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Research Paper

Efficacy of Smart Fertilizer for Combating Bacterial Wilt Disease in *Solanum Lycopersicum*

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Tomato plants are susceptible to bacterial wilting, which causes production losses varying from 10 to 100%. In Malaysia, there are more than 35 families of plants are affected by this disease, and the major economic host including potato, tomato, eggplant, chili, ginger and groundnut. Although there was no published data on the exact losses in Malaysia caused by *Ralstonia solanacearum*, however, this disease caused tomato yield losses up to 70 % of farmers' income. This work had the objective of evaluating "smart fertilizer" for controlling and/or preventing bacterial wilt disease and to study the overall growth of tomato plants in control environment. The plants height was increased from week-4 to week-12 with the average of 104.92 cm in treatment T_1 (formulated phage fertilizer); 93 cm in treatment T_2 (fertilizer alone) and only 43.9 cm in treatment T_3 (common commercial fertilizer). The growth performance of tomato plants was dwarfed in T_3. There are similar results in stem observation, fresh weight, dry weight and total fruits produced. The total fruits yield in T_3 was lesser than one third in both T_1 and T_2 due to a very high mortality rate in T_3 by wilting especially at the fruiting stage of the plants. Finally, we concluded that the used of smart fertilizer can be used as an alternative for the bacterial wilt control.

Key words: *Ralstonia solanacearum*, smart fertilizer, bacterial wilt, *Solanum lycopersicum*, bacteriophage.

INTRODUCTION

The world's population is increasing every year. In order to meet the demands of an ever expanding human population, global crop production needs to double by 2050; however, current estimates are far below what is needed (Ray *et al.*, 2013). Plant diseases, insects, and weeds decrease the production of crops worldwide by 36%, and diseases alone have been shown to reduce crop yields by 14% (Agrios, 2005). Thus, the control of plant diseases contributes to increased crop production. Among plant diseases, soil-borne diseases are considered to be more limiting than seed-borne or air-borne diseases in the production of many crops and account for 10–20% of yield losses annually (USDA, 2003). Tomato (*Solanum lycopersicum*) is one of the most cultivated crops in Brazil, and is produced in all States. However, it is frequently affected by several diseases, the bacterial diseases being responsible for large yield losses (Peixoto, 1997). Among these,the bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*(1995) is the most important, being able to cause crop losses from 10 to 100 % (Lopes and Santos,

1994), under environmental conditions of high temperature and high soil humidity (Hayward, 1991; Lopes and Santos, 1994). In the other country, this disease incidences have been reported in fresh market in Taiwan, causing losses exceeded 12 million U.S. dollars annually (Hartman *et al*., 1991).

Ralstonia solanacearum is the one of top ten bacterial species have been listed based on their scientific and economic importance in plant diseases (Mansfied *et al.,* 2012). It has a worldwide distribution, high genetic variability and with a wide host range within more than 33 botanical families (Kurozawa and Pavan, 2005). *R. solanacearum* (Smith) Yabuuchi et al. (1995) causes a vascular wilt disease and has been ranked as the second most important bacterial pathogen. It is one of the most destructive pathogens identified to date because it induces rapid and fatal wilting symptoms in host plants. *R. solanacearum* can survive in water, soil, and among the roots of non-susceptible plant hosts (Hayward, 1991). Tomatoes cultivation in lowlands has been limited by the wide spread incidence of bacteria wilt, caused by *R. solanacearum* (Yabuuchi *et al*., 1995). The infection takes place through the roots and exhibits strong tissue-specific tropism within the host, specifically invading and extensively multiplying in the xylem vessels (Hayward, 1991). The difficulties are associated with controlling this pathogen due to its abilities to grow endophytically, survive in soil, especially in the deeper layers, travel along water, and its relationship with weeds (Wang and Lin, 2005). It can survives for many years in association with alternate host (Yamada *et al*., 2007). In the susceptible host plants, this pathogen disrupts water transport, alters physiology and induces a severe wilting symptom (Hayward, 1991). Control of bacterial wilt is very difficult, due to the wide distribution of host plants, high genetic variability and its survival in soil for long periods (Hayward, 1991). The major control methods are preventive measures or cultural methods which aim at avoiding or delaying of pathogen spread in production areas (Silveira *et al.,* 1996).

