SCREENING OF FOUR FOOD SPICES OF PLANT ORIGIN FOR ANTIFUNGAL ACTIVITY AGAINST A *FUSARIUM OXYSPORUM* SOIL ISOLATE

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ABSTRACT

Cold and hot water extracts of four food spices (Aframomum melegueta, Allium sativum, Zingiber officinale, and Monodora myristica) were studied for antifungal activity against a Fusarium oxysporum soil isolate. The study was necessitated by the current search for bioactive substances of plant origin in the bid to control disease-causing organisms. The Fusarium isolate was grown on Potato Dextrose Agar (PDA) and in Potato Dextrose Broth (PDB) containing 100, 50, 25 or 12.5% of each extract and incubated for 7 days (solid cultures) and 5 days (liquid cultures) at room temperature (28 ±2 ^{o}C). Mycelial radial growth was measured on the 7th day while dry weight of mycelium was determined after 5 days. Results showed that all tested spice plants had antifungal effect in the liquid cultures. Cold extracts were more effective as their percentage inhibition values were consistently, though insignificantly higher, than for hot extracts, except for A. sativum which had a significantly higher value (82.4% as against 55.5% for cold and hot extracts, respectively, at 100% concentration). Allium sativum had the highest inhibitory effect (82.4%) while M. myristica had the least (45.3%) for cold extracts in liquid cultures. For radial growth, Z. officinale, and M. myristica had no effect (cold extract) while M. myristica (hot extract) had no effect. These results indicate that the tested plants have antifungal potential that could be harnessed for the control of F. oxysporum. High temperatures seem to reduce the activity of the extracts. For future studies liquid cultures would be more effective.

Keywords: *Antifungal activity, Fusarium, plant extracts, plant pathogen*

INTRODUCTION

Recent studies have focussed on the general biology, ecology and natural means of controlling plant pathogens such as *Fusarium* since chemotherapy has negative effects on the environment (Dieter *et al*., 2007; Ghornany, Salary, 2004; Stompor-Chrzan, 2004). Some plant species in Nigeria have been reported to have antifungal activity (Abdulrahman *et al*., 2004; Okungbowa, Edema, 2006) and several herbal drugs are being tested for antifungal activity (Khan, Jain, 2000).

Ginger, the underground rhizome of *Zingiber officinale*, an annual herbaceous plant, is used as a common spice for soup, meat and drinks, in Nigeria. Extract from ginger has shown antibacterial activity (Chen *et al*., 2007) as well as antifungal action against *F. moniliforme*, *Aspergillus flavus* and *A. fumigates* (Nguefack *et al*., 2004). Garlic (a close ally of onion, Family Alliaceae) grows in the wild.

When crushed, garlic yields allicin, a sulphur-containing compound responsible for the pungent smell of garlic. Medicinally, garlic cloves are used as remedy for chest problems, hypertension,digestive disorder and fungal infections such as thrush (Shufford *et al*., 2005). *Monodora myristica* is the fruit of a tropical tree that belongs to the Family Annonaceae (Rehm, Espig, 1991).

The seeds contain the alkaloid annonaceine. All varieties are used locally as spice and it is a remedy for constipation and headache while the oil is a local stimulant for the gastrointestinal tract (Gill, 1992). *Aframomum melegueta* is a herbaceous perennial plant of the Family Zingiberaceae. The peppery taste of the seeds is due to the presence of aromatic ketones. Leaf extract of *A. melegueta* has been found to have antifungal property (Okigbo and Ogbonnaya, 2006).

Species of *Fusarium* are commonly found in the soil and they cause diseases in a variety of cultivated plants (Ghorbany, Salary, 2004). *Fusarium* species produce toxins in infected plants which can adversely affect man when such plants are consumed (Mathias *et al.,* 2003).

This study was undertaken to determine the effect of these four spices on the plant pathogen, *F. oxysporum* with a view to ascertaining their potential as sources of biocontrol substances for this pathogen that causes spoilage of a wide range of agricultural produce.

MATERIALS AND METHODS

Fusarium oxysporum isolate

The *F. oxysporum* soil isolate was obtained on agar slant from the Pathology Laboratory of the Nigerian Institute for Oil Palm Research (NIFOR) Benin City, and resuscitated in 10ml peptone water and then grown on fresh PDA for 5days. Re-identification of the isolate was done at the Mycology Unit of the Department of Plant Biology and Biotechnology of the University of Benin, Nigeria using previous descriptions (Barnett, Hunter,

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1998). Monoconidial fungal cultures of the pathogen were stored in sterile sand tubes at 4°C. Active cultures were obtained from small aliquots of sand culture plated on potato dextrose agar (PDA) and incubated at room temperature (28±2 °C) for 5 days with a 12 hours photoperiod of fluorescent light.

