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Evaluation of the Inhibitory Effects of Biological Agent (Trichoderma), Chemical Agent (Beltanol), and Nanocoatings (Nano-Chitosan) in Controlling *Fusarium solani* Fungus)

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ABSTRACT

This research assessed the inhibitory effects of Trichoderma species, the fungicide Beltanol, and nano-chitosan against Fusarium solani, with particular reference to reducing postharvest decay and ensuring the safety of tomato fruit in the food supply chain. Among the pathogens isolated, F4 showed the highest virulence as it significantly inhibited seed germination. In vitro antagonistic tests on Potato Dextrose Agar (PDA) showed Trichoderma harzianum (T2), T. longibrachiatum (T1), and T. harzianum (T3) to have substantial antagonistic activities (81.67% each) against F. solani. Beltanol exhibited irregular inhibition of Trichoderma isolates, where T1 was one of the least susceptible, and F. solani was very susceptible (74.46%). All fungal isolates were completely inhibited at 1.00 mL/L of Beltanol. Nano-chitosan exhibited dose-dependent antifungal activity, particularly against F. solani (67.30%). The combined treatments showed synergistic effects; the integration of Beltanol, nano-chitosan, and Trichoderma spp. resulted in the highest germination rate (97.70%), disease incidence, and root and shoot dry weights, which are parameters for fruit quality and postharvest shelf life. Furthermore, this combined treatment enhanced the activity of defense enzymes in the plant, which is very important for keeping fruits intact during storage as well as transportation to long distances. Trichoderma spp. + F. solani treatment showed the highest peroxidase activity of 5.30 units/mg. The maximum catalase activity of 1.85 units/mg was recorded in Trichoderma spp. and F. solani treatment. The highest polyphenol oxidase activity of 1.74 units/mg was observed in the combination treatment of Beltanol, F. solani, and nano-chitosan. These results indicate that a program involving chemical, biological, and nano-based methods is a good sustainable approach for the management of F. solani in improving tomato fruit quality, reducing postharvest losses, and ensuring food safety through the entire chain.

1-Introduction

Tomato (*Solanum lycopersicum*) is a very important vegetable crop known for its nutritional, economic, and health values as a member of the Solanaceae family. It contains dietary fibers and essential amino acids and some major minerals like phosphorus, iron, and calcium. It also contains vitamins A and C, sugars, proteins, and citric acid that make it an essential part of human diet [1]. Many researchers have reported that consumption of tomatoes may cure diseases like constipation, diabetes, and heart disease. For example, it has been proved that tomato juice reduces platelet activity in diabetic patients, therefore reducing the risk of fatal thrombosis [2,3].

The importance of tomato production is indeed reflected on a global scale in recent statistics published by the Food and Agriculture Organization, which estimated the total worldwide tomato crop to be well over 180 million tons for the season 2020–2021 [4]. In 2011, approximately 62,500 hectares were under tomato cultivation in Iraq, and the country produced 1,059,537 tons of the fruit. However, production has been gradually decreasing over the years and has reached a level of 630,160 tons in 2022 and an estimated 535,000 tons in 2023 [5]. This decline is most probably due to a combination of environmental stress factors and phytopathogenic pressures; both factors contribute significantly to overall crop productivity and fruit quality, which in turn directly affects postharvest supply chains and food safety. The tomato crop is subject to attacks by a large number of pests and phytopathogenic agents at various stages of its development and under different environmental conditions, thereby causing so many diseases which reduce the yield and spoil the quality of the fruits [6]. Of these, root rot ascribed to *Fusarium solani* is one of the most destructive fungal diseases on economically important crops like tomato. The disease leads to decay of the roots, damping-off of the seedlings, and wilting of the plant, which eventually results in enormous economic losses. Typical symptoms include yellowing of the leaves, stunted growth, and darkening of vascular tissues as a result of systemic infection [7]. In addition, soil-borne pathogens such as *F. solani* are considered a major threat to vegetable crops mainly because they can survive for long periods in soil and are associated with plant debris and organic matter [8].
Chemical fungicides have always been preferred in the fast management of fungicides over the years. However, its continuous application poses risks toward human and animal health, and also environmental health, disrupts ecological balance by affecting non-target organisms, and promotes the development of pathogen resistance [9]. Thus, there has been a sustainable and environmentally friendly alternative approach emphasized more recently, particularly the use of beneficial microorganisms such as fungi and bacteria. IPM (Integrated Pest Management) practices commonly incorporate these bioagents to reduce reliance on synthetic chemicals [10,11].

Some of the most promising bioagents belong to the genus Trichoderma, such as *T. harzianum, T. asperelloides, T. atroviride*, and *T. longibrachiatum*, which exhibit high levels of antagonistic activity against important soilborne pathogens such as *Fusarium* and *Rhizoctonia* spp. [12,13]. Recent developments in microbial biocontrol have focused on the ability of such agents not only to suppress diseases but also to induce systemic resistance in plants and enhance the quality of postharvest fruits [4]. Their efficacy as biocontrol agents is due to wide-spectrum antifungal action against the environment, via hydrolytic enzymes (chitinases, β-1,3-glucanases) and antifungal secondary metabolites. In addition to these fungal bioagents, nano-chitosan is a new type of non-toxic antimicrobial compound that is quite effective and biodegradable and also works as an inducer of plant defense systems. Nano-chitosan improves plant resistance by stopping spore germination and breaking the integrity of the cell membrane [14,15]. One area that has attracted great interest is the use of nanotechnology in food preservation and disease control in agriculture, with nano-chitosan being one of the most promising agents for microbial safety and shelf-life extension [6].
These properties make it particularly attractive for applications in postharvest preservation and food safety since it can help maintain fruit quality throughout the supply chain. From a food industry viewpoint, control of *F. solani* infection is important not only for crop yield but also for preservation of quality during storage, transport, and processing of the fruit. The decay is caused by fungal pathogens, which pose tremendous losses in the supply chain and compromise the safety of processed tomato products such as sauces, pastes, and juices besides shortening the shelf life of fresh-market tomatoes. Thus, integrated strategies involving chemical, biological, and nano-based interventions can provide a sustainable approach to the management of fungal infections postharvest losses as well as safety and quality improvement of tomato fruits intended for human consumption. Hence, this study was designed to achieve the following specific objectives: isolation and identification of *F. solani* associated with tomato seed rot and seedling damping-off; and efficacy testing of Trichoderma species, a chemical fungicide Beltanol, and nano-chitosan in controlling this pathogen. The study also aimed to determine the most effective synergistic combinations of these agents for managing *F. solani* infection and improving tomato plant growth, which could subsequently enhance postharvest fruit quality and food safety within the tomato supply chain.

2-Materials and Methods

This study comprised in vitro (Petri dishes) and in vivo (greenhouse pot of living tomato plants, *Solanum lycopersicum* L. cv. 'Wijdan') components.

2.1. Isolation and Identification of *Fusarium solani* from Field-Collected Infected Tomato Roots

Samples were collected during the 2023–2024 growing season from fields in Karbala (Al-Hurr and desert areas) and Najaf (Khan Al-Nus) Governorates. At each place, 30 randomly selected diseased tomato plants were sampled. Root samples were brought immediately to the Postgraduate Laboratory, Department of Plant Protection, College of Agriculture, University of Karbala, in sterile bags and placed in coolers with ice for integrity storage in transit.

