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FUSARIUM WILT DISEASE OF TOMATO: SCREENING FOR RESISTANCE AND *IN-VITRO* EVALUATION OF BOTANICALS FOR CONTROL; THE NIGERIA CASE

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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. The objectives of this study were to screen tomato varieties commonly cultivated in Nigeria to determine their host resistance status to *F. oxysporum*, identify fungi species associated with tomato plants showing Fusarium wilt symptoms and the *in-vitro* evaluation of aqueous and methanol extracts of four botanicals for bioactivity. Symptomatic sample plants were collected from three farms in tomato producing communities of Nigeria. Isolation was done by direct plating method on acidified Potato Dextrose Agar (PDA). Fungi isolates were identified by cultural and microscopic characteristics. Extracts were prepared and evaluated for bioactivity by agar dilution method. The experiment was laid in a Completely Randomized Design replicated three times. Data were analyzed by descriptive and statistical analysis. Significantly different means were separated using Least Significant Difference at 5% level of significance. Of the nine varieties evaluated, only Tomato Shanty^{+}N showed moderate resistance to *F. oxysporum*, while others were susceptible. Fungi isolated from symptomatic plants were *Colletotrichum spp, Curvularia lunata, Sclerotia rolfsii, Rhizopus spp, Pestalocia macrotrica, Aspergillus spp, and Fusarium oxysporum*. Extracts showed bioactivity against *F. oxysporum* at different concentrations. Methanol as solvent in preparing of *Azadirachta indica* leaf extract showed the highest inhibitory properties against *F. oxysporum* at low concentration. This was followed by *Morinda lucida* and *Tagetes erecta* at 37.5% and 25% concentration respectively. Tomato shanty^{+}N and low concentration methanol extracts of *Azadirachta indica* leaf (12.5%) are therefore recommended.

Keywords: *Fusariun oxysporum* f. sp. *lycopersici*, *Solanum lycopersicum*, Plant extracts, Bioactivity, *in-vitro*, Pathogenicity

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the Family Solanaceae. It is cultivated globally as an annual crop for its edible fruits. The fruit which is high in nutrients, vitamins (such as vitamins A and C) and minerals such as potassium, phosphorus, magnesium, calcium, and fair concentrations of protein and Niacin (**Norman, 1992; Kaushik *et al*, 2011; USDA, 2009**) has been used in Nigeria for a very long time as ingredients of meals, sauces, salads, soups and ketchup (**Nicola *et al*., 2009**). Despite numerous genetic improvements (**Ann, 2002**) in the development of varieties that are resistant to *F. oxysporum*, tomato is still susceptible to Fusariun wilt diseases caused by *Fusarium oxysporum* f. sp. *lycopersici*. The disease is reported to cause great losses in tomato production globally, but especially in Warmer climates (**Blancard, 1994**), presenting symptoms such as progressive chlorosis in part or all of the plant (beginning from lower leaves), stunting and wilting which, often become observable at maturity of the plant after flowering and initiation of fruit set. Losses due to Fusarium wilt in tomato can be severe sometimes resulting in total and complete loss of yield under greenhouse and field conditions **(Walker, 1971; Benhamou *et al.*, 1989)** The objectives of this study were to screen tomato varieties commonly cultivated in Nigeria for their host resistance status to *F. oxysporum*, identify fungi species associated with tomato plants showing Fusarium wilt symptoms in Nigeria and the *in-vitro* evaluation of aqueous and methanol extracts of four botanicals for bioactivity against the wilt causal pathogen.

MATERIALS AND METHODS

The experiment was conducted in the Phytopathology Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan. Samples of tomato plants showing wilt symptoms were collected in well labeled sample bags from farmers' fields in 'Saki' and 'Fiditi' in Oyo state and 'Ponlade' in Ogun states, Nigeria. The samples were taken to the laboratory where they were immediately prepared for isolation.

