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Pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) isolates in causing tomato wilt disease on two tomato (*Solanum lycopersicum* L) varieties

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Abstract
Pathogenicity test of *Fusarium oxysporum* isolates in causing tomato wilt disease (TWD) was carried out on two tomato varieties (UC 82B and Rio-grande). During the 2015 cropping season, the experiment was conducted in a screen house at the Federal University of Agriculture, Makurdi's Teaching and Research Farm. A 2 x 11 factorial was used in the experiment, which was replicated three times using a completely randomized design (CRD). Together with an uninoculated control, the *F. oxysporum* isolates that were evaluated were coded as FoAs1, FoAs2, FoAg, FoNb, FoSb, FoAm, FoAk, FoOr, FoAd, and FoUAM. All the isolates of *F. oxysporum* tested were pathogenic, causing wilt on the plants from 3 weeks after inoculation (WAI), with severity of wilt been significantly higher ($p \leq 0.05$) in FoUAM. Isolates of *F. oxysporum* showed significant difference ($p \leq 0.05$) both in incidence and severity compared with the control. Effect of *F. oxysporum* isolates on some agronomic characteristics such as plant height, number of fruits, fruit weight and number of branches on the two varieties of tomato at 12 weeks after sowing (WAS) was significantly different ($p \leq 0.05$) with the control experiment. Highest severity score of 5.00 was calculated in FoUAM while the least of 4.33 was in FoSb compare with uninoculated value of 2.50. It is therefore, concluded that the two tomato varieties are pathogenic to *F. oxysporum* isolates and illicit disease in tomato hence reduced the yield of the crop.

Keywords: Incidence, *Fusarium oxysporum*, isolates, pathogenicity, severity, tomato

1. Introduction
The crop known as tomatoes (*Solanum lycopersicum* L.) is thought to have come from the Andes of South America (Naika *et al*., 2005) [24]. With an anticipated production of 180,766,329 metric tonnes, it is one of the most significant vegetables grown worldwide (FAO, 2019) [11]. Nigeria is one of the world's top producers, coming in second place to Egypt as the continent's top producer (FAO, 2020) [12]. Tomatoes are eaten raw in salads or cooked in sauces, soups, and various meat or fish meals. They are also high in sugar, dietary fiber, vital amino acids, vitamins, and minerals (Naika *et al*., 2005) [24]. The crop is occasionally processed to make ketchup, juices, and purées. In some cases, it is canned and dried which can be preserved for a long period (Naika *et al*., 2005) [24].
Tomato is produced in large amount annually but it's availability throughout the year is limited by certain factors such as pests, diseases, storage facilities (Ahmed *et al*., 2013) [2]. Soil-borne diseases and pests present a major challenge to the production of horticultural and other crops grown in fields and in greenhouses inclusive.
According to several studies (Egel and Martyn, 2013; Sani and Gwa, 2018; Mamkaa and Gwa, 2018) [10, 21, 28], *Fusarium oxysporum* is one of the pathogens linked to producing major infections on a wide variety of sensitive hosts, including tomato, cowpea, pepper, sweet potatoes, cabbage, eggplant, and groundnut. Within the species, there are more than 100 host-specific formae speciales. For instance, *F. oxysporum* f. sp. *cubense* infects *bananas* while *F. oxysporum* f. sp. *batatas* affects sweet potatoes. Tomato vascular wilt is caused by *F. oxysporum* f. sp. *lycopersici. muskmelon* and cantaloupe are targeted by *F. melonis.*
Rots caused by pathogens on fresh tomatoes have been reported to be as high as 60% in Nigeria (Kutama *et al*., 2007; Sani and Gwa, 2018) [20, 28].

