

RESEARCH PAPER

## Screening of *Trichoderma* spp. isolates based on antagonism and chitinolytic index against *Xylaria* sp.

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### ABSTRACT

Sugarcane disease disturbance is one of the limiting factors in achieving high productivity. *Xylaria* sp. has become endemic to sugarcane plantations in Lampung and South Sumatra. The intensity of disease attacks in Indonesia by 25% and 26% can reduce sugar production 12.3 and 15.4%, respectively. Losses due to disease attacks are greater in ratoons because plant cane sugarcane which is attacked by this disease, in ratoon stage, there will be a decrease in the number of shoots. The aim of this study was to obtain isolates of *Trichoderma* spp. which have antagonistic abilities and have chitinase enzymes so that they are effectively used as biological agents against *Xylaria* sp. The research method of antagonist test used dual culture test and qualitative test of chitin substrate was used to assess the chitinolytic index of four isolates of *Trichoderma* spp. The parameters were the inhibition of *Trichoderma* spp. isolates and the chitinolytic index of the four isolates moreover the value of the chitinase enzyme activity of the selected isolates. Mechanism of antagonist T10 are competition, antibiosis and mycoparasitism. The results showed that the Indonesian Sugar Research Institute (ISRI) T10 isolate could be used as a biological agent against *Xylaria* sp. The isolate had an inhibitory ability 73.33% with a chitinolytic index value 1.15 and the activity value of the chitinase enzyme crude extraction at a dilution  $10^{-1}$  was 10.99 units/mL.

**Key words:** antagonism, chitinolytic, sugarcane, *Trichoderma* spp, *Xylaria* sp.

### INTRODUCTION

Plant disease disturbance is one of the limiting factors in achieving high productivity. Damage to certain parts of the plant can interfere with the physiological process of sugarcane plants which will affect the quality of sugarcane juice in the acquisition of yields. Root and stem rot disease was reported in Lampung Province at PT. Gunung Madu Plantation, in 1993. Losses by this disease in Taiwan range from 5% to 30% (Fang & Lee, 2000). The severity of the disease determines the level of decline in quality and sugar production. The intensity of disease attacks in Indonesia by 25 and 26% will reduce sugar production 12.3% and 15.4% (Sitepu et al., 2010). Losses due to disease attacks are greater in ratoons because plant cane sugarcane which is attacked by this disease, in ratoon stage, there will be a decrease in the number of shoots.

At the first time report, root and stem rot disease was only found in Taiwan, USA, and Puerto Rico. The

disease in Taiwan is caused by the fungus *Xylaria warburgii*, while in the USA and Puerto Rico it is caused by the fungus *Xylaria arbuscula* (Fang & Lee, 2000). The stroma of *Xylaria* sp. vary in color and shape. The variations of stroma depend on the part of the stage of stromal development (Lee et al., 2002). The disease symptom initially are yellowing leaves then transform to be a dried plant. Dead plants are easily removed because the roots die. If the base stem of sick cane begins to split out, it shows a light brown and reddish tissue, and there is a black line which is a characteristic of *Xylaria* sp. (Maryono et al., 2017).

Root and stem rot sugarcane disease control in Sumatra is using fungicides. Application of fungicides is less effective and expensive. The fungicides that have been used with active ingredients are benomyl, carbendazim, mankozeb, propinep, and maneb to control *X. warburgii*. Fungicides with active ingredients benomyl and carbendazim in PDA medium can inhibit the growth of pathogens, but the results of its application in the field can not control *X. warburgii* (Yulianti, 2017).

Antagonistic fungi *Trichoderma* spp. can be used to control various fungal pathogens that cause plant diseases (*Trichoderma* spp. can inhibit the fungus *Colletotrichum capsici*, *Fusarium* sp. and *Sclero-*

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*tium rolfsii* in vitro (Alfizar et al., 2013). *Trichoderma* antagonistic fungi have a broad spectrum in biological control. *Trichoderma* fungi mechanism in inhibiting the growth of pathogens indirectly through competition uptake nutrients and space, modifying environmental conditions or promoting plant growth and increasing plant resistance and antibiosis. The mechanism is directly through contact with antagonistic fungi, namely mycoparasitism (Berlian et al., 2013; Hastuti & Rahmawati, 2016).

The *Trichoderma* genus is selective for its pathogens. This occurrence correlated with the different levels of hydrolytic enzymes produced by each species of *Trichoderma* isolate when attached to the mycelium of the pathogenic fungus. *Trichoderma* spp. can produce extracellular lytic enzymes that are responsive to their antagonistic activity (Cherkupally et al., 2016).

Stability during fungal cell division and longitudinal growth of fungal hyphae are supported by cell walls. The presence of fungal cell walls is very important for fungal development. Moreover, in phytopathogenic fungi the cell wall is the first structure linked to the host plant. Fungi have a cell wall that is composed of chitin and N-acetyl glucosamine. The main constituent of fungal cell walls is chitin which is a polymer while the homopolymer of N-acetyl glucosamine. Chitin in the fungi is composed of a chain of glucan monomers (Langner & Göhre, 2016). Chitin is a polymer of N-acetylglucosamine which is a derivative of glucose. Chitin is a component of the outer cell wall of fungi (Gow et al., 2017). Chitin in fungi plays an important role, specifically in the formation of hyphal barriers, cell division, growth of hyphal tips, response to environmental stress and development of pathogenicity (Cui et al., 2013). Chitinases can have a scavenging function in cell wall plasticization or may act more specifically during cell fission, gain of nutrient chitin, or competitive interactions with other fungi. Therefore, this study aims to find an alternative control using *Trichoderma* spp. which has high inhibitory power and has a chitinase enzyme that plays a role in pathogenicity against pathogenic *Xylaria* sp.

