



Antifungal Activity of *Gliocladium viride* against *Fusarium oxysporum*

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ABSTRACT

Fusarium oxysporum is a cosmopolitan fungal species that can cause wilt disease in various plants. This study aimed to determine the antifungal activity of *Gliocladium viride* against *F. oxysporum* and identify the antifungal compound produced by *G. viride*. An antagonistic test of *G. viride* against *F. oxysporum* was carried out in dual culture. The antifungal activity of *G. viride* extract on the growth of *F. oxysporum* was determined using the paper disc diffusion method. The results showed that *G. viride* inhibited the growth of *F. oxysporum* colonies, with inhibition percentages ranging from 92.93% to 93.92%. The extract has antifungal activity, with the diameter of the inhibition zone formed being categorized as strong inhibition, which ranges from 19.3 mm to 24.5 mm. *G. viride* extract contains eleven compounds that have antifungal activity, namely cyclopropanecarboxylic acid; 2-nonanone; 2,3-butanediol; 2-heptanone; acetoin; 2,3-dimethylpyrazine; carbamic acid, phenyl esters; pyridine, 2,3,4,5-tetrahydro; 2-furancarboxaldehyde, 5-methyl; caryophyllene; and 1,2, benzenedicarboxylic acid. The results of this study provide information that *G. viride* can be used as a biological agent to control wilt in banana, vanilla, tomato and chili plants.

Keywords: *Gliocladium viride*, antifungal activity, *Fusarium oxysporum*

INTRODUCTION

Fusarium oxysporum fungus is a soil-borne pathogen that has more than 100 formae speciales (f.sp.) that have been known to cause disease in different hosts (Baayen *et al.* 2000). Some of the formae speciales of *F. oxysporum* are *F. oxysporum* f.sp. *cubense*, *F. oxysporum* f.sp. *vanillae*, *F. oxysporum* f.sp. *lycopersici*, and *F. oxysporum* f.sp. *capsici*. The fungus *F. oxysporum* f.sp. *cubense* is a pathogen in banana plants. Warman and Aitken (2018) reported that the cause of wilt disease in banana plants is *F. oxysporum* f.sp. *cubense*. The fungus *F. oxysporum* f.sp. *vanilla* is a pathogen in vanilla plants. Mosqueda *et al.* (2019) reported that the cause of wilt disease in *Vanilla planifolia* plants is *F. oxysporum* f.sp. *vanillae* which can cause stem and root rot. The fungus *F. oxysporum* f.sp. *lycopersici* is a pathogen in tomato plants. Srinivas *et al.* (2019) reported that the cause of wilt disease in tomato plants (*Lycopersicon esculentum* Mill.) is *F. oxysporum* f.sp. *lycopersici*. Meanwhile, the fungus *F. oxysporum* f.sp. *capsici* is a pathogen in chili plants. Gabrekiristos and Demiyo (2020) reported that the cause of wilt disease in *Capsicum annum* L plants. is the fungus *F. oxysporum* f.sp. *capsici*.

Currently, fusarium wilt disease control still uses synthetic fungicides. Song *et al.* (2003) reported that fungicides with active ingredients prochloraz, carbendazim, thiram, toclofos methyl, hymexazol, azoxystrobin, and carboxin are effective in controlling fusarium wilt disease. Nevertheless, the continuous use of synthetic fungicides can affect soil health. According to Roman *et al.* (2021), high doses of fungicides can affect the community of microorganisms in the soil, causing a decrease in the population of soil microorganisms. One of the efforts to reduce synthetic fungicides is to use the biological agent *Gliocladium viride*. One of the mechanisms of *G. viride* fungi in controlling pathogenic fungal populations is by producing antifungal compounds. As is the case with the fungus *Trichoderma asperellum* reported by Srinivasa *et al.* (2017), it can produce antifungal compounds viridin, viridiol, ferulic acid, gliovirin which have been proven to inhibit the growth of *Sclerotium rolfsii* fungus with an inhibitory percentage of 80.04%. Meanwhile, *T. harzianum* was reported by Siddiquee *et al.* (2012) can produce antifungal compounds 2,3-butanediol, 3-methyl-2,5-furandione, phenylethyl alcohol, carbamic acid, phenyl ester, and decahydro-1,6-dimethyl-naphthalene. The genus *Gliocladium* has been known as one of the fungi antagonists to *F. oxysporum*, but the antifungal compounds produced are not yet known. This study aims to test the antifungal activity of *G. viride* against the fungus *Fusarium oxysporum* f.sp. *cubense*, *F. oxysporum* f.sp. *vanillae*, *F. oxysporum* f.sp. *lycopersici*, *F. oxysporum* f.sp. *capsici* and identify antifungal compounds produced by the *G. viride*.

