



Mineral Fertilizer Effects on *Anthraco* Disease Caused by *Colletotrichum Gloeosporioides* Penz, Tuber Rot by *Botryodiplodia Theobromae* Pat and yield in *Dioscorea Rotundata* Variety Oniyere



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Abstract

Mineral nutrition effect on anthracnose caused by *Colletotrichum gloeosporioides*, tuber rot by *Botryodiplodia theobromae* and yield in *Dioscorea rotundata* var. Iseosi was investigated in this study. The field plot used had been mono-cropped to *Dioscorea* species in the two previous consecutive years with only calcium nitrate fertilizer. But nitrogen and calcium in the plot soil were still below the critical (2% and 0.6 cmol kg⁻¹ respectively) determined for optimum yam production (South West Nigeria). The field design was RCBD with 3 treatments of 0, 0.6, 1.2ml/l⁻¹ Bounty fertilizer applied at 3 MAP, and three replicates. Bounty fertilizer favoured higher concentration of the phytoanticipins (with antimicrobial activity) flavonoid, lignin and tannin over the control. Leaf N and Mg had significant correlation with leaf flavonoid content ($r = 0.7634, 0.6786$). Leaf flavonoid had significant correlation with leaf lignin content, $r = 0.7189$. Leaf lignin in turn had significant correlation with leaf tannin content, $r = 0.7556$. Leaf Sulphur correlated significantly with leaf flavonoid and leaf phosphorus, $r = 0.7218$ and 0.8467 respectively. Bounty fertilizer also favoured higher leaf magnesium 234.73 and 230.27mg/100gdm (0.60ml/l⁻¹ and 1.20ml/l⁻¹) than the control, 182.40mg/100gdm. Leaf nitrogen, phosphorus and sulphur increased with bounty fertilizer treatment, then the control. Only leaf potassium was higher in the control (116.72mg/100gdm) than in the Bounty-fertilized plants with 87.31 and 87.53mg/100gdm in the 0.60ml/l⁻¹ and 1.20ml/l⁻¹ treatments.

The following correlations were also significant respectively: Leaf N and Leaf P, S, $r = 0.8484$ and 0.9836 respectively.

Leaf Ca and leaf Mg, $r = 0.9192$

Anthraco incidence did not increase after the 3 MAP bounty fertilizer soil amendment. It was 11%, 6% and 0% in the control, 0.60ml/l⁻¹ and 1.20ml/l⁻¹ treatments respectively at 5 MAPS. It reduced to 5.6% (control and 0.60ml/l⁻¹ treatment) and remained 0% in the 1.20ml/l⁻¹ treatment at 6MAP. Severity of anthracnose was 1, category of resistant, both at 5 MAP and 6 MAPS across all the three treatments. The interplay/enhancement of leaf minerals with the three phytochemicals/anticipants, as well as the latter with one another should be responsible for the low disease incidence and resistant severity status of variety Oniyere. Mean tuber weight loss after inoculation and incubation with *B. theobromae* for 2 weeks was 0% and infection range over the three treatments was low, that is 1.21 to 2.95% with no significant differences. Mean tuber weight per plant was in the range 0.88kg to 1.35kg with no significant differences between treatments. The 1.20ml/l⁻¹ bounty fertilizer treatment gave equivalent yield of 13.46 tons ha⁻¹. Bounty fertilizer at 0.60ml/l⁻¹ or 1.20ml/l⁻¹ is recommended for local varieties of white guinea yam in South West Nigeria especially where monocropping is desired.

