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Effectiveness of the Combination *Trichoderma* spp. and Neem Leaf Powder (*Azadirachta indica* L.) Against *Fusarium Oxysporum* f. sp. *melonis* in Vitro

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ABSTRACT: *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *melonis* is a major factor reduces of melon *(Cucumis melo* L.) production in Indonesia. Control using chemical fungicides has not been effective and poses risks to the environment and health. The aim of this experiment was to find out more accurate treatment combination of *Trichoderma* spp. ***as biological control agents with*** neem leaf powder (Azadirachta indica) ***as a botanical fungicide*** for inhibiting the growth of *F. oxysporum* in vitro. The *Trichoderma* species used in this study were collected by the Plant Pathology Laboratory collection of Udayana University. ***The research was conducted using*** a Randomized Complete Design (RCD) ***with two factorial treatments comprising three Trichoderma species (*T. harzianum, T. koningii, *and* T. viride*) and four neem leaf powder concentrations (0*** g L^{-1}***, 15*** g L^{-1}***, 30*** g L^{-1}***, and 45*** g L^{-1}***) and*** 3 replications. ***Antagonistic activity was assessed using the dual culture method on potato dextrose agar (PDA) medium, with observations conducted over seven days*** observed parameters included pathogen colony growth and percentage of inhibition. The results showed that the combination of *Trichoderma* spp. and neem leaf powder significantly inhibited the growth of *F. oxysporum* in vitro, with varying effectiveness among Trichoderma species. The best inhibition was obtained from the combination of *Trichoderma koningii* and neem leaf powder at a concentration of 45 g L^{-1}, which completely inhibited the growth of *F. oxysporum* (100%) by day 5 without suppressing the growth of *Trichoderma*.

KEYWORDS: Trichoderma *spp., Neem Leaf Powder, biological control, Azadirachta indica, Fusarium oxysporum f. sp*. melonis*, in vitro*

INTRODUCTION

Melon (*Cucumis melo* L.) are a horticultural commodity with high economic value and increasing market demand in Indonesia. However, national melon production has shown a downward trend in the last three years (BPS, 2024). One of the main causes of this decline is *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *melonis*. This pathogen infects plants through the root system, disrupting xylem function and causing wilting and plant death, thereby significantly reducing productivity.

Control of *Fusarium* wilt disease at the farmer level is still dominated by the use of synthetic fungicides. This approach is not yet sustainable and has various negative impacts, such as pathogen resistance, environmental pollution, death of non-target organisms, and chemical residues in crops that pose a risk to human health (Janssen *et al*., 2024; Cruz *et al*., 2022). Therefore, environmentally friendly and sustainable control alternatives are needed.

One potential alternative is the use of *Trichoderma* spp. biological agents, which are known to have antagonistic activity against soil-borne pathogens. The control mechanism of *Trichoderma* spp. against *F. oxysporum* includes the production of antifungal compounds, competition for space and nutrients, colonization of the rhizosphere, and induction of systemic plant resistance (Li *et al*., 2018; Chen *et al*., 2019). In addition, a botanical approach using neem leaves (*Azadirachta indica*). Neem leaves contain active compounds including azadirachtin, nimbin, and salanin, which exhibit antifungal properties. Previous studies have demonstrated that neem leaf extracts or powders can significantly suppress *Fusarium* growth by damaging fungal cell structures and disrupting essential metabolic processes (Andini *et al*., 2023).

Although the use of *Trichoderma* spp. and neem leaf powder separately has been widely studied, research on the effectiveness of combining the two in controlling *Fusarium* wilt disease in melon plants is still limited. The combination of *Trichoderma* spp. and mimba leaf powder is expected to have a synergistic effect in suppressing pathogen development. Therefore,

this study aims to evaluate the effectiveness of the combination of *Trichoderma* spp. and mimba leaf powder and determine the optimal effective and environmentally friendly dosage as a strategy for controlling Fusarium wilt disease in melon plants.

RESEARCH METHODS

This study was conducted at Balai Penyuluhan Pertanian (BPP) Laboratory of Negara District, Jembrana Regency, Bali, from October to December 2025. The materials used in this experimental included three antagonistic fungal isolates of *Trichoderma* spp., namely *Trichoderma harzianum, Trichoderma koningii,* and *Trichoderma viride*, which were obtained from the collection of the Pest and Plant Disease Laboratory, Udayana University. The pathogenic fungus *Fusarium oxysporum* was isolated from melon plants infected with *Fusarium* wilt collected from farmer's fields in Subak Pemangket Awen Barat, Tegalbadeng Timur Village, Negara District, Jembrana Regency, Bali.

