



## BIOCONTROL ACTIVITIES OF SOME LOCAL NIGERIAN PLANT SPICES AGAINST SWEET POTATOES SPOILAGE FUNGI



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### Abstract

Sweet potato (*Iponmea batatas*) ranks as the fifth most important food crop in developing countries. It is mainly affected by fungal and bacterial diseases, which reduces its yields, quality, storage life and cause economic losses. Synthetic fungicides usually used to prevent this spoilage are toxic to man, crop and environment. This necessitated the search for the use of biological fungicides from plants, safe, biodegradable, economically and environmentally friendly and available. This study was aimed to determine the efficacy of *Murraya koenigii*, *Curcuma longa* and *Piper nigrum* on sweet potato spoilage fungi. Samples were purchased from Bodija market and sweet potatoes were purchased from Oje and Bodija market, Ibadan, Oyo state, Nigeria and were brought aseptically in sterile polythene bags to the laboratory. Fungal pathogens were isolated and identified based on growth pattern, color of mycelia, microscopic examination of vegetative and reproductive structure of the fungi. Extraction of bioactive ingredients in the plants were performed using methanol and distilled water. The isolates include *Aspergillus flavus* and *Aspergillus niger*, and they were subjected to pathogenicity test, severity test. *Aspergillus niger* showed more virulence and was subjected to inhibition by the plant extracts. The extracts significantly ( $p < 0.05$ ) inhibited the pathogen growth and the most effective extracts against the fungal pathogens among the three is that of *Murraya koenigii*. The zones of inhibition ranges between 0.84 to 1.53, 0.48 to 1.64 and 0.62 to 1.65 cm for Black pepper, Curry leaf and Tumeric respectively, they hence can be used in the control of sweet potato fungal pathogens.

### Keywords:

Fungi, fungicides, *Murraya koenigii*, *Aspergillus niger*, pathogen, biodegradable.

### Introduction

Sweet potato is among the world most important versatile, and under exploited food crops with more than 133million tons in annual production. Sweet potatoes rank sixth in the world in terms of value of roots and tubers based on fresh (Udemezue and Eluagu, 2021). Sweet potato is an outstanding source of nutrients, among which are vitamins, potassium, iron, calcium, and minerals. Derive from sweet potato is a natural source health-promoting compounds due to the presence of  $\beta$ -carotene and anthocyanins in it (Senthilkumar *et al.*, 2020). Its large starchy, sweet tasting, tuberous roots are a root vegetable. The desirable nutritional value of sweet potato is gaining recognition, as the understanding between diet and health increases. Various parts of the crop have been reported to contain both organic and mineral nutrients including vitamin A and C, Zinc, Potassium (K), Sodium (Na), Manganese, Calcium (Ca), Magnesium (Mg) and Iron (Fe) (Barbara *et al.*, 2014; Senthilkumar *et al.*, 2020).

Fungal and bacterial disease affecting the storage roots are important because they affect the yield, aesthetic quality, storage life and nutritional value of the storage roots, these pathogens create local discoloration and disruption of surrounding tissues of infected tubers (Snowdon, 2010; Clemente *et al.*, 2018), resulting in changes in appearances deterioration of texture and possibly flavour or taste. The activities of these pathogen results in post-harvest losses, reduction in the market value and misfortune to farmers. They also cause significant economic losses in the commercialization phase and are rendered unfit for human consumption.

Recent studies on the use of plant extracts have opened a new avenue for the control of plant diseases. These plant extracts have been reported to be safe, non-phytotoxic to man but effective against plant pathogens including in safeguarding food security.

All plants produce chemical compounds as part of their normal metabolic activities. These chemical compounds are called phytochemicals and they primarily metabolites such as sugars and fats, which are found all plants, serving a more specific function and secondary metabolite include phenol, saponin, flavonoids etc. many of these phytochemicals can be used effectively as antifungal agents (Kim *et al.*, 2003). It has been pointed out that such products from higher plants are relatively broad spectrum. They are bio-efficacies, economical and environmentally safe and can be ideal for use as agro-chemicals (Jayaprakash *et al.*, 2001). According to Okigbo and Ogbonaya (2006), the active principles in plants are influenced by many factors, which include the age of the plant, extracting solvent, method of extraction and time of harvesting plant materials. Many secondary metabolites which are produced and stored up in the plant had been reported to be effective in the control of plant diseases.

The high cost of synthetic chemicals is very predominant for the control of disease, apart from this it also has hazardous effect on the environment and are also toxic to man and animal. The negative effects of this chemical lead to the search for an alternative means which is easily feasible, degradable and safe to man and the environment. This research work is therefore targeted at determine the

antifungal activities of *Curcuma lonja*, *Piper nigrum* and *Murraya koenigii* on the fungal pathogen of sweet potato.

