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Research Article

Evaluating the biocontrol efficacy of beneficial soil microbes against the Fusarium wilt disease of tomato

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ABSTRACT

Tomato is one of the most important agricultural crops in the world as it is a rich source of vitamins A and C. The quality and the quantity of tomato fruits is severely affected by the fungal plant pathogens. Fungal diseases are among the most dangerous biological stresses that cause severe damage to tomato crops in many countries. Fusarium wilt disease in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, become a major concern of yield loss worldwide. This study evaluated the efficacy of multiple antagonistic biocontrol agents, including *Trichoderma* species and non-pathogenic *F. oxysporum*, in mitigating Fusarium wilt. Pathogenic *F. oxysporum* strains were isolated from diseased tomato plants, while biocontrol agents were cultured from rhizosphere soil of healthy tomato plants. In vitro dual-culture assays demonstrated that non-pathogenic *F. oxysporum* inhibited pathogenic strains by 86.8% (±4.8) at 7 days post-inoculation (dpi), significantly higher than *T. harzianum*, which achieved 62.8% (±6.2) inhibition ($p < 0.01$). Other Trichoderma strains, such as *T. viride* and *T. asperellum*, showed moderate inhibition levels of 54.3% (±2.3) and 51.9% (±5.9), respectively. Greenhouse pot trials reinforced these in vitro results, with non-pathogenic *F. oxysporum* achieving a significant disease reduction of 77.0% (±3.0) in Fusarium wilt incidence, followed by *T. harzianum* at 64.8% (±2.7) ($p < 0.01$). Statistically significant improvements in plant growth were also recorded in treated plants. *T. harzianum* increased shoot length by 25.6% (±3.1) and root weight by 22.4% (±2.9) compared to control plants, while non-pathogenic Fusarium enhanced root length by 30.2% (±3.4) and overall biomass by 28.7% (±3.8) ($p < 0.01$). Data were analyzed using ANOVA, followed by LSD for pairwise comparisons, confirming the efficacy of these biocontrol agents in reducing disease incidence and promoting plant growth. These findings underscore the potential of antagonistic biocontrol agents as viable, sustainable alternatives to chemical fungicides for managing Fusarium wilt in tomato production systems, supporting both plant health and environmental resilience.

Keywords: Fusarium wilt, Biocontrol agents, *Trichoderma*, Sustainable agriculture, Soil-borne pathogens.

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INTRODUCTION

Agriculture is very important for Pakistan's economy because it generates approximately 19.5% of the Gross Domestic Product (GDP) and provides employment for 42.3% of the labor force, showing its relevance to the balance of the economy and the food system (Khan et al., 2020). However, in recent times, the industry has faced several challenges, including lower productivity and limited land availability for the growing of vegetables (Adenle et al., 2018; Bhatti et al., 2024; Naqvi et al., 2024a). Tomatoes (*Solanum lycopersicum*) are one of the most crucial horticulture crops. They are valued not only economically but also due to their high importance in the dietary pattern and cooking habits of Pakistan (Poti et al., 2015). It can be seen that tomatoes are put together as an adjunct in many of Pakistan's traditional recipes. This vegetable crop is mainly eaten fresh, often raw (Poti et al., 2015). It can also be versatile as a material to apply in a wide range of culinary purposes, for example to make condiments like ketchup and many other types of sauces. As mentioned earlier, the tomato exhibits a notable concentration of vitamin C (31 mg per 100g) alongside vitamin A, calcium, iron, and other vital nutrients. Lycopene, a prevalent antioxidant, is naturally present in tomatoes and has been discovered to exhibit anti-carcinogenic characteristics, effectively impeding the progression of various types of cancer (Adenuga et al., 2013; Abbas et al., 2023). Despite their significance, tomato yields are frequently compromised by biotic stresses, especially soil-borne diseases caused by fungi like *Fusarium oxysporum* (Anwar et al., 2022; Rehman et al., 2023).

Fungal pathogens are responsible for approximately 80% of plant diseases. *Fusarium* wilt is a fungal pathogen primarily disseminated via the soil medium obstructing xylem vessels responsible for water transportation in plants. The obstruction results in the withering of plants and, in numerous instances, the death of the vegetation. The occurrence of Fusarium wilts can be attributed to the presence of pathogenic strains from various Fusarium species, including *F. eumartii, F. oxysporum, F. avenaceum, F. solani, F. sulphureum, and F. tabacinum*. These strains commonly demonstrate a notable specificity towards the plants they infect. However, *F. oxysporum* is the perpetrator that is most commonly encountered. *Fusarium* species encompass a collection of filamentous fungi classified within the genus *Fusarium*. *Fusarium* Schlechtendahl is a genus of filamentous fungi that belongs to the family Nectriaceae. Filamentous fungi, or molds, are a diverse group of microorganisms characterized by their elongated, thread-like structures called hyphae. These fungi play a significant According to (Pitt et al., 1994) description, Snyder and Hansen can be taxonomically classified as members of the Class Ascomycetes and Family Hypocreaceae**.** The fungus under consideration is an organism that resides in the soil and is found worldwide. Various species can produce mycotoxins, including fumonisins, zearalenones, and trichothecenes. This phenomenon leads to vegetation contamination and subsequent integration into the ecological food web. Mycotoxins pose significant health and safety hazards to various ecological components, including wildlife, livestock, agricultural commodities, and human populations (Arif et al., 2011; Balali and Iranpoor, 2006; Wang et al., 2011).

