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Evaluation of the antifungal activities of Zingiber officinale on fungi isolated from contaminated maize

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Abstract
One of the major challenges faced by farmers in developing countries is how to control seed-borne fungal pathogens leading to poor crop yields. The conventional control methods are expensive and, in most cases, resulted in environmental pollution and health hazards. The study seeks to examine the effects of ginger *Zingiber officinale* on fungi isolated from mouldy maize. This method could serve as an environmentally friendly and affordale way of checking this pathogen. The One hundred grams (100g) of the dried powder of *Zingiber Officinale wa*s weighed separately into 400 mL of the extracting solvent (Ethanol, methanol and ethylacetate) in different conical flasks, labelled accordingly and stored over a period of 72hours after which the antifungal properties for the plant extracts were determined using agar dilution method of Banso and Mann, 2016**.** Various concentrations (10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL) of the extract were also prepared and used to determine the minimum inhibitory concenteration and minimum fungicidal concentration. The test microorganisms used were *Rhizopus stolonifer*, *Mucor mucido* and *Fusarium solani*. The results of the study illustrated that the test organisms were susceptible to *Zingiber officinale* at different varying concenterations and this further implies that *Zingiber officinale* could serve as a form of preservative for stored grains leading to a significant product yield for farmers during the harvest season Control experiments were performed without the extracts.

Keywords: *Zingiber Officinale*, concenteration, extracts, fungicidal

Introduction
Ginger (*Zingiber officinale*) was originally known to be cultivated in India but has spread all over the world. Ginger was widely used by the ancient Roman Empire but was almost lost after the fall of the Roman Empire and later rediscovered in Europe (Chuku *et al*., 2010) [5]. Ginger is known to reduce cholesterol, regulate blood pressure and blood sugar, prevent artiosclerosis thereby reducing the risk of heart attack or stroke (George 2008; Chuku *et al*., 2010) [7, 5]. Maize is highly susceptible to contamination by fungi and mycotoxins, this result to high economic loss and health challenges. Therefore, the objective of the study was to investigate the effect of *Zingiber officinale* on the growth of fungi isolated from mouldy maize.

Materials and Method
Ginger (*Zingiber officinale)* and Maize (*zea mays*) were used in this study. They were obtained from New market, Bida Niger State.

Test Microorganisms
The microorganism used in this study were *Rhizopus stolonifer*, *Mucor mucido* and *Fusarium solani*. The organism were isolated from maize. They were identified after staining with lactophenol cotton blue.The organisms were maintained on potato dextrose agar slants and stored in the refrigerator until required.

Preparation of Plant Extracts
The One hundred grams (100g) of the dried powder of *Zingiber Officinale wa*s weighed separately into 400mlof the extracting solvent (Ethanol, methanol and ethylacetate) in different conical flasks the sample were labeled accordingly.
They were stored intermittently over a period of 72hours. The extracts were filtered into different conical flasks after which the extracts obtain were evaporated to dryness using a water bath (Barring tom 125) at 80℃. The extract were assayed immediately after preparation (Banso, 2016) [1].

Determination of Antifungal Properties for Extracts
The antifungal properties of the extract were determined using agar dilution method of Banso and Mann, 2016 [3]. Different volume of the extract were introduced into Mccartney bottles containing appropriate volume of sterile potato dextrose agar (PDA). The mixture was poured into different petri dishes and allowed to solidify. The plate was inoculated with 5mm diameter of the fungal culture. Control experiment was performed without the extracts.
The plates were incubated at 28±2℃ of 48hours. The antifungal properties were expressed in terms of diameter of growth (mm).

Determination of Minimum Inhibitory Concentration
Various concentrations (10mg/ml, 20mg/ml, 30mg/ml and 40mg/ml) of the extract were prepared. Each of these was added to 18ml of potato dextrose broth in test tubes and inoculated with 0.1ml spores of the spore suspension of *Rhizopus stolonifer*, *Mucor mucido* and *Fusarium solani*diluted in order to give a final spore suspension of 10^6 spores/ml. the mixture were then incubated for 48hours at 28±2°C. The least concentration of the plant extract that does not permit the growth of the inoculated test

organism was regarded as the minimum inhibitory concentration. Control experiments were performed without the extracts (Banso, 2016) [1].

