bark compost recorded significantly lowest disease severity index (1.2) and disease incidence (40%).

Evaluation of potting mixes showed that the potting mix with composition of peat:sand: tree bark compost in the ratio of (5:3:2) gave the best soil physical properties and plant growth. In the greenhouse experiment, the incorporation of tree bark compost in potting mixes of peat:sand:compost in ratio of 5:3:2 added with antagonist Pa II (*Pseudomonas aeruginosa*) and Kts 26 (*Pseudomonas putida*) individually, and Kt8 (*Pseudomonas aeruginosa*) + B333 (*Pseudomonas aeruginosa*) in combination antagonists significantly gave lower disease severity index and disease incidence of bacterial wilt of tomato compared to other antagonists tested. In the field trial using the three antagonists showed that the tree bark compost potting mix added with Kt8 + B333 in combination gave 57% reduction of bacterial wilt disease of tomato which was better compared to Pa II (50%) and Kts 26 (50%) individually.

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

**PEMBENTUKAN KOMPOS DAN CAMPURAN PASU PENINDAS PENYAKIT  
UNTUK KAWALAN PENYAKIT LAYU BAKTERIA TOMATO**

**Oleh**

**MASYITAH**

**Disember 2004**

**Pengurus** : **Profesor Madya Datin Hiriyati Abdullah, PhD**

**Fakulti** : **Pertanian**

Di Malaysia, penyakit layu bakteria yang disebabkan oleh *Ralstonia solanacearum* adalah satu faktor utama yang menghadkan produksi pada tanah rendah. Penggunaan kompos telah menindas penyakit secara efektif. Oleh itu pembentukan dan penilaian kompos untuk menindas penyakit layu bakteria dilakukan. Tiga jenis kompos yang dibuat dari batang jagung, jerami padi dan kulit kayu, masing-masing dicampur dengan tahi ayam dikompos menggunakan kaedah pengudaraan secara paksaan. Kematangan, kualiti dan penindas penyakit dinilai dalam kajian di makmal, rumah hijau, dan lapangan.

Uji kaji menunjukkan bahawa ekstrak kompos batang jagung dan jerami padi mengurangkan secara signifikan ( $P < 0.01$ ) percambahan, panjang akar dan indeks percambahan dibandingkan dengan kawalan. Bagaimanapun kesan dari ekstrak kompos

kulit kayu adalah berbeza di mana pada konsentrasi 10% and 30% tidak mempengaruhi percambahan, panjang akar, dan indeks percambahan tomato.

Pada bioasai pokok, kompos batang jagung dan jerami padi mengurangkan secara signifikan ( $P < 0.01$ ) peratus percambahan dan berat segar tomato dibandingkan dengan kawalan. Walaupun demikian kompos kulit kayu sehingga 50% tidak mempengaruhi peratus percambahan biji benih dan berat segar tomato.

Satu kajian tentang penindasan penyakit lecah *Pythium* menunjukkan bahawa kompos kulit kayu memberikan penindasan penyakit tomato yang tertinggi iaitu 80.58% dan 71.96% pada masing-masing sawi dan tomato. Kajian rumah hijau tentang penindasan penyakit layu bakteria tomato yang disebabkan oleh *R. solanacearum* menunjukkan bahawa seluruh kompos mengurangkan secara signifikan ( $P < 0.01$ ) penyakit tetapi rawatan dengan kompos kulit kayu mencatatkan indeks keterukan penyakit dan kejadian penyakit paling rendah.

Penilaian campuran pasu menunjukkan bahawa campuran pasu dengan komposisi gambut:pasir:kompos kulit kayu pada nisbah 5:3:2 memberikan sifat fizikal campuran pasu dan pertumbuhan pokok yang paling baik. Pada percubaan rumah hijau, pencampuran kompos kulit kayu dalam campuran pasu gambut:pasir:kompos kulit kayu pada nisbah 5:3:2 yang ditambah dengan antagonis Pa II (*Pseudomonas aeruginosa*) dan Kts 26 (*Pseudomonas putida*) secara berasingan, dan Kt8 (*Pseudomonas aeruginosa*) + B333 (*Pseudomonas aeruginosa*) secara kombinasi memberikan kesan signifikan indeks

