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Tuber Minerals and Phenolic Compounds in Calcium Nitrate Foliar-Sprayed *Dioscorea Alata* Var. Agbodo and *D. rotundata* Var. Amula

Otusanya, M.O.

ABSTRACT

Calcium Nitrate foliar spray for the management of anthracnose disease was assessed in *Dioscorea alata* variety Agbodo and *D. rotundata* var. Amula in two experiemental plots in COLPANT Farms, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Ogun State in Nigeria. The design in each plot was randomized complete block (RCBD) with three replications. Mounds were 1 metre (m) by 1m and about 80cm high, with inter-replicate inter-row and intra-row spacing of 1m, 1m and 0.5m respectively. One metre (1m) clear border was maintained on each of the four sides of each plot. Each replicate had 18 plants. Calcium Nitrate foliar spray at the rates 0mgl^{-1}, 5mgl^{-1} and 10mgl^{-1} was applied without surfactant at twelve weeks after planting (12 WAP). Anthracnose incidence and severity results are presented in another paper. But tubers in the plots were sampled at 24 WAP in order to determine, Calcium nitrate foliar spray of 5mgl^{-1} and 10mgl^{-1} without surfactant, effect on tuber minerals, namely Calcium (Ca), Magnesium (Mg), Nitrogen (N), Phosphorus (P), Potassium (K) and Sulphur (S), as well as Tuber Total Phenolic Compounds (TPC) and yield. The results of yield, TPC and the tuber minerals are presented in this paper. Statistical analysis was carried out separately for each plot. Tuber Number Per Plant (TNPP) and Tuber Weight Per Plant (TWPP) were similar across treatments for water Yam Agbodo and white guinea yam Amula. TNPP overall means are 4.43 and 3.16 for Agbodo and Amula respectively. TWPP overall means are 2.00kg (or 20.00 tonsha^{-1}) and 1.91kg (or 19.10 tonsha^{-1}) for Agobdo and Amula respectively. Yield (tuber fresh weight) is similar to that obtained in a report of Calcium nitrate foliar spray for anthracnose control in *Dioscorea* species (two improved and one local variety), but which had relatively higher spray concentrations of 0 to 12000 milligram per litre and with surfactant in the spray liquid. Tuber Ca, Mg, N and P were significantly higher in the treatments than the control in water yam Agbodo as follows: Ca in mg/100g dm is 21.48 (10mgl^{-1}) > 19.16 (5mgl^{-1}) > 16.31 (Control); Mg in mg/100gdm is 24.53 (10mgl^{-1}) > 21.22 (5mgl^{-1}) > 19.02 (control); N in percent (%) is 1.86 (10mgl^{-1}) > 1.37 (5mgl^{-1}) > 0.95 (control); P in mg/100g dm is 56.27 (10mgl^{-1}) > 53.14 (5mgl^{-1}) > 49.76 (control). K in mg/100g dm in Agbodo is 807.70 (10mgl^{-1}) higher than 770.60 (control). S and Total Phenolic Compounds (TPC) of Agbodo were overall means 4.68% and 5.58mg Gallic Acid Equivalent per gram dry matter (5.58mgGAE/gdm) respectively. In white guinea Yam Amula, TPC and five of the minerals were higher significantly in the treatments than the control as follows: TPC in mgGAE/gdm is 5.92 (10mgl^{-1}) > 5.39 (5mgl^{-1}) > 4.82 (control); Ca in mg/100gdm is 18.22 (10mgl^{-1}) > 16.45 (5mgl^{-1}) > 14.49 (Control); N in % is 1.80 (10mgl^{-1}) > 1.11 (5mgl^{-1}) > 0.91 (control); P in mg/100gdm is 54.82 (10mgl^{-1}) > 50.84 (5mgl^{-1}) > 48.59 (control); K in mg/100gdm is 812.44 (10mgl^{-1}) > 793.29 (5mgl^{-1}) > 759.22 (control); S in % is 6.42 (10mgl^{-1}) > 5.23 (5mgl^{-1}) > 4.48 (control).

