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Application of Bio P60 and Bio T10 in Combination Against Phytophthora Wilt of Papaya

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Abstract

Papaya is one of the most widely cultivated horticultural plants. Phytophthora wilt is an important papaya disease which results in production losses. This research aimed to determine the effect of Bio P60 (raw secondary metabolites of *Pseudomonas fluorescens* P60) and Bio T10 (raw secondary metabolites of *Trichoderma harzianum* T10) application in combination on Phytophthora wilt and on growth of pepaya. The research was conducted on Californian pepaya farm, Linggasari Village, Kembaran District, Banyumas Regency. Randomized block design was used with six replicates and five treatments consisted of control (Mancozeb 80%), Bio T10 flush + Bio T10 spray, Bio T10 flush + Bio P60 spray, Bio P60 flush + Bio T10 spray, and Bio P60 flush + Bio P60 spray. Variables observed were percentage of healthy leaves, infection rate, number of healthy leaves, and number of healthy pepaya fruit. Result of the research showed that application of Bio P60 and Bio T10 effectively cured Phytophthora wilt with the percentage of healthy leaves as 69.19% compared to control. The combination was able to increase the number of healthy leaves the number of healthy papaya fruit. The novelty of this research is that raw secondary metabolites of biological agents proven to be able to overcome the papaya diseases, which so far cannot be solved. The implication for the development of science is one step ahead in overcoming plant diseases biologically by utilizing raw secondary metabolites. The benefits for the community can overcome papaya plant diseases organically, safely, and environmentally friendly, and inexpensive.

How to Cite

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INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most widely cultivated horticultural plants because it has high economic value and nutritional content. This plant has fruits that are rich in vitamins C, A and B as well as carotene, so it is widely used as a dessert and a popular "table fruit". In addition, the roots, stems, and leaves can also be used as medicines (Yogiraj et al., 2014). According to Wijaya & Chen (2013), papaya is one of five major tropical fruits world production after banana, mango, and pineapple. Indonesia is one of important papaya production countries after India and Brazil. One of the reasons for the decline in papaya production is due to Phytophthora wilt which is often found in papaya farm, caused by *Phytophtora* spp. (Chliyeh et al., 2014; Oliveira et al., 2018). Phytophthora wilt disease is an important disease in papaya plants which results in losses reaching 40-65% (Dianese et al., 2010).

The existence of this fungus attack makes one of the limiting factors that cause a decrease in papaya production. The spread of the Phytophthora fungus is very fast and can spread to other plants by infecting the roots of plants by sporangium intermediaries (Mahadevi et al., 2016). Plant roots can be infected directly through the root tissue, or various host plants and spread with the help of water that flows above the surface of the soil (Muktiani, 2011). The Phytophthora fungus will continue to secrete blackish brown mucus and stink. Mucus that comes out continuously will make the plant run out of nutrients, so the plants die (Alvarez et al., 2009). So far, Indonesian farmers still rely on synthetic chemical pesticides to control diseases in the crops they cultivate. Excessive and continuous use of synthetic pesticides can have negative impacts on the environment, including the death of useful insects and natural enemies (Brittain et al., 2011). In addition, the use of other methods is also carried out, such as with vegetable pesticides (Ilondu, 2011), but the results have not been satisfactory. Therefore, it is necessary to find alternative controls that are safe and environmentally friendly, which are directed at biological control, including the use of the fungus *Trichoderma* spp. (López-Mondéjar et al., 2011) and *Pseudomonas* spp. (Couillerot et al., 2009).

According to Tarntip & Thungkao (2011), *P. fluorescens* is able to produce protease and chitinase. *Trichoderma* sp. can produce chitinase and β-1,3-glucanase (López-Mondéjar et al., 2011). Chitinase enzyme which hydrolyses chitin from the pathogenic fungal hyphae wall causing hyphae lysis in pathogenic fungal hyphae (Parani & Saha, 2012). Frederiksen et al. (2013) also said that chitinase is an important enzyme in virulence for the control of pathogens because the activity of this enzyme can cause decomposition of hyphal cell walls and changes in the cytoplasmic composition of pathogenic fungal cells, which infect plants and stimulate responsiveness of plants. Chitinase produced by *Trichoderma* sp. is more effective than the chitinase produced by other organisms to inhibit various plant pathogenic fungi, so as to suppress the growth and development of these pests, which in turn can reduce attack rates (López-Mondéjar et al., 2011).

This research was conducted with the aim to find out 1) response of Bio P60 (raw secondary metabolites of *Pseudomonas fluorescens* P60) and Bio T10 (raw secondary metabolites of *Trichoderma harzianum* T10) against Phytophthora wilt and 2) towards California papaya plant growth. This study has provided important information of controlling the papaya diseases organically that recently are still difficult to control.

METHODS

The research was carried out in California papaya farm, Linggasari Village, Kembaran District, Banyumas Regency, with an altitude of ± 110 m above sea level. California papaya plants used were papaya plants ± 8 months old in the field and symptomatic phytophtora wilt disease with a minimum disease intensity of 50%.