In the laboratory, roots were washed thoroughly under running tap water to get rid of soil particles and debris. They were then cut into small segments (2–3 cm long) and surface-sterilized with 1% solution of sodium hypochlorite (NaClO) for 2 minutes. The fragments were rinsed three times with sterile distilled water (30 seconds each) and dried using sterile filter paper.

Root segments were cultured on Potato Dextrose Agar (PDA) medium. The PDA medium was supplemented with 125 mg/L amoxicillin to inhibit bacterial contamination. The plates were incubated at 25 ± 2°C for three days. Small samples were aseptically taken from hyphal tips of emerging fungal colonies and transferred to fresh PDA plates with the same antibiotic to obtain pure cultures; sub-culturing was continued until purity was established. Fungi were isolated on the basis of morphological characters like color, mode, and structure of the margin by using the taxonomic keys of Leslie and Summerell [16].

It was later confirmed that the morphological identification was correct by molecular means. Genomic DNA was extracted from 7-day-old mycelial cultures using a CTAB-based protocol. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR products were sequenced and their sequences were compared with those in the NCBI GenBank nucleotide database by BLASTn. Isolate F4 was confirmed to be Fusarium solani with ≥99% sequence identity and the sequence was deposited in GenBank under accession number. The source of this isolate is a local field isolate obtained from diseased tomato roots in Al-Hurr district, Karbala Governorate, Iraq.

2.2. Source of *Trichoderma* spp. Used in This Study

The present study involved six *Trichoderma* spp. isolates, namely: T1, *T. longibrachiatum*; T2, *T. harzianum*; T3, *T. harzianum*; T4, *T. reesei*; T5, and *T. viride*. These isolates are strains from the environment (non-commercial) and were obtained from soil samples collected in studies previously characterized. Confirmation of species identity was through ITS region sequencing. Prior characterization at the molecular level by Muteab (2) deposited the five isolates in the NCBI GenBank under accession numbers PV114839 (*T. longibrachiatum* T1), PV113441 (*T. harzianum* T2), PV112458 (*T. harzianum* T3), PV114821 (*T. reesei* T4), and PV114819 (*T. viride* T5). The College of Agriculture, University of Kufa, provided the sixth isolate, *T. humatum* (T6). All fungal isolates were cultured on PDA medium and stored at 4°C in PDA plus 60% glycerol for future use. For in vivo experiments, Trichoderma inoculum was prepared as a conidial suspension by flooding 7-day-old PDA cultures with sterile distilled water, gently scraping the surface, and filtering through sterile cheesecloth. The concentration was adjusted to 1×10^7 conidia/mL using a hemocytometer.

2.3. Pathogenicity Testing of Fungal Isolates Against Tomato Seed Germination in Plastic Pots

The experiment was carried out in the greenhouse of the College of Agriculture, University of Karbala. A mixture of soil and peat moss in the ratio 1:2 was used. The mixture was autoclaved twice at 121°C and 15 psi for 60 minutes each time. The fungal inoculum was prepared on millet seeds at a concentration of 1% and then sterilized. It was then added to the soil, thoroughly mixed in plastic bags, and finally transferred into plastic pots at the rate of 1 kg soil per pot. The soil was lightly sprayed with water, covered with perforated polyethylene bags, and then left for 2 days for the fungus to establish. The procedure for preparing the negative control treatment was similar to that for preparing the inoculated treatments except that in this case, the fungal inoculum was not added. The positive control was prepared by using the pathogen F. solani alone. Tomato seeds were surface-sterilized using 1% sodium hypochlorite (NaClO) solution with two final rinses of sterile distilled water. Ten seeds were planted per pot and there were ten pots per treatment. Pots were watered as necessary without overwatering. The germination percentage was recorded 14 days after planting and the inhibition percentage was calculated relative to the control. Disease severity was assessed 21 days after planting by using a 0-5 scale for root rot symptoms, where: 0 = no visible symptoms; 1 = 1-10% root discoloration; 2 = 11-25% root discoloration; 3 = 26-50% root discoloration; 4 = 51-75% root discoloration; 5 = >75% root discoloration or dead plant.

2.4. Antagonistic Activity of *Trichoderma* spp. Isolates Against *F. solani* on Potato Dextrose Agar (PDA)

The dual culture technique was used to assess antagonistic effects. Petri plates (9 cm diameter) containing 20 mL of PDA were divided into two equal sections. A 0.5 cm mycelial plug from the actively growing margin of a 7-day-old Trichoderma culture was placed in the center of one section and a similar plug of *F. solani* in the center of the other section at a distance of 4.5 cm between the two inocula. Three biological replicates were maintained for each treatment. The negative control treatment, which contained only the pathogen without any Trichoderma, was also included. The plates were incubated at 25±2°C for 7 days until the pathogen reached the edge of the dish in the control. Colony diameter was measured daily along two perpendicular axes using a digital caliper. Pathogen radial growth was measured along two perpendicular diameters, and the inhibition percentage was calculated using Abbott's formula [17]:

$$\text{Inhibition Percentage (\%)} = [(C - T) / C] \times 100$$

where C is the colony diameter (mm) of the pathogen in the control plate and T is the colony diameter (mm) of the pathogen in the dual culture plate. The interaction zone between the two colonies was examined microscopically at 400× magnification to observe mycoparasitic interactions (coiling, penetration).

2.5. Evaluation of the Combined Efficacy of *Trichoderma* spp. Isolates in Suppressing *F. solani* on PDA

To determine the cumulative inhibitory activity of multiple Trichoderma isolates against *F. solani*, 20 mL PDA was dispensed into Petri dishes which were then divided into two equal sections. A 0.5 cm disc of actively growing *F. solani* was placed in the first half while the second half received different discs [18-20] of the Trichoderma isolates at 1 cm apart. In the negative control treatment, the first section had the pathogen alone and the second section had no inoculation. The positive control was *F. solani* alone. The plates were then incubated at 25 ± 2°C for a week or until the pathogen in the control reached the edge of the plate. The radial growth of *F. solani* was measured and the percentage inhibition was calculated by using the formula of Abbott [18].

2.6. Effect of Beltanol Concentrations on the Growth of *Trichoderma* spp. and *F. solani* on PDA

An in vitro test was carried out using the method of agar incorporation to assess the effect of Beltanol at 0.25, 0.50, 0.75, 1.00, and 1.25 mL/L against the isolates of *F. solani* and *Trichoderma* spp. The formulation of Beltanol is that of a commercial liquid fungicide; its active ingredient is 8-hydroxyquinoline sulfate at a concentration of 5% (w/v) (50 g/L). This product was made by Sumitomo Chemical Co., Ltd. of Japan. Its Batch No. is [insert], and its Expiry Date is [insert]. The chemical formula of the active ingredient is $C_9H_7NO \cdot H_2SO_4$. The preparation of the test concentrations was done by direct dilution of the commercial product in molten PDA. This is because the product is water-soluble, and therefore, a solvent-only control is not necessary. The sterile PDA was prepared in 500 mL flasks, autoclaved (121°C, 15 psi, 20 min), and then cooled to about 45–50°C before use. In this case, the negative control medium was PDA with no Beltanol (0 mL/L). The modified media were poured into fresh Petri dishes. A 0.5 cm fungal plug was inoculated at the center of each dish with three replicates per concentration. The plates were then incubated at 25 ± 2°C for 7 days until the control plates were fully covered. The radial colony growth was measured and the percentage of inhibition was calculated using the formula of Abbott [18].

