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Original Article

The application of *Trichoderma viride* -T1sk rice straw compost to suppress Fusarium wilt on banana seedling

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Abstract

Fusarium wilt is one of the most destructive diseases in banana that needs to be addressed. We used *T. viride* -T1sk, an antagonistic fungus as well as a decomposer to decompose rice straw, and applied the resulted compost to banana seedlings infected by Fusarium wilt to find a dosage that potential to suppress the disease. A greenhouse study was conducted in RBD with seven treatments and four replications. The treatments were the dosages of *T. viride*-rice straw compost (g/seedling), they were 60, 80, 100, 120, 140, 0 (Control I), and without compost (Control II). The parameters were the density of *T. viride*-T1sk in seedling's rhizosphere, the appearance of the first symptom, the percentage of symptomatic leaves, and the intensity of corm damage. The result showed that the density of *T. viride* in rhizosphere of banana seedlings before planting differed significantly among the dosages. The highest density was found at the dose of 140 g/seedling (8.23×10^4) which was not significantly differ with the doses of 120 and 100 g/seedling. Two months after planting, the density of *T. viride* in rhizosphere was increased. The highest density was found in the treatment of 120 g/seedling compost (9.85×10^4) which was not differed with the doses of 140 and 100 g/ seedling.

Keywords: Banana seedling, Fusarium wilt, Rice straw compost, *T. viride*-T1sk

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Introduction

Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp *cubense* (Foc) is an important disease that reduces banana's production economically. The disease has spread widely throughout the world's banana cultivation area (Ploetz, 2015). The pathogen is difficult to control since it forms chlamydospores which survive to approximately 40 years in the soil (Buddenhagen, 2009; Widyantoro et al., 2019).
Foc is known for its high genetic diversity. Until recently, four races of Foc have been identified with race-4 as the most pathogenic that infect all banana cultivars (Stover, 1990; Ploetz et al., 2015).
Various strategies for controlling Foc have been explored including the use of healthy seeds derived from tissue culture, eradication, and sanitation (Nasir and Jumjunidang, 2002). Eradication is considered to be ineffective in the areas previously infected by Foc (Ploetz et al., 2015). Another alternative that can be done to control Foc is to apply Trichoderma fungi as biological agents. The species within this genus are

found in many habitats such as soil, organic matter, decaying wood, animal waste, rice straw, and plant rhizosphere (Samuel, 1996; Harman, 2004; Druzhinina et al., 2006; Vinale et al., 2008). Trichoderma inhibits the growth of plant pathogens through direct competition in space and nutrient, the production of secondary metabolites which function as antimicrobial, and its parasitic nature against plant pathogens (Howell, 2003; Vinale et al., 2008).

T. viride - T1Sk, one of the species of Trichoderma from personal collection, showed high ability to colonize the roots of banana seedling, this might prevent contact between Foc and roots that leads to the avoidance of plant infection (Nurbailis et al, 2016). *Trichoderma* spp. has also been reported to induce plant resistance against a number of pathogens. In addition to acting as a biological agent, this fungus also act as decomposer. *Trichoderma* spp. was able to decompose organic materials containing cellulose to be used as the source of nutrition (Naher et al., 2014) such as rice straw and the bagasse of sugar cane.

Rice straw from harvested rice is usually left or burned by farmers in the field or being used for animal feed. In Indonesia, the average nutritional compositions of rice straw are 0.4% N, 0.02% P, 1.4% K and 5.6% Si. Based on the composition, rice straw is potential to be used as organic fertilizer. However, due to the cellulose, hemicellulose, and lignin within rice straw, natural decomposition of rice straw takes more time. The involvement of microbes that enhance the decomposition process with the production of enzymes is needed (Golueke, 1991 cit. Karumuri, 2017). *Trichoderma* is a potential fungus to decompose materials that contains lignin (Trengerdy et al., 2003; Noor et al., 2012), while composting rice straw using *T. harzianum* as decomposer has succeeded in reducing the C/N ratio to 17.5 within 90 days (Yacoob et al., 2017).

The problem of Fusarium wilt on banana needs to be addressed to reduce the economic loss. With the abundance of rice straw in Indonesia, we explored the potency of *T. viride* isolate T1sk as an antagonistic and a decomposer fungus in composted rice straw to suppress Fusarium wilt on banana seedling in green house scale. This study was conducted to know the density of *T. viride*-T1sk in composted rice straw and to find the potential dosage of *T. viride* -T1sk-rice-straw compost to suppress Fusarium wilt in banana seedling.

