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RESEARCH ARTICLE
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Potential of Endophytic Fungi, *Trichoderma harzianum* Th-B18 and *Dichotomomyces cejpii* in Controlling *Fusarium oxysporum*, the Cause of Wilt Disease in Shallots (*Allium ascalonicum* L.) in Vitro

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ABSTRACT

Fusarium oxysporum is a pathogenic fungus that damages shallot crops by causing Fusarium wilt disease. Managing this disease using antagonistic fungi offers an environmentally sustainable approach and a promising alternative to chemical treatments. This study aimed to evaluate the effectiveness of several unidentified endophytic fungi, *Trichoderma harzianum* Th-B18 and *Dichotomomyces cejpii*, as antagonistic agents for inhibiting the growth of *F. oxysporum*. The experiment was conducted using a completely randomized design (CRD) with a single factor, endophytic fungi (B6, Si AA 10; Si AA 11), *T. harzianum* Th-B18, and *D. cejpii* as the antagonistic fungi, applied across six treatment levels. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. Additionally, descriptive analysis was also performed to evaluate the antagonistic properties of the tested fungi. The findings revealed that *T. harzianum* Th-B18 exhibited the most significant antagonistic activity, achieving the highest colony diameter (7.18cm) and inhibition rate of 75.14% on the twelfth day of observation, indicating a significant level of antagonistic activity. The endophytic fungi SiAA11 and B6 demonstrated moderate inhibition. Based on these results, *T. harzianum* Th-B18, along with endophytic fungi Si AA 11 and B6, show potential as biological control agents for managing *F. oxysporum*. The use of these fungi represents an effective and environmentally friendly strategy for combating *Fusarium* wilt disease.

Keywords: Biocontrol agent, Antagonistic fungi, *Fusarium oxysporum*, Inhibition rate.

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INTRODUCTION

Shallots (*Allium ascalonicum* L.) are one of the horticultural commodities that farmers have intensively cultivated. In addition, it is a superior vegetable commodity that is consumed and needed every day by the community as an additional ingredient and mixture in cooking. Therefore, the need for this commodity is increasing day by day (Ministry of Agriculture, 2017). Shallots in Indonesia have experienced a decline in productivity caused by many factors, one of which is plant disease. An important disease that attacks a lot and also causes many losses to some shallot production is Fusarium wilt disease, known as moler disease, caused by *Fusarium oxysporum*. Fusarium wilt disease can cause various damage to shallot plants and cause a decrease of around 50% (Adhi

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& Suganda, 2020). For farmers, Fusarium wilt disease is one of the serious diseases of shallot plants and is difficult to control. Efforts to control Fusarium wilt until now are still emphasized on control techniques using fungicides. The use of chemicals results in resistance in a relatively short period. In addition, excessive use of fungicides and continuous use will certainly pollute the soil, can even damage the balance of nature and cause disease resistance to the use of certain fungicides that are often used can make the disease more resistant (Deden & Umiyati, 2017; Wang et al., 2023).

More environmentally friendly technology is needed to overcome the issue, one of which is the use of biological agents in the form of endophytic and saprophytic fungi; besides, the production results are safer if consumed. The potential of endophytic and saprophytic fungi as antagonistic fungi that have preventive properties against plant disease attacks has made these fungi increasingly widely used by farmers in efforts to control Plant Pest Organisms (Ministry of Agriculture, 2011). Based on the potential of these biological agents, it is necessary to optimize the use of biological agents from nature. Therefore, it is necessary to isolate and utilize these biological agents to control plant diseases. So, it is necessary to research antagonistic fungi tests in vitro to control *Fusarium oxysporum* wilt disease in shallots (*Allium ascalonicum* L.) (Anum et al., 2024).

The shallot plant (*Allium ascalonicum* L.) is an important horticultural plant that has high economic value in various parts of the world (Ministry of Agriculture, 2017; Andayani et al., 2021; Tori & Kholil, 2023; Sopha et al., 2023). The decrease in shallot production productivity in Indonesia is caused by many factors, one of which is plant disease. Diseases caused by fungi can attack shallot plants from planting to post-harvest (Prakoso et al., 2017). Plant pathogens are one of the obstacles to be overcome in shallot crops. These include disease infections caused by fungi, bacteria and viruses that can reduce the quantity and quality of shallot production by up to 30-40% (Rahman & Umami, 2019). According to Udiarto et al. (2005), the loss of shallots due to disease could be as high as 24-100%. Some common diseases affecting shallots are purple spot (*Altenaria porri*), Moler (*Fusarium oxysporum*), Leaf rot or anthracnose (*Collectricum gloeosporiodes*); Leaf spot (*Cercospora* sp.); Onion mosaic virus (Onion yellow dwarf virus); Shoot death (*Phytophthora porrif*); Feather dew (*Peronaspora destructor*) (Sari & Inayah, 2020).

Effectiveness in controlling plant diseases is determined by the accuracy of information on infecting pathogen species, factors affecting reproduction, and factors promoting disease spread. Fusarium wilt is a disease that affects onion plants either in the growing season or out of season (rainy season) and can reduce crop yield by 27-75% (Adiyoga et al., 2000). Furthermore, according to Adhi and Suganda (2020), this disease causes a reduction of about 50%. The symptoms of plants affected by Fusarium wilt disease are pseudo-stems, pale green leaf color, leaf lengthening, and twisting. The onion bulb turns white and rots in high-intensity attacks, leading to death and even crop failure. For farmers, Fusarium wilt is one of the most serious diseases of shallots and is difficult to control. Efforts to control Fusarium wilt are still focused on controlling the disease using fungicides. The use of chemicals leads to resistance in a relatively short time. In addition, excessive and continuous use of fungicides will certainly pollute the soil, may even damage the balance of nature and may lead to disease resistance to the use of certain fungicides that are frequently used, making the disease more resistant (Deden & Umiyati, 2017; Maulana et al., 2024).

Plant diseases caused by phytopathogenic fungi can cause huge losses in agriculture and, therefore, remain a threat to global food security (Xu et al., 2021). Globally, about 16% of crop losses are due to plant diseases. Therefore, management measures must be implemented to reduce losses and ensure food production. Besides traditional management, induced resistance, and biological control have developed rapidly in agriculture because of their potential. Endophytic fungi colonize plant tissues and have the potential to act as control agents, such as biological agents or triggers in the process of induced resistance and abiotic stress mitigation (Fontana et al., 2021). Biological control is expected to provide a solution to chemical control, which is harmful to the plant environment and human health. Endophytic fungi have a short adaptation time to their new environment. Endophytic fungi can suppress the growth of pathogens that cause disease in shallots by producing antibiotics and competing for space and nutrients. In addition, endophytic fungi can also increase plants' resistance by producing alkaloid compounds and mycotoxins (Pitasari et al., 2018). Chemical investigation of endophytic fungi has yielded a large number of antifungal natural products with potential use in biopesticide development (Xu et al., 2021; Wiyatiningsih et al., 2024).