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METHODS

Materials and Equipment

This research was carried out at the Plant Disease Science Laboratory, Faculty of Agriculture, Udayana University, and the Bali Police Forensic Laboratory from April to June 2023. The materials used were the fungus *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici*, biological agent *G. viride*, and potato dextrose agar (PDA) media. The instruments included test tubes, micropipettes, digital scales, Ose needles, microscopes, laminar flow cabinets, and gas chromatography-mass spectrometry (GCMS).

In vitro Antagonistic Test of *G. viride* against *F. oxysporum* Fungus

The antagonistic test of *G. viride* against the fungi *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici* *in vitro* was carried out based on Khalimi and Wirya (2009) procedure. The test of *G. viride* antifungal activity against the growth of fungi *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici* began by preparing the growth medium by pouring 10 mL of PDA media on a petri dish. Furthermore, the mushrooms *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici* were inoculated in the center of a petri dish that already contained PDA media, then *G. viride* was inoculated in 4 positions, flanking the fungus *Fusarium* spp. with 2 cm distance. The design used in this study was a Complete Randomized Design (RAL) with 8 treatments and 4 replicates.

The determination of the percentage of inhibition of biological bacteria to fungal growth was determined by the following formula:

$$\text{Inhibition} = \frac{\text{Control colony area} - \text{Treatment colony area}}{\text{Control colony area}} \times 100\%$$

The growth rate of a fungus colony was determined using the following formula:

$$\text{Colony growth rate} = \frac{\text{Control colony area at last observation}}{\text{Time lapse}}$$

Antifungal Activity Test of *G. viride* Extract against *F. oxysporum* *in vitro*

G. viride extract was produced from dual cultures of *G. viride* and *F. oxysporum*. *F. oxysporum* colonies were removed first, followed by *G. viride* cultures dissolved in methanol at a ratio of 10 *G. viride* culture cultures per 100 mL of methanol in petri dishes. Furthermore, it was macerated for 48 hours before being extracted with a vacuum rotary evaporator (Model HEA-02). The disc paper diffusion method was used to test the antifungal activity of *G. viride* extract against fungus *F. oxysporum* *ssp.* *cubense*, *F.*

oxysporum *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, and *F. oxysporum* *ssp.* *capsici*. The test started with preparing the growth medium by putting 1 mL of each *F. oxysporum* suspension into a petri dish. Next, 10 mL of PDA medium was added to a petri dish, then inoculated the solidified medium with a 6 mm diameter disc paper that had previously been soaked in *G. viride* extract. Inhibition zones were defined as areas that did not appear to be overgrown with fungus. Tendencia (2004) classified inhibition zones in disk diffusion tests into three categories: (1) weak inhibition, less than 14 mm, (2) medium inhibition, 15–18 mm, and (3) strong inhibition > 18 mm.

Identification of Antifungal Compounds from *G. viride* Extract

Antifungal compounds of *G. viride* extract were evaluated by GCMS (7890A GC-system 5975C inert XL E1/C1 MSD model G3174A, Agilent Technologies, Wilmington, DE, USA) using Ahamed and Ahring's (2011) approach. A sample of 2 μ L extract was put into the GCMS. The injector temperature was held at 240°C for 26 min. Antifungal compounds were identified using a comparison of the 2023 NIST database library, and the chemical names used in this research were consistent with the NIST database nomenclature.