Seed collection

Seeds of nine tomato varieties (seven exotic varieties and two local varieties) commonly cultivated in Nigeria obtained from the University of Ibadan, and certified seed retail outlets in Nigeria (Table 1)

Planting and transplanting

Seeds of each tomato variety were planted on well labeled seed trays containing sterilized top soil and river sand mixed to a ratio of 1:1 and placed inside the screen house. Transplanting of tomato seedlings were done two weeks after planting, into experimental pots each containing 10 kg of sterile soil.

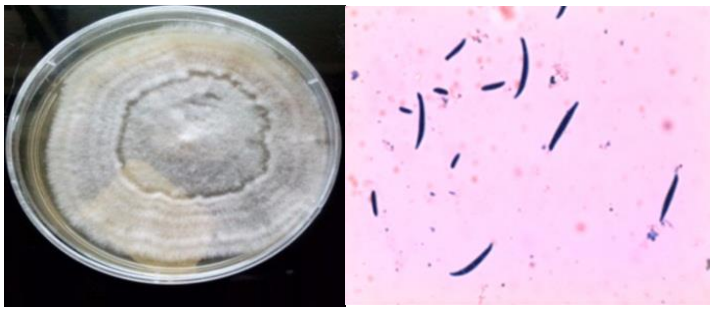
Table 1Type, source and general characteristics of tomato varieties commonly cultivated in Nigeria

S/N	Tomato variety	Source of seeds	Type	General characteristics
1	Beske	Dept. of CPEB, UI	Local	Sturdy, high yield potential, intermediate maturity period
2	Ibadan local	Dept. of CPEB, UI	Local	Sturdy, high yield potential, intermediate maturity period
3	Roma VF	Agro-tropic limited,	Exotic	Determinate, medium fruit size, resistant to Fusarium wilt and Verticilium wilt
4	Roma Savanna	Agro-tropic limited,	Exotic	Resistant to *Verticillium dalhiae*, *Fusarium oxysporum* sp. *lycopersici* race 0.
5	Tima	Premier Seed Limited	Exotic	Dry season cultivation, erect, oval shape, resistant to Verticillium, matures 80 DAT
6	UC82B	Premier Seed Limited	Exotic	Resistant to Verticillium and *Fusarium oxysporum* sp. *lycopersici* race 0, Determinate, mainly dry season cultivation, matures 70-80 days after transplant.
7	Tropimech	Agro-tropic	Exotic	Determinate, high fruit setting, maturity: 65-68 DAT, tolerant to nematodes &TYLCV
8	Rio Grande	Rade Farms Limited	Exotic	Determinate, cylindrical elongated fruits, harvest: from 5/6 months
9	Tomato Shanty+N	Rade Farms Limited	Exotic	Disease resistance: FHR: Vd, Fol (RACE 1, 2), Pst, TSWV. IR:SI, TYLCV. Very high yield potential, long shelf life.

Legends: CPEB, UI= Crop Protection and Environmental Biology, University of Ibadan. S/N = variety serial number

Inoculation of nine tomato varieties for resistance to *Fusarium oxysporum* f. sp. *lycopersici*

The 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 5 g mycelia plug of 7 days old pure culture of *F. oxysporum* f. sp. *lycopersici* face-down close to the root of the seedling and covered with soil. The seedlings were then observed for symptoms of infection.



A **B**

Figure 1 A: 7 day old pure culture of *F. oxysporum* **B:** Photomicrograph of *F. oxysporum* spores

