



RESEARCH ARTICLE

In-vitro efficacy of biopesticides against *Fusarium oxysporum* f. sp. *psidii* causing guava wilt

Suman Karmakar¹, Abhilasha A Lal¹, Swarnali Maiti¹ & Nayan Kishor Adhikary^{2*}

¹Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj 211 007, Uttar Pradesh, India

²School of Agricultural Sciences, Nagaland University, Medziphema, 797 106, Nagaland, India

*Correspondence email - nayan.bckv@gmail.com

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Abstract

Fusarium oxysporum f. sp. *psidii*, the causal agent of guava wilt, represents a significant constraint to guava (*Psidium guajava* L.) cultivation, particularly in the Prayagraj region of Uttar Pradesh, India. The present study was conducted in the year 2024 and aimed to evaluate the *in vitro* antagonistic efficacy of selected microbial biocontrol agents and botanical extracts against the pathogen. Two fungal antagonists, *Trichoderma viride* and *Trichoderma harzianum*, were assessed using the dual culture technique. Among them, *T. harzianum* demonstrated superior antagonistic activity, achieving a maximum mycelial inhibition of 70.55 %. Concurrently, aqueous extracts from four medicinal plants -*Azadirachta indica* (neem), *Solanum indicum* (black nightshade), *Moringa oleifera* (moringa) and *Ageratum conyzoides* (goat weed) were evaluated at concentrations of 5 %, 10 % and 15 % using the poisoned food technique. Neem extract exhibited the highest antifungal activity, with 96 % inhibition at 15 % concentration on the seventh day after inoculation. A dose-dependent inhibitory effect on mycelial growth was observed across all plant extracts. These findings underscore the potential of integrating both microbial and plant-based agents as sustainable alternatives to synthetic fungicides in the management of guava wilt. However, the outcomes are preliminary and necessitate validation through extended field trials to establish practical applicability.

Keywords: guava; *in-vitro*; management; plant extract; *Trichoderma*; wilt

Introduction

Guava, scientifically known as *Psidium guajava* (L.), is a widely cultivated fruit crop that thrives in various tropical and sub-tropical regions. This small tree is native to Mexico, Central America, the Caribbean and northern South America and belongs to the *Myrtaceae* family (1). It plays a significant role in global agriculture as it is full of nutrients. It is often called the poor man's apple. According to the National Institute of Nutrition, guavas' nutritional composition includes protein (2.55 g), crude fiber (5.44 g), carbohydrates (14.32 g), calories (68 Kcal), phosphorus (40 mg/100 g), iron (0.26 mg/100 g), vitamin B₃ (1.083 mg/100 g) and magnesium (22 mg/100 g). Guava is also rich in vitamin A, vital for maintaining healthy skin and eyes (2). Guava leaves contain carotenoids and polyphenols like (+) gallo catechin and leucocyanidin. The fruits colour is influenced by these phytochemicals, with red-orange guavas typically possessing higher polyphenol and carotenoid content than their yellow-green counterparts (3). The cultivation of guava in India encompasses an estimated area of around 315 thousand ha, resulting in a substantial production of 45.16 Mmt. Among the Indian states, Uttar Pradesh holds the top position in guava production, followed by Madhya Pradesh and Bihar. In Uttar Pradesh alone, the cultivated area spans 29 thousand hectares, yielding an impressive production of 9.83 million metric tons. Prayagraj district in Uttar Pradesh has gained renown for producing the finest guava, not only within the country but also on a global

scale (4). Numerous diseases impair the production of guava, but *Fusarium* wilt, which is sparked by *Fusarium oxysporum* f. sp. *psidii*, is the most serious disease of guava, causing devastating loss worldwide. Guava wilt was first reported in Taiwan in 1926 (5). In India, the first reported instance of guava wilt dates back to 1935 in the Allahabad region (6). The initial external symptoms manifest after the rainy season, around October to November, with yellowing and curling of leaves to drying of the whole plant. The cortical region of roots develops brown discolouration and fruits become hard and stony (7). Accurate and contemporary assessments of the losses attributed to wilt disease are currently lacking. The incidence of wilt disease in guava can result in production losses ranging from 5 % to 60 % in India and this disease is particularly prevalent in Uttar Pradesh, Bihar and Uttarakhand, where it frequently surpasses the economic threshold due to favourable climatic conditions (8). The extent of losses due to this disease has prompted farmers to transition to cultivating other orchard plants (9). There are numerous methods to manage wilt disease, with chemical methods being among the most effective. However, concerns regarding environmental pollution, residual toxicity of fungicides and adverse effects on non-target organisms have restricted their widespread and long-term use. Thus, plant-derived antifungal compounds and bio-control agents are the most suitable option to develop an eco-friendly management of guava wilt. Plant-derived antifungal compounds are particularly advantageous due to their lower toxicity, biodegradability and environmental compatibility.