## MATERIALS AND METHODS

**Research Site.** The research location is in the Plant Disease Laboratory and the Integrated Analysis Laboratory of the Indonesian Sugar Research Institute, Pasuruan, East Java.

**Morphological Observations of Four Isolates of *Trichoderma* spp.** *Trichoderma* isolates were grown

on potato dextrose agar (PDA) medium for 4 days. After growing, the morphology of the antagonist fungus was observed. After the isolates were 7 days, observations were made on the color and shape of the colonies of each isolate. Small conidia and hyphae were observed under a binocular microscope with a magnification of 1000 times. Parameters observed were conidiophores, phyalid, conidia, and hyphae forms (Suanda & Ratnadi, 2017).

**Selection of Four Isolates of *Trichoderma* spp.** The selection of *Trichoderma* spp. isolates were carried out based on the percentage of inhibition in the antagonism test and the value of the chitinolytic index. Isolates with a high percentage of inhibition value and a high chitinolytic index value were selected as biological agents. The selected isolates were measured on the activity of the chitinase enzyme with an incubation period of 1–6 days.

**Antagonism Test Four Isolates of *Trichoderma* spp.** The isolates of *Trichoderma* spp. (T1, T4, T10, and T15) and *Xylaria* sp. (Xu) were derived from isolates collected from the Indonesian Sugar Plantation Research Center. Fungal antagonism test was carried out by double culture method on PDA media. *Trichoderma* spp. and pathogens were grown in the same petri dish by facing each other with a distance of 3 cm between them. Antagonistic fungi were grown simultaneously on PDA media (Figure 1). Observations were made every day until the growth of the fungus *Xylaria* sp. as a control fulfill the petri dish. Observation of pathogen diameter as control and treatment by measuring in 4 line, that is W, Y, Z, and X (Figure 2). Determining of the inhibition percentage using the formula Amaria et al. (2013); Izzatinnisa et al. (2020) as follows:

$$P = \frac{C - T}{C} \times 100\%$$

- P = percentage of inhibition;
- C = diameter mean of the pathogen in control;
- T = diameter mean of the pathogen in the treatment by *Trichoderma* spp..

The experimental design in the antagonism test is a complete random design. The experiment was repeated with 3 replications. Observations were made every day until the control fulfilled the petri dish. Microscopic observations to the hyphae in the antagonist test. It is a test conducted by growing pathogens and antagonists on a glass object with PDA. Isolation of

pathogens and antagonists 2 days apart. Observations conducted 1 day after isolation on the object glass.

**Two-sided Antagonism Test (Inhibition by Volatile Compounds).** The antagonism test of inhibition by volatile compounds was carried out by growing antagonist fungi and pathogenic fungi on different sides. The lower side was antagonistic fungi and the upper side was pathogenic fungi. Both are folded so that there is a bottom and top. Observations were made on the diameter of the pathogen which was compared with the diameter of the control. Calculation of the percentage of inhibition using the formula Amaria et al. (2013); Izzatinnisa et al. (2020).

**Colloidal Chitin and Chitin Extraction.** Chitin isolation process consists of 2 stages, namely deproteinase and demineralization (Herdyastuti et al., 2010). Stage 1 deproteinization: 5 g of shrimp or crab shell with 50 mL of 3.5% NaOH (w/v) were heated at 65 °C for 2 hours. The results were cooled and filtered, resulting from filtration, namely residues. Stage 2 demineralization: the residue from the deproteinization was added

50 mL of 1 M HCL, stirred for 30 min at room temperature. Then filtered to obtain the residue. The residue was rinsed by distilled water until the pH was neutral. The residue was dried at 60 °C, obtained as chitin. Its chitin that is isolated from the shells of shrimp or crab is used to make colloidal chitin.

Colloidal chitin was prepared by means of 20 g chitin added by 300 mL of concentrated HCl and homogenized. Then incubated in the refrigerator for 24 hours. The solution was added with 200 mL of distilled water which was ready at 4 °C for one night, then filtered with glass wool. The precipitate was neutralized with 12 N NaOH until pH 7. Its solution was centrifuged at 4000 rpm for 10 min. The precipitate obtained was rinsed with sterile distilled water then centrifuged at 4000 rpm for 10 min (Saskiawan & Handayani, 2011).

**Chitin Substrate Qualitative Test and Determination of Chitinolytic Index.** Composition of colloidal chitin media with pH 7 are MgSO<sub>4</sub> 0.15 g, (NH<sub>4</sub>)SO<sub>4</sub> 1.5 g, KH<sub>2</sub>PO<sub>4</sub> 1 g, citric acid 0.5 g, agar 7 g, 40 mL colloidal chitin 5%, NA 1.6 g, bromocresol purple 0.15

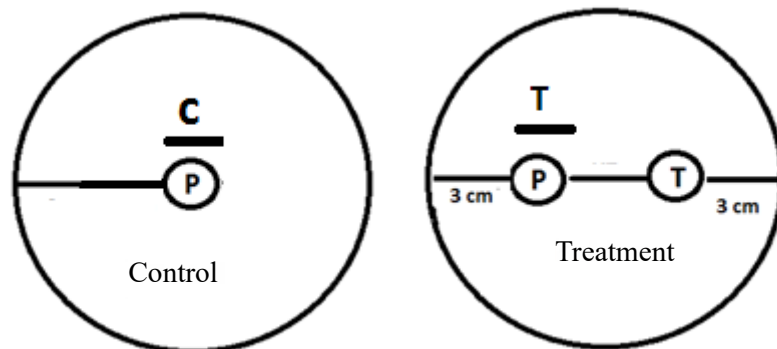


Figure 1. Test of antagonism of *Xylaria* sp.. (P) With the antagonist fungus *Trichoderma* spp., (C) Is the diameter of the pathogen in control (P), (T) Is the diameter of the pathogen (P) in the treatment of *Trichoderma* spp. isolates. (T) (Izzatinnisa et al., 2020).

