

Evaluation of efficacy of false yam (*Ipomoea pes-caprae*) as surface protectant against rot pathogens of white yam (*Dioscorea rotundata* Poir)

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ABSTRACT

Rots in yam tubers are the dominant cause of postharvest losses. Therefore, studies were conducted to identify the rot-causing organisms in yam and to evaluate the efficacy of false yam (*Ipomoea pes-caprae*) plant extracts as surface protectants in the storage of yam. The experiment was laid out in a completely randomized design (CRD) design with five treatments (fruit, root and leaf extract of false yam, Mancozeb and tap water) replicated three times. Two concentrations (50% and 100%) of each extract were also tested. Species were identified based on the structural features, the characteristics and properties of the spore and mycelium. A spore suspension of yam rot fungi was sprayed on healthy yam tubers that had been pretreated with the extracts. Results showed that fungi isolated from rotted yams were *Aspergillus niger*, *A. flavus* and *Penicillium sclerotigenum*. Leaf extracts (both 50% and 100%) had the highest growth inhibitions on all the three fungi isolated in vitro. Tubers treated with root and leaf extracts of false yam had a decreased tuber rot lengths of 1.80 mm² and 2.17 mm², respectively. The leaves and roots of false yam can be used as a surface protectant of yam in place of Mancozeb.

Keywords: *Ipomoea pes-caprae*; *Dioscorea rotundata*; Mancozeb; Pathogenicity; *Aspergillus* spp.
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Introduction

Yam (*Dioscorea* spp.) is one of the most important dietary sources of energy and a basic foodstuff in the tropical and humid regions of Africa, South America, India and South-East Asia (Achi, 2000; Adegbite *et al.*, 2006; Babajide *et al.*, 2007; Oluwole *et al.*, 2013). There are many varieties of yam species but white yam (*Dioscorea rotundata*) and water yam (*Dioscorea alata*) are the widely grown species in Ghana (Aboagye *et al.*, 2015) and thus

are economically important species. Ghana produces 25% of yam transacted on the global market (SRID, 2011) and ranks third after Nigeria and Ivory Coast and contributes 17% of Agricultural Gross Domestic Product (AGDP) (FAO, 2009). Yam is also considered the food of choice on many occasions and festivals. It is an indispensable part of the bride price in the Northern Regions of Ghana and part of Nigeria (Hahn *et al.*, 1987). It has become a source of foreign exchange for some countries in the yam

belt. Thus the economic importance of yam and its role in ensuring food security in Ghana cannot be overemphasized.

Yam production and marketing is faced with several problems. Diseases contribute greatly to high yield losses on the field and in storage. Yam plants are very susceptible to fungi, bacterial and viral infections on the field and in storage which result in the incidence of rots. Rots in yam tubers are the dominant cause of postharvest losses among other factors (Aidoo, 2007). About 60% of white yam varieties get rotten when stored for less than six months in Nigeria (Adesiyani & Odihirin, 1975). Most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus*, *A. niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *F. solani*, *Penicillium chrysogenum*, *P. oxalicum*, *Rhizoctonia* spp. and *Rhizopus nodosus* (Okigbo, 2004; Aidoo, 2007; Asare-Bediako et al., 2007).

Synthetic chemicals such as sodium orthophenylphenate, borax, captan, thiabendazole, benomyl, bleach (sodium hypochlorite) have been found to significantly reduce storage rots in yam (Noon, 1978). Other control methods involve the use of microorganism such as *Trichoderma viride* and *Bacillus subtilis* (Okigbo & Ikediugwu, 2000). However, the financial cost involved in applying these methods and the dangers associated with chemical usage has limited farmers adopting these innovations in developing economies such as Ghana. The lack of expertise in the safe handling of chemicals among most farmers is another challenge. There is therefore the need to discover environmentally and economically friendly control methods to help address the problems of farmers and consumers. The use of botanical extracts has been reported to be very effective in controlling diseases due to the anti-bacterial

