UDC 632

EXPLORATION OF TRICHODERMA SPP. AND FUNGAL PATHOGEN THAT CAUSES A STRAWBERRY ANTHRACNOSE AND EXAMINATION OF IN VITRO ANTAGONISTIC ACTIVITY

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ABSTRACT
Strawberry (*Fragaria Vesca* L.) is a kind of fruit that has high economic value. The obstacles that often arise in strawberry cultivation are pests and diseases. One of the alternatives to control the anthracnose is a biological agent known as *Trichoderma* spp. *Trichoderma* spp is antagonistic to the pathogen. The objective of this research is to explore the *Trichoderma* spp. as an antagonistic agent to the fungal pathogen that causes anthracnose disease in strawberry. The *Trichoderma* spp. was isolated by *pour plate* method while the pathogen was isolated by a *direct plating* method. The antagonistic activities of *Trichoderma* spp. were tested *in vitro* towards fungal pathogen by *dual culture* assay. The pathogen and antagonistic agent were paired by using three methods of pairing. The fungal pathogen has a similarity to the members of the Genus *Colletotrichum* and the two isolated antagonists have a similarity to the member of the Genus *Trichoderma*. The two *Trichoderma* spp. (TKL1 and TKL2) have significantly inhibited a radial growth of the pathogen. An introduction of the antagonists before the pathogen has given the best growth inhibition of the pathogen. The inhibitory effect of the two *Trichoderma* spp. is not significantly differed from each other at p>0,05. The microscopic examination showed that the most common mode of the action was mycoparasitism. The *Trichoderma* hyphae grew alongside and coiled compactly around the hyphae of the fungal pathogen isolates.

KEY WORDS
Antagonist, *Colletotrichum*, strawberry, *Trichoderma.*

Strawberry (*Fragaria Vesca* L.) is a fruit that has high economic value. The obstacles that often arise in strawberry cultivation are the pests and diseases that can cause damage to roots, leaves, flowers, and fruit. The main diseases on strawberry one of which is caused by anthracnose pathogen that are the members of Genus *Colletotrichum* (Semangun, 2003). Almost all parts of strawberry attacked by a pathogen will result in brown spots, rotten fruits, and rotten root. The incidence of anthracnose in Ohio, United States have caused a crop loss due to an increase in strawberry anthracnose. Even though the disease occurred sporadically or rarely, but it would destroy the harvest up to 100% in one attack (Ellis &Erincik, 2008).

An effort to control anthracnose disease is to use synthetic fungicides because they are practical, easy to get and show rapid effect. However, the fungicide application often leaves a residue on plants and environment that is harmful to human health and the environment itself especially through fresh and/or processed fruit (Duriat, 1994). One alternative to control the anthracnose is by using a biological agent that is *Trichoderma* spp. *Trichoderma* spp is antagonistic to the pathogen. *Trichoderma* spp. is a saprophyte mold soil that is able to attack the pathogens naturally in plants. *Trichoderma* spp. has a very fast growth rate and does not cause a disease to plants (Trianto & Sumantri, 2003 *in* Purwantisari & Hastuti, 2009).

The mechanism of the antagonistic *Trichoderma* spp. towards the pathogenic fungi is carried out in several ways such as the competition for space and nutrients, the production of metabolites that can inhibit the germination of spores of the pathogen, and the interaction of

pathogen through direct contact and synthesis of hydrolytic enzymes that are toxical and kill the cells by its antibiosis (Benitez *et al*., 2004). Under these conditions, the process of isolation, identification, and determination of the level of similarity needs to be done in between the isolated pathogenic fungi and genus *Colletotrichum* fungi and in between the isolated antagonistic *Trichoderma* fungi. The next important step that needs to be done is the test of antagonists in *Trichoderma spp.* towards pathogenic fungi to determine the potential of *Trichoderma* spp. in inhibiting the growth of pathogenic fungi as an effort to control the anthracnose disease on strawberry plants.

MATERIALS AND METHODS OF RESEARCH

Isolation and identification of pathogens and antagonists. The pathogens, *Colletotrichum* spp., were isolated by using direct plating techniques (Malloch, 1997) from naturally infected leaves, stems, and fruits of strawberry which were cut and taken to the laboratory to be isolated. Several smaller tissues from the infected parts were cut and sterilized by soaking it in 10 % NaOCl and then rinsed in three changes of sterile distilled water. The processed tissues were picked onto sterile filter papers to be dried for several minutes. Then, those were plated on Potatoes Dextrose Agar (PDA) containing 50 mg/L Terramycin in 9 cm diameter sterile Petri plates and incubated for 7 days at 28^{o}C. The methods used to isolate the *Trichoderma* species were the soil dilution plate method (Srilakshmi *et al*., 2001). Pure cultures of the *Colletotrichum* were obtained by monospores subculture. The *Trichoderma* species were compared with identified species from Indonesian Citrus and Subtropical Fruits Research Institute, Batu, East Java, Indonesia.