Other materials used included neem leaf powder, Potato Dextrose Agar (PDA) medium, 70% alcohol, distilled water, cotton, aluminum foil, plastic wrap, and chloramphenicol. The equipment used consisted of Erlenmeyer flasks, Petri dishes, measuring cylinders, micropipettes, cover glasses, an autoclave, an oven, an inoculating loop, a digital balance, a magnetic stirrer, a microscope, a laminar airflow cabinet, a gas stove, a cork borer, a stirring spoon, and a Bunsen burner.

The experiment was arranged in a Completely Randomized Design (CRD) with two treatment factors. The first factor was the type of antagonistic fungus (*Trichoderma* spp.), consisting of three levels: *Trichoderma harzianum* (Th), *T. koningii* (Tk), and *T. viride* (Tv). The second factor was the concentration of neem leaf powder, consisting of three levels: 15 g L^{-1} (N15), 30 g L^{-1} (N30), and 45 g L^{-1} (N45). A control treatment using PDA medium without neem leaf powder was included for comparison. Each treatment was replicated three times, resulting in a total number of experimental units experimental units according to the factorial arrangement.

Isolation of the Fungal Pathogen *Fusarium oxysporum*

Fusarium oxysporum isolates were obtained from melon plants exhibiting typical symptoms of *Fusarium* wilt in farmers' 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 water to remove adhering soil and then cut into approximately 1 cm segments. The tissue segments were surface-sterilized with 70% ethanol for approximately 30 seconds, rinsed twice with sterile distilled water, and dried using sterile filter paper.

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

Subculture of Fungal Isolates

The isolates *T. harzianum, T. koningi, T. viride* and *F. oxysporum* were subsequently rejuvenated on sterile Potato Dextrose Agar (PDA) medium. Subculturing was conducted aseptically in a laminar airflow cabinet 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 effectiveness test was evaluated in vitro using the dual culture method. Potato Dextrose Agar (PDA) medium was mixed with neem leaf powder according to the treatment doses and then poured into 9 cm diameter Petri dishes. Inocula of the antagonistic fungus and the pathogen were obtained using a sterile cork borer with a diameter of 0.5 cm. Inocula of *Trichoderma* spp. and *Fusarium oxysporum* were placed on opposite edges of the PDA medium at a distance of approximately 3 cm and incubated at room temperature. Observations were carried out on the colony growth of *Trichoderma* spp. and *F. oxysporum* from day 1 to day 6 after inoculation. The observed variable was the diameter of pathogen colony growth under each treatment and control.

Observed Variables

Percentage Inhibition of Radial Growth

The percentage inhibition of *F. oxysporum* growth was calculated following the formula described byDharmaputra *et al.* (1999):

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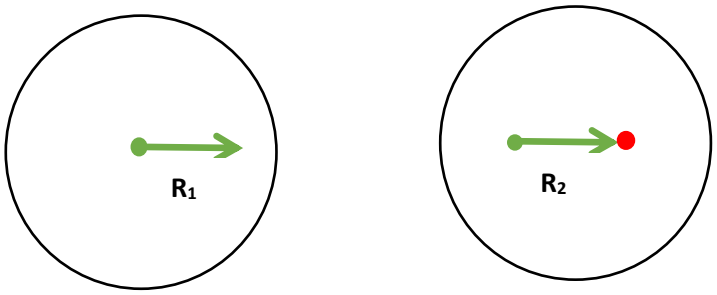
$$P = \frac{R1 - R2}{R1} \; x \; 100\%$$



Description:
P : Percentage inhibition (%)
R_1 : Radius of pathogen growth in the control treatment (mm)
R_2 : Radius of pathogen growth in the treated samples (mm)

RESULTS

Growth of *Fusarium oxysporum* f.sp *melonis* on the Medium Suplemented with Neem Leaf Powder

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

Treatment	Growth of *Fusarium oxysporum* (mm) Day-													
	1		2		3		4		5		6		7	
N0	2.67	a	11.00	a	16.67	a	20.00	a	25.00	a	31.00	a	34.00	a
N15	1.67	a	9.67	a	18.00	a	21.67	a	26.33	a	35.00	a	37.67	a
N30	1.00	b	6.33	b	11.00	b	16.00	b	16.67	b	21.67	b	24.33	b
N45	0.67	b	5.33	b	9.33	b	14.67	b	18.67	b	21.67	b	23.00	b

Description: N0= without neem leaf powder, N15= 15 g L^{-1} neem leaf powder, N30= 30 g L^{-1} neem leaf powder, N45= 45 g L^{-1} neem leaf powder.

