Bio-Science Research Bulletin
Volume 39, Number 2
July-December 2023: P.60-68
Original Research Article

Print version ISSN 0970 0889
Online version ISSN 2320 3161
DOI: 10.48165/bpas.2023.39.2.3
http://www.bpasjournals.com

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) in a screen house located at the Teaching and Research Farm of Federal University of Agriculture, Makurdi during 2015 cropping season. The experiment was a 2 x 11 factorial laid out in Completely Randomized Design (CRD) and replicated three times. F. oxysporum isolates tested were coded as: FoAs1, FoAs2, FoAg, FoNb, FoSb, FoAm, FoAk, FoOr, FoAd and FoUAM together with an uninoculated control. 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≤0.05) in FoUAM. Isolates of F. oxysporum showed significant difference (P≤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≤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*

Received on 20.08.2023, Revised on 21.09.2023, Approved on 30.10.2023, Accepted on 26.11.2023, Published on 24.12.2023

How to cite this article: Abdulkadir, H.K., Ekefan, E.J. and Gwa, V.I. (2023). Pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Isolates in Causing Tomato Wilt Disease on Two Tomato (*Solanum lycopersicum* L*)* Varieties. *Bio-Science Research Bulletin*, 39(2), 60-68.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a crop that is believed to have originated from South American Andes (Naika *et al*., 2005). It is one of the most important vegetables cultivated throughout the world with an estimated yield of 180,766,329 metric tonnes (FAO, 2019). Nigeria is one of the largest producers in the world and ranked 14th and second largest producer after Egypt on the continent of Africa (FAO, 2020). Tomatoes are rich in minerals, vitamins, essential amino acids, sugars and dietary fibers and are consumed in some instances fresh as salad or cooked in sauces, soups and several meat or fish dishes (Naika *et al*., 2005). The crop can sometimes be processed into purées, juices and ketchup. In some cases, it is canned and dried which can be preserved for a long period (Naika *et al*., 2005).

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). Soil-borne diseases and pests present a major challenge to the production of horticultural and other crops grown in fields and in greenhouses inclusive.

Fusarium oxysporum is one of the pathogens implicated in causing serious diseases on a wide varieties of susceptible hosts such as tomato, cowpea, pepper, sweet potatoes, cabbage, eggplant, groundnut (Egel and Martyn, 2013; Sani and Gwa, 2018; Mamkaa and Gwa, 2018). There are over 100 formae speciales within the species which are host-specific. For example; *F. oxysporum* f. sp. *batatas* affects sweet potato; *F. oxysporum* f. sp. *cubense* causes Panama disease on banana. *F. oxysporum* f. sp. *lycopersici* causes vascular wilt in tomato. *F. melonis* attacks muskmelon and cantaloupe.

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). It has been reported that several pathogens caused diseases in tomato out of which *Fusarium solani* f. sp. *Eumartii* and *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) (*Fusarium* wilt) are the most important (Akrami and Yousefi, 2015). *Fusarium* wilt alone has been reported to cause as much as 30 to 40% yield loss in tomatoes (Anita and Rabeeth, 2009). Fusarium stem and root rot is a soil-borne tomato disease caused by *Fusarium oxysporum* Schlect f. sp. radicis-lycopersici roots and quite frequent in greenhouse tomato production (Roberts *et al*., 2001; Pavlou and Vakalounakis, 2005). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is a soil borne fungal pathogen that infect plants through the roots at all stages of plant growth and cause major economic losses by inducing necrosis and wilting symptoms in many crop plants (Cotxarrera *et al*., 2002).

There are obviously few varieties of tomato reported 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

Experimental Site

The location falls within Latitude 7^{o} 45′ North and Longitude 8^{o} 37′ East with altitude average 97m mean above sea level in the Southern Guinea Savannah Agro-ecological zone of Nigeria.

Preparation of Culture Medium

Potato Dextrose Agar (PDA) medium was prepared according to manufacturer's recommendation by dissolving 39g of PDA in 1 liter of distilled water. The mixtures were sterilized in an autoclave at 33Kg P.S.I and 121^{o}C for 15 minutes (Ritchie, 1991). The molten medium was allowed to cool to about 40^{o}C, after which 0.16g/L of Streptomycin sulphate was added to prevent growth of bacteria and shaken gently in order to mix well before pouring 20ml into 9cm Petri dishes and allowed to solidify.

