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In vitro assessment of the fungicidal properties of some spices for the control of the Tomato Wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici*

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ABSTRACT*:*An assessment of the fungicidal properties of *Garcinia kola*, *Monodora myristica*, *Ocimum gratissemum*, *Syzygium aromaticum* and *Aframomum septrum* showed they had significant effect on mycelia diameter of *Fusarium oxysporum* f. sp. *lycopersici*. The *in vitro* evaluation of concentration effects of all spices showed day-7 mycelial diameters of 7.43, 5.25 and 2.96cm for control, 1g/100ml and 10g/100ml concentrations respectively. The most effective spice in this study was *S. aromaticum* followed by *O. gratissemum* both resulting in 100% and 77.6% mycelia inhibition in the 10g/100ml concentration. Multiple comparison of means from all treatment showed that *S. aromaticum* had a significance value of -2.85556 and -5.00 against *O. gratissemum* and control respectively. Evaluation of the toxicity of the spices to tomato seeds resulted in *O. gratissemum* having percentage germinations of 97 from the 1g/100 ml which was not significant to that from the control (96%).

Key words: Spices; tomato wilt; *Fusarium oxysporum* f. sp. *lycopersici*

Introduction

Fusarium oxysporum f. sp *lycopersici* (Sacc) W.C Snyder and H.N Hans is an important soil borne pathogen that is favoured by warm soil temperature and low soil moisture and causing wilt in tomato which is the major host . Other host of the pathogen includes sweet potato, onion, eggplant, cucumber, muskmelon, watermelon, cucurbits etc. The virulent strains of *Fusarium oxysporum* f. sp *lycopersici* have been demonstrated to produce pectin lyase and other pectolytic enzymes (Osagie et al. 2002; Obuekwe and Osagie, 1989).

Vascular wilt disease causes serious economic losses to tomatoes and many agricultural and floricultural crops in warm areas. The Fusarium wilt pathogens show a high level of host specificity as such they are classified based on plant species and plant cultivars, they can infect into more than 120 formae speciales and races. (Armstrong and Armstrong (1981) in Fravel et.al., 2003).

Disease management in Fusarium wilt is mainly through chemical soil fumigation and resistant cultivates. Chemicals such as methyl bromide are used for fumigation but they are environmentally damaging (Fravel *et al.*, 2003; Ristaino and Thomas, 1997), and their uses are banned in some countries.

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The use of resistant varieties and breeding for resistance is difficult although some resistant varieties to common races of *Fusarium oxysporum* f. sp *lycopersici* exist. The difficulty in breeding is because no dominant gene has been identified and new races of the pathogen can develop to overcome the resistance (Dobinson et.al., 1996; Fravel *et al.*, 2003).

Because of these challenges in the control of fusarium wilt, there is an ongoing research into biological means of control. As such, this study is aimed at identifying plants that may be used in the preparation of extracts for the control of the pathogen *Fusarium oxysporum* f sp. *lycopersici.*

Materials and Method

Collection of Samples

Spices used in this study were bought from the Uselu market, in Benin City. The five spices viz. *Ocimum gratissemum* (scent leaf); *Garcinia kola* (bitter kola); *Syzygium aromaticum* (clove); *Monodora myristica* (African nutmeg); and *Aframomium septrum* (grains of paradise) were bought from the local market, and air dried on laboratory benches. When samples were dry, they were ground using a corona corn hand mill and were stored in clean mayonnaise bottles and stored in the refrigerator at about 4^0C.

Culture of Target Organism.

The culture of the pathogen *Fusarium* f. sp *lycopersici* was collected from the Pathology Department of Ahmadu Bello University, Zaria. The vial of the pathogen was stored in the refrigerator. Cultures were made on Potato Dextrose Agar (PDA).

Bioassay of Spice Amended Media

Before sterilization at 121^{o}C for 15 min, 1g and 10g quantity of each spice was added to 100ml of dissolved PDA. The spice amended PDA was allowed to cool to about 45^{o}C, and about 15ml quantity was dispensed per plastic Petri dish. The spice amended media were seeded with 1cm^3 plug of 7-day old culture of the target organism *Fusarium oxysporum* f.sp *lycopersici* and mycelia growth was observation.

Data Collection and Statistical Analysis

Measurement was taken of the mycelial diameter (cm) for one week. Pictures of the plates were taken at the end of the one week of observation. Analysis of variance and mean separation were carried out using the GENSTAT statistical software.

Results

The analysis of variance carried out for the effects of concentration, days and spice on the growth rate of the pathogen and interactions showed significant differences at 5% level of probability (Table 1).

