

Investigation of biosuppression of wilt disease in tomato using cell-free culture filtrate of  
phytopathogenic *Fusarium oxysporum* f.sp *lycopersici* ▼

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UNDER PEER REVIEW

## Abstract

Potency of cell-free culture filtrate of pathogenic *Fusarium oxysporum* f.sp. *lycopersici* as systemic defense inducer against wilt disease in tomato was tested by treating seedlings with the filtrate before pathogen challenge. Infective conidia and the cell-free culture filtrate were prepared from *F. oxysporum* (Sensu lato) previously isolated from wilt diseased affected plants. Growth relations of the isolates were characterized under ambient temperature ( $25 \pm 2^{\circ}\text{C}$ ) and viability of the infective conidia of the two isolates were evaluated. Treatments were done as follows: (a) treatment of seedlings with infective conidia only (Treatment-A) (b) culture filtrate treatment only (Treatment-B) (c) treatment with culture filtrate followed by inoculation with infective conidia (Treatment-C) and (d) the control which consisted of plants sprayed with sterile distilled water only (Treatment-D). Effects of fungal conidia on tomato seed germination as well as some agronomic characters of the tomato plants and disease incidence under treatments A, B, C and D were evaluated. The growth and sporulation rates of the *F. oxysporum* F-isolate-1 were  $12.1 \text{ mm day}^{-1}$  and  $6.5 \times 10^4 \text{ conidia cm}^{-2}$  colony area respectively and the values for the second isolate, F-Isolate 2 were  $6.1 \text{ mm day}^{-1}$  and  $5.4 \times 10^4 \text{ conidia cm}^{-2}$  colony area. The germination rates of the infective conidia of F-isolate 1 and F-Isolate 2 after 24 hours incubation period at ambient temperature were 70% and 85% respectively. Treatment of tomato seeds with conidia suspension containing  $1.0 \times 10^6$  had no statistically significant effects on seed germination [ $F(2,12)=0.148$ ,  $P=0.64$ ]; the mean percentage germination of the seeds treated with F-isolate 1, F-isolate 2 conidia and the control were 52%, 46% and 52% respectively after five days. In the Treatment-A (Infective conidia only), F-isolate 1 and F-isolate 2 caused 100% and 80% wilt of the plant populations at 7 weeks post-inoculation, when the tomato plants were 9 weeks old and no wilt was recorded in the control. There was no wilt recorded under Treatment-B and Treatment-C also, but there was reduced flowering and the mean percentage number of leaves showing chlorosis were significantly higher than the control [ $F(4, 192) = 6.641$ ,  $P=0.001$ ]. When the plants were 10 weeks old, withered branches at the lower vegetal strata and the senescent leaves in the control were comparable with that recorded under Treatment-B and Treatment-C [ $F(4, 159) = 3.563$ ,  $P=0.08$ ].

**Key words:** Resistance, Infective conidia, Sporulation, Seed germination, Tomato plants

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## Introduction

Fusarium wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* is a serious constraint to tomato production and several physiological races that are capable of attacking specific cultivars of tomato have been described (Biju et al. [2017](#); Nirmaladevi et al. 2016). The existence of physiological races within the specialized forms of Fusaria is a serious hurdle in breeding of resistant cultivars, limiting the success of resistant hybrids of tomato in commercial production (Bawa, [2016](#)). Studies on plant-pathogen interactions have shown strong evidence that pathogenic strains of *F. oxysporum* are capable of secreting Avr-genes to evade the defense system of the host, which may limit the success of resistant hybrids of tomato (Essarioui et al.2016).

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*Fusarium oxysporum* produces three types of asexual spores; microconidia, macroconidia and chlamydospores (Ohara et al. [2004](#)) that are capable of surviving saprophytically in soil for several years and are responsible for rapid dispersal in soil (Wong 2003). The bioecology of the fungus, especially its ability to subsist in soil for long periods by switching from pathogenic mode into saprobic growth in the absence of a susceptible host renders cultural control and crop rotation ineffective. Direct elimination of the pathogen with fungicides also often fail when the fungus has established in the host. Thus, adequate management is not currently available regardless of chemical, biological and cultural approaches once tomato plants are infected.

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For disease to occur, the infective conidia of the pathogen pass through series of transitions which involve germination, penetration of host tissue while avoiding plant-pathogen interactions that could trigger the host's defensive responses, followed by progressive establishment of systemic infection and eventual wilt (Srinivas et al. 2019). The defense mechanism in tomato plants thus requires the perception of the pathogen through receptors of pathogen-associated molecular patterns domiciled in the plasma membrane, which deploys basal defense systems (Essarioui [et al., 2016](#)). However, upon

successful recognition of the pathogen, resistant tomato plants show increased polyphenol oxidase activities, overexpression of chitinase and deposition of calcium at the entry point of the pathogen, which confer systemic resistance (Rai et al. 2011). Similar responses have been reported in susceptible

tomato seedlings treated with biotic inducers, various organic and inorganic chemicals. In some studies, the concentrations of the bioactive metabolites detected in the tissues positively correlated with levels of resistance (Mandal et al., 2009). When tomato plants are treated with systemic defense inducer, before pathogen attack, higher resistance and lower disease rates were observed (Borisade et al., 2017). Resistance of plants to disease after treatment with systemic defense inducers is often described in relation to concentrations of resistance-conferring metabolites detected in the tissues.