Isolation and identification

Isolation was done using the direct plating method of **Okhuoya *et al.*, (2012)**. Plant parts sampled (root, stem, leaf) were washed thoroughly in running water, cut into small pieces with a sterile scalpel, surface-sterilized in 1% sodium hypochlorite, rinsed in three changes of sterile distilled water, air-dried on sterile filter paper and then plated on Petri-dishes containing Potato Dextrose Agar (PDA) acidified by addition of few drops of lactic acid per 100 ml of media. The inoculated plates were labeled and incubated at 28^{o}C ± 2^{o}C for 72 hours. Sub-culturing based on different cultural characteristics (color of culture, growth rate, sporulation rate and color etc.) was done by picking little pinch of mycelial mass from isolates and inoculating into new petri-dishes containing sterilized PDA with the aid of a sterilized inoculating needle and was incubated at 28^{o}C ± 2^{o}C. After 7 days, a second tier sub-culturing of the hyphal ends (Hyphal tipping) of each fungal isolate was done to ensure that the cultures obtained were pure. Identification was done based on morphological and cultural characteristics exhibited by the various fungal isolates and comparing with the description of **Barnett and Hunter (1998)**.

Pathogenicity test

The root dip method as described by **Nirmaladevi and Srinivas (2012)** was used to ascertain the causal organisms for wilt symptoms observed in the field. 2 weeks old tomato plants were inoculated at the roots. The roots were first thoroughly washed in running water after which lateral roots was trimmed with sterile scissors before dipping them in a beaker containing the spore suspension of the various fungi after which it was left for 30 minutes before transplanting is done. The plants were closely monitored for expression of symptoms.

Extract preparation

Botanicals were prepared using the procedures of **Amadioha, (1999)**. Fresh leaves of *Azadirachta indica*, *Morinda lucida* and *Targetes erecta*, were collected and air dried for 14 days after which they were ground with the aid of a mechanical grinder to powder. *Azadirachta indica* fruit was also collected, de-pulped and threshed. The seeds were grounded to powder. Two solvents (methanol and water) were used in extract preparation at 12.5, 25, 37.5 and 50% concentrations. Aqueous extracts were prepared by adding 12.5, 25, 37.5 and 50g of leaf and seed powder into a beaker and adding sterile distilled water until the 100 ml mark on the beaker. The contents in the beaker were thoroughly mixed and left to stand for 24 hours. Thereafter it was filtered with a Whatmann No.1 filter paper into a receptacle for use. Methanol extracts were prepared by weighing fifty grams (50g) of dried leaf and seed powder into a 1000 ml conical flask. Methanol (200 m) was then added and vigorously stirred and mounted on a mechanical shaker for 30 minutes at 50 rpm and left undisturbed for 24 hours. The mixture obtained was filtered with Whatmann No.1 filter paper. The filtrate was then evaporated to dryness using a Rotary vacuum evaporator. Stock solution was then prepared by diluting 2 g of the extract in 20 ml of sterile distilled water. Concentrations of 12.5, 25, 37.5 and 50 % methanol extracts were prepared by diluting 2.5, 5, 7.5, 10 ml in 17.5, 15, 12.5 and 10 ml of sterile distilled water respectively.

***In-vitro* evaluation for bioactivity**

In-vitro evaluation for bioactivity was done using the agar dilution method described by **Okhuoya, (2012)**. Two perpendicular lines were drawn at the bottom of each Petri-dish (**Amadioha and Obi, 1999)**. One (1) ml of extract was introduced into sterile Petri-dishes, then PDA (10 ml) was then added to the Petri-dishes containing the extracts and gently swirled to ensure homogeneity of mixture. Five (5) mm agar plug of 7 day old *F. oxysporum* f. sp. *lycopersici* culture was placed at the center of each petri-dish containing PDA (at the intersection of the two perpendicular lines). The radial growth was measured at 24 hours interval over a three day period.

