Comparative Study of Biological and Chemical Control for Fusarium Wilt in Tomato

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Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol), severely affects tomato crops worldwide. This study evaluated the effectiveness of chemical and biological control methods on the susceptible tomato variety A-Z-54-F1, under laboratory and field conditions. Four Fol isolates (F1–F4) were obtained from infected tomato fields, while *Bacillus subtilis* was isolated from rhizosphere soil and *Trichoderma harzianum* was obtained from Tasneem Company. In vitro, Hemixazol completely inhibited Fol growth (100%), followed by *B. subtilis* (64.53%) and *T. harzianum* (47.97%). Field experiments showed Hemixazol significantly reduced infection rate from 70% to 10% and severity from 81% to 15%, achieving the highest control efficacy (81.48%). *B. subtilis* and *T. harzianum* reduced infection to 26% and 30%, respectively, and reduced severity to 31% and 44%, respectively, with control efficacies of 61.73% and 45.68%, respectively. Hemixazol was the most effective, though biological agents also offered promising protection. It can be concluded that both chemical and biological treatments can reduce the incidence of Fusarium wilt disease on tomato plants.

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Introduction

Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is considered one of the most dangerous diseases affecting tomato plants worldwide (Al-mourrh *et al.,* 2024a), including in the United States (Pandey *et al.,* 2024). This fungal disease significantly impacts plant growth, leading to wilting and death (Hellman *et al.,* 2024). The disease spreads through contaminated soil or water carrying the fungal spores (Muhorakeye *et al.,* 2024), making it difficult to control in open agricultural environments (Kayim *et al.,* 2022). Fusarium wilt causes significant economic losses in tomato production, with farms in the United States losing millions of dollars annually due to reduced yields and the cost of control measures. Recent estimates indicate that tomato production in the U.S. is particularly affected in regions with environmental conditions favorable for fungal spread (Pandey *et al.,* 2024).

Over the past decades, farmers have used chemical fungicides to combat Fol, These fungicides inhibit the growth of pathogenic fungi, providing farmers with a quick and effective way to counter disease outbreaks in the fields (Al-mourrh *et al.,* 2024b).

Agrios (2005) noted that fungicides like benzimidazole and dicarboxim are widely used to control *Fol*, showing effectiveness in limiting the spread of the disease when applied correctly. However, the repeated use of these fungicides can lead to the emergence of resistant fungal strains (Akbari *et al.,* 2024). Over time, the fungus may become less responsive to fungicides, reducing their effectiveness and complicating disease control(Al-mourrh *et al.,* 2024a).

Martínez-Cano *et al.* (2022) found that the repeated use of benzimidazole fungicides, with limited use of fungicides, contributed to the development of resistance in Fol to many commonly fungicides. Also found that several strains of Fol have become capable of surviving and reproducing despite the use of these fungicides. Researchers emphasize the need to monitor fungicide use and integrate it with other techniques, such as integrated pest management (IPM), to avoid further resistance development and extend the effectiveness of available fungicides (Brent and Hollomon 2007).

Fungicides threaten the environment and the health of those who handle them. In nature, they can contaminate soil, water, and food, causing respiratory problems, skin disorders, hormonal disturbances, and sometimes serious diseases such as cancer.

Therefore, biological control has emerged as a sustainable and environmentally friendly alternative to limit the spread of plant diseases, including Fusarium wilt in tomatoes. This control relies on the use of natural organisms such as fungi and bacteria to combat plant diseases (Hillman *et al.,* 2024).

As it reduces reliance on harmful chemicals and helps maintain ecological balance (Akanand *et al.,* 2024). For example, studies have shown that the use of *Bacillus* species as biological agents can effectively reduce the spread of Fusarium wilt in tomatoes without harming the ecosystem (Karačić *et al.,* 2024). These organisms improve plant health by stimulating its natural defense mechanisms and reducing the presence of pathogenic fungi in the soil, thereby minimizing the need for chemical intervention (El Ariebi *et al.,* 2016).

