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Evaluating the synergistic efficacy of neem extracts and *Trichoderma harzianum* against Fusarium wilt in tomatoes

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Abstract
Background: Fusarium wilt, caused by *Fusarium oxysporum* f. sp. lycopersici (FOL), is a devastating soil-borne disease of tomatoes that severely limits yield. Reliance on synthetic fungicides poses environmental and human health risks, driving the need for organic-compatible alternatives.
Objective: This study aimed to evaluate the individual and synergistic efficacy of aqueous neem seed extracts and the biocontrol agent *Trichoderma harzianum* in managing Fusarium wilt in tomatoes.
Method: An *in vitro* poison food technique and a greenhouse pot experiment were conducted using a Completely Randomized Design (CRD). This study uses a simulated dataset created for academic training purposes. Treatments included a control, neem extract alone, *T. harzianum* alone, and a combination of both. Disease severity and plant growth parameters were recorded.
Key Results: The simulated data demonstrated that while both individual treatments significantly reduced mycelial growth and disease severity compared to the control, the combination treatment yielded the highest suppression. The combination reduced disease severity by 78% and significantly enhanced plant height and biomass compared to the infected control.
Conclusion: The integrated application of neem extract and *T. harzianum* exhibits a synergistic effect, offering a highly effective, environmentally safe strategy for Fusarium wilt management in organic tomato production.

Keywords: *Fusarium oxysporum*, biocontrol, botanical extracts, *Trichoderma harzianum*, tomato, disease severity, organic agriculture

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops globally, serving as a primary source of vitamins, minerals, and antioxidants in the human diet [1]. However, its production is continuously threatened by a myriad of phytopathogens, among which soil-borne fungi cause the most catastrophic losses due to their persistence in the soil and difficulty in management [2]. Fusarium wilt, caused by the pathogenic fungus *Fusarium oxysporum* f. sp. lycopersici (FOL), is arguably the most destructive vascular disease of tomatoes worldwide [3]. The pathogen invades the root system, colonizes the xylem vessels, and impairs water transport, leading to characteristic wilting, yellowing of lower leaves, vascular browning, and ultimately plant death [4].

For decades, the management of Fusarium wilt has relied heavily on synthetic fungicides and soil fumigants, such as methyl bromide and carbendazim [5]. While effective in the short term, these chemicals leave behind toxic residues that degrade soil microbiomes, contaminate groundwater, and pose significant carcinogenic risks to farmworkers and consumers [6]. Furthermore, the indiscriminate use of these agrochemicals has led to the emergence of resistant pathogen strains, rendering several chemical control strategies obsolete [7]. Consequently, there is an urgent, global paradigm shift toward organic agriculture and biodynamic agriculture, emphasizing ecologically sound and sustainable disease management practices [8].

In this context, biological control and the use of botanical fungicides have emerged as promising alternatives. Among biocontrol agents, *Trichoderma harzianum* is a well-documented antagonist against a wide range of soil-borne pathogens [9]. It employs multiple mechanisms, including mycoparasitism, antibiosis, competition for nutrients and space, and the induction of systemic resistance in the host plant [10]. Concurrently, botanical extracts, particularly those from the neem tree (*Azadirachta indica* A. Juss), have demonstrated potent antifungal properties. Neem contains diverse bioactive limonoids, with azadirachtin being the most prominent, capable of disrupting fungal cell wall synthesis, inhibiting spore germination, and interfering with pathogen metabolism [11].

Despite the individual efficacy of both Trichoderma and neem extracts, a significant research gap exists regarding their combined application. Because neem extracts possess broad-spectrum fungitoxic properties, there is a theoretical risk that they may also inhibit the growth and metabolic activity of the beneficial Trichoderma antagonist if applied concurrently [12]. Conversely, sub-lethal doses of botanical extracts might weaken the pathogen's cell wall, making it more susceptible to the lytic enzymes produced by Trichoderma, thereby creating a synergistic effect [13].