The pathogenic fungi (*Colletotrichum* spp.) and *Trichoderma* spp. were identified based on phenotypic characters and observed macroscopically and microscopically. The parts of the fungus were observed by microscopic observation and were stained by using lactophenol cotton blue (LCB). This was observed with Olympus BX51 microscope that is connected to a camera Evolution^{TM} LC Color Olympus U-PMTVC magnifications ranging from lowest to highest magnification. The characters of the fungus numerically analyzed by using CLAD 97 program to determine the value of phenotypic similarity. The value was determined by simple matching method (SSM) that is proposed by Sembiring (2002 in Suharjono, 2008).

Antagonists test among Trichoderma spp. towards the pathogens in vitro. The experiments to test the antagonism or inhibition of *Trichoderma* spp. towards pathogenic fungi were performed by using a factorial randomized block design with three replications. The combination treatment was the kind of *Trichoderma* fungus with the time of inoculation. Each bioagent (*Trichoderma*) was paired with pathogen in 90 mm diameter Petri dishes by using the method described by Sobowale (2005): (a) 5 mm plug of the pathogens was placed 30 mm away from the edge of the plate and at the same time, separately, 5 mm plug of the antagonists was placed 30 mm away from the edge of the plate. (b) 5 mm plug of each bioagents was placed 30 mm away from the edge of the plate, and after 48 hours, the pathogens were placed 30 mm away at the other edge of the same petri dish. (c) 5 mm plug of the pathogens was placed 30 mm away from the edge of the petri dish, and after 48 hours, the bioagents were placed at the other edge of the plate individually. The parameters observed were the percentage of inhibition of *Trichoderma* towards the growth of fungus pathogens. The percentage of inhibition was calculated by using Equation 1 (Anggraeni & Suharti, 1996 in Dewi, 2000).

$$P = ((R_1 - R_2) / R_1) \times 100\% \qquad (1)$$

Where: P = the percentage of inhibition of *Trichoderma* spp. against pathogenic fungi; R1 = the distance to control the growth of pathogen fungi; R2 = the growth distance of pathogen mold inoculated with *Trichoderma* spp.

The observation of interaction between Trichoderma spp. with the pathogens. The test of interaction or inhibition mechanism of pathogen fungi by *Trichoderma* spp. performed by using the theory of Aryantha & Guest (2006) was modified. It was carried out by using a

sterile glass object inserted in a sterile petri dish and then poured with PDA. At the edge of the petri dish, those were inoculated with pathogenic fungi by using a needle graft and on the opposite side, those also inoculated with antagonistic fungi (*Trichoderma*). The petri dish was then covered and incubated at 28°C for seven days. The lactophenol cotton blue (LCB) was dropped when the pathogens met the antagonistic fungi hyphae and then covered with a glass cover. This was observed by using Olympus BX51 microscope that is connected to a camera Evolution^{TM} Color LC Olympus U-PMTV.

RESULTS AND DISCUSSION

Pathogen isolates. Six of ten isolates of pathogenic fungi (Table 1) have the macroscopic and microscopic characteristics such as *Colletotrichum spp*. (fungus that causes anthracnose).

Table 1 – Pathogen isolates

Isolates code	Origin	Cultivar	Organ
TLT1	Tlekung, Batu	Local Brastagi	stalk
TLT2	Tlekung, Batu	Local Brastagi	Stalk
PRD2	Pandanrejo, Batu	California	Leaf
TLD1	Tlekung, Batu	Local Brastagi	Leaf
PRB1	Pandanrejo, Batu	California	Fruit
PRB3	Pandanrejo, Batu	California	Fruit

The characteristics of pathogenic colonies on PDA (Potato Dextrose Agar) after seven days of incubation at 28°C show white brownish pigmentation, setae absent in culture, smooth texture in some colonies, and rough texture in other colonies (Figure 1). Akhter *et.al*., (2009) mentioned that acervuli and setae are produced after three weeks of incubation at 28°C on PDA. Setae size ranges between 24-80µm and 4-6µmin. The color of C. *fragariae* colony on PDA is beige (beige to dark gray), C.*gloeosporioides* colony is white and then dark gray, while the C.*acutatum* colony is white and for the next 4-5 days will turn grayish brown. Based on that fact, after seven days of incubation, the fungal colonies grow at an average rate of 5,9 to 6,9 cm in diameter. According to Smith and Black (1990), *Colletotrichum gloeosporioides*, C. *acutatum* and C. *fragariae* can be distinguished by their growth on PDA medium, especially at 32°C with an average diameter on the fifth day of C. *acutatum*, C. *gloeosporioides* and C. *fragariae* respectively 13 mm, 63 mm, and 69 mm.