Extensive research in recent years has focused on extracting and screening various plants for their antifungal properties, yielding valuable results. These plant-based compounds provide a promising solution for sustainable plant disease management, reducing dependence on hazardous chemical treatments and fostering ecological balance (10). Among these, botanicals such as *Azadirachta indica* (neem) and other medicinal plants have been extensively studied for their antifungal potential. Neem contains bioactive constituents including azadirachtin, nimbin, salannin and flavonoids, which inhibit fungal growth by disrupting cell wall formation, membrane integrity and spore germination. Previous studies have demonstrated the inhibitory effects of neem and other plant extracts against soil-borne pathogens, including *Fusarium* spp.; however, their efficacy on *Fusarium* varies with extraction method and concentration. Therefore, systematic evaluation of different plant extracts at varying concentrations is essential to find out effective and reliable botanical treatments. Besides plant extracts biocontrol agents such as *Trichoderma* sp., *Aspergillus niger*, *Penicillium citrinum*, *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* are commonly found in the rhizospheric area of guava plant roots. Different species of the genus *Trichoderma* are extensively utilised as biological control agents because of their capacity to restrict the growth of plant pathogens through diverse mechanisms, including mycoparasitism, production of inhibitory metabolites, competition for resources and imitation of host defence responses. Among the commonly studied species, *Trichoderma viride* and *T. harzianum* have been shown to possess strong antagonistic activity against *F. oxysporum*; however, published reports show differences in identifying either species as the more effective antagonist. Such inconsistencies indicate that the performance of *Trichoderma* species may be influenced by pathogen variability and experimental conditions. Although botanical extracts and *Trichoderma* spp. have been widely investigated, comparative studies assessing the effectiveness of different plant extracts at multiple concentrations alongside the relative antagonistic potential of *T. viride* and *T. harzianum* against *F. oxysporum* are scanty. Therefore, the present investigation was conducted to assess the antifungal activity of selected plant extracts, including neem and to comparatively evaluate *T. viride* and *T. harzianum* under *in vitro* conditions to identify dependable and environmentally safe management practices for guava wilt. (8,9).

Materials and Methods

Isolation of test pathogen

Samples of guava plant roots showing the typical symptoms of wilt disease were collected and small pieces (2-3 mm) of infected roots were cut with the help of a sterilised scalpel to isolate the pathogen and were sterilised by using 1 % sodium hypochlorite (NaOCl) solution for 10–20 sec, then the bits were repeatedly washed in sterilised distilled water. Then the bits were transferred to PDA medium under aseptic conditions and incubated at 25 ± 2 °C. The isolated fungal pathogen was brought into pure culture using single spore isolation (10–11).

Identification of the test pathogen

The isolated fungal pathogen was identified as *F. oxysporum* by ITCC (Indian Type Culture Identification), IARI, New Delhi, with the identification number 12055.24.

Evaluation of biocontrol agents by dual culture technique

Two fungal biocontrol agents, viz., *Trichoderma harzianum* and *Trichoderma viride*, were tested against *F. oxysporum* in a dual culture approach. The isolates of *Trichoderma* species were obtained from the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Parayagraj, U.P. These bioagents and the pathogen were maintained in PDA medium. The dual culture technique was performed by pouring 20 mL of PDA medium into sterile petri plates and allowing it to solidify. Then, from the previously grown 5-day-old cultures of both fungal bioagents and the pathogen, 5 mm discs of the test fungus and respective bioagents were aseptically transferred to the Petri plates, leaving sufficient space between the two discs. Seven replications were maintained for each treatment. The petri plates were then incubated at 25±2 °C until the pathogen colony in the control plate reached the periphery (12). Colony diameter of *F. oxysporum* was measured in millimetres and percent inhibition of mycelium was calculated by the formula (13). The observations were taken at intervals of 24 hr.

$$I = \frac{C - T}{C} \times 100 \quad (\text{Eqn. 1})$$

Where, I = Percent inhibition of mycelial growth, C = mycelial growth in control and T = mycelial growth in treatment.

Evaluation of selected plant extracts against *Fusarium oxysporum*

By using the poison food technique, four locally available plant extracts were tested against *F. oxysporum* at 5 %, 10 % and 15 % concentration to determine their efficacy. Information on the plant extracts that were tested against the pathogen is mentioned (Table 1).

The collected plant parts of black nightshade, ageratum weed (Goat weed), neem and moringa (Sehjan) were washed under tap water followed by distilled water. Washed samples were soaked in sterilised blotting paper to remove excess water. Then 100 g of each plant part was crushed manually with the help of a mixture grinder by adding 100 mL of distilled water (1:1 W/V). Then the obtained pastes were filtered through muslin cloth, followed by filter paper filtration with shaking motion. The processed extracts were poured into the flasks and plugged with cotton separately. In the very next step, the flasks were heated at 50 °C for 15 min to avoid contamination. Then, different concentrations, viz., 5 %, 10 % and 15 % of plant extracts, were made and incorporated into the PDA medium for inoculation of the test pathogen in sterilised petri dishes. These concentrations were selected as per prior studies that reported exceptional antifungal activity in a similar range. This helps to ensure comparability of results and allows to investigate dose dependent efficacy of the plant extracts. Each Petri plate was inoculated with a 2 mm disc of

Table 1. List of plants that were used in this study to check their efficacy

Botanical name	Local name	Family	Plant part used	Medicinal use	Reference
<i>Solanum indicum</i>	Black Night Shade	Solanaceae	Whole plant parts	Antifungal	(23)
<i>Ageratum conyzoides</i>	Billy Goat Weed	Asteraceae	Leaf and Stem	Antifungal	(24)
<i>Azadirachta indica</i>	Neem	Meliaceae	Young twigs	Antimicrobial	(23)
<i>Moringa oleifera</i>	Sehjan	Moringaceae	Leaves	Antifungal	(10)

the test pathogen in the centre of the plate separately and incubated at 28 ± 2 °C for 7 days and four replications were maintained for each treatment. The radial growth of the colony was recorded on the 3rd, 5th and 7th day of intervals and percent (%) inhibition of mycelial growth was calculated over the control with the help of the formula as per Equation 1.

Statistical analysis

The data collected during the experimental investigation were subjected to statistical analysis using the Analysis of Variance technique to arrive at conclusions (14). The F (Variance Ratio) test was utilised to determine the significance and non-significance of the treatments and the critical differences at the 5 % probability level were used to test for significant differences between the means. The experimental design included a completely random design (CRD).

