

Effectiveness of the Combination of *Gliocladium* Spp. and Betel Leaf (*Piper Betle* L.) In Inhibiting *Fusarium Oxysporum* F. Sp. *Melonis* in Vitro

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ABSTRACT: *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *melonis* represents a major constraint in melon (*Cucumis melo* L.) production worldwide, causing substantial economic losses. The intensive use of chemical fungicides for disease management has raised serious environmental and health concerns, necessitating the development of sustainable alternative control strategies. This study aimed to evaluate the in vitro effectiveness of combining *Gliocladium* spp. as biological control agents with betel leaf (*Piper betle* L.) powder as a botanical fungicide in inhibiting *F. oxysporum* f.sp. *melonis*. The research was conducted using a completely randomized design with two factorial treatments: three *Gliocladium* species (*G. aureum*, *G. roseum*, and *G. viride*) and four betel leaf powder concentrations (0, 25, 50, and 75 g/L). Results demonstrated that betel leaf powder at concentrations of 50-75 g/L significantly inhibited *F. oxysporum* growth, reducing colony diameter compared to the control. **Interaction between *Gliocladium* species and betel leaf powder dosage significantly influenced *F. oxysporum* f.sp. *melonis* suppression with a strong species-dependent interaction, in which *G. roseum* delivered the most effective.** *G. roseum* exhibited the highest antagonistic activity, achieving inhibition percentages exceeding 91% across all betel leaf powder concentrations, with the maximum inhibition of 94.17% observed at 75 g/L. These findings indicate that the integrated application of *Gliocladium* spp., particularly *G. roseum*, with betel leaf powder represents a promising environmentally sustainable strategy for managing *Fusarium* wilt in melon cultivation, offering an effective alternative to conventional chemical fungicides.

KEYWORDS: *Gliocladium* spp., *Piper betle* L., biological control, *Fusarium oxysporum* f. sp. *melonis*, botanical fungicide

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* is one of the major constraints in melon (*Cucumis melo* L.) production. This pathogen infects the vascular tissues of the plant, disrupting water and nutrient transport, which ultimately leads to irreversible wilting symptoms and plant death if not effectively controlled (Chai et al., 2025). *Fusarium* wilt has been reported to cause substantial yield losses in major melon-producing regions, both under open-field cultivation systems and greenhouse conditions (Sarica & Karaca, 2024).

To date, the management of *Fusarium* wilt has been predominantly reliant on the application of chemical fungicides. However, the intensive and prolonged use of synthetic fungicides poses serious risks to the environment, non-target organisms, and human health (Burandt et al., 2023). Consequently, the development of alternative disease management strategies that are safer, environmentally friendly, and sustainable has become increasingly important, particularly through the application of biological control agents and plant-based natural products (He et al., 2021). Among biological control agents, *Gliocladium* spp. have been widely reported to suppress the growth of *F. oxysporum* through multiple antagonistic mechanisms, including competition for space and nutrients, production of antifungal metabolites, and mycoparasitic activity (Khalimi & Leana, 2025). In addition, betel leaf (*Piper betle* L.), a traditional medicinal plant, is known to be rich in bioactive compounds such as hydroxychavicol, eugenol, and flavonoids, which exhibit strong antifungal properties and therefore hold promise as sources of environmentally friendly botanical fungicides (Nayaka et al., 2021).

Considering the antagonistic mechanisms exhibited by *Gliocladium* spp. and the antifungal properties of betel leaf, the combination of these two agents is expected to generate synergistic effects and provide more effective inhibition of *F. oxysporum* f.sp. *melonis* under in vitro conditions. Nevertheless, integrated studies evaluating the effectiveness of combined applications of *Gliocladium* spp. and betel leaf against this pathogen remain limited. Therefore, further investigation is required to assess the

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potential of this combination as an effective and environmentally sustainable alternative strategy for the management of *Fusarium* wilt in melon cultivation.

RESEARCH METHODS

This study was conducted at Balai Penyuluhan Pertanian (BPP) Laboratory, Negara District, Jembrana Regency, Bali, Indonesia, from October to November 2025. The experimental materials consisted of three antagonistic fungal isolates of *Gliocladium* spp., namely *Gliocladium viride*, *Gliocladium aureum* and *Gliocladium roseum*, obtained from the Plant Pests and Diseases Laboratory, Universitas Udayana. The pathogenic fungus *F. oxysporum* was isolated from melon plants exhibiting *Fusarium* wilt symptoms collected from farmer's fields in Subak Pemangket Awen Barat, Tegalbadeng Timur Village, Negara District, Jembrana Regency, Bali.

