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Research Article

Biological Control of Fusarium Wilt on Tomato Using Avirulent *Fusarium oxysporum* f. sp. *cepae* Combined with Siam Weed Compost

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a major disease that results in significant yield loss in tomato production. Currently, chemical control of Fusarium wilt using fungicides has yielded unsatisfactory results and has caused negative effects. Biological control using antagonistic microorganism combined with compost is a promising and environmentally friendly alternative management method for this disease. This research aimed to evaluate the effectiveness of an avirulent *Fusarium oxysporum* f. sp. *cepae* biological agent combined with siam weed compost to manage Fusarium wilt and increase growth and yield in tomato productions. This research was conducted using a completely randomized design with two factors and three replicates. The first factor was the dose of the avirulent *F. oxysporum* f. sp. *cepae* biological agent i.e 0 (control), 80, and 100 mL/plant. The second factor was dose of siam weed compost i.e. 0 (control), 300, and 600 g/plant. Disease intensity, plant growth and yield variables were evaluated. Data collected were analyzed using an ANOVA followed with Duncan's Multiple Range test at 95% confidence level. The results showed that the use of avirulent *F. oxysporum* f. sp. *cepae* biological agent was able to manage Fusarium wilt disease in tomato productions and increase growth and yield of tomato. The best treatment for increasing tomato yield was the combination of 100 mL/plant of avirulent *F. oxysporum* f. sp. *cepae* with 600 g/plant of siam weed compost, resulting in a yield of 110.41 g/plant.

Keywords: major disease; organic agriculture; organic fertilizer; soil-borne pathogen

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) has an important role in human diet because of its high content of bioactive compounds including lycopene and carotenoid, making in an important cash crop worldwide (Dobrin *et al*., 2019; Gatahi, 2020). However, plant disease still remains a constrain in tomato productions. Moustaine *et al*. (2023) documented at least 15 fungal diseases on tomato starting from seedling disease, such as damping-off, to fruiting disease like anthracnose.

Indonesia's low tomato productive (18.67 ton/ha) (BPS, 2025) has potential to be increased to 68.81 ton/ha (Pratiwi & Soegianto, 2023) by improving disease management. Fusarium wilt on tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the main diseases where its management may result in significantly increased tomato yield. This disease is characterized by the wilting and leaf yellowing during vegetative stages until fruit rot symptom during generative phase (Bawa, 2016). Hassan (2020) and López-Zapata (2021) stated that *F. oxysporum* f. sp. *lycopersici* has high economic and ecological impacts due to its potential to cause yields loss of up to 100%.

Fusarium wilt on tomato is managed mainly using synthetic fungicides. However, management using this method have been reported to unsatisfactory (Mohammed *et al*., 2019). Borrero *et al*. (2004) stated that there is not yet an effective chemical method to manage this disease. Biological control using antagonistic microorganism is a potential and good alternative method to control this disease

(Heriyanto, 2019). Biological control on Fusarium wilt had been done using various antagonistic microorganisms whether in single or in a combination application. Biological control agents may reduce the disease intensity by reducing inoculum production of the pathogen, inhibiting spore germination, and surviving through antibiosis mechanism (McGovern, 2015). In general, biological control is capable of controlling Fusarium wilt and increase tomato yield.

One of biological agents which can be used to control plant diseases is avirulent microorganisms or non-pathogenic microorganisms such as avirulent *F. oxysporum*. Avirulent strain of *F. oxysporum* is known to be able to protect plants from pathogenic attack by *F. oxysporum*. Iida *et al.* (2022) showed that pre-inoculation using avirulent *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici* mutants reduced disease intensities of Fusarium wilt on melon and tomato, respectively. It indicates that avirulent variants of *F. oxysporum* are potential methods to manage Fusarium wilt.

Fusarium oxysporum is a soil inhabitant fungus known as plant pathogen causing wilt and root rot disease on several important crops (Kaur *et al.*, 2010). Soil borne pathogens are affected by several factors such as the other microorganisms, plant roots, and the compost or organic matters content in the soil. Compost may affect the soil-borne pathogens through its chemical and physical properties, presence of microflora, ability to stimulate existing antagonistic microflora around plant roots which later can induce plant health (Patil *et al.*, 2018; van Elsas & Postma, 2007). Compost application is useful in managing soil-borne pathogens and later increasing crop yields in sustainable agricultural systems.

