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Identification and Control of Postharvest Fungal Contaminants of Sweet Potato (*Ipomea batatas* L.) in Lafia

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Abstract: Postharvest rot leads to huge yield losses of potato annually. A study was conducted to identify and control fungi causing postharvest tuber rot of sweet potato in Lafia. Infected tubers were collected from sales points in Lafia, and conveyed to the Plant Science Laboratory, Federal University of Lafia. Infected tissues were collected from the rotted tubers using a sterile scalpel, surface sterilised in 70% ethanol, and cultured for 3-5 days on PDA. Based on cultural and microscopic morphology, fungi isolated were identified under four genera, namely *Mucor, Aspergillus, Candida, Fusarium.* A total of 13 isolates were obtained, of which *Aspergillus niger* and *Candida* sp. had the highest number of isolates 4(30.77%), followed by *Mucor racemosus* 3(23.08) and *Fusarium* sp. 2(14.39). Pathogenicity test of the identified fungi showed that of the four identified species, *Aspergillus niger* produced tissue rot on the tubers, beginning from the first week (8.67 mm) to second week after inoculation (10.00 mm). Tuber rot produced by *A. niger* differed significantly from the other fungi as well as the uninoculated control ($P \leq 0.05$). Test of bioefficacy of aqueous and ethanolic extracts of *Moringa oleifera* in the control of *A. niger*showed that ethanolic extracts of *Moringa oleifera* produced higher and significantly different growth inhibition of *A. niger* compared to the extracts of *Zingiber officinale* ($P<0.05$). The study revealed that extracts of *Moringa oleifera* and *Zingiber officenale* hold promising prospects in the development of biofungicides for the management of postharvest tuber rot of sweet potato.

Keywords: Identification, pathogenicity, plant extracts, biocontrol, tuber rot, sweet potato

Introduction

The sweet potatoes (*Ipomoea batatas*) are dicotyledonous root vegetable plants that belong to the morning glory family, *Convolvulaceae* [1]. Cultivars of the sweet potato have been bred to bear tubers with flesh and skin of many colors, white, yellow and orange flesh is common with a darker skin [2]. Sweet potatoes are underground tubers that have admirable quantity of beta carotene which our bodies can convert into vitamin A [3]. According to Abebe, the potato crop is a staple food that is rich in carbohydrates, protein, vitamin C, vitamin A, zinc, iron and minerals, which alleviate the problem of malnutrition in subsistence farmers and towns [4]. They come in different shapes and their peels ranges from creamy white, yellow-orange, tan, reddish-purple and red colours [5]. The health benefits of sweet potato include improvement of blood sugar regulation, maintaining healthy blood pressure levels, improving digestion, reducing risk of cancer, and it serves as a good source of provitamin A in the form of beta-carotene, important for protecting eye health [6].

Storage of sweet potato tubers after harvest is imperative, as this practice may prevent a surfeit of potatoes entering the food markets at any given time and prolong the period of fresh tuber availability, especially when the crop is not in season or when the economic circumstances and/or regional climates in a particular area of production dictate its production during the year. Sweet potatoes are one of the most important vegetables and they are susceptible to a variety of field and storage diseases. They are vulnerable to deterioration because of their high water and nutrient content [7].

Sweet potatoes are affected by a number of diseases, both fungal and bacterial [8]. Commonly observed postharvest diseases caused by fungi include black rot (*Ceratocystis fimbriata*), dry rot (*A. niger* and *Diaporthe batatas*), Fusarium surface rot (*F. oxysporum*), Fusarium root and end rot (*F. solani*), foot rot (*Plenodomusdestruens*), soft rot (*Rhizopus stolonifer* and *Rhizopus oryzae*), blue mold (*Penicillium* spp.), java black rot (*Botryodiplodiatheobromae*), circular spot (*Sclerotium rolfsii*), charcoal rot (*M. phaseolina*) and storage rot (*Mucor* spp.) [9-11].