Additional materials used in this study included betel leaf powder, Potato Dextrose Agar (PDA), 70% ethanol, distilled water, cotton, aluminum foil, plastic wrap, and chloramphenicol. The equipment utilized comprised Erlenmeyer flasks, Petri dishes, graduated cylinders, micropipettes, cover glasses, an autoclave, an oven, inoculation needles, a digital balance, a magnetic stirrer, a microscope, a laminar airflow cabinet, a gas stove, a cork borer, stirring spoons, and a Bunsen burner.

The experiment was arranged in a completely randomized design (CRD) with two treatment factors. The first factor was the antagonistic fungal species (*Gliocladium* spp.), consisting of three levels: *G. viride* (Gv), *G. aureum* (Gv) and *G. roseum* (Gr). The second factor was the concentration of betel leaf powder, comprising three levels: 25 g/L (S25), 50 g/L (S50), and 75 g/L (S75). A control treatment using PDA medium without betel leaf powder supplementation was included for comparison. Each treatment was replicated three times, resulting in a total number of experimental units according to the factorial arrangement.

Isolation of the Fungal Pathogen *F. oxysporum*

F. oxysporum isolates were obtained from melon plants exhibiting typical symptoms of *Fusarium* wilt in farmer's fields. Plant samples consisting of roots and basal stem tissues were collected from infected plants, placed in sterile plastic bags, and transported to the laboratory for isolation.

The root and basal stem samples were washed under running tap water to remove adhering soil particles and then cut into approximately 1 cm segments. The tissue segments were surface-sterilized with 70% ethanol for approximately 30 second, rinsed twice with sterile distilled water, and dried on sterile filter paper.

The sterilized tissue segments were then plated onto Potato Dextrose Agar (PDA) supplemented with chloramphenicol to suppress bacterial growth. The Petri dishes were incubated at room temperature for 5–7 days until fungal colonies emerged. Colonies exhibiting morphological characteristics typical of *Fusarium* were subsequently purified by transferring hyphal tips onto fresh PDA medium to obtain pure isolates of *F. oxysporum*.

Subculture of Fungal Isolates

The obtained isolates of *G. viride*, *G. roseum*, *G. aureum*, and *F. oxysporum* were subsequently subcultured on sterile Potato Dextrose Agar (PDA) medium. Subculturing was performed aseptically under laminar airflow conditions and the cultures were incubated at room temperature for 5–7 days until optimal mycelial growth was achieved and the isolates were ready for use as test inocula.

In Vitro Antagonistic Activity Assay

The antagonistic activity was evaluated in vitro using the dual culture method. Potato Dextrose Agar (PDA) medium was amended with betel leaf powder according to the respective treatment concentrations and poured into 9 cm diameter Petri dishes. Inocula of the antagonistic fungus and the pathogen were obtained using a sterile cork borer (0.5 cm in diameter).

Mycelial plugs of *Gliocladium* spp. and *F. oxysporum* were placed on opposite edges of the PDA medium at a distance of approximately 3 cm and subsequently incubated at room temperature. Observations were conducted on the colony growth of *Gliocladium* spp. and *F. oxysporum* from day 1 to day 6 after inoculation. The measured parameter was the radial growth (colony diameter) of the pathogenic fungus under each treatment and control condition.

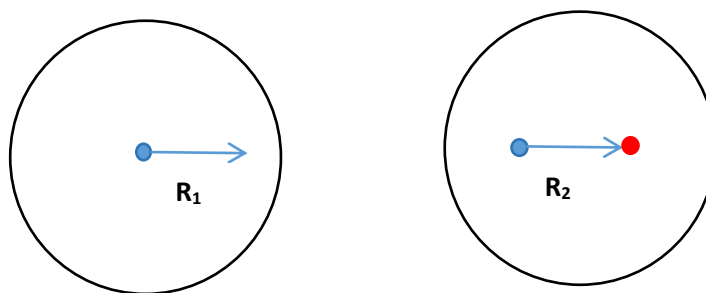
Observed Variables

Percentage Inhibition of Radial Growth

The percentage inhibition of *F. oxysporum* growth was calculated following the formula described by Hidayat et al. (2020):

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

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Description:

P : Percentage inhibition (%)

R₁ : Radius of pathogen growth in the control treatment (mm)

R₂ : Radius of pathogen growth in the treated samples (mm)

RESULTS

Growth of *Fusarium oxysporum* f.sp. *melonis* on the Medium Supplemented with Betel Leaf Powder

The results of observations on the growth of *F. oxysporum* on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of betel leaf powder are presented in Table 1.

