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Rhizopus Rot Controlwith Calcium Nitrate in *Dioscorea Rotundata* Var. Apepe

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ABSTRACT

This study investigated the response of *Dioscorea rotundata* variety Apepe to tuber rot by *Rhizopus stolonifer* with Calcium nitrate foliar spray. The experiment was a randomized complete block (RCBD) with three replications. In the pre-planting soil analysis, Calcium, Magnesium and Potassium were 31.58cmol kg^{-1}, 2.34cmol kg^{-1}and 0.52cmol kg^{-1} respectively in the soil of the experimental site, higher than the criticals reported for the three elements for optimum production of yam in South West Nigeria. However, Nitrogen and Phosphorus were 1.06% and 11.79ppm respectively, below the criticals. Calcium nitrate at 0mgl^{-1}, 750mgl^{-1}, and 12000mgl^{-1} was sprayed 16 weeks after planting (16WAP), and then four weekly until 28WAP. Tubers inoculated at 32WAP with *R. stolonifer* for four weeks, had weight loss not significantly different across the three treatments. It was 0.26% for the control, 1.23% for the 750mg l^{-1} treatment and 5.50% for the 12000mgl^{-1} treatment. Infection in the 750 mgl^{-1} treatment was 2.26% significantly lower than the control which was 6.66%. The 750mgl^{-1} treatment was lower than the control by 66%. But infection in the 12000mgl^{-1} treatment was 60.29%, higher than the control and is

thus not suitable for the control of *R. stolonifer* tuber rot. However, 750mgl^{-1}calcium nitrate foliar spray is recommended for control of *R. stolonifer* tuber rot in variety Apepe. Tuber yield at 32WAP was the equivalent of 35.80tonsha^{-1}, 32.50tonsha^{-1} and 31.10tonsha^{-1} in the 0mg^{-1}, 750mgl^{-1} and 12000mg^{-1}treatments respectively. Overall mean yield of 33.13tons ha^{-1}is similar to the optimum of 34.20tons ha^{-1}reported for economic production of yam in South West Nigeria.

INTRODUCTION

Yam (*Diosciorea.*species) is a tuber crop belonging to the family of Dioscoreaceae with over 600 species, and white guinea yam (*Dioscorea rotundata* Poir.) is one of the six most important in the tropics (Onwueme, 1978, Hahn *et al.,* 1993). The tuber is an important source of carbohydrate/calories in many developing nations, especially Africa (Maroya, 2014). Nigeria accounts for over 75% of world production (FAO, 2014). Despite its importance in the country, it has suffered many setbacks in production as a result of both abiotic and biotic factors. One of the most important is disease, especially by fungi which attack yam both in the field and in storage.

Rhizopus stolonifer Ehrenb. is reported to be the second highest occurring fungal rot pathogen of yams in Nigeria (Dania *et al.*, 2012). It causes soft rot, with the fungal mycelia invading the cells, collapsing their walls, with a characteristic foul smell within a short period. Fungicides for yam rot control are no longer encouraged because of their adverse effects on man, animals and environment. Effort to store yams in cold or low temperature environment damaged yam tissue and were too expensive to maintain (Adesuyi, 1973; Osunde, 2008). Biological agents have also been used for control (Okigbo, 2004). Resistance of plants or plant products to disease is being improved by nutrient fertilization. Calcium foliar sprays have been used to reduce *Alternaria* rot and bitter rot pathogens in apple fruits (Biggs *et al.*, 1993, Biggs, 1999). Calcium foliar sprays have been used to increase calcium content of strawberry to reduce grey mould infection (Cheour *et al.,* 1990).Calcium fertilization has been reported to control damage caused by storage pathogens on both potatoes and two improved yam lines (Ozygen *et al.*, 2003, Otusanya *et al.*, 2016). The effect of calcium nitrate foliar spray in yam anthracnose control has also been recently reported (Otusanya *et al.*, 2017).This study was carried out to investigate the effect of foliar-sprayed calcium nitrate on tuber rot by *Rhizopus stolonifer* in a popular white guinea yam variety from South West Nigeria known as Apepe.

MATERIALS AND METHODS

Source of Planting Material

Tubers of *Dioscorea rotundata* var. Apepe were sourced from the Lafenwa yam market in Abeokuta, Ogun State, Nigeria.

Soil Sampling and Analysis

Soil samples were collected from the experimental plot with a soil auger. Samples were air-dried, sieved and bulked according to standard methods (Page *et al*, 1982). Analysis of mineral elements in the samples was also according to standard methods (Page *et al.*, 1982).

Field Experiment

The field experiment was carried out at the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. A randomized

complete block design (RCBD) was used with three replications. Each replicate block had thirty mounds. Mounds were 1metre (m) by 1m and 80cm high. Each mound was established with a 2m stake. Inter-row, intra-row and inter-replicate spacing were 0.5m, 0.5m and 1m respectively. There was a field border of 1m around the plot.

