

RESEARCH ARTICLE

Effect of Storage Methods and Management of Sweet Potato on the Incidence of Tuber Rot Induced by *Rhizopus stolonifer* in Kano, Nigeria

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Abstract

Different storage methods and management practices of sweet potatoes in Kano state, northwestern Nigeria, were investigated to find out how they may affect the incidence of tuber rot. Three local government areas were selected for the sample collection. In each local government area, two sweet potato farming communities were selected, infected and uninfected tubers were sampled and taken to the laboratory for further studies. Pathogenicity test confirmed *Rhizopus stolonifer* as the causal pathogen responsible for tuber rot and this fungus was used for the inoculation of fresh sweet potato tubers in all the storage and management methods used in the experiment. Tubers preserved using ash + sand and fungicide + sand showed the least incidence of tuber rots. Similarly, it was also observed that storage of tubers with sand alone showed a reduction in the severity of the disease across the storage weeks. There was no significant difference in the incidence and severity of tuber rot between the two inoculation methods with both the treatment combinations. Our result showed that the incubation period of *R. stolonifer* to infect sweet potato tubers started at two days after inoculation with about 25% infection. The result also indicated that none of the tubers treated with fungicide, ash, and eucalyptus sprouted at both the storage weeks, this may be attributed to the possibility of having some inhibitory properties against sprouting.

Keywords: *Ipomea batatas*, *Eucalyptus camaldulensis*, wood ash, sand, tricyclazole, post-harvest deterioration

Introduction

Sweet potato [*Ipomea batatas* (L.) Lam] is presently ranked the seventh most important crop in the world with a total of about 103 million tonnes in 2013 (FAO, 2015; Sugri et al., 2017). Asia accounts for close to 76% of total world production, followed by Africa (19.5%). The top five sweet potato producing countries in the world are China, Nigeria, Uganda, Indonesia, and Tanzania with China being the leading producer with about 75.6 million tonnes, followed by Tanzania and Nigeria that produce around 3.57 and 2.73 million tonnes, respectively (Sugri et al., 2017). In Nigeria, sweet potato is one of the six most important root and tuber crops, other root crops are cassava, yam, Irish potato, cocoyam, and ginger. Although the sweet potato leaves are edible, the starchy tubers are by far the most important product (Bidzakin et al., 2014). The roots are mostly boiled, fried, roasted or baked for their rich sources of dietary energy (Oduola et al., 2018; Olaitan, 2012), quite essential for their beta carotene and vitamin C (Motsa et al., 2015; Naico and Lusk, 2010), and it is an imperative source of antioxidants and anthocyanidins (Oladoye et al., 2013). However, sweet potato is a highly perishable food source that is susceptible to destruction by micro-organisms, metabolic spoilage, physical destruction and pest infestation after harvesting. The production of perishable products in Nigeria can be improved by increasing productivity and avoiding losses caused by storage rots (Echerenwa and Umechuruba, 2004).

Postharvest loss becomes a major limiting factor in prolonging storage duration of sweet potato (Ravi et al., 1996; Xie et al., 2018). During storage, the tubers are very perishable because they contain high moisture content (60-75%), and due to high respiratory rate and the resultant heat production

soften the tuber textures which make them susceptible to damage and microbial decay (Munish et al., 2006). Postharvest quality deterioration emanates from weight loss, weevil damage, sprouting and attack by microorganisms (Singh et al., 2016) with postharvest rots being predominantly reported as the main cause of post-harvest loss (da Silva and Clark, 2013; Olaitan, 2012). A wide variety of microorganisms, mostly moulds, have been implicated in tuber spoilage but few are implicated as primary pathogens (da Silva and Clark, 2013). The fungi associated with sweet potato rots comprises; *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Botryodiplodia theobroma* and *Penicillium* sp. (Olaitan, 2012).