Siam weed compost (*Chromolaena odorata* (L.) R.M.King & H.Rob.) can be a compost source with properties to manage plant disease and to provide plant nutrients. Abolusoro *et al.* (2020) showed that the use of siam weed compost could effectively manage *Meloidogyne incognita* and increase yield in tomato productions. Dewi *et al.* (2018) found that siam weed compost application at dosage of 40 ton/ha was able to improve physical soil properties and several growth parameters of red chili. Siam weed compost can be produced by fermenting the chopped biomass for 3 days that were applied with 5% EM4 solution. However, the combined use of avirulent variant of *F. oxysporum* f. sp. *cepae* with siam weed compost to manage Fusarium wilt on tomatoes has not been studied. Thus, objective of this research was to determine the effectiveness of biological agent of avirulent *F. oxysporum* f. sp. *cepae* combined with the use of siam weed compost to manage Fusarium wilt and increase growth and yield of tomato.

MATERIALS AND METHODS

Study area

This research was conducted from September 2022 to March 2023 in Research Garden, Faculty of Agroindustry, University of Mercu Buana Yogyakarta located in Gunung Buluh, Sedayu, Yogyakarta at the elevation of 114 m above sea level.

Treatment and Experimental Design

This study was set as a completely randomized experiment with two factors and three replicates was used. The first factor was dose of biological control agent i.e. A0 = control, A1 = 80 mL/plant, and A2 = 100 mL/plant. The second factor was dose of siam weed compost i.e. B0 = control, B1 = 10 ton/ha, and B2 = 20 ton/ha. The treatment combination tested were A0B0, A0B1, A0B2, A1B0, A1B1, A1B2, A2B0, A2B1, and A2B2. Each combination consisted of 10 plants with a total plant population of 270 plants.

Preparation of Biological Control Agent and Fusarium Wilt Pathogen

Biological agent of avirulent *F. oxysporum* f. sp. *cepae* and Fusarium wilt pathogen of *F. oxysporum* f. sp. *lycopersici* were obtained from the isolate collections of the Laboratory of Plant Protection, Study Program of Agrotechnology, University of Mercu Buana Yogyakarta. Biological control agent was obtained by using monospore isolation technique as described by Nugroho (1996) and was patented under IDP000067297. Both isolates were cultured using the same method. Inoculum production of the two isolates was done by cutting the edge of rejuvenated colonies. The cuts were then inoculated in Erlenmeyer flasks containing 100 mL of Potato Dextrose Broth (PDB) and incubated unshaken for 2 weeks at 25 °C (Nugroho, 2015).

Preparation of Siam Weed Compost

Two kilogram of siam weed leaves and young shoots were chopped and applied evenly with EM4 solution. EM4 solution was prepared by diluting 5 mL of EM4 and 8 g sucrose into 1 L of tap water. The sprayed siam weed was then fermented in the plastic bag for 4 days. After fermentation, the fermented siam weed was sun dried and macerated until it reached powder form.

Seedling Preparation

Tomato var. *Fortuna* plants were planted in 5 × 5 cm polybags containing vertisol soil, sand, and cow manure with the ratio of 1:1:1 (v/v). The soil used was unsterilized soil which has never been used to plant tomatoes. Each polybag was planted with one tomato seed. Seedling were maintained until they have 4−5 leaves.

Tomato Planting and Pathogen Inoculation

Four week-seedlings old with 4−5 leaves were transplanted into 40 × 40 cm polybags containing vertisol soil. The soil used was the same soil as used in the seedling preparation. Before transplanting, the planting media was inoculated with suspension of *F. oxysporum* f. sp. *lycopersici* spore with the concentration of 10^6 spore/mL and the dose of 20 mL/polybag.

Application of Biological Agent and Siam Weed Compost

Before the tomato seedlings were planted, avirulent *F. oxysporum* f. sp. *cepae* was applied using designated treatments with concentrations of 10^6 spore/mL. Siam weed compost was thoroughly mixed into the planting media following the previously designated treatments. No biological agent and no siam weed compost were used in the control.

