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Evaluation of a new method for testing the pathogenicity of molds to yam tubers

Frank Chukwunwike Ogbo, Kingsley Chukwuebuka Agu

ABSTRACT

Aims: The Objective of this study was to compare methods involving cutting of yam, with a method developed in our lab, which exposes the yams to the pathogens but without cutting the protective skin as well as to estimate the weight loss and percentage severity of rots caused by rot-causing molds of yam tubers. Methods: A total of thirteen test molds viz., *Aspergillus* sp., *Aspergillus tamarii*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium solani*, *Lasiodiplodia theobromae*, *Mucor circinelloides*, *Paecilomyces* sp., *Fonsecaea* sp., *Phialophora* sp., *Graphium* sp., *Saccharomyces* sp. and *Exophiala* sp., as identified using the partial ITS rDNA sequencing analysis and a BLAST search using the GenBank sequence database, isolated from previous works from five yam varieties namely: *Dioscorea dumetorum*, two varieties each of *D. alata* and *D. rotundata*, were used for this study. Comparative pathogenicity tests were carried out with fresh, healthy yam tubers by the methods described by Okigbo and Nmeka, (2005) and a method developed in our laboratory. The percentage weight loss over a period of four weeks, symptoms observed and percentage severity of rots produced by the pathogenic molds were all studied. Results: The ability of both tests to detect severity of spoilage were studied and recorded as + (symptoms noticed), – (No symptom noticed) and +/C (symptoms noticed and consistent). Only seven isolates were pathogenic by the method of Ogbo and Agu, whereas all thirteen isolates were pathogenic by the method of Okigbo and Nmeka (2005). There was no significant difference $p>0.05$ between the weight losses and percentage severity of rots amongst the test molds with both methods except in the case of *D. alata* var. abana mmee tested with *Fusarium solani* which displayed high percentage rot severity of 450.00 by the method of Ogbo and Agu as against 153.85 displayed by the method of Okigbo and Nmeka (2005). Results obtained helped to ascertain the state of the molds (opportunistic pathogens or pathogens). Conclusion: Only molds capable of breaching the protective cover of yam tubers and eliciting original symptoms consistent with those seen in previous studies and reported in other literatures were actual yam pathogens.

Keywords: Fungal rots, Fungal spoilage, Pathogenicity tests, Yam varieties

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INTRODUCTION

Yams (*Dioscorea* spp.) are perennial herbaceous vines grown for the consumption of their starchy tubers in West Africa, Asia, Carribean, Latin-America, Oceania islands of the South Pacific and some parts of Brazil [1]. Out of the World production of 50 million tonnes of yams per annum, West and Central Africa account for about 94% of the world's production, with Nigeria ranking highest by producing 35 million tonnes [2, 3]. In spite of this, the demand of yam tubers in Nigeria has always exceeded its supply as a result of post-harvest losses emanating from fungal rots. After poverty per se, the third world's most burning challenge is food scarcity. The International Food Policy Research Institute [4] predicted a dismal future for the world's food condition. Post-harvest storage loss is one of the important sources of food insecurity in West Africa and is a major concern even to the United Nations which brought it to international focus when it announced in 1975 that "further reduction of post-harvest food losses in developing countries should be undertaken as a matter of priority" [5, 6]. In Nigeria, post-harvest spoilage of yam tubers ensuing in microbially-induced rot is one of the principal influences affecting commercial production of yam [7].

Various fungi have been recognized by several researchers as causative agents of numerous yam diseases including, *Colletotrichum gloeosporioides* as a fungal pathogen infecting minisettes through infested tubers. *Botryodiplodia theobromae*, *Aspergillus niger*, *A. tamarii*, *Cladosporium herbarum*, *Cylindrocarpon radicola*, *Gliocladium roseum*, *Geotrichum candidum*, *Gliomastix convoluta*, *Macrophomina phaseoli*, *Mucor circinelloides*, *Fusarium moniliforme*, *F. solani*, *Penicillium cyclopium*, *P. sclerotigenum*, *Rhizoctonia solani*, *Rhizopus nigricans*, *R. stolonifer*, and *Rosellinia* spp. and other fungal pathogens associated with rots of yam tubers [8–12].

