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SHORT COMMUNICATION

Comparative Studies on the Susceptibility of Three Tubers of *Dioscorea* Species to Dry Rot in Anyigba, Kogi State

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ABSTRACT

Various yam species are cultivated but white yam (*Dioscorea rotundata* Poir.), water yam (*Dioscorea alata L.*) and yellow yam (*Dioscorea cayenensis* Lam.) are among the most cultivated in Nigeria. The Susceptibility of three tubers of *Dioscorea* species (*D. rotundata, D. alata* and *D. cayenensis*) to dry rot pathogens was investigated. Isolation was made from rotted tuber tissues, followed by pathogenicity test and identification of isolates; two fungal species *Aspergillus niger* and *Rhizopus stolonifer* were isolated and identified as the major fungi causing yam tuber dry rot in the study area. There was significant difference (P<0.05) in the susceptibility of the various *Dioscorea* tuber species studied to rot caused by *Aspergillus niger* [*D. rotundata* (20mm), *D. alata* (11mm) and *D. cayenensis* (3.5mm)] and *Rhizopus stolonifer* [*D. rotundata* (17.5mm), *D. alata* (10mm) and *D. cayenensis* (2mm)]. The phytochemical contents of the three tubers of *Dioscorea* species examined also varied significantly, with the bioactive substances of *D. cayenensis* and *D. alata* relatively higher when compared to *D. rotundata*. Therefore, this research reveals that three metabolites; Saponin, Flavonoid and Tanin constitute the major trait for resistant ability of *D. cayanesis* and D. *alata* to dry rot causing pathogen. Therefore, it is necessary that the differences in susceptibility be considered when developing yam barns. Also, researchers should work on genetically improving the susceptible white yam (*D. rotundata)* which is commonly eaten by millions of Nigerians with a possibility of increasing the quantities of the deficient phytochemicals.

Keywords: *Dioscorea alata, Dioscorea cayanensis*, *Dioscorea rotundata,* dry rot, Susceptibility, phytochemical.

1 Introduction

Yam (Dioscorea spp) is commonly grown in many parts of the world especially the Tropics, parts of Asia and America [1]. Out of over 600 Species of yam: *Dioscorea rotundata, Dioscorea alata, Dioscorea cayenensis, Dioscorea dumetorum, Dioscorea bulbifera and Dioscorea esculentus* are of economic importance in West Africa [2]. Nigeria is regarded as the leading producer of yam in the world; accounting for 68% out of the 93% global production, they are mostly consumed as food and also serve as source of income for most rural dwellers [3]. Bacterial, Viral, and Fungal are among the pathogens that cause yam tuber rot [3] although rot due to fungi infestation remain a major challenge to yam production all over the world [1]. Gwa et al., [4] reported that about 50% of yam tubers produced in Nigeria are lost to post harvest diseases. This trend is the greatest threat to attaining food security through yam production, negating the possibility for exports. Some of the fungi pathogens associated with yam rot includes: *Aspergillus flatus,* F*usarium oxysporum, Fusarium solani, Aspergillus Niger, Botryodiplodia theobromae, Collectotrichum spp*, *Geotrichum scandium, Penicillius chrysogenum, Rhizoctonia spp*, *Penicillium oxalicum, Pennicillium digatum, Trichoderma viride* and *Rhizopus nodosus* [5].

Various studies have established the causes of yam rot but only few to the best of our knowledge have looked into the susceptibility of various yam tuber species to different rot causing organism with an aim of providing sustainable method of control. According to Eleazu el al., [6] Several control measures have been employed to control postharvest losses of yam tubers due to fungi infestation, some of which includes the use of fungicides and certain microorganisms as predators.

These, however, have negative effect on the environment and might be difficult to adopt in a developing country like Nigeria; because of its high cost implication and technical knowhow [6]. It has been reported that certain bioactive substances present in plant possess fungitoxic properties which can inhibit the growth of fungi both in vitro and in vivo [6], this explains the demonstration of antifungal activity by the plant extracts as reported by Gwa and Richard, [7] who suggest that *Azadiracta indica, Carica papaya, Piper guineense, Zingiber officinale,* and *Nicotiana tabacum* contain bioactive substances that can inhibit the growth of rot causing fungi.

