ANTI-FUNGAL EFFECTS OF GINGER RHIZOME EXTRACTS ON MYCELIAL GROWTH OF SOME FUNGAL PATHOGENS OF Dioscorea rotundata IN TARABA STATE, NIGERIA

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ABSTRACT
The objective of this study is to isolate, identify and to determine the effect of various concentrations of aqueous and ethanol extracts from the rhizome of the test plant Zingiber officinale in the control of fungal isolates from yam rot in Wukari Nigeria. Rotted yam tubers were obtained from three markets in Wukari. The ingredients of test plant were extracted by aqueous and ethanol solvents. Rhizome extracts at different concentrations (0%, 20%, 40% and 60%) of aqueous and ethanol extraction of the test plant was poisoned to growth media prior to inoculation. The fungi associated with the spoilage of the sample of the yam tuber were identified base on their morphological characteristics. Of all the samples studied, three species of fungi were found to be associated with the yam rots. The most commonly isolated fungi were Aspergillus niger and others are Aspergillus flavus and Rhizopus stolonifer. All concentrations used suppressed the mycelia growth of the tested pathogens except the control treatment. The effect was proportional to concentrations and inhibition value was highest at 60% concentration, for aqueous extraction, Zingiber officinale was more effective on Aspergillus flavus, for both aqueous and ethanol extractions, Zingiber officinale was more effective on Rhizopus stolonifer. Phytochemical analysis showed that the extracts contain, tannins, saponins, terpenoids, alkanoids, steroids and those absent phenols, glycosides, anthraquinones, anthracenes and flavonoids. The presence of these compounds supports the use of the extracts as antimicrobial agents which can prolong the shelf–life of yam under storage.

KEY WORDS: Antifungal effects, Extracts, Phytochemicals, Zingiber officinale.

INTRODUCTION
The yam tuber belongs to the genus Dioscorea in the family of Dioscoreaceae and is monocotyledonedous. It is one of the highly rated and commonest food crops of the tropical world. The edible varieties of yam are important food crop and serve as an important carbohydrate staple for millions of people in both the tropical and subtropical regions in West Africa, The Caribbean, Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Coursey, 1967; Okigbo and Ikediugwu, 2000). The FAO (1989) estimated that the world production is around 20 million tons per year. Nigeria alone produces three quarter of the world total output of yams. Of the ten cultivated species, the six most important in Nigeria are Dioscorea rotundata Poir (white yam), Discocrea. cayennensis Lam (yellow yam), Discocrea alata L. (Water yam), Discocrea dumetorum (Cluster, or bitter yam), Discocrea excudents (Loir) bark (Chinese yam) and Discocrea bulbipera L. (aeria yam) (Adeniji, 1970; Okigbo, 2004). The principal microorganisms associated with yam in Nigeria include Aspergillus niger, Van, Tiegh, Hendersomula rotuloidea, Macrophomina phaseoli, Rhizopus nodosus Namyslowski, Botrodiploida theobrome, Fusarium monoliform varsubglutinamus Wollenw and Reinking.Pencillum sclerotigenum Yamamoto, and Rosellina bundodes (Berk and Br) Sacc. (Ogundana et al., 1970; Adeniji, 1970; Ogundana, 1972; Okigbo and Ikediugwu, 2000). Other fungi which have been reported as secondary invaders are Fusarium oxysporum Schlecht, Cladosporium spearo spermum, Fusarium solani, Geotrichum candidum (Okafor, 1966; Coursey, 1967; Adeniji, 1970). Fusarium solani, Rhizopus stolonifera, Botrodiploida theobrome, Geotrichum canadicum (Okafor, 1966; Coursey, 1967; Adeniji, 1970) some such as Trichoderma and Billus subtilis are also effective in the control or reducing storage rot in yam (Okigbo, 2005). The uses of synthetic chemicals such as sodium orthophenylenphenate, borax, captan, thiobendazole, benomyl, bleach (sodium hypochlorite) have been found to significantly reduce storage rot in yam (Booth, 1974; Noon, 1978.). Other control methods involve the use of microorganism such as Trichoderma viride and Bacillus subtilis (Okigbo and Ikediugwu, 2000; Okigbo, 2002). However, farmers in developing economies such as Nigeria have hardly adopted these findings, because the majority of them cannot afford the financial cost. Moreover, chemical pesticides have the additional potential disadvantages of accumulation in the ecosystem and of induction of pesticide resistance in pathogens (Adeniji 1970; Okigbo and Ikediugwu, 2000; Okigbo, 2004). There is also the problem of lack of expertise in the safe handling of pesticides among most of the farmers.
The use of synthetic chemicals such as sodium orthophenylphenate and borax has been found to reduce storage rot yam (Booth, 1974). But biological control is generally favoured as a method of plant disease management (Okigbo and Ikediugwu, 2000; Okigbo, 2002; 2005). Kuhn and Hargreaves (1987) observed that substances found fungicidal in vitro in almost all cases kill the fungus in vivo. Plant extracts have been used to control yam diseases (Okigbo and Ogbonna, 2006). Plants with such fungicidal properties include Zingiber officinale (Maurice, 1993). Z. officinale (family: Zingiberaceae) is a herbaceous perennial plant which has an upright stems and narrow medium, green leaves arranged in two ranks on each stem.

