

Full Length Research Paper

Bioactivity of *Zingiber officinale* and *Piper nigrum* plant extracts in controlling post-harvest white yam (*Dioscorea rotundata*) tuber rot fungi

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Rotten white yam (*Dioscorea rotundata*) tuber samples were collected from farms in three communities. Nine fungal organisms were isolated by direct tissue plating on potato dextrose agar medium. These were: *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp., *Lasiodiplodia theobromae*, *Trichoderma viride*, *Rhizopus* spp., *Pestalotia guepini* and *Alternaria solani*. Each of these isolates caused rot lesions when inoculated into healthy yam tubers. These organisms were re-isolated, identified and compared with the initial isolates to confirm their pathogenicity. *L. theobromae*, *A. niger*, *Rhizopus* sp. and *A. solani* were the most frequently isolated fungi species from the rotten yam tuber samples, with frequency of isolation of 30.07, 16.08, 16.08 and 12.59%, respectively. *Zingiber officinale* rhizome and *Piper nigrum* seeds were tested against *L. theobromae* and *F. oxysporum* *in vitro*. The botanical extracts were prepared by cold water extraction method at a concentration of 60% w/v. The two extracts showed significance in inhibiting the growth of the two fungi when data was statistically analysed ($p = 0.05$) using Genstat 9.2 package. This implies that they have some anti-fungal properties which need to be further investigated *in vivo* to establish their suitability in protecting yam tubers from storage rot. However, *P. nigrum* showed to be much stronger than ginger in terms of bioactivity.

Key words: *Zingiber officinale*, *Piper nigrum*, bioactivity, yam tuber rot, rot fungi.

INTRODUCTION

Rot is a major factor limiting the post-harvest life of yams and losses can be as high as 60% in storage (Adesiyun and Odihirin, 1975). Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. The quality of yam tubers is affected by rot which makes them unappealing to consumers.

Some white yam varieties like 'pona' that are preferred

by most consumers in Ghana, do not store for a long time due to attack by rot organisms. Because of their poor storability, farmers sell produce just after harvest to avoid losses, and this result in low income and reduced profits.

Nine fungal species including *Aspergillus flavus*, *Aspergillus niger*, *Lasiodiplodia theobromae*, *Fusarium culmorum*, *Fusarium oxysporum*, *Penicillium brevicompactum*, *Penicillium oxalicum* and *Rhizopus*

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stolonifer, have been identified to be associated with yam tuber rot in Ghana (Aboagye- Nuamah et al., 2005). Ezeibekwe and Ibe (2010) reported that *F. oxysporium*, *L. theobromae* and *F. solani* were associated with yam rot diseases in Nigeria.

Fungal pathogens causing rot in yam often gain entry into tubers through wounds caused by insects, nematodes or poor handling before, during and after harvest (Amusa et al., 2003). Rot of fleshy parts of plants that develop as tissues are disintegrated by the action of microorganisms. Extra-cellular enzymes such as hydrolases and lyases are produced in advance of fungal hyphae of the attacking pathogens. The affected tubers become hydrotic and soft, turn brown, emit offensive odour and exhibits a sharp demarcation between a healthy intact tissue and a diseased tissue.

Some of the control measures studied over the years include minimising physical damage of tubers, the use of chemicals, the use of crop rotation, fallowing and planting of healthy materials, destruction of infected crop cultivars, wood ash application and breeding for resistance (Oduro et al., 1991; Nyadanu et al., 2014). Some plants possess fungicidal properties.

Okigbo and Nmeka (2005) showed that extract of *Xylopiya aethiopicum* and *Zingiber officinale* controlled post-harvest yam rot. Pesticides of plant origin are specifically more biodegradable, readily available, cheaper and environmentally friendly than synthetic chemicals. In this report, the bioactivity of the extracts of *Z. officinale* rhizome and *P. nigrum* seed in controlling the growth of some yam tuber rot fungi were studied.

MATERIALS AND METHODS

Collection of diseased yam tubers

About twenty rotten tubers of white yam 'pona' were collected randomly from farms in three districts, namely Kintampo North, Wenchi and Tain districts and sent to the Plant Pathology Laboratory, CSIR-Crops Research Institute, Kumasi, in polythene bags. Collected samples were kept in a refrigerator at 4°C until required.