The colony diameter of F. *oxysporum* f.sp *lycopersici* at day-7 was 7.4 cm, 5.25cm and 2.96cm for control 1g and 10g concentration respectively (Table 2). Treatment with *S. aromaticum* resulted in 0cm growth of the pathogen and was the most effective of all five spices evaluated. There were significant differences in the mycelia growth at the two concentrations for all spices except *A. septrum* (Table 3).

Treatment effect over days showed that by the 7th day of inoculation, G. kola (1g/100ml) resulted in mycelia diameters of 7.5cm which was higher (not significant) than from the control (7.43cm). The 1g/100ml concentration was not as effective as the 10g concentration for all the spices except for *S. aromaticum* and *A. septrum* where there was no significant difference in their effects (Table 4). Multiple comparison of effects showed that *G. kola* (-1.02778) and *A. septrum* (-1.31667) were the least effective on pathogen growth

Seed germination:

Analysis of germination percentage of treated tomato seeds: in the two concentrations; showed that the *O. gratissemum* treatment resulted in the highest percentage germination (97%) and this was not significant to that from the untreated control (96%). The lowest germination of the seeds was from the *M. myristica* 10g/100 ml treatment.

Table 1: Treatment effects of concentration day after treatment and spice used on mycelial growth of *Fusarium oxysporun* f.sp. *lycopersici*.

Source of variation	Mean square	F cal	Pr
Concentration	48.98255	2266.01	<.09
Days	72.18465	2339.38	<.001
Spice	36.40013	1683.93	<.001
Concentration days	3.71812	172.01	<.001
Concentration * spice	12.72778	588.81	<.001
Days * spice	3.13653	145.10	<.001
Concentration * days * spice	1.22622	56.73	<.001

Table 2: Concentration effect on mycelial diameter of *Fusarium oxysporum* f.sp *lycopersici* on plant extract amended PDA.

Concentration	Mycelial Diameter (cm)		
(g/100ml)	Day-3	Day-5	Day-7
0	2.43	5.13*	7.43*
1	1.77	3.56	5.25
10	0.93	1.93*	2.96*

LSD Concentration*day 2.137

Table 3: Concentration effect of five spices used as media amendment on mycelia growth of *Fusarium oxysporum* f.sp *lycopersici*

Spice	Concentration (g/100ml)		
	0	1(% I)	10(% I)
Garcinia kola	5.0	5.04(-.8)	2.9(42)
Monodora myristica	5.0	4.20(16)	2.13(47.4)
Ocimum gratissemum	5.0	4.59(8.4)	1.12(77.6)
Syzygium aromaticum	5.0	0(100%)	0(100%)
Aframomium septrum	5.0	3.79(24.2)	3.58(28.4)

LSD concentration 1.092; LSD spice 1.221; LSD conc*spice 1.410
% I – percentage inhibition

Table 4: Assessment of concentration effect over days for the five spices used as media amendment on mycelia growth of *Fusarium oxysporum* f.sp *lycopersici*

Spice	Concentration (g/100ml)	Day-3	Day-5	Day-7
Garcinia kola	1	2.5	5.04	7.50
Garcinia kola	10	1.37	5.04	4.47
Monodora myristica	1	2.0	4.20	6.37
Monodora myristica	10	0.93	4.20	3.40
Ocimum gratissemum	1	2.33	4.59	6.80
Ocimum gratissemum	10	0.67	4.59	1.57
Syzygium aromaticum	1	0	0	0
Syzygium aromaticum	10	0	0	0
Aframomium septrum	1	2.0	3.79	5.50
Aframomium septrum	10	1.0	3.79	5.37
Control		2.43	5.13	7.43

LSD conc 0.1072; LSD day 0.0723; LSD spice 0.1198; LSD conc*day 0.1857; LSD day*spice 0.2076; LSD conc*day*spice 0.2397

Table 5: Table of significant effect using multiple comparisons of spice effects on mycelia growth of *Fusarium oxysporum* f.sp *lycopersici*

Spice Comparison	Significance
Syzygium aromaticum * *Garcinia kola*	-3.97222
Syzygium aromaticum * *Monodora myristica*	-3.16667
Syzygium aromaticum * *Aframomium septrum*	-3.68333
Syzygium aromaticum * *Ocimum gratissemum*	-2.85556
Syzygium aromaticum * Control	-5.00000
Monodora myristica * Control	-1.83333
Ocimum gratissemum * Control	-2.14444
Garcinia kola * Control	-1.02778
*Aframomium septrum** Control	-1.31667

