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Calcium Nitrate Fertilizer Effect on Anthracnose Disease Caused by *Colletotrichum Gloeosporioides* penz. and Tuber Rot by *Botryodiplodia Theobromae* pat. in Dioscorea Alata Variety Alakisa



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Abstract

Three or more years fallow may not replenish enough for optimum quality of Dioscorea (food cultivars) tubers. This study indicated this because Calcium and Nitrogen of values 0.47cmol kg $^{-1}$ and 0.2% respectively were still below the critical determined for optimum yam production (South West Nigeria) even after three years fallow followed by two consecutive years of calcium nitrate fertilization to Dioscorea on the field plot used in this study. The experiment was a 3 by 3 factorial, arranged in RCBD and 3 replicates. Ca $(NO_3)_2$ soil amendment at 0, 1, 2kg ha $^{-1}$ of calcium was the main plot treatment applied at 3 MAP (months after planting) and one foliar spray only at the rates 0, 100 and 500mg I^{-1} Ca $(NO_3)_2$ applied at 5 MAP. Ca $(NO_3)_2$ soil amendment (1 and 2kg ha $^{-1}$) and 100mg I^{-1} Ca $(NO_3)_2$ foliar spray all promoted more leaf phytoanticipins namely flavonoid, lignin and tannin over the control. Leaf minerals namely N, P, Fe and S increased in the soil amendment treatments over the control and N, P and Fe in turn correlated significantly with the three phytoanticipins with their antimicrobial activities.

The $2 \text{kg ha}^{-1} \times 0 \text{mg l}^{-1}$, $0 \text{kg ha}^{-1} \times 100 \text{mg l}^{-1}$ gave anthracnose severity score of 1 which is resistant level. The $1 \text{kg ha}^{-1} \times 500 \text{mg l}^{-1}$, $1 \text{kg ha}^{-1} \times 100 \text{mg l}^{-1}$ both gave anthracnose severity score of 2 which is moderately susceptible. Only the highest fertilizer interaction of $2 \text{kg ha}^{-1} \times 500 \text{mg l}^{-1}$ gave susceptible severity score of $3 \text{cm}^{-1} \times 500 \text{mg}$ leave susceptible severity score of $3 \text{cm}^{-1} \times 500 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety the $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible variety susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave susceptible. Only the highest fertilizer interaction of $3 \text{ma}^{-1} \times 300 \text{mg}$ leave suscepti

Introduction

Yam (Dioscorea species) is a climbing, vigorously twinning plant. They are perennial through root system but are grown as annual crops [1]. The food cultivars are staples with socio-economic importance in Africa, the Caribbean, Asia and Latin America [2]. West Africa grows over 90% of the worldwide production (40tonnes fresh tubers/year), followed by the West Indies where Jamaica is the leading producer [3]. The popular cultivated species in Nigeria are the white guinea yam (D. rotundata), water yam (D. alata), yellow guinea yam (D. cayenensis) and trifoliate yam (D. dumetorum). Yam is an important staple food crop second to cassava in terms of land area under cultivation in Nigeria (Chukwu and Ikwell, 2000). Most of the world production of yam is from West Africa (over 90%) with Nigeria alone accounting for nearly 75% of the total world production [4]. One of the major constraints to the production of yam is disease. Economic diseases occurring on yams from planting to harvest include Anthracnose (Colletotrichum gloeosporioides), seed tuber rot (Aspergillus spp, Botryodiplodia

theobromae, Rhizopus spp, Penicillium spp., Fusarium oxysporum and F. solani), root rot (F. oxysporum and Phythium sp.), stem basal canker (F. oxysporum), vascular wilt (F. oxysporum), virus diseases (shoe string and mosaic viruses). Leaf spots (Curvularia eragrostidis, C. geniculate and Cercospara sp.) [5].

Anthracnose caused by the fungus *Collectrotrichum gloesoporioides* is the single largest constraint for yam production in the tropical and temperate regions [6]. *Collectrotrichum gloesoporioides* Penz. infects mainly leaves, although it causes anthracnose symptoms on all plant parts, which appear initially as small brown spots that extend and cause extensive blackening of the leaves as the disease progresses [7]. It reduces the photosynthesis efficiency of yam plants, which severely limits tuber production and results in heavy yield loss (80%, 85%) in susceptible genotypes [6-8]. Postharvest deterioration and rot caused by various microorganisms especially fungi are also seen as the single most important factor militating against commercial yam production

in Nigeria. Yam storage rots account for loses of 7 million metric tonnes annually (Taiga, 2001). *Botryodiplopodia theobromae* is one of the four most important pathogens responsible for tuber deterioration in stored yams [9].