Mg in white Yam Amula was overall mean of 20.19mg/100gdm, similar across treatments. A numerical comparison may be made of the two species (two varieties) because statistical analysis was carried out separately in the two experimental plots. Thus numerically (not statistically) overall means were higher in Agbodo in yield (TNPP and TWPP), TPC, Ca, Mg, N, P and K, than Amula. Amula was numerically higher in Sulphur only than Agbodo. Phenolic compounds reports of association with defence mechanisms in crop plants because they exhibit anti-fungal, anti-bacterial and anti-viral properties is discussed. Phenolic compounds reports of exhibition of antioxidant or free radical scavenging properties that boost immunity against diseases caused by free radical reactions such as cancer, diabetes, aging and cardio vascular diseases, as well as analgesic and anti-inflammatory properties is also discussed. This is to draw attention to inputs during Crop Protection Research such as this study which will effect synthesis of, or increase phenolic compounds in the edible food yams for disease resistance as well as for health benefits. Increase in calcium in this study would enhance disease resistance due to calcium role in the enhancement of the integrity of intracellular cementing materials. Magnesium and phosphorus requirement in yams is reported to be low, thus the increase in this study will ensure yams receive the optimum of the two elements. Lastly, Nitrogen and Potassium increase is to be emphasized for recommendation of 5mgl^{-1} and 10mgl^{-1} calcium nitrate foliar spray in *Dioscorea* species as the two are the most important for optimum yield in yams.

INTRODUCTION

Calcium Nitrate Foliar Spray for control of Anthracnose disease in two improved and one local variety of *Dioscorea* species was reported, with five concentrations between 0mgl^{-1} and 12000mgl^{-1} (Otusanya *et al.*, 2017). Yield obtained in the first year was similar across treatments. Yield in the second year however, was significantly higher than the control, after a change in rate and time of the foliar spray. In a second study, relatively lower concentrations of 5 and 10 milligram per litre of calcium nitrate foliar spray without surfactant were evaluated for anthracnose control in two Nigerian local varieties of *Dioscorea alata* and *D. rotundata* namely Agbodo and Amula respectively. The results of Yield, Tuber Total Phenolic Compounds and six tuber minerals (Calcium, Magnesium, Nitrogen, Phosphorus, Potassium and Sulphur) in the study are presented in this paper.

MATERIALS AND METHOD

Planting Material

Tubers of *Dioscorea alata* variety Agbodo and *D. rotundata* variety Amula were sourced from the Osiele Yam market in Abeokuta, Ogun State, Nigeria. Agbodo is a Yoruba (South West Nigeria) local name and the variety is widely grown in the South West region of Nigeria. Amula is widely grown in Benue State in the middle belt region of Nigeria (Shiriki *et al.*, 2015). But Amula has also been adopted in South West Nigeria and other parts of the country as farmers refer to it as a good yielding variety.

Field Experiment

Two experiments were carried out in two separate plots in COLPLANT (College of Plant Science and Crop Production Fatms), Federal University of Agriculture, Abeokuta FUNAAB, Ogun State, Nigeria. Each was RCBD (Randomized Complete Block) with three replications. Mounds in each replicate were 1metre (m) by 1m, and about 80cm high, with inter-replicate, inter-row, and

intra-row spacing of 1m, 1m and 0.5m respectively. A clean border of 1m space was maintained on each of the four sides of each plot. Each replicate had eighteen mounds. The two experiments were of calcium nitrate foliar spray at the rates 0mgl^{-1} (control) 5mgl^{-1} and 10mgl^{-1} applied without surfactant at 12WAP (Weeks after planting), to evaluate calcium nitrate effect on anthracnose incidence and severity, and the results have been presented elsewhere.

However tubers from the plots were analysed for effect of the spray solution on tuber quality in terms of mineral content and total phenolic compounds. The tuber analysis results as well as tuber yield at 24 WAP are presented in this paper.

Tuber Sampling and Processing for Analysis at 24 WAP

Tuber sampling was along the diagonal transect of each plot replicate. Wooden spoons were used to move soil away from tubers within the mound and the shoots attached cut away with a sharp stainless steel knife. Mounds were then closed up with soil for the final harvest the following day. Tubers were removed in triplicate per treatment per replicate. Sampled plants were tagged so as to have a correct record of total number of tubers produced by the plant and tuber weight per plant in the final harvest. The tubers were cleared of soil, washed in tap water and left to dry on top of laboratory benches under low-speed ceiling fans. After drying, they were sliced into thin chips with stainless steel knives and left to dry in labelled plastic trays on the bench tops for about four days. They were milled after drying with a Hammer and Bull Mill. The milled samples were transferred to the Biological Tetfund Laboratory in FUNAAB and the Biotechnology centre in FUNAAB for tissue analysis. Total phenolic compounds and mineral analysis of Calcium (Ca) Magnesium (Mg) Nitrogen (N) Phosphorus (P) Potassium (K) and Sulphur (S) in the samples was according to standard methods of the A.O.A.C (1990/2000).