## Materials and Method

### Source of Collection

Sweet potato tubers with symptoms of deterioration were purchased from Bodija and Sango markets in Ibadan, Oyo State, Nigeria. The samples were collected separately in sterile polythene bags and labelled appropriately. They were then brought to the laboratory for further study.

### Isolation of Fungi

Disease tubers were washed under running tap and surface sterilized with 70% ethanol for one minute to remove surface contaminants. It was then rinsed in three changes sterile distilled water and then blotted dry with sterile filter paper. The infected part was cut into a small fragment and plated into Potato Dextrose Agar (PDA) in petri dishes. The plates were incubated at 28-2°C for 3-5 days and were examined daily. Mixed cultures were subcultured until pure cultures of the isolates were obtained and maintained on PDA slants McCartney bottles. Fungal isolates were identified based on growth pattern colour of mycelia and microscopic examinations of vegetative and reproductive structures of the fungus (Odebode and Unachukwu, 1997; Barnett and Hunter. (1998).

### Pathogenicity Test

All fungal isolates were tested for their ability to induce rot in healthy sweet potato tuber. The modified method of Narayan (2021) was adopted. Healthy sweet potato were surface sterilized using 75% ethanol for 60 seconds and rinsed in three changes of sterile distilled water. A hole of 5mm was aseptically bored into each tuber using 5mm diameter Cork borer and then core was carefully removed. Mycelium plug of 5days old culture was cut from the culture growing, part with the aid of 3mm Cork borer and inserted into the hole in the sweet potato tubers, the cork was then replaced. The wound was sealed with petroleum jelly. Control sample was treated in the same manner with inoculated PDA. The treated samples and control were placed individually in sterile polythene bags and incubated at 28-2°C for 11 days at 2 days intervals, the sample were sterilized through the site by inoculation and examined for lesion development. Infected portions were aseptically transferred onto Potato Dextrose Agar to confirm that the infection was caused by the inoculants.

### Severity Test

All fungal cultured were tested for their ability to induce rot in healthy sweet potato tuber. The modified method of Narayan *et al.* (2021) was adopted. Healthy sweet potato tuber were surface sterilized using 70% ethanol for 60 seconds and rinsed in three changes of sterile distilled water. A hole of 5mm was aseptically bored into each tuber using 5mm diameter cork borer. The core was carefully removed. Mycelium plog of 5days culture was cut from the growing part with the aid of 3mm diameter Cork borer and inserted into the hole in the sweet potato tuber. The core was then replaced. The wound was sealed with petroleum jelly. Control sample were treated in the same manner with inoculated PDA. The treated samples and control were placed individually in sterile polyethylene bags and

incubated at 28-2°C for 11 days. At 2 days intervals, the samples were examined for lesion development.

### Plant Materials Collection

The Tumeric (*Curcuma longa*), Spreng leaf (*Murraya koenigii*) and Black pepper (*Piper nigrum*) seed/fruits were purchased from Bodija market, Ibadan, Oyo State. The spices were soaked in 70% ethanol for 30seconds for surface disinfection and rinsed in three changes of sterile distilled water and air dried in a pathogen free environment. The spices were milled into coarse powder using sterile mortar and pestle with a sterile blender. The powder was kept in an air-tight container for further uses.

### Extraction Procedure

The modified method of Oluduro, (2012) was used for extracting the active principle of the spices. Methanol and aqueous extracts of ground fruits of black pepper and leaves of curry were prepared by weighing 100g of each fruit and leaves into 250ml of methanol and distilled water in separate beakers. The mixtures were covered and stirred every 24 hour using a sterile glass rod and allowed to stand for three days for aqueous extraction and five days for methanolic extraction. The mixture was filtered 3 times using sterile muslin cloth. The concentrations used were 750mg/ml, 500mg/mL and 100mg/mL

### Effect of the extract on the Radial Growth of the Fungal Pathogens

The method employed was Odebode *et al.* (2006), the medium was prepared by dissolving 39.5g of PDA in one litre (1l) of distilled water and sterilized at 121°C in an autoclave for 15minutes. Aseptically 1ml of each plant extract at different concentration was pipetted into sterilized petri dish and overlaid with 10ml of molten potato dextrose agar and mixed thoroughly. Using a 3mm cork-borer, an agar block containing a 5-days old fungal culture was introduced into the freshly prepared medium. The culture plates were incubated at 28-2 C in an incubator the diameter were replicated 3times. Sterile aqueous water and methanol were both used as control in the determination of radial growth of the fungal pathogen.

