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Analysis of Chitinase Enzyme Trichoderma sp. in Degrading Fusarium oxysporum

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KEYWORDS

Biological control; enzyme chitinase; *Fusarium oxysporum*; *Trichoderma asperellum.*

Abstract The chitinase enzyme in Trichoderma sp. play an important role in pathogen control. This is because chitin is the main component of the fungal cell wall which the chitinase enzyme can degrade. One Trichoderma sp. isolate was obtained (UBPK6) with the highest percentage of inhibition against Fusarium oxysporum, 76.71%. In addition, UBPK6 isolates also showed the highest bromocresol purple reagent test results (indicating the presence of chitinase enzymes). Chitinase enzyme activity was measured for Trichoderma asperellum isolates. The results showed that the optimum incubation period for enzyme production was the 4th day, with an enzyme activity value of 4.05 U/mL. It indicates that this time is the right time for harvesting enzymes. Furthermore, the effect of pH on the chitinase enzyme activity of Trichoderma asperellum fungus. The test results showed that the optimum value was produced at pH 5 with a value of 3.4 U/mL and decreased afterward. The pH five treatment was the best in inhibiting the growth of pathogens, with an inhibition value of 60.63%. The higher the content of the chitinase enzyme, the higher its ability to degrade damage to the germination of pathogenic spores, thus causing the growth of the pathogen to be inhibited.

Introduction

Fusarium wilt disease in chilies caused by the fungus Fusarium oxysporum is an important limiting factor for optimal chili yields (Andriastini et al., 2018). The final stage of this disease is pallor of the veins followed by rolling of old leaves (epinasti), and then the whole plant withers which then causes the plant to die (Anggraeni and Usman, 2015), which of course will have an impact on decreasing yields. Control of F. oxysporum needs to be done to inhibit the spread and yield loss in cultivated plants.

Control of disease attacks that have been carried out so far uses fungicides that have a negative impact on the environment. Using fungicides that are not appropriately managed causes fungicide residues on chilies and can endanger health. This fungicide residue was present since the benomyl fungicide compound was not completely hydrolyzed (Palladino et al., 2021). Alternative biological control is needed to overcome dependence on chemicals, one of which is using enzymes that can degrade pathogens from Trichoderma sp.

The chitinase enzyme is an extracellular enzyme whose production process is influenced by environmental factors such as substrate concentration, inoculum, environmental pH, temperature, and incubation time (Ting and Chai, 2015). The highest chitinase activity was obtained at an acidic pH, namely pH 5.5, and an optimum temperature of 50^{o}C, the highest chitinase activity was achieved at a pH of 5.5 and decreased slowly at a pH below and above 5.5. The pH range for chitinase activity is between pH 5-6.5 (Eslahi et al., 2021). Chitinase is a

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hydrolytic enzyme that can hydrolyze chitin at its β-1,4-glycosidic bond by producing chitin derivatives such as chitin oligomers. Chitinolytic *Trichoderma* sp. consists of many enzymes and their components are rapidly renewed as new enzymes and genes. Chitinases are divided into 1,4-β-acetylglucosaminidases (GlcNAcases), endochitinases, and exochitinases. The chitinase enzyme produced by *Trichoderma* sp. is also thought to play a role in inhibiting and killing pathogens, this enzyme can damage and lyse the cell walls that make up pathogenic hyphae, as stated by Alfizar et al. (2013) that the percentage of inhibition of *Trichoderma* sp. which is caused by the soil-borne pathogen *F. oxysporum* which can cause damage reaching a level of 60%, therefore the fungus *Trichoderma* sp. can suppress the development of *F. oxysporum*, due to *Trichoderma* sp. can produce chitinase enzymes which soil-borne pathogens are the target of the chitinase enzyme *Trichoderma* sp.

Antibiosis is a mechanism for controlling pathogens by releasing compounds, including the chitinase enzyme. According to (Almeida et al., 2022), the mycelium on *Trichoderma* sp. capable of producing various enzymes, for example, chitinase, cellulase, and glucanase. These enzymes are used to control pathogens. This is because enzymes can degrade cells in pathogenic fungi, so the cells undergo osmosis and then die. The mechanism for controlling antagonistic fungi against *F. oxysporum* begins with the hyphae walls of *F. oxysporum* degrading, causing nutrients in the pathogenic hyphae to be absorbed by the hyphae of *Trichoderma* sp., thus causing the pathogenic hyphae to become flat and the nutrients in the hyphae will come out. The absence of nutrients in pathogenic fungi causes inhibition of the pathogen's metabolism, disrupting colony growth and death of hyphal cells (Hoang et al., 2020). Trichoderma sp.'s ability to produce cell wall degrading enzymes (chitinolytic, cellulolytic, and glucanolytic) has become the concern of researchers. According to (Zhang et al., 2017) *Trichoderma* sp. can produce chitinase enzymes. Chitinase in agriculture is used as an antifungal, nematocidal, and biopesticide compound. Chitinase acts as a biological control agent for plant diseases caused by fungi.

The control of fusarium wilt disease in chilies by utilizing the chitinase enzyme using biological agents has not been done much, so research is needed to analyze the chitinase enzyme *Trichoderma* sp. at different pH media on controlling *F. oxysporum* to get potential microbes.