In addition to environmental benefits, biological control also helps reduce production costs in the long term by improving soil quality and increasing crop productivity in a sustainable way *(*Woo *et al.,* 2023). This makes the shift to biological control an important step toward achieving safer and more sustainable agriculture in the future, as fungi develop resistance to conventional fungicides, switching to more sustainable and environmentally friendly control methods, such as biological control, helps reduce reliance on chemical fungicides and enhances natural plant immunity (Alsalmo *et al.,* 2025). This research aimed to isolate and identify the fungi causing wilt in tomato plants. And study the use of *Trichoderma* sp. fungus and local strain *Bacillus subtilis*. Bacteria in biological control of Fusarium wilt in tomatoes compared with the chemical fungicide Hemixazole.

Materials and Methods

The Tomato variety

In this study, we used A-Z-54-F1, a *Fol*-susceptible variety from the Turkish company A.Z TOHUM. This high-yield, climbing plant produces medium-sized fruits (240-280 g).

The tools used

Several tools used to conduct laboratory and field experiments, as shown in Table (1):

Table 1. Tools used for conducting laboratory and field experiments

No.	Tool	Use
1	Sterilized scissors or scalpel	Cutting infected plant parts (leaves, stem, roots)
2	Sterilized inoculation needle	Transferring fungal samples to the growth medium
3	Sterilized petri dish	Cultivating fungal isolates on growth media
4	Growth medium (e.g., PDA)	Providing a suitable environment for fungal growth
5	Sterilized test tubes	Storing fungal isolates after isolation
6	Autoclave	Sterilizing tools and media before use
7	Light microscope	Examining microscopic characteristics of isolated fungi
8	Ethyl alcohol (70%)	Sterilizing tools and work surfaces to prevent contamination
9	Flasks and cylinders of various sizes	Preparing suspensions and solutions

Chemicals growth media and Chemicals material

The main chemicals and growth media used to identify *Bacillus subtilis*. Bacteria isolated from the rhizosphere of tomato plants, along with materials for isolating Fusarium fungi and preserving fungal isolates, are shown in Table 2:

Table 2. Chemicals and growth media used in the experiment

No.	Chemical	Use
1	Gram Crystal Violet	Primary stain for Gram staining
2	Safranin	Counterstain for Gram staining
3	Hydrogen peroxide (H2O2)	Catalase test to determine enzyme activity in *Bacillus*
4	Methylene blue	Stain for examining bacteria under the microscope
5	Nitrate medium	Nitrate reduction test to assess the respiratory capability
6	Potato Dextrose Agar (PDA)	Growth medium for isolation and cultivation of *Fusarium* fungi
7	Nutrient Agar	General growth medium for bacteria
8	Sabouraud Agar	Specialized medium for fungi and yeast growth
9	Hemexazol	Hemexazol Fungicide 99% TC 70% WP, 30% SL, 15% (C10H17O7).

Sample Collection

A field survey was conducted in the Azaz area of Aleppo Governorate, covering 10 tomato fields as follows: F1 (36.621247, 37.112865), F2 (36.609173, 37.115698), F3 (36.596620, 37.138841), F4 (36.583118, 37.135527), F5(36.588417, 37.086890), F6 (36.525625, 37.117578), F7 (36.548357, 37.084666), F8 (36.564637, 37.235024), F9 (36.600278, 37.214119), Samples were collected from plants showing symptoms of Fusarium vascular wilt. The sampling period for isolation extended from May to August 2023. Samples were obtained from fields in the Azaz area, which is part of the Aleppo Governorate. The isolation procedures were carried out at the Faculty of Agriculture, Idlib University.

Isolation and Identification of the Fungus

Samples of roots were washed with running water, cut into 1 cm pieces, and surface sterilized with a 0.5% NaOCl solution for 2 minutes, then rinsed twice with distilled water. The roots were then placed on potato dextrose agar (PDA) medium and incubated at 25°C in dark conditions. After 5 days of incubation, a cork borer was used to extract 5 mm discs and five discs were transferred to the middle of new 9 cm PDA plates containing 15 mL of PDA medium. The plates were incubated at 25°C for 7 days.

Fusarium oxysporum f. sp. *lycopersici* was identified by the Department of Plant Pathology, Faculty of Agriculture, University of Idlib, based on its described morphological characteristics reported by (Leslie and Summerell 2006).

Fungal Biomass Production

Fungal biomass was produced by cultivating the fungus on sterilized barley grains. Conical flasks containing sterilized barley were autoclaved at 121°C and 1.5 kg/cm² pressure for 30 minutes, twice. After sterilization, the flasks were inoculated with the fungus (*Fusarium oxysporum*), using 10 fungal discs per 250 ml flask, and incubated at 25 ± 2°C for two weeks.

