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Microbial biocontrol of Fusarium oxysporum in tomato using Papiliotrema terrestris, Bacillus amyloliquefaciens and Trichoderma harzianum under greenhouse conditions

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Abstract: *Fusarium oxysporum* is a persistent soil-borne pathogen that causes severe vascular wilt in tomato and remains difficult to manage under intensive greenhouse production. This study evaluated the individual and combined performance of three microbial antagonists, *Papiliotrema terrestris* PT22AV, *Bacillus amyloliquefaciens* BO7, and *Trichoderma harzianum* T22, against the aggressive isolate FO23 under controlled greenhouse conditions. Tomato seedlings of the susceptible cultivar San Marzano Nano were grown in pathogen-infested substrate and treated with microbial agents individually or as a consortium. Plant response was assessed through symptom expression, chlorosis, plant height, leaf area, vigor, and overall disease suppression. Among the single agents, PT22AV showed the strongest performance, combining visible disease suppression with a clear biostimulant effect. BO7 also reduced pathogen damage and improved plant development, whereas T22 provided only modest protection under the tested conditions. The microbial consortium composed of PT22AV, BO7, and T22 produced the best overall plant response, with improved vigor, greener foliage, and reduced symptom severity compared with the pathogen-only treatment. The observed differences among microbial treatments indicate that biological control performance depends not only on pathogen suppression but also on the capacity of the antagonist to improve plant physiological status. The integration of complementary microorganisms within a consortium may therefore represent a promising strategy for sustainable greenhouse tomato production under high disease pressure.

Keywords: *Fusarium oxysporum*; tomato wilt; biological control; *Papiliotrema terrestris*; *Bacillus amyloliquefaciens*; *Trichoderma harzianum*; microbial consortium; greenhouse production

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops cultivated under protected conditions, where intensive production systems allow high yields but also favor the accumulation of soil-borne pathogens. Among these pathogens, *Fusarium oxysporum* is particularly problematic because it colonizes the root system and vascular tissues, causing chlorosis, wilting, reduced vigor, and plant death [1, 3, 5].

Management of Fusarium wilt is difficult because the pathogen can persist in soil and plant debris for long periods. Repeated greenhouse cropping, limited crop rotation, and favorable environmental conditions may increase disease pressure and reduce the effectiveness of single control measures [2, 3, 5]. Therefore, sustainable alternatives that complement cultivar resistance and hygiene practices are needed.

Biological control using beneficial microorganisms is a promising approach for the management of soil-borne diseases [2, 7, 8, 11, 16]. Microbial antagonists may suppress pathogens through competition for nutrients and space, production of antifungal compounds, mycoparasitism, stimulation of plant defense responses, or indirect promotion of plant growth.

2. Object and subject of research

The object of this research was the biological control of *Fusarium oxysporum*-induced tomato wilt under controlled greenhouse conditions. The study focused on the capacity of selected beneficial microorganisms to reduce disease symptoms and improve the physiological status and vigor of tomato seedlings exposed to the aggressive FO23 isolate.

The subject of research consisted of three microbial antagonists representing different biological groups: *Papiliotrema terrestris* PT22AV as a yeast, *Bacillus amyloliquefaciens* BO7 as a bacterium, and *Trichoderma harzianum* T22 as a filamentous fungus. These agents were tested individually and as a combined microbial consortium against *Fusarium oxysporum* FO23.

The main shortcoming addressed by this research was that single biological control agents may provide inconsistent disease suppression under high pathogen pressure. A combined microbial strategy may offer more stable protection if the selected microorganisms express complementary mechanisms of action.

3. Target of research

The target of this research was to evaluate the individual and combined effects of *Papiliotrema terrestris* PT22AV, *Bacillus amyloliquefaciens* BO7, and *Trichoderma harzianum* T22 against the aggressive isolate FO23 of *Fusarium oxysporum* in tomato under controlled greenhouse conditions.

The specific tasks were to compare the biocontrol performance of each antagonist, assess their effects on plant height, leaf area, chlorosis, and vigor, evaluate the performance of the microbial consortium, and determine which treatment provided the most useful disease suppression and growth support.