Material and methods

MGMK media

Preparation of solid chitin minimum salt medium (MGMK) composed of 0.3 grams KH2PO4, 0.7 grams K2HPO4, 0.5 grams MgSO4.7H2O, 0.01 grams FeSO4.7H2O, 0.001 grams ZnSO4, 0.001 grams MnCl2, 20 grams colloidal chitin, 20 grams of agar, 10N HCl, 10N NaOH, 1000 ml of distilled water. Then making liquid MGMK is the same as making solid MGMK, but no agar is added.

Preparation of 3.5-dinitrosalicylic acid (DNS) reagent

DNS reagent is prepared by dissolving 1 gram of 3,5-dinitrosalicylic acid in 50 ml of sterile distilled water, then adding 30 grams of sodium potassium tartrate until the mixture is milky yellow. Then 20 ml of 2N NaOH was added, then the color of the mixture would change to orange. Add distilled water until the final volume becomes 100 ml, then store it in a dark bottle in cold conditions.

Chitinase tests of Trichoderma sp. isolates with bromocresol purple reagent

Fungal culture of *Trichoderma* sp. which has been rejuvenated, inoculated on MGMK media which has been added with bromocresol

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purple reagent as much as one cork borer, and placed in the middle of the surface of the media in each petri dish. Fungal cultures were incubated at room temperature for seven days. Observations were made at seven his to see the color change in the petri dish. If a clear zone appears and changes color from yellow to purple, it can be concluded that the fungus *Trichoderma* sp. produces chitinase enzymes that can degrade *F. oxysporum* chitin. The chitinolytic index is determined using the formula (Suryadi et al. 2013):

$$\text{chitinolytic indes} = \frac{\text{clear zone diameter}}{\text{colony diameter}}$$

Molecular identification of Trichoderma sp. the chosen

Trichoderma sp. isolates the selected ones were grown in EKG media, then placed in a tube and labeled. The initial step of DNA purification is adding ammonium acetate 0.1 times the sample volume and absolute ethanol 2.5 times the sample volume. Then the homogenization was turned over 10-20 times and incubated at -20°C for 24 hours.

PCR was used for amplification of fungal DNA. Extraction of fungal DNA genomes was amplified using universal primers, namely forward primer ITS1 (5'-TCCG TAGG TGAA CCTG CGG-3') and reverse primer ITS4 (5'-TCCT CCGC TTAT TGAT ATGC-3') with a target size of ± 550 bp (White et al. 1990). This was done by pre-denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, then annealing at 51.9°C for 30 seconds, then elongation at 72°C for 2 minutes, and final elongation at 72°C for 7 minutes. It was repeated for 30 repetitions (Sandy et al. 2015).

Chitinase enzyme production and characterization at different pH conditions using chitinase enzyme production media

Preparation of production media for the chitinase enzyme using a modified method (Suryadi et al. 2013) containing 2.4 grams of PDB, 1.5 grams (NH4)2SO4, 1 grams of KH2PO4, 1 grams of yeast extract, 1 grams of peptone, 0.15 grams of MgSO4.7H2O, 1 grams of NaCl, and 50 ml of colloidal chitin and then sterilized by autoclave. Furthermore, 1500 µl of liquid culture of *Trichoderma* sp. inoculated into 30 ml of chitinase production medium. Each medium was tested at different pH conditions. Moreover, the isolates were incubated at room temperature, namely ± 27°C for 96 hours, using an incubator with a shaking speed of 150 rpm (Kurniawan et al. 2014). Enzyme extraction was centrifuged at 12.000 rpm at 4°C for 10 minutes. The resulting supernatant is the crude chitinase enzyme extract.

Chitinase enzyme activity test

This test was carried out by growing *Trichoderma* sp. with a pH of 5, 6, 7, 8, and 9 colloidal chitin 5 grams/ml then incubated for 7 days at room temperature ±27°C. Then 10 ml of solution was centrifuged at 5.000 rpm for 20 minutes at room temperature ±27oC. Furthermore, 1 ml of the supernatant formed was reacted with 1 ml of DNS, then heated at 100°C for 10 minutes to stop the reaction, after which 1 ml of distilled water was added.

The absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 584 nm. This test is carried out to determine the optimum pH of chitinase, which will be used for further tests. The absorbance values obtained were entered into the standard curve regression equations made with N-AGA (100, 200, 300, 400, 500, 600, 700, 800, 900, 1.000 µ/ml). The formula calculates enzyme activity:

$$\text{chitinase enzyme activity 10-3} = \frac{\text{(reduced N-AGA) x 1000 x FP}}{\text{molecular mass (221) x time}}$$

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Data analysis

This test was carried out by growing ANOVA analysed all data with a level of 5%. If it showed a significant effect, proceed with the LSD (Least Significance Different) further test. All data obtained were processed using Microsoft Excel 2019 software, then presented in graphs and tables.

Result and Discussion

Screening of Trichoderma sp. isolates with the antagonist test

The results of the antagonist test of the five isolates showed different inhibitory abilities. The data in (Table 1) is the percentage of inhibition data taken on the 5th to 7th day of observation, according to the statement of Rachmawati et al. (2016) that inhibition of pathogenic colonies starts from 4-7 hsi.