Isolation of *Bacillus* Bacteria

Bacillus bacteria were isolated from tomato root soil samples and mixed with distilled water to form a suspension. Serial dilutions were performed, and the final dilution of 10-6 was applied to nutrient agar. Plates were incubated at 37°C for 48 h to grow bacterial colonies. Pure Bacillus isolates were obtained and identified using enzyme assays (as shown in Table 4) and staining tests (Hellany, 2024).

Isolation of *Trichoderma*

Trichoderma fungus isolate was obtained from Tasnim Agricultural Company, where ready-made powder of *Trichoderma harzianum* fungus is used to control plant diseases at a rate of 1g L^{-1} (according to the manufacturer's recommendations). *Trichoderma* fungus is characterized by its ability to attack pathogenic fungi and enhance plant health. It can be applied directly as a preventive or curative treatment, often mixed with soil. When used preventively, *Trichoderma* colonizes the rhizosphere and competes with pathogens for space and nutrients, while also producing antimicrobial compounds and inducing systemic resistance in plants (Harman *et al.,* 2004; Benítez *et al.,* 2004).

Pathogenicity Test

The pathogenicity test followed the method of Haggag and Al-Jamal. Infected barley grains were mixed with sterile soil (3% of soil weight), and four-week-old tomato seedlings were planted in the infested soil. Disease incidence was recorded 40 days after planting.

Preparation of Solutions

Fol fungal inoculum was cultured on PDA for 8 days. The spores were suspended in 5000 ml of sterile water to achieve a concentration of 1×10^{6} CFU (Corral Melgoza *et al.,* 2024). Similarly, *Bacillus* suspensions were prepared at 1×10^{8} CFU. A chemical fungicide, Hemexazol Fungicide 99 % TC 70% WP,30% SL,15% ($C_{10}H_{17}O_7$), and *Trichoderma* powder was dissolved in water (1 g L^{-1}).

In Vitro Bioassay

The inhibitory activity of *Trichoderma* sp. and *Bacillus* sp. against *Fusarium* was tested using dual culture techniques on petri dishes (Ghasemi *et al.,* 2019). Fol discs (5 mm) were placed on the opposite side of *Trichoderma* sp. and *Bacillus* sp. inoculation, and plates were incubated. The fungicide Hemixazol was applied by mixing 100 µL into the medium (Malandrakis *et al.,* 2018). The inhibition percentage was calculated using the formula (Skidmore and Dickinson, 1976):

$$Inhibition\ (\%) = \frac{C\text{-}T}{T}$$

C: Colony diameter of control, T: Colony diameter of treated colony.

Field Bioassay

The field experiment was conducted in Azaz. Soil mix (3 parts soil: 1 part peat moss) was sterilized with formalin (5%) and ventilated for 3 days before planting five-week-old tomato seedlings. The soil was mixed with *Fusarium* spores grown on barley grains at a rate of 3 grams per liter of soil. Plant seedlings took off, and then the roots were dipped in *Fol* spore suspension before transplanting into 3-liter pots. Next day the plants were treated with 300 ml of *Trichoderma harizanum*, *Bacillus subtilis*., or Hemixazol solution. Control infection plants received sterile water. The experiment was conducted with five replicates per treatment, and disease incidence was recorded after 40 days.

The infection rate was calculated as (Al-mourrh *et al.,* 2024a), using the formula:

$$\text{Infection } (\%) = \frac{n}{N} \times 10$$

N: Number of infected plants in control, n: Number of infected plants in treatment.

Effectiveness of the biological agent (EFA), or fungicide was calculated as (Almasoudi *et al.,* 2025), based on the treatment: $$EFA\ (\%) = \frac{N-n}{N} \times 10$$

N: number of infected plants in control, n: number of infected plants in treatment.

The disease severity was calculated according to (El Shamy *et al.,* 2024) by the following formula:

$$Severity\ (\%) = \sum \left(\frac{a \times b}{N \times K}\right) \times 100$$

a: Number of infected plants at each infection level.

b: Infection rating according to the scale five degree (0: No infection, 1: 1-25% severity, 2: 26-50% severity, 3: 51-75% severity, 4: 76-100% severity).