The soil-borne pathogen, *Fusarium oxysporum* f. sp. *lycopersici* that causes the Fusarium wilt of the plants retards water transportation through wilting and killing of the plants. It has threatened the quality of tomato for centuries causing immense yield loss (Lahlali et al., 2022). Traditional control methods such as chemical fungicides are harmful to the environment and induce resistance in the pathogens, which manifests the urgency of developing more sustainable approaches (Tang et al., 2021; Naqvi et al., 2024b). Biological control agents (BCAs) are amongst the environmentally friendly alternatives for Fusarium pathogens management. Therefore, the biological control of plant pathogens using the fungal biocontrol agents (Ali et al., 2021a; Ali et al., 2023a), beneficial edaphophytes (Tabbasum et al., 2022; Ali et al., 2023b), bacterial biocontrol agents (Azeem et al., 2020; Ali et al., 2022; Ayub et al., 2024), plant extracts (Ali et al., 2020; Ali et al., 2021b), yeast species (Ali et al., 2024a), green synthesized nanoparticles (Ali et al., 2024b), and RNA interferences based management strategies (Ali et al., 2024c) has gained popularity in recent years due to their effectiveness and eco-friendly sustainability. Other antagonistic microorganisms that are classified under beneficial bacteria and fungi can suppress pathogens growth. These microorganisms are beneficial in terms of maintaining good health in the plants, (Azeem et al. 2020; Cheng et al. 2021). Evidence is lacking for the efficacy of fungicides against Fusarium wilts, but the momentum of increasing support for sustainable agriculture production gains (Lemanceau and Alabouvette, 1993).

Cultural practices can help control soil pathogens, including enhancing host resistance, adding other organisms, fumigants, and using native microflora without environmental damage (Deacon, 1988). The *Trichoderma* spp have been known for ages as effective biocontrol agents against fungal phytopathogens. It indirectly impacts through competing for available resources, environmental modulation, plant growth stimulation, plant defense mechanisms activation, and antibiosis. It also affects directly by mycoparasitism (Howell, 2003; Papavizas, 1985). *Trichoderma*

spp differ from each other by fast growth, long-living conidia, and wide tolerance to substrates. According to Hjeljord et al. (2000), these microorganisms are very efficient in competition both for nutritional resources and habitat. Moreover, Benítez et al. (2004) reported that *Trichoderma* spp. possess a significant ability to produce siderophores strongly binding iron and inhibiting the growth of many other fungal species. Hence, the attributes of soil exert an influence on the efficacy of *Trichoderma* as a biocontrol agent. Several mechanisms have been proposed to explain the phenomenon of biocontrol, with the main contributing factor believed to be the enhanced competitive ability of antagonistic microorganisms in nutrient competition compared to pathogens (Couteaudier and Alabouvette, 1990; Lemanceau, 1989; Schuster and Schmoll, 2010). The putative mechanism of action against *Fusarium oxysporum* may entail the production of lytic enzymes by antagonistic microorganisms. (Mitchell and Alexander, 1961) documented the utilization of bacterial strains that demonstrate lytic enzyme production to control *Fusarium* wilt. The idea of employing nonpathogenic Fusaria to manage *Fusarium* wilts emerged from the study of soils that exhibit inherent suppressive characteristics against *Fusarium* wilts. This study evaluates various antagonists as biocontrol agents against *Fusarium oxysporum* f. sp. *lycopersici*, aiming to identify effective, sustainable methods for managing Fusarium wilt in tomato production.

MATERIAL AND METHODS

This study was conducted in the Biological Sciences, Lab at Virtual University, Gujranwala Campus, Pakistan. Below is a detailed description of the methodologies utilized for sample collection, pathogen isolation, preparation of biocontrol agents, and in-vitro as well as greenhouse antagonistic testing.

Field Survey and Sample Collection

A field survey was conducted across tomato fields in various locations in Gujranwala to collect diseased samples based on visible symptoms. Plants showing signs of wilting were selected, and infected vascular tissues from the roots and stems were collected. Root samples were gathered by excavating soil from a depth of 5–30 cm, then placed in labeled zipper bags. Stem tissues were collected by removing the outer peel with a sterile knife, preserving sections with brown lesions. All samples were transported to the lab and stored at 4°C.

Sample Preparation

To prepare the collected samples, wilted root and stem tissues were washed with tap water to remove soil particles and sterilized with 1% sodium hypochlorite (NaOCl) for 2–3 minutes. Samples were then cut into 2–3 mm pieces, rinsed twice with distilled water, and air-dried on blotting paper. Root samples were placed in sealed bags and stored at an appropriate temperature until use.

Pathogen Isolation and Culture Preparation

Potato Dextrose Agar (PDA) was prepared by combining 250 ml potato starch, 15 g agar, 20 g dextrose, and 750 ml distilled water to produce 1 liter of media. The media was sterilized at 121°C for 20 minutes, poured into Petri dishes, and allowed to cool. Surface-sterilized root segments were placed on PDA plates, incubated at 25°C for 3–4 days, and then transferred to fresh media for purification. Pure cultures were identified by observing colony morphology under a microscope.

Pathogen Purification and Characterization

After incubation, colonies with distinctive morphologies were isolated on fresh PDA media to obtain pure cultures. Identification was performed based on colony color, diameter, and appearance, as well as microscopic characteristics, including septation and reproductive structures.

Pathogenicity Assay

To confirm pathogenicity, tomatoes were surface-sterilized with a 1% sodium hypochlorite solution and rinsed with distilled water. Sterile needles were used to inoculate the fruits with 5 mm PDA plugs of fungal isolates. Symptoms were monitored for seven days, and lesion development data were recorded.

Collection and Isolation of Biocontrol Agents

Biocontrol agents were isolated from rhizosphere soil samples taken from healthy tomato plants. The soil was diluted in sterile distilled water (SDW) to achieve progressive dilutions (10^{-1} to 10^{-5}). From each dilution, 1 mL of the solution was spread on PDA plates. Plates were incubated at 28 °C for 72–96 hours. Distinct colonies were subculture to isolate fungal and bacterial strains. Trichoderma isolates were purified through single-spore isolation and observed using a scanning electron microscope.

In-vitro Antagonistic Activity of Biocontrol Agents

The antagonistic efficacy of fungal and bacterial isolates against *Fusarium oxysporum* was assessed via the dual culture technique. Biocontrol agents and the target pathogen were placed 3 cm apart on 90 mm PDA plates. Mycelial growth was measured at 5, 7, and 9 days post-inoculation (dpi) to calculate inhibition percentages.

$$PercentageInhibition\ (PI) = \frac{Control\ (C) - Treatment\ (T)}{Control\ (C)} \times 100$$

Greenhouse Pot Trial

A greenhouse trial was conducted to test biocontrol efficacy in-vivo. Sterilized soil (75% soil, 25% silt) was used to fill pots. Tomato roots were soaked in a spore suspension of 1 × 10^6 CFU/mL for 1 hour before transplanting. Untreated plants served as controls. Pathogen inoculation was achieved by placing 5 mm PDA discs with pure fungal cultures in the pots. Disease progression was monitored, and results were recorded.

