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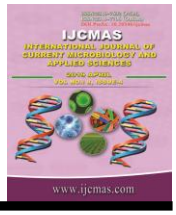

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Secondary Metabolite Production by *Trichoderma* spp and its Potential as Antibacteria

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A B S T R A C T

Trichoderma can produce secondary metabolites which act as anti-bacteriawhichpotential to be used for controlling the plant pathogen like *Xanthomonas axanopodis* pv. alii. The purpose of this study was to known the best interaction beetween the filtrates of *Trichoderma* spp and cocentration of the filtrate for reducing the growth of X. axanopodis pv. alii caused leaf blight disease on red onion. The method of secondary metabolite production of *Trichoderma* spp was the single culture. The design used was factorial in a complete randomized design with 2 factors and 4 replications. The first factor was the filtrate originating from *Trichoderma* spp, i.e. *T. harzianum, T. koningii* and *T. viride* and the second factor was the concentration of *Trichoderma* spp, i.e. 0%, 25%, 50%, 75% and 100%. Parameters observed were: wide of clear zone, the amount of colony and the growth rate of of *X. axanopodis*. The result show that all *Trichoderma* filtrate can inhibit the growth of *X. axanopodis* pv. *alii*. The higher of concentration the more depressed the pathogen growth. The interaction between the filtrate and the concentration indicated that the filtrate of *T. harzianum* with a concentration of 100% could inhibit the total growth of *X. axanopodis pv. alii.*

Introduction

Bacterial leaf blight disease caused by *Xanthomonas axanopodis* pv. alii (*Xaa*) is an important disease on onion (Kadota *et al*, 2000; Roumanag *et al*, 2004; Habazar *et al.*, 2007). Loss of the yield due to the attack of this pathogen can reach 100% especially if suitable environment (Schwart and Gent, 2006).

Some methods that have been carried out for controlling this pathogen are: crop rotation with the non-hosts, resistant varieties, healthy seeds and chemical control using bactericides (Paulraj and Garro, 1993; Schwartz and Gent, 2006). In Indonesia, information about controlling this pathogen is still limited. For this reason, it is necessary to develop various research methods that environmentally friendly, one of which is to use a biopesticide derived from *Trichoderma* spp.

Trichoderma is one of the soil fungi that is antagonistic to various pathogens that cause plant disease (Cook and Baker, 1983; Nurbailis, 1992; Harman, 2006; Pusvapavathi *et al.,* 2016). This mechanism of antagonism

is competition, mycoparasites, and antibiosis (Cook and Baker, 1983; Howell, 2003; Nurbailis, 2008). Nurbailis *et al.,* (2006) reported that *T. viride* and *T. harzianum* isolates from banana rhizosphere were able to inhibit the growth of *F. oxysporum* f.sp. *cubense* with the antibiosis mechanism.

Antibiosis is a mechanism of antagonistic fungi that can inhibit the growth of pathogens with antagonistic chemical products that produced and released by *Trichoderma* into their environment, There are extra cellular enzyme systems, antibiotics which damage the pathogens (Cook and Baker 1983; Leelavathy *et al.,* 2014) Antibiotic compounds can be used as an alternative to substituting artificial pesticides for controlling plant pathogens

Trichoderma spp produces secondary metabolites which act as antifungal and antibacterial such as polyketides, pyrones, and terpenes (Naher *et al.,* 2014). Leelavathy *et al.,* (2014) reported that crude extracts of *T. harzianum* with different concentrations can inhibit the growth of various pathogenic bacteria. Effective concentration in inhibiting the growth of *Staphylococcus aureus*, *Escheria coli* and *Klebsiella* was 100 µl / ml with clear zone area 1.8 - 2.0 cm. Basiriya *et al.,* (2017) report that secondary metabolites (crude extract) from *Trichoderma* spp indigenoes mangrove rhizosphere inhibited the growth of *S. aereus*, *E. coli* and *Pseudomonas auregenesa*. The best isolates were *T. harzianum* (1) and *T. viride*.