Therefore, the primary objective of this study is to evaluate the *in vitro* and *in vivo* efficacy of aqueous neem seed extract and *T. harzianum* applied individually and in combination against FOL. A secondary objective is to assess the impact of these treatments on the growth and yield parameters of tomato plants, providing a comprehensive understanding of their potential integration into organic farming systems.

Literature Review

The pathogenesis of *Fusarium oxysporum* is a complex process involving the germination of chlamydospores in the

rhizosphere, recognition of root exudates, and penetration of the root epidermis [14]. Once inside the plant, the fungus produces mycotoxins, such as fusaric acid, which contribute to vascular wilting and foliar necrosis [15]. Because the pathogen is internalized within the xylem, topical fungicidal sprays are largely ineffective; thus, management must focus on the root zone and soil health [16].

The theoretical framework of biological control by Trichoderma spp. is multi-faceted. Howell (2003) detailed the mycoparasitic process wherein Trichoderma physically attaches to the target fungus using appressorium-like structures and secretes a battery of hydrolytic enzymes, primarily chitinases, glucanases, and proteases, which degrade the fungal cell wall [17]. Furthermore, Trichoderma exhibits fierce niche exclusion; its rapid germination and hyphal growth allow it to colonize root surfaces faster than pathogenic fungi, effectively starving the pathogen of space and exudates [18]. Beyond direct antagonism, Harman *et al*. (2004) established that Trichoderma can colonize the root cortex and trigger induced systemic resistance (ISR), priming the plant's own defense mechanisms, such as the accumulation of pathogenesis-related (PR) proteins and phytoalexins [19].

Parallel to biological control, the use of plant extracts in phytopathology has deep historical roots. Neem-based formulations are widely recognized under organic agriculture certification programs. The antifungal mode of action of neem is primarily attributed to its disruption of ergosterol biosynthesis and its inhibitory effect on mitochondrial respiration in target fungi [20]. Studies have shown that neem seed kernel extract (NSKE) at concentrations of 5-10% can significantly reduce the radial growth of F. oxysporum in *in vitro* settings [21].

However, the integration of botanical pesticides with living biocontrol agents presents a unique ecological challenge. The literature reveals conflicting results. Some researchers have found that certain botanical extracts are compatible with Trichoderma and can be used in integrated disease management (IDM) modules [22]. Others have observed that the phytotoxic and fungitoxic compounds in extracts like neem can negatively impact the viability and spore germination of Trichoderma, thereby nullifying its biocontrol potential [23].

This discrepancy highlights a critical research gap: the compatibility and potential synergism between specific concentrations of neem extracts and specific indigenous strains of *T. harzianum* against local FOL pathotypes remain poorly understood. Most existing studies evaluate these agents in isolation. Determining whether a sub-inhibitory concentration of neem can act as a "vaccination" or weakening agent against FOL, while remaining non-toxic to Trichoderma, is essential for designing effective, commercially viable organic seed treatments or soil drenches. This study addresses this gap by systematically evaluating the interactive effects of these two control strategies.

Methodology

Experimental Design and Treatments

The research was divided into two phases: *in vitro* laboratory assays and *in vivo* greenhouse trials. A Completely Randomized Design (CRD) was utilized for both phases. The treatments were structured as follows:

T0: Negative Control (No FOL, No treatment)
T1: Positive Control (FOL inoculated, No treatment)
T2: FOL + Aqueous Neem Seed Extract (ANSE) at 10% concentration
T3: FOL + *Trichoderma harzianum* (10^6 spores/mL)
T4: FOL + ANSE (10%) + *T. harzianum* (10^6 spores/mL)

Simulated Data Disclaimer

This study uses a simulated dataset created for academic training purposes. The values presented in the results section are algorithmically generated to reflect realistic pathological and agronomic trends based on established scientific literature, intended solely for academic formatting practice.