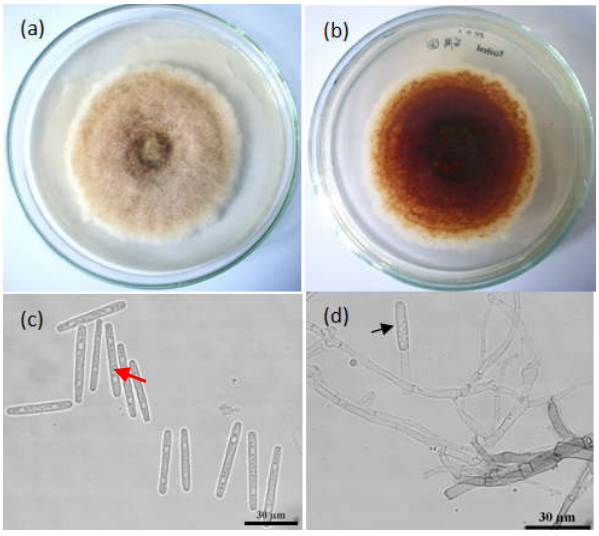

Figure 1 – Characteristics of the pathogenic fungi: (a &b) cultured pathogen on PDA, 28°C, 7th day, upper and lower colony surface, (c) conidia of pathogens; (d) conidia (black arrow) arises from conidiophores (red arrow) (magnification by 400x)

Based on the macroscopic characteristics of the pathogenic fungi that have been isolated, it can be indicated that *Colletotrichum* spp. has approached the C. *fragariae* character.

Microscopic observations showed that the conidia of the six pathogen isolates had the same characteristics, hyaline color, obovate and straight conidia with a length between 38,7 to 43,9 µm x 4-5 µm for width while the measure of conidia that arise from conidiophores was 17-18 µm x 4 to 5,1 µm (Fig. 5c and 5d). Hyaline, septate, and branched hyphae are the shape of *Colletotrichum* conidia according to Gunnell & Gubler (1992). Meanwhile, C. *fragariae* have obovate, straight, or sometimes slightly curved shape. C. *acutatum*, on the other hand, has elliptic shape to fusiform shape. The conidia of C. *gloeosporioides* is longitudinal with a blunt, straight, shorter, and wider tip than the conidia on the other two isolates. The hyphae of C. *fragariae*, C. *acutatum*, and C. *gloeosporioides* was septate, branched, and hyaline.

Based on the characteristics description above, it is suspected that the pathogens were the members of Genus *Colletotrichum.* However, when compared to the size of conidia, there is a difference in the average size of the three members of the genus *Colletotrichum* species by Gunnell & Gubler (1992) which is 18 µm x 4 µm, 15,5 µm x 3,7µm and 15µm x 4,3µm respectively for C.*fragariae*, C.*acutatum*, and C.*gloeosporioides*. The conidia of the isolated pathogens are two times longer than the description of Gunnell and Gubler. Yet, the conidia which arise from conidiophores have same relative size to the size of conidia C. *fragariae*.

The phenotypic similarity among the six isolates with the reference of *Colletotrichum gloeosporioides* (Gunnell &Gubler, 1992) was analyzed based on macroscopic and microscopic characteristics. The constructed dendrogram (Figure 2) shows that PRB1 isolates have a similarity value by 86% to C.*gloeosporioides* and PRD2 has a similarity value by 80% to C.*gloeosporioides* reference. Based on the value of similarity, it is clear that the pathogenic isolates of PRB1 and PRD2 are in the Genus *Colletotrichum* but not in C.*gloeosporioides* species.

