

Research article

In Vitro Evaluation of the Pathogenicity of *Fusarium oxysporum* f. sp. *cucumerinum* and the Antifungal Efficacy of Hymexazol and *Trichoderma viride*

Mays Muhsin Almagsoosi and Jawadayn Talib Alkooranee

Department of Plant Protection, College of Agriculture, Wasit University, Wasit 52001, Iraq

ARTICLE INFO

Keywords:

Cucumber;
Fusarium oxysporum f.
sp. *cucumerinum*;
Trichoderma viride;
Hymexazol.

Article history:

Received: 23th July 2026
Accepted: 7st August 2026
Online: 30 Sep 2026

ABSTRACT

The present study aimed to evaluate the pathogenicity of *Fusarium oxysporum* f. sp. *cucumerinum* and to investigate the effectiveness of Hymexazol and *Trichoderma viride* as chemical and biological control agents under laboratory conditions. Laboratory experiments confirmed that the fungal isolate was highly pathogenic to cucumber. Seed germination of the susceptible local cultivar decreased to 60%, whereas the resistant imported cultivar maintained 90% germination, compared with 100% in the uninoculated control. Typical *Fusarium* wilt symptoms were successfully reproduced, and Koch's postulates were confirmed by re-isolation of the pathogen from infected plants. Hymexazol exhibited strong antifungal activity, completely inhibiting the radial growth of *F. oxysporum* f. sp. *cucumerinum* at 2000 ppm, while having no inhibitory effect on the growth of *T. viride*. Furthermore, *T. viride* showed strong antagonistic activity against the pathogen, completely overgrowing the pathogen colony and being classified as Class I according to Bell's rating scale. These findings demonstrate that Hymexazol and *T. viride* are compatible control agents and represent promising components of integrated management strategies for *Fusarium* wilt of cucumber.

1. Introduction

Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop cultivated throughout the world because of its high nutritional value and strong consumer demand. It is widely grown under both open-field and protected cultivation systems and is consumed either fresh or processed. Despite its economic importance, cucumber production is threatened by several diseases, particularly those caused by soil-borne pathogens, which can markedly reduce plant growth and yield. Among these diseases, *Fusarium* wilt is regarded as one of the most destructive (Abbey et al., 2017; Aziz & Hussien, 2023; Javid et al., 2024).

Fusarium wilt of cucumber is caused by *Fusarium oxysporum* f. sp. *cucumerinum*, a soil-borne vascular fungus capable of surviving in the soil for many years. Infection begins through the roots, after which the pathogen colonizes the xylem vessels, restricting the transport of water and mineral nutrients. As a consequence, infected plants exhibit yellowing, wilting, vascular discoloration, reduced growth, and considerable yield losses. Under severe infection, complete plant death may occur. The disease is particularly problematic in continuously

✉ *Corresponding author:
alkooranee@uowasit.edu.iq (Jawadayn Alkooranee)



cultivated cucumber fields and under environmental conditions that favor pathogen development (Leslie & Summerell, 2006; Sun et al., 2017; Vorob'eva & Toropova, 2020).

For many years, chemical fungicides have been the principal means of controlling *Fusarium* wilt. Nevertheless, intensive reliance on these chemicals has resulted in the emergence of fungicide-resistant pathogen populations and has raised increasing concerns regarding environmental safety and public health. Consequently, interest has shifted toward integrated disease management (IDM), which combines chemical control with biological approaches and enhancement of the plant's own defense mechanisms (Panth et al., 2020; Hou et al., 2023; Jayawardana & Fernando, 2024).

Induced systemic resistance (ISR) represents one of the most effective natural defense strategies in plants. Beneficial microorganisms stimulate the plant to activate its defense system more rapidly and efficiently following pathogen attack. Besides suppressing pathogens directly, these microorganisms prime host defense responses, thereby reducing disease severity and improving tolerance to biotic stress. Because of these advantages, ISR has become an important component of sustainable plant disease management (Conrath et al., 2006; Pieterse et al., 2014; Kovalchuk, 2023; Gong et al., 2023).

Species of *Trichoderma* are among the most widely used biological control agents against plant diseases. Their effectiveness is attributed to several mechanisms, including competition for nutrients and space, mycoparasitism, and the production of antifungal metabolites and cell wall-degrading enzymes. In addition, *Trichoderma* spp. promote plant growth and activate induced systemic resistance, resulting in improved nutrient uptake, greater plant vigor, and reduced disease severity (Druzhinina et al., 2011; Alkooranee et al., 2017; Guzmán-Guzmán et al., 2023).

Hymexazol is a systemic fungicide that has proven effective against a wide range of soil-borne fungal pathogens, including *Fusarium* species. It inhibits spore germination and mycelial growth and is readily absorbed by plant roots before being translocated throughout plant tissues. Several studies have demonstrated that integrating Hymexazol with biological control agents provides more effective disease suppression than either treatment alone. This improved performance is attributed to the combined action of direct pathogen inhibition by the fungicide and activation of plant defense responses by the biological agent (Myresiotis et al., 2012; Zhang et al., 2021; Báez-Sañudo, 2022).

Despite the increasing use of biological control agents and fungicides for the management of soil-borne diseases, further studies are required to evaluate their efficacy under laboratory conditions. Therefore, the present study aimed to evaluate the pathogenicity of *F. oxysporum* f. sp. *cucumerinum*, its effect on cucumber seed germination, the inhibitory activity of Hymexazol against the pathogen, and the antagonistic potential of *T. viride* under in vitro conditions.

