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Fusarium oxysporum f. sp. *lycopersici* biomass variations under disease control regimes using *Trichoderma* and compost

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Summary. A comprehensive understanding of population dynamics of pathogens and bioagents in plant rhizospheres is important for improving organic farming. *Fusarium oxysporum* f. sp. *lycopersici* (FOL30) causes Fusarium wilt of tomato. In this study, we compared biomass variations of FOL30 under different disease control regimes, using *Trichoderma asperellum* TA23 strain, compost, or their combination. Biomass variations of FOL30 and TA23 were observed for 13 weeks using quantitative real-time PCR. Separate applications of TA23, compost, and their combination all reduced FOL biomass when compared to experimental controls. Regression analyses of the qPCR data showed that FOL populations fitted curvilinear polynomial order 3 regression models (R^2 = 0.87 to 0.95). Areas under the population dynamic curves (AUPDCs; $\log_{10}$ ng DNA week^{-1} g^{-1} soil) were: 43.8 from FOL30 alone, 36.6 from FOL30 plus TA23, 25.4 from FOL30 plus compost, and 25.5 from FOL30 plus TA23 plus compost. These results indicate that the individual applications of TA23 or compost, or their combination, decreased the FOL biomass. The negative correlation between TA23 and FOL30 populations showed that the compost and biocontrol agent reduced FOL pathogen populations. This study demonstrates that compost fortified with *T. asperellum* TA23 decreased FOL populations and reduced disease, and that their use is a promising strategy for managing Fusarium wilt of tomato in organic farming.

Keywords. Area Under Population Dynamic Curve (AUPDC), biological control, quantitative PCR.

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INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici* (FOL) is an important soil-borne pathogen, causing serious wilt disease of tomato (*Lycopersicon esculentum*) plants (Srinivas *et al*., 2019). The pathogen is difficult to control with standard cultural and chemical methods. Wilt resistant varieties of tomato are avail-

able, but that resistance can be overcome by the development of new FOL races due to ability of the fungus to evolve in different ways under selection pressure (Biju *et al*., 2017). Growing awareness of the potential hazards from the use of agrochemicals has also led to increased research on alternative methods for effective disease control, including the use of biological control agents. The antagonistic activities of many soil microorganisms against plant pathogens, including *Trichoderma*, *Clonostachys*, *Bacillus* spp., and fluorescent *Pseudomonas* spp., can offer alternative approaches to manage many plant diseases, including Fusarium wilt of tomato (Alabouvette *et al*., 1993; Larkin and Fravel 1998; De Cal *et al*., 1999; Sánchez-Montesinos *et al*., 2021). Biological control is ecologically safe and compatible with different agricultural practices including organic and integrated pest/pathogen management programs (Baker *et al*., 2020).

Organic farming is considered a sustainable and climate-friendly agriculture system, with a possibility to feed the world with organic products. Saudi Arabia is one of 181 countries that strongly promote and adopt organic farming, to reduce water usage, save environments and alleviate negative consequences of chemical-based agriculture (IFOAM, 2020). Application of organic matter such as compost and manure can improve soil quality by increasing water-holding capacity and organic content, along with maintaining exchangeable cations (Reeves, 1997; Etana *et al*., 1999; Balesdent *et al*., 2000; De Corato, 2023). A major challenge with implementing biocontrol strategies is how to maintain stable populations of biocontrol agents throughout crop growing seasons (Lewis and Papavizas, 1984; Waage and Greathead, 1988; Chammem *et al*., 2022). Amendment of organic substrates with biocontrol agents offers a promising solution, where organic substrates have been shown to support the survival of biocontrol agents in soil near plant roots (Hoitink and Boehm, 1999). Composts are naturally suppressive of plant diseases, especially if the composts are amended with biocontrol microorganisms (Abbasi *et al*., 2002; Spadaro and Gullino, 2005).

Understanding the ecology and population dynamics of bioagents and pathogens in the host plant rhizospheres/rhizoplanes provides insights on significance of bioagents for management of crop diseases (Gangwar *et al*., 2013). DNA-based assays are used to monitor populations of microorganisms in soil (Zhang *et al*., 2017), and quantitative real-time PCR (QPCR) can be used to detect, characterize and quantify nucleic acids for numerous applications. QPCR has been widely used to study population dynamics of microorganisms, including pathogenic fungi (Moya-Elizondo *et al*., 2011; Sui *et al*., 2022), bacteria (Hu *et al*., 2013), and to monitor spatial and temporal responses of soil microorganisms to abiotic stresses (Pereira e Silva *et al*., 2012).

Mathematical descriptions of microbial population dynamics can be used to reduce the amount of measured data, to explain observed patterns, to compare growth rates and patterns, and to predict population growth (Karkach, 2006). These descriptions can be modeled using techniques such as empirical, mechanistic and polynomial regression statistics. Empirical models are derived from measures of population size and age, while mechanistic models are derived from differential equations relating growth rates to population size (France and Thornley, 1984). Modelling the development of pathogens, establishing thresholds, and monitoring pest populations facilitate the implementation of integrated disease management in greenhouse crop production systems (Marchand *et al*., 2020).