keterukan penyakit dan kejadian penyakit layu bakteri tomat yang lebih rendah dibandingkan antagonis lain yang dikaji. Pada percobaan ladang menggunakan ketiga-tiga rawatan antagonis diatas, menunjukkan campuran pasu kompos kulit kayu yang ditambah dengan antagonis dalam kombinasi Kt8 + B333 memberikan pengurangan 57% penyakit layu bakteri tomat yang lebih baik dari Pa II (50%) dan Kts 26 (50%) secara berasingan.

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I certify that an Examination Committee met on 15<sup>th</sup> December 2004 to conduct the final examination of Masyitah on her Master of Science thesis entitled “Development of Disease Suppressive Compost and Potting Mix for Control of Bacterial Wilt of Tomato” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Kamaruzzaman Sijam, PhD**

**Associate Professor**

Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Inon Sulaiman, PhD**

Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Radziah Othman, PhD**

**Associate Professor**

Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Mohamad Omar, PhD**

**Professor**

Monash University  
(Independent Examiner)

---

**ZAKARIAH ABD. RASHID, Ph.D.**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for degree of Master of Science. The members of the Supervisory Committee are as follows:

**DATIN HIRYATI ABDULLAH, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**CHE FAUZIAH ISHAK, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**MOHD. RAZI ISMAIL, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## **DECLARATION**

**I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.**

**MASYITAH**

Date:

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## LIST OF ABBREVIATION

AAS	: Atomic Absorption Spectrophotometer
BIRC	: Bio Integral Resource Centre
BWD	: Bacterial wilt disease
CEC	: Cation Exchange Capacity
CHB	: Composted Hardwood Bark
CPG	: Casaminoacid Potato Glucose
CS	: Corn Stalk
DI	: Disease Incidence
DSI	: Disease Severity Index
EC	: Electrical Conductivity
FAO	: Food and Agriculture Organization
FAMA	: Federal Agricultural Marketing Authority
LOI	: Loss On Ignition
MARDI	: Malaysia Agricultural Research and Development Institute
MSW	: Municipal Sewage Waste
MT	: Malaysia Tomato
PGPR	: Plant Growth Promoting Rhizobacteria
RS	: Rice Straw
TB	: Tree Bark
TZC	: Tetrazolium Chloride
U.S.EPA	: United State Environmental Protection Agency
WAT	: Week After Transplanting



## CHAPTER I

### INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely distributed vegetables grown in various cropping systems and locations throughout the world. In Malaysia, the planting hectareage and production of tomatoes has rapidly increased during 1999 to 2004 (FAO, 2004). Tomato is a key food crop for low-income farmers and is also a valuable cash crop in the tropics and subtropics. Bacterial wilt disease caused by *Ralstonia solanacearum* E.F. Smith (Syn. *Pseudomonas solanacearum*) is a major limitation to production of tomatoes. Heavy losses frequently occur, with additional losses occurring through restrictions to further production in infested areas (Prior *et al.*, 1994).

In Malaysia, bacterial wilt is a major disease, especially in the lowlands. It was first reported on potato and tomato in 1910 (Bancroft, 1910). The bacterium had been reported on 43 hosts distributed over a wide range of families including crop plants, ornamentals and weeds (Abdullah, 1992). The most common hosts being tomato, potato, eggplant, ginger and groundnut.

Control measures that are commonly employed to control bacterial wilt includes resistant or tolerant varieties or cultivars, cultural practices, biological, and chemical control. The use of compost had been reported for controlling some soil-borne

pathogens. Hoitink *et al.* (1991) indicated that composts were as effective as fungicides for the control of root rots. Diseases that have been shown to be effectively suppressed by compost includes those caused by *Fusarium*, *Pythium*, *Phytophthora* and *Rhizoctonia solani* (Hoitink *et al.*, 2001).

