INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0026/2024/32–4–308–314
DOI: 10.17957/IJAB/15.2206
http://www.fspublishers.org

Full Length Article

The Antagonistic Ability of Fungal Isolates from Oyster Spent Media Substrate against *Fusarium oxysporum* in Chili Plants

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Received 16 January 2024; Accepted 04 June 2024; Published 21 August 2024

Abstract

One of the main diseases of chili plant in Indonesia is Fusarium wilt caused by the fungus *Fusarium oxysporum*. This study aims to determine whether fungal isolates isolated from oyster spent media substrate (OSMS) have antagonistic ability against *F. oxysporum* and are not pathogenic in chili plants. The research was conducted by isolating, identifying and characterizing fungal isolates isolated from OSMS, then conducting dual culture tests between OSMS isolates and *F. oxysporum* in the laboratory, as well as conducting trials in a seedling house to see the effect of inoculation of OSMS fungal isolates on the pathogenicity and growth of chili plants. The results showed that the fungal isolates successfully isolated from OSMS were identified as the genus *Trichoderma* and *Gliocladium*. The antagonistic ability of isolates from OSMS in inhibiting *F. oxysporum* ranged from 45.3–57.53%. The results of the trial in the seedling house showed that the OSMS isolates statistically did not affect plant growth and were not pathogenic in chili plants. Overall, the results of this study indicate that the application of *Trichoderma* and *Gliocladium* obtained from OSMS can be an alternative initial foundation in controlling *F. oxysporum* in chili plants.

Keywords: Biocontrol; Chili; Competition; Fusarium; Pathogen; Spent mushroom; Sustainable agriculture

Introduction

Chili plants in Indonesia are one of the leading horticultural commodities that are often used as seasonings. During chili cultivation activities, many farmers are still often faced with disease attacks that can cause crop failure. One of the diseases in chili plants is fusarium wilt, which is generally caused by *Fusarium oxysporum* (Agrios 2005; Fatma *et al*. 2021). The presence of fusarium wilt disease in the period from germination to mature plants can cause crop failure of up to 50% (Afriani *et al.* 2019). *F. oxysporum* is also reported to be the main disease in chili pepper crops in Asian countries such as Thailand, India, China, and Indonesia (Ali 2006).

Disease control efforts that have been carried out at the chili farm level in Indonesia still rely on synthetic pesticides. For example, most chili farmers in Bali Province and East Java Province, Indonesia use synthetic pesticides with an application frequency of 2–3 times per week for 5–8 weeks (Mariyono *et al*. 2018). However, such control has been widely reported to cause adverse effects on human health, environmental pollution, ecological disturbances, resistance and resurgence in pests or pathogens, killing of natural enemies, and leaving residues in crops (Tudi *et al.* 2021; Lahati and Ladjinga 2022).

To prevent and overcome the negative effects of using synthetic pesticides, biological control can be done by utilizing antagonistic microorganisms. Microorganisms that are antagonistic to plant pathogens can be found in organic materials (Bonanomi *et al.* 2020). One of sources of organic material that can be utilized in efforts to control plant diseases is spent mushroom media substrate (SMS) (Rinker 2017; Adhi 2023).

Spent mushroom media substrate (SMS) is a waste media from the production of consumer mushrooms such as straw mushroom, oyster, shiitake, and others. Based on the results of research by Verma *et al*. (2020), the content of organic matter in Oyster Spent Media Substrate (OSMS) is still quite high reaching 20%. The content of organic matter has an important role directly and indirectly on the presence and activity of microorganisms (Babur *et al.* 2022).

