

Proximate Content, Phenol and Mineral Fertilizer Effects on Infection and Weight Loss in *Dioscorea Rotundata* (Three Varieties) and *D. Alata* (One Variety) Incubated with *Botryodiplodia Theobromae*



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Abstract

Resistance to tuber rot by *Botryodiplodia theobromae* in relation to tuber phenol and proximate content was investigated in this study in variety Agbodo of *Dioscorea alata* and varieties Oniyere, Iseosi and Abuja of *D. rotundata*. The four varieties harvested from a field plot where they had been fertilized with an 8-mineral content fertilizer (Bounty), were incubated with *B. theobromae* for 4 weeks along with their counterpart (the same four varieties) sourced from farmers' plots. The experiment was a 4x3 factorial, with 4 main factor treatments (variety), 3 subfactor treatments for phenol and mineral content in a 4 by 2 factorial experiment, as resistance to tuber infection by *B. theobromae* had correlated significantly with phenol and mineral uptake in white guinea yam (unpublished). Mineral fertilizer effects in infection (2.27%) and weight loss (2.11%) was evident as both were 97% lower and 67% lower respectively than those recorded for two improved *Dioscorea* varieties fertilized with calcium carbonate in literature. However, 0.36% (infection)/0.49% (weight loss) of FPT tubers were significantly lower than those of the fertilized tubers. Lower moisture and higher phenol content in Oniyere did not reflect in lower weight loss or infection than the other varieties. Analysis of tuber phytochemicals including phenol/phenolic compounds generally as well as minerals in the tuber which are all implicated in infection reduction should explain varietal response.

Introduction

Yam *Dioscorea* species is a staple food in the tropics and humid regions of the world. Major production is in West Africa and Nigeria had been the world's largest producer for several decades. The importance of yam is exhibited in it being a food security and income generating crops, as well as one with components useful for pharmaceuticals. Yams also display a variety of uses socially and in ethnic religion in many areas of the world such as West Africa where some species are Indigenous, such as the guinea yams (*D. rotundata* Poir and *D. cayenensis* Lamk.) *Dioscorea alata*, water or greater yam is popular along with the white and yellow guinea yams in Nigeria. Higher levels of protein as well as various Vitamins and minerals make yams more nourishing than cassava - the poor man's food. Production is more labour intensive than for cassava and constraints therein makes it lag demand both locally and in international foreign markets. Major losses in stored tubers are caused mainly by fungi [1] such as *Aperpillus species*, *Penicillium species*, *Botryodiplodia theobromae*, *Rhizopus nodulans* and *R. stolonifer* among others. These annually losses in millions of metric tons weight was estimated at 7million [2]. Minerals nutrition for disease resistance remain relatively cost effective than chemical

control with attendant-environmental/health hazards [3]. This study investigated the response of 3 common white guinea yam varieties and 1 water yam variety in South West Nigeria to infection after mineral fertilization, compared to these same varieties sourced from farmer's plots. The objective of the study was:

- To determine phenol and proximate content in bounty-fertilized *D. rotundata* vars Oniyere, Iseosi and Abuja and *D. alata* var. Agbodo, after incubation with *Botryodiplodia theobromae* for 3 weeks.
- To relate phenol content and moisture in the fertilized tubers to infection and weight loss, after incubation with *Botryodiplodia theobromae*.

Materials and Methods

Source of Tubers

One set of tubers of the three varieties Oniyere, Iseosi and Abuja of *Dioscorea rotundata* and var. agbodo of *D. Alata* were sourced from a yam project plot at the Teaching and Research Farms of DUFARMS (Directorate of University Farms), Federal University of

Agriculture, Abeokuta, Ogun State, Nigeria. The tubers had been fertilized in the yam project plot with bounty fertilizer containing eight minerals namely Calcium 7% w/w, Magnesium oxide 0.50% w/w, manganese 1% w/w, Iron 2% w/w, Zinc 2% w/w, Copper 0.50% w/w, Boron 0.0025% w/w, and Nitrogen 15% w/w. Rate of fertilization was 0.60 ml l⁻¹ and 1.20 ml l⁻¹ of Bounty fertilizer. Another set of the four varieties were sourced from farmers plots within Ogun State (South West region of Nigeria).

