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In Vitro Antifungal Activities of Three Aromatic Plant Extracts Against *Fusarium Oxysporum* Schlechtend. Fr. F. Sp. *Lycopersici* (Sacc.) Causal Organism of Fusarium Wilt In Tomato

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Abstract

In vitro assay of aqueous extracts of *Allium sativum*, *Zingiber officinale* and *Dysphania ambrosioides* were evaluated against *Fusarium oxysporum*. Five concentrations of cold and hot water aqueous extracts were obtained by infusing 4, 8, 12, 16, and 20 g of powder in 20 ml of sterile distilled water for 24 hours to obtain 20, 40, 60, 80 and 100% concentration, the hot water extracts were obtained using similar procedure with each flask placed in a water bath at 90°C for 90 mins. Food poisoned technique was employed for investigating the fungi toxicity of the plants essential oils (EO) against *Fusarium oxysporum* growth. An aliquot of 0.1 of the EO was dissolved in 0.9 ml of Tween-20 and mixed with 15 ml of Potato Dextrose Agar medium to achieve 20, 40, and 60% concentration, while the undiluted oil was recorded as 100%. The plates were inoculated with a 6mm assay disc cut from 7 day old culture fungus; observations were recorded on 7th day of incubation. Results indicate a reduction in *F. oxysporum* growth in the culture media, with hyphae becoming visible 48-50h after inoculation. *A. sativum* at 100% gave the highest inhibitory effect (71.24%) on mycelial growth, and compete favourably with Carbendazim (80.38%). The hot water extraction revealed better antifungal effects (45.86%) on *F. oxysporium* than cold water extract (33.80%). *A. sativum* showed the highest inhibition of 71.24% and 66.92% at 80% and 100% respectively, after Carbendazim (80.38%) at 0.5 mg/ml which is the standard. However, all extracts recorded the lowest mycelia growth at 20% concentration level.

Keywords: *Fusarium oxysporum*; Tomato; antifungal; Aromatic plant; Carbendazim

Introduction

Tomato (*Solanum lycopersicum* Mill.) is the second most important vegetable, widely cultivated in tropics and sub-tropics region of the world. It belongs to Solanaceae family, and cultivated for its edible fruits and can be processed to other usable form [1]. It is a source of vitamins A and C and serve as a source of income for tomato growers. Tomato cultivation has become increasingly popular since mid-nineteenth century with world production of 100 million tons fresh fruits [2]. In Africa, Nigeria is the second largest producer with production output estimated at 1.7 million tonnes in 2008 after Egypt and the highest producer in sub-Saharan Africa [1].

Tomato is cultivated majorly in Southwestern and Northern part of Nigeria by smallholder farmers. The production is throughout the year and the largest production comes from north central states during dry season when the weather is cooler and the incidence of insect pests and diseases is minimal [1]. The production output in Nigeria declined from 1.7 million tonnes to 687,611 tonnes in 2010 [2]. This decline in production output in Nigeria compared with those of the temperate zones is being attributed to differences in environmental conditions, lack of high-yielding varieties, cultural practices applied to the crop on the field and attack from insect pests and diseases [2]. Of these, disease attack is the most important factor contributing to decline in tomato production in Nigeria, and the fluctuation of tomato production worldwide with some estimates putting losses as high as 75% to 95% of total production. The major diseases of tomato in Nigeria are caused by bacteria, nematodes, viruses and fungi. Among the fungi diseases affecting tomato productivity, Fusarium wilt caused by the fungal pathogen *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.). Snyder and Hans, is reported to be one of the most devastating diseases of tomato (*Lycopersicon esculentum* Mill.) worldwide [3]. The fungus causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele [4]. Control of wilt diseases depends mainly on fungicides [5,6]. Synthetics fungicides have been used for the management of plant pathogens including fusaria [7,8], and the effectives ones that are eco-friendly is rare. Also, the occurrence of fungicide-resistance pathogen, high cost, and accumulation of this chemicals in the environment has led to resurgence of interest in alternative approach [9]. Alternative

methods of controlling diseases have been studied with emphasis on novel compounds derived from plant sources [10,11]. Plant extracts and plant essential oils have been reported to be effective antimicrobials agent against food and grain storage fungi, foliar pathogens and soil-borne pathogens [4]. Several plants and their bye-products have been reported to possess pest control properties. These are good alternatives to chemical pesticides, as they are biodegradable in nature. The aim of this study was to evaluate, the *in vitro* efficacy of aqueous extracts of *Allium sativum*, *Zingiber officinale* and *Dysphania ambrosioides* against the mycelia growth of *Fusarium oxysporum in vitro*.

Materials and Methods

Pathogen isolation

Fusarium oxysporum was isolated from an infected tomato leaves that had typical fusarium wilt disease symptoms in the Crop Soil and Pest Management Department, Federal University of Technology, Akure, Ondo State. The infected leaf portion were cut into 2 mm pieces, surface sterilized with 70% ethanol and rinsed with sterile water. Four pieces were placed per Petri dishes containing 20 ml PDA (Merck KGaA, Darmstadt, Germany) embedded with streptomycin and then incubated at 28+2°C for 5 days. The pure culture of *Fusarium oxysporum* was maintained in PDA slants and placed in refrigerator at 18°C until needed.