**MATERIALS & METHODS**

**Collection of Yam**

Ten yam tubers with symptoms of rot were obtained from new market, Old market of Wukari, Taraba state, Nigeria.

**Isolation of Spoilage Fungi from Rotted Yam**

Potato dextrose agar were routinely used for culturing fungi respectively during the study, laco-phenol cotton blue stain was also use for microscopic examination of fungi. All materials used were adequately sterilized.

**Collection and Preparation of Plant Extracts**

The method of Ijato (2011) was used to prepare both aqueous and ethanol extracts. Zingiber officinale rhizomes (Plate 1) were collected from New Market of Wukari local Government, Taraba State. The plants were taken to the Biological Sciences Department, Federal University Wukari, Taraba State. The collected plant parts were washed thoroughly under running tap water and were allowed to air dry for 7 days. These were being grinded separately. Thirty grams of each sample was added to 1 ml of distilled water in separate conical flasks. This was vigorously shaken and left to stand for 24 hours. The samples were filtered with 3 layers cheese cloth and filtrate extract preparation of 60%, 40% and 20% concentrations were used as the aqueous extract. The same procedure was used for 60%, 40% and 20% ethanol extract.

**Pathogenicity Test**

Pathogenicity test was carried out using techniques of Okigbo et al. (2009). Healthy yam tuber was washed with sterile distilled water, wiped dry using Whatman No.1 filter paper and surface sterilized with 0.1 % mercury chloride solution to remove surface contaminants and rinsed in three changes of sterile distilled water. A sterile 2 mm cork borer was used to make a 2 mm cut on the yam tuber and then culture of the isolates were inoculated into the open cut surface and the removed tissue was replaced with the core borer and sealed with Vaseline jelly. Yam tuber was inoculated in three replicates. The yam tuber was incubated for 5 days. On establishment of disease symptoms, the infected yam tissue was taken and cultured until pure cultures were obtained (Plates 2 and 3). The morphological and microscopic characteristics of the Isolates were compared with the original isolate.

**PLATE 1: Zingiber officinale rhizomes**

**PLATE 2: Infected Yam Tubers**
PLATE 3: Yam Tubers showing rot after 14 days of inoculation

**Effect of Plant Extracts on Fungal Mycelia Growth**

The approach of Ijato (2011) was used to evaluate the effect of the extract on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were poured into the flask, plugged with cotton wool and wrapped with aluminum foil to avoid contamination (Madari and Singh, 2005). About 2 mls each of extract of *Zingiber officinale* was separately introduced into the Petri-dishes containing the media and the pure isolates (poisoned food method). Control experiments were without addition of any plant extract but sterile distilled water. Fungitoxicity was determined in terms of percentage colony inhibition % : (Nene and Thalpiyal, 2000).

\[
\text{Percentage Colony inhibition} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100
\]

**Phytochemical Analysis of Plant Materials**

Qualitative analyses of the constituents of the plant extracts were carried out. The presence of biological active ingredients in the *Zingiber officinale* was investigated using standard methods as described by Edeoga et al. (2012).