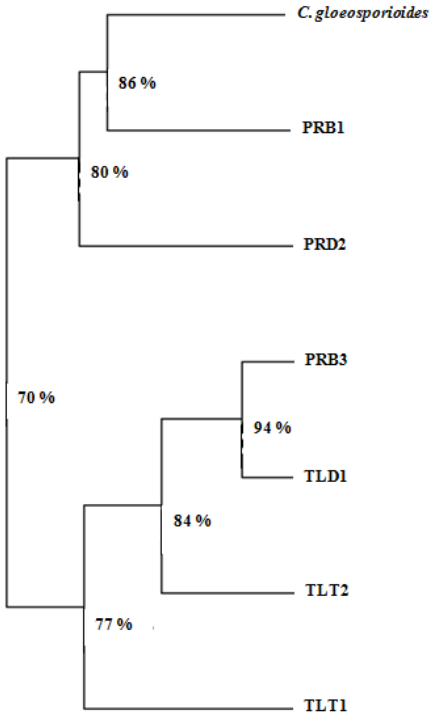

Figure 2 – Dendrogram construction among six isolates of pathogenic fungi

Dendrogram construction or similarity matrix is made to show the coefficient correlation based on the individual percentage of isolates similarity (Priest, 1993). The similarity has a

specific range of values that can be grouped into species, genus, or family based on its percentage. The value of phenotypic similarity that is over 80% indicates that these isolates are in the same genus, while the value of phenotypic similarity that is less than 80% indicates that these isolates not in a single genus (Prescott *et al.,* 2002).

Trichoderma spp. isolates. Trichoderma spp. was isolated from soil samples in Kliran. The characteristics of two *Trichoderma* cultures (TKL1 and TKL2) on PDA incubation for seven days at 28°C show green colony pigmentation. TKL1 isolates have a radial line and grow faster than TKL2 isolates. Based on microscopic observations, TKL1 isolates have spherical shape conidia while TKL2 has oval shape conidia, chlamydospore was found in two isolates and branched conidiophores (Figure 3).

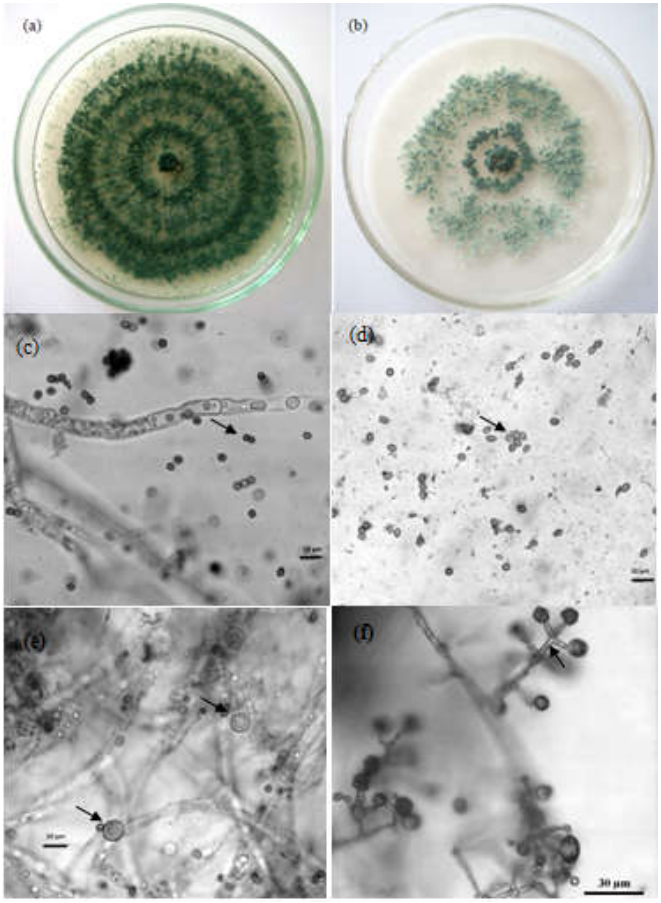

Figure 3 – Characteristics of fungi Trichoderma spp. the isolated:
(a). culture of TKL1, 7 days of incubation, 28°C, PDA, (b) culture of TKL2, 7 days of incubation, 28°C, PDA, (c) spherical conidia of TKL1; (d) oval conidia of TKL2; (e) chlamydospore (arrows), (f) branching conidiophores (arrows) (magnification by 400x) Bar= 10µm(ce), 30 µm, (f). TKL1 isolates estimated T.*harzianum*and/or as T.*viride* while TKL2 isolates characterized leading to the T.*koningii*. The results were obtained by a comparison of the macroscopic and microscopic descriptions among isolates (TKL1 and TKL2) referencing to T.*harzianum*, T.*koningii* and T.*viride* isolates.