RESULTS AND DISCUSSION

In vitro Antagonistic Test of *G. viride* against *F. oxysporum*

The results of the *G. viride* antagonistic test against *F. oxysporum* *in vitro* showed that *G. viride* inhibited the growth of fungal colonies of *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici*, with the % inhibition ranging from 92.93% to 93.92% (Table 1).

The fungal colonies of *F. oxysporum* *ssp.* *cubense*, *F. oxysporum* *ssp.* *vanillae*, *F. oxysporum* *ssp.* *lycopersici*, *F. oxysporum* *ssp.* *capsici* in the control treatment grew normally, with the colony growth rate ranging from 802.62 mm²/day to 890.20 mm²/day (Figure 1). Meanwhile, fungal colonies in *G. viride* treatment were inhibited in growth. It can be seen in the fungus colony area's low value, which manifests fungal growth. The lower the value of the area of the fungal colony, the higher the value of the inhibition of biological bacteria to the fungus. Fungal growth in *G. viride* treatment was inhibited due to the presence of antifungal compounds produced by *G. viride* through the antibiotic mechanism. The growth of fungi in *G. viride* treatment was inhibited, with colony growth rates ranging from 50.86 mm²/day to 59.69 mm²/day. Demirci *et al.* (2011) showed that *G. viride* ME-7 can inhibit the growth of *Rhizoctonia solani* fungus with an inhibitory percentage of 35%. Meanwhile, Agustina *et al.* (2019) reported that *Gliocladium* sp. inhibits the

Table 1 Results of the *G. viride* antagonistic test against fungi *F. oxysporum* *fsp. cubense*, *F. oxysporum* *fsp. vanillae*, *F. oxysporum* *fsp. lycopersici*, *F. oxysporum* *fsp. capsici* *secara in vitro*

Treatment	Area of fungal colonies (mm ²)	Colony growth rate (mm ² /day)	Inhibition (%)
<i>F. oxysporum</i> <i>fsp. cubense</i>	4013.11 a*	802.62 a	-
<i>G. viride</i> + <i>F. oxysporum</i> <i>fsp. cubense</i>	283.38 b	56.67 b	92.93
<i>F. oxysporum</i> <i>fsp. vanillae</i>	4183.26 a	836.65 a	-
<i>G. viride</i> + <i>F. oxysporum</i> <i>fsp. vanillae</i>	254.34 b	50.86 b	93.92
<i>F. oxysporum</i> <i>fsp. lycopersici</i>	4415.62 a	883.12 a	-
<i>G. viride</i> + <i>F. oxysporum</i> <i>fsp. lycopersici</i>	268.66 b	53.73 b	93.91
<i>F. oxysporum</i> <i>fsp. capsici</i>	4451.02 a	890.20 a	-
<i>G. viride</i> + <i>F. oxysporum</i> <i>fsp. capsici</i>	298.49 b	59.69 b	93.29

Remarks: *Values in the same column followed by the same letter do not differ significantly ($p > 0.05$) according to the Duncan Multiple Distance Test at the 5% level.



Figure 1 Results of *G. viride* antagonistic test against *Fusarium* spp. fungus *in vitro*. A: Control *F. oxysporum* *fsp. vanillae*, B: *G. viride* + *F. oxysporum* *fsp. cubense*, C: *G. viride* + *F. oxysporum* *fsp. vanillae*, D: *G. viride* + *F. oxysporum* *fsp. lycopersici*, E: *G. viride* + *F. oxysporum* *fsp. capsici*.

growth of *Botryodiplodia theobromae* fungus with an inhibition percentage of 84.56% in *in vitro* testing.

