

Morphological Characteristics and Antagonist Test *Trichoderma* sp. Against Fungi *Helminthosporium* sp. Causing Leaf Blight Disease in Corn Plants *In Vitro*

(Karakteristik Morfologi dan Uji Antagonis *Trichoderma* sp. Terhadap Jamur *Helminthosporium* sp. Penyebab Penyakit Hawar Daun pada Tanaman Jagung Secara *In Vitro*)

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(Article History: Received-Feb 10, 2025; Revised-May 08, 2025; Accepted-July 30, 2025)

ABSTRACT

Helminthosporium sp. is an organism that causes leaf blight disease in corn plants, thus becoming one of the causes of declining production in the agricultural sector. Lack of awareness and low knowledge of farmers in control efforts that still use synthetic pesticides that have a negative impact on the environment and health, therefore the use of antagonistic microbes that live around plant roots such as *Trichoderma* sp. is one way to control environmentally friendly soil-borne pathogens. This study aims to analyze the characteristics of *Trichoderma* sp. and *Helminthosporium* sp. macroscopically and microscopically and to test the antagonistic ability of *Trichoderma* sp. fungi based on competition tests, antibiosis tests and mycoparasitism tests against *Helminthosporium* sp. fungi isolate Tondegesan. *Trichoderma* sp. fungal antagonist test against *Helminthosporium* sp. fungi using the duo plate assay method in Potato Dextrose Agar (PDA) media *in vitro*. The results showed that *Trichoderma* sp. fungi. has macroscopic morphological characteristics of dark green colonies with fine cotton fibers and microscopic morphological characteristics of green septate hyphae, upright conidiophores and many branches. *Helminthosporium* sp. fungi have macroscopic morphological characteristics of flat fibrous colony surfaces, oval shapes with slightly brownish white colors and microscopic morphological characteristics of fairly thick hyphal walls with yellowish brown colors. Based on the antagonist test, *Trichoderma* sp. fungi have an inhibition percentage of 71% against *Helminthosporium* sp. fungi on the seventh day.

Keywords: *Helminthosporium* sp.; Corn; Antagonist Test; *Trichoderma* sp.

ABSTRAK

Helminthosporium sp. adalah organisme penyebab penyakit hawar daun pada tanaman jagung sehingga menjadi salah satu penyebab produksi dari sektor pertanian menurun. Kurangnya kesadaran dan rendahnya pengetahuan para petani dalam upaya pengendalian yang masih menggunakan pestisida sintetik yang memiliki dampak negatif pada lingkungan dan kesehatan oleh karena itu pemanfaatan mikroba antagonis yang hidup di sekitar akar tanaman seperti *Trichoderma* sp. merupakan salah satu cara pengendalian patogen tular tanah yang ramah lingkungan. Penelitian ini bertujuan untuk menganalisis karakteristik jamur *Trichoderma* sp. dan jamur *Helminthosporium* sp. secara makroskopis dan mikroskopis serta menguji kemampuan antagonis jamur *Trichoderma* sp. berdasarkan uji kompetisi, uji antibiosis dan uji mikoparasitisme terhadap jamur *Helminthosporium* sp. isolat Tondegesan. Uji antagonis jamur *Trichoderma* sp. terhadap jamur *Helminthosporium* sp. menggunakan metode duo plate aessay di dalam media *Potato Dextrose Agar* (PDA) secara *in vitro*. Hasil penelitian menunjukkan bahwa jamur *Trichoderma* sp. memiliki karakteristik morfologi secara makroskopis koloni berwarna hijau tua berserat halus seperti kapas dan karakteristik morfologi secara mikroskopis hifanya bersekat berwarna hijau, konidiofor yang tegak dan banyak bercabang. Jamur *Helminthosporium* sp. memiliki karakteristik morfologi secara makroskopis permukaan koloni datar berserabut, bentuknya oval berwarna putih sedikit kecokelatan dan karakteristik morfologi secara mikroskopis dinding hifa yang cukup tebal berwarna kuning kecokelatan. Berdasarkan uji antagonis jamur *Trichoderma* sp. memiliki persentase hambat sebesar 71% terhadap jamur *Helminthosporium* sp. pada hari ke-tujuh.