Resistance breeding in yam anthracnose control is continuous as varietal resistance breaks down with environment and anthracnose pathogenic strains may develop resistance [10]. Cultural practices and fungicides, where affordable are being used also to manage anthracnose disease. Management of yam storage rot pathogens has also been through usage of fungicides, avoidance of wounds/injuries that promote microbial attack, cultural practices such as improved storage methods and biological control [11]. Managing crop disease through mineral nutrition deemphasizes pesticide usage with their attendant hazards and is cost effective in sustainable agriculture [12]. Calcium fertilization or calcium foliar feeding has been reported to enhance resistance to disease [13]. Combination of soil application of Ca $(NO_3)_2$ and foliar spray of fertilizer containing Ca2+ and Mg2+ resulted in significant reductions in severity and incidence of Phytophthora infestans, Septoria lycopersici and Alternaia solani on Solanum lycopersicum (tomato) vars. Rio de Grenier and Rona in Cameroon [14]. Apple fruit quality and fruit N/Ca and K/Ca ratios were improved by soil application of calcium nitrate fertilizer [15]. Soft rots by Erwinia carotovora subsp. Atrospetica in potatoes, Solanum tuberosum have been reduced by calcium sulphate and calcium nitrate fertilizers [16]. Most recently CaCO₃ and NPK reduced tuber rots by A. niger and B. theobromae in three varieties of Dioscorea species [17]. This study aimed at determining the effect of soil amendment and foliarsprayed Calcium nitrate on Anthracnose disease and tuber rot caused by Botryodiplodia theobromae in D. alata var. Alakisa.

Materials and Methods

Field Layout

The field plot has been monocropped to Dioscorea species for two previous consecutive years (2015 and 2016) with Ca $(NO_3)_2$ fertilizer. The experiment was a 3 by 3 factorial arranged in RCBD (randomized complete block design) with three replications. The plot size was 14m by 9.5m, with 1m by 1m mounds, 80cm high. A 2m bamboo stake was established per mound. Intra-row distance was 0.5m while inter-row and inter-replicate distance were 1m each. A field border 1m all round was maintained. Eighteen (18) plants occupied a replicate with a total of 54 plants.

Soil Sampling and Analysis

Soil samples were collected by replicate with a soil auger before mound making. Twenty (20) core samples were collected per replicate lifted in the zig-zag pattern at 20cm depth in a slanting position (to include top and inner soil) into new labelled polythene bags (Page et al. 1982). They were transferred to the laboratory, airdried and sieved with a 2mm sieve, after which they were bulked together per replicate for analysis. Exchangeable cations were analyzed using standard methods, of the A.O.A.C. [18].

Fertilization with Calcium Nitrate

The three soil amendments rates were $0 kg ha^{-1}$, $1 kg ha^{-1}$ and $2 kg ha^{-1}$ Ca $(NO_3)_2$ applied at 3 MAP (months after planting) in a groove

of 25cm radius, about 20cm deep, around the plant on the mound [19]. The groove was covered well with soil after even sprinkling of the fertilizer in the groove. Foliar spray of Ca $(NO_3)_2$ dissolved in deionized water was administered once at 5 MAP, at the rates 0mg l^{-1} , 100mg l^{-1} and 500mg l^{-1} , with the surfactant polysorbate 20/Tween 20 $(C_{58}H_{14}O_{26})$ at the rate of 0.01%.

Leaf Sampling and Analysis at 6 MAP

Leaves were sampled along a diagonal transect across the field plot 4 weeks after the foliar spray. Four (4) leaves each from the top, middle and lower portion were collected into new large labelled paper envelopes in the early hours, 7:00 to 8:00am, and transferred to the laboratory. They were washed in 2 changes of deionized water, before spreading in new labelled food paper packs to dry, on raised wooden storage structures in the COLPLANT (College of Plant Science) screen house. The dried leaves were milled in a 4-speed Saisho blender and re-packed in new labelled paper envelopes for analysis in the Biological Sciences Laboratory and Biotechnology Centre, Federal University of Agriculture, Abeokuta. Three phytochemicals namely flavonoid, lignin and tannin and seven minerals namely Nitrogen, Magnesium, Potassium, Calcium, Iron, Phosphorus and Sulphur were analyzed using standard methods [18].

Disease Incidence and Severity Assessment

Anthracnose disease incidence was assessed at 3 MAP before soil amendment fertilization. It was also assessed four (4) weeks after foliar-spray fertilization of 5 MAP. Disease severity was determined on an Anthracnose severity scale of 1 (resistant) to 5 (susceptible) in Figures 1-3 [20] at 6 MAP.

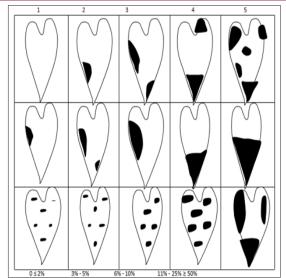


Figure 1: Yam leaf anthracnose disease assessment diagram+lesions on petiole and stem.

No symptom/ $0 \le 2\%$ severity/resistant/highly resistant Necrotic spots 1.00mm-2.00mm/3-5% severity/moderately resistant (tolerant)

Necrotic spots 2.01mm-3.00mm/6-10% severity/moderately susceptible

Necrotic spots 3.01mm-4.00mm/11-25% severity/susceptible

Necrotic spots >4.00mm/50% severity/very susceptible Modified from Green (1994)

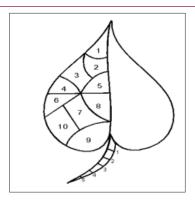


Figure 2: Leaf anthracnose assessment.

Petiole $1/5 \times 100 = 20\%$

Leaf BLADE 1/10 = 5%, 2/10 = 10% ETC.

