Antifungal Potency of Essential Oil Components of African Ginger- *Zingiber officinale* (Roscoe)

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Abstract. The antifungal potency of essential oil of *Zingiber officinale* (Roscoe) (African Ginger) was investigated using the hyphal extension bioassay. The essential oil (vacuum distillate) was obtained through vacuum distillation. Gas chromatography-mass spectrometry analysis of the oil revealed 27 compounds with six compounds (1,8-Cineole, α -Pinene, Camphene, (*E,E*)- α -Farnesene, Geranial and Zingiberene) showing major biological activity. Results of antifungal screening of the bioactive blend from these compounds and vacuum distillate in comparison with four synthetic fungicides revealed that the bioactive blend was more effective against the test pathogen – *Botryodiplodia theobromae* (Pat.) in culture allowing the shortest hyphal lengths of 1.12cm, 0.28cm and 0.18cm at 50, 75 and 100 μ g/ml concentration compared with 3.39cm, 0.77cm and 0.28cm respectively obtained for forcelet (the best fungicide tested). Vacuum distillate was comparable ($P < 0.05$) in performance to the bioactive blend. These two plant-based chemicals were persistent in their action against *B. theobromae* at all levels of concentration throughout the course of the experiment.

Introduction

Ginger (*Zingiber officinale* Roscoe.) is a perennial, tuberous root or rhizome which belongs to the family Zingiberaceae. It is native to South-East Asia and has been cultivated for thousands of years as a spice and for its medicinal uses [1]. The stems are erect, oblique, round, annual, covered by smooth sheaths of leaves, and is about 2 to 3 feet in height. The most economically viable part of the plant is the perennial rhizome which is prepared and used in various forms across the globe, including, India, Europe and Asian countries like China and Japan. In Chinese Traditional Medicine (TCM), its usage dates as far back as 2500 years ago [2]. Medicinally, ginger has been reported to be effective in the treatment of conditions like rheumatologic conditions, muscular discomfort, atherosclerosis, migraine headaches, rheumatoid arthritis, chronic inflammatory conditions, high cholesterol, ulcers, depression and impotency [3-8].

Besides its multifarious medicinal uses, ginger is a valuable spice commonly used in cooking worldwide. It is also believed to provide relief to common cold, flu-like symptoms, and even painful menstruation in women [9].

Ginger is rich in an array of chemical compounds such as pungent volatile oils and non-volatile compounds. The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumen (18%) and farnesene (10%), with lesser amounts of bisabolene and β -sesquiphellandrene. A small percentage of at least 40 different monoterpenoid hydrocarbons are present with 1,8-cineole, linalool, borneol, neral, and geraniol being the most abundant [2]. Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. The non-volatile pungent compounds is a collection of biologically-active constituents including the non-volatile pungent principles, such as the gingerols, shogaols, paradols and zingerone that produce a “hot” sensation in the mouth.

The usefulness of ginger transcends its effectiveness in the cure of various human ailments as the potency of aqueous, ethanolic extracts and volatile oils of several plant products including ginger has been reported in the control of various insect pests [10, 11] and plant pathogenic diseases of fungal and bacterial origin [12-19]. As effective as these plant products have been reported in plant disease control, it is rather unfortunate that most farmers are still using costly synthetic chemicals with the dire consequences of environmental pollution [20].

According to FAO Statistics [21] for root crops, yam is regarded as one of the most important root crops worldwide. It is a major food crop with a variety of uses. Besides its usefulness as food, it is a good source of medicine, alcohol, industrial starch and feed for animal. However, the activity of pathogenic fungi as rot-causing agents in yam tubers has remained one of the most serious setbacks to yam production worldwide resulting in about 50% losses within a season [22]. One of the most virulent fungi is *Botryodiplodia theobromae* and has been implicated in many reports of yam rots [23-25].

Information on the use of essential oil for the management of plant disease is not extensive. This paper evaluates the biological activity of volatile compounds in essential oil of ginger (*Zingiber officinale*) for the control of *B. theobromae* causing rot of white yam.