4. Literature analysis

Biological control of *Fusarium oxysporum* has been widely investigated because chemical and cultural measures alone are often insufficient under intensive greenhouse production [2, 3, 5]. Beneficial microorganisms may reduce pathogen development directly through antibiosis, competition, parasitism, or indirectly through plant growth promotion and induced resistance [8, 10, 11].

Bacillus species are frequently associated with rhizosphere competence, antimicrobial metabolite production, and induction of systemic resistance [8, 10, 12]. *Bacillus amyloliquefaciens* BO7 has been associated with antifungal lipopeptide production and inhibitory activity against *Fusarium oxysporum* [13, 14].

Trichoderma species are among the most widely used fungal biocontrol agents, but their field performance may vary depending on strain adaptation, substrate conditions, and pathogen pressure [6, 15]. Beneficial yeasts, including *Papiliotrema terrestris*, are receiving increasing attention because they may combine antagonistic activity with biostimulant effects [7, 17]. Microbial consortia may be advantageous when complementary mechanisms are required for stable disease suppression.

5. Research methods

Pathogen isolate

The aggressive isolate FO23 of *Fusarium oxysporum* was used as the pathogen in the biocontrol assays. FO23 was selected because previous pathogenicity tests identified it as one of the most damaging isolates on tomato seedlings, producing strong chlorosis, growth reduction, and mortality under controlled conditions.

Microbial antagonists

Three microbial antagonists were tested: *Papiliotrema terrestris* PT22AV, *Bacillus amyloliquefaciens* BO7, and *Trichoderma harzianum* T22. These microorganisms were selected because they represent different functional groups of biological control agents: yeast, bacterium, and filamentous fungus, respectively. Biological control products and related materials were sourced through AgroVentures, Albania.

Table 1. Microbial antagonists evaluated in the biocontrol assay.

Microbial agent	Biological group	Application form	Expected role
Papiliotrema terrestris PT22AV	Yeast	Cell suspension	Biostimulation and disease suppression
Bacillus amyloliquefaciens BO7	Bacterium	Cell suspension	Growth support and pathogen suppression
Trichoderma harzianum T22	Filamentous fungus	Conidial suspension	Fungal antagonism and root-zone protection
PT22AV + BO7 + T22	Microbial consortium	Combined treatment	Complementary biological control mechanisms

Greenhouse biocontrol assay

The greenhouse experiment was conducted between April and August 2024 using tomato seedlings of the susceptible cultivar San Marzano Nano. Seedlings were transplanted into pots containing a 50:50 mixture of universal substrate and perlite. The pathogen FO23 was incorporated into the substrate at 2×10^7 conidia / g soil 24 h before transplanting.

PT22AV and BO7 were applied at 1×10^8 cells / ml, while T22 was applied at 5×10^7 conidia / ml. Treatments included non-infested control plants, plants inoculated only with FO23, individual microbial treatments in the presence or absence of FO23, and a consortium treatment composed of PT22AV, BO7, and T22. The biocontrol assays were repeated three times under controlled greenhouse conditions.

Plant response assessment

Plant response was assessed using both visual and quantitative criteria. The main variables evaluated were plant height, leaf area, chlorosis, general vigor, and visible symptom severity. Individual microbial treatments were compared with the pathogen-only control to determine their ability to reduce disease symptoms and improve plant growth. The consortium treatment was assessed to determine whether combined microbial application produced stronger protection than individual agents.

Statistical analysis

Experimental data were evaluated using descriptive and comparative statistical analysis. Mean values were calculated for plant height, leaf area, chlorosis severity, and overall treatment response. The biocontrol assays were repeated three times, and treatment consistency was evaluated across experimental replicates. Where quantitative replicated data were suitable for comparison, treatment means were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test at $P < 0.05$. Semi-quantitative symptom responses were interpreted conservatively on the basis of stable and reproducible treatment patterns under controlled greenhouse conditions.

6. Research results

Biocontrol performance of Papiliotrema terrestris PT22AV

Among the tested microbial antagonists, *Papiliotrema terrestris* PT22AV showed the strongest individual performance. Plants treated with PT22AV alone displayed a clear biostimulant effect, with greater average height and improved vegetative vigor compared with the water-treated control. When PT22AV was applied together with FO23, plants performed better than those treated with the pathogen alone, showing increased plant height, larger leaf area, and visibly reduced chlorosis [7, 17].