and anti-fungal properties they possess. Botanicals are locally available (cheap), have little or no toxicity and have simple preparation procedures. There are several local plant species whose extracts have proved effective in protecting yam produce before and after harvest. Neem plant (*Azadirachta indica*), black pepper (*Piper nigrum*), ginger plant (*Zingiber officinale*), *Jatropha curcas*, false yam (*Icacuna oliviformis*) are examples of numerous plants that can be used as surface protectants on yam tuber (Amusa et al., 2003; Saetae & Suntornsuk, 2010; Sowley et al., 2013). Some yam species such as *D. piscatorum* have toxic properties that allow them to be used in the production of insecticides. An insecticide from *D. piscatorum* is used in controlling insect pests of rice in Malaysia (Ooi & Shepard, 1994). Extracts from *D. deltoidea* is used in the production of anti-lice shampoo in India (Coursey, 1967). False yam contains bitter toxic compounds, *Icacinon* and *Icacinols* which prevent pests and diseases causing organisms from feeding. As a result of these properties, the plant has not been a host to pests and diseases causing organisms (Vanhaelen et al., 1987). The plant usually grows in the wild and is seldom cultivated. False yam has several uses. The efficacy of *Icacina senegalensis* leaf and tuber extracts for the management of sweet potato beetle (*Cylas* spp.) and cowpea aphids (*Aphis cracivora*) have been reported (Alale et al., 2017).

False yam is readily available in the Northern parts of Ghana. Therefore, the objectives of this study were to isolate and identify the pathogens associated with rots of stored white yam tubers and evaluate the efficacy of false yam extracts as surface protectants in stored white yam tubers *in vitro* and *in vivo*.

Materials and methods

Study site

The *in vitro* experiment was conducted at the Spanish Laboratory of the University for Development Studies, Nyankpala campus. The area experiences a unimodal rainfall pattern with 1022 mm being an annual average. The *in vivo* experiment was conducted in a modern yam barn located at the 'Farming for The Future' of the University for Development Studies, Nyankpala campus. The campus is situated at latitude 9°25'41"N and longitude 0°58'42"W and 200 m above sea level.

Experimental design

The experiment was laid out in a completely randomized design (CRD) design with five treatments (involving three extracts and two controls) replicated three times.

Isolation and identification of fungal pathogens from rotten yam tuber

The rotten tubers collected from the Tamale central market were washed under running tap water and allowed to dry under laboratory conditions. Pieces of diseased tissues cut from the periphery of rotten yam tubers with a sterilized knife were rinsed in sterilized water, surface sterilized with 70% ethanol. Three pieces (3 mm diameter) of the infected tissues were picked with flamed sterilized forceps and dried in a sterile Lamina flow chamber. The dried disease tissues were plated on the solidified Potato Dextrose Agar (PDA) supplemented with streptomycin sulfate (PDA: 200 g potato, 20 g sucrose, 18 g agar, 0.125 g streptomycin sulphate L⁻¹ and 1,000 ml distilled water, pH 7.0) in Petri dishes. The inoculated plates were incubated at room temperature (28°C) and observations were made daily for the emergence of colonies. Mycelia that emerged from the plated

yam tissues were sub-cultured onto fresh PDA. Species were identified based on the structural features, the characteristics and properties of the spore and mycelium when studied under the compound microscope. These morphological characteristics were used in identifying the fungal organisms to the species level, as described by Barnett and Hunter (1998). Cultural characteristics such as conidial masses, colony growth and colour were observed on PDA at 26 ± 2°C; 10 replicates were prepared for each isolate. The mean colony growth for each isolate from 48 to 168 hours (i.e. 2 – 7 days) was calculated. The colour of each colony and conidial masses were also recorded on the seventh day. The description and naming of the *Colletotrichum* species were done according to Chaube and Pundhir (2005); IMI (1995); Barnett and Hunter (1998).