Accurate species identification is considered the first step in the chracterisation and control of fungal pathogens of crop plants [12, 13]. Therefore, the objective of the present study was to identify and control fungi causing postharvest tuber rot of sweet potato in Lafia using plant extracts. The findings of this study will compliment ongoing efforts in the use of environmentally friendly approaches in the management of fungal diseases of crops.

Materials and Methods

Sample collection

The white variety of sweet potato cultivars most commonly cultivated by farmers in Nasarawa State was bought from the farmers in Lafia, Nasarawa state. In each case, collected sweet potato tubers with fungal rot and the healthy sweet potato tubers were packaged in clean polyethylene bags and jute bags respectively. The samples were labeled with date, collection site and sample number and taken to the

Plant Science and Biotechnology Laboratory, Federal University of Lafia, for further analysis. Sweet potato tubers collected were cleaned and swabbed with 70% ethanol solution to remove external contaminants.

Fungi isolation and identification

A sterile scalpel was used to cut small pieces (1-2 cm^2) of *Ipomoea batata* from the region of the infected tubers, ensuring to include the healthy and infected regions. The tissues were surface sterilised in 70% ethanol for 2 minutes, rinsed in two changes of sterile distilled water, plated on sterile PDA at room temperature for 3-5 days, and observed for the emergence of fungal growth.

When growth was established, different fungal colonies shown on a 3-5 days old cultures were sub-cultured through point inoculation to obtain pure cultures.Fungi identification was based on morphological characteristics described by Mathur and Kongsdal [14], Samson *et al.* [15], and Scot [16].

Pathogenicity test of isolated fungi

Healthy potato tubers were slightly wounded by gently piercing with a syringe needle, and 7 mm diameter mycelial discs obtained from actively growing regions of three days old cultures on PDA using a cork borer were placed on the wounded portions. The inoculated points were sealed with moistened sterile cotton wool, incubated at room temperature for two weeks, and observed for the commencement and advancement of tissue rot. Diameter of tuber rot was obtained using a meter rule.

Preparation of plant extracts

The leaves of *Moringa oleifera* and the rhizomes of *Zingiber officinale* were washed and dried under room temperature for 12 days. Thereafter, the dried plant materials were ground to powder using amortal and a pestle. For extraction, 50 g of the powdered plant materials were soaked in 500 mL of Ethanol (70%) and 500 mL of water, and the mixtures were agitated at different intervals for three days.The extracts were filtered using a muslin cloth, and the filtrates were stored at room temperature in sterile glass bottles.

Effect of plant extracts on pathogenic fungi associated with sweet potato tubers

This was determined using the modified method of Terna and Simon [18]. Five mL from each of the concentrations of leaf extracts of (0, 25, 50, 75 and 100 percent) were dispensed separately into 9 cm diameter Petri dishes and mixed gently with 15 mL of sterile molten PDA to form Potato Dextrose Leaf Extracts Agar (PDLEA). The medium was allowed to solidify and inoculated centrally with 7 mm diameter mycelia plugs of test fungi obtained from 5 days old cultures, using a sterile cork borer. Test fungi growing on PDA plates inoculated with 5 mL sterile distilled water served as control. All cultures were incubated at room temperature, and colony diameters measured for 3, 5, and 7days after inoculation, using a transparent meter rule. The percentage radial growth inhibition of the pathogen was calculated using the formula;

$$PI=(C-T/C) \times 100\%$$

Where: PI= Percent inhibition of fungi growth; C= Colony diameter of fungus in the control plate; T= Colony diameter of fungus in the treated plate

Experimental design and data analysis

The experimental units were set up in Completely Randomized Design (CRD), with three replicates. Data obtained were subjected to Analysis of Variance (ANOVA) at 5% level of probability using the Minitab software Version 17. Means were separated using the Tukey's Honestly Significant Difference.

Results and Discussion

Table 1 presents the morphological characteristics of the fungi isolated from rotted sweet potato tubers in Lafia. Based on cultural and microscopic examination, the fungi isolated from the rotted tubers were identified under four genera, namely *Mucor*, *Aspergillus*, *Candida*, and *Fusarium.* A total of 13 isolates were recovered, of which *A. niger* and *Candida* sp. had the highest number of isolates 4(30.77%), followed by *Mucor racemosus* 3(23.08%), and *Fusarium* sp. 2(14.39%).