2.7. Effect of Nano-Chitosan Concentrations on *F. solani* and *Trichoderma* spp. Growth on PDA

Nano-chitosan was incorporated into PDA to assess its effect on fungal growth. PDA was prepared in 500 mL glass flasks, sterilized and then cooled to a temperature just above its gelling point. Nano-chitosan was added at 50, 100, and 1000 ppm and mixed. Control plates were prepared using PDA without nano-chitosan. The medium was poured into Petri dishes and allowed to solidify. A 0.5 cm disc from a 3-7-day-old colony of *F. solani* or *Trichoderma* spp. was placed at the center of each dish, with three replications per treatment. The plates were then incubated at 25 ± 2°C until the control set reached the edge of the plate. Radial growth was measured, and percentage inhibition was calculated using the formula of Abbott (18).

2.8. Effects of Beltanol, Nano-Chitosan, *Trichoderma* spp., and *F. solani* on Tomato Seed Germination, Seedling Growth, Dry Weight, and Enzyme Activity

The study was carried out at a farm in Karbala Governorate to assess the impact of biological and chemical treatments applied to *F. solani* on tomato seed germination and subsequent development of the seedlings two parameters that directly affect the quality of the harvested fruit and, hence, sustainability of the supply chain. The soil was mixed with peat moss at a ratio of 1:2, autoclaved at 121°C and 15 psi for 60 minutes. The same

treatment was given again the next day for the full elimination of microorganisms.

The final inoculum concentration was 1×10^4 CFU/g of soil. The pots used had a diameter of 19 cm and each was filled with 2 kg of soil. This soil was watered and left for 48 hours to allow the fungus to establish. Ten tomato seeds sourced locally were surface-sterilized with 1% sodium hypochlorite for 2 minutes, rinsed three times with sterile distilled water, and then planted per pot. The treatments were arranged in a CRD with three replicates per treatment. The entire experiment was repeated once independently to confirm reproducibility. The negative controls were uninfected soil. The positive controls were *F. solani*-inoculated soil without any treatment.Treatments were as follows:

Treatment Code	Description
1	Uninfected soil (negative control)
2	*F. solani* alone (pathogen control)
3	Beltanol + *F. solani*
4	Nano-chitosan + *F. solani*
5	*Trichoderma* spp. (T2 isolate, *T. harzianum*, 1×10^7 conidia/mL) + F. solani
6	Nano-chitosan + Beltanol + *F. solani*
7	*Trichoderma* spp. + Beltanol + *F. solani*
8	Nano-chitosan + *Trichoderma* spp. + *F. solani*
9	*Trichoderma* spp. + nano-chitosan + Beltanol + *F. solani*
10	Beltanol + uninfected soil
11	Nano-chitosan + uninfected soil
12	*Trichoderma* spp. + uninfected soil

Concentrations used were 0.25 mL/L for Beltanol (equivalent to 12.5 mg active ingredient/L) and 50 ppm for nano-chitosan. The inoculum of *Trichoderma* spp. (*T. harzianum* T2) was applied as a soil drench, applying 50 mL of conidial suspension (1×10^7 conidia/mL) per pot at the time of planting. The percentage of seed germination was taken on the 24th day after planting. The percentage of seedling mortality was recorded 40 days after sowing. Activities of defense enzymes in plants (peroxidase, catalase, and polyphenol oxidase) were measured. Seedlings, roots, and shoots were weighed after drying. Roots were washed free from soil particles, tied in paper bags, and dried in an oven at 60°C until a constant weight was obtained. Disease severity was assessed as described in Section 2.3.

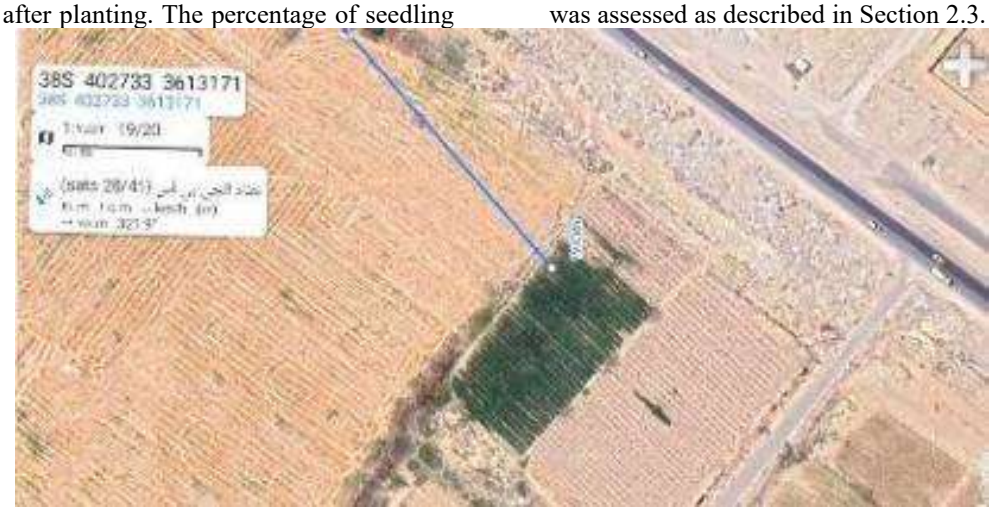

Fig. 1. Location of the study site in Arab Saleh area – Al-Hurr District

2.9. Measurement of Plant Defense Enzymes

2.9.1. Polyphenol Oxidase Enzyme (PPO)

PPO activity was assayed as described by Zhang [19]. Approximately 1 g of leaf tissue from 50-day-old plants was homogenized in 20 mL of 0.1 M sodium phosphate buffer at pH 6.5. The homogenate was centrifuged at 16,000 rpm for 15 min at 4°C; the supernatant was used as the crude enzyme extract. The assay mixture contained 1.5 mL of 0.1 M sodium phosphate buffer at pH 7.0, 200 µL of 0.01 M catechol, and 200 µL of the enzyme extract. The reaction rate was measured spectrophotometrically at 495 nm for every 30 s during 3 min. Enzyme activity was calculated as:

Enzymatic activity = (Spectrophotometric Reading) / [(Sample weight (g) / Extraction volume (mL)) × Volume used in assay (mL)] × 100

2.9.2. Peroxidase Enzyme (POD)

Peroxidase activity was assayed by the method of Hammerschmidt and Kuć [20]. One gram of tomato leaf tissue was ground in 20 mL of 0.1 M sodium phosphate buffer (pH 7.0) and then filtered through two layers of muslin. The homogenate was centrifuged at 16,000 rpm for 15 min at 4°C. The assay mixture contained 0.5 mL of enzyme extract, 0.5 mL of 0.05 M pyrogallol, and 1.5 mL of distilled water. The absorbance was taken at 420 nm at an interval of 30 seconds for 3 minutes. Enzyme activity was expressed as the change in absorbance per minute per gram of fresh weight, calculated using the same formula as that for PPO.