Preparation of plant extracts and their *in vitro* evaluation

The efficacy of cold and hot water extracts of garlic bulb (*Allium sativum*), ginger rhizome (*Zingiber officinale*), and *Dysphania ambrosioides* were tested to control *F. oxysporum* isolated from diseased tomato *in vitro*. The plant materials were air dried, powdered separately using mortar and pestle and then blender. Four, eight, twelve, sixteen and twenty grams of dried powder of *A. sativum*, *Z. officinale*, and *D. ambrosioides* were dissolved separately in a cold 20 ml sterile distilled water to obtain 20%, 40%, 60%, 80% and 100% concentrations, respectively".

The hot water extracts were prepared similar to the cold water except that each flask was placed in a water bath at 90°C for 90 mins. The extracts was filtered and incorporated into PDA used to culture *F.oxysporum*. Petri dishes were poured with 15 ml of PDA extract and allowed to solidify. Inoculum disc of 6mm diameter obtained from the edge of a seven day old culture of *F. oxysporum* on PDA were inoculated facing upside down at the centre of each of the different plant extract plates. PDA without any plant extract served as control, while PDA with synthetic fungicide- Carbendazim was used as standard. Each treatment was replicated three times and arranged on a laboratory bench at room temperature (28+2°C) using completely randomized design (CRD). Mycelial growth was determined by measuring culture size with vernier callipers along with two diameters at 2-7 days after inoculation (DAI).

Extraction of the essential oil of the plant samples

Fresh leaves of *A. sativum*, *Z. officinale* and *D. ambrosioides* were cut into small pieces and washed thoroughly with sterilised water. The plant materials were then placed in the round bottom flask of the hydro-distillation apparatus. The ratio between the plant materials and water in the flask was maintained as 1:3. Water was heated to produce steam that carried the most volatile fractions of the aromatic materials with it. The essential oils (EO) were found to float on the top of the distillate and were separated via separating funnels. The extracted oil was dehydrated by the addition of anhydrous sodium sulphate, followed by thorough shaking and standing for 6-8 hours and filtration [12].

Fungitoxic properties of the essential oils (EO) against *Fusarium oxysporum*

Poisoned food technique method was employed for this investigation [13]. 0.1 ml of the EO was dissolved in 0.9 ml of 0.1% Tween-20 and then mixed with 15 ml of PDA medium to get different concentration viz, 20, 40, 60, and while undiluted oil was recorded as 100%. For the negative control test, the requisite amount of sterilized 0.1% Tween-20 was added to the medium in place of the EO, while Carbendazim (0.5 mg/ml) was used as positive control. The plates were inoculated aseptically with a 6 mm assay disc cut from the periphery of 7day old culture of test fungus and observations were recorded at 7th day of incubation at 28+2°C.

Data analysis

All the data collected were subjected to analysis of variance (ANOVA) according to the procedure outlined by Steel and Torrie (1980) for completely randomized design (CRD). Test for significant difference among treatment means was performed using least significant difference at 5% level of probability.

Results

Effect of plant extracts and Carbendazim at different concentrations on the mycelia growth inhibition of *Fusarium oxysporum*

The results were discussed in the form of mean diameter of the treated fungi of the different extracts at different concentrations, expressed as percentages, compared with untreated (control) of fungi diameter. The growth of *F. oxysporum* in the culture media was slow, with the hyphae becoming visible after 48-50 hours after inoculation. **Tables 1 and 2** showed the results of antifungal screening of aqueous extracts used. The results revealed the inhibition activity of the plant extracts against *F. oxysporum*. The inhibitory effects of the plant extracts on the mycelia growth is as follow; *A. sativum*, *D. ambrosioides* and *Z. officinale*. Extract of *A. sativum* showed better antifungal activity on the mycelium growth compare to Carbendazim. *Allium sativum* at 100% gave the highest inhibitory effect (70.24%) on mycelial growth out of all the plant

extracts and compete favourably with Carbendazim which gave 80.38% inhibition. It was observed in

Table 2 that the higher the concentration the higher the inhibitory effect.

Table 1 Effect of plant extracts at different concentrations on mycelia growth of *F. oxysporum.*

Treatment	Concentration (g/100 ml)	Mycelia growth inhibition (%)
sativum rhizome extract	20	16.70^{b}
	40	17.81^{b}
	60	30.35^{ab}
	80	66.92^{ab}
	100	71.24^{a}
Z. officinale bulb extract	20	39.99^{c}
	40	43.56^{bc}
	60	60.65^{abc}
	80	70.24^{a}
	100	65.92^{ab}
Dysphania ambrosioides	20	29.80^{a}
	40	39.77^{a}
	60	42.10^{a}
	80	31.40^{a}
	100	23.72^{a}
Carbendazim	20	80.38^{a}
Control	0	
Level of significant		
SE+		0.05

Values in the same columns followed by the same letter(s) are not significantly different P=0.05 according to Least Significant Difference (LSD).