Plant-associated endophytes are an untapped source of new natural and bioactive products. Over 20,000 substances have been described (Ownley et al., 2010), of which 51% have novel structures and 80% have biological activities (Yang et al., 2012). For example, some have antimicrobial, antioxidant, and antitumor activities (Liu et al., 2008; Tejesvi et al., 2011). This can be explained by ecological theory, which states that the production of these metabolites depends on the ecological niche in which the microorganism is found and the resulting biotic and abiotic interactions (Strobel and Daisy 2003). Based on the findings of Tumangger et al. (2018), endophytic fungi are often found in plant roots, stems, and leaves and have high potential as bioprotectants against the pathogen Fusarium sp. The ability of endophytic fungi to inhibit pathogens is related to their ability to produce chemical compounds that can suppress their growth. Furthermore, research by Fitriani et al. (2019) showed that the use of biocontrol agents can reduce the development of Fusarium wilt disease on shallot, reducing disease incidence by 40%. Meanwhile, Sudantha and Abadi (2007) reported that endophytic fungi can be antagonistic to Fusarium oxysporum by producing antibiotics.

In addition, among the commonly used antagonistic fungi, *Trichoderma harzianum* is a saprophytic fungus

known to be an effective antagonistic biocontrol agent against a number of phytopathogenic fungi, *Dichotomomayces cejpii* is also an antagonistic fungus with the potential to inhibit the development of pathogenic fungi (Gveroska & Jugoslav, 2011). Currently, there is an urgent need to understand synergistic interactions between Trichoderma, plants, and pathogenic microorganisms in induced disease resistance at the cross-genome scale to develop Trichoderma and other microbial symbionts that can cure diseases and pests and to develop new biostimulatory products based on Trichoderma metabolites (Yao et al., 2023; Dewi et al., 2025). Species of the genus Trichoderma inhabit various environments and interact with many different organisms. Mycoparasitic Trichoderma species have been successfully used as bio-fungicides due to their ability to protect plants. They are prolific producers of secondary metabolites, accompanied by enrichment of secondary metabolism-related genes in their genomes (Zeilinger et al., 2016).

Based on the potential of these biological agents, it is necessary to optimize the use of biological agents found in nature. Therefore, it is necessary to isolate and use these biological agents to control plant diseases. Therefore, it is necessary to conduct research on the testing of antagonistic fungi as biological control agents in the control of *Fusarium oxysporum* wilt disease of shallots (*Allium ascalonicum* L.) in vitro and to obtain isolates of fungi that have the potential to be developed as biological agents. The analysis revealed the potential to develop endophytic fungi into innovative bio-formulations such as biofertilizers, biostimulants, and biopesticides, paving the way for their integration into sustainable and more resilient future agricultural systems (Fite et al., 2023).

MATERIALS & METHODS

Experimental Design

The research was conducted using a single-factor experimental method consisting of 6 treatments. The design used was a Completely Randomized Design (CRD) with 4 replications, namely: A0 = Control *F. oxysporum*; A1 = B6 + *F. oxysporum*; A2 = Si AA 10 + *F. oxysporum*; A3 = Si AA 11 + *F. oxysporum*; A4 = T. harzianum + *F. oxysporum*; A5 = *D. cejpii* + *F. oxysporum*

Rejuvenation of Endophytic Fungi Isolates, Trichoderma harzianum, *Dichotomomyces cejpii* and *Fusarium oxysporum*

Endophytic fungi come from the base of the stems of ferns and cantigi plants from the Bogor Botanical Gardens, namely endophytic fungi isolates B6, Si AA 10, Si AA 11 (collection of the Center for Horticulture and Plantation Research, Agricultural and Food Research Organization, BRIN). *Trichoderma harzianum* Th-B18, *Dichotomomyces cejpii*, and *Fusarium oxysporum* isolates are collections of the Basic Agrotechnology Laboratory, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto (Hajoeningtijas, 2018). Rejuvenation of endophytic fungal isolates, *Trichoderma harzianum*, *Dichotomomyces cejpii,* and *Fusarium oxysporum* using Potato Dextrose Agar (PDA) media. The rejuvenated fungi were incubated for 7 days at room temperature (25-27°C) until the fungal mycelium became abundant (Halwiyah et al., 2019).

Fungal Antagonist Test against Pathogenic Fungus *Fusarium oxysporum*

Antagonist test was conducted using the dual culture method (Fig. 1 & 2). *Fusarium oxysporum* isolates and fungal isolates were inoculated onto PDA media in a petri dish facing each other at a distance of 3cm. The petri dish containing the isolate was incubated for 7 days at a temperature of 28°C (Alfizar et al., 2013).

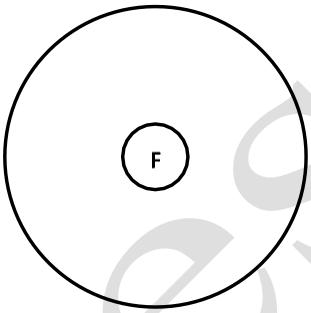

Fig. 1: Control treatment scheme; F= *F. oxysporum* colony.

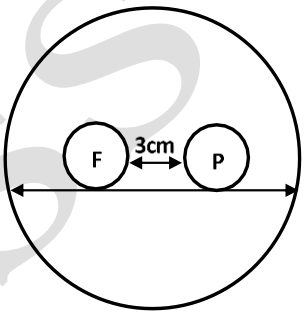

Fig. 2: Antagonist test scheme using dual culture method F= Antagonist fungi colony, P= *F. oxysporum* colony.

Fungal and *Fusarium oxysporum* Characteristics Test

The microscopic characterization of fungal isolates was conducted using prepared fungal samples observed under a microscope. The procedure began by cleaning glass slides with 70% alcohol to ensure sterility. A 1 x 1cm piece of PDA (Potato Dextrose Agar) medium was then placed on the glass slide. A single loop of fungal hyphae attached to the edge of the PDA medium was carefully transferred to the slide, which was subsequently covered with a cover slip. The prepared slides were placed in sterile petri dishes and incubated for three days to allow hyphal growth. Following incubation, the coverslip with overgrown hyphae was removed and transferred to a clean glass slide. A drop of methylene blue was added to the sample to enhance visualization, and the fungal structures were then observed under a microscope.

Observation Variables

Characteristics of Fungal Isolates

The characteristics of fungal isolates were observed using a binocular microscope with a magnification of 40x. Fungal characteristics were examined based on both macroscopic and microscopic features. Macroscopic characteristics included the color of the colony (both top and bottom), colony texture, type of colony growth, and colony shape. Microscopic characteristics encompassed the shape of the hyphae (whether septate or non-septate) and the morphology of the spores/conidia (Aji et al., 2022).

Characteristics of *Fusarium oxysporum* Isolates

The characteristics of *Fusarium oxysporum* isolates were observed using a binocular microscope with a magnification of 40x. Fungal characteristics were examined

based on both macroscopic and microscopic features. Macroscopic characteristics included colony color (both top and bottom), colony texture, growth pattern, and colony shape. Microscopic characteristics focused on the morphology of the hyphae (septate or non-septate) and the shape of the spores/conidia (Aji et al., 2022).