Isolation of *Fusarium oxysporum*

Stem samples with visible wilt symptoms were washed under running tap water to remove any residues, for each stem about 5mm pieces were cut, immersed in 5% Sodium hypochlorite for 2 minutes for surface purification and then washed in four successive changes of sterile distilled water and dried on sterile filter paper (Gwa and Akombo, 2016, Gwa and Ekefan, 2017a). Four pieces were plated on each Petri dish containing Potato Dextrose Agar (PDA) on a laminar air flow chamber. The plates were incubated at ambient condition for three days.

After three (3) days, different emerging fungi mycelia were aseptically sub-cultured with a flamed inoculation needle onto newly prepared Potato Dextrose Agar plate. Fungal growths typical of *F. oxysporum* were sub-cultured. Pure culture of *F. oxysporum* was subsequently identified with the aid of a compound microscope using an identification guide

(Agrios, 2005). The *F. oxysporum* isolates were coded based on collection site as follows: 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 *F. oxysporum* isolates obtained above was tested 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). The 2 x 11 factorial set of treatments were arranged in Completely Randomized Design (CRD) replicated three times. 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 at two weeks after sowing (WAS) and the control 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 (Hassanein *et al*., 2010).

Data Collected

(a) Disease incidence and severity.

Fusarium wilts incidence and severity were taken at weekly intervals, starting from three weeks after sowing (WAS) to 12 WAS.

(i). Disease incidence was recorded by counting the number of infected plants through physical observation and dividing it with the total number of plants assessed in each treatment. The results obtained were converted to percentage using the formula:

Disease Incidence

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

(ii). The severity of the disease on tomato plants were scored using 1-5 scale developed by De-Cal *et al*, (1995) as described below:

1 - All leaves green.
2 - Lower leaves yellow.
3 - Lower leaves dead.
4 - Lower leaves dead and upper leaves wilted.
5 - Dead plant.

b. Plant height, number of branches, number of fruits and weight of fruits were collected weekly starting from 3WAS to 12WAS.

Statistical analysis

All data collected were subjected to analysis of variance using Genstat. Statistical Package (Discovery Edition 12) and significantly different means were separated at 5% level of probability using Fisher's Least Significant Difference (Cochran and Cox, 1992).

RESULTS

Pathogenicity of Isolates of *Fusarium oxysporum* on Tomato Plants

Effect of isolates of *F. oxysporum* on incidence and severity of fusarium wilt on Rio grande

Figure 1 shows the disease progress curves of incidence of Fusarium wilt induced by isolates of *Fusarium oxysporum* on tomato over a period of 12 weeks. Fusarium wilt was first observed at 3 weeks after sowing (WAS). Incidence of the disease was lowest in the control where the plants were not inoculated. All the isolates of *F. oxysporum* tested were pathogenic, causing wilt on the plant. At 12 WAS the incidence of Fusarium wilt was 100% in plant inoculated with FoAk, FoAm, FoAd, FoNb, FoOr and FoUam while the uninoculated control had the least Fusarium wilt incidence of 66.67%.

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.

Effect of isolates of *F. oxysporum* on incidence and severity of fusarium wilt on UC 82B

Figure 3 shows the disease progress curve of incidence of Fusarium wilt induced by isolates of *Fusarium oxysporum* on UC 82B over a period of 12 weeks. Fusarium wilt was first observed at 3 weeks after sowing (WAS). Incidence of the disease was lowest in the control where the plants were not inoculated. All the Isolates of *F. oxysporum* tested were pathogenic causing wilt on the plant. At 12 WAS the incidence of Fusarium wilt was 100% in plant inoculated with FoAk, FoAm, FoAd, FoNb, FoOr, FoAs2 and FoUam.