Numerous pathogens have been documented to cause illnesses in tomatoes, the most significant of which are *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) (Fusarium wilt) and *Fusarium solani* f. sp. *Eumartii* (Akrami and Yousefi, 2015) [4]. According to reports, fusarium wilt alone can reduce tomato yield by up to 30 to 40% (Anita and Rabeeth, 2009) [5]. *Fusarium oxysporum Schlect* f. sp. *radicis-lycopersici* roots, the cause of fusarium stem and root rot, is a soil-borne tomato disease that is commonly found in greenhouse tomato production (Roberts *et al*., 2001; Pavlou & Vakalounakis, 2005) [27, 25]. All plant growth stages are susceptible to fungal pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), which enters plants through the roots and causes significant economic losses by producing wilting and necrosis in a variety of crop plants (Cotxarrera *et al*., 2002) [6].

Evidently, only a few number of tomato cultivars have been found to be resistant to *F. oxysporum* f. sp. *lycopersici* (Sacc.). It is therefore, necessary to know which of the *F. oxysporum* isolates are more pathogenic in causing tomato wilt disease and responsible in reducing growth on the two varieties of tomato.

Materials and Methods

Site of experimental

The area is in Nigeria's Southern Guinea Savannah Agro-ecological zone, with latitudes of 7º45' North and longitudes of 8º37' east. The average elevation above sea level is 97 meters.

Preparation of Culture Medium

The manufacturer's instructions were followed to prepare the Potato Dextrose Agar (PDA) medium, which involved dissolving 39 g of PDA in 1 liter of distilled water. The mixes were autoclaved for 15 minutes at 121 ºC and 33 kg P.S.I. (Ritchie, 1991) [26]. After allowing the molten medium to cool to around 40 ºC, 0.16 g/L of streptomycin sulfate was added to stop bacterial growth. The mixture was then thoroughly mixed and 20ml was poured into 9cm Petri plates, where it was allowed to harden.

Isolation of *Fusarium oxysporum*

After removing any residues, stem samples exhibiting symptoms of wilt were cleaned under running tap water. Each stem was then cut into approximately 5mm pieces and submerged in 5% sodium hypochlorite for two minutes to purify the surface. The samples were then rinsed in four separate changes of sterile distilled water and allowed to dry on sterile filter paper (Gwa and Akombo, 2016, Gwa and Ekefan, 2017a) [13-14]. On a laminar air flow chamber, four pieces were plated on each Petri dish with Potato Dextrose Agar (PDA). For three days, the plates were incubated at room temperature**.**

Following duration of three (3) days, various mycelia of developing fungus were aseptically sub-cultured onto freshly prepared Potato Dextrose Agar plates using a flamed inoculation needle. Sub-cultures of *F. oxysporum*-typical fungal growths were used. Then, using an identification guide and a compound microscope, a pure culture of *F. oxysporum* was recognized (Agrios, 2005) [3]. The following codes were assigned to the *F. oxysporum* isolates based on the location of collection: Asase1 (FoAs1), Asase2 (FoAs2), Agromiller (FoAg), Northbank (FoNb), Southbank (FoSb), Amih (FoAm), Orduen (FoOr), Adudu (FoAd), and University of Agriculture Makurdi (FoUAM).

Test of Pathogenicity of *Fusarium oxysporum* Isolates

Pathogenicity test of the isolates obtained above was done in a screen house at the Federal University of Agriculture, Makurdi. The treatments consisted of two tomato varieties (UC 82B and Rio- Grande) and ten (10) isolates of *Fusarium oxysporum* a control inoculated with sterilized distilled water (SDW). Three replications of the 2 x 11 factorial set of treatments were set up in a completely randomized design (CRD). The pots were filled with 5kg sterilized sandy loamy soil and watered using sterilized distilled water. The seeds were sowed in three-liter capacity pots, one pot made up a treatment with two plants per pot. The soil was inoculated separately with 5ml of spore suspension of the different isolates of *F. oxysporum* containing $1x10^6$ spores/ml with syringes two weeks following sowing and the control experiment were inoculated with 5ml SDW. Spore concentrations were determined using a haemocytometer. The inoculated soils were covered with plastic bags for 48 hours after inoculation to maintain high relative humidity and create condition suitable for infection.