Material and Methods

The study was carried out in the Phytopathology Laboratory and the greenhouse of the Faculty of Agriculture, Universitas Andalas from August 2012 to January 2013. Randomized Block Design (RBD) with seven treatments and four replications was employed in this study. The treatments were the dosages (g/seedling) of *T. viride*-T1sk rice straw compost, namely: A = 60, B = 80, C = 100, D = 120, E = 140, F = rice straw compost without *T. viride* -T1sk (Control I), and G = Without Compost (Control II). The data were analyzed by variance and continued with Duncan's New Multiple Range Test (DNMRT) at a 5% significance level.

Rejuvenation and propagation of *T. viride*-T1sk and Foc

The *T. viride* and Foc isolates were the collection of the Biological Control Laboratory of the Faculty of Agriculture, Universitas Andalas. Both isolates were rejuvenated in Potato Dextrose Agar (PDA) and incubated at room temperature for 7 and 14 days consecutively. Bagasse was used as substrate for *T. viride* propagation. It was cut into the size of 1 cm^2, moistened, packed in plastic and autoclaved at 121^0C, 1 atm. Cooled bagasse was then inoculated with a piece of *T. viride* culture (1 cm^2) and incubated for 14 days at room temperature.

On the other hand, Foc was mass-propagated on rice. The rice was washed, wrapped inside a piece of muslin cloth and dipped into the boiling water for 3 minutes. This half-cooked rice was then distributed into the plastic bags to a weight of 250 g. These bags of rice were then autoclaved at 121^0C, 1 atm for 15 minutes. Sterilized bags of rice were left to cool prior to fungus inoculation. Two pieces of Foc from the isolate, each of 0.5 mm in diameter were transferred into each bag. All bags were incubated for 14 days at ambient temperature.

T. viride-T1sk- rice straw compost

A hundred kg of rice straw previously chopped into small pieces, were carefully layered onto a tarpaulin sheet. One layer was consisted of 100 x 100 x 20 cm^3 chopped straw, and a total of five layers were stacked into a 100 cm tall rice-straw stack. A mixture of 200 g *T. viride*- T1sk in bagasse medium and 5 liter of water was evenly poured onto each layer prior to stacking.

Finally, these layers were covered with a sheet of black plastic and topped with a piece of wood and heavy stones to tighten them up. The compost was left untouched for 7 days, and afterwards the layers were inverted every 2 weeks. The compost was harvested after 6 weeks when the straw has turn brown and softened and no foul smell was detected.

To count the density of *T. viride*-T1sk from compost, 10 g of compost was mixed with 100 ml of distilled water, stirred evenly, and then diluted to 10^3. One ml of the dilution was transferred to petridish containing 9 ml of McFadden and Sutton's RB-S-F, specific media for *Trichoderma*. The culture was incubated for 3 days at room temperature. Herr's formula (1959), cit Busnia et al. (1990) were used to calculate the fungus.

$$A = \frac{K(100 + KA)\ x\ P}{100}$$

A = the density of *T. viride*-T1sk/g compost,
K = the density of *T. viride*- T1sk/dish,
KA= the moisture content
P = the dilution used in the preparation

Soil treatments and planting of banana seedlings

Ambon Hijau banana variety originated from Tropical Fruit Research Institute Solok - Indonesia, was chosen for this study. The seedlings were product of tissue culture that had underwent a two month acclimatization. As planting media, oven (dry)-sterilized (2 hours, 150°C) soils were used. Cooled soils were distributed into polybags, each contained 5 kg of them. The compost was introduced into the soils, 8 cm below the surface according to the treatments. Seven days later, the banana seedlings were planted into each polybag. The inoculation of Foc was conducted 14 days after planting. The soil around the base of the stem was dug to the depth of 8 cm. Ten g of Foc on rice was evenly spread into this gutter and covered with the dug soil. The seedlings were watered and weeded as needed throughout the study.

The calculation of the density of *T. viride* in soil was conducted twice, viz. 7 and 60 days after application. Forty g of soil around the rhizosphere were taken from four points and mixed evenly. The same formula as that on rice straw compost was used in the calculation.

Fusarium wilt symptom

The seedlings were observed every day to anticipate the appearance of the first symptom. The observation was started from the third day after Foc inoculation and continued for 2 months. Furthermore, the percentage of symptomatic leaves was observed at the interval of 7 days for two months by counting the number of symptomatic leaves on each seedling. The percentage of symptomatic leaves was calculated with the following formula:

$$Pd = \frac{Di}{D} \text{ x } 100\%$$

Pd= The percentage of symptomatic leaves
Di= The number of symptomatic leaves per seedling
D = The number of leaves per seedling

Intensity of corm damage

The observation of corm damage was conducted at the end of the study. The damage was scored according to discoloration on corm tissue (Table 1). The complete corm was removed from the soil, the roots were cut off and excess soil was removed. Transverse section was made on the corm to obtain five slices of equal thickness per corm. The upper surface of each cut section was examined and the extent of vascular discoloration was scaled (Orjeda, 1998). [Table 1]

The intensity of corm damage was calculated using the following formula:

$$DS = \sum \frac{(ni\ x\ Vi)}{(Z\ x\ N)}\ x\ 100$$

Ds = the percentage of damage intensity
n_i = the number of infected corms in each scoring criteria
V_i = was the numerical value of each scoring criteria, Z was the highest numerical value of each scoring criteria, and N was the number of observed corms.

