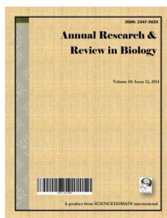

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Preliminary Report on *Fusarium oxysporum* f. sp. *lycopersici* (*Sensu lato*) From Some Tomato Producing Agroecological Areas in Southwestern Nigeria and Susceptibility of F1-Resistant Tomato Hybrid (F1-Lindo) to Infection

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author YIU managed the analyses of the study and literature searches. Author AES collected the infected tomato plants for the experiments. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate *Fusarium oxysporum* f.sp. *lycopersici* from infected tomato plants in selected tomato producing agroecological areas in Southwestern Nigeria and evaluate susceptibility of F1-resistant tomato hybrid (F1-Lindo) to infection, to understand the propensity of adopting the hybrid.
Methodology: Seven *F. oxysporum* f.sp. *lycopersici* were isolated from infected tomato plants collected from some tomato producing agroecological areas in the Southwestern Nigeria. The isolates were identified using their morphological features and characterized based on growth,

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sporulation rates, lag-time and virulence. Growth rate was estimated using a linear model for estimation of fungal growth. Conidia of five of the isolates were standardized to 1.0 x 10^6 conidia/mland tested for virulence against Race-1-resistant tomato hybrid, F1-Lindo. Pathogenicity of the fungal isolates were ranked according to severity of damage to the tomato hybrid.

Results: The growth, sporulation rates and the lag time of the isolates showed statistically significant difference, F (16, 4) =249.16, P=0.001. The isolate, ADO-1 had the slowest rate of growth (0.24 mm day^{-1}), while ADO-2 had the fastest growth (1.6 mm day^{-1}) among the seven isolates. The lag time of the isolates showed no statistically significant difference, F (6, 281.06) =0.98, P=0.479 and they were between 4.6 hours to 10.5 hours. Five of the isolates were tested for pathogenicity to tomato and the mean percentage damage recorded (53-61%) were not statistically different F (4, 40384.9) =1.993, P=0.98.

Conclusion: The tomato hybrid, F1-Lindo was considered susceptible to the isolates and it was suggested that the isolates of *F. oxysporum* f. sp. *lycopersici* evaluated for virulence were probably other races to which F1-Lindo was susceptible.

Keywords: Fusarium oxysporum f. sp lycopersici; growth rates; tomato hybrid; lag time; resistance.

1. INTRODUCTION

Tomato (*Lycopersicum esculentum*) is an important fruit vegetable and the most widely cultivated member of the family Solanaceae in Nigeria. Commercial production in the Northern agro-ecological zones under irrigation systems have been relatively successful compared with the Southern savanna and the Rainforest zones. Pests [1] and diseases [2,3] are serious constraints to tomato cultivation in Nigeria, but restrictions to commercial production in the Southwest are more associated with wilt diseases caused by fungal pathogens [4]. Among the wilt diseases, the vascular wilt caused by soil borne *F. oxysporum* f.sp. *Lycopersici* is the most prevalent, particularly where landraces of tomato that are highly susceptible are cultivated.

Generally, phytopathogenic disease incidents are often much severe in the wet season and where infective fungal conidia-nematode complex exist in the soil [5]. The fungus gain entry into plant roots with its sporangial germ tube through root tips, exposed tissues caused by wounds or at points of development of lateral roots. This is followed by invasion of xylem tissues by the mycelia. Subsequently, micro-conidia are produced and transported upwards through the sap stream. The spread of the fungal growth within the xylem tissues impair water supply to upper parts of tomato plants, causing wilt and death of highly susceptible hosts. Characteristic symptoms of *Fusarium* attack include leaf chlorosis and vascular wilt that progress into partial or total wilting of tomato plants and darkening or browning of the vascular system [6].

Fusarium oxysporum is capable of persisting in soil for many years without a host, growing as a saprophyte on plant debris. Thus, the traditional control practices [7] that are used in the Nigerian cropping systems, such as removal of infected crops and crop rotation are ineffective for the management. Chemical control of the pathogen is relatively effective but problems of environmental toxicity and the challenge of keeping pesticides residues within acceptable levels in fresh fruits are of serious concern [8,9]. Biological control by the use apathogenic-pathogenic fungal interactions [10,11,12,13] has shown promising results in *in vitro* studies, but challenges are expected under field conditions, where abiotic interactions: temperature, water availability and the presence of other biotic factors may limit bio-efficacy. Abiotic interactions are capable of modulating growth characteristics, establishment of biocontrol fungi [14] and infectivity [15], such that *in vitro* results may be inconsistent with the outcome of field trials. Thus, there may be limited success in the use of eco-friendly biocontrol options for the management of Fusarium wilt disease, particularly under topical climatic conditions.