In the current study, there is interest to evaluate agronomic performance and aspects of pathology of tomato plants pre-treated with cell-free fusarium culture filtrate (as systemic defense inducer) before infection, as it may affect real-time expression of disease symptoms, growth, flowering, fruiting and the quality of fruits. The metabolites secreted by plants in response to infection often confer broad resistance but they are likely to be more specific to the pathogen that triggers the response. This study was conducted to investigate biosuppression of wilt in tomato using crude culture filtrate of phytopathogenic isolates of *F. oxysporum* f.sp. *lycopersici* applied to rhizosphere of seedlings in nursery to elicit innate defense response before pathogen challenge in the field.

## MATERIALS AND METHODS

### Source of *Fusarium* isolates

Two pathogenic strains of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) *sensu lato*, were isolated from susceptible local landrace of tomato root using PDA (Sigma-Aldrich, UK) amended with 0.05% Chloramphenicol as the selective medium, henceforth referred to as CPDA. Field-cultivated tomato plants showing advanced symptoms of Fusarium wilt disease; chlorosis of lower leaves and loss of turgor

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(wilt), were uprooted and roots were severed from the plants. The roots were rinsed in stream of tap water to remove soil particles and cut into approximately 10mm portions. This was followed by washing with 0.05% Hypochlorite solution and left for ten minutes before rinsing with sterile distilled water containing 0.02% Tween-80 and placed on sterile filter paper inside Laminar Flow cabinet to dry. The disinfected root-cuts were sprayed with 80% ethanol, transferred unto the prepared CPDA and incubated for 3-5 days at 25±2 °C. The infecting pathogen, *Fusarium*, grew out of infected roots into the CPDA. The outgrowths were subcultured into freshly prepared PDA and purified using the single spore isolation technique as described by Zhang et al. (2013). Details of the microscopical characteristics used for identification and evaluation of pathogenicity of the two isolates to tomato plants were described elsewhere (Borisade *et al.*, 2017; Borisade and Uwaide, 2018). In this study, the isolates were designated as *Fusarium* Isolate-1 and *Fusarium* Isolate-2.

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#### Evaluation of growth and sporulation rates

Characterization of growth and sporulation rates was done on Potato Dextrose Agar (PDA) media in 9 cm disposable Petri-dishes inoculated at the centre with 5 µl of conidia suspension containing  $1.0 \times 10^4$  conidia ml<sup>-1</sup> using micropipette (Eppendorff 1-20 µl). Triplicate plates (Replicates 1-3) designated as R1, R2 and R3 were inoculated, sealed with Parafilm to prevent moisture loss from the agar surface and incubated at ambient temperature in the dark. Radial extension was measured along pre-marked orthogonal axes after initial 48 hours incubation period and this continued daily for 14 days or until the surface of the plate was fully covered. Radial extension (mm) against the incubation period (days) was fitted into a linear model to estimate growth rates (Borisade and Magan, 2014). The triplicate cultures used for estimation of growth were incubated for additional one week. Thereafter, 1cm<sup>2</sup> agar plugs were cut from 5 random positions on each Petridish and transferred into Universal bottle. Ten milliliter sterile de-ionized water containing 0.02% Tween 80 was poured into the bottle and vortexed to dislodge the conidia. The conidia suspension was decanted, serial dilutions were made and counting was done

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using Improved Neubauer Haemocytometer under X40 magnification of Microscope (OLYMPUS BHTUBH-2). The mean number of conidia per cm<sup>2</sup> colony area was determined by simple arithmetic average.

#### Evaluation of conidia viability

Harvested conidia from 14 days old culture were suspended in sterile distilled water containing 0.02% Tween 80, vortexed and standardized to  $1.0 \times 10^4$  conidia ml<sup>-1</sup>. Ten microliter of the conidia suspension was spreadplated on standard PDA media in 9 cm Petridish and replicated three times. The Petridishes were kept in Laminar flow Cabinet for 30 minutes to dry, and sterile coverslips (2 cm<sup>2</sup>) were placed randomly at three positions on the spread plate. The conidia in each coverslip area was counted, thus representing three different fields for counting per Petridish. The set-up was incubated at 25°C and the number of germinated conidia among 50 randomly counted under microscope in each coverslip field after 24 hours was estimated. The counting was repeated after 48 and 72 hours and conidia viability was expressed as shown in Equation 1:

$$\text{Viability of conidia (\%)} = \frac{\text{Number of germinated conidia}}{\text{Total number of counted conidia per coverslip field}} \times \frac{100}{1} \text{ Equation 1.}$$

#### Effect of infective conidia of *F. oxysporum* on viability of tomato seeds

Twenty seeds of tomato were introduced into 10 ml conidial suspension of the *F. oxysporum* isolates containing 0.02% Tween 80, vortexed and used to set up germination experiment as summarized: the seeds were separated from the suspension and placed on sterile cotton wool, moistened with sterile distilled water inside 9 cm Petridishes. The Petridish was covered with the lid and kept under 12 hours alternating light and darkness at  $25 \pm 2^\circ\text{C}$  for 5 days. The germination experiment was replicated three times, each Petridish contained 20 seeds. The control experiment contained disinfected tomato seeds

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which were prepared by spraying with 0.05% hypochlorite solution and rinsed with sterile distilled water after 10 minutes. The number of germinated seeds in each dish was counted after 5 days and the recorded values were averaged and expressed as a percentage. Isolation of *Fusarium oxysporum* (Fol) from 5-days old sprouts was done by placing 1 cm portions of the sprout aseptically on CPDA and incubating for 3-5 days. Ten sprouted tomatoes were randomly selected from each replicate and evaluated for infection.