Table-1: Scoring criteria of banana corm damage due to Fusarium wilt (Orjeda, 1998).

Symptoms	Scoring
Corm completely clean, no vascular discoloration	1
Isolated points of discoloration in vascular tissue	2
Discoloration of up to 1/3 of vascular tissue	3
Discoloration of between 1/3 and 2/3 of vascular tissue	4
Discoloration greater than 2/3 of vascular tissue	5
Total discoloration of vascular tissue	6

Results and Discussion

The density of *T. viride*-T1sk

Both composted rice straws, either previously inoculated with *T. viride* or not, were found to contain *T. viride* (Table 2). Six weeks after inoculation, the density of *Trichoderma* in rice straw decomposed with *T. viride* was 7.63 x 10^4 cfu/g fungus, while without *T. viride* was found to be harboured by 2.11 x10^4 cfu/g fungus. This indicated that *Trichoderma* was present in rice straw before it was composted, even though the population was lower than composted with *T. viride*. According to Druzhinina et al, (2006), *Trichoderma* can be found in various regions with different climates. Habitat of the fungus includes various types of soil, decaying wood, manure and rice straw. Rice straw composted with *T. viride* had higher amount of *T. viride* six weeks after inoculation than without *T. viride* with the ratio of the density 3.6:1 (Table 2). This showed that *T. viride* was able to grow and to develop on rice straw. *Trichoderma* itself is a potential fungus to decompose materials that contain lignin (Trengerdy et al, 2003; Noor et al., 2012) and cellulose (Yaacob et al., 2017). The fungus degraded rice straw with their enzymes, especially those that play the role as cellulose and lignin remodeler (Samuel, 1996; Druzhinina et al., 2006) to obtain nutrition.

Table-2: The density of *Trichoderma viride* -T1sk in rice straw compost, six weeks after inoculation

Treatment	*T. viride*-T1sk (cfu/g)
Rice straw composted with *T. viride* -T1sk	7.63 x 10^4
Rice straw composted without *T. viride* -T1sk	2.11 x 10^4

The density of *T. viride* in the rhizosphere of banana seedlings before planting differed significantly among the dosages (Table 3). The highest density was found at the dosage of 140 g/seedling (8.23 x 10^4) which was not significantly differ with the dosage of 120 (7.93 x 10^4) and 100 g/seedling (7.63 x 10^4). The lowest density was found in rice straw without *T. viride* (2.48 x 10^4) and without compost treatment (2.30 x 10^4).

Two months after planting, the density of *T. viride* in rhizosphere was increased (Table 3). The highest density was found in the treatment of 120 g/seedling compost (9.85 x 10^4) which was not differed with 140 and 100 g/seedling (9.18 x 10^4). The lowest density was found in the treatment without compost (3.15 x 10^4).

Table-3: The density of *Trichoderma viride* -T1sk in seedlings' rhizosphere treated with various dosage of rice straw compost before and 2 months after planting.

Dosage (g/seedling)	*T. viride* -T1sk (cfu/g)	
	Before planting (7days after compost application)	**Two months after planting (60 days after planting)**
140	8.23 x 10^4 a	9.18 x 10^4 a
120	7.93 x 10^4 ab	9.85 x 10^4 a
100	7.63 x 10^4 ab	9.18 x 10^4 a
80	6.33 x 10^4 b	6.65 x 10^4 ab
60	4.35 x 10^4 c	5.18 x 10^4 b
Control I (Compost without *T. viride*)	2.48 x 10^4 d	5.23 x 10^4 b
Control II (Without compost)	2.30 x 10^4 d	3.15 x 10^4 b

Figures followed by the same letter are not significantly different at 5% level according to DNMRT

Rice straw compost applied to the soil, the seedlings' planting media, in general increased the density of fungus. Seven days after compost application, the density of fungus that was applied with compost into the soil were yet to raise. This might be due to the adaptation period in the soil before it started to develop. *T. viride* which was applied to the soil undergoes an adaptation period and then started to decompose organic matters in the soil and made them it source of nutrition. The increase of density occurred after the seedlings planted. The highest density of the fungus was found in the dosages of 100, 120, 140 g fungus per seedling. The growth of *T. viride* was basically supported by the compost before it was able to use other organic matters that ended up around the rhizosphere. According to Vinale et al. (2008), besides having a saprophytic nature, *Trichoderma* is also antagonistic to various plant pathogens and can colonize plant rhizosphere.