Determination of Minimum Fungicidal Concentration

The content of the tubes that showed no visible fungal growth in the minimum inhibitory concentration (MIC) experiments were cultured into freshly prepared potato dextrose agar plates to assay for the fungicidal effect of the extracts. The plates containing the test fungi was incubated at $28 \pm 2^{o}C$ for 72hours. The lowest concentration of the extract that does not yield any visible fungal growth on the solid medium was regarded as the minimum fungicidal concentration (Banso *et al*., 2010) [2].

Phytochemical Screening of Extracts

Extracts of spice used in this study were screened for active ingredient as described by Banso *et al*., 2010 [2].

Test for Sterols

Two millitres of distilled water was added to 0.3g of the extract to form a solution. To 1ml of the solution a few drop of 10% ferric chloride was added and observed. The absence of green precipitate indicate the presence of sterols.

Test for Flavonoids

Five militres of distilled water was added to 0.3g of the extract and 10% lead acetate solution was also added. A precipitate indicate the presence of flavonoids.

Test or Tannins

The two milliliters of distilled water was added to 0.3 of the extract to form a solution. to 1ml of the solution, a few drop of 10% ferric chloride was added and observed for the presence of tannins. The presence of green precipitate indicates the presence of tannins.

Test or Phenols

The three milliliters of distilled water was added to 0.3g of the extract. one milliliter of ferric chloride and 1ml of 1% potassium ferric cyanide were added and observed for the presencs of phenols. Development of a blue green color indicate the presence of phenols.

Test for Anthroquinones

Three milliliters of distilled water was added to 0.3g of the extract to 1ml of the solution, 0.2ml of dilute sulphuric acid (H_2S0_4) and 9ml of benzene were added. Upon separation of benzene layer into another test tube, three drops of dilute ammonium solution was added. Presences of pink colour in the ammonium solution phase indicate negative result.

Test for Saponins

Two milliliters of distilled water was added to 0.3g of the extract and shaken. development of frothing that persisted on warming was a primary identification of saponins.

Results

Antifungal Properties of Extract of *Zingiber officinale*

The result of the antifungal properties of *Zingiber officinale* are shown in the table 1. The mean diameter of the growth of *Rhizopus stolonifer* when ethanol extract of *Zingiber officinale*was assayed against *Rhizopus stolonifer* was 11.0±0.2mm. the value recorded against methanol was 12.0±0.1mm while 10.5±0.1mm was recorded against chloroform extract.

A value of 10.0±0.1mm was recorded against *Mucor mucido* when ethanol extract of *Zingiber officinale* was assayed against the test fungi. The value recorded against methanol extract was 11.5±0.1mm while a value of 9.5±0.2mm was recorded against chloroform extract.

A value of 11.0±0.2mm was recorded against *Fusarium solani* when methanolic extract of *Zingiber officinale* was assayed against the test fungi. The value recorded against ethanolic extract was 10.5±0.1mm while a value of 8.5±0.2mm was recorded against chloroform extract.

Table 1: Antifungal properties of extracts of Zingiber officinale

Diameter of growth (mm) ±SD				
Organism	**Control**	**Ethanol extract**	**Methanol extract**	**Chloroform extract**
		n =3	n =3	n =3
Rhizopus stolonifer	13.0±0.1	11.0±0.2	12.5±0.1	10.5±0.2
Mucor mucido	*12.0*±0.1	10.0±0.1	11.5±0.1	9.0±0.2
Fusarium solani	*12.5*±0.2	10.5±0.1	11.5±0.1	8.5±0.2