Dendrogram construction of the five *Trichoderma* isolates (Figure 4) which is based on the characteristics of each isolate showed that TKL2 isolates have a value of 79% similarity with T.*koningii*, while TKL1 isolates have a value of 84% similarity with T.*viride* and T.*harzianum*. The similarity value between isolates (T.*viride* and T.*harzianum*) by 88% is assumed that these isolates are in the same genus. However, the value of similarity among isolates referencing to T.*koningii* with T.*viride* and T.*harzianum* by 57% do not meet the assumption that the three isolates are in the same *Trichoderma* Genus. This is due to the less widely used and less specific characteristics. According to Priest (1993), approximately 50-200 characters are required which include biochemical characteristics (including

sensitivity to antibiotics), morphology, and the character of the colony to determine the degree of similarity between some of the organisms in the fracture of numerical taxonomy (taxonomy frenetic).

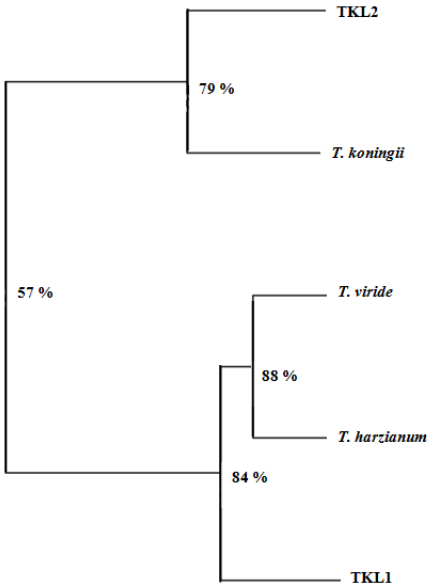

Figure 4 – Dendrogram construction among isolates of *Trichoderma* spp. with isolates reference to antagonism between *Trichoderma* spp. (TKL1 and TKL2) and pathogen (PRB3) in vitro

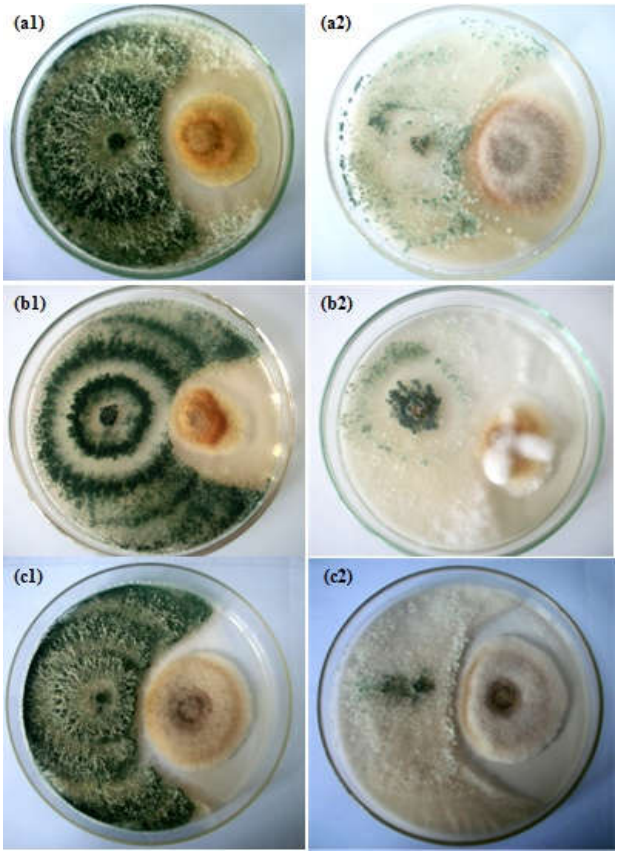

Figure 5 – Antagonism of *Trichoderma* spp. against pathogenic fungi:
(a1-a2) Inoculation of pathogenic and antagonist fungi at the same time, (b1-b2) antagonist fungus inoculation in advance (48hours), (c1-c2) inoculation of pathogenic fungi in advance (48hours). (code number 1) an antagonist that is used in TKL1, (code number 2) TKL2

The pattern of inhibition between the two antagonistic isolates (green colonies) against pathogenic isolates (brownish white colonies) was observed on the 7th day of incubation (Figure 5).

Two inoculated antagonistic fungus (*Trichoderma* spp.) can inhibit the growth of pathogenic fungi (PRB3) at three different treatments. Both of these antagonists inhibit the growth of pathogenic fungi without forming a clear zone as the zone of inhibition. However, the colony of pathogenic fungus stopped growing when the colony of antagonist fungus began to approach. This is possible to happen because of the compounds released by antagonist fungus as a mechanism of inhibition. These results are consistent with the studies that have been conducted by Zivkovic *et al.* (2010).