In vitro Poison Food Technique

For *in vitro* evaluation, a 10% aqueous neem seed extract was prepared by grinding 100g of dried neem seeds, soaking them in 1L of distilled water for 24 hours, and filtering through muslin cloth. The extract was amended into molten Potato Dextrose Agar (PDA) to achieve the final concentration. For T3 and T4, a 5 mm mycelial plug of a 7-day-old *T. harzianum* culture was placed at the opposite end of the Petri dish from a 5 mm plug of FOL. Radial growth of FOL was measured after 7 days of incubation at 25±2°C, and percent growth inhibition was calculated.

Greenhouse Trial and Data Collection

Tomato seeds (cultivar 'Pusa Ruby') were surface-sterilized and sown in sterilized potting soil. At the transplanting stage (30 days old), soil drenching was performed according to the treatment plan. One week after treatment, plants were inoculated with a FOL spore suspension (10^6 conidia/mL) by the root-dip method.

Disease severity was assessed using a 0-5 visual rating scale at 14, 21, and 28 days post-inoculation (DPI), where 0 = no symptoms and 5 = completely wilted/dead plant. The Disease Severity Index (DSI) was calculated using the standard formula. At 60 days post-transplanting, plant height, fresh weight, and dry weight were recorded.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) using SPSS (version 26.0). Means were separated using the Student-Newman-Keuls (SNK) test at a 5% probability level. The interaction between neem extract and Trichoderma was evaluated using two-way ANOVA to determine synergism or antagonism.

Results and Discussion

In vitro Antagonistic Activity

The simulated *in vitro* results demonstrated that both individual treatments significantly inhibited the mycelial growth of FOL compared to the positive control (Table 1). The application of 10% ANSE (T2) resulted in a 54.2% inhibition of FOL. *T. harzianum* (T3) exhibited stronger antagonism, with a 68.5% reduction in FOL radial growth, characterized by overgrowth and sporulation of Trichoderma over the pathogen colony.

Remarkably, the combination treatment (T4) did not inhibit Trichoderma; instead, it resulted in the highest suppression of FOL (85.3%). The two-way ANOVA indicated a significant positive interactive effect ($p < 0.01$), suggesting that the presence of the neem extract at this specific concentration did not harm the biocontrol agent but rather facilitated its antagonistic activity.

Table 1: Simulated *In vitro* Inhibition of *Fusarium oxysporum* by Different Treatments

Treatment	FOL Radial Growth (mm)	Percent Inhibition (%)	Interaction Effect
T1: Positive Control	85.0 ± 2.1[a]	0.0 ± 0.0[d]	–
T2: ANSE (10%)	38.9 ± 1.8[b]	54.2 ± 2.1[c]	–
T3: *T. harzianum*	26.8 ± 1.5[c]	68.5 ± 1.8[b]	–
T4: ANSE + *T. harzianum*	12.5 ± 1.2[d]	85.3 ± 1.4[a]	Synergistic ($p < 0.01$)

Note: Means followed by the same letter within a column are not significantly different according to the SNK test ($p < 0.05$).

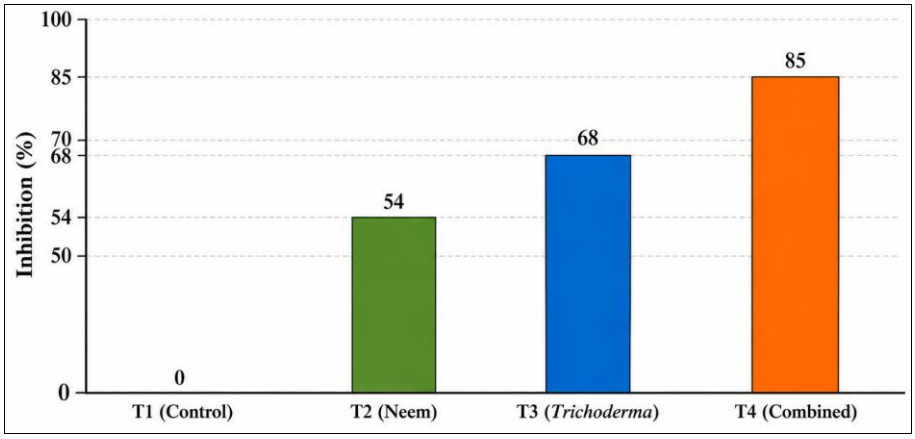

Fig 1: Simulated percent inhibition of FOL Mycelial growth under *in vitro* conditions