Data Collected

(a) Disease incidence and severity.

The incidence and severity of fusarium wilts disease were measured every week from three weeks after sowing (WAS) to twelve WAS.

(i). The number of diseased plants was counted physically, and the resulting number was divided by the total number of plants evaluated in each treatment to determine the disease incidence. Using the following formula, the results were converted to a percentage.

$$\text{Disease Incidence} = \frac{\text{Number of Diseased plants}}{\text{Total number of plants}} \times \frac{100}{1}$$

(ii). A 1–5 scale created by De-Cal *et al*. (1995) was used to rate the disease's severity on tomato plants, as explained below:

1. Every leaf is green.
2- Yellow lower leaves.
3. Dead lower leaves.
4-The upper leaves faded and the lower leaves were dead.
5. A dead plant.

(b) Plant height, branch count, fruit count, and fruit weight were recorded every week beginning at 3WAS and continuing until 12WAS.

Statistical analysis

Genstat was used to do an analysis of variance on all the obtained data. Fisher's Least Significant Difference (Cochran and Cox, 1992) [7] was used to separate substantially different means at the 5% level of probability using the Statistical Package (Discovery Edition 12).

Results

Pathogenicity test of *Fusarium oxysporum* isolates on Tomato Plants

Effect of *F. oxysporum* isolates on the incidence and severity of fusarium wilt in Rio Grande

The disease progression curves for Fusarium wilt caused by *Fusarium oxysporum* isolates on tomato over a period of 12

weeks are displayed in Figure 1. The first signs of fusarium wilt appeared three weeks after sowing (WAS). Incidence of the disease was lowest in the control where the plants were not inoculated. Each and every *F. oxysporum* isolate tested were pathogenic, causing wilt on the plant. At 12 WAS the plant treated with Fusarium wilt exhibited a 100% incidence from isolates of FoAk, FoAm, FoAd, FoNb, FoOr and FoUam while the uninoculated control had the least Fusarium wilt incidence of 66.67%.

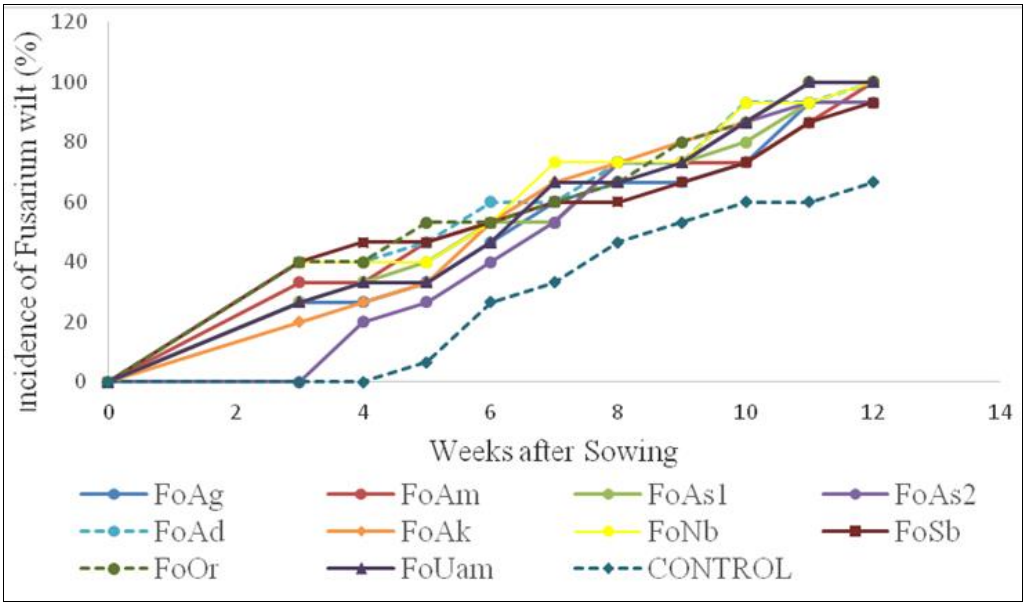

Fig 1: Disease Progression Curves Showing Incidence of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on Rio Grande over a Period of 12 Weeks

Figure 2, Shows the disease severity of Fusarium wilt induced by ten isolates of *F. oxysporum* on tomato over a period of 12 weeks. The trend in disease severity was similar to that of incidence showing highest severity score of 5 in FoAd, FoOr, FoUam and FoAm in Rio Grande variety. The lowest severity was recorded in the control.