10 Leaves UPPER PORTION (young = tender/lighter texture)

10 Leaves MIDDLE PORTION (maturing = green/expanded/big)

10 Leaves LOWER PORTION (fully expanded + well-formed Cuticle/texture leathery)

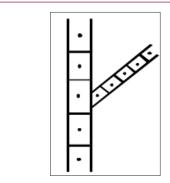


Figure 3: Vine and Petiole anthracnose assessmentPetiole

Tuber Sampling for Infection and Weight loss Experiment

The 6 MAP tuber sampling for tissue analysis and the infection/weight loss experiment was done one day before the 6 MAP harvest. Sampling was along the second diagonal transect (the first was that used in the leaf sampling) across the field plot in the early morning (7.00am-8.00am). Plants were labelled, and treatments were also randomized along the diagonal transect. Three unbruised tubers each were selected per treatment for tissue analysis and for the infection and weight experiment. Sampling in a mound was done by moving soil away carefully with the wooden spoon from the roots of the plant until at least six tubers/or more were exposed. Each tuber chosen, was then turned clockwise until it was free from the shoot. Tubers were freed from soil with a soft cloth.

Infection and Weight Loss Experiment

Triplicate tubers set aside for the infection and weight loss experiment were labelled and weighed with a top-loading mettler balance, before inoculation. Inoculation of tubers was done inside the Inoculation Hood Structure of the Crop Protection Laboratory (COLPLANT Building), FUNAAB. Before inoculation, the point to be inoculated on each tuber (inoculation site) was surface-sterilized with cotton wool soaked in 80% ethanol. Two cork borers (6mm

and 4mm diameter), scalpel and a pair of forceps were surfacesterilized, by dipping in 80% ethanol and flaming over a lighted spirit flame bottle until red-hot. They were then cooled, slanted against another cork borer. The sterile and cooled 6 mm cork borer was then used to make a 20mm to 25mm deep incision (based on the size of the tuber) through the inoculation site of each tuber. After this, the incised yam tissue was freed and lifted out with the sterilized scalpel and forceps. A 4mm agar (potato dextrose agar) disc of a 7-day old pure culture of Botryodiplodia theobromae growing inside a 9cm petri-dish, was then removed with the surface-sterilized 4mm cork borer and put in the hole inside the tuber. The incised tissue was then replaced, and the top of the inoculation site sealed with Vaseline (petroleum jelly). Inoculated tubers were labelled and transferred again to the yam storage structures in the COLPLANT Screenhouse for an incubation period of 2 weeks. After this, the tubers were transferred to the Crop Protection Laboratory. Each tuber was cleared of Vaseline with a spatula and cotton wool. Their weights were recorded again with a top loading mettler balance. Thereafter, each tuber was cut into two across the inoculation site with a steel knife. Infected tissue from each half was cut into a pre-weighed plastic Petri dish with a scalpel. Infected tissue weight was measured with an electronic balance.

Percent weight loss was determined with the formula:

% Weight loss
$$(Y) = \left(\frac{A-B}{A}\right) 100$$

where A and B are weight of the tuber at the beginning and at the end of the experiment respectively.

Percent infection was determined with the formula:

% infection =
$$\left(\frac{C}{A}\right)100$$

where C and A are weight of infected tissue corrected for normal weight loss in the tuber and weight of the tuber at the beginning of the experiment respectively.

C was determined with the formula =
$$\frac{100X}{100-Y}$$

where X and Y are, weight of infected tissue and percent weight loss respectively [21].

Yield Assessment at 6 MAP

Harvest was early morning (7:00 to 8:00am) with smooth wooden spoons to avoid bruising of tubers. Tubers were freed from the yam shoots in each mound in the same manner as for the sampling for the infection and weight loss experiment (section 2.6). n the harvested tubers were left to dry for about 3 hours on the mounds, after which number of tubers per mound were counted and total weight of tubers per plant was then measured with a top loading mettler balance (field scale).

Data Analysis

Data were subjected to analysis of variance (ANOVA). Percent data were transformed appropriately before analysis of variance. Means were separated with Tukey's (HSD) test.

Results

Soil Analysis

Results of the soil analysis is presented in Table 1. Soil pH was 7.33. Calcium and Nitrogen in the soil site of the values $0.47 \, \mathrm{cmol} \, \mathrm{kg}^{-1}$ and 0.199% respectively, were both below the critical determined for optimum yam production in South West Nigeria [22]. Values for potassium, magnesium and phosphorus in the soil site were $0.66 \, \mathrm{mol} \, \mathrm{kg}^{-1}, 0.51 \, \mathrm{cmol} \, \mathrm{kg}^{-1}$ and $46.52 \, \mathrm{mg} \, \mathrm{kg}^{-1}$ respectively.