**Test for tannins**

Two millilitres each of the aqueous and ethanolic extracts were separately boiled for ten minutes in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride were added to each test tube and observed for 10 minutes for a brownish green or a blue black coloration (Okwu, 2005). This test was repeated once to confirm the results.

**Test for phlobatannins**

Two millilitres of dilute ammonia solution was added to 3 ml each of the aqueous and ethanolic extracts, followed by the addition of concentrated tetraoxosulphate (VI), (H2S04). A yellow coloration was taken as evidence for the presence of phlobatannins (Okwu, 2005). The test was repeated once as above to confirm results.

**Test for saponins**

Frothing test according to Trease and Evans (1989) was adopted. About 5 ml each of the aqueous and ethanolic extracts of the samples were shaken with equivalent amount of water in a test tube for 5 minutes. This was boiled in the water bath for 5-10 minutes. Frothing that persists on warming was taken as evidence of the presence of saponins (Trease and Evans, 1989). The procedures were repeated as well to confirm the results.

**Test for flavonoids**

About 5 ml of dilute ammonia solution was added to 3 ml each of the aqueous and ethanolic extracts, followed by the addition of concentrated tetraoxosulphate (VI), (H2S04). A yellow coloration was taken as evidence for the presence of flavonoids (Okwu, 2005). This test was repeated again to confirm results.

**Test for alkaloids**

About 2 ml each of the aqueous and ethanolic extracts were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath for 10 minutes; 1 ml of the extract was treated with a few drops of Mayer’s reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids. The method was repeated again to confirm the results (Sofowora, 1993).

**Test for steroids**

One millilitre each of the aqueous and ethanolic extracts was dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish brown colour formed at the interphase indicates the presence of a steroid ring (Sofowora, 1993). The same procedures were repeated once to confirm the results.

**Test for terpenes**

One millilitre each of the aqueous and ethanolic extracts were added to 2 ml of chloroform and treated with five drops of acetic anhydride along with 2 drops of concentrated sulphuric acid. A pink colour formed at the interphase indicated a positive test of terpenes (Sofowora, 1993). This test was repeated once to confirm results.

**Experimental Design and Data Analysis**

The experimental layout was completely randomized design containing aqueous and ethanol extract treatments each at 0%, 20%, 40% and 60% concentrations. The
Ginger extracts on growth of some fungal pathogens of *Dioscorea rotundata*

experiment was replicated three times. All the data was analyzed using analysis of variance (ANOVA) according to Gomez and Gomez (1984). Least Significant Difference (LSD) according to Scheff (1953) was being used to separate the means where there was significant difference.

**RESULTS & DISCUSSION**

**Isolation and Identification of the Pathogens**

Three fungi were found associated with rotting of the yam tubers in all the three markets surveyed. The isolated fungi from Yam tubers were identified as *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifer*. The frequency of occurrence from the three markets (Table 1) shows that *Aspergillus niger* has the highest percentage frequency of occurrence of 40%, followed by *Rhizopus stolonifer* (33.33%). These fungal species isolated and identified in this study corroborate those reported by Ogaraku and Usman (2008). The pathogenicity test (Table 2) confirmed the natural pathogens responsible for the rot disease in the sampled yam tubers. The intrinsic ability of some exposed yam tubers has equally been reported (Okigbo and Ogbonna, 2006; Oyelana et al. 2011). The average spread of the rotted area at 14 days after incubation (1.10 – 6.80cm) was observed for all the fungal species. *Aspergillus niger* exhibited a wider area (6.80 cm) followed by *Aspergillus flavus* with 5.70 cm spread (Table 2). A significantly different result was reported by Oyelana et al. (2011) in which they observed that *Penicillium chrysogenum* exhibited a 62 mm spread and a 60 mm and 55 mm spread by *Fusarium solani* and *Aspergillus flavus* respectively. These implicated organisms posed a significant threat to the revenue of farmers and the health of consumers.

