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Research Article

BIOCIDAL FUNCTION OF *TRICHODERMA*-DERIVED SECONDARY METABOLITES AGAINST FUSARIUM WILT OF PEA

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ABSTRACT

The current research investigates the potential of secondary metabolites extracted from *Trichoderma* species for managing Fusarium wilt in pea plants. Crude extracts of secondary metabolites from *T. viride* and *T. harzianum* were obtained using ethyl acetate and methanol through solvent extraction methods and evaluated for their antifungal activity against *Fusarium oxysporum* f.sp. *pisi* under various experimental conditions. *In vitro* assays were performed using the poisoned food technique on potato dextrose agar (PDA) medium, employing Completely Randomized Design (CRD) with three concentrations of secondary metabolites (250 ppm, 350 ppm, and 500 ppm). Greenhouse experiments were also conducted in a CRD setup, where pea plants were inoculated with a spore suspension (1×10^6 spores/ml) of the pathogen and treated with different concentrations of metabolites through soil drenching. For field trials, a Randomized Complete Block Design was utilized to evaluate the most effective concentration (500 ppm) of metabolites, applied via hand sprayer and soil drenching. Results showed that the combination of *T. viride* and *T. harzianum* metabolites significantly reduced mycelial growth (10.11 mm) under laboratory conditions and decreased disease incidence to 27.08% in greenhouse trials and 28.15% in field trials. These findings highlight the potential of *Trichoderma*-derived secondary metabolites as a sustainable approach for managing Fusarium wilt in pea plants, offering promising prospects for integrated disease management strategies.

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INTRODUCTION

Pea (*Pisum sativum* L.), belonging to the botanical family Leguminosae, is a globally significant vegetable crop (Husnain et al., 2019). This short-duration, self-pollinating crop thrives primarily in temperate regions worldwide (Sirwaiya et al., 2018). Peas are a nutrient-rich food source, offering proteins with essential amino acids (tryptophan and lysine), carbohydrates, vitamin B, dietary fiber, key minerals (calcium, potassium, and iron), and starch (Atiq et al., 2016). Additionally, peas boast a rich phytochemical profile, containing a variety of compounds such as catechins, and acids like coumaric, caffeic, vanillic, and ferulic acids, along with pisatin, protocatechuic acid, proanthocyanidins, steroids, phytohormones, and tannins. These compounds exhibit antioxidant properties, contributing to the prevention of

diseases such as cancer and cardiovascular ailments (Dai and Mumper, 2010; Bello et al., 2018).

Pea production is influenced by both biotic (fungi, bacteria, nematodes) and abiotic (stress, injury, temperature) factors, with fungal pathogens being the primary constraints to successful production (Chaudhary et al., 2021). Significant diseases impacting pea production worldwide include damping-off, fusarium wilt, powdery mildew, ascochyta blight, root-knot nematode, and rust (Khulbe and Sharma, 2020). Among these, pea wilt caused by *Fusarium oxysporum* f.sp. *pisi* (Fop) is particularly devastating, leading to yield losses of up to 100% under favorable environmental conditions, severely affecting both the quality and quantity of peas (El-Sharkawy et al., 2021).

Fusarium wilt is challenging to manage due to the presence of resistant chlamydospores that can persist in the soil for extended periods, even in the absence of a host plant. Various strategies are employed to manage Fusarium wilt in peas, including the use of chemicals, plant extracts, biocontrol agents, nanoparticles, and plant defense activators (Mukhtar et al., 2013, 2023). The most reliable method for managing plant diseases is the use of resistant varieties (Jimenez-Diaz et al., 1993; Jagtap et al., 2012). Although the use of resistant varieties is undoubtedly the most effective approach, in cases where the disease reaches epidemic levels, farmers often resort to synthetic chemicals due to their rapid action and direct impact on the targeted pathogen (Pancholi et al., 2022). However, although these chemicals act quickly, they have significant drawbacks, including their lack of eco-friendliness and potential negative effects on human health. Furthermore, the excessive use of synthetic chemicals can lead to the development of pathogen resistance, making these chemicals less effective over time (Segaran and Sathiavelu, 2019).

Scientists are increasingly focused on developing eco-friendly management strategies due to the residual effects of synthetic chemicals. In this context, the use of biocontrol agents (BCAs) has emerged as an effective method for managing various pathogenic diseases caused by bacteria, fungi, and nematodes (Tariq et al., 2020). These BCAs are non-toxic and easily target specific pathogens (Sharma et al., 2013). Among these, *Trichoderma* stands out as a significant fungus widely employed as a biocontrol agent in plant disease management (Iqbal and Mukhtar, 2020; Azeem et al., 2021; Mukhtar et al., 2021). This saprophytic fungus, with a global distribution, is renowned for synthesizing diverse compounds with potent antimicrobial activity (Gonzalez et al., 2020).

The symbiotic association of *Trichoderma* enhances plant resistance against pathogens by secreting compounds such as peptides, proteins, and alkaloids. These substances not only improve plant growth and yield but also facilitate nutrient absorption, thereby promoting overall plant health (Sood et al., 2020; Mukhtar, 2018). However, changing climatic conditions can adversely affect the activity of antagonistic organisms, potentially reducing their effectiveness in managing plant diseases (Jacobsen et al., 2020).

Trichoderma produces a variety of low molecular weight secondary metabolites, including polyketides, nonribosomal peptides, terpenoids, nafuredins, pyrones, peptaibols, alkaloids, and flavonoids. These compounds have been shown to inhibit the growth of fungal and bacterial pathogens (Buddhika and Abeysinghe, 2021; Maheshwary et al., 2022). Although the potential of *Trichoderma* species as biocontrol agents has been extensively studied, most research has focused on their general efficacy against a range of plant pathogens (Maurya et al., 2024).

However, there remains a significant gap in understanding the specific metabolites produced by *Trichoderma* spp. and their precise effects against *Fusarium oxysporum* f.sp. *pisi* (*Fop*). Although previous studies have identified various bioactive compounds, including polyketides, peptaibols, and alkaloids, which contribute to the antagonistic activity of *Trichoderma*, detailed insights into their individual and synergistic effects are still lacking (Singh et al., 2024). This includes examining how these metabolites function under different conditions, such as in laboratory, greenhouse, and field settings. This study, therefore, aims to bridge these knowledge gaps by thoroughly analyzing the secondary metabolites of *Trichoderma* spp. and their specific biocidal effects on *Fop*.

MATERIALS AND METHODS

Survey and Sample Collection

A survey was conducted in pea fields across district Faisalabad to collect diseased plant samples, specifically focusing on roots. Samples were gathered from several locations, including the vegetable area at AARI (Latitude: 31.3981 N, Longitude: 73.0503 E), the Horticulture field

at UAF (Latitude: 31.4341 N, Longitude: 73.0769 E), Chak # 281 RB (Latitude: 31.2180 N, Longitude: 72.8240 E), Chak # 76 JB (Latitude: 31.3279 N, Longitude: 72.8843 E), and Chak # 227 RB (Latitude: 31.4071 N, Longitude: 73.2103 E) within the Faisalabad region (Figure 1). The collected samples were carefully placed in 14×22 cm brown paper bags. Each bag was labeled with the collection time and location before being transported to the Plant Pathology Laboratory, UAF for pathogen isolation.