Introduction

White guinea yam, *Dioscorea rotundata* Poir is one of the three more popular species of yams in Nigeria, the largest world producer [1]. Production of this food security and income generating crop is constrained especially by necessary fallow periods as it drains heavily on soil nutrients [2,3]. High export and commercialization are also hampered by storage losses of the tune of 7million metric tons annually [4]. *Anthraco* disease now prevalent on all cultivated *Dioscorea* species also reduces yield as it affects the leaves responsible for photosynthesis and

subsequent tuber bulking. Yield losses are reported to be high, 80 to 85% in susceptible varieties in Nigeria [5]. Resistance breeding, regular fungicide usage where affordable and good cultural practices such as improved storage are management practices to combat yield losses in the field and in storage. Mineral nutrition for disease reduction in agricultural crops is regarded as cost effective management of crop loss compared to pesticide usage with environmental /health hazards [6]. Biofortification of crops through the application of mineral fertilizers combined

with breeding varieties with an increased ability to acquire mineral elements is also advocated as an immediate strategy not only to increase mineral concentrations in edible crops but also to improve yields in infertile soils [7,8].

This project aimed at investigating the effect of the fertilizer Bounty, which is composed of 8 mineral elements (N, Ca, Mg, Mn, Zn, Cu, Fe and B) on anthracnose disease and tuber rot by *Botrydiploia theobromae* in *Dioscorea rotundata* var. Oniyere, a popular white guinea yam variety in South West Nigeria.

Materials and Methods

Field layout, soil sampling and planting

The plot used had been monocropped to *Dioscorea* species for two previous consecutive years (2015 and 2016) with only calcium nitrate fertilizer. It was sited at the Teaching and Research Farms of the Directorate of University Farms (DUFARMS), Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State, Nigeria. The soil site was sandy-loamy soil. The plot was 14m by 9.5m with 1m field border on all four sides. Soil samples were collected using standard methods from each of 3 replicates in the plot [9]. Twenty core samples were lifted at 20cm depth with a slanting soil auger, into labeled polythene bags. They were transferred to the laboratory for air-drying and sieving (2mm sieve) before

bulking per replicate for analysis. Soil analysis of exchangeable cations was by the method of [10].

After soil sampling, mounds of 1m by 1m size and 80cm high were made, 18 per replicate. Intra-row inter -row distances were 0.5m and 1m respectively. 2m length robust bamboo stakes were established per mound. Planting of whole seed yams of 0.4kg to 0.5kg average size was at the onset of heavy rains (April 5, 2017).

Fertilization with bounty fertilizer

At 3MAP, three treatments of Bounty fertilizer per replicate, of 1.20ml/l⁻¹, 0.60ml/l⁻¹ and 0ml/l⁻¹ (control) was applied in a 15cm deep groove, at 25cm radius from the plant roots in the mound [3]. Fertilizer was mixed in deionised water and poured evenly into the groove, which was thereafter covered well with soil.

Disease incidence and severity assessment

Incidence of *Anthracnose* was assessed at 5MAP and 6MAPS. Percent incidence was determined using the formula:

$$\% \text{Incidence in a replicate plot} = \left(\frac{\text{Number of plants with Anthracnose symptoms}}{\text{Total number of plants in the replicate plot}} \right) 100$$

Anthracnose severity was determined on a scale of 1 (resistant) to 5 (very susceptible) according to the method [11] shown in (Figures 1-3).

