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Fusariosis level (*Fusarium oxysporum*) in tomato plants (*Solanum lycopersicum*) treated with *Trichoderma asperellum* Jc01 and *Bacillus subtilis* ANT01

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ABSTRACT

Background/Objective. The production and quality of tomato (*Solanum lycopersicum*) crops are severely reduced by the fungus *Fusarium oxysporum*, the causal agent of fusarium wilt. Chemical fungicides are conventionally applied, although in cases of severe infection, the entire harvest is lost. Favorable environmental conditions increase the incidence, infection rate, and spread of this pathogen. Biological control is a useful strategy for combating this type of pathogen. The objective of this research was to determine the incidence of fusarium wilt (*Fusarium oxysporum*) in field-grown tomato plants treated with *Trichoderma asperellum* Jc01 and *Bacillus subtilis* ANT01.

Experimental development. *Trichoderma asperellum* Jc01 and *Bacillus subtilis* ANT01 either alone or in combination, were weekly applied to the drip zone of tomato plants (*Solanum lycopersicum*). Disease incidence was periodically sampled in both treated and untreated plants to monitor its progression over 15 weeks. Additionally, the number of flowers and fruits produced on the experimental plants was recorded.

Results. At the end of the field trial, plants treated with *B. subtilis* ANT01 showed 60% less incidence compared to the control and a total of 47.9 fruits produced per plant, while plants treated with *Trichoderma asperellum* Jc01 or the combination of both microorganisms showed 16 and 28% less incidence than the control, and 18.0 and 27.3 fruits produced per plant, respectively.

Conclusion. The results show at least partial evidence of the potential of the ANT01 strain as a biocontrol agent of *F. oxysporum* in tomato plants.

Keywords: Biocontrol, Incidence, Phytopathogen, Microorganisms

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is cultivated across all states of the Mexican Republic on approximately 49 000 ha, with an average yield exceeding 70 t ha^{-1}. Mexico ranks first among tomato-exporting countries, holding a market share of more than 19% in the global market (Montaño *et al.*, 2021). Of the total area under tomato cultivation, Chiapas contributed 3.3% (1 638 ha) and 2.4% (82 729 t) of national production, ranking eighth and fourteenth in these categories, respectively (SIAP, 2023). Conventional management of tomato pests and diseases relies on synthetic chemical formulations (McGovern, 2015).

Bacteria such as *Bacillus subtilis* and fungi such as *Trichoderma* spp. are considered biological control agents of plant diseases with broad potential in modern agriculture (Prabhakaran *et al.*, 2015). Both can be applied as live or dormant cells to soil, foliage, or seeds (Minchev *et al.*, 2021). *Trichoderma* spp. is ubiquitous in the environment, easy to isolate and culture, capable of growing on diverse substrates, and able to control a wide range of phytopathogenic microorganisms. In tomato plants, *Trichoderma* spp. has been reported to reduce the incidence of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* by 85–90% (Alshammari *et al.*, 2022). Similarly, *Bacillus* sp. reduces wilting caused by *Fusarium oxysporum* f. sp. *lycopersici* race 2 by 75% (Qi *et al.*, 2016). In light of the above, the objective of the present study was to determine the level of Fusarium wilt (*Fusarium oxysporum*) in tomato (*Solanum lycopersicum*) using *Trichoderma asperellum* Jc01 and *Bacillus subtilis* ANT01, two microorganisms with biocontrol potential.

MATERIALS AND METHODS

Study site. The study was conducted at the "Ayol" Agroecological Station, Municipality of Tapachula, Chiapas, Mexico (14°49'46" N; 92°17'42" W; mean annual rainfall 2,600 mm; relative humidity 80%; mean annual temperature 26 °C).

Culturing of *T. asperellum* Jc01 and *B. subtilis* ANT01. The strains were obtained from the culture collection of the Institute of Biosciences of the Autonomous University of Chiapas. *T. asperellum* Jc01 was grown to sporulation on Potato Dextrose Agar (PDA) at pH 5.6 ± 0.2 and 25–28 °C in Petri dishes. For mass spore production, a disk of actively growing mycelium (1 cm diameter) was suspended in 10 mL of isotonic solution, and 150 µL of the suspension was spread onto PDA in 500 mL Roux bottles. The microorganism was grown under the conditions described above until sporulation. Spore harvesting was performed using 10 mL of isotonic solution containing 0.5% Tween 80. Spore concentration was determined by direct counting with a Neubauer hemocytometer. *B. subtilis* ANT01 was cultured in Potato Dextrose Broth (PDB). A single colony with the characteristic morphology of the strain was transferred with a bacteriological loop into 5 mL of PDB in a 16 × 100 mm test tube and incubated with rotary shaking (150 rpm) at 25 °C for 24 h. The culture was then transferred to 100 mL of PDB and incubated under the same conditions. Bacterial concentration was determined by serial dilution using the Most Probable Number (MPN) method. Following incubation under the conditions described above, microbial growth was assessed spectrophotometrically at 560 nm against uninoculated PDB.