Results and Discussion

Efficacy of bio-control agents

The study examined how effectively *Trichoderma viride* (T₁) and *Trichoderma harzianum* (T₂) suppressed fungal growth over time, showing that both treatments significantly reduced mycelial development compared to the untreated control. At 48 hr, *T. harzianum* showed 39.32 % inhibition, slightly more effective than *T. viride*, which showed 31.01 %. This trend continued across all observation periods. At 72 hr, inhibition increased to 52.80 % (T₂) and 46.92 % (T₁). By 96 hr, T₂ and T₁ reached 65.75 % and 62.22 % inhibition, respectively. At 120 hr, the suppression further improved, with T₂ showing 67.97 % and T₁ 63.77 % inhibition. Finally, at 144 hr, *T. harzianum* maintained the highest efficacy with 70.55 % inhibition, while *T. viride* achieved 64.27 %. Overall, *T. harzianum* consistently outperformed *T. viride* in controlling mycelial growth throughout the study (Table 2; Fig. 1).

Eventually, it was found that T₂ (*T. harzianum*) was superior to T₁ (*T. viride*) as a bio-control agent against *F. oxysporum*, with the average mycelium inhibition of 59.27 % and 53.63 %, respectively. Research on *Trichoderma*, mediated biocontrol was initiated following the observation that these fungi are capable of parasitising other plant pathogenic fungi (15). It was not until forty years later that *Trichoderma*'s potential in suppressing fungal plant diseases was demonstrated (16). Subsequent publications have

emphasised mycoparasitism as a crucial mechanism of biological control (17). The suppression of diseases mediated by *Trichoderma* involves a combination of mycoparasitism and antibiosis (18). In the context of direct antibiosis, secondary metabolites or secreted enzymes produced by *Trichoderma* inhibit the growth or germination of pathogens (19). Species of *Trichoderma* are prolific producers of secondary metabolites, which function not only in antagonism (through antibiosis and mycoparasitism) but also play roles in plant interactions (20). A similar finding was reported in which *Trichoderma harzianum* was used as a bio-agent *in vitro* (9). They observed that *T. harzianum* inhibited the mycelium of *F. oxysporum* by 73 %, which aligns closely with the results of the present study. Furthermore, research indicates that *T. harzianum* reduced the growth of *F. oxysporum* by 75.9 %, while *T. viride* exhibited a 67.7 % inhibition rate. These findings affirmatively support the results of the present study (21).

Efficacy of plant extracts

Antifungal activity of four plant extracts, viz., black nightshade (*Solanum indicum*), ageratum weed (*Ageratum conyzoides*), Neem (*Azadirachta indica*) and moringa (*Moringa oleifera*) were evaluated for their efficacy against *F. oxysporum* at different concentrations, of 5 %, 10 % and 15 %. All the plant extracts at 5 % (T₁), 10 % (T₂) and 15 % (T₃) concentration were found statistically significant at 5 % level of significance over control (T₀) on 3rd, 5th and 7th day after inoculation (Table 3–5) where neem leaf extract showed maximum mycelium inhibition on 3rd, 5th and 7th day after inoculation followed by Black night shade, Moringa and least mycelium inhibition was found in Ageratum weed extract. The mycelium growth of control (T₀) was 32.85 mm on 3rd DAI, 54.72 mm on 5th DAI and 76.32 mm on 7th DAI. The mycelium growth and inhibition percentage at each interval are discussed below (Fig. 2–4).

On the 3rd DAI, Neem leaf extract showed the maximum inhibition of mycelium growth of *F. oxysporum* at every concentration viz., 5 % (19.85 mm, 39.57 %), 10 % (6.45 mm, 80.36 %) and 15 % (2.6 mm, 92.08 %) followed by Black Nightshade at 5 % (24.3 mm, 26.02 %), 10 % (18.02 mm, 45.14 %) and 15 % (14.82 mm, 54.88 %). Moringa extract showed inhibition at 5 % (25.07 mm, 23.68 %), 10 % (21.55 mm, 34.39 %) and 15 % (19.05 mm, 42 %). The least mycelium inhibition was found in Ageratum weed extract at 5 % (26.85 mm, 18.26 %), 10 % (23.05 mm, 29.83 %) and 15 % (19.87 mm, 39.51 %).

Table 2. Mycelium growth inhibition of *Fusarium oxysporum* by *T. viride* and *T. harzianum*

Bioagents	48 hr		72 hr		96 hr		120 hr		144 hr		Avg. inhibition (%)
	Mycelium growth (MG) in mm and growth inhibition (MI) in %										
	MG	MI	MG	MI	MG	MI	MG	MI	MG	MI	
Control (T ₀)	21.54 ^a	0	36.78 ^a	0	59.86 ^a	0	72.07 ^a	0	81.07 ^a	0	0
<i>T. viride</i> (T ₁)	14.86 ^b	31.01	19.52 ^b	46.92	22.61 ^b	62.22	26.11 ^b	63.77	28.96 ^b	64.27	53.63
<i>T. harzianum</i> (T ₂)	13.07 ^c	39.32	17.36 ^c	52.8	20.5 ^c	65.75	23.08 ^c	67.97	23.87 ^c	70.55	59.27
C.D. (5 %)	1.76		1.79		2.52		2.03		2.22		

Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Table 3. Efficacy of plant extracts against *Fusarium oxysporum* on 3rd DAI

