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Storage Rot of Ginger Rhizomes (*Zingiber officinale* Roscoe) and their control with African Nutmeg (*Monodora Myristica* Gaerth) Seeds

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ABSTRACT

Ginger grown for its edible rhizome commonly used as spice for food and drinks. It is also known for its medicinal values; however, protecting harvested rhizomes from deterioration is important to enhance storability. Ginger is attacked by fungi which cause destructive storage rot of harvested rhizomes. Effect of storage rots constitute hindrance to meeting current demands for ginger production. Therefore, effect of African nutmeg, (*Monodora myristica*) for control of fungi causing rot of ginger rhizomes was evaluated. Laboratory studies were carried out, infected ginger rhizomes were sourced from Umudike and Ori-ugba markets; taken to the laboratory for fungi isolation and identification using standard laboratory procedures. In vitro studies of effect of African nutmeg on four fungal species isolated from ginger rhizomes was evaluated. Data collected were analysed using descriptive statistics and ANOVA at $\alpha 0.05$. *Fusarium solani*, *Fusarium oxysporium* and *Rhizopus oryzae* were controlled in vitro and in vivo using crude African nutmeg extract. *Fusarium solani*, *F. oxysporium*, *R. oryzae* and *A. flavus* were isolated. Incidence of *F. solani* was (50-84%), followed by *F. oxysporium* (30-78%), *R. oryzae* (11-30%) and *A. flavus* (3-11%). *F. oxysporium*, mycelial growth was reduced between 3.33-6.05%; in the first 4 days after inoculation and 21-30% to 74.98% from 5-6 days after inoculation. There is significant difference ($P=0.05$) in mycelial growth reduction between treated and the control from 5 days after inoculation on the African nutmeg amended PDA. Mycelial growth reductions for *R. oryzae* decreased with increase in days of incubation. Highest growth reduction (51.67%) was recorded on the first day, while the least (22.14%) was recorded on fourth day. Crude extract of *M. myristica* considerably reduced growths of fungi studied and could serve as a good safe alternative to chemical fungicides in ginger storage.

Key words: ginger rhizomes, postharvest rot, African nutmeg, incubations,

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is cultivated in the tropical and sub-tropical regions of the world as a spice and medicinal crop. It has obtained a significant stature among the spices worldwide due to its universal appeal. Ginger is one of the first spices to have been exported from Asia, arriving to Europe with the species trade and used by ancient Greek and Roman. About half of its world's production comes from India, whereas, its cultivation is extended to China, Australia, Malaysia, Nigeria, Fiji, Brazil and Mexico (Sanderson *et al.*, 2002). India and China are the major suppliers of the species all over the world.

Ginger is now grown as a cash crop in Africa and Latin America and has entered many local cuisines. Nigeria is one of the world's leading producers of ginger in 2016, Nigeria recorded a production of 522,964 tons of ginger to place second in the world's 10 top ginger producing

countries having 16% share of world ginger production (www.factfish.com).

With the recent development in science and modern technology in food productions, ginger has been formulated in several products including ginger tea, ginger bear, ginger powder, ginger candies and ginger juice (Srinivasan, 2017; Malu *et al.*, 2019). Ginger has been used as a food, spice, supplement and flavouring agent.

As dietary supplements, it has been made into various preparations such as medicinal liquor or liquor plus honey, pills, capsules and tablets. The health benefits of ginger for human health are mainly attributed to bioactive compounds such as phenolic compounds, flavonoids, terpene and some volatile compounds contained in the essential oils of ginger. It has been discovered that ginger is suitable to cure ailments like nausea, vomiting after chemotherapy, asthma, impotence, gout, diarrhea, dysentery, peptic ulcer and diabetes. Ginger extract can control

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disorders caused by oxidative stresses as a strong anti-oxidant. Ginger has numerous effects such as those on metabolic syndrome, cholesterol levels, lipid metabolism and inflammation, revealed by epidemiological studies (Toda *et al.*, 2016).