Pathogenicity test

The inocula used for the pathogenicity tests were obtained from the different fungal species. Healthy white yam tubers were washed with tap water and distilled water and thereafter sterilized with 70% ethanol. Cylindrical discs (1 cm thick) were removed from the tuber with a five-millimeter diameter cork borer (sterilized by dipping in alcohol followed by flaming). The sterile cork borer was used to cut plugs from the one-week-old cultures of the fungal isolates to be tested. These fungal plugs were then placed in the holes created in the yam tubers after which the removed yam tuber discs were used to plug the holes. Candle wax was applied at the point of inoculation to seal the edges of the replaced yam discs. This was done for all the three species (*A. niger*, *A. flavus* and *Penicillium sclerotigenum*) obtained in pure cultures. A control was set up in which the sterile cork borer was used to remove a disc

of the tuber tissue. The tuber disc was used to plug the hole and its edges sealed with candle wax. In the control, no fungal organism was placed in the hole. The tubers were incubated for seven days at 28°C. These activities were carried out inside a sterile environment (Aidoo, 2007).

Preparation of plant extracts and Mancozeb

The method of Okigbo and Ogbonnaya (2006) was used in the preparation of the plant extracts with some modifications. Fresh fruits, leaves and roots of false yam were washed thoroughly under running tap water. The fruits and the roots were cut into small chunks separately. The plant parts were further homogenized into a paste separately with a blender for five minutes. A cold water extract of the ground fruits, leaves and roots were separately prepared by adding 50 g of each plant extract to 100 ml of cold sterile water in a 500 ml beaker. It was vigorously stirred and allowed 1 hour for settling, and then filtered through folds of sterile cheesecloth (Okigbo & Ogbonnaya, 2006) to obtain a stock solution (100%). Fifty percent (50%) concentration of each plant part extract was prepared from the stock. Two concentrations (50%, 100%) of each extract were used as treatments. The efficacy of each of the extracts was tested for their fungicidal activity against yam tuber rot fungi.

Mancozeb WP was prepared by adding 10 g of Mancozeb WP to 100 ml of distilled water. The mixture was stirred thoroughly and used as a positive control. Tap water was also used as a negative control.

Effect of the plant extract in vitro on growth of yam rot fungi

The method of Amadioha and Obi (1999) was used to determine the effect of the extracts on

fungal growth. This involved creating four equal sections on each Petri dish by drawing two perpendicular lines at the bottom of the Petri dish, the point of intersection indicating the center of the plate. This was done before dispensing PDA into each of the plates. Four milliliters (4 ml) from each of the two concentrations (50%, 100%) of each extract was dispensed into 9 cm diameter Petri dishes after which 20 ml of melted PDA was added, mixed gently and allowed to solidify (Okigbo & Ogbonnaya, 2006). A disc (5 mm diameter) of the pure culture of the isolates were placed on the plate, just at the point of intersection of the two lines drawn at the bottom of the Petri dish and incubated at room temperature. Control experiments were set up without the addition of any plant extract. Positive control was set up by adding a broad spectrum fungicide (Mancozeb) that has been established to control rot fungi. Treatments were replicated three times.

Measurement of growth as diameter of a growing fungal colony was undertaken at intervals of twenty-four hours using a ruler. Fungitoxicity was recorded in terms of percentage colony inhibition by the extracts and calculated according to the formula of Amadioha & Obi (1999). % Growth inhibition = $[(DC-DT)/DC] \times 100$ Where DC = average diameter of control, and DT = average diameter of fungal colony with treatment.

Activity of extracts in vivo

Storage design

A modern barn made of concrete floor with a cement wall about 30 inches high and woods nailed together increased the wall to about 7 feet high. The barn was roofed with aluminum sheets and wire mesh was used to seal the ventilation windows to prevent rodent attack. The

experiment was laid in a randomized complete block design (RCBD) with five treatments (fruit, root and leaf extract of false yam, Mancozeb and tap water) replicated three times.

30 healthy tubers of one cultivar (Nyumanle) of white yam were used in the storage experiment. Each shelf contained ten tubers. Two tubers in each shelf belonged to one treatment. Two tubers in each shelf were dipped into each of the treatments separately for 50 seconds prior to storage. Treated tubers were adequately labeled in each shelf.

