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Short Communication

Antifungal properties of some locally used spices In Nigeria against some rot fungi

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Water extracts of three spices (*Allium sativum*, *Afromumum melegueta* and *Zingiber officinale*) were tested for antifungal activities against three rot fungi (*Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*) using mycelial growth extension method. The extracts showed varying degrees of antifungal activities against the test fungi. *P. notatum* was observed as the most susceptible fungus while *A. niger* was the least susceptible. There was no significant difference (P=0.05) in the antifungal activities of the three spices against the test fungi. The effectiveness of the spices extracts increased with increase in extract concentrations. The result of this study indicated that the three spices can be used as alternatives to chemical preservatives of agricultural products against fungal rots caused by the three fungi in storage.

Keywords: Antifungal properties*, Allium sativum, Afromomum melegueta Zingiber officinale, Aspergillus niger, Aspergillus flavus, Penicillium notatum*.

INTRODUCTION

Spices and herbs have been used to improve the flavour of food for hundreds of years. Spices have been items of international commerce for many hundreds of years. Their popularity lie in the interest they give to almost tasteless foods such as rice and many other food items. They also disguised the flavour of unpleasant tasting foods such as rotten meats. Some spices aid digestion and some have antitoxic properties (Leslie, 1976). Their essential oils are extracted through distillation and are used in flavouring, perfuming and in soft drink production (Maclaren, 1979).

Apart from using some spices as food, many local people and traditional healers use spices in combination with other plant materials to cure and heal different diseases. The use of spices goes beyond ordinary food items, but as medicine to treat some diseases. In some tribes in Nigeria, particularly the Uhrobo people of Delta State, spices are used to prepare pepper soup with fish (fresh or dry) and meat for people suffering from malaria fever. These are relieved of the fever after taken the pepper soup for some times. Spices contain some microbial properties that can be used to treat diseases caused by micro-organisms (Odoemena et al., 2007, Iwalokunet al., 2004). Many scientists have worked on the anti-microbial properties of several plants in Nigeria (Akpulu et al., 1994, Rojas et al., 1992, Akueshi et al., 2002, Danalap and Akueshi, 2005, Okungbowa and Edema, 2007). The microbial activities of many spicies on pathogenic bacteria have been reported (Iwalokun et al., 2004, Odoemena et al., 2007). It is on this note that we tried to investigate the antifungal properties of some locally used spices in Nigeria. The results obtained may contribute to the alternative use of plant materials for preservation of agricultural products in storage against rot fungi.

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MATERIALS AND METHODS

Collection of plant materials

The three spices used were *Allium sativum (*Garlic*), Afromumum melegueta (*Aligator pepper*)* and *Zingiber officinale (*Ginger). The spices were purchased in Abraka main market in Delta State, Nigeria. They were identified in the Department of Botany, Delta State University, Abraka, by Professor M. Idu. The spices were washed separately several times with sterile distilled water and then crushed and pulverized separately. The pulverized spices (10 g each) were then separately soaked in 100 ml sterile distilled water and shaken continuously for 24 h using a rotary shaker following the method of Onwuliri and Wonang (2005). Crude aqueous extracts

Table 1. Inhibitory effect of the crude extracts of *Allium sativum, Zingiber officinale* and *Afromumum melegueta* on mycelial growth of *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum* (mm).

Spice	Fungi		
	A. niger	***A. flavus***	***P. notatum***
Control	23.41 ± 0.01	26.10±0.02	18.31±0.03
Allium sativum	9.05±0.02	10.01±0.04	6.20±0.01
Zingiber officinale	10.03±0.1	10.50±0.3	6.80±0.05
Afromumum melegueta	11.70±0.02	13.61±0.01	13.40_0.05

Each value is mean of 3 replicates ± standard error

were obtained by filtering through a sterile Whatman No 1 filter paper and stored at about 4°C in the refrigerator until when used.

Test Organisms

A. niger, A. flavus and *P. notatum* isolates which are known fruit and vegetable rot fungi were collected from the mycology/ pathology unit of the Department of botany, Delta State University, Abraka, Nigeria. The pure cultures of the isolates were maintained on Potato Dextrose Agars (PDA) and kept in the refrigerator until when used.

Determination of antifungal properties

The effect of the spice extracts were determined by measuring the mycelial extension of the fungi on PDA media. Two milliliters of each spice extract was mixed with 20 ml of sterile PDA medium in test tubes and poured into sterile Petri dishes. Streptomycin, 0.5 mg/litre was prepared and added to the mixture of the PDA and the spices to suppress the growth of bacteria. The plates were allowed to cool and solidified before inoculating with the test organisms. Mycelia discs (5 mm in diameter) were collected from the periphery of actively growing fungi cultures using sterile cork borer and placed at the centre of the PDA mixtures. Three plates were prepared for each spice and for each fungus under aseptic conditions. The control plates without the spice extracts were similarly inoculated with the fungi. The set up were incubated at room temperature (30 ± 2°C) for five days in the laboratory. The radial growth less the diameter of the initial inoculums was measured in two directions along the perpendicular lines and the means were calculated for each plate and for each fungus.