For culinary intent, ginger is appropriate for all dishes both sweet such as drinks, cakes, puddings, apple pie, candies, breads etc. and savoury puddings, grills, roasts (Oludoyin and Adegoke, 2014).

Ginger is attacked by several biotic entities including fungi, oomycetes, bacteria, and viruses. Soft rot caused by *Pythium* spp, predominantly *Pythium myriotylum* and bacteria wilt caused by *Ralstonia pseudosolanacearum* are the major production constraints in ginger worldwide. A number of fungal species have been reported worldwide to cause diseases in ginger (Dohroo, 2005, 2016). These fungi belong to different genus including *Aspergillus*, *Penicillium*, *Pythium*, *Fusarium* and *Phyllosticta*, (Moreira *et al.*, 2013, Meenu and Kaushal, 2017; Meenu and Jebasingh, 2019). All parts of the plant including sprouts, roots, rhizomes and the collar region of the pseudo stem are prone to infection. The examples of fungi disease of ginger are Soft rot, leaf spot, Dry rot, yellow/wet rot and storage rots. The effect caused by the diseases mentioned above constitute hindrance to ginger in the meeting the current demands for ginger production (Gupta and Jennyson, 2019).

Efforts to identify the fungi diseases affecting ginger production will go a long way in improving availability of diseases free rhizomes for consumption and other uses. Soft rot of ginger is caused by *pythium* spp and *Fusarium* spp. The fungi diseases are destructive diseases of ginger. It can reduce the ginger yield by 50-90% if not controlled (Dahroo, 2005). Therefore, the Objective of the study are to: identify fungi causing rot of ginger rhizome and evaluate effect of African nutmeg (*Monodora myristica Gaerth*) for control of fungi causing rot of ginger rhizomes.

MATERIALS AND METHODS

Experimental location

This study was carried out in the laboratory of the Department of Plant Health Management, College of Crop and Soil Sciences in Michael Okpara University of Agriculture, Umudike, Abia State; located on 5022 N latitude, 7033 E Longitude and altitude of 122m above sea level (NRCRI, 2016).

Source of materials

The healthy (uninfected) and infected ginger rhizomes were randomly purchased from the two

different Markets from four different sellers at Orié Ugba Umuahia Abia State. The seeds of African nutmeg (*Monodora myristica*) were purchased from the same market. Samples were collected in a sterile polythene bag and taken to the laboratory for isolation and identification. Each was collected in a separate sterile polythene bag and brought to the laboratory for further investigation in the Department of Plant Health Management. The samples were surface-sterilized with 1% sodium hypochlorite to remove the external contaminants which might be at the surface of the ginger rhizome and rinsed in 3 changes of sterile distilled water.

Isolation and Identification of Fungal Pathogens

Thirty nine (39) gram of Potato Dextrose Agar (PDA) was dissolved in 1litre of distilled water autoclaved at 15Ibs pressure for 20 minutes then allowed to cool to 40°C before dispensing into Petri dishes. The Chiejina (2008) isolation method was used. Thin sections (3 mm diameter) were cut from the Periphery of diseased ginger rhizomes and surface sterilized with 1% sodium for 2-3 min, after which they were rinsed in three changes of sterile distilled water. Streptomycin (0.2g) was added to the potato dextrose agar solution before pouring into sterile Petri plates under aseptic conditions to prevent bacterial growth. This pouring was done in a sterile inoculation chamber to avoid contamination. The medium was allowed to solidify before the bits were plated on solidified potato dextrose agar medium, under aseptic conditions. The inoculated Petri dishes were incubated at 25±1°C for fungal growth for 5 days. The isolates were sub cultured to obtain pure cultures. During the sub culturing each colony in the original culture was transferred into a fresh plate containing potato dextrose agar and then incubated for 5 days at room temperature 27°C. Four distinct organisms were identified by their varying colours and were used for pathogenicity test. The identification of the isolated fungi was done macroscopically and microscopically. Physiological identification was done by observing culture growth patterns and mycelial colour. Small portions of the fungal cultures were collected, teased and mounted in lactophonel in cotton blue on clean slides and cover slips and then viewed with the microscope. Fungal identification was confirmed by comparison with books by Barnett and Hunter (1999), Alexopoulos *et al.* (2002) and Agrios (2005).