Planting of resistant varieties or hybrids may be among the most promising management options, particularly where the race of *Fusarium* that is endemic within a cropping area is known. However, *F. oxysporum* are forma specialis [16] that are pathogenic to specific plant species and consist of different physiological races within the specialized forms. Existence of different races of the forma specialis is thus likely to be a hurdle in selection and breeding of *Fusarium*-resistant tomato as well as determining the suitability of a

hybrid for cultivation in a particular *F. oxysporum*-endemic area. The biodiversity of races of *F. oxysporum* causing tomato wilt disease [17] is expected to vary widely depending on the agro-ecological region, land use practices, climatic and soil conditions and the cultivated tomato landraces. However, there are commercially available tomato hybrids that are resistant to different races of *F. oxysporum* [18].

The diversity of host specific races of *F. oxysporum* causing tomato wilt in the Southwestern Nigeria have not been reported and the possibility of adopting resistant tomato hybrids in Fusarium wilt disease affected areas has not also been considered. In this study, *F. oxysporum* was isolated from tomato plants showing strong evidence of Fusarium-wilt disease in some of the major tomato producing areas in Ekiti State Nigeria. The objectives of this study was to test six of the *Fusarium* isolates against F1-resistant tomato hybrid, F1-Lindo to assess susceptibility and the possibility of adopting the variety in the tomato farming communities from where the pathogens were isolated. The tomato Lindo F1 is a hybrid with strong resistance to bacterial wilt caused by *Ralstonia solanacearum,* Tobacco mosaic virus, TMV (O), *F. oxysporum* (fol.0) and it is capable of reaching maturity at 65 days after transplanting.

2. MATERIALS AND METHODS

2.1 Source of Tomato Hybrid, Infected Plants, Isolation and Identification of Pathogen

The hybrid of tomato F1-Lindo was purchased from TECHNISEM®, Tomato hybrid seeds supplier in France. Tomato plants showing advanced symptoms of *Fusarium* attack: vascular wilt or withered plants were collected from seven tomato fields in Ekiti state Nigeria in the month of July, 2016. The sample collection points and their coordinates are shown in Table 1. The foliage were clipped off and washed under running tap water to remove sand and other dirt particles. Thereafter, about 5-6 cm portions of the stem showing vascular discolouration were washed in 1.5% hypochlorite solution for 1 minute, rinsed three times in sterile distilled water and dried on sterile filter paper. About 1 cm portion of the surface-sterilized stem was placed on half-strength Potato Dextrose Agar modified with 0.02% chloramphenicol (CPDA). Chloramphenicol was added to the media to suppress bacteria growth. The Petridish was sealed with Parafilm and incubated for 14 days at ambient temperature (25 ± 2°C) in the dark. Pure cultures were prepared by transferring a single conidia into standard PDA. The single spore isolation method is described by Choi et al. [19]. Slides were prepared from pure cultures and identification was based on colony colour and morphology, phyalid type and characteristics of conidia under microscope.

2.2 Growth Characteristics and Sporulation Rates of Fungal Isolates

One centimeter agar disk of the pure culture was transferred into standard PDA media inside 9 cm Petridish. The Petridish was sealed with Parafilm and incubated at ambient temperature. Radial extension of the colony was measured along pre-marked orthogonal axes on the base of the Petridish after 24 hours and this continued for 5-6 days or until surface of the plate was fully covered. The data of radial extension (mm) recorded from three replicate plates was fitted into a linear model ($y = mx + c$) to estimate growth rate (mm day^{-1}) [14] and lag time. Conidia from 14 days old culture were harvested into standard bottles using sterile distilled water containing 0.02% polyoxyethylenesorbitan monooleate (Tween 80®, Sigma-Aldrich, UK) as a surfactant. Spore numbers were estimated using improved Neaubaur Haemocytometer under x40 objective of light microscope [20].

