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Screening of Some Commonly Cultivated Tomato Varieties Against Fusarium Wilt in Jama'are, Bauchi State, Nigeria

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Abstract

Fusarium wilt disease of tomato is caused by Fusarium oxysporum f.sp. lycopersici and is a limiting factor to tomato production in Nigeria. This research was conducted to assess the effect of Fusarium oxysporum on some tomato varieties cultivated in Jama'are, Bauchi state. The objectives of this research work were to isolate and identify pathogen (Fusarium oxysporum) from the soil, to determine the incidence and severity of Fusarium wilt on different varieties of Tomato grown in Jama'are, and to determine the most resistance variety among four different varieties. The experiment was laid in a Completely Randomized design (CRD) with four replications. The four tomato varieties (Roma Savanna, Rio Grande, UC82B and Tropimech) were inoculated at the root region by making shallow groove around the base of the plant in the root region and placing 3 g mycelia plug of 7 days old pure culture of F. oxysporum face-down close to the root of the seedling and covered with soil. At two weeks intervals after treatments the seedlings were then observed for symptoms of wilt infection. Data on disease incidence (%), were collected based on observation after which they were analyzed using Analysis of Variance (ANOVA) test. The Disease Incidence Percentage for Roma Savanna, Rio Grande, UC28B and Tropimech were evaluated to be 51.6%, 18.2 %,38.4 % and 43.3 % respectively while the Disease Severity Percentage were 72.1%, 24.8%, 40.9% and 62.3% respectively. The findings of this research reveals that; there is a significant effect of F. oxysporum on the tomato varieties, all the varieties were severely infected to some degree, and that of all species, Roma savanna was moderately resistant to F. oxysporum to some degree. This agrees with other findings in the literature, as Fusarium oxysporum affects different varieties of tomato at different growth stages. Also, it was observed that screening excises involving selecting of non-infected seedlings with Fusarium species and other fungi should be done prior to transplanting.

Keywords: *Fusarium oxysporum*, Tomato, screening, transplanting, Jama'are

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INTRODUCTION

Tomato has become an important cash and industrial crop in many parts of the world (Ayandiji *et al*., 2011). Tomato production can serve as a source of income for most rural and urban producers in most developing countries. The tomato industry has been identified as an area that has the ability for poverty reduction because of its potential for growth and employment creation (Anang *et al*., 2013). Tomato (*Lycopersicon esculentum* Mill.) is the most commercially grown vegetable in Africa and parts of Northern Nigeria (Schippers, 2000). Its importance lies not only in profit, but also in the income generated in local economies for farmers and agricultural workers (Villarreal *et al.,* 2003). Among the diseases of this crop, fungal diseases are economically important and the most common diseases in vegetable production throughout the world (Kutama et. al., 2007). *Fusarium oxysporum f. sp. lycopersici(FOL)* has become one of the most damaging pathogen wherever tomatoes are grown intensively because it grows endophytically and persists in infested soils (Agrios, 1997). *Fusarium wilt* is one of the major problems of tropical vegetation which leads to damages to the seedbeds (Dean *et al*., 2012). *Fusarium oxysporum* is a soil borne fungal pathogen which has the ability to infect plants at all stages of plant growth through roots, causing necrosis and wilting symptoms in many crop plants which leads to major economic losses (El-Khallal, 2007). *F. oxysporum* f. sp.*lycopersici* (Fol) is the cause of Fusarium wilt of tomato which is considered a major restrictive factor in the production of tomato (Ignjatov *et al*., 2012). *Fusarium wilt* is one of the major problems of tropical vegetation which leads to damages to the seedbeds (Dean *et al*., 2012). *Fusarium oxysporum* affects tomato plant during all life stages (seedling stage, flowering stage, and fruiting stage), it can also affect the whole plant parts and causes great loss (Decal *et al*., 2000). Furthermore, it enters the epidermis of root, later spreads through vascular tissue and inhabits plant xylem vessels (Singh *et al.,* 2007). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), a soil borne plant pathogen in the class Hyphomycetes, causes *Fusarium* wilt specifically on tomato. This disease was first described by G.E. Massee in England in 1895. The pathogen has three physiological races (1, 2, and 3, hereafter r1, r2 and r3) and are distinguished by their specific pathogenicity on tester plants carrying dominant race-specific resistance genes. The disease is of worldwide importance where at least 32 countries had reported the disease, which is particularly severe in countries with warm climate. The *Fusarium* fungus is a known pathogen of tomato plant which is present in all important tomato growing regions of the world (Umar *et al*., 2013) and produces three types of asexual spores; microconidia, macroconidia and chlamydospores. Some strains of *Fusarium oxysporum* are not pathogenic and may even antagonize the growth of pathogenic strains and can be used as biological agents. Symptoms of attack first appear as slight vein clearing on the outer portion of the young leaves followed by epinasty of the older leaves. This symptom often occurs on one side of the plant or on one shoot. Successive leaves yellow, wilt and die, often before the plant reaches maturity. As the disease progresses, growth is typically stunted, and little or no fruit develops. If the main stem is cut, dark brown streaks may be seen running lengthwise through the stem. The browning of the vascular system is characteristic of the disease and generally can be used for its identification. On the outside of affected stems, white, pink or orange fungal growth can be seen especially in wet conditions (Hadiza *et al*., 2018).