To cite this paper: Adhi SR, Nuraeni, Sugiarto, L Afifah, W Rianti, I Wicaksana (2024). The Antagonistic ability of fungal isolates from oyster spent media substrate against *Fusarium oxysporum* in chili plants. *Intl J Agric Biol* 32:308–314

Antagonistic microorganisms from fungi and bacteria can be found in SMS. Based on the results of Adedji and Aduramigba (2016), antagonistic fungi with species of *Trichoderma viridae*, *Pencillium chrysogenum*, *Penicillium oxalicum* and *Aspergillus terreus* were isolated from OSMS bath water. Fungal isolates found in SMS are reported to have the ability to suppress several plant pathogens. Herawati and Istifadah (2018) discovered that isolates of the *Aspergillus* and *Trichoderma* from substrate made from discarded oyster and shitake mushrooms were able to prevent tomato plant damping off disease growth by 57.5–100%. SMS water extract of *Hericium erinaceus* was also able to suppress mycelial growth of several pathogenic fungi in tomato plants such as *Alternaria solani* by 72.2%; *Botrytis cinerea* by 23.1%; *Corynespora cassicola* by 58.3%; *F. oxysporum* by 70.4%; *Phytophthora capsica* by 100%; *Rizoctonia solani* by 46.6%; and *Sclerotinia sclerotiorum* by 43.2% (Lee *et al.* 2015).

Although the antagonistic ability of some fungal species from SMS in suppressing diseases in several crops has been reported, research on the identification of antagonistic fungal species and their antagonistic ability from OSMS against fusarium wilt disease of chili plants has not been reported so far. Therefore, the objective of this study was to identify and evaluate the antagonistic ability of fungal isolates of OSMS origin against *F. oxysporum* of chili plants. The main focus of this study was to determine whether fungal isolates isolated from OSMS have antagonistic ability and can be used as a more environmentally friendly biological control method for Fusarium wilt disease.

Materials and Methods

The research was conducted at the Laboratory of Biotechnology and seedling house, Faculty of Agriculture, Universitas Singaperbangsa Karawang from May to August 2023.

Sample collection

Oyster Spent Mushroom Substrate (OSMS) is obtained from oyster mushroom cultivation waste, namely Berkah Jamur 17 in Kp. Sukasari Karangjaya Village, Tirtamulya District, Karawang Regency, West Java, Indonesia, 41372. The sampling location is at an altitude of 24 m above sea level with coordinates 6˚22'39.5 "S 107˚27'05.1 "E. The isolate of pathogenic *F. oxysporum* is a collection of Plant Pests and Diseases Laboratory, Faculty of Agriculture, Universitas Singaperbanga Karawang.

Isolation and Characterization of fungal from OSMS

OSMS samples that have been composited from the sampling location are isolated to obtain antagonistic fungi. The isolation method uses a modified method from Ristiari *et al.* (2018), which begins with a multilevel dilution by taking 10 g of OSMS samples that have a fine texture and suspended in 90 mL of sterile distilled water and homogenized for 15 minutes using a vortex. Next, 1 mL of suspension was taken and re-suspended in 9 mL of sterile distilled water, then multilevel dilutions were continued from 10^{-1} to 10^{-5} (Sutari 2020).

1 mL was taken using a sterile pipette from each dilution result 10^{-1} to 10^{-5}, then put into a Petri dish, then added 10 mL of potato dextrose agar (PDA) media and shaken to mix and incubated at room temperature 28–30°C for 7 days. Colonies that grow well on PDA media and have differences from color, different macroscopic shapes are further purified (sub-culture) repeatedly until a pure culture is obtained (Adedji and Aduramigba 2016).

Characterization and identification of pure isolates of sub-cultured fungi are based on macroscopic and microscopic characteristics. Differences in colony shape and color become the basis in supporting microscopic observations. Microscopic observations were made by looking at the shape of the hyphae, the size of the conidia or conidiophores, and the shape of the conidia and conidiophores, then compared with the help of the determination key book Illustrated Genera of Imperfect Fungi by Barnett and Hunter (1987) and the Pictorial Atlas of Soil and Seed Fungi Third Edition book by Watanabe (2010) to identify the isolates for knowing the genus.