### Antifungal Screening

The antifungal activities of the plant extracts were determined by Radial Growth Techniques described by Alum and Umeh, (2020). For the purpose of assessing antifungal efficacy of plant extracts, 125, 250, 500 and 750 mg/ml concentrations of each plant extract were assayed. A four equidistant section was created on each Petri dish by drawing two perpendicular lines at the reverse bottom of the plate, the point of intersection indicating the centre of the plate. An aliquot of 1 ml each of the extracts was separately introduced into the Petri dish containing 9ml PDA, carefully rotated to ensure even distribution of extract and allowed to set. A 4-mm cork borer was used to inoculate a disc of a 5 day old pathogen culture on the medium containing extract just at the point of intersection of the two previously drawn lines at the bottom of the Petri dish in three replicates. Inhibitory effect of the extract was expressed as percentage inhibition and calculated using the formula:

$$\% \text{ Mycelial Inhibition} = (XC - YT) \times 100 / XC$$

Where: XC = Average diameter of control YT = Average diameter of fungal colony with treatment

All the data obtained in this study were analyzed using Statistical Analysis System (SAS version 9.1) software and subjected to the analysis of variance, while means were separated at 5% confidence interval, using Duncan multiple range test (DMRT).

## Results

### Data Analysis

**Table 1: Pathogenic effect of fungi associated with spoilage of sweet potato (cm)**

Pathogenic	Day 3	Day 5	Day 7	Day 9	Day 11
<i>A. niger</i>	5.64 <sup>a</sup>	9.70 <sup>a</sup>	12.20 <sup>a</sup>	14.12 <sup>a</sup>	16.43 <sup>a</sup>
<i>A. flavus</i>	1.12 <sup>b</sup>	1.33 <sup>b</sup>	3.17 <sup>b</sup>	4.3 <sup>b</sup>	5.60 <sup>b</sup>
Control	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	1.10 <sup>c</sup>	2.17 <sup>c</sup>

Means with different letters are significantly ( $p < 0.05$ ) different across each column

**Table 2: Biocontrol Effect of Extracts and Extraction Solvents on the Mycelia Inhibition of the Pathogens (cm)**

Parameters	Variables	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
<b>Extracts</b>	Tumeric	0.62 <sup>a</sup>	0.80 <sup>a</sup>	0.96 <sup>a</sup>	1.09 <sup>a</sup>	1.33 <sup>a</sup>	1.43 <sup>a</sup>	1.47 <sup>a</sup>	1.58 <sup>a</sup>	1.65 <sup>a</sup>
	Curry leaf	0.48 <sup>a</sup>	0.66 <sup>b</sup>	0.84 <sup>a</sup>	0.92 <sup>b</sup>	1.23 <sup>a</sup>	1.42 <sup>a</sup>	1.46 <sup>a</sup>	1.54 <sup>a</sup>	1.64 <sup>a</sup>
	Black pepper	0.48 <sup>a</sup>	0.83 <sup>a</sup>	0.94 <sup>a</sup>	1.09 <sup>a</sup>	1.22 <sup>a</sup>	1.31 <sup>a</sup>	1.37 <sup>a</sup>	1.44 <sup>a</sup>	1.53 <sup>a</sup>
<b>Solvents</b>	Methanol	0.32 <sup>b</sup>	0.52 <sup>b</sup>	0.65 <sup>b</sup>	0.76 <sup>b</sup>	0.97 <sup>b</sup>	1.09 <sup>b</sup>	1.14 <sup>b</sup>	1.24 <sup>b</sup>	1.31 <sup>b</sup>
	Water	0.87 <sup>a</sup>	1.17 <sup>a</sup>	1.36 <sup>a</sup>	1.49 <sup>a</sup>	1.71 <sup>a</sup>	1.80 <sup>a</sup>	1.86 <sup>a</sup>	1.94 <sup>a</sup>	2.03 <sup>a</sup>
	LSD	0.11	0.11	0.12	0.12	0.14	0.14	0.15	0.15	0.15

Means with different letters are significantly ( $p < 0.05$ ) different across each column  
Where LSD is the lowest significant different.