The rapid growth of *Trichoderma* spp. is shown by extensive colonies that almost fill the petri dish on the 7th day. This rapid growth provides benefits in terms of getting nutrition when it is paired with pathogenic fungi. It is an early mechanism before *Trichoderma* secrete mycotoxins (Barbosa *et al*., 2001). According to Aryantha & Guest (2006), the structure of the hyphae of *Colletotrichum* isolates contains toxic compounds when it is grown together with *Trichoderma harzianum*. *Trichoderma* spp. known to produce a number of antibiotics such as trichodermin, Trichoderma, trichotoxin, harzianum A, and harzianolide (Dennis & Webster, 1971 in Zivkovic *et al*., 2010). The chemical component has a major role to inhibit the growth of *Colletotrichum* and another phytopathogen (Zazzerini & Tosi, 1985; Gupta et al., 1995 in Zivkovic *et al*., 2010).

Three treatments were used significantly ($p<0,05$) on the growth inhibition percentage of pathogenic fungi by antagonistic fungi, but the TKL1 and TKL2 isolates were not significantly different ($p>0,05$) to the value of inhibition percentage. The treatment combination of antagonistic fungus also did not show a significant and different value for the percentage inhibition of pathogenic fungi by antagonistic fungi. The percentage constraint value by two antagonistic fungi to pathogenic fungus after the 7th day of observation are listed in Table 2.

Table 2 – Growth inhibition percentage of pathogenic fungus in the three treatments with two different antagonists

Treatment (time inoculation)	*percentage inhibition (%)	
	TKL 1	TKL2
Antagonist and pathogen at the same time	58,90b	54,43b
Antagonist in advance	71,07a	65.27a
Pathogen in advance	31,40c	31.37c

* Numbers followed by the same letter are not significantly different ($p>0,05$) in between treatment.

The antagonist that is inoculated before the pathogen (treatment B) has the highest value to inhibit the pathogenic growth (71,07% of TKL1 isolates and 65,27% of TKL2 isolates) compared with the other two treatments based on inoculation time. The lowest value is found in treatment C (31,4% and 31,37% of TKL1 and TKL2 isolates respectively). In treatment A, the value for TKL1 percentage inhibition is 58,9% and amounted to 54,43% of TKL2 isolates. Two isolates of *Trichoderma* (TKL1 and TKL2) indicate a potential as antagonistic for the three treatments. It means that the ability of each *Trichoderma* isolates to inhibit the growth of pathogen mycelium does not depend on the time of application. Giving one of the two isolates of *Trichoderma* will be able to suppress the growth of pathogenic fungi. The results of antagonism among *Trichoderma spp.* against pathogenic fungi (PRB3) in vitro studies suggest that the introduction of the antagonists before the pathogen gave the best growth inhibition of the pathogen. This indicates that it would be better if the existence of antagonistic fungi in the field had existed before the emergence of the pathogen. This is expected to suppress the growth of pathogenic fungi or as a measure of precaution (prevention) before the disease in plants happens.

Mechanism of Inhibition (interaction) between the antagonist and pathogens. Based on the observation of interaction test in between antagonistic fungi with pathogenic fungi, the

hyphae of the antagonists (*Trichoderma* spp.) is able to make contact with the hyphae of the pathogenic fungus through the mycoparasitism mechanism.