The average inhibition percentage of the five Trichoderma sp. isolates increased (Table 1). Table 1 shows that the five isolates could suppress the growth of F. oxysporum. The highest inhibition was observed at seven hsi, namely UBPK6 isolate of 76.71%, the growth of Trichoderma sp. which quickly mastered the substrate so that it was able to beat F. oxysporum in competition for space and nutrients (Pardede et al., 2022). Inhibition occurs from the hyphae of Trichoderma sp., which parasitizes the hyphae of F. oxysporum and releases secondary metabolites, including antifungal compounds (Al-ani, 2018). Antifungal compounds indirectly affect the growth of the fungus F. oxysporum. F. oxysporum also secretes secondary compounds that cause Trichoderma sp. produce antifungal compounds. According to Vicente et al. (2022), there is a relationship between the production of secondary metabolites from Trichoderma sp. and F. oxysporum. Therefore, Trichoderma sp. has high inhibitory ability, so it suppresses F. oxysporum.

Mechanisms of Trichoderma sp. was found during the observation, namely mycoparasites, antibiosis, and competition. At 96 hours of observation, it was seen that there was a competition mechanism for space and nutrients, where the growth of F. oxysporum colonies was inhibited, and colonies of Trichoderma sp. had a larger area. Then the mechanism of mycoparasitism can be seen in the picture where the attachment of the hyphae of Trichoderma sp. on F. oxysporum. In addition, the mycelium of Trichoderma sp. parasites the mycelium of F. oxysporum by penetration and absorption until lysis. Microscopically, more and more of the mycelium of F. oxysporum underwent lysis.

Table 1. *Trichoderma* sp. vs *F. oxysporum* inhibitory test

Isolates	average inhibitory at days of observation (%)		
	five days	six days	seven days
UBPK6	72.1_{ef}	74.4_{fg}	76.71_{fg}
UBPK7	71.11_{d}	73.32_{d}	75.81_{cd}
UBPK8	72.12_{fg}	74.34_{ef}	76.67_{ef}
UBPK9	71.42_{de}	73.75_{de}	76.11_{de}
UBPK10	65.5_{a}	68.22_{a}	71.14_{a}

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the LSD test at an error level of 5%

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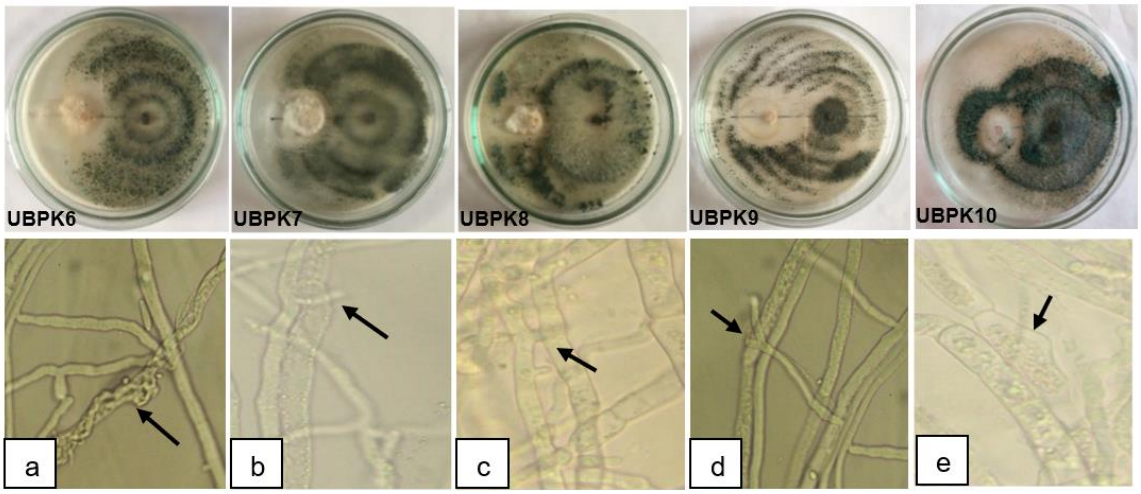

Figure 2. The antagonistic mechanism of *Trichoderma* sp. vs *F. oxysporum* (7 hsi). a. a purchase occurs, b. competition occurs, the hyphae branch of *Trichoderma* sp. attached to the hyphae of *F. oxysporum,* and penetration occurs, c. hyphae of *Trichoderma* sp. penetrate the hyphae of *F. oxysporum*, lysis occurs, d. hyphae branch of *Trichoderma* sp. attached secrete cell degradation enzymes to hyphae *F. oxysporum* lysis, e. swelling of *F. oxysporum* hyphae (lysis)

Qualitative test of Trichoderma sp. isolates on chitin media

The qualitative test results (bromocresol purple reagent test) are shown in (Figure 3), namely the color change in the media. The qualitative test was expressed in terms of the chitinolytic index, which is the ratio between the diameter of the color change and the diameter of the Trichoderma sp. isolate. The results of the bromocresol purple reagent test for the five isolates of Trichoderma sp. showed that these isolates can produce chitinase enzymes.