In Malaysia, more than 35 families of plants are affected by this disease, and the major economic host includes potato, tomato, eggplant, chili, ginger and groundnut (Hamidah and Lum, 1992; Hayward, 2000). Direct yield losses by *R. solanacearum* vary widely according to the host, cultivar, climate, soil type, cropping pattern, and strain. For example, yield losses vary from 0 to 91% in the tomato, 33 to 90% in the potato, 10 to 30% in tobacco, 80 to 100% in the banana, and up to 20% in the groundnut (Elphinstone, 2005). This disease was first reported in Peninsular Malaysia in 1910 on potato and tomato (Abdullah, 1992; Masyitah, 2004). The interest in biological control has increased due to concerns over the general use of chemicals (Whipps, 2001). The mechanisms employed by biological control agents (BCAs) are sustained by various interactions such as competition for nutrients and space, antibiosis, parasitism, and induced systemic resistance (Cook and Baker, 1983; Agrios, 2005). The possible suppression mechanisms of these species are competition, induced systemic resistance, antibiosis, and the production of enzymes that degrade the cell wall and siderophores. Hyakumachi *et al.* (2013) recently revealed that *B. thuringiensis*, a famous bioinsecticide-producing bacterium, induced defense-related genes, such as PR-1, acidic chitinase, and beta-1,3-glucanase and showed resistance against a direct inoculation with *R. solanacearum*. The expression of several salicylic acid-responsive defense-related genes was con-firmed to be specifically induced (Takahashi *et al.,* 2014), and also that suppression by *B. thuringiensis* may differ from the induced systemic resistance (ISR) elicited by many plant growth-promoting rhizobacteria (PGPR), in which jasmonic acid and ethylene-dependent signaling pathways mediate plant resistance to pathogens (Hyakumachi *et al.,* 2013). From the previous study (Tan *et al*., 2010), there were five local phage isolates which caused inhibition against *R. solanacearum* in agar diffusion method. The pathogenic activity of *R. solanacearum* also reduced after infected by phages and at the same time increase the resistance response of tomato plant against the bacterial wilt disease. Glasshouse studied also showed significant different between treated and non-treated with phage cocktails (Tan *et al*., 2009; 2010).

Organic amendments (is the one of the BCAs) to soil have direct impacts on plant health and crop productivity. They are advantageous because they improve the physical, chemical, and biological properties of soil, which can have positive effects on plant growth (Bailey and Lazarovits, 2003). The degradation of organic matter in soil can directly affect the viability and survival of a pathogen by restricting available nutrients and releasing natural chemical substances with varying inhibitory properties (Bailey and Lazarovits, 2003). Carbon released during the degradation of organic matter contributes to increasing soil microbial activity and thereby enhances the likelihood of competition effects in the soil (Bailey and Lazarovits, 2003). Organic amendments to soil have been shown to stimulate the activities of microorganisms that are antagonistic to pathogens (Akhtar and Malik, 2000). In addition, organic amendments often contain biologically-active molecules such as vitamins, growth regulators, and toxins, which can affect soil microorganisms.

Youssef and Tartoura (2013), recently reported that plant resistance against the bacterial wilt pathogen was enhanced through the augmented activities of ascorbate peroxidase, mono dehydro-ascorbate reductase, dehydroascorbate reductase, and glutathione reductase following the application of compost. The smart fertilizer will be formulated with the components of nitrogen, phosphorus and potassium, beneficial microbes from the group of *Lactobacillus*, *Bacillus*, certain yeasts, bacteriophages and slow release cofactors with certain

formulation to maintain the consistent supply of nutrients to the plants. Thus, this study will focus on the effectives of newly smart fertilizer on tomato application under glasshouse condition with certain parameters to be determined.

