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Research Article

Antifungal Activities of Some Medicinal Plants in the Control of Fungi Associated With Spoilage of Three Varieties of Cocoyam (*Colocasia esculenta*)

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ABSTRACT

The antifungal effects of ethanolic and aqueous extracts of *Vernonia amygdalina*, *Gongronema latifolium* and *Ocimum gratissimum* were investigated against fungi associated with the spoilage of cocoyam. Standard mycological and agar-well diffusion methods were used to isolate the fungi associated with the spoilage of cocoyam and to determine the antimicrobial effect of the plant species. Five fungi species were isolated from spoiled cocoyam which included *Aspergillus flavus, Aspergillus fumigatus, Mucor hiemalis, Aspergillus versicolor* and *Rhizopus stolonifer.* The fungitoxic effect of the plant extracts was determined by zone of inhibition (mm) at three concentrations of 100mg/ml, 75mg/ml and 50mg/ml. The diameter zones of inhibition for *Ocimum gratissimum* extract ranged from 6mm - 24mm, *Vernonia amygdalina* 6mm - 22mm, while that of *Gongronema latifolium* ranged from 6mm - 22 mm. The diameter of growth inhibition zone was different at varying concentrations for the plant extracts used. However, extract of *O. gratissimum* showed higher diameter growth inhibition zones than extract of *G. latifolium* and *V. amygdalina* at all concentrations used. It was observed that the inhibitory activity of the plant extract increased with increase in the concentration of the extracts. The fungicidal attribute evidenced in this study can be useful to identify the plant extracts that would act as fungicides in the control of fungal spoilage of cocoyam.

Keywords: Antifungal activity, Cocoyam, *Vernonia amygdalina*, *Gongronema latifolium*, *Ocimum gratissimum*.

Introduction

Cocoyam belongs to the family of Araceae and has been reported to have superior nutritional value over other major root tubers in terms of their protein digestibility and mineral composition (Lim, 2016). However, irrespective of the nutritional potential that cocoyam provides to its consumers, it has been largely neglected and underutilized in Nigeria. Nevertheless, if generally accepted, cocoyam can help to provide nutritional benefits capable of balancing certain dietary requirements hence, improve food security. Chair *et al*. (2016) reported that cocoyam is an important root crop widely cultivated for its edible corms and cormels. The post-harvest loss of root and tuber crops has been a very serious problem to farmers, as more than 40% of their harvest maybe lost because of decay (Osuji, 2013).

It is estimated that in the tropics each year between 25% and 40% of stored agricultural products are lost because of inadequate farm and village-level storage (Okigbo *et al*., 2015). Cocoyam has a very short post-harvest storage life due to fungi causing spoilage associated with it. Many studies have shown that a wide range of microorganisms such as molds have been associated with cocoyam decay especially during storage.these organisms can make food crops unfit for consumption, by changing their nutritional value or producing mycotoxins that are harmful for human and animal health. A wide variety of microorganisms, particularly molds, have been implicated in tuber spoilage, relatively few are implicated as primary pathogens (Onifade *et al*., 2004). These fungal organisms have also been reported to be the major cause of storage rots of other root and tuber crops which includes yam, cassava and sweet potatoes

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(Yusuf and Okusanya, 2008, Okigbo *et al*., 2010; Okigbo and Nwakamma, 2005; Ogaraku and Usman 2008; Amienyo and Ataga, 2007; Obire *et al*., 2016). However, studies have shown that plant extracts can effectively control various plant pathogens under laboratory conditions (Talibi *et al*., 2012). Presently, considerable efforts are directed at exploring the potentials of botanicals (plant extracts - phytochemicals) as alternatives or complimentary to synthetic chemicals. Botanicals have the advantage of not only being readily available and affordable but are also sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics, hence environment friendly (Akueshi *et al.,* 2002). The present study seeks to investigate the antifungal effects of *Vernonia amygdalina, Gongronema latifolium* and *Ocimum gratissimum* against fungi associated with the spoilage of cocoyam.