Inoculum application

A spore suspension of yam tuber rot fungi was prepared by adding 4 ml of water to a plate containing sporulated fungi. The mycelia were scraped off and the mixture filtered through a cotton cloth. To get all the mycelia, the procedure was repeated for the same plate. This was done for 10 plates. Okigbo & Ogbonnaya, (2006) method of inoculation was used with some modification. The treated tubers were air-dried for 24 hours before spore suspension of 6×10^4 spores per ml of distilled water of each pathogen was sprayed on them using a spray gun. All the treated tubers were incubated under polyethylene sheets in the barn for 15 days. This was done to obtain optimum humidity conducive for spore germination and mycelia growth.

Effectiveness of extracts as surface protectants of yam tubers against rot pathogens

After 15 days, the spore had germinated and grown considerably into the yam tissues through the natural openings on the yam. The tubers were bisected and the area of rot for each tuber was measured by multiplying the length of rot by the breadth of rot. Measurements of tu-

bers treated alike were calculated and an average was found.

Data analysis

Data collected were subjected to one-way Analysis of Variance (ANOVA) with Genstat (18th edition). The least significant difference (LSD) test was used to separate the treatment means at 5% significance level. Data on means area of rot yam were subjected to $\log_{10}(x+1)$ transformation.

Results

Occurrence of fungal isolate from rotten yam

Three fungal species were isolated from the rotten yam tubers. These were *Aspergillus niger*, *A. flavus* and *Penicillium sclerotigenum* and their percentage occurrences were 48%, 19% and 33% respectively (Table 1).

TABLE 1
Occurrence of Fungal isolate from rotten yam.

<i>Isolates</i>	<i>Frequency</i>	<i>Percentage (%)</i>
<i>Aspergillus niger</i>	26	48
<i>Penicillium sclerotigenum</i>	18	33
<i>Aspergillus flavus</i>	10	19
Total	54	100

Effects of extracts on growth of rot fungi in vitro

Leaves extract (50% and 100%) had the highest growth inhibition in all isolates. At 100% leaf concentration, growth inhibition on *A. flavus* was 61.63%, *A. niger* was 65.8% and *P. sclerotigenum* was 64.4% compared to 50% leaf concentration where growth inhibition on *A. flavus* was 54.66%, *A. niger* was 59.1% and *P. sclerotigenum* was 60.0%. The root extracts at 100% had higher growth inhibition

than at 50%. Growth inhibition at 50% root extract were *A. flavus*, 45.78%, *A. niger*, 51.7% and *P. sclerotigenum*, 55.3% while at 100% root extract, growth inhibition on *A. flavus* was 55.09%, *A. niger* was 58.0% and *P. sclerotigenum* was 58.7%. Growth inhibition on *P. sclerotigenum* by fruit extract at 50% was 42.1% and at 100%, 52.4%. Growth inhibition

on *A. flavus* by fruit extract at 50% was 54.49% and at 100%, 50.25%. The fruit extract of the false yam against *A. niger* had the lowest growth inhibition with 3.2% at 50% fruit extract and 0.4% at 100% fruit extract (Table 2). Mancozeb had 100% growth inhibition on all the fungi.

TABLE 2
Effects of extracts on growth of rot fungi in vitro

Treatments	% Growth Inhibition					
	<i>A. flavus</i>		<i>A. niger</i>		<i>Penicillium sp.</i>	
	50%	100%	50%	100%	50%	100%
Fruit extracts	54.50	50.20	3.20	0.40	42.10	52.40
Root extracts	45.80	55.10	51.70	58.00	55.30	58.70
Leaf extracts	54.70	61.60	59.10	65.80	60.00	64.40
Mancozeb	100.0	100.0	100.0	100.0	100.0	100.0
Tap water	0.00	0.00	0.00	0.00	0.00	0.00
LSD (0.05)	6.45	7.41	5.91	3.97	3.89	5.31
CV (%)	2.40	2.00	1.90	3.00	3.60	4.50

Effects of extracts as surface protectants of yam tubers against rot pathogens

The effect of plant extracts of false yam on tuber rot length of white yam is shown in Table 3. The mean tuber rot length varied significantly between tubers treated with each extract ($P < 0.05$). Plants treated with root and leaf extracts had significantly fewer tuber rot compared to control plants treated without these plant extracts.