The pathogen enters the plant through root tips and can remain viable in the soil for up to 30 years. The mycelium grows in the xylem vessels where they cut off water supply resulting to wilting. There is often an association of *Fusarium* wilt and nematode colonization where the nematodes provide entry route for the fungus. Enzymes may also facilitate *Fusarium* penetration into plant host. Infection and disease development in *Fusarium* wilt is favoured

by warm soil temperature and low soil moisture. The disease tends to be most severe in sandy soils and generally less of a problem in heavier clay soils (Kutama *et al*., 2013).

The control of *Fusarium* wilt of tomato is important in maintaining plant vigour and fruit quality and quantity. Though *Fusarium* wilt is a difficult disease to control. Numerous strategies have been proposed to control this fungal pathogen. However, attempts to control the disease have experienced limited success due mainly to emergence of new pathogenic races. Documented methods that are used in the control of the disease include cultural, biological, use of resistance, chemical and use of natural products (Kutama *et al*., 2013).

Jama'are town of Bauchi state, Nigeria is blessed with fertile irrigation land and a large portion of the irrigated fields are put to tomato cultivation seasonally. However, in recent years the production of this valuable commodity has drastically declined and farmers are losing a lot of commercial yield of this crop. The total yield loss has been rising over the years and it was later attributed to disease infection seasonally which reduces the yield of the crop. Amongst the common diseases discovered that affects this crop was wilt caused by *Fusarium oxysporum*. Therefore, the aim of this research was to screen four tomato varieties commonly grown in Jama'are against fusarium.

MATERIALS AND METHODS

Collection of soil sample, Isolation and Identification of *F. oxysporum* from soil

Soil samples of infected Tomato plants were obtained from farmers' fields in Jama'are irrigation sites and taken to the Biological Sciences Laboratory, Federal University Dutse for isolation of pathogen (*F. oxysporum*) from the soil. Two types of fertilizer N P K (15:15:15) and UREA were obtained from retail-shop in Jama'are market.

Experimental design

Field experiment was laid out on a Complete Randomized Design (CRD) with four replications each of the four tomato varieties.

Media preparation

Potato Dextrose Agar (PDA) was prepared using the method of Smith and Onion (1983). Exactly 7.5 grams of PDA powder was dissolved in 192 ml of distilled water in a sterile conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and autoclaved at 121 ^{0}C for 15 minutes under a pressure of 100kPa. The medium was cooled after autoclaving and dispensed aseptically into sterile Petri dishes. Streptomycin (30mg/l) was added to the medium to prevent the growth of bacteria.

Culturing of fungal pathogen

The serial dilution plating method was used to dilute the soil sample as described by Waksman (1920).The soil samples were inoculated in Potatoes Dextrose Agar (PDA) and then incubated at room temperature (25 ^{0}C) for 4-7 days. The resulting mixed culture was sub cultured on PDA to obtain the pure cultures of few fungal isolates that appeared on the plates.