2. Materials and Methods

2.1. Sample Collection

Collected cucumber plant samples on April 4, 2025, from protected cultivation houses in Al-Kardiyah area, south of Kut City, Wasit Province, Iraq (32.555850° N, 45.938264° E). The sampled plants exhibited typical symptoms of *Fusarium* wilt, including leaf yellowing and wilting. In addition, longitudinal and transverse sections of the stems revealed dark-brown discoloration of the xylem vessels. The collected samples were placed in polyethylene bags to preserve them during

transportation. Information including the sampling date, collection site, and disease incidence was recorded on each bag. The samples were stored at 4°C in the Microbiology Laboratory, Department of Field Crops, College of Agriculture, University of Wasit, until fungal isolation was performed.

Seeds of the imported cucumber cultivar Ghazir, produced by Monsanto Holland B.V. (Seminis), and seeds of a local cucumber cultivar were used in the experiments. *Trichoderma viride* fungus was obtained from the Plant Pathology Laboratory at the College of Agriculture, Wasit University.

The fungicide Himex SL, containing 30% Hymexazol as the active ingredient, was used in this study. It is manufactured by MIDMAC, Jordan, and registered with the Iraqi Ministry of Agriculture under Registration Certificate No. (1921). Himex SL is a systemic fungicide with both protective and curative activity and is used against a broad spectrum of soil-borne fungal pathogens affecting vegetable and gramineous crops. The fungicide was purchased from local commercial markets in Kut City, Wasit Province, Iraq.

2.2. Isolation and Purification of the Pathogen from Cucumber Roots and Stem Bases

To isolate the causal pathogen, the collected symptomatic root systems were thoroughly washed under running tap water to remove adhering soil and debris. Small pieces (approximately 1 cm in length) were cut from the roots and stem bases using a sterile scalpel. The tissue pieces were surface-sterilized in 10% sodium hypochlorite (NaOCl) solution for 2 min, rinsed three times with sterile distilled water for 3 min, and dried on sterile filter paper for 2 min.

Potato Dextrose Agar (PDA) medium was prepared by dissolving 39 g of commercial PDA powder in 1 L of distilled water. The medium was sterilized in an autoclave at 121°C for 20 min under 15 psi pressure. After cooling to approximately 45–50°C, tetracycline was added to suppress bacterial contamination. The medium was thoroughly mixed and poured into sterile 9-cm Petri dishes.

Following solidification of the medium, three surface-sterilized tissue pieces were aseptically placed onto each Petri dish, ensuring sufficient spacing between the explants. Three replicate plates were prepared for each sample under aseptic conditions. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Emerging fungal colonies were subsequently purified by transferring hyphal tips onto fresh PDA plates. Pure cultures were maintained on PDA slants in test tubes and incubated at 28°C for 3 days, after which they were stored at 4°C until use. Cultures were periodically subcultured to maintain their viability.

2.3. Morphological and Microscopic Identification of *Fusarium oxysporum* f. sp. *cucumerinum*

The isolated fungal culture was initially identified based on colony morphology, including colony color, surface and reverse pigmentation, and growth characteristics. Microscopic identification was subsequently performed using a compound light microscope by Prof. Dr. Jawadayn Talib Abd. Identification to the species level was based on the morphological characteristics of the reproductive structures, including the size and shape of the conidiophores, the number of septa in the spores, and the morphology of macroconidia, microconidia, and chlamydospores, according to the taxonomic keys described by Booth (1977) and Leslie and Summerell (2006). The identity of the isolate was further confirmed using molecular characterization.

2.4. Germination Test of Two Cucumber Cultivars

To evaluate seed viability of the resistant and local cucumber cultivars and to determine the effect of *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c.) on seed germination, sterile 9-cm Petri dishes were lined with sterile medical gauze moistened with sterile distilled water. Six Petri dishes were

prepared for each cultivar. Three dishes received 10 mL of a fungal suspension (2×10^3 spores mL⁻¹) prepared from cultures grown in Potato Sucrose Broth (PSB), whereas the remaining three dishes received only sterile distilled water and served as the control.

Cucumber seeds were washed thoroughly under running tap water to remove surface impurities, surface-sterilized in 50% ethanol for 30 s, followed by immersion in sodium hypochlorite solution for 1 min. The seeds were then rinsed five times with sterile distilled water and dried on sterile filter paper.

One hour after adding the fungal suspension, ten seeds were placed evenly in each Petri dish in a circular arrangement approximately 1 cm from the edge of the plate, leaving equal spacing between adjacent seeds. The plates were incubated for three days under aseptic laboratory conditions, and sterile distilled water was added whenever necessary to maintain adequate moisture. Germination percentage and the effect of fungal infection were determined using the following equation:

$$\text{Germination (\%)} = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100$$

2.5. Pathogenicity Test of *Fusarium oxysporum* f. sp. *cucumerinum*

The pathogenicity of the *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c.) isolate was evaluated to confirm its ability to induce Fusarium wilt symptoms on cucumber plants according to the procedures described in *The Fusarium Laboratory Manual*. The fungal isolate was cultured on Potato Dextrose Agar (PDA) at $25 \pm 2^\circ\text{C}$ for 5–7 days. A conidial suspension was prepared by adding sterile distilled water to the fungal colonies and gently scraping the colony surface to release the spores. The spore concentration was adjusted to approximately 1×10^6 spores mL⁻¹ using a hemocytometer.

Six healthy cucumber seedlings (2–3 weeks old) of both cultivars were carefully uprooted, and the soil adhering to the roots was removed. The roots of three seedlings of each cultivar were immersed in the conidial suspension for 10 min, whereas control seedlings were dipped in sterile distilled water only. The seedlings were then transplanted into pots containing sterilized soil and maintained under growth chamber conditions.

Disease development was monitored during the experimental period based on the appearance and progression of typical Fusarium wilt symptoms, including leaf yellowing, wilting, severe wilting, and plant death. Disease severity was assessed using a 0–4 rating scale, where 0 = no visible symptoms; 1 = slight yellowing or wilting; 2 = moderate yellowing and wilting; 3 = severe wilting affecting most of the plant; and 4 = complete wilting or plant death. Disease severity was expressed as a percentage and calculated according to the following equation:

$$\text{Disease severity (\%)} = \frac{\sum(n \times v)}{N \times V} \times 100$$

where n is the number of plants in each disease category, v is the corresponding disease rating, N is the total number of evaluated plants, and V is the maximum disease rating (4).