MATERIALS AND METHODS

Experimental site and materials

Pot experiments were conducted in a glasshouse at Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (latitude 3.0077, longitude 101.7026, altitude 16.6 m.a.s.l.) with the temperature: 33°C and 83% RH, relative humidity). The experimental crop was tomato (*Lycopersicon esculentum* Hybrid F1).

Glasshouse experiment

Experiment was carried out to evaluate the efficacy of smart fertilizer against bacterial wilt in tomato grown in a glasshouse under real cultivating conditions. The experimental design was using completely randomized design (CRD) with three treatments and five replications (10 plants/treatment/replicate). There are three different treatments as follow:

Treatment 1 (T_1): ACI Fertilizer (15:15:15) plus phages cocktail (6-9 Log PFU/ml)
Treatment 2 (T_2): ACI Fertilizer (15:15:15) without phages cocktail
Treatment 3 (T_3): Commercial fertilizer NPK as control.

The data recorded for this study including data on soil analysis before the start of experiment such as pH, cation-exchange capacity (CEC), total N, total P, soluble P, and exchangeable K, Ca and Mg. Plant height were measured at 4, 8 and 12 weeks after transplanting and stem diameter were determined at 8 and 12 weeks after transplanting. Fruit weight per plant also quantified for yield assessment. Disease scoring (wilting symptom) before incorporated-phages fertilizer application and after application was also done. The statistical analysis software used in this study was the RStudio Version 0.98.1103. The test used was Kruskal-Wallis Test.

RESULTS AND DISCUSSION

Before the experiment had been carried out, the soil analysis needs to be done to determine the fertility status of the soil. Soil pH was 4.8 and organic carbon content was 20.83%. The contents of total nitrogen (N), phosphorus (P) and potassium (K) were 1.300 g/kg, 0.586 g/kg and 0.265 g/kg respectively. The essential mineral elements are absorbed through the roots in either their cationic or anionic form from the solution in rooting media. These elements are grouped into two categories, the major elements [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S)] and the micronutrients [boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn)]. The result for a few major elements is as shown in (Table 1).

Table 1. Some chemical properties of cultivated soil.

Parameter	Unit
CEC	5.984 meq/100 gm
Ca	0.361 meq/100 gm
Mg	0.248 meq/100 gm
Soluble P	9.46 ppm
pH	4.8
Organic C content	20.83%
Total N	1.300 g/kg
Total P	0.586 g/kg
K	0.265 g/kg

Tomato is moderately tolerant to a wide range of pH, but grows well in soils with a pH of 5.5 - 6.8 (DOA, 2000). Liming was applied according to the calculated amount of lime requirement to bring the pH to optimum range. The other three major elements requirements were fulfilled with the addition of fertilizers from different formulations as recommended by Department of Agriculture Malaysia (DOA, 2000).

The average plant height and girth for T_1, T_2 and T_3 were as presented in (Figures 1 and 2). The plants in treatment T_1 showed the best growth compared to plants in treatment T_3. These two parameters correlated with the disease incidence as it was known that *R. solanacearum* disrupted water transport (xylem tissue), altered the physiology and induced severe wilting symptom. Preventive methods are essential for maintaining fields that are free of bacterial wilt. Since *R. solanacearum* is a soil-borne bacterium, they can survive for prolonged periods in soil, water, and plant materials (López and Biosca, 2005). Plant diseases caused by soil-borne pathogens such as *R. solanacearum* result from the multiple and complex interactions, including both biotic and abiotic factors, they have with plants. Abiotic factors such as nutrient (organic matter and minerals) conditions, soil type, pH, anaerobic conditions, temperature, and moisture content influence the development of *R. solanacearumin* soil (Yuliar *et al*., 2015). Biotic factors are related to microorganisms, flora, fauna in the soil, and plants that can affect *R. solanacearum*. Previous studies investigated the biotic factors controlling *R. solanacearum* such as the microbial community in soil, introduction of