Colony Diameter

Observations of colony diameter were conducted on the 12th day, focusing on fungal colonies and *Fusarium oxysporum* colonies growing on PDA media in a petri dish. The colony diameter was measured using a ruler, and the results were recorded. To measure the diameter of fungal colony growth, the radial direction of the fungal mycelium was assessed along four straight lines (Fig. 3). The following calculation formula was used, as outlined by Risdianto et al. (2007):

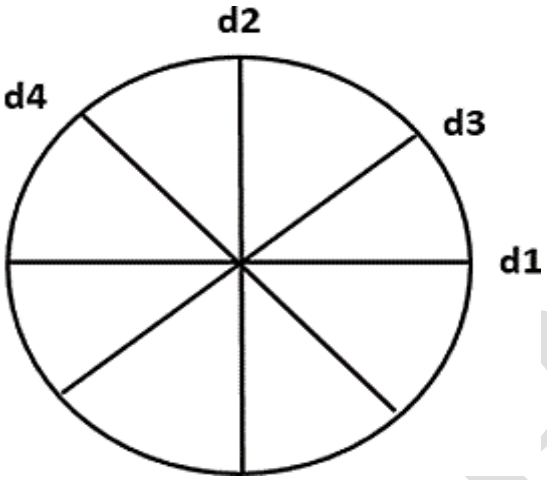

Fig. 3: Scheme of colony diameter measurement in the radial direction.

$$\text{Radial direction diameter} = \frac{d1 + d2 + d3 + d4}{4}$$

Description: d1: Axis diameter 1; d2: Axis diameter 2
d3: Axis diameter 3; d4: Axis diameter 4

Fungal Colony Growth Rate Per Day

Observation of colony growth rate was carried out every day on fungal colonies growing on PDA media in a petri dish. Colony growth rate measurement was carried out for 12 days and measured using a ruler, and the results were calculated by:

Colony growth rate = $b - a$

Description:

a : previous day diameter

b : observation time diameter

Growth Rate of Fusarium oxysporum Colony Per Day

Observation of colony growth rate was carried out every day on F. oxysporum colonies growing on PDA media in a petri dish. Measurement of colony growth rate was carried out for 12 days and measured using a ruler, and the results were calculated by:

Colony growth rate = $b - a$

Description:

a: previous day diameter

b: Observation time diameter

Percentage of Inhibitory Power

The Percentage of fungal inhibition power against the growth of *Fusarium oxyporum* was calculated every day until 12 Days After Incubation (DAI). The Percentage of inhibition was calculated based on the formula:

$$PI = \frac{R1 - R2}{R1} \times 100\%$$

Description:

PI = Percentage of mycelial growth inhibition (%)

R1= Mycelial diameter of F. oxysporum on a control petri dish (cm)

R2 = Mycelial diameter of F. oxysporum on treatment petri dish (cm)

Criteria for Percentage of Growth Inhibition (%) (Amaria et al., 2013):

- High inhibition percentage: 70-100%
- Medium inhibition percentage: 40-69%
- Low inhibition percentage: 0-39%

Data Analysis

Data analysis was conducted descriptively based on observations of the characteristics of fungi and *Fusarium oxysporum*, which were presented in the form of images. Observations of colony growth rates were presented in graphs. Data from colony diameter and in vitro inhibition tests for each treatment were analyzed using Analysis of Variance (ANOVA) with a 5% confidence level. Treatments that showed significant differences were further tested with the Duncan Multiple Range Test (DMRT) with an α level of 5% to determine the differences between treatments. Data analysis was performed using SPSS (Statistical Package for the Social Sciences) analysis.

RESULTS & DISCUSSION

Characteristics of Antagonistic Fungi Isolates

Isolate B6

Based on the results of observations (Fig. 4), macroscopically, the upper surface has a brownish-white colony with brown edges, while the bottom of the colony is white with brown edges. The surface texture of the colony is rather smooth; the distribution pattern is irregular and the hyphae density is less dense and does not form concentric circles.

Microscopic observations revealed that the fungus possesses non-septate hyphae and branched conidiophores. Conidia were observed emerging from round, transparent conidiophores and were dispersed around the hyphae and conidiophores, forming clusters. Additionally, some conidia were arranged in chains.

Isolate Si AA 10

Based on the observational results (Fig. 5), the colony appeared white on both the upper and lower surfaces. The colony's surface texture was smooth, with a round and even distribution pattern. The hyphal density was thick and dense, and no concentric circles were observed.

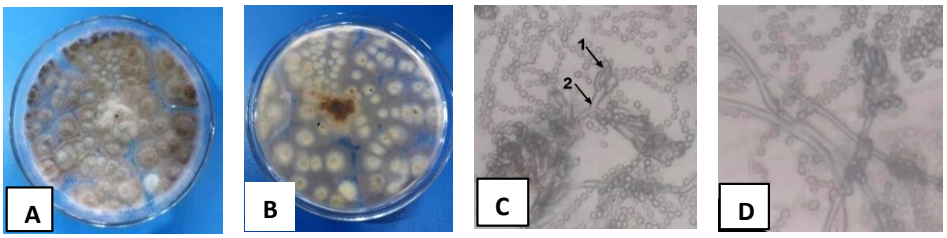

Fig. 4: Isolate B6 (A) Upper surface of B6 colony (B) Lower surface of B6 colony: (C) 1. Conidia 2. Conidiophores (D) Non-septate hyphae.

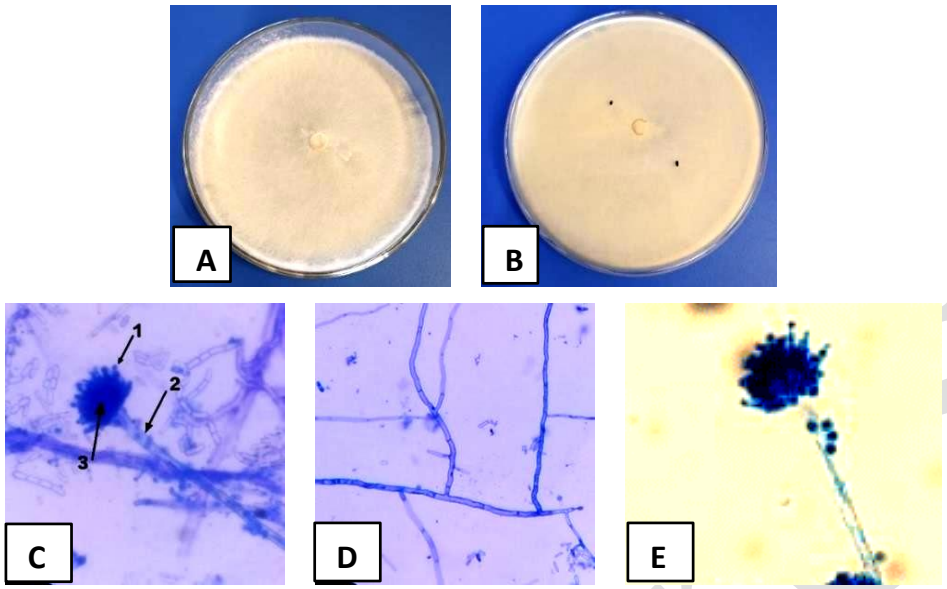

Fig. 5: Isolate SI AA 10 (A) Upper surface of colony Si AA 10 (B) Lower surface of colony Si AA 10(C) 1. Conidia 2. conidiophores 3. vesicles (D) Insulated hyphae (E) Aspergillus sp. (Diba et al., 2007).