RESULTS

Characterization of pathogen

In the present study, *F. oxysporum* f. sp. *Lycopercisi* from tomatoes was isolated and characterized by morphological characteristics. Pathogenic *Fusarium* species were characterized by colony morphology and microscopic observation. Seven isolates were isolated from diseased roots of tomatoes and were tentatively identified as belonging to the genus *Fusarium*. The microscopic examination revealed distinctive feature including the presence of both microconidia and macroconidia, characteristic of the *Fusarium* species, along with dense whitish mycelial mate. Further morphological and colony characteristics such as septate conidia and slightly curved macroconidia, confirmed their identity as *F. oxysporum*. Minor variation in colony color and shape among the isolates were observed, but these did not deviate from the defining traits of *F. oxysporum*.

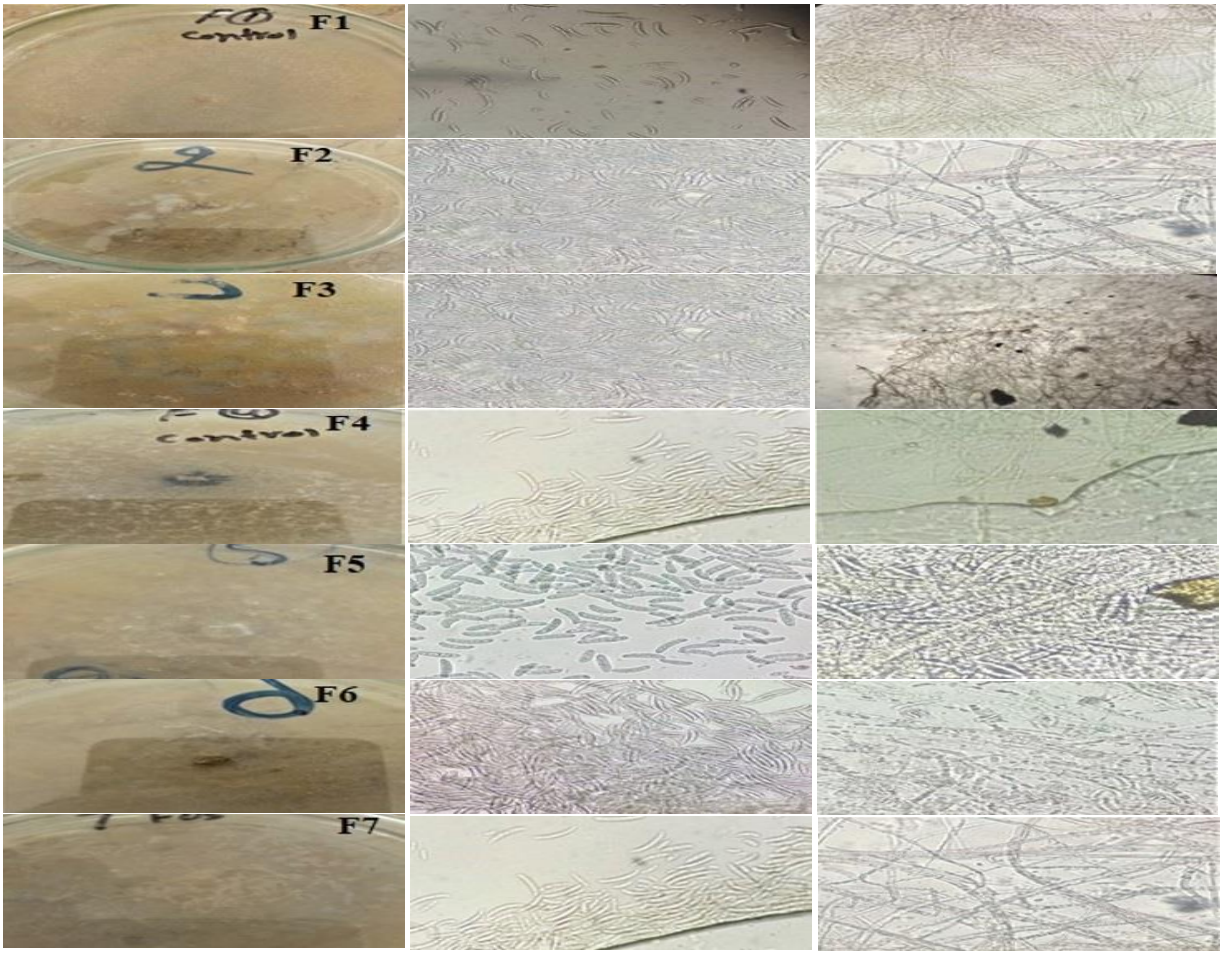

Figure 1. Morphological observations of *Fusarium* isolates from the roots of tomatoes. *Fusarium oxysporum* isolates from F1 to F7; front view of culture in the first column, microscopic view of spores in second column, and microscopic view of spores.

Morphological identification *Fusarium* spp.

Fungal isolates were grown on a PDA medium at 25±2℃ for seven days to observe the morphological and macroscopic characteristics. On the seventh day of inoculating pure culture, the diameter of the colony and color were recorded. The finding revealed a diversity of morphological characteristics among the isolates, including the variation in colony growth pattern, pigmentation, and spore structures. Despite this variability, all isolates displayed key diagnostic features characteristics of *Fusarium* spp., such as the production of microconidia and macroconidia with septate hyphae, confirming their identity as members of the *Fusarium* genus.

Table 1. Morphological characterization of *Fusarium* spp.