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#### Culture of *Fol* and preparation of cell-free culture filtrate

Standard Potato Dextrose Agar (PDA) media (Sigma-Aldrich,  $a_w=0.995$ ) inside 9 cm disposable Petridish was lined with sterile cellophane film. Conidia suspension of 14 days old PDA culture of the *Fol* isolates were prepared with sterile distilled water containing 0.02% Tween 80 and standardized to  $1.0 \times 10^4$  conidia  $\text{ml}^{-1}$ . The cellophane-lined PDA plates were inoculated at the center with 10  $\mu\text{l}$  of the prepared fungal conidia and spread using bent glass rod. Ten replicate plates were produced for each *Fol* isolate. The set-up was incubated for 14 days at 25°C in the dark. After the 14 days incubation period, fungal mycelia were separated from the agar by removing the colonies growing on the cellophane film overlay. The intoxicated agar, containing secondary metabolites secreted by the cultured *Fol*, was sliced using scalpel and 40 g sub-samples of the agar was weighed into 100 ml conical flasks. The crude metabolite was extracted from the agar with 30 ml Dichloromethane: Ethyl acetate (1:1, v/v). The flasks containing the agar and the solvent were kept on rotary shaker and agitated at 50 rpm for 48 hours at 25°C. Thereafter, the solvent was decanted into standard glass bottles and evaporated in a fume cupboard to dry. The standard glass bottle was rinsed with 5 ml absolute methanol, poured into glass vials as stock crude filtrate. The stock filtrate was mixed with sterile distilled water in ratio 1:3 (filtrate: water), passed through microfilter and stored at 4°C for 3-4 days until required for use.

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#### Treatment of tomato seedlings with culture filtrate and inoculation with infective conidia

The soil used for cultivation of tomatoes in the nursery and growing pots was moistened, filled into autoclave bags, autoclaved at 121°C, 15 Psi for 30 minutes and allowed to cool overnight before distributing into nursery trays and growing pots. Seeds of fusarium wilt disease-susceptible landrace of tomato, tiwantiwa, were sown in nursery trays and two weeks old seedlings were transplanted into growing pots in green house at the rate of two seedlings per pot. The prepared fusarium culture filtrate was applied to the root area of the seedlings in the growing pots at the rate of 4 ml filtrate per pot. After 5 days, 1 ml conidia suspension of either isolate of *Fol* (F-Isolate 1 or F-Isolate 2) containing  $10^4$  conidia  $\text{ml}^{-1}$  was applied to the tomato root. Each treatment was replicated 20 times and the treatment combinations are shown in Table 1.

Table 1. Treatment combinations

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Treatment combinations						
F-Isolate 1 Culture Filtrate + conidia	F-Isolate 2 Culture Filtrate + Conidia	F-Isolate 1 Culture Filtrate	F-Isolate 2 Culture Filtrate	Sterile Water + F-Isolate 1 conidia	Sterile Water+ F-Isolate 2 conidia	Sterile Water (Control)

### Assessment of infection in tomato seedlings

Petioles of leaves were randomly clipped off from the first three lower vegetal strata of all the treated plants and the control (1-10 cm up the stem) at 4 weeks post-inoculation and washed under running tap water to remove sand particles. Plants were also uprooted and approximately 1 cm root and petiole portions were excised from all the plants. These were rinsed in 0.05% hypochlorite solution for 1 minute to surface-sterilize, rinsed in sterile distilled water and dried on sterile filter paper. The root and petiole portions were transferred into half-strength Potato Dextrose Agar modified with 0.05% chloramphenicol (CPDA) in 9 cm Petridish. Chloramphenicol was added to the media to suppress bacteria growth. The Petridish was sealed with Parafilm and incubated for 3-5 days at ambient temperature ( $25 \pm 2^{\circ}\text{C}$ ) in the dark. Growth or no growth data was recorded and the image of representative sample plate was photographed.

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### Assessment of agronomic and pathological characters of tomato plants

Agronomic and pathological characters of the treated and the control tomato plants were assessed at 5 weeks after treatment (First sampling, Plant age=7 weeks old), coinciding with flowering period and onset of fruiting and repeated at the 6th week (Second sampling, Plant age=8 weeks old). Number of flowers, leaves showing symptoms of Fusarium wilt disease (chlorosis of the lower leaves), withered branches and wilted plants were counted for 20 replicate plants. Assessment of fruiting and the pathological characters was done when the tomato plants were 10 weeks old and repeated one week later (Plant age=11 weeks old), when the effect of fusarium attack is often most severe on infected tomato plants. The number of completely wilted plants was recorded weekly.