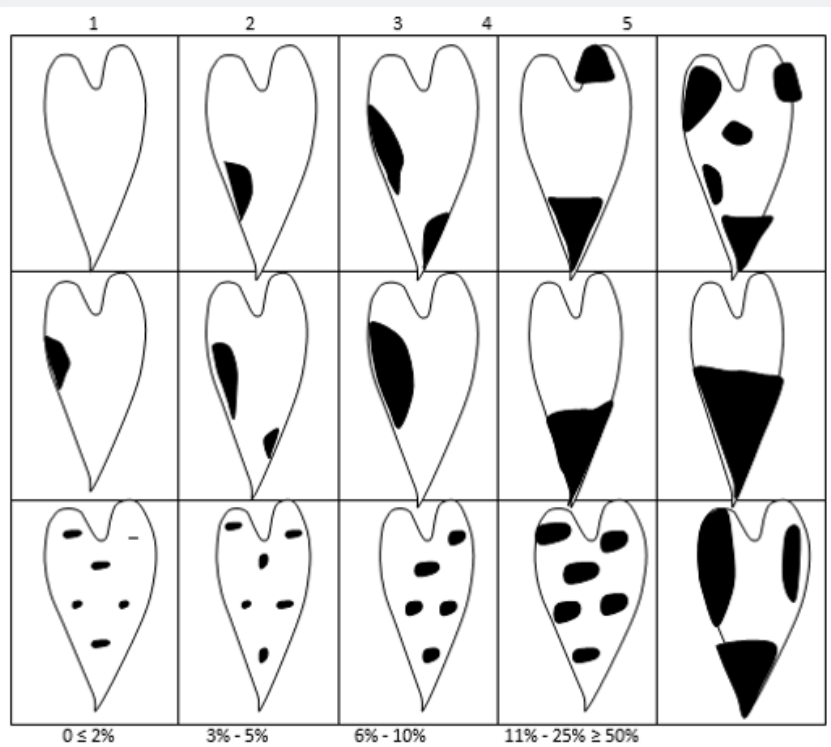


Figure 1: Yam leaf anthracnose disease assessment diagram + lesions on petiole and stem

- 1- No symptom/0 ≤ 2% severity/resistant/highly resistant
- 2 - Necrotic spots 1.00mm - 2.00mm/3-5% severity/moderately resistant (tolerant)
- 3 - Necrotic spots 2.01mm - 3.00mm/6-10% severity/moderately susceptible
- 4 - Necrotic spots 3.01mm - 4.00mm/11-25% severity/susceptible
- 5 - Necrotic spots > 4.00mm/50% severity/very susceptible

Modified from Green (1994)

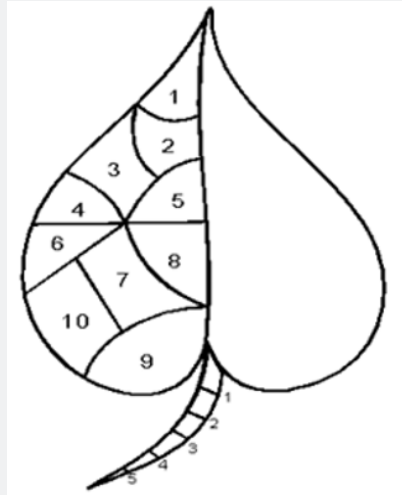


Figure 2: Leaf anthracnose assessment.

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)

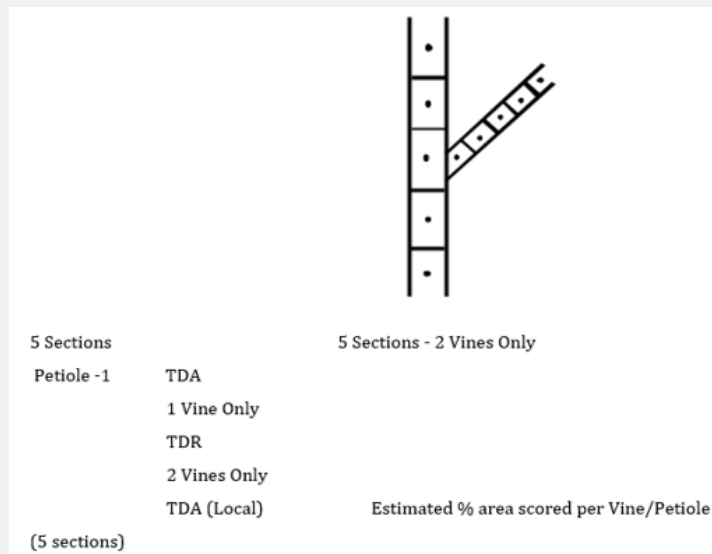


Figure 3: Vine and Petiole anthracnose assessment.

Leaf sampling and analysis

Leaf sampling was done at 6MAP, along a diagonal transect in the field plot. Sampling was done according to treatments, and 12 leaves (from top middle and lower portion) were plucked with the petiole attached from each sampled plant, into new paper envelopes, in the early morning (7:00am). They were transferred to the Crop Protection Laboratory, COLPLANT (College of Plant Science and Crop Production), FUNAAB. They were washed in three changes of distilled deionized water and left to dry on new food paper packs under a low speed fan, for an hour. They were

transferred for subsequent drying on raised wooden structures in the COLPLANT Screen house under shade for 2 days. They were then milled with a waring 2 speed Saisho blender after drying and repacked into new paper envelopes for analysis. Analysis of the three phytochemicals flavonoid lignin and tannin was carried out in the Biological Sciences Tet fund Laboratories, FUNAAB, using standard methods of the [12].