There is a need to have precise tests to determine pathogenicity of those mold species and their roles in yam spoilage. Knowledge will help develop sufficient preservation methods. Slices of potato tubers arranged on a filter paper placed inside a Petri dish and subsequent inoculation of fungal spores have been used to assay for the pathogenicity of *Fusarium* isolates [13] whereas, the boring of holes of various sizes in healthy yam tubers and incorporation of several days old fungal cultures into the holes and subsequent replacement of the removed tissue and sealing with petroleum jelly have been performed by various researchers including [14–17]. However, it is well known that the protective cover of foods play a major role in the prevention of microbial invasion and subsequent attack.

The aim of this work, therefore, is to compare methods involving cutting of yam, with a method developed in our lab, which exposes the yams to the pathogens but without cutting the protective skin. Results which reveal pathogens with ability to spoil intact yam tubers would be accepted.

MATERIALS AND METHODS

Yam Collection and Identification

A total of twenty tubers comprising the five varieties were obtained from farmers in Awgbu, Orumba North Local Government of Anambra State, Nigeria about two weeks after their harvest. Yams were selected on the basis of their good health, absence of cuts on their skin and fair uniformity in weight. The yam species were identified by Dr. Mbaekwe, Prof. Izundu and Prof. Okigbo, of the Department of Botany, Nnamdi Azikiwe University, Awka as (*Dioscorea rotundata* var. ''abi" and *Dioscorea rotundata* var. ''adaka"), water yams (*Dioscorea alata* var. ''abana ocha" and *Dioscorea alata* var. ''abana mmee") and trifoliate yam (*Dioscorea dumetorum*).

Test Molds

The test molds viz. *Aspergillus tamarii*, Aspergillus sp., *Aspergillus niger*, *Aspergillus flavus*, *Mucor circinelloides*, *Lasiodiplodia theobromae* and *Fusarium solani* were obtained from a previous study and used to inoculate similar fresh healthy yam varieties.

Pathogenicity Studies

Yams were split into two sets. One set of yams were subjected to pathogenicity tests as described by Okigbo and Nmeka, (2005). This test compromised fresh and healthy. These tubers were weighed, washed with sterile distilled water and then disinfected with 70% ethanol. Thereafter, cylindrical discs were removed from the tubers with sterile 4 mm cork borers. Then, 4 mm discs of five days old cultures of the suspected rot-causing isolates were used to plug the holes created in the tubers and the discs of the yam were replaced after 4 mm pieces had been removed to compensate for the thickness of the fungal culture.

The developing symptoms and rate of spoilage were monitored weekly for a period of one month and recorded. Yam tuber impregnated with only agar was also set up as the control and monitored weekly for a period of one month [14].

A second method developed in our laboratory involved obtaining five-day old pure cultures of the suspected rot-causing fungi and transferring them in sterile bandages.

Thereafter, the culture-bandage arrangements were used to dress selected healthy yam tubers that were surface sterilized and having nearly similar weights with those used for the previous study. A control containing only bandage and un-inoculated agar was also set up. The yam tubers were then stored in a yam barn until rots developed. Observations about rate of decay and weight loss were also recorded weekly for a period of one month.

The results obtained from the two methods were matched with those obtained from a previous study "Role of Fungal Rots in Post-harvest Storage Losses in Some Nigerian Varieties of Dioscorea Species" (Ogbo and Agu).

Symptoms of Yam Rot

Hand feel and visual examination, followed by examination of the interior of suggestive tubers was used to ascertain the symptoms of tuber rots. Rots were categorized using the scheme of Amusa et al. [18] as follows:

Dry rot: The infected tissues became hard and dry with varying discolorations depending on the causative agent.

Soft rot: The infected tissues became soft and ramified by the fungal mycelium.

Wet rot: This was typified by the exudation of whitish fluid from the infected yam tissue when pressed between the fingers.

Percentage Severity of Rots

The severity of rot seeks to measure the magnitude of the infection as well as the rate of the pathogenicity of the rot-causing fungi. This was determined by obtaining the rotted portions off the whole tubers and taking the final weight of the individual yam tuber. The percentage severity of rot (Sr %) was calculated thus:

$$Sr\ (\%) = \frac{FW - w \times 100}{w}$$

where, FW = Final weight of infected yam tuber, w = weight of rotted tuber portion.

