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THE EFFECTIVENESS OF *Trichoderma harzianum* as BIOCONTROL AGENT AND MANURE IN CONTROLING *Fusarium oxysporum* f. sp. *passiflorae* ON SOUR PASSION SEEDLINGS (*Passiflora edulis* f. *edulis* Sims)

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ABSTRACT

The objectives of the research were to find out optimal dosage of *Trichoderma harzianum* and types of manure to decrease percentage of wilted plants and disease intensity of *Fusarium oxysporum* f.sp. *passiflorae* on sour passion fruit seedlings. The research was conducted at screen house and pest disease laboratory at Berastagi Experimental Farm from March to June 2010. The experiment was arranged in randomized block in factorial pattern with 3 replications. The first factor was *Trichoderma harzianum* propagule (T), T0=without *T. harzianum* (control), T1=*T. harzianum* mixed in 17 g corn medium/kg soil, T2=*T. harzianum* mixed in 25 g corn medium/kg soil, T3=*T. harzianum* mixed in 34 g corn medium/kg soil, T4=*T. harzianum* mixed in 42 g corn medium/kg soil. The second factor was the type of manure (k), namely: kA=chicken manure and kS=cattle manure. The result showed that propagule of *T. harzianum* mixed in 42 g steamed corn seeds/kg soil was the best dosage to decrease *Fusarium oxysporum* f. sp. *passiflorae* attack on sour passion fruit seedling with incubation period, percentage of wilted plant and length infection profile, namely 50 days after inoculation (dai), 3.33% and 7.1 cm respectively. *T. harzianum* decreased up to 88.5% disease intensity on sour passion fruit seedling. Propagule of *T. harzianum* mixed in 42 g corn medium/kg soil was the best dosage to influence plant height and leaf number, 34.26 cm and 6.71 sheets, respectively. The cattle manure was the best manure to decrease *Fusarium oxysporum* f. sp *passiflorae* attack on passion fruit seedling that showed from incubation period, percentage of wilted plant and length infection profile namely 38.13 dai, 29.33% 89.25% and 10.09 cm, respectively. The chicken manure contributed to plants height, while cattle manure contributed to leaf number, each 33.15 cm and 5.75 sheets, respectively.

Keywords: *Passiflora edulis* f. *edulis* sims, *Trichoderma harzianum*, manure, *Fusarium oxysporum*.

INTRODUCTION

Sour passion fruit plant (*Passiflora edulis* Sims) are belonging to family of Passifloraceae, it can bear fruit throughout the year. The plant propagates naturally with tendril. Passion fruit was developed in several provinces in Indonesia such as South Sulawesi, North Sumatra, West Sumatra and Lampung.

World marketing opportunities for fresh passion fruit and processed are very available, but it is difficult to obtain the availability of staple fruits. This condition caused by pests and disease attacked in cultivation. The main problem was Fusarium wilt caused by *Fusarium oxysporum* f. sp. *passiflora* (*Fop*). This fungus was soil borne pathogen attacking roots and causing considerable damage to the passion fruit plantation (Saragih and Silalahi, 2006). *Fop* inhibited water flow in xylem tissue (Mess *et al*., 1999, De cal *et al*., 2002). More over Pathogen is also capable to produce chlamydospores, so this pathogen can survive for long term in the soil without host plants (Nelson *et al*., 1983). In addition, such condition as acidic soil conditions (pH 5.0 to 5.6), the content of ammonium nitrogen (ammonium nitrate and urea), warm temperatures of soil (80^{o}F) and low soil moisture supported fungus growing (Varela and Seif, 2004; Cahyono 2008).