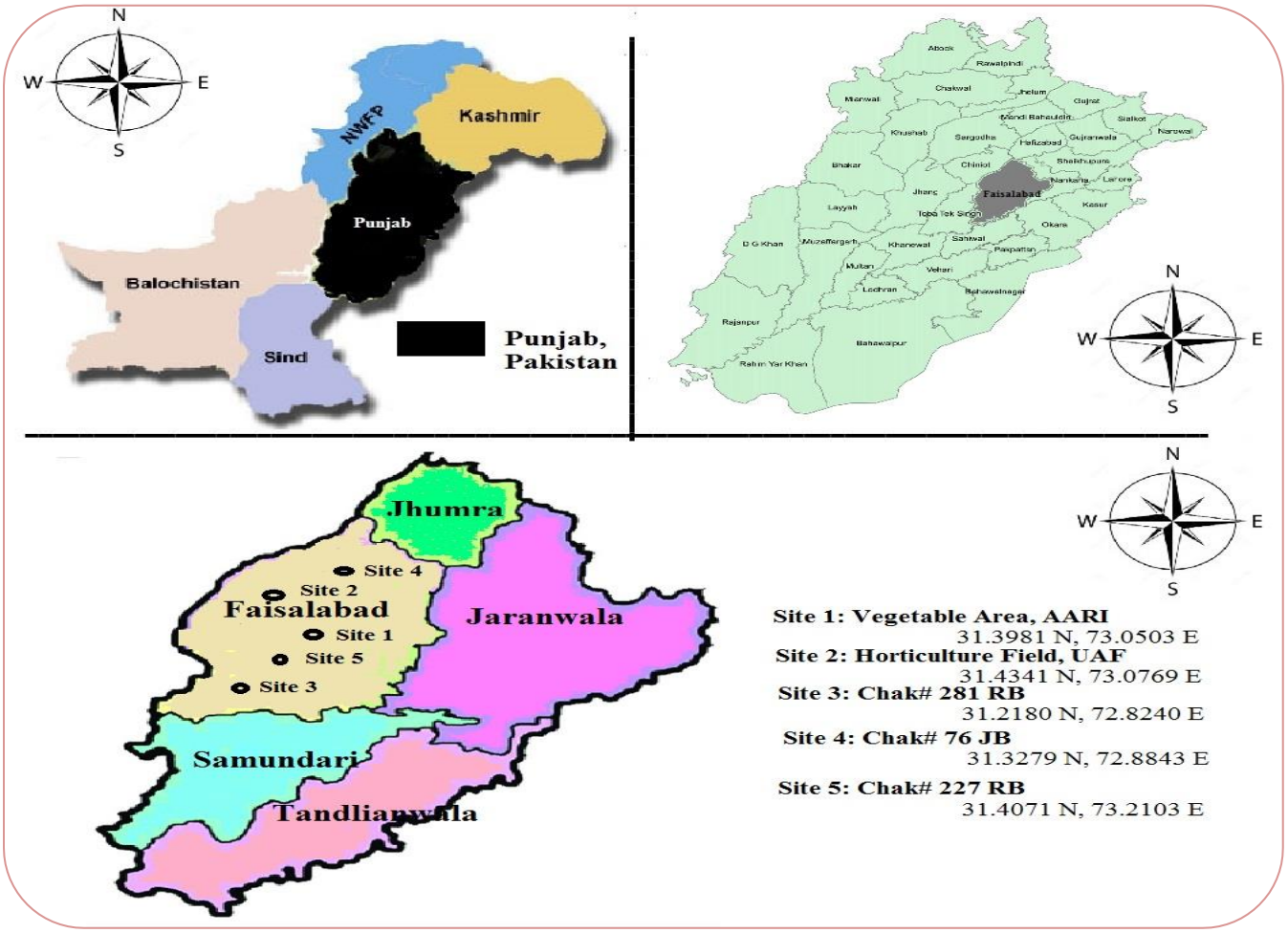

Figure 1. Survey of pea fields in district Faisalabad for collecting *Fusarium* wilt-infected samples.

Isolation and purification of pathogen

Potato dextrose agar (PDA) was used to isolate the fungal pathogen (Ali et al., 2024). Infected root samples were first washed with tap water to remove soil debris. The samples were then cut into smaller pieces (4-5 mm) using sterilized scissors. These pieces were surface-sterilized with 1% sodium hypochlorite (NaOCl) solution for 30 sec, followed by three consecutive rinses with sterilized distilled water to eliminate any residual sodium hypochlorite. The samples were subsequently dried on sterilized filter paper.

Approximately 3-5 ml of PDA was poured into sterilized Petri dishes (90 mm). The Petri dishes were then opened, and 3-5 pieces of the diseased samples were placed onto the PDA using sterilized forceps. The dishes were sealed with paraffin tape to prevent contamination. All procedures were conducted within a laminar flow chamber (Robus Technology, UK) to maintain sterility. The Petri dishes were incubated at 25±5°C, and fungal growth was observed after 2-3 days (Rehman et al., 2024).

The fungal culture was purified using the hyphal tip method. A single hypha was carefully picked from the edge of the growing fungal colony using a sterilized needle and transferred to a new Petri dish containing fresh PDA. The dish was then sealed with paraffin tape and incubated at 25°C. Fungal growth was observed after 3-5 days.

Identification and preservation

The fungal pathogen was identified based on its morphological characteristics, including colony color, growth pattern, and type of sporulation (Sun et al., 2018). For identification, temporary slides were prepared using fresh culture plates. A small amount of the fungal colony was carefully picked with a sterilized needle and placed in a drop of water on the slide. A cover slip was then gently placed on top to avoid trapping air bubbles. The prepared slides were observed under a light microscope at 4×, 10×, and 40× magnifications.

To preserve the pathogen, PDA medium was poured into test tubes and allowed to solidify in a slanted position, providing a larger surface area for pathogen growth. A pure culture was transferred into the test tubes and incubated at 25±2°C for a few days. The culture tubes were labeled and stored at -4°C to maintain viability for maximum preservation time.

Pathogenicity test

Koch's postulates were followed to test pathogenicity. For this purpose, sandy loam soil was sterilized using formalin and filled into pots (13×15 cm) as the growth medium. Seeds of the susceptible pea variety 'Meteor' were sown. A spore suspension (1×10^6 spores/ml) was prepared from a 7-day-old mature culture using a hemocytometer (Model T20B05) (Aberkane et al., 2002). After the seedlings were established, the spore suspension was applied to the pea plants through the soil drenching method. One week later, wilting symptoms appeared. The pathogen was then isolated from the infected plant (root) samples and compared with the original pathogen based on morphological and taxonomical characteristics.

Extraction of secondary metabolites from *Trichoderma* species

Trichoderma species (*T. viride* and *T. harzianum*) were obtained from the Department of Microbiology, the University of Agriculture, Faisalabad. To extract crude secondary metabolites, these strains were cultured on PDA plates and incubated at 28°C for three days to encourage fungal growth. Agar plugs containing fungal mycelium were then transferred to 400 ml of Potato Dextrose Broth (PDB) in a 500 ml volumetric flask. The flasks were incubated under static conditions at 28°C for 30 days. The culture was filtered through gauze and Whatman filter paper (125 mm) to separate the mycelial biomass from the aqueous phase.

The filtrate was extracted twice with ethyl acetate ($C_4H_8O_2$) in a 1:1 (v/v) solvent-to-sample ratio. The mycelial biomass was mechanically disrupted using a pestle and mortar, followed by ultrasonic disruption for 10 min. This was followed by an extraction using a dichloromethane (CH_2Cl_2) and methanol (CH_3OH) mixture in a 1:1 (v/v) ratio. After removing the dichloromethane and methanol via rotary evaporation, the remaining aqueous phase was extracted three times with ethyl acetate.

The total ethyl acetate extract was obtained by concentrating the combined extracts under reduced pressure. Solvent extraction was performed using equal volumes of ethyl acetate and methanol (1:1 v/v). The mixture was thoroughly agitated for 10 min and then allowed to separate into two distinct layers for 5 min. The upper layer, containing the extracted compounds, was separated using a separating funnel. The solvent was evaporated, and the resultant compound was dried using a rotary vacuum evaporator, yielding the crude metabolite (Figure 2) (Zhao et al., 2002; Bhardwaj et al., 2015).

To prepare the media, 1 L of PDA was prepared in a 1000 ml media bottle. Stock solutions of the desired concentrations (250 ppm, 350 ppm, and 500 ppm) were then prepared from the crude metabolites extract. Specifically, 1 ml of the crude secondary metabolite extract was added to 100 ml of distilled water to create the stock solutions. To achieve the final concentrations, 2.5 ml, 3.5 ml, and 5 ml of the stock solution were added to 100 ml of PDA media, respectively. These prepared concentrations were then added to separate media bottles containing 100 ml of PDA, while the media bottle without any amendment served as the control treatment.