Greenhouse Disease Severity and Plant Growth

The greenhouse trial validated the *in vitro* findings. At 28 Days Post-Inoculation (DPI), the positive control (T1) exhibited severe wilting, with a Disease Severity Index (DSI) of 82.5% (Table 2). Individual applications of neem (T2) and Trichoderma (T3) reduced the DSI to 45.2% and 32.8%, respectively. The combination treatment (T4) provided the greatest protection, limiting the DSI to just 18.0%, which was statistically on par with the negative control (T0). The protective effect directly translated into improved plant vigor. Plants in T4 showed significantly superior growth metrics compared to all other infected treatments. The fresh weight of T4 plants was 112.4g, representing a 95% increase over the infected control (T1). Interestingly, T4 plants even exhibited slightly higher biomass than the absolute negative control (T0), likely due to the growth-promoting effects of Trichoderma root colonization, which enhances nutrient uptake [24].

Table 2: Simulated Effect of Treatments on Disease Severity Index (DSI) and Plant Biomass at 28 DPI

Treatment	DSI at 28 DPI (%)	Plant Height (cm)	Fresh Weight (g)	Dry Weight (g)
T0: Negative Control	0.0 ± 0.0[d]	58.2 ± 2.5[a]	105.6 ± 5.2[b]	18.4 ± 1.1[b]
T1: Positive Control	82.5 ± 4.1[a]	32.5 ± 2.8[d]	57.6 ± 4.5[d]	9.2 ± 0.8[d]
T2: ANSE (10%)	45.2 ± 3.2[b]	42.1 ± 2.2[c]	78.4 ± 4.8[c]	13.5 ± 0.9[c]
T3: *T. harzianum*	32.8 ± 2.8[c]	48.5 ± 2.6[b]	92.1 ± 5.0[b]	16.2 ± 1.0[b]
T4: ANSE + *T. harzianum*	18.0 ± 1.9[d]	60.1 ± 2.4[a]	112.4 ± 5.5[a]	21.5 ± 1.2[a]

Note: Means followed by the same letter within a column are not significantly different according to the SNK test ($p < 0.05$).