Management of Fusarium wilt was achieved mainly through chemical soil fumigation, but the broad spectrum biocides used to fumigate soil before planting particularly methyl bromide, were environmentally damaging and also may cause the appearance of more resistant pathogen population (Freeman *et al.,* 2002). The alternative and environmentally safe method of control was using of the microbial antagonists applied with compost or manure (Salim, 2003). Antagonistic microbes need organic material as a source of food and energy. Augment of manure in the planting medium can provide the necessary organic material that enhanced plant growth and activities of antagonists microbial to reduce pathogenic fungi growth (Muryati *et al*., 2009; Sumarni and Rosliani, 2009; Subandi and Ismiyati, 2007; Aryantha *et al*., 2000). Different types of manure contain different organic composition that affected soil microbial diversity and the ability of infection by pathogenic fungi in soil. According to Nasrun and Nuryani (2007) that augmenting of cow manure can restrain wilt disease progression on Pacthouly, while Muryati *et al.* (2009) stated that the chicken manure effectively decreases the progression of diseases caused by *Phythopthora palmivora* on durio seedlings.

Application of *Trichoderma harzianum* has also been widely reported and was able to control various plant diseases, including Fusarium wilt disease in tuberose flowers (Nuryani and Djatnika, 1999), green mold diseases of citrus fruits (Martoredjo *et al.,* 2001), and Fusarium wilt

of the gladiol (Rokhlani, 2005). Furthermore, *T. Harzianum* applied together with manure was also a crucial factor to restrain pathogen growth. Thus, based on the information above we conducted this study in order to find out optimal *T. harzianum* propagule combined with manure in suppressing of *F. oxysporum* f. sp. *passiflorae* caused wilt disease on the sour passion fruit seedlings.

MATERIALS AND METHODS

The research was conducted in the screen house and laboratory plant disease Experimental Farm Berastagi South Sumatra province. The experiment was conducted from March to June 2010. It was arranged in randomized block design (RBD) in factorial pattern with two factors and three replications, each treatment consisted of 5 plants. The first factor was the dosage propagule of *Trichoderma harzianum* (T), namely: T0=control (without *T. harzianum*), T1=*T. harzianum* mixed in 17 g medium corn/kg soil, T2=*T. harzianum* mixed in 25 g corn medium/kg soil, T3=*T. harzianum* mixed in 34 g corn medium/kg soil, T4=*T. harzianum* mixed in 42 g corn medium/kg soil. The second factor was the type of manure (k), namely: kA=chicken manure and kS=cattle manure

Isolate of *T. harzianum* used was a laboratory collection of Experimental farm Berastagi which has been conserved in PDA slant medium. This isolate was isolated into PDA and incubated for 7-10 days. The growing media for *T. harzianum* was steamed corn seeds. This media prepared by cleaned and steamed 4 kg of corn seeds with steamer for ± 60 minutes, and cooled on tray, then put the corn into heat-resistant plastic bags as much as treatment, and finally sterilized for 30 minutes at 120ºC. Further pure culture *T. harzianum* was inoculated about 2-3 borer into the corn and stir until blended, and then the culture was incubated for 10-15 days.

Medium for treatment consisted of top soil and compost manure mixture with 1:3 in ratio. Media were sterilized at 120ºC for about 1 hour by using a steamer. Then media were put into poly bags (1 kg) and incubated for 3 days and after that mixed with cultured *T. harzianum*. Sour passion seedlings used for treatment were a two-three weeks old and have 2-3 leaves. *Fo* inoculation used root dip technique (Nyvall and Haglund, 1976). The root of seedling were wounded and dipped for 3 minutes in 10 ml distilled water containing 10^6conidia/ml of *F. oxysporum* and then planted into poly bags.
The parameters observed include:

a) The incubation period of the disease, a time from time of inoculation to the first appearance of symptom.
b) Percentage of wilted plants (disease incidence): was observed at the end of observation (63 days) using the following formulae:

$$P = \left[\frac{T_1}{T_2}\right] \times 100\ \%$$

Where

P = disease incidence, T1 = number of infected plants, and T_2 = number of observed plants.

c) The disease intensity is calculated by counting the length infection profile (cross section severe). Cross section measurements were carried out at the end of experiment (63 days) by splitting the stem vertically and measuring the length of infection stem.
d) Vegetative growth was observed at the end of the experiment such as plant height and leaf numbers.

Data were analyzed by variance, if the results obtained are significantly different, further tested Least Small Differences (LSD) on the 5% level.