Treatments	Conc.	Mycelium growth (MG) of <i>F. oxysporum</i> with plant extracts (mm) and mycelium inhibition (MI) (%)							
		Black night shade		Neem		Moringa		Ageratum	
		MG	MI	MG	MI	MG	MI	MG	MI
T ₀	Control	32.85 ^a	00	32.85 ^a	00	32.85 ^a	00	32.85 ^a	00
T ₁	5 %	24.30 ^b	26.02	19.85 ^b	39.57	25.07 ^b	23.68	26.85 ^b	18.26
T ₂	10 %	18.02 ^c	45.14	6.45 ^c	80.36	21.55 ^c	34.39	23.05 ^c	29.83
T ₃	15 %	14.82 ^d	54.88	2.60 ^d	92.08	19.05 ^d	42	19.87 ^d	39.51
CD (5 %)		0.95		0.99		1.12		1.13	

Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Table 4. Efficacy of plant extracts against *Fusarium oxysporum* on 5th DAI

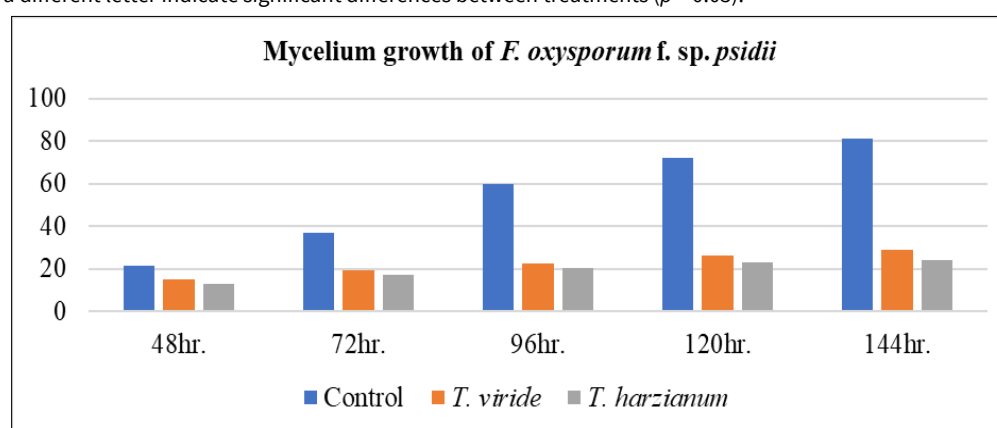
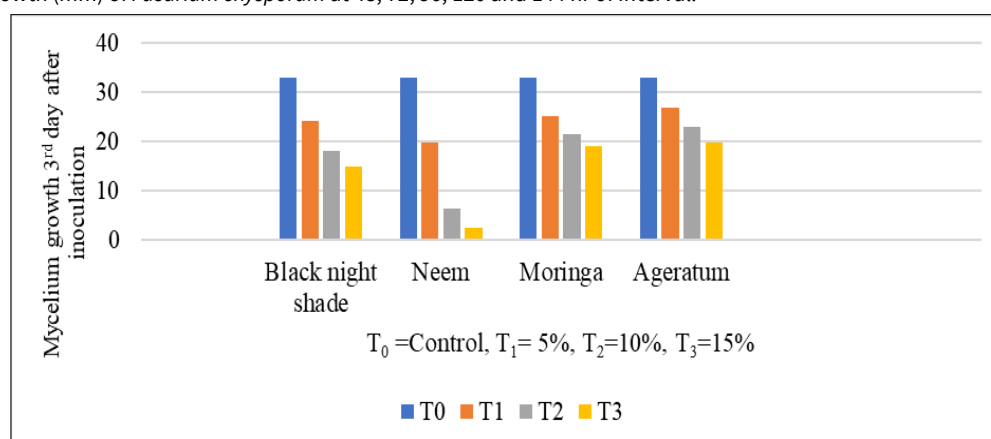
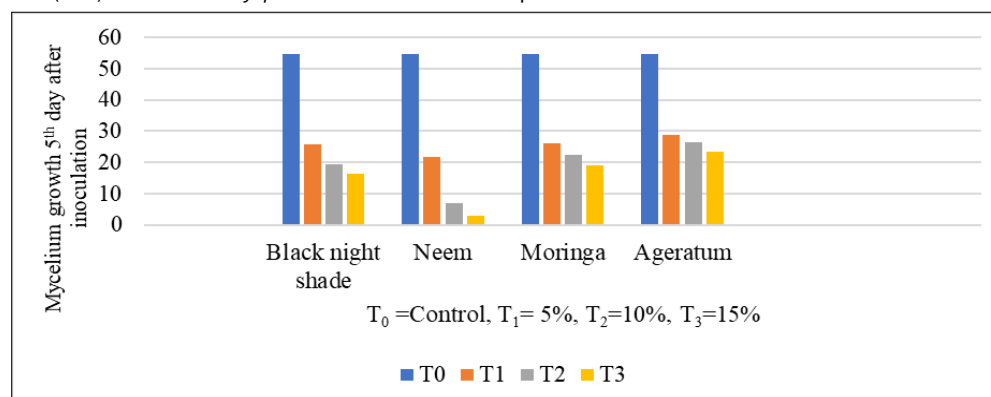
Treatments	Conc.	Mycelium growth (MG) of <i>F. oxysporum</i> with plant extracts (mm) and mycelium inhibition (MI) (%)							
		Black night shade		Neem		Moringa		Ageratum	
		MG	MI	MG	MI	MG	MI	MG	MI
T ₀	Control	54.72 ^a	00	54.72 ^a	00	54.72 ^a	00	54.72 ^a	00
T ₁	5 %	25.77 ^b	52.90	21.72 ^b	60.30	26.20 ^b	52.11	28.62 ^b	47.69
T ₂	10 %	19.42 ^c	64.51	6.82 ^c	80.36	22.47 ^c	58.93	26.25 ^c	52.02
T ₃	15 %	16.42 ^d	69.99	3.00 ^d	92.08	19.05 ^d	61.80	23.3 ^d	57.41
CD (5 %)		1.10		1.10		1.21		1.09	

Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Table 5. Efficacy of plant extracts against *Fusarium oxysporum* on 7th DAI

Treatments	Conc.	Mycelium growth (MG) of <i>F. oxysporum</i> with plant extracts (mm) and mycelium inhibition (MI) (%)							
		Black night shade		Neem		Moringa		Ageratum	
		MG	MI	MG	MI	MG	MI	MG	MI
T ₀	Control	76.32 ^a	00	76.32 ^a	00	76.32 ^a	00	76.32 ^a	00
T ₁	5 %	26.95 ^b	64.68	23.1 ^b	69.73	27.2 ^b	64.36	30.62 ^b	59.87
T ₂	10 %	20.3 ^c	73.40	7.05 ^c	90.76	23.7 ^c	68.94	28.37 ^c	62.82
T ₃	15 %	17.37 ^d	77.24	3.05 ^d	96	21.4 ^d	71.96	26.22 ^d	65.64
CD (5 %)		0.86		0.75		0.79		0.75	
R (-)		0.855		0.902		0.828		0.819	
R ²		0.732		0.813		0.686		0.671	

Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

**Fig. 1.** Mycelium growth (mm) of *Fusarium oxysporum* at 48, 72, 96, 120 and 144 hr of interval.**Fig. 2.** Mycelium growth (mm) of *Fusarium oxysporum* at different conc. of plant extracts on 3rd DAI.**Fig. 3.** Mycelium growth (mm) of *Fusarium oxysporum* at different conc. of plant extracts on 5th DAI.

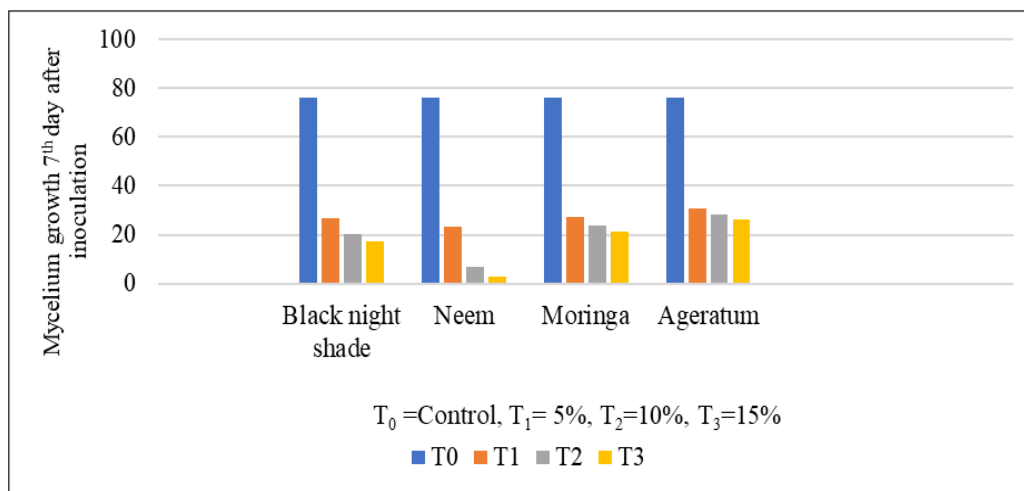


Fig. 4. Mycelium growth (mm) of *Fusarium oxysporum* at different conc. of plant extracts on 7th DAI.

On the 5th DAI, the maximum mycelium inhibition was found in Neem leaf extract at 5 % (21.72 mm, 60.30 %), 10 % (6.82 mm, 87.53 %) and 15 % (3 mm, 94.51 %) followed by Black nightshade extract at 5 % (25.77 mm, 52.9 %), 10 % (19.42 mm, 64.51 %) and 15 % (16.42 mm, 69.99 %). Moringa leaf extract showed inhibition at 5 % (26.2 mm, 52.11 %), 10 % (22.47 mm, 58.93 %) and 15 % (19.05 mm, 61.80 %). The least mycelium inhibition was recorded in Ageratum weed extract at 5 % (28.62 mm, 47.69 %), 10 % (26.25 mm, 52.02 %) and 15 % (23.3 mm, 57.41 %).

On the 7th DAI, Neem leaf extract showed maximum inhibition at 5 % concentration (23.1 mm, 69.73 %), 10 % (7.05 mm, 90.76 %) and 15 % (3.05 mm, 96 %) followed by Black Nightshade at 5 % (26.95 mm, 64.68 %), 10 % (20.3 mm, 73.40 %) and 15 % (17.37 mm, 77.24 %), Moringa at 5 % concentration (27.2 mm, 64.36 %), 10 % (23.7 mm, 68.94 %) and 15 % (21.40 mm, 71.96 %). The least mycelium inhibition was recorded in Ageratum weed

extract at 5 % (30.62 mm, 59.87 %), 10 % (28.37 mm, 62.82 %) and 15 % (26.22 mm, 65.64 %) (Fig. 5–8).

All treatments showed a negative correlation (-R) with their concentrations, indicating that as the concentration of plant extracts increased, the mycelium growth of *F. oxysporum* decreased (Table 4). A clear trend was observed that higher extract concentrations corresponded to increased percent inhibition of the pathogen. The correlation between the mycelium growth of the pathogen and the concentration of plant extracts after 7 days of inoculation is illustrated in Fig. 9–12. This demonstrates the efficacy of higher concentrations of plant extracts in suppressing the growth of the pathogen over time.