Determination of Percentage Weight Loss

Weight loss (%) was calculated from a comparison of the weight of individual yam samples at the commencement and at the end of experiment.

Statistical Analyses

All measurements were carried out in triplicate and data subjected to Analyses of Variance (ANOVA) using IBM SPSS Statistics version 20.

RESULTS

Test tubers were observed for developing symptoms and reported as +, – or +/C.

There was no significant difference in the percentage weight loss and percentage severity of rots in the yam tubers tested by both methods as seen in (Tables 1–7). Apart from percentage severity of rot of *Fusarium solani* which was significantly different in both methods as seen in Table 8.

Table 1: Ability of both Tests to Detect Severity of Spoilage

Yam Tubers	Isolates	Pathogenicity Testing Methods	
		Okigbo and Nmeka (2005)	Ogbo and Agu
Dioscorea dumetorum [1]	*Aspergillus tamarii*	+	+/C
Dioscorea alata var. abana mmee [2]	*Fusarium solani*	+	+/C
Dioscorea alata var. abana mmee [3]	*Aspergillus* sp.	+	+/C
Dioscorea alata var. abana ocha [4]	*Lasiodiplodia theobromae*	+	+/C
Dioscorea rotundata var adaka [5]	*Aspergillus niger*	+/C	+/C
Dioscorea rotundata var adaka [6]	*Aspergillus flavus*	+/C	+/C
Dioscorea rotundata var abi [7]	*Mucor circinelloides*	+/C	+/C
Dioscorea rotundata var abi [8]	*Paecilomyces* sp.	+	–
Dioscorea dumetorum [9]	*Fonsecaea* sp.	+	–
Dioscorea alata var. abana mmee [10]	*Phialophora* sp.	+	–
Dioscorea alata var. abana ocha [11]	*Graphium* sp.	+	–
Dioscorea rotundata var adaka [12]	*Saccharomyces* sp.	+	–
Dioscorea rotundata var abi [13]	*Exophiala* sp.	+	–

KEY:
+ = Symptoms noticed
– = No symptom noticed
+/C = Symptoms noticed and consistent

Table 2: Comparative Rate/ Symptoms of Spoilage of *Dioscorea dumetorum* Tubers Inoculated with *Aspergillus tamarii*

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea dumetorum					150.00
1	Nil	8.00	127.27	Nil	18.00	
2	Hard and dry, with massive greenish discoloration of infected tissues	25.00		Nil	20.00	
3	Hard and dry, with massive greenish discoloration of infected tissues	28.00		Hard and dry, with massive reddish-brown discoloration of infected tissues	28.00	
4	Hard and dry, with massive greenish discoloration of infected tissues	56.00		Hard and dry, with massive reddish-brown to dark-brown discolorations with pinkish borders on the infected tissues.	60.00	

Table 3: Comparative Rate/ Symptoms of Spoilage of *Dioscorea alata* var. abana mmee Inoculated with *Aspergillus* sp.

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea alata var. abana mmee					37.04
1	Nil	5.79	49.38	Nil	6.67	
2	Soft, with massive greenish discoloration of infected tissues	14.05		Nil	26.67	
3	Soft, with massive greenish discoloration of infected tissues	19.01		Hard and dry, with brown discoloration of infected tissues	33.33	
4	Soft, with massive greenish discoloration of infected tissues	33.06		Hard and dry, with brown to fawn discolorations observed on infected tissues	55.00	

Table 4: Comparative Rate/ Symptoms of Spoilage of *Dioscorea alata* var. abana ocha Inoculated with *Lasiodiplodia theobromae*

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea alata var. abana ocha					194.00
1	Nil	22.00	203.03	Nil	20.00	
2	Hard and dry, with grey to black discolorations of the infected tissues	40.00		Hard and dry, with massive black discolorations of the infected tissues	34.00	
3	Hard and dry, with grey to black discolorations of the infected tissues	48.00		Hard and dry, with massive black discolorations of the infected tissues	42.00	
4	Hard and dry, with grey to black discolorations of the infected tissues	67.00		Hard and dry, with massive black discolorations of the infected tissues, which flaked off when felt with the hand	66.00	