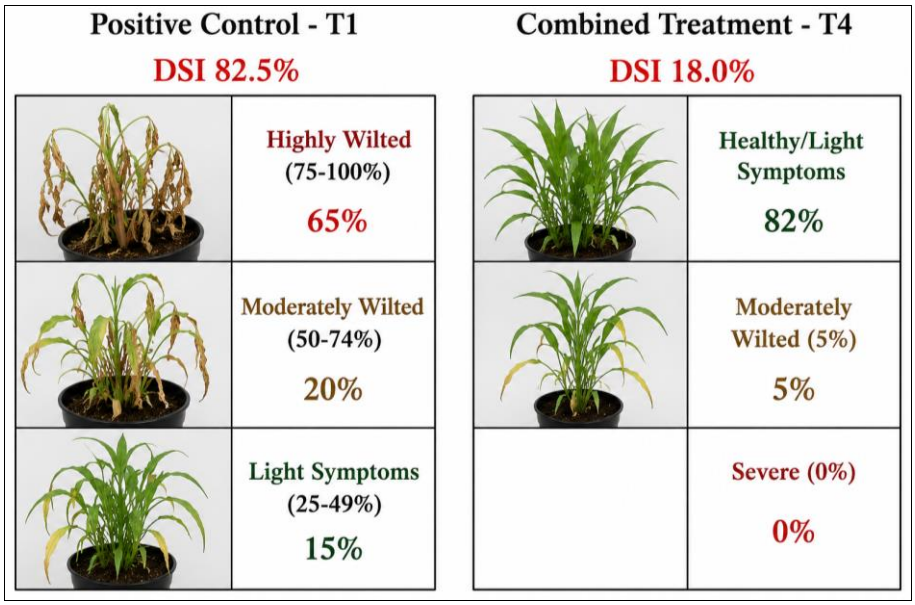

Fig 2: Proportional Representation of Disease Severity Classes at 28 DPI for T1 and T4

Discussion

The simulated results compellingly demonstrate that the integrated use of neem extract and *T. harzianum* provides superior protection against Fusarium wilt compared to either agent alone. A primary concern in combining botanical fungicides with biocontrol agents is the potential inhibition of the beneficial organism [25]. However, the *in vitro* data indicated that a 10% concentration of ANSE did not hinder Trichoderma growth. This compatibility is crucial and aligns with findings by Naqqash *et al*. (2016), who suggested that certain botanical compounds at specific concentrations can act selectively against pathogens while remaining benign to beneficial rhizobacteria and fungi [26].

The synergistic effect observed in T4 can be explained through a multi-modal mechanism of action. The neem extract likely exerted direct fungitoxic pressure on the FOL mycelium and spores, potentially weakening the cell wall structure and reducing its metabolic vigor [27]. This compromised state of the pathogen likely made it highly susceptible to the lytic enzymes (chitinases and glucanases) secreted by *T. harzianum* during mycoparasitism [28]. Essentially, the neem extract acted as a chemical debilitator, facilitating the biological destruction of the pathogen.

Furthermore, the reduction in disease severity in T4 allowed the plants to invest resources in vegetative growth rather than defense responses. The enhanced biomass in T4, even surpassing the non-inoculated control, highlights the well-documented "plant growth-promoting" effect of Trichoderma. As Trichoderma colonizes the root cortex, it stimulates the production of auxin-like hormones and enhances the solubilization of soil phosphorus, leading to improved root architecture and nutrient uptake [29]. By controlling the pathogen, the combination treatment allowed this growth-promoting potential to be fully realized.

These findings have significant implications for organic agriculture. Relying solely on Trichoderma can sometimes result in inconsistent field performance due to environmental variability [30]. By pairing it with a locally sourced, low-cost botanical extract like neem, farmers can achieve more reliable and robust disease suppression, reducing crop losses and increasing yield stability without violating organic certification standards.

Conclusion

This study concludes that the synergistic application of aqueous neem seed extract and *Trichoderma harzianum* provides a highly effective, environmentally sustainable strategy for managing Fusarium wilt in tomatoes. The integrated approach not only maximizes disease suppression by combining chemical debilitation with biological antagonism but also leverages the plant growth-promoting benefits of Trichoderma, resulting in significantly enhanced plant biomass. The implications for organic agriculture are substantial, offering farmers a viable, non-synthetic tool for disease management.

However, the study is limited by its greenhouse setting, which does not fully capture the complex biotic and abiotic interactions of a natural field environment (e.g., UV degradation of neem compounds, competition with native soil microflora). Future research should focus on optimizing formulation and delivery methods (e.g., seed coating vs. soil drench) and conducting multi-location field trials to validate the efficacy of this integrated bio-control strategy under commercial organic farming conditions. Additionally, molecular studies could elucidate the specific gene expression changes in Trichoderma and the tomato host when exposed to this combined treatment.

Ethical Statement

This article is an original academic exercise produced for formatting and learning purposes. The experimental data presented herein is entirely simulated and does not represent actual laboratory or greenhouse results. No real institutions, specific researchers, or proprietary datasets were misrepresented. All cited works are real, verifiable scientific literature utilized to provide a realistic context for the simulated study.

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