Collection of spices

All four spices (*Aframomum melegueta K.*Schum, *Allium sativum* L.,*Zingiber officinale* Roscoe and *Monodora myristica* Dunal) were purchased from a local market in Benin City and identified at the Department of Plant Biology and Biotechnology of the University of Benin, Nigeria, where samples were also deposited in the herbarium. The materials were taken to the Environmental Laboratory of the Ministry of Environment and Public Utilities, Benin City, Edo State, Nigeria, where this work was done.

Plant extracts

Cold and hot water extractions were carried out. The *A. sativum* and *Z. officinale* were washed in distilled water and air-dried. The seed - coat of *M. myristica* seeds was removed with a kitchen knife while the pods of *A. melegueta* were split open and the seeds removed with a spatula. Then 100 g of each spice was ground with an electric blender (Mixer Model 830 L, Hong Kong) and mixed with 500 ml sterile distilled water in a conical flask and left to stand for 2 hours. For hot water extraction, the conical flask containing the mixture of distilled water and ground spice was placed in a water bath with temperature maintained at 90 °C for 2 hours. The mixture was then filtered with a Whatmaan Number 1 filter paper, and cooled to room temperature (28 ±2 °C).

Serial dilution of extract

Serial dilution of extract was done according to the broth macro-dilution technique (Akinyemi *et al*., 2005). Each extract (5 ml) was serially diluted with sterile distilled water in a 1:1 ratio to get concentrations of 50, 25 and 12.5% (v/v). The original (undiluted) extract-distilled water mixture was designated as 100% concentration. The positive control was 10% Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate, Sigma) while distilled water containing neither plant extract nor benomyl served as a negative control.

Inoculation of plates for radial growth determination

The Pour-Plate method was used. Using a calibrated 2 ml pipette, 0.2 ml of mixture for each concentration was transferred separately into each plate. Then 15ml of molten PDA (40 °C) was poured into the plate and the plate swirled gently for even mixing, and then left to solidify. Each plate was inoculated at the centre with a 0.1 ml fungal spore suspension ($2.5x10^6$/ml) and incubated at room temperature 28 ±2 °C for 7 days. Plates, including controls, were in triplicates. Mycelial radial growth was measured on the 7th day with a metre rule (Okwu *et al*., 2007. Mean values were calculated.

Effect of extracts on mycelial dry weight

A volume of 100 ml of mixture for each concentration in a 250 ml conical flask was inoculated with a 3 mm diameter fungal mycelial disc from a 4 day- old *F. oxysporum* pure culture, and incubated at room temperature (with shaking) for 5 days. Mycelia were harvested on pre-weighed filter paper, dried in a hot air oven at 80 °C and weighed. Percentage inhibition in liquid media was then calculated using the modified formula (Amadioha, 2003).

$$I = 100(WC-WT)/WT$$

where

I = Percentage inhibition of mycelia growth with respect to control;

W C = Dry weight in control medium

WT =Dry weight in treated medium

Statistical analysis

Data were analysed using student t-Test at a probability level of 0.05.

RESULTS AND DISCUSSION

The study was carried out to determine the antifungal activity of the four spices on the plant pathogen, *F. oxysporum* with a view to ascertaining their potential as possible sources of biocontrol substances for the pathogen.

Extracts of all plants tested in liquid cultures had antifungal activity (Figure 1).

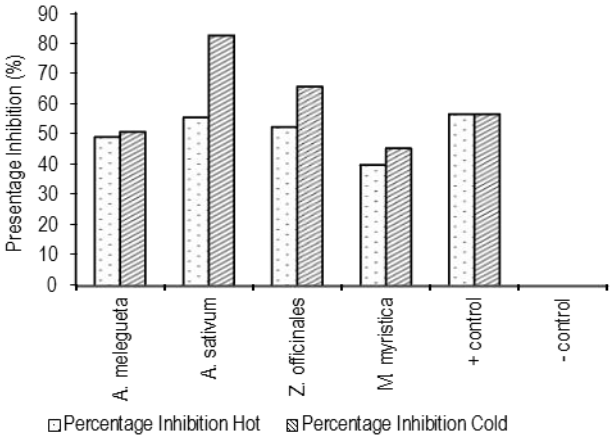

Figure 1. Mean Percentage Inhibition of extracts of food spices on growth of *F. oxysporum* incubated at 28±2ºC for 5days.