Table 1. Radial Growth of *Fusarium oxysporum* f.sp. *melonis* on PDA with Various Betel Leaf Powder Concentration

Treatment	Growth of <i>Fusarium oxysporum</i> (mm)													
	Day-													
	1	2	3	4	5	6	7							
S0	4.00	a	10.33	a	14.67	a	18.67	b	21.00	b	28.33	a	34.33	a
S25	3.67	a	10.33	a	15.67	a	23.00	a	27.67	a	30.67	a	34.67	a
S50	2.33	b	9.00	b	14.00	a	17.67	b	21.67	b	24.00	a	26.33	b
S75	2.33	b	8.67	b	12.33	b	15.67	b	17.67	b	20.00	a	24.00	b

Description: S0= without betel leaf powder, S25= 25 g/L betel leaf powder, S50= 50 g/L betel leaf powder, S75= 75 g/L betel leaf powder.

Growth of *Gliocladium* spp. on the Medium Supplemented with Betel Leaf Powder

The growth of *G. aureum*, *G. viride*, and *G. roseum* on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of betel leaf powder is presented in Table 2.

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Table 2.Radial Growth of *Gliocladium* spp. on PDA with Various Betel Leaf Powder Concentration

Treatment		Growth of <i>Gliocladium</i> spp. (mm)													
		Day-													
		1	2	3	4	5	6	7							
Ga	S0	1.00	b	4.00	bc	5.33	c	5.67	c	6.67	c	6.67	c	6.67	c
Ga	S25	1.00	b	4.33	bc	4.67	c	4.67	c	4.67	c	4.67	c	4.67	c
Ga	S50	1.00	b	4.67	bc	5.67	c	6.00	c	6.00	c	6.00	c	6.00	c
Ga	S75	1.00	b	4.33	bc	5.67	c	6.00	c	6.00	c	6.00	c	6.00	c
Gr	S0	10.00	a	50.00	a	54.00	a	55.00	a	55.00	a	55.00	a	55.00	a
Gr	S25	11.00	a	50.33	a	52.00	a	53.33	a	53.33	a	53.33	a	53.33	a
Gr	S50	10.00	a	41.00	a	48.33	a	51.33	a	51.33	a	51.67	a	51.67	a
Gr	S75	9.67	a	45.67	a	53.33	a	55.00	a	55.00	a	55.00	a	55.00	a
Gv	S0	9.33	a	23.00	b	25.67	b	31.67	b	34.00	b	34.67	b	36.67	b
Gv	S25	8.33	a	20.00	b	25.67	b	33.33	b	35.00	b	35.67	b	38.33	b
Gv	S50	7.33	a	19.33	b	22.67	b	28.33	b	30.67	b	32.67	b	36.00	b
Gv	S75	7.00	a	18.00	bc	21.33	b	26.00	b	30.00	b	33.33	b	33.33	b

Description: Ga= *Gliocladium aureum*, Gr= *Gliocladium roseum*, Gv=*Gliocladium viride*, S25= 25 g/L betel leaf powder, S50= 50 g/L betel leaf powder, S75= 75 g/L betel leaf powder.

Interaction between *Gliocladium* spp. and Betel Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

The result of the ANOVA test to determine the significance of the interaction between *Gliocladium* spp. and betel leaf powder against *F. oxysporum* f.sp. *melonis* are presented in Table 3. Furthermore, the DMRT post hoc test of interaction between *Gliocladium* spp. and betel leaf powder in inhibiting the growth of *F. oxysporum* f.sp. *melonis* are presented in Table 4.

Table 3. Significance of the Interaction between *Gliocladium* spp. and Betel Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

Source of Variation	df	SS	MS	F-value	p-value	Significance
<i>Gliocladium</i> species (A)	3	5,325.229	1,775.076	417.67	<0.0001	**
Betel leaf powder dosage (B)	3	68.063	22.688	5.34	0.0043	**
Interaction (A×B)	9	207.188	23.021	5.42	0.00016	**
Error	32	136.000	4.250	-	-	-
Total	47	5,736.479	-	-	-	-

Statistical Significance:

** = Highly significant ($p < 0.01$)

* = Significant ($p < 0.05$)

ns = Not significant ($p \geq 0.05$)

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Table 4. Interaction between *Gliocladium* spp. and Betel Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

Treatment	Growth of <i>Fusarium oxysporum</i> (mm)			
	S0	S25	S50	S75
G0 (control)	34.33 a	34.67 a	26.33 b	24.00 b
Ga	19.33 c	19.00 c	19.00 c	18.67 c
Gv	7.33 d	7.00 d	8.33 d	8.00 d
Gr	2.67 e	3.00 e	2.67 e	2.00 e

Description: variable = radial growth of *F. oxysporum* f. sp. *melonis* (mm); the same letters in the table indicate not significant difference (DMRT 5%).