Infection and Weight Loss Determination After Inoculation and Incubation with *Botryodiplodia Theobromae*

A 4 by 3 factorial experiment was carried out arranged in RCBD (randomized complete block design) with 3 replicates. Main plot factor was variety (four) and the 3 subplot factors were fertilizer namely 0.60 ml l⁻¹ bounty fertilizer, 1.20ml l⁻¹ bounty fertilizer and no bounty or farmers plot tuber (FPT). Tubers were inoculated with a 5mm agar disc of a 7-day old pure culture of *Botryodiplodia theobromae*. The 5mm discs of *B. theobromae* were introduced into the surface sterilized tubers with sterilized scalpel, forceps and 5mm cork borers; after 10-12mm deep holes had been made in the tubers with sterilized 6 mm cork borers. The inoculation site of each tuber was sealed with pure petroleum jelly after the cut-out tissue had been inserted into the hole. Arrangement of inoculated tubers was in ventilated, wire-netted wooden yam storage structures inside the COLPLANT (College of Plant Science and Crop Production), Federal University of Agriculture (FUNAAB) screenhouse for 3 weeks. Tubers were weighted before inoculation with a top loading mettler balance. After 3 weeks, weights of the tubers were again recorded. The infection site on each tuber was cut open after the Vaseline was cleared with spatula and cotton wool, with a steel knife. Infected tissue was carefully removed with a sharp scalpel on to pre-weighted aluminum foil. Infected tissue weight was determined with an electronic balance.

Weight loss was determined using the formula % Weight loss (Y) = ((A-B)/A)100, Where A and B are weight of tuber before inoculation and at the end of the experiment respectively. Infection was determined using the formula % Infection = (C/A)100, Where C = corrected weight of infected tissue.

And $C = 100X / (100 - Y)$, [4].

Where X = weight of infected tissue and Y = % Weight loss.

And A = Weight of tuber before inoculation.

Data Analysis

Percent data were transformed appropriately before analysis of variance. Means were separated using Tukey's Studentized range (HSD) test.

Results

Infection and Weight Loss in Two Species of *Dioscorea* (Four Varieties) From a Bounty-Fertilized Yam Project Plot and From Farmers Plots After 3 Week Incubation with *Botryodiplodia Theobromae*

The range of infection in the four varieties was 1.028% to 2.119% with no significant difference (Table 1). Range of infection in the subfactor means, that is fertilizer rate including farmer's plot, was 0.364% to 2.714%. The farmers plot tubers had significantly lower infection of 0.364%, than the 0.60 ml l⁻¹ and 1.20 ml l⁻¹ Bounty-fertilized tubers which had 1.817% and 2.714% respectively (Table 1). Interaction of variety x fertilizer, or variety x FPT (farmers' plot) had no significant differences in infection as shown in table 1. Varietal weight loss range in the 3 weeks was 1.37% to 1.94% with no significant differences (Table 1). Tubers from farmers; plot however had lower weight loss of 0.49% than the 1.20 ml l⁻¹ and 0.60 ml l⁻¹ bounty-fertilized tubers which had 2.11% and 2.10% respectively. The bounty-fertilized tubers were not significantly different from one another in weight loss. Interaction of Agbodo x FPT (farmers plot) was 0% for weight loss. It was significantly different (lower) than the bounty-fertilized tubers of Abuja which had 2.48% (Abuja x 1.20 ml l⁻¹ and 2.51% (Abuja x 0.60 ml l⁻¹) weight loss. The bounty fertilized tubers of Abuja also had significantly higher weight loss than Oniyere x FPT which had 0.32% weight loss. The seven remaining interactions (weight loss of 0.32% to 2.06%) were not significantly different from Agbodo x FPT as shown in (Table 1).

Table 1: Infection and weight loss in two *Dioscorea* spp. (four varieties) from a Bounty-fertilized yam plot and from farmers' plots after 3 weeks incubation with *Botryodiplodia theobromae*.