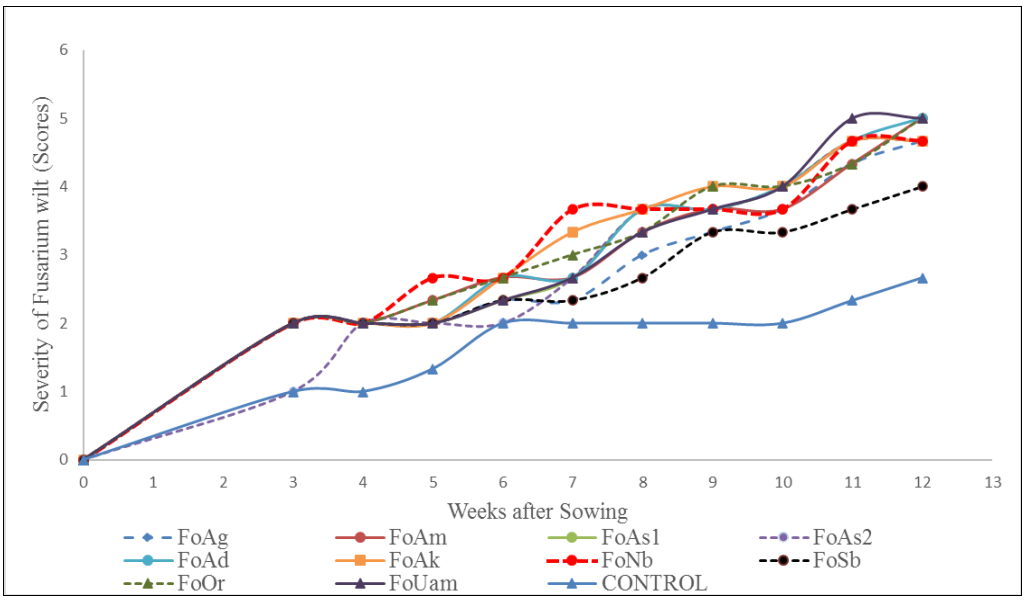

Fig 2: Disease Progression Curves Showing Severity scores of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on Rio Grande over a Period of 12 Weeks.

Effect of *F. oxysporum* isolates on the incidence and severity of Fusarium wilt in UC 82B

The disease progression curve of Fusarium wilt incidence caused by *Fusarium oxysporum* isolates on UC 82B over a 12-week period is displayed in Figure 3. The first signs of Fusarium wilt were noticed three weeks after sowing (WAS). The control, where the plants weren't inoculated, had the lowest disease incidence. Every isolate of *F. oxysporum* that was tested proved to be pathogenic and caused the plant to wilt. When the plant was inoculated with FoAk, FoAm, FoAd, FoNb, FoOr, FoAs2, and FoUam at 12 WAS, the incidence of Fusarium wilt was 100%.