The growth response observed after PT22AV treatment is summarized in fig. 1.

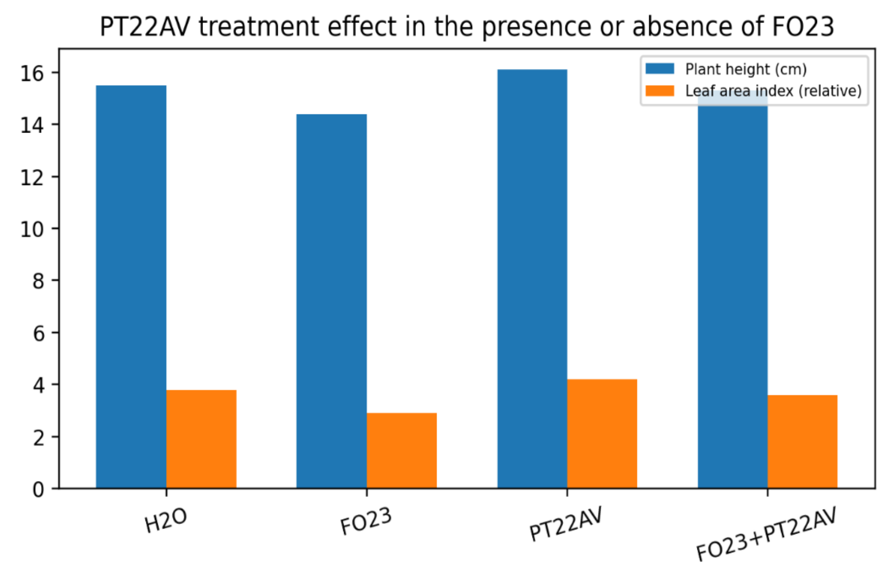

Figure 1. Plant height and leaf area of tomato seedlings treated with *Papiliotrema terrestris* PT22AV in the presence and absence of FO23.

Representative visual responses are presented in fig. 2.

Figure 2. Visual biocontrol effect of *Papiliotrema terrestris* PT22AV against FO23 in tomato seedlings.

Biocontrol performance of Bacillus amyloliquefaciens BO7

The bacterial antagonist *Bacillus amyloliquefaciens* BO7 showed a useful suppressive effect against FO23, although its biostimulant effect was weaker than that observed for PT22AV. BO7 applied alone did not strongly increase plant growth, but when combined with FO23 it improved plant height and reduced chlorosis relative to the pathogen-only control [12-14].

Quantitative effects of BO7 on plant growth are presented in fig. 3.

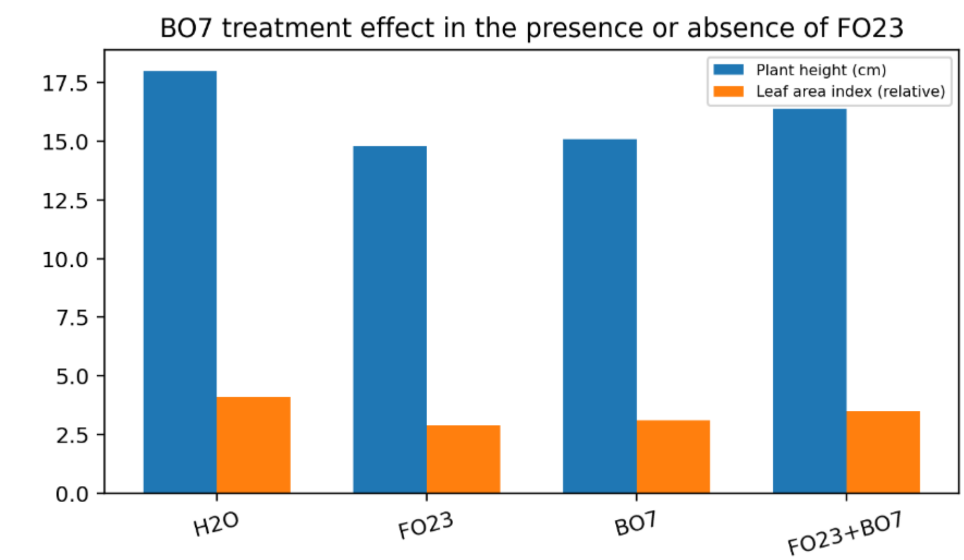

Figure 3. Plant height and leaf area of tomato seedlings treated with *Bacillus amyloliquefaciens* BO7 in the presence and absence of FO23.