Table 1: Morphological characteristics and assigned identities of fungi isolated from diseased sweet potato tubers

Isolate Group	`Morphological characteristics				Morphologically assigned identity
	Cultural characteristics	Conidia	Conidiogenous cells	Hyphae	
1	Cultures were grayish with profuse fuzzy growth and aerial mycelia	Sporangiospores were smooth-walled, ellipsoidal to subglobose	Sporangiophores were elongate, hyaline, having an obovoid to ellipsoidal columella with a truncate base	Scantily branched and aseptate	*M. racemosus* (3 isolates)
2	Colonies were blackish-brown, flat, circular, with whitish margins and whitish to gray pigmentation on the reverse	Mostly globose and nearly smooth-walled	Conidiophore was radiate, biseriate, with cylindrical phialides	Rough-walled, aseptate, and scantily branched	*A. niger* (4 isolates)
3	Colonies were creamy, slimy, flat, and irregular, with a pale to white pigmentation on the reverse	Oval shaped ballistoconidia found	Cylindrical to oval-shaped budding cells found	Absent	*Candida* sp. (4 isolates)
4	Colonies were whitish to gray in appearance, irregular, fluffy, raised, with purple mixed with brown pigmentation on reverse	Slightly curved cylindrical to sub-globose conidia	Slender and elongated phialides were observed	Hyphae were septate, branched, and unpigmented	*Fusarium* sp. (2 isolates)

Table 2 presents the results of the pathogenicity of fungi isolated from rotted sweet potato tubers from Lafia. Of the four different fungal species, namely *Mucor racemosus*, *Candida* sp., *Fusarium* sp., and *Aspergillus niger*, isolated from the rotted tubers, only *A. niger* was able to produce tissue rot on the tubers, beginning from the first week (8.67 mm), to the second week after inoculation (10.00 mm). Tuber rot produced by *A. niger* differed significantly from the other fungi as well as the uninoculated control (P≤0.05).

Table 2: Pathogenicity of fungi isolated from rotted sweet potato tubers

Isolate	Diameter of Tuber Rot (mm)	
	Week 1	Week 2
Mucor racemosus	0.00[a]	0.00[a]
Candida sp.	0.00[a]	0.00[a]
Fusarium sp.	0.00[a]	0.00[a]
Aspergillus niger	8.67[b]	10.00[b]
Control	0.00[a]	0.00[a]

[a]Means followed by the same superscripts within the same column are not significantly different (P>0.05)
[b]Means followed by different superscripts within the same column are significantly different (P≤0.05)

Table 3: Effects of different concentrations of aqueous and ethanolic leaf extracts of *Zingiber officinale* on the radial growth of *Aspergillus niger*

Extract	% Concentration	Day 3	Day 5	Day 7
Aqueous	25	0.00[c]	0.00[d]	2.38[b]
	50	0.00[c]	0.00[d]	0.00[b]
	75	11.80[c]	0.00[d]	8.10[b]
	100	0.00[c]	0.00[d]	0.00[b]
Ethanolic	25	66.67[b]	28.88[c]	12.76[b]
	50	85.93[ab]	63.02[b]	21.40[ab]
	75	100[a]	81.03[a]	46.09[a]
	100	100[a]	84.83[a]	15.23[b]

[a]Means followed by the same superscripts within the same column are not significantly different (P>0.05)
[b]Means followed by different superscripts within the same column are significantly different (P≤0.05)

Table 4: Effects of different concentrations of aqueous and ethanolic leaf extracts of *Moringa oleifera* on the radial growth of *Aspergillus niger*