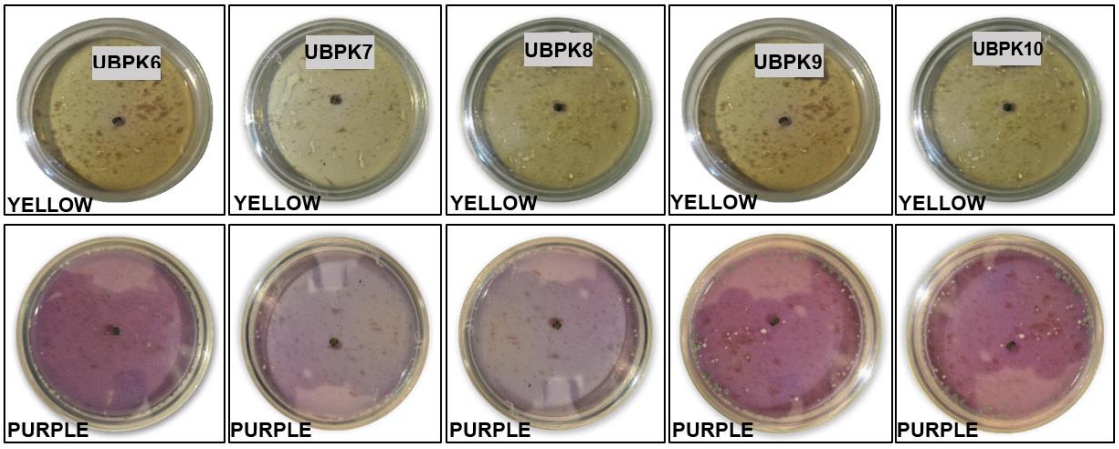

Figure 3. Test bromocresol purple reagent

The color change on the chitin substrate indicates the presence of the chitinase enzyme. (Figure 3) shows that the five isolates experienced a color change caused by the activity of the chitinase enzyme. The color change was shown from yellow to purple in the administration of bromocresol purple reagent due to a change in pH due to enzyme reactions from low to high pH. The color change

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occurred when the MGMK medium was added to the bromocresol purple reagent in the petri dish and then adjusted to pH 5. Polysaccharide molecules separated from hydrochloric acid (HCl) induced colloidal chitin can form the basis for forming hydrogen bonds between the chitin matrix and the dye. Bind sites for anionic dyes such as bromocresol purple to give the bound complex (yellow color). Determination of chitinase activity was seen in MGMK media containing bromocresol. *Trichoderma* sp. which was inoculated on the media, caused the breakdown of chitin into N-acetyl glucosamine resulting in a shift in pH and a change in the color of the pH indicator dye (BCP) from the yellow to the purple zone around the inoculated isolate (Figure 3).

Molecular identification of Trichoderma sp. isolates the chosen

The results of the highest inhibition and bromocresol tests were on UBPK6 isolates. The isolates were later used to treat chitinase enzyme activity and observation of inhibition (crude chitinase extract). Identification of UBPK6 isolates was carried out molecularly based on genetic analysis using ITS1 and ITS4 primers (Matas-Baca et al., 2022) (Figure 4).

The BLAST classification results concluded that the sample being analysed was *Trichoderma asperellum* because it is in the same clade as the sequences of the fungus *Trichoderma asperellum*. These results were followed by the results of the phylogenic tree construction which showed that the samples had high similarity with *Trichoderma asperellum* species. The use of the MEGA application to obtain the results of phylogenic tree reconstruction to see the kinship of *Trichoderma* sp. isolates with the sequences listed in the BLAST program list (Pradhan et al., 2023). Filegenic analysis results on the fungus *Trichoderma* sp. consists of two groups, namely Clad I (*T. viride, T. hamatum, T. asperelloides, Trichoderma asperellum*). Likewise, in Clad II some species have a sisterhood relationship, namely *Trichoderma asperellum* strain MLT5J1 and *Trichoderma asperellum* strain MLT3J2.

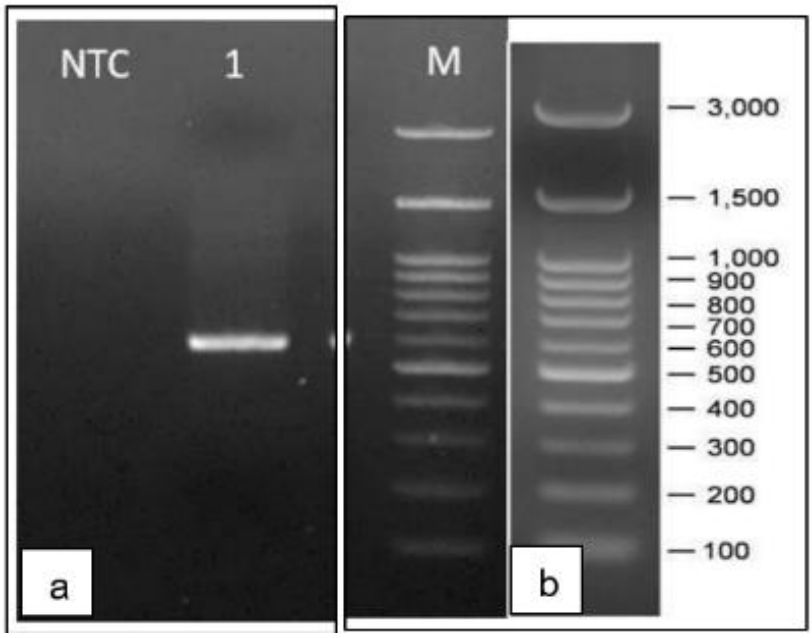

Figure 4. Determination of similarity of electrophoresis results. a. electrophoresis of PCR results (UBPK6 isolates) and markers, b. determining the size of UBPK6 isolates of approximately 600 bp.