Fusarium wilt on banana seedlings

The appearance of the first symptoms of Fusarium wilt on banana seedling treated with various dosages of *T. viride*-T1sk rice straw compost showed significantly different (Table 4). The longest appearance of the symptom was detected in the treatments of 100 and 120 g/seedling, which appeared at 13.50 days after inoculation. On the other hand, the earliest symptom appeared in the control II (without compost application). This was at 6.25 days after inoculation. The compost dosage of 100 g/seedling was able to delay the appearance of the symptom to one week (6 days). This showed that the dosage has potency to be an effective to delay the appearance of the first symptoms of Fusarium wilt in banana seedlings. Hoitink and Fahy (1986) explained the importance of the initial population of an antagonistic fungus as the factor determines the antagonistic ability to pathogenic fungi. Syatrawati (2008) reported that the presence of antagonistic agents in rhizosphere slows the contact and penetration of pathogens to their hosts due to the competition in space and food. [Table 4]

The percentage of symptomatic leaves caused by Fusarium wilt was showed significantly different (Table 4). The highest percentage was found in the treatment without compost, which reached 49% of the total, while the lowest was found in the treatment dosage of 120 g/seedling (26%) which was not significantly differed from the dosage of 100 g/seedling (28%) and 140 g/seedling (35.5%). However, the treatment of 120 g/seedling was significantly differed to 80 and 60 g/seedling compost and both controls.

The intensity of corm damage however, also showed various results (Table 4). The highest intensity was found in the treatment without compost which was 66.67%, while the lowest was in the treatment with the dosage of 100 g/seedling, which was 26.38%. Similar to the percentage of symptomatic leaves, the treatment of 100 g/seedling was significantly differed to that of 80 and 60 g/seedling compost and both controls.

The infestation of Foc in banana seedlings which can be observed from the percentage of affected leaves and the intensity of corm damage were suppressed with the application of compost. This was especially seen at the dosage of 100 and 120 g/seedling compost. The suppression was in turn influenced by population density of *T. viride* in seedling's rhizosphere. The addition of rice straw compost with the dosages of 100, 120, 140 g/seedling showed an increase in the density of *T. viride* during the growth of banana seedling (Table 3). From those results, the potency of the treatment of two months old banana seedling with 100 g/seedling of *T. viride*- T1sk rice straw compost around its rhizosphere was the most effective and potential dosage to delay the appearance of the first symptom, the suppressing of the percentage of the symptomatic leaves, as well as the percentage of corm damage intensity.

T. viride which growth in the rhizosphere of banana seedling competed Foc in terms of space, nutrition, and other limited factors needed by these two microbes to live and thrive in plant rhizosphere. Furthermore, *Trichoderma* spp. also produces secondary metabolites that able to inhibit the growth and parasitic to pathogens (Howell, 2003; Vinale, 2008). Nurbailis et al. (2016) reported that *T. viride* - T1sk has a high ability to colonize the roots of banana seedling to inhibit the contact between Foc and banana roots, hence suppressing the development of Foc.

Table-4: The appearance of the first symptoms, the percentage of symptomatic leaves and the intensity of corm damage caused by Fusarium Wilt on banana seedlings treated with *T. viride*-T1sk rice straw compost.

Treatment	Appearance of the first symptom (d)		Symptomatic leaves (%)		Intensity of corm damage (%)	
Control II (without compost)	6.25	D	49.00	A	66.67	A
Control I = Rice straw (without *T. viride*)	9.50	C	41.50	Ab	52.78	B
60 g/seedling	10.25	Bc	41.25	Ab	52.78	B
80 g/seedling	10.75	Bc	38.25	Abc	44.44	Bcd
140 g/seedling	12.25	Ab	35.50	Bcd	38.89	cde
100 g/seedling	13.50	A	28.25	Cd	26.39	e
120 g/seedling	13.50	A	26.00	D	34.72	de

Figures followed by the same letter are not significantly different at 5% level according to DNMRT

Conclusion

The addition of *T. viride*-T1sk inocula in rice straw as a decomposer increased the density of the antagonistic fungi in the compost. The highest density was found at the dose of 140 g/seedling (8.23 x 104) which was not significantly differ with the doses of 120 and 100 g/seedling. Two months after planting, the density of T. viride in rhizosphere was increased. The highest density was found in the treatment of 120 g/seedling compost (9.85 x 104) which was not differed with the doses of 140 and 100 g/ seedling.

Contribution of Authors

Nurbailis: Conceived idea, conducted experiment and write up of article
Ningrum ED: Helped in experiment and article write up
Trisno J: Helped in experiment, compilation of results and statistical analysis

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