Isolates	Colony diameter (mm)	Color colony front	Color colony back	Incidence in the moist chamber (%)	Incidence in pot essay (%)
F-1	28.5	White	Pale	100	80
F-2	29.9	White	Creamy white	80	100
F-3	27.4	White	Pale brown	60	100
F-4	25.15	Creamy	Pinkish white	80	80
F-5	30.5	Pale white	Creamy white	100	100
F-6	22	Creamy	White	100	80
F-7	26.6	White	Pinkish white	80	80

Virulence of pathogenic *Fusarium* isolates

To confirm the pathogenic potential of isolates, the surface-sterilized healthy leaves were inoculated with pathogenic media plugged into a moist box. In the second step of an experiment to justify Koch's postulates, surface sterilized tomato fruits were placed in a moist box. Fungal media plugs were applied on fruits and leaves to check the disease incidence due to *Fusarium* isolates. A control set, consisting of leaves and fruits treated with sterile media plugs was maintained under the same condition in separate boxes to ensure proper comparison of disease incidence caused by *Fusarium* isolates. of controls for comparison. The results showed that F-1, F-2, F-4, and F-5 grew comparatively faster, while F-3, F-6, and F-7 grew slower in circular colonies. F-1, F-2, F-4, and F-5 primarily affect the leaves and tomatoes. While F-3, F-6, and F-7 have minimum effects on leaves and tomatoes. Nonpathogenic *Fusarium* isolates were evaluated in DC assay for evaluation of their biocontrol potential.

Characterization of bio-control *Trichoderma* spp.

The fungal antagonists used in this study were *Trichoderma harzianum, Trichoderma virens*, *Trichoderma atroviridae*, *Trichoderma asperellum, Trichoderma* sp., and nonpathogenic *Fusarium oxysporum.* The fungal biological control agents were morphologically identified based on colony morphology. Moreover, microscopic observations were also conducted to confirm the biocontrol strains at the genus level.

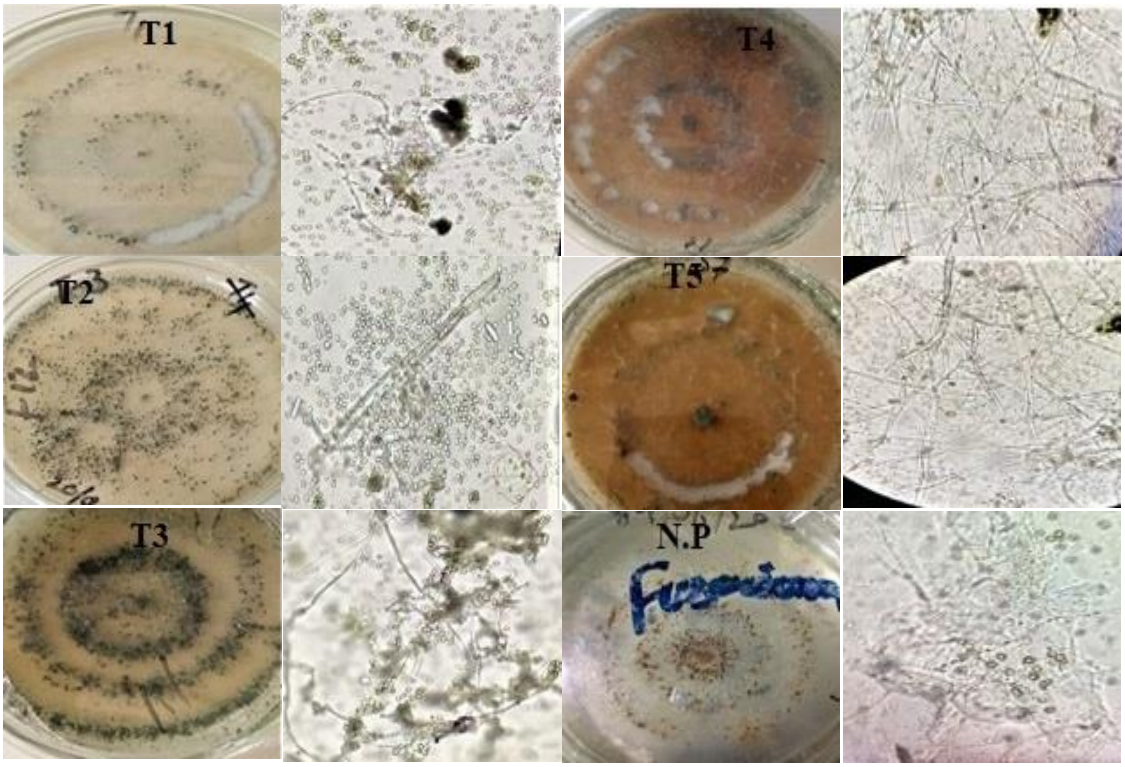

Figure 2. Morphological and microscopic characterizations of *Trichoderma* spp. and Nonpathogenic *F. oxysporum.*

Potential of bio-agents *Trichoderma* spp. against pathogenic *Fusarium*

In order to assess the effectiveness of various *Trichoderma species* against *Fusarium F1*, a series of in-vitro dual culture assays were performed. After a period of seven days, during which potato dextrose agar was inoculated and incubated at a temperature of 25±2℃, the observed outcome indicated that *Trichoderma spp.* effectively impeded the growth of the mycelium of the most potent strains of *Fusarium oxysporum*. Non-pathogenic *Fusarium oxysporum* inhibited the maximum growth rate of pathogenic *Fusarium* to 86.8% as compared to all other six isolates. On the 2nd number, T2 gives 62.8% best inhibition of pathogen. On the lowest level, T4 gives a minimum growth rate of 51.9% for pathogen inhibition.