Kanu *et al.,* [8] reported that Yam tubers contain good quantities of phytochemicals such as: Flavonoids, Saponins, Alkaloids, Tannis, Glycoside Steroids, Anthraquinones and Phenols. These phytochemicals have been reported by previous researches [9, 10] to confer resistance to plant against fungi invasion.

The aim of this study is to compare the susceptibility of three tubers of *Dioscorea* species namely *D. rotundata, D. cayanensis* and *D. alata* to dry rot causing pathogens and to investigate what is responsible for variation (if any) in the susceptibility of tubers to the rot causing fungi.

2 Materials and Methods

The three tubers of *Dioscorea* species that were used in this research are *Dioscorea rotundata, Dioscorea cayeneensis* and *Dioscorea alata*. Healthy and infested yam tubers of the three species were bought from Anyigba market and all the healthy and infested tubers were washed under running tap water, dried and observed under a binocular for possible rot symptoms.

Fresh healthy yam tubers were peeled washed and 250g was weighed and boiled for 30min in 1litres of tap water in a clean pot. The infusion was filtered and allowed to cool. To the infusion, 20g of agar-agar and 15g of dextrose were added to prepare PDA medium. The infusion was made up to 1litre with distilled water before the medium was autoclaved at 1.02 kg/cm3 pressures for 15 minutes. Streptomycin was added to the 1litre of sterilized media just before it was poured into sterile Petri dishes, to prevent the growth of bacteria [11].

About 0.5-1cm pieces was cut at the periphery of the infested tissues of yam tubers with the aid of sterile scalpel and inoculated into a Potato Dextrose Agar (PDA). Three replicate plates each was pleated out and three non-inoculated plates serve as control. The inoculated plates were incubated at room temperature for up to 5 days and microbial growth was monitored and each microbe was identified. Pure culture of each isolate was aseptically transferred to a freshly prepared medium (PDA). Wet mount of each isolate in latophenol blue was microscopically examined and identification was on the basis of characteristics spores with reference to illustrated genera of imperfect fungi [5].

Pathogenicity test was carried out to establish which of the fungal isolates cause the rot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulate which was also used by [4].

Susceptibility of yam tubers to fungi attack was determined by using sterile 5mm cork borer to bore holes on each of the healthy tubers and 4mm cork borer was used to inoculate spores of each of the fungal isolates into the healthy tubers, and covered with Vaseline to prevent the entry of external contaminants. The inoculated tubers were incubated at room temperature for three weeks, after which their rot depth was determined by using a knife to cut open the inoculated yam tubers from the point of inoculation to obtain identical halves. A transparent ruler was used to measure the depth of rot. The actual rot depth was determined by subtracting the inoculation depth (5mm) from the final depth (i.e final depth – inoculation depth = actual depth) using the formula as modify by [7].

2.1 Phytochemical Analysis

Phytochemical analysis was carried out on D. *rotundata, D. cayanensis and D. alata* healthy tubers to check for their Phytochemical Constituent. The yam tubers were peeled washed and sun

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dried for four days. After drying, the samples were ground into powder and stored in airtight bottles before analysis. The methods reported by [12, 13] was used to determine the Alkaloid, Saponin, Phenols, Tanin and Flavoniod content of the various yam tuber species studied.

2.2 Statistical Analysis

Completely Randomized Design was used and the analysis of variance (oneway anova) to separate the means using least significance difference (LSD) to determine levels of significance (p>0.05).

3 Results

3.1 Growth Rate of Fungi on PDA Medium

Table 1 shows the radial growth of the isolated fungal species *Apergillus niger* and *Rhizopus stolonifer* were measured daily. Their growth increased with a corresponding increase in the number of days of incubation. It took *Aspergillus niger* 6 days and *Rhizopus stolonifer* 4 days to completely colonize the petri-dishes respectively.