<u>Table 1:</u> Mean values of soil properties of the experimental site.

pH (in water)	7.333±0.11	
Ca (cmol k ⁻¹)	0.467±0.005	
Mg (cmol k⁻¹)	0.514±0.003	
K (cmol k ⁻¹)	0.663±0.005	
N (%)	0.199±0.007	
P (available mg k⁻¹)	46.516±0.62	

Disease Incidence and Severity at 6 MAP

Main plot means for disease incidence (DI) at 6 MAP was in the range 61.11% to 77.78% as shown in Table 2, and subplot means was in the range 55.56% to 83.33%. There were no significant

differences between the treatments. Interactions of treatment combinations $M_{_0}T_{_2}$ (0kg ha^-1 x 500mg l^-1), $M_{_1}T_{_0}$ (1kg ha^-1 x 0mg l^-1) and $M_{_2}T_{_2}$ (2kg ha^-1 x 500mg l^-1) gave significantly higher DI of 100% than other treatments. Treatment combination of significantly lower DI of 66.67% was recorded in $M_{_0}T_{_0}$, $M_{_0}T_{_1}$, $M_{_2}T_{_0}$ and $M_{_2}T_{_1}$ as shown in Table 2. The third lowest DI of 50% was recorded in the 1kg ha^-1 x 500mg l^-1 ($M_{_1}T_{_2}$) treatment and the lowest of 33.33% was recorded in the $M_{_1}T_{_1}$ (1kg ha^-1 x 100mg l^-1) treatment combination.

Mean main plot and subplot disease severity score was 2.41 (moderately resistant/tolerant) Table 2. Interaction treatments 0kg ha⁻¹ x 100mg l⁻¹ and 2kg ha⁻¹ x 0mg l⁻¹ had the score of 1 (resistant). The 1kg ha⁻¹ x 100mg l⁻¹ and 1kg ha⁻¹ x 500mg l⁻¹ had a score of 2 (tolerant/moderately resistant). 0kg ha⁻¹ x 0mg l⁻¹, 0kg ha⁻¹ x 500mg l⁻¹, 1kg ha⁻¹ x 0mg l⁻¹ and 2kg ha⁻¹ x 100mg l⁻¹ had the score of 2.67 to 3.33 (moderately susceptible). Lastly the highest soil amendment x foliar spray treatment, 2kg ha⁻¹ x 500mg l⁻¹ had the score of 3.67 (susceptible). There were significant differences in disease severity score in main plots, subplot or interaction treatments. Disease severity score correlated positively and significantly with disease incidence, leaf phosphorus and leaf potassium, r=0.4373, r=0.4350 and r=0.4054 Table 3.

Table 2: Disease incidence and severity in calcium-fertilized Dioscorea alata variety Alakisa at 6 months after planting.

Fertilizer (Soil Amended)	Disease Incidence (%) at 6MAP	Disease Severity Score at 6MAP					
	Ca (NO ₃) ₂ kg ha ⁻¹						
$(M_0) 0$	77.78a	2.333a					
(M ₁) 1	61.11a	2.333a					
(M ₂) 2	77.78a	2.556a					
	Foliar Spray (CaNO ₃) ₂ in Mg L ⁻¹						
(T_0) 0	77.78a	2.444a					
(T ₁) 100	55.56a	2.000a					
(T ₂) 500	83.33a	2.778a					
	Interaction						
M _o T _o (0kg ha ⁻¹ X 0mg L ⁻¹)	66.67b	3.33a					
M _o T ₁ (0kg ha ⁻¹ X 100mg L ⁻¹)	66.67b	1.00a					
M _o T ₂ (0kg ha ⁻¹ X 500mg L ⁻¹)	100.00a	2.67a					
$M_1^{}T_0^{}$ (1kg ha ⁻¹ X 0mg L ⁻¹)	100.00a	3.00a					
M ₁ T ₁ (1kg ha ⁻¹ X 100mg L ⁻¹)	33.33d	2.00a					
M ₁ T ₂ (1kg ha ⁻¹ X 500mg L ⁻¹)	50.00c	2.00a					
M ₂ T ₀ (2kg ha ⁻¹ X 0mg L ⁻¹)	66.67b	1.00a					
M ₂ T ₁ (2kg ha ⁻¹ X 100mg L ⁻¹)	66.67b	3.00a					
M ₂ T ₂ (2kg ha ⁻¹ X 500mg L ⁻¹)	100.00a	3.67a					

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

Table 3: Three anticipins and mineral content (mg/100g dry matter) in leaves of calcium-fertilized *Dioscorea alata* variety Alakisa, at 6 MAP.

Fertilizer	Calcium	Mg	К	Flovonoid	Tannins	Lignin	Fe	N	P	S
Ca (NO ₃) ₂ Kg ha ⁻¹										
$(M_0) 0$	1751.8a	341.60a	94.84ba	1205.00a	4.77b	1410.0b	1.59a	831.7c	84.76a	1088.00b
(M ₁) 1	2185.7a	332.25a	63.31b	1166.67a	5.06a	1491.67a	1.64a	869.3b	84.19a	1124.67ba
(M ₂) 2	1589.3a	316.52a	105.47a	118.33a	5.10a	1496.7a	1.76a	930.7a	84.41a	1159.83a