Table 2. Applications of *Trichoderma* spp. and Nonpathogenic *Fusarium* against Pathogenic *Fusarium*

Treatments	3rd day	5th day	7th day
T1	38.3±4.0	48.3±5.0	62.0±3.1
T2	38.3±1.3	59.7±7.5	62.8±6.2
T3	46.5±2.3	44.3±3.6	54.3±2.3
T4	47.3±3.6	49.3±3.6	51.9±5.9
T5	48.8±2.3	57.4±1.3	54.3±4.8
NF	73.6±1.3	75.2±3.6	86.8±4.8

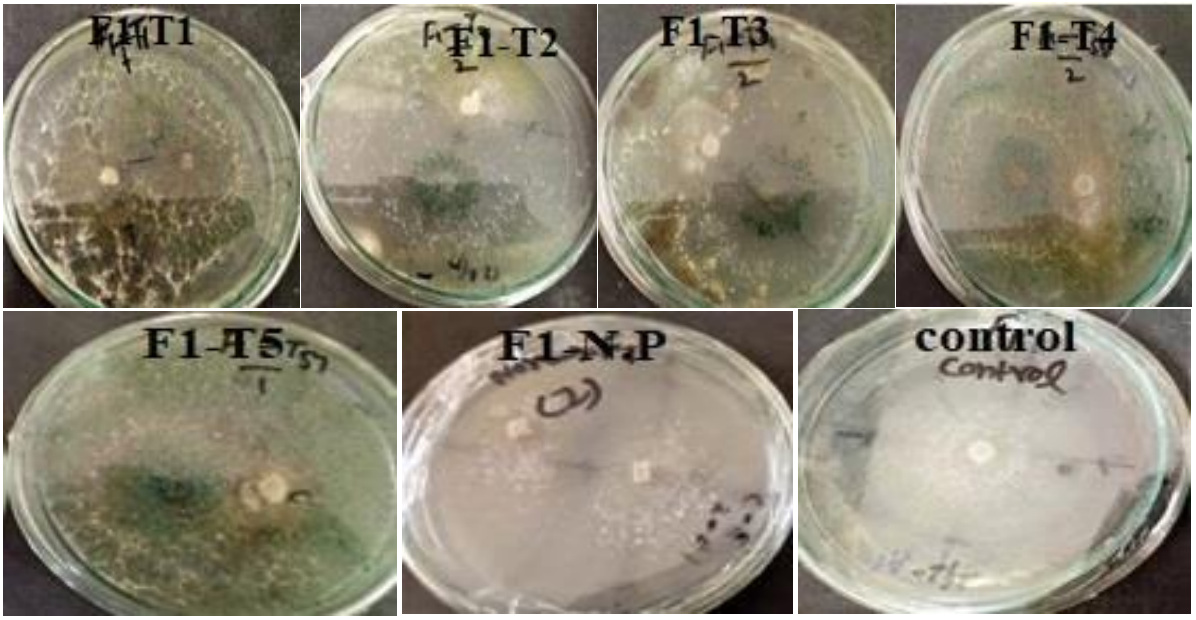

Figure 3. Dual culture of pathogenic *Fusarium* with *Trichoderma* spp., Nonpathogenic *Fusarium* and control.

To check the efficiency of different *Trichoderma* spp. against *Fusarium* F2, dual culture assays were conducted. On the 7th day of inoculation on potato dextrose agar at 25±2℃, the result notable difference in growth among different *Trichoderma* spp. Nonpathogenic gives a maximum growth rate of 85.3% against *Fusarium*1, on the 2nd number T5 gives the best growth rate of 67.4% and on the lowest number, T2 gives a 63.6% growth rate against *Fusarium*.

Table 3. Application of *Trichoderma* spp. and non-pathogenic *Fusarium oxysporum*

Treatments	3rd day	5th day	7th day
T1	41.7±2.5	55.0±2.0	66.0±3.6
T2	43.2±2.9	55.0±3.1	63.6±1.2
T3	48.1±1.3	48.1±1.3	64.1±2.9
T4	45.0±2.6	53.0±4.7	64.8±2.7
T5	44.7±3.9	50.4±3.6	67.4±4.7
NF	68.2±4.8	79.8±3.6	85.3±4.8

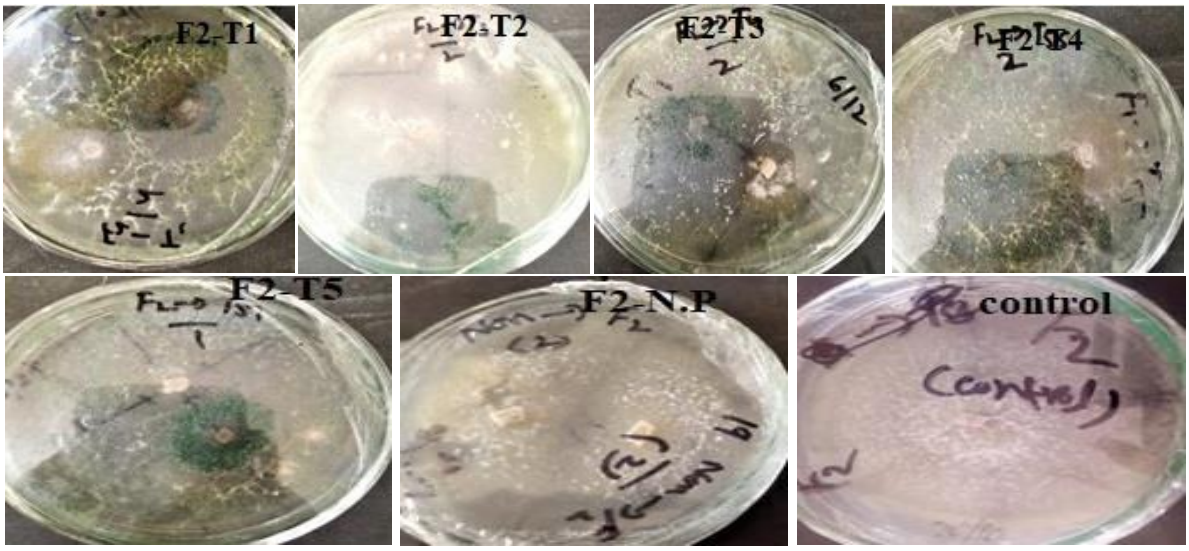

Figure 4. Dual culture of *Trichoderma spp*., Non-pathogenic *Fusarium oxysporum*, and control

To test the potential of different *Trichoderma* spp. against *Fusarium F*4, the PDA media was engaged in dual culture assay. On the 7th day of inoculation on potato dextrose agar at 25±2℃the result notable difference in growth among different *Trichoderma* spp. And nonpathogenic gives a maximum growth rate of 77% the 2nd number T5 gives the best growth rate against *Fusarium* on the last T2 gives a 58.2% growth rate against the pathogen.