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### Statistical Analysis

The agronomic performance and the pathological characters data of the treated and the control plants were subjected to Analysis of Variance (ANOVA) Test. One way between-groups analysis of variance was conducted to explore the effect of Fusarium inoculum on germination rates (%) of the tomato seeds.

Where the ANOVA results showed significant difference, a post-hoc test was conducted to separate the means using Tukey's Honestly Significant Difference (HSD) statistical procedure,  $P > 0.05$ .

#### **Growth and sporulation rates of *F. oxysporum* isolates and viability of infective conidia**

A one-way between-groups analysis of variance was conducted to compare the growth rates of the two *F. oxysporum* isolates, F-Isolate 1 and F-Isolate 2. There was a statistically significant difference in the mean growth rates of the two isolates [ $F(1,4) = 320.43$ ,  $P = 0.0001$ ], F-Isolate 1 being the isolate with the faster growth ( $12.1 \text{ mm day}^{-1}$ ) and the mean growth rate of F-Isolate 2 was  $6.1 \text{ mm day}^{-1}$ . The rate of growth of F-Isolate 1 was approximately twice the rate of growth of F-Isolate 2 (Figure 1). The fusarium isolate, F-Isolate 1 also produced greater number of infective conidia ( $6.5 \times 10^4$  conidia per  $\text{cm}^2$  colony area) compared with F-Isolate 2 ( $5.4 \times 10^4$  conidia per  $\text{cm}^2$  colony area) (Figure 2). The mean germination rates of the infective conidia of F-Isolate 1 after 24, 48 and 72 hours incubation period were 70%, 93% and 100% respectively while the second isolate, F-Isolate 2 had 85%, 98.3% and 100% germinated conidia (Figure 3).

#### **Germination rates of tomato seeds inoculated with *F. oxysporum* conidia**

Germination rates of tomato seeds separately inoculated with the infective conidia of F-Isolate 1 and F-Isolate 2 and the control (containing no inoculum) after 5 days incubation at ambient temperature in alternating light and darkness (12: 12 hrs.) is shown in Table 2. There was no statistically significant difference ( $P < 0.05$ ) in the rates of germination of the seeds treated with *Fusarium* conidia suspension and the control  $F(2, 12) = 0.148$ ,  $P = 0.864$ . The effect of size, calculated using eta squared was 0.02. The mean percentage germination of the inoculated seeds and the control are: F-Isolate 1 ( $M = 52\%$ ,  $SD = 21.68$ ), F-Isolate 2-treated seeds ( $M = 46\%$ ,  $SD = 11.40$ ) and the control ( $M = 50\%$ ,  $SD = 18.9$ ). The sprouts of the tomato seeds yielded no growth on CPDA after 5 days incubation period.

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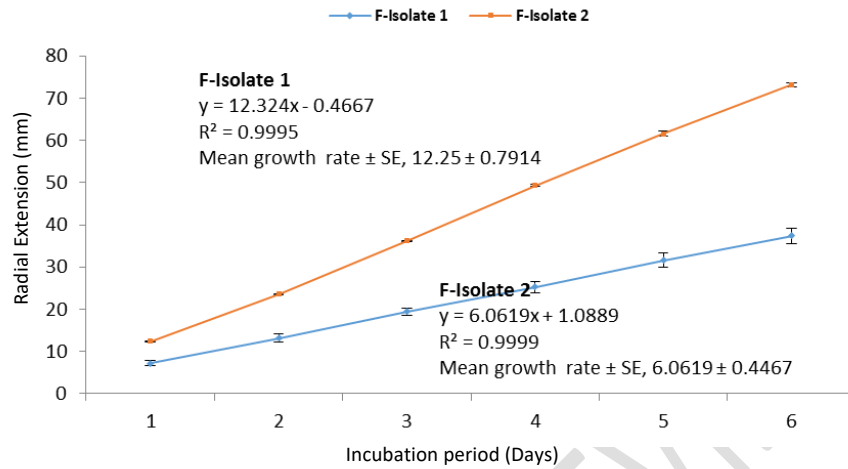