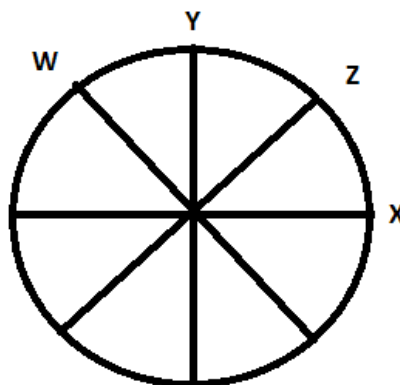


Figure 2. Measuring the mean colony diameter of the pathogen (Halwiyah et al., 2019).

g, 80 mL distilled water, afterwards sterilized by autoclave. *Trichoderma* spp. culture in PDA media was transferred to colloidal chitin agar media. Then incubated at 30 °C for 4 days. The activity of the chitinase enzyme was characterized by a change in the color of the chitinolytic medium from brown to purple. (Agrawal & Kotasthane, 2012). The determination of the chitinolytic index is performed by the formula (Suryadi et al., 2013; Khikmah et al., 2016):

$$\text{Chitinolytic index} = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

**Measurement of Chitinase Enzyme Activity.** Colloidal chitin media pH 7 was made by composition of MgSO<sub>4</sub> 0.15 g, (NH<sub>4</sub>)SO<sub>4</sub> 1.5 g, KH<sub>2</sub>PO<sub>4</sub> 1 g, citric acid 0.5 g, agar 7 g, colloidal chitin 5% 40 production media with pH 7 contained PDB 2.4 g, (NH<sub>4</sub>)SO<sub>4</sub> 1.5 g, KH<sub>2</sub>PO<sub>4</sub> 1 g, yeast extract 1 g, peptone 1 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.15 g, and NaCl 1 g, colloidal chitin 5% 50 mL, sterilized by autoclave. A total 1500 µL of *T. viride* liquid culture was inoculated into 30 mL of chitinase production media. Then incubated at a temperature 28–32 °C. Enzyme extraction was performed by centrifugation at 10,000 rpm at 4 °C for 15 min (Wirawan & Herdyastuti, 2013). Incubation was performed for 6 days so that the enzyme extraction results were obtained from 1 day after incubation to 6 days after incubation. The

resulting supernatant is an extract of the crude enzyme chitinase.

Supernatant or crude enzyme extract of 2 mL plus 2 mL of 1% substrate (b/v) colloidal chitin and phosphate buffer pH 7 in the test tube was incubated at 37 °C for 30 min. The centrifugation of the solution was carried out for 15 min at a speed of 10,000 rpm. The result is supernatant 2 mL added with 1 mL of DNS reagents. It was heated at 100 °C for 10 min. The mixture was cooled and measured absorbance using UV Vis spectrophotometer at a wavelength of 540 nm with N-acetyl glucosamine as a standard solution (Nafisah et al., 2017). The concentration of crude enzyme extract was determined using the equation in the standard curve solution (Halim et al., 2018).

## RESULTS AND DISCUSSION

**The Morphological Character of Four *Trichoderma* spp. Isolates.** The appearance of *Trichoderma* isolates used in this study has macroscopically different forms. In general, *Trichoderma* spp. isolates have a type of colonies from white, cream white, whitish green until dark green. The colony form has a circular shape with a small circle standing out in the middle (Figure 3). The distinguishing between four colonies lies in the color and form of the circle in the middle. Microscop-