Identification of fungal pathogen

Identification of isolated fungi was conducted by comparing morphological appearance of fungal isolates with the atlas of *Fusarium*, as described by Leslie *et al.* (2006) and Samsun *et al.* (2008).

Sourcing of Four Tomato Varieties
Four different varieties of tomato seed namely; Roma Savanna, Rio Grande, UC28B and Tropimech) were obtained from IITA (International Institute of Tropical Agriculture) Kano station and these were raised in nursery as recommended by IITA (2012) and FMARD (2018).

Clearing of land and bed preparation
A portion of 25m^2 of land was obtained from Jama'are tomato irrigated hot spot fields, measured, cleared and tilled thoroughly and the soil was kept loose for construction of good seed beds. Seed beds were wide and long as possible and incorporated with 10g of N.P.K. (15:15:15) (FMARD, 2018).

Watering of beds
After germination of the seeds, watering continued at two days intervals for three weeks with the aid of watering can to prevent the seedlings from drying off.

Transplanting of seedlings
After the permanent site was pre irrigated about an hour before transplanting, in the evening, three weeks old seedlings were transplanted on the ridges employing 60cm x 45cm spacing (FMARD, 2018).

Inoculation of Pathogen in to tomato seedlings
The four tomato varieties were inoculated at the root region as described by Ford *et al.* (2015) by making shallow groove around the base of the plant in the root region and placing 3 g mycelia plug of 7 days old pure culture of *F. oxysporum* face-down close to the root of the seedling and covered with soil. The seedlings were then observed for symptoms of infection.

Data Collection
The following data was collected at week's interval after inoculations;

i. Disease incidence
ii. Disease Severity

Data Analysis
The Microsoft EXCEL was used to organize the raw data generated from the study and then subjected to Analysis of Variance (ANOVA). Data from individual cultivar was subjected to One way Analysis of variance (ANOVA) using Genstat, a statistical software package. Significant difference among treatment means was determined using Least Significant Difference (LSD) at 5% level of significance.

Disease Incidence
The disease scoring and data of disease incidence was determined from each selected stand were taken at two weeks intervals, starting from 6 weeks after Inoculation (WAI) to 10 WAI in order to find the percentage of infection cause by disease on the plants. Disease incidence was recorded by counting the number of infected plants and dividing it with the total number of plant assessed in each treatment. The result obtained was converted to percentage by using the following formula of disease incidence:

Disease Incidence Percentage [DIP] = $\frac{\text{number of infected plants}}{\text{Total number of plant}}$ X 100 %

Or, $DIP = \frac{n}{N} \times 100$ %

Where: n= number of plants showing wilts symptoms
N = Total number of plant sampled (Michel *et al.,* 2006).

Disease Severity

After making keen observations on how severe the disease infected a plant or not at all, the Disease Severity Percentage was calculated using the formula;

$$\text{Disease Severity Percentage (DSP)} = \frac{\text{Number of Individual Ratings}}{\text{Number of Plants Assesed}} \times 100\ \%$$

(Michel *et al.,* 2006).

The Disease Severity Rating was assigned using a scale of 1 – 5 as described by Kutama *et al.* (2010b).

Where: 1= no visible symptoms, 2 = 1 – 10 % slightly severe, 3 = 11 – 25 % moderately severe, 4 = 26 – 59 % severe infection 5 = 59 – 90 % highly susceptible, necrosis and complete death of plant occur.

Results and Discussion

Results

The tables below presents a data of all the measurement and calculation made as well as statistics taken which are paramount for this research.

Disease incidence percentage (DIP), Disease Severity Percentage (DSP) and Disease Severity Rating (DSR), at 2 weeks interval after transplanting.