Pathogenicity test

Eight healthy, uninfected ginger rhizomes were washed with running tap water and then with 1 % sodium hypochlorite and sterile water and dried

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with sterile paper towel. Holes were made on each using a sterilized cork borer (5mm diameter) the various cultures containing the isolates 5mm discs using the cork borer and 5 mm disc of the isolates was placed in the holes created on the healthy gingers (Amadioha and Uchendu, 2003; Amadioha, 2004) The disc of the ginger rhizomes was then used to cover the fungal disc. After covering the hole, vaseline was applied round the point of inoculation to prevent contamination by other microorganisms. The inoculated gingers were kept in a micro humidity chamber to ensure that the relative humidity for fungal growth is supplied. They were incubated for about 6 weeks at room temperature, after which a longitudinal section was made on the infected gingers. The gingers that showed rot where selected and pieces of each rotted ginger was re-isolated on a fresh plate and compared with original isolate to confirm the pathogen causing the rot was the same and there was no contamination.

Effects of botanical on fungal growth in culture

Five drops of African nutmeg extract were added to sterilized PDA and well spread before solidification. Sterile distilled water was used instead of extract in control experiment. A disc of 5mm diameter of the pure culture of each test fungus was placed separately on the extract-PDA medium at the point of intersection of two perpendicular lines draw under the Petri dish. Control plates were without any plant extract but sterile distilled water was added. The diameter of the colony was measured with meter rule until the growth of the isolate in the control experiment had reached the edge of the Petri dish. This was replicated 3 times and laid out in completely randomized design (CRD). The percentage growth inhibition by the extract was calculated using the formula reported by Amadioha (2003).

Where: DC= Average diameter of fungal colony with control.

DT= Average diameter of fungal colony with treatment.

Fungi rot development as affected by botanical treatment *in vivo*

The method adopted by Amadioha, (2004) was used for the study. Healthy ginger were surface sterilized with 1% sodium hypochlorite; rinsed in three changes of sterile water and dried with sterile paper towel. The ginger were treated with the extract and allowed to stand for one hour before inoculation with fungi. The treated ginger were incubated for 39 days and observed for rot development and percentage rot development was recorded at the end of the incubation period. This experiment was laid out in CRD with 3 replicates.

STATISTICAL ANALYSIS

Data collected from the experiments were analyzed using analysis of Variance (ANOVA) Using SPSS version 23 and Significant means were separated using least significant difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

Incidence of fungi species in rotted ginger market samples

Figure 1 shows the percentage incidence of four fungal species isolated from infected ginger rhizomes. *Fusarium solani* (50.0 -84.0%) was the most predominant fungus species encountered in the ginger samples studied, followed by *Fusarium oxysporium* (30.0 -78%), *Rhizopus oryzae* (11-31%), while *Aspergillus flavus* (30-11%) was be least (Figure 1).

The effect of various fungi isolated from rotted ginger (pathogenicity test)

The pathogenicity of the different fungi isolated from ginger is shown in Table 1. *Aspergillus flavus* did not induce rhizome rot in healthy ginger rhizomes just like control in which no fungi was inoculated. However, *Rhizopus* and *Fusarium* spp both caused 40% rot of the healthy rhizomes inoculated although the rots took up to 6 weeks to develop (Table 1).