Rhizopus stolonifer (Zygomycetes), commonly known as the black bread mould, is a fungus which survives in soils and debris in the farm or around houses. Postharvest infection by this secondary pathogen commonly occurs in fruits or tubers pierced by insects or affected by physiological disorders or mechanical injuries (Agrios, 2005). Once a spore lands on a wounded tissue, it germinates and starts growing on the surface, producing a thick mycelium which at the same time produces cell degrading enzymes (amylases and pectinases) that denature the tissue, thus results in infection (Bautista-Baños et al., 2008).

Currently, both the traditional and modified traditional methods of storage are practiced in most of sub-Saharan Africa and some developing countries (Abidin et al., 2016; Sowley et al., 2015). These include in-ground storage, heap storage, platform storage, pit storage, and under shade covering with grass amongst others (Dandago and Gungula, 2011). In some instances, sawdust and ash are added to improve the shelf-life (Amoah et al., 2011). In addition, fungicides such as Dichloronitroaniline are used to protect tubers against *Rhizopus* soft rot. However, the use of synthetic fungicides, apart from their potential danger to both the farmer and the environment, are unaffordable by most farmers (Obagwu et al., 1997). Based on these notions this study was conducted in order to evaluate the efficacy of using locally available materials and fungicide (used as a control) in the management of tuber rots on sweet potato.

The main objectives of this study were to identify the fungus associated with post-harvest deterioration in sweet potato in selected areas of Kano state, Nigeria, and to evaluate the efficacy of locally available materials in the storage and the management of tuber rot of sweet potato.

Materials and Methods

Experimental Site

The experiment was conducted between November 2014 and February 2015 at the Pathology Laboratory of Crop Protection Department, Faculty of Agriculture, Bayero University, Kano, located at latitude 11° 58' N and longitude 8° 25'E situated at 475 m above sea level in Sudan savannah ecological zone of Nigeria.

Collection of Sweet Potato Tubers

Tubers were collected from the six sweet potato producing areas of Kano state. Three local government areas were selected for the sampling, with two locations selected from each local government; namely Kumbotso L.G.A. (Tsamawa and Riga Fada), Rimin Gado L.G.A. (Gulu and Asakala) and Ungogo L.G.A. (BUK new site and Tudun fulani). Both the infected and uninfected tubers were collected as samples then taken to the laboratory for further work.

Collection of Materials for Tuber Rot Treatments

Fresh leaves of *Eucalyptus camaldulensis*, coarse sand and wood-ash were collected from the Faculty of Agriculture environs, Bayero University, Kano, while fungicide (Tricyclazole 75% WP) was purchased from Sabon Gari market, in Kano metropolis.

Isolation and Identification of Fungal Pathogens Associated with Potato Tuber Rot

Infected potato tubers were cut toward the advance margin between the healthy and infected portions of the tubers with a surface sterilized knife; then were further cut into smaller portions of about 5 mm with a sterilized scalpel. The sections were surface sterilized in 1% sodium hypochlorite and rinsed with several changes of sterile distilled water. They were then plated on petri dishes containing potato dextrose agar (PDA) incorporated with streptomycin. The culture plates were incubated at room temperature (28° ± 2°C) for seven days and observed daily for fungal development. The developing fungus was sub-cultured and pure cultures were obtained and kept for further use. The fungus was viewed under a microscope and identified according to the morphology using an illustrated genera of imperfect fungi, fourth edition identification manual as a guide (Barnett and Hunter, 1998). Based on the morphology, *Rhizopus stolonifer* was identified to be associated with the deteriorating sweet potato tubers.

*Pathogenicity and Determination of Incubation Period of *Rhizopus stolonifera**

To determine the pathogenicity of *Rhizopus stolonifera* fresh, healthy sweet potato tubers were inoculated with the fungus. This was done by washing the tubers with tap water, rinsed with distilled water and surface sterilized with 60% ethanol before a 5mm cylindrical disc was removed from the tuber using a cork borer. A disc of a seven days old culture of the isolated fungus was transferred into the holes created in the tubers. Cello tape was used to completely seal the opening of the hole created. The inoculated tubers were then incubated at room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for seven days, and examined for infection and disease development.