The PDA was subsequently poured into Petri plates, and small mycelial plugs were inoculated onto the media. The Petri plates were sealed with paraffin tape and incubated at 28 ± 1°C in a REICO incubator. The experiment followed a completely randomized design (CRD) with three replications for each treatment, and the Petri plates without any treatment were used as the control.

Mycelial growth inhibition was recorded at 48, 72, and 96-h intervals. Data were analyzed using the Least Significant Difference (LSD) test to compare the means of different treatments. Correlation analysis was also performed to determine the relationships between treatment concentrations and mycelial growth inhibition. Polar heatmaps were generated to visualize the treatment effects over time.

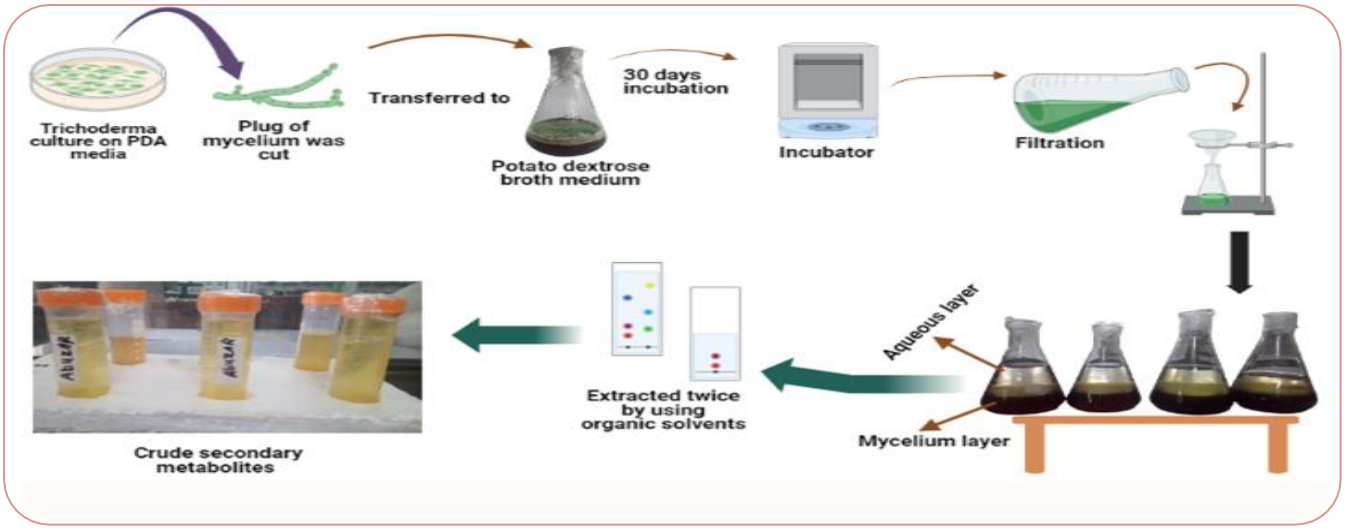

Figure 2. Extraction of secondary metabolites from *Trichoderma* spp. through solvent extraction method.

Evaluation of SMs of antagonistic organisms towards Fusarium wilt of pea under glasshouse condition

Secondary metabolites of *T. viride* and *T. harzianum*, both individually and in combination, were evaluated at different concentrations (250 ppm, 350 ppm, and 500 ppm) for their efficacy against Fusarium wilt of pea under greenhouse conditions. For this study, seeds of the susceptible pea variety 'Meteor' were sown in pots (13×15 cm). Stock solutions were prepared from crude metabolite extracts by adding 1 ml of the extract to 100 ml of distilled water. To achieve the desired concentrations, 25 ml, 35 ml, and 50 ml of the stock solution were each diluted in 1000 ml of distilled water to make the 250 ppm, 350 ppm, and 500 ppm solutions, respectively.

Under greenhouse conditions, artificial inoculation was carried out using a spore suspension of the pathogen, adjusted to 1×10^6 spores/ml using a hemocytometer (Model-T20B05). Once seedlings were established, the spore suspension of the fungal pathogen was applied through soil drenching. The prepared concentrations of secondary metabolites from *Trichoderma* species were then applied to the soil in the same manner. The experiment was designed using CRD, with each treatment replicated three times to minimize error. Disease incidence (%) was measured at three one-week intervals and calculated using the formula suggested by Ahmad et al. (2010).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \times 100$$

The LSD test was used to compare means among different treatments. Correlation analysis was conducted to assess the relationship between metabolite concentrations and disease incidence. Polar heatmaps and dendrograms were utilized to visualize the effects of treatments and interactions over time.

Evaluation of secondary metabolites of antagonistic organisms towards Fusarium wilt of pea under field conditions

The secondary metabolites of *T. viride* and *T. harzianum*, both individually and in combination, at a significant concentration (500 ppm) under laboratory were evaluated under field conditions. To achieve this, pea varieties were cultivated in the research area of the Department of Plant Pathology using a Randomized Complete Block Design with three replications. For artificial inoculation in the field, a spore suspension (1×10^6 spores/ml) was prepared using a hemocytometer (Model T20B05). After seedling establishment, the inoculum was applied through soil drenching.

Subsequently, the most effective concentration (500 ppm) of crude metabolite extracts from *T. viride* and *T. harzianum*, both individually and in combination, was applied using a hand sprayer in the morning (when the maximum number of stomata were open) and through soil drenching. Disease incidence data were recorded three times at one-week intervals using the formula provided by Ahmad et al. (2010).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \times 100$$

The LSD test was employed to identify significant differences between treatment means. Correlation analysis was performed to assess the relationship between treatment concentrations and disease incidence. Polar heatmaps were used to illustrate the effects of treatments over time.

RESULTS

Assessment of SMs of antagonistic organisms towards *F. oxysporum* f.sp. *pisi* under lab conditions

Results indicated that among all treatments, the combination of *T. viride* + *T. harzianum* SMs exhibited the lowest mycelial growth (10.118 mm), followed by the SMs of *T. harzianum* (12.944 mm) and the secondary metabolites of *T. viride* (14.356 mm), compared to the control (30.954 mm) (Figure 3). The interaction between treatment and concentration (T×C) revealed that the minimum fungal growth was observed at a 250ppm concentration of secondary metabolites of *T. viride* (16.694 mm), compared to *T. harzianum* SMs (14.832 mm) and the combination of *T. viride* + *T. harzianum* SMs (11.724 mm). At a 350ppm concentration, these treatments resulted in fungal colony growth of 13.928 mm, 12.842 mm, and 10.089 mm, respectively. At a 500ppm concentration, the fungal colony growth was 12.447 mm, 11.159 mm, and 8.540 mm for these treatments, respectively (Figure 4). The interaction between treatment and days (T×D) showed that the combination of *T. viride* + *T. harzianum* SMs inhibited the maximum fungal colony growth (7.329 mm, 10.384 mm, and 12.640 mm) after 48, 72, and 96 h, respectively, followed by *T. harzianum* SMs (9.239 mm, 12.434 mm, and 17.160 mm) and *T. viride* SMs (10.384 mm, 14.149 mm, and 18.536 mm) (Figure 5). Polar heatmap was also generated to visualize the effect of different treatments and their concentrations on mycelial growth over time (Figure 6). Correlation analysis expressed the relationship between treatments and mycelial growth (Figure 7).

