Antagonistic Test of Dark Septate Endophyte (DSE) Against *Colletotrichum gloeosporioides* on Chili Plantation (*Capsicum annuum*)

Krisna Suzana[1(✉)], Apriwi Zulfitri[2], Muhammad Ilyas[3], Titik Kartika[4], and Ikhsan Guswenrivo[4]

[1] Department of Biology, Padjadjaran University, Jl. Raya Bandung Sumedang No Km. 21, Hegarmanah, Jatinangor, Sumedang, West Java 45363, Indonesia
krisna18001@mail.unpad.ac.id

[2] Directorate of Repositories, Multimedia, and Scientific Publishing, National Research and Innovation Agency (BRIN), Jakarta, Indonesia

[3] Indonesian Culture Collection (InaCC), Research Center of Biosystematic and Evolution, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia

[4] Research Center of Applied Zoology, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor No Km. 46, Cibinong, Bogor, West Java 16911, Indonesia

Abstract. Dark Septate Endophyte (DSE) is an endophytic fungus with dark, septate hyphae and has the potential as a biocontrol agent in controlling pathogenic fungi in plants. The purpose of this study was to determine the antagonism of DSE fungi isolated from chili roots against *Colletotrichum gloeosporioides* fungi in vitro. This research is descriptive exploratory and experimental. Three isolates of DSE fungi isolated from chili roots were tested for antagonism against *C. gloeosporioides* in vitro using dual culture 1 and dual culture 2 tests. The dual culture 1 test was by growing the DSE fungi first before meeting *C. gloeosporioides* and the dual culture 2 test was by growing the DSE fungi and *C. gloeosporioides* directly on agar media. The percentage of inhibition was analyzed using the ANOVA test and Duncan's advanced test. The results showed the highest percentage of inhibition of *C. gloeosporioides* by isolate CA7 was 80.83% (dual culture 1) and CA1 isolate was 51.33% (dual culture 2). The results of the ANOVA analysis showed that there was an effect on the treatment performed ($p < 0.05$). Thus, DSE fungi isolated from the roots of chili has the potential to inhibit *C. gloeosporioides* fungi.

Keywords: Antagonistic · Anthracnose · Chili · *Colletotrichum gloeosporioides* · DSE

1 Introduction

Chili is one of the important agricultural commodities in Indonesia. Based on data from the Trade Assessment and Development Agency (BPPP) (2019), chili consumption in Indonesia from 2016 to 2019 tends to increase. Chili cultivation in Indonesia often

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results in a decrease in production, one of which is anthracnose. Anthracnose is the main disease in chili cultivation caused by the pathogenic fungi *Colletotrichum* sp. [1]. The anthracnose attack phase varies, starting from the vegetative phase (germination) and the generative phase (fertilization). Anthracnose disease can appear in the postharvest period due to the latent period of *Colletotrichum* sp. namely the time when the pathogen is already in the plant tissue and then develops and infects the plant [2].

Prevention and control of anthracnose in chili is currently generally using synthetic fungicides, the reason being that the results are visible faster. Excessive use and administration of synthetic fungicides both in terms of dosage and frequency can cause negative impacts on non-target organisms, human health and environmental pollution [3]. The use of biological agents that have active ingredients such as endophytic fungi is currently attracting attention because it can support environmentally friendly and sustainable agricultural activities [4]. He *et al*., (2019) [5] stated that endophytic fungi such as Dark Septate Endophyte (DSE) can be used as growth promoters or biological fertilizers in plant management, environmental conservation, and functional food production [6].

DSE fungi has dark mycelium on agar media, dark hyphae, septates and is able to colonize plant roots intercellularly and intracellularly without causing disease [7]. DSE fungi have been reported to be able to symbiotically with plants and are able to live in conditions exposed to biotic stresses (attacks from pests and diseases) and abiotic stresses (drought, high salinity, heavy metal pollution and etc.) so that plants are able to adapt and grow normally in these conditions [8].

Several species of DSE fungi have been known to suppress the growth of several pathogenic fungi such as *Rigidoporus microporus, Pyricularia oryzae*, and *Fusarium* sp. [9]. The mechanism of DSE fungi in fighting pathogenic fungi includes competition between space and nutrients, producing allelochemical compounds that can inhibit pathogens and induce systemic resistance in host plants. DSE fungi is expected to be an alternative solution as a biological agent in controlling *Colletotrichum* sp. cause of anthracnose disease in chili plants. The purpose of this study was to determine the antagonism of DSE fungi isolated from chili roots against *C. gloeosporioides* fungi in vitro.