Seven minerals were analyzed in the leaves namely Calcium (Ca), Magnesium (Mg), Potassium (K), Nitrogen (N), Phosphorus (P), Iron (Fe) and Sulphur (S) using standard methods [12].

Yield at 6MAP

Careful harvest with smooth wooden spoons was undertaken to avoid bruising /wounding of tubers. Tuber number per plant was assessed after freeing of all tubers in a mound from soil. Thereafter they were left to dry for about 3 hours, when total tuber weight per mound was recorded with a top loading field mettler balance.

Infection and weight loss experiment

Triplicate tubers from treatment plots were also used in the infection and weight loss experiment. Inoculation of tubers was carried out in an Inoculation Hood Structure in the Crop Protection Laboratory, COLPLANT Building, FUNAAB. Three tubers per treatment were surface-sterilized by swabbing a point on the yam surface (inoculation site) with cotton wool dipped in 80% ethanol. Two cork borers (6mm and 4mm), scalpel and pair of forceps were surface-sterilized by dipping into 80% ethanol and flaming until red-hot over a lighted spirit flame bottle. They were thereafter cooled, slanting against another cork borer. The surface-sterilized 6mm borer was then used to bore a 10 to 12mm (depending on the size of the tuber) hole through the inoculation site in each tuber. The incised portion was lifted out with the sterilized scalpel and forceps. A 4mm disc from a 7-day old pure culture (growing on potato dextrose agar) of *Botryodiplodia theobromae* in a 9cm petri-dish was removed with the sterile 4mm cork borer and placed inside the hole created in the tuber. The incised tissue was replaced and the incision on the inoculation site sealed with vaseline (petroleum jelly). Each tuber weight was recorded before inoculation. Inoculated tubers were transferred into the raised wooden netted and ventilated yam storage structures in the COLPLANT Screenhouse for a period of 2 weeks. After the 2week incubation period each tuber weight was again measured with a field scale. Percent weight loss in the tuber was determined with the formula:

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

where A and B are the weight of the tuber before inoculation and at the end of incubation respectively. Percent infection was determined with the formula:

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

where C and A are, corrected weight of infected tissue and weight of the tuber at the beginning of the experiment (before inoculation) respectively. Also "C" is determined with the

$$formula\ C = \frac{100X}{100 - Y}$$

where X and Y are, weight of infected tissue and percent weight loss respectively [13].

Data analysis

Percentage data obtained were transformed appropriately (arcsine, square root or logarithm) before analysis of variance. Means were separated with Turkey's Studentized (HSD) test.

Results

Soil analysis

Nitrogen and Calcium in the field plot soil were 0.14% and 0.48cmol/kg-1 Table 1, below the critical required for optimum yam production in South West Nigeria [14]. Phosphorus, potassium and magnesium were 42.51mg/kg⁻¹, 0.67cmol/kg⁻¹ and 0.54cmol/kg⁻¹ respectively Table 1.

Table 1: Mean values of Soil properties of the experimental site.

Mean values of Soil properties of the experimental site	
pH (in water)	7.40 ± 0.200
Ca (cmol k ⁻¹)	0.45 ± 0.003
Mg (cmol k ⁻¹)	0.51 ± 0.004
K (cmol k ⁻¹)	0.68 ± 0.004
N (%)	1.17 ± 0.50
P (available mg k ⁻¹)	44.47 ± 0.54

Leaf tissue analysis of phytochemicals and minerals at 6MAP

Flavonoid content, 920mg/100gdm in the 1.20ml l⁻¹ soil amendment treatment was higher than the control, 876.67mg/100gdm, and not significantly different from that of the 0.60ml/l⁻¹ soil amendment (916.67 mg/100gdm) as shown in Table 2.