Materials and Methods

Samples Collection

Infected cocoyam corms with symptoms of softness were randomly procured locally from markets within Obingwa LGA of Abia State. Ten samples were collected randomly from different sellers at the different markets, placed in sterile polythene bags and conveyed to the laboratory for fungal isolation and subsequent identification. Plant leaves of *Vernonia amygdalina* (Bitter leaf), *Gongronema latifolium* (Utazi leaf) and *Ocimum gratissimum* (Scent leaf) were freshly bought from Umungasi market in Aba north LGA of Abia State. The plants were authenticated by the Botanist Dr. K. O. Ndukwe, the HOD Department of Biology/Microbiology, Abia State Polytechnic, Aba. The plant samples were placed in a polythene bag and taken to the Biology/ Microbiology laboratory of Abia State; Polytechnic Aba for plant extraction.

Extraction of Plant Material

The fresh and healthy plant leaves of *Vernonia amygdalina, Gongronema latifolium* and *Ocimum gratissimum* collected were thoroughly washed with tap water and then with sterile distilled water (SDW) and were naturally dried under room temperature at 37ºC for 3 weeks to a point they were dried enough for milling. The dried samples were separately grinded in the laboratory using the laboratory manual blender.

The different milled plant leaves were separated stored in different bottles and kept for extraction. The air-dried and powdered leaves were extracted by separately macerating 200 grams of the dried pulverized leaves at room temperature for 48 hours in 2 litres of water, and of 96% ethanol. The beaker was covered with aluminum foil, shaken and left to stand for 48 hours with regular stirring with a glass rod. After 48 hours, the suspension was filtered using Whatman No. 1 filter paper and the filtrate solution was evaporated in a water bath at 70°C to obtain the extract. The extracts were reconstituted by diluting with sterile water to get different concentrations of 100mg/ml, 75mg/ml and 50mg/ml respectively for *Gongronem alatifolium, Vernonia amygdalina* and *Ocimum gratissimum.*

Cultivation and Isolation of Fungi

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions, following the techniques described by Arora and Arora, (2008). 80mg of Gentamycin, an antibiotic was added to each 500 ml preparation of the agar to inhibit probable bacteria growth. Diseased portion of the tomato fruits were cut out to collect the infected area under aseptic conditions into small bits into a sterile dish with the aid of sterile scissors (flamed over a Bunsen burner flame) and dipped inside methylated spirit. The bits were sterilized with 70% ethanol and placed on Petri dishes containing already prepared solidified potato dextrose agar (PDA). The solidified plates were incubated at room temperature (28±2°C) in the dark until visible growths were seen on the plates. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure cultures were obtained and kept in stock for antifungal assay.

Antifungal Susceptibility Test (Bioassay Procedure)

The agar-well diffusion method was used to test for the antifungal activities of the ethanolic and aqueous extracts of the test plants against the test fungal organisms isolated from the cocoyams. About 20 ml of PDA were added to sterile petri-dishes. After the agar solidified, each fungal isolate was evenly spread on the surface of the medium to create "agar lawn".

Four (4) wells of 5 mm each were bored in each plate using a sterile cork borer. About 4.0 mls of each plant extract at the varying concentrations of were used to fill the well using a sterile micropipette.

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Control experiment was set up using fluconazole 250mg for the fungal assay. The plates were incubated at 25°C for 48 hours for the fungi. All inoculation procedures were conducted under aseptic conditions. With the aid of a transparent ruler, the diameters of the zones of inhibition around the wells were measured in millimeter as an indication of antifungal activity of the plant extract. Results the diameters of the zones of inhibition were interpreted as Resistant or Intermediate or Sensitive according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2017).