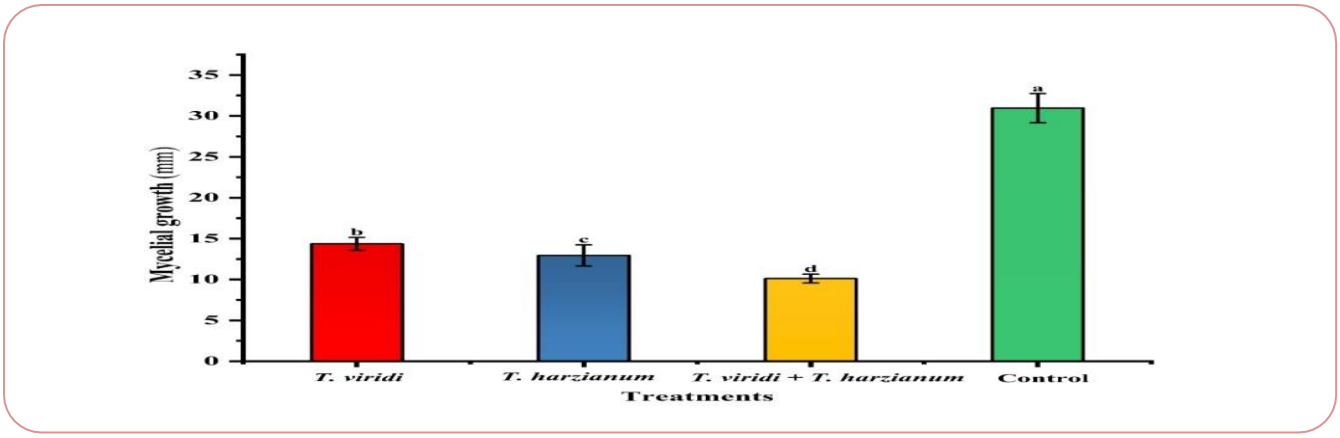

Figure 3. Evaluation of secondary metabolites of *Trichoderma* species towards *F. oxysporum* f.sp. *pisi* under lab conditions.

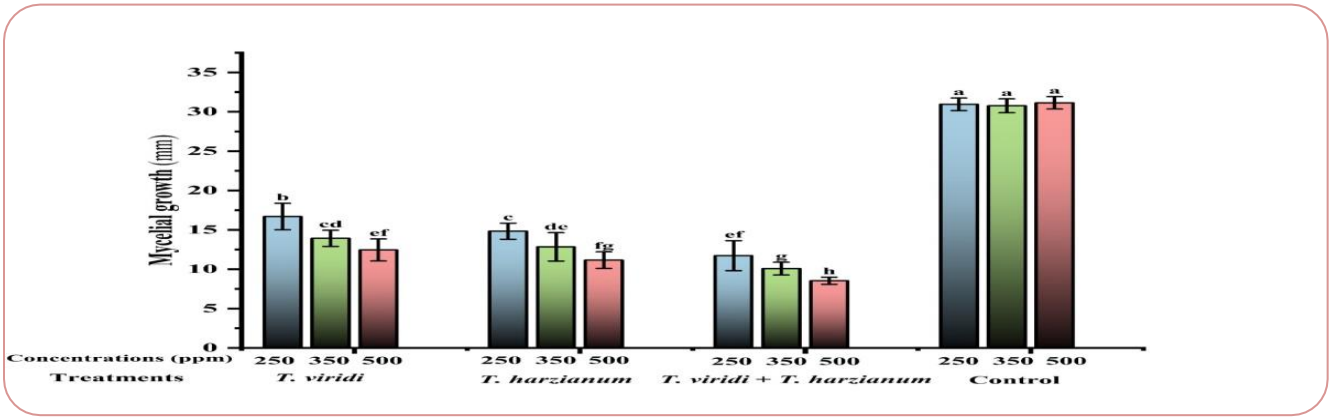

Figure 4. Impact of interaction between treatments and concentrations on the mycelial growth of *F. oxysporum* f.sp. *pisi*.

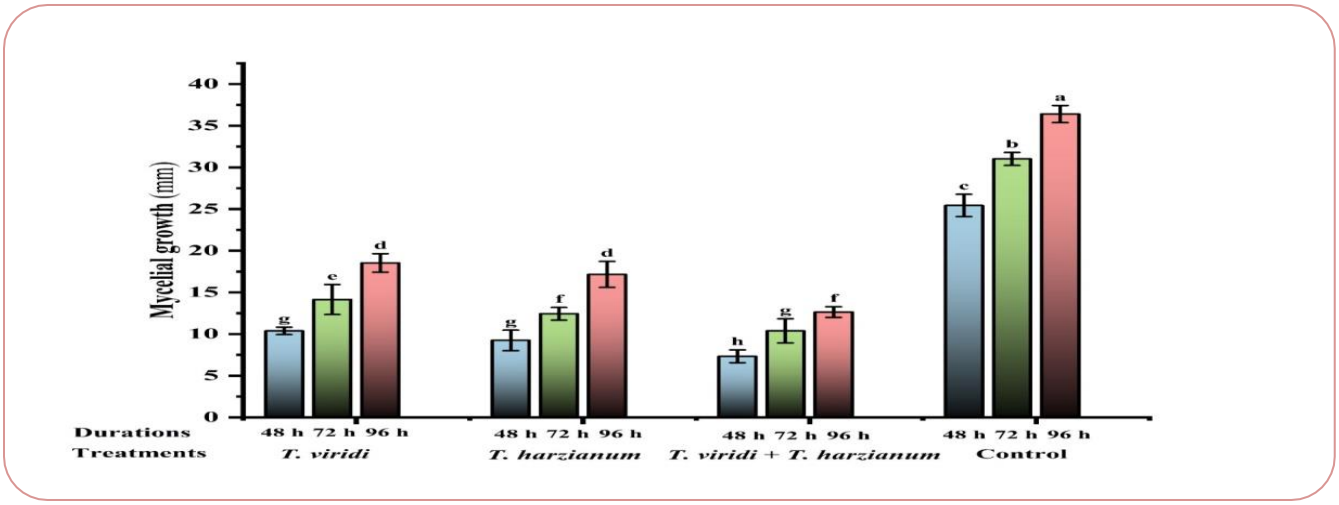

Figure 5. Effect of interaction between treatments and durations on the mycelial growth of *F. oxysporum* f.sp. *pisi*.

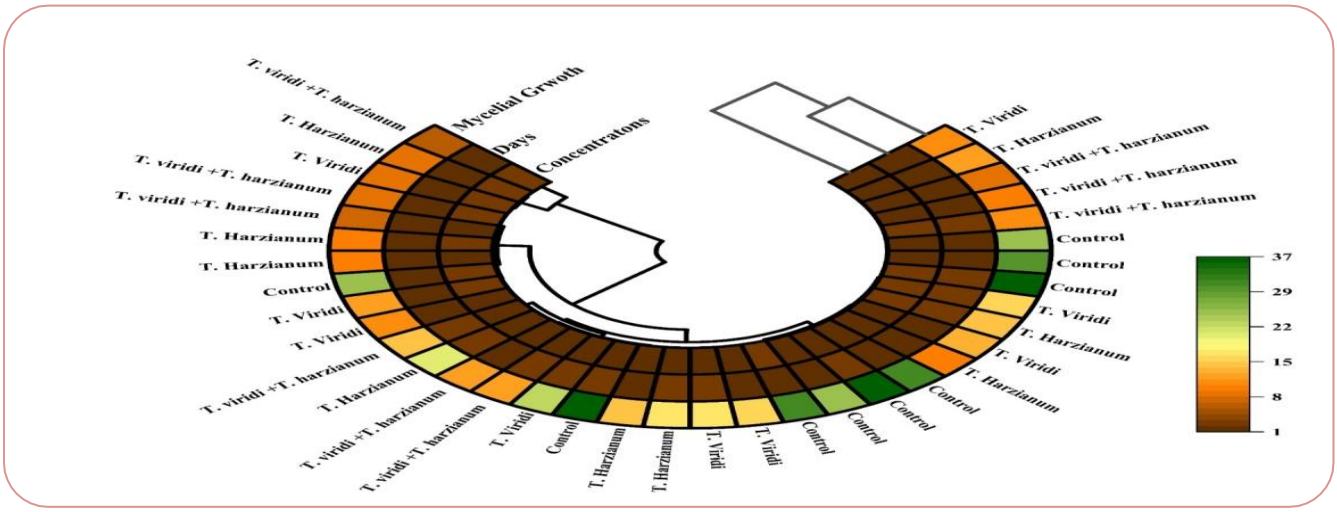

Figure 6. Polar heatmap with dendrogram indicating the effect of different treatments and their concentration on mycelial growth over time.