To fulfill Koch's postulates, the pathogen was re-isolated from symptomatic cucumber tissues. Small pieces of symptomatic tissues were cultured on PDA, and the recovered fungal colonies were examined based on their cultural and microscopic characteristics. These characteristics

were compared with those of the original F.o.c. isolate to confirm the identity of the re-isolated pathogen.

2.6. Effect of Different Concentrations of Hymexazol on the Growth of *F. oxysporum* f. sp. *cucumerinum*

Hymexazol was evaluated at concentrations of 500, 1000, 1500, and 2000 ppm. A stock solution of 20,000 ppm was prepared by mixing 2 mL of Hymexazol with 98 mL of sterile distilled water. Aliquots of 2.5, 5.0, 7.5, and 10 mL of the stock solution were separately added to 97.5, 95.0, 92.5, and 90.0 mL of molten PDA medium, respectively.

The amended media were poured into sterile 9-cm Petri dishes with three replicates per concentration. PDA plates without fungicide served as the control. After solidification, a 0.5-cm mycelial disc taken from a 3-day-old culture of *F. oxysporum* f. sp. *cucumerinum* was placed at the center of each plate. Plates were incubated at 28°C until the control colonies completely covered the Petri dishes.

The percentage inhibition of mycelial growth was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{[(\text{Colony diameter in the control} - \text{Colony diameter in the treatment}) / \text{Colony diameter in the control}] \times 100}{}$$

2.7. Effect of Different Concentrations of Hymexazol on the Growth of *T. viride*

PDA medium amended with Hymexazol was prepared as described in Section 2.6. A 0.5-cm mycelial disc obtained from a 3-day-old culture of *T. viride* was placed at the center of each Petri dish. Three replicates were prepared for each fungicide concentration. PDA plates inoculated with *T. viride* but without Hymexazol served as the control. All plates were incubated at 28°C, and the percentage inhibition of mycelial growth was calculated using the equation described in Section 2.6.

2.8. Antagonistic Activity of *T. viride* Against *F. oxysporum* f. sp. *cucumerinum*

The antagonistic activity of *Trichoderma viride* against *Fusarium oxysporum* f. sp. *cucumerinum* was evaluated using the dual culture technique. Sterile PDA medium was poured into 9-cm Petri dishes. Two 0.5-cm mycelial discs, one from *T. viride* and the other from *F. oxysporum* f. sp. *cucumerinum*, both obtained from 3-day-old cultures, were placed on opposite sides of the same plate along one diameter, approximately 0.5 cm from the plate edge. Three replicates were prepared for each treatment.

The plates were incubated at 28°C, and colony growth was monitored daily by measuring colony diameters. Antagonistic interactions were evaluated according to the five-point scale described by Bell et al. (1982):

1. *Trichoderma* completely overgrew the pathogen and covered the entire plate.
2. *Trichoderma* overgrew approximately three-quarters of the plate.
3. *Trichoderma* and the pathogen each occupied approximately half of the plate.
4. The pathogen occupied approximately three-quarters of the plate.
5. The pathogen completely overgrew the plate.

Trichoderma viride was considered to exhibit strong antagonistic activity when the antagonism rating was ≤ 2 .

2.9. Experimental Design and Statistical Analysis

All laboratory were conducted using a Completely Randomized Design (CRD). The collected data were analyzed using GenStat software (Version 12), and treatment means were separated using the Least Significant Difference (LSD) test at the 5% probability level according to Al-Rawi and Khalaf Allah (2000).

3. Results and Discussion

3.1. Isolation and Identification of *F. oxysporum* f. sp. *cucumerinum*

Fungal isolation from infected cucumber roots and stem bases collected from protected cucumber fields in Al-Kardhiyah, south of Kut City, resulted in the growth of fungal colonies on Potato Dextrose Agar (PDA) medium. After purification, the majority of the recovered isolates were identified as belonging to the genus *Fusarium*. Colonies became visible after 5–7 days of incubation and initially exhibited abundant white cottony mycelial growth, which gradually changed to pale pink or violet with colony maturation. A characteristic grayish-white pigmentation also developed on the reverse side of the culture medium (Fig. 1 A, B).

3.2. Morphological and Microscopic Identification of *F. oxysporum* f. sp. *cucumerinum*

Morphological examination of the fungal isolates grown on Potato Dextrose Agar (PDA) showed rapid colony development, with colonies completely covering the Petri dishes within seven days of incubation. The colony surface was characterized by dense cottony mycelia with a violet-pink appearance, whereas the reverse side exhibited a grayish-white coloration (Fig. 1 A, B).

Microscopic examination revealed the production of abundant microconidia that were oval to kidney-shaped, single-celled, and measured approximately $7.0 \times 3.5 \mu\text{m}$. These spores were borne on short, slender, unbranched monophialides. Macroconidia were slightly curved, elongated, and ellipsoidal with tapered ends, predominantly containing three septa, and measured approximately $18.5 \times 4.0 \mu\text{m}$.

The cultural and microscopic characteristics of the isolate were consistent with the diagnostic descriptions of *Fusarium oxysporum* reported by Leslie and Summerell (2006), confirming its identity as this species. Subsequently, the isolate was identified to the forma specialis level using molecular characterization (Fig. 1 C, D).