K: Highest infection rating on the scale.

N: Total number of plants.

Statistical analysis

The laboratory experiment was designed as a completely randomized design with five replications for each biological agent, five replications for the fungicide Hemixazol, and five replications for the control treated with distilled water. The field experiment was IBM Corp (2021). IBM SPSS Statistics for Windows, Version 27.0. designed as a completely randomized design with six replications for each biological agent, six replications for the fungicide Hemixazol, and six replications for the control treated with distilled water.

The means were compared using LSD according to the ANOVA test at a 1% significance level for the laboratory experiment, and 5% for the field experiment.

Results and Discussion

Results of Isolating the Wilt-Causing Fungus in the Study Area

During a field survey of 10 tomato fields in the Azaz region, the fungus *Fusarium* was isolated Four isolates were obtained, identified as *Fusarium oxysporum* according to Leslie and Summerell, (2006). Based on the morphological characteristics of the colony, the macroconidia, microconidia, and chlamydospores (Figure 1).

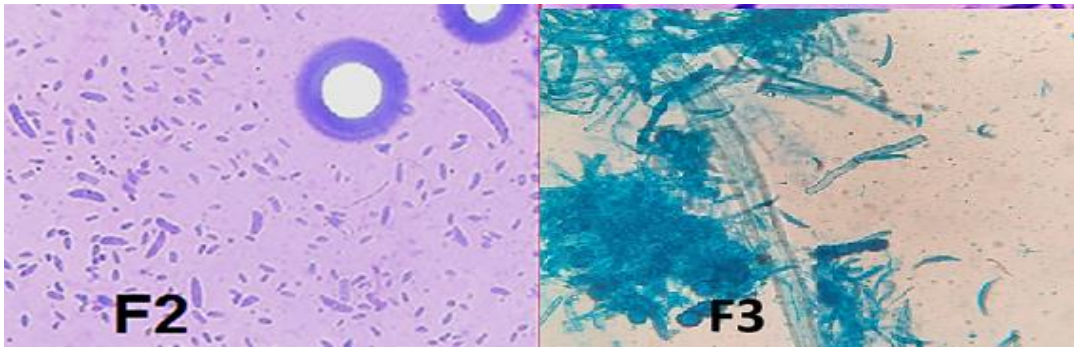

Figure 1. Macroconidia and microconidia were observed in *Fusarium* isolates F2 and F3

This identification is consistent with the findings of Al-mourrh *et al., (*2024a), (Figure 2).

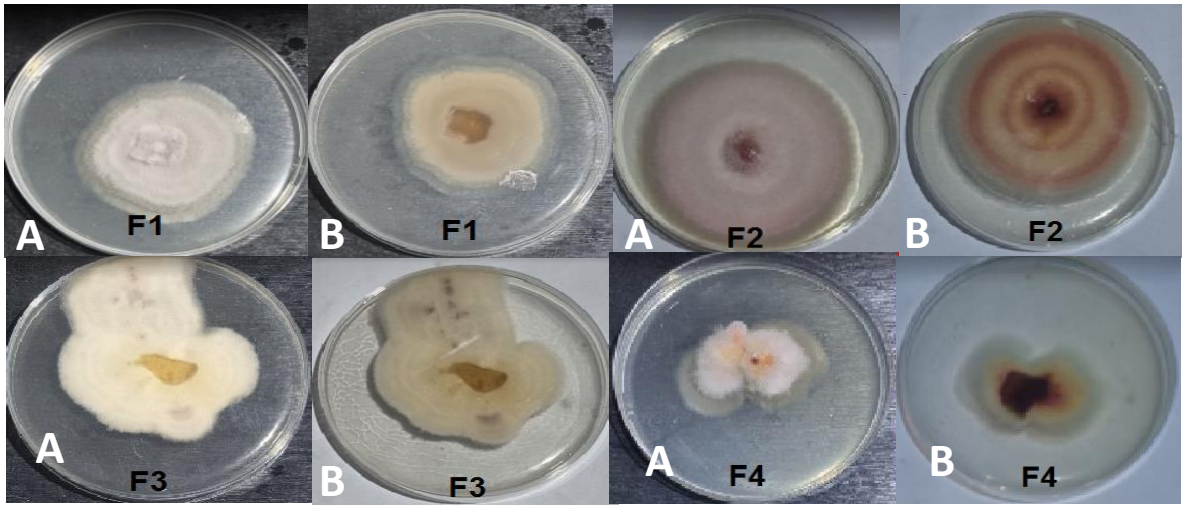

Figure 2. A, The upper and B, lower surfaces of *Fusarium* colonies F1 (A&B) F2 (A&B) F3 (A&B) and F4 (A&B)

These colonies were characterized by their rapid growth on PDA medium, (Table 3).

