Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.12, 2015

Some Fungal Pathogens of Yam (*Dioscorea* Spp.) In Storage And The Effects Of Their Infection On The Nutrient Composition

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ABSTRACT
The study isolated and identified three fungal pathogens of yam (Dioscorea spp.) in storage and investigated the effects of their infection on the yam nutrient composition. The pathogens were *Aspergillus niger* Van Tiegh, *Botryodiplodia theobromae* Pat and *Sclerotium rolfsii* Sacc. Results showed that the nutrient contents of the two species of yam, *D. rotundata* Poir. And *D. alata* L. were quantitatively altered by *A. niger, B. theobromae* and *S. rolfsii* during pathogenesis. The moisture content of both species significantly ($P < 0.05$) increased, whereas carbohydrate content significantly ($P < 0.05$) decreased in quantity during the period of incubation. The total protein and fat contents of both species were also lowered quantitatively, while the fibre and ash contents were increased by the rot causing organisms. The depletion of carbohydrate, protein and fat contents was more in *D. rotundata* than in *D. alata*. The results obtained suggests that the presence of carbohydrate, protein and fat is of primary importance for the survival and successful establishment of the pathogens within the tissue of yam.
Keywords: Fungal pathogen, nutrient composition, *Dioscorea rotundata Dioscorea alata*.

INTRODUCTION

Yams (*Dioscorea* spp.) are important food crops for millions of people in many tropical and sub-tropical countries in Africa, Caribbean, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Okigbo and Ikediugwu, 2000) and a variety of palatable dishes are prepared from yam tubers (Aluko *et al.,* 2003; Fasasi and Fasina, 2005; Oladebo and Okanlawo, 2010). Yam is essentially a carbohydrate food with low protein and ascorbic acid contents (Igyor *et al*., 2004). Coursey (1983) gave the chemical composition of the edible part of fresh yam tubers water 72.4%, carbohydrate 24.1%, protein 2.4%, fat 0.2%, fibre 0.6%, calcium 22mg/100g, iron 0.8 mg/100g, thiamine 0.09 mg/100g, riboflavin 0.03mg/100g, niacin 0.50 mg/100g and ascorbic acid 10mg/100g.

Several yam species are cultivated. White yam (*D. rotundata* Poir.) water yam (*D. alata* L.) and yellow yam (*D. cayenensis* Lam.) are the most widely cultivated in Nigeria where post harvest losses due to microbial rot is heavy affecting 20 -39.5% of tubers in storage (Okigbo, 1999). Microbial deterioration of stored products have been known to reduce the eating quantity and market value of yam (Ravinduram and Wanasindera, 1992; Amusa *et al.,* 2003), cassava (Ekundayo and Daniel, 1973; Booth, 1976), sweet potato (Arinze *et al*., 1975; Amadioha, 2001) and fruits (Ladele *et al.,* 1984; Amusa *et al.,* 2002).

Studies have shown that fungal rot is the greatest cause of tuber loss in storage (IITA, 1993; Cornelius and Oduro, 1999; Amusa *et al.,* 2003). The principal species of microorganisms associated with yam rot in Nigeria are: *Botryodiplodia theobromae* Pat., *Fusarium oxysporum* Schlencht, *Penicillium oxalicum* Currie and Thom., *Sclerotium rolfsii* Sacc, *Aspergillus niger* Van Tiegh and *A. tamarii* Kita (IITA, 1993; Amusa and Baiyewu, 1999; Amusa *et al.*, 2003; Okigbo, 2005). The present study isolated and identified some fungal pathogens of yam and investigated the effect of their infection on the nutrient composition.

MATERIALS AND METHODS

Source of yams

Two species of yam were used in this study. They were *D. rotundata* Poir. (white yam) and *D. alata* L. (water yam). Healthy yam samples and those with symptoms of post-harvest rot were obtained locally from five different towns: Asaba, Issele-uku, Umunede, Agbor and Kwale in Delta State, Nigeria. They were placed in polythene bags and taken to the laboratory for analysis.

Isolation and identification of fungi associated with the spoilage of yam tubers

Twenty four infected tubers each of *D. rotundata* and *D. alata* from each town were used for this study. The tubers were washed in tap water and cut into $2mm^2$ segments at the junction between healthy and infected portions of the tuber using a sterilized scalpel. The cut tissues were surface sterilized for 2 minutes in 10% sodium hypochlorite (NaOCL) solution to remove surface contaminants and rinsed twice in sterile distilled water (Okigbo *et al.,* 2009).