Plant material. *S. lycopersicum* seedlings were raised in a compost–vermicompost mixture (4:1) in 70-cell trays. Seeds were surface-sterilized with sodium hypochlorite

(1.5%) for 10 min, rinsed three times with sterile water, and allowed to dry on absorbent paper. Three seeds per cell were sown at a depth of 1 cm. Germinated seedlings were selected and maintained for 30 days under constant moisture through regular irrigation until reaching a height of 10–12 cm.

Field crop establishment. For open-field cultivation, four planting beds were prepared, each 12 m long, 45 cm wide, and 30 cm high, with 200 cm between beds. Healthy seedlings (30 per treatment) with well-developed green leaves, an erect habit, and a well-developed white root system were transplanted at 30 cm between plants.

Treatment design. A complete 2^2 factorial treatment design was used (Table 1), with *B. subtilis* ANT01 (1×10^6 CFU g^{-1} soil) and *T. asperellum* Jc01 (1×10^4 spores g^{-1} soil) as factors, each at two levels: absence (−1) and presence (+1). Treatments were applied weekly within a 30 cm radius around each plant to a depth of 30 cm, from the day of transplanting through fruit harvest (15 applications in total).

Crop management. Soil fertilization was carried out with 2 L of biol every 7 days throughout the crop cycle, and 1 kg of compost plus 2 kg of vermicompost every 56 days. Biofertilizers were applied at the drip line of each plant. Weed control was performed manually, and pest control was achieved by spraying a 10% aqueous extract of neem (*Azadirachta indica*) leaves every 7 days. Irrigation to field capacity was applied as needed, and stakes were placed to support plant growth.

Response variables. Plants in all treatments were inspected weekly for the characteristic visual symptoms of Fusarium wilt (yellowing and wilting of lower leaves, vascular discoloration of the stem) resulting from natural infection. Disease incidence (I) was calculated for each treatment using the following formula: I = (number of plants showing Fusarium wilt symptoms) × (total plants)$^{-1}$. In addition, the total number of flowers and fruits produced per plant was recorded for each treatment.

Data analysis. Incidence, flower, and fruit production data were subjected to analysis of the effects of *B. subtilis* ANT01 and/or *T. asperellum* Jc01, and the statistical significance of treatment effects on Fusarium wilt incidence was determined in accordance with the procedures established for a complete factorial design.

Six weeks after transplanting (WAT) into the field, the first plants displaying characteristic symptoms of Fusarium wilt were observed. In plants treated with *B. subtilis* ANT01, the first symptomatic plants were not observed until 9 WAT, whereas in plants treated with *T. asperellum* Jc01 or with both microorganisms, they were recorded at 6 and 7 WAT, respectively. As the crop cycle progressed, the number of symptomatic plants continued to increase; plants in the untreated control reached the highest incidence (0.50), while plants in the *B. subtilis* ANT01, *T. asperellum* Jc01, and dual-inoculation treatments showed 60, 16, and 28% lower incidence at 15 WAT, respectively, compared to the control (Figure 1).

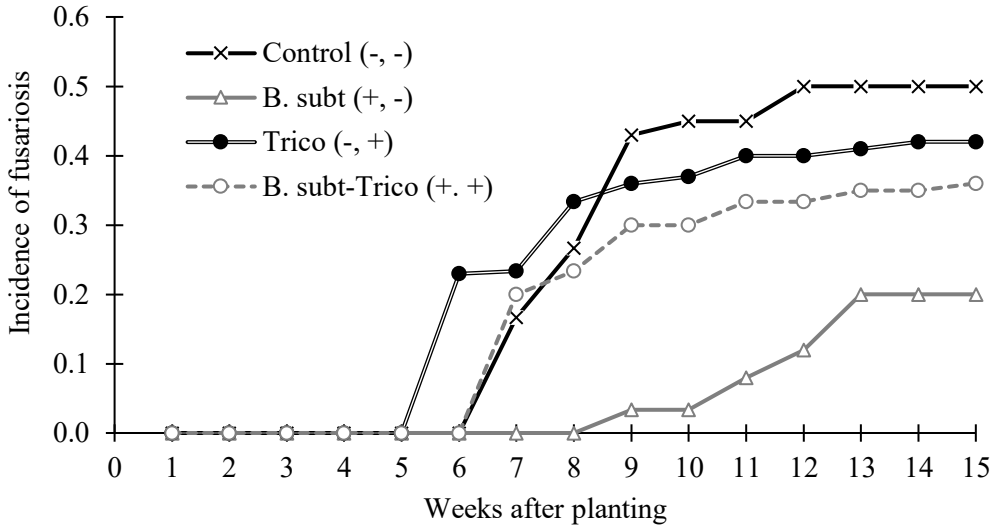

Figure 1. Incidence (represented as proportion on a 0-1 scale) of fusariosis-associated symptoms in field-grown tomato plants and subjected to different biological control treatments.