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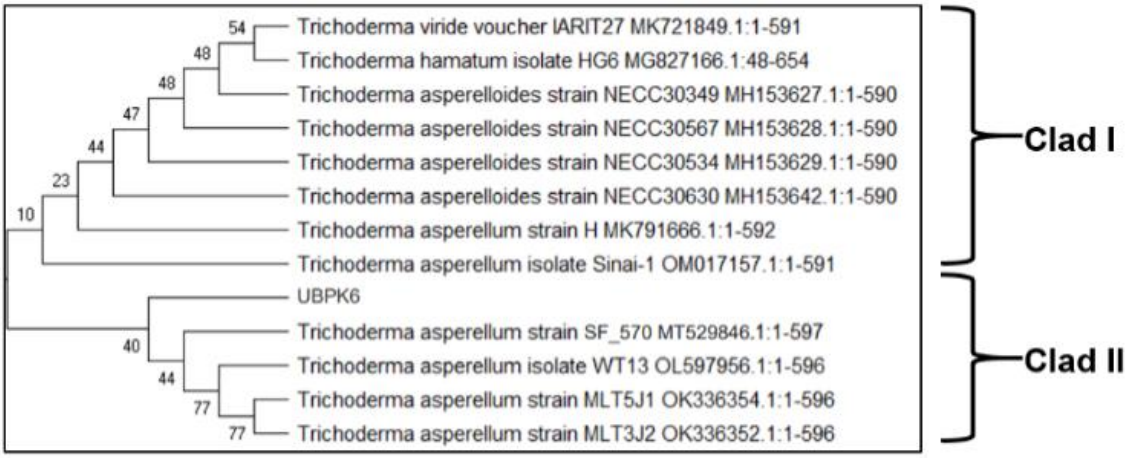

Figure 5. The phylogenetic tree of *Trichoderma* sp.

Production and activity of *Trichoderma asperellum* chitinase enzyme

Chitinase enzyme activity was measured for *Trichoderma asperellum* isolates. The chitinase enzyme incubation test showed that the incubation period differed from day 1 to day 7. The results showed that the optimum incubation period for enzyme production was the 4th day, with an enzyme activity value of 4.05 U/mL (Figure 6). Chitinase enzyme activity measures the amount of product resulting from a breakdown of chitin substrate (Deng et al., 2019). Figure 6 shows that this time is the right time for harvesting enzymes.

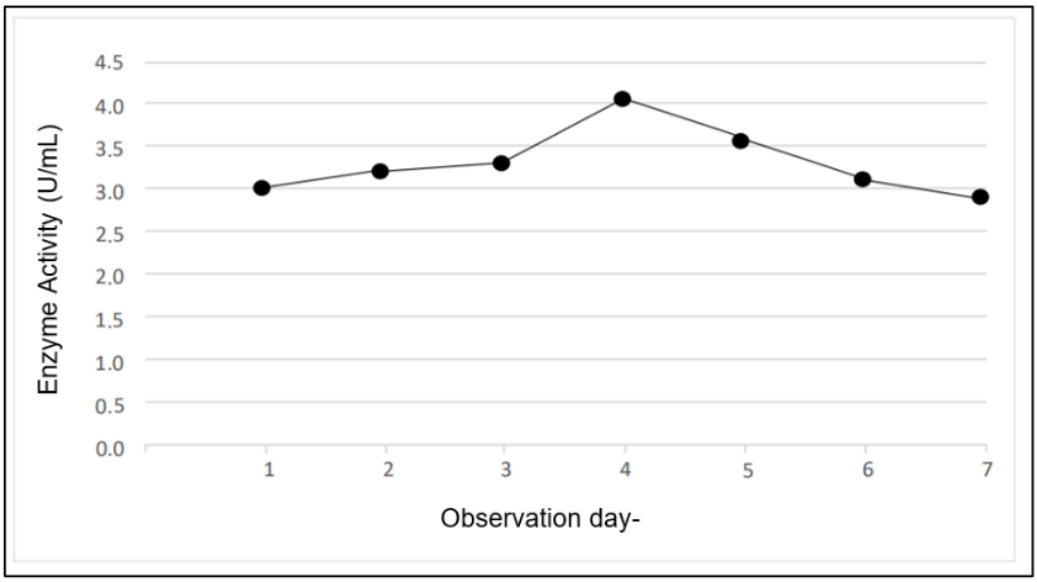

Figure 6. Chitinase enzyme activity test 7 days of incubation

Enzyme harvesting is usually carried out in the log phase because in this phase the fungal cells grow quite rapidly with a large number of cells. Elawati et al. (2018) stated that day 0 to day 3 is the log (exponential) phase. In this phase, the microbial cells divide rapidly following a logarithmic curve. The peak of incubation time occurs in the stationary phase, which is the 4th day. Day 5 of incubation showed a decrease in the number of microbial cells. If the number of growing cells is large, the enzymes produced are also large because each cell secretes enzymes (Karso et al., 2014). After

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reaching the optimum time, the activity of the enzyme decreases due to the accumulation of hydrolysis products, which can further inhibit the activity of the enzyme (Purkan et al., 2014).

The optimum incubation time for enzyme production for each microorganism is different. Verena's study (2008) shows that the production time for the chitinase enzyme from *Streptomyces* sp. was six days, indicated by the highest activity of 34 U/mL. Rachmawaty and Madihah (2013) reported that chitinase production from *Trichoderma virens* produced the highest chitinase activity of 0.194 U/g after three days of fermentation using microwave-treated shrimp waste substrate. Chitinase is an extracellular enzyme that plays a role in hydrolyzing chitin. Extracellular enzymes are enzymes that are produced inside cells but are released into the growth medium.