Microscopic observations revealed that the hyphae are septate, with round conidia dispersed around the conidiophores. At the ends of the conidiophores, vesicles were present, and the conidiophores appeared straight and unbranched. Based on these characteristics, isolate Si AA 10 is suspected to belong to the genus *Aspergillus* sp. These findings align with the description provided by Mizana et al. (2016), which states that *Aspergillus* sp. exhibits septate and branched hyphae, as well as round conidia. Conidia serves as the asexual reproductive structures of *Aspergillus* sp. additionally, conidiophores and vesicles are present; conidiophores are rod-shaped, unbranched, and upright, while the round vesicles consist of phialides.

Isolate Si AA 11

Based on the observational results (Fig. 6), the upper surface of the colony is white, while the lower surface exhibits a yellowish-white coloration. The surface texture of the fungus is relatively smooth. The colony distribution pattern is round, evenly spread, and fairly dense, with no concentric circles observed. Microscopic observations reveal that the hyphae are septate, and conidia are dispersed around the hyphae.

Isolate *Trichoderma harzianum* Th-B18

Microscopic observations reveal that the fungus produces round conidia with smooth walls, which are distributed around the hyphae. The conidiophores are upright, and the hyphae are non-septate (Fig. 7). These findings align with previous research on *Trichoderma harzianum* Th-B18, which describes its microscopic morphology as having unicellular, non-septate, and transparent hyphae. The fungus possesses conidiophores, which serve as the supporting structure for the hyphae that produce conidia (asexual spores). The conidiophores are generally upright and may be branched or unbranched. The conidia are round, transparent, and possess smooth cell walls.

Isolate *Dichotomomyces cejpii*

Based on macroscopic observations (Fig. 8), the upper and lower surfaces of the colony appear yellowish-white. The surface of the yellow colony is scattered with grains, and the surface texture is rough. The hyphal density is relatively thick and dense, and the colony distribution is round, with no concentric circles observed. Microscopic observations reveal that the conidia are oval-shaped and located at the ends of the conidiophores. The conidiophores are slender, upright, and exhibit septate hyphae. According to Varga et al. (2007), this fungus produces colonies that range from white to cream in color, with abundant spore balls. It also exhibits racket-shaped hyphae (septate), with conidia produced from the branches of the conidiophores. The conidiophores are long, with smooth walls.

Characteristics of *Fusarium oxysporum* Isolates

Based on macroscopic observations of the characteristics of *Fusarium oxysporum* isolates (Fig. 9), it was observed that the upper and lower surfaces of the colony during initial growth are pink. As the colony ages, the color gradually changes to purple. The colony's surface texture is rough and fibrous, with loose hyphae. The distribution pattern is even, with wavy edges forming concentric circles. According to Semangun (2004), the morphology of *F. oxysporum* is characterized by a pink or purple colony surface, a rough and fibrous texture, and wavy edges.

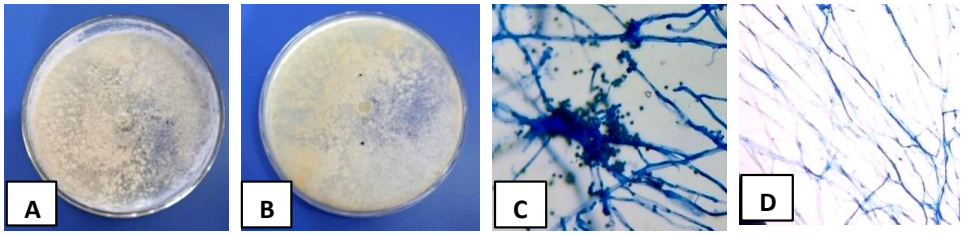

Fig. 6: Si AA 11 (A) Upper surface of the Si AA 11 colony (B) Lower surface of the Si AA 11: colony (C) Conidia (D) Insulated hyphae.

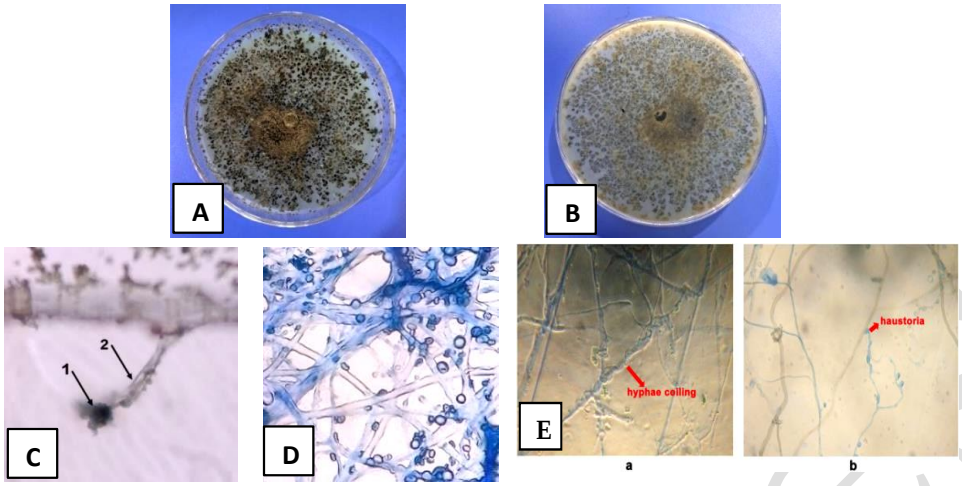

Fig. 7: Trichoderma harzianum isolate Th-B18(A) Upper surface of T. harzianum colony (B) Lower surface of T. harzianum colony, (C) 1. Conidia 2. Conidiophores (D) Non-septate hyphae (E) T. harzianum wrapping across Rhizoctoniasolani hyphae, b) Macrophomina sp. development is inhibited by the production of haustoria of T.harzianum (Pani et al., 2021).