Folair – spray (Ca (NO ₃) ₂ Mg L ⁻¹										
$(T_0) 0$	1717.5a	341.77a	87.81a	1125.0b	4.87a	1523.33a	1.49c	884.67a	83.75b	1090.67b
(T ₁) 100	1661.3a	312.75a	77.63a	1200.0a	4.94a	1453.33b	1.68b	879.67a	84.52ab	1135.17ab
(T ₂) 500	2148.0a	335.85a	98.19a	1230.0a	5.07a	1421.67b	1.82a	894.33a	85.10a	1146.67a
Interaction										
M _o T _o (0 kg ha ⁻¹ X 0 mg L ⁻¹)	1828.0c	335.10d	137.24a	1170.0ab	4.71d	1465.0c	1.48i	818.0f	85.17ab	1013.5f
M _o T ₁ (0 kg ha ⁻¹ X 100 mg L ⁻¹)	1628.0e	310.55f	48.26c	1240.0a	4.69d	1415.0cd	1.59f	834.0ef	84.18ab	1120.0e
M _o T ₂ (0 kg ha ⁻¹ X 500 mg L ⁻¹)	1799.5c	379.15a	99.03abc	1205.0ab	4.91bcd	1350.0d	1.73d	843.0def	84.93ab	1130.5d
M ₁ T ₀ (1 kg ha ⁻¹ X 0 mg L ⁻¹)	1548.5f	335.35d	63.35bc	1075.0b	4.85cd	1565.0a	1.50h	921.5bac	82.90b	1114.0e
M ₁ T ₁ (1 kg ha ⁻¹ X 100 mg L ⁻¹)	2001.0b	360.00b	63.59bc	1190.0ab	5.00abc	1470.0c	1.62e	875.0cde	84.57ab	1127.0d
M ₁ T ₂ (1 kg ha ⁻¹ X500 mg L ⁻¹)	3007.5a	301.40g	63.00bc	1235.0a	5.19a	1440.0c	1.79c	892.5bcd	85.12ab	1133.0d
M ₂ T ₀ (2 kg ha ⁻¹ X 0 mg L ⁻¹)	1776.0c	354.85c	62.84bc	1130.0ab	5.05abc	1540.0ab	1.52g	914.50bac	83.17ab	1144.5c
M ₂ T ₁ (2 kg ha ⁻¹ X 100 mg L ⁻¹)	1355.0g	267.70h	121.05ab	1170.0ab	5.14a	1475.0bc	1.83b	930.0ab	84.82ab	1158.5b
M ₂ T ₂ (2 kg ha ⁻¹ X 500 mg L ⁻¹)	1637.0d	327.00e	132.00a	1250.0a	5.10ab	1475.0bc	1.94a	947.5a	85.24a	1176.5a

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

Leaf Tissue Analysis (Phytochemicals) at 6 MAP: Flavonoid content in the range 1166.67 to 1230mg/100gdm in the mainplot treatments were not significantly different from one another Table 3. It was significantly lower in the control subplot 1125mg/100gdm than the 100 and 500mg l^{-1} treatments, 1200 and 1230mg/100gdm respectively Table 3. Tannin content of 5.06 to 5.10mg/100gdm was significantly higher in the 1kg ha⁻¹ and 2kg ha⁻¹ main plots than the control 4.77mg/100gdm.

Tannin content in the 2kg ha⁻¹ x 100mg l⁻¹, 1kg ha⁻¹ x 500mg l⁻¹ treatments were significantly higher (5.14mg/100gdm and 5.19mg/100gdm), than four others, 0kg ha⁻¹ x 0mg l⁻¹, 0kg ha⁻¹ x 100mg l⁻¹, 0kg ha⁻¹ x 500mg l⁻¹ and 1kg ha⁻¹ x 0mg l⁻¹ which were in the range 4.69 to 4.91mg/100gdm. Leaf lignin content was significantly higher, 1491.67 and 1496.70mg/100gdm, in the Ca (NO₃)₂ soil-amendment treatments (2kg ha⁻¹ and 1kg ha⁻¹), than the control (0kg ha⁻¹), 1410mg/100gdm. The control foliar-sprayed plants however had higher lignin content 1523.33mg/100gdm than the Ca (NO₃)₂ foliar-sprayed plants (100mg l⁻¹, 500mg l⁻¹) which were 1453.33mg/100gdm and 1421.67mg/100gdm.

Leaf Mineral Content: Leaf calcium was higher in the $1 \text{kg ha}^{-1} \text{ x } 500 \text{mg l}^{-1} \text{ treatment (} 3007.5 \text{mg/} 100 \text{gdm)} \text{ than other treatments,}$

Table 3. The lowest leaf calcium content of 1355mg/ 100gdm was in the 2kg ha-1 x 100mg l-1 treatment, while the seven other treatments varied significantly from one another as shown in Table 3. Calcium was not significantly different in the main plot or subplot treatments. Leaf Mg was not different in main plot or subplot treatments, but eight of the nine treatments, but eight of the nine treatment interactions were significantly different from one another, in increasing order as follows: 379.15mg/100gdm (0kg ha⁻¹ x 500mg l⁻¹), <360mg/100gdm (1kg ha⁻¹ x 100mg l⁻¹), < 354.85mg/100gdm (2kg ha⁻¹ x 0mg l⁻¹), < over 335mg/100gdm $(1 \text{kg ha}^{-1} \times 0 \text{mg l}^{-1} \text{ and } 0 \text{kg ha}^{-1} \times 0 \text{mg l}^{-1}), < 327 \text{mg}/100 \text{gdm}$ (2 kg $ha^{-1} \times 500 mg l^{-1}$ < 310.55 mg/100 gdm (0kg $ha^{-1} \times 100 mg l^{-1}$) < 301.4 mg / 100 gdm (1kg ha⁻¹ x 500 mg l⁻¹) < 267.7 mg/100 gdm (2kg ha-1 x 100mg l-1). There were no articulate significant differences in K (potassium) in the treatments. Leaf iron (Fe) content was significantly higher, 1.82mg/100gdm in the 500mg l-1 treatment than the 100mg l-1 treatment which was 1.68mg/100gdm, and this was significantly higher, 1.49mg/100gdm than in the 0mg l⁻¹.