Catalase Enzyme (CAT) Catalase activity was determined by the method of Aebi, with modifications described by Al-Shammari [21,22]. Reagents were prepared as follows:

Assay Procedure:

- Not much detail was given about the assay procedure. Most of the details are on the preparation of the two solutions used in the assay. This can be a limitation because the paper does not give all the details of the procedures used in carrying out the assay.
- One gram of leaf tissue was homogenized in 2 mL of extraction buffer and then filtered through two layers of muslin cloth. The filtrate was centrifuged at 10,000 rpm for 10 minutes to pellet the debris. To 2 mL of freshly prepared 10 mM hydrogen peroxide solution, the reaction was started by adding 40 µL of enzyme extract, mixed, and then incubated for one minute at room temperature; the decrease in absorbance was then recorded at 240 nm. Enzyme activity was calculated as:

Statistical Analysis Catalase Activity (Units) = (Abs × Reaction Volume × Reaction Time × 2) / 0.01 × 100 where Abs = difference between first and second absorbance readings, and Reaction Volume = 2.04 mL.

2.10. Statistical Analysis

The in vitro work was conducted as a Completely Randomized Design (CRD) with three replicates. The field (in vivo pot) experiments were also carried out as a Completely Randomized Design (CRD). Each experiment was repeated at least once independently. The three replicates are biological replicates, i.e., independently prepared cultures or pots. The data were subjected to analysis of variance to determine significant differences between treatments by using the least significant difference (L.S.D.) post-hoc test at the 5% level of probability. The experimental procedures were based on those described by Al-Rawi and Khalaf-Allah [23]. The data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances with Levene's test before subjecting it to analysis. GenStat (version 2020) was used to perform the statistical analysis, while Microsoft Excel was used to prepare the graphical representations.

3-Results and Discussion

Isolation and morphological identification of *F. solani*.

Fourteen fungal isolates were acquired from the lowermost parts of tomato plants with typical symptoms of Fusarium wilt, such as root rot, yellowing of leaves, wilting, stunted growth, and vascular discoloration. The morphological characterization was carried out according to Leslie and Summerell [16]. They showed marked differences in colony morphology and pigmentation on Potato Dextrose Agar (PDA). The colors of their colonies ranged from white to pink and some of the isolates produced diffusible pigments that made the color of the surrounding medium pink to violet. Microscopic examination revealed that all the isolates belong to the genus Fusarium. They had septate mycelia and produced two types of conidia. The macroconidia were relatively broad, falcate, thick-walled, and usually five to seven septate. The microconidia were one to two-septate and had globose, oval, reniform, and fusiform forms. Chlamydospores were also observed, appearing as globose to oval and often in chains. Molecular identification through ITS rDNA sequencing confirmed the species identity of the most virulent isolate F4 as F. solani; the sequence was deposited under accession number.

3.2. Pathogenicity of Fungal Isolates on Tomato Seed Germination in Plastic Pots

As depicted in Table (1) and Figure (2), the isolates of Fusarium spp. showed a very devastating effect on seed germination as the germination percentages varied from 20.0% to 75.0% against 100.0% in the negative control treatment. Isolate F4 was the most virulent with the minimum germination percentage (20.0%), while isolate F9 was the least virulent (75.0% germination), followed by F11 (73.3%) and F5 (70.0%). The average diameter of the colony of *F. solani* F4 on PDA at 7 days was 85.0 ± 2.0 mm (Table S1). These results confirm that isolate F4 was the most pathogenic among all the Fusarium isolates tested. Results obtained are in line with those of Al-Tamimi [24], Al-Asadi [12], and Muteab and Al-Abidi [17]. For this reason, isolate F4 was chosen for all subsequent experiments as the most virulent *F. solani* isolate.

Table (1): Pathogenicity of Fungal Isolates on Tomato Seed Germination in Plastic Pots

ISOLATE NUMBER	GERMINATION	ISOLATE NUMBER	GERMINATION
Control	100.00	F8	63.3
F1	63.3	F9	75.0
F2	63.3	F10	53.3
F3	50.0	F11	73.3
F4	20.0	F12	60.0
F5	70.0	F13	60.0
F6	50.0	F14	43.3
F7	66.3	-	-

$LSD_{0.05}$ = 21.38

Fig.2 Pathogenicity of fungal isolates against tomato seed germination in plastic pots. (A) Soil inoculated with *F. solani*. isolate F4; (B) Uninoculated control soil.

3.3. Morphological Characterization of the Most Virulent Isolate, *Fusarium solani* (F4), on Tomato Seeds and Seedlings

The taxonomic key of Leslie and Summerell [16] was used for isolate F4 to identify the causative agent of rot and damping-off symptoms morphologically. This isolate grew rapidly on PDA, having the colonies first white and later changing to creamy-white as growth continued. Septate mycelia and two types of conidia were observed microscopically. The macroconidia were relatively broad, straight to slightly curved with rounded apices, thick-walled, and 3–6 septate. Most of the microconidia were oval, fusiform, reniform, or globose shaped and 1–2 septate. The chlamydospores were abundant, mostly globose to oval and often formed in chains. In contrast, isolate F9 developed pinkish-red colonies after seven days of incubation on PDA (Figure 3). All morphological and microscopic traits of isolate F4 quite agreed with those of *F. solani* as reported by Qiu et al. [25]. *F. solani* produces macroconidia that are straight or slightly curved, 3-5 (rarely 6) septate, with rather broad central cells, and the apical cells are blunt, the basal cells being notched off or distinctly foot-shaped. Microconidia are mostly oblong to oval. Chlamydospores are numerous, with rough walls and are mostly globose.

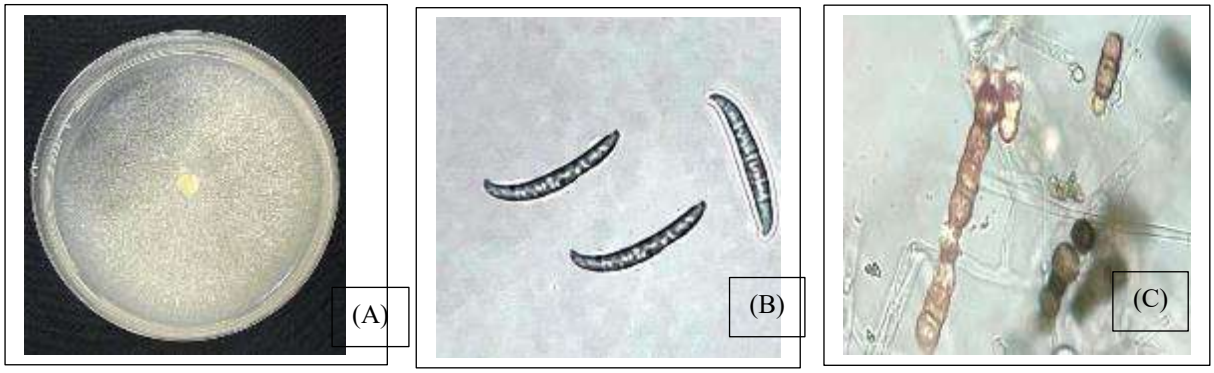

Fig.3 Morphological and microscopic characteristics of *F. solani* isolate F4. Colony of isolate F4 on Potato Dextrose Agar (PDA); (B) Macroconidia and microconidia; (C) Chlamydospores.