Table 3. the color of the colonies, the shape of the apical cell of the macroconidia, the growth rate on PDA medium, and the site of isolation from plants

Isolate	Colony Color	Shape of the Apical Cell of Macroconidia	Growth Rate (days/mm)	Plant Isolation Site	Result
F1	Whitish Pink	Tapered	6.4	Crown Region	F o*

F2	Pink	Tapered	7.3	Crown Region	F o
F3	White	Tapered	4.3	Roots	F o
F4	Yellowish White	Rounded	5.2	Roots	F s**

Fo*: *Fusarium oxysporum*. F s**: *Fusarium solani*.

Pathogenicity Test for Fusarium Isolates

All the isolates caused disease infection, but showed varying levels of severity, (This variation is likely due to differences in virulence factors, such as toxin production and enzymatic activity, as well as possible genetic diversity among the isolates. Environmental conditions and interactions with the host genotype may also have contributed to these differences) (El Shamy *et al.,* 2024) and isolate number 2 *Fusarium oxysporium* being the most virulent, causing an infection rate of 48% (Figure 3).

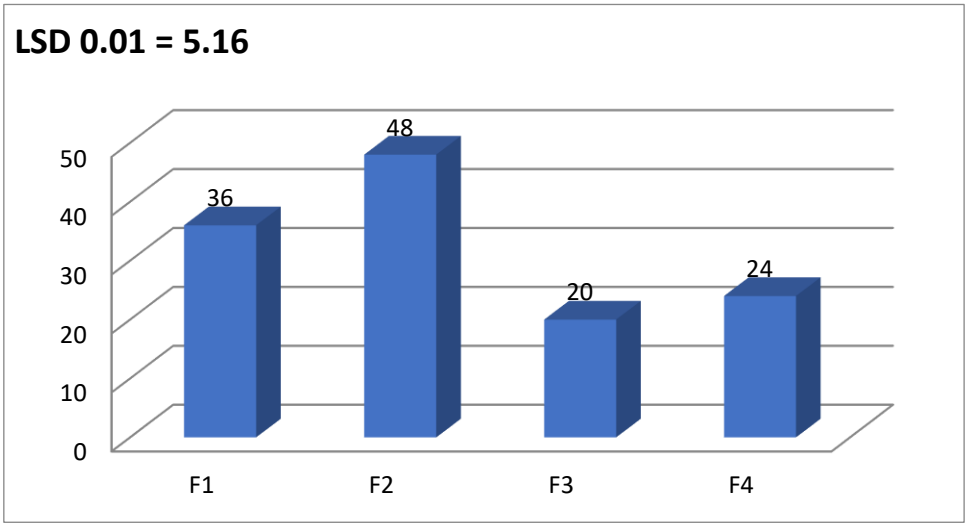

Figure 3. The percentage of infections caused by *Fusarium* isolates

Isolation of *Bacillus* from Soil around Tomato Roots

By culturing soil samples diluted to 10^{-6} from around tomato plant roots, different bacterial genera were obtained. Several isolates were tested, and one isolate, which showed clear inhibitory activity in Petri dishes against the Fusarium fungus, was identified. It was found to belong to the Bacillus bacteria through enzymatic and biological tests, as shown in Inhibition tests showed that Bacillus had a colony inhibition rate of 64.53%, demonstrating its clear effectiveness in reducing fungal spread, agree with (El-Mougy and Abdel-Kader, 2019). While Trichoderma fungus showed a moderate inhibition rate of 47.97%, agree (Abd-El-Kareem *et al.,* 2021), (Table 4).

