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Correlation between Arbuscular Mycorrhiza (AM) fungi and plant growth of two cassava (*Manihot esculenta* Crantz) clones under Bentex 'T'(Benomyl+Thiram) soil treatments

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Growth response of two clones of *Manihot esculenta* Crantz (Cassava), TMS 30555 and TMS 30572 to Bentex T soil treatment was studied. Mycorrhizal root colonization in relation to growth parameters such as stomata size, plant water content, plant foliation, as well as height and stem circumference was examined. Bentex T, a fungicide which could be used to limit the growth of Arbuscular Mycorrhizal (AM) fungi was added to soil at the concentrations of zero 0 (control), 50, 100, 500 and 1000 µg a.i. /g soil. Growth parameters had minimal variations (p> 0.05) between treatments in both clones of the plant. However, clonal differences at (p< 0.01) occurred in some of the growth parameters. The level of root colonization by the AM fungi affected the growth response of the plant. The untreated soil (control) with the highest AM fungi root colonization (84%) had the least plant foliation (15 and 16) and height (34.1 and 28.5 cm) for TMS 30572 and TMS 30555, respectively. The highest values obtained for stomata size (width and length) were at 50 µg/g bentex concentration; 0.040 and 0.019mm for TMS 30572 and 0.017 and 0.007 for TMS 30555, respectively. The least value obtained for the stomata size was at the zero (0) µg/g bentex concentration. Plants from soil treated with 100 µg/g bentex T concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555. The untreated soil had plants with the least amount of water. Implications of Bentex T soil treatment of cassava plants was discussed in relation to mycorrhizal colonization rating and some growth parameters of the test plant.

Key words: AM fungal colonization rating, growth response, cassava, Bentex T Soil Treatment

INTRODUCTION

Manihot esculenta Crantz (Cassava) is a dicotyledonous plant belonging to the Euphorbiaceae. The plant originated in North East Brazil and has spread to various parts of the world. In Nigeria, it exhibits great potential in alleviating food shortage problems due to its high yielding ability, wide ecological adaptability and low input

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requirements (Onwueme, 1978). Cassava is one of the most exploited crops in world agriculture, occupying approximately 20 million hectares with a production of about 276 million tons of roots (FAO, 2014). To achieve the higher productivity needed to meet current and future demand, agriculture must literally, return to its roots by rediscovering the importance of healthy soil, drawing on natural sources of crop nutrition since the overuse of mineral fertiliser in agricultural production has carried significant costs to the environment. Crop nutrition can enhance by such biological associations between plant roots and soil mycorrhizae (FAO, 2012). Cassava can grow and produce reasonable yields on soils where other crops would fail. It is highly tolerant of soils with low levels of phosphorus and can generally grow even with no application of phosphorus fertilizer. That is because cassava has formed a mutually beneficial association with a group of soil fungi called arbuscular mycorrhizae (AM) fungi (Howler, 2017). Present in practically all natural soils, mycorrhizae penetrate the cassava roots and feed on the sugars it produces, in exchange for enhanced phosphorus uptake by roots of the plant. Commercial inoculants of AM fungi have also been used. The combined inoculation of *Glomus clarum*, fungi in a study was significant in cassava and fostered better performance in plant growth over time (Lopes et al., 2019). However, one major drawback in the use of commercial inoculants is that the species used might not survive the competition with local AM fungi communities (Rodriguez and Sanders, 2015).

Arbuscular Mycorrhizal (AM) fungi symbiotic associations form when host roots and compatible fungi are both active in close proximity and soil conditions are favorable. The ability of cassava to yield reasonably well in soils low in phosphorus is reported to be due mainly to the crops responsiveness to AM fungi (Kang et al., 1980). It is well known that cassava obligately depends on AM fungi for phosphorus uptake and that this is increased with mycorrhizal associations (Howeler, 2017). In addition to the nutritional function they provide, AM fungi can enhance plant tolerance to both biotic and abiotic stresses (Augé et al., 2015). An ideal experimental system for the study of AM fungi and their effect on cassava growth rate would involve a fungicide that could be used specifically to eliminate or reduce AM fungi, with little or no effect on the remaining biota (Schreiner and Bethlenfalvay, 1996). One of the most widely used of such fungicide is Bentex T (20% benomyl (methyl 1-(butyl carbamoyl) benzimidazol -2-yl carbamate plus 20% Thiram). The measurement of mycorrhizal contribution to crop growth and phosphorus uptake by comparing with a control with inhibited or decreased AM fungi formation has its inherent problem. The main problems in this approach concerns soil sampling, creation of a non-mycorrhizal control and choice of test plant. However, creating the non-mycorrhizal control by benomyl turned out to decrease mycorrhization in a satisfactory degree.