Effect of pH on chitinase enzyme activity of Trichoderma asperellum fungus

The results of the chitinase enzyme activity test with a UV-Vis spectrophotometer showed that different pH in the growth media could affect the activity of the chitinase enzyme. Determination of the optimum pH aims to determine the optimum conditions for enzyme activity so that it can determine the use of enzymes according to their characterization. The effects of pH and temperature on enzymes can affect the isoelectric point of enzymes. The enzyme activity of *Trichoderma asperellum* showed that the optimum activity value was produced at pH five at room temperature (±27^{o}C) with an enzyme activity value of 3.4 U/mL and decreased after that, the lowest enzyme activity at pH 9 was 2.65 U/ mL (Figure 7).

Based on (Figure 7) showed that the highest activity was found in live media at pH 5. The analysis of the chitinase enzyme by Gueye et al. (2020) showed a significant effect on chitinase activity analysis by *Trichoderma* sp. strain TG4, namely the optimal pH at pH 6. In addition, based on the decrease in chitinase enzyme activity, the more alkaline the media, the lower the chitinase enzyme activity. Research Cheba et al. (2016) stated that at a higher pH level, the stability of the chitinase enzyme was reduced by 57 to 67%. According to Mukherjee et al. (2022), chitinase production will increase at acidic pH (pH 5). Acidic pH is a vital growth parameter for chitinase production in the mechanism of *Trichoderma asperellum* mycoparasitism. A study by Kamaruzzaman et al. (2021) showed that the maximum production of *Trichoderma asperellum* chitinase was obtained at pH 5 - 5.5.

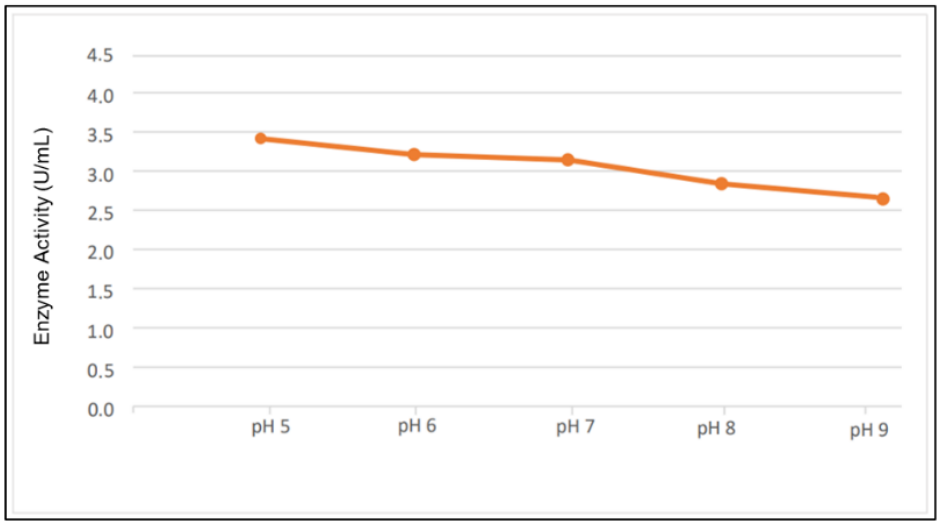

Figure 7. Chitinase activity of *Trichoderma asperellum* during six days of incubation

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The result of this different activity is because each enzyme can hydrolyze substrates at different optimum pH. Luis et al. (2012) showed that at the optimum pH there was a change in ionization in the ionic group of the enzyme on its active site. The proton donor and acceptor groups are in the desired ionization level so that the conformation of the active site becomes effective in binding and converting the substrate into products. Changes in environmental pH can affect changes in the ionization of enzymes, substrates, or enzyme-substrate complexes, resulting in decreased enzyme activity.

Influence of temperature and pH there was a progressive increase in the average chitinase production as the temperature increased. High-temperature variations cause thermal inactivation of metabolic enzymes, while low temperatures change membrane permeability and limit nutrient flow (Iftikhar et al., 2008). In addition, the moderate pH for maximum enzyme production is 5 – 5.5. Each fungus has a difference in producing enzymes because temperature and pH conditions affect the ability of fungi to hydrolyze substrates. Some research results show that chitin hydrolyzing enzymes generally work optimally in the acidic to neutral pH range (Sharaf et al., 2012). The optimal temperature and pH for the chitinolytic activity of *T. viride* is 30^{o}C and pH 5. Chitinase produced by the strain of the fungus *Trichoderma* sp. is stable at room temperature and pH 5-7 (Ekundayo et al., 2016).

A pH value that is too high or too low will cause instability in the conformation of the enzyme, causing the enzyme activity to decrease. The optimum pH state is related to the condition when the important proton donor or acceptor on the catalytic side of the enzyme is in the desired ionization level (Lehninger et al., 2004). Each enzyme generally has maximum activity at a certain temperature, enzyme activity will increase with increasing temperature until the optimum temperature is reached. After that, further temperature increase will cause the enzyme activity to decrease. This causes changes in the conformation of the enzyme-substrate so that it experiences obstacles to the binding. In addition, high temperatures will damage the structure of the enzyme, so it experiences denaturation (Lehninger et al., 2004).

Test of inhibitory power of chitinase crude extract of Trichoderma asperellum mushroom against F. oxysporum in vitro pathogen

Enzyme testing was carried out to confirm that the cell culture was free of *Trichoderma* sp. contained cell wall-degrading enzymes (CWDEs) responsible for fungal cell wall degradation, while chitinase and β-1,3 glucanase activities were tested with 3,5-dinitro salicylic acid. All testing of the crude extract of the chitinase enzyme *Trichoderma asperellum* could inhibit the growth of the pathogen in the petri dish test. The pH five treatment was the best in inhibiting the growth of pathogens, with an inhibition value of 60.63% in the 7th week after incubation. At pH nine it has the lowest ability to inhibit the pathogen *F. oxysporum* at 37.50% (Figure 8).