Table 4. Results of biochemical and enzymatic tests for *Bacillus* sp. bacteria

Isolate	Test	Result
Bacillus sp.	Gram Stain	+
Its shape is rod-shaped. Short,	Spores	+
	Motility	+
	Growth in 7% saline solution	+

Gram-positive, chalky colonies. Single, double, or in chains.	Oxidase	+
	Catalase	+
	Starch Hydrolysis	-
	Gelatin Hydrolysis	+
	Anaerobic Growth	-
	Casein Hydrolysis	+
	Sucrose Fermentation	+
	Voges/Proskaver (VP) test	+

The *Bacillus* bacteria were characterized by a chalky white colony color and a rod-shaped form, with the presence of a central spherical spore, which aligns with the findings of (del Fresno *et al.,* 2021; Hellany, 2024).

Inhibition of Fusarium fungal colony growth by *Bacillus* bacteria and *Trichoderma* fungus in vitro

The results showed that the Hemixazol treatment was the most effective in inhibiting the growth of the Fusarium fungal colony, achieving a 100% inhibition rate, indicating its complete ability to prevent fungal growth (Geiger *et al.,* 2010).

In contrast, the *Bacillus* treatment achieved a colony inhibition rate of 64.53%, reflecting its clear inhibitory effectiveness (Figure 4). Meanwhile, the *Trichoderma* treatment demonstrated an inhibition rate of 47.97% against the Fusarium colony (Nakova, 2010). On the other hand, the average diameter of the Fusarium colony reached 59.2 mm after 5 days of incubation at 25°C on PDA medium (Figure 4).

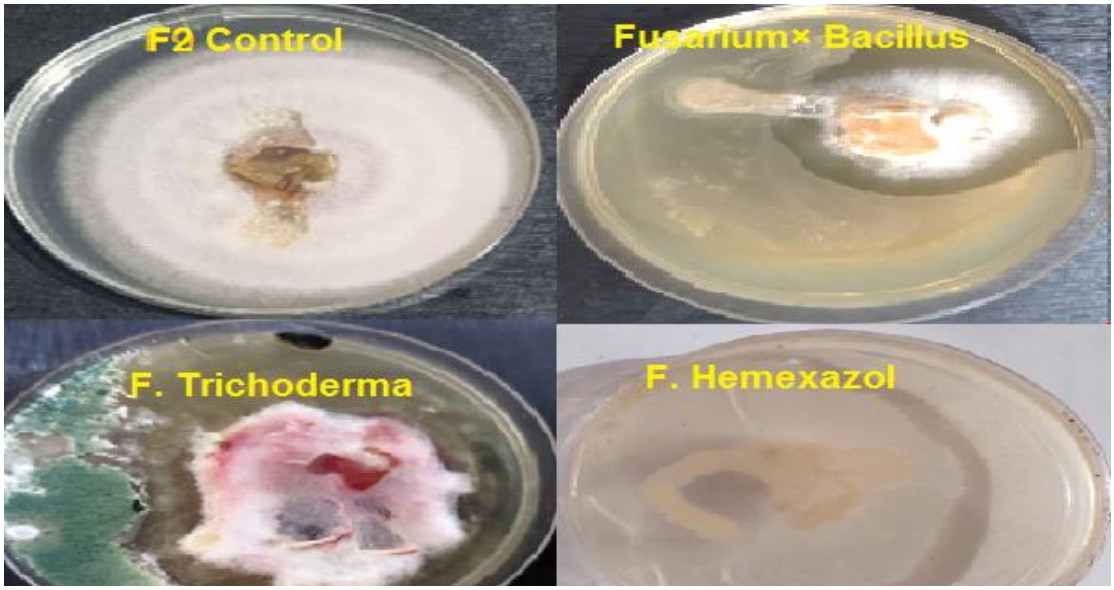

Figure 4. The inhibition of (*Hemexazol* × *Fusarium*), (F×B: *Fusarium*×*Bacillus* (and F×T: *Fusarium* × *Trichoderma* (F: Fo= infected control)

Table 5: Demonstrates the inhibition rate of diameter Fusarium colony growth/ cm by Bacillus bacteria, Trichoderma fungus, and the chemical fungicide Hemixazol in dual culture on PDA medium for 5 days at 25°C.