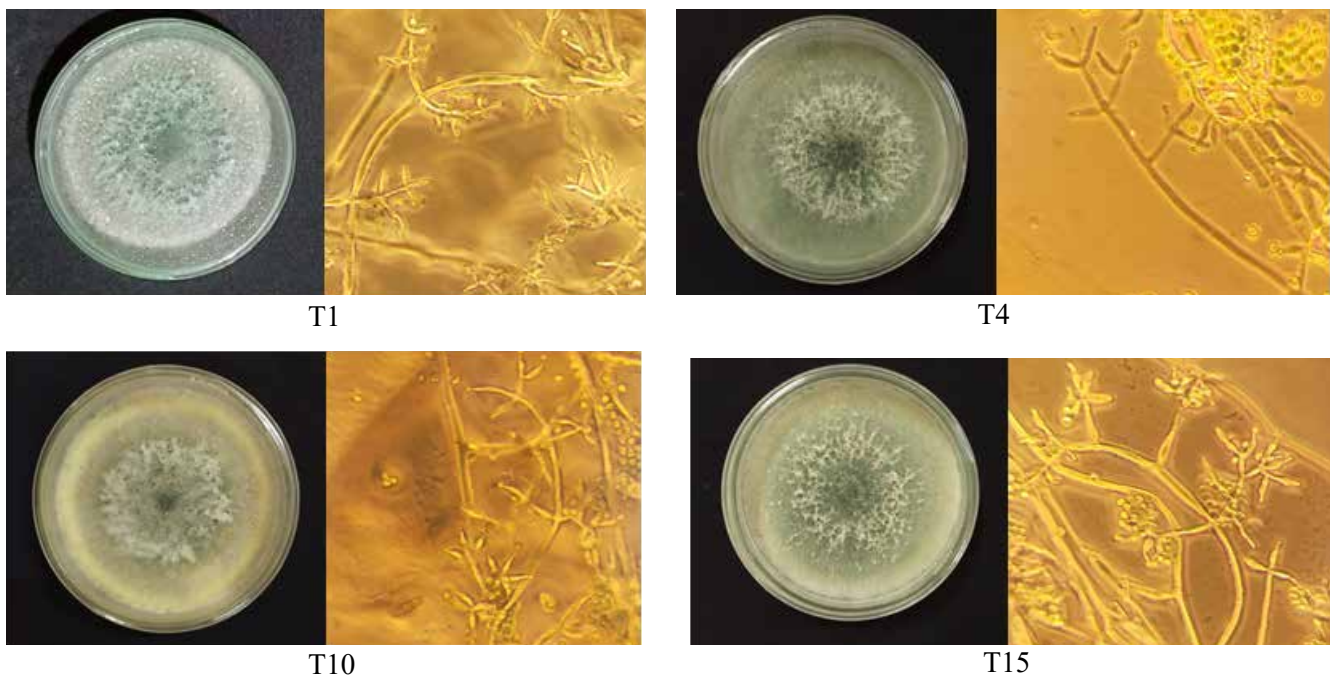


Figure 3. The morphological character of four *Trichoderma* spp. isolates by macroscopically and microscopically. T1 *Trichoderma* spp. isolate, T4 *Trichoderma* spp. isolate, T10 *Trichoderma* spp. isolates and T15 *Trichoderma* spp. isolate.

ic characters are almost similar among these four isolates. The distinguishing characteristic of microscopic lies in the conidia and fialid forms (Table 1). Based on morphological characteristics in each isolate probably they were a different strain or species.

**Antagonism Test of Four *Trichoderma* spp. Isolates.**

The results of the observation of the inhibition zone in the isolation antagonist test performed simultaneously showed that there was a growth inhibition of the pathogen *Xylaria* sp. compared to controls (Figure 4). Based on Figure 2, isolates that have high inhibitor ability is

T10 isolate. The isolate that had the lowest inhibitor ability was T15 isolate. The growth of T15 isolate was faster than T10, but the slower growth of T10 isolates could suppress the growth of *Xylaria* sp. since the beginning. The growth of *Xylaria* at T15 was initially suppressed, but *Xylaria* hyphae over time were able to override the hyphae of *Trichoderma* T15 isolate.

The antagonism at the same isolation time showed that the isolate T10 had the highest inhibition zone compared to T1, T4 and T15. T10 isolates were not significantly different from T1 and significantly different from T4, T15 and controls. The percentage

Table 1. Morphological characteristic of isolates T1, T4, T10 and T15 by macroscopically and microscopically

Isolate	Macroscopic			Microscopic		
	Colour	Shape	Conidio-phores	Fialid	Conidia	Hyphae
T1	Thick white hyphae, whitish green spores in the inner circle	Round that filled the culture medium, hyphae shaped like thick cotton, spore colonies form a circle that stands out in the middle and jagged at the edge of the circle	Hyaline and branched	Lageniform to ampuliform and hyaline	Subglobose shape	Septum
T4	Thin white hyphae, dark green spores	Round that filled the culture medium, hyphae shaped like thin cotton, spore colonies form a circle that stands out in the middle and it was jagged until the center of the circle	Hyaline and branched	Ampuliform and hyaline	Subglobose shape to ellipsoidal	Septum
T10	White to thick creamy white hyphae, dark green spores	Round that filled the culture medium, hyphae shaped like thick cotton, spore colonies form a circle that stands out in the middle and it was jagged until the center of the circle	Hyaline and branched	Lageniform and hyaline	Globose to subglobose shape	Septum
T15	Creamy white hyphae, dark green spore	Round that filled the culture medium, hyphae shaped like thin cotton, spore colonies form a circle that stands out in the middle and jagged at the edge of the circle.	Hyaline and branched	Ampuliform and hyaline	Subglobose shape	Septum

of inhibition between T4 and T5 was not significantly different but significantly different from the control. Based on the data in Table 2. The highest percentage of inhibition is T10 with the percentage of inhibition reaching 73.33%.

The percentage value of inhibition with simultaneous incubation time T10 > 60%, therefore it is possible to be a candidate for biological agents against *Xylaria* sp. As stated by Suanda (2019) that the percentage of inhibition of *Trichoderma* spp. with the dual culture method simultaneously inoculation if more than 60% it will have a good effect on disease suppression. The growth of *Trichoderma* spp. is faster than *Xylaria* sp. resulting in increased competitiveness.