In vitro screening of antagonism ability to pathogen

Antagonism screening assay was conducted by dual culture method on 90 mm Petri dish containing 10 mL PDA media. Antagonism screening of fungal isolates from OSMS that had been characterized and identified the genus was then grown together with pathogenic *F. oxysporum* isolates in a completely randomized design. OSMS and *F. oxysporum* fungal isolates were placed 30 mm next to each other with 30 mm between isolates and from the edge of the Petri dish.

The experiment was conducted using a completely randomized design. Observations were made by observing the diameter (cm) of *F. oxysporum* colonies for 7 days after the incubation period both in the control treatment (without OSMS fungal culture) and in the dual culture experiment. Colony diameter data obtained on day 7 was used as the basis for calculating the percentage of inhibition through the formula adopted from Adedji and Aduramigba (2016):

$$I (\%) = (C-T)/C \times 100$$

Where, I = percentage inhibition of mycelia growth, C = radial growth of pathogen in control, T = radial growth of pathogen in dual culture.

Seedling house trials

The seedling house experiment was conducted using a group randomized design. This experiment was conducted to determine the effect of OSMS fungal isolate inoculation on chili seed germination as a selection step for non-pathogenic isolates. Experiments in the seedling house were conducted on seed trays using planting media with a composition of 15% soil, 15% burnt husk, and 65% compost. Tests were carried out using the method modified from Astiko *et al.* (2017) by inoculating culture pieces of each OSMS isolate measuring 8 mm into the planting medium with a depth of ± 1 cm on the seedling tray 3 days before the seeds were planted. After 3 days, 4 chili seeds were planted on each OSMS isolate treatment tray. Observations were made for 14 days after inoculation by looking at whether the seeds grew normally and healthily without any symptoms of rotting and calculating the percentage of germination.

Data analysis

Screening assay antagonism and seedling house trials were statistically analyzed through analysis of variance (ANOVA), followed by a post hoc comparison of means by Duncan Multiple Range's Test (DMRT) in IBM SPSS 21 statistical software.

Results

Characteristics of OSMS fungal isolates

Based on the results of isolation and characterization obtained 10 fungal isolates from oyster spent media substrate (OSMS) which showed different morphological characteristics (Fig. 1). Ten isolates consisted of 8 isolates belonging to the genus *Trichoderma* and 2 isolates of the genus *Gliocladium* (Table 1). The identification results are based on comparisons on macroscopic and microscopic characteristics produced if the *Trichoderma* genus has general characteristics of green colonies with irregularly branched conidiophores, hyaline conidia and ovoid (globose). While *Gliocladium* has general characteristics of green and gray-green colonies, its conidiophores are branched as in *Penicillium* (Penicilliate brances), hyaline conidia.

Antagonism ability of OSMS isolate to *Fusarium oxysporum*

Based on the results of dual culture testing between OSMS isolates and *F. oxysporum*, it was found that ten OSM isolates had an antagonistic ability to suppress the growth of *F. oxysporum* colonies with a percentage of ability to inhibit 45.39–57.53% (Table 2). The percentage of the highest inhibition was found in the OSMS8 (*Trichoderma* sp. 6) treatment (Fig. 2). However, other OSMS isolates (in the same genus) had abilities that were not significantly different based on the results of statistical tests in suppressing the growth of *F. oxysporum*.

Effect of OSMS on chili seedling

The observation of the effect of OSMS isolates inoculation on chili seed germination showed that the germination percentage was in the range of 91.67–100% (Table 3). Although based on the results of statistical tests did not show significant differences ($P > 0.05$), but it can be seen from 10 OSMS isolates there are 7 isolates that do not affect the germination power of chili seeds because they show the percentage value of germination that is not below the control treatment. OSMS isolates that do not affect chili seed germination are *Trichoderma* sp.1, *Trichoderma* sp. 3, *Trichoderma* sp. 4, *Trichoderma* sp. 5, *Trichoderma* sp. 6, *Trichoderma* sp. 7, and *Trichoderma* sp. 8. Meanwhile, isolates that caused germination percentage values below the control treatment were *Gliocladium* sp. 1, *Gliocladium* sp. 2, and *Trichoderma* sp. 2. The data suggests that the inoculation with these specific OSMS fungal isolates does not significantly impact chili seed germination compared to the control group.