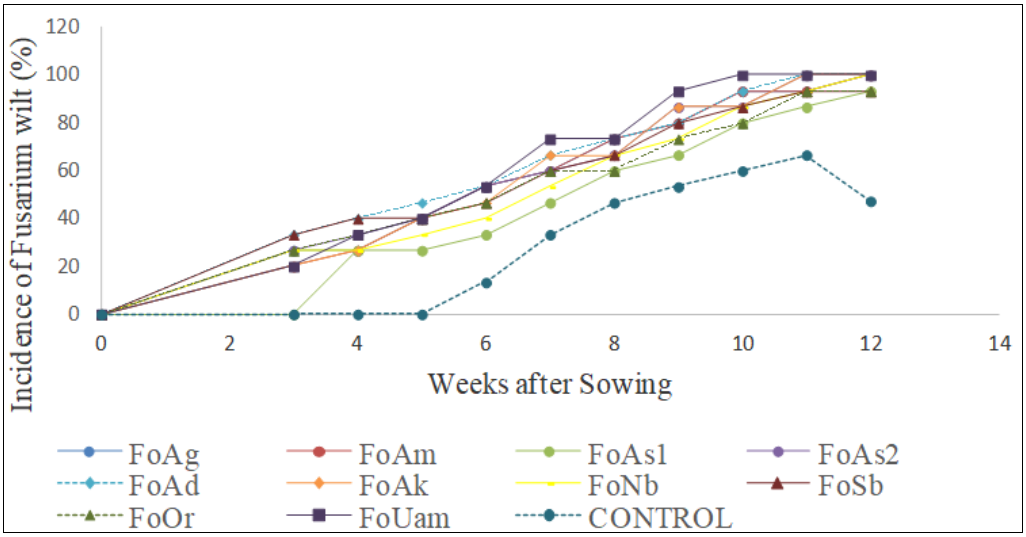

Fig 3: Disease Progression Curves Showing Incidence of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on UC 82B over a Period of 12 Weeks

Figure 4. Shows the disease sverity of ten isolates of *Fusarium oxysporum* on tomato over a period of 12 weeks on UC 82B. the control had the lowest value of 2.33 while the highest severity value occurred in FoAg, FoAs2, FoAsl, FoNb and Fosb with a value of 4.67 for each.

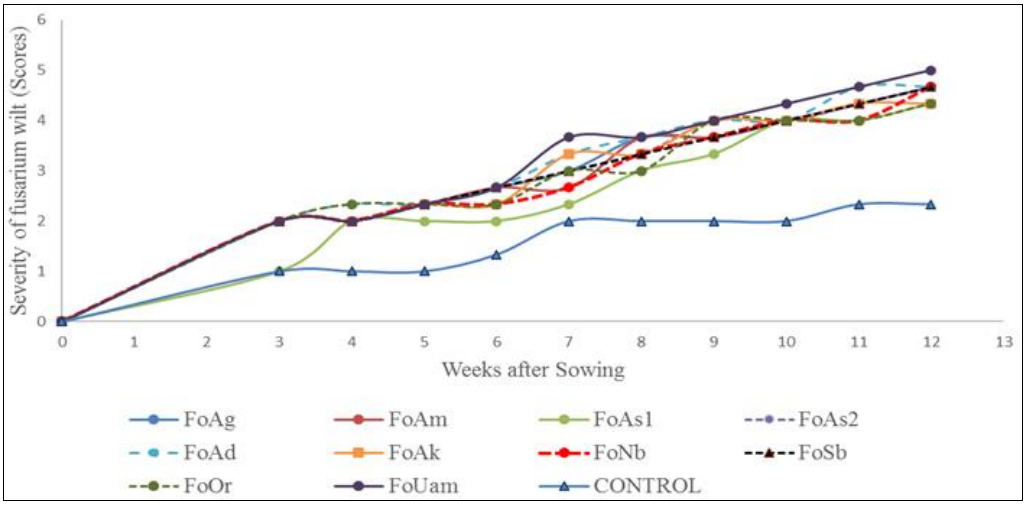

Fig 4: Disease Progression Curves Showing Severity scores of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on UC 82B over a Period of 12 Weeks.

The incidence and degree of severity of Fusarium wilt in tomatoes at 12 weeks post-sowing are displayed in Table 1. When plants treated with different isolates of *Fusarium oxysporum* were compared to the uninoculated control, the incidence of fusarium wilt was considerably (p<0.05) lower in the former. The incidence of Fusarium wilt caused by the several Fusarium isolates that were investigated did not differ significantly. Each and every isolate had a pathogenic potential. The disease was substantially more severe (p<0.05) in FoUAM than in the other categories.