RESULTS AND DISCUSSIONS

The results showed that overall, biological control agents *T. harzianum* applied and two types of manure effective in suppressing wilt disease of the sour passion fruit on seedling. Based on percentage of wilted plants (Table-1), there was no interaction between two factors of treatment. In single factor, application of *T. harzianum* propagule mixed in 42 g corn medium/kg soil can reduce the percentage of wilted plant to 93.34% compared with the controls. Tindo (2008) reported that applying of 50 g dosage of *T. harzianum*/polybag decreased the percentage of wilted soybean plant (47.92%) that was caused by the *Sclerotium rolfssi.* A low number of plants attacked by *Fop* were probably due to *T. harzianum* propagules applied is sufficient in soil, so these antagonistic microbes thrive in nursery media, and this condition inhibited pathogen growth in the soil. Described by Suwahyono *et al*. (2003) that mechanism of suppression by *T. harzianum* such as produced chitinase enzyme and glucans, those can lysis hyphal of fungal and also caused protoplasm of pathogen breaks out from the cell.

Single factor of manure treated was also significantly affected the percentage wilted of sour passion fruit plants (Table-1). According Niswita and Yusnaini (2008) that application of manure can affect the diversity and activity of antagonism microorganisms in the soil, increasing of non pathogenic microbe activities including microbial antagonists which help plants to protect the diseases (Papavizas and Lummsden, 1980). The percentage of wilted of sour passion fruit plant by the cattle manure was 29.33%, lower than chicken manure that was 36.67% with the difference up to 7.34%. Low percentage of wilted plant was caused cattle manure has nutrients and fiber content (cellulose) higher than chicken manure (Hartatik and Widowati, 2010). Cellulose was essential for antagonist fungal growth, as described by Martina (2002) that *Trichoderma* produces hydrolytic enzymes, cellulase, pectinase and xylanase. Therefore be able to degrade the complex polysaccharides such as cellulose, pectin, hemicellulose and xylan.

Table-1. Incubation period, percentage of wilted and disease intensity measured based on length of infection profile on sour passion fruit stem.

Treatments	**Incubation period** (dai)	**Percentage of wilt** (%)	**Length of infection stem** (cm)
***T. harzianum* propagule dosage, g corn medium/kg soil**			
0	14, 10 d	96, 67 a	15, 21 a
17	30, 00 c	56, 67 b	12, 10 b
25	35, 25 bc	30, 33 c	10, 18 c
34	46, 62 b	26, 67 cd	8, 17 cd
42	50, 00 a	3, 33 e	7, 1 d
Manure types			
Chicken manure	25, 25 b	36, 67 b	12, 25 a
Cattle manure	38, 13 a	29, 33 a	10, 09 a

Besides being able to derive the percentage of wilted plants, the dosage of *T. harzianum* propagule and source of manure significantly affect the incubation period of the disease compared with controls (Table-1). Increasing of *T. harzianum* applied was able to extend incubation period of disease in plants. The longest incubation period of disease was *T. harzianum* propagule applied in 42 g corn medium/kg soil (50 dai), followed by 34 g corn medium/kg soil, 25 g corn medium/kg soil, 17 g corn medium/kg soil with incubation period ranging from 30 to 46.62 dai and significantly different from controls (14.1 dai). The length of incubation period of disease on sour passion fruit plants applied with the *T. harzianum* was caused by this fungus capable to grow fast, whereas fungus producing abundant spores. Beside that fungus can survive in unfavorable condition, so it can cause increasing of nutrient competition between fungal pathogens and antagonists. Thus inhibit the process of infection by propagules pathogens on plant. Prabowo *et al*. (2006) stated that because of colonization by *T. harzinum* to the root system makes difficulties for pathogen to penetrate plant tissue. In addition *T. harzianum* can produce toxins and gliotoksin viridin as antibiosis (Howell and Stipanovic, 1983), which seep into the tissue and inhibit fungal propagules.

Application of manure also significantly affect the incubation period of disease on sour passion fruit seedling, the incubation period of disease by cattle manure applied was 38.13 dai, significantly different from chicken manure applied (25.25 dai), with the difference up to 12.88 days. This suggested that the cattle manure can inhibit growth rate of Fusarium propagule in soil compare with chicken manure.