Extract	% Concentration	Day 3	Day 5	Day 7
Aqueous	25	21.20[b]	27.40[bc]	28.60[a]
	50	50.00[ab]	42.50[abc]	42.90[a]
	75	12.63[b]	19.90[c]	19.80[a]
	100	100.00[a]	95.97[a]	48.41[a]
Ethanolic	25	60.38[ab]	42.50[abc]	16.90[a]
	50	81.18[a]	83.56[ab]	38.40[a]
	75	100.00[a]	86.30[ab]	33.80[a]
	100	100.00[a]	100.00[a]	42.60[a]

[a]Means followed by the same superscripts within the same column are not significantly different (P>0.05)
[b]Means followed by different superscripts within the same column are significantly different (P≤0.05)

Table 3 presents the results of the effects of different concentrations of aqueous and ethanolic extracts of *Zingiber officinale* on the radial growth of *A. niger*. The highest radial growth of the pathogen (100.00%) was observed after three days of post-application of 75% and 100% ethanolic extracts of *Zingiber officinale*, followed by 50% concentration of ethanolic extracts (85.93%). Differences in radial growth inhibition of *A. niger* were significant among the different extract concentrations and extraction solvents (P≤0.05). Table 4 presents the effects of different concentrations of aqueous and ethanolic extracts of *Moringa oleifera* on the radial growth of *A. niger*. The highest radial growth of the pathogen (100.00%) was observed after three days of post-application of 100% aqueous extracts, 75% and 100% ethanolic extracts of *Moringa oleifera*, and five days of post-application of 100% concentration of ethanolic extracts, followed by five days of post-application of 100% concentration of aqueous extracts (95.97%). Differences in radial growth inhibition of *A. niger* were significant among the different extract concentrations and extraction solvents (P≤0.05).

Figure 1 presents the comparative effect of aqueous extracts of *Zingiber officinale* and *Moringa oleifera* in the growth inhibition of the potato tuber rot pathogen, *A. niger*. Aqueous extracts of *Moringa oleifera* were more effective in inhibiting the growth of the fungal pathogen (42.44%), compared to *Zingiber officinale* (1.86%). The differences in radial growth inhibition of the rot fungus were significant (P≤0.05) among the two plant extracts.

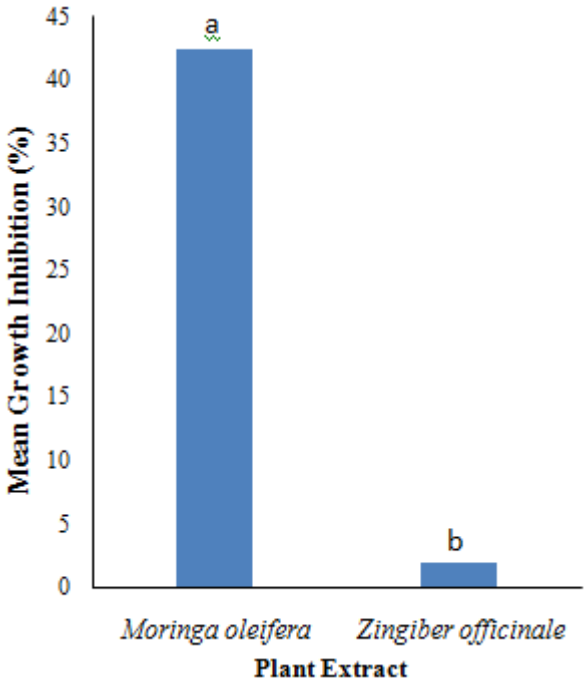

Figure 1: Comparative antifungal effect of aqueous leaf extracts of *Moringa oleifera* on *A. niger*