Table 4. Application of *Trichoderma* spp. non-pathogenic *Fusarium oxysporum*

F4	3rd	5th	7th
T1	48.8±2.3	51.9±3.6	59.7±3.8
T2	42.2±2.2	48.3±3.2	58.2±4.3
T3	55.0±3.6	57.7±3.8	62.1±3.9
T4	54.8±3.5	56.8±3.7	61.2±3.9
T5	52.5±2.2	59.3±5.3	64.0±5.8
NF	60.0±2.3	66.0±2.4	77.0±3.0

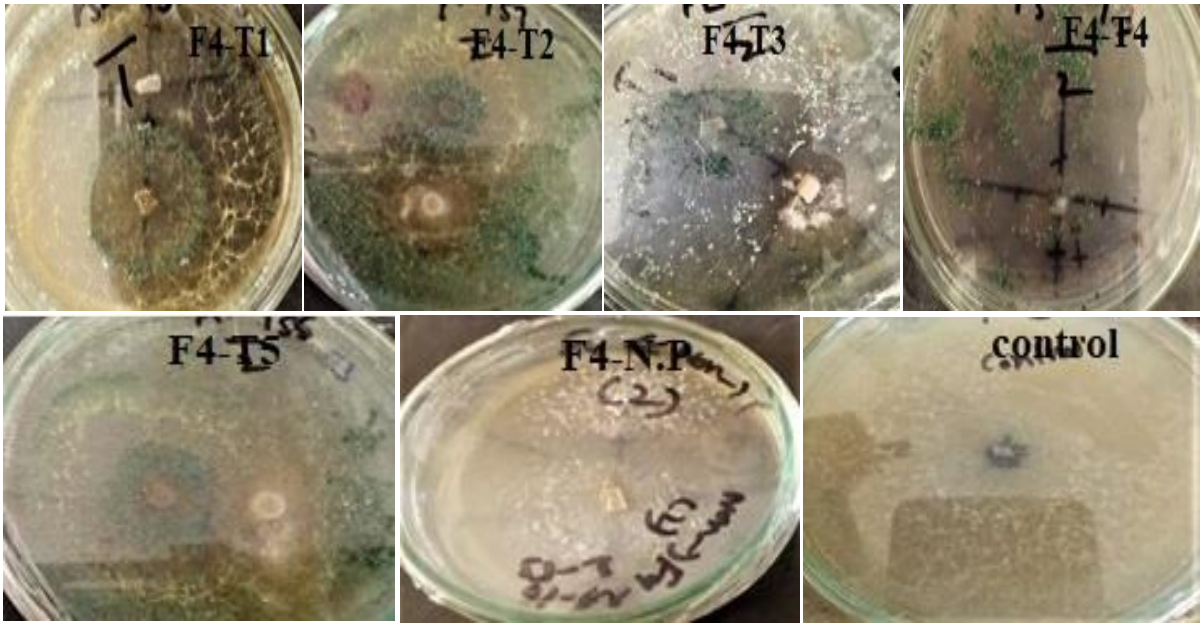

Figure 5. The dual culture assay of *Trichoderma* spp., Non-pathogenic *Fusarium oxysporum*

To evaluate the potential of different *Trichoderma* spp. against *Fusarium* F5, dual culture assays were conducted. On the 7th day of inoculation on potato dextrose agar at 25±2℃ the result notable difference in growth among different *Trichoderma* spp. Non-pathogenic gives a maximum inhibition rate of 81.4% against Fusarium F5. On the 2nd number, T2 gives the best inhibition rate of 60.2% on the lower number T3 gives a minimum inhibition rate of 49.2% against the pathogen.

Table 5. Application of *Trichoderma* spp. And non-pathogenic *Fusarium oxysporum*

Treatments	3rd	5th	7th
T1	52.3±3.5	54.7±3.6	70.3±3.8
T2	50.4±2.6	57.1±3.0	60.2±5.5
T3	43.7±1.3	45.9±2.8	49.2±7.0
T4	49.6±2.7	50.4±2.9	52.9±3.8
T5	51.2±4.0	53.7±4.4	56.9±5.4
NF	71.3±1.3	73.9±2.4	81.4±2.7

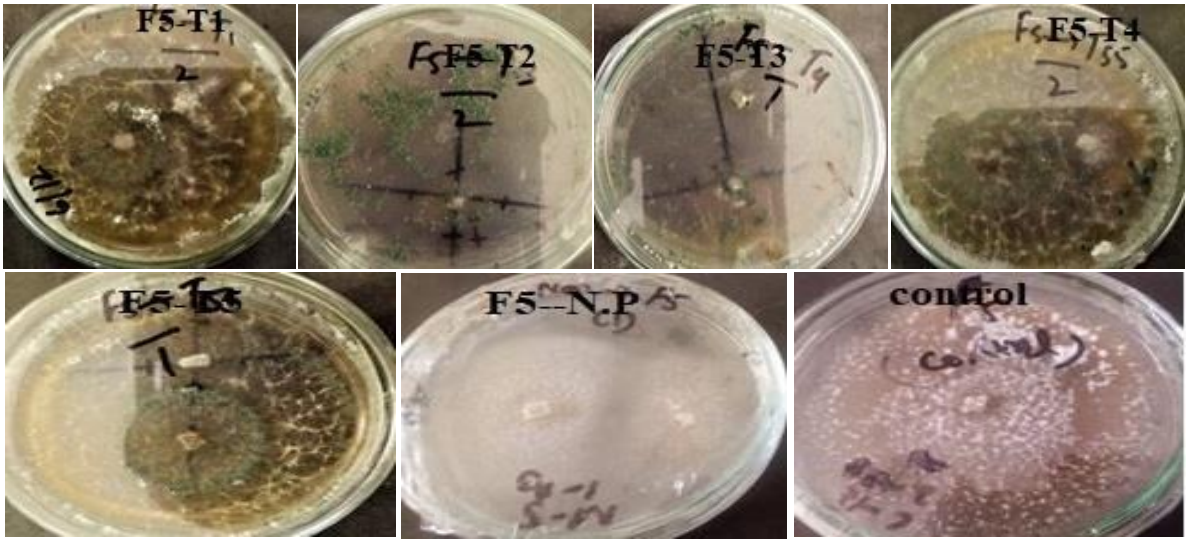

Figure 6. The dual culture assay with *Trichoderma* spp., Non-pathogenic *Fusarium oxysporum* and control