Variety	% infection	% weight loss
Isesoi (<i>D. rotundata</i>)	2.119a	1.40a
Oniyere (<i>D. rotundata</i>)	1.844a	1.57a
Abuja (<i>D. rotundata</i>)	1.536a	1.94a
Agbodo (<i>D. alata</i>)	1.028a	1.37a
Fertilizer (Bounty)		
F1 (0.60ml l ⁻¹)	1.817a	2.10a
F2 (1.20ml l ⁻¹)	2.714a	2.11a
FPT (Farmers' Plot)	0.364b	0.49b
Interaction		
Iseosi X F1 (0.60ml l ⁻¹)	2.893a	1.71abc
Iseosi X F2 (1.20ml l ⁻¹)	3.163a	1.67abc
Iseosi X FPT (farmers' plot)	0.300a	0.82abc
oniyere X F1 (0.60ml l ⁻¹)	1.753a	2.20ab
Oniyere X F2 (1.20ml l ⁻¹)	3.497a	2.20ab
Oniyere X FPT (farmers' plot)	0.283a	0.32ab
Abuja X F1 (0.60ml l ⁻¹)	1.260a	2.51a
Abuja X F2 (1.20ml l ⁻¹)	2.997a	2.48a
Abuja X FPT (farmers' plot)	0.350a	0.82abc
Agbodo X F1 (0.60ml l ⁻¹)	1.360a	2.06abc
Agbodo X F2 (1.20ml l ⁻¹)	1.200a	2.06abc
Agbodo X FPT (farmers' plot)	0.523a	0.00c

Means in any column followed by a common letter are not significantly different at P = 0.05 (Tukey's test).

Proximate and Phenol Content Analysis

Table 2: Proximate content (% dry matter) and Phenol (mg/100g dry matter) in two Dioscorea Species (four varieties) from a bounty-fertilized Yam plot after 3 week incubation with *Botryodiplodia theobromae*.

Yam variety	Moisture	Dry matter	Fat	Ash	Crude fiber	Crude protein	Carbohydrate	Phenol
ABUJA (AB)	13.90a	86.10b	0.55b	2.78a	8.75a	4.16b	83.80ab	135.26b
AGBODO (AA)	13.59a	86.41b	0.62ab	2.73a	8.80a	4.04b	83.84a	133.87b
ISEOSI (IS)	12.78a	87.22b	0.63a	2.72a	8.61a	4.05b	84.09a	122.89b
ONIYERE (ON)	8.38b	91.70a	0.59ab	2.73a	9.04a	4.92a	83.38b	163.55a
Bounty Fertilizer (ml l ⁻¹)								
1.2	12.76a	87.25a	0.60a	2.75a	8.73a	4.20a	83.73a	138.83a
0.6	11.58a	88.43a	0.59a	2.72a	9.16a	4.38a	83.83a	142.45a
Interaction								
AB X 1.20	15.16a	84.85c	0.57a	2.81a	9.07b	3.82d	83.73a	126.99cd
AB X 0.60	12.65ab	87.35bc	0.53a	2.75ab	8.46d	4.49bc	83.87ab	143.52bc
AG X 1.20	14.23ab	85.77bc	0.61a	2.73ab	8.82bc	4.00d	83.86ab	132.00bcd
AG X 0.60	12.96ab	87.05bc	0.63a	2.74ab	8.75bcd	4.09cd	83.81ab	139.46bc
IS X 1.20	13.42ab	86.58bc	0.62a	2.72ab	8.60cd	3.98d	84.07a	137.90bcd
IS X 0.60	12.15b	87.85b	0.65a	2.71ab	8.63cd	4.11cd	84.11a	107.88d
ON X 1.20	8.22c	91.78a	0.60a	2.74ab	8.45d	5.02a	83.24b	158.42ab
ON X 0.60	8.55c	91.47a	0.57a	2.68b	10.81a	4.82ab	83.52ab	178.93a