Preparation of Materials for the Root Rot Treatments

The collected leaves of *Eucalyptus camaldulensis* were thoroughly washed with tap water and rinsed in sterile water. These leaves were then dried and grounded using a household blender (Binaton model). Twenty grams (20g) of each sample (*Eucalyptus* leaves, wood ash, and fungicide) were added to one litre of distilled water separately. These suspensions were vigorously stirred and later used in treating the tubers.

Evaluation of Storage Methods

In this experiment, two storage methods were used; treated tubers and stored in sand, untreated tubers and stored in sand, and control (untreated tubers and not stored in sand).

Treated Tubers and Sand Storage

Two hundred grams (200 g) of sweet potato tubers replicated four times were immersed into the suspensions/solutions of *Eucalyptus* leaves, wood ash or fungicide then allowed to stay for 10 minutes, removed and dried under shade. These tubers were then placed in plastic containers either containing 2/3 filled coarse sand or left in the container without any treatment and not covered with sand (control). These treatments were left for three weeks before observation. The treatment combinations are

- Treatment 1: sand + tuber treated with fungicide
- Treatment 2: sand + tuber treated with eucalyptus
- Treatment 3: sand + tuber treated with ash
- Treatment 4: sand alone
- Treatment 5: control (untreated tubers stored in container without sand)

Efficacy of the Materials Used in Storage on Injured and Uninjured Tubers

One-hundred grams of sweet potato tubers were washed with tap water and rinsed with sterile water, dried under shade and surface sterilized with 10% ethanol. These tubers were treated with the suspension treatments prepared from *Eucalyptus*, wood ash and fungicide respectively. Injury on the tubers were inflicted artificially using a sterile cork borer, the fungus *Rhizopus stolonifera* was then introduced into the tuber and finally sealed with cello tape to avoid external contaminants. Another set of tubers were treated also with the suspensions but the fungus was smeared on the surfaces of the tubers. Data was collected at two days interval for ten days. The experimental units were arranged using a Complete Randomized Design (CRD) and the treatments were replicated four times.

Determination of Incidence and Severity on Sweet Potato Inoculated Tubers

The disease incidence (expressed as a percentage) and severity of the tubers infected with rot were recorded. Disease incidence was calculated using the formula:

$$\text{Disease incidence} = \frac{\text{Number of visible infected tubers}}{\text{Total number of tubers assessed}} \times 100\%$$

A scale of 1 to 5 employed by Eze and Mauegesi (1990) was used with slight modifications to determine the disease severity as indicated below:

- 1: No disease
- 2: 1% area affected
- 3: 10% area affected
- 4: 25% area affected
- 5: >50% area affected

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using an SAS software version 9.1.3 and means were separated using Fisher's protected least significant difference test at 5% level of significant ($P \leq 0.05$) and standard error (SE) where necessary.

Results and Discussion

Identification of the Fungus and Pathogenicity Test

Based on the fungal morphology by Barnett and Hunter (1998) we identified *Rhizopus stolonifera* as the fungus associated for the tuber rot of sweet potato. The pathogenicity test confirmed the fungus as the

causal agent of the tuber rot.

Effect of Different Inoculation Methods on Treated tubers

Effect of Storage Methods on the Incidence of Tuber Rot

Incidence on Tuber Rots on Inoculated Tubers

As shown in Table 1, there was no symptoms of tuber rots observed in week one. However, treating the tubers with Eucalyptus + Sand showed a significantly high disease incidence (100%) as compared to the control which showed no disease incidence (0%). In week three, ash + sand, fungicide + sand showed the least incidence of tuber rot (0%) however, they were

There was no significant difference between the two inoculations methods in the incidence of tuber rot at both the treatments combinations (Table 3).