Materials and Methods

Sample collection

Fresh rhizomes of *Zingiber officinale* (ginger) were obtained from Marian market in Calabar Metropolis, Cross River State, Nigeria. The identification/authentication of the fruit was carried out at the herbarium of the Department of Biological Sciences, Cross River University of Technology (CRUTECH), Calabar and at the Department of Botany, University of Calabar, Calabar, Nigeria. Fresh uninfected and rotted yam tubers were obtained from three markets (Watt, Marian and Akim) in Calabar Metropolis. Infected yams were collected between the months of March-May, 2018 when fungal attacks are usually severe. The infected samples were collected in sterile cellophane bags and taken to the laboratory for isolation.

Preparation of culture medium

Potato Dextrose Agar (PDA) was used as a culture medium in this study. Thirty– nine grams of powdered PDA was dissolved in one litre of distilled water, amended with 1000mg of chloramphenicol (to suppress bacterial growth), thoroughly stirred and dispensed into 1000 ml conical flasks. The medium was sterilized at 121⁰C (105 kg/ cm²) for 15 minutes in an autoclave, allowed to cool and 20 ml of the sterilized medium was dispensed into 9cm diameter sterile disposable Petri dishes [24, 26].

Isolation and identification of isolates

Yam tissues (5mm) were cut out from infected white yam tubers after surface sterilization with 70 % ethanol for 10 seconds, blotted dry with sterile paper towel, inoculated on chloramphenicol-amended PDA and incubated for seven days at 28⁰ C. Pure cultures of fungal isolates obtained was identified by microscopy. The axenic culture of the isolates were identified based on morphological characteristics as described in the illustrated genera of fungi and with literature on identification of pathogenic fungi by [27].

Pathogenicity test

To confirm the pathogenicity of the isolates, axenic cultures of each of the isolates were separately inoculated on three tubers of yams respectively. A 5 mm diameter mycelial agar plug of a 4-day-old culture of each isolate was inoculated into the healthy white yam tubers, sealed with petroleum jelly and incubated at 28⁰C. After symptoms development 15 to 21 days post inoculation (dpi), tissue at the margin of the healthy and diseased part was excised, surface-sterilized,

inoculated on freshly prepared PDA and incubated at 28⁰C for four days and identified as those previously isolated and pathogenicity was ascertained.

Severity Index (SI)

Upon observation of symptoms 15 to 21 days post inoculation (dpi), rot severity index (SI) was assessed on a scale of 0- 4, (0 = no disease, 1=1-25% rot, 2=25- 50% rot, 3=50-75% rot and 4=75-100% rot), using a modified formula of [28].

$$SI = \frac{(X_0 \times Y_0) + (X_1 \times Y_1) + (X_2 \times Y_2) + (X_3 \times Y_3) + (X_4 \times Y_4)}{(Y_0 + Y_1 + Y_2 + Y_3 + Y_4)}$$

where X = Disease scale; Y = Volume of rot (cm³).

Preparation of *Zingiber officinale* A. melegueta vacuum distillate

Fresh rhizomes of ginger (*Zingiber officinale*) were thoroughly washed with distilled water, surface sterilized with 70% ethanol, sliced and sun-dried. Fifty grams (50g) of the dried sample was crushed and weighed with Sartorius weighing balance and extracted in diethyl ether (50ml) in an ultra-sonic bath at room temperature for 5 min. The content of the bath was transferred to a round bottom flask connected to a vacuum distillation apparatus equipped with a high vacuum pump (Edwards, England), and distilled under a vacuum of <0.05 mmHg for 24h [29]. The distillate was evacuated, dried using MgSO₄, filtered and concentrated to obtain 4 ml oil [30]. The distillate was stored in the freezer for GC analysis and GC-MS [31].