**TABLE 1:** Frequency of Occurrence (%) of the Pathogens Isolated from the Yam Tubers

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Frequency of occurrence in different locations</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>New Market 3, Old Market 5, Yam Market 4</td>
<td>12, 40</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>New Market 4, Old Market 3, Yam Market 1</td>
<td>8, 26.67</td>
</tr>
<tr>
<td><em>R. stolonifer</em></td>
<td>New Market 3, Old Market 2, Yam Market 5</td>
<td>10, 33.33</td>
</tr>
<tr>
<td>Total</td>
<td>10, 10, 10</td>
<td>30, 100</td>
</tr>
</tbody>
</table>

LSD = Least Significant Difference

**TABLE 2:** Pathogenicity Test of the Pathogens Isolated from Yam Tuber

<table>
<thead>
<tr>
<th>Days</th>
<th>Mycelia growth of fungal organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. niger</em></td>
</tr>
<tr>
<td>1</td>
<td>0.00(^a)</td>
</tr>
<tr>
<td>2</td>
<td>0.00(^a)</td>
</tr>
<tr>
<td>3</td>
<td>0.00(^a)</td>
</tr>
<tr>
<td>4</td>
<td>1.50(^d)</td>
</tr>
<tr>
<td>5</td>
<td>2.30(^g)</td>
</tr>
<tr>
<td>6</td>
<td>3.60(^f)</td>
</tr>
<tr>
<td>7</td>
<td>4.50(^f)</td>
</tr>
<tr>
<td>8</td>
<td>5.20(^c)</td>
</tr>
<tr>
<td>9</td>
<td>5.95(^c)</td>
</tr>
<tr>
<td>10</td>
<td>6.10(^b)</td>
</tr>
<tr>
<td>11</td>
<td>6.35(^b)</td>
</tr>
<tr>
<td>12</td>
<td>6.50(^b)</td>
</tr>
<tr>
<td>13</td>
<td>6.55(^b)</td>
</tr>
<tr>
<td>14</td>
<td>6.80(^b)</td>
</tr>
</tbody>
</table>

LSD(=0.0001) 0.47 0.43 0.46

**Effect of the Zingiber officinale extract at different concentrations on the fungal isolates**

*Z. officinale* extracts at various concentrations on the growth of pathogens are shown in Table 3. The results showed that the extracts *Z. officinale* significantly (p<0.05) reduced the radial growth of the pathogens in both aqueous and ethanol media (Table 3). All concentrations of aqueous and ethanol ginger rhizome extract suppressed the mycelial growth of the 3 tested pathogens. The effect was proportional to concentration of the extract. The inhibition was highest at 60% concentration and the lowest at 20% concentration. This is in agreement with the works of Trease and Evans (1989); Amadioha and Obi, (1999) and Udo et al. (2001) who reported the high potency of plant extracts for the control of pathogenic fungi of other crops. Results of the phytochemical analysis revealed the presence of Tannins, phlobatannins, steroids, terpenes Saponins, flavonoids and alkaloids in *Zingiber officinale* extracts (Table 4). The presence of these phenolic compounds in this extract indicates that this plant can serve as antimicrobial agent. This is because phenol and phenolic compounds have been extensively used in disinfection and remain the standard with which other fungicides are compared (Doherty et al., 2010). Phenolic compounds act as electron donors and are readily oxidized to phenolate ion or quinine, an electron acceptor.
et al., 2010). The antifungal activity of the oil is believed to be associated with the phytochemical components of these plants (Matasyoh et al., 2007) which diffuse into and damage cell membrane structures. Velluti et al. (2004) highlighted that generally, one of the critical things to consider for commercial applications is that the levels of essential oils and their compounds necessary to inhibit the microbial growth were higher in foods than in culture media. This is due to interactions between the phenolic compounds and the food matrix (Nuchas and Tassou, 2000). Extracts from rhizomes of Z. officinale therefore have potent antiseptic, bactericidal and fungicidal properties. These findings support the use of these extracts for prevention of infections as also reported by Okwu (2004). Results of this work suggest that fungitoxic compounds are present in Z. officinale extracts since they were able to control the growth of the fungal pathogens tested. This is in agreement with the work of Udo et al. (2001) who worked on the inhibition of growth and sporulation of fungal pathogens in Ipomoea batatas and Dioscorea sp by garlic extracts. The antimicrobial activity of these plants also agrees with the work of Adejumo and Langenkmper (2012), which showed that methanolic extracts of leaves of botanicals possessed antimicrobial properties.