Table 6: Assessment of toxicity of the spices on tomato seeds treated

Spice	Concentration (g/100ml)	Day-2(%G)	Day-4(%G)	Day-6(%G)
Monodora myristica	1	9.0(45)	8.0(40)	10.20(51)
Monodora myristica	10	1.8(.09)	2.20(11)	3.80(19)
Ocimum gratissemum	1	17.8(89)	19.00(95)	19.40(97)
Ocimum gratissemum	10	7.60(38)	3.40(17)	12.40(62)
Syzygium aromaticum	1	5.6 (28)	11.40(57)	9.00(45)
Syzygium aromaticum	10	1.40 (7)	10.00(50)	6.00(30)
Control	14.40(72)	17.80(89)	19.20(96)	

LSD conc*day 1.909; LSD conc* days*spice 2.339
%G – Percentage germination

Discussion

In this study, extraction from some plant species; *Monodora myristica (*"Ikpoza"), *Ocimum gratissimum* (Scent leaf), *Garcinia kola* (Bitter kola), Syzygium *aromaticum* (clove) and *Aframomium septrum* (grain of paradise) showed varied antifungal potentials when tested.

Results obtained from the analysis of variances between the various interactions shows that there are significant differences, across all levels of interactions. For control, it was observed that the growth rate was uniform across days (7 days), and that the culture was growing smoothly, fluffy white, purplish pink colour was observed. It was observed that some of the APDA (amended potato dextrose Agar) used slowed down the growth rate of the fungal (Figure 1-5). For *Syzygium aromatcum* (clove) there was no growth at all in the two levels of concentration used which suggested within the limit of the study that it might be an effective biological control agents for the fungal disease.

Plant extracts have been used successfully to control diseases in plants and tuber crops (Amadioha and Obi, 1999; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). Taiga and Olufolaji (2007) reported the effectiveness of 25, 50, 75 and 100% cold water extract concentration of *Nicotiana tabacum* in the complete inhibition of *Fusarium oxysporum* mycelia.

In this study, all five plants evaluated reduced mycelia growth in the 10g/100ml concentration by 28 -100%. Concentration effects were significant for all but *S. aromaticum* and *A. septrum*. Other research has shown that concentration is significant (Al-Abeed, 1992; Fabry et. al., 1996; Ogbebor and Adekunle, 2005). *Ocimum basilicum* when used to control *Corynespora cassicola* showed increased mycelia inhibition with increase in concentration (Ogbebor and Adekunle, 2005). In this study, *O. gratissimum* effectiveness increased with concentration from 8%-77% inhibition at 1g/100 and 10g/100ml concentrations respectively.

A. septrum and *S. aromaticum* did not show significant difference from concentration. *Syzingium cordatum* has been shown to have strong in vitro activity against gram positive and gram negative bacteria (Samie et al., 2005). There has been demonstrated control of damping-off pathogens using clove oil (Locke, 2005) and *Clostridium botulism* (Ismaiel and Pierson, 1990).

Bowers and Locke (2000) using products based on formulations of plant extract and essential oils as soil amendment from pepper, mustard cassia and clove extracts effectively reduced the population density of *Fusarium oxysporum* f. sp. *chrysanthami*.

There is a large reservoir of natural fungicides in plants and micro-organisms, which with continued research would provide safe and effective alternatives to synthetic fungicides. These compounds would have to be identified and formulated for application in the control of plant pathogens.

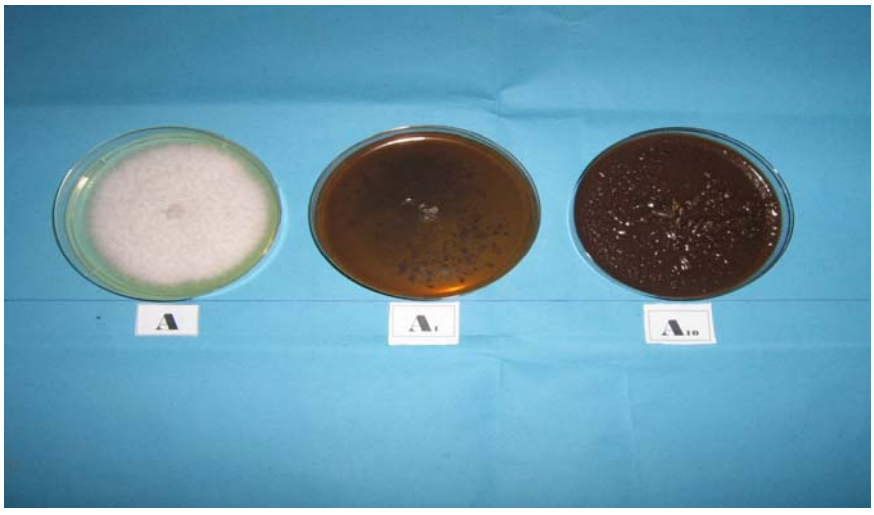

Figure 1. *Fusarium oxysporum* f.sp *lycopersici* on APDA *Syzygium aromaticum* (clove).