**Table 3: Effect of Tumeric Extract Concentration on the mycelia inhibition of the pathogens**

Concentration (mg/ml)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0.73 <sup>a</sup>	0.93 <sup>a</sup>	1.02 <sup>a</sup>	1.07 <sup>a</sup>	1.42 <sup>a</sup>	1.27 <sup>a</sup>	1.35 <sup>a</sup>	1.46 <sup>a</sup>	1.58 <sup>a</sup>
125	0.61 <sup>ab</sup>	0.86 <sup>ab</sup>	1.04 <sup>a</sup>	1.14 <sup>a</sup>	1.21 <sup>ab</sup>	1.50 <sup>a</sup>	1.54 <sup>a</sup>	1.60 <sup>a</sup>	1.66 <sup>a</sup>
250	0.46 <sup>bc</sup>	0.75 <sup>ab</sup>	0.86 <sup>a</sup>	0.99 <sup>a</sup>	1.22 <sup>ab</sup>	1.42 <sup>a</sup>	1.46 <sup>a</sup>	1.50 <sup>a</sup>	1.59 <sup>a</sup>
500	0.48 <sup>bc</sup>	0.67 <sup>b</sup>	0.90 <sup>a</sup>	1.07 <sup>a</sup>	1.32 <sup>ab</sup>	1.42 <sup>a</sup>	1.49 <sup>a</sup>	1.56 <sup>a</sup>	1.63 <sup>a</sup>
750	0.41 <sup>c</sup>	0.70 <sup>b</sup>	0.84 <sup>a</sup>	1.01 <sup>a</sup>	1.15 <sup>b</sup>	1.27 <sup>a</sup>	1.32 <sup>a</sup>	1.46 <sup>a</sup>	1.54 <sup>a</sup>

Means with different letters are significantly ( $p < 0.05$ ) different across each column

**Table 4: Effect of Curry Leaf Extract Concentration on the mycelia inhibition of the pathogens**

Concentration (mg/ml)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0.73 <sup>a</sup>	0.93 <sup>a</sup>	1.02 <sup>a</sup>	1.07 <sup>a</sup>	1.42 <sup>a</sup>	1.27 <sup>a</sup>	1.35 <sup>a</sup>	1.46 <sup>a</sup>	1.16 <sup>a</sup>
125	0.51 <sup>ab</sup>	0.68 <sup>ab</sup>	0.96 <sup>a</sup>	1.09 <sup>a</sup>	1.01 <sup>ab</sup>	1.20 <sup>a</sup>	1.14 <sup>a</sup>	1.32 <sup>a</sup>	1.22 <sup>a</sup>
250	0.42 <sup>bc</sup>	0.71 <sup>a</sup>	0.77 <sup>a</sup>	0.86 <sup>ab</sup>	1.12 <sup>ab</sup>	1.22 <sup>a</sup>	1.36 <sup>a</sup>	1.20 <sup>a</sup>	1.29 <sup>a</sup>
500	0.46 <sup>bc</sup>	0.67 <sup>b</sup>	0.82 <sup>a</sup>	1.04 <sup>a</sup>	1.21 <sup>ab</sup>	1.12 <sup>a</sup>	1.29 <sup>a</sup>	1.26 <sup>a</sup>	1.33 <sup>a</sup>
750	0.39 <sup>c</sup>	0.69 <sup>b</sup>	0.76 <sup>a</sup>	1.00 <sup>a</sup>	1.03 <sup>b</sup>	1.07 <sup>a</sup>	1.12 <sup>a</sup>	1.22 <sup>a</sup>	1.12 <sup>a</sup>

Means with different letters are significantly ( $p < 0.05$ ) different across each column

**Table 5: Effect of Black Pepper Extract Concentration on the mycelia inhibition of the pathogens**

Concentration (mg/ml)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0.73 <sup>a</sup>	0.93 <sup>a</sup>	1.02 <sup>a</sup>	1.07 <sup>a</sup>	1.42 <sup>a</sup>	1.27 <sup>a</sup>	1.35 <sup>a</sup>	1.46 <sup>a</sup>	1.58 <sup>a</sup>
125	0.41 <sup>ab</sup>	0.28 <sup>ab</sup>	1.02 <sup>a</sup>	1.11 <sup>a</sup>	1.01 <sup>ab</sup>	1.50 <sup>a</sup>	1.54 <sup>a</sup>	1.10 <sup>a</sup>	1.33 <sup>a</sup>
250	0.36 <sup>bc</sup>	0.55 <sup>ab</sup>	0.78 <sup>a</sup>	0.69 <sup>a</sup>	1.02 <sup>ab</sup>	1.33 <sup>a</sup>	1.46 <sup>a</sup>	1.20 <sup>a</sup>	1.39 <sup>a</sup>
500	0.38 <sup>bc</sup>	0.46 <sup>b</sup>	0.88 <sup>a</sup>	1.01 <sup>a</sup>	1.02 <sup>ab</sup>	1.32 <sup>a</sup>	1.49 <sup>a</sup>	1.26 <sup>a</sup>	1.23 <sup>a</sup>
750	0.31 <sup>c</sup>	0.56 <sup>b</sup>	0.64 <sup>a</sup>	1.01 <sup>a</sup>	1.12 <sup>b</sup>	1.22 <sup>a</sup>	1.32 <sup>a</sup>	1.26 <sup>a</sup>	1.44 <sup>a</sup>