Minimum Inhibitory Concentration (Mic) of the Extract of *Zingiber officinale*

The result of the minimum inhibitory concentration of the extract of *Zingiber officinale*against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani* are shown in table 2. Minimum inhibitory concentration value recorded against *Rhizopus stolonifer, Mucor mucido*and *Fusarium solani* when ethanol extract of *Zingiber officinale* was assayed against the fungi were 20mg/ml, 20mg/ml and 20mg/ml respectively. Minimum inhibitory concentration value of 30mg/ml, 30mg/ml and 20mg/ml were recorded against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani* respectively when methanol extract of *Zingiber officinale* was assayed against the fungi. Minimum inhibitory concentration value of recorded against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani* when chloroform extract of *Zingiber officinale* was assayed against the test fungi were 30mg/ml, 20mg/ml and 20mg/mlrespectively. The result of the minimum inhibitory concentration of the extract against the two organisms correlate with the report that microorganisms varied in the degree of their susceptibility to antifungal agents.

Table 2: Minimum inhibitory concentration of Zingiber officinale

Minimum inhibitory concentration (mg/ml)			
Organism	**Ethanol extract**	**Methanol extract**	**Chloroform extract**
Rhizopus stolonifer	20	30	30
Mucor mucido	30	30	20
Fusarium solani	20	20	30

Minimum Fungicidal Concentration of Extracts of *Zingiber officinale* Against the Test Organisms

The result (table 3) show that the minimum fungicidal concentration of *Zingiber officinale* against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani*range between 30mg/ml and 40mg/ml. A minimum fungicidal value of 30mg/ml was recorded

against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani*. Value of 30mg/ml, 30mg/ml and 30mg/ml was recorded against all the test fungi when ethanol extract of *Zingiber officinale* was assayed against the fungi. Minimum fungicidal concentration value of 40mg/ml, 40mg/ml and 30mg/ml were recorded against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani* respectively when methanol extract of *Zingiber officinale* was assayed against the test fungi.

Table 3: Minimum fungicidal concentration of extract of Zingiber officinale

Minimum inhibitory concentration (mg/ml)			
Organism	**Ethanol extract**	**Methanol extract**	**Chloroform extract**
Rhizopus stolonifer	30	40	40
Mucor mucido	30	40	40
Fusarium solani	20	30	40

Phytochemical Constituents of *Zingiber officinale*

Table 4 shows the result of the phytochemical screening of ethanol extract of *Zingiber officinale.* Ethanol extract of *Zingiber officinale* contains flavonoids, tannins, anthroquines and saponins. Phenol and steroids were absent in the extraction. These constituents may be responsible for the antifungal activity of the extracts used in this study. Biological active substance have been found to be responsible for antifungal activities of certain plants (Banso, 2016) [1].

Discussion

Antifungal test of extract of *Zingiber officinale* showed that the extracts exhibited antifungal activity against the test fungi (table 1). This may be as a result of active principles in the plant materials. Plant generally produce many secondary metabolites which constitute an important source of microbiocide, pesticide and pharmaceutical drugs (Banso *et al*., 2010) [2]. Spices contain phenolic essential oil which are inhibitory to microorganisms (Banso, 2016) [1]. The effect of microorganisms may depend on the type as well as the medium (Banso and Ayodele, 2005) [2]. The result of this study justify the traditional use of *Zingiber officinale* as food preservative. Earlier, some work have shown that *Zingiber officinale*have antimicrobial activities against some microorganisms (Banso *et al., 2010*) [2]. The result of the minimum inhibitory of the extracts against the test fungi correlate with the report that microorganisms varies in the degree of their susceptibility to antifungal agent (Banso *et al.*, 2010; Banso and Ayodele, 2005) [4, 2]. Antifungal agent with low activity against an organism have high minimum inhibitory concentration. *Zingiber officinale* contains flavonoids, tannins, phenols, anthroquines and saponins which are inhibitory to microorganisms (Banso and Ayodele, 2005) [2]. Reported the antifungal effect of flavonoids and anthroquinons.

Conclusion

The plant (*Zingiber officinale*) extracts used in this study demonstrated inhibitory effects against *Rhizopus stolonifer, Mucor mucido* and *Fusarium solani* isolated from mouldy maize. Therefore extracts of *Zingiber officinale* promises be an important source of preservative for maize.

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