Evaluation of *Trichoderma asperellum* Effect toward Anthracnose Pathogen Activity on Red Chili (*Capsicum annum* L.) As Ecofriendly Pesticide

Sutarman, Triana Setiorini, Arrohmatus Syafaqoh Li'aini, Purnomo and Ali Rahmat

***Abstract*—The use of synthetic pesticides has been widely practiced by farmers to control plant diseases. However, it can promote negative impacts such as environmental pollution, threatening human health, and making pathogens more resistant. This study aims to determine the inhibition of *Trichoderma* Tc-Jjr-02 *in vitro* against *Colletotrichum* sp. and test its ability as a biopesticide and biofertilizer agent in suppressing the growth of anthracnose disease and chili production. The results show that, there was no interaction effect between the application of *Trichoderma* Tc-Jjr-02 as a biopesticide and biofertilizer agent. The *in vitro* test reveals that *Trichoderma* Tc-Jjr-02 could inhibit the growth of pathogenic colonies up to 61.4% at 11 days after inoculation. *In vivo* test show, that application of *Trichoderma* 6 hours before inoculation of pathogens (T1) and *Trichoderma* inoculation concurrent pathogens (T3) can reduce the symptoms intensity of anthracnose attacks 70% and 43%, respectively, then increasing the number of fresh fruit 62.66% and 76.58%, respectively, also increasing fresh fruit weights 84.83% and 91.90% respectively, compared with the pathogen inoculation treatment six hours before the application of *Trichoderma*. Thus, *Trichoderma* is more effective when applied before inoculation of pathogens and more suitable as a prevention agent. Using *Trichoderma asperellum* as biopesticed can protect the environment.**

Index Terms*—Anthracnose, *Colletotrichum* sp., red chili, *Trichoderma asperellum.

I. Introduction

Chili is one of the strategic commodities in Indonesia, which at certain times is in short supply. Various ways have been tried to increase the production, such as crop rotation and intercropping. However, the problem of soil fertility and threats from pests and plant diseases caused by pathogens are difficult to avoid.

In almost all over the world, chili always gets dangerous pathogenic disorders, including *Colletotrichum* spp., which causes losses up to 80% [1], [2]. *Colletotrichum* is a pathogenic fungus that is the main cause of damage and loss of chili yields in the tropics and subtropics regions [3], mostly related to anthracnose disease [4]. Fungi from the *Colletotrichum* group can attack fruit in the post-harvest period [5]. The threat of this fungus cannot be ignored due to its life cycle pattern that can change from necrotrophic to hemibiotrophic, making it difficult to detect and control it [6].

Applying synthetic fungicides to control pathogens can negatively affect to the environment. The residue pesticide is recognized as the main component that pollutes the environment and promotes a serious threat to human health such as a trigger for cancer and neurotoxicity. The use of fungicide continuously cannot guarantee increased the production, and on the contrary, can cause resistance to pathogens that cause anthracnose disease [7]. *Colletotrichum* is resistant to benomyl [8] the active ingredients most often used in Indonesia. The use of chemicals is often not feasible and is not economical to control soil borne diseases [9]. In addition, using chemical fungicide have a negative impact on beneficial soil microbial life, fungicide residues can also reduce food safety [10]. For this reason, it is necessary to find alternatives in the use of effective biocontrol organisms and other alternatives that can improve plant health. The application of *Trichoderma* both as a biofertilizer agent and as a potential biopesticide agent applied to plant canopy is one of the answers to the challenges of controlling anthracnose pathogens by *Colletotrichum* spp.

The use of biological agents in the last two decades, especially *Trichoderma*, has been increasingly developed. In several research results it is known that *Trichoderma* is one of the fungi that have the potential to protect plants and attack fungal pathogens [11]-[13]. More than 60% of biofungicides with active ingredients from *Trichoderma* are used to bring up complex mechanisms that involve interactions between Plant-*Trichoderma*-Pathogens [14] in order to control pathogens. *Trichoderma* has also been used as a biofertilizer given its ability to degrade organic matter to produce nutrients for plants and growth regulating compounds [13], [15] and their activities in roots can induce systemic plant resistance [16]. Thus the use of *Trichoderma* today is not only as a biofungicide which means saving the use of fungicidal chemicals but also as a biofertilizer that can improve plant performance, health, and production [17], [18].

On the other hand, the ability of several types of *Trichoderma* to help plants overcome soil acidity [19], making this fungus potential to be applied as a biofertilizer in dry land to increase soil productivity agronomically [20] in acidic soil.

However, there are many reports about the success of Trichoderma application more in the context of controlling the soil-borne disease. Meanwhile, applications in the canopy,

Manuscript received August 22, 2021; revised November 10, 2021. This work was supported the Ministry of Research and Technology and Higher Education for the 2019 Applied Research Higher Education grant awarded under contract number 009/II.3.AU/14.00C/PRY/III/2019.

Sutarman and T. Setiorini are with Agrotechnology Department, Muhammadiyah University of Sidoarjo. Jl. Raya Mojopahit 666B, Sidoarjo, East Java, Indonesia (e-mail: sutarman@umsida.ac.id, triana@gmail.com).

A. S. Li'aini and Ali Rahmat are with Badan Riset dan Inovasi Nasional, Indonesia (e-mail: syafa.liaini@gmail.com, alyrahmat@yahoo.com).