The present study has used QPCR to investigate biomass variations of the pathogenic FOL30 strain and the biocontrol strain *T. asperellum* TA23, under different soil regimes. Polynomial regression and population dynamic rate models were used to determine the effects of biocontrol agent and compost on FOL populations in soil. The correlation between FOL biomass variations and disease intensity was also measured.

MATERIALS AND METHODS

Fungal strains and composting material

A pathogenic FOL30 strain was recovered from naturally infected roots of tomato plants showing wilt symptoms, and was morphologically and molecularly characterized by Khan *et al*. (2020). The strain was maintained on Petri plates containing potato dextrose agar (PDA; Difco). The *T. asperellum* strain TA23, originally isolated from soil samples from Riyadh region, Saudi Arabia (El_Komy *et al*., 2015), was obtained from cryogenic storage in the fungal collection at the Fungal and Bacterial Plant Diseases Laboratory, Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, and was maintained on PDA plates.

Compost material used in this study was the commercial compost Al-Reef (Al-Reef Organic Fertilizers Co., Riyadh, Kingdom of Saudi Arabia), with composition of 80% cow manure and 20% vegetable materials.

Preparation of fungal inocula for soil infestation

FOL30 inoculum was prepared by inoculating 500 mL capacity Erlenmeyer flasks each containing 100 mL

of potato dextrose broth (PDB; Difco) with mycelial discs from 10-d-old FOL30 cultures. Inoculated flasks were fitted on a shaker set at 200 rpm and incubated at 25°C for 7 d. Mycelial/conidial suspensions were filtered through a sterile sintered glass funnel (pore size 100 µm) to separate mycelia from conidia. The resulting conidial suspension was then centrifuged at 2000 × *g* for 15 min. The resulting conidial pellets were washed twice with sterile distilled water, vortexed and then re-centrifuged. The final conidial pellets were suspended in sterile distilled water, and suspensions were adjusted to 10^5 conidia mL^{-1} using a haemocytometer (Hawkley Ltd). This inoculum was applied to infest soils to achieve a final concentration of 10^5 conidia g^{-1} soil.

Trichoderma asperellum strain TA23 was grown on PDA in 90 mm diam. Petri dishes, which were incubated at 25°C for 10 d. Erlenmeyer flasks (500 mL capacity), each containing 100 mL of PDB, were then inoculated with 5 mm diam. mycelium plugs from these 10-d-old cultures. Inoculated flasks were fitted on a shaker set at 200 rpm and incubated at 25°C for 7 d. The contents of the flasks were filtered through four layers of sterile cheesecloth, and the resulting conidial suspensions were centrifuged at 2000 × *g* for 15 min. Conidial pellets were washed twice with sterile distilled water, vortexed and re-centrifuged. After washing, the pellets were suspended in sterile distilled water, and conidial suspensions were adjusted to 10^6 conidia mL^{-1} using a haemocytometer. The inoculum was applied to soils to achieve a final concentration of 10^6 conidia g^{-1} soil.

Soil infestation and plant material

'Tristar' tomato seeds (Sorouh Agricultural Co.) were surface-sterilized for 30 s in 1% sodium hypochlorite and then rinsed three times with sterile distilled water. The surface-sterilized seeds were pre-germinated in germinating trays containing an autoclaved potting mix of soil, peat moss, and perlite (2:1:1, v:v:v). Then these seeds were incubated in a growth chamber with a 16 h day (24°C) and 8 h night (20°C) cycle at 70% relative humidity. The seedlings were irrigated as needed and fertilized twice each week with 1 g L^{-1} of 20-20-20 (N-P-K) fertilizer (Alahmari Group). The subsequent experiments were carried out on 3-week-old tomato seedlings that had 3-5 fully expanded leaves. Plastic pots (16 cm diam.) were filled with either autoclaved sandy clay soil (1:1 v/v) or a mixture of autoclaved sandy clay soil and Al-Reef Ltd organic compost at a ratio of 4:1. Control pots were filled with 100% autoclaved sandy clay soil.

For soils inoculated with FOL30, the pots were infested by mixing conidial suspension of the fungus with soil at concentration of 10^5 conidia g^{-1} soil, and were then left for 1 week to allow establishment of the pathogen. The TA23 strain was applied at concentration of 10^6 conidia g^{-1} soil alone or in combination with compost, and also left for 1 week for the establishment of the fungus. Following the establishment period for FOL30 and TA23, three 3-week-old tomato seedlings were transplanted into each pot.