Observation of parasitism was conducted by looking at the hyphae grown together on the glass object between *Xylaria* sp. and *Trichoderma* spp. micro-

scopically observed. Parasitism occurs when there is entanglement and damage to the hyphae of *Xylaria* sp.. All treatments showed a parasitism mechanism (Figure 5), it can be seen that the control hyphae *Xylaria* sp. look intact. In isolates T1, T4, T10 and T15, the hyphae of *Xylaria* sp. and *Trichoderma* spp. intertwine with each other and expansion of the hyphae occurs (Tasik et al., 2015). The mechanism of parasitism takes place physically, namely the presence of hyphae intertwined between antagonistic fungi and pathogens (Adnan et al., 2019).

Antagonism test to determine the percentage of inhibition by volatile compounds or secondary metabolites by *Trichoderma* was carried out using the two-sided method. In the lower position was *Trichoderma* spp. and on the upper side was *Xylaria* sp.. On the control, the bottom side of the PDA is empty and the top side

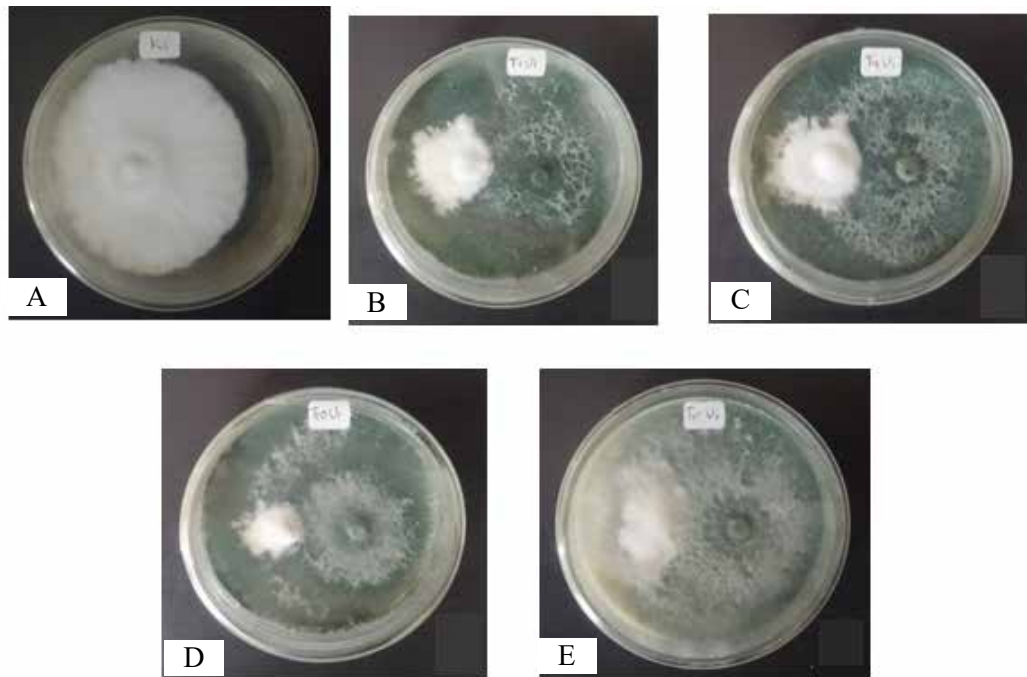


Figure 4. Isolation of antagonists simultaneously. (A) Control; (B) *Trichoderma* isolate T1; (C) *Trichoderma* isolate T4; (D) *Trichoderma* isolate T10; (E) *Trichoderma* isolate T15.

Table 2. The results of antagonist test are undertaken simultaneously

No.	Isolates	Simultaneous inoculation
1	Control	0.00 c
2	T1	69.00 a
3	T4	58.33 b
4	T10	73.33 a
5	T15	52.33 b
		LSD = 10.387

Numbers accompanied by the same letter in the same column represent no significant difference based on Duncan’s test at an 5% error rate.



was *Xylaria* sp.. The results of the antagonist test by volatile compounds released by *Trichoderma* can inhibit the growth of *Xylaria* sp. Figure 6. shows the barriers between treatment and control. The overall results of the inhibitory test due to volatile compounds were not significantly different in the percentage of inhibition zones between treatment and control between 41.6% to 54.33% as shown in (Table 3).

Antibiotic mechanism was not seen in the dual culture antagonist assay because of the clear zone at the end of the observation period. In the 2-sided test, it was seen that there was an inhibition of the growth of the pathogen compared to the control. This proves

that *Trichoderma* spp. releases secondary metabolites which are volatile so that it can be categorized as an antibiosis mechanism (Soesanto et al., 2013). Most of the *Trichoderma* species was tested by Qualhato et al. (2013) produced some volatile metabolites wick having a significant effects on the growth and development of the pathogens. The volatile chemical compounds of *Trichoderma* comes from the component (6-pentyl-a-pyrone) which is a derivative of (iso-cyanide) which is characterized by a distinctive odor (Kumar et al., 2017).