Purnomo is with Plant Protection Department, Faculty of Agriculture, University of Lampung. Jl. Sumantri Brojonegoro No.1 Bandarlampung 3514, Indonesia (e-mail: purnomo.1964@fp.unila.ac.id).

including fruit, have not been widely used during the production process of plants. Pathogenic disorders in fruit also require more attention, including chilli as one of the strategic commodities of horticulture.

This study aims to determine the inhibition of *Trichoderma asperellum* against pathogen *Colletotrichum* spp. both *in vitro* and *in vivo* through its application as a biopesticide agent and as a biofertilizer agent, each of which is applied to the surface of red chillies (canopy) and into the soil and to determine its effect on the growth index of anthracnose disease and curly red chili production.

II. Research Method

A. Preparation of Colletotrichum and Trichoderma Isolates

Pathogen isolates were isolated from anthracnose symptomatic red chilies from farmers' land in Sidoarjo, East Java. Koch's Postulate was performed, and it was shown that the isolate was *Colletotrichum* sp. then labeled as Col-Sb-01 isolate. *Trichoderma* Tc-Jjr-02 isolate was the collection of Microbiology Laboratory, Muhammadiyah University of Sidoarjo. *Trichoderma* and *Colletotrichum* sp. propagated in the PDA-chloramphenicol (PDA-c) media. After being incubated for 10 days, isolates were used for *in vitro* and *in vivo* experiments.

B. In vitro Inhibitory Assay

To assay the *in vitro* inhibitory effect, *Colletotrichum* sp. Col-Sb-01 and *Trichoderma* sp. Tc-Jjr-02 were taken using a 0.5 cm diameter cork borer then placed face to face on PDA-c media. The fungal then incubated for 120-144 hours. As a comparison, each isolate of *Colletotrichum* sp. and *Trichoderma* sp. was grown on PDA-c media individually. *Trichoderma* inhibitory effect against *Colletotrichum* calculated by the formula (1):

$$D = ((a\text{-}b)/a) \times 100\% \quad (1)$$

With the following provisions; a: the radial growth of *Colletotrichum* colony without *Trichoderma*, b is the distance of growth from the center of *Colletotrichum* colony towards *Trichoderma* at the same time. Observation of colony radii is done every 24 hours to 120 hours after inoculation.

C. Preparation of in Vivo Test Materials

Soil treatment was made from ready-to-use compost formulated with *Trichoderma* sp. Tc-Jjr-02 as much as 10.5 L of water and 350 ml *Trichoderma* suspension was mixed with 50 kg of compost then aerobically incubated for one month. After incubation, the population density of *Trichoderma* was measured and shown 6.3×10^8 CFU/g. Meanwhile, to assay *in vivo* inhibitory effect, *Trichoderma* and *Colletotrichum*, which have been incubated for 12 days, were then harvested and diluted at 2×10^8 CFU/ml.

D. Plant Preparation

Preparation of test plants was carried out in a greenhouse using sterile soil. The soil was obtained from a paddy field in Purwojati Village, Ngoro, Mojokerto, East Java. The soil was sterilized in an autoclave for 20 minutes at 121 ℃. Before the soil was used, the average soil pH was measured at 4.7. Chilli seeds were sown in a tray for one week then transferred to a 5 cm diameter polybag. At 35 days after planting, the seedlings were transferred to a 30 cm diameter polybag.

E. In Vivo Biocontrol Efficacy

At 60 days after planting, a total of 150 g of compost that has been formulated with *Trichoderma* Tc-Jjr-02 was added to the chili growing media. Compost without *Trichoderma* Tc-Jjr-02 was used as control. After 2 weeks, the inoculation of *Colletotrichum* and *Trichoderma* were carried out. Two healthy chilies with the average length up to 10–11 cm in each plant were sprayed with sterile distilled water, scratched using a cotton bud and carborandum to form a 5 mm wound, then sterilized again using sterile distilled water, then air-dried.

Every 3 ml of *Trichoderma* and *Colletotrichum* suspensions were inoculated on each injured chili using a hand sprayer. *Colletotrichum* inoculation was conducted in 3 ways; before, after, and simultaneously with the *Trichoderma* application. For the simultaneous method, every 3 ml of *Trichoderma* and *Colletotrichum* were mixed in a hand sprayer. Each plant then covered using a clear plastic bag to avoid the transfer of fungal spores. After the application of *Trichoderma* and *Colletotrichum*, the plant watering process was conducted at the growing media without touching the canopy.

F. In Vivo Test Trial Design

In vivo inhibitory assay arranged factorially in a completely randomized design (CRD). The first factor was the application of *Trichoderma* as biofertilizer; (1) non-*Trichoderma* fertilizer, and (2) *Trichoderma* biofertilizer. The second factor was the inoculation methods of *Trichoderma* and *Colletotrichum*; (T0) plants inoculated by *Colletotrichum* used as control, (T1) inoculation of *Trichoderma* sp. 6 hours before *Colletotrichum*, (T2) inoculation of *Trichoderma* sp. 6 hours after *Colletotrichum*, and (T3) inoculation of *Colletotrichum* and *Trichoderma* sp. simultaneously. Each combination of the two factors was repeated 4 times, so there were 32 experimental units. Each experimental unit consists of four plants, so there were 128 plants used in this study.