Bio P60 and Bio T10 materials were each produced by Loekas Soesanto, at the Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. Bio P60 was made with the basic ingredients of the antagonist *Pseudomonas fluorescens* P60 (Soesanto et al., 2013); whereas Bio T10 from the basic ingredients of antagonist *Trichoderma harzianum* ginger isolate (Soesanto et al., 2018).

The design used was a non-factorial randomized block design with 5 treatments and each treatment was repeated 6 times. The treatments tried include: control (flush and spray), Bio T10 (flush) + Bio T10 (spray), Bio T10 (flush) + Bio P60 (spray), Bio P60 (flush) + Bio T10 (spray), and Bio P60 (flush) + Bio P60 (spray). The application was carried out 7 times with an interval of 1 week. Control treatment was using a fungicide (Mancozeb 80%) with recommended dosage. Concentrations of Bio T10 and Bio P60 were 10 and 5 mL per liter, respectively. Flush application used 1 L volume and 0.5 L spray per plant.

The percentage of healthy leaves was cal-

culated starting from the time of the first observation made 1 day before application. Furthermore, the development of the percentage of healthy leaves occurred. The percentage of healthy leaves was calculated every 5 days using the following formula (Oniha & Louis, 2015):

PDS=∑((DS-DSK))/DS X 100 %

Note: PDS = percentage of healthy leaves, DS = number of healthy leaves, DSK = number of diseased leaves.

Infection rate was obtained using a formula (Van der Plank, 1963):

r= 2,3/t (log 1/(1-Xt)-log 1/(1-X_0)) unit per day

Note: r = infection rate, t = observation time, X_0 = proportion of diseased tissue at initial time, and X_t = proportion of diseased tissue at time t.

The number of healthy leaves and the number of healthy fruit was measured every 5 days. The number of healthy leaves was calculated by counting the number of initial healthy leaves before treatment to the final number after treatment. The number of healthy fruits was calculated by counting the number of healthy fruits in the beginning before treatment to the final number after treatment.

Data were analyzed statistically using the F test. If there are real differences, followed by Duncan Multiple Range Test (DMRT) (≤0.05).

RESULTS AND DISCUSSION

The effect of treatments on components of pathosystem

The percentage of healthy leaves and the rate of infection in California papaya plants treated with Bio P60 and Bio T10 in full is presented in Table 1. The results of the statistical analysis explained that the treatments and controls were significantly different. The combined treatment of Bio P60 and Bio T10 was not significantly different or all the same combined effect in treating Phytophthora wilt in California papaya plants, but each treatment different in the level of decrease in the percentage of healthy leaves. The increase in the percentage of healthy leaves is thought to be because Bio P60 and Bio T10, which are secondary metabolites of antagonistic microbes (*P. fluorescens* P60 and *T. harzianum* T10), have been widely used as biological agents control of plant pathogens. In addition, both types of antagonists produce growth hormones, so that each is also known as Plant Growth Promoting Rhizobacteria (PGPR) (Sivasakthi et al., 2014; Tahmasbi et al., 2014) and Plant Growth Promoting Fungi (Oskiera et al., 2015).

Combined secondary metabolites of the two microbial antagonists are able to produce a better effect than a single application (Siddiqui & Akhtar, 2009). The synergistic interactions that occur from this combination concern the synergism of secondary metabolite compounds produced. One of the compounds in secondary metabolites is an enzyme. Bio P60 contains protease and chitinase (Soesanto et al., 2011); while Bio T10 contains chitinase and (Soesanto, 2013). López-Mondéjar et al. (2011) and Sharma et al. (2011) revealed that the enzyme can hydrolyze chitin from the pathogen fungal hyphae wall, thus causing lysis of fungal hyphae. Chitinase is an important enzyme that can cause decaying hyphal cell walls and changes in the cytoplasmic composition of pathogenic fungal cells that infect plants and stimulate the response of resistance from plants (Pattanapipitpaisal & Kamlandharn, 2012).

An increase in the percentage of healthy leaves of California papaya by Bio P60 (flush) + Bio T10 (spray) is suspected because secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 produce several compounds that affect the development of Phytophthora wilt. This is because compounds contained in secondary metabolites inhibit the development of pathogenic fungi, resulting in an increase in the number of healthy leaves of California papaya. This is consistent with the statement of Couillerot et al. (2009) that the secondary metabolite of *P. fluorescens* inhibits

Table 1. The effect of treatment on percentage of healthy leaves

Treatments	Percentage of Healthy leaves (%)	Infection rate (unit day^{-1})
control (fl and Sp)	49.93a	0.00069
Bio T10 (fl) + Bio T10 (Sp)	66.91b	0.00138
Bio T10 (fl) + Bio P60 (Sp)	62.35b	0.00069
Bio P60 (fl) + Bio T10 (Sp)	69.19b	0.00092
Bio P60 (fl) + Bio P60 (Sp)	65.58b	0.00100

Note: Numbers followed by the same letters show significantly different in DMRT (≤0.05); fl = flush and Sp = Spray.

various phytopathogens *in vitro* and around the plant rhizosphere and enters plant tissue through nutrient transport and nutrition.