Data collection

Data was collected on: plant height, number of branches per plant, number of flowers, disease incidence and severity, and host status ratings, percentage diseases incidence

Given as:

$$D = \frac{n}{N} \times 100$$

Where: n= number of plants showing wilts symptoms

N = Total number of plant sampled **(Michel *et al.*, 2006)**

Severity ratings for *Fusarium oxysporum* inoculated plants were done using a scale of 1-6 as described by **Marley and Hilocks (1996).**

Where: 1= no symptoms, 2= chlorosis and wilting of the first branches, 3= chlorosis and wilting of second and third branches, 4= chlorosis above third, second and third branches may be lost, 5= chlorosis and partial desiccation, 6 = complete death of plant

Host status ratings for *Fusarium oxysporum* was done using a scale of 1-5 as adopted by **Silme and Cagirgan, (2010)** which was based on infection percent as follows:

1-10% = 1, 11-20% =2, 21-30% = 3, 31-50% = 4, 51-100% = 5

Where: 1= resistant (R), 2 = moderately resistant (MR), 3 = moderately susceptible (MS), 4= susceptible (S), 5= highly susceptible (HS)

After which the percentage growth inhibition was calculated according to **Pandley *et al.* (1982)**

$$\text{Bioactivity/growth inhibition (\%)} = \frac{Dc-Dt}{Dc} \times 100$$

Where: Dc =Average diameter of the pathogen in the control plates

Dt = Average diameter of the pathogen in the treatment plates

RESULTS AND DISCUSSION

Disease incidence, severity and host status of tomato evaluated varieties

The lowest disease incidence was observed in tomato shanty^{+}N with 33% at the 10th week after planting. Tropimech and UC82B had 66% incidence each while all other varieties had 100% disease incidence (Figure 1). The highest disease severity was observed in Rio grande and Tima with severity ratings of 4.66 and 4.67 respectively. The lowest disease severity was observed in Shanty^{+}N with a severity rating of 3 (Figure 2). Tomato shanty^{+}N had a host status rating of

moderately resistant (2). Tima and UC82B are moderately susceptible while other varieties tested are susceptible to *Fusarium oxysporum* (Table 2). The tomato varieties evaluated in this study were susceptible to *Fusarium oxysporum* f. sp. *lycopersici* with the exception of tomato shanty^{+}N which was moderately resistant with a host rating of 2. The environmental conditions such as the high day time temperature observed in the screen house may have been responsible for the break down of resistance of the various tomato varieties; this is in line with **Alexandrov, (2005)** who reported that extremely low or high temperature leads to a noticeable breakdown in the level of resistance of plants to pathogens.

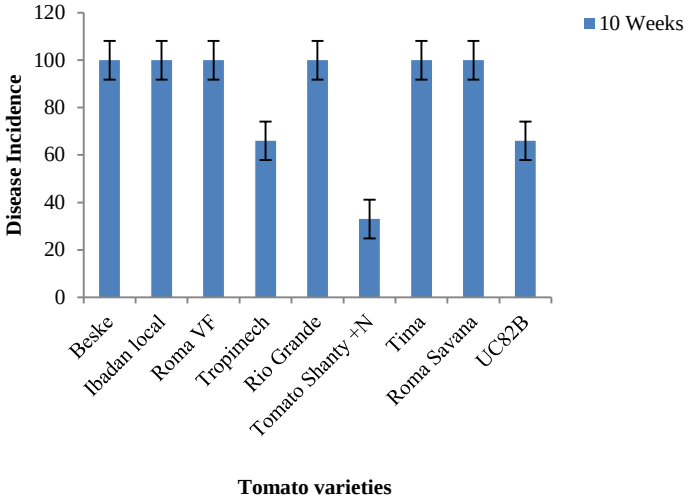


Figure 1 Disease incidence of *Fusarium oxysporum* f. sp. *lycopersici* inoculated tomato plants at 10 weeks after planting