Severity of Tuber Rots on Inoculated Tubers

There was no significant difference in the severity of

Table 1. Effect of preservation and storage methods on the incidence of tuber rot

Treatment	Disease incidence* (%)		
	Week 1	Week 2	Week 3
Ash + sand	0.00	0.00a	0.00a
Eucalyptus + sand	0.00	100.00b	100.00c
Fungicide + sand	0.00	0.00a	0.00a
Sand alone	0.00	25.00a	25.00ab
Control	0.00	0.00a	75.00bc
SE	0.00	15.00	22.36

Note: *Values of the same letter(s) within the same column are not significantly different using Fisher's Protected Least Significant Difference test at 5%; SE: standard error

statistically similar to sand alone. Sand alone and control are also statistically similar in week three.

the treatments in the two inoculation methods (Table 4).

Severity of Tuber Rots after Inoculation

Determination of Incubation and Infection Period of Sweet Potato Tuber Rot

The result of the severity of sweet potato tuber rot (Table 2) showed that there was no significant difference between the treatments in week one. In week two, all the treatments were statistically similar except for eucalyptus + sand. In week three, sand + ash had the lowest severity of tuber rot however it was not significantly different from fungicide + sand and sand alone.

We evaluated the incubation period of the sweet potato rot on the inoculated tubers. The result indicated that there was no infection shown one day after inoculation (Figure 1). However, after two days of inoculation 25% of the tubers were infected and by the fourth day, 50% of tubers inoculated showed sign of rot. By day five, 70% of the tubers were infected and were completely rotten.

Table 2. Effect of preservation and storage methods on the severity of tuber rot

Treatment	Disease severity (%)		
	Week 1	Week 2	Week 3
Ash + Sand	1.00	1.00a	1.00a
Eucalyptus + Sand	1.00	2.25b	3.250b
Fungicide + Sand	1.00	1.00a	1.00a
Sand alone	1.00	1.25a	1.250a
Control	1.00	1.00a	2.250ab
SE	0.00	0.22	0.53

Note: Values of the same letter(s) within the same column are not significantly different using Fisher's Protected Least Significant Difference test at 5%; SE: standard error.

Table 3: Effect of different inoculation methods and treatments on disease incidence

Treatments	Percentage incidence / days after inoculation*				
	2	4	6	8	10
Smear					
Ash	0.00	0.00	25.00	100.00	100.00
Eucalyptus	25.00	50.00	75.00	75.00	100.00
Fungicide	0.00	25.00	25.00	75.00	75.00
Control	0.00	25.00	50.00	50.00	100.00
SE	17.68	32.30	36.80	32.30	17.68
Injured					
Ash	0.00	0.00	0.00	25.00	25.00
Eucalyptus	0.00	25.00	50.00	50.00	75.00
Fungicide	0.00	25.00	50.00	75.00	75.00
Control	0.00	0.00	50.00	75.00	100.00
SE	0.00	25.00	35.40	36.80	30.60
LSD	ns	ns	ns	ns	ns

Note: * ns= values within the same column are not significantly different using Fisher's Protected Least Significant Difference test at 5%; SE: standard error

Table 4. Effect of different inoculation methods and treatments on disease severity

Treatments	Percentage severity / days after inoculation				
	2	4	6	8	10
Smear					
Ash	1.00	1.00	1.25	1.75	2.50
Eucalyptus	1.25	1.50	2.00	2.25	3.50
Fungicide	1.00	1.25	1.50	2.00	2.50
Control	1.00	1.25	1.50	2.00	2.75
SE	0.17	0.32	0.53	0.69	0.76
Injured					
Ash	1.00	1.00	1.00	1.25	1.50
Eucalyptus	1.00	1.25	1.75	2.25	3.70
Fungicide	1.00	1.25	1.50	1.75	2.50
Control	1.00	1.00	1.50	2.00	2.50
SE	0.00	0.25	0.45	0.65	0.92
LSD	ns	ns	ns	ns	ns

Note: ns= values within the same column are not significantly different using Fisher's Protected Least Significant Difference test at 5%; SE: standard error.

Effect of Preservation and Storage Method

The result in Table 5 indicated that in the first week some of the tubers treated with sand alone and the control started to sprout and this continued on in the third week. It was also observed that none of the tubers treated with fungicide, wood ash and eucalyptus sprouted.