Variable Observed and Data Analysis

Data were measured from five sample plants. The variables observed were disease intensity, plant height, leaf number, fresh and dry weight of plant, fruit weight per plant, fruit number per plant, and fruit diameter. Data were analyzed using ANOVA and a Duncan's Multiple Range Test (DMRT) post hoc with 95% confidence level was performed if a significant effects were detected.

The disease intensity was recorded on a 0−4 scale with the following descriptions: 0 = no infection; 1 = yellowing of two or three leaves; 2 = 50% leaves wilting; 3 = yellowing on all leaves; 75% leaves wilting and growth was inhibited; 4 = yellowing of whole leaves, 100% leaves wilting, and plant died. The disease intensity (IP) was then calculated using the following formula (Song *et al.*, 2004).

$$IP = \frac{\Sigma(n \times v)}{Z \times N} \times 100\%$$

n = scale, v = number of plant infected, Z = highest scale, and N = total number of plants.

RESULTS AND DISCUSSION

Disease Intensity

This research demonstrated that the biological control agent, avirulent *Fusarium oxysporum* f. sp. *cepae*, combined with siam weed compost was able to reduce the disease intensity and affect several growth and yield parameters of tomato plants. However, there were no interaction between the two factors used in this study (Table 1). Interaction between two factors can be affected by the dose combinations used for each treatment in this study. Masyitah *et al.* (2023) found that there was no interaction between *Bacillus thuringiensis* and *Pseudomonas aeruginosa* when the two antagonists were used in a combination to control Fusarium wilt on melon.

The siam weed compost was able to suppress the disease development. There was no plant death during the research. According to Ikechi-Nwogu *et al.* (2018), siam weed has antimicrobial properties because of its content of tannin, saponin, flavonoid, alkaloid, phenol, glycoside and terpenoid. Okigbo and Nnadiri (2017) demonstrated that siam weed extract was able to inhibit the growth of pathogenic fungus of *Botryodiplodia theobromae*.

Biological control agent of avirulent *F. oxysporum* f. sp. *cepae* used in this research showed ability to suppress Fusarium wilt development indicated by decreasing disease intensity until no more symptom were observed on the last observation (Table 1). Decrease in disease intensity was due to the pre-inoculation using the biological control agent which could induce tomato resistance against plant pathogens. Similar result was found by Garcı´a-Bastidas *et*

Table 1. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on Fusarium wilt intensity on tomato plants.

Treatments	Disease intensity (week)				
	2nd	3rd	4th	5th	6th
Siam weed compost (g/plant)					
Control	4.33±2.73a	4.44±5.09a	4.44±3.47a	2.78±1.57a	3.33±1.93a
300	0.56±0.96b	0.56±0.96b	0.56±0.96b	0.00±0.00b	1.67±0.96a
600	1.55±0.94ab	0.00±0.00ab	0.00±0.00b	0.00±0.00b	0.00±0.00a
Biological control agent (mL/plant)					
Control	3.89±2.55p	3.89±5.36p	3.33±1.47p	1.67±2.36p	5.00±1.67p
80	2.11±3.53p	1.11±1.92p	1.11±2.42p	0.56±0.79p	0.00±0.00q
100	0.44±0.77p	0.00±0.00p	0.56±3.14p	0.56±0.79p	0.00±0.00q
P-value					
Siam weed Compost (SWC)	0.243				
Biological control agent (BCA)	0.026				
Interaction (SWC×BCA)	0.247				

Note: (-) No interaction. Numbers followed by different letters in the same column are significantly different based on DMRT test of *a* = 0.05.

al. (2022) which successfully induced banana resistance against Fusarium wilt banana by using avirulent strain of *Fusarium* sp. race 1. Pamekas *et al.* (2023) found that the increase in shallot resistance against *moler* was correlated to the increase in salicylic acid content due to *Trichoderma* sp. applications.

Research done by Basco *et al.* (2017) used vermicompost fortified with *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* was able to suppress Fusarium wilt on tomato. Non-fortified vermicompost only provided disease suppression during early growth stages of tomato even though disease intensity was still lower compared to the control.

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is well known over the world as a major disease on tomato both in green house and in the field (Mohammed *et al.*, 2019; Mostafa *et al.*, 2022). López-Zapata *et al.* (2021) stated that the disease has the ability to result in 100% tomato yield loss.