Growth of *Trichoderma* spp. on the Medium Suplemented with Neem Leaf Powder

The growth of *T. harzianum*, *T. koningii*, and *T. viride* on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of betel neem powder is presented in Table 2.

Table 2. Radial Growth of *Trichoderma* spp. on PDA with Various Neem Leaf Powder Concentration

Treatment		Growth of Trichoderma spp. (mm) Day-													
		1		2		3		4		5		6		7	
Th	N0	4.33	b	22.67	b	39.00	b	42.33	b	48.67	b	49.67	b	49.67	b
Th	N15	6.33	b	20.67	b	28.33	c	41.67	b	48.33	b	49.33	b	49.33	b
Th	N30	5.00	b	15.00	c	23.00	c	37.00	b	43.00	b	49.00	b	49.67	b
Th	N45	2.67	c	13.00	c	14.67	d	39.33	b	44.00	b	46.33	b	49.67	b
Tk	N0	8.33	a	26.00	a	44.00	a	45.33	a	48.00	b	51.67	a	52.00	a
Tk	N15	11.33	a	28.00	a	43.33	a	47.33	a	51.33	a	54.00	a	54.00	a
Tk	N30	10.67	a	27.67	a	42.33	a	47.00	a	51.33	a	55.00	a	55.00	a
Tk	N45	10.00	a	22.00	b	41.67	a	50.67	a	55.00	a	55.00	a	55.00	a
Tv	N0	2.67	c	20.67	b	38.67	b	40.33	b	40.67	b	42.33	c	45.00	c
Tv	N15	5.00	b	17.67	c	26.67	c	28.67	c	36.67	c	43.00	c	44.33	c

Tv	**N30**	3.33	**c**	22.00	**b**	23.00	**c**	32.00	**c**	36.00	**c**	40.67	**c**	41.67	**c**
Tv	**N45**	2.00	**c**	9.00	**d**	12.67	**d**	29.67	**c**	34.67	**c**	37.67	**d**	38.33	**d**

Description: Th= *Trichoderma harzianum*, Tk= *Trichoderma koningii*, Tv= *Trichoderma viride*, N0= Potato Dextrose Agar (neem leaf powder 0%), N15= 15 g L^{-1} neem leaf powder, N30= 30 g L^{-1} neem leaf powder, N45= 45 g L^{-1} neem leaf powder.

Interaction between *Trichoderma* spp. and Neem Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

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

Source of Variation	df	SS	MS	F-value	p-value	Significance
Trichoderma species (A)	3	5,768.062	1,922.687	138.572	<0.0001	**
Nee leaf powder dosage (B)	3	131.562	43.854	3.161	0.0379	*
Interaction (A×B)	9	458.354	50.928	3.671	0.0030	**
Error	32	444.000	13.875	-	-	-
Total	47	6,801.979	-	-	-	-

Statistical Significance:

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

* = Significant ($p < 0.05$)

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

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

Treatment	Growth of *Fusarium oxysporum* (mm)			
	N0	**N15**	**N30**	**N45**
T0 (control)	34.00 **a**	37.67 **a**	24.33 **b**	23.00 **b**
Th	5.00 **c**	4.00 **c**	4.67 **c**	4.33 **c**
Tk	1.67 **d**	0.33 **e**	0.00 **e**	0.00 **e**
Tv	5.00 **c**	4.33 **c**	5.33 **c**	6.00 **c**

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 *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *melonis* on Various Neem Leaf Powder Concentrations

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

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

Treatment	Growth of *F. oxysporum* (mm)		% Antagonism
Th N0	20.00	**a**	75.00
Th N15	18.00	**a**	77.78
Th N30	22.00	**a**	78.79
Th N45	19.33	**a**	77.59
Tk N0	9.67	**c**	89.66
Tk N15	9.00	**c**	96.30
Tk N30	7.00	**c**	100.00
Tk N45	7.00	**c**	100.00
Tv N0	11.33	**b**	55.88

Tv	**N15**	11.67	**b**	62.86
Tv	**N30**	14.00	**b**	61.90
Tv	**N45**	14.67	**b**	60.42

Description: Th= *Trichoderma harzianum*, Tk= *Trichoderma koningii*, Tv= *Trichoderma viride*, N15= 15 g L^{-1} neem leaf powder, N30= 30 g L^{-1} neem leaf powder, N45= 45 g L^{-1} neem leaf powder.