Analysis of the effects of *B. subtilis* ANT01 and *T. asperellum* Jc01 on Fusarium wilt incidence showed that *B. subtilis* ANT01 was the only factor with negative values, whereas the values for *T. asperellum* Jc01 and the *B. subtilis* ANT01 × *T. asperellum* Jc01 interaction were positive (Table 1).

The *B. subtilis* ANT01 treatment yielded an average of 70 flowers plant^{-1}, while the *B. subtilis* ANT01 + *T. asperellum* Jc01, *T. asperellum* Jc01, and control treatments produced 42.1, 30.7, and 30.0 flowers plant^{-1}, respectively. Fruit production in the *B. subtilis* ANT01 treatment was 47.9 fruits plant^{-1}, whereas the control, *T. asperellum* Jc01, and *B. subtilis* ANT01 × *T. asperellum* Jc01 treatments produced 7.6, 18.0, and 27.3 fruits plant^{-1}, respectively.

Table 1. Effect of *Bacillus subtilis* ANT01 and *Trichoderma asperellum* Jc01 on fusariosis incidence, and on flowers and fruits production in tomato plants.

Effect	Incidence	Flowers plant^{-1}	Fruits plant^{-1}
Total	0.266	40.8	25.2
B. subtilis ANT01	-0.064	10.5	12.4
T. asperellum Jc01	0.025	-9.2	-2.6
B. subtilis ANT01+*T. asperellum* Jc01	0.051	-9.5	-7.7

Analysis of the effects of *B. subtilis* ANT01 and *T. asperellum* Jc01 and their interaction on flower and fruit production in tomato plants showed that *B. subtilis* ANT01 was the only factor that improved production of both variables (Table 1). The Fusarium wilt incidence observed in the present study (Figure 1) compared favorably to that reported for squash (*Cucurbita maxima*) using *B. subtilis* SQR 9 as a biocontrol agent, which achieved only a 55% reduction (Cao *et al.*, 2011). The biocontrol activity of *B. subtilis* ANT01 against Fusarium wilt may result from efficient colonization of tomato roots, as suggested by Cao *et al.* (2011) for *B. subtilis* strain SQR 9 and by Zhao *et al.* (2013) for *B. subtilis* strain Y-IVI; from induction of systemic resistance in the plant, as reported for *B. subtilis* strains CBMT51, B2, and IAGS174 (Mejía-Bautista *et al.*, 2016; Corrales *et al.*,

2012; Akram *et al.*, 2014); from the production of antibiotic compounds by *B. subtilis* strain B2 (Corrales *et al.*, 2012); or from nutrient competition or production of lytic enzymes by *B. subtilis* strain TV-125 (Wang *et al.*, 2018; Senol *et al.*, 2014).

On the other hand, the failure of *T. asperellum* Jc01 to control Fusarium wilt (Figure 1) merely indicates that this strain likely does not produce metabolites or enzymes capable of inhibiting *F. oxysporum* growth, and exhibits neither competitive ability nor capacity to induce systemic resistance in the plant. Nevertheless, *T. asperellum* Jc01 appears to exert a negative effect on the activity of *B. subtilis* ANT01; although the interaction reduced Fusarium wilt incidence, the reduction was not statistically significant (Table 1). Finally, the fruit yield (fruits plant^{-1}) obtained in this study falls within the range reported for this crop, which varies depending on cultivar, planting density, irrigation, and whether production takes place under greenhouse or open-field conditions, among other factors (Márquez-Hernández *et al.*, 2013). Further studies are needed to characterize the fungus associated with Fusarium wilt through morphological and molecular identification, and to determine whether it exhibits resistance to the biocontrol organisms evaluated in this study.

CONCLUSIONS

Bacillus subtilis ANT01 reduced Fusarium wilt incidence associated with *Fusarium oxysporum* in tomato (*Solanum lycopersicum*) plants grown under field conditions by 60%, while *Trichoderma asperellum* Jc01 and the combination of both microorganisms reduced incidence by 16 and 28%, respectively. The lower Fusarium wilt incidence and severity observed in tomato plants treated with *Bacillus subtilis* ANT01 translated into greater flower and fruit production per plant, with 70 flowers and 48 fruits recorded, respectively.

Limitations. The present report demonstrates the potential of strain ANT01 as a biocontrol agent against the pathogen; however, further field and laboratory trials are needed to corroborate its efficacy. Additionally, the molecular identification of the antagonistic strains has not been published and has only been reported at the thesis level. Furthermore, the molecular taxonomic identification of the pathogen is still lacking, as it has been characterized solely on the basis of morphology and plant symptomatology. Finally, although all plants in the field experiment were assumed to be exposed to the same inoculum density in the soil and uniform sowing conditions, these variables should be more rigorously controlled in future studies.

Conflict of interest. The authors declare no conflict of interest.

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