Currently, Fusarium wilt control is done mainly using chemical fungicides which have several negative effects. Biological control using antagonistic microorganism is a promising method as an alternative to chemical control (Basco *et al.*, 2017). Research done by Basco *et al.* (2017) found that biological control agent of *Trichoderma harzianum* formulated in vermicompost was effective in suppressing Fusarium wilt.

Tomato Plant Growth

Avirulent *F. oxysporum* f. sp. *cepae* dose had no significant effect on tomato plants height, while siam weed compost was able to increase it and was observed since the first observation at 2 weeks after planting (Table 2). Effect of biological control agent on tomato plant leaf number was observed variable at 2 and 3 weeks after planting (Table 3 and Table 4). An interaction effect was observed at 2 weeks after planting with the best combination of 100 ml/plant of biological control agent with 600 g/plant of siam weed compost with the leaf number of 7.13 (Table 3).

Increases due to the use of siam weed compost was also observed in tomato plants fresh and dry weight. The dose of 600 g/plant gave the highest tomato plant fresh and dry weight. However, the use of biological control agent did not increase tomato plants fresh and dry weight of tomato although it increased its leaf number (Table 5 and 6). The increase in tomato plants fresh and dry weight may be associated with nutrient availability of siam weed compost. According to Babajide *et al.* (2018), siam weed compost could increase nitrogen, phosphor, and potassium uptake in maize. Meanwhile Suryanto *et al.* (2020) found that siam weed compost increased soil organic matter and soil moisture content.

Table 2. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on tomato plant height at five observations.

Treatments	Weekly plant height (cm)				
	2	3	4	5	6
Siam weed compost (g/plant)					
Control	21.60±1.54b	23.49±1.70c	26.67±1.33c	30.55±1.17c	34.41±0.60c
300	25.22±1.51a	31.26±2.41a	35.56±10.46b	49.16±4.63b	59.63±6.80b
600	23.52±2.10a	30.92±3.37b	42.27±3.60a	53.82±3.35a	67.49±2.91a
Biological control agent (mL/plant)					
Control	21.67±2.12p	26.11±4.01p	29.35±8.77p	41.53±11.34p	50.54±16.17p
80	23.82±1.13p	28.64±3.63p	36.66±8.01p	45.07±11.86p	54.94±17.32p
100	24.85±2.28p	30.92±5.60p	38.49±9.84q	46.94±14.04p	56.05±19.08p
					(-)

Note: (-) No interaction. Numbers followed by different letters in the same column are significantly different based on DMRT test of $a = 0.05$.

Table 3. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on tomato plant leaf numbers 2 weeks after planting.

Siam weed compost (g/plant)	Biological control agent (mL/plant)			Average
	Control	80	100	
Control	5.13±0.31cd	4.87±0.50d	5.2±0.53cd	5.07
300	5.93±0.95b	6.6±0.35ab	6.07±0.12b	6.20
600	5.48±0.20cd	5.93±0.50bc	7.13±0.23a	6.18
Average	5.51	5.80	6.13	(+)

Note: (+) There was interaction between the two factors. Numbers followed by different letters in the same column are significantly different based on DMRT test of $a = 0.05$.

Table 4. The effect of siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on leaf number of tomato plant at observation of 3rd to 6th week.

Treatments	Leaf number on week			
	3rd	4th	5th	6th
Siam weed compost (g/plant)				
Control	5.67±0.23a	6.64±0.25a	7.89±0.15a	7.93±0.64c
300	8.31±0.96a	10.16±0.64a	11.33±0.57b	11.98±0.57b
600	9.07±0.75b	10.73±0.47b	12.20±0.48c	13.33±0.55a
Biological control agent(mL/plant)				
Control	7.47±1.49p	8.93±1.79p	10.20±1.92p	10.91±2.11p
80	7.20±1.64q	9.09±2.27p	10.42±2.30p	11.04±3.44p
100	8.38±2.25q	9.51±2.59p	10.80±2.63p	11.29±3.00p
				(-)

Note: (-) No interaction. Numbers followed by different letters in the same column are significantly different based on DMRT test of $a = 0.05$.

The tomato plants were not able to produce fruits without siam weed compost applications (control), however they still gave the fruits when the biological control agent was absent (Table 7 and 8). This was related to the lower content of nutrient in the control due to no fertilizer application.