Antagonistic Activity of *Gliocladium* spp. against *Fusarium oxysporum* f.sp. *melonis* on Various Betel Leaf Powder Concentrations

The inhibitory effects of different *Gliocladium* spp. on the growth of *F. oxysporum* f. sp. *melonis* on Potato Dextrose Agar (PDA) medium supplemented with various concentrations of betel leaf powder are presented in Table 5.

Table 5. Inhibitory Effect and Percentage Antagonism of *Gliocladium* spp. Against *Fusarium oxysporum* f. sp. *melonis* on PDA Medium Supplemented with Different Concentrations of Betel Leaf Powder

Treatment		Growth of <i>F. oxysporum</i> (mm)		% Antagonism
Ga	S0	19.33	a	43.69
Ga	S25	19.00	a	44.66
Ga	S50	19.00	a	44.66
Ga	S75	18.67	a	45.63
Gr	S0	2.67	c	92.23
Gr	S25	3.00	c	91.26
Gr	S50	2.67	c	92.23
Gr	S75	2.00	c	94.17
Gv	S0	7.33	b	78.64
Gv	S25	7.00	b	79.61
Gv	S50	8.33	b	75.73
Gv	S75	8.00	b	76.70

Description: Ga= *Gliocladium aureum*, Gr= *Gliocladium roseum*, Gv= *Gliocladium viride*, S25= 25 g/L betel leaf powder, S50= 50 g/L betel leaf powder, S75= 75 g/L betel leaf powder.

DISCUSSION

Growth Response of *Fusarium oxysporum* f.sp. *melonis* to Betel Leaf Powder

Observations of *F. oxysporum* growth on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of betel leaf powder demonstrated that the addition of betel leaf powder significantly affected the colony growth rate of *F. oxysporum*. In general, increasing concentrations of betel leaf powder resulted in a reduction in colony diameter compared with the control treatment without betel leaf powder (S0).

During the early observation period (days 1 and 2), differences in colony growth among treatments were not pronounced. However, from day 3 to day 7, PDA media supplemented with 50 g/L (S50) and 75 g/L (S75) betel leaf powder consistently produced smaller colony diameters than the control treatment (Table 1). In contrast, the growth of *F. oxysporum* under the S25 treatment did not differ markedly from that of the control (S0), indicating that a concentration of 25 g/L betel leaf powder was insufficient to suppress *Fusarium* growth. Duncan's multiple range test at the 5% significance level confirmed that, on several observation days, the S50 and S75 treatments differed significantly from the control, demonstrating a clear inhibitory effect of betel leaf powder on *F. oxysporum* growth.

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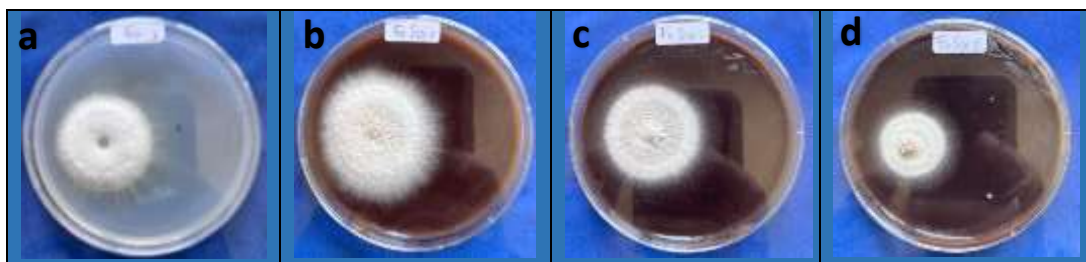


Figure 1. Growth of *Fusarium oxysporum* f.sp. *melonis* on media with Different Concentration of Betel Leaf Powder at day 7th; a). 0 g/L (control), b). 25 g/L, c). 50 g/L, d). 75 g/L.

The reduction in *F. oxysporum* colony growth is closely associated with the presence of bioactive compounds in betel leaves, particularly phenolic compounds such as eugenol, hydroxychavicol, and flavonoids. These compounds are known to exhibit antifungal activity through mechanisms including disruption of fungal cell membrane integrity, increased membrane permeability, and interference with metabolic processes and cell wall biosynthesis (Marchese et al., 2017; Nayaka et al., 2021).