Antifungal Activity Test of *G. viride* Extract against *F. oxysporum*

The results of the antifungal activity test of *G. viride* extract against the fungus *F. oxysporum* *in vitro* showed that the extract had antifungal activity against the fungus *F. oxysporum* *fsp. cubense*, *F. oxysporum* *fsp. vanillae*, *F. oxysporum* *fsp. lycopersici*, *F. oxysporum* *fsp. capsici*, with the inhibition zone's diameter, is categorized as strong resistance and ranges from 19.3 mm to 24.5 mm. The extract was able to inhibit the fungus *F. oxysporum* *fsp. cubense* with an inhibitory zone diameter of 19.3 mm, while the diameter of the inhibitory zone on *F. oxysporum* *fsp. capsici*, *F. oxysporum* *fsp. lycopersici*, and *F. oxysporum* *fsp. vanillae* were 19.8 mm, 20.2 mm, and 24.5 mm, respectively. The extract has antifungal activity, indicated by forming an inhibition zone around the disc paper (Figure 2). The inhibition zone is caused by the presence of antifungal compounds produced by the fungus *G. viride*. According to Ahamed and Ahring (2011), the identification of chemical compounds in *Gliocladium* sp. 62724 extract using GC-MS-SPME shows that the extract contains benzene, heptane, 1-octene, octane, *m*-xylene, 3-methylnonane, dodecane, tridecane, hexadecane, and nonadecane.

Identification of Antifungal Compounds from *G. viride* Extract

Based on the results of the GCMS analysis, *G. viride* extract contained 19 compounds. They are 2-hexanone; cyclopentanol, 3-methyl; propanal, 2,3-dihydroxy; propanoic acid, 2-hydroxy ethyl ester; propanoic acid, 2-hydroxy ethyl ester; 4-nitro-3-oxobutyric acid, methyl ester; benzene, 1,4-dimethyl; cyclopropanecarboxylic acid; cyclopropanecarboxylic acid; 2-nonanone; 2,3-butanediol; 2-heptanone; acetoin; 2,3-dimethylpyrazine; carbamic acid, phenyl ester; pyridine, 2,3,4,5-tetrahydro; pyridine, 2,3,4,5-tetrahydro; 2-furancarboxaldehyde, 5-methyl; caryophyllene; dan 1,2, benzenedicarboxylic acid (Table 2).

According to GCMS analysis, *G. viride* extract contains 11 compounds, including cyclopropanecarboxylic acid, 2-nonanone, 2,3-butanediol, 2-heptanone, acetoin, 2,3-dimethylpyrazine, carbamic acid, phenyl ester, pyridine, 2,3,4,5-tetrahydro, 2-furancarboxaldehyde, 5-methyl, caryophyllene, and 1,2-benzenedicarboxylic acid. Cyclopropanecarboxylic acid molecules were found at peaks 7 and 8, with retention time of 3.361 min and 3.552 min, respectively, and area of 5.98% and 5.69%. The 2-nonanone was discovered at peak 9, with a retention time of 3.596 min and an area of 11.16%. The molecule 2,3-butanediol was identified at peak 10

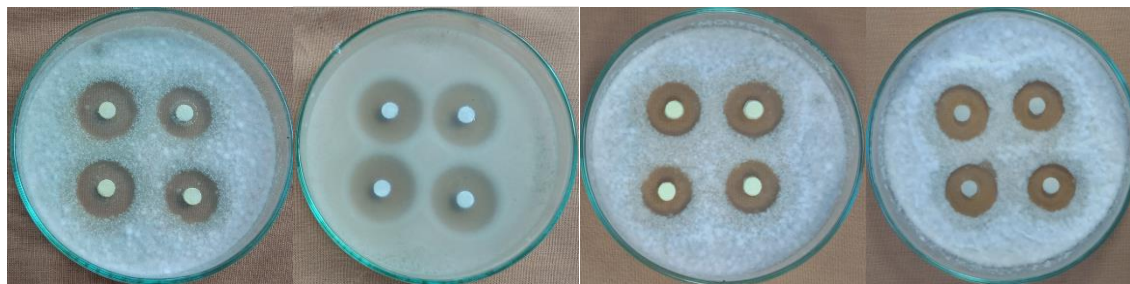


Figure 2 Results of antifungal activity test of *G. viride* extract against *F. oxysporum*. A: *F. oxysporum* fsp. *ubense*, B: *F. oxysporum* fsp. *vanilae*, C: *F. oxysporum* fsp. *lycopersici*, D: *F. oxysporum* fsp. *capsici*.