2.3 Preparation of Inoculum and Pathogenicity Assay

Conidia from 14 days old cultures grown on standard PDA were scrapped into McCartney bottles containing 1 ml sterile distilled water. The conidia suspension was vortexed intermittently for 1-2 minutes and made up to 10 ml. Serial dilutions were made as necessary and the conidia concentration was estimated using Haemocytometer and microscope. Final conidia concentration was standardized to 1×10^6 conidia ml^{-1}. Pathogenicity of the isolates was tested on four weeks old healthy tomato seedlings raised outdoor on autoclaved (121°C, 15 psi for one hour) soil samples in seedling pots. One ml of 10^6 conidia suspension of each isolate was introduced to the tomato roots using sterile disposable syringe. This was replicated six times and left for two weeks for infection to take place, during which sterile distilled water was applied ad-libitum to irrigate the plants. Six other

replicate potted plants were set up without the inoculum to serve as the control. The control was necessary to ensure that development of disease was due to the fungal inoculum rather than infection through contaminated soil or seeds. Should disease symptoms develop on the control plants, the results of the study would be invalidated. After three weeks post-inoculation, counting of leaves showing yellowish appearance (Chlorosis) on the six replicate treatment plants was done at seven days intervals. At six weeks post-inoculation, counting of diseased leaves was discontinued while estimation of wilted branches commenced and this was done at four consecutive times in weekly intervals. Damage was expressed as the percentage of the leaves or branches per plant that was affected by wilt disease.

2.4 Disease Severity Index

The percentages of the leaves showing symptoms of disease and the wilted branches were averaged and considered as the mean % damage (MPD) (Equation 1):

$$MPD = \frac{(\%\ leaves\ showing\ disease\ symptoms + \%\ wilted\ branches)}{2} \quad (1)$$

Severity of disease was based on MPD and rated with reference to Amini [21] and Bora et al. [22] which were slightly modified as briefly summarized in Table 2.

3. RESULTS

3.1 Source of Infected Plants, Isolation and Identification of Fungi

The diseased tomato samples collected from the seven locations yielded whitish mycellial growth on the PDA after 4-5 days incubation period. Older cultures of some of the isolates developed pinkish taint that was visible on the reverse side of the Petridish. Microscopic examination showed variable conidiophores and two distinct kinds of conidia in five of the isolates (Fig. 1). The microconidia were numerous, one celled and ovoid, borne singly and hyaline. The microconidia were boat shaped with slightly pointed ends, spare to abundant, variable in size with some 2-5 septate and hyaline [23]. The isolates were identified as *F. oxysporum* f.sp. *lycopersici.* Because of unavailability of facilities for molecular characterization, only five of the isolates with clearly distinct morphological features *F. oxysporum* were used for eventual pathogenicity studies.

3.2 Growth, Sporulation Rates and Lag Time

Table 3 shows the rates of growth, sporulation and lag time of the *F. oxysporum* isolates. There was a statistically significant difference F (6, 14) = 249.16, P=0.001 in the growth rates of the seven isolates. The rate of growth of ADO-1 was significantly the lowest (0.24 mm day^{-1}) while Ado-2 had the fastest growth, being 1.6 mm day^{-1}. The rate of growth of the five other isolates was between 0.43-0.56 mm day^{-1} without statistically significant difference. The lag time of the isolates were between 4.6-10.5 hours and they were not significantly different F (6, 281.06) =0.98, P = 0.479. After incubation for 24 hours, mycellial growth on the media were visible from the base of the Petridish. There were statistically significant variabilities in the sporulation rates of the isolates.

3.3 Pathogenicity of Isolates and Plant Damage

Pathogenicity of the five isolates tested were comparable and showed no statistically significant variabilities, F (4, 40384.9) = 1.993, P=0.98. However, the results showed that the disease progressed significantly with time; statistically significant difference in the level of damage was recorded at the three sampling periods, F (2, 40384.90 =50.995, P =0.001) (Table 4). The minimum and the maximum values of tomato leaves that showed disease symptoms were 52.3% and 62.75% respectively among the treated samples (Fig. 2). Wilted branches in the treatments were between 54% and 62.3% while the control plants showed no symptom of *Fusarium* infection. The tomato hybrid was rated as susceptible to all of the isolates based on the disease severity indices of 53-61.2% that was recorded (Fig. 3).