The mechanism of inhibition includes competition for nutrients, secondary metabolite production, and by producing chitinase enzymes which the formation of clear zones can characterize. Control mechanisms of *Trichoderma asperellum* vs *F. oxysporum* on MGMK media include antibiosis and mycoparasites. Microscopic observation was carried out to observe any changes in the morphology of the pathogenic hyphae after being tested for antagonist with *Trichoderma asperellum*. The morphological interactions of pathogenic hyphae and *Trichoderma asperellum* are presented in (Figure 9). The results showed that the hyphae of *Trichoderma asperellum* grew above and attached to the pathogenic hyphae. The hyphae of

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Trichoderma asperellum also coil around the pathogenic hyphae. It was also observed that the hyphae of *Trichoderma asperellum* penetrated the pathogenic hyphae. It causes morphological deformation and lysis of the pathogenic cell wall structure. The activity of the mycoparasite *Trichoderma asperellum* caused the mycelial wall to be destroyed, the hyphae to be damaged and the growth of the colony of *F. oxysporum* to be stunted. It is because *Trichoderma asperellum* secretes enzymes, one of which is the chitinase enzyme, which can damage cell walls and cause lysis.

During antagonistic activity, *Trichoderma asperellum* compounds secrete a secret in the chitinase enzyme, degrading the hyphal cell walls of pathogenic fungi and disrupting their growth. *Trichoderma* sp. It also secretes secretions in the form of enzymes, namely β-1,3 glucanase and chitinase, which can degrade compounds that make up the cell walls of chitin pathogenic fungal hyphae which are glucose polymer derivates of N-acetylglucosamine which form myofibril bonds and consist of a matrix in the form of galactomannoprotein (Madigan et al., 2012). Compounds produced by *Trichoderma* sp. have the potential to inhibit the growth of pathogenic fungi.

Then observation of inhibition of germination of *F. oxysporum* spores (at pH 5). This test was carried out at various suspension concentrations of the crude extract of the chitinase enzyme *Trichoderma asperellum* (control, 10%, 25%, 50%, 100%) at pH 5, because the most effective pH for producing the chitinase enzyme was pH 5. The results of the observations showed that the percentage of germination increased at each observation time (Table 2). The results of observations of *F. oxysporum* spore germination at 24 hours after incubation, the highest spore germination was in the control treatment, with a percentage of 74%. At the same time, the lowest percentage of spore germination occurred in the suspension treatment of the crude extract of the chitinase enzyme *Trichoderma asperellum* (100% concentration) with a percentage of 25%.

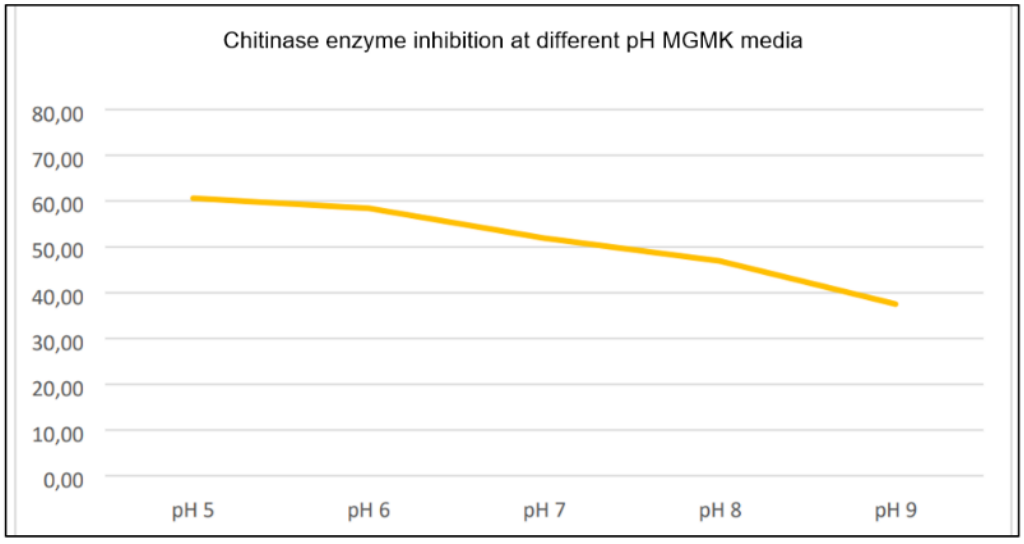

Figure 8. Chitinase enzyme inhibition at different pH MGMK media

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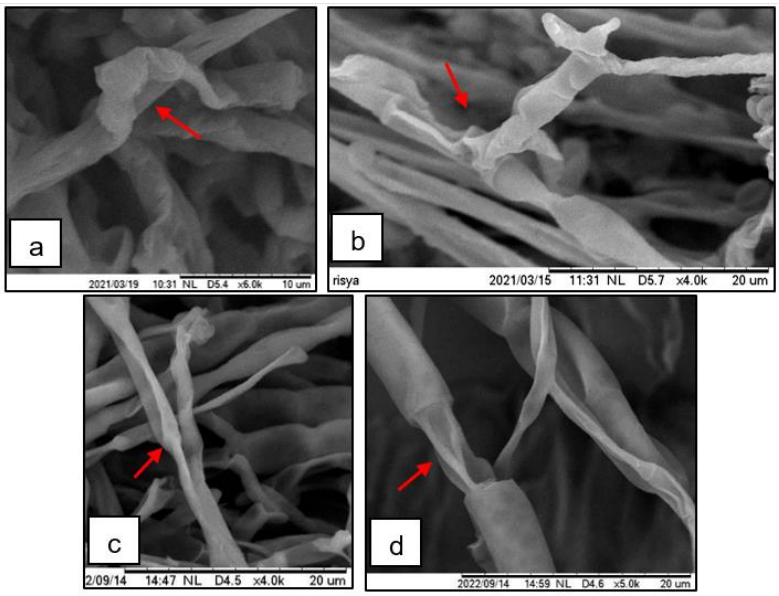