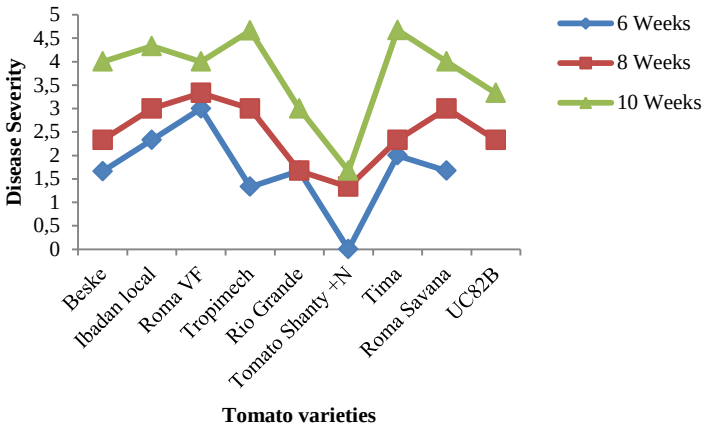


Figure 2 Disease severity of F. oxysporum inoculated tomato plants at 6, 8 and 10 weeks after planting

Table 2 Severity ratings (At 10th weeks after planting), host ratings and status of evaluated tomato varieties

Variety	Severity ratings(1-6)	Host ratings(1-5)	Status
Beske	4	4	Susceptible
Ibadan Local	5	4	Susceptible
Roma V.F	5	4	Susceptible
Tropimech	5	4	Susceptible
Rio Grande	4	4	Susceptible
Tomato Shanty^{+}N	3	2	Moderately resistant
Tima	3	3	Moderately susceptible
Roma Savana	4	4	Susceptible
UC82B	4	3	Moderately susceptible

Fungi associated with fusarium wilt disease

The fungi species isolated from tomato plants showing fusarium wilt symptoms were *Fusarium oxysporum, Curvularia lunata, Pestalocia macrotrica, Bispora spp, Sclerotia rolfsii, Rhizopus spp, Aspergillus spp, Colletotrichum gloesporoides, Blastomyces spp, Chaonepora spp, and Rhizoctonia spp. Fusarium oxysporum* had the highest frequency of occurrence of 37% in 'Fiditi' while *Sclerotia rolfsii* and *Pestalocia macrotrica* had the lowest frequency of occurrence of 9% each. Frequency of occurrence of *Fusarium oxysporum, Rhizopus spp* and *Aspergillus spp* in 'Ponlade' were 30%, 14% and 14% respectively. *Bispora spp* and *Fusarium oxysporum* had the highest frequency of occurrence in 'Saki' of 30% while *Curvularia lunata* had a frequency of 10%. (Table 3)

Table 3 Fungal isolates associated with tomato wilt disease from three locations in south western Nigeria

Location	S/N	Organism	Plant part	Frequency of occurrence (%)
'Fiditi'	1	*Fusarium oxysporum*	Root, stem	37%
	2	*Colletotrichum gloesporoides*	Root	27%
	3	*Bispora spp.*	Root	18%
	4	*Sclerotia rolfsii*	Root	9%
	5	*Pestalocia macrotrica*	Leaf	9%
'Ponlade'	1	*Rhizopus spp.*	Root, stem	14%
	2	*Aspergillus spp.*	Root, stem, leaf	14%
	3	*Colletotrichum gloesporoides*	Root, stem	21%
	4	*Sclerotia rolfsii*	Root	7%
	5	*Blastomyces spp.*	Leaf, root	7%
	6	*Fusarium oxysporum*	Stem, root, leaf	30%
	7	*Chaonephora spp.*	Leaf,	7%
'Saki'	1	*Bispora spp*	Root, stem	30%
	2	*Fusarium oxysporum*	Leaf, stem, root	30%
	3	*Curvularia lunata*	Root, stem	10%
	4	*Rhizopus spp*	Root, stem	20%
	5	*Rhizoctonia spp.*	Root	10%

Legend: S/N = Isolate serial number

Table 4 shows the mycelial growth inhibition of *Azadirachta indica* leaf extracts on *F. oxysporum* three days after application. There was an inverse relationship in the bioactivity of methanol and aqueous extracts of *A. indica* leaf. The highest inhibition of methanol extracts was recorded at the lowest concentration of 12.5% (13.00), further increase in concentration applied resulted in non-significant decline in the mycelial growth inhibition, with the highest concentration of 50% been the least effective (17.36). Whereas, the mycelial inhibitory potential for aqueous extracts of *A. indica* leaf increased per increase in concentration applied with the highest mycelial growth inhibition (24.33) recorded for the highest concentration (50%)