Figure 1. Growth rates of two *F. oxysporum* f.sp. *lycopersici* isolates

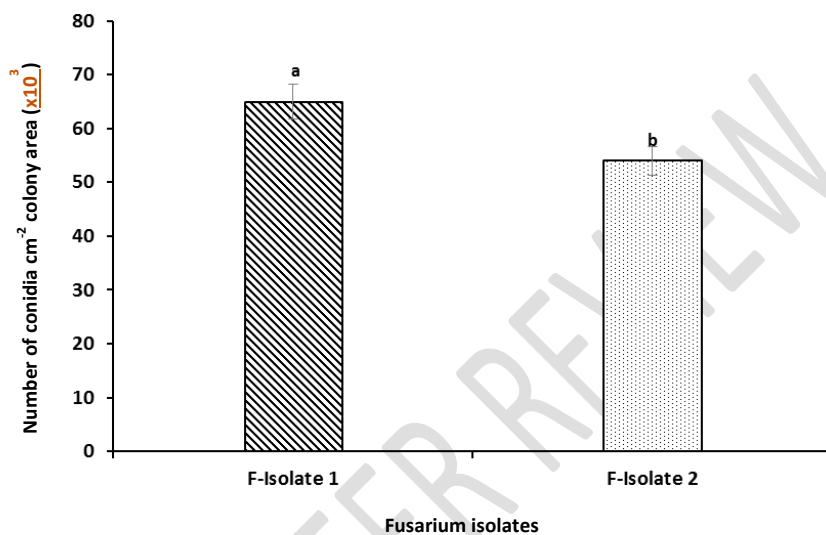


Figure 2. Sporulation rates of two *F. oxysporum* isolates on PDA after incubation for 14 days at ambient temperature (25±2°C)

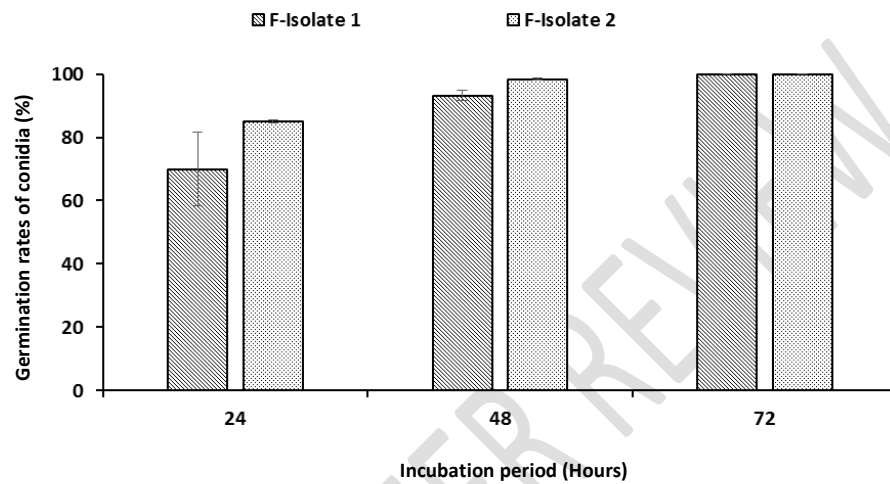


Figure 3. Germination rates of the infective conidia of two *F. oxysporum* isolates, F-Isolate 1 and F-Isolate 2 after 24, 48 and 72 hours incubation.

**Table 2. Germination rates of tomato seeds separately inoculated with infective conidia of two *Fusarium oxysporum* f.sp. *lycopersici* isolates, F-Isolate 1 and F-Isolate 2**

	Treatments		
	F-Isolate1 conidia	F-Isolate 2 Conidia	Sterile distilled water
Tomato seed germination rates (%) (Mean $\pm$ SE of Mean)	52 $\pm$ 10a	46 $\pm$ 5a	52 $\pm$ 11a

### Assessment of infection, agronomic and pathological characters of tomato plants

The incubated portions of the root and the vegetative parts yielded *Fusarium* growth (Sample image: Figure 4), indicating that infection occurred in all the inoculated plants regardless of filtrate pretreatment before inoculation with the infective conidia of the two isolates. Figure 5. shows the survival curve of the tomato plants inoculated with the infective conidia only, without prior treatment with the culture filtrate of the invading pathogens, F-isolate 1 and F-isolate 2. Wilt was first recorded at 4 weeks after infection (Plant age=6 weeks old): sixty percent of the plants (Total number of plants, N=20) inoculated with F-isolate 1 wilted and F-isolate 2 caused 40% (N=20) wilt over a period of 4 weeks. At 7 weeks (Plant age=9 weeks), there was 100% wilt of plants inoculated with F-isolate 1, while 80% percent wilt occurred in the tomato plants inoculated with F-isolate 2 conidia and the left-over plants all showed chlorosis.

The assessment of agronomic performance conducted on the tomato plants at age 7-8 weeks showed that the treatments: culture filtrate only or culture filtrate + *Fusarium* inoculum significantly reduced flowering (Table 3). The number of leaves showing symptoms of *fusarium* attack (necrosis/yellowing) among the plants treated with F-isolate 1 culture filtrate + F-isolate 1 inoculum and F-isolate 2 culture filtrate only were comparable at the first sampling period (Plant age = 7 weeks old), significantly higher than the values recorded in other treated plants and greater than number of senescent leaves in the control [ $F(4, 192) = 6.641, P = 0.0001$ ]. The numbers of withered branches at the lower vegetal strata in all the treated plants were comparable to the number of senescent leaf branches in the control.

Assessment of rates of fruiting at crop age 10-11 weeks old in the treated plants and the control showed no statistically significant difference (Table 4). The pathological characters; leaves showing necrosis or yellowing in relation to *fusarium* disease development and dead branches at the lower vegetal strata were comparable to the control, where yellowing and loss of branches were associated with senescence [ $F(4, 159) = 3.563, P = 0.08$ ].