Four segments of the sterilized tissue pieces were plated out on potato dextrose agar (PDA). The plates were incubated at 28°C for 5 days and observed daily for the development of fungi. Fungi growing from them were transferred into fresh plates of PDA and incubated at 28°C. Several subcultures were made on PDA until pure cultures were obtained. The resulting pure cultures were used for identification of the fungi with the aid of a

compound microscope and identification guide (Barnett and Hunter, 1999).

Pathogenicity test of the isolated fungi

Three fungal isolates (*Botrypdiplodia theobromae, Aspergillus niger and Sclerotium rolfsii*) obtained from the rotted tubers were inoculated into healthy tubers of yam. Twenty fresh healthy yam tubers were washed under running tap water and surface-sterilized in 10% sodium hypochlorite (NaOCL) solution for 2 minutes after which they were rinsed twice in sterile distilled water. Cylindrical discs (5mm diameter) were removed from the tubers (one per tuber) with a sterile cork borer. Mycelial discs (4mm diameter) were made from 5 day-old cultures of the fungi and each fungal disc was put into a hole in each of 5 tubers. The yam discs were replaced and inoculation sites smeared with petroleum jelly. The remaining 5 yam tubers served as the control with each hole receiving a disc of sterile PDA medium and plugged with the yam disc. The inoculated sites were treated as earlier stated. The inoculated tubers were place separately in their groups of 5 in sterile polythene bags containing cotton wool soaked in sterile distilled water. The bags were properly labelled and incubated at 28°C for 14 days. Disease symptoms produced by artificial inoculation after the incubation period were compared with those observed on the naturally infected tubers collected from the towns. The fungi were re-isolated from the inoculated diseased yam tubers and cultured on PDA plates. The morphology of each pathogenic fungus was compared with that of the original culture.

Effects of fungi infection on nutrient composition

The effect of fungi infection on nutrient content was studied on yam tubers artificially inoculated with the post-harvest pathogens isolated from diseased yam tubers and incubated at 28°C for 16 days. The pathogens were *A. niger, B. theobromae* and *S. rolfsii.* The Uninoculated yam tubers served as the control.

Nutrient composition analysis

One hundred grammes (100g) each of peeled and sliced healthy and pathogen-infected tissues of yam were used for the study. The samples were dried in an oven at 85°C, cooled and milled into powder with a hammer mill. The powdered samples (in triplicates) were analysed for moisture, crude protein, crude fibre, fat, ash and carbohydrate using the official method of analysis (AOAC, 2005) employing the Kjeldahl's method for crude protein determination:

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS 2002 and significant means were separated with the Duncan's Multiple Range Tests (DMRT) ($P < 0.05$).

RESULTS

Three fungi were found associated with rotting of the yam tubers. These fungi were *Aspergillus niger* Van Tiegh, *Botryodiplodia theobromae* Pat., and *Sclerotium rolfsii* Sacc. The range of occurrence were between 11.67% (S. *rolfsii*) and 65% (*A. niger*) Table 1. The production of typical symptoms in tubers inoculated separately with the fungi isolated from naturally infected yam tubers and the re-isolation of identical fungi fulfills Koch's postulates and establishes the pathogenicity of the isolates.

Generally, there was a sharp decrease after infection by *B. theobromae, S. rolfsii* and *A. niger* in the quantity of carbohydrate which is the major nutrient in both species of yam. The moisture, fibre and ash contents increased, whereas protein and fat decreased in quantities (Tables 2 and 3). The healthy yam tubers of *D. rotundata* and *D. alata* were found to contain carbohydrate 27.23% and 19.73% and moisture 60% and 65.94% respectively. Total carbohydrate content of tubers of *D. rotundata* infected with the rot-causing pathogens decreased significantly within the range of 19.23 – 22.16%, whereas total carbohydrate of *D. alata* dropped significantly within the range of 13.81 – 15.54% during the 16-day incubation period. The moisture content of *D. rotundata* increased significantly within the range of 66.04 – 68.94%, whereas that of *D. alata* increased significantly within the range of 70.28 – 72.06% after the 16-day period of incubation.

