






MANAGEMENT OF FUSARIUM WILT DISEASE OF TOMATO (*Solanum lycopersicum* L.) USING SELECTED PLANT EXTRACTS

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ABSTRACT

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen, is considered the most widespread, prevalent and economically damaging fungal disease of tomato in Nigeria. This study was conducted to isolate and identify fungal pathogens associated with tomato wilt disease, and also evaluate the efficacy of four plant extracts in the management of wilt disease *in vitro* and *in vivo*. Two varieties of tomato, Roma VF and UC 80 were used for the biocontrol assay. Four plant extracts, *Zanthoxylum zanthoxyloides* roots, *Distemonanthus benthamianus* stems, *Azadirachta indica* seeds and *oryza sativa* husk were evaluated against *Fusarium oxysporum* f. sp. *lycopersici*. Three concentrations (0.03, 0.04 and 0.05 g/mL) of each extract were investigated using the agar dilution method for radial growth inhibition assay. Of the four plant extracts evaluated, *Oryza sativa* husk extract was the most effective in inhibiting the growth of *Fusarium oxysporum*, both *in vitro* and *in vivo*. Rice husk extract (0.05 g/mL) had the highest inhibition rate (100%), followed by *A. indica* extract (80.32%) at 0.05 g/mL level of concentration, while *Z. zanthoxyloides* and *D. benthamianus* had the inhibition rates of 71.83% and 61.41% respectively, at 0.04 g/mL. Tomato plants treated with rice husk extract (0.05 g/mL) had the least wilt severity scores of 1.25 and 1.50 for both UC 80 and Roma VF varieties, respectively. Rice husk extract was the most effective against *Fusarium* wilt for both varieties and could serve as an alternative to synthetic chemicals in controlling *F. oxysporum* f. sp. *lycopersici*.

Contribution/Originality: The primary contribution of this research investigation is establishing the retention of significant antifungal activities in the aqueous extracts of *Zanthoxylum zanthoxyloides* roots, *Distemonanthus benthamianus* stems, *Azadirachta indica* seeds and *oryza sativa* husk, for the biocontrol of tomato-wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a fleshy important vegetable crop (Hadian, Rahnama, Jamali, & Eskandari, 2011) it belongs to the family Solanaceae and is the world's most cultivated vegetable (Saeed-Awan, Hussain, Tanveer Abbas, & Karim, 2012). Tomato is ranked second among the economic important vegetable crops worldwide. It is widely grown in Nigeria and ranked as the 6th most important crop after cereal, root and tuber

crops. Nigeria is the largest producer of tomato in Sub Saharan Africa, the second largest producer in Africa after Egypt and 13th producer in the world (FAOSTAT, 2016) with an annual estimated production of 3.94 million tonnes (FAO, 2020). Tomato is a very versatile plant and it could either be grown for fresh market tomatoes or for processing tomatoes, in which mechanical processes are involved. Tomatoes play a vital role in human diet and are good source of vitamins and minerals (Olaniyi & Ajibola, 2008). Low calories, high contents of vitamins A and C, beta-carotene, potassium and the presence of lycopene make tomato fruit highly beneficial to human health (Shidfar et al., 2011).

Tomato production is faced with several constraints which include high temperature, rainfall, insect pests, and diseases. The major diseases of tomato recorded all over the world are wilt diseases (Agbenin & Erinle, 2001). Wilt diseases of tomatoes can be caused by fungi, bacteria and nematode, as well as by abiotic factors. Fusarium wilt caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici* initially causes a yellowing and wilting of lower leaves on infected parts (Edward & William, 2009) which progresses upwards as the pathogen further invades the host tissues. The problems caused by synthetic pesticides and their residues have increased the need for effective biodegradable pesticides with greater selectivity. Continuous use of these chemicals may pose ecological problems (Adedeji & Aduramigba, 2016; Akaeze & Aduramigba-Modupe, 2017; Enikuomehin & Peters, 2002; Okigbo & Emoghene, 2003). However, some extracts from plants have been reported to possess different antimicrobial metabolites with biocontrol potentials against phytopathogens (Giordani et al., 2020).

Therefore, this study was conducted to isolate the fungal species associated with tomato plant wilt disease and evaluate the efficacy of selected plant extracts in the control of Fusarium wilt disease *in vitro* and *in vivo*.