Stickers 1, 2, 3 and 4 on the plate are portions of the stem excised at 2, 4, 6 and 8 cm along the

**Figure 4. Phytopathogenic isolate of *Fusarium oxysporum* f.sp. *lycopersici* growing out of root (center) and stem portions of tomato plants placed of CPDA medium.**

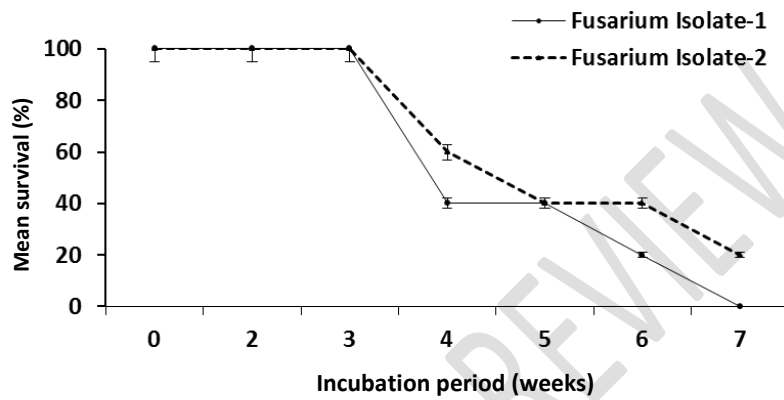


Figure 5. Mean survival (%) of tomato plants inoculated with infective conidia of two different *F. oxysporum* f. sp. *lycopersici* isolates, F-Isolate 1 and F-Isolate 2. The number of plants tested with each *Fusarium* isolate, N=60.

**Table 3. Effects of *Fusarium* culture filtrate and inoculum on agronomic and pathological characters at early stage of flowering in tomato**

Agronomic & Pathological Parameters	Treatments					
	Sampling Periods	F-Isolate 1 Culture Filtrate + F-Isolate 1 Inoculum	F-Isolate 2 Culture Filtrate + F-Isolate 2 Inoculum	F-Isolate 1 Culture Filtrate	F-Isolate 2 Culture Filtrate	Sterile Distilled Water (Control)
Number of flowers	1st	5a	15b,c	15 b,c	5a,b	30c
	2nd	12a	20a,b	22a,b	21a,b	40b
Leaves Showing Symptoms	1st	4a	14a,b	22b	8a	4a
	2nd	10a	10a	10a	9a	8a
Withered Branches	1st	0a	0a	0a	0a	0a
	2nd	3a	3a	5a	5a	3a

**Table 4. Effects of *Fusarium* culture filtrate and inoculum on agronomic and pathological characters at onset of fruiting in tomato**

Agronomic & Pathological Parameters	Sampling period	Treatments				
		F-Isolate 1 Culture Filtrate + Inoculum	F-Isolate 2 Culture Filtrate + Inoculum	F-Isolate 1 Culture Filtrate	F-Isolate 2 Culture Filtrate	Sterile Distilled Water (Control)
Number of fruits	1st	16a	16a	24a	18a	16a
	2nd	10a	15a	15a	11a	12a
Leaves Showing Symptoms	1st	9a,b	14a	16a	15a	0b
	2nd	11a	12a	10a	8a	1a
Withered Branches	1st	1a	1a	1a,b	0a,b	0ab
	2nd	1a	1a	1a	1a	2a

## DISCUSSION

The *Fusarium oxysporum* f.sp. *lycopersici* (Sensu lato) strains used in this study were originally isolated from roots of tomato plants killed by wilt disease. The results demonstrated that the two isolates are pathogenic to the tomato plants, considering foliar symptoms expression and wilt in the control specimens. The infective conidia of the two isolates germinated after 24 hours incubation, grew and sporulated differentially on PDA media at ambient temperature, indicating they are different strains or distinct races. F-Isolate 1 produced higher number of spores and the rate of growth was faster compared with F-Isolate 2. Growth relations, infectivity and pathogenicity are important characteristics that are related to ecophysiology of phytopathogenic *Fusarium* species and they are useful in differentiation (Vakalounakis and Fragkiadakis 1999). In several studies, it has been shown that pathogenicity and host range of physiologically distinct races of *Fol* are divergent and host susceptibility can be employed in racial classification of *F. oxysporum* (Amini 2009; Henrique et al. [2017](#))

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The germination rates of the tomato seedlings was not affected by the *Fol* inoculum and the excised subsamples of the sprout yielded no growth. The infective conidia of the two *Fol* isolates germinated (70-100% germination) at 24 - 72 hours incubation period as indicated in Figure 3 but no infection of the sprouted seedlings occurred after 5 days incubation. This suggests that further plant-pathogen interactions may be required for infection to occur in the presence of active inoculum. While spore germination may be spontaneous and dependent on favourable interacting abiotic factors, the requirements for infection are likely to be more dependent on host-pathogen interactions that lead to host recognition. Tomato root exudates secreted into the rhizosphere and recognized by *Fol* are likely to switch the pathogen from saprobic into pathogenic mode and production of such metabolites are known to be related to plant's age (Steinkellner et al. 2005; Steinkellner et al. 2008).