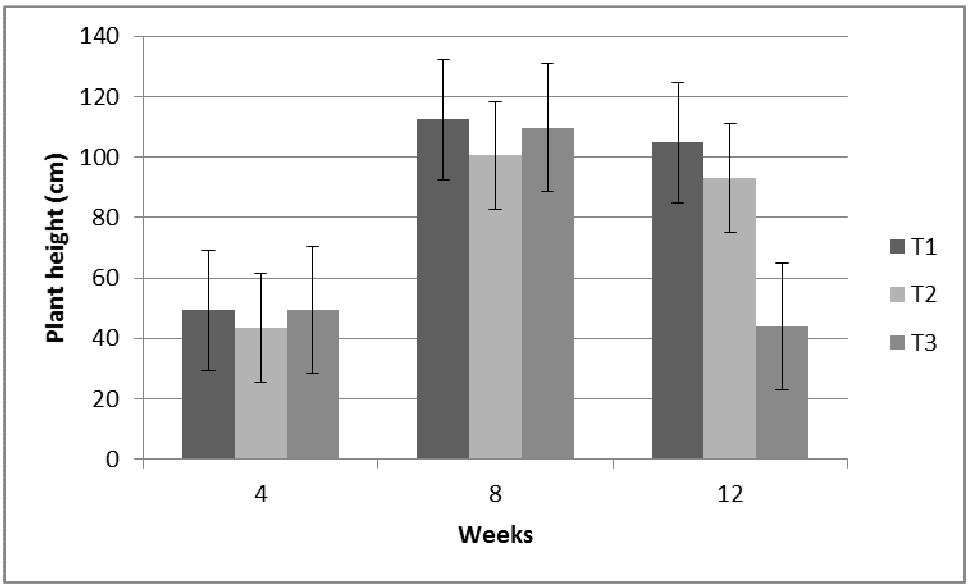

Figure 1. The height of tomato plants at week 4, week 8 and week 12 under different treatments. The error bars represent the standard deviation from the arithmetic mean.

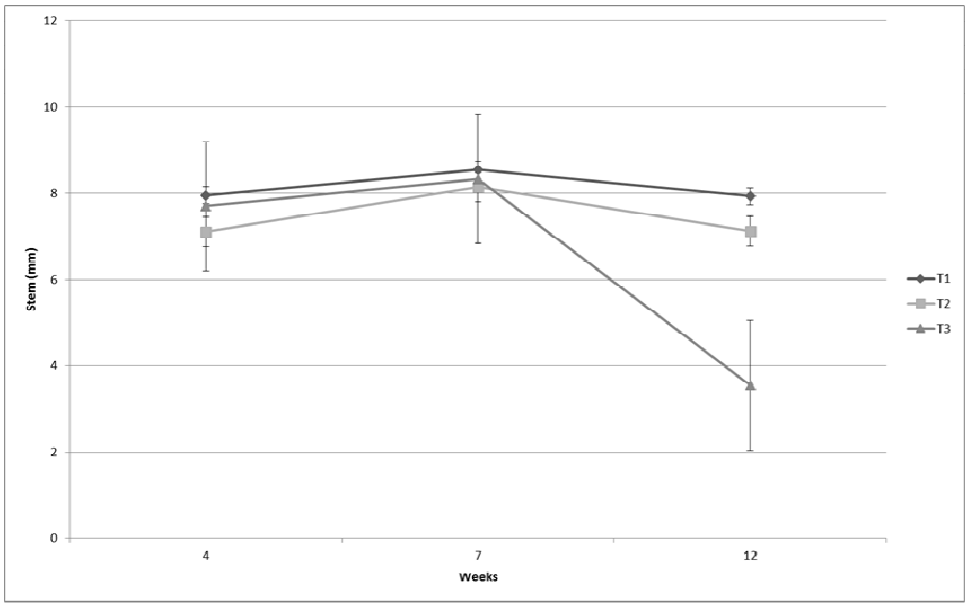

Figure 2. The stem diameter (girth) of tomato plants at week 4, week 7 and week 12. The error bars representing the standard deviation from the arithmetic mean.