2. MATERIALS AND METHODS

2.1. Sources of Materials

Tomato plants showing wilt symptoms were randomly collected from tomato farm in Ogere Remo in Ogun State, Nigeria. *Zanthoxylum zanthoxyloides* roots and *Distemonanthus benthamianus* stems were obtained from Apete market in Ibadan, Oyo State, Nigeria. *Azadirachta indica* seeds were obtained at the Faculty of Agriculture and Forestry, University of Ibadan and rice husk was obtained from the International Institute of Tropical Agriculture (IITA) in Ibadan. Tomatoes (Roma VF and UC 80) were obtained from National Horticultural Research Institute (NIHORT), Ibadan.

2.2. Isolation and Identification of Fungal Pathogens Associated with Tomato Wilt

Leaves, stems and roots parts showing wilt symptoms were washed under running water, cut into small pieces, surface sterilized with 0.5% sodium hypochlorite for 1 minute and rinsed thrice with sterile distilled water (SDW). The pieces were placed on sterile filter paper to dry for few minutes, then plated on Petri dishes containing acidified Potato Dextrose Agar. These were incubated at a temperature of 28°C for 3-4 days, and were then examined for fungal growth. The isolates were thereafter, sub-cultured to obtain pure cultures and identified (Carmona et al., 2020). Identification of the fungi isolates were done using cultural and morphological characteristics with different identification keys and methods developed by Barnett and Hunter (1998).

2.3. Pathogenicity Test

Three weeks old tomato seedlings were inoculated by standard root dip inoculation method. Seedlings were removed from the pots in the nursery, shaken to remove the adhering particles and washed carefully under running water. Thereafter, lateral roots were trimmed with sterile scissors before dipping them in a beaker containing the spore suspension (2.5×10^6 spore/mL) of each fungus and left for 30 minutes before transplanting was done. The plants were subsequently monitored for expression of symptoms (Carmona et al., 2020).

2.4. Preparation of Plant Extracts

Plants were prepared according to the method of Amadioha and Obi (1999). Selected parts of *Zanthoxylum zanthoxyloides*, *Distemonanthus benthamianus*, *Azadirachta indica* and rice husk Table 1 were rinsed thoroughly with SDW, air-dried (to constant dry weight) at room temperature ($28\pm 2^\circ\text{C}$) for two weeks. After which, they were grounded to powdered form with the aid of a mechanical grinder and stored in air-tight containers until use. Twenty grammes each of powdered plant samples were separately poured into 100 mL of sterile distilled water. The preparation was thereafter heated in water bath at 100°C for 1hr. It was allowed to cool and sieved with sterile muslin cloth (Wokocho & Okereke, 2005).

2.5. In Vitro Screening of Plant Extracts against *Fusarium oxysporum* f. sp. *lycopersici*

The experiment was laid out in completely randomized design with three replicates. The method of Amadioha and Obi (1999) was used to determine the effect of plant extracts on *Fusarium oxysporum* f. sp. *lycopersici*. This was done by drawing two perpendicular lines at the bottom of the plates, thereby making four equal sections on each Petri dish. Plant extracts, at different concentrations (0.03, 0.04 and 0.05 g/mL), were dispensed in the plates. Ten millilitres each of PDA was added and swirled gently to evenly distribute the mixture and allowed to gel. A disc of 10 day old *F. oxysporum* f. sp. *lycopersici* culture was placed at the centre of the plate using a 5 mm Cork borer and incubated at $28\pm 2^\circ\text{C}$ for 5 days. The radial growth of each inoculum was measured at 24 hours interval (Singh, Bhatnagar, & Tomar, 2019). Control plates consisted of PDA with no plant extract and was inoculated with the test fungus. Colony diameter was taken as the mean growth along two pre-drawn perpendicular lines on the reverse of the plates and percentage inhibition of *F. oxysporum* f. sp. *lycopersici* was determined as illustrated by Dhaliwal, Pathlak, and Vega (1990).

$$\% \text{MGI} = [(\Delta \text{Dc} - \Delta \text{Dt}) / \Delta \text{Dc}] \times 100$$

Where MGI= Mycelial growth inhibition; ΔDc = Average mycelial growth diameter of control sets and ΔDt = Average mycelial growth diameter of the treatment sets.

Table-1. Plant extracts and part of the plant used.

Botanical name	Family	Common name/Local name	Parts
<i>Zanthoxylum zanthoxyloides</i>	Rutaceae	Prickly-ash (Orin ata)	Roots
<i>Distemonanthus benthamianus</i>	Leguminosae	African Satinwood (Aayan)	Stems
<i>Azadirachta indica</i>	Meliaceae	Neem (Dongoyaro)	Seeds
<i>Oryza sativa</i>	Poaceae	Rice	Husks

Source: Islasa et al. (2020); Olushola-Siedoks, Igbo, Asieba, Damola, and Igwe (2020); Tiwari, Yadav, and Dikshit (2020).