Leaf lignin content was also significantly higher 1943.33 mg/100gdm in the 0.60 ml/l⁻¹ treatment than the control, which was 1763.33mg/100gdm Table 2. The 1.20 ml/l⁻¹ treatment leaf lignin (1876.67 mg/100gdm) was not significantly different from the 0.60ml⁻¹ treatment. Leaf tannin content in the 0.60 ml⁻¹ soil amendment was higher (4.16mg/100gdm) than the control (3.39mg/100gdm). The control and the 1.20ml l⁻¹ soil amendment treatments were not significantly different in leaf lignin. Leaf calcium and iron were not significantly different in the three treatments Table 2. The 0.60 and 1.20ml/l⁻¹ soil amended plants were higher in leaf magnesium (234.73 and 230.27mg/100gdm) than the control which is 182.40mg/100gdm Table 2. Leaf nitrogen phosphorus and Sulphur increased with bounty fertilizer treatment, then the control Table 2 in the following manner:

- a) Leaf nitrogen: 707.67mg/100gdm (control) < 748.33mg/100gdm (0.60ml⁻¹) < 777.33mg/100gdm (1.20ml/l⁻¹).
- b) Leaf Phosphorus: 76.81mg/100gdm (control) < 76.95mg/100gdm (0.60ml⁻¹) < 78.52mg/100gdm (1.20ml/l⁻¹).
- c) Leaf sulphur: 809mg/100gdm (control) < 834.33mg/100gdm (0.60ml⁻¹) < 854.33mg/100gdm (1.20ml/l⁻¹).

Only leaf potassium content was higher in the control (116.72mg/100gdm) than in the bounty-fertilized plants which had 87.31mg/100gdm and 87.53mg/100gdm respectively in the 0.60ml/l⁻¹ and 1.20ml/l⁻¹ treatments Table 2.

Table 2: Photochemicals (mg/100gdm) and minerals (mg/100gdm) in leaves of Bounty-fertilized *Dioscorea rotundata* variety *Oniyere* at 6MAP.

Bounty-Fertilized Treatment ML L ⁻¹	Photochemicals			Minerals							
	Lignin	Flavonoid	Tannis	Ca	Mg	K	N	S	N	P	Fe
0.00 (control)	1763.33 ^b	876.67 ^b	3.39 ^b	1104.0 ^a	182.40 ^b	116.72 ^a	707.67 ^c	809.33 ^c	707.67 ^c	76.81 ^c	1.17 ^a
0.6	1943.33 ^a	916.67 ^{ab}	4.16 ^a	1360.0 ^a	230.27 ^a	87.31 ^b	748.33 ^b	834.33 ^b	748.33 ^b	76.95 ^b	1.37 ^a
1.2	1876.67 ^a	920.00 ^a	3.93 ^{ab}	1325.3 ^a	234.73 ^a	87.53 ^b	777.33 ^a	854.33 ^a	777.33 ^a	78.52 ^a	1.24 ^a

Means in a column followed by the same letter are not significantly different at P ≤ 0.05 (Tukey's HSD test)

Disease incidence and severity

Incidence of Anthracnose was not high before the 3MAP soil amendment with Bounty fertilizer. After this, the plants were observed closely so as to administer foliar-spray of Ca(NO₃)₂ if necessary. However, anthracnose symptoms did not increase and at 5MAP was 11.11% in the control 5.56% in the 0.60ml⁻¹ treated

plants and 0% in the 1.20ml l⁻¹ treated plants as shown in Table 3. It reduced further at 6MAP to 5.56%/ 5.57% in the control and 0.60ml l⁻¹ treated plants and was still 0% in the higher treatment of 1.20 ml/l⁻¹ Table 3. Anthracnose severity was low both at 5MAP and 6MAP, when all three treatments including the control had a score of I in the category of resistant Table 3.

Table 3(a): Anthracnose disease incidence in *Dioscorea rotundata* variety ONIYERE at 5MAP and 6MAP.