3.4. Antagonistic Activity of *Trichoderma* spp. Isolates Against the Pathogenic Fungus *Fusarium solani* on PDA

The results revealed that all *Trichoderma* spp. isolates significantly inhibited the growth of the highly virulent *F. solani* isolate compared to the control. The highest inhibition percentages were recorded for *T. longibrachiatum* (T1; 73.6%), *T. harzianum* (T2; 81.6%), and *T. harzianum* (T3; 72.0%), followed by *T. humatum* (T6; 64.3%). In contrast, *T. reesei* (T4) and *T. viride* (T5) exhibited the lowest inhibition rates at 60.4% and 55.4%, respectively, as illustrated in Figures (4) and (5). This finding is more relevant to postharvest applications since Trichoderma species have been successfully used as biocontrol agents against fruit decay at the time of storage and transportation, hence extending shelf life and reducing wastage of the food [26].

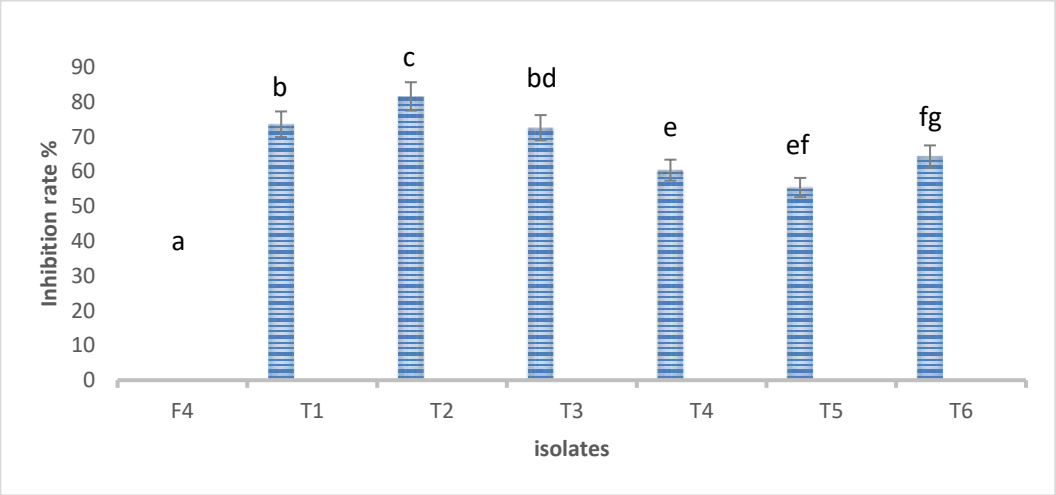

Fig.4 Antagonistic activity of *Trichoderma* spp. isolates against the pathogenic fungus *Fusarium solani* on PDA medium. *T. longibrachiatum* (T1), *T. harzianum* (T2), *T. harzianum* (T3), *T. reesei* (T4), *T. viride* (T5), *T. humatum* (T6), and *F. solani* (F4). Error bars represent the standard error of the mean (±SE). Different letters above the bars indicate significant differences among treatments according to the LSD test at **P ≤ 0.05**.

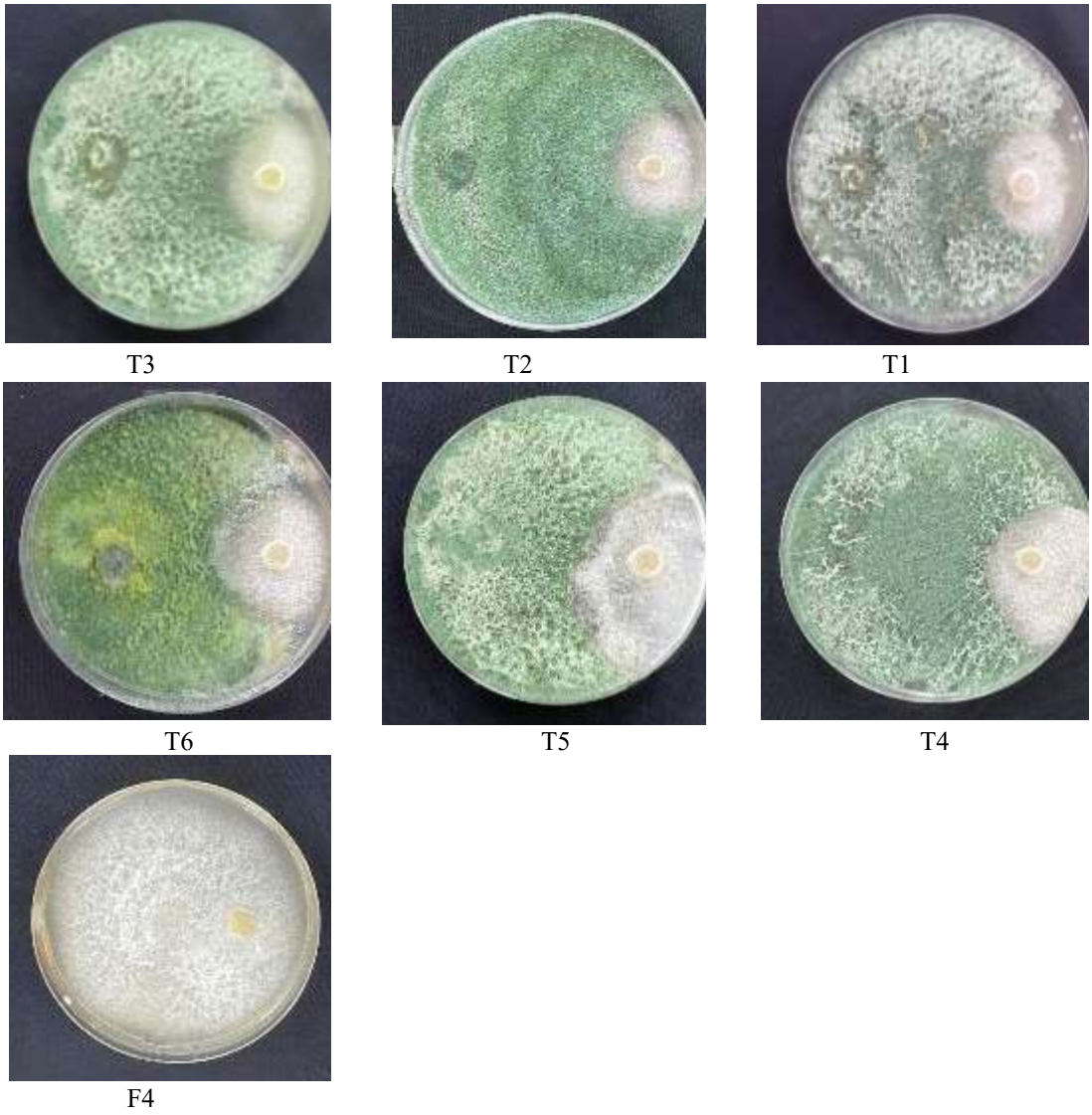

Fig.5 Antagonistic activity of *Trichoderma* spp. isolates against the pathogenic fungus *F. solani* on PDA medium. *T. longibrachiatum* (T1), *T. harzianum* (T2), *T. harzianum* (T3), *T. reesei* (T4), *T. viride* (T5), *T. humatum* (T6). *F. solani*(F4).