Minimum inhibitory concentration (MIC) of spices extracts

Twenty milliliter of PDA was pour into sterile Petri dishes containing different percentages (0, 10, 30, 50, 70 and 100) of the respective extracts of the spices. Ten milliliters of the crude extract of each spice + 90 ml of distilled water gives 10% extract solution. Inoculation and determination of the mycelia growth was as described above. The Minimum Inhibitory Concentration (MIC) was determined as the least percentage concentration of the spice extract that shows an inhibitory effect on the mycelia growth of the test fungi when compared with the control using radial growth method (Cheesbrouh, 1994).

RESULTS AND DISCUSSION

Varying degree of antifungal activities by the three spice extracts were observed at different concentrations. The spices showed antifungal activity against the fungi tested. *P. notatum* was observed as the most susceptible fungus while *A. flavus* was the least susceptible fungus to the crude extracts of the spices (Table 1). Results showed that the three spices have similar antifungal properties against the test fungi (Table 2). The spices also have similar minimum inhibitory concentrations (MIC) as shown in Table 2. *A. melegueta*, however had the highest minimum inhibitory concentration against *P. notatum* at 10% compared with the other two spices which have minimum inhibitory concentrations at 50% against *A. flavus* and *P. notatum*. This result is similar to the report of Odoemena et al. (2007) who observed significant inhibitory effect of *A. melegueta* against some pathogenic bacteria. The antifungal potency of these spices also supported the work of Udo et al. (2001) who reported that methanol and ethanol extracts of *A. sativum* have high potency for the control of pathogenic fungi on potato and yam tubers. They however reported that the potency of aqueous extracts were low compared to the methanol and ethanol extracts. In this study, it was observed that the higher the concentration of aqueous extracts of the spices, the higher the potency on the inhibition of mycelia growth of the fungi tested (Table 2). According to Iwalokun et al. (2004), the aqueous Garlic extract was effective against some multidrug-resistant bacteria and Candida species.

Onwuliri and Wonang (2005) also reported the antibacterial activities of *Z. officinale* and *A. sativum* on some bacteria (*Escherica coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylcoccus aureus*) even at low concentration.

The observation in this study supported the reports of Iwalokun et al. (2004) and Onwuliri and Wonang (2005) Udo et al. (2001) attributed the antifungal properties of *A. sativum* to its phytochemical components. The anti-fungal properties of these spices may also be due to their phytochemical contents which caused inhibitory effects on the mycelia growth of the fungi. However, the inhibittory potency may depend on the mode of extraction and the concentration of the extracts used. The phytoche-mical screening of these spices are therefore encouraged so that they can be properly harnessed and standardized for effective use as alternatives to chemical preservatives in the preservation of agricultural products against fungal rots in storage.

Table 2. Minimum inhibitory concentration (MIC) in % of crude *Allium sativum, Zingiber officinale* and *Afromumum melegueta* on mycelial extension (mm) of *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum* (mm).

Spice	Concentration (%)	*A. niger*	*A. flavus*	*P. notatum*
Allium sativum	0	23.40±0.01	26.10±0.02	18.31±0.03
	10	22.10±0.03	25.80±0.04	18.10±0.04
	30	18.61±0.01	23.05±0.01	16.91±0.01
	50	15.82±0.02	17.02±0.05	12.08±0.03
	70	11.32±0.04	16.03±0.01	6.60±0.05
	100	9.24±0.01	10.01±0.04	6.22±0.01
Zingiber officinale	0	23.40±0.01	26.10±0.02	18.31±0.03
	10	27.04±0.01	26.00±0.04	11.10±0.03
	30	26.01±0.05	20.40±0.01	7.42±0.04
	50	19.02±0.04	15.01±0.04	7.00±0.01
	70	18.06±0.01	16.02±0.01	6.21±0.01
	100	10.03±0.01	10.51±0.03	6.12±0.02
Afromumum melegueta	0	23.40±0.01	26.10±0.02	18.31±0.03
	10	19.21±0.05	21.12±0.04	17.02±0.03
	30	13.62±0.03	13.41±0.01	15.11±0.05
	50	13.01±0.01	12.00±0.04	15.05±0.01
	70	12.01±0.04	12.06±0.03	12.83±0.02
	100	11.70±0.02	12.61±0.01	11.96±0.05

Each value is mean of 3 replicates ± standard error.

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