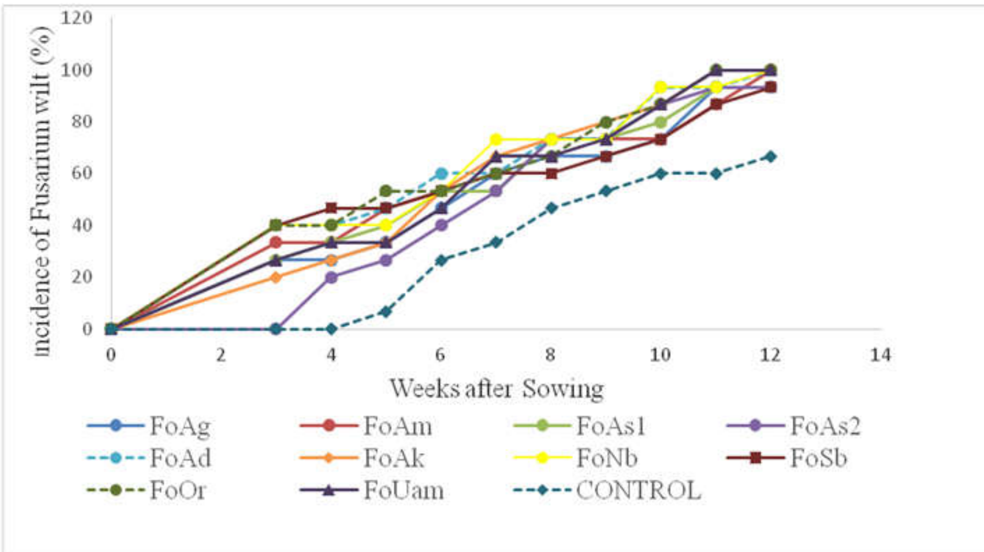

Figure 1: **Disease Progression Curves Showing Incidence of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on Rio Grande over a Period of 12 Weeks.**

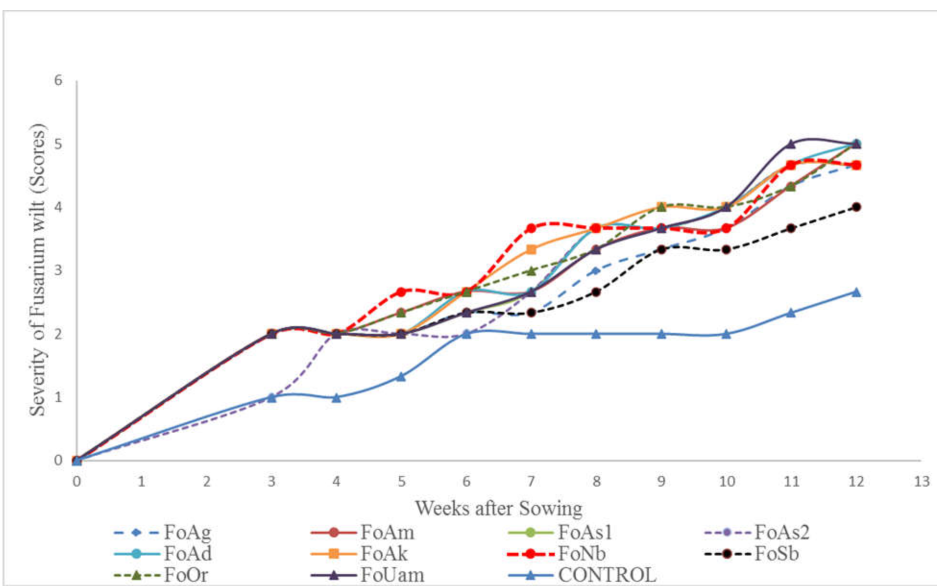

Figure 2: **Disease Progression Curves Showing Severity scores of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on Rio Grande over a Period of 12 Weeks.**