Cold extracts were more effective as their percentage inhibition values were consistently higher than for hot extracts, except for *A. sativum*. Also, *A. sativum* had the highest inhibition (82.4%) and *M. myristica* the lowest (45.3%) for cold extracts in liquid cultures. In solid cultures, cold extracts of *Z. officinale*, and *M. myristica* had no effect (Table 1). Hot extracts of all plants (except *M. myristica*) significantly repressed radial growth of mycelium and *A. sativum* exhibited strong inhibition (Table 2). Nguefack *et al*. (2004) reported *M. myristica* shows inhibitory effect on *F. monliliforme*. Also, *M. myristica* has the lowest activity, and *Z. officinale* has a moderate activity, compared to other tested plants; this report corroborates our observations in this work. Earlier workers (Gur *et al*., 2006) reported that *Z. officinale* contains fatty acids which inhibits the growth of some bacteria. It was also found that *A. sativum* extracts had high inhibitory effects on *F. oxysporum* (Benkeblia, 2004). An

inhibitory effect of 50% for *A. melegueta* against *Epidermophyton floccosum* was reported earlier (Okungbowa, 2006) while the leaves of *A. melegueta* had some inhibitory effect on various fungi including *F. oxysporum* (Okigbo, Ogbonnaya, 2006). The latter report also showed that cold extract of *A. melegueta* leaves had more effect than hot extract, supporting our findings in this work.

Table 1. Effect of Cold Water Extract on the Radial Growth of *F. oxysporum* incubated at 28±2ºC for 7days

Extract	*Mean Radial Growth ± Sx (cm)			
	100%	50%	25%	12.5%
A. melegueta	2.4 ± 0.1[a]	2.1 ± 0.3[a]	2.4 ± 0.1[a]	2.5 ± 0.1[a]
A. sativum	2.2 ± 0.2[a]	2.4 ± 0.2[a]	1.9 ± 0.5[a]	2.5 ± 0.2[a]
Z. officinale	4.2 ± 0.1[b]	4.2 ± 0.1[b]	4.2 ± 0.0[b]	4.2 ± 0.1[b]
M. myristica	4.1 ± 0.0[b]	4.2 ± 0.1[b]	4.3 ± 0.0[b]	4.3 ± 0.0[b]
Control (+ve)	4.2 ± 0.0[b]	4.2 ± 0.0[b]	4.2 ± 0.0[b]	4.2 ± 0.0[b]
Control (-ve)	4.2 ± 0.0[b]	-	-	-

Sx = Standard error of mean of three replicates.
*Data along the same column bearing different letters in superscript are significantly different (p≤ 0.05).

The generally higher efficacy of cold extracts over hot extracts could be attributed to the effect of temperature on some of the active components which might be some protein that got denatured at high temperature (Jabeen *et al*., 2008). For example, *A. sativum* extract lost its antifungal activity at temperatures above 60 ºC (Samuel and Jabeshree, 2000). It has also been reported that temperature does not alter the activity of some plant extracts (Doughari, 2006; Salama, Marraiki, 2009). The choice of temperature of extraction is, therefore, dependent on active components of the extracts concerned. The differences between mycelial radial growth for treated and control were significant for cold extracts (t-test at p≤ 0.05) except *Z. officinale* and *M. myristica*. Also, mycelia radial growth was significantly different between treated and control for hot extracts (t-test at p≤ 0.05) except for *M. myristica*.

These results show that the tested plant parts are potential sources of antifungal substances that can be used to control *F. oxysporum* a pathogen of serious concern in agriculture. This potential should be harnessed.

Table 2: Effect of Hot Water Extract on the Radial Growth of *F. oxysporum* incubated at 28±2ºC for 5 days.

Extract	*Mean Radial Growth ± Sx (cm)			
	100%	50%	25%	12.5%
A. melegueta	2.2 ± 0.2[a]	2.0 ± 0.3[a]	2.3 ± 0.8[a]	1.9 ± 0.4[a]
A. sativum	2.5 ± 0.1[a]	2.5 ± 0.2[a]	2.8 ± 0.1[a]	2.3 ± 0.1[a]
Z. officinale	2.2 ± 0.4[a]	2.5 ± 0.1[a]	1.7 ± 0.0[a]	2.2 ± 0.1[a]
M. myristica	4.1 ± 0.0[b]	4.2 ± 0.0[b]	4.2 ± 0.0[b]	4.2 ± 0.0[b]
Control (+ve)	4.2 ± 0.0[b]	4.2 ± 0.0[b]	4.3 ± 0.0[b]	4.2 ± 0.0[b]
Control (-ve)	4.2 ± 0.0[b]	-	-	-

Sx = Standard error of mean of three replicates.
*Data along the same column bearing same letters in superscript are not significantly different (p≤ 0.05).

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