Hydroxychavicol, one of the major bioactive components of betel leaves, has been reported to significantly inhibit *Fusarium* mycelial growth even at low concentrations by damaging cell membranes and causing leakage of cellular contents (Singburadom, 2015). In addition, eugenol plays a role in inhibiting ergosterol biosynthesis, an essential component of fungal cell membranes, thereby disrupting cellular physiological functions and suppressing the colony growth of *F. oxysporum* (De et al., 2013; Didehdar et al., 2022).

Overall, these results confirm that betel leaf powder has strong potential as a botanical antifungal agent capable of inhibiting *F. oxysporum* growth under in vitro conditions. However, the inhibitory effect was dose-dependent, with higher concentrations providing greater suppression of pathogen growth.

Growth Response of *Gliocladium* to Betle Leaf Powder

Observations of *Gliocladium* spp. colony growth on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of betel leaf powder revealed differential growth responses among *Gliocladium* species as well as among betel leaf powder concentrations. These results indicate variation in tolerance levels of individual *Gliocladium* species to the bioactive compounds present in betel leaves.

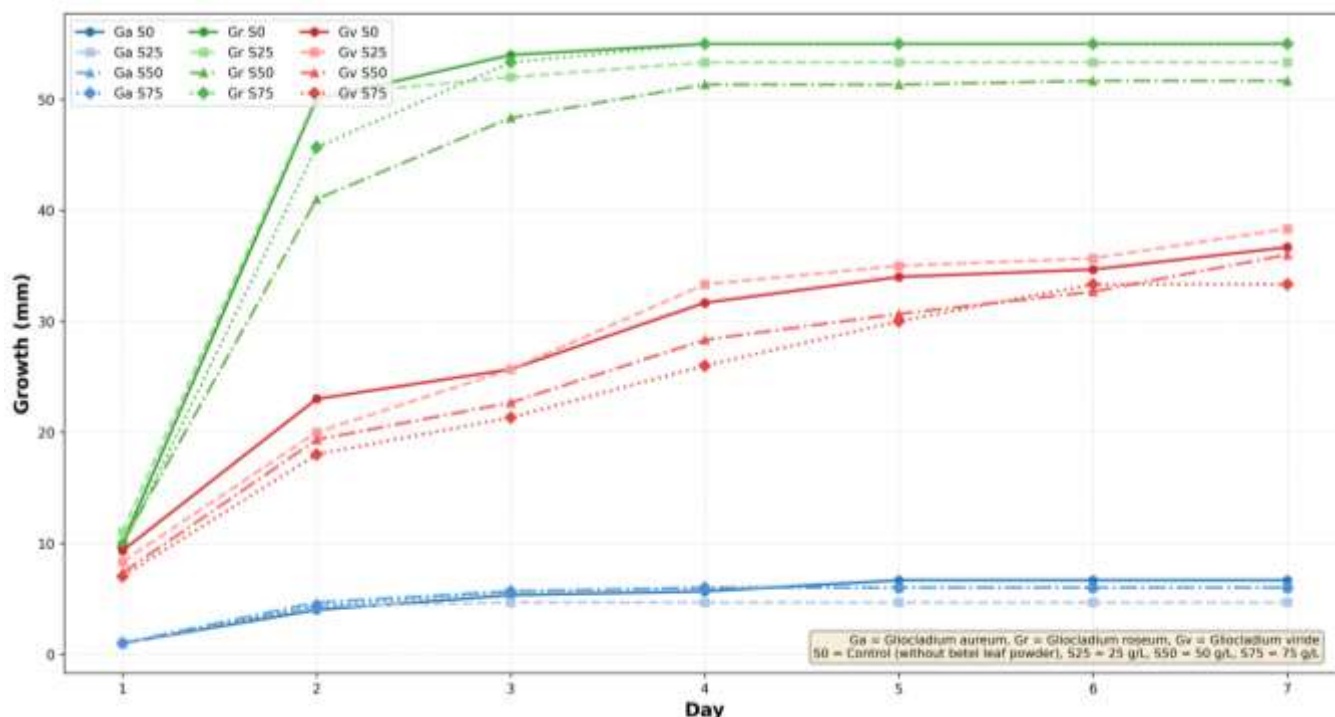


Figure 2. Growth Trends of *Gliocladium* spp. on Media with Various Betel Leaf Powder Concentration

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Overall, all tested *Gliocladium* species were able to grow on media supplemented with betel leaf powder at concentrations up to 75 g/L, although growth rates varied among species. *G. roseum* exhibited the highest growth performance and showed no apparent growth inhibition at any betel leaf powder concentration, as evidenced by large and consistent colony diameters throughout the observation period (Table 2). *G. viride* displayed relatively rapid but less aggressive colony growth, whereas *G. aureum* exhibited slower growth, particularly on media containing higher concentrations of betel leaf powder.