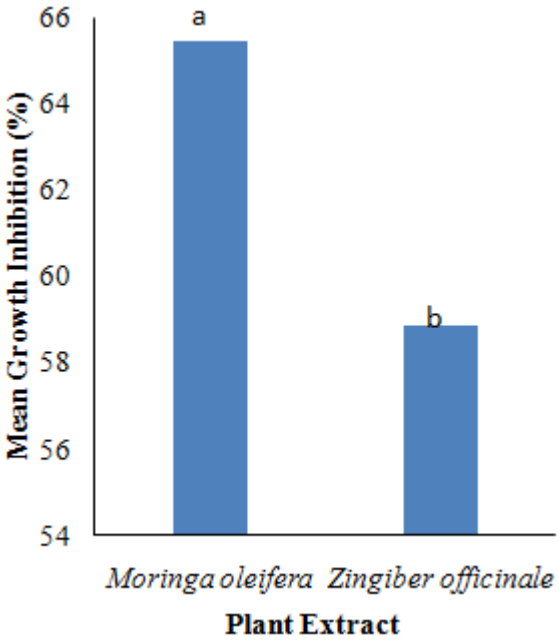

Figure 2: Comparative antifungal effect of ethanolic leaf extracts of *Moringa oleifera* and *Zingiber officinale* on *A. niger*

Figure 2 presents the comparative inhibitory effect of ethanolic extracts of *Zingiber officinale* and *Moringa oleifera* on the growth of the potato tuber rot pathogen, *A. niger*. Ethanolic extracts of *Moringa oleifera* were more effective in inhibiting the growth of the fungal pathogen (65.46%), compared to *Zingiber officinale* (58.82%). The differences in radial growth inhibition of the rot fungus were significant ($P \leq 0.05$) among the two plant extracts.

In the present study, fungi associated with rotted sweet potato tubers were identified as *Aspergillus niger*, *Mucor racemosus*, *Fusarium* sp.and *Candida* sp. Results of pathogenicity test further showed that *A. niger* was the only fungal species that produced rot on artificially inoculated tubers. The proliferation of these fungi on the infected potato tubers could be as result of the abundant moisture content of potato tubers, worsened by poor storage facilities in the study area. Similarly, Harrigan *et al*. [16] stated that insufficient drying and precarious conditions of storage could promote growth of *Aspergillus.* Jay [17] further reported that some fungal species particularly *Aspergillus niger* in sweet potato have been linked to mycotic infection of both human and animals. In a similar study, Amienyo and Ataga [18] identified *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodiatheobromae*, and *Fusarium solani*as the predominant species of fungi responsible for postharvest rot of sweet potato tubers. Oladoye *et al*. [7] suggested that once successfully established in the surface tissue of the tuber, spoilage fungi can easily infect the deep tissues causing tissue spoilage.

Results of the present study showed that aqueous and ethanolic extracts of *Moringa oleifera* were more effective in inhibiting the growth of the pathogen, thus confirming the report of Ijato [19] that *Maringa oleifera* leaves possess antifungal activity. Furthermore, studies of Busani *et al*. [20] and Foidl *et al*. [21] also confirmed that*Moringa oleifera* leaf extracts were effective in the control of fungal pathogens.

The inhibitory effect of the extracts tested in the present study was shown to vary with plant species, extract concentration, and extraction solvent. With respect to extract concentration, increasing the extract concentrations to 100 mg/mL, and 75 mg/mL caused significantly higher percentage inhibition of the test fungi than lower concentrations. This finding is in agreement with findings of Amionye and Ataga [18] and Alhussaen *et al*. [22] who reported that undiluted garlic extracts showed a high control activity with no growth, whereas, diluted garlic extracts at 10 and 5% reduced the fungal growth to 15.5 and 41%, respectively. Increasing concentrations of these extracts implied an increase in the active ingredients of the solutions which act on the fungi thereby affecting their physiological processes and consequently lowering their growth.

Conclusion

In the present study, fungi associated with postharvest rot of sweet potato were identified under four genera namely; *Mucor racemosus*, *Aspergillus niger*, *Candida* sp and *Fusarium* sp. Among the identified fungi, *A. niger* was the only fungal species that produced tissue rot on artificially inoculated tubers. Test of the bioefficacy of ethanolic and water extracts of *Moringa oleifera* and *zingiber officenale* in the control of *A. niger* revealed that although both plants contained antifungal properties, ethanolic and aqueous extracts of *Moringa oleifera* were most effective in inhibiting the growth of the rot fungus. The study revealed that extracts of *Moringa oleifera* and *Zingiber officenale* hold promising prospects in the development of biofungicides for the management of postharvest tuber rot of sweet potato.

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