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Root Colonization of Some Crops by Arbuscular Mycorrhizal Fungi in Rhizosphere Soils of University of Calabar Teaching and Research Farm, Calabar

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ABSTRACT

Root colonization with arbuscular mycorrhizal fungi (AMF) can enhance the uptake of nutrients, especially Phosphorus (P), Nitrogen (N) and improve plant growth, reduce the amount of fertilizer required by plant. Arbuscular mycorrhizal fungi are soil-borne organisms that form symbiotic associations with many plant roots and are thought to play a critical role in plant nutrition, growth, and fitness. AMF colonization of ten selected crops under field condition was investigated in research farm in Calabar. The results showed that the soils were sandy loam textured, strongly acid (pH = 5.5) and were characterized by low OC (0.99%), total N (0.084%), exchangeable Ca (4.31 cmol kg^{-1}), K (0.09 cmol kg^{-1}) and Na (0.059cmol kg^{-1}), moderate in exchangeable Mg (1.54 cmol kg^{-1}) and high available P (33.94 mg kg^{-1}) and base saturation (92.32%). The results further indicated that sweet potato (96.43%) had the highest AMF colonization whereas bitter leaf (58.93 %) showed the least colonization. Rhizospheric root colonization in sweet potato were not significantly different ($p>0.05$) from those associated with groundnut, cassava, cocoyam and fluted pumpkin but were significantly different ($p<0.05$) from those observed in okro, maize, millet, pepper and bitter leaf. This research identified that plant species differs in their degree of AMF colonization.

Keywords: *Arable crops, Arbuscular mycorrhizal Fungi, Chemical properties, Root infection*

INTRODUCTION

Research attention has been geared up towards understanding the potential of my corrhizae in enhancing crop productivity. The symbiotic association between mycorrhizal fungi and the roots of plants is widespread in the natural environment (Gosling *et al*., 2006). There are a number of different types of fungi that form these associations, but for agriculture, it is the arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota (Schüßler *et al*., 2001) which are mostly important. They form highly branched hyphal structures, called arbuscules, in root cortical cells, and spread intercellularly (*Arum*-type) or intercellularly (*Paris*-type) (Dickson *et al*., 2007). The formation of arbuscules has been attributed to the unique morphological characteristic of this symbiosis responsible for nutrient exchange between the host plant and AMF (Smith and Smith, 2011). The AMF are obligate symbionts which are solely dependent on the living plant hosts for their carbon needs (Fitter and Merry weather, 1992), and the living plant host benefits immensely from arbuscular mycorrhizal association in the areas of nutrient and water uptake (Okon *et al*., 1996; Fagbola *et al*., 1998).

AMF also plays an essential role in plant performance (Munkvold *et al*., 2004; Smith and Read, 2008) at individual and ecosystem levels. Moreso, they are relevant regulators of ecosystems dynamics and functionality, enhancing phosphorus acquisition and soil aggregation, structure and fertility (van der Heijden, Bardgett and van Straalen, 2008). In addition, AMF are of great significance in increasing the ability of the host plant to absorb fixed or immobile ions such as zinc, phosphorus and copper in alkaline or acidic soils as well as in the absorption of nitrogen and water (Sharif and Moawad, 2006). In most agricultural soils, arbuscular mycorrhizal fungi are found (Ryan and Angus, 2003). They are broad-host ranged and colonize the roots of most crops and weeds. Yaseen *et al.* 2016 and Kheyrodin (2014) reported that more than 80% of cultivated agricultural crops species have been found to be colonized with mycorrhizal fungi, but in soils of Cross River State, the extent of colonization is unknown.

Nevertheless, arbuscular mycorrhizal have been involved with alkaline phosphates and acid production for hydrolysis of organic phosphates thereby facilitating its availability for roots of plant (Tarafdar and Marschner, 1994). Improved nutrition, increase in plant growth and crop yield is as a result of enhanced nutrient and water uptake. In recent times, much prominence is given on sustainable agriculture, wherein AMF association with crops is regarded to be relevant in sustainable agriculture (Gianinazzi *et al.*, 2002; Sunilkumar *et al.*, 2012). This association could also facilitate resistance to soil-borne pathogens and also promote resistance to soil pollutants including heavy metals and hydrocarbons in some cases (Achakzai *et al.*, 2012). It is undisputable that colonization of rooting system is common among all AMF. Several studies have evaluated the efficacy of arbuscular mycorrhiza through inoculation without the assessment of native AMF level in the soil intended to be utilized for crop cultivation to know if there was any need for the soil to be augmented with starter cultures.