Planting and Foliar Spray Application

Whole tubers without bruises were selected and cut into setts of 0.5kg size. Sett cut surfaces were air-dried before planting, one per mound, at the onset of rains in 2016. At 16WAP, Calcium Nitrate (analar grade) concentrations of $750mg1^{-1}$, $12000mg1^{-1}$ were prepared with distilled de-ionized water and 0.01% Polysorbate 20 (Tween 20) as surfactant. Foliar spray rate of $0mgl^{-1}$ (control), $750mg1^{-1}$, and $12000mg1^{-1}$ calcium nitrate was sprayed, randomized in each replicate using a two-litre Presto Hand Sprayer, in the early morning hours (6.00am to 7.00am). Spraying was repeated at 20WAP, 24WAP and 28WAP.

Field data collection

Assessment of number of tubers per plant and fresh tuber weight per plant were taken at 32WAP, to determine any difference in treatments before physiological maturity (36WAP), when shoot above the mound would have senesced. Measurement of tuber weight was with a top-loading field mettler balance (field scale).

Source of Pathogen

Pure isolates of *Rhizopus stolonifer* were obtained from infected tubers from a market (Omida in Abeokuta) yam lot. Seven-day old pure cultures of the pathogen were prepared according to standard methods (CMI, 1983) in petri-dishes for the inoculation experiment.

Inoculation of Tubers and Inoculation Experiment

The inoculation experiment was a completely randomized design (CRD) with 3 replicates. Tubers without bruises were selected from each treatment per replicate and weighed using a field scale. Cotton wool soaked in 80% methylated spirit was used to surface sterilize the middle portion of the selected tubers. Then a 5mm diameter hole was bored into the surface-sterilized portion (inoculation site), using a flame-sterilized and cooled cork borer. The yam core from the hole was removed with the aid of a flame-sterilized and cooled pair of forceps. A four millimetre (4mm) disc of *R. stolonifer* was transferred using a flame-sterilized (and cooled) cork borer from the pure culture into the hole bored in the tuber. The yam tissue core was then plugged back into the hole, and the inoculation site sealed with pure petroleum jelly. The inoculated tubers were transferred in plastic trays into raised, ventilated, wooden, netted structures designed for the experiment (Otusanya *et al.*, 2016) in the College screen house. This was for four weeks incubation period. After the incubation the inoculated tubers were weighed again after the petroleum jelly had been cleared from the inoculation site of each tuber (with spatula and cotton wool). Each tuber was cut through its inoculation site with a sharp stainless steel knife and infected tissue within pared away with a scalpel, on to a pre-weighed aluminium foil. Weight of infected tissue were recorded with an electronic balance. Percentage infection and percentage weight loss were calculated using the corrected weight of infected tissue (Otusanya, 1994).

STATISTICAL ANALYSIS

Data analysis was done using SAS (2000) statistical package and means were separated using Tukey's Honest Studentized Range (HSD) test.

RESULTS

Nutrient concentrations in the soil of the Experimental site

Calcium (Ca), Magnesium (Mg) and Potassium (K)in the soil of the experimental site were 31.58cmol kg^{-1}, 2.34cmol kg^{-1} and 0.52cmol kg^{-1} respectively (Table 1), all above the criticals of 0.65cmol kg^{-1}, 0.45cmol$kg^{-1}$and 0.40cmol kg^{-1} respectively reported for optimum yam production in South West Nigeria (Ibedu *et al.*, 1988). Nitrogen (N) and available Phosphorus (P) were 1.06% and 11.79ppm respectively (Table 1), below the criticals of less than 2% and 25mg kg^{-1} (Ibedu *et al.,* 1988). Sodium (Na) was 0.15cmol kg^{-1}

Yield in calcium nitrate foliar-sprayed *D. rotundata* var. Apepe

Tuber number per plant of 1.65 in the 12000mgl^{-1} treatment was significantly higher than the control which was 1.40 and also higher than the 750mgl^{-1} treatment which was 1.49(Table 2).The control and the 750mgl^{-1} treatment were not significantly different in tuber number per plant (Table 2). Tuber weight per plant which was 3.58kg (control), 3.25kg (750mgl^{-1}) and 3.11kg (12000mgl^{-1})were similar across treatments (Table 2). The equivalent for yield in tons ha^{-1} are as shown in Table 2, with overall mean of 33.13 $tonsha^{-1}$.

Infection and Weight Loss in *D. rotundata* var. Apepe tubers inoculated with *R. stolonifer* for four weeks

Infection was 60.29% in the 12000mgl^{-1}treatment significantly higher than the two other treatments (Table 3).Infection of 2.26% in the 750mgl^{-1}treatment was significantly lower than the control which was 6.66% (Table 3). Weight loss values of 0.26% (control), 1.23% (750mgl^{-1}) and 5.50% (12000mgl^{-1}) were not significantly different.