4. DISCUSSION

This study has demonstrated that the prevalent pathogen responsible for field grown tomato wilt in the agro-communities is *F. oxysporum* f. sp. *lycopersici.* All the collected samples of the diseased tomatoes yielded *F. oxysporum.* The

tested tomato plant, F1-Lindo was a hybrid with a strong level of resistance to *F. oxysporum,* Race 1 (F1). However, the level of damage recorded (52-63%) showed that the tomato was susceptible to all the isolates tested in this study. It can be suggested that the result has excluded

Table 1. Source of infested tomato plant samples and geographical coordinates

Isolates of *F. oxysporum* (*Sensu lato*)	Plants infected (Source)	Agro-ecological origin of isolates	Coordinates
IGEDE-1	Tomato	Igede	N7°40’13.8792”, E 5°7’29.8776”
ERIO-1	Tomato	Erio	N7°43’57.648”, E 5°0’23.4144”
ERIO-2	Tomato	Erio	N7°43’55.1964”, E 5°0’42.57”
ADO-1	Tomato	Ado	N 7◦39’3.294”, E 5◦13’44.0112”
ADO-2	Tomato	Ado	N 7◦35’56.5116”, E 5◦13’22.026”
AWO-1	Tomato	Awo	N 7◦42’58.41”, E 5◦8’54.312”
AWO-2	Tomato	Awo	N 7◦42’38.6496”, E 5◦9’1.2816”

Table 2. Mean percentage damage on tomato and disease severity index

MPD levels	Severity Ratings
No symptoms	Resistant (R)
<25% MPD	Intermediate resistant (IR)
26-50% MPD	Tolerance (T)
51-75% MPD	Susceptible (S)
76-100% MPD	Very susceptible (VS)

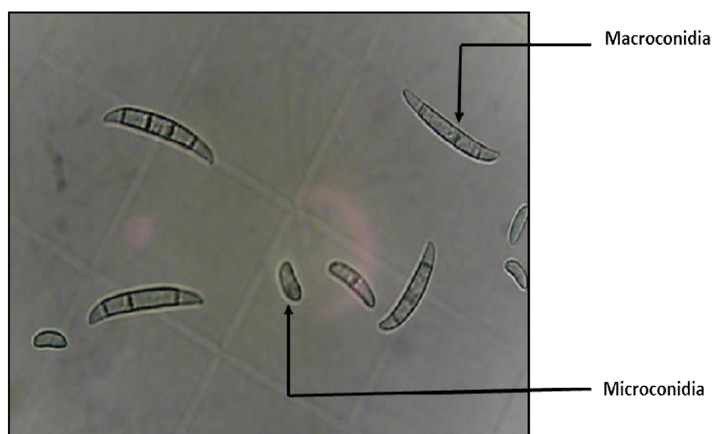

Fig. 1. Microconidia and macroconidia of *F. oxysporum* f. sp. *lycopersici*

Table 3. Growth, conidiation rates and lag time of seven *F. oxysporum* isolates

Isolates of *F. oxysporum* (*Sensu lato*)	Growth rate (mm day^{-1})	Lag time (Hours)	Conidiation rate Log_{10} (Conidia ml^{-1})
IGEDE-1	0.43a	4.56a	7.86a
ERIO-2	0.52a	7.14a	7.18b
ADO-2	1.60b	6.55a	*
ERIO-1	0.56a	10.27a	7.57ab
AWO-2	0.54a	7.31a	7.24bd
AWO-1	0.51a	10.50a	7.21bc
ADO-1	0.24c	10.27a	6.24be

*Note: Values in the same column and sub-table not sharing the same alphabet are significantly different at P=0.05, * Sporulation rate was not evaluated, IGEDE-1 produced higher spore numbers compared with all other isolates, while the least spore producer was ADO-1*