Gas Chromatography (GC) analysis of vacuum distillates and fractions

1 µl of ether extract vacuum distillate of *Z. officinale* was analyzed on a Hewlett-Packard 5890A gas chromatography equipped with a cold on-column injector, a flame ionization detector (FID) and a 50 m x 0.32 i.d. HP-1 bonded phase fused silica capillary column. The oven temperature was maintained at 40⁰ C for 2 min. and then programmed at 10⁰ C/min. to 250⁰C. The carrier gas was hydrogen. It was used at a constant flow rate of 1ml/min. Characterization, identification and determination of the relative amounts of the essential oils from the plant sample were done through Gas chromatography – Mass spectrometry and Gas liquid chromatography – co-injection of the essential oils with authentic standards [32].

Coupled Gas Chromatography – Mass spectrometry Analysis

Vacuum distillates of *Z. officinale* was obtained through Gas chromatography-Mass Spectrometry analysis performed using a fused silica capillary column (50 m x 0.32 mm i.d. film thickness HP-1) fitted with an on-column injector and directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments, Manchester, UK). Ionization was by electron impact (EI⁺) mass spectra recorded at 70eV and 250⁰C. Helium was the carrier gas. The oven temperature was maintained at 30⁰C for 5 min. and then programmed at 5⁰/min up to 250⁰ C. Tentative identification by GC-MS was confirmed by peak enhancement with authentic samples [31].

Antifungal Assay

Hyphae extension bioassay was used for the investigation of the antifungal activity of essential oil [32]. The medium used was Potato Dextrose Agar (Lab M.). The test fungi were *Rhizopus stolonifer*, *Penicillium expansum* and one of the most virulent rot fungi – *Botryodiplodia theobromae* Pat. Fungicidal compounds used in the test as controls were coacobre (56% w/w of cuprous oxide, equivalent of 50% w/w of pure copper metal), Ridomil (66% WP) metalaxyl-M, Mancozeb (75% WP) and forcelet (Carbendazim, 50% WP). The bioactive blend was made up of synthetic version of the six biologically active compounds, namely, 1,8 – cineole, 2 – pinene, Camphene, Farfescene, Geranial and Zingiberene. These chemicals were purchased as pure compounds from Sigma-Aldrich (Gillingham, Kent, UK). They were weighed using Sartorius

electronic balance and prepared in diethyl ether to make up the desired concentrations as in *Z. officinale* natural extracts. The essential oils and their bioactive blends and the synthetic fungicides were made into four (4) levels of concentrations as follows: 25µg/ml, 50µg/ml, 75µg/ml and then 100µg/ml of diethyl ether. The antifungal test was carried out adopting the method by [33]. Data are means of radius of 10-day old growth colony in a 9 cm diameter Petri dish. Means were separated using LSD ($P < 0.05$). The influence of period of incubation on the efficacy of the treatments on the growth of *B. theobromae* in culture was assessed using a 100% of each treatment and data were taken at two days interval for 10 days. Each of the experiments was replicated three times.

Results

Isolation and identification of fungi isolates

In this study, seven fungi were isolated and identified as the causative agents of yam rot obtained from various markets. The identified fungi and their frequency of isolation are presented in (Table 1). Of the seven identified isolates, *Aspergillus niger* was absent in yams obtained from Watt market while *Fusarium solani* was absent in yams obtained from Marian market. All the seven identified isolates were observed in yams obtained from Akim market. Severity index assessment of the pathogens showed that *R. stolonifer* was the most severe followed by *B. theobromae* with values of 82.5 and 57.5 respectively (Table 1).

Table 1. Fungi isolated from yam tubers, their frequency of occurrence and rot severity in the study area

Pathogens Isolated from yam	Markets and frequency of occurrence (%)			Mean	Severity
	Marian	Akim	Watt		
<i>Fusarium moniliformes</i>	21.00	47.00	10.00	26.00	32.50
<i>Fusarium solani</i>	0.00	3.00	25.00	9.30	11.25
<i>Rhizopus stolonifer</i>	70.00	87.00	42.00	66.30	82.50
<i>Aspergillus niger</i>	13.00	31.00	0.00	14.67	34.50
<i>Penicillium expansum</i>	41.00	57.00	10.00	36.00	45.00
<i>Penicillium sclerotigenum</i>	3.00	23.00	5.00	10.30	12.50
<i>Botryodiplodia theobromae</i>	42.00	81.00	16.00	46.30	57.50