Kata kunci: *Helminthosporium* sp.; Jagung; Uji Antagonis; *Trichoderma* sp.

INTRODUCTION

One of the horticultural commodities that is the second food source after rice and has a fairly high economic value is corn. In several countries, corn is used as the main source of carbohydrates such as animal feed, raw materials for cooking oil, flour industry and snacks (Hanif & Susanti, 2019). Tondegesan Village is one of the villages in Minahasa Regency which has an area of 3.25 km² with a total agricultural land area of 307 ha. The average Tondegesan Village community works as farmers. One of the superior commodities owned is corn, the area of corn land in 2013 according to the commodity was 899.4 ha. One of the obstacles in efforts to increase corn production is the attack of plant pathogens which causes decreased production, crop failure and even losses.

Diseases that often attack corn plants include leaf blight. This disease attacks corn crops with the most damage in hot and humid tropical areas (Ram Bhandari et al., 2017). In 2013, *Helminthosporium turcicum*, a pathogen that causes leaf blight disease, was found in areas with an altitude of > 1500 masl with high humidity and low temperatures in the Barastagi and Malino areas, its distribution reached 93.33% (Muis et al., 2019). In general, the first spots will appear on old leaves and then develop towards young leaves. Gray leaf blight that looks like it is drying or burning indicates a fairly severe infection symptom, which can cause plants to die quickly (Lea & Bhalu, 2022). One of the control techniques usually carried out by farmers is using synthetic pesticides by spraying them, the lack of understanding of farmers has a negative impact, especially if it is used excessively without paying attention to the rules for safe pesticide use (Siahaan, 2022).

One of the antagonistic microbes and plant fungicides that has the potential to be developed is *Trichoderma* sp. (Nurjannah, 2020). In nature, *Trichoderma* is a type of fungus that grows rapidly, is a productive producer of spores and is also a strong producer even under very competitive environmental pressures for space, nutrients, and light (Rajesh et al., 2016). Based on several studies, *Trichoderma* sp. is also able to inhibit pathogenic fungi that are harmful to plants, such as the results of research from Sharma et al. (2016) *Trichoderma* sp. can inhibit the growth of pathogens *Fusarium oxysprum*, *Altenaria brassicicola*, and *Sclerotinia sclerotiorum*.

Based on this background, this study was conducted to analyze the morphological characteristics of *Trichoderma* sp. and *Helminthosporium* sp. macroscopically and microscopically followed by testing the antagonistic ability of *Trichoderma* sp. based on competition tests, antibiosis tests and mycoparasitism tests against *Helminthosporium* sp. fungi that cause leaf blight disease in corn plants

METHODS

Time and Place of Research

This research was conducted from June 2023 to July 2023. Antagonistic tests and morphological identification were carried out at the Biological Agent Laboratory at the Center for Protection and Quality Testing of Food Crops and Horticulture (BP2MTPH) Kalasey, North Sulawesi Province.

Tools and materials

The tools used include ose needles, bunsen lamps, laminar, plastic bags, test tubes, markers, measuring pipettes, slides, rulers, analytical scales, 1 ml dropper

pipettes, petri dishes, cork drills (diameter 0.5 cm), microscopes, sampling spoons, scalpels, matches, beakers, micropipettes, Erlenmeyers, shovels, haemocytometers, pans, jet sprayers, stirring rods, hand gloves, cover glasses, and magnetic stirrers. The materials used are PDA (*Potato Dextrose Agar*), 70% alcohol, chloramphenicol, tissue, cotton, sterile distilled water, label paper, plastic wrap, *Trichoderma* sp. samples, and *Helminthosporium* sp. samples. (ISO Team, 2015).

Research Design

The research design used the duo plate assay method. *Trichoderma* sp. fungal colonies and *Helminthosporium* sp. fungal colonies were inoculated in PDA media in the same petri dish but their placement was given a distance of 4 cm between each other (Lelana & Anggraeni, 2015). There were 3 treatments and each treatment was repeated 3 times, namely:

- *Helminthosporium* sp. control.
- *Trichoderma* sp. control.
- *Trichoderma* sp. and *Helminthosporium* sp.