Some *Trichoderma* species produce fungitoxic compounds in design to cell wall destroying enzymes

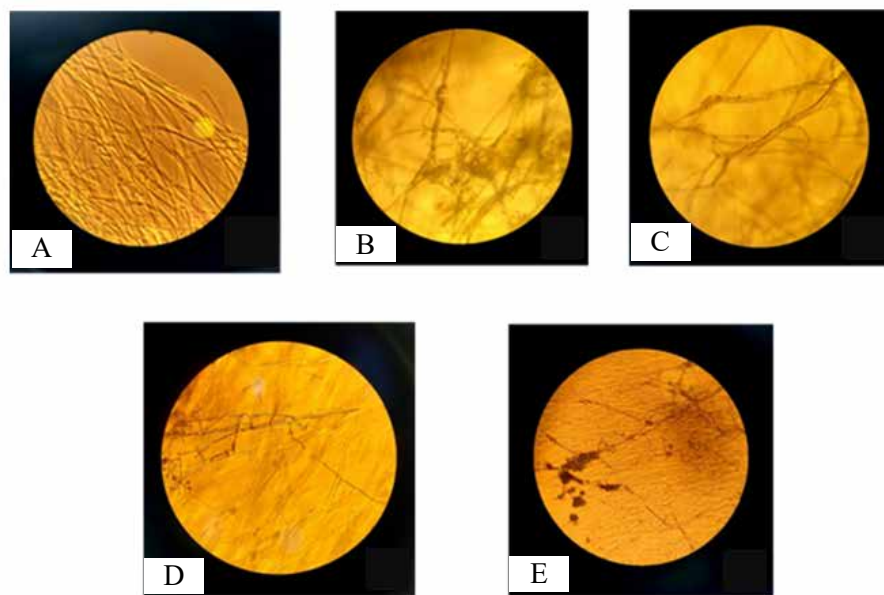


Figure 5. Parasitism of *Trichoderma* spp. against *Xylaria* sp. (A) Control; (B) *Trichoderma* isolate T1; (C) *Trichoderma* isolate T4; (D) *Trichoderma* isolate T10; (E) *Trichoderma* isolate T15.

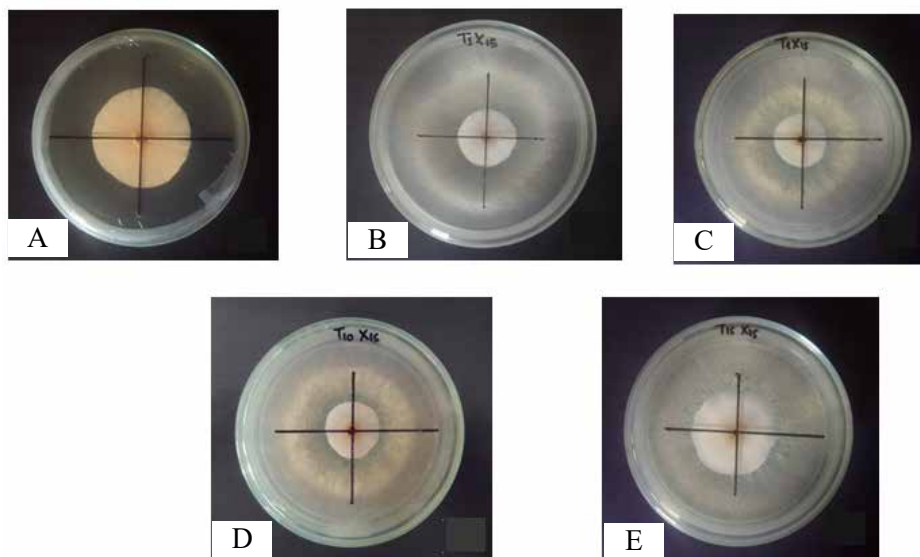


Figure 6. Inhibition by antagonists volatile compound. (A) Control; (B) *Trichoderma* isolate T1; (C) *Trichoderma* isolate T4; (D) *Trichoderma* isolate T10; (E) *Trichoderma* isolate T15.

and also have antibiotic peptides (peptaibol) which are secondary metabolites. Some of the peptaibols belonging to *Trichoderma* species such as: *T. atroviride* produces atroviridins, *T. asperellum* produces trichotoxin A, *T. harzianum* produces trichokindins, *T. koningii* produces trichokonins (Daniel & Filho, 2007). The compound produced by *Trichoderma* has the potential to inhibit the growth of pathogenic fungi (Muhibbudin et al., 2021). The mechanism of mycoparasitism occurs because the *Trichoderma* fungus secretes proteins or enzymes that destroy cell walls. The enzyme is the chitinase enzyme.

**Chitin Substrate Qualitative Test and Chitinolytic Index.** The results of the qualitative test are shown in Figure 7 which exhibit a color change in the substrate medium. In the control that is not overgrown with

fungus, the media is orange. The results exhibit that there was a color change from orange to purple after the *Trichoderma* fungus was grown on chitin colloidal substrate media. The qualitative test was expressed in the chitinolytic index, namely the ratio between the diameter of the color change compared to the diameter of the *Trichoderma* spp. The results of the calculation of the chitinolytic index can be seen from the size of the diameter of the colony (Figure 8). The smaller the diameter of the colony, the greater the value of the chitinolytic index. In isolate T10, the value of the chitinolytic index was the highest and the value of the chitinolytic index in T15 was the lowest. This indicated that the T10 isolate produced more enzymes than the other isolates.

The color change in the chitin substrate medium indicates the presence of the chitinase enzyme. All

Table 3. Percentage of inhibition due to volatile compounds *Trichoderma* spp.

No.	Isolates	Percentage of inhibition (%)
1	Control	0.00 b
2	T1	53.00 a
3	T4	52.00 a
4	T10	54.33 a
5	T15	41.67 a
		LSD =18.6

Numbers accompanied by the same letter in the same column show no significant difference based on the Duncan test at a 5% error rate.