2 Methods

2.1 Research Procedures

Media Preparation

The preparation of the media began by weighing 19.5 g of PDA powder dissolved in 500 ml of distilled water and homogenized until dissolved on a hotplate.

Cultivation of Isolates DSE and C. gloeosporioides fungi

Three isolates of DSE fungi with codes CA1, CA7, and CB isolated from chili roots were cultivated by planting mycelium on new media and incubated for 7–14 days in an incubator. Likewise, the isolates of C. gloeosporioides obtained from the Indonesian Culture Collection Microorganisms Storage Institute (Ina CC), the National Research

Fig. 1. Schematic of dual culture test of DSE fungi with *C. gloeosporioides*

and Innovation Agency (BRIN), Cibinong were cultivated on new media and incubated for 7 days.

Antagonistic Test (Dual Culture)

The dual culture test used in the antagonist test follows [10], namely the dual culture 1 test (DC 1) and the dual culture 2 test (DC 2). DC 1 test, DSE fungi were first grown on PDA media and incubated for 5 to 7 days. The pathogen *C. gloeosporioides* was grown on the other edge with a distance of 3 cm with the DSE fungi. All treatments were incubated for 14 days. DC 2 test, DSE fungi and pathogen *C. gloeosporioides* were grown at the same time with different edges (3 cm apart) and the treatments were incubated for 14 days. Observations were made by measuring the colony radius of *C. gloeosporioides* (cm size) every 2 days until 14 days. Control treatment was carried out by growing *C. gloeosporioides* on PDA media without DSE fungi (Fig. 1).

The calculation of the percentage of inhibition of DSE fungi against *C. gloeosporioides* follows the formula of Rahayu *et al.,* (2021) [10]:

$$\mathrm{P}(\%) = \frac{R1 - R2}{R2} \times 100\%$$

Note:
P(%) = Percentage of inhibition of mycelium growth
R1 = *C. gloeosporioides* colony radius away from DSE
R2 = *C. gloeosporioides* colony radius towards/near DSE
(DSE fungi which has an inhibitory power of $> 50\%$, has antagonistic properties).

2.2 Data Analysis

The data obtained were analysed using Analysis of variance (ANOVA) with a 95% confidence level using the Statistical Package for the Social Science (SPSS) software version 25 and if the results were significantly different, then tested with Duncan's Multiple Distance Test at a level of 5%.

3 Result and Discussion

The DSE fungi that has been isolated from chili roots contained three isolates obtained from 320 chili root segments. The three DSE isolates were coded CA1, CA7, and CB which can be seen in Fig. 2.

DSE fungi appear dark in color on agar media and tend to grow slowly (<5 mm per day), in accordance with Surono and Narisawa (2017) [7] that DSE fungi have dark pigmentation on media and colony growth tends to be slow. There are several factors that affect the biodiversity of DSE fungi such as plant age which affects the pattern of plant roots forming which can be in symbiosis with DSE fungi [11]. In addition, environmental factors such as soil fertility conditions, vegetation type, geographical conditions of the location also affect the diversity of DSE fungi that are symbiotic with certain host plants [12].

The characteristics of DSE fungi can be seen by macroscopic, microscopic, and molecular observations which can be seen in Table 1.

Based on Table 1, macroscopically the three DSE fungal isolates had a filamentous shape, cottony surface, greenish brown color on CA1 and CB isolates, and blackish brown color on CA7 isolates. Microscopic observation showed that the three DSE fungal isolates had dark, septate hyphae, and only CA7 isolates had pyriform conidia. The results of molecular analysis using ITS rDNA PCR, the three isolates of DSE fungi were homologous to the *Trichocladium pyriforme* species with a percentage of more than 90%. The characteristics of DSE fungi are dark hyphae, septate, have conidia or sterile. The three DSE isolates will be used in the antagonism test.