The experiments were conducted with completely randomized designs, each with five replicates for each treatment (15 plants per replicate). The treatments were: soil (designated T1), soil + compost (T2), soil + TA23 (T3), soil + FOL30 (T4), soil + FOL30 + TA23 (T5), soil + FOL30 + compost (T6), and soil + FOL30 + TA23 + compost (T7). The pot experiments were carried out in the greenhouse of the Plant Protection Department, College of Food and Agricultural Sciences, King Saud University. At the end of the 13th week post FOL-infestation, disease severity (DS) was assessed using the following scoring system of visual foliar symptoms: 1 = no symptoms (i.e., healthy plants with green leaves); 2 = light wilting, one or two yellow leaves; 3 =moderate wilting, three or more yellow leaves; 4 = extensive wilting, dead lower leaves with some wilted upper leaves and stunting; and 5 = dead plants (Horinouchi *et al*., 2007). Disease scores were converted to DS using the following formula: DS = [(A × 1) + (B × 2) + (C × 3) + (D × 4) + (E × 5)]/(total number of plants) × 100, where A, B, C, D, and E are the numbers of plants corresponding, respectively, to scores of 1, 2, 3, 4, and 5.

Quantification of FOL30 and TA23 strains

Extraction of total DNA from fungal cultures. Two 15 mL capacity Corning tubes, each containing 10 ml of PDB (one tube had 6×10^9 conidia of FOL30 and the other had 2×10^9 conidia of TA23) were centrifuged at 2300 × *g* in swing bucket centrifuge (Eppendorf, model 5810R). The pelleted conidia were then re-suspended in 300 µL Microbead solution buffer (MO BIO Laboratories Inc), and DNA isolation was carried out according to instructions for the MO BIO UltraClean® Microbial DNA Isolation Kit. At the final step, DNA was eluted in 50 µL of MD 5 solution. The DNA concentration was measured spectrophotometrically (Nanodrop 2000, Thermo Scientific), and also estimated using agarose gel stained with acridine orange. Each DNA sample was quantified three times and the average was used. Following the DNA quantification, 10-fold serial dilutions (10^0 to 10^{-5} ng μL^{-1}) were made of each DNA stock, using ultrapure molecular water (Genekam, Biotechnology).

Isolation of total DNA from soil. Soil samples (2-3 cm depth) were collected from tomato rhizospheres after 1, 2, 4, 6, 8, 11, or 13 weeks post tomato seedling planting in FOL-infested and non-infested soils. Three biological replicates were collected from each treatment for QPCR analyses. Total DNA was isolated from soil samples using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc.). Soil (250 mg) was transferred to each Power Bead Tube® and then 60 µL of C1® solution were added. The tubes were vortexed for 10 min, and were then centrifuged at 10,000 × *g* for 30 sec. Approx. 450 µL of supernatant were transferred to a 2 mL capacity clean tube. Two hundred and fifty µL of C2® solution were added to the supernatant. The mixtures were vortexed for 5 s and then incubated at 4°C for 5 min. The tubes were centrifuged at 10,000 × *g* for 1 min at room temperature. Six hundred µL of supernatant were transferred to a 2 mL capacity clean tube, and 200 µL of C3® solution were added to each tube. The tubes were briefly vortexed and incubated at 4°C for 5 min. The tubes were centrifuged at 10,000 × *g* for 1 min, and the supernatants were transferred to clean 2 mL capacity tubes and were each mixed with 1200 µL of C4® solution. Supernatant (650 µL) was then transferred to clean spin filter and centrifuged at 10,000 × *g* for 1 min, and the supernatant was discarded. This washing step was repeated three times by adding additional 650 µL of C4® solution each time. Five hundred µL of C5® solution were then added to the spin filter and centrifuged at 10,000 × *g* for 1 min. The supernatant was discarded, and the spin filter was centrifuged once more. The filter was carefully transferred to another clean 2 mL capacity collection tube. To elute DNA, 100 µL of C6® solution were added to the centre of each white filter membrane and the tube was centrifuged at room temperature for 30 sec at 10,000 × *g*. The flow through solution containing DNA was retained and the spin filter was discarded.

QPCR reaction and program. Standard curves were constructed based on cycle thresholds (C_t) of 10-fold dilution series (12×10^0, 12×10^{-1}, 12×10^{-2}, 12×10^{-3}, 12×10^{-4} and 12×10^{-5} ng µL^{-1}) for *F. oxysporum* genomic DNA preparations, using primer pair Fef1F/Fef2R (Haegi *et al*., 2013), and dilution series (1×10^0, 1×10^{-1}, 1×10^{-2}, and 1×10^{-3} ng µL^{-1}) for *T. asperellum* genomic DNA preparations, using primer pair TGP4F/TGP4R (Kim and Knudsen, 2008). C_t values were determined using the Applied Biosystem QPCR software program. The logarithm ($\log_{10}$) of the concentration of each 10-fold dilution series of fungal genomic DNA was plotted along the X axes and the respective C_t values were plotted along the Y axes. The standard curves were constructed using the linear regression equation y = mx + b. Standard curves were established from at least four dilution factors, each with three replicates. Each QPCR reaction contained 3 µL DNA (4 ng µL^{-1} - 40 fg µL^{-1}), 7.5 µL of 2′ SYBR green (Applied Biosystems), 0.15 µL of each 10 µM primer (Fef1F/Fef2R for *F. oxysporum* and TGP4F/TGP4R for *T. asperellum*), and 4.2 µL of ultrapure molecular water (Genekam). Negative control reactions contained the same mixtures, each with 2.0 µL of sterile water replacing the DNA template. The QPCR programs each consisted of one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 sec, and 56°C for 1 min. QPCRs were run using the 7500 Real-Time PCR system (Applied Biosystems). For quantifying FOL30 and TA23 in soil, each QPCR mixture contained 5 µL of DNA extracted from soil, 7.5 µL of 2′ SYBR green, 0.15 µL of each 10 µM primer, and 2.2 µL molecular grade water. The QPCR program was as described above. Three technical replicates were conducted for each biological replicate.