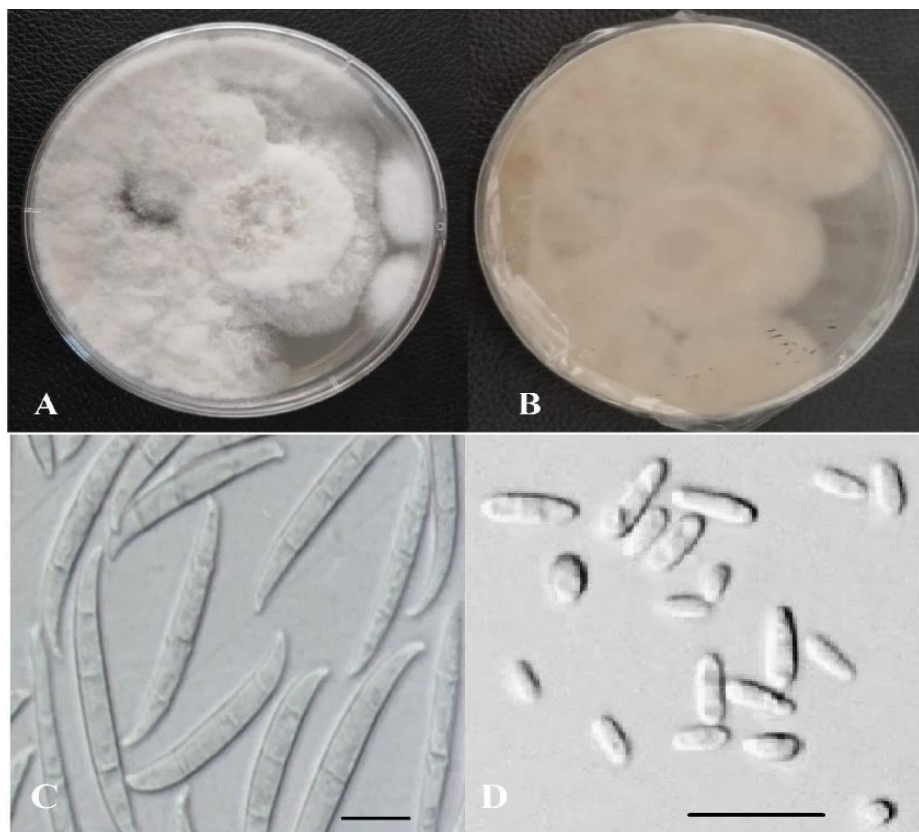


Figure 1. Morphological characteristics of *F. oxysporum* f. sp. *cucumerinum*. (A) Upper surface of the colony. (B) Reverse side of the colony. (C) Macroconidia. (D) Microconidia

3.3. Effect of *F. oxysporum* f. sp. *cucumerinum* on Seed Germination of Two Cucumber Cultivars

The germination test revealed a clear difference in the response of the two cucumber acultivars (local susceptible and imported resistant) to inoculation with *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c.). In the uninoculated control treatment, seeds of both cultivars exhibited 100% germination, indicating high seed viability and the absence of factors affecting germination.

Following inoculation with the pathogen, seed germination decreased in both cultivars, although the extent of reduction differed markedly between them. The imported resistant cultivar maintained a germination percentage of 90%, whereas germination of the local susceptible cultivar declined to 60% (Fig. 2).

These results demonstrate that *F. oxysporum* f. sp. *cucumerinum* adversely affected cucumber seed germination, with a substantially greater impact on the susceptible local cultivar than on the resistant imported cultivar. The higher germination observed in the resistant cultivar suggests a greater capacity to tolerate pathogen infection during the early stages of germination, indicating genetic differences in susceptibility between the two cultivars.

The reduction in germination may be attributed to the ability of the pathogen to infect seeds or embryonic tissues during the initial stages of germination. The fungus secretes

several cell wall-degrading enzymes, including cellulases and pectinases, together with phytotoxic metabolites that damage embryonic tissues, thereby impairing water and nutrient uptake and consequently delaying or preventing seed germination (Hazirah et al., 2020).

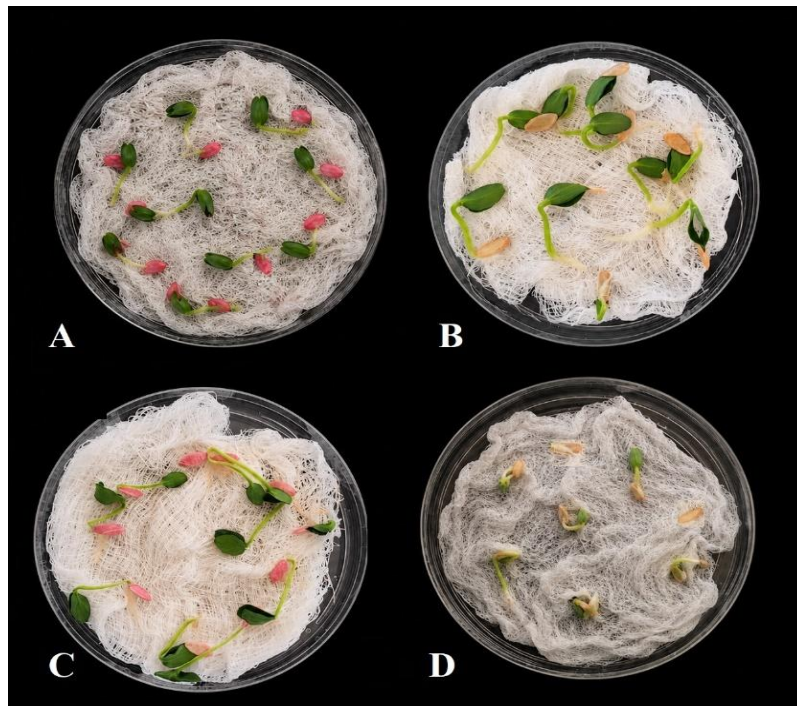


Figure 2. Comparison of seed germination between the imported resistant and local susceptible cucumber cultivars under control conditions and following inoculation with *F. oxysporum* f. sp. *cucumerinum*. (A) Imported cultivar without pathogen. (B) Local cultivar without pathogen. (C) Imported cultivar inoculated with the pathogen. (D) Local cultivar inoculated with the pathogen

The present findings are consistent with those of Hazirah et al. (2020), who reported that *F. oxysporum* infection significantly reduced seed germination, inhibited seedling growth, and increased seedling mortality in cucumber. Furthermore, the current results agree with the observations of Agrios (2005), who indicated that susceptibility to fungal pathogens is largely determined by the genetic background of the host cultivar. Resistant cultivars possess more effective defense mechanisms that restrict pathogen penetration and colonization, thereby explaining the superior germination performance of the imported cultivar observed in the present study.