All the inoculated plants yielded *Fusarium* growth when they were examined for infection at 2-4 weeks after inoculation. The infection was monitored in the root and up to 10 cm along the stem portions (Figure 5). However, severe disease conditions and wilt occurred in the inoculated plants at about flowering and fruiting stages, despite that infection took place earlier. It can be suggested that in as much as the host fails to produce the chemical cues that are recognized by *Fol*, infection would not occur and other hormonal changes during flowering trigger the processes that lead to wilt eventually in addition to breakdown of the plant's vascular system. It is also likely that differentiations exist in metabolites that trigger initial infection and those that are responsible for pathogenicity. Fungal metabolites that are related to infection and development of wilt disease are yet to be isolated from tomato tissues but the metabolites deployed by the host in response to infection are well known (Abbattista et al. 1988; Stout et al. 2008). Future genetic manipulation of tomato plants to produce non-host recognizable metabolites could become a sustainable management method, which could complement varietal resistance techniques.

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The culture filtrate of F-Isolate 1 and F-Isolate 2 significantly reduced flowering of the tomato plants. Variabilities in the rates of flowering were further modulated by infection and flowering was significantly lower in the treated plant populations compared with the control. Pathogenic forms of *Fol* are known produce proteinaceous toxins such as fusaric acid and lycomarasmin which are recognized as wilt protein (Mustafa et al. 2019; Srinivas et al. 2019). The filtrate used for priming the seedlings may be responsible for the repressive effects on flowering. Protoplasts of tomato lines that are susceptible to specific *Fol* isolates are more sensitive to the toxins present in the culture filtrate of the same isolates than non-hosts. Nevertheless, the proteinaceous toxins induce responses which include ethylene biosynthesis and cell death, as commonly observed in plants after treatment with elicitors. Probably, the concentration of the filtrate tested in this study was below the critical level capable of causing wilt but sufficient to prime the plant ready for an active defense, more so it was introduced to the root area.

It has been reported that a trade-off exist between agronomic performances and development of resistance when plants are induced by exogenously applied elicitors (Vallad and Goodman 2004). The tomato plants used in this study were grown in pots without soil enrichment treatments, such as fertilizer application. In addition to trade-offs, aspects of nutrition might have contributed to the poor agronomic performances. It would be interesting if aspects of soil fertility and nutrition are assessed in future studies.

The absence of wilt in the treated plants indicated that systemic resistance was invoked by the *Fol* culture filtrate and eventually resulted into active resistance to the pathogen, which lasted the entire growth cycle of the tomato plant. In several other studies where organic or inorganic substances were employed to induce systemic resistance in tomatoes and other crops, resistance was measured by the amount of resistance-related metabolites produced by the treated plants (Abbattista et al 1988; Stout et al., 2008). Resistance-related metabolites have been shown to vary in treated plants depending on the type of applied elicitor, its concentration and the plant cultivars (Vanitha et al 2009; Raja et al. 2016).

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However, in those studies, the duration of elicited systemic defenses were not measured over a period of time in order to generate data on the activity span of the induced resistance, peak periods of resistance and the decline, when the plant would be potentially prone to wilt. The tomato variety used in this study often succumb to wilt disease towards flowering and fruiting, making timing of elicitation of systemic and active defenses crucial in *Fusarium* wilt management. The plants would require a space of time to deploy its defenses between the application of defense inducers and pathogen challenge. Systemic resistance in tomatoes evoked by the use of organic or inorganic inducers as well as apathogenic species of *Fol* have been shown to prevent wilt disease differentially (Gupta and Acharya, 2018).

In this study, agronomic and pathological characters of the tomato plants were observed over the entire growth cycle after treatment with the filtrate (organic elicitor) and the post infection responses were

also recorded. The Filtrate-treated and Filtrate + inoculum treated plants showed some signs of necrosis in the lower vegetal strata. This suggests that development of necrosis may be more related to immunological responses of the plants to fungal toxins although biotoxicity of the filtrate to plant cells may also be a factor. All the treated plants yielded *Fusarium* growth when they were examined, indicating that the treatment did not stop infection but probably initiated systemic defenses that enhanced active defenses against the pathogen which lasted the entire growth cycle of the plant.

## Conclusion

The results showed that the treatment of the seedlings with cell-free culture filtrate conferred some resistance, such that eventual inoculation of the seedlings with the infective conidia caused no wilt. More importantly, the results indicated that treatments with the filtrate may not deter infection and there was a trade-off of agronomic performance for resistance. It may be interesting to introduce aspects of nutrition into future studies in order to evaluate effects of soil nutrients availability on pathological characters and agronomic performance of filtrate-treated tomato plants.