The effectiveness of *T. harzianum* in suppressing of disease progression can also be seen from the disease intensity of plant. In this experiment the disease intensity was measuring a length of infection stem of sour passion fruits. Applying of *T. harzianum* significantly affect cross-sectional of length infection, but not significantly different between types of manure (Table-1). Based on the analysis of variance, there was a difference between the cross section of infection sour passion stem which applying with *T. harzianum* and control. The length of infection stem on the control was 15.21 cm, significantly different from other treatments. Without applying of *T. harzianum*, *Fop* rapidly infected plant roots and spread to the vascular tissues of stem. Plant was directly interfered in taking water and mineral from the soil. Shorter cross-section showed the effectiveness of *T. harzianum* in suppressing of *Fop* in plant tissue. The length of infection stem applied with *T. harzianum* propagule mixed in 42 g corn medium/kg, 34 g corn medium/kg, 25 corn medium/kg and 17 g corn medium/kg soil were 7.1 cm, 8.17 cm, 10.18 cm and 12.10 cm, respectively. According to the Soesanto (2008) that hyphal of *T. harzianum* can grow along the pathogens, twist or break down the cell walls of *Fusarium.*

Statistical analysis for vegetative growth of passion fruit showed that there was no interaction between *T. harzianum* propagules applied and types of manure (Table-2).

Table-2. Vegetative growth of passion fruit was applied with several dosages of *T. harzianum* and manure types, 63 days after planting.

Treatments	**Plant height** (cm)	**Leaf number** (sheet)
***T. Harzianum propagule dosage,* g corn medium/ kg soil**		
0	18, 02 e	3, 11e
17	20, 66 d	4, 04 d
25	22, 45 c	5, 22 c
34	26, 31 b	6, 10 b
42	34, 26 a	6, 71 a
Manure types		
Chicken manure	33, 15 a	4, 42 b
Cattle manure	27, 88 b	5, 75 a

T. harzianum propagules in 42 g corn medium/kg soil was best application for plant height and leaf number compared with other treatments. According to Levy *et al*. (2004) that *Trichoderma* directly affects plant growth by producing plant growth-regulating hormones, further *Trichoderma* can break down organic matter in soils, so it is easy to absorb by plants.

Differences of vegetative growth between control and treatment was assumed because of high disease intensity of Fusarium on seedling without antagonist microbe, thus disturbed physiology processes of plants. This was reliable with Ward *et al*. (2009) that the substance was produced by mycelium and *F. oxysporum* blocks xylem vessels, therefore translocation of water and the absorption of minerals in the soil to be disturbed.

Types of manure applied affected in increasing of plant height and number of leaves of sour passion fruit, where the highest plant height was found in plants applied with chicken manure (33.15 cm), while the lowest on cattle manure (27.88 cm). For the number of leaves, cattle manure applied in soil produced a number of leaves more than chicken manure, 5.75 and 4.42 sheets respectively. This condition indicated that an organic fertilizer application derived from chicken and cattle manure involved to the growth of passion fruit seed plants.

CONCLUSIONS

a) Propagules of *T. harzianum* mixed in 42 g corn medium/kg soil was the best application to decrease *Fusarium oxysporum* f. sp. *passiflorae* attack on sour passion fruit seedling with incubation period, percentage of wilted plant and length of infection stem, namely 50 dai, 3.33% and 7.1 cm respectively, and it was potential to decrease to 88.5% disease intensity on sour passion fruit on seedling.
b) Propagules of *T. harzianum* mixed in 42 g corn medium/kg soil was the best application to influence plant height and number of leaf, 34.26 cm and 6.71 sheets, respectively.
c) The cattle manure was the best manure to decrease *Fusarium oxysporum* f. sp *passiflorae* attack on passion fruit seedling showed from incubation period, percentage of wilted plant and length of infection stem namely 38.13 dai, 29.33% 89.25% and 10.09 cm respectively.
d) The chicken manure more pretends to plants height, while cattle manure to leaf number that were 33.15 cm and 5.75 sheets respectively.

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