3.4. Pathogenicity of *F. oxysporum* f. sp. *cucumerinum*

The pathogenicity assay confirmed that the *F. oxysporum* f. sp. *cucumerinum* isolate was highly pathogenic and capable of inducing typical Fusarium wilt symptoms in both cucumber cultivars under growth chamber conditions (Fig. 3). Disease symptoms became evident a few days after inoculation, initially appearing as slight yellowing of the lower leaves, followed by progressive wilting and gradual spread of symptoms throughout the plant. As disease development progressed, infected plants exhibited reduced growth, general decline, and ultimately complete wilting and death in the susceptible cultivar. In contrast, control plants

treated only with sterile distilled water remained healthy throughout the experimental period

Assessment using the 0–4 disease rating scale revealed a clear difference in disease severity between the two cucumber cultivars. The local susceptible cultivar recorded a disease severity of 83.33 %, whereas the imported resistant cultivar exhibited a lower disease severity of 33.33 %. These results indicate that the F.o.c. isolate exhibited greater pathogenicity toward the local cultivar, while the imported cultivar showed a higher level of resistance to *Fusarium* wilt. The lower disease severity observed in the resistant cultivar was also associated with delayed symptom development and reduced progression of wilting compared with the susceptible cultivar (Fig. 3).

To complete Koch's postulates, the pathogen was re-isolated from symptomatic tissues and cultured on PDA medium. The recovered isolate exhibited cultural and microscopic characteristics consistent with those of the original isolate, supporting the identification of *F. oxysporum* f. sp. *cucumerinum* as the causal agent associated with the observed wilt symptoms.

The development of chlorosis followed by progressive wilting observed in the present study is consistent with previous descriptions of *Fusarium* wilt, in which vascular colonization is associated with disruption of water transport and subsequent wilting of infected plants (Vasić et al., 2025; Yu et al., 2025).

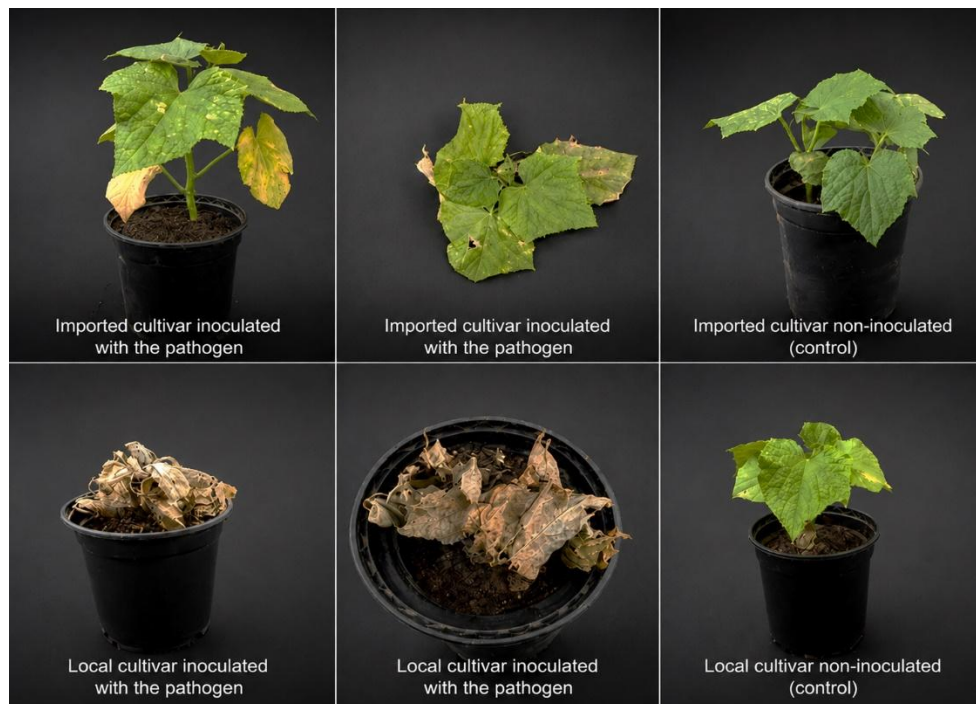


Figure 3. Comparison of *Fusarium* wilt symptoms in the imported resistant and local susceptible cucumber cultivars following inoculation with *F. oxysporum* f. sp. *cucumerinum*, compared with uninoculated control plants

3.5. Effect of Different Concentrations of Hymexazol on the Growth of *F. oxysporum* f. sp. *cucumerinum*

The effect of different concentrations of Hymexazol on the radial growth of *Fusarium oxysporum* f. sp. *cucumerinum* (F.o.c.) is presented in Figure 3. The results demonstrated a concentration-dependent inhibitory effect of the fungicide on fungal growth. No inhibition was observed in the untreated control (0 ppm), where the inhibition percentage was 0%.

Application of Hymexazol at 500 ppm resulted in 15% inhibition of mycelial growth. Increasing the concentration to 1000 ppm significantly enhanced fungal growth inhibition to 50%. A further increase to 1500 ppm raised the inhibition percentage to 85%, indicating a substantial reduction in radial growth. Complete inhibition (100%) was achieved at 2000 ppm, where no fungal growth was observed on PDA medium (Fig. 4).