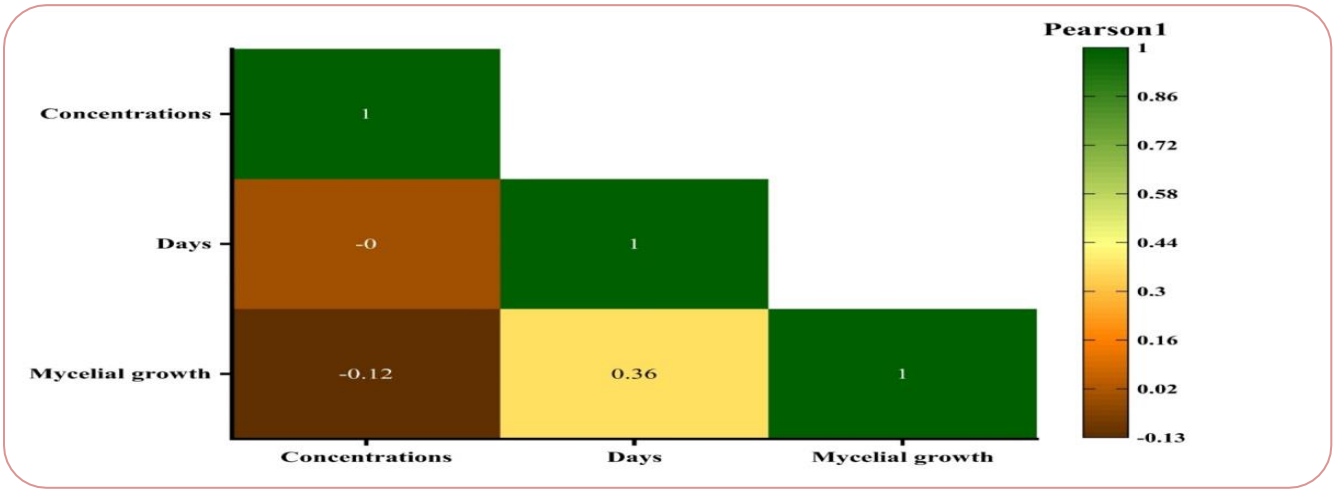

Figure 7. Correlation analysis between physiological and biochemical indexes of pea.

Assessment of secondary metabolites of *T. viride* and *T. harzianum* alone and in combination against *Fusarium* wilt of pea under greenhouse conditions

The results indicated that, among the secondary metabolites tested, the highest disease incidence (%) was observed with the secondary metabolites of *T. viridi* (36.850%), followed by *T. harzianum* (34.868%), and the combination of secondary metabolites from *T. viridi* and *T. harzianum* (27.082%) compared to the control under glasshouse conditions (Figure 8).

Interaction between treatments and concentrations (T×C) showed that the minimum disease incidence was recorded with a 500ppm concentration of the combination of secondary metabolites from *T. viridi* and *T. harzianum* (22.684%). This was lower compared to the secondary metabolites of *T. harzianum* (31.058%) and *T. viridi* (31.909%). At a 350ppm concentration, the disease incidences were 27.544%, 35.734%, and 38.008%, respectively, whereas at a 500ppm concentration, the incidences were 31.017%, 37.811%, and 40.632% (Figure 9).

The interaction between treatments and days (T×D) revealed that the secondary metabolites of *T. viridi* caused the highest disease incidences (39.889%, 37.453%, and 33.207%) after 7, 14, and 21 days, respectively. This was followed by the secondary metabolites of *T. harzianum* (38.058%, 35.062%, and 31.483%) and the combination of *T. viridi* + *T. harzianum* (30.572%, 27.686%, and 22.988%) for the same periods (Figure 10). The Polar heatmap expressed the effect of different treatments on disease incidence (Figure 11). Correlation analysis expressed the relationship between treatments and concentrations on the disease incidence with respect to days (Figure 12).

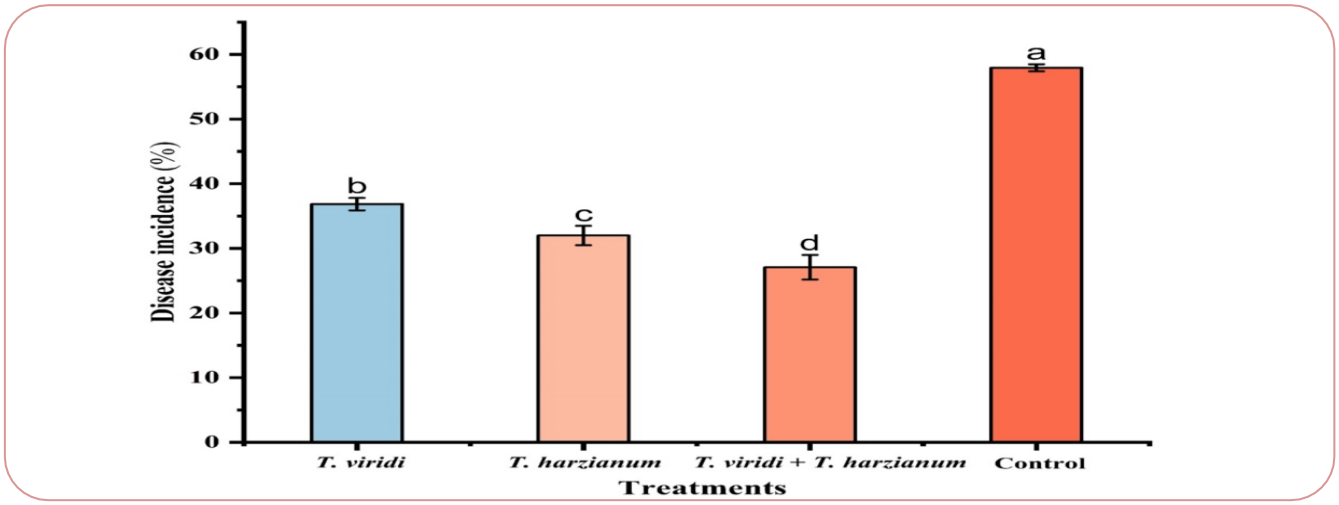

Figure 8. Evaluation of secondary metabolites of *T. viridi* and *T. harzianum* alone and in combination against Fusarium wilt of pea.

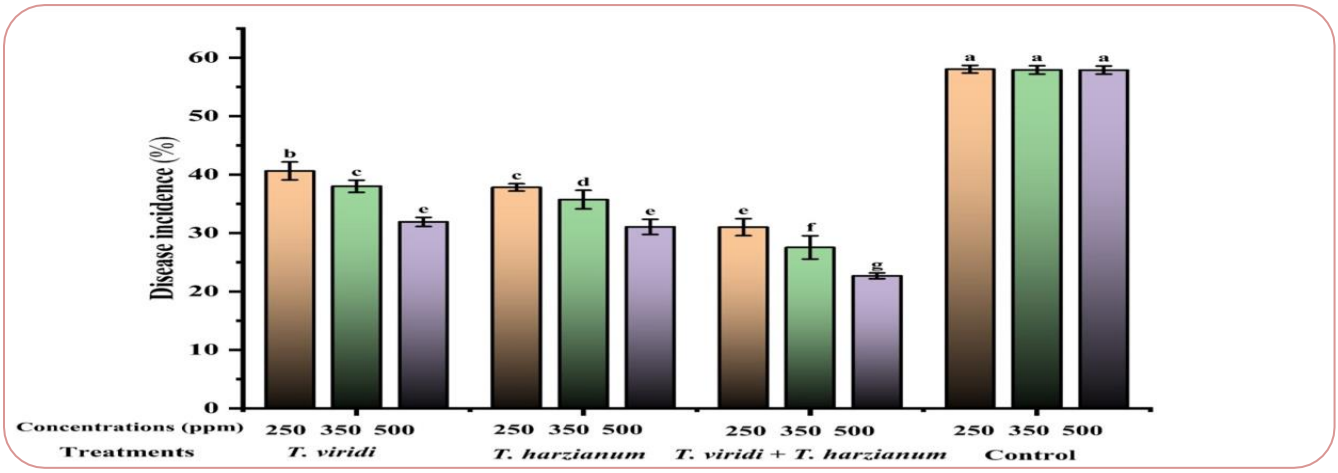

Figure 9. Impact of interaction between treatments and concentrations on the disease incidence of Fusarium wilt disease under greenhoue conditions.