Statistical analyses

The data of DS collected from greenhouse experiments were analyzed using analysis of variance (ANOVA) in SAS (SAS Institute) at $P < 0.05$ significance, followed by the least significant difference (LSD) tests. QPCR data were analyzed with Statistix 8.1 analytical software to compute ANOVA for treatments, time (weeks) and treatments × time interactions. Mean separation was accomplished using LSD at $\alpha = 0.05$. Pearson correlation analyses were conducted using Statistix 8.1. In addition, simple polynomial regression for FOL populations was generated from the $\log_{10}$ DNA data using Microsoft Excel 2010. The areas under population dynamic curves (AUPDC) were calculated as $\int_1^{13} at^3 + bt^2 + ct$, and were expressed as population size (ng week^{-1} g^{-1} soil).

RESULTS

Effects of treatments on disease severity and FOL30 biomass

In the pathogenicity experiments, none of the control plants (treatment T1) showed any disease symptoms throughout the experiments. In addition, tomato plants in treatments T2 (soil amended with compost) and T3 (soil amended with TA23) did not show any disease symptoms throughout the experiments (Table 1). However, plants in treatments T4 (soil infested with FOL30) and T5 (soil infested with FOL30 and amended with *T. asperellum* TA23) showed significant disease severity (Table 1). The T6 treatment (soil amended with compost)

Table 1. Mean disease severity scores and mean *Fusarium oxysporum* f. sp. *lycopersici* (FOL) biomasses for tomato plants inoculated with FOL and receiving different soil, compost or *Trichderma asperellum* treatments. The pathogenic FOL strain was FOL30 (Khan *et al.*, 2020), and the biocontrol agent *T. asperellum* strain was TA23 (El_Komy *et al.*, 2015).

Treatment	Disease severity	Biomass of FOL[a]
T1 (soil)	0.0 d [b]	0 d*
T2 (soil + compost)	0.0 d	0 d
T3 (soil + *T. asperellum*)	0.0 d	0 d
T4 (soil + FOL)	1.53 b	-1.7363 c
T5 (soil + FOL + *T. asperellum*)	2.0 a	-2.4175 b
T6 (soil + FOL + compost)	1.0 c	-3.205 a
T7 (soil + FOL + *T. asperellum* + compost)	0.0 d	-3.355 a

[a] Biomass of FOL30 was expressed as $\log_{10}$ ng DNA g^{-1} soil.
[b] Means accompanied by the same letter within each column are not significantly different ($P < 0.05$).

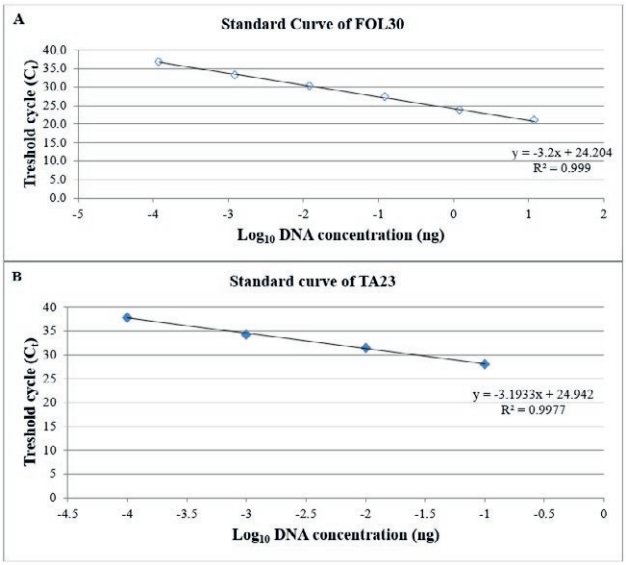

Figure 1. Standard curves of fungal DNA concentrations vs threshold cycles (C_t). C_t values were plotted against log-transformed DNA amounts and the linear regression equation was calculated. (A) FOL30 DNA concentration standards ranged from 120 fg to 12 ng, and (B) TA23 DNA concentrations ranged from 10 fg to 1 ng.

reduced mean disease severity ($P < 0.05$), while no disease symptoms were recorded on tomato plants in T7 treatment (soil infested with FOL30 strain and amended with both compost and TA23) (Table 1). Individual applications of compost, TA23 or their combination reduced FOL biomass ($P < 0.05$). The greatest reductions were recorded from T6 and T7 treatments, with no significant difference between these treatments. Application of TA23 alone also reduced the FOL biomass compared with the T4 treatment (Table 1).