Havest at 24 WAP

The final harvest was done one day after the tuber sampling for analysis. Dislodging tubers from within the mounds was in the same way as for the tubers harvested for analysis, to avoid bruising or wounding. Total number of tubers per plant was recorded as well as weight of tubers per plant using a field top loading mettler balance (field scale).

Statistical Analysis

Data obtained were analysed with SAS 2000 version 9.1.1 and means separated with Tukey's Honest Studentized Range (HSD) test.

RESULTS AND DISCUSSION

Dioscorea alata variety Agbodo and *D. rotundata* variety Amula were sprayed in two separate experimental plots in COLPLANT (College of Plant Science & Crop Production, Federal University of Agriculture, Abeokuta, Nigeria) FARMS with Calcium Nitrate rates 0mgl^{-1} (control) 5mgl^{-1} and 10mgl^{-1} for the management of Anthracnose disease. Tubers were sampled in the two plots at 24 WAP, to assess the effect of the spray solution on tuber minerals and tuber total phenolic compounds. In the first report of Calcium nitrate foliar spray to control Anthracnose in two improved varieties and one local variety of *Dioscorea* species in Nigeria, there were four differences in methodology namely variety as well as rate and time of spray solution application and addition of surfactant (Otusanya *et al.*, 2017). In the cited report, rate and time of calcium nitrate foliar spray were 0mgl^{-1} to 12000mgl^{-1} (5 treatments) at emergence and thereafter bi-weekly until 12 weeks after planting (12 WAP) in the first year. And 0mgl^{-1}to

12000mgl^{-1} calcium nitrate (4 treatments) applied at 12WAP and 20WAP in the second year. In this study rate and time of calcium nitrate foliar spray were 0mgl^{-1} to 10mgl^{-1} (3 treatments) applied at 12WAP only. The tuber fresh yield, tuber total phenolic compounds and tuber minerals, analysed separately for the two varieties are presented and discussed.

Tuber Number Per Plant (TNPP) and Tuber Weight Per Plant (TWPP) were not significantly different across treatments in the water yam Agbodo or in white guinea yam Amula. TNPP overall mean is 4.43 and 3.16 in Agbodo and Amula respectively as shown in Table 1. TWPP is overall mean 2.00kg (or 20tons ha^{-1}) and 1.91kg (or 19.10tons ha^{-1}) in Agbodo and Amula respectively (Table 1). Otusanya *et al.*, (2017) reported 1.85kg (or 18.50tons ha^{-1}) in the 750mgl^{-1} $Ca(NO_3)_2$ treatment and 1.76kg (or 17.60tons ha^{-1}) in the 12000mgl^{-1} treatment, both significantly higher than the control of 1.24kg (or 12.40tons ha^{-1}) in the second year. Yield in this study is similar to the cited report, despite the four differences mentioned earlier. Surfactant was not used in the spray solution in this study to avoid its scorching effects because the optimum had not been determined for Calcium nitrate in *Dioscorea* species. Significantly higher yield in treatments in the report of Otusanya *et al.*, (2017) was mainly due to interaction effect in the improved variety TDa 92-2, as there was no significant interaction in the second improved variety which is a different species, white yam TDr 131 or in the third variety, the local *D. alata* variety Iberekodo. This ability in the improved variety TDa 92-2, may be due to genetic specificity which explains variability in growth, carbon fixation and nutrient uptake between species (Diby *et al.,* 2004).

However, tuber TPC and minerals were higher in the treatments over the control in this study. Water yam Agbodo tuber Ca, Mg, N and P were significantly higher in the two $Ca(NO_3)_2$ treatments than the control as shown in Table 2 thus: Ca in mg/100gdm is 21.48(10mgl^{-1}) > 19.16 (5mgl^{-1}) > 16.31 (Control). Mg in mg/100gdm is 24.53(10mgl^{-1}) > 21.22 (5mgl^{-1}) > 19.02 (control). N in percent (%) is 1.86 (10mgl^{-1}) > 1.37 (5mgl^{-1}) > 0.95 (control). P in mg/100gdm is 56.27 (10mgl^{-1}) > 53.14 (5mgl^{-1}) > 49.76 (control). K in mg/100gdm in Agbodo is higher in the 10mgl^{-1} treatment than the control, 807.70 (10mgl^{-1}) > 770.60 (control), (Table 2). S and TPC were similar across treatments in Agbodo and have overall means of 4.68% and 5.58mg of Gallic Acid Equivalent per gram dry matter respectively (Table 2).