Pathogenicity test

Results of pathogenicity test showed that all the isolates were pathogenic on the yam tubers inoculated (Table 2). Four out of the seven fungi (*A. niger*, *P. expansum*, *F. solani* and *B. theobromae*) isolated caused the most extensive rot (> 50 mm diameter) under experimental conditions. The other three (*F. moniliformes*, *P. sclerotigenum* and *R. stolonifer*) were mildly pathogenic on the yam tubers. Re-isolated fungi from the inoculated yam tubers produced cultures identical to the original isolates. Based on the frequency of isolation and level of pathogenicity, *B. theobromae* was chosen and used throughout the course of this experiment as the test pathogen.

Table 2. Fungi isolated from stored and marketed yams tubers obtained from the study area and their pathogenicity on white yam (*Dioscorea rotundata* Poir)

Pathogens	Symptoms of infection	Pathogenicity (extent of rot)
<i>Fusarium moniliformes</i>	Soft rot	>50mm
<i>Fusarium solani</i>	“	<30mm
<i>Rhizopus stolonifer</i>	“	>50mm
<i>Aspergillus niger</i>	Dry rot	>50mm
<i>Penicillium expansum</i>	“	<30mm
<i>Penicillium sclerotigenum</i>	“	<30mm
<i>Botryodiplodia theobromae</i>	“	>50mm

Severity Index (SI)

The results of assessment of rot severity index on the yam tissues (Table 1) revealed that *Rhizopus stolonifer* recorded the highest rot severity index of 82.5% and was closely followed by *B. theobromae* (57.5%). *P. expansum* was next in virulence with a rot index of 45.0%. The other four pathogens tested recorded rot indexes within the range of 12.5% to 34.5% during the test period. Though *R. stolonifer* recorded the highest rot severity index, *B. theobromae* was however used as the test pathogen throughout the course of the experiment since *R. stolonifer* is an opportunistic organism (weak parasite).

Gas chromatography- Mass spectrometry (GC-MS) Analysis of vacuum distillates of *Z. officinale*

GC-MS analysis of *Z. officinale* vacuum distillates produced 27 compounds (Table 3). Of these 27 compounds, 6 showed major biological activity on the chromatogram corresponding to the major bioactive compounds based on the peak area (Fig 1). The six major bioactive compounds based on the peak area and the concentration used to prepare blend of essential oils of *Z. officinale* are presented in (Table 4).

Table 3. Compounds identified from coupled GC-MS analysis of vacuum distillate of *Zingiber officinale*

S/n	Compound	% Peak Area
1	Beta-phellandrene	1.71
2	Tricyclene	0.33
3	α -Pinene	27.06
4	Camphene	16.87
5	6-Methyl-5-hepten-2-one	0.05
6	Sabinene	0.84
7	β -Pinene	0.25
8	Myrcene	3.20
9	3-Carene	0.71
10	β -disasobolene	1.22
11	1,8-Cineole	22.63
12	Terpinolene	0.66
13	(R)-Linalool	0.83
14	Borneol	0.63
15	α -Terpineol	0.51
16	Neral	5.03
17	Geranial	11.25
18	Borneol acetate	0.15
19	Cyclosativene	0.20
20	Copaene	0.39
21	α -Curcumene	1.00
22	α -Muurolene	1.10
23	Zingiberene	10.63
24	(E,E)- α -farnesene	3.69
25	γ -Cadinene	3.21
26	β -Cadinene	3.38
27	6-Shogaol	3.07

Figure 1. Gas chromatography- Mass spectrometry (GC-MS) Analysis of vacuum distillates of *Z. officinale* essential oil based on peak area showing six major compounds

Table 4. Bioactive compounds used to prepare blend from *Z. officinale* essential oil