Use of compost is particularly valuable as a way to utilize what would often be considered waste products. While compost generally does not contain toxic or potentially harmful substances, it is critical that compost made from sewage sludge and related municipal or animal waste must undergo testing on a regular basis and it is carefully monitored to ensure that it will not pose any risk to human health or environmental quality. However, in general disease suppressive compost does not require any special care during use and handling (Segall, 1995; U.S. EPA, 1997).

Besides the suppression of the spread of disease at nurseries and field, disease suppressive compost also provide nutrients and organic matter, thereby eliminating or reducing the need for fertilizer or the use of expensive peat mixes. Compost can also improve soil structure which allows for better water transmission, thereby decreasing the potential for disease development.

In a composting process there are several factors which act against the survival of pathogenic organisms. These are: the high temperature reached; the time that the process takes; the release of ammonia during the process which has a direct effect on some organism and alters the waste from acid to alkaline conditions; the intense

competition for food, particularly during the cooling down stage; and competition and antibiosis between the microorganisms at the start of the maturing stage (Dalzell *et al.*, 1987).

Two classes of biological control mechanisms known as general and specific suppression have been described for compost-amended substrates. The mechanisms involved are based on competition, antibiosis, hyperparasitism, and the induction of systemic acquired resistance in the host plant (Hoitink *et al.*, 2001). Compost may alter resistance of plants to diseases. One of the beneficial properties of compost is the microbially induced suppression of soilborne plant pathogens and disease (Hoitink *et al.*, 1987). Chen *et al.* (1987) reported that suppressive compost possess a higher microbial activity than conducive ones. They suggested that a high microbial activity causes a depletion in essential nutrients for the survival and multiplication of the pathogen, thus preventing infection of the host. The beneficial microbes in compost and other decomposing organic matter can activate certain disease resistance systems in plants. When a pathogen infects a plant, the plant mobilizes certain biochemical defenses, but these are often too late to avoid the disease. Plants grown in compost appear to have these systems already running and this prevents the pathogen from causing disease. This mechanism, called systemic acquired resistance, is somewhat pathogen specific, but it opens the door for enhancing disease control through common farming practices (Hoitink *et al.*, 1993; Baker and Paulitz, 1996).

Disease suppressive potting mixes can be developed by: incorporating suppressive organic amendment such as certain types of peat moss and good quality composts, inoculating composts and or potting media with biocontrol microorganisms such as *Trichoderma*, *Gliocladium*, *Bacillus* and *Pseudomonas* or inoculating potting media with plant health promoting microorganism such as mycorrhizae (Boehm and Hoitink, 1992).

Based on the above, there is a dearth of information on suppression of bacterial wilt caused by *R. solanacearum* by the use of compost. This study was therefore conducted to develop disease suppressive compost and potting mixes for bacterial wilt control caused by *R. solanacearum* in tomato. Therefore this study was conducted with the following specific objectives:

1. To develop disease suppressive compost.
2. To develop disease suppressive potting mixes.
3. To evaluate the effectiveness of potting mixes for controlling bacterial wilt of tomato in the greenhouse and field conditions

## CHAPTER II

### LITERATURE REVIEW

#### Tomato Plant

Tomato or *Lycopersicon esculentum* Mill, is grown for its edible fruit. The genus *Lycopersicon* of the family *Solanaceae* is believed to have originated in the coastal strip of western South America, from the equator to about 30° C latitude south (Taylor, 1986; Papadopoulos, 1991). Tomatoes are freshly consumed, or processed and canned in the form of puree, paste, juice or peeled tomatoes. Tomato is a healthy food, low in fat and calories, and is cholesterol free. It is a good source of fiber and protein. In addition, tomato is rich in vitamin A and C, beta carotene, potassium, as well as the antioxidant lycopene (Gerster, 1997).

The demand for tomatoes has increased substantially worldwide. Tomato fruit for the fresh market and processing are produced worldwide on approximately 4.4 million hectares and yield of 115,951 metric ton. In Malaysia, the production of tomato was reported as 32,362 and 34,188 metric ton in 2003 and 2004, respectively (FAO, 2004).