Standard curves

The standard curves for DNA of FOL30 and TA23 showed strong relationships ($R^2 = 0.99$) between C_t and $\log_{10}$ DNA concentrations (Figure 1). These developed standard curves were suitable for detecting DNA at concentrations ranging from 10 to 10^{-4} ng. The standard curve slopes obtained for both FOL30 and TA23 were -3.2, with amplification efficiency (E) of 2.05 ($E = 10^{-(1/-3.2)}$), suggesting that the amounts of PCR products were probably doubled during each PCR cycle.

Tracking of FOL30 and TA23 populations in soil

Real-time QPCR showed that FOL30 was not detected in soil samples collected from treatments T1, T2 and T3, indicating that neither soil nor compost contained fungi related to *F. oxysporum*. However, FOL30 was detected in all FOL-infested soil samples collected from treatments T4 to T7 (Table 2). FOL30 populations at the first week after infestation were approx. 0.73 $\log_{10}$ DNA from T4 and T5, and 1.11 $\log_{10}$ DNA from T6 and T7. FOL30 decreased sharply at the second week after infestation, where $\log_{10}$ DNA amounts were -0.67 from T4 and T5 treatments, and -2.19 from T6 and T7 treatments. FOL30 populations continued to decease after 4 weeks post infestation, with $\log_{10}$ DNA values of -2.65 from T4, -1.95 from T5, -4.00 from T6, and -3.61 from T7. At the sixth week after infestation, FOL30 populations increased, with $\log_{10}$ DNA values of -1.47 from T4 and -2.92 from T6. However, for T5 and T7, FOL30 populations continued to decrease (-3.36 $\log_{10}$ DNA for T5 and -4.00 for T7). At the eighth week after infestation, FOL populations decreased in for T4 and T6, with $\log_{10}$ DNA values of -1.55 from T4 and -3.93 from T6. The FOL populations increased for T5 (-1.7 $\log_{10}$ DNA) and remained stable for T7 (Table 2). At the 11th week, FOL populations increased either slightly from T4 and T5, or considerably from T6 and T7. The FOL populations then decreased at the 13th week after infestation for treatments T4 to T7 (Table 2).

Populations of the biocontrol agent TA23 were established and detected from treatments T3, T5, and T7. The TA23 populations were relatively stable for T3 within the period of observation (Table 2). For T5, TA23 populations fluctuated, slightly increasing from the 4th week after infestation ($\log_{10}$ DNA -2.78) to the 6th week

Table 2. Tracking of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Trichoderma asperellum* populations in soil following different experimental treatments.

Treatment[a]	Weeks after treatments applied [b]							Population
	1	2	4	6	8	11	13	
T1 (soil)	-	-	-	-	-	-	-	-
T2 (soil + compost)	-	-	-	-	-	-	-	-
T3 (soil + *T. asperellum*)	-	-	32.39±1.51 B (-2.30±0.47)	34.52±3.66 B (-2.96±1.13)	34.22±1.40 A (-2.87±0.43)	32.43±0.88 B (-2.32±0.27)	33.28±3.74 A (-2.58±1.16)	TA23
T4 (soil + FOL)	21.86±7.04 a (0.73±2.20)	26.34±2.55 a (-0.67±0.80)	33.40±3.53 ab (-2.65±1.10)	30.52±4.02 a (-1.47±1.26)	29.15±4.37 a (-1.55±1.37)	26.90±2.90 b (-0.84±0.90)	28.83±2.75 a (-1.44±0.86)	FOL30
T5 (soil + FOL + *T. asperellum*)	-	-	33.91±2.01 B (-2.78±0.62)	33.47±1.97 B (-2.64±0.61)	35.59±1.04 A (-3.29±0.32)	35.30±1.60 A (-3.20±0.50)	33.09±2.15 A (-2.52±0.66)	TA23
	21.86±7.04 a (0.73±2.20)	26.34±2.55 a (-0.67±0.80)	30.45±3.93 b (-1.95±1.23)	34.95±2.75 a (-3.36±0.86)	29.63±2.50 a (-1.70±0.78)	29.26±4.12 ab (-1.58±1.29)	35.41±2.17 b (-3.50±0.68)	FOL30
T6 (Soil + FOL30 + compost)	-	-	-	-	-	-	-	TA23
	20.66±4.98 a (1.11±1.56)	31.22±3.66 a (-2.19±1.14)	37.00±0.00 a (-4.00±0.00)	33.53±3.88 a (-2.92±1.21)	36.77±4.56 b (-3.93±1.43)	32.10±2.69 a (-2.47±0.84)	35.29±2.64 b (-3.46±0.83)	FOL30
T7 (Soil + FOL+ *T. asperellum* + compost)	-	-	37.09±2.03 A (-3.76±0.63)	36.22±2.27 A (-3.49±0.70)	35.19±0.74 A (-3.17±0.23)	34.73±1.19 A (-3.03±0.37)	35.55±1.97 A (-3.28±0.61)	TA23
	20.66±4.98 a (1.11±1.56)	31.22±3.66 a (-2.19±1.14)	35.76±2.47 a (-3.61±0.77)	37.00±0.00 a (-4.00±0.00)	37.00±0.00 b (-4.00±0.00)	29.71±1.38 ab (-1.72±0.43)	32.83±2.35 b (-2.69±0.74)	FOL30

[a] The pathogenic FOL strain was FOL30 (Khan *et al.*, 2020), and the biocontrol agent was *Trichoderma asperellum* strain TA23 (El_Komy *et al.*, 2015).