Table 1: *Radial Growth Rate of Fungi on PDA Medium*

Incubation Period (Days)	***Aspergillus niger***	***Rhizopus stolonifer***
1	0.0	0.0
2	1.2	1.6
3	1.7	3.1
4	2.6	4.5
5	3.2	
6	4.5	

*Mean Value (cm)

3.2 Susceptibility Test Result

According to Table 2, all the three species examined (*D. roundata, D. alata and D. cayenensis*) were all susceptible to dry rot caused by *Aspergillus niger* and *Rhizopus stolonifer* isolates. Healthy yam species (control) that were artificially inoculated with sterile potato dextrose agar recorded no rot. There is significance difference (P≥0.05) in the susceptibility of the three tubers of *Dioscorea* species (*D. rotundata, D. alata and D. cayenensis*) inoculated with *Aspergillus niger* [*D. rotundata* (20mm), *D. alata* (11mm) and *D. cayenensis* (3.5mm)] and *Rhizopus stolonifer* [*D. rotundata* (17.5mm), *D. alata* (10mm) and *D. cayenensis* (2mm)].

Table 2: *Rot Depth (mm) after 21 days of Inoculation with Fungus*

Yam Species	***Aspergillus niger***	***Rhizopus stolonifer***
D. rotundata (white yam)	20.00 ± 1.00[a]	17.5 ± 0.50[a]
D. alata (water yam)	11.00 ± 1.00[b]	10.00 ± 1.00[b]
D. cayenensis (yellow yam)	3.5 ± 0.50[c]	2.00± 0.00[c]
Total	**11.5 ± 3.04**	**9.83 ± 2.85**
P Value	0.002*	0.001*

Mean in column with the same super script(s), do not differ significantly a P ≤ 0.05.

Rot caused by *Aspergillus niger* on *D. rotundata* (20mm) is higher than rot caused by *Rhizopus stolonifer* on *D.* rotundata (17.5mm), while rot caused by *Aspergillus niger* on *D.* alata (11mm) is higher than rot caused by *Rhizopus stolonifer* on *D. alata* (10mm). Also rot caused by *Aspergillus niger* on *D.* cayanensis (3.5mm) is higher than rot caused by *Rhizopus stolonifer* on *D. cayanensis* (2mm) (Table 2).

3.3 Phytochemical Test Result

Table 3 presents the results of phytochemical constituents of each *Dioscorea* tuber species studied. The Saponin, Tannin and Phenol level were very low in *D. rotundata*, whereas the level of Saponin in *D. cayenensis* were significantly higher with (17.2 mg/5g) even higher than the level in *Dioscorea alata* (6.88mg/5g). Both *D. Cayenensis* and *Dioscorea alata* recorded higher content of Saponin when compared with *Dioscorea rotundata* (2.88mg).

Table 3: *Phytochemical Composition of Dioscorea species*

Yam Species	**Saponin**	**Flavonoid**	**Tannin**	**Alkaloid**	**Phenol**
D. rotundata	2.88±0.02[c]	4.21±0.01[b]	0.00±0.00[b]	0.34±0.02[b]	0.02±0.00[b]
D. alata	6.88±0.02[b]	1.59±0.01[c]	0.07±0.01[c]	0.21±0.01[c]	0.03±0.01[c]
D. cayenensis	17.21±0.01[a]	6.81±0.01[a]	0.01±0.01[b]	0.76±0.01[a]	0.06±0.00[a]
Total	**8.99±2.70**	**4.20±0.95**	**0.03±0.01**	**0.44±0.11**	**0.04±0.01**
P value	0.000*	0.000*	0.011*	0.001*	0.033*

Mean in column bearing the same super script(s), do not differ significantly. (P ≤ 0.05)

The flavonoid content was significantly higher in *D. cayenensis* (6.81mg) compare to *D. rotundata* (4.21mg) while *D. alata* recorded low flavonoid content of (1.59mg). The Alkaloid content was higher in *D. cayenensis* (0.76mg) compare to *D. rotundata* (0.34mg) and D. *alata* (0.21mg). The Tannin content of D. *alata* (0.07) was higher compare to *D. rotundata* (0.00mg) and *D. cayenensis* (0.01mg). *D. cayenensis* has higher Phenol (0.06mg) compare to *D. alata* (0.03mg) and *D. rotundata* (0.02mg).