PDA Media

The agar media is made by putting 9.7 grams of PDA in powder form into a pan and adding 250 ml of water then heating it until it boils. Furthermore, the PDA solution is sterilized using an autoclave for 55 minutes at a temperature of 121°C followed by the pouring process into a petri dish and will be left to harden in solid form (ISO Team, 2015).

Isolation of Trichoderma sp. Fungus

To isolate the fungus *Trichoderma* sp. isolate Tondagesan first perform a dilution of 10^{-4} , where 25 grams of soil is put into a beaker then added 250 ml of sterile water, then stirred using a stirring rod for 15-20 minutes. The second dilution is to take 1 ml of solution put into a test tube by adding 9 ml of sterile aquades and vortexed until homogeneous after the dilution of 10^{-4} is obtained then taken as much as 1 ml from the test tube to be put into a petri dish to be poured into the PDA media using a micropipette. After that, let it stand and observe for 7 days (ISO Team, 2015).

Isolation of Helminthosporium sp.

Isolation of suspected *Helminthosporium* sp. disease can be done using the quadrant method, the vortexed *Helminthosporium* sp. sample is taken using an ose needle and scratched in quadrants on the surface of the PDA media, carried out near the Bunsen flame to minimize possible contamination (ISO Team, 2015).

Reisolation and Pure Culture Preparation

Reisolation is the process of transferring fungal isolates from one culture to a new medium. Pure cultures are made by taking fungal spores from isolated samples using an ose needle and cultured in a petri dish filled with PDA media. Pure cultures are made near a Bunsen burner to prevent contamination (ISO Team, 2015).

Antagonist Test

After observation of *Trichoderma* sp. fungal isolates that are seven days old are taken using a cork drill on the edge of the colony. The isolates that have been taken are placed on PDA agar media with the A mark (**Figure 1**). Furthermore, with the same treatment, it is carried out on *Helminthosporium* sp. isolates using a cork drill on the edge of the isolate colony, and then placed on PDA agar media without P (**Figure 1**). The treatment will be repeated three times to obtain maximum results. The observation process is carried out every day for one week. After that, the radius of the two fungal colonies in the petri dish is calculated (Lelana et al., 2015).

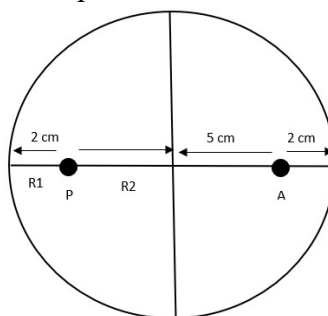


Figure 1. Description A = Antagonist (*Trichoderma* sp.); P = Pathogen (*Helminthosporium* sp.)

The mechanism of interaction between antagonistic fungi and pathogenic fungi according to Amaria et al. (2015) is:

- Competition test**
Competition occurs when the antagonist fungus colony grows faster than the pathogenic fungus colony so that the antagonist fungus looks more dominant filling the petri dish.
- Antibiosis test**
Antibiosis, if an empty zone is formed between the antagonist fungus and the pathogenic fungus, whether or not there is a color change on the lower surface of the test fungus colony due to antibiotic compounds produced by the test fungus.
- Parasitism test**
Parasitism, if the antagonist fungus hyphae grow above the pathogenic hyphae, in the contact area the antagonist fungus hyphae are found wrapped around the pathogenic hyphae, and undergo lysis observed microscopically.

Data Analysis

The growth rate of the fungus is known by measuring the diameter of the colony of each fungus every day after inoculation until the 7th day and the measurement is done using a ruler (ISO Team, 2015). The percentage of inhibition (%) is calculated on the 7th day after inoculation with the formula (ISO Team, 2015):

$$\text{Obstacle (\%)} = \frac{R1 - R2}{R1} \times 100\%$$

Information:

- R1 = The radius of the *Helminthosporium* sp. colony that moves away from the *Trichoderma* sp. fungal colony.
R2 = The radius of the *Helminthosporium* sp. colony approaching the *Trichoderma* sp. fungal colony.