Effect of Preservation and Storage Methods on Tuber Weight Loss

The tubers treated with Eucalyptus lost most of their weight (4.64%) as a result of rotting and drying; and also the same with fungicide treated tubers, even though the rate of rotten varies (Figure 2); the least weight was from the sand alone 1.86 g (0.93%) followed by the control 3.66 g (1.80%) as a result of sprouting from the tubers. Fungicide and ash seem to

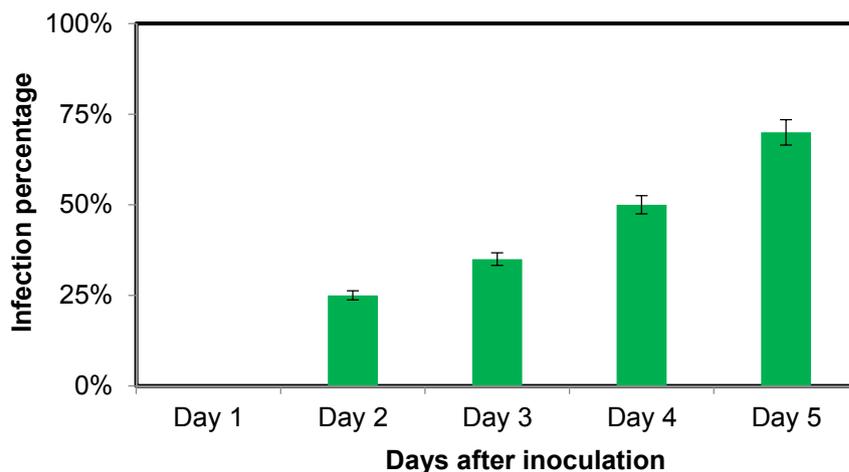


Figure 1. Determination of infection period of sweet potato tuber rot

Table 5. Effect of preservation and storage methods on sprouting

Treatment	Week 1	Week 2	Week 3
Fungicide	NS	NS	NS
Eucalyptus	NS	NS	NS
Ash	NS	NS	NS
Sand	SS	SS	SS
Control	S	SS	SSS

Note: S = Sprouting (25%); SSS = Sprouting (75%); SS = Sprouting (50%); NS = No sprouting

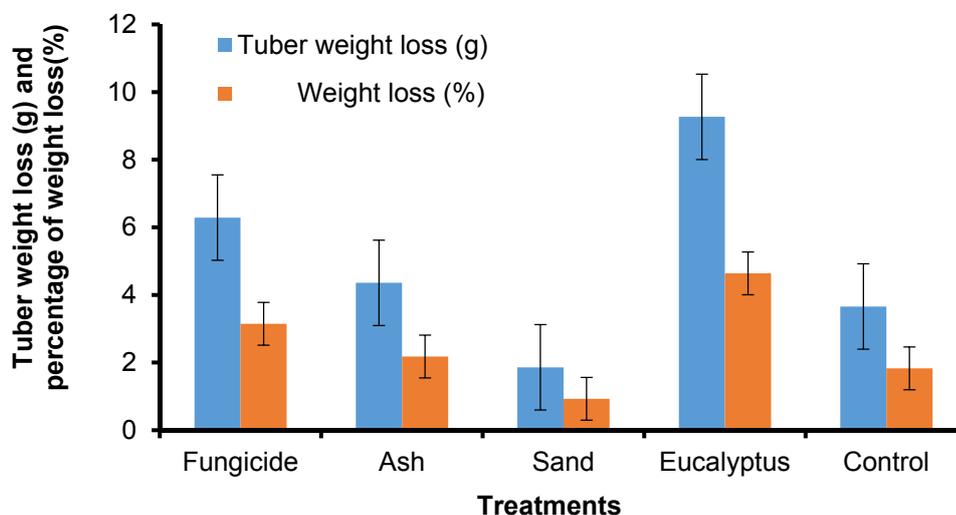


Figure 2. Effect of preservation and storage methods measured by tuber weight loss \pm SE (g) and percentage of weight loss \pm SE (%).

have inhibited sprouting; therefore the weight loss is only by drying which is less than 1% as that of sand and the control.