Table 1: Incidence and Severity of Fusarium Wilt on Tomato at 12 Weeks After Sowing

Isolate	Incidence (%)	Severity Scores
Control	57.00^{b}	2.50^{c}
FoAd	100.00^{a}	4.83^{ab}
FoAg	96.70^{a}	4.67^{ab}
FoAk	100.00^{a}	4.50^{ab}
FoAm	100.00^{a}	4.83^{ab}
FoAs1	93.30^{a}	4.50^{ab}
FoAs2	96.70^{a}	4.67^{ab}
FoNb	100.00^{a}	4.67ab
FoOr	96.70^{a}	4.67^{ab}
FoSb	93.30^{a}	4.33^{b}
FoUAM	100.00^{a}	5.00^{a}
FLSD(P≤0.05)	13.00	0.55

KEY
Adudu's *Fusarium oxysporum* is known as FoAd. *Fusarium oxysporum* from Agromiller is known as FoAg. Akor's *Fusarium oxysporum* is known as FoAk. Amih's *Fusarium oxysporum* is known as FoAm. FoAs1: Asase 1 *Fusarium oxysporum*. *Fusarium oxysporum* from Asase 2 (FoAs2). *Fusarium oxysporum* from Northbank is known as FoNb. FoOr: Orduen's *Fusarium oxysporum*. Southbank's *Fusarim oxysporum* is known as FoSb. *Fusarium oxysporum* from the University of Agriculture Makurdi is known as FoUAM. Fisher's Least Significant Difference, calculated at a 5% probability level.

Effect of Isolates of *Fusarium oxysporum* and Variety on Some Agronomic Characteristics of Tomato Plant at 12 weeks after sowing

Table 2 shows the effect of *F. oxysporum* isolates and types on a few agronomic traits of tomatoes 12 weeks after sowing. Plant height in the uninoculated control group was considerably ($p \leq 0.05$) higher than in the plants inoculated with different *F. oxysporum* isolates. Plant height varied significantly ($p \leq 0.05$) depending on which isolate was used to inoculate the tomato.

Plants inoculated with isolates of *F. oxysporum* produced substantially fewer fruits per plant than the uninoculated control ($p \leq 0.05$). Among the inoculated plants, there was no discernible variation in the quantity of fruits produced by each plant.

Fruit weight in the uninoculated control group was considerably ($p \leq 0.05$) higher than in the plants infected with the different *F. oxysporum* isolates. The fruit weights of the different *F. oxysporum* isolates varied significantly as well. When compared to the infected plants, the uninoculated control had a considerably ($p \leq 0.05$) larger number of branches. Table 2 shows that Rio-grande had significantly ($p \leq 0.05$) more branches than UC 82B, while UC 82B had significantly ($p \leq 0.05$) more plant height, fruits, and fruit weight than Rio-grande.

Table 2: Effect of *F. oxysporum* Isolates and Varieties on Some Agronomic Characteristics of Tomato Plant at 12 Weeks After Sowing

Isolate	Plant height (cm)	Number of Fruits/Pot	Fruit Weight (g)	Number of Branches
Control	141.20^{a}	3.17^{a}	105.30^{a}	27.67^{a}
FoAd	119.80^{bc}	0.67^{b}	21.30^{bc}	16.83^{cd}
FoAg	107.70^{c}	0.67^{b}	22.20^{bc}	18.17^{c}
FoAk	119.70^{bc}	1.67^{b}	50.00^{b}	17.83^{cd}
FoAm	109.50^{c}	0.67^{b}	14.90^{c}	16.00^{d}
FoAs1	112.80^{bc}	0.67^{b}	16.30^{c}	17.00^{cd}
FoAs2	113.30^{bc}	1.67^{b}	44.30^{bc}	20.00^{bc}
FoNb	120.70^{bc}	1.67^{b}	45.10^{b}	19.50^{bc}
FoOr	119.00^{bc}	0.50^{b}	13.60^{c}	21.17^{b}
FoSb	108.50^{bc}	0.67^{b}	14.50^{c}	16.33^{cd}
FoUAM	124.20^{b}	0.83^{b}	31.40^{bc}	18.83^{bc}
FLSD($p \leq 0.05$)	13.93	1.22	31.36	2.50
Varieties Rio-grande UC 82B	19.76^{b} 106.10^{a}	0.82^{b} 1.52^{a}	20.40^{b} 48.50^{a}	19.76^{a} 18.30^{b}
FLSD ($p \leq 0.05$)	5.94	0.52	13.37	1.07