G. Observation Variables and Statistical Analysis

Variables observed in the *in vivo* assay were disease severity, the number of healthy fruits, and fresh weight of healthy fruits per plant at the end of the observation. The disease index observed from 2 to 14 days after inoculation and calculated using the formula (2) [10].

$$DS = [\sum_{i=1}^{k=4}(i.ni)/(N.k)].100 \quad (2)$$

DS is disease severity (%), *i* is score of disease index, ni is the total of plants in *i* index, *N* is the number of plants observed, and k is the highest *i* index.

Disease index that used in this experiment consists of five severity scale; 0 indicates no symptom, 1 indicates 1–20% rotten area, 2 indicates 21–40% rotten area, 3 indicates the 41–60% rotten area, and 4 indicates more than 60% rotten area.

The data were analyzed by analysis of variance then

further analyzed with Tukey HSD test at 5% significance level. Statistical analysis uses the SPSS statistical analysis package (Version 10.0).

III. Result

A. In Vitro Inhibitory Assay

The result of *in vitro* inhibitory assay shows that *T. asperellum* Tc-Jjr-02 was able to inhibit the radial growth of *Colletotrichum* sp. (Fig. 1). The maximum inhibitory potential of *T. asperellum* Tc-Jjr-02 was performed by 120 hours after incubation (Table I).

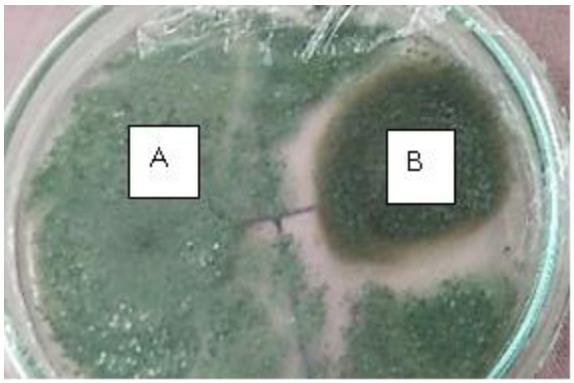

Fig. 1. Inhibitory effect of *Trichoderma asperellum* (A) toward *Colletotrichum* sp. (B) on PDA-chloramphenicol media at 144 hours/ 6 days after inoculation.

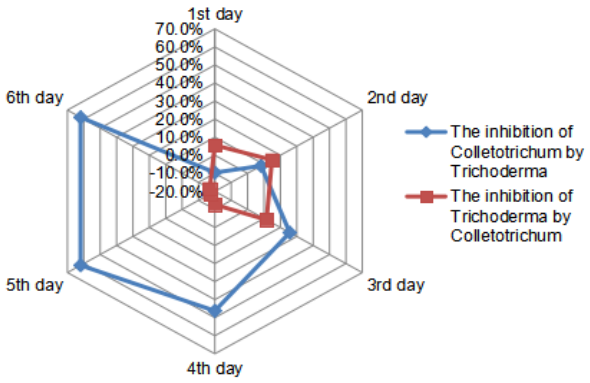

Fig. 2. *In vitro inhibitory* effect of *Trichoderma asperellum* against *Colletotrichum* sp. and *vice versa*.

From table II, it can be seen that the growth of Colletotrichum in single culture was faster than the growth of Trichoderma. Colletotrichum growth 48 hours after inoculation was 1.86 faster than Trichoderma and 3.88 were faster at 144 hours incubation. That indicated why Colletotrichum is difficult to control because of its fast growth ability. Fig. 1 show, that T. *asperellum* growth was suppressing *Colletotrichum sp* growth. Fig. 2, show the percentage of inhibitory effect T. *asperellum* against *Colletotrichum sp.* and vice versa day by day.

B. Disease Incidence

Trichoderma asperellum was used as biofertilizer and biopesticide to inhibit anthracnose on chili caused by *Colletotrichum* sp. As a result, the application of biofertilizer did not affect the disease development on chili. On the contrary, the disease severity was suppressed by the application of *T. asperellum* biopesticide. The application of *T. asperellum* 6 hours after the inoculation of *Colletotrichum* sp. on chili was significantly suppressed the disease severity (Table II). Application Trichoderma and Colletotrichum sp. simultaneously can reduce symptom of anthracnose disease (Fig. 3).

Fig 3. The symptom of anthracnose disease on chili at 13 days after inoculation. A: plants treated by *Trichoderma* and *Colletotrichum* sp. simultaneously; B: plants treated by *Trichoderma* 6 hours after *Colletotrichum* sp.

TABLE I: Colony Growth of Trichoderma Asperellum and Colletotrichum sp. on PDA-Chloramphenicol

Observation time (hours)	Dual culture colony growth (cm) ± SE		Single culture colony growth (cm) ± SE		Inhibitory effect (%) ± SE	
	Colletotrichum sp.	*T. asperellum*	*Colletotrichum* sp.	*T. asperellum*	*T. asperellum* against *Colletotrichum* sp.	*Colletotrichum* sp. against *T. asperellum*
24	0.43 ±0.03	0.68 ±0.11	0.64 ±0.09	0.48 ±0.03	-9.60 ±6.66	5.50 ±14.02
48	1.27 ±0.03	2.53 ±0.18	2.15 ±0.14	1.16 ±0.07	8.22 ±5.92	14.85 ±5.36
72	1.70 ±0.05	4.00 ±0.21	3.54 ±0.11	1.26 ±0.13	25.70 ±7.35	11.50 ±2.77
96	2.42 ±0.12	4.35 ±0.28	4.90±0.16	1.30 ±0.11	46.21 ±4.47	-12.64 ±3.63
120	3.52 ±0.13	4.43 ±0.11	5.18±0.03	1.34 ±0.10	61.97 ±2.93	-16.95 ±0.65
144	3.52 ±0.13	4.45 ±0.07	5.20±0.00	1.34 ±0.10	61.97 ±2.93	-16.85 ±0.00