Table 2 Results of GC-MS analysis of chemical compounds in *G. viride* extract

Peak	Retention time	Area (%)	Chemical compounds identified	Chemical formula	Chemical structure*
1	2.122	1.18	2-hexanone	C ₆ H ₁₂ O	
2	2.168	1.46	3-methyl cyclopentanol	C ₆ H ₁₂ O	
3	2.189	1.64	2,3-dihydroxy propanal	C ₃ H ₆ O ₃	
4	2.585	1.07	propanoic acid, 2-hydroxy ethyl ester 2-ethylhexyl (2S)-2-hydroxypropanoate	C ₅ H ₁₀ O ₃	
5	2.651	1.11	4-nitro-3-oxobutyric acid, methyl ester	C ₁₁ H ₁₁ NO ₅	
6	3.313	2.98	1,4-dimethyl benzene	C ₈ H ₁₀	
7	3.361	5.98	cyclopropanecarboxylic acid	C ₄ H ₆ O ₂	

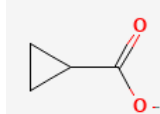
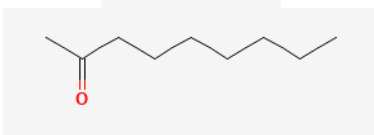
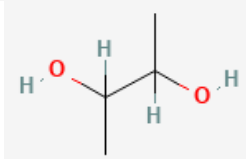
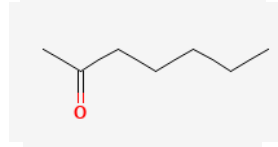
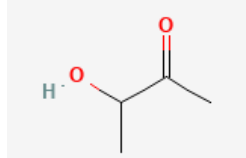
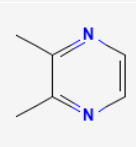
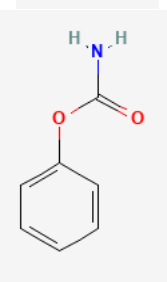
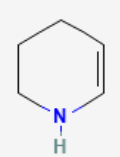
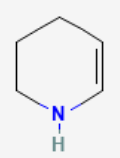
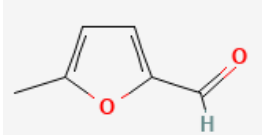
with a retention time of 3.842 min and an area of 2.30%.

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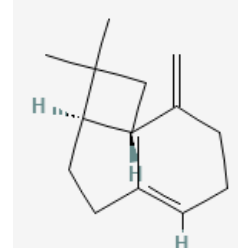
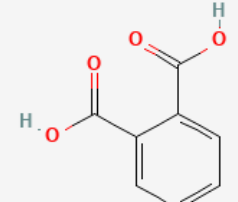
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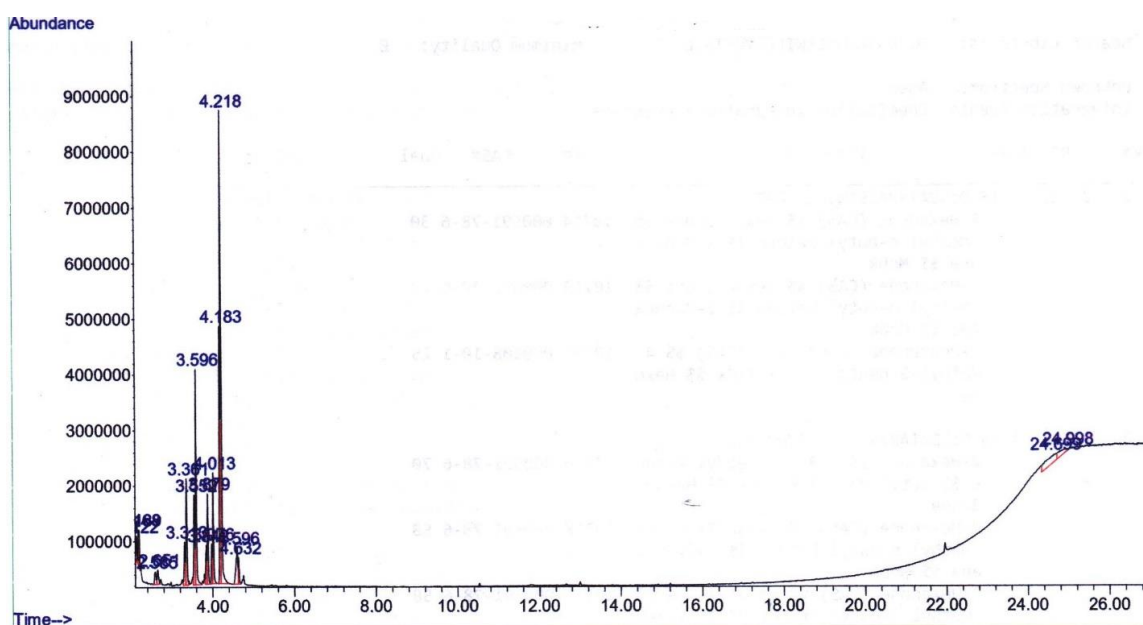
Table 2 Results of GC-MS analysis of chemical compounds in *G. viride extract* (advanced)