2.6. In Vivo Screening of Plant Extracts against *Fusarium Wilt*

The experiment was set up in a $4 \times 2 \times 2$ factorial experiment, laid out in completely randomized block design with four replicates. The soil was sterilized in an electric sterilizer at 160°C for 2 hrs. Sterile soil (3 kg) was poured into a 5 kg pot. Tomato seeds were surface sterilized with 0.5% sodium hypochlorite for one minute and rinsed thrice with sterile distilled water before planting. The plants were transplanted after 3 weeks. Inoculum was prepared by adding 50 mL of sterile distilled water to pure culture plates. The spores were scrapped off using sterilized scalpel and sieved with a sterilized cheese cloth folded into four layers. The spores were counted with haemocytometer and the concentration was adjusted by diluting it with water to 2.5×10^6 spores/mL. Root dip method was used, where the root of each tomato seedling was dipped into the prepared inoculum suspension for 30 minutes before transplanting (Nirmaladevi & Srinivas, 2012). Plant extracts at different levels of concentration (0.04 and 0.05 g/mL) were also applied at the rhizosphere around the root of each plant. Data were collected on *Fusarium* wilt severity and plant performance.

Disease severity was scored using the following rating scale by Marley and Hillocks (1996).

1 = no symptom, 2 = chlorosis and wilting of primary branches, 3 = chlorosis and wilting of second and third branch, 4 = chlorosis above third branch, second and third branch may be lost, 5 = chlorosis and partial desiccation, 6 = completely necrotic or dead plant.

All data obtained were analysed using descriptive analysis and Analysis of Variance (ANOVA). Means were separated by Least Significant Difference (LSD) test at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The fungi isolated were *Fusarium oxysporum*, *Trichoderma viride* and *Colletotrichum gloeosporoides* and *Fusarium oxysporum* f. sp. *lycopersici* was confirmed to cause wilt through pathogenicity test. Both *Azadirachta indica* and *Oryza sativa* inhibited the mycelial growth of *Fusarium oxysporum* as their concentration increases, with *Oryza sativa* having the highest inhibition (100%) at 0.05 g/mL Table 2. There were significant differences in mycelial growth inhibition recorded for the extracts at all levels of concentration, except *Distemonanthus benthamianus* with mycelial growth inhibition values of 61.41, 58.55% (at 0.04 g/mL and 0.05 g/mL, respectively), which were not significantly different. Mycelial growth inhibition of *Azadirachta indica* and *Oryza sativa* were significantly higher at 0.05 g/mL while *Distemonanthus benthamianus* and *Zanthoxylum zanthoxyloides* were higher at 0.04 g/mL level of concentration Table 2.

3.1. Inhibitory Effect of Plant Extracts on Disease Severity and Plant Height

Table 3 shows the effect of plant extracts on *Fusarium* wilt severity of Roma VF tomato variety. Rice husk treated plants had the least severity (1.75 and 1.50) at 7 WAT, at both 0.04 and 0.05 g/mL concentrations respectively, followed by *D. Benthamianus* and *A. indica*. The *Z. zanthoxyloides* treated plants had high wilt severity at both levels of concentration, while the severity score (3.50) at 0.05 g/mL was not significantly different from the recorded severity of control plants treated with *F. oxysporum* only. There were no symptoms of wilt disease on the control (uninoculated) plant. Plants with treatment of only *F. oxysporum* had the highest severity (4.00). It appeared that all the plant extracts were able to control *F. oxysporum* on Roma VF tomato variety at 0.04 g/mL.

Table 4 shows the effect of plant extracts on *Fusarium* wilt severity of UC 80 tomato variety. Rice husk (0.05 g/mL) treated plant had the least severity (1.25), *A. Indica* (0.4 g/mL) treated plants had an average severity score of 2.25 at 7 WAT, while the control (infected) plants were observed to have the highest wilt severity score (4.25). There was no significant difference amongst the wilt severity scores at all the levels of extract concentrations. As implied in Table 4, *A. indica* at 0.04 g/mL, rice husk and *Z. zanthoxyloides* at both 0.04 and 0.05 g/mL were all able to control *Fusarium* wilt disease of UC 80 tomato variety. For Roma VF variety Table 5 plant height recorded at 7 WAT showed no significant difference between the levels of extract concentrations but they were significantly lower than the height of control (uninfected) plants. There was no significant difference (in plant height) between the extract treatment and the *Fusarium*-only control plant sets. However, *Fusarium*-only plants had the least height (49.25 cm) for *Z. zanthoxyloides*, *D. Benthamianus* and *O. Sativa* extract treatments. Similar observations were recorded for UC 80 variety plant height; there were no significant difference between the two levels of extract concentrations in each case of the treatments. However, the observed height of control (uninoculated) plant was significantly higher than each treated plant set Table 6.