BCAs, cultivar resistance, and rotation, as described above. Various suppression mechanisms are considered to be biotic factors for the pathogen, such as enhanced microbial activity, which can suppress *R. solanacearum*, the release of antibiotics, enhanced competition, decreased in colonization, the induction of systemic resistance, and protection against or avoidance of pathogen contact with the host crop (Yuliar *et al.,* 2015). Several virulence factors such as extracellular polysaccharide (EPS) (Denny and Baek, 1991), type IV pili (Kang *et al*., 2002), flagella (Tans-Kersten *et al*., 2001), and cell-wall degrading polygalacturonases and cellulases (González and Allen, 2003) have been identified by loss of function analyses or by correlation to other plant pathosystems. It is likely that the 5.8 Mb genome of *R. solanacearum* (Salanoubat *et al*., 2002) includes many genes that confer adaptation to the host and enhance the ability to cause disease.

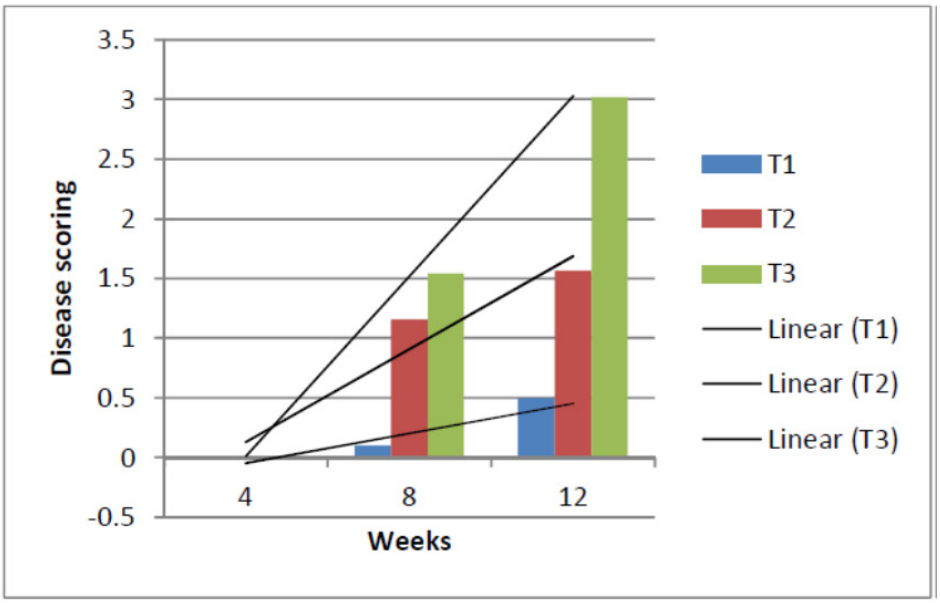

Figure 3. Disease scoring of tomato plants at week 4, week 8 and week 12.