DISCUSSION

Dioscorea rotundata var. Apepe a white guinea yam variety from South West Nigeria was foliar-sprayed with calcium nitrate from 16WAP, four weekly until 28WAP, for its response to *Rhizopus stolonifer*, tuber rot, one of the three most important storage pathogens of yams in Nigeria (Dania *et al.*, 2012). Calcium, Magnesium and Potassium of the values 31.58$cmolkg^{-1}$, 0.52$cmolkg^{-1}$, and 2.34$cmolkg^{-1}$ respectively in the soil of the experimental site were above the critical levels of 0.65$cmolkg^{-1}$, 0.45$cmolkg^{-1}$and 0.40$cmolkg^{-1}$ respectively of the three elements reported for optimum yam production in South West Nigeria (Ibedu *et al.*, 1988). Nitrogen and Phosphoruss were 1.06% and 11.76ppm, both below the criticals of less than 2% and 25$mgkg^{-1}$ respectively for the two elements reported for optimum yam production in South West Nigeria (Ibedu *et al.*, 1988). The quantity of phosphorus needed when the conventional NPK fertilizer is applied in Yam production is usually smaller relative to Nitrogen and Potassium because of the mycorrhizal association in yam roots which makes it readily available to the yam plant. Nitrogen was below the critical in the experimental soil site, but it was supplied from the calcium nitrate foliar spray. These facts may be the reason for the yield obtained in this study, which was overall mean of 33.13$tonsha^{-1}$ .In a previous report of calcium nitrate foliar spray on two improved varieties and a local variety of *D. rotundata* and *D. alata*, 750mgl^{-1} and 12000mgl^{-1} treatments gave significantly higher yield by 49.19 % and 41.94% over the control, but the yields were only the equivalent of 18.50$tonsha^{-1}$ (750mgl^{-1}) and 17.60 $tonsha^{-1}$ (12000mgl^{-1}),

(Otusanya *et al.*, 2017). The site history however in the cited report of Otusanya *et al.* (2017) is that it had been monocropped to yams (*Dioscorea spp.*) for three years without fertilizer. Fresh tuber yield in this study is the equivalent of 35.8tonsha^{-1}(control), 32.5tonsha^{-1}(750mgl^{-1}) and 31.1tonsha^{-1}(12000mgl^{-1}) similar to two reports. Firstly 34.20tons ha^{-1} reported to be optimum for an economic production of yam in South West Nigeria with use of 2.5tonsha^{-1}Cassava Peel Compost fortified with 450kgha^{-1} NPK 15:15:15 (Akanbi *et al.*, 2007). Secondly 24tonsha^{-1} obtained with 300kg ha^{-1} NPK 15:15:15 reported to be optimum for white guinea yam in rainforest locations of Nigeria (Law-Ogbomo and Remison, 2008). Tuber number per plant of 1.65 was significantly higher in the 12000mgl^{-1}treatment than the control (1.40) and the 750mgl^{-1} treatment (1.49). Tubers harvested at 8MAP and inoculated with *R. stolonifer* for a four-week period had 60.29% infection in the 12000mgl^{-1} treatment significantly higher than the control which was 6.66%. But infection in tubers of the 750mg l^{-1}treatment was 2.26% significantly lower than infection in the control by 66.07%. The 750mgl^{-1} Calcium foliar spray is suitable and is recommended for *R. stolonifer* tuber rot control in *D. rotundata var.* Apepe. The 12000mgl^{-1} calcium nitrate foliar spray is high and not suitable for the variety. The two levels of $Ca(NO_3)_2$ sprayed on the plants did not reduce weight loss over the control in the 4-week incubation with *Rhizopus stolonifer*. Weight loss was 0.26% in the control, 1.23% in the 750mgl^{-1} treatment and 5.50% in the12000mgl^{-1} treatment and was similar across the three treatments.

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Table 1: Nutrient Concentrations in the Soil of the Experimental Site

Parameters	
pH (in H_2O)	6.52
Calcium (cmol kg^{-1})	31.58
Nitrogen (%)	1.06
Phosphorus (ppm)	11.79
Potassium (cmolkg^{-1})	0.52
Magnesium (cmolkg^{-1})	2.34
Sodium (cmolkg^{-1})	0.15

Table 2: Fresh Tuber Yield at 32WAP of *D. rotundata* var. Apepe Foliar-Sprayed with Calcium Nitrate

Treatment of Calcium Nitrate (mg l^{-1})	**Number of Tubers Per Plant**	**Tuber Weight Per Plant (kg)**	**Tuber Weight in tons ha^{-1}**
0	1.40 b	3.58 a	35.80
750	1.49 b	3.25 a	32.50
12000	1.65 a	3.11 a	31.10
Overall Mean			**33.13**

Means with the same letter along the column are not significantly different at P ≤ 0.05 (Tukey's HSD test)

Table 3: Infection and weight loss after 4-week incubation with *Rhizopus stolonifer* in *D. rotundata* var. Apepe tubers

Calcium nitrate Mg l^{-1}	% Infection	% Weight Loss
0	6.66 b	0.26 a
750	2.26 c	1.23 a
12000	60.29 a	5.50 a
Overall Mean		2.33

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