DISCUSSION

Growth Response of *Fusarium oxysporum* f.sp. *melonis* to Neem 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 neem leaf powder significantly affected the colony growth rate of *F. oxysporum*. In general, increasing concentrations of neem leaf powder resulted in a reduction in colony diameter compared with the control treatment without neem leaf powder (N0).

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 30 g L^{-1} (N30) and 45 g/ L (S75) neem leaf powder consistently produced smaller colony diameters than the control treatment (Table 1). In contrast, the growth of *F. oxysporum* under the N15 treatment did not differ markedly from that of the control (N0), indicating that a concentration of 15 g L^{-1} neem 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 N30 and N45 treatments differed significantly from the control, demonstrating a clear inhibitory effect of neem leaf powder on *F. oxysporum* growth.

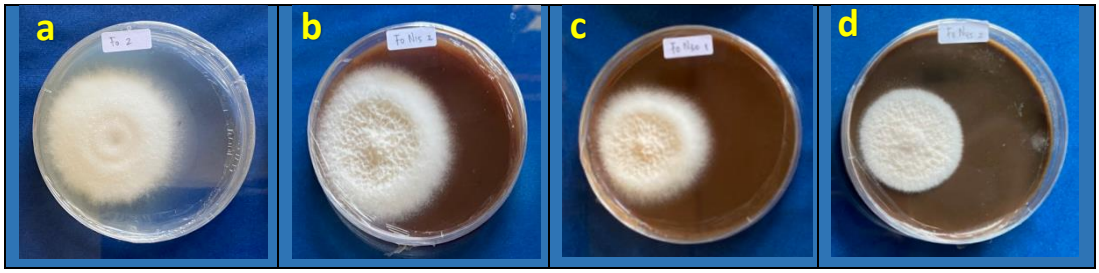


Figure 1. Growth of *Fusarium oxysporum* f.sp. *melonis* on media with Different Concentration of Neem Leaf Powder at day 7th; a). 0 g L^{-1} (control), b). 15 g L^{-1}, c). 30 g L^{-1}, d). 45 g L^{-1}.

Suppression of *Fusarium oxysporum* growth by neem leaf powder is closely related to the content of bioactive compounds azadirachtin and nimbin in neem leaves. These compounds are also accompanied by flavonoids that jointly work through several antifungal mechanisms, including disruption of cell membrane integrity, inhibition of cell wall synthesis enzymes, and induction of oxidative stress in fungal cells (Khare & Kamble, 2025). Disruption of the cell membrane causes leakage of cell contents and inhibits the basic metabolic processes that fungi need to grow and develop. The accumulation of phenolic compounds at high concentrations amplifies such inhibitory effects resulting in the growth *of F. oxysporum* is increasingly depressed at levels of 30 g L^{-1} and 45 g L^{-1}.

A similar pattern has also been reported in various other botanical fungicide studies, where the inhibitory effectiveness increases progressively with the addition of extract concentration (Awal *et al*., 2024). These findings confirm the importance of determining the optimal dose in the development of neem leaf powder as a botanical fungicide so that its effectiveness can be relied upon on a broader application scale.These results overall confirm the potential of neem leaf powder as a botanical fungicide capable of suppressing the growth of *F. oxysporum* at the right concentration. This potential becomes relevant considering that reliance on synthetic chemical fungicides has thus far posed ecotoxicological risks that drive the need for alternative controls that are safer for the environment and human health (Palmieri *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 *Trichoderma* spp. to Neem Leaf Powder

Observations of *Trichoderma* spp. colony growth on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of neem leaf powder revealed differential growth responses among *Trichoderma* species as well as among neem

leaf powder concentrations. These results indicate variation in tolerance levels of individual *Trichoderma* species to the bioactive compounds present in neem leaves.

All tested *Trichoderma* species were able to grow at all neem leaf powder concentrations up to 45 g L^{-1}, although the inhibitory effect varied among species and observation days. Significant differences among treatment combinations were more evident during the early stages of incubation (days 1-4). During the later observation period, colony growth of *T. harzianum* and *T. koningii* became increasingly similar as the colonies approached the edge of the Petri dish. In contrast, *T. viridae* continued to exhibit significant growth reduction at the highest neem leaf powder concentration (45 g L^{-1}), indicating greater sensitivity to the bioactive compounds present in neem leaf powder. Nevertheless, all three species remained capable of sustained colony development throughout the seven-day observation period, demonstrating their compatibility with neem leaf powder.