Isolation of fungal species from rotten yam tubers

Pieces of diseased tissues (50 mm³ average) cut from the periphery of the disease lesion on the tubers with a sterilized knife were surface-sterilized in 5% sodium hypochlorite solution for 2-3 min. The surface sterilized diseased tissues were washed three times using sterile distilled water. The tissues were allowed to dry in a sterile Lamina flow chamber (BASSAIRE, Duncan Road, Swandick, Southampton, SO3 7ZS) for about 30-45 min. The dried disease tissues were plated on potato dextrose agar (PDA) medium (Merck; Merck KGaA, 64271 Darmstadt, Germany). Two days after incubation, mycelia that grew from the plated yam tissues were sub-cultured onto fresh PDA. Further sub-culturing was carried out until pure cultures of single isolates were obtained. From these pure cultures, inocula of the different fungal species were obtained for the pathogenicity tests. Frequency of occurrence for each organism was determined by calculating the number of colonies of a fungus

out of the total number of fungal colonies, expressed as a percentage.

Identification of fungal isolates

Characteristics of fungal isolates from rotten yam tubers such as colour, pigment production, colony texture, spore or conidia-producing structures and spore shapes were documented. The characteristics were observed from fungal mycelia grown on PDA for one week or more, depending on the fungal species. Spore and mycelium characteristics were studied using the compound microscope. These characteristics were used in identifying the fungal organisms to the species level, following standards described by Mathur and Kongsdal (2003) and Barnett and Hunter (1972).

Pathogenicity test

One week old pure cultures of the fungal isolates obtained from rotten yam tubers produced on PDA were the source of inocula for the pathogenicity studies. Middle portion of healthy yam tubers (average 40 cm long) of 'pona' were inoculated with the fungal isolates identified (4cm interval). A 5-mm diameter cork borer was used to remove discs (1 cm thick) from the yam tuber surface after surface sterilization of the tubers with 5% sodium hypochlorite solution. The 5-mm diameter cork borer (sterilized by dipping in 100% alcohol followed by flaming) was used to cut plugs from the one week old cultures of the fungal isolates to be tested. These fungal plugs were put in the holes created in the yam tubers after which the removed yam tuber discs were used to plug the holes. Melted candle wax from a burning candle was used to seal the edges of the replaced yam discs. This process prevented contamination by other microbes. Each fungal isolate was replicated three times (on three different yam tubers) in a complete randomised design. Controls were set up whereby no fungal organism was placed in the hole. These activities were carried out inside a sterile hood. After 10 days of inoculation, the inoculated wholes were cut cross-sectionally to observe rot infection by inoculated fungi.

Preparation of plant extracts

Cold water extraction method was used for the preparation of the plant extracts. Fresh rhizomes of *Z. officinale* (ginger) and seeds of *P. nigrum* (black pepper) were washed thoroughly with distilled water. These were further blended into a fine paste separately for each botanical with a blender (Binatone, BLG-401, Hong Kong) at a speed of 4000 rpm for five to ten minutes. Extract concentration of 60% (w/v) was obtained by adding 40 ml of sterile distilled water to 60 g of each botanical paste in a beaker with vigorous stirring.

Anti-fungal bioactivity of plant extracts *in vitro* on yam rot organisms

Two test fungi, *L. theobromae* and *F. oxysporum*, obtained from rotten yam tissues, were used in this experiment. Surface coating of potato dextrose agar (PDA) medium with botanical extracts was the method used to investigate the bioactivity of the extracts. PDA medium was prepared by dissolving 39 g in one litre sterile distilled water and autoclaved at 121°C and 15 psi for 15 min. The medium was poured into sterilized Petri dishes and allowed to solidify. Five hundred microlitres (500 µl) of each botanical extract preparation was spread thinly on the surface of the PDA in Petri dishes. The extract was allowed to dry and the medium inoculated centrally with

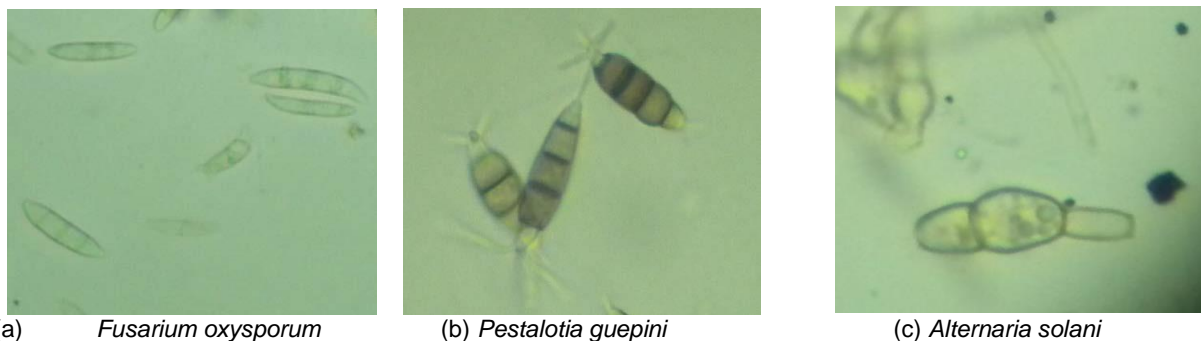


Plate 1. Conidia of some fungi identified (from rotten yam tissues) under compound microscope (x40/0.65 magnification).