Table 5: Comparative Rate/ Symptoms of Spoilage of *Dioscorea rotundata* var. adaka Inoculated with *Aspergillus niger*

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea rotundata var. adaka					221.43
1	Nil	20.88	225.00	Nil	22.22	
2	Hard and dry, with massive brownish discolorations of infected tissues	38.46		Hard and dry, with massive brownish discolorations on infected tissues	33.33	
3	Hard and dry, with massive brownish discolorations of infected tissues	51.65		Hard and dry, with massive brownish discolorations on infected tissues	44.44	
4	Hard and dry, with massive brownish discolorations of infected tissues	69.23		Hard and dry, with massive brownish discolorations with yellowish borders on infected tissues	68.89	

Table 6: Comparative Rate/ Symptoms of Spoilage of *Dioscorea rotundata* var. adaka Inoculated with *Aspergillus flavus*

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea rotundata var. adaka					163.17
1	Nil	19.19	175.00	nil	19.00	
2	Hard and dry, with massive greenish discoloration of infected tissues	34.07		Hard and dry, with massive greenish discoloration of infected tissues	28.00	
3	Hard and dry, with massive greenish discoloration of infected tissues	39.39		Hard and dry, with massive greenish discoloration of infected tissues	38.00	
4	Hard and dry, with massive greenish discoloration of infected tissues	63.64		Hard and dry, with massive greenish discoloration of infected tissues	62.00	

Table 7: Comparative Rate/ Symptoms of Spoilage of Dioscorea rotundata var. abi Inoculated with Mucor circinelloides

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**%Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea rotundata var. abi					400.00
1	Nil	21.57	410.00	Nil	16.00	
2	Soft, with brownish discolorations of infected tissues	41.18		Infected tissues appeared soft.	28.00	
3	Soft, with brownish discolorations of infected tissues	52.94		Soft, with brownish discolorations of infected tissues	40.00	
4	Soft and wet, with brownish discolorations of infected tissues	80.39		Soft and wet, with brownish discolorations of infected tissues	80.00	

Table 8: Comparative Rate/ Symptoms of Spoilage of *Dioscorea alata* var. abana mmee Inoculated with *Fusarium solani*

	Okigbo and Nmeka, (2005)			Ogbo and Agu		
Weeks	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**	**Observed symptoms of Spoilage**	**% Wt. loss**	**% Severity of rots (end of four weeks)**
	Dioscorea alata var. abana mmee					
						450.00
1	Nil	9.09	153.85	Nil	7.58	
2	Hard and dry, with grey discolorations of the infected tissues	24.24		Nil	24.24	
3	Hard and dry, with grey discolorations of the infected tissues	33.33		Hard and dry, with massive brownish discolorations of infected tissues	51.52	
4	Hard and dry, with grey discolorations of the infected tissues	60.61		Hard and dry, with massive brownish discolorations with pinkish borders on the infected tissues	81.82	

DISCUSSIONS

The ability of both tests to detect severity of spoilage were studied and recorded as + (symptoms noticed), – (No symptom noticed) and +/C (symptoms noticed and consistent) and presented in Table 1. Symptoms were observed in all the tubers studied by the Method of Okigbo and Nmeka (2005), but only three isolates viz. *Aspergillus flavus*, *Aspergillus niger,* and *Mucor circinelloides* were consistent with previous symptoms observed in the diseased yam tubers from where they were isolated. Seven out of the thirteen isolates elicited symptoms consistent with previous studies. This served as the yardstick for measuring the veracity of both pathogenicity testing methods employed in this study based simply on conformity of the symptoms observed with those reported in a previous study. The results of the symptoms observed in the former method were not in tandem with those observed in a previous study, whereas the symptoms observed in the latter method showed absolute conformity with the symptoms observed in a previous study. This could be suggestive of the fact that most of the molds that showed pathogenicity with the former method could have been secondary pathogens and were not able to out-compete the primary pathogens in establishing a disease in the presence of possible aerial contamination.