Effect of method of extraction of plant extracts on production of antifungal substances for mycelia growth inhibition of *F. oxysporum*

Evaluation of the efficacy of essential oil, cold and hot aqueous plant extracts was carried out *in vitro*, and it was observed that the three methods of extraction inhibited the growth of *F. oxysporum.* Results showed that hot aqueous extract has antifungal effect (44.87%) better than cold aqueous extract.

Effects of plants extracts at different concentrations on growth inhibition of *Fusarium oxysporum*

The results showed that, the mycelia growth inhibition increased with increase in the concentration of plant extracts. *A. sativum* showed the highest inhibition percentage of 71.24% and 66.92% at 80% and 100% concentrations respectively. It ranked next to 0.5 mg a.i/ml of Carbendazim (80.38%) which is the standard of comparison. There was no significant difference (p>0.05) between *A. sativum* at 80% (66.92%) and 100% (71.24%).

Table 2 The effect of plant extracts at 100% on mycelia growth inhibition of *F. oxysporum in vitro.*

Treatment	Mycelial growth inhibition (%)
Zingiber officinale	26.271^{d}
Allium sativum	54.223^{b}
Dysphania ambrosioides	34.247^{c}
Carbendazim	80.384^{a}
Level of significance	
SE +	0.05

Values in the same columns followed by the same letter(s) are not significantly different P=0.05 according to Least Significant Difference (LSD).

Discussion

Plants contain several phytochemicals known to play very important defensive roles against pathogens, rodents, and insects [14,15]. Medicinal aqueous plant extract has been reported to inhibit the growth of plant pathogenic fungi *in vitro* [16-18]. Mycelium growth inhibition assay results suggested that the aqueous extracts from *A. sativum*, *D. ambrosioides* and *Z. officinale* were active against the fungus *F. oxysporum*. These plants are extensively used in Nigeria due to their important usage in traditional medicine, and high content of polyphenols, flavonoids, phenolic acids, tannins, quinines, coumarins, terpenoids and alkaloids present in them [18-20]. The mechanism expected to be responsible for toxicity against pathogens may involves various targets viz; interference with the synthesis of cellular walls, alteration of cell permeability, interference with the transport of electron, the nutrient absorption, the adenosine triphosphatase and other metabolic processes of the cell, deactivation of various cellular enzymes and denaturation of cellular proteins [21-24]. *Allium sativum* contains flavonoids, phytic acid, tannins and phenols. Its aqueous extract promoted almost total inhibition of the mycelium. *Allium sativum* contains flavonoids, phytic acid, tannins and phenols. Its aqueous extract promoted almost total inhibition of the mycelium. Studies have shown that the presence of hydroxyl groups on the phenol groups are said to be related in their relative toxicity to microorganisms, which implies that increased hydroxylation results led to increased toxicity. Enzyme inhibition of the oxidized compounds are thought to be mechanism responsible for phenolic toxicity to microorganisms, possibly through reaction with sulfhydryl groups through more nonspecific interactions with proteins [25].

The result also indicates that extracts of *Z. officinale* and *A. sativum* possesses fungicidial properties that inhibit the growth of *F. oxysporum*. Researchers reported in their research a decrease in seed borne fungi disease of mustard with increase in dilution of garlic, neem, ginger and onion extract. It was also

reported in the experimental results that hot water method of extraction was the most effective way in promoting the action of plant extraction compared to cold water method of extraction. These findings supported the work of reserachers [13,26] which reported that extract of ether was more effective in reducing the *Alternaria alternate* and *Fusarium palliodoroseum* than the hot aqueous extract. Results revealed that the essential oils from *D. ambrosiodes*, *A. sativum L.* and *Z. officinale* plants showed more inhibitory properties on the mycelium growth of *F. oxysporum*, which showed a clear indication of their potential source over synthetic fungicides for the control of *F. oxysporum.*

Conclusion

The result of this study showed that the plant extracts had inhibitory effect on the mycelia growth of *F. oxysporum*, causative agent of Fusarium wilt of tomato crop. Thus, it can be recommended for use against the fusarium wilt of tomato. The extracts performed reasonably well at all levels of concentrations tested but the best performance was recorded at 80% concentration. The efficacy and effectiveness of these extracts coupled with their environmental friendliness, as presented these extracts for considerations as replacement for the synthetic fungicides against *F. oxysporum* infection in both biologically and integrated control programs. The development of natural pesticides would help to decrease the negative impact of synthetic agents such as residues, pathogen resistance and environmental pollution. In this respect, natural fungicides may be effective, selective, biodegradable, and less toxic to the environment as well as food and agriculture industries. Thus, it can be concluded that the use of extracts from medicinal plants should be considered as an antifungal agent to control *F. oxysporum* causing severe fungal disease such as rice blast.

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