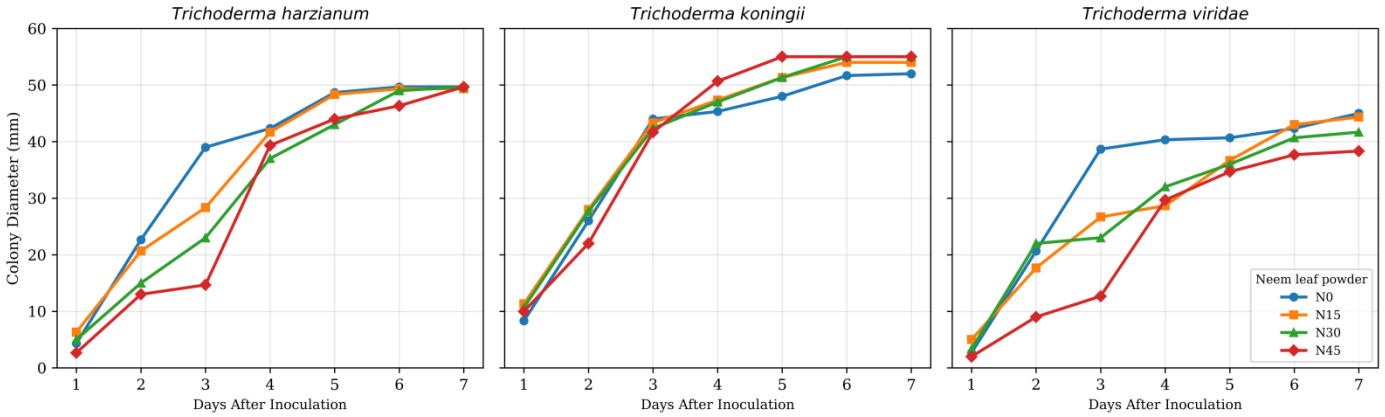


Figure 2. Growth Trends of *Trichoderma* spp. on Media with Various Neem Leaf Powder Concentration

Trichoderma koningii showed the fastest growth rate among the three tested species, especially in the early days of observation. *Trichoderma harzianum* exhibited an intermediate growth rate with a relatively stable pattern across all concentration levels of neem leaf powder. *Trichoderma viridae* exhibited the slowest growth rate, especially at the highest concentration of 45 g L^{-1}, with a seventh-day colony diameter that was significantly smaller compared to *T. harzianum* and *T. koningii* at the same concentration. The differences in growth rates among species indicates variations in the level of physiological tolerance to neem leaf phenolic compounds among the tested *Trichoderma* species.

The high tolerance of *Trichoderma* to neem leaf antifungal compounds is highly likely related to the physiological and biochemical adaptation capabilities possessed by this fungus. These adaptation mechanisms include the production of detoxification enzymes and secondary metabolites that help Trichoderma survive in unfavorable environmental conditions (Köhl *et al*., 2019). Trichoderma is also known to have a fast growth rate and high nutrient utilization efficiency, so it remains capable of developing even if the media contains inhibitory compounds (Sarnaik *et al*., 2025). The combination of these two traits makes Trichoderma spp. a compatible biological agent candidate to be applied together with botanical materials such as neem leaf powder without reducing its viability in the field.

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

Interaction between *Trichoderma* spp. and Neem Leaf Powder Against *Fusarium oxysporum* f.sp. *melonis*

Analysis of variance on the seventh day of observation confirmed that the *Trichoderma* species exerted a dominant influence on suppressing the growth of *Fusarium oxysporum* compared to the concentration of neem leaf powder. The much larger F-value for the *Trichoderma* species factor compared to the neem leaf powder concentration factor indicates that the selection of the antagonistic species is the main determinant of successful pathogen suppression. The highly significant interaction between the two factors indicates that the effect of neem leaf powder on suppressing *F. oxysporum* is not uniform across all *Trichoderma* species, but rather depends on the characteristics of each antagonistic species. This type of interaction pattern is commonly found in studies combining biological agents with botanical materials, where the success of the synergy highly depends on the compatibility of the mechanism of action between the two components (Zayton *et al.*, 2026).