These findings clearly demonstrate a positive relationship between Hymexazol concentration and inhibition of fungal growth. The fungicide became progressively more effective as its concentration increased, ultimately achieving complete suppression of fungal development at the highest concentration tested. This result indicates the high efficacy of Hymexazol against *F. oxysporum* f. sp. *cucumerinum* under laboratory conditions.

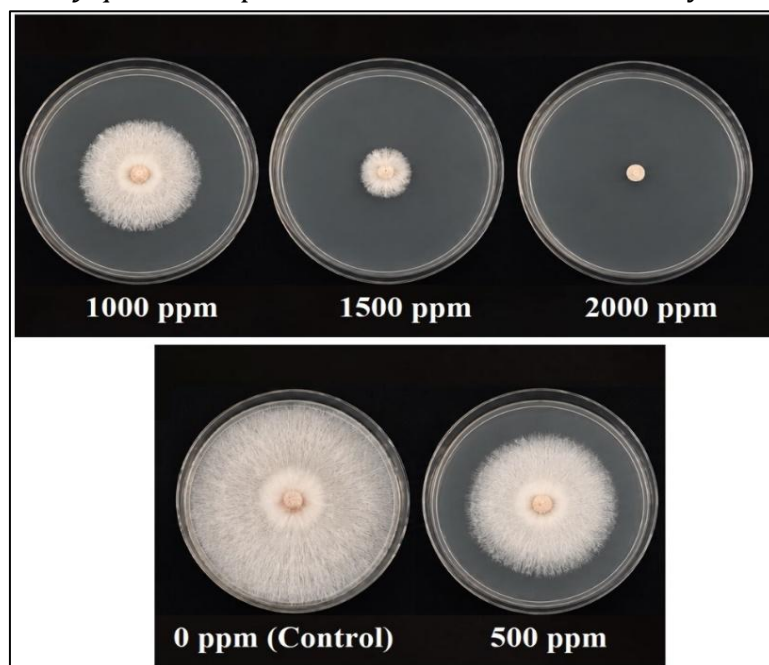


Figure 4. Effect of different concentrations of Hymexazol on the radial growth of *F. oxysporum* f. sp. *cucumerinum*

The concentration-dependent inhibition observed in the present study suggests that increasing the amount of active ingredient enhances the fungicidal activity of Hymexazol. As a systemic fungicide commonly used for the management of soil-borne fungal pathogens, Hymexazol suppresses mycelial growth and limits the ability of the pathogen to colonize nutrient media, resulting in a progressive reduction in colony diameter with increasing fungicide concentration. The complete inhibition observed at 2000 ppm further indicates the high sensitivity of the fungal isolate to Hymexazol under in vitro conditions.

These findings are consistent with the general principles described by Agrios (2005), who reported that the efficacy of fungicides largely depends on the concentration of the active ingredient and that increasing the concentration within the recommended range generally enhances suppression of fungal growth. Likewise, the reduction in radial growth

observed in the present study reflects the ability of Hymexazol to inhibit pathogen development during the early stages of infection.

3.6. Effect of Different Concentrations of Hymexazol on the Growth of *T. viride*

The results presented in Figure 5 showed that none of the tested Hymexazol concentrations (500, 1000, 1500, or 2000 ppm) had any detectable effect on the radial growth of *T. viride*. The fungus exhibited normal colony development comparable to that of the untreated control, indicating a high level of tolerance to the fungicide across all tested concentrations.

This tolerance may be attributed to the physiological and genetic characteristics of *Trichoderma* species, which possess a remarkable capacity to adapt to diverse environmental conditions. In addition, these fungi are equipped with detoxification systems and metabolic enzymes that enable them to tolerate a wide range of chemical compounds more efficiently than many plants pathogenic fungi (Druzhinina et al., 2011). Their rapid growth and efficient utilization of available nutrients may also contribute to maintaining normal growth even in the presence of certain fungicides (Alkooranee et al., 2017).

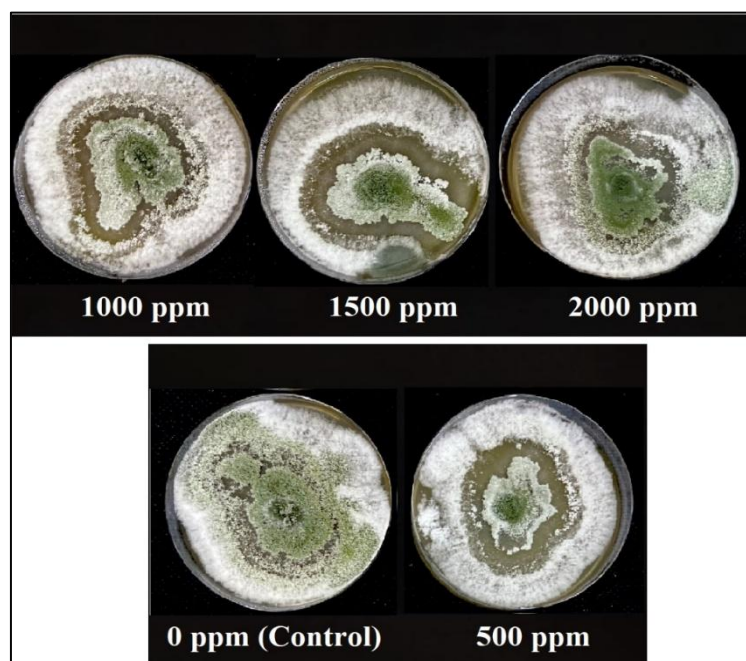


Figure 5. Effect of different concentrations of Hymexazol on the radial growth of *T. viride*

These findings have important practical implications, as they suggest that Hymexazol can be applied simultaneously with *T. viride* within integrated management programs for Fusarium wilt. Under such conditions, the fungicide suppresses the pathogen while preserving the activity of the biological control agent, thereby improving disease management and reducing reliance on chemical fungicides alone (Zhang et al., 2021).