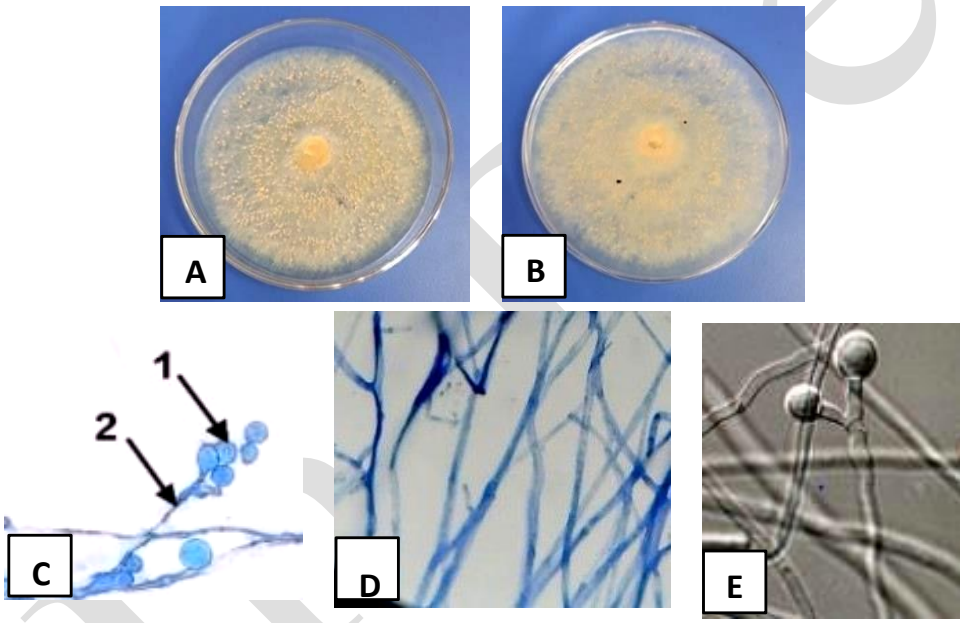

Fig. 8: Dichotomomyces cejpii isolate (A) Upper surface of D. cejpii colony (B) Lower surface of D. cejpii colony (C) 1. Conidia 2. conidiophores (D) Insulated hyphae (E) D. cejpii (Varga et al., 2007).

Microscopic examination reveals that the fungus produces oval-shaped microconidia, which are arranged in clusters at the tips of the conidiophores, with no macroconidia present. The conidiophores are upright, unbranched, and composed of hyaline-colored septate hyphae. Chlamydospores are also observed. According to Suryanti et al. (2015), most species within the *Fusarium* genus exhibit septate hyphae and produce asexual spores, including both macroconidia and microconidia. *Fusarium oxysporum* is known to produce three types of spores: microconidia, macroconidia, and chlamydospores. The microscopic morphology of *Fusarium oxysporum* observed in this study shows that several isolates feature elliptical or oval-shaped microconidia, which may possess one or two septa or be non-septate. These microconidia are located at the end of unbranched conidiophores.

Based on the results of the 5% Duncan's Multiple Range Test (DMRT) (Table 1 and Fig. 10), the comparison of colony diameters among antagonistic fungi showed no significant difference between treatments A2 and A3. However, both treatments were significantly different from treatment A4, which in turn was significantly different from treatment A5. Treatment A4 (*T. harzianum*) exhibited the largest colony diameter of antagonistic fungi (7.18cm), while treatment A5 (*D. cejpii*) displayed the smallest colony diameter (6.13cm). In terms of *F. oxysporum* colony diameter, no significant differences were observed among treatments A1, A2 and A3, but all three treatments were significantly different from treatments A0, A4, and A5. Treatment A0 (control) showed robust fungal growth due to the absence of antagonistic interactions. Treatment A5 produced the largest *F. oxysporum* colony diameter (6.74cm), whereas treatment A4 resulted in the smallest *F. oxysporum* colony diameter (2.02cm). Following the

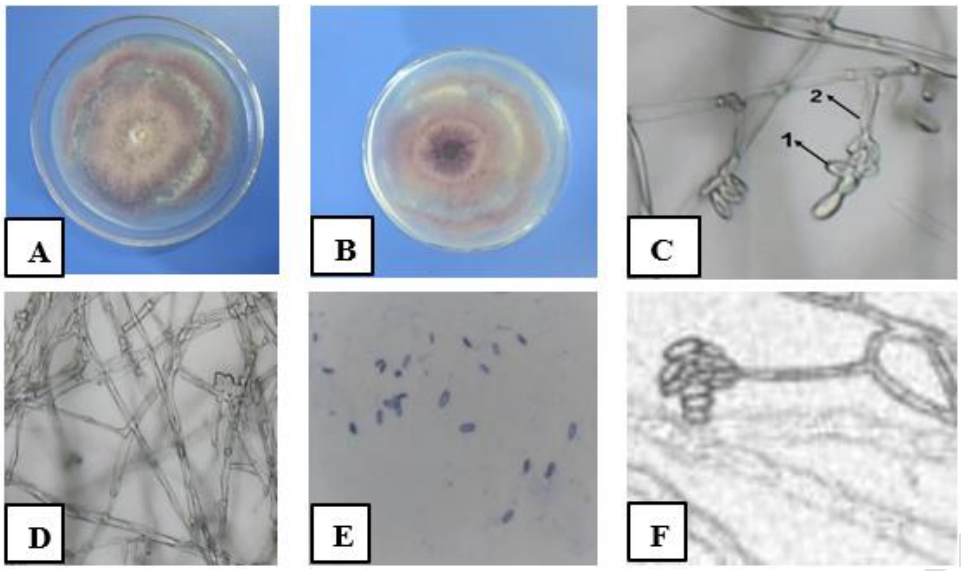

Fig. 9: *Fusarium oxysporum* isolate (A) Upper surface of *F. oxysporum* colony (B) Lower surface of F. oxysporum colony (C) 1. Microconidium 2. Conidiophores (D) Septate hyphae (E) Chlamydospores (F) *F. oxysporum* (Sutejo et al., 2008).

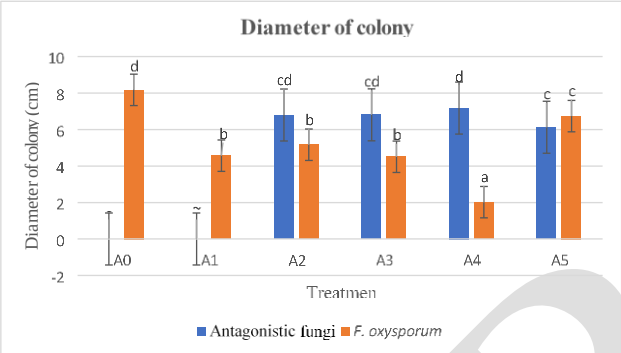

Fig. 10: Colony diameter graph of antagonistic fungi and *F. oxysporum*.