The value of the correlation determination (R^2) indicates the potential of each plant extract to inhibit pathogen growth (10). Neem extract had the highest R^2 value ($R^2 = 0.813$), demonstrating its highest potential to inhibit the mycelium growth of *F. oxysporum* followed by Black night shade extract ($R^2 = 0.732$) and Moringa leaf

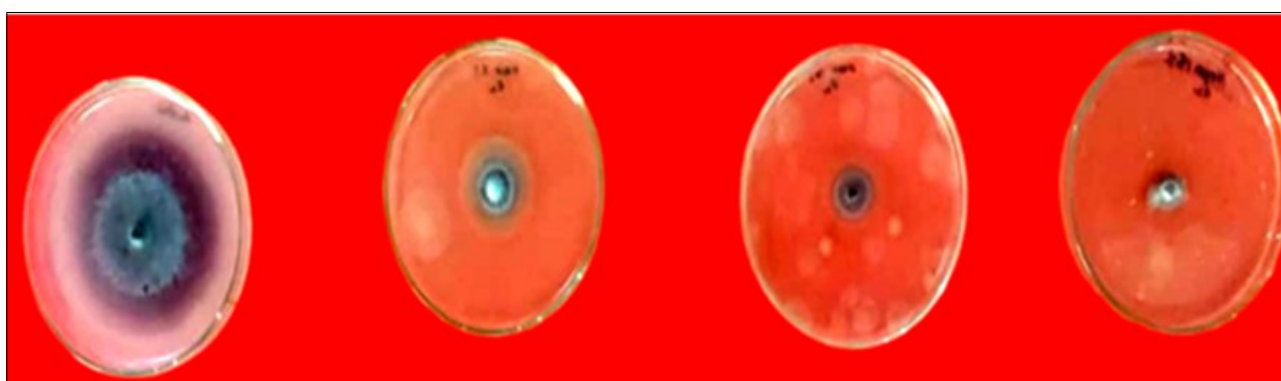


Fig. 5. Mycelium growth of *Fusarium oxysporum* on PDA media with neem leaf extract on 7th DAI (T₀ = Control, T₁ = 5 %, T₂ = 10 %, T₃ = 15 %).



Fig. 6. Mycelium growth of *Fusarium oxysporum* on PDA media with black Nightshade extract on 7th DAI (T₀ = Control, T₁ = 5 %, T₂ = 10 %, T₃ = 15 %).

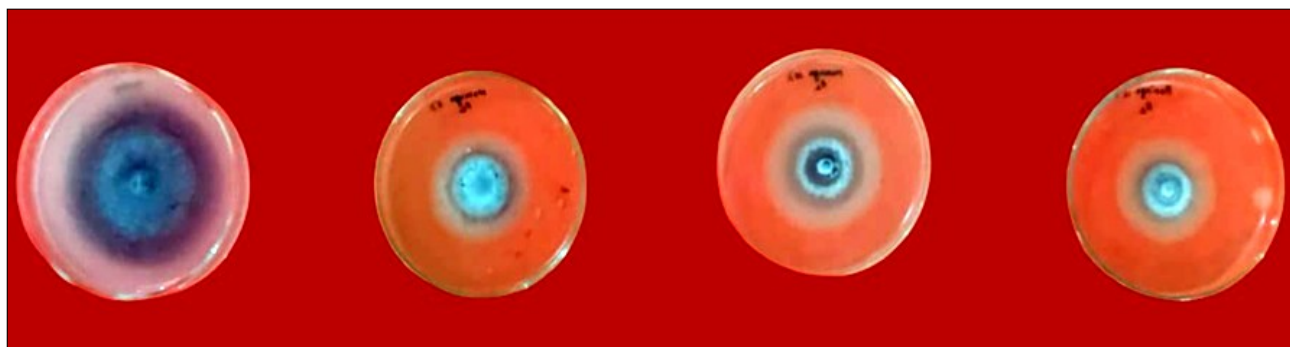


Fig. 7. Mycelium growth of *Fusarium oxysporum* on PDA media with moringa leaf extract on 7th DAI (T_0 = Control, T_1 = 5 %, T_2 = 10 %, T_3 = 15 %).

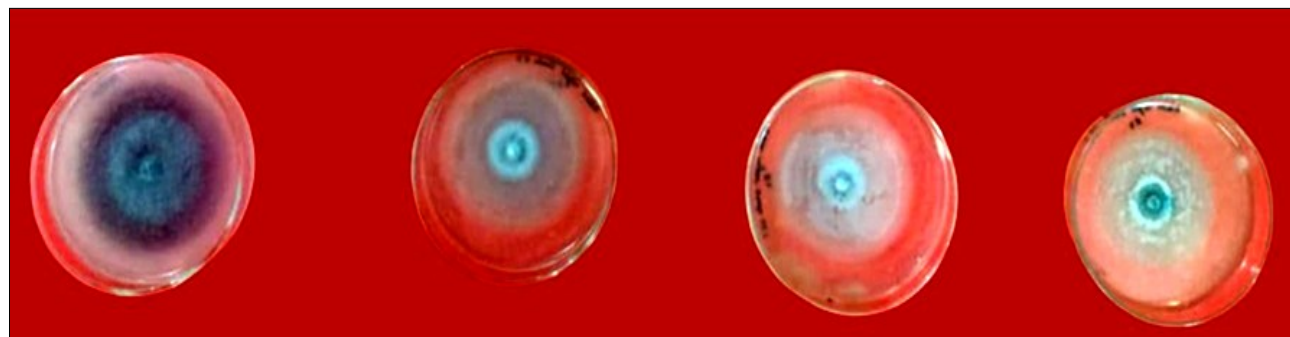


Fig. 8. Mycelium growth of *Fusarium oxysporum* on PDA media with ageratum weed extract on 7th DAI (T_0 = Control, T_1 = 5 %, T_2 = 10 %, T_3 = 15 %).