Significant differences were observed among betel leaf powder treatments in terms of *Gliocladium* colony growth; however, the inhibitory effect of betel leaf powder on *Gliocladium* spp. was markedly weaker than its effect on *F. oxysporum*. This finding suggests that *Gliocladium* spp. possess a higher tolerance to the antifungal compounds in betel leaf powder compared with the pathogenic fungus *Fusarium*.

The tolerance of *Gliocladium* spp. to betel leaf bioactive compounds is likely associated with the ability of these antagonistic fungi to undergo physiological and biochemical adaptations, including the production of detoxification enzymes and secondary metabolites that support growth and survival under unfavorable environmental conditions (Köhl et al., 2019). In addition, *Gliocladium* spp. are known for their rapid growth and efficient nutrient utilization, enabling them to maintain development despite the presence of inhibitory compounds in the growth medium (Sopialena et al., 2024).

These results provide an important basis for concluding that betel leaf powder, at the concentrations tested, is compatible with *Gliocladium* spp. and therefore has strong potential for use in integrated biological control strategies without significantly suppressing the growth of antagonistic agents.

Interaction between *Gliocladium* spp. and Betel Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

Analysis of variance revealed highly significant effects of both *Gliocladium* species ($F = 417.665$, $p < 0.0001$) and betel leaf powder dosage ($F = 5.338$, $p = 0.0043$) on the radial growth inhibition of *F. oxysporum* f.sp. *melonis* (Table 3). Critically, a highly significant interaction between these factors was observed ($F = 5.417$, $p = 0.00016$), demonstrating that the efficacy of betel leaf powder was not uniformly additive across *Gliocladium* species but rather exhibited species-dependent modulation. Among the tested biocontrol agents, *G. roseum* (Gr) demonstrated exceptional antagonistic activity with mean radial growth values ranging from 2.00 to 3.00 mm across dosage treatments, representing up to 94.2% growth inhibition compared to the untreated control (34.33 mm). This level of efficacy substantially exceeds previous reports of *Trichoderma* species against *F. oxysporum*, where inhibition rates typically range from 16.7% to 54.8% (Chen et al., 2021; Naglot et al., 2015). The superior biocontrol capacity of *G. roseum* can be attributed to its highly efficient mycoparasitic mechanisms, including aggressive hyphal coiling around pathogen hyphae, rapid appressoria formation, and prolific secretion of cell wall degrading enzymes (CWDEs) such as chitinase, β -1,3-glucanase, and protease (Prasad et al., 2016). Additionally, *Gliocladium* species are known to produce potent antifungal secondary metabolites including 6-pentyl- α -pyrone, gliotoxin, and viridin, which exert direct inhibitory effects on competing fungi through membrane disruption and metabolic interference (Tyśkiewicz et al., 2022).

The significant interaction effect unveiled three distinct response patterns that underscore the species specific nature of synergy between fungal biocontrol agents and botanical extracts. First, *G. roseum* exhibited a clear synergistic response wherein betel leaf supplementation enhanced antagonistic activity in a dose dependent manner. The pathogen radial growth decreased up to 2.00 mm (Table 4), with the most pronounced enhancement observed at the highest dosage (75 g/L). This synergism likely operates through complementary biochemical mechanisms which is hydroxychavicol and eugenol, the principal bioactive compounds in betel leaf, disrupt fungal membrane integrity and inhibit ergosterol biosynthesis (Ali et al., 2010; Singburaudom, 2015), thereby compromising the pathogen's structural defenses and rendering it more susceptible to enzymatic degradation by *Gliocladium* CWDEs. Furthermore, phenolic compounds may induce expression of biocontrol associated genes in *G. roseum*, analogous to the upregulation of chitinase and PR-protein genes observed when *Trichoderma* species interact with plant root exudates (Chen et al., 2021). Second, *G. aureum* (Ga) displayed a neutral interaction profile with remarkably consistent pathogen growth values (18.67-19.33 mm) across all dosage levels (Table 4). This pattern suggests that *G. aureum* operates at or near maximum biocontrol efficiency under these experimental conditions, such that botanical supplementation provides no additional benefit—a phenomenon potentially explained by saturation of available binding sites for CWDEs on the pathogen cell wall or an absence of compatible molecular targets for phenolic enhancement. Third, *G. viride* (Gv) exhibited a mild antagonistic interaction at intermediate and high extract concentrations, with pathogen growth increasing from 7.33 mm (S0) to 8.33 mm (S50-S75). This counterintuitive finding indicates that elevated phenolic levels may exert non-selective fungistatic effects that marginally impair *G. viride* growth or interfere with its metabolic pathways, thereby indirectly reducing biocontrol efficacy.