Plate 2. A cross-section of a tuber of yam inoculated with fungus showing rot lesion.

discs (5 mm diameter) obtained from one-week-old cultures of the test fungi, *B. theobromae* and *F. oxysporum*. Three replications were set for each treatment. Controls were set up in which PDA with no botanical extract were inoculated with test fungi. The organisms were incubated at 26-28°C and measurement of mycelial growth as radius of a growing fungal colony was undertaken and recorded at intervals of twenty-four hours using a ruler. All the analyses were done in triplicates and data obtained were statistically analysed using Genstat (Release 9.2) statistical package. Analysis of variance (ANOVA) test and least significant difference at 5% were used to compare the treatment values. Percent growth inhibition of fungal organisms due to the plant extracts was calculated and graphically presented to show the fungicidal action of the plant extracts using the formula of Pandey et al. (1982).

RESULTS

Rot fungi identified

Based on cultural and microscopic characteristics of the cultures, the nine isolates of fungi obtained from rotten tubers were identified as *F. oxysporum*, *A. flavus*, *A. niger*, *Penicillium* sp., *Lasiodiplodia theobromae*, *T. viride*, *Rhizopus* sp., *Pestalotia guepini* and *Alternaria*

solani. Plate 1 shows the conidia of some of the fungal isolates from rotten yam tissues produced on PDA.

Each of these isolates was able to cause rot lesions when inoculated into healthy yam tubers (Plate 2). *L. theobromae*, *A. niger*, *Rhizopus* spp. and *A. solani* were the most frequently isolated fungal species from the rotten yam tubers collected from the study districts. The frequency of isolation was in the order of 30.07, 16.08, 16.08 and 12.59%, respectively.

Anti-fungal bioactivity of plant extracts against *L. theobromae*

Table 1 shows the mean mycelial growth (mm) of *L. theobromae*. The statistical analysis (ANOVA) showed that there were significant differences among the treatments in the experiment. Mycelial growth of *L. theobromae* on the two plant extract amended PDA were significantly different from the control. However, there was a significant difference between ginger and black pepper at 72-h period although there was no difference between them at 24 and 48 h periods.

After 24 h of incubation, *Z. officinale* rhizome extract inhibited growth of *L. theobromae* by 76.12% when compared with the control. This bioactivity declined to 70.16% at the end of 48 h period and reduced to 64.64%, 72 h after incubation. *P. nigrum* extract at a concentration of 60% (w/v) inhibited growth of *L. theobromae* by 83.58, 80.65 and 81.23% after 24, 48 and 72 h incubation, respectively (Figure 1).

Anti-fungal bioactivity of plant extracts against *F. oxysporum*

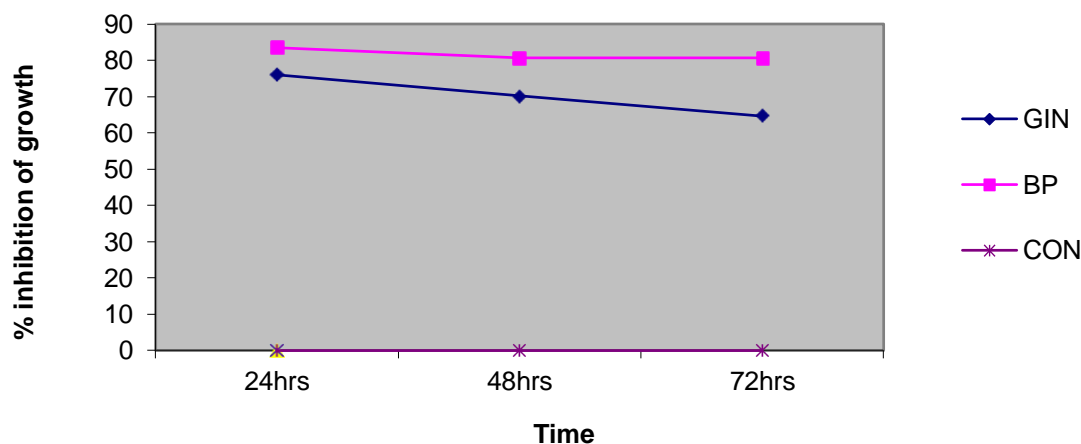
Similarly, there was significant difference among the treatments according to the ANOVA results. The two plant extracts showed significant differences from the control. The differences among the plant extracts were realised at 72 and 96 h period (Table 2).