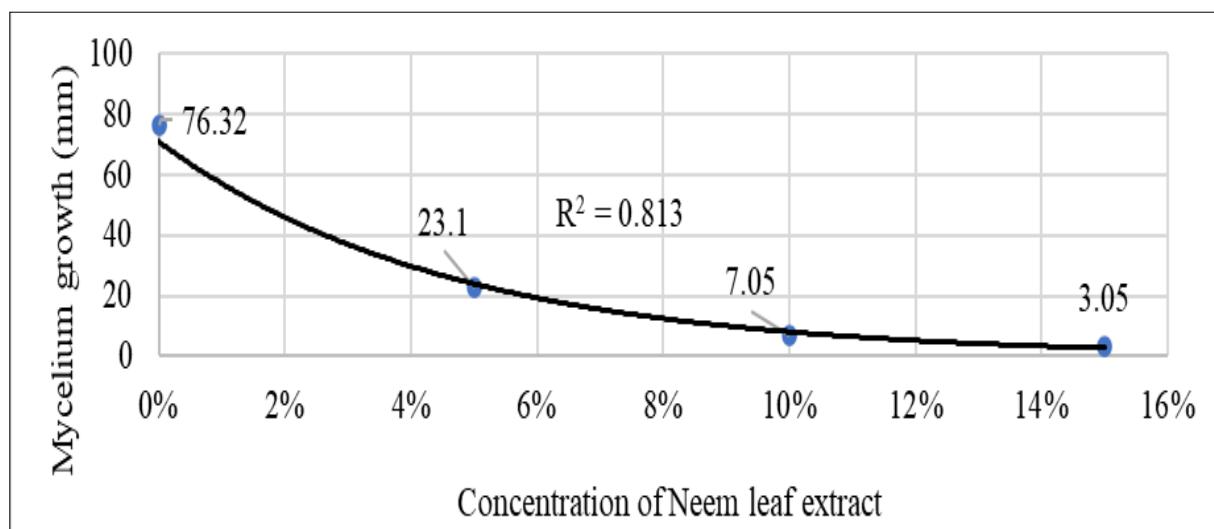


Fig. 9. Correlation between mycelium growth of *Fusarium oxysporum* and concentration of neem leaf extract.

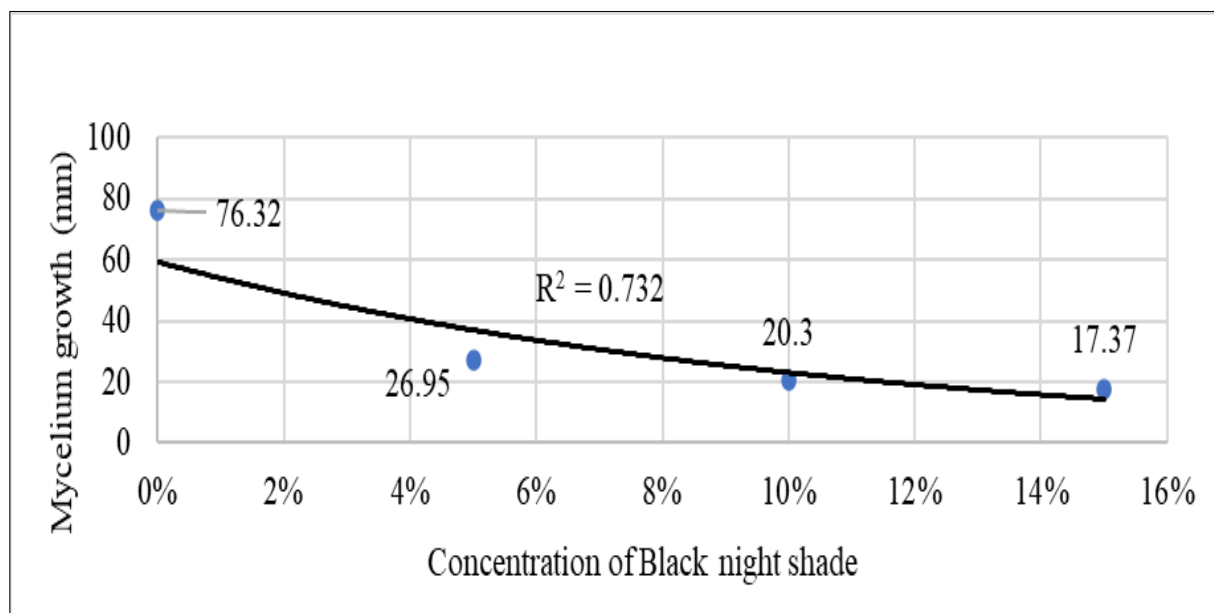


Fig. 10. Correlation between mycelium growth of *Fusarium oxysporum* and concentration of Black night shade leaf extract.