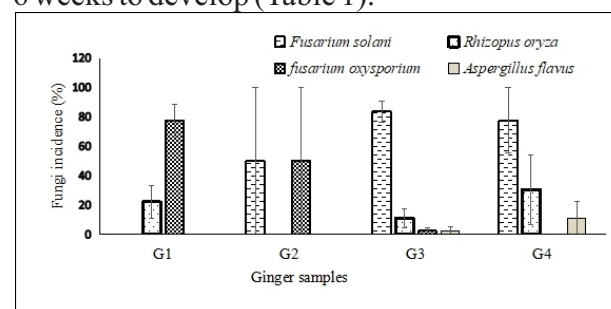


Figure 1: Percentage incidence of fungal species isolated from ginger rhizomes

Table 1: The pathogenicity of fungi isolated from ginger rhizomes.

Fungi	Percentage rot (%)	Rating
<i>Rhizopus</i> spp	30.00	3
<i>Aspergillus</i>	0.00	0
<i>flavus</i> <i>Fusarium</i>	40.00	4
<i>solani</i> Control	0.00	0

Table 2: Percentage reduction of mycelial growth of fungi by crude extracts of botanicals *in-vit*

Treatments	Fungi spp	Days after treatment										Mean
		1	2	3	4	5	6	7	8	9	10	
Ehuru	<i>Fusarium oxysporium</i>	5.00	3.33	5.21	6.05	21.30	66.49	68.13	67.76	74.20	74.98	39.25
	<i>Rhizopus</i> sp	51.67	44.44	26.67	22.14	0.00	0.00	0.00	0.00	0.00	0.00	14.49
Fusarium	<i>Fusarium solani</i>	100.00	100.00	50.45	55.56	69.19	79.51	89.18	85.10	85.35	84.57	79.89
	<i>Fusarium oxysporium</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	<i>Rhizopus</i> sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	<i>Fusarium solani</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD(p=0.05)		15.31	17.57	11.46	10.53	2.44	3.16	3.77	1.84	1.48	1.09	



Plate 1: *Fusarium solani* growth on ginger rhizomes treated with African nutmeg extract

Table 3: Rot development on ginger rhizomes as influenced by crude extract of African nutmeg (*Monodora myristica*) in-vivo

Botanical treatment	Fungi	Percentage Rot (%)
African nutmeg	<i>Rhizopus</i> sp	38.89
	<i>Fusarium oxysporium</i>	63.89
	<i>Fusarium solani</i>	73.89
	<i>Fusarium oxysporium</i>	0.00
Control	<i>Rhizopus</i> sp	0.00
	<i>Fusarium solani</i>	0.00
	LSD(p=0.05)	29.44

Effect of *Monodora myristica* on mycelial growth of fungi

Table 2 presents the effect of African nutmeg (*Monodora myristica*) on four fungal species isolated from ginger rhizomes in-vitro. For *Fusarium oxysporium*, mycelial growth was reduced between 3.33 -6.05 %. In the first 4 days after inoculation and 21-30 % to 74.98 % from 5-6 days after inoculation. There is significant difference (P=0.05) in mycelial growth reduction from 5 days after inoculation on the African amended PDA. There was significant difference (P=0.05) between the treated and the control. Mycelial reductions for *Rhizopus oryzae* decreased with increase in days of incubation. The highest growth reduction (51.67 %) was recorded on the first day, while the least (22.14 %) was recorded on the fourth day (Table 2).

Rot development reduction percentage (%) by *Monodora myristica*

The results for effect of African nutmeg on rot development reduction percentage in treated ginger rhizomes (in- vivo trial) is presented on Table 3. The effect of extracts of *Monodora myristica* on the mycelial growth of *Fusarium oxysporium* and *Rhizopus oryzae* on ginger rhizome before and after inoculation are presented in Table 3. Percentage growth of the fungal pathogens in-vivo were significantly (p=0.05) influenced by African nutmeg extracts. The growth rate of *Fusarium oxysporium* on ginger rhizome reduced up to 63.89 % before and after treatment with *Monodora myristica* extract,

respectively. The differences in growth reduction differed significantly (p=0.05) from the control. A similar trend was observable in *Rhizopus oryzae* *Monodora myristica* significantly reduced fungal growth by > 38 % in ginger samples with treatments (Table 3).