[b] Fungal populations were expressed as C_t values and $\log_{10}$ DNA; Up: average of C_t ± Std. dev.; Down: average of biomass ($\log_{10}$ DNA ± Std. dev.). Values accompanied with the same letter (capitals for TA23 and lower case for FOL30) within a column are not significantly different (LSD, α = 0.05).

($\log_{10}$ DNA -2.64), and then decreasing sharply at the 8th week ($\log_{10}$ DNA -3.29), and increasing again in the 11th and 13th weeks after infestation (Table 2). In T7, TA23 populations slightly increased through the 13 weeks of observations (Table 2).

Analysis of the average C_t values of FOL30 and TA23 from the 4th to the 13th sampling weeks showed negative correlation for T5 but positive correlation for T7. For T5 (Soil + FOL30 + TA23), the correlation analysis showed negative correlation (r = -0.90; P = 0.035), while for T7 (Soil + FOL30+ TA23 + compost), this analysis showed positive correlation (r = 0.54; P = 0.35). The positive correlation for the T7 treatment indicates that the compost treatment acted as a substrate promoting and maintaining TA23.

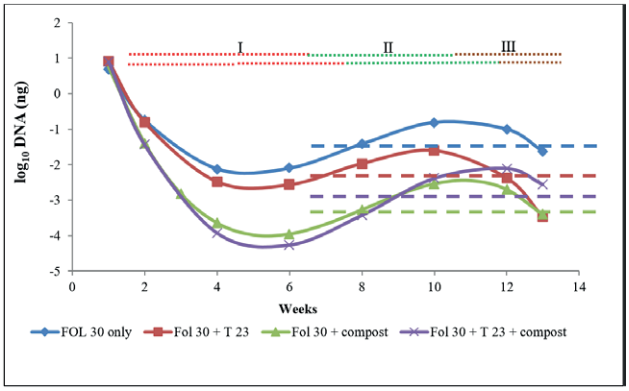

Figure 2. Polynomial regression model for FOL30 populations following different experimental treatments. Dash lines represent predicted pathogen capacity. Dotted lines represent three predicted phases: I adaptation (lag) phase, II log (growth) phase, and III stationary phase.

Polynomial regressions of FOL30 populations

Data collected from the T4, T5, T6 and T7 treatments of FOL30 populations fitted a third order polynomial regression model ($at^3 + bt^2 + ct + d$), with R^2 values of 0.87 for T4, 0.92 for T5, 0.90 for T6, and 0.95 for T7 (Table 3, Figure 2). According to the polynomial regression analysis, FOL30 populations from T4 decreased up to the 5th week from infestation, and increased from the 6th week and reached a maximum at the 10th week

Table 3. Mathematical descriptions of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) populations in soil receiving different treatments.

Treatment [a]	Polynomial regression model [b]						Population dynamic rate model [c]	
	Function (f(t))	Y *max*		Y *min*		R^2	AUPDC [d]	(*dy*/*dt*)	
		Value	Time (week)	Value	Time (week)		ng week/g soil	ng/week	Slope (m)
T4 (soil + FOL)	$= -0.015t^3 + 0.356t^2 - 2.3944t + 2.7361$	-0.81	10	-2.3	5	0.87	43.84 c	$-0.0456t^2 + 0.712t - 2.3944$	0.073
T5 (soil + FOL + *T. asperellum*)	$= -0.019t^3 + 0.4273t^2 - 2.8712t + 3.3825$	-1.59	10	-2.6	5	0.92	36.59 b	$-0.057t^2 + 0.8566t - 2.8712$	1.057
T6 (soil + FOL + compost)	$= -0.0196t^3 + 0.4767t^2 - 3.4323t + 3.7162$	-2.4	11	-3.9	5	0.90	25.41 a	$-0.0588t^2 + 0.9534t - 3.4323$	1.13
T7 (soil + FOL + *T. asperellum* + compost)	$= -0.0202t^3 + 0.5104t^2 - 3.7371t + 4.1515$	-2.08	11	-4.0	5	0.95	25.46 a	$-0.0606t^2 + 1.0208t - 3.7371$	1.172

[a] The pathogenic FOL strain was FOL30 (Khan *et al.*, 2020), and the bioagent *Trichoderma asperellum* strain was TA23 (El_Komy *et al.*, 2015).
[b] Model was constructed from 63 data points of $\log_{10}$ DNA (ng) at 1, 2, 4, 6, 8, 11 or 13 weeks
[c] Plotted time value in differential equation (*dy*/*dt*) generated linear trend line for the period from 1st to 13th weeks (see Figure 3b).
[d] Area Under Population Dynamic Curve (AUPDC) was calculated as $\int_1^{13} at^3 + bt^2 + ct$, expressed as population size ($\log_{10}$ ng DNA week^{-1} g^{-1} soil), ($P < 0.05$).