Table 5. The effects of the *bacillus* and *Trichoderma* and Hemexazole on the Diameter of *Fusarium* colony growth and the percentage of inhibition after 5 days of incubating at 25c°

Tretment	Mean (*Fusarium* colony Diameter)	Inhibition growth after 5 days of incubating (%)
Trichoderma	30.8	47.97
Bacillus	21	64.53
Hemixazol	0	100.00
Fusarium	59.2	0.00
$LSD_{0.01}$	12.22	20.64

The Effect of Bacillus Bacteria and Trichoderma Fungus on the Percentage of Fusarium Infection in the field

In the field, the Hemixazol treatment was the most effective in reducing Fusarium infection, with infection rates dropping to 10% compared to the control, which recorded an infection rate of 70% (Figure 5).

The Bacillus treatment also showed good effectiveness by reducing infection to 26%, followed by the Trichoderma treatment, which recorded an infection rate of 30% compared to the control.

All chemical and biological control treatments significantly contributed to reducing the infection rate, indicating the effectiveness of these biological and chemical agents in combating the fungus (Figure 5).

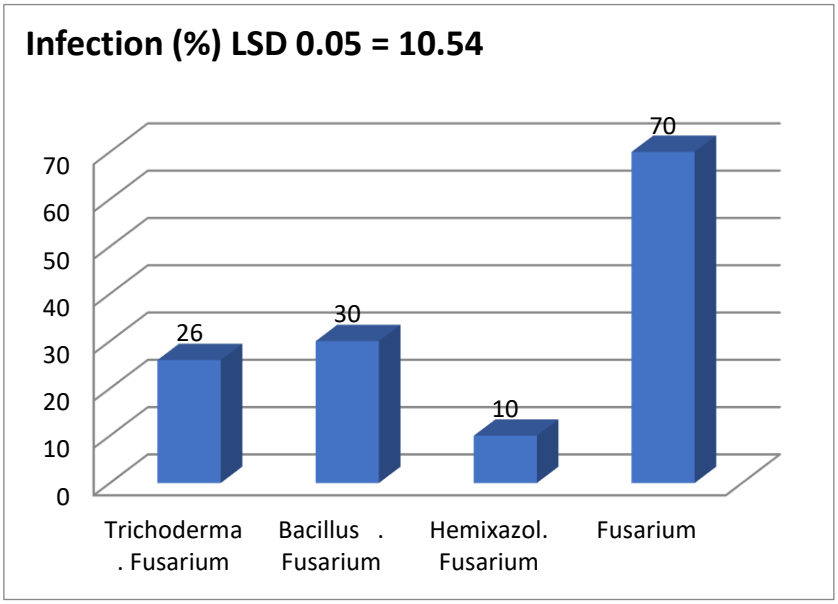

Figure 5. Shows the average percentage of plant infection in the field experiment

The Effect of Bacillus Bacteria and Trichoderma Fungus on the Severity of Fusarium Infection

The Hemixazol treatment reduced the infection severity to 15%, indicating its high effectiveness in reducing the severity of the infection, (Figure 6).

Bacillus treatment reduced the infection severity to 31%, with significant differences compared to the infected control *Fusarium* (81% severity). The *Trichoderma* treatment was less effective than

Hemixazol and *Bacillus*, recording an infection severity of 44%. The control (Fusarium without treatment) showed the highest infection severity, with an average of 81%. These results indicate that all treatments reduced the infection severity compared to the control, with Hemixazol fungicide showing clear superiority. Agree with Martínez-Cano *et al.* (2022); Akanand *et al.* (2024). Followed by Bacillus (20%), and Trichoderma (23%), the same (Ju *et al.,* 2020), (Figure 6).