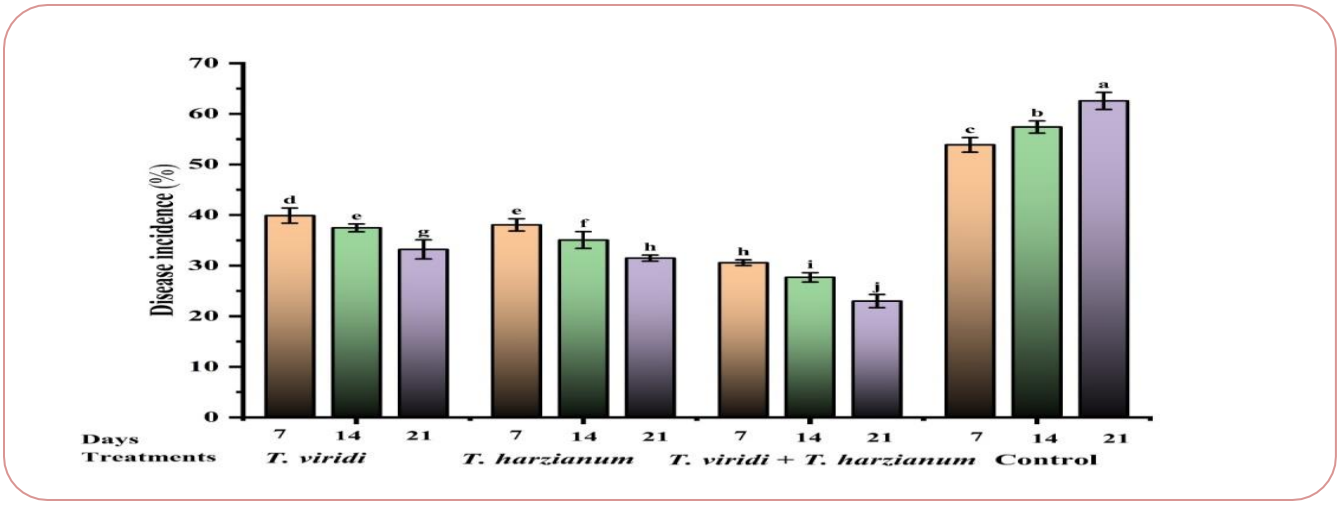

Figure 10. Effect of interaction between treatments and days on the disease development under greenhouse conditions.

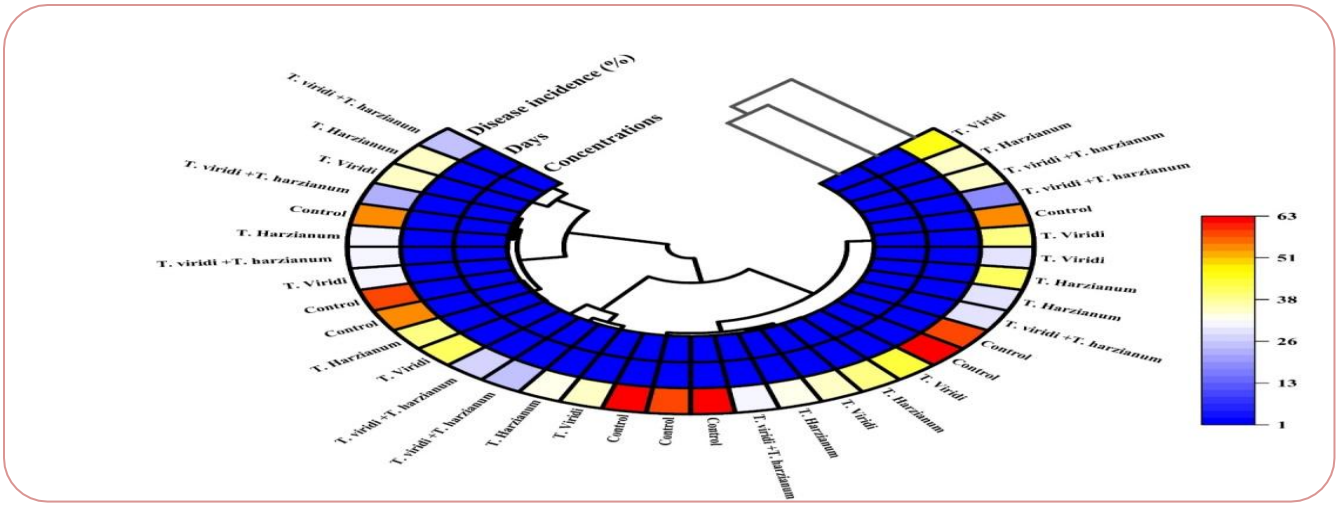

Figure 11. Polar heatmap with dendrogram expressing the effect of different treatments on the disease incidence of Fusarium wilt of pea under greenhouse conditions.

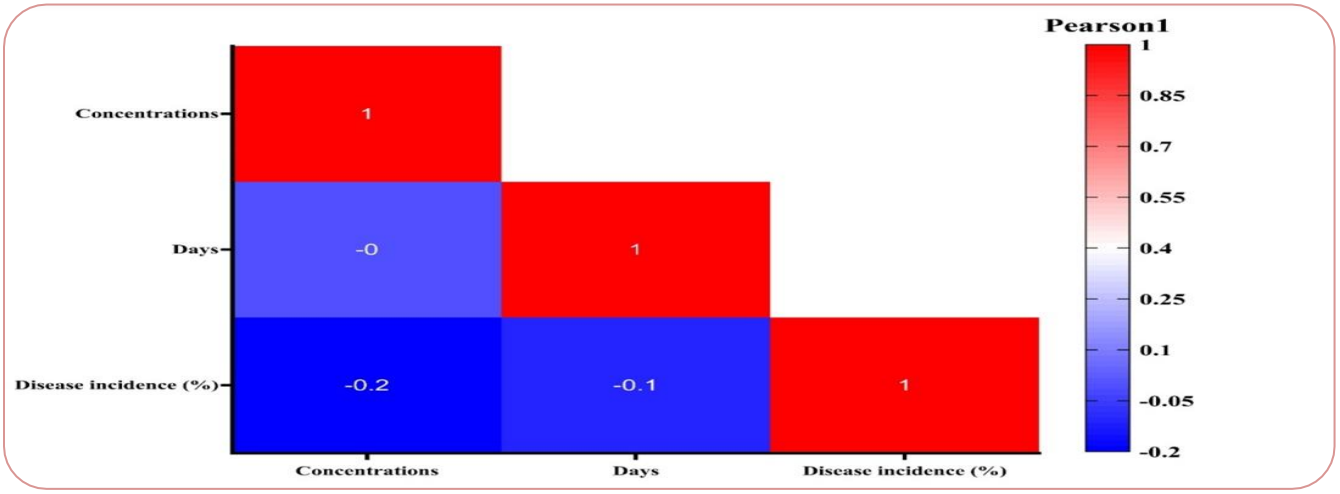

Figure 12. Correlation analysis between different concentrations of treatments on the disease incidence with days.

Evaluation of secondary metabolites of *T. viride* and *T. harzianum* alone and in combination against Fusarium wilt of pea under field conditions

The results indicated that, among the secondary metabolites of *Trichoderma* species, the combination of *T. viride* and *T. harzianum* exhibited the lowest disease incidence (28.15%). This was followed by the secondary metabolites of *T. harzianum* (34.91%) and *T. viride* (37.53%), compared to the control (Figure 13). The interaction between treatments and days (T×D) showed that, after 7, 14, and 21 days, the highest disease incidences were observed with the secondary metabolites of *T. viride* (44.01%, 37.03%, and 31.57%, respectively), followed by *T. harzianum* (41.02%, 34.87%, and 28.85%) and the combination of *T. viride* and *T. harzianum* (36.55%, 27.12%, and 20.78%), relative to the control (Figure 14). The Polar heatmap expressed the effect of different treatments on disease incidence of Fusarium wilt with days (Figure 15). Correlation analysis expressed the relationship between treatments and days on the disease incidence of Fusarium wilt (Figure 16).