Meanwhile, the aim of every farmer is to maximize profit. Acquiring of inoculums of AMF prepared from starter cultures would be an additional cost to the overall cost of production. Furthermore, information on mycorrhizal fungi infection in the root of predominant crops cultivated in the soil of University of Calabar Teaching and Research farm would facilitate researchers/farmers on the rational planning on soil development and use accordingly. This will also harness available agricultural land to their best sustainable use without waste of input resources. Several literatures (Sharif and Moawad 2006; Isobe *et al.*, 2007; Isobe *et al.*, 2008; Tchinmegni *et al.*, 2017) have documented the relationship between soil P and AM colonization. According to Miranda and Harris (1994), "high P levels in soil can reduce not only spore germination and hyphal growth from the germinated spores but also early colonization of the roots and growth of the extra-radical mycelium". Few studies have evaluated the levels of native AM colonization on agricultural soils and the response of root infection to soil P levels, with little or no attempts of implicating more investigation on it. It is therefore expedient not only to study the effect of inoculated AMF on crop growth and yield as reported in most studies, but also to investigate the level of infection in soil already planted with crops. This study aim at determining the level of AMF infection in the root of predominant crops in Calabar and how soil P levels react to the root infection.

Materials and Methods

Description of the Study Area

This study was conducted in Teaching and Research farm of University of Calabar. The farm is located between Latitudes 04^o 45´ and 04^o 57´N and Longitudes 08^o 21´ and 08^o 37´E. The area is characterized by distinct two tropical climates – rainy and dry seasons. The mean annual rainfall of the area exceeds 2500 mm with mean annual temperatures and relative humidity of 26.7^{o}C and 86% respectively. The farm is located on an undulating topography, underlain by the tertiary coastal plain sands parent material, usually referred to as acid sands. The soils are deeply permeable, poorly structured and low in fertility indicators, and are taxonomically classified as Kandiudults (Ultisols) according to USDA soil Taxonomy (Akpan-Idiok *et al.*, 2012), which is equivalent to Acrisol based on FAO-UNESCO legend. Physico-chemical analysis of the soil revealed that it is characteristically loamy sand consisting of 81% sand, 9.29% silt and 9.71% clay. The chemical composition of the soil was soil pH 5.5 indicating that the soil was strongly acid; 0.084% total N; 33.94 mg kg^{-1} P; 0.99% organic carbon and 1.7% organic matter.

The exchangeable cations and ECEC 6.62 cmol kg^{-1} were all low while available phosphorus 33.94 mg kg^{-1} and base saturation 92.32% were high (Table 1). The vegetation of the area is humid tropical rainforest, and is used predominantly for cultivation of horticultural and arable crops like maize, sweet potatoes, fluted pumpkin, okra, groundnut, pepper, waterleaf, cocoyam, melon, bitter leaf, watermelon, cassava, yam etc.

Table1. *Some Physico-chemical Properties of the Experimental soil*

Soil properties	Units	Values
Particle size distribution		
Sand		81.0

Silt	%	9.29
Clay		9.71
Textural class		Loamy sand
Chemical properties		
pH(H_2O)		5.5
Organic carbon	%	0.99
Organic matter		1.70
Total nitrogen		0.084
Available phosphorus	($mgkg^{-1}$)	33.94
Exchangeable cations		
Ca	cmolkg^{-1}	4.31
Mg		1.54
K		0.09
Na		0.059
Exchangeable acidity		
Al^{+3}		0.29
H^+		0.23
ECEC		6.62
BS	%	92.32

Roots and Soil Sampling

Roots of ten predominant crops were selected for this study and they were *Ipomea batatas*, *Arachis hypogea*, *Manihot esculenta*, *Colocasia esculenta*, *Telfairia occidentalis*, *Abelmoschus esculentus*, *Zea mays*, *Pennisetum glaucum*, *Capsicum spp.*, *Vernonia amygadlina.* Amongst these ten predominant crops were; three vegetable and three tuber crops, two leguminous crops, a cereal crop and a spice crop respectively. These crops which fell between 3-4 months old (juvenile age) on the farms were carefully uprooted with the roots attached to them, soil samples beneath these crops were collected as well with the aid of a soil auger at an angle of 45^0 perpendicular to the crops, and transferred immediately into labelled polyethylene bags and taken to the laboratory. The sampling was carried out in triplicate randomly selected locations within the site for the experiment.

Isolation / Quantification of Mycorrhizal Fungal

Gerdemann and Nicolson (1963) "Wet Sieving and Decanting Technique" were used for mycorrhizal fungal isolation. Clearing and staining were carried out as outlined by Kormanik *et al.* (1980). The percentage root colonization (infection) of each test crop was quantified using grid line intersect method (Gaur and Adholeya, 1994) and were expressed as:

$$\frac{\textit{Number} \text{ of root/gridline intersect with colonization}}{\textit{Total} \text{ number of roots/grid line intersect counted}} X \ 100$$

Statistical Analyses

Data collected were subjected to statistical analyses using the analysis of variance (ANOVA) procedures for Randomized Complete Block Design (RCBD). Mean values were compared using Duncan's Multiple Range Test (DMRT) at 5% level of probability when the F-ratio was significant using GenStat 12 statistical software package. Pearson correlation analysis between soil chemical properties and mycorrhizal infection in the study area were performed using RStudio package and correlation plot were generated with "corrplot".