Discussion

In this study we show evidence that fungal isolates of the genus *Trichoderma* and *Gliocladium* isolated from oyster spent media substrate (OSMS) have antagonistic abilities and are able to suppress the growth of *F. oxysporum* which causes fusarium wilt disease of chili plants. During the screening process, growth inhibition of *F. oxysporum* occurred due to the mechanism of competition, hyper parasitism and presumably the presence of antibiotic compounds released by OSMS isolates.

Trichoderma and *Gliocladium* are biological control agents that suppress the growth of *F. oxysporum*, the pathogen responsible for fusarium wilt disease in chili plants. It has been demonstrated that *Trichoderma* sp. inhibit *F. oxysporum* development by a number of mechanisms, such as mycoparasitism and the release of secondary metabolites that are antifungal (Purba *et al*. 2023). *T. asperellum* in particular has demonstrated high mycelial inhibition rates against *F. oxysporum* and has been found to produce bioactive compounds that inhibit the pathogen (Sehim *et al.* 2023). Additionally, both *In vitro* and *In vivo*, *Gliocladium* sp. has been beneficial in stopping the development of *F. oxysporum* (Farihadina 2022). *Trichoderma* and *Gliocladium* suppress the growth of *F. oxysporum* through their antifungal activity and the production of bioactive compounds, making them effective biological control agents against fusarium wilt disease in chili plants.

The interaction of *Trichoderma* on *F. oxysporum* resulted in a competitive interaction shown by the struggle for media space and nutrients in the Petri dish. The faster growth of *Trichoderma* compared to *F. oxysporum* on PDA media is due to the fact that *Trichoderma* is a fungus with a low level of metabolic stress even though it lives on a simple growth

Table 1: Characteristics of fungal isolates isolated from oyster spent media substrate (OSMS)

Isolate code	Characteristic	Genus
OSMS1	Colonies grayish green, mycelium grayish green. Septae hyphae fibrous, conidiophores hyaline, branched like Penicillium	*Gliocladium*
OSMS2	Colonies are dark green, mycelium is white, smooth surface. Septae hypae, conidiophores are hyaline, brances are irregular, ovoid conidia.	*Trichoderma*
OSMS3	Green colonies, green mycelium, septae hypae, conidiophores branched and Penicillium-like, spore mass formed only at the apex of conidiophores	*Gliocladium*
OSMS4	Colonies are green, mycelium is white, giving off reddish brown pigment on PDA media. Septae hyphae, conidiophores are irregularly branched and hyaline conidia are oval.	*Trichoderma*
OSMS5	Colonies are green, mycelium is green, surface is smooth like thin cotton, conodiphores irregularly branched and conidia are oval.	*Trichoderma*
OSMS6	Colonies are green, mycelium is green, surface is smooth like thin cotton, evenly distributed, septae hyphae, conidiophores are branched and conidia are oval.	*Trichoderma*
OSMS7	Colonies are dark green, mycelium is white with a smooth surface like thin cotton in the middle relatively thick hollow spread, septae hypae, conidiophores are irregularly branched, and conidia are oval.	*Trichoderma*
OSMS8	Colonies are gray-green, mycelium is greenish-white, surface is smooth like thin cotton, spreading, septae hyphae, conidiphores are branched and conidia are oval	*Trichoderma*
OSMS9	Colonies are green, mycelium is gray-green, the surface is slightly thick and smooth like cotton, evenly spreading, hyphae are concentrated, conidiophores are branched and conidia are oval.	*Trichoderma*
OSMS10	Yellowish green colonies, white cotton-like mycelium with smooth surface, thick, hollow spread. Septae hyphae, conidiophores are branched and conidia are oval.	*Trichoderma*