3.5. Combined Antagonistic Activity of Multiple *Trichoderma* spp. Isolates Against *Fusarium solani* on PDA

All *Trichoderma* spp. isolates demonstrated significant growth inhibition of the pathogenic fungus compared to the control. The inhibition percentage increased proportionally with the number of isolates used per plate, achieving a maximum of 94.5% inhibition when all six isolates (T1–T6) were combined. However, synergistic interactions were not significantly different when fewer isolates were used, with inhibition rates of 93.6% (T1–T5), 85.3% (T1–T4), and 82.6% (T1–T3). The lowest significant inhibition (71.8%) was observed when isolates T1 and T2 were used alone (Figures 6 and 7). A photo plate of representative combined dual culture plates is presented in Figure S3 (Supplementary Material).

Trichoderma spp. are well-known for their multiple mechanisms of fungal pathogen control, including mycoparasitism, antibiosis, and competition for nutrients (26). Work has been published proving the effectiveness of different Trichoderma species in controlling plant-pathogenic fungi, enhancing seed germination, accelerating tomato growth, and inducing systemic resistance against pathogens like *F. graminearum, R. solani*, and *Alternaria alternata* [27]. From the perspective of the food industry, these biocontrol agents represent a sustainable pre-harvest approach to reducing fungal contamination, which has a direct effect on the safety and quality of fresh and processed tomato products [11,13].

a

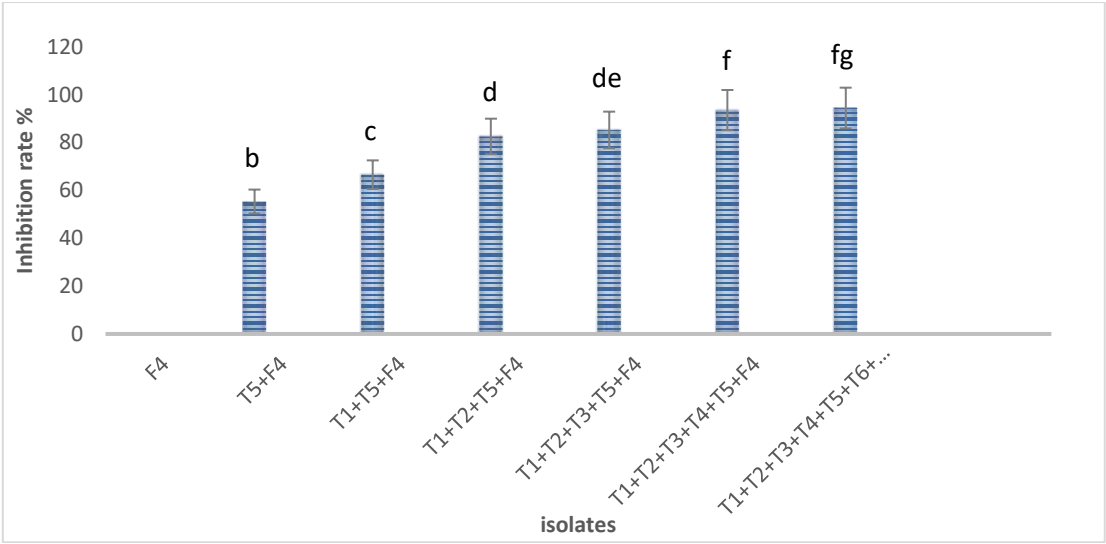

Fig.6 Combined effect of *Trichoderma* spp. isolates on the inhibition of *Fusarium solani* growth on potato dextrose agar (PDA). *T. longibrachiatum* (T1), *T. harzianum* (T2), *T. harzianum* (T3), *T. reesei* (T4), *T. viride* (T5), *T. humatum* (T6), and *F. solani* (F4). Error bars represent the standard error of the mean (±SE). Different letters above the bars indicate significant differences among treatments according to the LSD test at **P ≤ 0.05**.

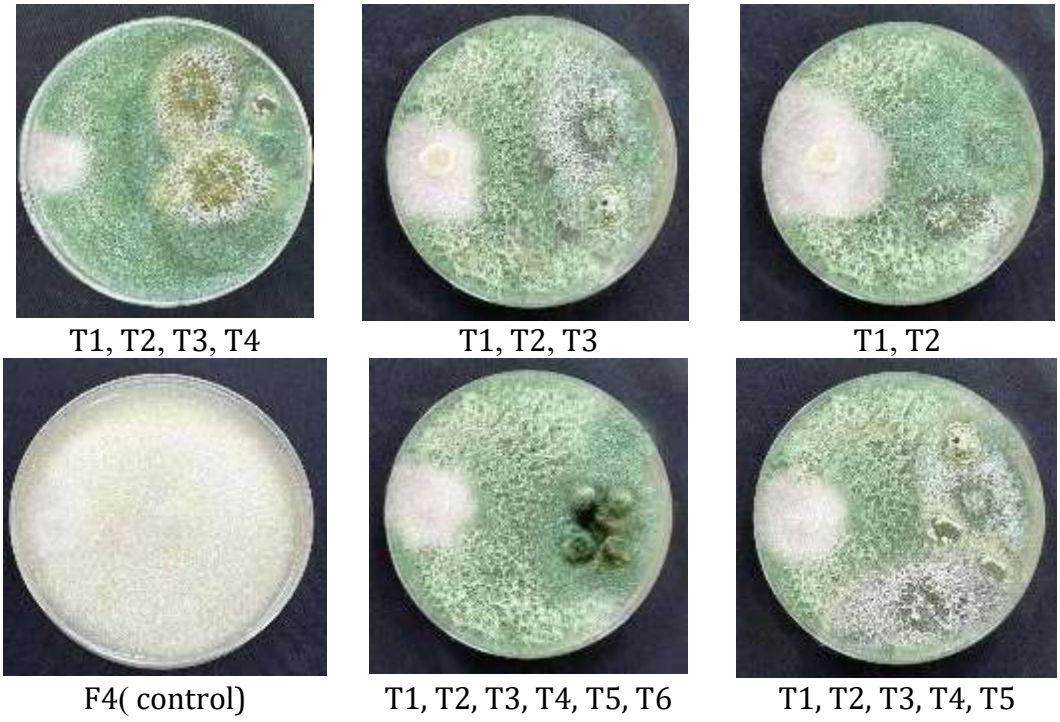

Fig.7 Combined effect of *Trichoderma* spp. isolates on the inhibition of *F. solani* growth on Potato Dextrose Agar.