Each of the nine interaction treatments were significantly different from one another also, in leaf iron as follows: 1.94 mg/100 gdm (2kg ha⁻¹ x 500 mg l⁻¹) < 1.83 mg/100 gdm (2

kg ha⁻¹ x 100mg l⁻¹) < 1.79mg/100gdm (1kg ha⁻¹ x 500mg l⁻¹) < 1.73mg/100gdm (0kg ha⁻¹ x 500mg l⁻¹) < 1.62mg/100gdm (1kg ha⁻¹ x 100mg l⁻¹) <1.59mg/100gdm (0kg ha⁻¹ x 100mg l⁻¹) < 1.52mg/100gdm (2kg ha⁻¹ x 0mg l⁻¹) < 1.50mg/100gdm (1kg ha⁻¹ x 0mg l⁻¹) < 1.48mg/100gdm (0kg ha⁻¹ x 0mg l⁻¹). Nitrogen content was significantly higher 930.7mg/100gdm in the 2kg ha⁻¹ treatment than the 1kg ha⁻¹ treatment which was 896.3mg/100gdm, which was in turn higher than the control (0kg ha⁻¹), 831.7mg/100gdm. Subplot treatments were not different in leaf Nitrogen content, but significant differences in the interactions are as shown in Table 3.

Leaf phosphorus was significantly higher $85.10 \, \text{mg}/100 \, \text{gdm}$ in the $500 \, \text{mg} \, \text{l}^{-1}$, Ca $(\text{NO}_3)_2$ treated plants than the control (0mg l⁻¹) which was $83.75 \, \text{mg}/100 \, \text{gdm}$ Table 3. Interaction effects in leaf phosphorus content $85.24 \, \text{mg}/100 \, \text{gdm}$ was significantly higher in 2kg ha⁻¹ x $500 \, \text{mg} \, \text{l}^{-1}$ than in 1kg ha⁻¹ x $0 \, \text{mg} \, \text{l}^{-1}$ which was $82.90 \, \text{mg}/100 \, \text{gdm}$. The remaining 7 interaction effects were similar Table 3. Sulphur content of $1159.83 \, \text{mg}/100 \, \text{gdm}$ in the 2kg ha⁻¹ Ca $(\text{NO}_3)_2$ treated plants was significantly higher than in

the control (0kg ha¹¹) which was 1088mg/100gdm. The higher foliar spray treatment, 500mg l¹¹ also had significantly higher sulphur content, 1146.67mg/100gdm than the control (0mg l¹¹), which was 1090.67mg/100gdm. All eight interaction effects were significantly higher than the control in sulphur content. The values are 1176.5mg/100gdm (2kg ha¹¹ x 500mg l¹¹) < 1158.5mg/100gdm (2kg ha¹¹ x 100mg l¹¹) < 1144.5mg/100gdm (2kg ha¹¹ x 0mg l¹¹) < 1127 to 1133mg/100gdm (1kg ha¹¹ x 500mg l¹¹, 0kg ha¹¹ x 500mg l¹¹, 1kg ha¹¹ x 100mg l¹¹) < 1120 to 1114mg/100gdm (0kg ha¹¹ x 100mg l¹¹, 1kg ha¹¹ x 0mg l¹¹) < 1013.5mg/100gdm (0kg ha¹¹ x 0mg l¹¹) Table 3.

Significant Correlations: Positive significant correlations between the leaf minerals are as shown also in Table 4. N significantly correlated with Fe and S, r=0.4662, 0.7043. P significantly correlated with Fe, r=0.4814. Fe with S, r=0.6841 and K with P, r=0.5794. The four minerals, N, Fe, S and P correlated positively and significantly with the phytochemicals Table 4, as follows:

Table 4: Significant Correlations of Disease incidence, severity, leaf anticipins, leaf minerals and yield in D. alata var. Alakisa.

	Flavonoid	Tannin	Lignin	Phosphorus	Potassium	Nitrogen	Sulphur	Disease incidence at 6 MAP	Total Tuber Number Per Plant
Lignin	-0.6231*								-0.3936*
Iron	0.4960*	0.6534*	- 0.3425*	0.4814*		0.4662*	0.6841*	0.6841*	
Nitrogen		0.7487*	0.5192*						
Potassium				0.5794*					
Sulphur		0.6681*				0.7043*			
Disease severity at 6 map				0.4350*	0.4054*			0.4373*	
Phosphorus		0.1282*							

^{*}Significant Correlation at P=0.001 to 0.05, n=27.