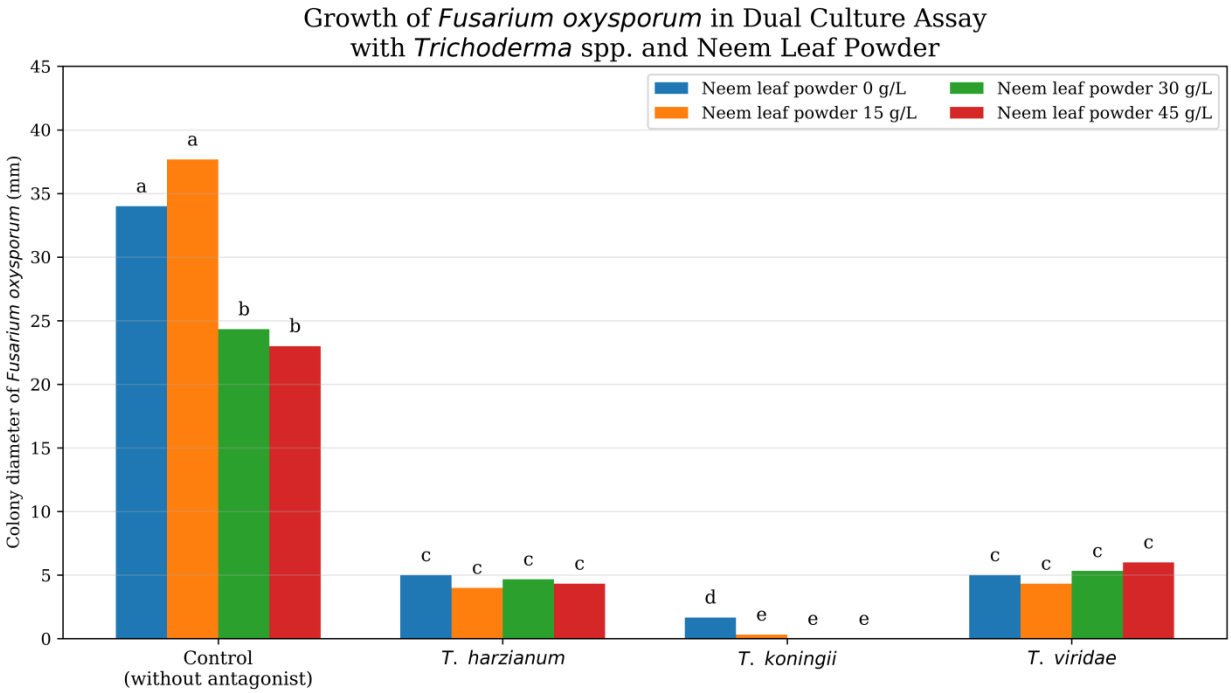


Figure 3. Growth of Fusarium of *Fusarium oxysporum* under Dual Culture with *Trichoderma* Species and Neem Leaf Powder Treatments

Trichoderma koningii showed the most prominent antagonism performance among the three tested species. Complete suppression of *Fusarium oxysporum* to 0.0 mm was achieved in combination with 30 g L^{-1} and 45 g L^{-1} neem leaf powder, whereas the 15 g L^{-1} treatment resulted in almost complete inhibition, with the pathogen colony diameter reduced to 0.33 mm. This result indicates that the side of the pathogen facing *T. koningii* experienced absolutely no further growth during the observation period. This superior antagonistic of *T. koningii* is in line with reports that this species produces the peptaibol trichokonin, a broad-spectrum antimicrobial compound active against various plant pathogens including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea* (Ji *et al*., 2023). The production of trichokonin alongside mechanisms of mycoparasitism and competition for growth space are thought to work synergistically with the antifungal compounds from neem leaf powder, producing a dual pressure that renders *F. oxysporum* entirely unable to develop on the side facing the antagonist.

Trichoderma harzianum produced a relatively high percentage of antagonism at most neem leaf powder concentrations, although not as high as *T. koningii*. The *Fusarium* colony diameter remained low throughout the treatments, ranging from approximately 4.0 to 5.0 mm by the end of the observation period. This result indicates a complex interaction between the dose of neem leaf powder and the antagonism activity of *T. harzianum*. The antagonism mechanism of *T. harzianum* generally involves the production of cell wall-degrading enzymes such as chitinase and glucanase, nutrient competition, and the induction of defense genes in host plants (Chen *et al*., 2021). The interaction between neem leaf phenolic components and the enzymatic system of *T. harzianum* at intermediate concentrations likely caused a temporary disruption to the working efficiency of these enzymes before their effectiveness increased again at the highest concentration.