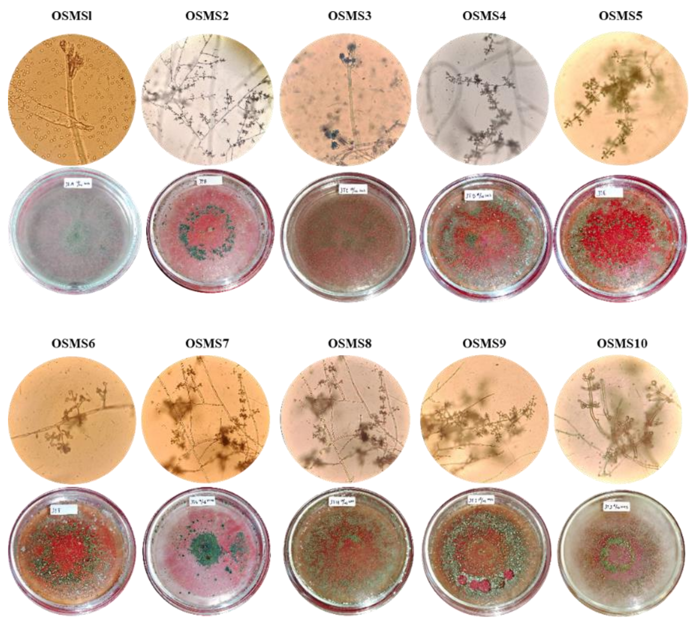

Fig. 1: Characteristics of isolates isolated from OSMS

substrate when compared to pathogenic fungi (Oszust *et al.* 2020). This is an advantage of *Trichoderma*, because *Trichoderma* is able to live in a wider environment, especially if used as a biocontrol fungus for plant disease control.

Another interaction that occurs is mycolysis in the form of hyphal swelling of *F. oxysporum* (Fig. 3a). Mycolysis occurs because *Trichoderma* produces extracellular cell wall degrading enzymes such as chitinase and β-1, 3 glucanase which are associated with lysis of the fungal cell wall (Shahid *et al.* 2015). Another enzyme in the form of cellulase produced by *T. virens* is also involved in the process of detoxification or degradation of mycotoxins produced by pathogens (Ghasemi *et al.* 2020).

Based on the observations, there is also an antibiosis mechanism in the form of a melanization zone (Fig. 3b) at the meeting area of *Trichoderma* mycelium and *F. oxysporum*. According to Mendoza *et al.* (2015), the inhibition zone in the form of discoloration in the dual culture test is due to the excretion of secondary metabolites between the tested fungi. Antibiosis is the production of anti-microbial compounds that play a role in protecting plants against pathogen attack (Bailey *et al.* 2008). The main mechanisms of *Trichoderma* sp. as a biocontrol fungus against plant pathogens are (i) recognition and invasion through cell wall disruption and absorption of released nutrients, known as mycoparasitism (Bhat 2017), (ii) induced plant resistance to diseases by altering root architecture during interaction with pathogens (Kumar *et al.* 2019).

Trichoderma sp. has long been reported as a biocontrol fungus capable of suppressing a wide range of

Table 2: Percentage inhibition between OSMS fungal isolates against *F. oxysporum*

Treatment	Inhibition (%)
Control (*F. oxysporum*)	0.00 a
OSMS1 (*Gliocladium* sp.1)	47.32 bc
OSMS2 (*Trichoderma* sp.1)	56.45 bc
OSMS3 (*Gliocladium* sp.2)	45.75 b
OSMS4 (*Trichoderma* sp.2)	45.39 b
OSMS5 (*Trichoderma* sp.3)	46.62 bc
OSMS6 (*Trichoderma* sp.4)	46.69 bc
OSMS7 (*Trichoderma* sp.5)	56.72 bc
OSMS8 (*Trichoderma* sp.6)	57.53 c
OSMS9 (*Trichoderma* sp.7)	47.07 bc
OSMS10(*Trichoderma* sp.8)	47.57 bc

Remark: Standar Error ± 3.395; Identical letter notations show that the differences between the results are not significant based on the 5% DMRT test