TABLE II: DISEASE SEVERITY OF ANTHRACNOSE ON CHILI CAUSED BY *COLLETOTRICHUM* SP. CONTROLLED BY *TRICHODERMA ASPERELLUM* BIOPESTICIDE

Treatments	Disease severity (DS) (%) on days after inoculation					
	7		10		13	
	DS	Δx	DS	Δx	DS	Δx
Control	6.25 a	81.25	6.25 a	83.78	12.50 a	70.00
T. asperellum before *Colletotrichum* sp.	6.25 a	81.25	8.33 a	78.38	12.50 a	70.00
T. asperellum after *Colletotrichum* sp.	33.33 b	-	38.54 b	-	41.67 b	-
T. asperellum and *Colletotrichum* sp. simultaneously	17.71ab	46.88	20.83ab	45.59	23.96ab	43.00

Note: The same column followed by different letters is significantly different according to the HSD test ($p < 0.05$). Δx showed comparing all treatments toward plants treated by T. asperellum 6 hours after the inoculation of Colletotrichum sp.

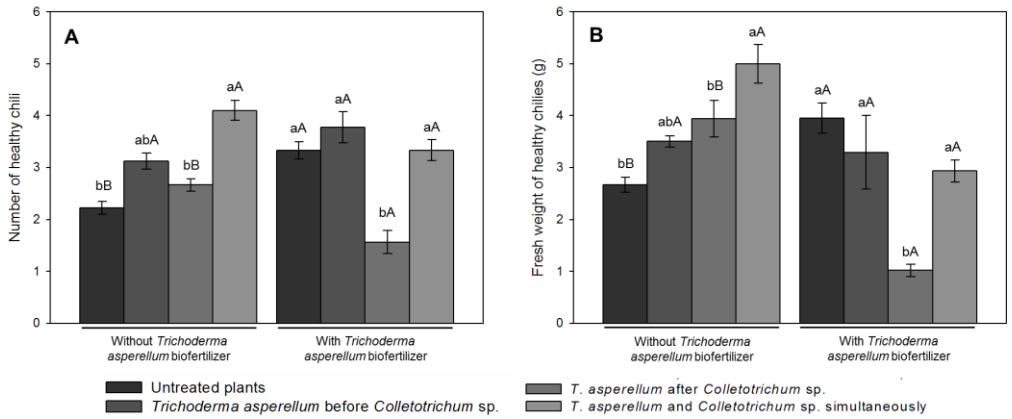

Fig. 4. Yield was observed by 90 days after planting. Panel A and Panel B reveals the number of healthy chili produced per plant and its fresh weight, respectively. Different small letters above the bars indicate significant different among the inoculation methods according to HSD test ($p < 0.05$). Different capital letter above the bars indicate significant different among the application of biofertilizer in the same inoculation method according to HSD test ($p < 0.01$).

C. Yield Observationss

Chili was harvested by 90 days after planting. The yield observation consists of the number of healthy chilies and their fresh weight. As shown in Fig. 4, the application of *T. asperellum* as a biopesticide through several application methods and its interaction with the application of *T. asperellum* as biofertilizer had a significant effect on the average number of healthy chilies per plant as well as its fresh weight.

Form Fig. 4 can be seen applying *Trichoderma* and *Colletotrichum* sp. simultaneously to the plant can produce a high number of healthy chilli and fresh weight healthy chilli, especially the treatment without Trichoderma as fertilizer.

IV. DISCUSSION

The isolate of *Trichoderma asperellum* was tested *in vitro* to assay the potential biocontrol activity against pathogenic fungi *Colletotrichum* sp. In this study, the growth of *Colletotrichum* sp. was inhibited by *T. asperellum* up to 61.97% (Table I). Similarly, *Trichoderma* sp. was able to inhibit the colonies of *C. dracaenophilum* in PDA media by 67.4% after 7 days of incubation [21]. *Trichoderma* has been widely employed as biocontrol agents of fungal phytopathogens, such as *Fusarium oxysporium*, *Fusarium guttiforme*, *Phytophthora capsici*, and *Pythium myriotylum* [22]. Furthermore, *T. asperellum* and *T. harzianum* were reported inhibited over 90% of the mycelial growth and conidial germination of *Botrytis cinerea*, while *T. viride* was documented to inhibit the growth of *Rhizoctonia solani*, *Macrophomina phaseolina*, *Alternaria alternata*, and *C. capsici* up to 70% [23].