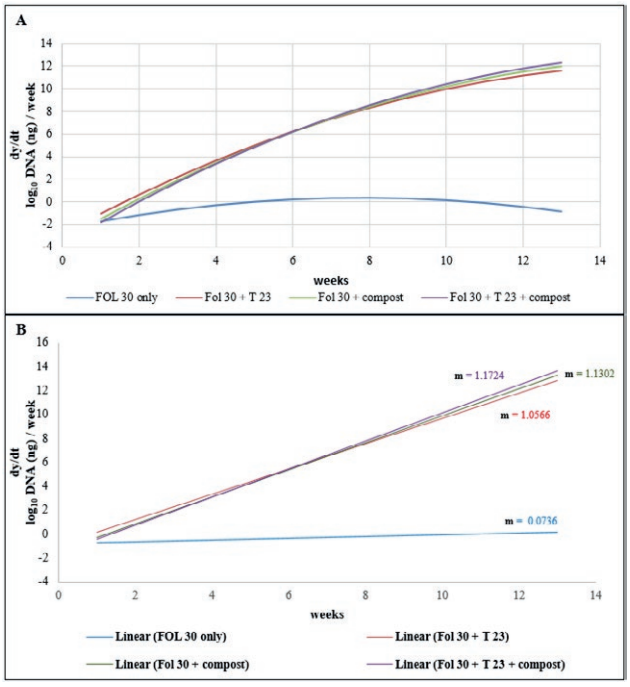

Figure 3. (A) Population dynamic rate model of FOL30 in different experimental treatments. Model was generated from differential (of polynomial models. (B) Linear trend line analysis on the model of FOL30. Straight lines indicate the continuous increasing rate. m = slope values.

(Table 3). For T5, T6 and T7, FOL30 populations also decreased up to the 5th week, then increased from the 6th week and reached maximum at the 10th week for T5 and at the 11th week for T6 and T7 (Table 3). The AUPDC values representing FOL disease potentials were: 43.84 for T4; 36.59 for T5; 25.41 for T6; and 25.46 $\log_{10}$ ng DNA week^{-1} g^{-1} soil for T7 (Table 3). Biomass variation rates from treatments T5 to T7 were obtained by differentiating the polynomials of the regression models in Table 3 with time (dy/dt). The biomass change rates were described as second order polynomials in the form $y = at^2 + bt + c$ (a, b, and c are constants and t is time in weeks), and were plotted against time (week) to obtained the curves in Figure 3 A. To precisely clarify the rates of change of FOL biomass with time, data in Figure 3 A were fitted with linear trend lines (Figure 3 B). This gave different slope values of 0.07 for T4, 1.05 for T5, 1.13 for T6, and 1.172 for T7 (Table 3, Figure 3 B). Slope values reflected continuously increasing dynamic rates of FOL30 biomass with time. Lesser slope values probably indicated stable conditions of the biosystems compared to the greater slope values.

DISCUSSION

QPCR is a powerful technique for detecting and quantifying nucleic acids in different environments (Filion *et al.*, 2003; Taberlet *et al.*, 2018). For example, Filion

et al. (2003) used QPCR to directly detect and quantify DNA of *F. solani* f. sp. *phaseoli* in different substrates, and found no significant differences between amounts of DNA extracted from spore suspensions or from soil infested with known concentrations of *F. solani* f. sp. *phaseoli* conidia. In the present study, biomass of the tomato pathogenic FOL30 strain and the biocontrol agent TA23 were estimated using QPCR. The standard curve constructed for the pathogen had an R^2 of 0.999, and that for the biocontrol agent had an R^2 of 0.997. The values confirmed the linearity of quantification between exponential increases of DNA concentrations and real-time PCR threshold cycles.

Our results confirmed that autoclaved soil contained very low amounts of DNA that could not be amplified by QPCR (C_t was low and non-repeatable). These results gave confidence as to freedom of the soil used from contamination of non-degraded DNA. Previous studies (Neate *et al.*, 2004; Taberlet *et al.*, 2018) have shown that DNA molecules do not persist in soil, especially under high temperature conditions.

Application of TA23 as a biocontrol competitor, alone or in combination with compost reduced FOL30 biomass when compared to treatment T4 (soil infested only with FOL30). These results were similar to those from previous studies, that have shown *Fusarium* biomass in rhizospheres was reduced due to application of biocontrol agents, e.g. *Trichoderma* and *Bacillus subtilis* fortified with compost (Jangir *et al.*, 2019; Cucu *et al.*, 2020). Similarly, our results demonstrate a significant reduction of FOL30 populations. There was a significant reduction in disease severity of tomato plants with the preventive applications of TA23 in combination with compost, and with compost applied alone. Sawant *et al.* (2017) showed that *Trichoderma* isolates overgrew *Erysiphe necator* and reduced powdery mildew of *Vitis vinifera* by up to 53%. However, the disease severity of tomato plants in FOL-infested soils and treated with TA23 was greater compared to the control plants in FOL-infested soils. This result indicates that the biocontrol TA23 strain increased FOL pathogenicity under certain conditions, e.g. nutrient shortage. Previous studies have shown that nutrient shortage may trigger *Trichoderma*, such as *T. saturnisporum* and *T. viridae*, to become pathogenic to seedlings of cucumber, pepper and tomato (Menzies, 1993; Marín-Guirao *et al.*, 2016).