RESULTS AND DISCUSSION

The results of the *Trichoderma* sp. fungus exploration were taken from soil samples on healthy banana plants by digging the soil to a depth of 15 cm. The soil samples were taken from a banana plantation location in Tondegesan Village, Minahasa Regency, North Sulawesi (**Figure 2**). The results of the exploration of *Helminthosporium* sp. fungus, the cause of leaf blight disease in sweet corn leaves, were taken from a corn plantation location in Tondegesan Village, Minahasa Regency, North Sulawesi (**Figure 3**).



Figure 2. Exploration of *Trichoderma* sp. fungus with Tondegesan isolate soil media.



Figure 3. Exploration of *Helminthosporium* sp. fungus on corn leaves isolate Tondegesan.

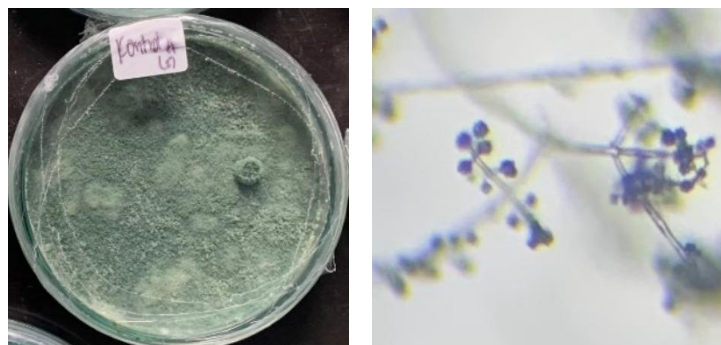


Figure 4. Macroscopic and microscopic morphology of fungal colonies suspected to be *Trichoderma* sp.

Macroscopic and microscopic morphological characteristics of *Trichoderma* sp. fungi from local Tondagesan isolates (**Figure 4**). Based on the results of macroscopic observations of the morphological characteristics of the *Trichoderma* sp. fungus (**Figure 4**) it was found that the colony was dark green, 9 cm in diameter, round in shape, and had a flat, thick, and fibrous surface. According to Simangunsong et al. (2019) the colony of rhizosphere fungi of the genus *Trichoderma* after the colony was 7 days old became green on all surfaces, the surface of the colony was thick with a rough texture.

Based on the results of microscopic observations of the morphological characteristics of the *Trichoderma* sp. fungus (**Figure 4**) it was found that the hyphae were green, septate and thin, the conidiophores were upright and irregularly branched, the conidia were round and green, and the phialids were short. The results of the study were not much different from the results of Suanda's study (2016) microscopic appearance of *Trichoderma* sp. namely green hyphae, short phialids, greenish conidia, round in shape, and the phialids had a size of $\pm 11.1\mu$ and the conidiophore branches were $\pm 13.4\mu$ long.

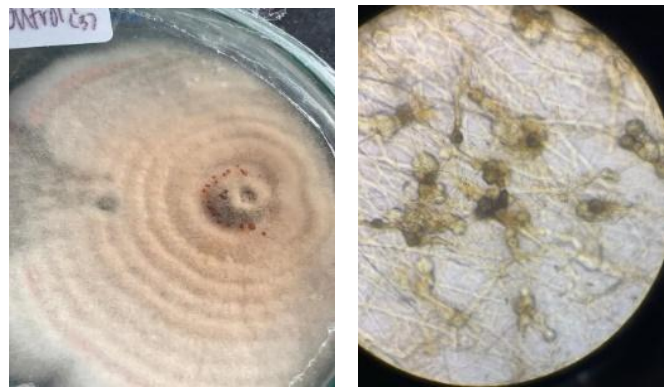


Figure 5. Macroscopic and microscopic morphology of fungal colonies suspected to be *Helminthosporium* sp.

Macroscopic and microscopic morphological characteristics of *Helminthosporium* sp. fungi from local Tondagesan isolates (**Figure 5**). Based on the results of macroscopic observations of the morphological characteristics of the *Helminthosporium* sp. fungus (**Figure 5**), it was found that the surface of the colony was brownish white, the diameter was 6.8 cm, the surface was thick, appeared fibrous like cotton, the shape was round, and there were circular partitions. Fadilah et al. (2021) this pathogen can grow well in areas with air humidity of around 97-98%. In addition to humidity, temperature also plays an important role in the development of the *Helminthosporium* fungus, the optimal temperature for this growth and development is around 20-30°C.