Table 1: Occurrence of fungi associated with yam tuber rot at different locations/town-markets

	Occurrence (%)									
	Dioscorea rotundata					*Dioscorea alata*				
Fungi	**Asaba**	**Issele-Uku**	**Umuneze**	**Abavo**	**Kwale**	**Asaba**	**Issele-Uku**	**Umuneze**	**Abavo**	**Kwale**
A. niger	65.00*	55.83	52.50	55.00	55.83	54.17	54.17	50.00	50.00	58.33
B. theobromae	23.33	32.50	30.00	31.67	29.17	25.00	33.33	37.50	33.33	29.17
S. rolfsii	11.67	11.67	17.50	13.33	15.00	20.83	12.50	12.50	16.67	12.50

Total isolation = 24/location
*Error in approximation = ±0.02%

Table 2: Effects of inoculating *Dioscorea rotundata* (white yam) tubers with *Aspergillus niger*, *Sclerotium rolfsii* and *Botryodiplodia. theobromae* on the nutrient composition 16 days after incubation at 28°C

	Nutrient composition (%)					
Treatment	**Moisture**	**Crude protein**	**Crude fibre**	**Fat**	**Ash**	**Carbohydrate**
Uninoculated (control)	60.00^{b}	5.84^{a}	2.13^{a}	0.49^{a}	4.28^{a}	27.23^{a}
A. niger	66.04^{a}	4.25^{a}	2.40^{a}	0.40^{a}	4.31^{a}	22.61^{b}
S. rolfsii	68.94^{a}	4.10^{a}	3.11^{a}	0.34^{a}	4.29^{a}	19.23^{b}
B. theobromae	67.72^{a}	4.10^{a}	2.97^{a}	0.38^{a}	4.30^{a}	20.54^{b}

Means in the same column with different superscript are significantly different (P < 0.05)

Table 3: Effects of inoculating *Dioscorea alata* (water yam) tubers with *Aspergillus niger*, *Sclerotium rolfsii* and *Botryodiplodia. theobromae* on the nutrient composition 16 days after incubation at 28°C

	Nutrient composition (%)					
Treatment	**Moisture**	**Crude protein**	**Crude fibre**	**Fat**	**Ash**	**Carbohydrate**
Uninoculated (control)	65.94^{b}	6.06^{a}	2.52^{a}	0.71^{a}	5.00^{a}	19.73^{a}
A. niger	70.28^{a}	5.85^{a}	2.64^{a}	0.67^{a}	5.04^{a}	15.54^{b}
S. rolfsii	72.06^{a}	5.71^{a}	2.76^{a}	0.66^{a}	5.02^{a}	13.81^{b}
B. theobromae	71.94^{a}	5.39^{a}	2.85^{a}	0.60^{a}	5.30^{a}	14.19^{b}

Means in the same column with different superscript are significantly different (P < 0.05)

Discussion

The fungi found associated with deteriorating yam tubers in storage were *Aspergillus niger, Botryodiplodia theobromae,* and *Sclerotium rolfsii.* Fungi have been reported to constitute the main spoilage micro organisms associated with post harvest deterioration of plants and food materials (Adeniji, 1996; Amusa, 2001; Amusa *et al.,* 2002; Nweke and Agbogidi, 2008). Micro organisms responsible for post-harvest rot of yam tubers have also been identified (Amusa and Baiyewu, 1999; Okigbo and Ikediugwu, 2000; Khadijat, 2003; Yusuf and Okusanya, 2008; Okigbo *et al.*, 2013). These fungi include the ones that were identified in this work.

Attack by pathogenic fungi on plant products is aimed at absorbing the nutrients available in such stored products for their cellular growth, survival and reproduction (Amadioha, 2001). In the present study, *A. niger, B. theobromae* and *S. rolfsii* utilized the carbohydrate, protein and fat contents of both species of yam, suggesting that the presence of these food substances is of primary importance for the survival and successful establishment of these pathogens within the tissue of yam. Depletion of nutrient contents in potato and yam by other pathogenic fungi have been reported (Ravinduram and Wanasindera, 1992; Amusa, 2001; Amadioha, 2001).

The quantity of carbohydrate in both species decreased during pathogenesis by the pathogens. This might be due to fermentation caused by the microbes and the respiratory loss of sugars as carbon dioxide. This is in conformity with observations made by Ravinduram and Wanasindera (1992) and Amusa *et al.* (2002). The decrease in protein and fat contents of the samples is in agreement with the findings of Amusa (2001) who reported that the percentage crude protein and fat of freshly processed yam chips were significantly higher than those of the stored ones. Most moulds have high lipolytic activities; therefore fats are broken down into fatty acids and partial glycerides during fungal deterioration of stored products. Increase in moisture and fibre contents were observed in both species during pathogenesis by the pathogens. Amusa *et al.* (2002) had reported

that the moisture and fibre contents of breadfruit (*Artocarpus communis*) increased with deterioration in storage.

Conclusion

The study revealed that *Aspergillus niger, Botryodiplodia theobromae* and *Sclerotium rolfsii* were fungal pathogens of yam in storage in The study also found out that the carbohydrate, protein and fat contents of yam were utilized by the rot causing pathogens during pathogenesis. This will provide the understanding of the physiology of growth and infection caused by the pathogens.

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