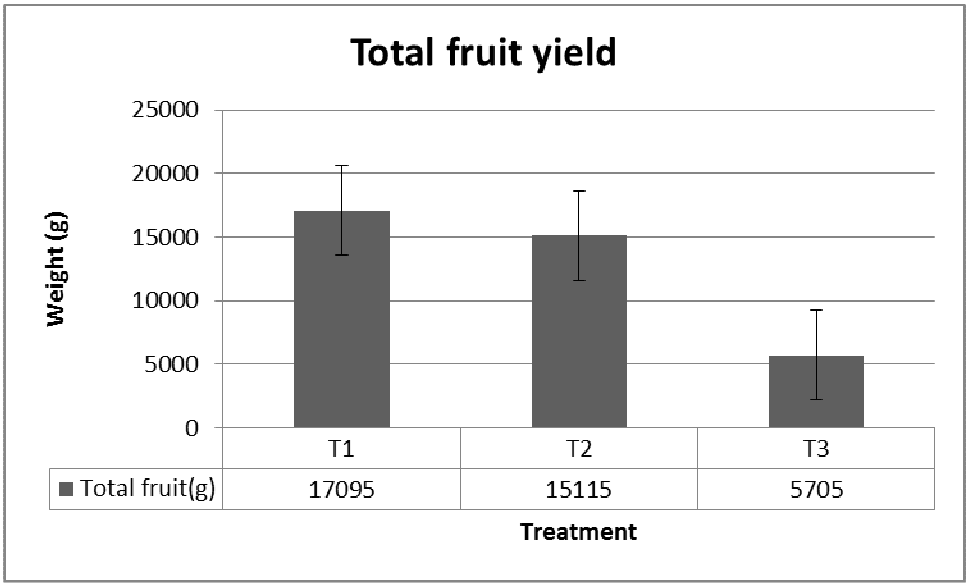

Figure 4. Total fruit yield for different treatments. The error bars represent the standard deviation from the arithmetic mean.

Moreover, many of these genes have a selectable phenotype which they are specifically induced in the host. These can be the main factor in pathogenecity of *R. solanacearum* inducing severe wilting symptom.

At the first four weeks, none of the plants showed wilting symptom (Figure 3). This is because *R. solanacearum* requires some time to infect and multiply within the host (only after eight weeks). The disease develops progressively, especially in T_3 (control). The disease incidence in T_1 was the lowest recorded as anticipated due to the presence of specific phages which possible to control the population of *R. solanacearum* in soil, preventing it from wilting. These phages, when presence in sufficient working quantity (above 10^6 pfu/gm of soil) will attack *R. solanacearum* as their host and multiply inside them. The lytic properties of these phages caused mass death of *R. solanacearum*, thus significantly reduced their number (Kurtboke, 2012).

The disease scoring in T_2 was lower than T_3 due to the presence of other beneficial microbes, such as *Bacillus*

Sp., *Lactobacillus sp.*, and certain yeasts in the bio-chemical fertilizer. Besides, some beneficial microbes such as mycorrhizae which live symbiotically within or outside the tomato roots may also protecting it from being penetrated with *R. solanacearum*. Three endomycorrhizal fungi (*Gigaspora margarita*, *Glomus mosseae*, and *Scutellospora* sp.) (Tahat *et al*., 2012) have been identified as biological control agents against *R. solanacearum*.

In addition, T_2 fertilizer contains certain organic matters which can directly affect the viability and survival of pathogen by restricting available nutrients and releasing natural chemical substances with varying inhibitory properties (Bailey and Lazarovits, 2003).

As known that carbon released during the degradation of organic matter contributes to increasing soil microbial activity and thereby enhances the likelihood of competition effects in the soil (Bailey and Lazarovits, 2003).

Organic amendments to soil have been shown to stimulate the activities of microorganism that are antagonistic to pathogens (Akhtar and Malik, 2000). The total fruit yield for T_3 was lesser than one third of both T_1 and T_2 (Figure 4).

This is due to very high mortality in T_3 by wilting especially at the fruiting stage of the plant. This can be translated to over 66% of losses, much higher than reported in Taiwan, with 29% loss of fresh fruit production in hybrid tomatoes (Hartman *et al*., 1991). This loss occurs as a result of extracellular polysaccharide (EPS) produced by *R. solanacearum* directly causes wilting by physically blocking water flow in the densely-colonized xylem vessels of infected hosts that disrupt the water transport system (Genin and Boucher, 2002).

It is well known that water is vital to the plant and its deficiency led to retardation as well as low productivity and yields.

Conclusion

This study showed that smart fertilizer can provide the nutrition requirements for tomato growth and also in overcoming the bacterial wilt disease of the crop. Hence, further study need to be carried out, especially on lengthening the shelf life of the fertilizer, since it involves microbes and chemicals attached each other.

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AUTHORS` DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the Journal.

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