Visual symptom reduction achieved by BO7 is shown in fig. 4.

Figure 4. Visual response of tomato seedlings treated with *Bacillus amyloliquefaciens* BO7 in the presence of FO23.

Biocontrol performance of Trichoderma harzianum T22

The fungal antagonist *Trichoderma harzianum* T22 showed the weakest individual performance among the tested microbial agents. Only minor differences were visible between the FO23 treatment and the FO23 + T22 treatment. Some reduction in chlorosis became visible at later observation stages, but the quantitative and visual improvement remained modest compared with PT22AV and BO7 [6, 15].

Growth responses associated with T22 treatment are summarized in fig. 5.

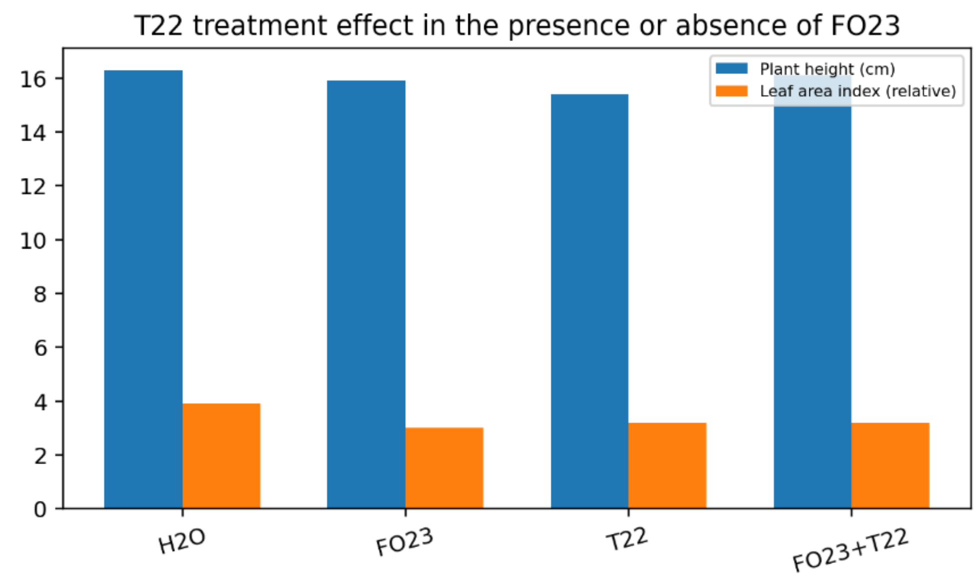

Figure 5. Plant response of tomato seedlings treated with *Trichoderma harzianum* T22 in the presence and absence of FO23.

Comparative symptom expression under T22 treatment is presented in fig. 6.