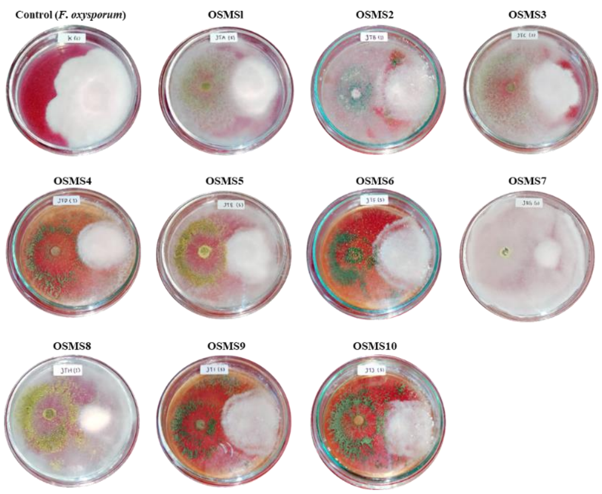

Fig. 2: Antagonism ability of OSMS isolates against *F. oxysporum*

plant diseases in agriculture. Biocontrol can be defined as the use of a living organism to suppress the development of pest populations in an environmentally friendly manner (Zin and Badaluddin 2020). Poudel *et al.* (2023) in their research reported that *T. harzianum* has a high antagonistic ability in killing *Alternaria brassicicola* by 70.35%; *Fusarium solani* by 70.82%; *Helminthosporium sorokinana* by 66.55%; *Rhizoctonia solani* by 78.58%; and *Sclerotium rolfsii* by 92.53%. Research by Khuong *et al.* (2023) also reported that 6 *Trichoderma* isolates were able to suppress wilt disease on sesame by 35%.

Gliocladium at the time of observation also had an antagonistic effect on *F. oxysporum*. According to Hadiwiyono *et al.* (2022) *Gliocladium* act as an antagonistic fungus that can suppress various diseases in plants because it has several antagonistic mechanisms. The production of metabolite compounds such as gliotoxin, pyridine, gliovirin, viridol, valinotrocin, viridin and heptelidic acid act as antifungal compounds that can suppress plant pathogens (Suryaminarsih *et al.* 2015; El-Saadony *et al.* 2022). *Gliocladium* was also reported to suppress several important diseases of ginger plants caused by *Fusarium oxyporum* f. sp. *zingiberi* and *Pythium*

Table 3: Percentage of chili seed germination due to inoculation of OSMS fungal isolates

Treatment	Germination (%)
Control (Without inoculation)	100.00 a
OSMS1 (*Gliocladium* sp.1)	91.67 a
OSMS2 (*Trichoderma* sp.1)	100.00 a
OSMS3 (*Gliocladium* sp.2)	91.67 a
OSMS4 (*Trichoderma* sp.2)	91.67 a
OSMS5 (*Trichoderma* sp.3)	100.00 a
OSMS6 (*Trichoderma* sp.4)	100.00 a
OSMS7 (*Trichoderma* sp.5)	100.00 a
OSMS8 (*Trichoderma* sp.6)	100.00 a
OSMS9 (*Trichoderma* sp.7)	100.00 a
OSMS10(*Trichoderma* sp.8)	100.00 a

Remark: Identical letter notations show that the differences between the results are not significant based on the 5% DMRT test