Rice husk, *Azadirachta indica*, *Distemonanthus benthamianus* and *Zanthoxylum zanthoxyloides* inhibited the fungus at varying concentrations *in vitro*. This implies that plants.

Commonly used in human medicine also possess antimicrobial effects on plant pathogenic fungi. This conformed to the observations of Olufolaji and Adeyeye (2002); Olufolaji. and Ojo (2005) and Akaeze and Aduramigba-Modupe (2017) in their studies on the bioassay of some plant extracts on selected fungal pathogens. Rice husk extract had the highest inhibitory activities against *F. oxysporum* f. sp. *lycopersici*.

Table-2. Percentage inhibition of four plant extracts against mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*

Extract Concentration	Mycelial inhibition (%)			
	<i>A. indica</i>	<i>D. benthamianus</i>	<i>Oryza sativa</i>	<i>Z. zanthoxyloides</i>
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.03 g/mL	51.77 ± 1.39	52.15 ± 2.04	54.86 ± 0.67	54.86 ± 0.67
0.04 g/mL	66.82 ± 0.39	61.41 ± 2.04	71.06 ± 0.67	71.83 ± 0.37
0.05 g/mL	80.32 ± 1.16	58.55 ± 0.00	100.00 ± 1.16	67.98 ± 0.39
LSD	3.02	4.71	1.54	1.41

Note:

Values are means of three replicates ($P \leq 0.05$).

LSD: Least significant difference.

Table-3. Effect of four plant extracts on *Fusarium* wilt severity of tomato (Roma VF variety)

Plant treatment (F. oxysporum + extract)	Wilt severity			
	<i>A. indica</i>	<i>Z. zanthoxyloides</i>	<i>D. benthamianus</i>	<i>Oryza sativa</i>
Uninoculated	1.00	1.00	1.00	1.00
F only	4.00	4.00	4.00	4.00
F + 0.04 g/mL	3.00	3.25	3.00	1.75
F + 0.05 g/mL	3.50	3.50	3.00	1.50
LSD	0.77	0.59	0.63	0.54

Note:

Values are means of three replicates ($P \leq 0.05$).F: *F. oxysporum* inoculation.

LSD: Least significant difference.

Table-4. Effect of four plant extracts on *Fusarium* wilt severity of tomato (UC 80 variety).

Plant treatment (F. oxysporum + extract)	Wilt severity			
	<i>A. indica</i>	<i>Z. zanthoxyloides</i>	<i>D. benthamianus</i>	<i>Oryza sativa</i>
Uninoculated	1.00	1.00	1.00	1.00
F only	4.25	4.25	4.25	4.25
F + 0.04 g/mL	2.25	3.00	3.50	1.50
F + 0.05 g/mL	3.35	2.75	3.50	1.25
LSD	1.11	0.54	0.97	0.70

Note:

Values are means of three replicates ($P \leq 0.05$).F: *F. oxysporum* inoculation.

LSD: Least significant difference.

Table-5. Effect of four plant extracts on plant height of tomato (Roma VF variety).

Plant treatment (F. oxysporum + extract)	Plant height (cm)			
	<i>A. indica</i>	<i>Z. zanthoxyloides</i>	<i>D. benthamianus</i>	<i>Oryza sativa</i>
Uninoculated	76.00	76.00	76.00	76.00
F only	49.25	49.25	49.25	49.25
F + 0.04 g/mL	53.00	57.50	59.00	58.50
F + 0.05 g/mL	48.75	55.75	53.50	60.00
LSD	11.86	13.46	14.23	11.00

Note:

Values are means of three replicates ($P \leq 0.05$).F: *F. oxysporum* inoculation.

LSD: Least significant difference.

Table-6. Effect of four plant extracts on plant height of tomato (UC 80 variety).