The colony diameter of *T. asperellum* was wider in dual culture plate than single culture. This suggests that the growth of *T. asperellum* was triggered by the existence of phytopathogen. In addition, the clear zone was only formed in dual culture plate (Fig. 1). A clear zone of inhibition exhibited the antibiosis of antagonist against pathogen. The ability of antagonist as biocontrol agent was conducted through a complex process made up of several successive steps generally initiated by remote sensing of the pathogen which stimulates directed growth, consequently a contact that made between antagonist and pathogen surface. This step provides the specific recognition event involving the hydrophobic interaction and the interaction between complementary molecules present on both antagonist and pathogen [23]. Correspondingly, we expected that the inhibitory mechanisms of *T. asperellum* against *Colletotrichum* sp. were antibiosis and competition of space

and nutrients. Previous study showed that the inhibition of *P. myriotylum* occurs immediately after contact with *T. asperellum*, but the cessation of growth was examined due to the mycoparasitic activity [22]. *Trichoderma* produces several secondary metabolites such as gliotoxins [24] as well as hydrolytic enzymes and able to paralyze pathogen that are induced by responses to molecules (extracellular) released by the pathogen [25]. Moreover, several enzymes produced by the biocontrol agent are also responsible for the suppression of phytopathogen by breaking down the polysaccharides, chitin, and β-glucans that lead to the destruction of pathogen cell wall integrity, or disintegrate the hydrolytic enzymes into peptide chains and/or their constituent amino acids and thereby destroy the capacity of pathogen to act on plant cells. The concept of enzyme biosynthesis as a mechanism of biocontrol has been expanded to include synergism with antibiosis ability [26].

Previous study exhibited that *T. asperellum* extract as biopesticide or its combination with the application of *T. asperellum* as biofertilizer was effectively reduced the damping-off disease on cowpea [27]. On the contrary, in this study, we found that the application of biofertilizer did not affect the disease development on chili. Otherwise, the disease severity (DS) was suppressed by the application of *T. asperellum* biopesticide. The effectiveness of *T. asperellum* as biocontrol agent was evaluated by observing anthracnose disease symptoms on chili induced by *Colletotrichum* sp. The biocontrol efficacy was conducted in 3 different strategies of pathogen inoculation, including preventing and scavenging treatment, and the inoculation of pathogen and biocontrol agent simultaneously. Preventing treatment indicates the application of *T. asperellum* 6 hours before the inoculation of *Colletotrichum* sp., while scavenging treatment indicates *T. asperellum* was applied 6 hours after the inoculation of *Colletotrichum* sp. As a result, the DS of anthracnose caused by *Colletotrichum* sp. was significantly suppressed by *T. asperellum* (Table II). As expected, the application of *T. asperellum* before *Colletotrichum* sp. delivering the lowest DS (12.50%) compared to the scavenging treatment (41.67%) and simultaneously treatment (23.96%). Comparatively, the highest reduction in the *Fusarium* wilt disease severity on sesame was achieved when either *T. viride* or *T. harzianum* were applied 7 days before challenged with the *F. oxysporum* f.sp. *sesami* (Mahmoud & Abdalla, 2018). However, we found that DS showed in the preventing treatment was similar to the DS on untreated plants. Previously, Herrera-T ᷉lez et al. [28] demonstrated that pretreatment plants with *T. asperellum* resulted in fewer disease symptoms than untreated plants. On the contrary, Asad et al. [29] demonstrated that, under laboratory conditions, disease incidence in untreated plants (0.00%) was significantly lower than treated plants. However, they also demonstrated that the ability of disease suppression by *T. asperellum* against *R. solani* on bean in preventing treatment (19.3%) was similar to the scavenging treatment (19.7%). Additionally, wilting and damping-off diseases on beans caused by *F. oxysporum* and *R. solani* was significantly suppressed by *T. harzianum* [30], *T. harzianum* and *T. viride* were exhibited strong biocontrol activity against *Meloidogyne javanica* on tomato [31]. Correspondingly, we demonstrated that *T. asperellum* could be used as a biocontrol agent to suppress anthracnose disease caused by *Colletotrichum* sp. on chili.

Artificial wound in chilies does not indicate the invasion of pathogens. In the preventing treatment, the index reaches 8.33% on day 10 post inoculation. Previously, on day 10 post inoculation, the preventing treatment of *Trichoderma* sp. challenged with *Phythopthora palmivora* on cocoa leaves were able to suppress the disease index lower than scavenging treatment; 16.7 and 35.71%, respectively [32]. This indicates that *T. asperellum* Tc-Jjr-02 showed a good ability in terms of predisposition and performance as a competitor of phytopathogen in the plant tissue. When the houstorium is established and develops in the infected tissue, the initial pathogen hyphal cells will become an inhibitor for Tc-Jjr-02 sprouts in predisposing. Meanwhile, hypha cells at the bottom of the cells and fruit tissue do not have obstacles to carry out the invasion. *Trichoderma* is known to be endophytic [33] and is a good colonizer of plant remains or dead plant tissue [34]. In the injured tissue there is carbohydrate-rich biomass which can be a suitable habitat for *Trichoderma* to carry out the conidiospores germination process [35]. *Trichoderma* also has a high ability in spatial control competition and produces the enzyme chitinase [36] which can damage the cell walls of pathogenic fungal hyphal. With its secondary metabolites and their chitinases, *Trichoderma* is also able to recycle the cells that die next to the host fungal cells [37]. *Trichoderma* as endophytes deposited in the intercellular tissue space can actually increase plant resistance to pathogens [38].