Trichoderma viridae exhibited the most varied response pattern among the three tested species to the presence of neem leaf powder. The negative antagonism value in the combination with a concentration of 15 g L^{-1} indicates that the growth of *F. oxysporum* towards *T. viridae* was actually greater compared to its growth in the opposite direction in that combination. This condition likely occurred because a low concentration of neem leaf phenolic compounds provided a mild stimulatory effect on the initial growth of *F. oxysporum* without being accompanied by an adequate strengthening of *T. viridae* antagonism activity to counterbalance it. The improved performance of *T. viridae* at concentrations of 30 g L^{-1} and 45 g L^{-1} indicates that this species requires a higher active compound concentration threshold compared to *T. koningii* and *T. harzianum* before its antagonism activity can be consistently optimized.

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

The antagonistic interaction between *Trichoderma* spp. and *F. oxysporum* was significantly influenced by fungal species, neem leaf powder concentration, and their interaction (Table 3). Among the tested species, *T. koningii* exhibited the highest

antagonistic activity, with inhibition percentages ranging from 89.66-100%, significantly higher than those of *T. harzianum* (75.00–78.79%) and *T. viridae* (55.88–62.86%).

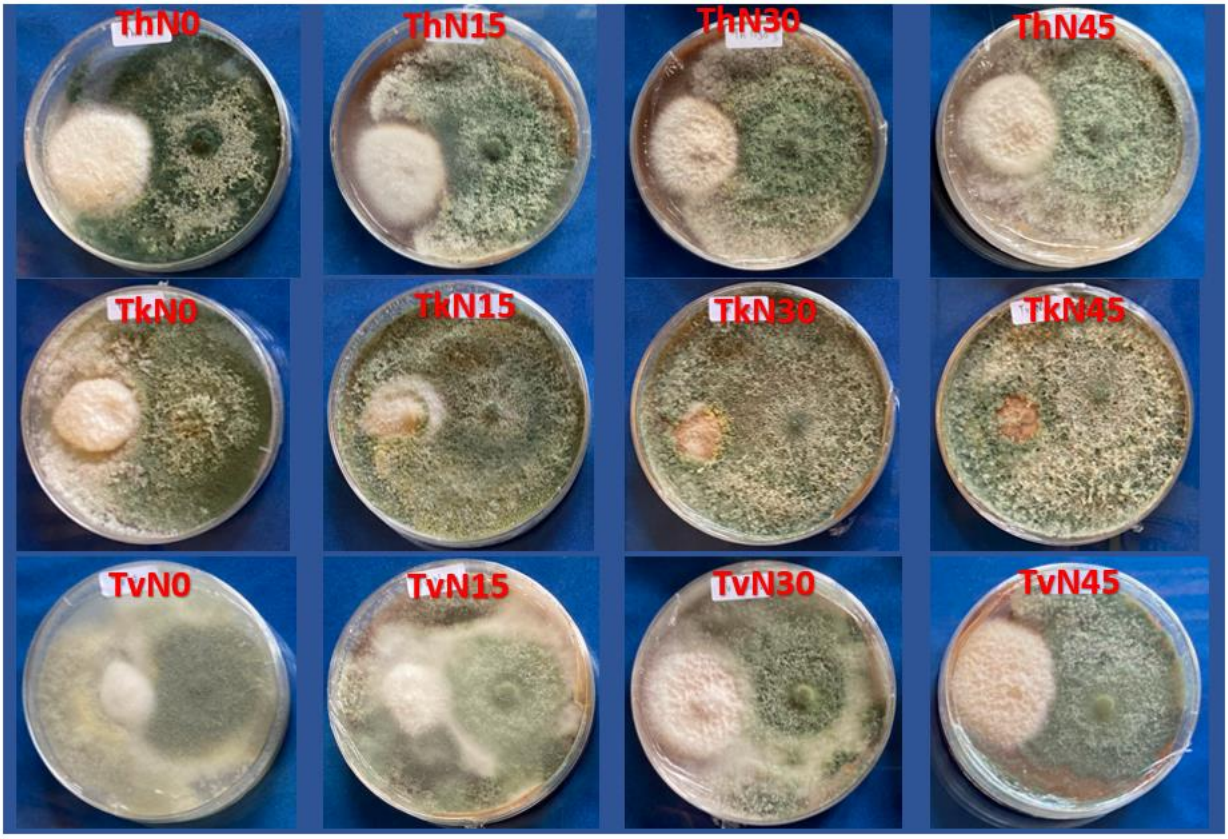


Figure 4. Antagonistic Interaction between*Trichoderma* spp. and *Fusarium oxysporum* f. sp. *melonis* at Different Neem Leaf Powder Concentrations.