Figure 9. Mycoparasite *Trichoderma asperellum* against *F. oxysporum*. a. hyphae of *Trichoderma asperellum* attached to pathogenic hyphae, b. the process of twisting and lysis, c. Pathogenic hyphae undergo lysis, d. lysis.

Table 2. Germination of *F. oxysporum* spores

Treatments	spore germination (%) after suspension preparation (hours)			
	6 hours	9 hours	12 hours	24 hours
Control	50_c	59_c	71_c	74_c
100%	18_a	18_a	20_a	25_a
50%	14_a	20_a	24_a	33_a
25%	36_b	44_b	48_b	55_b
10%	36_b	42_b	46_b	58_b

Note: Numbers accompanied by the same letter in the same column are not significantly different based on the LSD test at an error level of 5%

The seed tube is a growth product spore fungi produce during germination. The germination tube is considered as an essential requirement for the occurrence of a disease, from spore germination, seed tube elongation, and appressorium formation during plant-pathogen interaction. Based on the experimental results, it was shown that there was a decrease in the percentage of spore germination at various concentrations of the suspension of the crude extract of the chitinase enzyme *Trichoderma asperellum*. The higher the concentration of the crude extract of the enzyme, the lower the percentage of spore germination. The following is a picture of the highest and lowest percentages of spore germination observations with control treatment (Figure 10).

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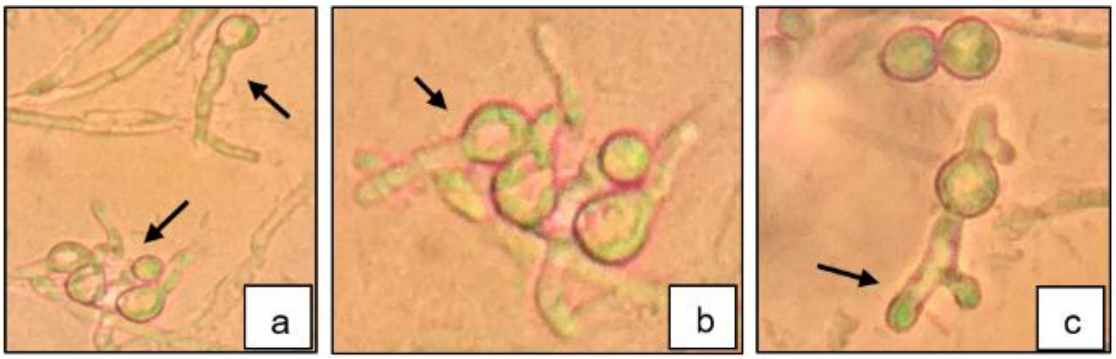

Figure 10. Microscopy of *F. oxysporum* germ tubes. a. control, b. concentration of 10%, c. 100% concentration

*Trichoderma asperel*lum as a biocontrol agent used in controlling a broad spectrum of pathogens. Living in the soil allows *Trichoderma* asperellum to be influenced by environmental conditions, which, based on (Table 2) shows that optimal conditions at pH 5 can have a low percentage of spore germination. However, at alkaline pH it will cause an increase in the percentage of spore germination. Abeyratne and Deshappriya's research (2018) found that the ability of Trichoderma sp. at pH 5 has a low percentage of spore germination on pathogens.

The ability of Trichoderma sp. inhibited by the pH of the media causes the growth of pathogens to be higher. Therefore, land conditions that are too high in soil organic matter and high humidity will potentially result in an increase in soil pH, resulting in pathogens growing freely with minimum inhibition. Inhibition is affected by the production of the chitinase enzyme, which is lower than the optimal pH (Hung et al., 2015). In addition, secondary metabolites in *Trichoderma* sp. will also have an effect.

Conclusion

In conclusion, this study has found *Trichoderma* sp. is an antagonistic fungus that can produce chitinase enzymes as a mechanism for inhibiting the growth and development of the *Fusarium oxysporum* pathogen, then obtained molecular identification of one *Trichoderma* sp. isolate the best is *Trichoderma asperellum*. The optimum incubation period of *Trichoderma asperellum* for enzyme production was the 4th day with an enzyme activity value of 4.05 U/mL. The pH in the living medium of the fungus *Trichoderma asperellum* can affect the activity of the chitinase enzyme, where the greatest activity is at the optimum pH, namely pH 5 with an enzyme activity value of 3.4 U/mL and the smallest at pH 9 with an enzyme activity value of 2.65 U/mL. The inhibitory power of the crude extract of the chitinase enzyme *Trichoderma asperellum* against *Fusarium oxysporum* increased with the chitinase enzyme's content. The pH of the *Trichoderma asperellum* media also affects the inhibition.

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