Strains of *F. oxysporum* have different abilities to colonize soils. These abilities depend on factors related to the strains or to the substrate environment (Couteaudier and Steinberg, 1990; Fravel *et al.*, 2003). In our study, FOL populations from treatment T4 fluctuated through the period of observation. The populations decreased by up to 50% until the 4th week post infestation, then later increased. The decreases in detectable FOL30 DNA indicated that the FOL30 populations adapted to the environment. However, FOL30 populations also decreased after application of treatments T5, T6 and T7. In the presence of the TA23 strain, FOL30 populations showed negative correlations with statistically significant reductions ($r = -0.90$; $P = 0.035$). In a previous study, strain TA23 had high antagonistic activity against FOL isolates under laboratory conditions (El_ Komy *et al.*, 2015). With compost, FOL30 populations also decreased. In general, amendments of soils with compost increases suppressiveness against soil-borne pathogenic fungi (Hoitink and Changa, 2004; Vida *et al.*, 2016). The greatest reduction in FOL30 populations recorded in our study was with the combination treatment of TA23 and compost. However, there were no significant differences in reduction of FOL30 populations between the application of a combination of compost with TA23 and the application of compost alone.

Besides increasing the soil suppressiveness, compost can also be a substrate to establish, promote and maintain biocontrol agents (Leandro *et al.*, 2007; Xu *et al.*, 2011; Gava and Pinto, 2016; Vida *et al.*, 2016). The use of *Trichoderma* strains as biocontrol agents may require formulated products and suitable substrates, e.g. compost, in order to establish and survive in field soils (Leandro *et al.*, 2007). In our study, populations of *T. asperellum* biocontrol strain TA23 fluctuated during all the experiments. Consequently, the use of *Trichoderma* strains as biocontrol agents may require appropriate formulated products and suitable substrates for establishment and survival in field soils (Leandro *et al.*, 2007).

Both mathematical modelling and description have been used to investigate population dynamics of plant pathogens and their biocontrol agents (Couteaudier and Steinberg, 1990; Jeger and Xu, 2015). In the present study, a model of FOL populations under different control regimes best fitted an order 3 polynomial regression model. Polynomial models have been widely used to summarize information from data sets, since these models are simple to fit to experimental data, and statistical distribution properties of the parameters are simpler to calculate when fitted to samples of individuals, than for logistic curves (Goldstein, 1979). In the present study, all the treatments gave similar curves of polynomial regressions, which could be interpreted into three phases. These were adaptation (lag phase), growth phase (exponential phase) and stationary phase. Using polynomial regression models, optimum populations of the FOL pathogen that can be sustained by a soil ecosystem could be predicted in particular time periods.

The present study showed that the pathogen capacity of FOL in the T4 was greater than from the other treatments (disease control regimes), where FOL capacity reduced. This result indicates that disease development was influenced by the pathogen "carrying capacity" of the environment or the host plants (Aylor, 2003; Savarya *et al*., 2018). Application of compost alone or in combination with TA23 may have prolonged the FOL30 growth period, indicating that both the compost and TA23 delayed and reduced the growth of the pathogen. The prolonged lag phase may be an indicator of cellular stress (Hamill *et al*., 2020). Based on the AUPDC values, the individual applications of TA23 or compost, and their combination, reduced FOL30 population size that was expressed as $\log_{10}$ ng DNA week^{-1} g^{-1} soil. Population size may reflect the potential for a pathogen to cause disease.

Dynamic rate models were also constructed from differential equations (dy/dt) of a polynomial regression model for FOL populations. The FOL populations after different potential disease control regimes (T5, T6 or T7) gave high slope values of dynamic rates compared to the control treatment (T4). High slope values of dynamic rate models could be positive indicators of the effectiveness of disease control regimes. The high slope value of the dynamic rate model was possibly achieved because of different factors, e.g. continuous population increase with time, large gaps between minima and maxima FOL30 biomasses, rapid fluctuations in FOL30 populations in certain periods, and/or impacts of the biocontrol agent and compost.

In conclusion, the locally available compost, applied alone or combined with TA23, decreased FOL biomass, and reduced disease severity caused by *F. oxysporum* on tomato plants. Use of the local compost and indigenous *Trichoderma* could therefore be promising environmentally friendly approaches for control of Fusarium wilt in tomato under organic farming systems in Saudi Arabia. The present study also showed that mathematical descriptions provided comprehensive understanding of the population dynamics of the *F. oxysporum* pathogen of tomato.

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