Aside from its ability as an antagonist against wide-broad spectrum plant pests and diseases, *Trichoderma* species was known as a potential plant growth regulator. The germination rate of *F. oxysporum*-contaminated bean treated with *T. koningii* and *T. reesei* was significantly higher than other treatments [39]. Mahmoud & Abdalla [40] found that the seedling emergence on sesame was significantly increased by the application of *T. harzianum* and *T. viridae*. Moreover, the highest seedling emergence was exhibited in the treatment of biocontrol agents that applied 7 days before challenged with *F. oxysporum f. sp. sesami*. Recently studies showed that *Trichoderma* species effectively increased the fresh weight of pathogen-contaminated tomato and wheat [29], [28], [31]. In this study, we found that yield evaluation that enumerated by the number of chili and its fresh weight was affected by the application of *T. asperellum* as biopesticide and biofertilizer (Fig. 4). The lowest yield evaluation was recorded in the treatment of *T. asperellum* after *Colletotrichum* sp., both in soil treated with and without *T. asperellum* biofertilizer. This result was correlated with *in vivo* biocontrol efficacy. The scavenging treatment exhibited the highest disease severity, subsequently to the lowest chili number and its fresh weight. Thus, it appears that *T. asperellum* acts as a barrier to the disposition and invasion of pathogens in the plant tissue. Correspondingly, we demonstrated that the application of *T. asperellum* could be used as a potential plant growth regulator to detract the yield losses on chili. The significant contribution of *T. asperellum* to promote the plant growth due to its ability to digest substrates like other types of *Trichoderma* known as good saprophytes such as *T. atroviridae*, *T. reesei*, and *T. virens* [35]. *Trichoderma* carries out its distinctive activity of producing extracellular compounds that are beneficial to plants [13], [14].

Trichoderma can induce the activity of the peroxidase enzyme, polyphenol oxidase, and superoxide [12], [36], [41]. Endophytic fungi on leaves, stems, and skin of *Xylocarpus granatum* mangrove plants, namely *Trichoderma* sp. Xy24 turned out to produce (9R, 10R)-dihydro-harzianone and harzianelactone that play a role in plant resistance [10] as well as the β-1,3-glucanase, cellulase, and peroxidase enzymes [42]. Glutathione peroxidase enzyme produced by *Trichoderma* can protect the structure and function of the plant cell membrane [43]. Thus, *T. asperellum* used in this study was not only inhibited pathogen (in those that were inoculated earlier or together with *Trichoderma*), yet induce plant growth.

V. Conclusion

Trichoderma asperellum Tc-Jjr-02 was able to inhibit the growth of *Colletotrichum* sp. on agar plate. The anthracnose disease severity on chili was only suppressed by the application of T. *asperellum* as a biopesticide. Application of *Trichoderma* 6 hours before inoculation of pathogens (T1) and *Trichoderma* inoculation concurrent pathogens (T3) can reduce the intensity of the symptoms of anthracnose attacks 70% and 43%, respectively, then increasing the number of fresh fruit 62.66% and 76.58%, also increasing fresh fruit weights 84.83% and 91.90% respectively, compared with the pathogen inoculation treatment six hours before the application of *Trichoderma*. Application of T. *asperellum as* biopesticide can be use as prevention agent.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

Acknowledgments

The Authors thank the Ministry of Research and Technology and Higher Education for the 2019 Applied Research Higher Education grant awarded under contract number 009/II.3.AU/14.00C/PRY/III/2019.

Author Contributions

Sutarman, T. Setiorini, Arrohmatus Syafaqoh Li'aini: Design the experiment, field observation, analysis, interpretation data and drafting manuscript. Purnomo: critical reviewing in drafting, Ali Rahmat: Critical reviewing and final approval of the last version to be submitted. all authors had approved the final version.

References

[1] A. Katoch, P. Sharma, and P. N. Sharma, "Identification of *Colletotrichum* spp. associated with fruit rot of *Capsicum annuum* in North Western Himalayan region of India using fungal DNA barcode markers," *Journal of Plant Biochemistry and Biotechnology*, vol. 26, no. 2, pp. 216–223, 2017.

[2] A. Maharaj and S. N. Rampersad, "Genetic differentiation of *Colletotrichum gloeosporioides* and *C. truncatum* associated with anthracnose disease of papaya (*Carica papaya* L.) and bell pepper (*Capsicum annuum* L.) based on its pcr-rflp fingerprinting," *Molecular Biotechnology*, vol. 50, no. 3, pp. 237–249, 2012.

[3] N. N. Bhat *et al*., "Microsatellite mining in the genus *Colletotrichum*," *Gene Reports*, pp. 84–93, 2018.

[4] N. N. Bhat *et al*., "Compendium of *Colletotrichum graminicola* responsive infection-induced transcriptomic shifts in the maize," *Plant Gene*, 2019.

[5] A. Campos-Mart ńez *et al*., "Antagonistic yeasts with potential to control *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* J.H. Simmonds on avocado fruits," *Crop Protection*, vol. 89, pp. 101–104, 2016.

[6] T. S. Porto *et al*., "Immobilization of pectinase from *Aspergillus aculeatus* in alginate beads and clarification of apple and umbu juices in a packed bed reactor," *Food and Bioproducts Processing*, vol. 109, pp. 9–18, 2018.

[7] A. Chechi *et al*., "Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples," *Pesticide Biochemistry and Physiology*, 2019.