The effects of fungicide targeted on AM fungi are of interest to agriculture since the inhibition of these beneficial microorganisms may counteract benefit derived from them (Schreiner and Bethlenfalvay (1996). It is on the basis of this that this work sought to establish the relationship between arbuscular mycorrhizal colonization rating and cassava growth rate in two clones (TMS 30555 and 30572) of the plant.

MATERIALS AND METHODS

Screened house experiment

Ten-day old seedlings from 2-bud node stem cuttings of two clones of *M. esculenta* Crantz, TMS 30555 and TMS 30572, obtained from a farm stead in Benin City were transplanted from moist sawdust into fungicide-treated soil in polyethene bags at the Department of Crop Science, University of Benin, Benin City. A complete randomized experimental design (CRD) in which five different treatments (bentex T dilutions) were replicated four times and duplicated for each clone of cassava giving a total of 40 samples per clone. Each polybag contained 5 kg of sandy soil taken from a field plot 100 meters from the screened house were the experiment was carried out. The fungicide used for the study Bentex 'T' contained 20% benomyl (methy 1-(butylcarbamoyl) benzimidazole-2-yl carbamate) and 20% Thiram (Tetramethylthiuram disulfide) a seed protectant fungicide as active ingredients (a.i). Fungicide was added to soil at the rate of zero (0), 50, 100, 500 and 1000 µg/g a.i. These rates constituted the five treatments in the study with the zero titre as control.

Measurement of stomatal size of plant

Fresh leaf sample detached from the parent plant was immediately painted with a quick-drying substance, cosmetic nail vanish (Theons UK), to make a leaf impression on the ad axial surface. The leaf impression was carefully removed and placed on a clean slide. A drop of glycerine was added and a cover slip placed on the impression (Hsiao and Fischer, 1975). The size of the stomata aperture was measured under a microscope (x40). The width and the length of the pores were obtained using a calibrated eye piece. Stomata pores appeared as holes.

Determination of plant water status

Shoots of plant were harvested as 08.00 h in the morning and the fresh weight determined. Plants were placed in appropriately labeled sealable paper and over dried at 80°C for 3-5 days. The weights of the dried plants were determined as follows:

$$\%\ water\ content = \frac{fresh\ weight - dry\ weight}{fresh\ weight} \times 100$$

Differences in the plant water status between treatment levels were assessed using the analysis of variance test (Slavik, 1974).

Evaluation of the effects of Bentex T soil treatment on plant foliation, height and stem girth

At the 14th week after planting measurements were taken of the foliation, height and stem circumference of 4 test plants from each treatment levels. Stem circumference data were taken at 2 cm from

Table 1. Stomata size (width and length) and number per unit surface area of cassava grown in soil treated with different concentrations of Bentex T for TMS 30572.

Mean values	Soil treatment (bentex titre – µg/g soil)		
	Zero (control)	50	100
Width of stomata (mm)	0.026	0.040	0.029
Length of stomata(mm)	0.008	0.019	0.013
Number of stomata(per mm^2 leaf area)	11.25	12.0	8.0
Statistical significance		0.040	

$p > 0.05$ = Significant.

Table 2. Stomata size (width and length) and number per unit surface area of Cassava grown in soil treated with different concentrations of Bentex T for TMS30555.

Mean values	Soil treatment (bentex concentration – µg/g soil)		
	Zero (control)	50	100
Width of stomata(mm)	0.015	0.017	0.014
Length of stomata(mm)	0.006	0.007	0.006
Number of stomata(per mm^2 leaf area)	10.75	12.75	9.75
Statistical significance		0.514	

$p > 0.05$ = Not Significant.

ground level, while height measurements were from the base of the plant to the tip of the school using a long meter rule. Foliation was done by a manual counting of leaves. Differences in height, foliation and girth data were assessed for significance, using the analysis of variance (ANOVA) test.

Estimation of Mycorrhizal colonization rating in cassava rootlets

The method of Philip and Hayman (1970) was used Root segments of cassava cut 1cm each and fixed in F.A.A. (13 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol) were heated at 90°C for about 1 hour in 10% KOH. This removed the host cytoplasm and most of the nucleus and the roots became clear with the vascular cylinder distinctly visible. The roots were then rinsed in water and acidified with dilute HCl. They were stained by simmering for 5 min in 0.05% trypan blue in lactophenol. Root segments were then mounted on slides temporarily in lactophenol. Slight pressure on the cover slip flattened KOH – treated roots for observation within a limited range of focus (x10). Quantitative estimates of root infection were made on 1cm segments by recording the number of segments with any infection and the amount of infection per unit length of root (Philips and Hayman, 1970).