3.6. Effect of Beltanol on *Trichoderma* spp. and *Fusarium solani* Growth on PDA

Beltanol exhibited differential inhibitory effects on *Trichoderma* spp. isolates and *F. solani*. *T. longibrachiatum* (T1) was the most tolerant, with only 33.6% inhibition, while other *Trichoderma* isolates (T2–T6) showed 53.1–74.5% inhibition. *F. solani* was the most sensitive, with 74.5% inhibition (Table 2). Increasing Beltanol concentrations (0.25, 0.50, 0.75, and 1.00 mL/L) resulted in higher inhibition rates across all fungal isolates. Complete inhibition (100%) was achieved at 1.00 mL/L for both *Trichoderma* spp. and *F. solani*. At 0.25 mL/L, *Trichoderma* spp. exhibited low inhibition (9.3–52.9%), while *F. solani* was strongly inhibited (72.3%). The EC_{50} value of Beltanol against F. solani was estimated at 0.18 mL/L.

Beltanol's antifungal efficacy is attributed to its active component, 8-hydroxyquinoline, which chelates copper, disrupts cell membranes, and affects sclerotia formation (30–33). This result is consistent with previous findings, which showed that complete inhibition of the fungi was recorded at 1 mL/L and lower efficacy at reduced concentrations [34]. Chemical fungicides, however, are effective for disease control after the crops have been harvested; residues on fresh produce raise concerns about food safety. Therefore, an integrated approach is used to minimize chemical usage while maintaining fruit quality [9].

Concentration (mL/L) / Isolate	Inhibition (%)					
	Control	0.25	0.50	0.75	1.00	Mean
T1	0.00	9.33	29.40	29.47	100.00	33.64
٢T	0.00	52.90	65.70	82.27	100.00	60.17
٣T	0.00	27.40	52.90	85.29	100.00	53.12
٤T	0.00	30.88	100.00	100.00	100.00	53.12
٥T	0.00	19.17	100.00	100.00	100.00	63.83
T6	0.00	16.43	91.33	100.00	100.00	61.35
F4	0.00	72.30	100.00	100.00	100.00	74.46
Mean	0.00	32.63	76.90	85.29	100.00	
LSD 0.05		Isolates 1.061		Concentrations 1.085		Interaction 2.807

3.7. Effect of Nano-Chitosan on the Growth of *Trichoderma* spp. and *Fusarium solani* on PDA

Trichoderma isolate T2 exhibited the highest tolerance to nano-chitosan, with the lowest inhibition (49.8%), while other isolates (T1, T3–T6) showed inhibition percentages ranging from 53.7% to 60.9%. F. solani was more susceptible, with 67.3% inhibition (Table 3). Increasing nano-chitosan concentrations (50, 100, and 1000 ppm) resulted in dose-dependent fungal inhibition, with complete inhibition (100%) achieved at 1000 ppm. The bulk (non-nano) chitosan control at 1000 ppm showed 78.3 ± 3.2% inhibition of *F. solani*. This was significantly lower ($P \leq 0.05$) than the 100% inhibition recorded for the same concentration of nano-chitosan. It confirmed the better antifungal efficacy of the nano-formulation. The antifungal activity of nano-chitosan is based on its particle size in the nanometric range (mean 85.3 ± 12.7 nm), which allows it to enter the cell wall of fungi, thus provoking hypertrophy and the bursting of hyphae. Moreover, electrostatic interactions made possible by the positive zeta potential (+32.5 ± 2.1 mV) destroy the integrity of the membrane and interfere with cellular as well as enzymatic synthesis [14]. These are characteristics that make nano-chitosan very appealing for postharvest use because it can be added as an ingredient in edible coatings for fresh tomatoes to extend shelf life while maintaining safety standards for consumption as food [15]. Other workers have reported that nano-chitosan not only inhibits pathogenic fungi but also enhances plant defense enzymes, including peroxidase, chitinase, and β-1,3-glucanase [36]. The EC_{50} value of nano-chitosan against *F. solani* was estimated at 42 ppm.

Table (3): Effect of different Nano-Chitosan concentrations on the growth of *Trichoderma* spp. isolates and the pathogenic fungus *F. solani* on Potato Dextrose Agar (ppm)

Concentration (mL/L) / Isolate	Inhibition (%)					
	Negative control (0 ppm)	50 ppm	100ppm	1000ppm	Bulk chitosan (1000 ppm)	Mean
T1	0.00	41.60	77.71	100.00	71.25	54.91
T	0.00	33.30	65.70	100.00	65.80	49.75
٣T	0.00	49.77	78.90	100.00	72.10	57.17
٤T	0.00	59.57	84.23	100.00	77.40	60.95
٥T	0.00	42.43	72.47	100.00	68.30	53.73
T6	0.00	35.43	78.20	100.00	69.55	53.41

F4	0.00	81.47	87.73	100.00	78.30	67.30
Mean	0.00	49.13	77.85	100.00	71.81	

LSD $_{0.05}$	Isolates	Concentrations	Interaction
	3.102	2.501	6.331

3.8. Impact of Beltanol, Nano-Chitosan, and *Trichoderma* spp. Isolates, Alone and in Combination, on Tomato Seed Germination, Seedling Growth, and Root and Shoot Dry Weights

Results presented in Table (4) show that *F. solani* (positive control) significantly reduced tomato seed germination (33.3%) and negatively affected root and shoot dry weights, confirming its high pathogenicity against young plant tissues. Beltanol treatment significantly improved germination (70.0%), reduced root rot, and increased root and shoot dry weights — clear signs the fungicide's working well against the pathogen. It was, however, unacceptable from a food safety point of view to rely on chemical fungicides due to residues in fresh and processed tomato products [9].

Germination was higher (72.7%) with nano-chitosan treatment, root rot was lower at 15.9%, and the dry weights of roots and shoots were increased, thus showing its dual role in inhibiting the growth of the fungus and that of the plant through induced resistance [14,15]. The germination was further enhanced by *Trichoderma* spp. treatment (80.3%), with root rot decreasing to 10.0%, and the dry weights were better; this was attributed to the production of hydrolytic enzymes and growth-promoting compounds [11,13].

Synergistic effects were observed in all combination treatments. The quadruple treatment (Beltanol + nano-chitosan + *Trichoderma* spp. + *F. solani*) resulted in the highest germination rate (97.7%), complete suppression of root rot (0.0%), and maximum root and shoot dry weights. This combination was the most effective strategy for controlling F. solani while promoting plant growth. The synergies observed with half-dose Beltanol (0.25 mL/L) in combination with nano-chitosan (50 ppm) and *Trichoderma* spp. were comparable to or even better than full-dose chemical treatment alone in terms of disease suppression, hence indicating a potential significant reduction in chemical fungicide use. The integration of chemical, biological, and nanotechnological components further enhance resistance against *F. solani* by complementary mechanisms: chemical inhibition by Beltanol, biological stimulation by Trichoderma, and immune activation by nano-chitosan [28,35,36]. These results are of prime importance to the food industry because enhanced seed germination and seedling vigor will lead to high-quality fruits with lowered susceptibility to postharvest decay. Biocontrol agents and nano-chitosan offer a sustainable alternative in the reduction of chemical inputs with the added advantage of maintaining food safety across the supply chain [10,11].