N with lignin and tannin, r=0.5192 and 0.7487 respectively. Fe with flavonoid and tannin, r=0.4960 and 0.6534 respectively. P with tannin, r=0.1282; S with tannin, r=0.6681. Significant negative correlations were between Lignin and flavonoid, r=-0.6231; Iron (Fe) and lignin, r=-0.3425.

Infection and Weight Loss Experiment

Tubers sampled at 6 MAP, inoculated and incubated with

Botryodiplodia theobromae for 2 weeks had mean infection of 0.89% to 1.17% (main plot means) and 0.68% to 1.17% as shown in Table 5 for subplot means. They were not significantly different from one another. Interaction effects gave a range of 0.50% to 1.95% in infection and no significant differences between them Table 5. Weight loss was 0.00% for main plot and subplot means, as well as for interactions Table 5.

<u>Table 5:</u> Infection (%) and weight loss (%) in tubers of D. alata var. Alakisa after two weeks incubation with *Botryodiplodia theobromae*, yield at 6 months.

Fertilizer (Soil Amended)	Infection (%)	Weight Loss (%)
Ca (NO ₃) ₂ kg ha⁻¹		
(M_0) 0	1.172a	0.00
(M ₁) 1	1.019a	0.00
(M ₂) 2	0.898a	0.00
Foliar spray (CaNO $_3$) $_2$ in Mg L $^{\cdot 1}$		
(T ₀) 0	0.684a	0.00
(T ₁) 100	1.428a	0.00

(T ₂) 500	0.978a	0.00
Interaction		
M _o T _o (0kg ha ⁻¹ X 0mg L ⁻¹)	0.95a	0.00
M _o T ₁ (0kg ha ⁻¹ X 100mg L ⁻¹)	1.86a	0.00
M _o T ₂ (0kg ha ⁻¹ X 500mg L ⁻¹)	0.70a	0.00
M ₁ T ₀ (1kg ha ⁻¹ X 0mg L ⁻¹)	0.50a	0.00
M ₁ T ₁ (1kg ha ⁻¹ X 100mg L ⁻¹)	1.34a	0.00
M ₁ T ₂ (1kg ha ⁻¹ X 500mg L ⁻¹)	1.24a	0.00
M ₂ T ₀ (2kg ha ⁻¹ X 0mg L ⁻¹)	0.59a	0.00
M ₂ T ₁ (2kg ha ⁻¹ X 100mg L ⁻¹)	1.10a	0.00
M ₂ T ₂ (2kg ha ⁻¹ X 500mg L ⁻¹)	1.00a	0.00

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

Yield at 6 MAP

Tuber number per plant was 3.17 to 9.06, with no significant differences in main plots, subplots or interactions Table 6. Tuber weight per plant was 2.51 kg in the control (main plots), 1.459 kg in the $1 kg \ ha^{-1}$ treatment and 1.50 kg in the $2 kg \ ha^{-1}$ treatment. It was

2.109 kg, 1.427 kg and 1.937 kg in the $0 mg \, l^{-1}$, $100 mg \, l^{-1}$ and $500 mg \, l^{-1}$ subplot treatments respectively. Tuber weight per plant was in the range 0.88 to 2.85 kg in the treatment interactions. Tuber weight in tons per hectare is given in the last column in Table 6. Means for main plot, subplot and interaction are 18.24 tons per ha, 18.24 tons per ha and 18.46 tons per ha respectively Table 6.

<u>Table 6:</u> Yield at 6 months after planting in *D. alata* var. Alakisa.

	_		
Fertilizer (Soil Amended)	Tuber Weight (kg) Per Plant	Tuber Number Per Plant	Tuber Weight in Tons Per Hectare
Ca (NO ₃) ₂ kg ha ⁻¹			
(M_0) 0	2.510a	9.058a	25.10
(M ₁) 1	1.459a	3.724a	14.59
(M ₂) 2	1.503a	4.167a	15.03
Foliar spray (CaNO $_3$) $_2$ in Mg L $^{-1}$			
(T_0) 0	2.109a	3.500a	21.09
(T ₁) 100	1.427a	4.113a	14.27
(T ₂) 500	1.937a	5.336a	19.37
Interaction			
M _o T _o (0kg ha ⁻¹ X 0mg L ⁻¹)	2.85a	3.50a	28.50
M _o T ₁ (0kg ha ⁻¹ X 100mg L ⁻¹)	2.32a	4.03a	23.20
M _o T ₂ (0kg ha ⁻¹ X 500mg L ⁻¹)	2.60a	8.17a	26.00
$M_{1}T_{0}$ (1kg ha ⁻¹ X 0mg L ⁻¹)	1.55a	3.17a	15.50
M ₁ T ₁ (1kg ha ⁻¹ X 100mg L ⁻¹)	0.88a	3.67a	8.80
M ₁ T ₂ (1kg ha ⁻¹ X 500mg L ⁻¹)	1.90a	4.33a	19.00
M ₂ T ₀ (2kg ha ⁻¹ X 0mg L ⁻¹)	1.92a	4.33a	19.20
M ₂ T ₁ (2kg ha ⁻¹ X 100mg L ⁻¹)	1.34a	4.67a	13.40
M ₂ T ₂ (2kg ha ⁻¹ X 500mg L ⁻¹)	1.25a	3.50a	12.50