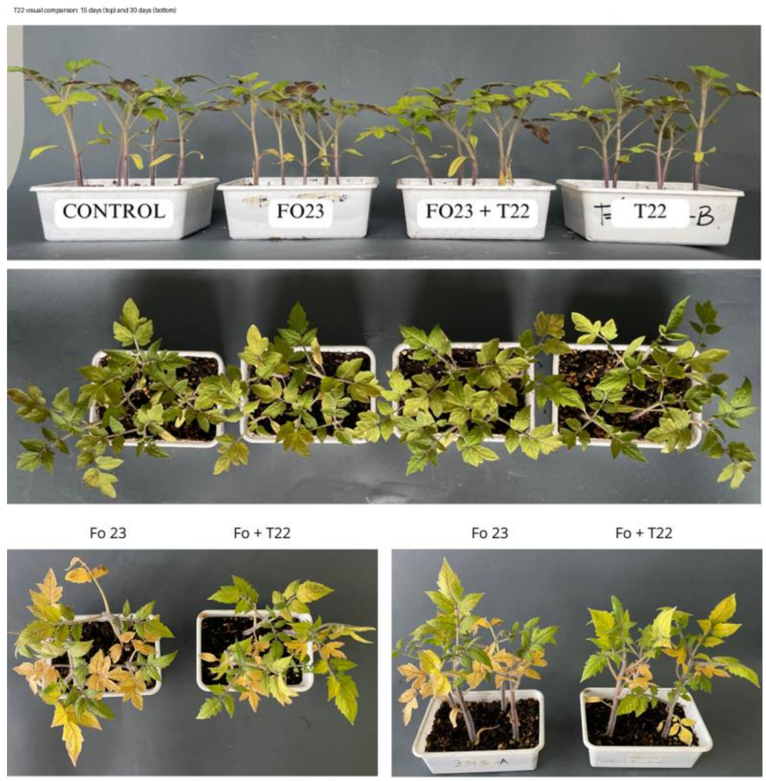

Figure 6. Comparative symptom expression in tomato seedlings treated with *Trichoderma harzianum* T22 under FO23 infection conditions.

Performance of the microbial consortium

The microbial consortium composed of PT22AV, BO7, and T22 produced the strongest overall plant response. Plants treated with the consortium in the presence of FO23 showed better vigor, greener foliage, and reduced symptom severity compared with plants treated with the pathogen alone or with individual antagonists. The consortium treatment therefore provided the most balanced and consistent improvement across disease suppression and plant growth parameters.

The superior performance of the consortium suggests that the three microorganisms acted in a complementary way within the same biological system. The combined treatment likely integrated different mechanisms, including growth support, pathogen suppression, and partial compensation of pathogen-induced stress.

Table 2. Comparative performance of microbial biocontrol treatments against FO23 in tomato seedlings.

Treatment	Biostimulant effect	Symptom reduction	Chlorosis reduction	Overall effectiveness
FO23 only	None	None	None	Severe damage reference
PT22AV only	Strong	—	—	Strong plant stimulation
FO23 + PT22AV	Strong	Clear	Clear	Best single-agent treatment

Continuation of Table 2

BO7 only	Weak	—	—	Limited biostimulation
FO23 + BO7	Moderate	Moderate	Moderate	Useful suppressive effect
T22 only	Weak or none	—	—	Minimal stimulation
FO23 + T22	Weak	Slight	Slight	Modest protection
Consortium only	Strong	—	—	Strong stimulation potential
FO23 + Consortium	Strong	Strong	Strong	Best overall treatment

The overall effect of the microbial consortium is illustrated in fig. 7.

Figure 7. Effect of the microbial consortium composed of PT22AV, BO7, and T22 on tomato seedlings inoculated with FO23.

The results demonstrate that microbial antagonists can reduce the negative effects of *Fusarium oxysporum* FO23 on tomato seedlings, but their performance differed substantially among treatments. The strongest individual performance was observed with *Papiliotrema terrestris* PT22AV, which combined disease mitigation with clear biostimulant activity.

Bacillus amyloliquefaciens BO7 showed a moderate but useful suppressive effect, whereas *Trichoderma harzianum* T22 showed only modest protection under the tested conditions. The microbial consortium produced the strongest overall response, indicating that combined microbial applications may offer broader and more stable disease management than single-agent treatments.

7. Prospects for further research development

Future research should validate the performance of PT22AV, BO7, T22, and their consortium under commercial greenhouse conditions, where substrate composition, irrigation regime, temperature, and pathogen pressure may differ from controlled experimental settings.

Further development should investigate the timing, frequency, and mode of application of microbial consortia in greenhouse tomato production. Additional work should also clarify microbial compatibility, establishment in the rhizosphere, persistence during the crop cycle, and integration with resistant cultivars, sanitation, and other components of integrated Fusarium wilt management.

8. Conclusions

Papiliotrema terrestris PT22AV was the strongest individual antagonist against *Fusarium oxysporum* FO23, combining visible disease suppression with improved plant growth and reduced chlorosis.

Bacillus amyloliquefaciens BO7 provided moderate disease suppression and improved plant performance under pathogen pressure, whereas *Trichoderma harzianum* T22 showed only limited protection under the tested conditions.

The microbial consortium composed of PT22AV, BO7, and T22 produced the best overall plant response, suggesting that combined microbial applications may provide stronger and more stable protection than single antagonists.

These results support the use of microbial biocontrol, particularly consortium-based approaches, as a sustainable component of integrated Fusarium wilt management in greenhouse tomato production.

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