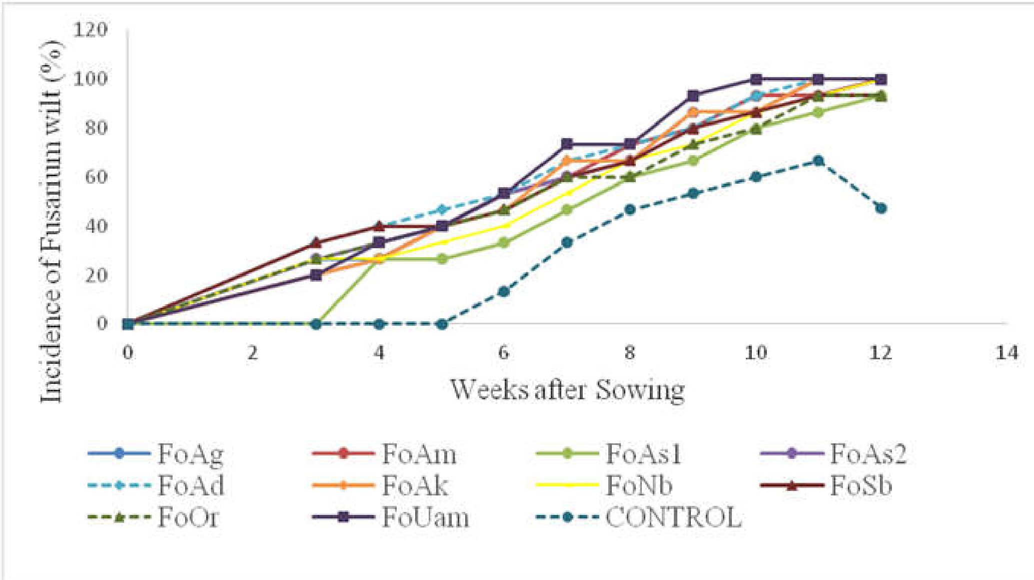

Figure 3: **Disease Progression Curves Showing Incidence of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on UC 82B over a Period of 12 Weeks**

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.

Table 1 show the incidence and severity of Fusarium wilt on Tomato at 12 weeks after sowing. Fusarium wilt incidence was significantly ($p \leq 0.05$) lower in the uninoculated control compared with plants that were inoculated with the various isolates of *F. oxysporum*. There was no significant difference in incidence of Fusarium wilt induced by the various isolates of *Fusarium* tested. All the isolates were pathogenic. With respect to severity, the disease was significantly higher ($p \leq 0.05$) in FoUAM compared with others.

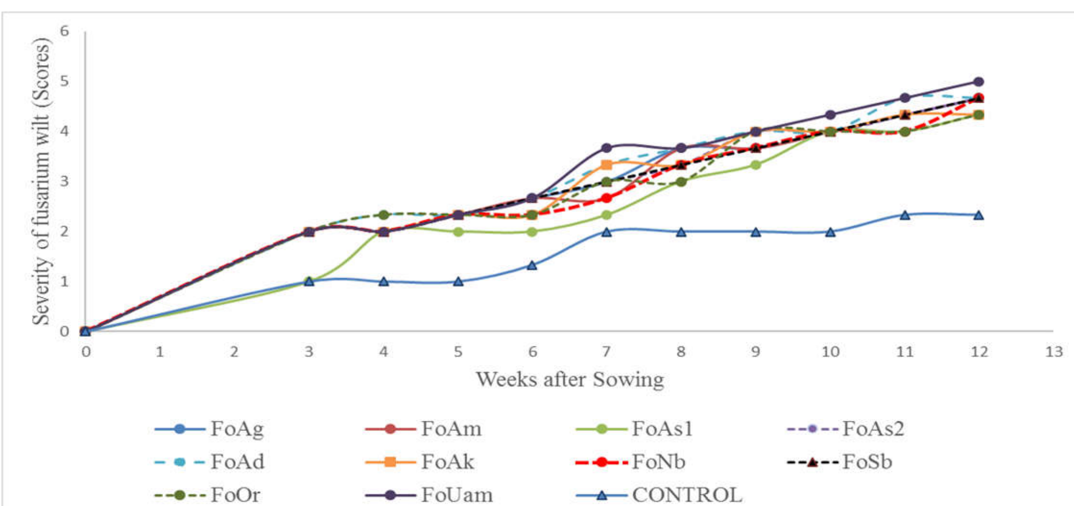

Figure 4: **Disease Progression Curves Showing Severity scores of Fusarium Wilt Induced by 10 Isolates of *Fusarium* on UC 82B over a Period of 12 Weeks.**

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:
FoAd – *Fusarium oxysporum* from Adudu. FoAg – *Fusarium oxysporum* from Agromiller. FoAk – *Fusarium oxysporum* from Akor. FoAm – *Fusarium oxysporum* from Amih. FoAs1 – *Fusarium oxysporum* from Asase 1. FoAs2 – *Fusarium oxysporum* from Asase 2. FoNb – *Fusarium oxysporum* from Northbank. FoOr – *Fusarium oxysporum* from Orduen. FoSb – *Fusarim oxysporum* from Southbank. FoUAM – *Fusarium oxysporum* from University of Agriculture Makurdi. FLSD –Fisher's Least Significant Difference at 5% level of Probability.