Based on the results of microscopic observations of the characteristics of the *Helminthosporium* sp. fungus (**Figure 5**), it was found that the hyphal walls were thick, yellowish brown in color, the septa were branched, single elliptical conidia had 6-8 partitions, and the conidiophores were short and partitioned. A similar thing was also expressed by Asad et al. (2020) that the conidia of *Helminthosporium* sp. were slightly curved, slightly yellowish brown in color, had 2-13 partitions, and an average size of 38.3-65.8 μm x 12.3- μm .

Antagonist Test

The growth rate of *Trichoderma* sp. and *Helminthosporium* sp. fungi based on the analysis results obtained the average diameter of the *Trichoderma* sp. fungal colony growth is faster when compared to the average diameter of the *Helminthosporium* sp. fungal colony, both from the test results and from the control treatment (**Table 1**).

Table 1. Average diameter of *Trichoderma* sp. and *Helminthosporium* sp. fungi

Types of mushrooms	Average colony diameter (cm) on day 2 of growth						
	1	2	3	4	5	6	7
<i>Trichoderma</i> sp.	1,1	4,18	6,02	6,42	6,72	8,06	8,82
<i>Helminthosporium</i> sp.	0,3	1,18	1,7	1,96	2,28	2,7	2,96

Percentage of Inhibition of *Trichoderma* sp. and *Helminthosporium* sp. Fungi

Based on the percentage of inhibition of *Trichoderma* sp. fungi against *Helminthosporium* sp. fungi for 7 days after inoculation (HSI) obtained significant results can be seen in the bar chart (**Figure 6**). Based on the analysis of the inhibition test of *Trichoderma* sp. fungi against *Helminthosporium* sp. fungi, it has a fairly large inhibitory effect, where the percentage of the smallest inhibition on the first day is 33% and the percentage of the largest inhibition on the seventh day is 71% (**Figure 7**). So it can be concluded that the percentage of the antagonist test of *Trichoderma* sp. fungi can inhibit the growth of the *Helminthosporium* sp. pathogen by 71%.



Figure 6. Antagonistic test of *Trichoderma* sp. and *Helminthosporium* sp. fungi 7 HSI

The growth rate of antagonistic fungi is an indicator of the mechanism of competition for space and nutrients, where the faster the growth of antagonistic fungi, the more effective it is in inhibiting the growth of pathogenic fungi (Amaria et al., 2015). The very limited supply of nutritional needs in PDA media causes nutritional competition between *Trichoderma* sp. and *Diplodia* sp. fungi (Sundari et al., 2014). According to Sayang & Kumalasari (2022), the enzymes produced by *Trichoderma* sp. can dissolve cell walls and also produce toxins such as gliotoxin and viridian, both of which can interact to suppress pathogenic fungi.

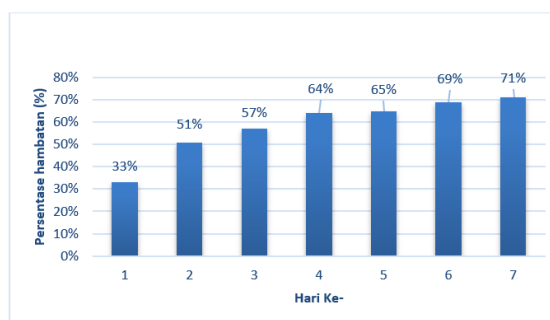


Figure 7. The percentage of inhibition of *Trichoderma* sp. fungi against *Helminthosporium* sp. fungi.

Table 2. T-Test: Paired Two Sample for Means against *Helminthosporium* sp. fungus

Types of Mushrooms	Day 7	
	Average diameter	Sig.(2-tailed)
Control <i>Helminthosporium</i> sp.	5	0,03
Treatment <i>Helminthosporium</i> sp.	2,80	

The results of the T-Test: Paired Two Samples for Means against *Helminthosporium* sp. fungus (**Table 2**) based on the data above, the average of 3 repetitions was taken on the 7th day to see whether or not there was a significant difference in the treatment. The sig. (2-tailed) value was obtained on the seventh day, which was 0.03. So from the results of the sig. (2-tailed) value above, it can be concluded that there was a significant difference because the sig. (2-tailed) value < 0.05 . Based on these data, it shows that the treatment of *Helminthosporium* sp. using *Trichoderma* sp. fungus shows a real difference and can also inhibit the growth of the pathogenic fungus *Helminthosporium* sp.