Table 5 shows the effect of *A. indica* seed extract on radial growth of *F. oxysporum*. Methanol extracts of *A. Indica* seed shows bioactivity at lower concentration of 12.5% (19.00). This value was not significantly different from higher concentrations (25, 37.5 and 50%) at 5% level of probability. Aqueous extract of *A. indica* seeds showed highest inhibition of 33.30 at the highest concentration (50%). This was not significantly different from other levels of concentration but was significantly differeny from the control (45.00).

Methanol and aqueous extracts of *Morinda lucida* showed anti-microbial potentials at all levels of concentration (Table 6). Higher inhibition of radial growth was observed with the methanol extract. The level of concentration applied did not significantly influence the level of mycelial growth inhibition at 50, 37.5 and 25%, but was significantly lower at 12.5% concentration at 5% level of probability. The aqueous extract of *M. lucida* was most effective for the control of *F. oxysporum* at 50% (31.33). this value was not significantly different from 12.5% (33.00), but was significantly lower than control, 25% and 37.5%

Methanol extracts of *Tagetes erecta* leaf significantly inhibited myceial growth of *F. oxysporum* at all level of concentration. Methanolic extract at 25% gave the highest inhibition with a mean radial growth of 19.00. This value was significantly lower than control (45.00), 12.5% (27.33) and 50% (24.00) but was not significantly different from 37.5%. Irrespective of the concentration applied, aqueous extracts of *T. erecta* significantly inhibited radial growth of *F. oxysporum*, though higher inhibition was observed at the highest concentration of 50% (24.33) (Table 7)

Table 4 Mycelial growth inhibition of *Azadirachta indica* leaf extract against *Fusarium oxysporum* f. sp. *lycopersici*

Treatment concentration	Solvent	
	Methanol	Aqueous
Methanol solvent (control)	6.66 ± 1.08	--
Water solvent (control)	45.00 ± 1.41	45.00 ± 1.41
12.5%	13.00 ± 1.22	31.60 ± 0.81
25%	16.33 ± 0.41	30.00 ± 0.70
37.5%	16.69 ± 1.47	28.70 ± 2.16
50%	17.36 ± 0.81	24.33 ± 1.45
LSD (P ≤ 0.05)	**4.34**	**3.73**

Legend: values presented in tables are means of radial growth (cm) of *Fusarium oxysporum*, three days after extracts application

Table 5 Mycelial growth inhibition of *Azadirachta indica* seed extract against *Fusarium oxysporum* f. sp. *lycopersici*

Treatment concentration	Solvent	
	Methanol	Aqueous
Methanol solvent (control)	6.66 ± 1.08	--
Water solvent (control)	45.00 ± 1.41	45.00 ± 1.41
12.5%	19.00 ± 2.54	34.30 ± 0.81
25%	20.33 ± 1.08	34.00 ± 0.70
37.5%	22.63 ± 1.63	33.39 ± 1.08
50%	22.60 ± 1.77	33.30 ±1.77
LSD (P ≤ 0.05)	**5.32**	**3.15**

Legend: values presented in tables are means of radial growth (cm) of *Fusarium oxysporum*, three days after extracts application

Table 6 Mycelial growth inhibition of *Morinda lucida* leaf extract against *Fusarium oxysporum* f. sp. *lycopersici*

Treatment concentration	Solvent	
	Methanol	Aqueous
Methanol solvent (control)	06.66 ± 1.08	--
Water solvent (control)	45.00 ± 1.41	45.00 ±1.41
12.5%	26.33 ± 0.40	33.00 ± 0.70
25%	21.33 ± 0.40	38.30 ± 1.08
37.5%	19.62 ± 1.08	34.60 ± 1.08
50%	21.00 ±0.70	31.33 ± 1.87
LSD (P ≤ 0.05)	**4.02**	**2.82**