[8] A. C. Velho *et al*., "New insights into the characterization of *Colletotrichum* species associated with apple diseases in southern Brazil and Uruguay," *Fungal Biology*, vol. 119, no. 4, pp. 229–244, 2015.

[9] A. Abbas, D. Jiang, and Y. Fu, "*Trichoderma* spp. as antagonist of *Rhizoctonia solani*," *Journal of Plant Pathology & Microbiology*, vol. 8, no. 3, 2017.

[10] J. You *et al*., "Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato," *Biological Control*, vol. 101, pp. 31–38, 2016.

[11] P. Pruksakorn *et al*., "Trichoderins, novel aminolipopeptides from a marine sponge-derived *Trichoderma* sp., are active against dormant mycobacteria," *Bioorganic & Medicinal Chemistry Letters*, vol. 20, 2010.

[12] R. Srivastava *et al*., "Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt," *Biological Control*, vol. 53, no. 1, pp. 24–31, 2010.

[13] X. Hu *et al*., "Components of a rice-oilseed rape production system augmented with *Trichoderma* sp. Tri-1 control *Sclerotinia sclerotiorum* on oilseed rape," *Phytopathology*, vol. 105, no. 10, pp. 1325–1333, 2015.

[14] A. Singh *et al*., "Review on plant-*Trichoderma*-pathogen interaction," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 02, pp. 2382–2397, 2018.

[15] C. Buysens *et al*., "Inoculation of *Medicago sativa* cover crop with R*hizophagus irregularis* and *Trichoderma harzianum* increases the yield of subsequently-grown potato under low nutrient conditions," *Applied Soil Ecology*, vol. 105, pp. 137–143, 2016.

[16] J. T. Alkooranee *et al*., "Detecting the hormonal pathways in oilseed rape behind induced systemic resistance by *Trichoderma harzianum* TH12 to *Sclerotinia sclerotiorum*," *PloS One*, vol. 12, no. 1, p. e0168850, 2017.

[17] T. Glare *et al*., "Have biopesticides come of age?" *Trends in Biotechnology*, vol. 30, no. 5, pp. 250–258, 2012.

[18] A. Miftahurrohmat *et al*., "Initial growth response of shallot on *Trichoderma* formulated in oyster mushroom cultivation waste," *IOP Conference Series: Materials Science and Engineering*, vol. 420, p. 12064, 2018.

[19] R. Yasmeen and Z. S. Siddiqui, "Physiological responses of crop plants against *Trichoderma harzianum* in saline environment," *Acta Botanica Croatica*, vol. 76, no. 2, pp. 154–162, 2017.

[20] J. Ribera *et al*., "Effect of *Trichoderma*-enriched organic charcoal in the integrated wood protection strategy," *PLoS ONE*, vol. 12, no. 8, pp. 1–14, 2017.

[21] A. A. Morsy and I. E. Elshahawy, "Anthracnose of lucky bamboo *Dracaena sanderiana* caused by the fungus *Colletotrichum dracaenophilum* in Egypt," *Journal of Advanced Research*, vol. 7, no. 3, pp. 327–335, 2016.

[22] J. B. Mbarga *et al*., "*Trichoderma asperellum*: a potential biocontrol agent for P*ythium myriotylum*, causal agent of cocoyam (*Xanthosoma sagittifolium*) root rot disease in Cameroon," *Crop Protection*, pp. 18–22, 2012.

[23] B. K. Mishra *et al*., "Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L.," *Archives of Applied Science Research*, vol. 3, no. 2, pp. 361–369, 2011.

[24] P. K. Mukherjee *et al*., "*Trichoderma* research in the genome era," *Annual Review of Phytopathology*, vol. 51, pp. 105–129, 2013.

[25] M. R. Omann *et al*., "The seven-transmembrane receptor Gpr1 governs processes relevant for the antagonistic interaction of *Trichoderma atroviride* with its host," *Microbiology (Reading, England)*, pp. 107–118, 2012.

[26] C. R. Howell, "Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts," *Plant Disease*, vol. 87, no. 1, pp. 4–10, 2003.