The percent growth inhibition of *F. oxysporum* by *Z.*

Table 1. Effect of plant extracts on mean mycelial growth (mm) of *L. theobromae*.

Treatment	Incubation period (hours)		
	24	48	72
Control	22.33±1.16 ^a	41.33±0.58 ^a	60.33±2.31 ^a
Ginger	5.33±1.53 ^b	12.33±2.31 ^b	21.33±2.52 ^b
Black pepper	3.67±3.22 ^b	8.00±4.36 ^b	11.67±4.16 ^c
LSD(0.05) = 4.966 SED (0.05) = 2.343 CV% = 13.9			

*Values followed by the same letter within a column are not significantly different ($p=0.05$).

**Figure 1.** Percent growth inhibition of *B. theobromae* by plant extracts.**Table 2.** Effect of plant extracts on mean mycelial growth (mm) of *F. oxysporum*.

Treatment	Incubation period (hours)			
	24	48	72	96
Control	2.00±1.00 ^a	10.33±1.53 ^a	14.67±2.08 ^a	19.33±2.52 ^a
Ginger	0.00±0.00 ^b	0.00±0.00 ^b	1.67±0.58 ^b	5.67±1.16 ^b
Black pepper	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^c
LSD (0.05) = 1.571 SED (0.05) = 0.758 CV% = 20.7				

*Values followed by the same letter within a column are not significantly different ($p=0.05$).

officinale extract (60% concentration) after 24 and 48 h incubation was 100%. Inhibition was 88.64 and 70.69% at the end of 72 and 96 h, respectively. One hundred percent (100%) growth inhibition of *F. oxysporum* was achieved with *P. nigrum*, even at 96 h of incubation (Figure 2).

DISCUSSION

Seven of the identified fungi in this work have been isolated and identified to be rot-causing organisms in other research works done (Okigbo and Ikediugwu, 2002;

Aboagye-Nuamah et al., 2005). *L. theobromae* and *Penicillium oxalicum* were reported to cause dry rot of yam (IITA, 1993), whilst *Rhizopus* spp. causes soft rot. These fungi are soil-borne pathogens and this confirms that soils adhering to harvested tubers contain many microorganisms that could be pathogenic to the tubers (Ezeibekwe and Ibe, 2010).

Pesticides of plant origin are known to be more specific, biodegradable, cheaper, more readily available and environmentally friendly than synthetic chemicals. The efficacy of the two botanical extracts (*Z. officinale* and *P. nigrum*) in controlling yam tuber rot fungi was significant. This confirms the work done by Okigbo and

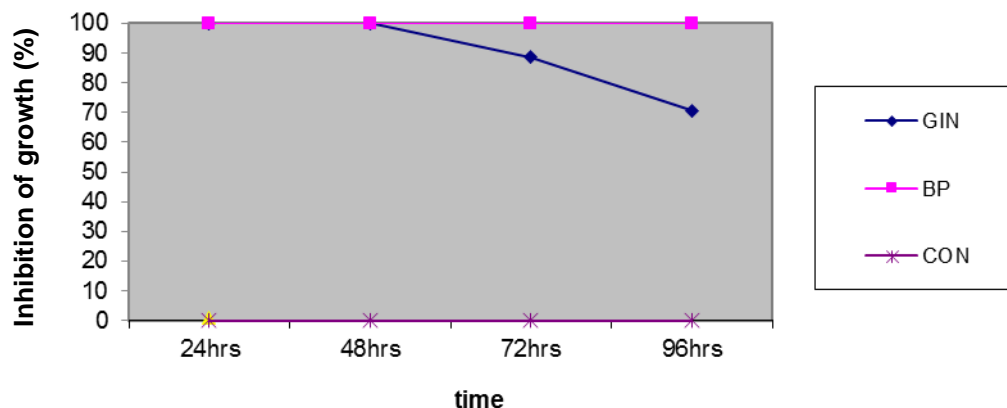


Figure 2. Percent growth inhibition of *F. oxysporum* by plant extracts.

Nmeka (2005) that *Z. officinale* suppresses the growth of rot fungi in culture. *Z. officinale* contains an active ingredient called *gingerol*. Ginger extracts have been shown to possess a broad range of biological activity against fungi (Foster and Yue, 1992). *P. nigrum* has shown to possess anti-fungal properties (Kuhn and Hargreaves, 1987). In this study *P. nigrum* exhibited a stronger and persistent bioactivity as compared to *Z. officinale*.

Conclusion

In conclusion, plant extract based technologies can be developed in the near future to control these organisms on yam tubers. It is recommended that the anti-fungal properties of the two botanicals are further investigated *in vivo* and phytochemical analyses done to establish their suitability in protecting yam tubers against rot fungi.

Conflict of interests

The author declares that there is no conflict of interest.

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