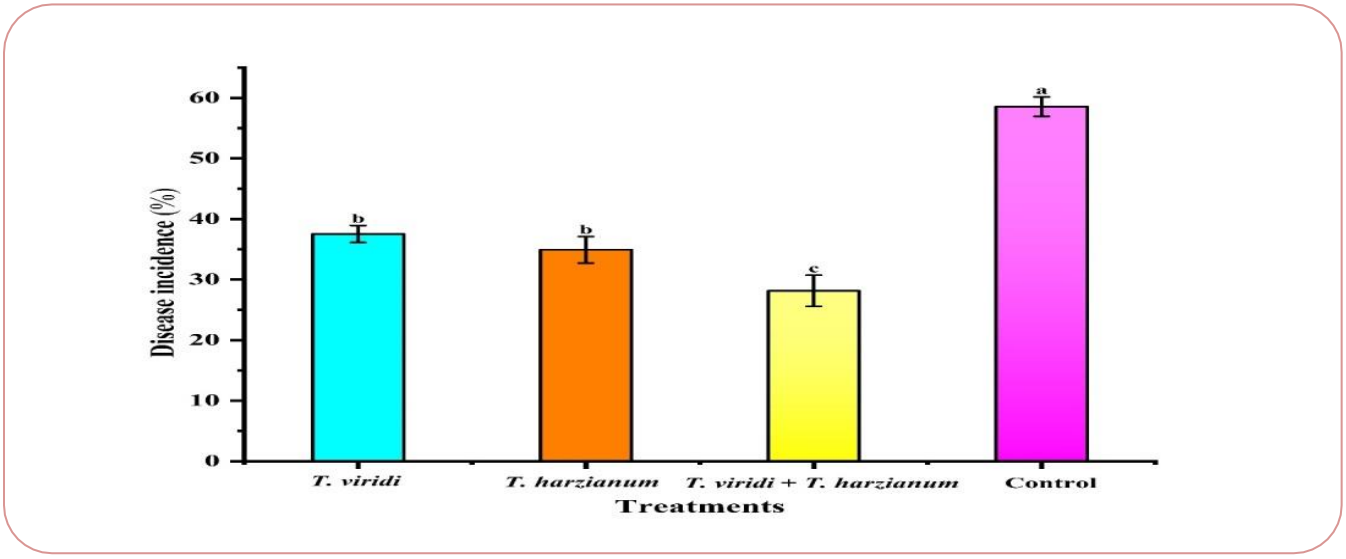

Figure 13. Evaluation of secondary metabolites of *T. viride* and *T. harzianum* alone and in combination against Fusarium wilt of pea under field conditions.

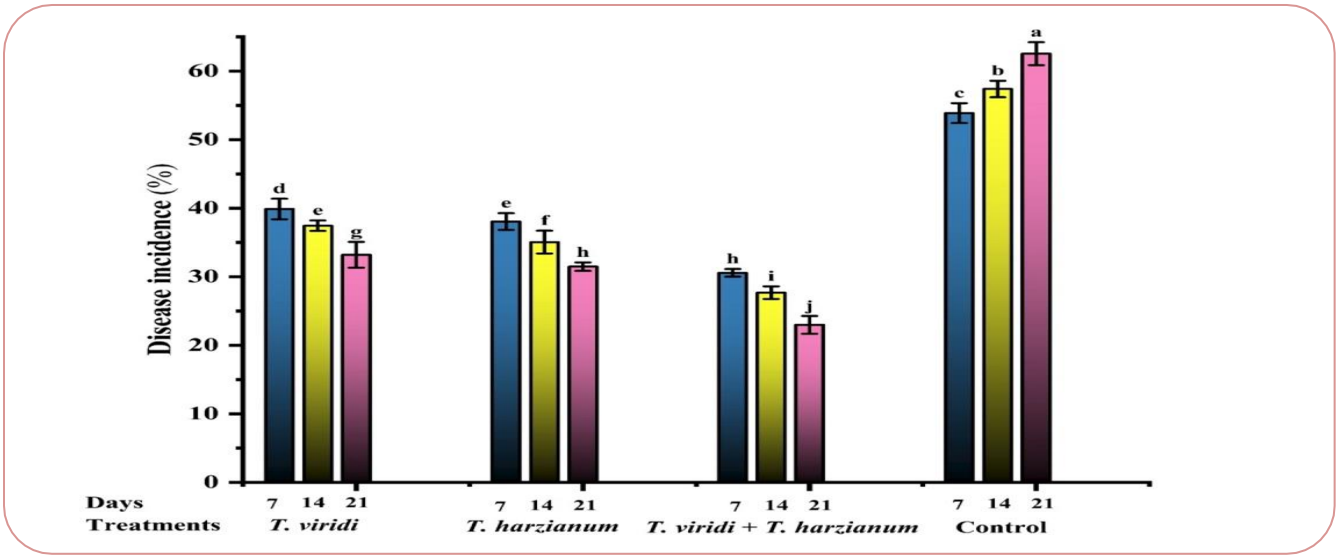

Figure 14. Effect of interaction between treatments and days (T×D) on the development of Fusarium wilt disease of pea under field conditions.

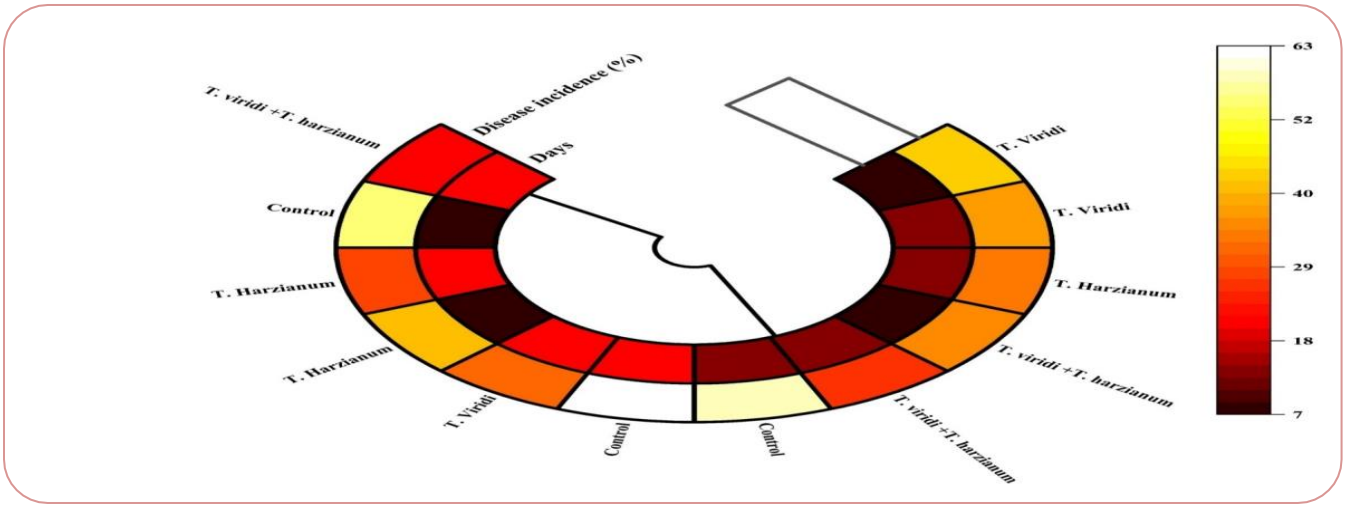

Figure 15. Effect of different treatments on the disease incidence of Fusarium wilt with days.