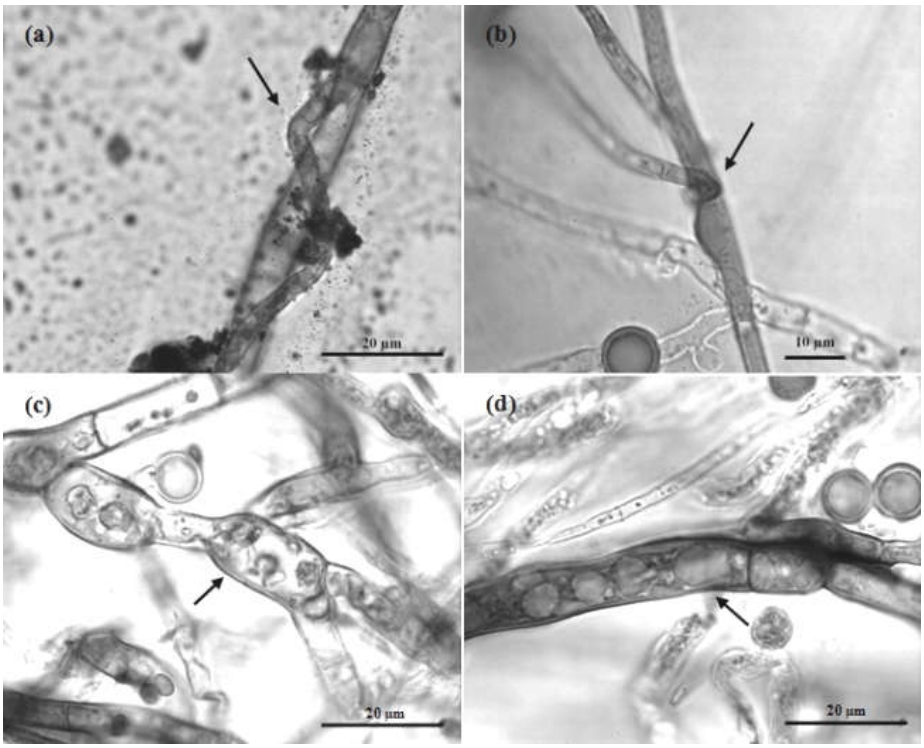

Figure 6 – Mycoparasitism *Trichoderma* spp. against pathogenic fungi:
(a& b) direct contact of the hyphae of pathogenic fungi and the hyphae of antagonistic fungi (arrows),
(c&d) damage structure of hyphae in the pathogen (arrows) (magnification by 1000x).
Bar = 20µm (a, c, d) and 10µm (b)

The mechanisms of mycoparasitism in the hyphae of antagonist were twine or wrapped in the hyphae of the pathogen (Fig. 6a and 6b). The penetration in the hyphae of antagonistic fungi to the hyphae of pathogenic fungi cannot be seen in this observation but the signs of deterioration in the hyphae of pathogenic fungi can be seen in the observation (Fig. 6c and 6d).

According to Cook & Baker (1983 in Sudantha *et al*., 2011), in general mechanism of antagonism, *Trichoderma spp*. suppressed the pathogenic with mycoparasitism and acts as an aggressive competitor. The process of antagonism by ***Trichoderma spp.*** through mycoparasitism is done with the growth of mycelia that is twisted, elongated, and penetrated to the hyphae of the pathogen. Furthermore, the hyphae of antagonistic fungus grow in the hyphae of the pathogen.

CONCLUSION

Based on the results of this study, it can be concluded that:

Based on phenotypic characteristics and PRB1 PRD2 mold, it is alleged that the member of the genus *Colletotrichum* have a value of more than 80% similarity, whereas the antagonistic fungi of *Trichoderma* genus TKL1 are suspected to have the similarity value by 84%.

TKL1 and TKL2 isolates could inhibit the growth of pathogenic fungi PRB3 respectively by 71,07% and 65,27% on the 7th day of observation in vitro treatment with the treatment of antagonist fungus on the first hand.