The development of using *Trichoderma* spp which indicates the presence of an antibiosis mechanism for controlling *Xaa* on red onion requires the research about Utilization of secondary metabolites from *Trichoderma* spp to inhibit the growth of Xaa. The purpose of this research were to obtain superior *Trichoderma* isolates which is capable to produce secondary metabolites which act as antibacterial compounds and the best concentration for inhibiting the growth of *Xanthomonas axonopodis* pv. *allii.*

Materials and Methods

The research was conducted at the Microbiology laboratory of the Faculty of Agriculture Andalas University, Padang from April to November 2018.This research used factorial in Complete Randomized Design which consist of 2 factors, 15 treatment combinations and 3 replications was employed in this study. The first factor was the filtrate (secondary metabolite) of *Trichoderma* spp, ie: *T. viride*, *T. harzianum* and *Trichoderma* PP3. The second factor were the filtrate concentration which consist of 0%, 25%, 50%, 75% and 100%. The data were analyzed by variance and with continued Duncan's multiple distance test (DNMRT) at a 5% significance level

Implementation

Propagation of *Trichoderma* spp.

Trichoderma spp.: *T. viride*, *T. harzianum*, *T. koningii*, which had been shown antibiosis mechanism, were propagated in Potato Dextrosa Agar medium and incubatedin room temperature for 7 days.

Propagation of *Trichoderma* spp in liquid culture

Trichoderma was propagated in liquid culture in Potato Dextrosa Broth medium. For every 1 liter of medium used as much as 100 ml of starter (10% total volume) and incubated for five days at room temperature, then the culture was incubated using a shaker at a speed of 180 rpm for 7 days. (Kumar *et al*, 2014).

Preparation of secondary metabolite of *Trichoderma* spp

Trichoderma spp were propagated in a liquid medium as mentioned above, used to obtain filtrate by separating the liquid culture between the hifa and the filtrate by using What man filter paper, then centrifuged at 4000 rpm for 30 minutes. The filtrate was filtered again with What man paper into another test tube, finally a milipore filter membrane (0.2 µm) was used for filtering the filtrate.

Preparation of *Xanthomonas axonopodis* pv. allii Culture

Xantthomonas axanopodis pv. alii was obtained from the collection of the Laboratory of Microbiology, Faculty of Agriculture, Andalas University, were rejuvenated on Nutrient Glucose Agar (NGA) medium by scratching method and incubated for 48 hours at room temperature

Treatment of *Trichoderma* filtrate against *Xanthomonas axonopodis* pv. allii

Trichoderma filtrate was prepared with various concentrations, each of filtrate was taken 1 ml and mixed evenly with 9 ml of NGA medium which was still hot (45^0C), then the medium was cooled. The *Xanthomona saxonopodis* pv. allii (10^{-4} cells / ml), Spread on the medium and incubated for 48 hours at room temperature.

Testing of *Xanthomonas axonopodis* pv. allii growth inhibition carried out byusing sterile disc paper, filter paper is cut circularly with a diameter of 0.5 cm,soaked into each filtrate for 5 minutes, placed on streaks of *Xanthomonas axonopodis* pv. allii in petri dishes and incubated for 24 hours at room temperature, for a control was used sterile aquades.

Observation

Inhibitory Power of *Trichoderma* Filtrate againts *X. axanopodis* pv. alii Growth

The inhibitory power of *Trichoderma* filtrate against *X. axanopodis* pv. Alii *X. axanopodis* pv. alii growth is done by carving a clear zone formed on paper discs that contain Trichoderma spp filtrate. Measurements are made by drawing the area of the clear zone formed on transparant plastic and measured with a ruler.

Number of *X. axanopodis* pv. alii colonies by treatment with *Trichoderma* filtrate

Observation of the number of colonies was carried out by counting the number of *X. Axanopodis* pv. alii colonies by using colony counter, observations carried out at 12, and 24 hours after inoculation.

Results and Discussion

Growth inhibition of *X.axanopodis* pv. alii that treated with *Trichoderma* filtrate

In general, secondary metabolites of*Trichoderma* spp could form a clear zone for inhibition of *X.axanopodis* pv. alii growth. The area of clear zone is different between isolates and concentrations (Table 1).

The formation of a clear zone indicates that the secondary metabolites produced by *Trichoderma* spp contains anti-bacterial compounds. *T. viride* and *T. harzianum* isolates form higher clear zones compared with *Trichoderma* PP3. According to Naher *et al* (2012) some secondary metabolites produced by *Trichoderma* spp such as polyketides, pyrones, and terpenes act as antibacterial and anti fungal. Basiria *et al* (2017) reported that *T. harzianum* (1) and *T. viride* could inhibit the growth of gram +

staphylococcus aureus and gram negative *Escheria coli*.

The number of *Xanthomonas axanopodis pv.alii* colonies treated with the *Trichoderma* spp. filtrate

The number of *X. axanopodis pv.alii* colonies with the treatment with various concentrations of *Trichoderma* spp. showed significant differences between 12 hours and 24 hours incubation (Table 2 and 3).