Peak	Retention time	Area (%)	Chemical compounds identified	Chemical formula	Chemical structure*
8	3.552	5.69	cyclopropanecarboxylic acid	C ₄ H ₆ O ₂	
9	3.596	11.16	2-nonanone	C ₉ H ₁₈ O	
10	3.842	2.30	2,3-butanediol	C ₄ H ₁₀ O ₂	
11	3.879	4.70	2-heptanone	C ₇ H ₁₄ O	
12	3.976	2.16	acetoin	C ₄ H ₈ O ₂	
13	4.013	5.95	2,3-dimethylpyrazine	C ₆ H ₈ N ₂	
14	4.183	13.75	carbamic acid, phenyl ester	C ₇ H ₇ NO ₂	
15	4.218	26.35	2,3,4,5-tetrahydro pyridine 1,2,3,4-tetrahydro pyridine	C ₅ H ₉ N	
16	4.596	3.64	2,3,4,5-tetrahydro pyridine	C ₅ H ₉ N	
17	4.632	1.45	5-methyl 2-furancarboxaldehyde	C ₆ H ₆ O ₂	

The 2-heptanone was discovered at peak 11 with a

Table 2 Results of GC-MS analysis of chemical compounds in *G. viride* extract (advanced)

Peak	Retention time	Area (%)	Chemical compounds identified	Chemical formula	Chemical structure*
18	24.699	5.24	caryophyllene	C ₁₅ H ₂₄	
19	24.998	2.19	1,2-benzenedicarboxylic acid	C ₈ H ₆ O ₄	

Figure 3 GC-MS chromatographic representative data from *G. viride* extract.

with a retention time of 3.842 min and an area of 2.30%.

The 2-heptanone was discovered at peak 11 with a retention time of 3.879 min and an area of 4.70%. Acetoin was found at peak 12 at 3.976 min, having an area proportion of 2.16%. The 2,3-dimethylpyrazine was identified at peak 13 with a retention time of 4.013 min and an area of 5.95%. The carbamic acid compound phenyl ester was discovered at peak 14 with a retention time of 4.183 min and an area of 13.75%. The pyridine 2,3,4,5-tetrahydro was discovered at peaks 15 and 16 with retention time of 4.218 and 4.596 min, respectively, and area of 26.35% and 3.64%. The 2-furancarboxaldehyde was identified at peak 17 with

a retention time of 4.632 min and an area of 1.45%. The 5-methyl-caryophyllene was discovered at peak 18 with a retention time of 24.699 min and an area of 5.24%. Compound 1,2-benzenedicarboxylic acid was discovered at peak 19 with a retention time of 24.998 min and an area of 2.19% (Figure 3).