Tomato plants grow under a wide range of environmental conditions, but for the optimum production they require plenty of sunshine, moderately cool night temperatures, warm days and well-drained soil. They also require plenty of water but

not excessive because tomato roots will not function under anaerobic conditions. When the moisture level surrounding the roots is too high, epinasty, poor growth, later flowering, fewer flowers and lower fruit set will occur (Peet and Willits, 1995).

## **Bacterial Wilt Disease**

### **The Disease**

One of the most devastating plant diseases in the tropics is bacterial wilt, caused by the bacterium *Ralstonia solanacearum*. In susceptible host plants this pathogen disrupts water transport, alter physiology and induces a severe, usually fatal wilt. One plant species that is seriously affected by bacterial wilt is tomato, *Lycopersicon esculentum* Mill., and efforts to grow tomatoes widely in the tropics have generally been hampered by this disease (Hayward, 1991).

Bacterial wilt disease caused by *R. solanacearum* has been recognized as a major bacterial disease. It is considered as one of the most important, widespread and lethal bacterial diseases of plants in the world (Buddenhagen and Kelman, 1964). Bacterial wilt is predominantly a disease of tropical, subtropical and warm temperate regions. The pathogen has a very wide host range. The very extensive host range includes several hundred species of plants representing 44 families (He *et al.*, 1983). Among the common hosts are chili, eggplant, irish potato, ginger, groundnut, tomato and tobacco.

The ornamental hosts include *Heliconia sp.*, *Helianthus annuus L.* and *Petunia sp.* (Sequeira and Averre, 1961; Buddenhagen and Kelman, 1964).

The disease was first reported in Peninsula Malaysia in 1910 on potato and tomato (Bancroft, 1910). In Malaysia, more than 35 families of plants are affected by the disease, and the major economic host include potato, tomato, eggplant, chili, ginger and groundnut (Abdullah, 1982; Hamidah and Lum, 1992). The disease can be found throughout the year. The bacterium also infects weed hosts. Weed hosts that had been reported in Malaysia, include *Croton hirtus*, *Hyptis capitata*, *Euphorbia prunifolia*, *Euphorbia hirta*, *Ageratum conyzoides* and *Synedrella nodiflora* (Abdullah, 1980, 1983; Abdullah and Sabudin, 1986). The disease occurred on plants grown in many types of mineral soils (clay, loam and sand) and organic soils i.e. muck and peat (Abdullah, 1992).

In India, the disease caused losses of 80% in brinjal (Rao, 1976) and 90% in tomato (Kishun, 1985). Ramesh and Ansari (1989) reported that the disease became the major constraint in the tomato production in Andaman and Nicobar island of the Northeastern Indian Ocean. The reduction of yield could be as high as 82 to 90% at certain times of the year. The disease caused 29% loss of fresh fruit production in hybrid tomatoes in Taiwan (Hartman *et al.*, 1991).

## **The Pathogen – *Ralstonia solanacearum***

*R. solanacearum* is the causal agent of bacterial wilt which is a devastating soil borne pathogen. It is classified in the family of *Pseudomonadaceae*. It has been characterized in Bergey's Manual of Determinative Bacteriology (Sneath *et al.*, 1986) as a Gram-negative, rod shape and non-spore forming bacterium. Its average size is (0.5 – 0.7) x (1.5 – 2.5)  $\mu\text{m}$ . It is motile with one to four polar flagella for the avirulent form while the virulent forms are mainly non-flagellated and non-motile (Hayward, 1976). It is an aerobic obligate organism and the optimum growth temperature is 25 to 35 °C (Kelman, 1953).

*R. solanacearum* has a strictly respiratory type of metabolism, with oxygen as the terminal electron acceptor and fluorescent pigment is not produced. Less virulent or avirulent mutants readily appear in subcultures on artificial media while the pathogenic wild strains form fluidal opaque colonies on agar plate and cells are non-flagellated, whereas attenuated mutant strains form butyrous transparent colonies and cells are flagellated (Goto, 1992).

***Kelman (1954) first discovered 43 years ago that *R. solanacearum* produced two different kinds of colonies on complex media; one kind is smooth, fluid and elevated, which was called wild type; the other is somewhat rough, dry and flat which was designated as a mutated or avirulent type. When a wild type strain undergoes a change***