Plant treatment (F. oxysporum + extract)	Plant height (cm)			
	<i>A. indica</i>	<i>Z. zanthoxyloides</i>	<i>D. benthamianus</i>	<i>Oryza sativa</i>
Uninoculated	70.00	70.00	70.00	70.00
F only	54.00	54.00	54.00	54.00
F + 0.04 g/mL	58.00	56.50	51.25	59.75
F + 0.05 g/mL	46.75	56.75	52.00	59.75
LSD	15.72	7.06	9.97	11.25

Note:

Values are means of three replicates ($P \leq 0.05$).F: *F. oxysporum* inoculation

LSD: Least significant difference

This is in line with the report of Godakumbura, Kariyawasam, Arachchi, Fernando, and Premakumara (2017) who investigated the *in vitro* antimicrobial activities of crude extracts from five local rice varieties; they reported very potent inhibitory activities against selected pathogens. The antimicrobial activity was attributed to the presence of various bioactive compounds including flavones, anthocyanins, tannin, phenolics, sterols, tocopherols, γ -oryzanol, amino acids and essential oils present in the rice extracts. Lee et al. (2010) also reported that acetic acid produced from pyrolytic extract discouraged the growth of *Penicillium spp.* and white rot fungus, *Pycnoporus sanguineus* in timber wood. Extracts from *Azadirachta indica* and *Oryza sativa* were more effective against *F. oxysporum* at high concentration (0.05 g/mL), while *Distemonanthus benthamianus* and *Zanthoxylum zanthoxyloides* had better inhibition against the fungus at 0.04 g/mL.

The antimicrobial activities of these extracts may be due to the presence of secondary metabolites such as tannins, alkaloids, saponins or flavonoids Adekunle and Odukoya (2006). In a similar study, Giordani et al. (2020) investigated the *in vitro* antimicrobial properties of extracts obtained from 14 medicinal plants. They observed that the extracts exhibited significant levels of antifungal activities against selected mycopathogens. Chelerythrine, berberine and canthin-6-one were also indicated as antifungal components of *Z. zanthoxyloides*. *Azadirachta indica* seeds were reported to contain an antimicrobial substance (nimbidin) which was effective against the growth of *Fusarium oxysporum*, *Alternaria tenuis* and *Rhizoctonia nodulosum* (Girish, 2020; Islasa et al., 2020).

The presence of antifungal substances in the extracts could be responsible for the observed inhibition of radial growth, as well as possible inhibition of spore germination *in vitro*. This agrees with earlier reports on antimicrobial plant extracts (Akaze & Aduramigba-Modupe, 2017; Dhaliwal et al., 1990; Enikuomehin, 1998; Giordani et al., 2020). Rice husk extract was the most effective of all the botanicals tested, followed by *Azadirachta indica*. *Distemonanthus benthamianus* and *Zanthoxylum zanthoxyloides* were also effective, both *in vitro* and *in vivo*.

Antifungal plant metabolites like apigenin, alkaloids, flavonoids, saponins, tannins, ferulic acid, phlobatanins, terpenoids, phenols, anthraquinone and pyrolytic acid, may also be attributed to the *in vivo* antimicrobial potentials of plant extracts (Adekunle & Odukoya, 2006; Giordani et al., 2020; Mulugeta et al., 2020). Such metabolites could inhibit *F. oxysporum* tissue invasion, resulting in the reduction of tomato wilt severity, as observed on the extract-treated, infected plants in this study. In their report on the importance of botanicals and plant health for tomato cultivation in Africa, Mulugeta et al. (2020) concluded that antimicrobial extracts from several plant species, such as *A. indica* and *C. citratus*, could promote plant growth, tolerance to abiotic stress and resistance to phytopathogenic fungi associated with tomato plants.

4. CONCLUSION

This study highlighted the potentials of plant extracts in the control of fusarium wilt disease on tomato. Aqueous extracts of all the plants possess antifungal properties against *F. oxysporum* f. sp. *Lycopersici*. Extracts from *Azadirachta indica* and *Oryza sativa* were more effective against the pathogen at high concentration (0.05 g/mL), while *Distemonanthus benthamianus* and *Zanthoxylum zanthoxyloides* had better inhibitory effects at 0.04 g/mL. However, rice husk was the most effective in controlling fusarium wilt disease of tomato, both *in vitro* and *in vivo*. These plant extracts could serve as safer, cost-effective, narrow spectrum, biodegradable alternatives to the use of synthetic fungicides in the control of mycopathogens. Also, re-application of extracts could be done for effective control of microbial pathogens. However, cultural control methods, like the use of healthy planting materials and good farm sanitation should also be employed in addition to botanicals in the management of fusarium wilt disease of tomato. Further studies could also be carried out to identify and optimise the active antifungal components of these botanicals, as well as to establish their biocontrol potentials on the field.

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