S/n	Compound	Rel. Conc. (ng/ μ l)
1	1.8-Cineole	22.63
2	α -Pinene	27.06
3	Camphene	540.58
4	(<i>E,E</i>)- α -Farnesene	102.07
5	Geranial	724.98
6	Zingiberene	339.98

Antifungal assay

Assessment of the antifungal activity of the essential oils of *Z. officinale* and synthetic fungicides on the radial growth (hyphae) of the test fungus are presented in (Fig. 2). Result showed that increase in the concentration of the extract/chemicals positively correlated with the growth inhibitions of the test pathogen that were observed. At 25 μ g/ml concentration, coacobre was the most effective in inhibiting the growth of the test fungus in culture with a hyphal length of 1.61 ± 0.03 cm. The inhibitory activity was closely followed by bioactive blend of *Z. officinale* with a hyphal length of 3.00 ± 0.20 cm. Forcelet was the least effective in the inhibition of the pathogen with a hyphal length of 3.74 ± 0.20 cm which is not significantly ($P < 0.05$) different from the control (4.19cm). Bioactive blend of *Z. officinale* and coacobre were the best and showed significant ($P < 0.05$) activity at 50 μ g/ml, while mancozeb and natural oil of *Z. officinale* were not significantly different ($P < 0.05$) in their inhibitory activity with hyphal lengths of 2.01 ± 0.10 cm and 1.60 ± 0.10 cm respectively. However, beyond this concentration level, forcelet, *Z. officinale* bioactive blend and natural oil showed a significant effect ($P < 0.05$) as the most effective growth inhibitor of the test pathogen. The inhibitory activity was however not significantly ($P < 0.05$) different from the inhibition activity recorded by coacobre and mancozeb at 75 μ g/ml (1.01 ± 0.23 cm and 1.16 ± 0.12 cm) and 100 μ g/ml (0.96 ± 0.11 cm and 0.69 ± 0.01 cm) respectively. Generally, ridomil had the least effect in the inhibition of *B. theobromae* when compared with the control.

Figure 2. Effect of different concentrations of essential oils of *Z. officinale*, bioactive blend and synthetic fungicides on the growth of *B. theobromae* in culture

The effect of incubation period on the growth inhibitory capacity of bioactive blend and the vacuum distillate of *Z. officinale* was assessed in conjunction with synthetic fungicides (Table 5). Results obtained showed that, bioactive blend and the vacuum distillate of *Z. officinale* were the best as there was a significant effect ($P < 0.05$) in their inhibitory activity throughout the duration of the experiment except on the 10th day where a slight decline was observed.

Table 5. Effect of incubation period on the efficacy of the vacuum distillates of *Z. officinale* and the bioactive blends on the growth of *B. theobromae* in culture

Treatments	Period of incubation (days) and growth (cm)				
	2	4	6	8	10
<i>Z. officinale</i> bioactive blend	0.94±0.11	1.26±0.04	1.27±0.05	1.13±0.12	1.38±0.11
<i>Z. officinale</i> natural oil	1.57±0.02	0.93±0.18	1.41±0.17	1.90±0.11	2.20±0.10
Forcelet	1.43±0.12	1.57±0.02	1.72±0.07	1.77±0.02	1.82±0.30
Mancozeb	1.39±0.30	1.43±0.01	1.44±0.19	1.37±0.03	1.59±0.13
Ridomil	1.82±0.15	1.86±0.13	2.12±0.10	2.54±0.01	2.32±0.07
Coacobre	0.87±0.01	1.08±0.03	1.00±0.20	1.30±0.11	1.16±0.08
Control(no treatment)	3.05±0.10	4.40±0.13	4.50±0.06	4.50±0.04	4.50±0.15
LSD	0.36	0.50	0.43	0.42	1.64