Table 1: Diameter of Antagonistic Fungi Colonies and *F. oxysporum* colony (12 DAI)

Treatment	Diameter of Antagonistic Fungi Colonies (cm)	Diameter of *F. oxysporum* colony (cm)
A0	-	8.18±0.37d
A1	-	4.57±1.10b
A2	6.80±0.00cd	5.17±0.43b
A3	6.81±0.05cd	4.51±0.31b
A4	7.18±0.14d	2.02±0.70a
A5	6.13±0.38c	6.74±0.05c
DMRT 5%	*	*

Note: Numbers followed by different notations in the same column indicate significant differences according to the DMRT test at the 5% level, ~ (infinity), - (none).

statement of Rotasouw et al. (2020), fungi that grow faster will outperform in controlling space so that they can suppress the development of rival fungi. Competition that occurs is caused by the need for nutrients contained in the media for its survival. In line with Wahyuni and Noviani's (2019), antagonistic fungi can be used as biological agents to inhibit the growth of *F. oxysporum* because of faster fungal growth. Fast fungal growth can indicate competition for nutrients and growth space, besides producing antibiotic compounds that can inhibit pathogen growth. This phenomenon was particularly evident in treatment A4 (*T. harzianum*).

Trichoderma uses a variety of complex direct or indirect mechanisms against fungal pathogens, which usually interact as a whole in biological control. Direct effects against pathogens include the production of cell wall degradation enzymes (CWDE), synthesis of antimicrobials, competition for space and nutrients (mainly carbon, nitrogen and iron) and establishment of direct parasitism relationships with fungal pathogens (Benítez et al., 2004; Srivastava et al., 2016). Furthermore, the effects of necrotrophic mycoparasites of Trichoderma on fungal pathogens include prey sensing and chemotaxis, adhesion to the host, and physical attack through heavy branching and twisting around host hyphae. In addition, Trichoderma can form penetrating structures such as appressoria, which are homologs of the appressoria of pathogens (Mukherjee et al., 2012; Moreno-Ruiz et al., 2020). Chemical attack and degradation of the pathogen cell wall by hydrolytic enzymes and antifungal compounds produced by Trichoderma is the final stage of the mycoparasite interaction, ultimately leading to host death (Seidl-Seiboth et al., 2014; Mukherjee et al., 2012).

The larger colony diameter of T. harzianum observed in treatment A4 compared to F. oxysporum can be attributed to the production of secondary metabolites, including antibiotics that inhibit the germination of F. oxysporum spores. Suwahyono (2000) reported that T. harzianum produces two types of antibiotic compounds—viridin and glycotoxin—which effectively suppress pathogen growth.

Growth Rate of Antagonistic Fungal Colonies

Based on the growth rate curve (Fig. 11), treatments A2 and A3 exhibited an initial growth phase, as indicated by the increase in the curve from day 1 to day 3. In contrast, treatment A5 entered the initial growth phase from day 1 to day 2. During this phase, the growth rate was relatively low due to the cells undergoing an adaptation process. Treatment A4 (*T. harzianum*) demonstrated an exponential growth phase from day 1 to day 2, as evidenced by the steep increase in the curve, reflecting a rapid rise in cell numbers. The exponential phase represents the optimum growth period, characterized by a significant increase in cell population. By days 3 to 12, all antagonistic fungi entered the stationary phase, as indicated by the plateauing of the growth rate curve. During this phase, cell growth slows,

and the rate of living cells becomes nearly equal to the rate of dying cells, primarily due to the depletion of nutrients in the media. Following the stationary phase, the fungi entered the death phase, where the growth rate declined to zero, resulting in a constant colony diameter. In this phase, the number of fungal cells decreased due to nutrient exhaustion in the media and the depletion of cellular food reserves (Srikandace et al., 2007). Treatment A4 (*T. harzianum*) exhibited the fastest growth rate, as indicated by the steeper slope of its early growth curve. Conversely, treatment A5 (*D. cejpii*) showed a relatively slower colony growth rate, as evidenced by the gentler slope of its curve. The observed growth rate is directly related to pathogen inhibition, wherein the faster growth rate of antagonistic fungi suppresses pathogen growth, thereby achieving a higher percentage of inhibition. Widi et al. (2015) suggested that the growth rate of antagonistic fungi serves as an indicator of their competitive mechanisms for space and nutrients against pathogens. The faster the growth of the antagonistic fungi, the more effective they are in suppressing the growth of pathogenic fungi.

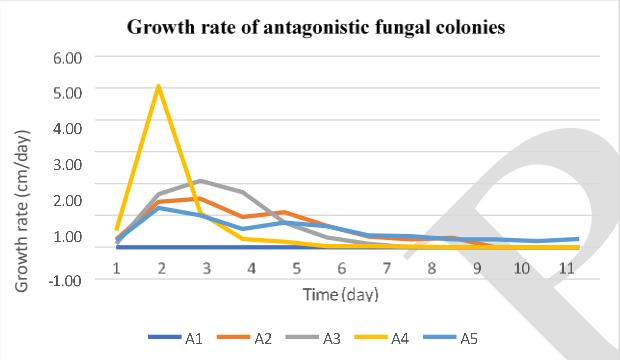

Fig. 11: The curve of growth rate of antagonistic fungal colonies.

The Growth Rate of Antagonistic Fungal Colonies of *Fusarium oxysporum*

In treatment, A5, *F. oxysporum* exhibited a faster colony growth rate, as indicated by the higher curve (Fig. 13). In contrast, treatment A4 displayed a slower growth rate, as shown by the lower curve. The internal conditions of the fungus itself influence the rate of fungal growth. When sufficient nutrients are available, the fungus continues to grow unabated. The growth rate of *F. oxysporum* directly impacts the Percentage of inhibition; a faster growth rate makes it more challenging for antagonistic fungi to suppress the pathogen. According to Apriliahetty et al. (2023), the growth rate varies depending on the conditions of each isolate and is further influenced by favorable environmental factors. According to Hasanah (2018), during the exponential phase, fungi undergo rapid division following a logarithmic growth curve. In this phase, the growth rate of fungi is significantly influenced by the growth medium, including its nutrient content and environmental conditions, such as temperature. During this stage, microorganisms require higher energy levels compared to other growth phases, rendering them highly sensitive to environmental conditions. Conversely, the death phase occurs when nutrients in the medium are depleted or the cells exhaust their energy reserves. The duration and onset of this phase are determined by factors such as environmental conditions, nutrient availability, and the specific characteristics of the microbial species.

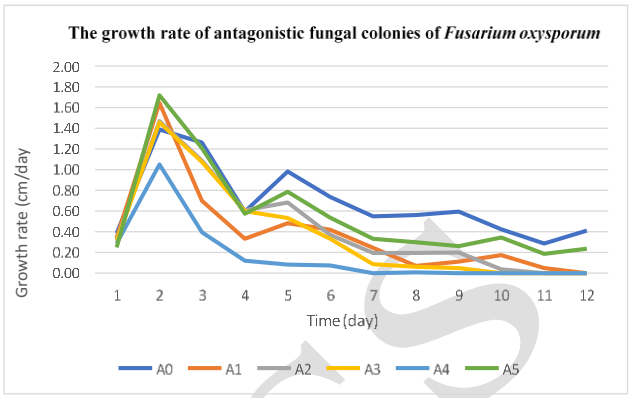

Fig. 13: The curve of growth rate of antagonistic fungal colonies *F. oxysporum*.