RESULTS

Plant stomatal size

Data obtained on the size of stomata opening and number of stomata per leaf area for cassava clone TMS 30522 is given in Tables 1 and 2. The highest values obtained for stomatal size (width and length) were at the 50 µg/g bentex concentration; 0.040 and 0.019 mm for TMS 30522 and 0.017 and 0.007 for TMS 30555. The least value obtained for stomata size (width and length) were at the zero (0) bentex concentration (control); 0.026 and 0.008 for TMS 30572 and 0.015 and 0.006 for TMS 30555. Mean number of stomata per lower leaf area obtained ranged between 8.0 -12.0 per mm^2 leaf area for TMS 30572 and 9.75- 11.75 per mm^2 leaf area for TMS 30555.

Plant water content

Data obtained on the water content of two clones of cassava plant (TMS 30572 and 30555) are shown in Table 3. Plant water content varied significantly at $p < 0.05$ between treatment levels in both clones of the plant. Plants from soil amended with 100 µg/g bentex concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555. Analysis of variance carried out on the data showed a significance difference at ($p < 0.05$) between treatments in water content of the cassava clones.

Plant foliation, height and stem circumference

Plant treated with 500 and 1000 µg/g bentex concentration had evident phytotoxic symptoms; chlorotic dry leaves, stunted growth and heavy defoliation characterized these plants. These plants eventually withered and died after about 8 weeks of planting. Data

Table 3. Water content (%) of cassava plant (TMS 30572 and 30555) grown in soil treated with different concentrations of Bentex T.

Cassava	Soil treatment (bentex titre-µg/g soil)		
Clones	Zero (control)	50	100
TMS 30572	72	72	75
TMS 30555	73	72	76
Statistical significance		0.001	

$p < 0.05$ = Significant.

Table 4a. Cassava seedlings foliation, stem circumference and height of plant for TMS 30572.

Mean values	Soil treatment (bentex concentration- µg/g soil)			
	Zero (control)	**50**	**100**	**Statistical sig.**
Plant foliation	15.3	18.3	16.5	0.086
Stem circ. (cm)	1.00	1.12	0.95	0.091
Plant height (cm)	35.1	34.1	37.3	0.499

$p > 0.05$ = Not significant.

Table 4b. Cassava seedlings foliation, stem circumference and height of plant for TMS 30555.

Mean values	Soil treatment (bentex titre -µg/g soil)			
	Zero (control)	**50**	**100**	**Statistical sig.**
Plant foliation	16.0	17.0	16.5	0.045
Stem circ. (cm)	1.01	0.05	0.78	0.005
Plant height (cm)	28.5	38.5	35.5	0.052

$p > 0.05$ = Not significant.

obtained on the number of leaves per plant of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting shows that plants from soil treated with 50 µg/g bentex had the highest number of leaves; 18 for TMS 30572 and 17 for TMS 30555. The untreated soil (control) had plant with the least number of leaves, 15 for TMS 30572 and 16 for TMS 30555 (Tables 4a and b). On the mean height of two clones of cassava plant (TMS 30572 and 30555) at the 14th week of planting. Plants from soil amended with 50 µg/g bentex concentration had the highest plant height; 37.3 cm for TMS 30572 and 38.5 cm. The untreated soil (control) however have the least plant height; 34.1 and 28.5cm for TMS 30572 and 30555 respectively (Tables 4a and b). Differences in measurements between treatments at $p > 0.05$ were not significant in both clones of the plant. Measurement of stem girth of the two clones of cassava plant (TMS 30572 and 30555) at 14 weeks planting ranged between 0.95-1.12 in TMS 30572 and 0.73-1.01 cm in TMS 30555 (Table 4a and b). Differences in measurements between treatments was not significant ($p > 0.05$) in TMS 30572.