The experimental evaluation demonstrates significant variation in the biocontrol efficacy of three Trichoderma species against *Fusarium oxysporum* f. sp. *melonis*. The superior antagonistic performance of *T. koningii* was consistent across all neem leaf powder concentrations, indicating that its biocontrol efficacy was not compromised by the presence of neem leaf bioactive compounds. *T. koningii* suppressed the radial growth of the pathogen to 9.67 mm with 89.66% inhibition in the untreated control. Suppression levels reached 100.00% when *T. koningii* was combined with 30 g L^{-1} and 45 g L^{-1} of neem leaf powder. Sood et al. (2020) confirmed that *Trichoderma* species exhibit diverse inhibitory potential against soilborne phytopathogens through specialized enzymatic mechanisms. Variations in biocontrol activity depend strongly on the specific species and strain compatibility under different environmental conditions (Joo & Hussein, 2022).

Trichoderma harzianum and *Trichoderma viride* exhibited moderate antagonistic activity compared to *T. koningii* across all botanical treatments. Mycelial growth for *T. harzianum* remained between 18.00 mm and 22.00 mm with percentage antagonism ranging from 75.00% to 78.79%. *T. viride* recorded lower antagonistic capabilities with inhibition values ranging from 55.88% to 62.86%. Jamil et al. (2020) observed similar differential growth inhibition among Trichoderma isolates when facing aggressive vascular wilt fungi. Intraspecific metabolic diversity determines the rate of chitinase and glucanase secretion needed to dismantle fungal cell walls effectively (Mustofa & Hastuti, 2024).

Neem leaf powder contains active tetranortriterpenoid compounds that suppress fungal development. Bioactive metabolites such as azadirachtin and nimbin disrupt cellular membranes and restrict spore germination in target pathogens. Higher dosages of botanical extracts increase phenolic concentrations within the culture medium to inhibit fungal enzymes. Jamil *et al*. (2020) demonstrated that *Azadirachta indica* extracts cause severe hyphal alteration and structural disintegration in *F. oxysporum*. Botanical extracts act as primary fungistatic barriers that slow down pathogen proliferation before complete biological parasitism occurs (Joo & Hussein, 2022).

The combination of *T. koningii* and neem leaf powder creates a strong synergistic effect against *Fusarium oxysporum* f. sp. *melonis*. Supplements at 30 g L^{-1} and 45 g L^{-1} enabled *T. koningii* to achieve total radial restriction of the target fungus. High physiological tolerance enables *T. koningii* to assimilate nutrient sources while resisting botanical limonoids in the growth medium.

Sood *et al*. (2020) highlighted that integrating bioagents with plant phytochemicals enhances overall pathogen suppression beyond single-treatment levels. Integrated application strategies provide an effective eco-friendly solution for sustainable management of soilborne wilt diseases in agricultural crops (Mustofa & Hastuti, 2024).

CONCLUSION

Based on the research results and discussion above, several things can be concluded as follows. (1) Neem leaf powder (*Azadirachta indica*) showed a significant inhibitory effect on the in vitro growth of *Fusarium oxysporum*, with effective inhibition observed at concentrations of 30 g L^{-1} and 45 g L^{-1}. (2) The three tested Trichoderma species, namely *T. harzianum, T. koningii*, and *T. viridae*, were able to grow on media supplemented with neem leaf powder up to a concentration of 45 g L^{-1}, which indicates a high tolerance to the bioactive compounds of neem leaves, with *T. koningii* showing the fastest and most stable growth among the three tested species. (3) The interaction between *Trichoderma* species and the concentration of neem leaf powder had a highly significant effect on suppressing the growth of *F. oxysporum* with a species-dependent response pattern, where *T. koningii* provided the most consistent results across all tested concentration levels. (4) The highest inhibition against *F. oxysporum* was achieved by the combination of *T. koningii* with neem leaf powder at concentrations of 30, and 45 g L^{-1}, with antagonism percentages reaching 100% at all three concentration levels. These findings indicate that the combination of *T. koningii* and neem leaf powder has great potential as an environmentally friendly biological control strategy against *Fusarium* wilt, serving as an effective alternative to synthetic chemical fungicides. Further research on a greenhouse and field scale is needed to validate the effectiveness and stability of this combination in controlling *Fusarium* wilt under actual cultivation conditions.

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