In white guinea yam Amula, TPC and five of the six minerals were significantly higher in the two calcium nitrate treatments than the control (Table 2) as follows: TPC in mg GAE/gdm is 5.92 (10mgl^{-1}) > 5.39 (5mgl^{-1}) > 4.82 (control); Ca in mg/100gdm is 18.22 (10mgl^{-1}) > 16.45 (5mgl^{-1}) > 14.49 (control); N in % is 1.80 (10mgl^{-1}) > 1.11 (5mgl^{-1}) > 0.91 (control); P in mg/100gdm is 54.82 (10mgl^{-1}) > 50.84 (5mgl^{-1}) > 48.59 (control); K in mg/100gdm is 812.44 (10mgl^{-1}) > 793.29 (5mgl^{-1}) > 759.22 (control); S in % is 6.42 (10mgl^{-1}) > 5.23 (5mgl^{-1}) > 4.48 (control). The sixth mineral Mg was similar across treatments in Amula, with overall mean of 20.19mg/100gdm as shown in Table 2.

Separate experimental plots were used for Agbodo and Amula thus values/results obtained for the two species may only be compared numerically. Generally numerically not statistically, overall means were higher in water yam Agbodo in Yield (both tuber number per plant and tuber weight per plant), total phenolic compounds and five of the six minerals (calcium, magnesium, nitrogen, phosphorus and potassium) than white yam Amula (Table 2). Amula was numerically higher only in sulphur (5.38%) content than Agbodo (4.68%), (Table 2).

Phytochemicals or secondary metabolites such as phenolic compounds occur naturally in plants and form part of the plant defence mechanisms against disease because they exhibit anti-microbial, anti-bacterial and anti-viral properties. Marie *et al.*, (2005) reported 79.3mg/kg phenolic compounds in variety St. Vincent of *D. alata* in Jamaica, which is lower than that obtained in *D. alata* var. Agbodo in this study. Martin Kouakou *et al.*, (2010) reported that higher phenolic compound content of over 200mg/100g dry weight of the proximal portions of the two yam species *D. alata* variety Florido and *D. cayenensis-rotundata* complex variety Krengle was responsible for higher resistance to microbial attack in those portions than the distal portions, which had lower phenolic compound content. The phenolic compound value obtained in the latter report is also lower than that in this study for either water yam Agbodo or white yam Amula. Phenolic compounds extracted (acqeous and ethanolic extracts) from the Yam Peels of tubers of *Dioscorea bulbifera* and *D. alata* (collected from Nat. Root Crop Res. Inst., Umudike Yam Barn in South East region of Nigeria) exhibited various anti fungal actions in a dose-dependent manner, and at 40% concentration exhibited higher inhibitory action on *Botryodipodia theeobromae* than standard Griseofulvin a fungicide (Eleazu *et al.*, 2013). *B. theobromae* is one of the three important economic storage pathogens of yams in Nigeria (Dania *et al.*, 2012). The phenolic compound Catechin of the value 12 to 69mg catechin/100g fresh weight were reported in cultivars Abii, Ekpe, Nwopoke, Obiaturugo and Okwocha of *Dioscorea rotundata*, and 304 to 513mg/100g fresh weight of catechin in cultivars Ominelu and UM 680 of *Dioscorea alata* (Ozo *et al.*, 1984). The concentrations are of the cultivar fresh weight and are lower than the TPC obtained in this study. Ozo *et al.*, (1984) also reported that the two cultivars Uminelu and UM 680 of *D. alata*, cultivar Adu of *Dioscorea bulbifera*, cultivar Oku of *Dioseorea cayenensis* and cultivar Ona of *Dioscorea dumetorum*, all had higher phenolic compound content than the five cultivars Abii, Ekpe, Nwopoke, Obiaturugo and Okwocha of *Dioscorea rotundata*. The ten cultivers of five species in the latter report (of Ozo *et al.*, 1984) are edible food yams from the South East region of Nigeria. Graham – Acquaah *et al.* (2014) reported a similar range as that of Ozo et al., 1984, of 20-37mg catechol/100g in Puna and Bayere Fitaa in Ghana. The latter two cultivars are important and cultivar Puna is the most preferred white guinea yam cultivar for export and local consumption in Ghana (Erio William Cornelius, 1998). Polyphenols have also been reported to be associated with antioxidant activity or free radical scavenging properties which boost immunity against diseases caused by free radical reactions such as cancer, cardiovascular diseases, diabetes and aging (Lubag *et al.*, 2008; Sonibare and Abegunde 2012; Bhandarii and Kawabata 2004; Cornago *et al.*, 2011). Others regulate processes such as the reduction of cholesterol absorption, and others exhibit analgesic and anti-inflammatory properties (Avula *et al.*, 2012, 2014; Alamu *et al.*, 2014; Zang *et al.*, 2014; Padhan *et al.*, 2020; Price *et al.*, 2017). Total phenolic compounds increase in this study and the facts stated in the foregoing cited reports, draw attention to phenolic compounds synthesis in the edible Food yams (and some potentially useful wild relatives) during Yam Crop Protection research, during Yam Breeding Research and during Yam Soil Fertility Research, which have enormous potential for development of drugs for human health benefits. Gibney *et al.*, 2009 and Lanham – New *et al.*, 2010 report that research is on to establish recommended daily intake (RDA values) for these Bioactive Compounds (Phenolics) but it is generally agreed currently that they have positive influence on human health, and that when they are consumed as part of a balanced diet, the benefits are significant (Halliwell and Gutteridge, 2007).