APPENDIX 1 – THE CHARACTERISTICS OF ANTAGONIST FUNGI

Table L1 – The characteristics of the reference and isolated antagonist fungi

Character	Isolates						
	TLD1	PRD2	PRB1	PRB3	TLT1	TLT2	*C. gloeosporioides*
Filament culture	+	+	+	+	+	+	+
circular colony	+	+	+	+	+	+	+
Erose Margin	+	-	-	+	-	+	-
Wolly Margin	-	+	+	-	+	-	+
Color change of the media	+	-	-	+	-	+	-
Color change of the brown media	+	-	-	+	-	-	-
Row Mycelium	+	-	-	+	+	+	-
Soft Mycelium	-	+	+	-	-	-	+
Radial line	+	+	+	+	+	+	+
Clear radial line	-	+	+	-	+	-	+
Thin radial line	+	-	-	+	-	+	-
Lower radial line	+	+	+	+	+	+	+
Tight mycelium	-	+	+	-	-	-	+
Loose mycelium	+	-	-	+	+	+	-
Umbonate elevation	+	-	-	+	+	-	-
Convex elevation	-	+	+	-	-	+	+
White-brown upper pigmentation	+	+	-	+	+	+	+
White upper pigmentation	-	-	+	-	-	-	-
Brown lower pigmentation	+	+	-	+	-	+	-
Brown-white lower pigmentation	-	-	+	-	+	-	+
Non-*acervulli* in culture	+	+	+	+	+	+	+
Non-setae in culture	+	+	+	+	+	+	+
Septate Hyphae	+	+	+	+	+	+	+
Thick Septate Hyphae	+	+	+	+	+	+	+
Thin Septate Hyphae	-	-	-	-	-	-	-
Hyaline Hyphae	+	+	+	+	+	+	+
Branched Hyphae	+	+	+	+	+	+	+
Non-Branched Hyphae	-	-	-	-	-	-	-
Round Hyphopodia	+	+	+	+	-	+	+
Chained Hyphopodia	+	+	+	+	-	+	+
Spore-forming body: conidia	+	+	+	+	+	+	+
Spore-forming body: conidia	+	+	+	+	+	+	+
obovate conidia	+	+	+	+	+	+	-
eliptical conidia	-	-	-	-	-	-	-
fusiform conidia	-	+	-	-	-	-	-
cylindrical conidia	-	-	-	-	-	-	+
hyaline conidia	+	+	+	+	+	+	+
1 type conidia	+	-	+	+	+	-	+
2 type conidia	-	+	-	-	-	+	-
core cell conidia	+	+	+	+	+	+	+
1 cell conidia	+	+	+	+	+	+	+
non-septate conidia	+	+	+	+	+	+	+
Conidia Length by 38,7 µm	-	-	+	-	-	-	-
Conidia Length by 43,9 µm	-	-	-	+	-	-	-
Conidia Length by 39,7 µm	-	+	-	-	-	-	-
Conidia Length by 41,1 µm	+	-	-	-	-	-	-
Conidia Length by 40,3 µm	-	-	-	-	+	-	-
Conidia Length by 40,5 µm	-	-	-	-	-	-	-
Conidia Length by 15 µm	-	-	-	-	-	-	+
Conidia Width by 4,5 µm	-	-	+	-	-	-	-
Conidia Width by 4,07 µm	-	-	-	+	-	+	-
Conidia Width by 5,3 µm	-	+	-	-	-	-	-
Conidia Width by 4,9 µm	+	-	-	-	+	-	-
Conidia Width by 5,0 µm	-	-	-	-	-	+	-
Conidia Width by 4,3 µm	-	-	-	-	-	-	+

APPENDIX 2 – THE CHARACTERISTICS OF ANTAGONIST FUNGI

Table L2 – The characteristics of the reference and isolated antagonist fungi

Character	*T.Harzianum*	*T.viride*	*T.koningii*	TKL 1	TKL 2
Filament	+	+	+	+	+
Thick filament	-	-	-	-	+
Thin filament	+	+	+	+	-
Radial line	+	+	-	+	-
Upper radial line	+	+	-	+	-
Lower radial line	+	+	-	+	-
Blur radial line	-	+	-	-	-
Clear radial line	+	-	-	+	-
Thick green colony pigment	+	+	-	+	-
Light green colony pigment	-	-	+	-	+
Granular colony	+	+	+	+	+
Ordered granula on radial line	+	+	-	+	-
Fast growth of colony	+	+	-	+	-
Slow growth of colony	-	-	+	-	+
Margin wavy/undulate	+	+	-	+	+
Margin irregular/erose	-	-	+	-	-
Curved elevation	+	+	+	+	+
Color change of the media	+	+	+	-	-
Brown-ish media	+	+	+	-	-
Branched Hyphae	+	+	+	+	+
Septate Hyphae	+	+	+	+	+
Thick Septate Hyphae	-	-	-	-	-
Thin Septate Hyphae	+	+	+	+	+
Hyaline Hyphae	+	+	+	+	+
Conidia 1 type	+	+	+	+	+
Round Conidia	+	+	-	+	-
oval conidia	-	-	+	-	+
hyaline conidia	-	-	-	-	-
dark conidia	+	+	+	+	+
1 cell conidia	+	+	+	+	+
3,7 µm conidia	-	+	-	+	+
3,8 µm conidia	+	-	-	-	-
4,1 µm conidia	-	-	+	-	-
Core conidia	-	-	-	-	-
Chlamydospores	+	+	+	+	+
Hyaline Chlamydospores	+	+	+	+	+
2 layers of chlamydospores	+	+	+	-	+
1 layer of chlamydospores	-	-	-	+	-
9,02 µm chlamydospores	+	-	-	-	-
9,4 µm chlamydospores	-	-	-	+	-
9,6 µm chlamydospores	-	-	-	-	+
10,8 µm chlamydospores	-	-	+	-	-
Branched conodiofor	+	+	+	+	+
hyaline conodiofor	+	+	+	+	+
Mycelium dominated with white	-	-	+	-	+
Mycelium dominated with green	+	+	-	+	-

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