Mycorrhizal colonization ratings

The result of mycorrhizal colonization ratings in cassava rootlets after the 14th week of planting in two clones of cassava plant (TMS 30555 and 30572) revealed that the degree of infection by AM fungi infection varied proportionally with the amount of Bentex T applied (Table 5). The infection rates in both clones of the plant were highest among roots of the untreated soil (control); 70 and 84% for TMS 30555 and 30572 respectively and lowest in roots of plants with soil amended with 100 µg/g Bentex T; 48 and 58% for TMS 30555 and 30572 respectively. Endophytes infection ratings for plants grown in soil treated with 500 and 1000 µg/g Bentex T could not be determined because of the phytotoxic effect Bentex T had on the test plant at those titre. Analysis of variance (ANOVA) carried out on the infection data

Table 5. Mycorrhizal colonization ratings (%) of roots segments from 14 weeks old cassava (TMS 30572 and 30555) grown in soil treated with different concentrations of Bentex T.

Sample	Soil treatment (Bentex titre µg/g soil)			
	Zero (control)	50	100	Statistical significance
TMS 30572	84	72	58	0.243
TMS 30555	70	52	48	0.093

C infected number out of 100 root segments examined (%).
p > 0.05 = Not significant.

Table 6. ANOVA for Mycorrhizal colonization rates in both TMS 30572 and 30555.

Source of variation	SS	df	MS	F	P-value$^{\alpha}$	F crit.
TMS	1231.7	1	1232.7	75.983	*0.0070	4.4139
Treatment	2360.3	2	1180.2	72.750	*0.0024	3.5546
Interaction	112.33	2	56.167	3.462	^{NS}0.0534	3.5546
Within	292.00	18	16.222			
Total	3397.3	23				

α* significant at p < 0.01, F = frequency p = calculated value.
NS= Not Significant, F crit. = table value, SS = sum of squares, df = degree of freedom, MS = mean square.

showed no significant difference at (p> 0.05) in the mean mycorrhizal infection between the treatments in both clones of the plant; AM fungi root infections from the unamended soil was not significantly different from those of 50 and 100 µg/g Bentex T treated soils. However, clonal differences were significant at p<0.01 in the mycorrhizal colonization rating (Table 6).

DISCUSSION

The level of root colonization of arbuscular mycorrhiza (AM) fungi affected the growth responses (stomatal size, plant water content, plant height, foliation and stem circumference) of the test cassava plant in both clones of the plant. This is in line with report in a study carried out by Séry et al. (2016) who noted that there was a difference in the way the two native arbuscular mycorrhiza fungi species impacted cassava plant growth in green house conditions stating that AM fungi contributed to the growth and development of the test cassava plant. This study however reports the contrary, as AM colonization of test cassava plant led to growth depression. Habte (1994) reports that the growth response of *M. esculenta* Crantz (cassava) to fungicide treatment ranged from growth promotion to growth depression. Plant growth responses to colonization by mycorrhizal fungi have been observed to have a similar trend (Allen, 1992). This is consistent with this study. Under low Phosphorus (P) conditions, inoculation with in-vitro produced AM fungi inoculants has been effectively employed to reduce P fertiliser requirement for cassava and improve yield (Aliyu et al., 2019). The same response was observed for cotton plants grown in upland Asian soils (Gao et al., 2020). However, as earlier stated, the presence of AM fungi lead to growth depression in the test cassava plant as was observed in the study. The 50µg/g bentex treated soil at the 14th week of cultivation thrived more than the untreated test plant. This can be attributed to competition for carbon and nitrogen between plant and fungus (Hodge and Storer 2015). Plant stomata size was highest at the 50 µg/g bentex concentration and the least value obtained for stomata width and length were at the zero (0) µg/g bentex concentration (Tables 1 and 2). Plant water content varied significantly at p < 0.05 between treatment levels in both clones of the plant. Plants from soil treated with 100 µg/g bentex concentration had the highest amount of water; 75% for TMS 30572 and 76% for TMS 30555 (Table 3a). Augé et al. (2015) reported similarly that arbuscular mycorrhiza symbiosis alters stomatal conductance of host plant more under drought than under amply watered conditions. Mycorrhizal colonization has been reported to improve plant growth and yield by enhancing nutrient absorption in cassava, increase water stress tolerance and nematode resistance (Séry et al., 2016); however, exudes from the hyphae of fungi can stimulate growth of other soil organisms such as bacteria, and this can change the overall structure of the communities (Varela-cervero et al., 2016). This may also be responsible for the depression in growth in the untreated soil (control) reported in this study. Analysis of variance carried out on the data showed significance difference at (p< 0.05) between treatments in water content of the cassava clones (Table 3). Studies have indicated that when water is available, cassava maintains a high stomatal

conductance and can keep internal CO_2 concentration high, but when water is limiting, it closes stomata in response even small decreases in soil water potential (Cock, 1985). This observation is consistent with the study.