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

Effect of *F. oxysporum* isolates and varieties on some agronomic characteristics of Tomato at 12 weeks after sowing, are presented in Table 2. Plant height was significantly ($p \leq 0.05$) higher in the uninoculated control compared with the plants that were inoculated with various isolates of *F. oxysporum*. There was equally significant difference ($p \leq 0.05$) in plant height when tomato was inoculated with the various isolates.

Number of fruits per plant was significantly ($p \leq 0.05$) lower in plants inoculated with isolates of *F. oxysporum* compared with the uninoculated control. There was no significant difference in number of fruits per plant amongst the inoculated plants.

Fruit weight was significantly ($p \leq 0.05$) higher in the uninoculated control compared with the plant that were inoculated with the various isolates of *F. oxysporum*. There was also significant difference in fruit weight amongst the various isolates of *F. oxysporum*.

The number of branches in the uninoculated control was significantly ($p \leq 0.05$) higher compared with the inoculated plants.

The number of branches in Rio-grande was significantly ($P \leq 0.05$) higher compared with UC 82B (Table 2) while plant height, number of fruits and fruit weight were significantly ($p \leq 0.05$) higher UC 82B compared with 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≤0.05)	**13.93**	**1.22**	**31.36**	**2.50**
Varieties				
Rio-grande	19.76[b]	0.82[b]	20.40[b]	19.76[a]
UC 82B	106.10[a]	1.52[a]	48.50[a]	18.30[b]
FLSD(P≤0.05)	**5.94**	**0.52**	**13.37**	**1.07**

KEY: FoAd – *Fusarium oxysporum* from Adudu. FoAg – *Fusarium oxysporum* from Agromiller. FoAk – *Fusarium oxysporum* from Akor. FoAm – *Fusarium oxysporum* from Amih. FoAs1 – *Fusarium oxysporum* from Asase 1. FoAs2 – *Fusarium oxysporum* from Asase 2. FoNb – *Fusarium oxysporum* from Northbank. FoOr – *Fusarium oxysporum* from Orduen. FoSb – *Fusarim oxysporum* from Southbank. FoUAM – *Fusarium oxysporum* from University of Agriculture Makurdi. FLSD –Fisher's Least Significant Difference at 5% level of Probability.

DISCUSSION

Tomato cultivation is affected by a number of diseases among which wilt disease caused by *Fusarium oxysporum* is serious in major tomato-growing areas of the world (Anita and Rebeeth, 2009). Pathogenicity test of ten isolates of *Fusarium oxysporum* in this study showed that all the isolates were pathogenic on tomato plants causing rot in tomato fruits. The isolate FoUAM was the most virulent, causing severe infections on tomato. This result is similar with those of Joshi *et al*. (2013), who in their pathogenicity test found majority of *F. oxysporum* isolates to be pathogenic on tomato. They found that isolates generated wilt symptoms on at least one tomato plant; however wilt did not develop when non-pathogenic isolates were inoculated on the tomato plant. Varietal resistance is a choice to control fusarium wilt disease of tomato with the use of commercially bred varieties from the resistance genes (McGovern (2015). Fusarium wilt disease causes wilting of the whole plant by entering from the roots and spreading via xylem fibers, blocking the movement of water and thus causing wilting (Cox *et al.,* 2019). The high incidence of between 93% and 100% reported in this research corresponds to earlier findings of Heydari *et al.* (2007) and McGovern (2015). They authors reported 100% incidence of Tomato Wilt Disease (TWD) in USA. On the other hand, Houterman *et al.* (2007) reported fewer incidences of between 0% and 29% on resistant cultivars but 100% on susceptible cultivars in USA. Similar result was obtained by Abdel Fattah and Al-Amri (2012) reported 53-71% disease incidence while 47-78% disease severity of TWD in Egypt. Findings in this study deviate with Ishikawa *et al.* (2005) reported 50% disease severity in Japan, while Mandal *et al.* (2009) reported 40% TWD incidence in Japan. Results from this study indicate the incidence and severity of Fusarium wilt disease and damage on tomato crop in Benue state, Nigeria. The disease can reduce yield and undermine food sustainability in the country and increase cost of production.

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
The authors declared that there is no conflict of interest regarding the publication of this paper.

Funding Acknowledgement
This research received no specific grant from any funding agency in the public, commercial or not-for- profit sectors.

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