The results of the Comparative pathogenicity tests using the methods of Okigbo and Nmeka, (2005) as well as the method Ogbo and Agu (Tables 2–8) has shown that high percentage severity of rots were recorded in the former method except for *D. dumetorum* and *D. alata* var. abana mmee. This may be as a result of the ability of the pathogens to utilize the nutrients of the tubers as substrates for growth and development. This result is similar to the report on fungi associated with Nigeria yam [19]. In both methods, however, there were high percentage weight loss of inoculated tubers during the four weeks pathogenicity test period. Again, this may be due to the fact that the nutrients viz. carbohydrate, moisture, protein, fiber and fat of the tubers were being degraded by the pathogenic molds as well as depletion emanating from auto-spoilage during storage.

This finding is in consonance with more recent report by Ikediobi CO and Oti E [20] and even more recent report by Frank CO and Kingsley CA [12] they showed that perhaps, the most prominent physiological change occurring in the tuber during storage is respiration. This leads to the loss of dry matter and quality of the tuber as food.

Again, El-Hassan et al. [13], and Osunde ZD and Orhevba BA [21] reported that the physiological changes occurring within the tuber, which determine the shelf life of yams primarily, arise from sprouting, transpiration, which is essentially a physical evaporation process and respiration. This phenomenon is significant because among other effects, it leads to the shriveling and loss of the culinary quality of the tuber.

The former method revealed that all the test fungi were pathogenic to the yam tuber samples. This may be

as a result of the fact that the tubers were first wounded and the fungi inoculated into them, in conjunction with the fact that fungi are amylolytic and yam tubers are good sources of carbohydrates. Thus, even the non-pathogenic molds, became opportunistic pathogens. Owing to this limitation a second method developed in our laboratory was employed which brought the integrity of the whole tuber into focus. Thus, the second method proved to be a better method for testing the pathogenicity of fungal pathogens of yams and will help yam pathologists in various yam growing zones of the world, to establish original yam pathogens thereby boosting the overall output of yam production and minimizing post-harvest loss of yam production to fungal pathogens. This method revealed only seven out of the thirteen isolates to be pathogenic. Pathogenicity was based on the ability of isolates to be able to colonize, penetrate and elicit symptoms and possibly establish the disease it caused originally. This data fairly agrees with the findings of Amusa et al. [18] they carried out a survey of the micro organisms associated with the stored and marketed yam tubers obtained from the tropical forest region of South-western Nigeria and their pathogenicity on yams and reported that dry rot is considered as the most devastating of all the storage diseases of yam tubers as well as dry rot of yams alone causes a marked reduction in the quantity, marketable value and edible portions of tubers and those reductions are more severe in stored yams

It is also in consonance with more recent reports [22, 23]. *Aspergillus tamarii* exhibited the highest percentage severity of rot (300 %), while *Aspergillus flavus* displayed the lowest (100%).

This is in contrast with the mild pathogenicity (++) recorded by Amusa et al. [18]. There was no significant difference $p>0.05$ between the weight losses and percentage severity of rots amongst the test molds with both methods except in the case of *D. alata* var abana mmee tested with Fusarium solani which displayed high percentage rot severity of 450.00 by the method of Ogbo and Agu as against 153.85 displayed by the method of Okigbo and Nmeka (2005).

CONCLUSION

The comparative pathogenicity tests carried out by the method of Okigbo and Nmeka, (2005) and a method developed in our laboratory helped determine the actual pathogens from the opportunistic pathogens and the non-pathogens, while the former method demonstrated that all the thirteen initial isolates were pathogenic, the later proved that only seven out of the thirteen isolates were actually pathogenic.

It was, however, concluded that since molds were amylolytic in nature the opportunistic pathogens may have capitalized on the wounds created on the tubers to elicit disease symptoms. Therefore, pathogenicity was built on the ability of isolates to be able to colonize, penetrate and elicit the original disease symptom it was isolated from. This knowledge would be helpful to yam pathologists to find out more accurate pathogenicity testing methods which would be useful in the reduction of post-harvest loss of yams.

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Author Contributions
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Guarantor
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Conflict of Interest
Authors declare no conflict of interest.

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