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Antagonistic Activity of *Gliocladium* spp. Against *Fusarium oxysporum* f.sp. *melonis* in the Presence of Betel Leaf Powder

The antagonistic interaction between *Gliocladium* spp. and *F. oxysporum* was significantly influenced by fungal species, betel leaf powder concentration, and their interaction (Table 3). Among the tested species, *G. roseum* exhibited the highest antagonistic activity, with inhibition percentages ranging from 91.26% to 94.17%, significantly higher than those of *G. viride* (75.73–79.61%) and *G. aureum* (43.69–45.63%).

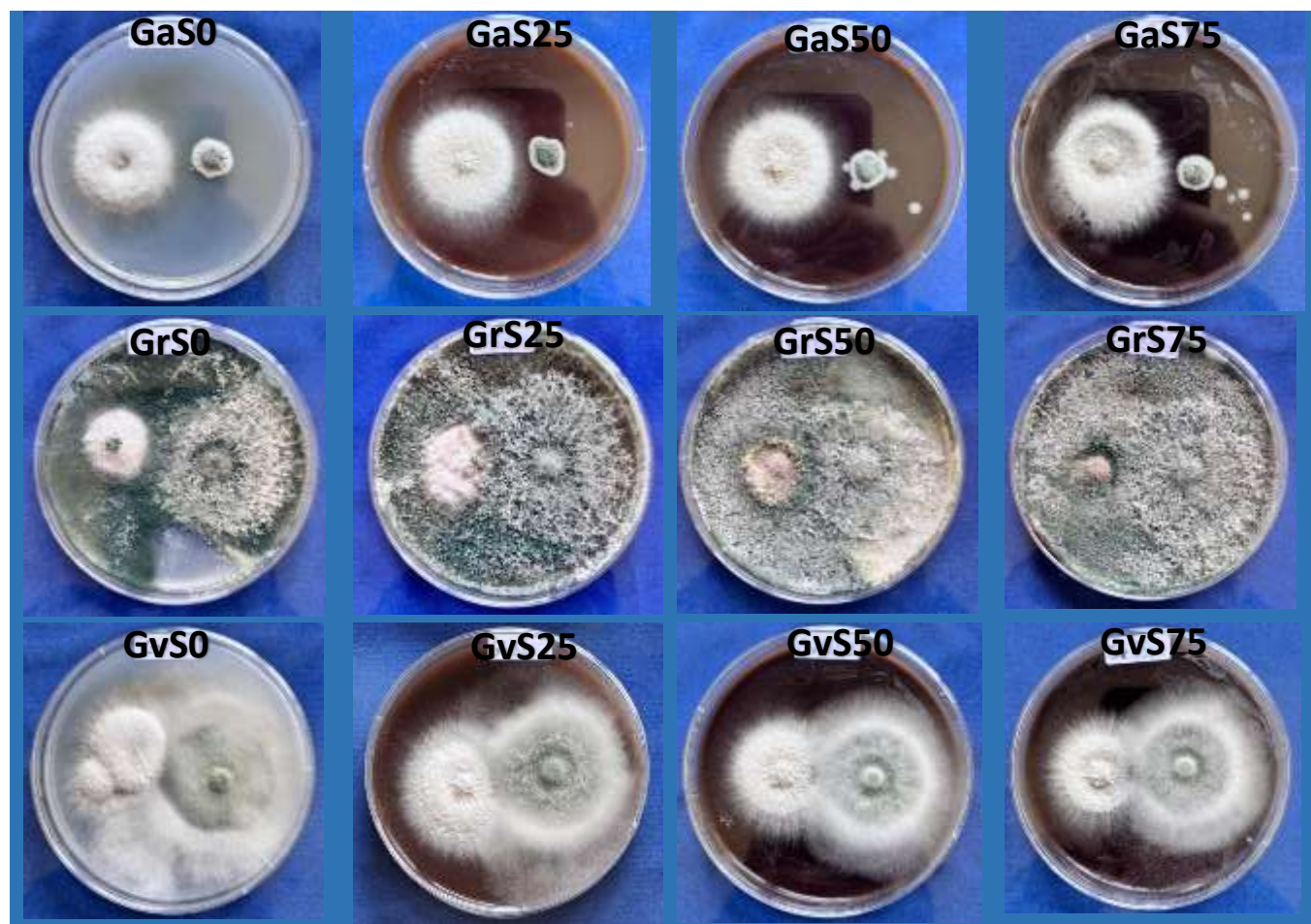


Figure 2. Antagonistic Interaction between *Gliocladium* spp. and *Fusarium oxysporum* f. sp. *melonis* at Different Betel Leaf Powder Concentrations.

The superior antagonistic performance of *G. roseum* was consistent across all betel leaf powder concentrations, indicating that its biocontrol efficacy was not compromised by the presence of betel leaf bioactive compounds. The highest inhibition was observed in the *G. roseum* and S75 treatment with inhibition percentage 94.17% (Table 3), suggesting a synergistic effect between the biological control agent and the botanical antifungal.

In *G. viride*, antagonistic activity did not increase consistently with higher betel leaf powder concentrations, suggesting that elevated doses may exert mild inhibitory effects on the antagonist itself, thereby reducing the net biocontrol effect. Conversely, *G. roseum* maintained high antagonistic efficiency even at the highest betel leaf powder concentration, highlighting its robustness and suitability for combined application.

The antagonistic mechanisms of *Gliocladium* spp. include antibiosis, competition for space and nutrients, and mycoparasitism. *Gliocladium* species are known to produce antifungal metabolites such as gliotoxin, gliovirin, and viridin, as well as lytic enzymes including chitinase and glucanase, which degrade the cell walls of pathogenic fungi (Howell et al., 1993; Wilhite & Straney, 1996; Köhl et al., 2019). In addition to antibiosis, competition for space and nutrients played a crucial role in suppressing *F. oxysporum* growth. *Gliocladium*, particularly *G. roseum*, exhibited rapid and aggressive colony expansion, allowing it to colonize available space more efficiently than the pathogen. This rapid growth limits nutrient availability for *F. oxysporum*, thereby restricting its ability to proliferate. Furthermore, mycoparasitism likely contributed to the strong antagonistic effect observed in this study. Mycoparasitism involves direct physical interaction between the antagonist and the pathogen, including hyphal contact,