In this study, we focused on the different methods of storing sweet potato tubers as well as identifying the best possible control practices to limit post-harvest loss due to tuber rots. Our result indicated that *Rhizopus stolonifer* was the causal agent for the sweet potato tuber rot. This is in line with several

reports showing tuber rot of sweet potato caused by *Rhizopus stolonifer* during storage (Olaitan, 2012; Onuegbu, 2002; Oyewale, 2006), and they also suggest that the pathogen has the potential to cause severe damage to sweet potato tubers during post-harvest handling when the condition is favourable. The effect of the preservation methods and the storage methods used on the incidence of tuber rot showed no significant difference at week one in all the treatments (Table 1). At week two, all the treatments

are statistically the same except Eucalyptus + sand which showed a high incidence of the disease. The best preservation method was observed in ash + sand, fungicide + sand at three weeks after storage. For the effects of these storage methods on the severity of tuber rot, the same trend was observed as in incidence at week one and two respectively (Table 2). On the third week, sand + ash had the lowest severity of the disease, even though it did not differ from fungicide + sand and preserving on sand alone. The result of this study confirms the finding of Amoah et al. (2011) who reported the effectiveness of wood ash and sawdust in the storage performance of sweet potatoes. Similarly, storing sweet potatoes in fresh river sand showed good storage potential for up to 5 months without been damaged or infected (Dandago and Gungula, 2011). Our result showed that treatment with wood-ash + sand or sand alone is capable of preventing tuber rot, signifying that ash and or sand may have fungi toxic effects which probably explain why it was able to prevent microbial growth than any other treatment combination.

No significant difference was observed between the severity and incidence of tuber rot in all the treatments combination in both smears and injured inoculation methods across the post- inoculation days (Table 3 and 4). The mechanism behind non-significant differences on these two inoculation methods is not clearly known. It may be attributed to the short duration of the experiment coupled with treatments combination that may have likely suppressed the activities of the microbes. However, differences were observed between the means as compared by the standard error.

Our result showed the incubation period of *Rhizopus stolonifer* to infect sweet potato started at two days after inoculation with 25% infection (Figure 1). Infected tubers were found to be completely rotten at five days after inoculation with about 70% level of infection. A similar pattern of disease infection with increasing days of storage was observed on sweet potato tubers stored after treatments for over a time (Oladoye et al., 2016). We suggested that *Rhizopus stolonifer* can infect within the shortest possible time as fast as 48hours, therefore management strategies should be applied even when tubers are to be stored for a short period of time. The result also indicated that none of the tubers treated with fungicide, ash, and eucalyptus sprouted at both the storage week (Table 5), this may be attributed to the possibility of these treatments having some inhibitory properties against sprouting. This is in line with Okigbo and Nmeka, (2005) who reported the efficacy of *Xylopiya aethiopica* and *Zingiber officinale* leaf extracts in controlling tuber rot of yam. Tuber treated with Eucalyptus lost most of

their weight as a result of rotting and drying and the same scenario was also observed with the fungicide treated tubers. Sand treated tubers recorded the least weight loss of less than 1% (Figure 2). This is similar to the finding of Dandago and Gungula (2011) that fresh river sand is a good storage method used to keep sweet potato tubers for a long time without losing weight or reduction in quality or taste.

Conclusion

The results of this study revealed that *Rhizopus stolonifer* was found to be responsible for the sweet potato tuber rot. Based on the study, wood-ash + sand, fungicide + sand and sand alone are the more effective methods in suppressing *Rhizopus stolonifer* during storage of sweet potato. Results of this study can be useful to farmers in the postharvest storage of sweet potato so as to minimize losses caused by *Rhizopus stolonifer*. It was also found that the *Rhizopus stolonifer* can infect tubers as low as two days post inoculation period, therefore management strategies should be applied when tubers are to be stored even for a short period of time to avoid damage or infection. A mixture of fungicide with ash and eucalyptus leaf extract was found to suppress the sweet potato tubers from sprouting, suggesting that these two treatments are effective in managing the tuber rots successfully.

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