T. harzianum filtrate showed the better inhibition of *X. axanopodis pv.alii*colonization growth than *T.viride* filtrate and *Trichoderma* PP3. The treatment of *T. harzianum* filtrate with the concentration of 25% could reduce the number of *X. axanopodis pv.alii* colonies compared with without filtrate (control). Increased the concentration of *Trichoderma* spp. filtrate 50% and 100% the growth of *X. axanopodis pv.alii* colonies becomes zero or *X. axanopodis pv.alii* could not grow. This showed that the secondary metabolites produced by *T. harzianum* contain anti-bacterial compounds that could inhibit the growth of *X. axanopodis pv.alii*. Leelavathy *et al.*, (2014) reported that the secondary metabolites or crude extracts of *T. harzianum* could inhibit the growth of various pathogenic bacteria Staphylococcus aureus, E coli, Klebsiella, effective concentration of 100 µl / ml aquades.

Table.1 Area of clear zone of growth inhibition of *Xanthomonas axanopodis pv.alii* with various concentrations of *Trichoderma* spp filtrate in 12 hours incubation

The Kind of Filtrate	Area of Clear Zone (mm)				
	Filtrate cocentration (%)				
	0	**25**	**50**	**75**	**100**
T. harzianum	0.00	1.0 ab	6.0 b	6.0 b	6.0 b
T. viride	0.00	6.0 b	8.0 ab	6.0 b	8.0 ab
***Trichoderma* PP3**	0.00	4.0 b	4.0 b	1.0 ab	4.0 ab

The numbers followed by the same lowercase letter on the same lane are not significantly different according to DMNRT level 5%

Table.2 The number of *Xanthomonas axanopodis pv.alii* colonies treated with various concentration of *Trichoderma* spp. filtrate in 12 hours incubation

The Kind of Filtrate	The amount of *Xanthomonas axanopodis pv.alii* colony 10^4cell/ml				
	Filtrate cocentration (%)				
	0	25	50	75	100
T. harzianum	33.66 a	10.66 ab	0.00 c	0.00 c	0.00 c
T. viride	33.66 a	33.66 a	26.66 ab	12.00bc	0.00 c
***Trichoderma* PP3**	33.66 a	27.66 ab	14.00bc	16.66 bc	26.66 ab

The numbers followed by the same lowercase letter on the same lane are not significantly different according to DMNRT level 5%

Table.3 The number of *Xanthomonas axanopodis pv. alii* colonies treated with various concentration of *Trichoderma* spp. Filtrate in 24 hours incubation

The Kind of Filtrate	The amount of*Xanthomonas axanopodis pv.alii* 10^4cell/ml				
	Filtrate Cocentration (%)				
	0	25	50	75	100
T. harzianum	59.00 a	22.33cd	0.00 c	0.00 c	0.00 c
T. viride	59.00 a	74.00 a	45.66 ab	24.66 cd	0.00 c
***Trichoderma* PP3**	59.00 a	44.66 bc	34.00 bc	38.66 bc	49.66 ab

The numbers followed by the same lowercase letter on the same lane are not significantly different according to DMNRT level 5%

The secondary metabolites produced by *T. viride* at the concentration of 25% were not able to inhibit the growth of *X. axanopodis pv.alii*. This can be seen in the incubation period of 12 hours the number of *X. axanopodis pv.alii* colonies was the same as the control treatment, namely 33.66. 10^4sel / ml suspension. This shows that the concentration of 25% did not affect the growth of *X. axanopodis pv.alii*. Increasing the cocentration 50% - 75% caused a decrease the growth of *X. axanopodis pv.alii* to 45.66 and 24.66 cells / ml suspension and at concentration 100% *X. axanopodis pv.alii* growth to be zero. Basiriya *et al.,* (2017) report that secondary metabolites (crude extract) of *T. harzianum* and *T. viride* indigenoes mangrove rhizosphere were the good isolatesin inhibiting the growth of *S.aereus*, *E. coli* and *Pseudomonas auregenesa*.

In conclusion, *Trichoderma*s pp. filtrate couldinhibit the growth of *X. axanopodis* pv. *alii*. The higher of concentration the more depressed the pathogen growth.The interaction between the filtrate and the concentration indicated that the filtrate of *T. harzianum* with a concentration of 100% could inhibit the total growth of *X. axanopodis*pv. alii

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