Inhibitory Power Test of Two *Trichoderma* Isolates in In Vitro Way Againts *Fusarium oxysporum* The Cause of Red Chilli Stem Rot

A Wachid* and Sutarman

Departement of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sidaorjo, Sidoarjo, Indonesia

*awachid@umsida.ac.id

Abstract. This study aims to determine the control power of two isolates of *Trichoderma* obtained from the forest floor soil to the activity of *Fusarium oxysporum* pathogen causing stem rotation of red chili plant in vitro. The experiment was arranged in complete randomized design using 3 kinds of treatment of *Trichoderma* sp., *T. harzianum* isolate and control (without *Trichoderma* fungi isolate) tested for its antagonistic ability against pathogen; each treatment was repeated 4 times. The observed variable is pathogen inhibition (%). The observational data were analyzed by using variance followed by 5% HSD test to know the difference between treatments. The results showed that *T. harzianum* (Tc-Jjr-02 isolate) and *Trichoderma* sp. (Tc-Pjn-01 isolate) were able to inhibit *F. oxysporum* pathogens respectively 76.9% and 77.1% at 108 hours after inoculation. Both of these *Trichoderma* fungal isolates can be developed into biocontrol agents for *F. oxysporum*.

1. Introduction

Red Chili is one of strategic horticulture commodities in Indonesia. There are varies disturbance on its production. One of them is disease attack, which caused the most losses. Therefore, there were many studies that provided protection technology for plant healthiness against pathogen disturbance. Climate change, on the other hand, can lead to co-evolution between pathogens and plants [1]. The process of co-evolution can lead to new diseases [2]. *Fusarium oxysporum* isolate collection of the Mycology Laboratory (Bogor Agricultural University or IPB) which isolated from red pepper plant showed symptoms of stem rot and potentially born dangerous disease in Indonesia. *F. oxysporum* fungi has been widely recognized as a dangerous pathogen of important crops in different parts of the world.

Various studies have shown that *Trichoderma* fungi are potentially utilized as control agents of various cultivated plants. Important *Trichoderma* fungi characters are: capable of producing secondary metabolites that can induce plant pathogen resistance, produce plant hormones, and nutrients to increase growth of plants and crops [10-13]. Currently the Microbiology Laboratory of the Faculty of Agriculture Universitas Muhamamdiyah Sidoarjo has collected at least 30 *Trichoderma* isolates. Four of the 30 isolates were selected because of their potential as biocontrol agents and...
biofertilizer agents [2]. The test results are an opportunity to prepare potential isolates as biocontrol agents against *F. oxysporum* as an effort of support to the production of red chillies for both consumption purposes and for producing seeds.

This study aims to explore the inhibitory power of *Trichoderma* sp. isolate Tc-Clkt-01 and *T. harzianum* isolate Tc_Jjr-02 against *F. Oxysporum* which isolated from red chilli.

2. Materials and methods

Study held in Microbiology Laboratory of Agriculture Faculty of Universitas Muhammadiyah Sidoarjo, Candi-Sidoarjo.

Fungi *F. oxysporum* (collection of Microbiology Laboratory, IPB) and *Trichoderma* sp isolates Tc-Clkt-01 and *T. harzianum* isolates Tc-Jjr-02 (collection of Microbiology Laboratory of Faculty of Agriculture, Universitas Muhammadiyah Sidoarjo) were grown on PDA media given chloramphenicol to prevent bacterial contaminants [14]. After culture was incubated for 8 days, we taken a round shot by 5 mm diameter from one type of *Trichoderma* culture and one from pathogen culture using a *cook borer*. Both samples were taken using an ose needle and placed face to face on the surface of PDA-chloramphenicol medium in a petri dish with a distance of 5 cm. Meanwhile, in parallel way, a footage of a 5 mm diameter pathogen culture was placed in the center of the media surface. Furthermore the edge of the cup after being heated over a bunsen flame is coated with plastic tape to prevent contamination. Each of these experimental units is repeated four times. All petri dishes in this experiment were incubated for 108 hours at 28 ± 2 ° C. The observations were performed every 12 hours by measuring the diameter of *F. oxysporum* isolate colony to *Trichoderma* fungi isolate and compared to cultured *F. oxysporum* diameter without biocontrol agents as controls. The comparison between the colony diameter of the control pathogen and diameter of pathogen colony which inhibited by *Trichoderma* against the diameter of control pathogen colony is the pathogen inhibitory power that expressed in the percentage [15, 16]. The experiments were arranged in a complete randomized design and the data were analysed by 5% ANOVA followed by 5% HSD test.

3. Results and discussion

Variance analysis result showed a significant difference on treatments effect which at once showed inhibition against pathogen by both *Trichoderma* isolates. The inhibitory power growth pattern of biocontrol agent from 24 HAI to 108 HAI is shown in Figure 1.
The inhibitory growth of the two pathogens varied slightly to 48 HAI, but from 60 to 108 HAI became the same. From 36 HAI, it appears that both \textit{Trichoderma} biological agents have inhibition of pathogens. The mean inhibitory power of both \textit{Trichoderma} isolates was not significantly different as shown in Table 1.

Table 1: Mean percentage of inhibition against pathogen 36-108 HAI$^*$

| Treatment     | 36 HAI | 60 HAI | 84 HAI | 108 HAI |
|---------------|--------|--------|--------|---------|
| Tc-Clkt-01    | 6.60 b | 26.43 b| 71.90 b| 76.85 b |
| Tc-Jjjr-02    | 7.85 b | 25.48 b| 72.23 b| 77.15 b |
| Control       | 0.00 a | 0.00 a | 0.00 a | 0.00 a  |
| BNT 5%        | 6.09   | 12.66  | 4.10   | 3.37    |

$^*$) HAI is hour after inoculation
$^{**}$ Numbers followed by the same letter in the same column show no significant difference in 5% HSD test.

The pattern of inhibitory growth was relatively similar between the two \textit{Trichoderma} isolates tested primarily from 60 to 108 HAI. This indicates that both isolates have the same ability to produce inhibitory performance against pathogens. This pathogen inhibiting ability is supported by \textit{Trichoderma}'s ability to produce secondary metabolites [17, 7] so as to have the ability to suppress pathogens [18, 8] with considerable inhibitory power of 76.9% and 77.1%, produced by isolates \textit{Trichoderma sp.} Tc-Clkt-01 and \textit{T. harzianum} Tc-Jjjr-02 respectively.

4. Conclusion
Fungi \textit{Trichoderma sp.} isolate Tc-Clkt-01 and \textit{T. harzianum} isolate Tc-Jjjr-02 have high in vitro inhibitory power and have potential as biocontrol agent for \textit{Fusarium oxysporum} which causing red
stem rot disease. The implication of the research is the Trichoderma fungi isolates can be developed to become the biocontrol agents.

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