Dioscorea alata var. Agbodo

Dioscorea rotundata vars. Abuja, Iseosi and Oniyere

Means in any column followed by a common letter are not significantly different at P = 0.05 (Tukey's test)

Proximate analysis showed that ash and Crude fiber were not significantly different among the four varieties, as shown in (Table 2). Carbohydrate was lower in Oniyere, 83.38%dm, than in Agbodo and Iseosi which are 83.84%dm and 84.09%dm respectively. It was not significantly different from Abuja which is 83.80%dm (Table 2). Crude Protein content of 4.92dm was higher in Oniyere than the other three varieties. Fat content of 0.63%dm in Iseosi was significantly higher than in Abuja which is 0.55%dm. Fat content in Abuja (0.55%dm), Agbodo (0.62%dm), and Oniyere 0.59%dm were not significantly different from one another. Dry matter content was higher significantly in Oniyere 91.70% than the other three varieties which were 86.10% (Abuja) 86.41% (Agbodo) and 87.22% (Iseosi) as shown in (Table 2). Moisture content of 8.38%dm Oniyere was significantly lower than in Abuja 13.90% dm), Agbodo (13.59% dm) and Iseosi (12.78% dm) (Table 2). Phenol content was higher in Oniyere, 163.55 mg/100gdm, than any of the other three varieties. The latter which had 135.26 mg/100gdm (Abuja), 133.87 mg/100gdm (Agbodo) and 122.89 mg/100gdm (Iseosi) are not significantly different from one another in phenol content (Table 2). There were no significant differences in any proximate component in terms of either 0.6 ml l⁻¹ or 1.2 ml l⁻¹ fertilizer treatment. In variety x fertilizer interactions phenol content of Oniyere x fertilizer (0.60ml l⁻¹) and Oniyere x fertilizer (1.20 ml l⁻¹) were 178.93mg/100gdm and 158.42mg/100gdm respectively significantly higher than

Iseosi x fertilizer (0.60ml l⁻¹) which is 107.88%dm and Abuja x fertilizer (1.20 ml l⁻¹) which is 126.99mg/100gdm. Phenol content in the remaining five interactions were not significantly different from one another as shown also in (Table 2).

Discussion

Proximate and phenol content is compared only in the tubers fertilized with an 8- mineral component (Ca, N, MgO, Mn, Zn, Cu, Fe, B) fertilizer namely Bounty. This is because resistance to the tuber rot pathogen *B. Theobromae* had been observed to correlate significantly with mineral uptake in white guinea yam (unpublished). However, both infection and weight loss were lower in the tubers from farmers plot (not Bounty fertilized) than in the bounty-fertilized tubers. Phenolics are often implicated in the resistance to microorganisms in plant species [5]. Phenol content was highest in variety Oniyere, but this did not reflect in a lower tuber infection compared to the other three varieties. But generally low infection of below 2% was recorded for each of the four varieties. Variety x fertilizer effects showed 0% weight loss in Agbodo x FPT and it was significantly lower than Oniyere x 0.60 fertilizer, Oniyere x 1.20 fertilizer, Abuja x 0.60 fertilizer and Abuja x 1.20 fertilizer all of which had weight loss of over 2%. *Dioscorea alata* is reported to have a longer storage of the fresh tubers than white guinea yam, [6]. *D. rotundata*. Lower moisture content has

been reported to enhance/prolong shelf life in *Dioscorea alata* [7]. Moisture content in all four varieties in this study was over 8% and was lowest in the white Yam Oniyere. Low moisture commensurate to low infection or low weight loss may be varietal as the cited report recorded moisture levels of only 6% in the varieties of water yam studied [7]. Analysis of total phenolic compounds and minerals which are all implicated in reduction of infection/disease [3,8] should explain varietal responses or response of tubers (storage organs) to pathogenic infection. However, mineral nutrition effects in reducing disease is evident as infection and weight loss in this study are only 97% and 67% respectively of that in a previous report, where Calcium carbonate reduced both infection and weight loss in three improved varieties of white and water yam (*Dioscorea* species) [9].

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