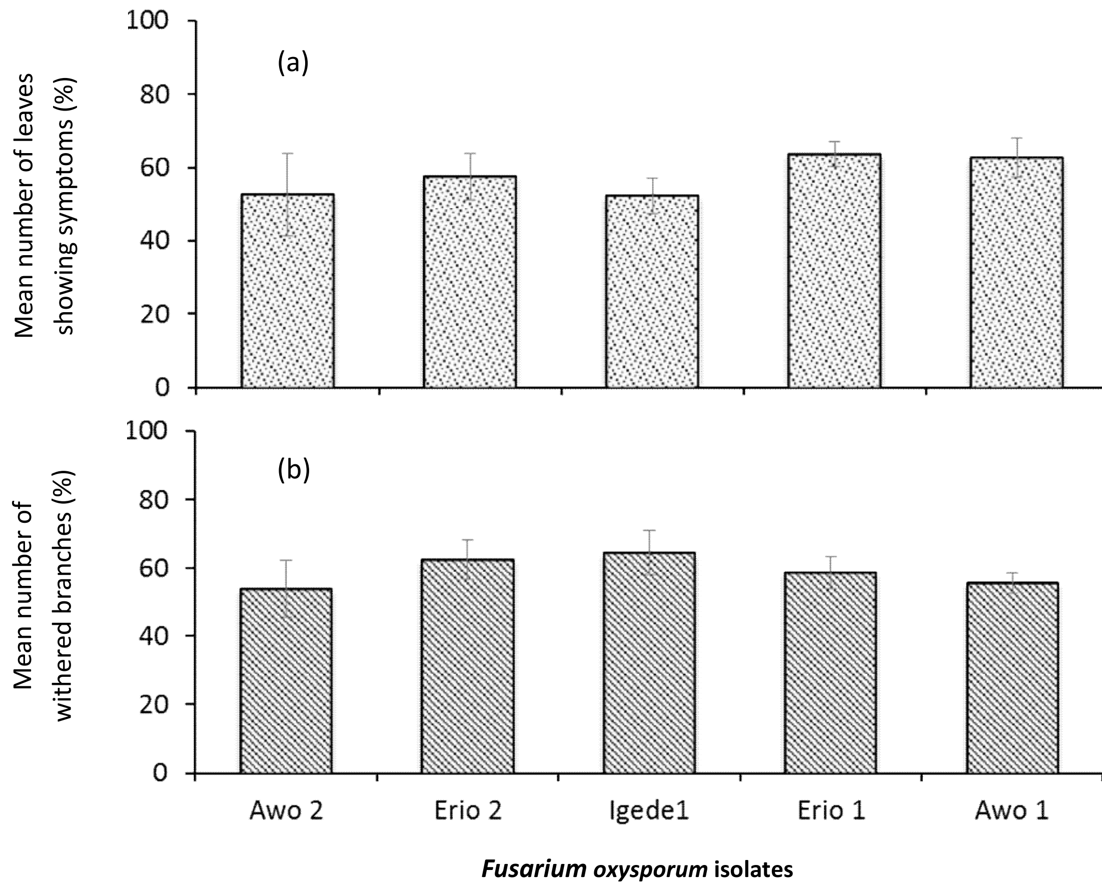

Fig. 2. Mean percentages of leaves showing symptoms of *F. oxysporum* infection and wilted tomato branches