Results

The results of the fungi isolated from the cocoyams and the antifungal activity of ethanolic and aqueous extracts of the three medicinal plants *Vernonia amygdalina*, *Gongronema latifolium* and *Ocimum gratissimum* against five pathogenic fungi are presented in Tables 1, 2, and 3 respectively. The antifungal activity of ethanolic and aqueous extracts of the three medicinal plants *Vernonia amygdalina*, *Gongronema latifolium* and *Ocimum gratissimum* against five pathogenic fungi showed that all the extracts were effective in reducing mycelial growth of fungal isolates studied. Five fungi species were isolated from spoiled cocoyam which included *Aspergillus flavus, Aspergillus fumigatus, Mucor hiemalis, Aspergillus versicolor* and *Rhizopus stolonifer***.**

The diameter of the zones of inhibition for *Ocimum gratissimum* extract ranged from 6mm - 24mm, as shown in Table 1, *Vernonia amygdalina* (06 - 22mm) as shown in Tables 2 while that of *Gongronema latifolium* ranged from (06 - 22 mm) as shown in Table 3. However, at all concentrations used, extract of *O. gratissimum* showed higher diameter growth inhibition zones than extract of *G. latifolium* and *V. amygdalina*. The inhibition of all the pathogens is higher in the control using conventional antifungal agent (Fluconazole) than the plant extracts used in this study.

Table 1: Antifungal activities (zones of inhibition - mm) of *Ocimum gratissimum* extracts on pathogenic fungi

Fungal Isolate	Ethanol Extract Concentration (mg/ml)			Aqueous Extract Concentration (mg/ml)			Control (Fluconazole mg/ml)
	100	75	50	100	75	50	250
Aspergillus flavus	-	-	-	-	-	-	14
Aspergillus fumigates	22	16	09	11	08	-	30
Mucor hiemalis	20	12	08	12	07	-	18
Aspergillus versicolor	24	18	12	14	08	06	32
Rhizopus stolonifer	20	13	10	10	07	-	30

Table 2: Antifungal activities (zones of inhibition - mm) of *Vernonia amygdalina* extracts on pathogenic fungi

Fungal Isolate	Ethanol Extract Concentration (mg/ml)			Aqueous Extract Concentration (mg/ml)			Control (Fluconazole mg/ml)
	100	75	50	100	75	50	250
Aspergillus flavus	18	11	06	11	07	-	14
Aspergillus fumigates	18	14	09	10	08	-	30
Mucor hiemalis	20	12	07	10	07	-	18
Aspergillus versicolor	22	15	08	12	08	06	32
Rhizopus stolonifer	18	13	10	10	07	-	30

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Table 3: Diameter of the zones of inhibition (Antifungal activities) of *Gongronem alatifolium* (Utazi leaf) extracts on pathogenic fungi

Fungal Isolate	Ethanol Extract Concentration (mg/ml)			Aqueous Extract Concentration (mg/ml)			Control (Fluconazole mg/ml)
	100	75	50	100	75	50	250
Aspergillus flavus	20	14	08	-	-	-	14
Aspergillus fumigates	17	10	06	11	08	-	30
Mucor hiemalis	15	12	08	09	07	-	18
Aspergillus versicolor	22	16	10	14	08	06	32
Rhizopus stolonifer	20	13	10	10	07	-	30

Discussion

The fungi in the spoilt cocoyam were identified as *Fusarium oxysporum, Penicillium chrysogenum, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates* and *Rhizopus stolonifer*. This rot due to *Aspergillus*, according to Okigbo *et al.* (2015) was extensive resulting in complete maceration of cocoyam tissues**.** Okigbo (2003) isolated *Rhizopus* and *Mucor* species which belonged to the group of fast-growing fungi that cause rot in cocoyam. Agu *et al*. (2015) also isolated *Aspergillus* and *Fusarium* species from spoilt cocoyams. However, one fungal specie (*Fusarium*) was not detected in this present study, these might be due to differences in the study area with certain environmental factors that favor the growth of this specific specie.