Bio P60 used is thought to be able to work systemically to suppress the population of *Phytophthora* spp. This condition is in accordance with the results of research by Soesanto et al. (2013), that *P. fluorescens* P60 was able to suppress the intensity of viral diseases in chili plants, namely to reduce the intensity of the disease by 73.37% and to increase the content of phenols (saponins, tannins and glycosides) qualitatively. Latifah et al. (2012) informed that the application of *T. harzianum* was able to suppress Fusarium wilt in shallot by 21.41%.

According to Soesanto et al. (2008), the combined treatment of *T. harzianum*, *Gliocladium* sp., and *P. fluorescens* P60 had a positive influence in suppressing Fusarium wilt in gladiolus plants by 53.98%. In addition, the results of *P. fluorescens* P60 application against *Fusarium oxysporum* f.sp. *lycopersici* in tomato plants showed that the application of *P. fluorescens* P60 for 5 times had a very significant effect in suppressing Fusarium wilt. This is indicated by a decrease in disease intensity and low final density of *F. oxysporum* (Soesanto et al., 2010). Meanwhile, secondary metabolite *T. harzianum* T10 was able to delay the incubation period and reduce the intensity of vascular streak dieback in cocoa seedlings by 24.97 and 62.17%, respectively (Soesanto et al., 2019).

The rate of disease infection can be known from the development of the percentage of healthy leaves. The treatment of Bio P60 flush and Bio T10 spray had a positive effect on the rate of infection. This is presumably because Bio P60 which is watered in the soil can act as a pathogen competitor in the soil and absorbed by plants, so that the roots are protected from Phytophthora disease and increase the growth of healthy leaves.

In accordance with the results of Soesanto's (2000) research, that strain *P. fluorescens* P60 produces antibiotics 2,4-diacylfluoroglusinol (Phl), which can inhibit the pathogenic wilt of *Verticillium dahliae* in potato and eggplant plants. This is in accordance with Oskiera et al. (2015) reported that the application of *Trichoderma* sp. horticulture plants not only protect against soil-borne fungus attacks, but can also produce growth regulators or Plant Growth Regulation (PGR).

The effect of treatments on component of Californian papaya growth

The effect of the treatment on growth components and yields of California papaya is presented in Table 2. Statistical analysis of the growth components of California papaya shows that there are significant differences (number of healthy leaves and number of healthy fruits) on the growth component.

This happens because the application of Bio P60 is able to treat plants that are attacked by pathogenic fungi, so that plants can grow and develop without attack from pathogens and can increase plant growth. This is consistent with the results of research by Couillerot et al. (2009) and Soesanto et al. (2013), namely that the application of *P. fluorescens* antagonists can reduce the level of pathogen populations in the soil and increase the growth component of test plants. In addition, there is an increase in the number of plant leaves due to the application of the secondary metabolite of *P. fluorescens* P60, which contains growth hormone compounds, so it is known as PGPR as well. Meanwhile, according to Soesanto et al. (2019), the secondary metabolite *T. harzianum* T10 is able to increase the growth component of cocoa seedlings. This is according to the statement of Khan et al. (2017), that *Trichoderma* is able to increase tomato production.

The number of fruits in the California papaya plant was significantly different in the control (fungicide) and the combination of Bio T10 + Bio P60, both flush and spray. This is thought to be because both Bio P60 and Bio T10 can increase the number of fruits. The combined ability is thought to be a synergism of hormone compounds, which influences better growth and ultimately produces better fruit as well. This is consistent with the statement of Garcia-Seco et

Table 2. Numbers of leaves and fruit of papaya affected by treatments

Treatments	Number of healthy leaves	Number of healthy fruis
control (fl and Sp)	15.79a	0.82a
Bio T10 (fl) + Bio T10 (Sp)	23.42b	1.08b
Bio T10 (fl) + Bio P60 (Sp)	19.50b	1.23b
Bio P60 (fl) + Bio T10 (Sp)	37.56b	1.13b
Bio P60 (fl) + Bio P60 (Sp)	22.63b	1.00b

Note: Numbers followed by the same letters in the same column show no significant difference in DMRT (≤0.05); Sr = flush and Sp = Spray.

al. (2015), that the physiological mechanism of flavonoid and phenylpropanoid biosynthesis is involved in the formation and quality of fruit by PGPR.

The novelty of this research is the use of raw secondary metabolites of biological agents proven to be able to overcome the problem of papaya plant diseases, which so far cannot be solved. The implication for the development of science is one step ahead in overcoming plant diseases biologically by utilizing raw secondary metabolites. The benefits for the community can overcome papaya plant diseases organically, safely, and environmentally friendly, and in inexpensive way.

CONCLUSION

The combined application of Bio P60 and Bio T10 both flush and spray is effective in treating Phytophthora wilt with a healthy leaf percentage of 69.19% in California papaya plants compared with the use of the Mancozeb fungicide. The combination is able to increase the growth of healthy leaves and the number of healthy fruits of California papaya plants.

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