As can be seen in Table 1, the result obtained showed that at 8WAT, RG infected with *Fusarium oxysporum* recorded the highest diseases incidence of 52% followed by UC infected with *Fusarium oxysporum* and TRP infected with *Fusarium oxysporum* (38% and 43.7%, respectively) while the lowest disease incidence was found on RS infected with *Fusarium oxysporum* (18.3% respectively). At 10WAT the data obtained showed that RG infected with *Fusarium oxysporum* has the highest valve rating as (60.3%), followed by TRP infected with *Fusarium oxysporum* and TRP infected with *Fusarium oxysporum* rating as (41.5% and 45% respectively) while RS infected with *Fusarium oxysporum* has the lowest valve rating as (18.5% respectively). At 10WAT the result obtained showed that RG infected with *Fusarium oxysporum* has the high infection followed by TRP infected with *Fusarium oxysporum* and UC infected with *Fusarium oxysporum* while RS infected with *Fusarium oxysporum* has the least fungal infection. The Disease Severity Rate (DSR) result obtained showed that. At 8WAT the high disease severity was observed in RG infected with *Fusarium oxysporum* rating of (70.0% respectively). The lowest disease severity was observed in RS infected with *Fusarium oxysporum* rating of (23.7.0% respectively) with an exception of control .At 10WAT the result obtained showed that RG infected with *Fusarium oxysporum* rating of (74.0% respectively) was highly susceptible followed by TRP infected with *Fusarium oxysporum* which was severe infection rating of (61.7% respectively)while RS infected with *Fusarium oxysporum* has least infection rating of (24.3% respectively) which was moderately severe and the control of each variety were slightly severe rating of (3.0%,3.7%,3.0% and 2.0%). At 12WAT RG infected with *Fusarium oxysporum* and TRP infected with *Fusarium oxysporum* were highly susceptible rating of (74% and 62% respectively) followed by UC infected with *Fusarium oxysporum* was severe infection rating of (43.5% respectively) while RS infected with *Fusarium oxysporum* was moderately severe and the control of each variety were slightly severe rating as (2.9%, 3.7%, 2.3 and 2.0%, respectively).

This finding suggests that the healthy tomato (control) were infected due to airborne diseases. In line with the work of Samia *et al.* (2017) reported that percentage of disease incidence of non infected plants were almost negligible and probably due to soil contaminations from neighboring inoculated soil and or airborne spore production of the infected plants. This work is also ssuggests that environmental condition plays an importance role in increasing high

disease incidence and severity. This is in line with the work of Alexandrove (2005) who reported that extremely low or high temperature lead to noticeable break down in the level of Resistance of plants to pathogen. In addition, in the recorded mean of all the disease parameters, the mean value of the above mentioned varieties, almost consistently, are next to the control in descending order. This result therefore validates the alternative inference of the second hypothesis since at least one of the varieties showed some resistance of some degree RS (Roma Savanna). And UC (UC82B) also have a low DSP of 22.6 % and 43.0 % respectively making their severity ratings to be 3 (moderately severe) and 4 (severe infection) respectively. However, all the varieties were observed to show severity of some degree. Therefore, the null hypothesis was rejected and the alternative hypothesis was accepted which states that, "The effect of *Fusarium* wilt is severe on the tomato varieties grown in Jama'are" the highest disease severity was observed in Rio Grande rating 0f 72.3% respectively compare with Rio Grande control and other three tomato varieties while the lowest disease severity was observed in Roma savanna infected with *fosarium oxyforum*. A research on wilt disease of tomato carried in Nigeria by Akaeze and Aduramig ba-Modupe (2017) recorded that a DIP of Tropimech and UC82B is 66 %. The difference in values of their findings with that of this research [UC (UC82B) = 43.5 % and TRP (Tropimech) = 64.3 %] could have emanate from geographical and agricultural factors. It is, nevertheless, noteworthy that the difference in the DIP values for Tropimech for both researches is not huge. Another research carried out in Eritrea recorded a DIP for 5 varieties of tomato ranging from 59.7 – 97.8 %and a DSP that ranges from 17.5 – 82.9 % in two farms (Rao *et al*., 2016). This finding suggest that the tomato varieties evaluated in this study were susceptible to *Fusarium oxysporum* with the exception of tomato Roma Savanna which is moderately resistant at some degree with rating valve of 24.8 %, respectively.

Table 1. Effect of *F. oxysporum* on Disease incidence percentage (DIP) of different variety of tomato cultivated at 2 weeks interval after transplanting.