3.7. Antagonistic Activity of *T. viride* Against *F. oxysporum* f. sp. *cucumerinum* in Dual Culture

The dual culture assay demonstrated a strong antagonistic interaction between *T. viride* and *F. oxysporum* f. sp. *cucumerinum* on PDA medium. *T. viride* exhibited a markedly faster

growth rate than the pathogen and rapidly colonized the culture medium. It completely overgrew the pathogen colony and subsequently occupied the entire surface of the Petri dish in all replicates, resulting in complete suppression of pathogen growth (Fig. 6).

According to the classification proposed by Bell et al. (1982), the isolate was assigned to Class 1, in which the antagonist completely overgrows the pathogen colony and occupies the entire culture medium. This classification indicates a very high antagonistic potential of *T. viride* against *F. oxysporum* f. sp. *cucumerinum*.

The superior antagonistic performance of *T. viride* can be attributed to the combined action of several biological control mechanisms, including rapid competition for nutrients and space, direct mycoparasitism of the pathogen, secretion of fungal cell wall-degrading enzymes such as chitinase, β -1,3-glucanase, and protease, and the production of numerous antifungal secondary metabolites. Together, these mechanisms disrupt the pathogen's hyphae and effectively suppress its growth.

The present findings are consistent with those reported by Dakheel (2021), who demonstrated that *Trichoderma koningiopsis* exhibited strong antagonistic activity against several root rot pathogens on PDA medium. In that study, inhibition of *Curvularia lunata*, *F. oxysporum*, and *T. griseum*, isolated from root rot of spider plant, reached 80.39%, 100%, and 100%, respectively. Likewise, inhibition of *F. oxysporum* and *F. equiseti* isolated from root rot of *Tradescantia* reached 74.5% and 100%, respectively. Complete inhibition (100%) was also recorded against *F. oxysporum*, *T. griseum*, and *Marcellina persoonii* associated with root rot of *Gazania*, whereas inhibition of *F. oxysporum* and *Lasiodiplodia theobromae* isolated from bleeding-heart plants reached 84.31% for both pathogens.



Figure 6. Dual culture assay on PDA showing the upper and reverse colony surfaces of *T. viride* and *F. oxysporum* f. sp. *cucumerinum*

The present results also agree with those of Harman et al. (2004), who reported that *Trichoderma* species suppress plant pathogenic fungi through multiple complementary mechanisms, including competition, mycoparasitism, secretion of hydrolytic enzymes, and production of antifungal metabolites, making them among the most successful biological control agents against plant diseases. Similarly, Druzhinina et al. (2011) suggested that the ecological success of *Trichoderma* is largely attributable to its extensive repertoire of genes

involved in the production of cell wall-degrading enzymes and secondary metabolites, together with its exceptional ability to adapt to and colonize diverse ecological niches. These characteristics explain its superior competitive performance against many plants' pathogenic fungi in dual culture assays.

4- Conclusion

The present study demonstrated that Hymexazol and *Trichoderma viride*, when evaluated separately under in vitro conditions, effectively inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum*. Hymexazol showed a clear concentration-dependent inhibitory effect, whereas *T. viride* exhibited strong antagonistic activity against the pathogen. These findings indicate the potential of both treatments as individual components for the management of cucumber Fusarium wilt. However, their compatibility and combined efficacy were not evaluated in the present study. Therefore, further studies under pot, greenhouse, and field conditions are required to determine their compatibility, combined effectiveness, and practical applicability in integrated disease management programs.

References

- Abbey, B. W., Nwachoko, N., & Ikiroma, G. N. (2017). Nutritional value of cucumber cultivated in three selected states of Nigeria. *Biochemistry & Analytical Biochemistry*, 6(3), Article 1000328. <https://doi.org/10.4172/2161-1009.1000328>.
- Agrios, G. N. (2005). *Plant pathology* (5th ed.). Elsevier Academic Press.
- Alkooranee, J. T., Aledan, T. R., Ali, A. K., Lu, G., Zhang, X., Wu, J., Fu, C., & Li, M. (2017). *Induced systemic resistance in Brassica napus mediated by Trichoderma harzianum against Sclerotinia sclerotiorum and Leptosphaeria maculans. Physiological and Molecular Plant Pathology*, 97, 71–77. <https://doi.org/10.1016/j.pmpp.2016.12.011>
- Al-Rawi, K. M., and Khalaf Allah. A. A. M., (2000). Design and Analysis of Agricultural Experiments. Dar Al-Kutub for Printing and Publishing, College of Agriculture and Forestry, University of Mosul.
- Aziz, N. J., & Huseen, K. M. (2023). An analytical economic study of the cucumber crop production systems in Sulaymaniyah Governorate for the agricultural season (2021–2022). *Journal of University of Human Development*, 9(4), 12–22. <https://doi.org/10.21928/juhd.v9n4y2023.pp12-22>.
- Báez-Sañudo, M. A., Jiménez-Ortega, L. A., & Bastidas-Bastidas, P. J. (2022). Assessment of residuality of hymexazol in strawberry (*Fragaria × ananassa*) crop by a modified QuEChERS method and liquid chromatography tandem-mass spectrometry. *Agronomy*, 12(12), 3110. <https://doi.org/10.3390/agronomy12123110>.
- Bell, D. K., Wells, H. D., & Markham, C. R. (1982). In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*, 72, 379–382.
- Booth, C. (1977). *Fusarium: Laboratory guide to the identification of the major species*. Commonwealth Mycological Institute.
- Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., & Mauch-Mani, B. (2006). Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions*, 19(10), 1062–1071. <https://doi.org/10.1094/MPMI-19-1062>

Dakheel, F. A. (2021). Integration of biological control agents and chemical fungicides for the management of root disease pathogens of ornamental plants in nurseries of Karbala and Babylon Governorates (Master's thesis). College of Agriculture, University of Karbala, Karbala, Iraq.