Efficacy of bio-control agents against *Fusarium* wilt during pot trials

The evaluation of potential biological agents screened during in-vitro assays was conducted in a greenhouse pot trial. The spore suspension of *Trichoderma* spp. and non-pathogenic *Fusarium* oxysporum was prepared by multiplying in liquid cultures. The spore suspension was calculated for the number of spores per ml of water. The roots of nursery plants were treated with spore suspension (1×109 per ml) of bio-control agents for 90 minutes. The untreated plants were considered as controls. The pot trial was conducted in a screen house, and data for disease incidence and plant traits was recorded after two months of inoculation. The result showed that tomato plants treated with *Trichoderma* spp. Showed less disease incidence and improved plant growth and root development. The treated plants showed wilting symptoms, while untreated plants showed no symptoms. The results showed that bio-control strains have good potential to protect tomato plants from wilt disease. The result showed that T3 and non-pathogenic strains used in the study proved to improve the length of the shoot of plant as compared to untreated control. Non-pathogenic showed maximum shoot length. T4 showed maximum root length the result showed that all strains used in this study proved to improve the plant root length as compared to control. The result showed that all strains used in this study proved to improve the plant root weight as compared to control. All the treatments give best response on number of leaves (Fig 7).

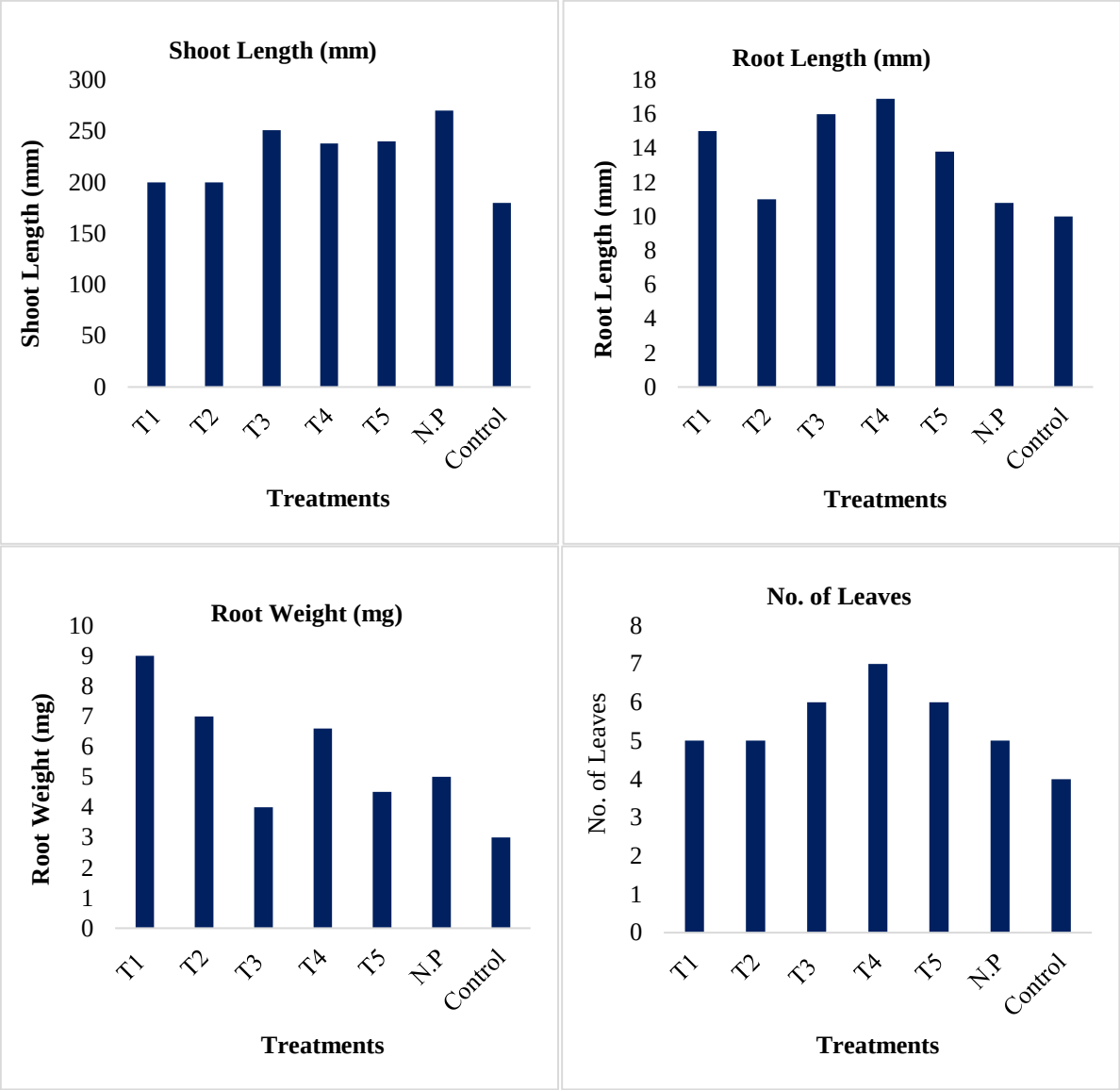

Figure 7. Effect of different *Trichoderma* spp. and non-pathogenic strains on shoot length, root length, leaves development and root weight of tomato plants