Discussion

Fungal Rot disease of yam is a major limiting factor in the production of edible white yam (*Dioscorea rotundata* Poir.) worldwide. Fungal rot losses and cost of controlling this disease constitute a significant burden in agricultural enterprise and has serious socio-economic and environmental consequences. Consequently, there is an urgent need for effective and sustainable control of this disease. The current trend in the effective and sustainable management of plant diseases requires the use of botanical pesticides which are non-phytoxic to human and are environmentally friendly. In this study the antifungal potency of essential oils components of African Ginger (*Zingiber officinale* Roscoe) was tested against a major yam rot fungal pathogen (*Botryodiplodia theobromae* Pat). Assessment of the effect of plant extract and synthetic fungicides on the radial growth of the test fungus showed that increase in the concentration of the chemicals

positively correlated with the growth inhibitions that were obtained (Fig. 2). Similar observations have been made by [34, 16, 35-37] using plant extracts; [38-41] on fungicides. The different levels of reductions of radial growth by the plant extracts and synthetic fungicides may probably be due to varying levels of interference of these chemicals on the metabolism of the fungi involved. Also, the effect of incubation period on the growth inhibitory capacity of bioactive blend and the vacuum distillate of *Z. officinale* assessed in conjunction with synthetic fungicides (Table 5) showed that, bioactive blend and the vacuum distillate of *Z. officinale* were best as there was a significant effect ($P < 0.05$) in their inhibitory activity throughout the duration of the experiment except on the 10th day where a slight decline was observed. This is clear indication of the stability and persistence of the component compounds of the essential oils of this plant. Report of similar results have earlier been given by several researchers who worked on extracts of some plants against rot pathogens of cocoyams [34], potatoes [42], cassava [17] and yams [24]. In a report, [43], demonstrated the inhibitory capacity of ginger extracts against 5 spoilage pathogens namely *Rhizopus stolonifer*, *Aspergillus niger*, *Pythium sulcatum* and *Fusarium sambucinum*. Aqueous extracts of ginger at concentrations of 0.05g/ml and 0.15g/ml showed a significant effect on the growth inhibition of *P. sulcatum* (100%) and *Rhizopus stolonifer* (56%) respectively within 7 days of incubation. [23], secured growth inhibition of *B. theobromae* in culture using 0.025µmg/ml of vacuum distillate and bioactive blend of *Aframomum melegueta* (Alligator pepper). These extracts persisted and were still effective within 10 days of incubation. The fungicidal nature and persistent nature of extracts of plant origin have also been reported by [34]. Extracts of *Allium sativum*, *Azadirachta indica*, *Garcinia kola* and *Carica papaya* were shown to persistently inhibit the growth of seven rot pathogens of cocoyam including *B. theobromae* after 7 days of treatment. The antimicrobial activity of the synthetic fungicides used in this study was significantly ($P < 0.05$) lower than that obtained for the bioactive blend and vacuum distillate of *Z. officinale*. The superior performance of plant products over that of synthetic fungicides has severally been documented. Aqueous extracts of *A. sativum* has been reported to cause 86.33% inhibition of *B. theobromae* compared to 79.46% obtained for Grisovid (a synthetic fungicide) [34]. [44] also demonstrated the efficacy of plant extracts over synthetic fungicides. They showed that hot water extracts of *Xylopiya aethiopica* gave 20% lesion reduction compared to 12.6% recorded for benomyl. The control experiment did not record any growth reduction activity against the test pathogen throughout the course of the experiment. The superlative growth inhibitory performance demonstrated by these plant-based fungicides is a clear pointer to the efficacy of these natural products as potent contact fungicides for the control of *Botryodiplodia* rot of white yam in storage. A major advantage of the use of these plant-based chemicals over the synthetic chemicals as storage protectants of yam is their non-poisonous nature [45] as they have been consistently used as culinary spice in many dishes globally [46].

Conclusion

Investigations on the antifungal activity of essential oils of the African Ginger (*Zingiber officinale* Roscoe) in comparison with some synthetic fungicides showed a significant inhibitory effect ($P < 0.05$) against *B. theobromae* causing rot of white yam. To this effect, timely spraying of yam tubers with essential oils extracts of *Z. officinale* during post-harvest storage will play a vital role in reducing the rot activities of the fungal pathogen and as such increase the shelf life of the yam tubers.

Conflict of Interest

The authors declare that there is no conflict of interest.

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