TREATMENT	MEAN DIS INC% IN WAT		
TRT COMBNT.	**DIS INC6**	**DIS INC8**	**DIS INC810**
R S (CTRL)	0.0^{d}	0.0^{d}	0.3^{e}
R S + F.O	17.7^{c}	18.3^{c}	18.5^{d}
R G (CTRL)	0.3^{d}	0.3^{d}	1.3^{e}
R G + F.O	42.3^{a}	52.3^{a}	60.3^{a}
UC(CTRL)	0.0	1.3^{d}	1.3^{e}
UC+F.O	35.7^{b}	38.0^{b}	41.5^{c}
TRP (CTRL)	0.2^{d}	0.3^{d}	0.5^{e}
TRP +F.O	41.3^{a}	43.7^{b}	45.0^{b}
LSD	**3.31**	**5.8**	**4. 7**

*The mean value within the column with the same latter are not significantly different at **($p \leq 0.05$)** levels using Fisher' 5 LSD (Least significant Difference)

Key to tomato varieties used:

RS,=Roma Savanna, RG =Rio Grande, UC = UC82B, and TRP= Tropimech

Table 2. Effect of *F. oxysporum* on Disease Severity Percentage (DSP) of different variety of tomato cultivated at 2 weeks interval after transplanting.

TREATMENT	MEAN DIS SEVERITY % IN WAT		
TRT COMBNT.	**DIS SEV8**	**DIS SEV10**	**DIS SEV12**
R S (CTRL)	1.7^{e}	3.0^{d}	4.0^{e}
R S + F.O	23.7^{d}	24.3^{d}	26.6^{d}
R G (CTRL)	2.6^{e}	3.7^{d}	3.7^{e}
R G + F.O	70.0^{a}	72.0^{a}	74.3^{a}
UC(CTRL)	1.6^{e}	2.3^{d}	2.7^{e}
UC+F.O	38.3^{c}	41.0^{c}	43.5^{c}
TRP (CTRL)	1.0^{e}	2.0	3.0^{e}
TRP +F.O	60.5^{b}	61.7^{b}	64.3^{b}
LSD	**5.11**	**4,91**	**3.5**

*The mean value within the column with the same latter are not significantly different at **($p \leq 0.05$)** levels using Fisher' LSD (Least significant Difference)

Key to tomato varieties used:

RS,=Roma Savanna, RG =Rio Grande, UC = UC82B, and TRP= Tropimech

Table 3. Disease incidence percentage (DIP), Disease Severity Percentage (DSP), Disease Severity Rating (DSR) and Status.
Tomato cultivated at 1 weeks interval inoculation.

	Mean Number of D .I and D.S			
	At 6 to 10 Weeks After Inoculation			
Treatment	**DIP (%)**	**DSP (%)**	**DSR***	**STATUS**
R G+ *F. O*	51.6	72.3	5	Highly susceptible
R S + *F. O*	18.6	24.8	3	moderately sever
UC28 + *F. O*	38.4	40.9	4	Severe infection
TRP + *F. O*	43.3	62.3	5	highly susceptible
Control	0.64	3.7	1	slightly severe

Key:

*Disease Severity Rating: 1= no visible symptoms, 2 = 1 - 10 % slightly severe, 3 = 11 – 25 % moderately severe, 4 = 26 – 59 % severe infection 5 = 59 – 90 % highly susceptible, necrosis and complete death of plant occur (Kutama *et al.* 2010b).

Key to tomato varieties used:

RS,=Roma Savanna, RG =Rio Grande, UC = UC82B, and TRP= Tropimech

Conclusion

The results of this research is an eye-opener on the effect of *Fusarium* wilt on the production of tomato. The result of this research revealed that spores of *Fusarium oxysporum* were present in the soil used in Jama'are irrigations sites for cultivation of many agricultural crops.The research discovered that inoculation of *Fusarium oxysporum* to healthy tomato plant lead to cause *Fusarium* wilt of tomato.

The research also established that, Percentage of disease Incidence for Roma Savanna, Rio Grande, UC28B and Tropimech were evaluated to be 51.6%, 18.2 %, 38.4 % and 43.3 % respectively while the Percentage disease Severity were 72.0%, 24.8%, 40.9% and 62.3% respectively. Therefore, Roma Savanna infected with *Fusarium oxysporum* was moderately

affected compared to Rio Grande and Tropimech infected with *Fusarium oxysporum* which were severely affected.

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