A: control, without concentration (spices)
A_1: 1g concentration of *Syzygium aromaticum* in PDA
A_{10}: l0g concentration of *Syzygium aromaticum* in PDA

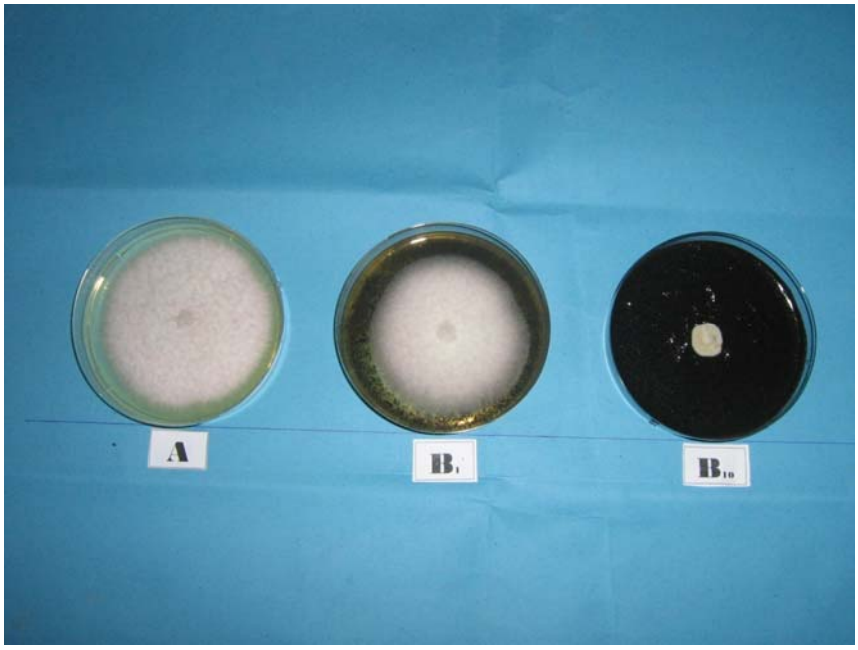

Figure 2. *Fusarium oxysporum* f. sp *Lycopersici* in APDA of *Ocimum gratissimum* (scent leaf)
A: control without concentration (spice)
B: 1g concentration of *Ocimum grattissimum* in PDA
B_2: 10g concentration of *Ocimum grattissimum* in PDA

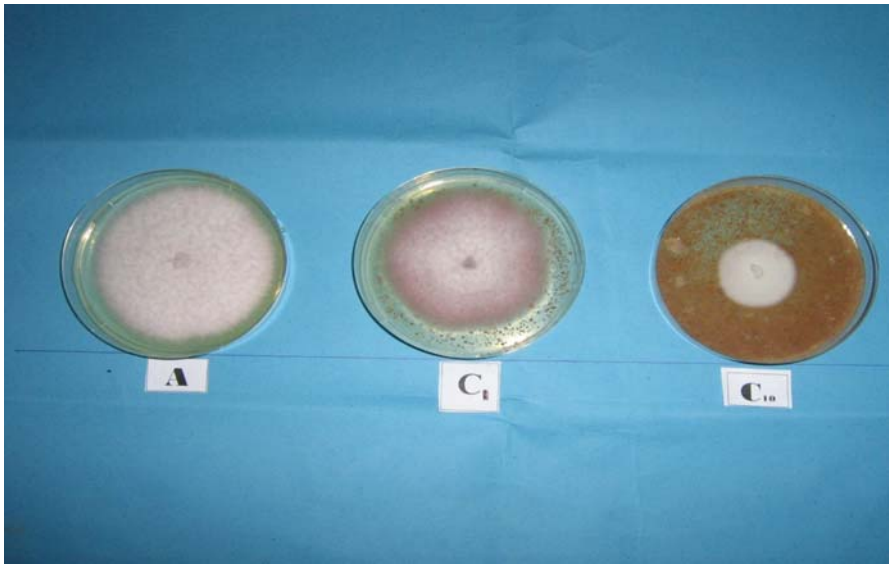

Figure 3. *Fusarium oxysporum* f. sp *lycopersici* in APDA of *Monodora myristica* (African nutmeg)