This antagonist test was carried out in vitro on agar media using 2 tests following Rahayu *et al.,* (2021) [10], namely the dual culture 1 (DC 1) test and the dual culture 2 test (DC 2). The principle of DC 1 test, DSE fungi were grown for 7 days on agar media and then *C. gloeosporioides* was inoculated on the other edge. While the principle

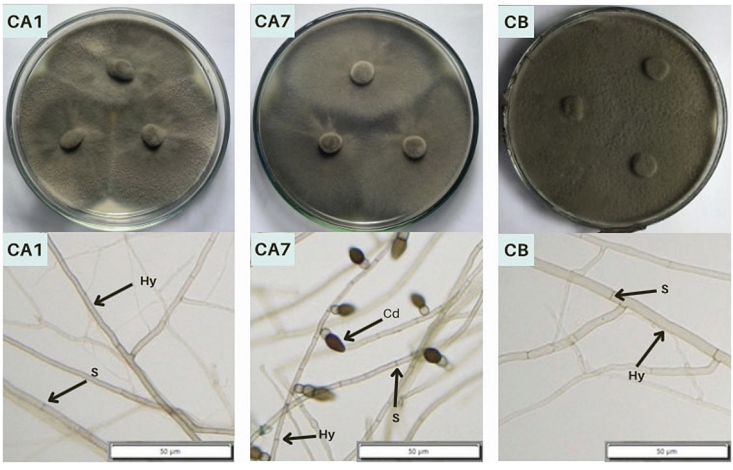

Fig. 2. Macroscopic (top) and microscopic at scale: 50 m (bottom) morphology of DSE fungi from chili root (*C. annuum*) on PDA media. Note: Hy, hyalin; S, Septa; Cd, Conidia.

Table 1. Characteristics of DSE fungi isolated from chili roots (*C. annuum*)

Identification		Isolate code		
		CA1	CA7	CB
Macroscopic	Shape	Filamentous	Filamentous	Filamentous
	Surface	Cottony	Cottony	Cottony
	Color	Brown greenish	Brown blackish	Brown greenish
Microscopic	Hyphae	Dark and septate	Dark and septate	Dark and septate
	Conidia form	–	Pyriform	–
Molecular		*Trichocladium pyriforme*	*Trichocladium pyriforme*	*Trichocladium pyriforme*

of the DC 2 test, DSE and *C. gloeosporioides* were inoculated together on PDA media with different edges. Each treatment was given a distance of 3 cm. The results of the antagonism test can be seen in Fig. 3.

Treatment using the dual culture test (Fig. 3) revealed that the fungal antagonist mechanism of DSE exhibits mycelium physical contact. In the DC I test, the growth of *C. gloeosporioides* was inhibited by the DSE fungi and did not go beyond the direction of the DSE fungi growth, while in the DC II test both *C. gloeosporioides* and DSE fungi both maintained space for mutual growth and the growth of *C. gloeosporioides* did not go beyond the DSE fungi growing direction. The mechanism of DSE fungi antagonist against *C. gloeosporioides* can be assumed as a mechanical mechanism due to the competition for space and nutrients that occurs between the two fungi. According to Atugala and Deshappriya (2015) [13], one of the pathogenic mechanisms by biocontrol

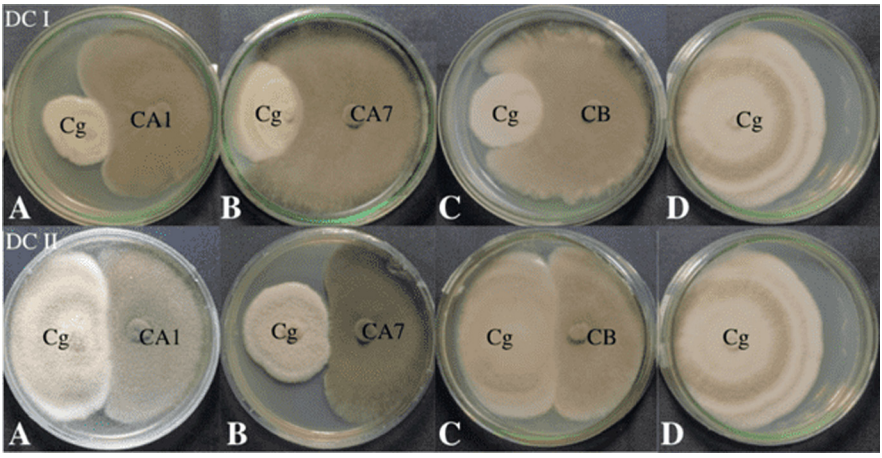

Fig. 3. Interaction between *C. gloeosporioides* and DSE fungi after 14 days incubation on PDA media. Note: DC 1, Dual Culture 1; DC 1, Dual Culture 2; A) DSE CA1; B) DSE CA7; C) DSE CB; D) Control; Cg, *C. gloeosporioides.*

agents is the ability to compete for space and nutrients. Endophytic fungi are known to tend to slow down the growth of pathogens compared to controls, besides competing for space and nutrients is possible by producing antifungal substances.