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

Discussion

From the soil analysis results, Nitrogen was below 2% and calcium was below 0.65cmol kg-1, which are critical levels determined for yam production in South West Nigeria [22]. Therefore, calcium nitrate soil amendment in this study was justified. The only reports in literature of soil amendment or foliar spray effect of calcium fertilizer in the control of any disease in *Dioscorea* species were with calcium carbonate plus NPK [17] and calcium nitrate foliar spray [20]. In the report of the effect of foliar

application of Ca $(\mathrm{NO_3})_2$ on susceptibility to anthracnose, the two improved varieties namely D. alata TDa 92-2 and D. rotundata TDr 131 and local variety *D. alata* variety Iberekodo were all susceptible late in the growth cycle that is after week 20 [20]. In this study, susceptibility was observed before the Ca $(\mathrm{NO_3})_2$ soil amendment at 3 MAP or 12 weeks after planting. Disease symptoms then increased, for which reason the only foliar spray of Ca $(\mathrm{NO_3})_2$ was administered at 5 MAP. Leaf analysis at 6 MAP indicated leaf lignin and tannin was higher in the calcium nitrate soil-amended plants, than the control. Leaf flavonoid content was also higher

in the Ca $(\mathrm{NO_3})_2$ foliar-sprayed plants than in the control. Preformed secondary metabolites or phytoanticipins such as lignin, flavonoid and tannin, which are formed on leaf surface cell layers or within leaf cells, have antimicrobial properties as they provide a chemical barrier which resists pathogen enzymatic degradation, or release toxic substances within the cells, conferring chemical protection against pathogenic metabolites (Dube, 2014). The increase of these three phytochemicals in the leaves by fertilization is acceptable with reference to their antimicrobial potential. Among the eight minerals, N, Fe, P and S increased with Ca $(\mathrm{NO_3})_2$ fertilizer application.

The positive correlation of N with lignin and tannin, Fe with flavonoid and tannin, S with tannin and P with tannin, implies higher antimicrobial effects by the phytoanticipins on foliage diseases including anthracnose with Ca (NO₂)₂ fertilizer. Positive correlation between N and Fe, and N and S implies that iron and sulphur may increase as N supply increases from Ca (NO₃)₂ fertilizer. Treatment interactions gave disease severity scores from 1 (resistant) to 3.67 (susceptible). The 2kg ha⁻¹ x 0mg l⁻¹ and 0kg ha⁻¹ x 100mg l⁻¹ had a score of 1.00 (resistant). The 1kg ha⁻¹ x 500mg l⁻¹ and 1 kg ha⁻¹ x 100mg l-1 had a score of 2 (moderately resistant/tolerant). The 2kg $ha^{\text{-}1}\,x\,100mg\,l^{\text{-}1},\,1kg\,ha^{\text{-}1}\,x\,0mg\,l^{\text{-}1},\,0kg\,ha^{\text{-}1}\,x\,500mg\,l^{\text{-}1}$ and $0kg\,ha^{\text{-}1}\,x$ 0mg l-1 had a score of 2.67 to 3.33 (moderately susceptible). The 2kg ha-1 x 500mg l-1 had a score of 3.67 (susceptible). Disease severity correlated positively with disease incidence implying that increase in incidence may result in increase in severity if anthracnose is not controlled. Variety Alakisa is susceptible early in the growth cycle and not late as for the three varieties in the cited report of Otusanya et al. [20]. The increase in susceptibility from 3 MAP suggests that apart from Ca (NO₃)₂ soil amendment at 3 MAP, Ca (NO₃), foliar sprays may be appropriate in this variety from 1 MAP (one month after planting), in future trials. The varietal response to monthly foliar spray applications may then be monitored, for the best interaction effects (soil amendment x foliar spray) which may reduce anthracnose to resistant level (score 1).

Mean infection in the tubers incubated with B. theobromae for 2 weeks was 1.03%. This infection level (1.03%) is 44% and 60% of that recorded (in 2 weeks) for the first and second year respectively in the two improved varieties (D. alata TDa 92-2 and D. rotundata TDr 131) and the local variety D. alata variety Iberkodo, in the report of the effect of CaCO₃ soil amendment to tuber infection by B. theobromae [17]. This is an improvement in infection probably due to better soil amendment fertilizer effect of Ca (NO₃)₂ than CaCO₃. Weight loss in variety Alakisa tubers in this study was nil (0%), which is also an improvement over the 3.16% (Year 1) and 4.10% (Year 2) recorded for the three varieties fertilized with CaCO₃ soil amendment in the cited report [17]. Mean tuber weight per plant at 6 MAP in this study is 18.24tons ha⁻¹ (main plot and subplot means), within the range of the potential of 16 to 25tons per hectare for water yam under good management of 400kg (8 bags) of NPK 15-15-15 per hectare (60kg N, 60kg P₂O₅ and 60kg K₂O per hectare) under continuous cultivation in Nigeria [23].

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