DISCUSSION

The result of this study indicates that *Rhizopus* and *Fusarium* species were the most prevalent fungi in encountered in ginger samples, accounting for over 50 % of the total fungi incidence. This high incidence of *Rhizopus* species could be attributed to the environmental conditions during storage, as *Rhizopus* species are known for their rapid growth and ability to thrive in various substrates. These fungi have been reported under traditional storage structures of ginger rhizomes (Ram and Thakur, 2010). *Fusarium* rot (*Fusarium* spp) can cause serious problems on stored ginger (Ram and Thakore, 2009). *Aspergillus* sp which was not pathogenic from the pathogenicity test was also recorded; they have been reported to grow on cut ends and injured areas, and although not parasitic, may lead to mycotoxin production (Overy and Frisvad, 2005).

The results of the antifungal activities of *Monodora myristica* on *Fusarium solani* and *Rhizopus oryzae* causing ginger rot showed that the plant extracts had inhibitory effects on growth of the fungi. From the results, the antifungal activities of the extract decreased with increasing days of inoculation, hence the inhibition activities of the extract of *M. myristica* was time dependent. This is in agreement with the report of Rahman (2016), Chiejina and Ukeh (2013) who stated that the antifungal activities were observed to correspond with period of extracts inoculation. Extract of *Monodora myristica* has been found to inhibit the growth of conidial organisms. According to Inderjit and Mukerji (2006), *Rhizopus* sp. can produce and release many kinds of allelochemicals participating in their defense against pathogens. In vitro result in the study showed that the African nutmeg extract exhibited fungistatic effect on the fungi pathogens. This agrees with the reports of (Ram and Thakore, 2009; Jadhav *et al.*, 2013) in which crude extracts from plant materials significantly inhibited mycelial growth of many pathogenic fungi. The significant inhibitory effect of the plant extracts in the control of *Fusarium solani* and *Rhizopus oryzae* showed that the fungi toxic components of these extracts with regards to the days of incubation after inoculation can effectively control the mycelial growth and sporulation density of the fungal pathogens. This is in line with the findings of Daouk *et al.* (1995) who reported that reduction in microbial population

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depends on days of inoculation which can completely inhibit the growth of microorganism.

The ethanolic extract of *Monodora myristica* seed possess broad spectrum antifungal activities against *Aspergillus* sp and also inhibited their mycelia growth (Ogunmoyole *et al.*, 2013). This generally confirmed that this seed is highly potent to activities of many microorganisms. The in vitro and in vivo effects of African nutmeg *Monodora myristica* extract was evaluated in order to develop cheaper methods of controlling post storage rot on ginger rhizomes.

CONCLUSION AND RECOMMENDATIONS

In this study, four fungi were isolated from ginger while two out of the four, *Fusarium solani* and *Rhizopus* sp caused deterioration of ginger rhizomes in the pathogenicity. The fungi pathogens include *Fusarium oxysporum*, *Rhizopus oryzae*, *Aspergillus flavus* and *Fusarium solani*. This was confirmed through a pathogenicity test which indicated that *Fusarium solani* had the highest rot advancement (40 %) followed by *Rhizopus oryzae* (30 %) followed by *Fusarium oxysporum* and *Aspergillus flavus* on the ginger rhizomes sourced from Orié Ugba at Umuahia. These fungi attack and reduce the nutritional and market values of gingers. African nutmeg extract was assessed and found to reduce the radial growths of the test fungi in culture. The antimicrobial potential of this plant could be employed in control of post - harvest of ginger caused by *Fusarium oxysporum* and *Rhizopus oryzae*.

Considering the inherent problem associated with use of synthetic chemicals. It is therefore recommended that African nutmeg extracts *Monodora myristica* can be exploited or used as substitute to synthetic fungicides in the control of the storage rot of ginger caused by these fungi.

Based on the findings of this study, plants extracts are environmentally friendly and has less financial implications to obtain. It implies that they can serve as a good alternative to chemical fungicides considering their safety and degradable qualities.

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