DISCUSSION

Fusarium wilt of tomato Caused by *Fusarium oxysporum f. sp. lycopersici* is a significant devastating disease of tomatoes and also its major yield-limiting factor. The prevalence of this disease extends across various regions worldwide (Kanwal et al., 2024). Elshahawy et al. (2017) described that biological control methods provide ecologically sustainable, non-toxic, and economically feasible alternatives to applying fungicides in agricultural practices. The study aimed to evaluate the effectiveness of different fungal biocontrol agents in suppressing the mycelial growth of *F. oxysporum* f. sp. *lycopersici*. The results indicated that these agents demonstrated varying efficacy levels in controlling the pathogen's growth. The pathogenicity of *Fusarium oxysporum* on tomato fruit was investigated. In current studies, isolates collected from tomato roots were inoculated to confirm pathogenicity. The decrease in the growth and multiplication of pathogens can be ascribed to the mycoparasitic behavior displayed by Trichoderma species. Trichoderma spp. Possess the ability to engage in resource competition with pathogens, particularly in terms of acquiring space and nutrients (Devi et al., 2012). Mycoparasitism is a biological phenomenon characterized by the utilization of Trichoderma spp. as a means to extract essential nutrients from the continually expanding hyphae of parasitic fungi. This process ultimately results in the pathogenic organism's gradual debilitation and eventual demise (Alwathnani and Perveen, 2012). They also reported that all *Trichoderma* strains were able to inhibit and overgrow *Fusarium.* In vitro test T1 controls the pathogen at 58.5%, T3 controls the pathogen at 38%, T4 controls the pathogen at 44%, T55 controls the pathogen at 20%, and T57 controls the pathogen at 17%.

Subsequently, the isolates of *Trichoderma* were subjected to rigorous examination to evaluate their capacity to generate antagonistic effects against Fusarium, specifically targeting oomycetes. Five strains of *Trichoderma* were found to inhibit tomatoes' Fusarium wilt effectively. This study applied the greenhouse pot trail. Trichoderma species penetrate the roots of plants, therefore enhancing nutrient uptake (Kleifeld and Chet, 1992). This phenomenon has been supported by studies conducted by (Shukla et al., 2018), and (Iqbal and Mukhtar, 2020). The most important reason for the variation in height is that of the biological agents increasing the production of indole acetic acid, according to the studies conducted by López-Bucio et al. (2015) and Paramanandham et al. (2017). Khan et al. (2017) mentioned that *Trichoderma* spp. enhances the level of lycopene which further enhances the yield of tomato crops. The studies showed that Trichoderma species produce different antifungal substances including trichodermin apart from enzymes such as endo-chitinase, β-glucosidase, and α-1,3-glucanase, which are involved in the pathogenicity (Jamil et al., 2021). (Li et al., 2018) confirmed that these compounds inhibit pathogens while promoting plant growth and yield. Studies by (Thompson and Huber, 2007) and (Li et al., 2018) conclude that Trichoderma has significantly increased the uptake of Manganese (Mn). The process makes the host resistant to pathogens through alteration of the rhizosphere or metabolic parts, reducing the infection. Srivastava et al. (2010) reported that *Trichoderma harzianum* reduces the virulence of *F. oxysporum* f. sp. *lycopersici* because of enhanced uptake of nutrients. Plants enhanced the growth parameter fresh weight, shoot and root length, soluble protein, chlorophyll, and phenolic content by a considerable level because of the *T. harzianum* treatment (Jangir et al. 2019). Plasmids of Trichoderma have been demonstrated to increase the expression of several photosynthesis-associated proteins in plants. According to Harman (2000), this phenomenon can promote photosynthesis, thereby improving crop yield and quality. Trichoderma spp. and nonpathogenic isolates produced the best results in laboratory tests. A pot trial validated their efficacy against Fusarium wilt and disease suppression in the field. These are important for plant growth as well as the biological control of soil pathogens. The bacteria involved here are nonpathogenic. They stimulate growth and suppress pathogens of the soil. Sharma et al. (2012) and Konappa et al. (2018) have demonstrated in their studies that inoculation of biocontrol agents in tomatoes increases their phenolic content and activates the enzymes of phenylpropanoid metabolism. Abd-El-Khair et al. (2019) reported that phenolic compounds played a major role in pathogen control, especially lignification-suberisation. Abo-Elyousr et al. (2014) Some shortcomings of the spread of pathogens are discussed. Resistance might be enhanced by the modifications in the pH of plant cell cytoplasm. This has led to a higher quantity of phenolic acids which have inhibitory effects on the proliferation of the pathogen, thus increasing resistance (Benhamou et al., 2000). Stereoscopy examinations demonstrated destructive impacts on the vascular structures of wilted plants, characterized by stunted growth, yellowing, and death. Actually, it has already been proved that Trichoderma is a microorganism, which in many cases accompanies the plant and is not pathogenic. To harness the full potential of Trichoderma in various applications, it is imperative to develop formulations that effectively enhance the activity and viability of these microorganisms. In contrast, the innovative notion of biocontrol necessitates an external environment beyond the confines of the laboratory in order to yield tangible outcomes within production systems.

CONCLUSION

The management of *Fusarium oxysporum f. sp. lycopersici* poses a significant challenge due to its capacity for seed and soil transmission, as well as its ability to persist as chlamydospores in the soil for extended periods. The current study has demonstrated that using biocontrol agents leads to favorable results in efficiently controlling wilt disease and enhancing the growth and productivity of tomato plants. Based on the study's findings, it can be inferred that *Trichoderma viride* exhibited the highest level of efficacy as a biocontrol agent, whereas *T. harzianum* was ranked second in terms of its effectiveness. Therefore, the current study supports the utilization of biocontrol agents as a feasible approach for the sustainable control of wilt disease. The research endorses the use of biocontrol agents as a viable method for the sustainable management of wilt disease. Future study should concentrate on investigating the synergistic effects of integrating different biocontrol agents and their application in field situations. Furthermore, examining the genetic foundation of biocontrol effectiveness in *Trichoderma* species may yield insights for the formulation of more resilient and precise biocontrol strategies.

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AUTHOR CONTRIBUTIONS

Naureen Anwar and **Muhammad Umer**: Project administration, Conducted the research trial and Writing– review and editing. **Amjad Ali and Parnaz Mortazavi:** Conceptualization, Finalization and Writing– review and editing. **Iqra Kanwal:** Software, Writing– review and editing. **Sara Anum, Humera Aslam, Tooba Khan, Eman Fatima** and **Muhammad Imran:** Formal analysis, Validation Visualization, Figure preparations, Writing original draft, Writing– review and editing.

COMPETING OF INTEREST

The authors declare that the research was carried without any commercial or financial relationships that could be construed as a potential conflict of interest.

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