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## References

- Abbattista Gentile I, Ferraris L, Matta A (1988). Variations of phenoloxidase activities as a consequence of stresses that induce resistance to *Fusarium* wilt of tomato. *Journal of Phytopathology* 122(1):45-53.
- Amini J (2009) Physiological race of *Fusarium oxysporum* f. sp. *lycopersici* in Kurdistan province of Iran and reaction of some tomato cultivars to race 1 of pathogen. *Plant Pathology Journal*. 8(2):68-73.
- Bawa I (2016) Management strategies of *Fusarium* wilt disease of tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.): A review. *Management*. 2016 May;2(5).
- Biju VC, Fokkens L, Houterman PM, Rep M, Cornelissen BJ. (2017) Multiple evolutionary trajectories have led to the emergence of races in *Fusarium oxysporum* f. sp. *lycopersici*. *Appl. Environ. Microbiol.* 83 (4):e02548-16.
- Borisade OA, Uwaidem YI (2018). Priming F1-resistant Tomato Hybrid (Lindo-F1) Seedlings with Copper-I-Oxide Metalaxyl Composite Fungicide before Infection Enhanced Resistance to *Fusarium* Wilt. *International Journal of Pathogen Research*. 14:1-3.
- Borisade OA, Uwaidem YI, Salami AE (2017). Preliminary report on *Fusarium oxysporum* f. sp. *lycopersici* (Sensu lato) from some tomato producing agroecological areas in Southwestern Nigeria and Susceptibility of F1-Resistant Tomato Hybrid (F1-Lindo) to Infection. *Ann. Res. Rev. Biol.* 18(2):1-9.
- [Borisade, OA., & Magan, N \(2014\). Growth and sporulation of entomopathogenic \*Beauveria bassiana\*, \*Metarhizium anisopliae\*, \*Isaria farinosa\* and \*Isaria fumosorosea\* strains in relation to water activity and temperature interactions. \*Biocontrol science and technology\*, 24\(9\), 999-1011.](#)
- Essarioui A, Mokrini F, Afechtal M (2016) Molecular interactions between tomato and its wilt pathogen *Fusarium oxysporum* f. sp. *Lycopersici*-a review. *Revue Marocaine des Sciences Agronomiques et Vétérinaires*. 4(1):66-74.
- Gupta NS, Acharya K (2018). Diluted Fungal Phytotoxin: An Immerging tool for Boosting Innate Immunity in Plants. *Research Journal of Pharmacy and Technology*. 11(10):4711-8.
- Henrique FH, Carbonell SA, Ito MF, Gonçalves JG, Sasserón GR, Chiorato AF (2016). Classification of physiological races of *Fusarium oxysporum* f. sp. *phaseoli* in common bean. *Bragantia*. 74(1):84-92.
- Mandal S, Mallick N, Mitra A (2009) Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *Plant Physiology and Biochemistry*. 47(7):642-9.
- Mustafa G, Masood S, Ahmed N, Saboor A, Ahmad S, Hussain S, Bilal M, Ali MA (2019). Seed Priming for Disease Resistance in Plants. In: *Priming and Pretreatment of Seeds and Seedlings* 333-362. Springer, Singapore.

Nirmaladevi D, Venkataramana M, Srivastava RK, Uppalapati SR, Gupta VK, Yli-Mattila T, Tsui KC, Srinivas C, Niranjana SR, Chandra NS. (2016) Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. *Scientific reports*. 6:21367.

Ohara T, Tsuge T. Fostua (2004) Encoding a basic helix-loop-helix protein, differentially regulates development of three kinds of asexual spores, macroconidia, microconidia, and chlamydospores, in the fungal plant pathogen *Fusarium oxysporum*. *Eukaryotic Cell*. 3(6):1412-22.

Rai GK, Kumar R, Singh J, Rai PK, Rai SK (2011). Peroxidase, polyphenol oxidase activity, protein profile and Phenolic content in tomato cultivars tolerant and susceptible to *Fusarium oxysporum* f. sp. *lycopersici*. *Pak. J. Bot.* 43(6):2987-90.

Raja M, Pandian RT, Sharma MA, Jambhulkar PP, Sharma PR (2016). Study of induced systemic resistance in tomato against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. *Indian Phytopathology* 69:542.

Srinivas C, Devi DN, Murthy KN, Mohan CD, Lakshmeesha TR, Singh B, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B (2019). *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity—A review. *Saudi Journal of Biological Sciences*.

**Deleted:** Srinivas C, Devi DN, Murthy KN, Mohan CD, Lakshmeesha TR, Singh B, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B (2019) *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity—A review. *Saudi Journal of Biological Sciences*. 2019 Jun 4.¶

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Steinkellner S, Mammerler R and Vierheilg H (2008). Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. *European journal of plant pathology* 122(3).395-401.

Steinkellner S, Mammerler R, Vierheilg H (2005). Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. *Journal of plant interactions* 1(1):23-30.

Stout MJ, Kurabchew H, Leite GL (2008). Host-Plant Resistance in Tomato. In: Sustainable Management of Arthropod Pests of Tomato. 217-236). Academic Press.

Vakalounakis DJ, Fragkiadakis GA (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. *Phytopathology*. 89(2):161-8.

Vallad, GE., & Goodman, RM. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop science*, 44(6), 1920-1934.

Vanitha SC, Niranjana SR, Umesha S (2009). Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. *Journal of Phytopathology*. 157(9):552-7.

Wong M (2003) *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) WC Snyder and HN Hans. PP728 Soilborne Plant Pathogen Class Project [cit. 201009-10].

Zhang, K, Yuan-Ying, S, Cai, L. (2013). An optimized protocol of single spore isolation for fungi. *Cryptogamie, Mycologie*, 34(4), 349-356.