Key
Adudu's *Fusarium oxysporum* is known as FoAd. *Fusarium oxysporum* from Agromiller is known as FoAg. Akor's *Fusarium oxysporum* is known as FoAk. Amih's *Fusarium oxysporum* is known as FoAm. FoAs1: Asase 1 *Fusarium oxysporum*. *Fusarium oxysporum* from Asase 2 (FoAs2). *Fusarium oxysporum* from Northbank is known as FoNb. FoOr: Orduen's *Fusarium oxysporum*. Southbank's *Fusarim oxysporum* is known as FoSb. *Fusarium oxysporum* from the University of Agriculture Makurdi is known as FoUAM. Fisher's Least Significant Difference, calculated at a 5% probability level.

Discussion
Many diseases impact tomato farming, with *Fusarium oxysporum*-caused wilt disease being one of the most dangerous in major tomato-growing regions of the world (Anita and Rebeeth, 2009) [5]. In this study, a pathogenicity test of ten *Fusarium oxysporum* isolates revealed that every isolate was pathogenic on tomato plants, resulting in fruit rot.

The isolate FoUAM was the most virulent, causing severe infections on tomato. This result is similar with those of Joshi *et al*. (2013) [19], who in their pathogenicity test found majority of *F. oxysporum* isolates to be pathogenic on tomato. They discovered that at least one tomato plant had wilt symptoms due to the isolates; however, when non-pathogenic isolates were injected into the tomato plant, wilt did not appear. Using commercially engineered tomato varieties derived from the resistance genes, one option for managing the fusarium wilt disease is varietal resistance (McGovern, 2015) [23]. Fusarium wilt disease enters a plant by its roots and spreads through its xylem fibers, obstructing water flow and resulting in withering of the entire plant (Cox *et al*., 2019) [8].

The high incidence of 93% to 100% found in this study is consistent with previous research by McGovern (2015) [23] and Heydari *et al.* (2007) [15]. The authors stated that Tomato Wilt Disease (TWD) is 100% common in the United States. Conversely, Houterman *et al.* (2007) [17] found that in the USA, there was less infection of between 0% and 29% on resistant cultivars but 100% on susceptible cultivars. AbdelFattah and Al-Amri (2012) [1] found a similar outcome, reporting 53-71% disease incidence and 47-78% disease severity of TWD in Egypt. The results of this investigation differ from those of Ishikawa *et al.* (2005) [18], who found a 50% disease severity in Japan, and Mandal *et al*. (2009) [22], who claimed a 40% incidence of TWD in Japan. The study's findings show the incidence, severity, and damage caused by Fusarium wilt.

Conclusion

Tomato is a crop that is affected by different isolates of *Fusarium oxysporum* in farmers' fields cutting across three Local Government Areas of Benue State which include Gboko, Makurdi and Tarka. Pathogenicity tests of all the ten isolates carried out on two healthy tomato varieties Rio-grande and UC 82B confirmed that the isolates were pathogenic causing tomato wilt disease on the two tomato varieties tested. It is therefore, recommended that appropriate measures be taken to reduce incidence and severity of tomato wit disease to increase yield and enhance food security.

Conflict of interest disclosure

There is no conflict of interest with relation to the publishing of this paper, according to the authors.

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