4 Discussion

Two fungal species *Aspergillus niger* and *Rhizopus stolonifer* were isolated and identified as the major fungi causing yam tuber dry rot in Anyigba kogi State Nigeria. This observation is in agreement with the report of [14] who isolated *Aspergillus niger* and *Rhizopus stolonifer* as a dry rot pathogen of *Dioscorea rotundata* in his research on the "Differential rate of dry rot in *Dioscorea rotundata* (white yam) along its tuber length". Patrice el al., [10] Revealed that *A. niger* is one of the most severe fungi that causes rot in storage; causing losses between 25-60% in storage. [7, 11] also isolated the same fungi in their research on the storage rot of yams tuber. Pathogenecity test reveals that these fungi are pathogenic, and they are responsible for dry rot of yam in the study area. The control samples produce a brownish thickened layer around the cut area probably due to the healing process in response to the cut since the tissue are still living and performing physiological functions [15]. *Rhizopus stolonifer* grew faster when compare to *Aspergillus niger in vitro* but *Aspergillus niger* grew faster than *Rhizopus stolonifer in vivo*. These might be as a result of various environmental and growth conditions that varies *in vitro* to *in vovo in* the yam tubers [15]. The severity of dry rot in the tubers of *Dioscorea species* studied is directly proportional to the fungal species causing the rot; two different fungal species caused varied rot depths on the same yam tuber species. There was significant difference in the susceptibility of tubers of *Dioscorea* species (*D. rotundata, D. alata and D. cayenensis*) to rot caused by *Aspergillus niger* and *Rhizopus stolonifer*. This result is in agreement with the report of [12] which state that severity of post-harvest loss appears to vary among the different varieties of yams. [7] Also suggested this variance in susceptibility; has they have reported that susceptibility of white yam to rot pathogen varies within the head and the tail region. The significant difference in susceptibility observed can be trace to the uneven distribution of phytoalexins in the yam tubers [16]. Because [7] pointed out that the presences of phytochemicals confer resistance in plants against bacteria, fungi and pest. They also inhibit the growth of pathogens at various stages of growth and in storage [7]. The Phytochemical screening of all the three tubers of *Dioscorea* species studied tested positive for all the phytochemicals tested (Alkaloid, Flavoid, Saponin, Tanin and Phenol) at varied composition. There was significance difference in the phytochemical contents of the three tubers of *Dioscorea* species studied; *D. cayenensis* contain high level of Saponin, Flavonoid and Alkaloid compared to the quantities found in *D. alata* and *D. rotundata* respectively. These results are in agreement with the research of [13] on the "Evaluation of Phytonutrients, Mineral and Vitamin Contents of some Varieties of Yam (*Dioscorea sp.*)". The presence of high Saponin (17.21mg), Flavonoid (6.81mg) and Alkaloid (0.76mg) in *D. cayanensis* might be responsible for the resistance observed in the species against the low quantities in *D. rotundata* and *D. alata* respectively [16, 9].

5 Conclusions

The varieties of yams cultivated in Anyigba, Kogi State suffer rots from various fungi which are similar to those reported in other parts of the country. Severities of post-harvest losses appear to vary among the different species of yam tuber studied. The fungus/fungi causing the rot also plays a major role in severity. Data from this work suggests that *D. cayanensis* would store better than all other varieties studied. The phytochemical screening of D. *rotundata, D. alata and D. cayenensis* revealed that three metabolites; namely Saponin, Flavonoid and Tanin constitute the major trait for resistant ability of *D. cayanesis* and *D. alata* to dry rot causing pathogens. The phytochemicals content of *D. cayenensis* and *D. alata* quantitatively is higher than that of *D. rotundata*. This research therefore recommends that: The Development of storage techniques in future should take the chemical and physical differences among yam species into consideration. Plant breeders should carry out research through genetic engineering with the aim of improving on *D. rotundata* resistant ability. Since moisture content is an important factor in food preservation, quality and resistant to deterioration. Therefore, it will be necessary in future to also investigate the effect of moisture content on the susceptibility of *Diocorea* tuber species to dry rot pathogens.

6 Competing Interests

The authors report no conflicts of interest, the author solely responsible for the content and writing of this article.

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