[27] E. R. Hamed *et al*., "*Trichoderma asperellum* isolated from salinity soil using rice straw waste as biocontrol agent for cowpea plant pathogens," *Journal of Applied Pharmaceutical Science*, vol. 5, Suppl 2, pp. 91–98, 2015.
[28] V. I. Herrera-Tđlez *et al*., "The protective effect of *Trichoderma asperellum* on tomato plants against *Fusarium oxysporum* and *Botrytis cinerea* diseases involves inhibition of reactive oxygen species production," *International Journal of Molecular Sciences*, vol. 20, no. 8, pp. 1–13, 2019.
[29] S. A. Asad *et al*., "Biocontrol efficacy of different isolates of *Trichoderma* against soil born pathogen *Rhizoctonia solani*," *Polish Journal of Microbiology*, vol. 63, no. 1, pp. 95–103, 2014.
[30] T. Kamala and S. I. Devi, "Biocontrol properties of indigenous *Trichoderma* isolates from North-east India against *Fusarium oxysporum* and *Rhizoctonia solani*," *African Journal of Biotechnology*, vol. 11, no. 34, pp. 8491–8499, 2012.
[31] A. S. Al-hazmi and M. Tariqjaveed, "Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato," *Saudi Journal of Biological Sciences*, vol. 23, no. 2, pp. 288–292, 2016.
[32] Sutarman, Pengujian *Trichoderma* sp. sebagai pengendali hawar daun bibit kako yang disebabkan oleh *Phytopthora palmivora*. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, vol. 17, no. 1, pp. 45–52, 2017.
[33] G. E. Harman *et al*., "Special issue: *Trichoderma*-from basic biology to biotechnology," *Microbiology (Reading, England)*, vol. 158, issue Pt 1, pp. 1–2, 2012.
[34] F. Matarese *et al*., "Biocontrol of *Fusarium* head blight: Interactions between *Trichoderma* and mycotoxigenic *Fusarium*," *Microbiology (Reading, England)*, pp. 98–106, 2012.
[35] B. Metz *et al*., "Expression of biomass-degrading enzymes is a major event during conidium development in *Trichoderma reesei*," *Eukaryotic Cell*, vol. 10, no. 11, pp. 1527–1535, 2011.
[36] P. Chowdappa *et al*., "Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3," *Biological Control*, vol. 65, no. 1, pp. 109–117, 2013.
[37] S. Gruber and V. Seidl-Seiboth, "Self versus non-self: fungal cell wall degradation in *Trichoderma*," *Microbiology (Reading, England)*, vol. 158(Pt 1), pp. 26–34, 2012.
[38] I. Yedidia *et al*., "Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203," *Plant Physiology and Biochemistry*, vol. 38, no. 11, pp. 863–873, 2000.
[39] J. A. Otadoh *et al*., "Assessment of *Trichoderma* isolates for virulence efficacy," *Tropical and Subtropical Agroecosystems*, vol. 13, pp. 99–107, 2011.
[40] A. F. Mahmoud and O. A. Abdalla, "Biocontrol efficacy of *Trichoderma* spp. against sesame wilt caused by *Fusarium oxysporum* f. sp. *sesami*," *Archives of Phytopathology and Plant Protection*, vol. 51, no. 5–6, pp. 277–287, 2018.
[41] S. A. Youssef, K. A. Tartoura, and G. A. Abdelraouf, "Evaluation of *Trichoderma harzianum* and *Serratia proteamaculans* effect on disease suppression, stimulation of ROS-scavenging enzymes and improving tomato growth infected by *Rhizoctonia solani*," *Biological Control*, vol. 100, pp. 79–86, 2016.
[42] K. Saravanakumar *et al*., "Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*," *Biological Control*, vol. 94, pp. 37–46, 2016.
[43] O. Hassan *et al*., "Molecular and morphological characterization of *Colletotrichum* species in the *Colletotrichum gloeosporioides* complex associated with persimmon anthracnose in South Korea," *Plant Disease*, vol. 102, no. 5, pp. 1015–1024, 2018.

Sutarman was born in Indonesia. He is an associate professor of Agrotechnology study program, Faculty of Science and Technology, University of Muhammadiyah Sidoarjo. He graduated with his master's degree in agricultural science from Brawijaya University and graduated with his Ph.D. in Forest Pathology from IPB University, Indonesia. At the time of his doctoral education, Dr. Sutarman received the Post Graduated scholarships from the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia. He has had a career as a lecturer in the Agrotechnology study program since graduating with a Ph.D. at the Muhammadiyah University of Sidoarjo in 2004 until now. Currently, he is working in the fields of plant pathology and agricultural biotechnology.

Triana Setiorini was born in Indonesia. She was graduated from the Department of Agrotechnology and currently work as a research assistant at the Department of Agrotechnology, Faculty of Science and Technology, University of Muhammadiyah Sidoarjo.

Since completing her education in 2019, she has mostly carried out activities as a research assistant at the Laboratory of Pests and Plant Diseases and the Laboratory of Microbiology and Biotechnology, University of Muhammadiyah Sidoarjo.

Arrohmatus Syafaqoh Li'aini was born in Indonesia on April, 22nd 1992. She is a research assistant of Research Center for Plants Conservation and Botanic Gardens, Indonesian Institute of Sciences, who is currently served at Eka Karya Bali Botanic Garden. She graduated with her Master Dual Degree in biological science from National Pingtung University of Science and Technology, Taiwan, and agronomy from Brawijaya University, Indonesia on 2017.

She started her career as a lecturer at Muhammadiyah University of Sidoarjo on 2018, then moved to Indonesian Institute of Sciences on 2019. Recently, she published articles about antioxidant and antimicrobial activity of several plants extract and biological control against phytopathogens. She is currently conducting research in the fields of ecology, agronomy, and plant conservation such as invasive species, the use of plant growth promoter rhizobacteria, seed germination biology, and essential oil. She can be contacted through her email address at syafa.liaini@gmail.com.

Purnomo is one of the Professors at the Faculty of Agriculture, the University of Lampung in the Field of Plant Pest Science, born in Tanjungkarang in 1964. He is a Permanent Lecturer in the Department of Agrotechnology and Plant Protection. Masters and doctoral degrees were completed at the Bogor Agricultural University. Prof. Purnomo has published many of his researches both nationally and internationally.

Ali Rahmat was born in Indonesia, He is a Junior Researcher in Badan Riset dan Inovasi Nasional, Indonesia (National Research and Innovation Agency).He get Master and Doctoral Degree from Gifu University Japan in field Science of Biological Environment. His research close to soil, agro climatology, hydrology and environment.