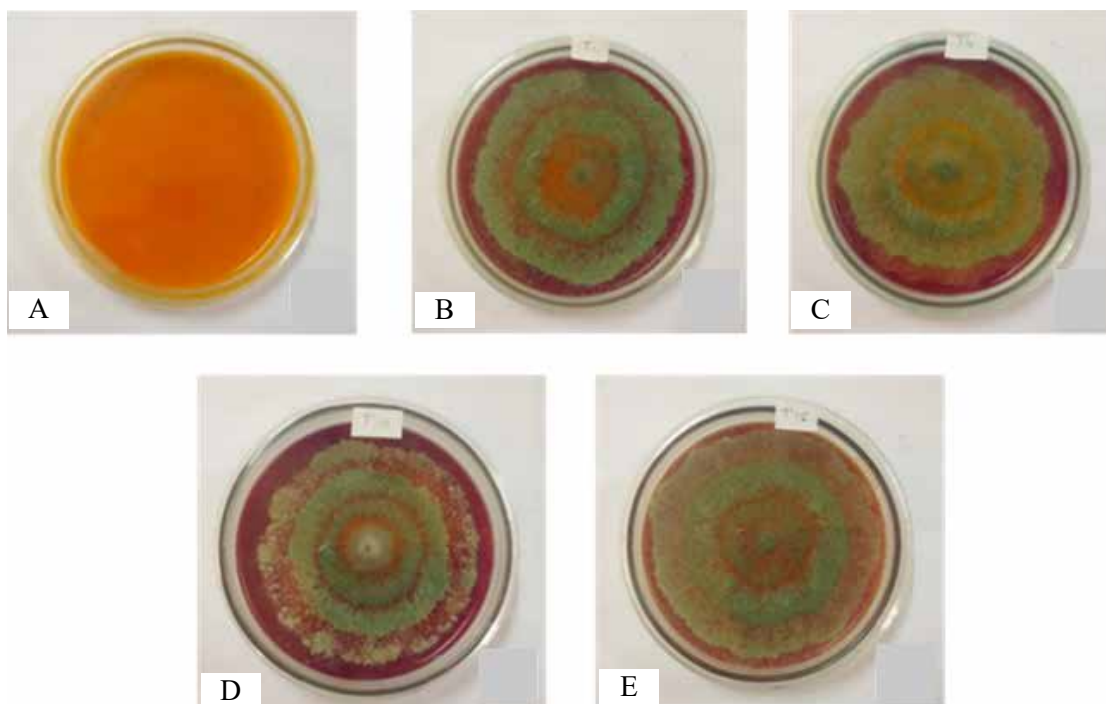


Figure 7. Chitinolytic qualitative test of *Trichoderma* spp. isolates. (A) Control; (B) *Trichoderma* isolate T1; (C) *Trichoderma* isolate T4; (D) *Trichoderma* isolate T10; (E) *Trichoderma* isolate T15.



isolates selected in the antagonist test had different amounts of the chitinase enzyme. The main factor in the lysis of pathogenic cell walls in the mycoparasitism process is the presence of various extracellular enzymes, one of which is the chitinase enzyme (Verma et al., 2007). Chitin in fungal cell walls protects against hyphae aging, autolysis and mycoparasitism attacks. The factor that affects the chitinase enzyme is the ability to access the substrate (Poria et al., 2021). The second largest polysaccharide sequence after cellulose is chitin, both of which have biopolymers. Components of chitin 1,4 linked to N acetyl glucosamine. Chitin is abundant in the exoskeleton of Ecdysozoa and in fungal cell walls which makes fungal cell walls stable. Chitin in fungi is a component of membranes and cell walls of hyphae, conidiophores, conidia, spores (El-soud & El Kady, 2019).

Selection of biological agents based on simultaneous antagonism and chitinolytic enzyme tests qualitatively, isolate T10 was the selected isolate that could be used as biological agent for controlling root and stem rot disease of *Xylaria* sp. The antagonist mechanism of the T10 isolate is nutritional competition because the growth of the T10 isolate is faster if there are pathogens. The second mechanism of antagonism is parasitism by the fungus *Trichoderma* spp. in inhibiting the growth of the fungus *Xylaria* sp. In this process, the chitinase enzyme plays a role in damaging the cell wall of the *Xylaria* sp. so that the hyphae become damaged and fungal growth stops. Chitinase enzyme activity was measured using a spectrophotometer with a wavelength of 540 nm.

**Enzyme Activity Measurement of Selected *Trichoderma* spp. Isolate.** The selected isolate in the antago-

nist test and the qualitative test of the chitinase enzyme are isolate T10. Chitinase enzyme activity test was implemented to determine the ability of the chitinase enzyme released by *Trichoderma* spp. in degrading pathogenic chitin. Based on the test results, the chitinase enzyme activity expressed in units/mL of chitinase enzyme activity reached 10.99 units/mL (Figure 9).

The activity of the chitinase enzyme on the first day of incubation until the third day showed an increase, the activity of the chitinase enzyme increased slowly on the first day to the third day, then the enzyme activity increased sharply on the fourth day. Enzyme activity decreased on the fifth and sixth days. This is in accordance with the research of Corneliyawati et al. (2018) that the activity of the chitinase enzyme after 5 days will decrease until the 10 days of incubation. The decrease in activity was due to a number of living cells decreasing in number so the substrate was also reduced. The higher value of the enzyme activity, it means a higher ability to degrade the chitinase of pathogenic fungi which causes damage to the cell wall of the pathogen, thereby inhibiting a pathogen growth. The higher enzyme activity, it means higher producing of N acetyl glucosamine, thereby the ability of the enzyme to degrade the substrate more optimally (Insani & Herdyastuti, 2016).