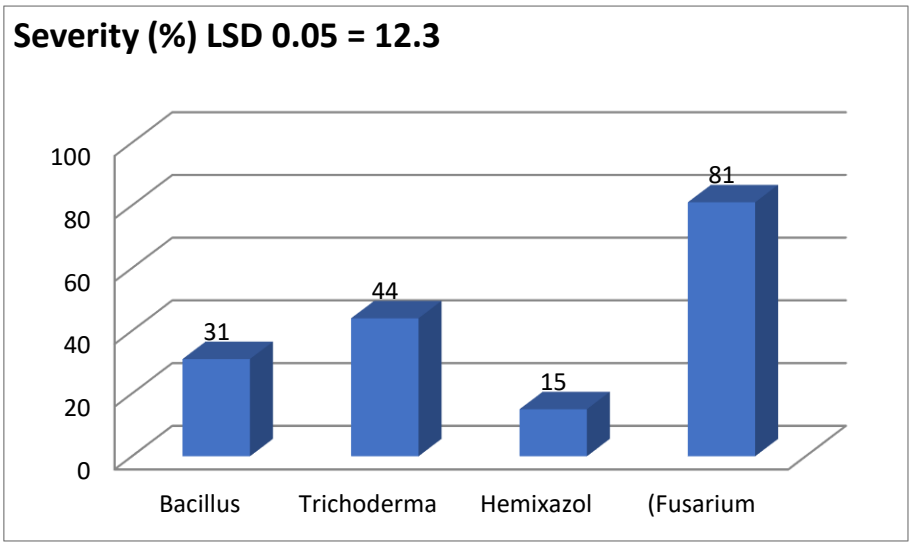

Figure 6. The average percentage of disease severity on the plants

The Effect of *Bacillus* Bacteria and *Trichoderma* Fungus on the Percentage of Fusarium Infection Reduction (EFA)

The results showed that the Hemixazol treatment was the most effective in reducing Fusarium infection, achieving a reduction rate of 81.48%. As shown in Figure 8, this was followed by the *Bacillus* treatment, which reduced the infection by 61.73%, reflecting a good effect in combating the fungus. Meanwhile, the Trichoderma treatment showed moderate effectiveness, with a reduction rate of 45.68% compared to the control (Fusarium), (Figure 7).

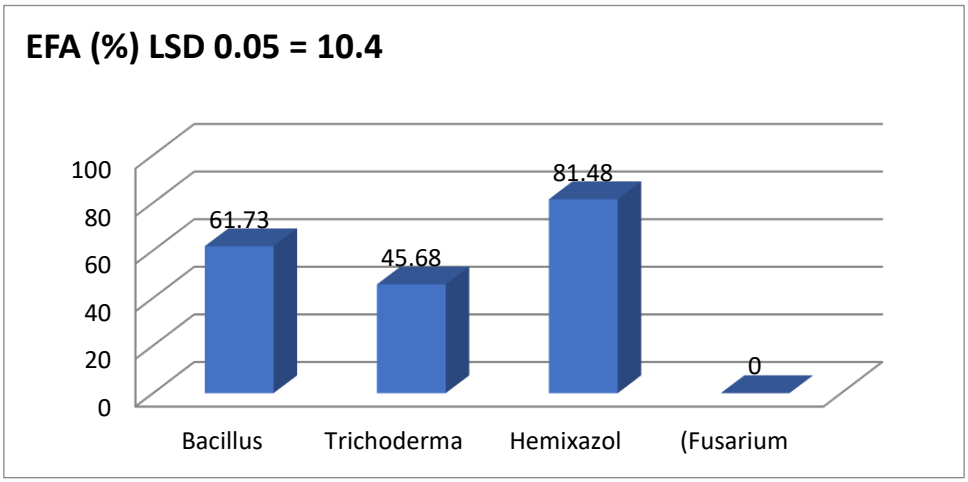

Figure 7. Shows the average percentage of Fusarium infection reduction on the plants in the field experiment

Conclusions

We obtained four isolates of *Fusarium oxysporum* from tomato fields, characterized by rapid growth on PDA medium. The isolates exhibited varying morphological characteristics, with differences in colony color and cell structure, reflecting the diversity of their growth patterns. Pathogenicity testing showed that the isolates had varying abilities to cause plant infection. The second isolate was found to be the most virulent, with a 48% infection rate. This indicates differences in the ability of these isolates to cause wilt. *Bacillus* bacteria were isolated from soil samples surrounding tomato roots, and one isolate showed significant inhibitory activity against Fusarium spp. This isolate was identified as a *Bacillus* sp. through biochemical and enzymatic tests.

Inhibition tests showed a 64.53% inhibition rate for Bacillus colonies. Meanwhile, Trichoderma bacteria showed an inhibition rate of 47.97%. Field experiments confirmed that the fungicide hemixazole was the optimal treatment, reducing the infection rate to 11%. Bacillus bacteria followed with an infection rate of 20%, followed by Trichoderma bacteria with an infection rate of 23%. These results indicate that both chemical and biological treatments can reduce the incidence of Fusarium wilt disease on tomato plants.

Conflict of Interests

The authors declare no competing interests.

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