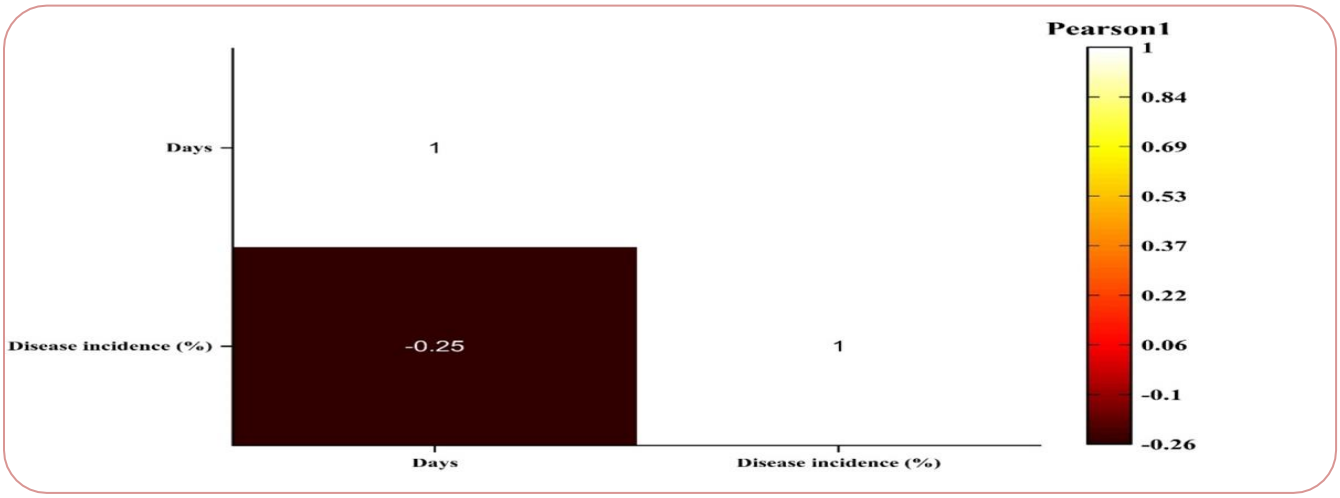

Figure 16. Correlation analysis between days and disease incidence of Fusarium wilt.

DISCUSSION

Fusarium wilt of pea is caused by *Fusarium oxysporum* f.sp. *pisi*, with distinct races categorized based on their virulence. As a soil-borne pathogen, it can persist in the soil for up to a decade, due to resilient, thick-walled chlamydospores (Zeeshan et al., 2023; Yaseen et al., 2024). Once the pathogen infects the soil or a plant, managing wilt becomes a formidable challenge due to its enduring presence and elusive nature (Gupta and Gupta, 2019). The excessive use of synthetic chemicals has traditionally been employed to manage this disease; however, concerns about human health and environmental risks have led to a shift towards more sustainable alternatives.

Among these alternatives, the use of biocontrol agents (BCAs) has emerged as a promising strategy, offering effective disease management with minimal ecological impact. Nonetheless, the effectiveness of BCAs can be affected by changing climatic conditions, highlighting the need for resilient solutions. Antagonistic organisms, particularly *Trichoderma* species, not only produce a range of low molecular weight secondary metabolites with potent antimicrobial properties but also provide ecological benefits such as enhancing soil health and promoting plant growth. Research suggests that the efficacy of these secondary metabolites remains relatively stable despite environmental fluctuations, making them a reliable foundation for sustainable disease management strategies.

In this study, we aimed to evaluate the antifungal efficacy of secondary metabolites extracted from *Trichoderma* species against Fusarium wilt of pea. Our

findings indicate that the combination of *T. viride* and *T. harzianum* exhibited the most significant inhibition of mycelial growth. This was followed by individual metabolites from *T. viride* and *T. harzianum* under laboratory conditions, which also demonstrated the lowest disease incidence in greenhouse and field conditions. These results are consistent with previous research (Kumar et al., 2019), which reported that secondary metabolites from *T. viride* and *T. harzianum* significantly reduced the mycelial growth of *F. oxysporum*. Similarly, studies by Petrişor and Paica (2019) and Moutassem et al. (2020) have highlighted the inhibitory effects of volatile and non-volatile metabolites from *Trichoderma* spp. on the growth of *F. oxysporum* f.sp. *radicis-lycopersici* and *F. solani*, supporting the presnent findings.

Furthermore, a previous study demonstrated that *T. asperellum* strain CCTCC-RW0014 showed strong biocontrol efficacy against *F. oxysporum* f.sp. *cucumerinum*, reducing disease incidence by 71.67% in greenhouse trials. Our current investigation builds on this work, confirming its potent antagonistic activity under both *in vitro* and *in vivo* conditions. The analysis of synergistic effects between cell wall-degrading enzymes (CWDEs) and secondary metabolites reaffirmed its efficacy against *F. oxysporum* (Saravanakumar et al., 2016).

Trichoderma produces a diverse range of low molecular weight secondary metabolites, including polyketides, nonribosomal peptides, terpenoids, nafuredins, pyrones, peptaibols, alkaloids, and flavonoids. These metabolites exhibit potent antimicrobial properties, adversely affecting the growth of both fungal and bacterial pathogens. Their modes of action vary: polyketides and nonribosomal peptides often disrupt key cellular processes in pathogens, such as cell wall synthesis and protein production. Terpenoids and alkaloids may interfere with membrane integrity or enzymatic functions, while flavonoids can disrupt cellular signaling pathways. Peptaibols, being peptide-based, typically compromise membrane integrity, leading to cell lysis. Nafuredins and pyrones may inhibit essential metabolic pathways within the pathogens. This diverse array of secondary metabolites enables *Trichoderma* to employ a multifaceted approach to pathogen inhibition, establishing it as a potent biocontrol agent (Buddhika and Abeysinghe, 2021; Maheshwary et al., 2022).

These metabolites also play a crucial role in enhancing plant defense systems by acting as signaling molecules that regulate the expression of defense-related genes (Divekar et al., 2022). Such genes produce defense-related enzymes, including phytoalexins and pathogenesis-related proteins, which are activated in response to oxidative stress and further strengthen the plant's defense mechanisms. By orchestrating this complex molecular response, these secondary metabolites effectively increase the plant's resilience against pathogens and environmental stresses (Harman, 2000; Yedidia et al., 2000, 2003).

However, the compatibility of *Trichoderma*-derived secondary metabolites with host plants, particularly *T. harzianum* and *T. viride*, in the context of Fusarium wilt in peas requires further investigation. Some secondary metabolites have dual roles as both antifungal agents and plant growth regulators, benefiting plant defense mechanisms. Thus, this study is pivotal for understanding the potential of *Trichoderma* spp. as biocontrol agents against Fusarium wilt in pea plants. Current findings reveal significant biological implications, demonstrating how *Trichoderma*-derived metabolites might influence disease progression and metabolic pathways in pea plants. These metabolites could either enhance plant defenses or inhibit pathogen growth, offering valuable insights for developing effective biocontrol strategies and improving disease management. Nonetheless, further research is essential to evaluate the effects of *Trichoderma* spp.-derived metabolites on disease progression and metabolic pathways in pea plants afflicted with Fusarium wilt. Future studies should focus on identifying the active components of these metabolites and understanding their specific mechanisms of action.

CONCLUSION

In conclusion, the current findings underscore the significant potential of secondary metabolites derived from *Trichoderma* species in managing Fusarium wilt in pea plants. The combination of crude extracts from *T. viride* and *T. harzianum* was exceptionally effective, resulting in minimal mycelial growth and consistently low disease incidence rates across all tested conditions whether *in vitro*, in the greenhouse, or *in vivo*. The successful use of *Trichoderma*-derived secondary metabolites points to broader applications in integrated plant disease management, offering promising solutions for controlling a wide range of plant pathogens.

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AUTHORS' CONTRIBUTIONS

AH, and MA designed, formulated and laid out the study; NAR, AN, and WA conducted the experiments; MA, ZA, and AW collected, arranged and analyzed the data; MA, and NAR provided technical assistance; AH, MA, and NAR supervised the work; AH, AN, and WA wrote the manuscript; AI, and AU proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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