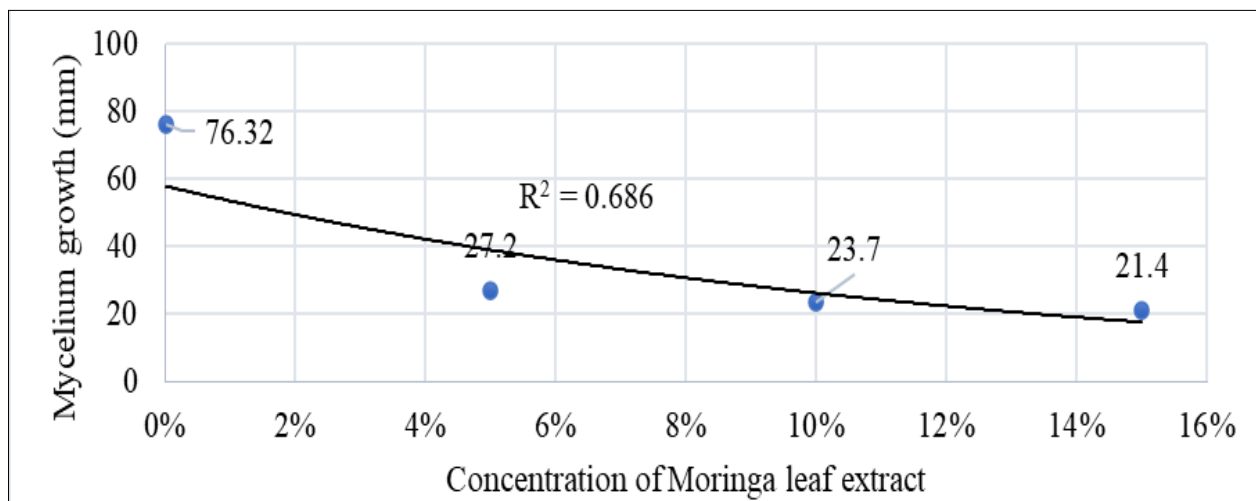


Fig. 11. Correlation between mycelium growth of *Fusarium oxysporum* and concentration of moringa leaf extract.

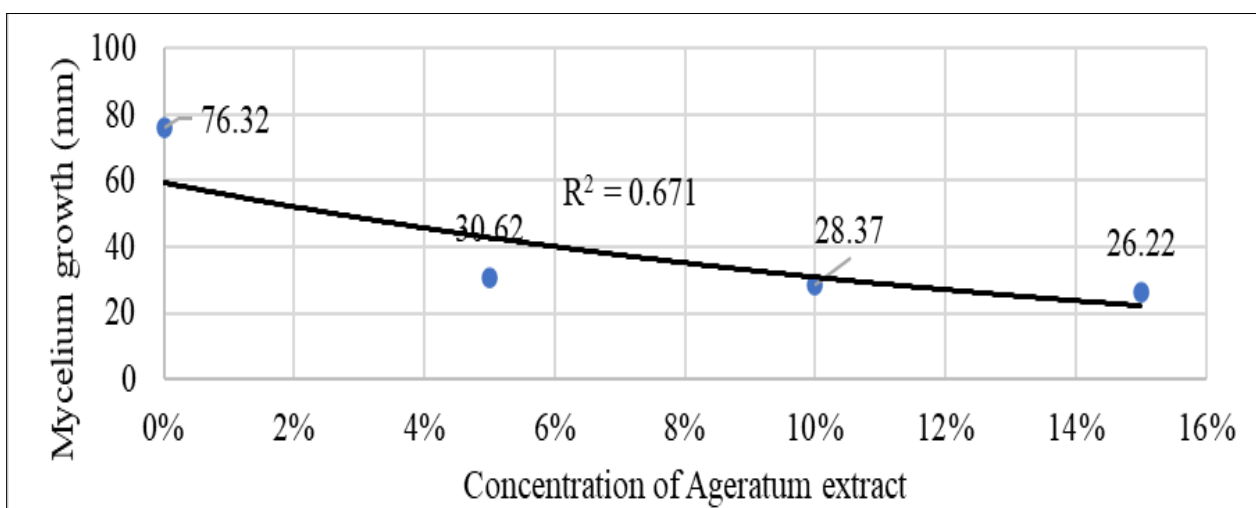


Fig. 12. Correlation between mycelium growth of *Fusarium oxysporum* and concentration of ageratum weed extract.

extract ($R^2 = 0.686$). The lowest R^2 value was observed in Ageratum weed extract ($R^2 = 0.671$), indicating that it has the least potential to inhibit mycelium growth among all the studied plant extracts.

The study highlights the growing interest in plant-based antifungal agents as safer and more eco-friendly alternatives to chemical fungicides. Among the tested botanical extracts, Neem (*Azadirachta indica*) leaf extract showed the highest mycelial inhibition (96 %) of *F. oxysporum* at 15 % concentration on the 7th day after inoculation. This was followed by Black Nightshade (*Solanum indicum*) and Moringa (*Moringa oleifera*), while Ageratum weed (*Ageratum conyzoides*) showed the least inhibition. A consistent trend of increasing antifungal activity with higher extract concentrations was observed.

Previous studies support these findings: Neems' efficacy is attributed to compounds like azadirachtin and has shown similar inhibition rates in another research (22, 23). Black Nightshade contains quercetin, a potent antioxidant and has demonstrated strong antifungal activity (24–26). Moringas' antifungal effect is linked to benzyl isocyanate (10). Although Ageratum showed lower inhibition in earlier studies, the higher inhibition in this study (62.82 % at 10 %) may be due to the combined use of all plant parts for extract preparation. Overall, the study reinforces the potential of phytochemicals in fungal disease management.

Conclusion

In the *in-vitro* assessment of biocontrol agents (*Trichoderma viride* and *Trichoderma harzianum*) against *F. oxysporum*, *T. harzianum* exhibited the highest mycelial inhibition. Similarly, among the tested plant extracts at different concentrations (5 % 10 % and 15 %), neem leaf extract proved to be the most effective in suppressing fungal growth across all concentrations. A distinct pattern was observed such as mycelial growth decreased as extract concentration increased, indicating a dose-dependent antifungal effect. Although the study demonstrates the potential of both plant extracts and biocontrol agents in managing *F. oxysporum* and guava wilt in the Prayagraj region, the short duration of the trial limits its applicability for practical disease management. These preliminary findings offer valuable insights but require further in-depth research and extended trials to confirm their reliability and to develop concrete, field-ready recommendations.

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Authors' contributions

SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. AAL and SM managed the analyses of the study. NKA managed the literature searches, modified and finalised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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