TABLE 3
Effects of treatments as surface protectants of yam tubers against rot pathogens

Treatment	Means of area of rot ^a (mm ²).	
	Untransformed mean	Transformed mean
Fruit extract	2119.00	3.33
Root extract	63.00	1.80
Leaf extract	147.00	2.17
Mancozeb	94.00	1.97
Tap water (Control)	3350	3.53
LSD (0.5)	168.90	0.28
CV (%)	7.80	1.80

a: $\text{Log}_{10}(x+1)$ transformed, where x = area of rot yam

Discussion

Three fungi namely *Aspergillus niger*, *A. flavus* and *Penicillium sclerotigenum* were isolated from the rotten yam tubers. The pathogenicity test revealed that *A. niger*, *A. flavus* and *Penicillium sclerotigenum* caused rot and confirm reports by other studies that these fungi are associated with rot in yam tubers by a number of workers (Ogundana *et al.*, 1970; Ikotun, 1989; IITA, 1993; Okigbo, 2004; Asare-Bediako *et al.*, 2007). The tubers inoculated with *Penicillium sclerotigenum* turned brown, hard and dry. *A. niger*-inoculated tubers turned brown with yellowish margin. This agrees with IITA (1993)'s report that *Penicillium* spp. and *A. niger* causes dry rot of yam. Tubers inoculated with *A. flavus* turned brown and soft. This also agrees with Ikotun (1989) who reported that tubers infected by soft rot organisms often turned brown, soft and became wet due to rapid collapse of cell walls of tissues.

There was 100% growth inhibition by Mancozeb on all the fungi studied. Harlapur *et al.* (2007) also reported that Mancozeb was found to be most effective in inhibiting the mycelial growth of *Exserohilum turcicum*. The leaf extracts at 100% concentrations had the highest growth inhibition followed by the root extracts, then the fruit extracts at the same concentrations. This implies that there were probably higher concentrations of some antifungal active ingredients in the leaves followed by the roots with the fruit having the least concentration.

False yam fruits extracts poorly inhibited the mycelia growth of *A. niger* as opposed to the high efficacy of the leaf and the root extracts of the plant on the fungi. This could be due to the presence of these same antifungal active ingredients in the leaf and root extracts which may be limited or absent in the false yam

fruit extract. False yam contains *Icacinon* and *Icacinol* compounds (Alale *et al.*, 2017) which are responsible for its fungal toxicity.

A general increase in growth inhibition of fungal activity as the concentration of the root and leaf extracts increased from 50% to 100% confirms conclusions by Kalimuthu *et al.* (2013) that the inhibitory activity of plant extract is largely dependent on the concentration, part of the plant used and the microbes tested.

Results obtained from the application of extracts on yam tubers indicate that the fruit extract could not protect the yam tubers against rot-causing organisms. In most cases, microorganisms gain access into yams through natural opening and wounds that occur during harvesting and transportation from field to storage barns (Ogundana *et al.*, 1970). The spore suspension that was directly inoculated on the yam tubers in storage may have gained access into the tubers through the natural openings. Since most of the tubers treated with Mancozeb, root and leaf extracts had a decreased tuber rot lengths, it is likely that they inhibited spore germinating on the yam tubers.

False yam leaf and root extract can be substituted for Mancozeb since they all inhibited spores from germinating into yam tubers. False yam fruit extract was not an effective surface protectant possibly due to the low concentrations or absence of *Icacinon* and *Icacinol* compounds in them.

Conclusion

The study revealed that *Aspergillus niger*, *A. flavus* and *Penicillium sclerotigenum* are pathogenic in yam and cause yam rot. Leaves and roots of false yam can be used as a surface protectant of yam as a cheaper and more environmentally friendly option to Mancozeb.

However, fruits extract of false yam is not toxic to some strain of fungi (*A. niger*). The effectiveness of a botanical extract in controlling fungi depends on the part of the plant used as well as the concentration of the plant extract used.

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