Calcium maintains the intergrity of intracellular cementing materials (Polycarp *et al.,* 2012). The increase in calcium in the two varieties in this study, thus resulted in enhancement of

resistance to nematodes and pathogenic attack. The latter especially microbial pathogens are responsible for the highest losses in stored yams (Cornelius and Oduru, 1999). Phosphorus is not a limiting nutrient in yam production because mycorrlizal associations (with fungi) in Yam roots allow uptake of the mineral element (Obigbesan and Agboola, 1978). Uptake of Magnesium by yams (*Dioscorea spp.*) is also reported to be low (Obligbesan and Agboola, 1978; O'Sullivan and Jenner, 2006). Thus Phosphorus increase in both varieties and Magnesium increase in variety Agbodo in this study means that $5mgl^{-1}$ and $10mgl^{-1}$ calcium nitrate foliar spray ensures optimum uptake of the two mineral elements by *Dioscorea* species tubers. Nitrogen and Potassium both increased in the two varieties in this study and these two mineral elements are the most important for optimum yield in *Dioscorea* species (Degras, 1993; Diby et al., 2011; O'Sullivan, 2008).

Calcium, Magnesium, Nitrogen, Phosphorus, Potassium and Sulphur are all essential dietary minerals in the human diet, playing vital roles in the body metabolic processes (Polycarp *et al.*, 2012).

Table 1: Tuber Yield in Calcium Nitrate Foliar – Sprayed *D. alata* var. Agbodo and *D. rotundata* var. Amula

	TUBER NUMBER PER PLANT (TNPP)		**TUBER WEIGHT PER PLANT (TWPP) kg**			
CALCIUM NITRATE FOLIAR SPRAY	**AGBODO**	**AMULA**	**AGBODO**		**AMULA**	
			Kg	**(Tonsha^{-1})**	**Kg**	**(Tonsha^{-1})**
0	4.80a	2.93a	1.62a	(16.20)	1.87a	(18.70)
5	4.15a	2.73a	2.48a	(24.80)	1.73a	(17.30)
10	4.34a	3.83a	1.89a	(18.90)	2.13a	(21.30)
OVERALL MEAN	**4.43**	**3.16**	**2.00**	**(20.00**	**1.91**	**(19.10)**

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD Test

Table 2: Tuber Total Phenolic Compounds and Tuber Minerals in Calcium Nitrate Foliar-Sprayed *D. alata* variety Agbodo and *D. rotundata* variety Amula

	AGBODO	**AMULA**
TPC mgGAE/gdm	5.58	5.34
$Ca(NO_3)_2$ 0 5 10 mgl^{-1}	5.61a 5.56a 5.57a	4.82c 5.39b 5.92a
Calcium mg/100gdm	18.98	16.35
0 5 10	16.31c 19.16b 21.48a	14.49c 16.45b 18.22a
Magnesium mg/100gdm	21.59	20.19
0 5 10	19.02c 21.22b 24.53a	20.10a 20.37a 20.09a
Nitrogen %	1.362	1.273
0 5 10	0.95c 1.37b 1.86a	0.91c 1.11b 1.80a
Phosphorus mg/100gdm	53.06	51.42
0 5 10	49.76c 53.14b 56.27a	48.59c 50.84b 54.82a
Potassium mg/100gdm	789.45	788.32
0 5 10	770.60b 790.05ab 807.70a	759.22c 793.29b 812.44a
Sulphur %	4.68	5.38
0 5 10	4.81a 5.02a 4.21a	4.48c 5.23b 6.42a

Means aligned horizontally followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD Test.

Overall means for the two *Dioscorea spp.* Are indicated in vertical columns.

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