The level of root colonization by AM fungi affected the growth responses of the cassava plant. Plants with higher mycorrization had reduced stomatal size, plant foliation, height and circumference as compared to the plant with less AM fungi root infection. The untreated soil (control) had plant with the least number of leaves, 15 for TMS 30572 and 16 for TMS 30555 and the least plant height; 34.1 and 28.5cm for TMS 30572 and 30555 respectively (Tables 4a and b). However, studies have shown that AM fungi have benefits that facilitate and improve soil fertility (Azcón-Aguilar and Barea 2015). Hence, as earlier stated, reduced growth responses in the untreated soils may be due to competition between symbionts. Data obtained on the number of leaves per plant of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting shows that plants from soil treated with 50 µg/g bentex had the highest number of leaves; 18 for TMS 30572 and 17 for TMS 30555. On the mean height of two clones of cassava plant (TMS 30572 and 30555) at 14 weeks of planting. Plants from soil treated with 50 µg/g bentex concentration had the highest plant height; 37.3 cm for TMS 30572 and 38.5 cm (Tables 4a and b). Differences in height measurements between treatments at $p>0.05$ were not significant in both clones of the plant (Tables 5a and b). Measurement of stem circumference of the two clones of cassava plant (TMS 30572 and 30555) at the 14th week planting ranged between 0.95 – 1.12 in TMS 30572 and 0.73 -1.01 cm in TMS 30555 (Tables 4a and b). Differences in measurements between treatments was not significant ($p >0.05$) in TMS 30572.

The result of mycorrhizal colonization ratings in cassava rootlets at the 14th week of planting in two clones of cassava plant (TMS 30555 and 30572) revealed that the degree of infection by AM fungi infection varied proportionally with the amount of Bentex T applied (Table 5). The infection rates in both clones of the plant were highest among roots of the untreated soil (control); 70 and 84% for TMS 30555 and 30572 respectively and lowest in roots of plants with soil treated with 100 µg/g Bentex T; 48 and 58% for TMS 30555 and 30572 respectively. The mycorrhizal infection ratings in both clones of the plant were highest among roots with no bentex application (control) and lowest in roots of plants with the highest amount of bentex application. This is consistent with previous findings (Boatman et al., 1978). Mycorrhizal infection ratings for plants grown in soil treated with 500 and 1000 µg/g Bentex T could not be determined because of the phytotoxic effect Bentex T had on the test plant at those titre. Analysis of variance (ANOVA) carried out on the infection data showed no significant difference at ($p>0.05$) in the mean mycorrhizal infection between the treatments in both clones of the plant. AM fungi root infections from the untreated soil was not significantly different from those of 50 and 100 µg/g Bentex T treated soils (Table 5). However, clonal differences were significant at $p<0.01$ in the mycorrhizal infection rating (Table 6). This is in line with studies done by Walder and Vander Heijden (2015) who reported that factors such as environmental conditions and functional diversity, can affect nutrient exchange between the fungi and plant partners. However, some studies carried out with different varieties of cassava suggest that the level of root colonization of AM fungi was probably more dependent on the environmental conditions than on the plant variety (Begoude et al., 2016).

Conclusion

The use of arbuscular mycorrhiza (AM) fungi inoculation in sustainable agriculture is now widespread. The potential of these AM fungi species to promote growth in two clones of cassava species TMS 30555 and 30572 was evaluated. Fungicide was added to soil at the rate of zero (0), 50, 100, 500 and 1000 µg active ingredient per gram in a complete randomized experimental design (CRD). Growth responses observed varied from treatment to treatment and from parameter to parameter. The highest root colonization by AM fungi was observed in the untreated soil. Again, the least value obtained for stomata size, water content, plant foliation, height and circumference was on the untreated soil (control). This clearly shows a level of growth depression in the untreated soil. This growth depression exhibited in the untreated soil due to root colonization by AM fungi may be as a result of competition for carbon and nitrogen between plant and fungus. Clonal variations were observed for most of the parameters studied in the two clones of the cassava plant. The degree of dependency on AM fungi by a host plant has been found to be related to the host species, especially the structure of the host root system, the fungus and soil conditions. Different plant species vary in the response to AM fungi. Even within cultivars and hybrids of the same plant, response to AM fungi may differ.

The measurement of mycorrhizal contribution to crop growth and phosphorus uptake by comparing with a control with inhibited or decreased AM fungi formation has its inherent problem. The main problems in this approach concerns soil sampling, creation of a non-mycorrhizal control and choice of test plant. Mycorrhiza technology should therefore be employed in such a way that it will benefit the plant so its use is not counterproductive.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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