Siam weed compost increased fruit diameter and weight respectively to the higher dose of siam weed compost. The highest fruit diameter (3.39 cm) and weight (26.65 g per fruit) were obtained at the dose of 600 g/plant. Research by Dewi *et al.* (2018) showed that siam weed compost contained NH_4

Table 5. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on tomato plant fresh weight.

Siam weed compost (g/plant)	Biological control agent (mL/plant)			Average (g)
	Control	80	100	
Control	11.12	15.38	8.23	11.58±3.60c
300	35.42	42.22	41.35	39.66±3.70b
600	64.88	51.10	86.283	67.42±17.73a
Average (g)	37.14±26.92p	36.23±18.60p	45.29±39.18p	**(+)**

Note: (+) There was an interaction between the two factors. Numbers followed by different letters in the same column are significantly different based on DMRT test of *a* = 0.05.

Table 6. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on tomato plant dry weight.

Siam weed compost (g/plant)	Biological control agent (mL/plant)			Average (g)
	Control	80	100	
Control	1.71	1.18	1.26	1.38±0.29c
300	5.10	5.95	6.70	5.92±0.80b
600	9.02	6.99	10.80	8.94±1.90a
Average (g)	5.28±3.66p	4.71±3.10p	6.25±4.78p	**(+)**

Note: (+) There was an interaction between the two treatments. Numbers followed by different letters in the same column are significantly different based on DMRT test of *a* = 0.05.

Table 7. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on tomato yield.

Treatments	Yield variables		
	Fruit number/plant	Fruit diameter (cm)	Weight/fruit (g)
Siam weed compost (g/plant)			
Control	0.00a±0.00	0.00±0.00c	0.00±0.00c
300	2.580.28a	3.11±0.06b	22.55±0.123b
600	2.980.85a	3.39±0.03a	26.65±1.76a
Biological control agent (mL/plant)			
Control	1.63±1.41p	2.13±1.85p	16.09±14.25p
80	1.85±1.60p	2.19±1.90p	15.97±13.85p
100	2.08±1.98p	2.18±1.89p	17.14±15.04p
P-value			
Siam weed Compost (SWC)	0.32		
Biological control agent (BCA)	0.49		
Interaction (SWC×BCA)	0.60		

Note: No interaction. Numbers followed by different letters in the same column are significantly different based on DMRT test of *a* = 0.05.

and NO_3 higher than cow manure. Quantitatively, siam weed contain higher nutrients of N, P, dan K (Nugroho *et al.*, 2019).

Interaction effect between avirulent *F. oxysporum* f. sp. *cepae* with siam weed compost occurred on the variable of fruit weight per plant. The highest yield of 110.41 g/plant was obtained at the combination of 100 ml/plant biological control agent with 600 g/plant of siam weed compost (Table 8). Soil-borne pathogens are affected by several factors such as the presence of other microorganisms, plant roots, and the content of compost or organic matter in the soil (van Elsas & Postma, 2007).

Table 8. The effect of combination between siam weed compost and avirulence *Fusarium oxysporum* f. sp. *cepae* biological control agent on fruit weight per plant.

Siam weed compost (g/plant)	Biological agent (ml/plant)			Average (g)
	Control	80	100	
Control	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00
300	53.97±11.25c	65.52±18.57c	53.16±10.11c	57.55
600	64.22±23.72c	66.08±7.81bc	110.41±34.44a	80.24
Average	39.40	43.87	54.53	**(+)**

Note: (+) There was an interaction between the two treatments. Numbers followed by different letters in the same column are significantly different based on DMRT test of a = 0.05.

CONCLUSIONS

Results demonstrated that the combined use of avirulent *Fusarium oxysporum* f. sp. *cepae* was able to manage Fusarium wilt disease in tomato while also increasing the growth and yield of tomato. The best treatment for increasing tomato yield was the combination of avirulent *F. oxysporum* f. sp. *cepae* at 100 mL/plant with siam weed compost at 600 g/plant resulting in a yield of 110.41 g/plant. Further research is needed to study the mechanism of the interaction between the biological control agent and Siam weed compost in controlling Fusarium wilt and in increasing tomato growth and yield.

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