The calculation of the percentage of inhibition of *C. gloeosporioides* by DSE fungi was statistically analyzed using Analysis of Variance (ANOVA). The results of ANOVA on the DC I and DC II tests showed that DSE fungal isolates had a significantly different effect on the percentage of inhibition of *C. gloeosporioides.* This means that the DSE fungal isolate affected the percentage of inhibition of *C. gloeosporioides.* Based on these results, to determine the extent of the influence of DSE fungal isolates on the percentage of inhibition of *C. gloeosporioides*, Duncan's Multiple Range Test was continued with the 5% level which is presented in Table 2.

Based on Table 2, the DC I and DC II tests showed a significant effect between DSE fungi treatment and control on the percentage of inhibition of *C. gloeosporioides.* The treatment of different DSE fungi did not significantly affect the inhibition of *C. gloeosporioides* in both the DC 1 and DC 2 tests, so that the administration of different DSE fungi did not significantly affect the percentage of inhibition of *C. gloeosporioides* in vitro.

Based on the DC 1 test, the three DSE isolates had antagonistic properties with the percentage of inhibition >50% and the DSE CA7 isolate had the highest percentage of inhibition. Based on the DC 2 test, only CA1 isolates had antagonistic properties with a percentage of inhibition >50% while isolates CA7 and CB had a negative percentage of inhibition (less able to inhibit) because the percentage of inhibition was <50%. According to Rahayu *et al.,* (2021) [10], DSE fungi which have inhibition percentage >50% have antagonistic properties.

The percentage of inhibition of DSE fungi against *C. gloeosporioides* was better in the DC 1 test. All DSE isolates used were able to inhibit the growth of *C. gloeosporioides* on agar media. This inhibition could occur because DSE fungal colonies were already formed when encountered with newly growing *C. gloeosporioides* colonies. This condition allows the DSE fungi to develop another mechanism to deal with *C. gloeosporioides*, in this case a mechanical barrier is formed and slows the development

Table 2. Duncan's Multiple Distance Test Results 5% Level on the Percentage of Growth Inhibition of *C. gloeosporioides* by DSE Fungi

Treatment	Inhibition percentage (%)	
	Dual Culture I	*Dual Culture* II
CA1	62,00^{b}	51,33^{c}
CA7	80,83^{c}	37,67^{b}
CB	75,33^{cb}	46,00^{cb}
Control	0,00^{a}	0,00^{a}

Note: Different Lowercase Letters Read in the Vertical Direction Show Differences in Statistical Significance according to Duncan's Multiple Distance Test ($p < 0.05$). A) DSE CA1; B) DSE CA7; C) DSE CB; D) Control.

of the pathogenic fungi then followed by hyphal connection between the two fungi or replacement of mycelium [14].

In the DC 2 test, the DSE fungi grew slower than *C. gloeosporioides*, so that the DSE fungi occupied a smaller space than *C. gloeosporioides*. All DSE fungi colonies generally started growing after three days of incubation and it took 25 to 30 days for the colonies to fill the petri dish (Ø = 9 cm). DSE fungi grows slowly (<5 mm per day), and its colonies begin to develop from 7 to 14 days after inoculation [7] while *C. gloeosporioides* grows relatively faster than DSE fungi which grows about 12 mm per day. And it takes 12 to 14 days for the colony to fill the petri dish (Ø = 9 cm) [1].

Based on the two dual culture tests used, the DC 1 test gave the best results, where each DSE fungi was grown before meeting the pathogen. According to Rahayu *et al.,* (2021) [10] in the application the use of DSE fungi which have antagonistic properties against pathogenic fungi can be applied by growing DSE fungi first on the planting medium before meeting the pathogens. This was due to the slow growth rate of DSE fungi, so that DSE fungi could first form an antagonist mechanism to fight pathogenic fungi. Therefore, to maximize the use of the three DSE fungi by growing them first as in the planting medium.

4 Conclusion

The results showed that the highest percentage of inhibition of DSE fungi isolated from chili roots against *C. gloeosporioides* by CA7 isolate was 80.83% in the dual culture 1 test and CA1 isolate was 51.33% in the dual culture 2 test. The potential of DSE fungi as a biocontrol agent for *C. gloeosporioides* can be applied by seed coating method on chili seeds or by growing DSE fungi on growing media. In addition, to identify the type of antifungal produced by the DSE fungi can use tools such as Pyrolysis-Gas Chromatography-Mass Spectrophotometry (Py-GCMS).

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