3.9. Effect of Beltanol, Nano-Chitosan, and *Trichoderma* spp. Isolates, Alone and in Combination, on the Activity of Selected Plant Defense Enzymes

There were significant differences in the activities of plant defense enzymes among treatments as presented in Table 5. The highest peroxidase (POD) activity recorded was 5.30 units/mg fresh weight in the treatment with *Trichoderma* spp. + uninfected soil and the lowest was 0.15 units/mg for the negative control (uninfected soil). This is in line with earlier reports which state that *Trichoderma* spp. can enhance defense mechanism since it activates gene expression for enzymes that scavenge oxidative stress; thus, this increase was due to the capability of the *Trichoderma* spp. The treatment *F. solani* + Beltanol + nano-chitosan also showed high enzyme activity (4.70 units/mg) which means that nano-chitosan induces resistance against stress in plants by promoting enzymatic defense mechanisms. This result is in conformity with the earlier workers who found that different species of Trichoderma increase POD activity under conditions of fungal infection.

The highest value was recorded in treatments of *Trichoderma* spp. and *F. solani* at 1.85 units/mg in catalase activity as compared to the negative control which was at 0.66 units/mg. Catalase is an important enzyme in the detoxification of accumulated H_2O_2 to protect cellular components from oxidative damage [21]. Nano-chitosan + *Trichoderma* spp. + F. solani also increased catalase activity, showing synergy in inducing the defense system of the plant. The treatment that gave the maximum CAT activity was *Trichoderma* spp. + nano-chitosan + Beltanol + *F. solani*, at 1.90 units/mg, thus further justifying the synergistic benefit of the integrated approach. These results also support those of Li et al. [37] who stated that combinations of biocontrol agents with pathogens induce the expression of defense-related genes including catalase. In the PPO assay, the highest value was observed in the treatment of Beltanol + *F. solani* + nano-chitosan + *Trichoderma* spp. (1.74 units/mg) and the lowest in the negative control (uninfected soil) (1.22 units/mg). The PPO process catalyzes phenolic compound oxidation to produce lignin. Lignin strengthens cell walls and reduces penetration by pathogens [19]. These results are in agreement with those of Dobbs et al. [38] who found that heightened activities of oxidative enzymes, including PPO and POD, represent major

physiological defense responses in plants exposed to biocontrol agents and safe pesticides. Similar findings were reported by Yadav [39] where he demonstrated that biocontrol fungi significantly reduce disease severity and stimulate the activity of defense-related enzymes SOD, APX, CAT, PPO, POD, PAL, and GPX.

To the food industry, higher defensive enzyme activity in plants means better fruit quality, a longer shelf life, and lower losses after harvesting. The biocontrol agent's combination with nano-chitosan offers an environmentally friendly approach to control fungal diseases with minimal chemical residues on fresh and processed tomato products. [10,11,15]

Table (5): Effect of the chemical fungicide Beltanol, Nano Chitosan, and *Trichoderma* spp. isolates and their interactions on the activity of Peroxidase (POD), Catalase (CAT), and Polyphenol Oxidase (PPO) enzymes.

Treatment	Peroxidase (POD)	Catalase (CAT)	Polyphenol oxidase (PPO)
Uninfected soil (control)	0.15	0.66	1.22
Pathogenic fungus *F. solani* (control)	3.30	1.20	1.47
Beltanol pesticide + *F. solani*	3.50	1.20	1.41
Nano-chitosan + *F. solani*	3.90	1.50	1.55
Trichoderma spp. + *F. solani*	4.80	1.80	1.70
Nano-chitosan + Beltanol + *F. solani*	3.50	1.70	1.43
Trichoderma spp. + Beltanol + *F. solani*	4.40	1.80	1.72
Nano-chitosan + *Trichoderma* spp. + *F. solani*	4.30	1.85	1.71
Trichoderma spp. + nano-chitosan + Beltanol + *F. solani*	4.70	1.90	1.74
Beltanol + uninfected soil	3.30	0.75	1.23
Nano-chitosan + uninfected soil	4.70	0.80	1.33
Trichoderma spp. + uninfected soil	5.30	1.30	1.65
$LSD_{0.05}$	**0.057**	**0.061**	**0.07**

4-Conclusion

The results of this study showed that *Fusarium solani* reduced seed germination, increased the risk to seedling development, and decreased the yield of tomatoes, which are parameters that directly affect the quality of fruit at harvest and safety in processed tomato products. *Trichoderma* spp. (*T. harzianum* T2, GenBank PV113441), Beltanol (active ingredient: 8-hydroxyquinoline sulfate, 5% w/v), and nano-chitosan (mean particle size 85.3 nm, PDI 0.32, zeta potential +32.5 mV) all singly retarded the pathogen; however, their combined treatment was the best synergistic response. This combination treatment gave the highest germination percentage (97.7%), complete suppression of root rot, and better plant growth. In particular, the combination of half-dose Beltanol with nano-chitosan and *Trichoderma* spp. was equal or better in controlling the disease compared to the full dose of the chemical treatment alone and offered a viable strategy through which chemical fungicide use could be reduced by up to 75%. In addition, the treatment increased the activity of some other important enzymes in plant defense (POD, CAT, PPO) that are associated with higher resistance to postharvest decay. To the best of our knowledge, this is the first report that highlights the synergistic effect of biological, chemical, and nano-based agents against *F. solani* infection in tomatoes to boost productivity. The current research would benefit stakeholders directly involved in the fresh and processed tomato value chain by reducing postharvest losses, increasing shelf life, and maintaining the safety of fresh and processed tomato products at all levels within the food supply chain. However, this study was limited to some extent. The fungicidal or fungistatic nature of the tested agents was not determined; hence, future studies should re-plate inhibited colonies on fresh medium to make this differentiation. A time-course analysis of inhibition kinetics would also provide insight into the duration of antifungal activity. Stability tests of nano-chitosan under different storage conditions should be conducted. The optimization of such integrated treatments for their commercial application and the effect on fruit quality parameters (nutrition, sensory attributes, and storage stability) would

be major thrust areas for future research. Field validation of these greenhouse results would require large-scale commercial production condition trials.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research received no external funding.

Data Availability

All data supporting the findings of this study are available within the manuscript.

Supplementary Material

Fig. S1. Transmission electron microscopy (TEM) image of nano-chitosan nanoparticles synthesized. Scale bar = 200 nm.

Fig. S2. Photo plate of dual culture assays showing the antagonistic activity of individual *Trichoderma* spp. isolates (T1–T6) against *F. solani* on PDA after 7 days at 25°C.

Photo Plate of Combined Dual Culture Assays Showing Antagonistic Activity of Multiple *Trichoderma* spp. Isolate Combinations (T1+T2, T1+T2+T3, T1+T2+T3+T4, T1+T2+T3+T4+T5, and T1–T6) Against *F. solani* on PDA after 7 Days at 25°C.

Table S1. Mean colony diameter (mm ± SD) of *F. solani* and *Trichoderma* spp. isolates under different treatments.

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