Similar, species were reported by Agu *et al*. (2016). The higher number of these fungi in this present studied could probably be due to environmental conditions such as temperature and relative humidity that favor the growth and activity of these fungi species in the study area. On the other hand, Okigbo (2003) and Agu *et al.* (2016) indicated that factors such as ambient temperature, light and air moisture as well as mechanical damage of tubers also accelerate the degradation of the tubers.

The fungitoxic effect of the plant extracts was determined by zone of inhibition (mm) at three concentrations of 100%, 75% and 50%. The diameter zones of inhibition for *Ocimum gratissimum* extract ranged from (6mm - 24mm), *Vernonia amygdalina* (6mm - 22mm) while that of *Gongronema latifolium* ranged from (6mm - 20 mm). For the plant extracts the diameter of growth inhibition zone was different at varying concentrations used.

However, at all concentrations used, extract of *O. gratissimum* showed higher diameter growth inhibition zones than extract of *G. latifolium* and *V. amygdalina*. It was observed that the inhibitory activity of the plant extract increased with increase in the concentration of the extracts. The fungicidal attribute evidenced in this study can be useful to identify the plant extracts that would act as fungicides to control the spoilage of cocoyam.

In-vitro antifungal activity of three plant extracts against five pathogenic fungi associated with deterioration of cocoyam reveals that almost all the test plants were effective to inhibit the mycelia growth of the test fungi studied. The ethanolic leaf extracts of *Ocimum gratissimum* at 100% was found to be effective to inhibit the growth of the test fungi. However, the ethanolic extract was more effective (24 mg/ml) to inhibit the mycelia growth of *Aspergillus versicolor,* while the least activity of 8 mm was shown by *Mucor hiemalis.* However, the aqueous leaf extract of *Ocimum gratissimum*at 100 mg/ml followed the same trend. At 100 mg/ml conc., the aqueous and ethanolic did not show any activity.

All the test organisms were inhibited by the control. Apart from 50 mg/ml for aqueous extract, the antifungi activity of *Vernonia amygdalina* was quite effective against the fungi isolate. The mycelia growth of *Aspergillus versicolor* was highly inhibited by the ethanolic extract of *Vernonia amygdalina*. Fluconazole (250 mg/ml) was tested against the fungi isolate of cocoyam. It was observed that it is more effective against the test fungi as compared to the plant extracts. It completely inhibited the growth of the test organisms.

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The comparative result of the plant extracts reveals that *Ocimum gratissimum* was more effective in controlling the mycelia growth of *Aspergillus versicolor* and *Aspergillus fumigates*. The variation noted in their potencies may be as a result of solubility of the active substance in the solvents used or the presence of inhibitor against fungicidal principles. The inhibitory activity of plant extract increased with concentration of the extract. This agrees with reports of Onifade (2002) and Okigbo and Ogbonnaya (2006). The plant extracts of bitter leaf, scent leaf and utazi leaf in aqueous and ethanol solvents were shown to exhibit inhibitory activity against the isolated fungi. The results of the present study support the folkloric usage of the plants extract and show that some of the plant extracts possess compounds with antimicrobial properties and that can be used as a botanical in the control of rot in cocoyam. The antifungal of leaf extracts *Ocimum gratissimum* on spore germination and mycelia reduction of the most commonly occurring fungal pathogen causing soft rot of cocoyam has been demonstrated by Okigbo and Ogbonnaya (2006).

In conclusion, this study evaluated the effect of three botanicals against fungi isolate associated with cocoyam and the results indicated that leaf extracts of all plants had inhibitory activity against the test fungi. This may be due to the presence of some chemical compounds in the plant extracts suggestingthat combining the plant extracts even at lower concentration will help in reducing mycelial growth of fungi. The extracts could hence, be used as protective pesticides, since mycelia inhibitions of the pathogen was effective and also plant extracts are easily biodegradable and eco-friendly compared to chemical pesticide.

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