The results show that the two pathogens steadily infect and spread gradually on the sweet potatoes as the number of the day increase after the inoculation (Table 1). The ability of the plant extracts to inhibit the spread of the fungi, as shown in table 2, revealed the gradual increment in the reduction of the spread of pathogens as incubation days increased. Tables 3, 4 and 5 depict that different concentrations of the plant extracts have varied levels of significant effects on the pathogens.

### Discussion

A vast percentage of plant produce are lost to invasion by microbial pathogens; including bacteria and fungi. Several workers had reported involvement these pathogens especially fungi in destruction of plantain, banana, Tomatoes. Many researchers have described fungal pathogens of pepper and potatoes that have become important disease-causing agents Akinyele *et al.*, 2020; Alum and Umeh, 2020). Narayan *et al.* (2021) had also observed the involvement fungi including *Aspergillus* sp in the spoilage of potato.

Over the years, the control of sweet potato plant disease was significantly managed by the use of fungicide, pesticide, and bactericide; but these chemicals leave residues on the treated plants, causing harmful effects on the treated plants (Okigbo and Nmeka, 2005).

*Aspergillus niger* and *Aspergillus flavus*, are microbes associated with sweet potato as reported by Ijato *et al.* (2011). Antifungal effectiveness of some tropical plants extracts in controlling several diseases had been reported (Agbolade *et al.*, 2020; Rani *et al.*, 2013).

This study showed that extracts of the test plants capable of reducing fungal load on agriculture products. Methanol and aqueous extracts of the test plants of *Curcuma longa*, *Piper nigrum* and *Murraya koenigii* were able to suppress the radial growth of the fungi mycelia. Their ability to reduce the growth of the fungal pathogens might not be unconnected with the phytochemicals contained in them (Igara *et al.*, 2016; Taoheed *et al.*, 2017; Mariselviand Manimegalai, 2017;). Ijato *et al.*, (2011) also carried out a similar test on water and ethanol extracts of *Azadirachta indica* and *Chromolaena odorata* against fungi pathogen of

rotten watermelon fruits and concluded that it has promising antifungal activity against the fungi isolated.

Adetunji *et al.* (2013) investigated the antimicrobial activity of ethanol extracts of *Vermonia amygdalina* and *Croton macrostachyus* against bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Salmonella typhi*, *Escherichia coli*), and two fungal pathogenic species (i.e. *Aspergillus flavus* and *Aspergillus niger*) were also studied. Similar studies were also conducted and the results revealed that all the extracts of the shrub had inhibitory effect on the tested organism (Selam and Sibhatu (2019). Investigation on antifungal properties of *Acalypha wilkesiana*, *Chromolaena odorata* and *carica papaya* was carried out. (Pedro *et al.* 2011; Katibi *et al.*, 2021). The author reported that the plant extracts possess some inhibitory components which caused significant reduction in mycelia growth of the fungi. This agrees with result of Olabiyi *et al.* (2022) who reported the efficacy of extracts from *Acalypha wilkesiana*, *Chromolaena odorata* and *carica papaya* among other extracts in reducing the mycelia growth of *Fusarium lateritium*. These confirmed the antimicrobial properties of *Acalypha wilkesiana* as reported in this study.

The strong inhibition potential of Ginger is attributed to the fact that it contains over 400 different compounds. A mixture of both volatile and non-volatile chemical constituents such as Zingerone, S'hogaols and gingerols, sesquiterpenoids ( Kizhakkayil 2012; Moon *et al.* 2018). These several chemical constituents increases its antimicrobial effectiveness.

The increase in concentrations of the plant extracts also increased the reduction in fungal growth significantly. This has been ascribed to the increase in the concentrations of phytochemical in the higher concentrations of the plant extracts. The higher the dilution the lower the inhibitory potentials. The results in this work is in conformity with observations of earlier reports (Amionye and Ataga, 2007; Alhussaen *et al.*, 2011).

### Conclusion

Spoilage of fruits is on the increase due to pathogenic microbes particularly fungi and this is causing serious economic loss especially to farmers. This work investigated the destruction of potato fruits and how the spoilage can be mitigated using plant materials. All the spices significantly inhibited the pathogen and therefore can be applied in their control.

### Conflict of Interest

The authors declared that there is no conflict of interests.

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