The stages of chitin degradation by the chitinase enzyme initiate by lytic polysaccharide monoxygenases (LPMOs) and endochitinase that destruct the chitin polymer at the fungal cell wall section and there are reduced and non-reduced parts and also loss of substrate. After the polymer chain is broken, the reduced part of the chain will attach to the exochitinase site. Part of the element of exochitinase is Chito oligosaccharides (CHOs) whose constituents are N-acetyl-glu-

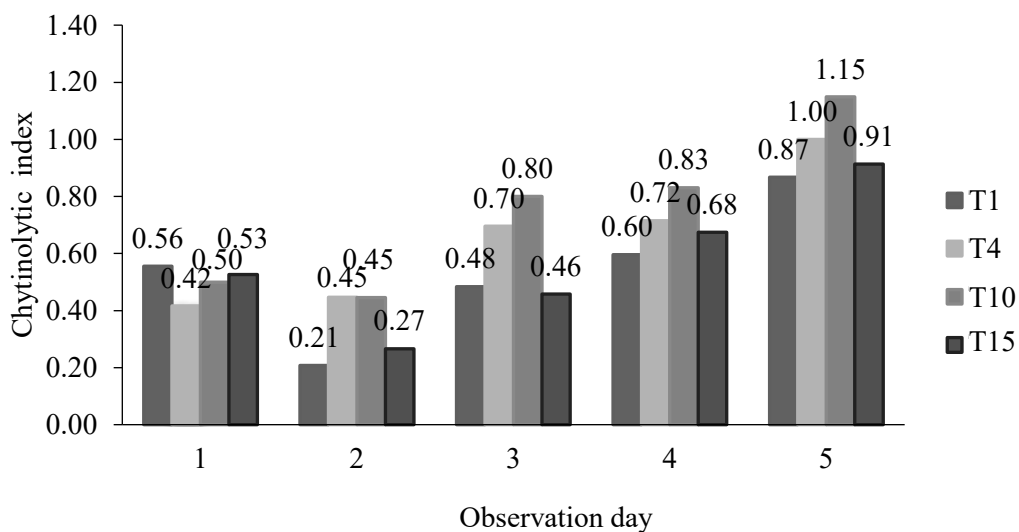


Figure 8. The development of the chitinolytic index value up to 5 days after isolation.

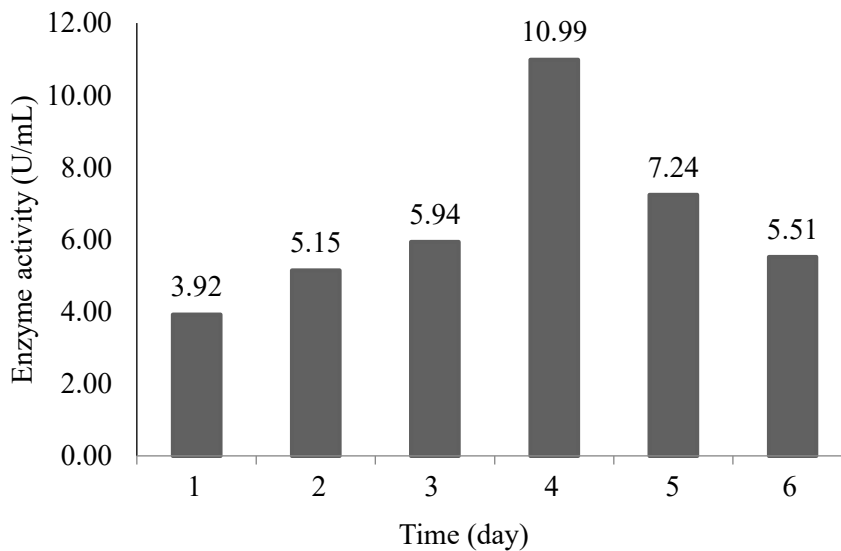


Figure 9. Results of chitinase enzyme activity *Trichoderma* spp. (isolate T10) for 6 days.

cosamineidase. These CHOs will provide a substrate for N acetyl glucosamine from other chitinases, both endochitinase and exochitinase so that the chitin polymer layer can be degraded (Langner & Göhre, 2016).

### CONCLUSION

The selection of *Trichoderma* spp. isolates from the Indonesian Sugar Research Institute (ISRI) collection resulted in isolates that have a potential as biological agents for the pathogen *Xylaria* sp. Selection with the antagonist test illustrates the ability of the antagonist to the competition, antibiosis and mycoparasitism. The selection of wall-degrading enzymes illustrates the ability of mycoparasitism related to the pathogenicity of antagonistic fungi. ISRI isolate T10 was the isolate that had the highest antagonism ability and had chitinase enzyme. The ability to degrade pathogenic chitin is quite high with the activity value of the coarse enzyme extract at a  $10^{-1}$  dilution is 10.99 units/mL.

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### AUTHORS' CONTRIBUTIONS

WWJ considered and planned the experiment. WWJ carried out the isolation of *Trichoderma* spp. for antagonistic test. I performed molecular work, analysis and interpreting the inhibition of *Trichoderma* spp. against *Xylaria* sp. WWJ prepared the manuscript, ALA, LQA, SD data interpretation and manuscript writing and editing. ALA as a promoter focus on mycology, LQA as a promoter focus on biotechnology and enzymes. SD as a promoter focus on biological control. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

### COMPETING INTEREST

Authors declares that there is no competing interest regarding to the publication of this manuscript.

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