Bounty-Fertilizer Treatment ml l ⁻¹	Disease Incidence (%) at 5MAP	Disease Incidence (%) at 6MAP
0	11.11 ^a	5.57 ^a
0.6	5.56 ^a	5.56 ^a
1.2	0.00 ^a	0.00 ^a

Means in a column followed by the same letter are not significantly different at P ≤ 0.05 (Tukey's HSD test).

Table 3(b): Anthracnose disease severity score in *Dioscorea rotundata* variety *Oniyere* at 5MAP and 6MAP.

Bounty-Fertilized Treatment mL L ⁻¹	Disease Severity (%) at 5MAP	Disease Severity (%) at 6MAP
0	1.00 ^a	1.00 ^a
0.6	1.67 ^a	1.00 ^a
1.2	1.00 ^a	1.00 ^a

Means in a column followed by the same letter are not significantly different at P ≤ 0.05 (Tukey's HSD test)

MAP = Months After Planting.

Correlations of anthracnose incidence/severity, leaf phytoanticipins, leaf minerals and yield

Only significant correlations in all parameters assessed are presented in Table 4. Leaf nitrogen correlated positively with leaf phosphorus and leaf Sulphur, r = 0.8484 and 0.9836. Leaf Nitrogen also correlated positively with leaf flavonoid content r = 0.7634. Leaf Calcium correlated positively with leaf magnesium,

rr = 0.9192 while leaf magnesium correlated positively with leaf flavonoid content, r = 0.6786. Leaf flavonoid content correlated positively with leaf lignin content, r = 0.7189. Leaf lignin content correlated positively with leaf tannin content, r = 0.7556. Leaf Sulphur correlated both with flavonoid and phosphorus, r = 0.7218 and r = 0.8467. Leaf tannin content correlated negatively with disease incidence at 5 MAPS Table 4.

Table 4: Significant correlations of Anthracnose incidence/severity, leaf anticipins/minerals and yield in *D. rotundata* var. *Oniyere*.

	Calcium	Magnesium	Flavonoid	Tannin	Lignin	Nitrogen	Phosphorus	Sulphur	Tuber Weight Per Plant
Calcium		0.9192*							0.8196*
Magnesium			0.6786*						0.8044*
Flavonoid					0.7189*				
Tannin					0.7556*				
Nitrogen			0.7634*				0.8484*	0.9836*	
Sulphur			0.7218*				0.8467*		
Lignin									

Disease Incidence at 5MAP				-0.6883*					
Tuber Number per plant									0.7411*

Significant Correlations at P = 0.001 or 0.05.

Infection and Weight loss experiment

Tubers inoculated and incubated with *Botryodiplodia theobromae* for 2 weeks had mean infection range of 1.213% to

2.95% across the three treatments. There were no significant differences in infection. Weight loss was 0% across all three treatments Table 5.

Table 5: Infection and weight loss in bounty-fertilized *D. rotundata* var. *Oniyere* incubated with *B. theobromae* for 2 weeks.

Bounty-Fertilizer Treatment ml l ⁻¹	Infection %	Weight loss %
0	1.923 ^a	0
0.6	1.213 ^a	0
1.2	2.950 ^a	0

Means in a column followed by the same letter are not significantly different at P ≤ 0.05 (Tukey's HSD test).

Yield at 6MAP

Tuber number per plant was in the range 1.443 to 2.333 Table 6. There were no significant differences across the three treatments. Tuber weight significantly correlated positively with tuber number per plant, r = 0.7411 Table 4. Tuber weight per

plant range was 0.877kg to 1.347kg. There were no significant differences between treatments. Tuber weight in tons per hectare is 8.77 to 13.47tons ha⁻¹ Table 4. Leaf calcium and leaf magnesium had significant positive correlation with tuber weight per plant, r = 8196 and r = 0.8044 respectively Table 6.

Table 6: Yield in Bounty-fertilized *Dioscorea rotundata* variety *Oniyere* at 6MAP.