Percent of Inhibition

The results of the 5% DMRT test indicate that the Percentage of inhibition at 12 hours after inoculation (HAI) for treatments A1, A2, and A3 was not significantly different. However, these treatments exhibited significant differences when compared to treatments A4 and A5. Treatment A0 (control) demonstrated an inhibition percentage of 0.00% because F. oxysporum was cultured on a PDA medium without the presence of antagonistic fungi, resulting in the absence of antagonistic activity (Table 2; Fig. 14). Among the treatments, A4 showed the highest Percentage of inhibition at 75.14%, while A5 exhibited the lowest inhibition percentage at 17.54%. The high inhibitory effect observed in treatment A4 can be attributed to the broader mycelial coverage produced by T. harzianum compared to F. oxysporum, which suggests that T. harzianum has a superior capacity to dominate space and utilize nutrients. However, this ability may also lead to antagonism against other fungi coexisting with the pathogenic fungus within the same growth medium.

In contrast, the low inhibitory effect observed in treatment A5 may be due to the limited competitive ability of D. cejpii to secure nutrients within the medium. Furthermore, its slower growth rate, likely influenced by genetic factors, results in a smaller mycelial area compared to F. oxysporum. According to Lubis and Wati (2022), variations in inhibitory power can be influenced by multiple factors, including the ability of fungal isolates to produce secondary metabolites or antibiotics in varying quantities, their genetic traits, growth rates, and competition for space and nutrients. Fungi that produce greater quantities of antifungal substances are more effective at suppressing the growth of other organisms. Secondary metabolites, which exhibit antibiotic properties, inhibit pathogen growth. In addition to producing antifungal compounds, endophytic fungi can also synthesize enzymes and mycotoxins that contribute to pathogen suppression.

Table 2: Percent of inhibition (12 DAI)

Treatment	Percent of inhibition (%)
A0	0.00±0.00a
A1	43.69±15.30c
A2	36.66±7.64c
A3	44.96±1.75c
A4	75.14±9.23d
A5	17.54±3.67b
DMRT 5%	*

Note: Numbers followed by different notations indicate significant differences according to the DMRT test at the 5% level.

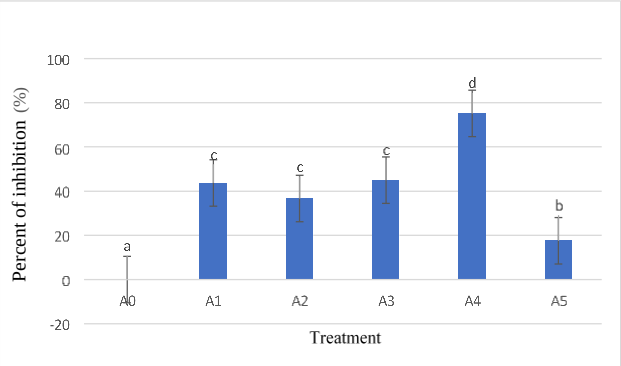

Fig. 14: Percent of inhibition.

Alfizar et al. (2013) reported that *T. harzianum* produces significant amounts of extracellular enzymes, including β-1,3-glucanase, chitinase, and cellulase. The presence of chitinase and β-1,3-glucanase can inhibit pathogen growth by degrading the cell walls of pathogenic fungi. Chitinase, specifically, decomposes chitin, a major structural component of fungal cell walls, facilitating this degradation process. Additionally, *T. harzianum* produces two types of antibiotics: viridin and glycotoxin. According to Piecková and Roeijmans (1999), *D. cejpii* also produces various metabolites with antibiotic properties, including glycotoxin, which serves as a biocontrol agent against pathogens. The efficacy of treatments was evaluated based on criteria established by Amaria et al. (2013). Treatments achieving 70–100% inhibition are classified as strong, 40–69% as moderate, and 0–39% as weak. Based on this classification, treatment A4 (*T. harzianum*) demonstrated the highest inhibition percentage and was classified as strong. Treatments A3 (SiAA11) and A1 (B6) exhibited moderate inhibition percentages, while treatments A2 (SiAA10) and A5 (*D. cejpii*) displayed weak inhibitory effects.

Moderate inhibition levels observed in treatments A3 and A1 can be attributed to the production of bioactive compounds by the fungal endophytes. These endophytes serve as biological control agents by either stimulating the plant's natural defenses or directly producing bioactive compounds that inhibit or prevent pathogen attacks (Jaber & Araj, 2018; Huang et al., 2019). These bioactive compounds, which target both fungi and bacteria, include terpenoids, flavonoids, alkaloids, quinols, chlorinated compounds, peptides, steroids, polyketides, and phenols (Moraes et al., 2020). Research has demonstrated that treating food crops with endophytic fungi reduces damage from various plant diseases and plant-parasitic nematodes after effective colonization. For instance, *F. oxysporum* f. sp. *cucumerinum*, the causal agent of cucumber wilt, was successfully suppressed by 30 species of endophytic fungi, including *Penicillium* sp. and *Hypocrea* sp., which inhibited mycelial growth and reduced disease severity (Abro et al., 2019).

Certain alkaloid compounds produced by fungal endophytes, such as 12-hydroxy-13-methoxyverruculogen TR-2, fumiteromorgin B, and verruculogen, have demonstrated antifungal efficacy against eight fungal species, including *B. cinerea*, *A. solani*, *A. alternata*, *C. gloeosporioides*, *F. solani*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *vasinfectum*, and *G. saubinettii*. These compounds exhibited minimum inhibitory concentrations (MICs) ranging from 13.7 to 100 mM, comparable to standard controls like carbendazim and hymexazol (Li et al., 2012). Additionally, terpenoids such as rhinomilisin B, diveronsol H, and triveronsol demonstrated moderate to potent antifungal activity against *Penicillium italicum*, *F. oxysporum*, *F. graminearum*, *C. musae*, and *C. gloeosporioides*, with MIC values superior to triadimefon, a conventional fungicide (Hu et al., 2019). Furthermore, polyketides, such as macrosporin, exhibited broad-spectrum antifungal activity against *F. oxysporum*, *F. graminearum*, *C. musae*, *P. italicum*, *R. solani*, and *C. gloeosporioides*, with MIC values ranging from 13.2 to 252.1 mM (Huang et al., 2017).

Although treatment A5 (*D. cejpii*) displayed low inhibition percentages, it could still serve as an effective biocontrol agent if its population density is increased. Bukhari and Safridar (2020) emphasized that the success of antagonistic fungi as biological control agents depends on establishing a robust population of the inoculated fungi. Siregar et al. (2022) stated that antagonistic fungi can suppress pathogen growth through several mechanisms, including degrading cell walls, producing antifungal compounds, and secreting hydrolytic enzymes. Moreover, endophytic fungi produce alkaloids and mycotoxins, which enhance plant resistance to diseases. The antagonistic mechanisms of fungi against *F. oxysporum* can be categorized into three main approaches: competition, parasitism, and antibiosis. During the competition, antagonistic fungi suppress the growth of pathogens by consuming nutrients more efficiently and outgrowing the pathogens, thereby occupying available space (Purwantisari & Hastuti, 2009). The antibiosis mechanism involves the production of active compounds, such as steroids, phenolics, alkaloids, terpenoids, isocoumarins, chromones, volatile substances, polypeptides, and aromatic compounds, which inhibit the growth of *F. oxysporum* (Kumar & Kaushik, 2012). Trichoderma species are particularly notable for producing hundreds of antimicrobial secondary metabolites, including trichomycin, gelatinomycin, chlorotrichomycin, and antibacterial peptides, which enhance their efficacy as biocontrol agents (Maruyama et al., 2020).