Legend: values presented in tables are means of radial growth (cm) of *Fusarium oxysporum*, three days after extracts application

Table 7 Mycelial growth inhibition of *Tagetes erecta* leaf extract against *Fusarium oxysporum* f. sp. *lycopersici*

Treatment concentration	Solvent	
	Methanol	Aqueous
Methanol solvent (control)	06.66 ±3.48	--
Water solvent (control)	45.00 ±1.41	45.00 ±1.41
12.5%	27.33 ±1.08	33.32 ± 0.40
25%	19.00 ± 0.70	36.68 ± 0.41
37.5%	22.33 ± 1.47	35.00 ± 1.87
50%	24.00 ± 2.44	35.33 ± 1.77
LSD (P ≤ 0.05)	**4.94**	**3.42**

Legend: values presented in tables are means of radial growth (cm) of *Fusarium oxysporum*, three days after extracts application

Plant extracts evaluated exhibited varied degree of anti-fungal properties. This trend is confirmed by the findings of **Olufolaji and Adeyeye (2002)** and **Olufolaji and Ojo (2005)** on the efficacy of the bioassay of some plants extracts on some fungi pathogens. **Dhaliwal *et al.* (1993)** also showed that the presence of anti-fungal substances in different extracts is responsible for the inhibition of radial growth and spore germination *in-vitro*. The superiority of *Azadirachta indica* extracts over other plant extracts for the control of *F. oxysporum* may be because of the presence of Nimbidin substances which may be effective against growth of *Fusarium oxysporum.* This is in agreement with the previous works which reported that *Azadirachta indica* extracts significantly inhibited mycelia growth of *F. oxysporum*, *Alternaria tennis*, *Rhizoctonia nodulosum* **(Suresh *et al*., 1997)**.

Effect of extracting solvent on efficacy of plant extracts

The varied inhibitory effect of the same plant materials in different extracting (methanol and water) solvent elucidates the idea that the active ingredients available in plants are influenced to a large extent by the extracting solvents **(Amadioha and Obi, 1999)**. Other factors such as age of plant, plant part used, method of extraction, and time of harvesting the plant materials may also affect the level of bioactivity of plant materials **(Okigbo, 2013)**. From this study, methanol extracts were the most effective. This is in agreement with the work of **Adeniyi *et al*. (2010)** who observed that methanol extracts had the highest inhibitory effect on mycelia growth of *Fusarium oxysporum* even at low concentrations. Although aqueous solvent showed lower bioactivity when compared with methanol extract, it still significantly inhibited growth of *Fusarium oxysporum.* This suggests that some of the compounds responsible for the antifungal properties in plant materials are water soluble **(Raji *et al*., 2005)**.

CONCLUSION

Nine tomato varieties evaluated responded differently when inoculated with *Fusarium oxysporum* f. sp. *lycopersici.* All but one (Tomato Shanty^{+}N) variety were susceptible to the Fusarium wilt pathogen. Eleven fungi isolates were isolated from tomato plants showing Fusarium wilt symptoms from different locations in Nigeria, they include *Fusarium oxysporum, Curvularia lunata, Pestalocia macrotrica, Bispora spp, Sclerotia rolfsii, Rhizopus spp, Aspergillus spp, Colletotrichum gloesporoides, Blastomyces spp, Chaonepora spp, and Rhizoctonia spp*. Generally, all plant extracts showed promising level of bioactivity against *F. oxysporum.* However, the efficacy of plant extracts where greatly influenced by either the extracting solvent used, the concentration of the extracts or both. Methanol extracts of *A.indica* at low concentration of 12.5% showed more bioactivity against *Fusarium oxysporum* f. sp. *lycopersici*, the causal organism for Fusarium wilt disease of tomato and is thus recommended as a possible alternative to the application of synthetic fungicide applied for the control of *Fusarium oxysporum*

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