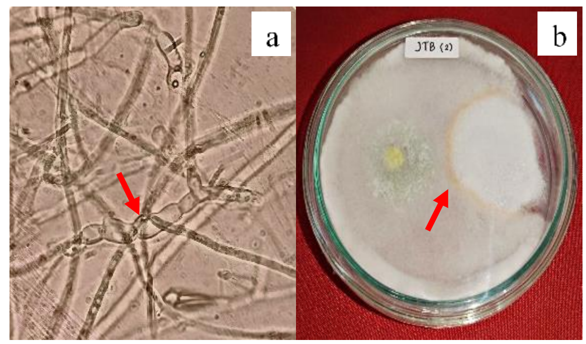

Fig. 3: Interaction of antagonistic fungi against *F. oxysporum*. (**a**) Swelling of the hyphae of *F. oxysporum,* (**b**) Melanization of the mycelium of *F. oxysporum* in the dual culture test

ultimum. Research by Afriani *et al.* (2019) showed that *Gliocladium virens* was able to suppress soybean stem base rot caused by *Sclerotium rolfsii* with up to 85% inhibition. Castillo *et al.* (2016) in their research reported that *Gliocladium* sp. was able to suppress *Sclerotium cepivorum* in shallot plants with the ability to inhibit 53.66% after 8 days of inoculation.

In seedling house trials, all isolates (*Trichoderma* and *Gliocladium*) of OSMS origin did not cause disease symptoms on chili plants during nursery. This indicates that the OSMS isolates tested have potential as biocontrol agents because they do not affect the growth of chili plants and are not phytopathogenic. Currently, research that reports the existence of *Trichoderma* and *Gliocladium* that act as phytopathogens in plants is still very limited and on the contrary, more research has discussed their role as biocontrol fungi for plant pathogens. Although it has been reported by Pfordt *et al*. (2023), if the species *Trichoderma afroharzianum* is able to act as a pathogen that causes rot disease of wheat and barley crops in Germany, France, and Italy, but the research is only the first report and the test is on a greenhouse scale.

Gliocladium and *Trichoderma* have been found to have both positive and negative effects on plant life. Positively, these fungi can increase plant resistance to a variety of plant diseases, boost plant growth, and improve nutrient usage efficiency, they can also function as biocontrol agents (Karadzhova and Georgieva 2023; Triasih and Widyaningsih 2023). Additionally, they can aid plants in overcoming abiotic stressors including heat, salt, cold, and drought (Zaid *et al.* 2022). However, there are also negative effects associated with these fungi. *Gliocladium* and *Trichoderma* strains that produce gliotoxin, a toxic fungal metabolite, can have negative effects on growth under certain laboratory conditions (Yao *et al.* 2023). The application of *Trichoderma* and *Gliocladium* fungi into the soil can help mitigate the negative effects. Overall, the effects of *Gliocladium* and *Trichoderma* on plant life depend on various factors such as strain, environmental conditions, and interactions with other microorganisms.

Although the results of this study show that OSMS isolates can suppress *F. oxyporum* on chili plants, there are research limitations that need to be followed up in further research. The limitation of this study is the limited data of test results related to identification to the species level based on molecular and genetic analysis of all isolates, and the type and amount of metabolite content that acts as an antagonistic compound on the pathogen has not been obtained. However, from the results of the existing literature review, research on the types of antagonistic fungal genus to those isolated from OSMS has never been reported so far, this is a challenge that we will complete in the next study.

Conclusion

Ten fungal isolates of the genus *Trichoderma* and *Gliocladium* isolated from Oyster Spent Media Susbstrate (OSMS) has a significant antagonistic ability in suppressing the growth of *F. oxysporum* of chili plants with the ability to inhibit 45.3–57.53%. Based on the results of seedling house trials, although there are 3 isolates that show the value of chili germination below the control treatment, it is not significantly different based on statistical tests, meaning that ten isolates are isolates that do not act as phytopathogens of chili plants. However, it should be noted that this study has limitations, such as the need for further molecular and genetic analysis for precise species identification and further research related to the content of metabolites that act as antagonistic compounds.

Acknowledgements

The first authors acknowledge the financial grant from Universitas Singaperbangsa Karawang Indonesia and Supported by Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) Unsika.

Author Contributions

SRA, N, and S planned the experiments; SRA, S, and LA, interpreted the results; SRA, N, S, and LA made the write up; WR and IW analyzed the data and made illustration.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

Funding Source

Hibah Prioritas Unsika (Hipka) 2023, Universitas Singaperbangsa Karawang

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