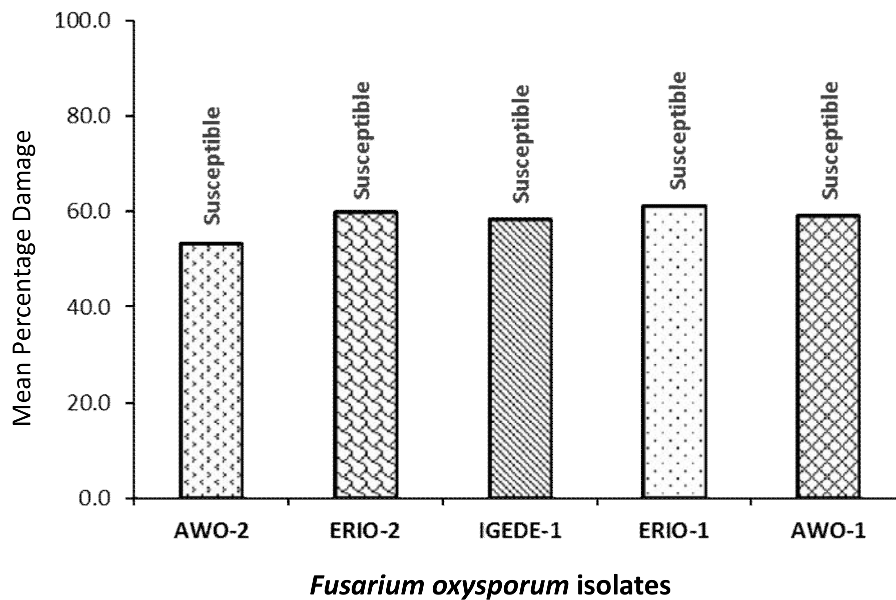

Fig. 3. Susceptibility ratings of F1-resistant tomato hybrid (F1-Lindo) to five pathogenic isolates of *F. oxysporum* f.sp. *lycopersici*

Table 4. Analysis of variance table showing pathogenicity of fungal isolates and effect of time on disease severity

Tests of between-subjects effects					
Dependent variable: Percentage damage					
Source	**SS**	**DF**	**Mean square**	**F**	**Sig.**
Corrected Model	49212.523[a]	29	1696.984	6.303	.000
Intercept	613897.296	1	613897.296	2280.175	.000
%Damage	43.601	1	43.601	.162	.688
%Damage * Sampling Periods	27459.236	2	13729.618	50.995	.000
% Damage * *Fusarium* isolates	2146.361	4	536.590	1.993	.098
Error	40384.883	150	269.233		
Total	703494.702	180			
Corrected Total	89597.406	179			

a. R Squared = .549 (Adjusted R Squared = .462)

the possibility of F1 being the causative agent of tomato wilt in the study area. Amini [21] successfully characterized *F. oxysporum* f.sp. *lycopersici* from different provinces in Iran into different races (F1-F4) using resistant tomato hybrids for the screening. It may also be possible that the concentration of the infective conidia in the experimental conidia suspension was higher than what could be encountered in the field under natural conditions, and probably the F-resistance quality of the tomato plant was compromised. There is no data to compare the relationship between fungal inoculum load and fitness (tolerance or resistance) of tomato hybrids, except the study conducted by Caligiore Gei [24] on *Fusarium* inoculum density and resistance screening tests in onion, where higher inoculum concentrations showed a positive correlation with plant susceptibility. Also, there are no established standards (inoculum load) at which fitness of hybrids to resist fungal infections would no longer be guaranteed.

It may be interesting to test the levels of fitness of some tomato hybrids that are resistant to the common phytopathogenic races (F1-F4) of *F. oxysporum* against the isolates that were used in this study at varied inocula concentrations. It might be possible to generate the first data on ‘tolerance or resistance boundaries’ of tomato hybrids in relation to inoculum load (conidia concentrations). In the present context, the data may be useful to provide partial clues to the hybrids of tomato that can be recommended for cultivation in the agro-communities from where the isolates were sourced.

The lag time, which is the time taken by a fungus to adjust to its environment before active growth and the eventual growth rates of the isolates were not different statistically. Thus, it was not surprising that their pathogenicity and rates of disease progress in the inoculated plants were comparable. The speed of germination, lag time and eventual rates of growth of pathogenic fungi may correlate with infectivity and virulence. The lag time of all the isolates were less than 24 hours and this may be important in their ability to overcome host defenses. Unfortunately, this could not be established in the current study since there were no statistically significant variabilities in the lag time and growth rates of the five isolates. There is also no data to compare the relationship between lag time and aggressiveness of pathogenic fungus. However, Borisade et al. [25] suggested that lag-time, as a component of growth behaviour, is an important factor in characterization of virulence of entomopathogenic fungal species.

Sporulation rates among the five isolates varied significantly. The ability of pathogenic fungus to produce infective conidia has a direct relationship with its secondary spread. Production of large numbers of conidia [26] by phyopathogenic fungi may enhance their spread in the secondary cycle.

5. CONCLUSION

The present study, being the first evaluation of susceptibility of F1-resistant tomato hybrid to local isolates of *F. oxysporum* f. sp. *lycopersici* has not sufficiently established the hybrid of tomato that may be planted within the study area. *Fusarium oxysporum* races are divergent and it may be practically impossible to perform comprehensive screening of *Fusarium* races within an area in order to select a resistant or compatible tomato hybrid. We therefore suggest

enhancement of F-resistance using other management methods in another study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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