### TABLE 3: Effects of Extracts of Zingiber officinale on the Growth of the Isolates

<table>
<thead>
<tr>
<th>Concentrations (%)</th>
<th>Solvent</th>
<th>A. flavus</th>
<th>A. niger</th>
<th>R. stolonifer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Aqueous</td>
<td>68.95</td>
<td>74.32</td>
<td>65.04</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>50.63</td>
<td>63.26</td>
<td>50.87</td>
</tr>
<tr>
<td>20</td>
<td>Aqueous</td>
<td>57.50</td>
<td>64.55</td>
<td>57.30</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>41.03</td>
<td>54.00</td>
<td>44.56</td>
</tr>
<tr>
<td>40</td>
<td>Aqueous</td>
<td>52.11</td>
<td>55.20</td>
<td>50.90</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>39.76</td>
<td>40.00</td>
<td>35.36</td>
</tr>
<tr>
<td>60</td>
<td>Aqueous</td>
<td>39.00</td>
<td>40.12</td>
<td>43.00</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>31.15</td>
<td>30.78</td>
<td>28.77</td>
</tr>
</tbody>
</table>

LSD (p=0.0001) 8.10 6.31 5.25

LSD = Least Significant Difference

### TABLE 4. Quantitative Determination of phytochemical Groups of Extract of Test Plant Leaves

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compounds</th>
<th>Zingiber officinale</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
</tr>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Phlobatannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Steroids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>9.</td>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++ = Present in high amount; + = Present in moderate amount; - = Absent

Also Okigbo amd Nmeka (2005) used leaf extracts of Xylopia aethiopica and Z. officinale to control yam tuber rot caused by Aspergillus niger, Aspergillus flavus and Fusarium oxysporum. A. melegueta extract was also used by Okigbo and Ogbonnaya (2006) in the control of F. oxysporum and A. niger rot in yam tubers. Also the effect of aqueous extract of ginger was evaluated by Stangarlin et al. (2011) at the concentrations of 1, 5, 10, 15, 20 and 25 % on Sclerotina sclerotiorum mycelial growth and sclerotia production, in vitro. The efficiency of protection of Z. officinale was also verified in lettuce plants. Besides the reduction in disease incidence, the authors reported that, the crop yield and the peroxidase induction were also analyzed in the plant tissues. This antimicrobial property of ginger in reducing the mycelial growth of fungal pathogens is in line with the results of this study. Ijato (2011) reported that extracts of Z. officinale and Ocimum gratissimum were mycotoxic to Fusarium oxysporum, Botrydioploida theobromae, Aspergillus flavus and Aspergillus niger of postharvest rot of yam tubers and that the effectiveness of the extracts increased with increase in concentration as was observed in this study (Table 3). Further studies on these effective botanical should gear towards fractionation of the extracts which will lead to the isolation of the compounds that is showing considerable antifungal activity. The continuation of study on the plant is essential to isolate, identify, characterize and elucidate the structure of the bioactive compounds responsible for the observed antifungal activities. From this result, it is essential to investigate the specific constituents which are responsible for this observed activity. The in vivo study is also required to confirm the usefulness of the obtained results.

### CONCLUSION

The conclusion drawn from these studies showed that A. niger, A. flavus and R. stolonifer are common pathogenic fungi which cause tuber rot in yam in the study area. The result from the pathogenicity test indicated that all the isolated fungi are pathogenic and attributed to the cause of yam tuber rot in Wukari. The inhibitory effect of the plant extracts against fungal isolates could be due to the
Ginger extracts on growth of some fungal pathogens of *Dioscorea rotundata*

presence of antifungal substances in the extract. Higher inhibition of fungal growth was observed at higher concentrations of the aqueous and ethanol extracts. The result also indicated that ethanol is better solvent than water for the extraction of active ingredients from this plant; the results of the present investigation are clear indications for the potential of plant extracts to control fungal pathogens. It is also clear from the result that the test plant extract significantly reduce the radial growth of isolated fungi and this finding is first of its kind in Wukari. It seems that the anti-fungi effects are the results of many compounds acting synergistically (Bediakao et al., 2007). This can be formulated and successfully produced as fungicides with local technology, which can be applied at both pre and post-harvest in yam rot /management.

REFERENCES