Antagonistic Mechanism

The results of microscopic observations show that *Trichoderma* sp. attacks *Helminthosporium* sp. by wrapping around the pathogenic hyphae and the chitinase enzyme produced by *Trichoderma* sp. is able to easily degrade the hyphal walls of *Helminthosporium* sp., after the chitinase enzyme enters directly, there is a nutritional competition between the *Trichoderma* sp. fungus and *Helminthosporium* sp. so that the pathogenic hyphae lose nutrients causing the pathogenic hyphae to shrink then break and die (**Figure 8**).

Mycoparasitism is one of the most important mechanisms for *Trichoderma* sp. against plant pathogenic fungi (Patty et al., 2021). According to Diwastuti et al. (2015), the antagonistic process occurs due to competition between the two types of fungi grown close together in one medium. The antagonistic mechanisms of *Trichoderma* in suppressing the growth of pathogenic fungi include competition, antibiosis and lysis. Competition occurs if in the same media, *Trichoderma* isolates compete for nutrients with pathogenic fungi. While antibiosis and lysis involve the production of toxic metabolites (toxins) or extracellular enzymes produced by antagonistic fungi (Dendang, 2015).



Figure 8. *Trichoderma* sp. fungal hyphae entwine *Helminthosporium* sp. fungal hyphae with a magnification of 10 x 10

The mechanism of antibiosis is indicated by the presence of antibiosis activity which is marked by the presence of a clear zone measuring 0.5–1 cm formed between the *Trichoderma* sp. fungal colony and the *Helminthosporium* sp. fungal colony (**Figure 9**). The clear zone is marked by the formation of an area between the antagonistic fungus and the fungus in one petri dish that is not overgrown by both (Asmi et al., 2022).



Figure 9. Clear zone in the antagonistic test of *Trichoderma* sp. fungus against *Helminthosporium* sp. fungus.

The formation of a clear zone is due to the breaking of the β -1,3 bond of N-acetylglucosamine homopolymer in chitin into N-acetylglucosamine monomer (Rupaedah, 2018). The antibiosis mechanism occurs due to the presence of secondary metabolites produced by microbes in the form of and mycotoxins (Meiniwati et al., 2014).

Thus, a series of antagonistic tests that have been tested on the basis of antagonism mechanisms consisting of competition, antibiosis and mycoparasitism have shown good results that environmentally friendly biological control agents, namely *Trichoderma* sp. fungi as biocontrol and its hyperparasitic properties which have been proven effective can inhibit the growth of *Helminthosporium* sp. fungal pathogens by up to 71%.

CONCLUSION

Based on the research results, it can be concluded that the *Trichoderma* sp. fungus isolate Tondegesan has the following macroscopic morphological characteristics: Dark green colonies, 9 cm in diameter, thick, flat, and fibrous surfaces. While microscopically, the hyphae are green, septate and thin, the conidiophores are upright and irregularly branched, the conidia are round and green, and the phialids are short. The *Helminthosporium* sp. fungus isolate Tondegesan has the following macroscopic morphological characteristics: Brownish white colonies, 6.8 cm in size, thick surfaces, cotton-like fibrous, round in shape, and there are circular septa. While microscopically, the hyphae are thick, yellow-brown in color, have branched septa, single conidia are elliptical, there are 6-8 septa, and short and septate conidiophores. The *Trichoderma* sp. fungus can inhibit the growth of the pathogenic fungus *Helminthosporium* sp. by 71% on the seventh day. Antagonistic mechanisms through competition tests, antibiosis tests, and mycoparasitism tests.

ACKNOWLEDGEMENT

The authors would like to thank the Center for Protection and Quality Testing of Food Crops and Horticulture (BP2MTPH) Kalasey, North Sulawesi Province for helping in completing this research.

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