The parasitism mechanism occurs when the mycelium of antagonistic fungi envelops the entire surface of the medium, including *F. oxysporum*, as observed in treatments A3 and A4 (Fig. 15). According to Kusumawardani et al. (2015), this process occurs when the antagonistic fungus

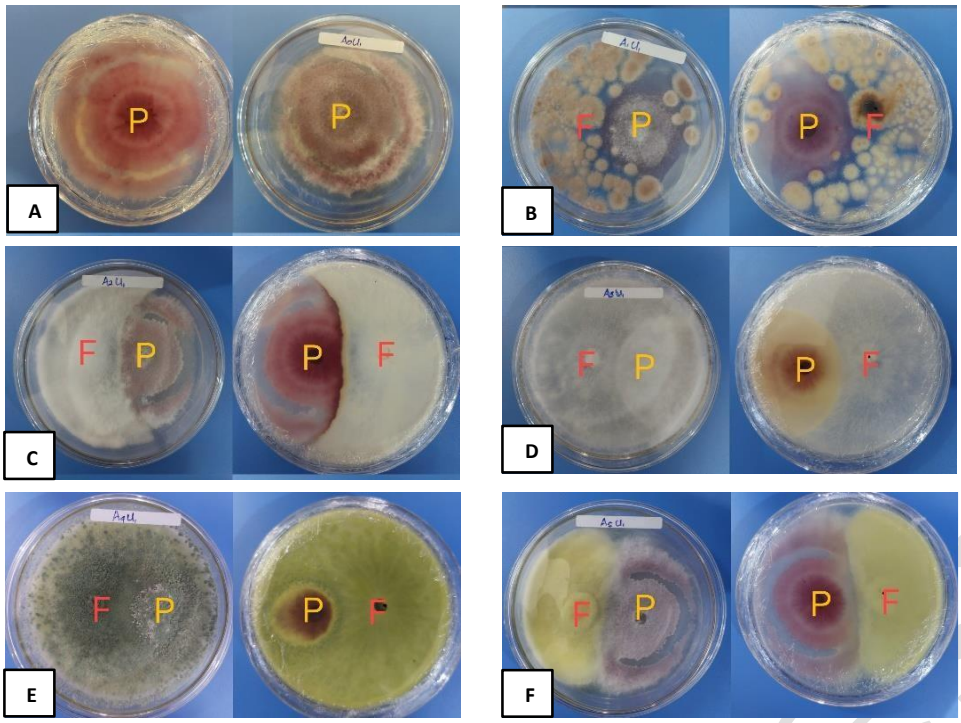

Fig. 15: Treatment for the antagonist fungi day 12 (A) Treatment A0: F. oxysporum control (B) Treatment A1: B6+*F. oxysporum* (C) Treatment A2: Si AA 10+*F. oxysporum* (D) Treatment A3: Si AA 11+*F. oxysporum* (E) Treatment A4: *T. harzianum*+*F. oxysporum* (F) Treatment A5: *D. cejpii*+*F. oxysporum*. Note: P= Pathogen F. oxysporum; F= Fungi antagonist.

surrounds the pathogen, inhibiting its growth through lysis of the pathogen's mycelium. The endophytic hyphae envelop the pathogen, absorbing its nutrients and causing visible discoloration. Endophytes employ direct mechanisms to protect plants from pathogens, which include antagonistic actions such as antibiotic production, competitive exclusion of pathogens, parasitism, and attenuation of pathogen virulence (Köhl et al., 2019). Antibiotic activity by endophytes involves the secretion of allelochemicals, including bacteriocins, lipopeptides, biosurfactants, cell wall-degrading enzymes, volatile organic compounds, and antibiotics. These substances disrupt the metabolism of plant pathogens, effectively halting their development (Raymaekers et al., 2020). Additionally, competition between endophytes and pathogens for nutrients and space contributes to reducing pathogen infections. Enzymes such as pectinases and chitinases further inhibit pathogen virulence by interfering with factors responsible for pathogenicity in phytopathogens (Wang et al., 2022).

Isolates A1, A2, and A3 did not show significant differences in the diameters of the antagonistic fungi or *F. oxysporum*. Based on characterization results, isolate A2 (SiAA10) appears to belong to the *Aspergillus* genus. Aldinary et al. (2021) reported that fungi such as *Aspergillus alabamensis*, *Aspergillus tubingensis*, and *Aspergillus oryzae* are effective in combating *Fusarium* wilt. Isolate A4 (*T. harzianum* Th-B18) demonstrated the highest inhibition rate (75.14%), consistent with findings by Toghueo et al. (2016), who observed similar inhibitory effects of *Trichoderma* spp. on *Fusarium solani*. In vitro studies revealed that endophytes inhibited and suppressed the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* more effectively than entomopathogenic fungi. For example, *Trichoderma asperellum* M2RT4, *Hypocrea lixii* F3ST1, *Trichoderma harzianum* KF2R41, and *Trichoderma atroviride* ICIPE 710 demonstrated suppression rates of 68.84–99.61%, significantly higher than the entomopathogenic fungi, which achieved inhibition rates of 27.05–40.63% (Muhorakeye et al., 2024). These findings align with the results of this study, confirming the potential of endophytic fungi and *Trichoderma harzianum* Th-B18 as biological control agents against *Fusarium oxysporum*.

Conclusion

Antagonistic fungi, as biological control agents, demonstrated significant efficacy in the A4 (*T. harzianum* Th-B18) treatment by inhibiting the growth of *F. oxysporum*. This inhibition is evidenced by the faster growth rate of the antagonistic fungi, reflected in a larger fungal diameter of 7.18cm compared to the diameter of *F. oxysporum*. Among the treatments tested, A4 (*T. harzianum* Th-B18) exhibited the highest inhibitory effect, with a percentage inhibition of 75.14%, categorizing it as highly effective or strong. Further studies are recommended to identify the endophytic fungi at the species level to understand their potential and mechanisms of action better. Antagonistic testing involving endophytic fungi and *D. cejpii* against *F. oxysporum*, the causative agent of shallot wilt disease, was conducted by increasing the population of these fungi. This approach aims to evaluate their potential as antagonistic agents for the biological control of *F. oxysporum*.

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with GPB. AA and NFER prepare draft articles, including template frameworks and modifications to the final submitted version. All authors also contributed to reading and reviewing the final manuscript.

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