A: Control, without concentration (spice)
C_1: 1g concentration of *Monodora myristica* PDA
C_{10}: 10g concentration of *Monodora myristica* PDA

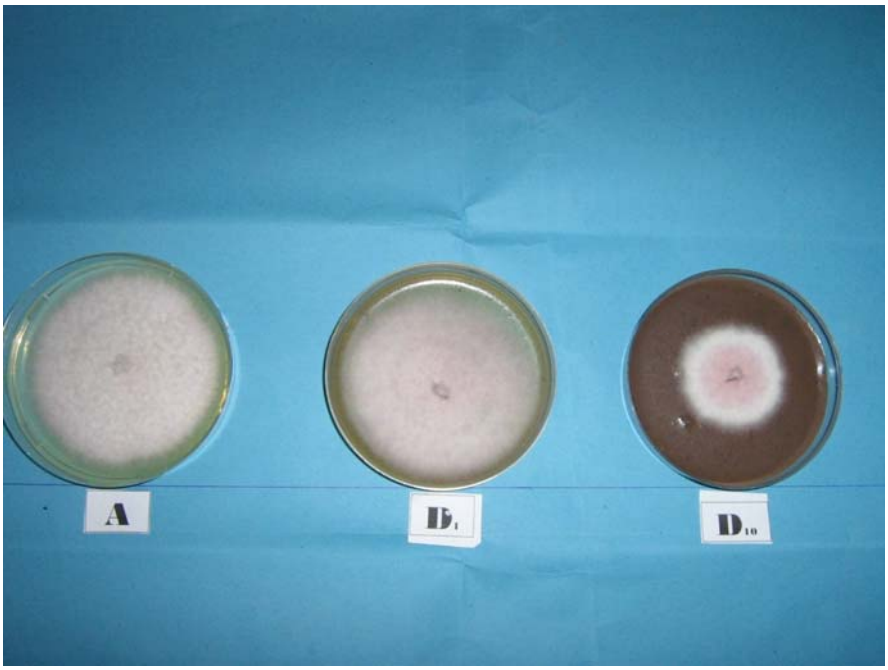

Figure 4. ***Fusarium oxysporum*** **F.sp** ***lycopersici*** **in APDA of** ***Garcinia Kola (Bitter Kola)***

A: Control, without concentration (spice)
D_1: 1g Concentration of *Garcinia kola* in PDA
D_{10} : 10g Concentration of *Garcinia kola in PDA*

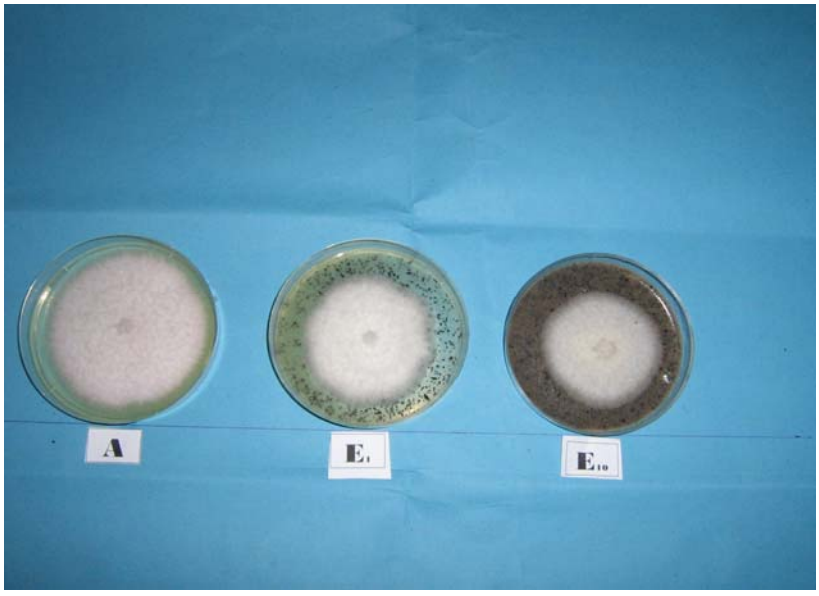

Figure 5. ***Fusarium oxysporum*** **f.sp** ***Lycopersici*** **on APDA** ***Aframomium Septrum*** **(grains of paradise).**
A: control, without concentration (spices)
E_1: 1g concentration of *Aframomium septrum* in PDA
E_{10}: l0g concentration of *Aframomium septrum* in PDA

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