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coiling, penetration, and enzymatic degradation of the pathogen's cell wall. *Gliocladium* spp. are known to produce lytic enzymes such as chitinases and β -1,3-glucanases, which degrade chitin and glucan components of fungal cell walls, leading to hyphal collapse and cell death (Köhl et al., 2019). The discoloration and apparent degeneration of *F. oxysporum* hyphae observed in dual culture treatments provide indirect evidence supporting the occurrence of mycoparasitic activity (Figure 3. GrS50 and GrS75). The combination of these mechanisms likely contributed to the strong inhibition of *F. oxysporum* observed in this study.

CONCLUSION

Based on the research results and discussion above, it can be concluded that: (1) Betel leaf (*Piper betle* L.) powder exhibited a significant inhibitory effect on the in vitro growth of *F. oxysporum* f. sp. *melonis*, with effective inhibition observed at concentrations of 50 g/L and 75 g/L (2) All tested *Gliocladium* species were able to grow on PDA medium supplemented with betel leaf powder, indicating tolerance to the bioactive compounds present in betel leaves. Among the evaluated species, *Gliocladium roseum* demonstrated the fastest and most stable growth across all tested concentrations, *G. viride* showed moderate tolerance, while *G. aureum* exhibited comparatively slower growth. (3) Interaction between *Gliocladium* species and betel leaf powder dosage significantly influenced *F. oxysporum* f. sp. *melonis* suppression with a strong species-dependent interaction, in which *G. roseum* delivered the most effective. (4) The greatest inhibition of *F. oxysporum* f. sp. *melonis* was achieved by the combination of *G. roseum* and 75 g/L betel leaf powder, resulting in an antagonistic inhibition of 94.17%. This finding suggests a synergistic interaction between *G. roseum* and betel leaf powder, supporting their potential combined use as an environmentally sustainable alternative to chemical fungicides. Further studies under greenhouse and field conditions are recommended to validate the efficacy and stability of this combination in controlling *Fusarium* wilt in melon production systems.

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