Several compounds, including cyclopropanecarboxylic acid, 2-nonanone, 2,3-butanediol, 2-heptanone, acetoin, 2,3-dimethylpyrazine, carbamic acid, phenyl ester, pyridine, 2,3,4,5-tetrahydro, 2-furancarboxaldehyde, 5-methyl caryophyllene, and 1,2-benzenedicarboxylic acid, have been shown to have antifungal properties. Guaranda *et al.* (2023) discovered that *Trichoderma reesei* extract (C2A) contains cyclopropanecarboxylic

acid with antifungal activity against *Moniliophthora perniciosa* and *M. roleri*. According to Calvo et al. (2020), *Bacillus velezensis* strains BUZ-14 extract contains 2-nonanone, 2,3-butanediol, 2-heptanone, acetoin, and 2,3-dimethylpyrazine, which have antifungal activity against *Botrytis cinerea*, *Monilinia fructicola*, *M. laxa*, *Penicillium italicum*, *P. digitatum*, and *P. expansum*. According to Yaseri et al. (2016), *Proteus mirabilis* extract contains 2-furancarboxaldehyde, which has antifungal action against *Aspergillus terreus*, *A. flavus*, *Candida albicans*, *Microsporum canis*, and *Trichophyton mentagrophytes*. Tabarestani et al. (2016) found that *Trichoderma virens* extract includes caryophyllene that show antifungal action against *F. oxysporum*. Siddiquee et al. (2012) discovered that *T. harzianum* strain FA1132 extract contains 2,3-butanediol, carbamic acid phenyl ester, and 1,2-benzenedicarboxylic acid, which have antifungal action against *Fusarium graminearum*, *Rhizoctonia solani*, and *F. oxysporum* f.sp. lycopersici. Dai et al. (2011) discovered that 6-alkyl-2,3,4,5-tetrahydropyridine exhibits antifungal properties against *Cryptococcus neoformans*, *Candida albicans*, *C. glabrata*, and *C. krusei*. According to Li et al. (2021), *Streptomyces* sp. H4 includes 1,2-benzenedicarboxylic acid, which shows antifungal activity against *Colletotrichum fragariae*.

G. viride produces eleven antifungal chemicals that work together to suppress the growth of harmful fungus. Compounds in the group of fatty acids such as cyclopropanecarboxylic acid, carbamic acid phenyl ester, and 1,2-benzenedicarboxylic acid work simultaneously in inhibiting fungal growth by interfering with cell membrane permeability, especially in cells with low sterol content, inhibiting the action of the topoisomerase enzyme, which plays an important role in the DNA replication process in the nucleus and the translation process in ribosomes, inhibiting the formation of N-myristoyltransferase, which plays an important role in the translation process in ribosomes, inhibits β -oxidation in mitochondria, inhibits the formation of triacylglycerol and sphingolipids in mitochondria, causing mitochondrial damage (Pohl et al. 2011).

The mechanism of 2-nonanone, 2,3-butanediol, 2-heptanone, acetoin, 2,3-dimethylpyrazine, pyridine 2,3,4,5-tetrahydro, 2-furancarboxaldehyde 5-methyl, and caryophyllene in inhibiting fungal growth is thought to be by inducing apoptosis, causing DNA and mitochondrial damage (Prasath et al. 2020), inhibiting the action of nitric oxide dioxygenase (Helmick et al. 2005), inhibits ergosterol biosynthesis (Hori et al. 2000), inhibits the formation of fungal cell membranes by methylating sterols and sterol esters (Ozdemir et al. 2010), inhibiting the formation of fungal cell walls by inhibiting the synthesis of β -glucan and chitin (Chudzik et al. 2019), and causes cell death by inhibiting the

action of enzymes that play a role in DNA and RNA synthesis in fungal cells (Pippi et al. 2017).

CONCLUSION

The results of this investigation show that *G. viride* fungus can limit the growth of *F. oxysporum* fungal colonies with inhibition ranging from 92.93% to 93.92%. *G. viride* extract possesses antifungal activity, with an inhibition zone diameter ranging from 19.3 mm to 24.5 mm, indicating significant inhibition. GC-MS analysis identified 11 antifungal compounds in the extract, including cyclopropanecarboxylic acid, 2-nonanone, 2,3-butanediol, 2-heptanone, acetoin, 2,3-dimethylpyrazine, carbamic acid, phenyl ester, pyridine, 2,3,4,5-tetrahydro, 2-furancarboxaldehyde, 5-methyl, caryophyllene, and 1,2-benzenedicarboxylic acid.

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