Descalzo, R. C., Rahe, J. E., & Mauza, B. (1990). Comparative efficacy of induced resistance to selected diseases of greenhouse cucumber. *Canadian Journal of Plant Pathology*, 12(1), 69–79. <https://doi.org/10.1080/07060669009501037>.

Descalzo, R. C., Rahe, J. E., & Mauza, B. (1990). Comparative efficacy of induced resistance to selected diseases of greenhouse cucumber. *Canadian Journal of Plant Pathology*, 12(1), 69–79. DOI: 10.1080/07060669009501037.

Dewan, M. M. (1989). Identify and Frequency of Occurrence of Fungi in Root of Wheat and Ryegrass and Their Effect on Take All and Host Growth. *Ph.D. Thesis, University of Australia*, 210 pp.

Dewan, M.M. (1989). Identify and Frequency of Occurrence of Fungi in Root of Wheat and Ryegrass and Their Effect on Take All and Host Growth. *Ph.D. Theses, University of Australia*, pp.210.

Druzhinina, I. S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B. A., Kenerley, C. M., Monte, E., Mukherjee, P. K., Zeilinger, S., Grigoriev, I. V., & Kubicek, C. P. (2011). Trichoderma: The genomics of opportunistic success. *Nature Reviews Microbiology*, 9(10), 749–759. <https://doi.org/10.1038/nrmicro2637>.

Gong, M., He, J., Kong, M., Huo, Q., Jiang, Y., Song, J., ... & Li, X. (2023). A microencapsulation approach to design microbial seed coatings to boost wheat seed germination and seedling growth under salt stress. *Frontiers in Plant Science*, 14, 1283590. <https://doi.org/10.3389/fpls.2023.1283590>.

Guzmán-Guzmán, P., Porras-Troncoso, M. D., Olmedo-Monfil, V., & Herrera-Estrella, A. (2019). Trichoderma species: Versatile plant symbionts and biological control agents. *Annual Review of Phytopathology*, 61, 201–224.

Hazirah Mohd Din, H., Rashed, O., & Ahmad, K. (2020). Prevalence of Fusarium wilt disease of cucumber (*Cucumis sativus* Linn.) in Peninsular Malaysia caused by *Fusarium oxysporum* and *Fusarium solani*. *Tropical Life Sciences Research*, 31(3), 29–45. <https://doi.org/10.21315/tlsr2020.31.3.3>.

Hou, Y., Guo, Y., Wang, L., He, S., Zheng, W., Liu, S., & Xu, J. (2023). Impact of phenamacril on the growth and development of *Fusarium pseudograminearum* and control of crown rot of wheat. *Plant Disease*, 107(12), 3843–3850.

Javid, H., Fatima, U., Rukhsar, A., Hussain, S., Bibi, S., Bodlah, M. A., Shahzad, H. H., Dilshad, M., Waqas, M., & Sharif, A. (2024). Phytochemical, nutritional and medicinal profile of *Cucumis sativus* L. (Cucumber). *Food Science and Engineering*, 5(2), 358–377. <https://doi.org/10.37256/FSE.5220244795>

Jayawardana, M. A., & Fernando, W. G. D. (2024). The mechanisms of developing fungicide resistance in *Fusarium graminearum* causing Fusarium head blight and fungicide resistance management. *Pathogens*, 13, 1012.

Kovalchuk, I. (2023). Role of epigenetic factors in response to stress and establishment of somatic memory of stress exposure in plants. *Plants*, 12(21), 3667. <https://doi.org/10.3390/plants12213667>.

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. Blackwell Publishing. <https://doi.org/10.1002/9780470278376>.

Mc Kinney, H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. 1. Agric. Res. 26: 195-210.

McKinney, H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research*, 26(5), 195–217.

Myresiotis, C. K., Vryzas, Z., & Papadopoulou-Mourkidou, E. (2012). Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. *Biodegradation*, 23(2), 297–310. <https://doi.org/10.1007/s10532-011-9509-6>.

Panth, M., Hassler, S. C., & Baysal-Gurel, F. (2020). Methods for management of soilborne diseases in crop production. *Agriculture*, 10(1), 16. <https://doi.org/10.3390/agriculture10010016>.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, 52, 347–375. <https://doi.org/10.1146/annurev-phyto-082712-102340>

Sun, Y., Wang, M., Li, Y., Gu, Z., Ling, N., Shen, Q., & Guo, S. (2017). Wilted cucumber plants infected by *Fusarium oxysporum* f. sp. *cucumerinum* do not suffer from water shortage. *Annals of botany*, 120(3), 427–436. <https://doi.org/10.1093/aob/mcx065>.

Vasić, T., Živković, S., Filipović, S., Mitra, D., & Jevremović, D. (2025). *Fusarium oxysporum*, the cause of Fusarium wilt on cucumber (*Cucumis sativus* L.) in Serbia. *Biologica Nyssana*, 16(1). <https://doi.org/10.46793/BiolNyss.16.1.9>.

Yu, M., Yuliana, R., Tumewu, S. A., Bao, W., Suga, H., & Shimizu, M. (2025). Efficacy of L-arabinose in managing cucumber Fusarium wilt and the underlying mechanism of action. *Pest Management Science*, 81(3), 1239–1250. <https://doi.org/10.1002/ps.8523>.

Zhang, C., Wang, W., Xue, M., Liu, Z., Zhang, Q., Hou, J., Xing, M., Wang, R., & Liu, T. (2021). The combination of a biocontrol agent *Trichoderma asperellum* SC012 and hymexazol reduces the effective fungicide dose to control Fusarium wilt in cowpea. *Journal of Fungi*, 7(9), 685. <https://doi.org/10.3390/jof7090685>.