Bounty-fertilizer Treatment ml l ⁻¹	Tuber Number Per Plant	Tuber Weight (kg) per Plant	Tuber Weight in Tons per Hectare
0	1.557 ^a	0.877 ^a	8.77
0.6	1.443 ^a	0.943 ^a	9.43
1.2	2.333 ^a	1.347 ^a	13.47

Means in a column followed by the same letter are not significantly different at P ≤ 0.05 (Tukey's HSD test).

Discussion

The field plot used in this study had been monocropped to yams, *Dioscorea* species with calcium nitrate fertilizer, for two previous consecutive years (2015 and 2016). However, soil Nitrogen and Calcium were still below 2% and 0.65cmol/kg-1, critical levels determined for optimum yam production in South West Nigeria [14]. Lands fallowed for 2 -3 years in Nigeria may still require soil testing for mineral replenishment for optimum yam production. Phosphorus and potassium were however above the critical. Yam, *Dioscorea* species drain heavily on soil nutrients [3]. This fact informed the choice of fertilizer containing 8 minerals, coupled with the aim of the study which was to investigate the effect of mineral nutrition on yam anthracnose and tuber rot, especially on a field plot monocropped to *Dioscorea* species with only Ca(NO₃)₂ fertilizer for two previous consecutive years. Performed secondary metabolites or phytoanticipins such as flavonoid, lignin and tannins which are formed on the surface cell layers or within leaf cells have antimicrobial properties as they provide a chemical barrier that resists pathogen enzymatic degradation, or release toxic chemicals within the cells, conferring chemical protection

against pathogen [15]. The three phytoanticipins (flavonoid, lignin, tannins) were higher in the leaves of the Bounty-fertilized plants in this study than the control. Furthermore, nitrogen and magnesium supplied from the fertilizer increased flavonoid content in the leaves (positive significant correlation). Nitrogen also promoted increase in phosphorus and Sulphur (both of which were not supplied from the fertilizer) in the leaves, while Sulphur in turn promoted leaf flavonoid content. Calcium supplied from the fertilizer promoted leaf magnesium content (significant positive correlation). Leaf flavonoid correlated significantly positively with lignin and lignin with tannin. The interplay/enhancement of the aforementioned minerals with phytoanticipins (three of them) and the latter with one another should be responsible for the low incidence of anthracnose at 5MAP and a lower incidence at 6MAP, and the resistance of the variety (severity score of 1 = resistant, at 5MAP and 6MAP). *Anthracnose* severity score, of 3, moderately susceptible was reported for an improved white yam variety *D. rotundata* TDr 131 at 6MAP, in the only report of use of Ca (NO₃)₂ foliar spray to control anthracnose in *Dioscorea* species in literature [11].

Mean infection in *D. rotundata* var. *Oniyere* tubers harvested at 6MAP, inoculated and incubated with *B. theobromae* for 2 weeks was 2.03%. This infection is 43% lower than that obtained in an improved variety of white guinea yam *D. rotundata* TDr 131 inoculated with *B. theobromae* after fertilization with CaCO₃ and NPK [16]. Weight loss in Variety Oniyere in this study was 0% whereas *D. rotundata* TDr 131 had 3.4% weight loss in the cited report [16]. Bounty fertilizer with eight minerals may be recommended for management of both anthracnose disease and tuber rot by *B. theobromae* in *D. rotundata* var. *Oniyere*. At the 6 MAP harvest, tuber weight was 0.877kg, 0.94kg and 1.347kg per plant in the control, 0.60 ml/l⁻¹ and 1.20ml/l⁻¹ Bounty-fertilized plants respectively. The higher treatment of 1.2ml/l⁻¹ Boutny fertilizer gave the yield equivalent of 13.47tons ha⁻¹ which is higher than the 9-12 metric tons/ha in smallholder yam cultivation in shifting agricultural systems without external input in West Africa [17], and it is within the expected range of 10-15t/ha for white guinea yam under good management of NPK/NPK+Urea [18]. Bounty fertilizer at 1.20ml/l⁻¹ is recommended for the production of local white guinea yam varieties such as variety *Oniyere* by smallholder farmers in Nigeria, after soil testing, especially where monocoping is desired.

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