RESULTS

Mycorrhizal Infection in Test Crop Roots

The result of the study showed that rhizospheric soil samples of the ten crops studied were infected with AMF and there were significant differences in percent infection (Table 2). The root infection varied with different crops. The highest AMF colonization (96.43%) was associated with sweet potato while the least colonization of 58.93% was observed in bitter leaf. Rhizospheric soil colonization in sweet potato was not significantly different ($p>0.05$) from those observed in groundnut, cassava, cocoyam and fluted pumpkin but was significantly different ($p<0.05$) from those observed in okro, maize, millet, pepper and bitter leaf. Similarly, the rhizospheric soil colonization in okro did not differ significantly ($p>0.05$) from those observed in maize and millet but were significantly different ($p< 0.05$) from those observed in pepper and bitter leaf. No significant difference in AMF root colonizations was observed for pepper and bitter leaf.

Table2. *Mycorrhizal infection in test crop roots*

Test crop		**Percent colonization (%)**
Common Name	Scientific name	
Sweet potato	*Ipomoea batatas*	96.43a
Groundnut	*Arachis hypogaea*	92.85a
Cassava	*Manihot esculenta*	91.07a
Cocoyam	*Colocasia esculenta*	89.28a
Fluted pumpkin	*Telfairia occidentalis*	85.71ab
Okro	*Abelmoschus esculentus*	76.78bc
Maize	*Zea mays*	69.64cd
Millet	*Pennisetum glaucum*	67.75cd
Pepper	*Capsicum Spp*	64.28e
Bitter leaf	*Vernonia amygadlina*	58.93e

Means followed by the same letter (s) within a column are not significantly different (p<0.05) according to Duncan's multiple range test.

Soil Chemical Properties Affecting Mycorrhizal Infection in the Experimented Soil

The relationship between percent mycorrhizal infection and soil chemical properties is presented in Figure 1. Strong positive correlations were observed between percent mycorrhizae infection and exchangeable K ($r = 0.471$, $p > 0.05$) and percent mycorrhizae infection and exchangeable acidity (Al^{3+}) ($r = 0.77$, $p < 0.01$) and percent mycorrhizae infection and available phosphorus

($r = 0.532$, $p > 0.05$). Similarly, among the negative correlations, those that indicates strong negative relationship were percent mycorrhizae infection and exchangeable Na ($r = -0.41$, $P > 0.05$), percent mycorrhizae infection and exchangeable acidity (H^+) ($r = -0.414$, $p > 0.05$) and percent mycorrhizae infection and base saturation ($r = -0.569$, $p > 0.05$). The observed relationships from the correlation analysis were indicative of the intricate connections among the various soil chemical properties and percent mycorrhizae infection which can hardly be observed directly in the field. The chemical properties of the experimented soil indicated that exchangeable acidity Al^{3+} ($cmol\ kg^{-1}$) was the only chemical property that strongly and significantly ($r = 0.70$, $p < 0.05$) influences the root infection by AMF in the studied soil.

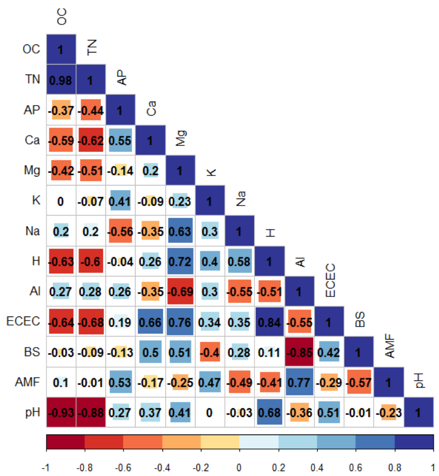

Fig1. *Relationship between percent mycorrhizal infection and soil chemical properties*

Note. *OC = Organic Carbon (%), TN = Total Nitrogen (%), AP = Available Phosphorus (mg kg-1), Ca = Exchangeable Calcium (cmol kg-1), Mg = Exchangeable Magnesium (cmol kg-1), K = Exchangeable Potassium (cmol kg-1), Na = Exchangeable Sodium (cmol kg-1), H+ (cmol kg-1), Al3+ (cmol kg-1), ECEC = Effective Cation Exchange Capacity (cmol kg-1) and BS = Base saturation (%), AMF = Arbuscular Mycorrhizal Fungi percent infection*

Discussion

The present study showed that sweet potato, groundnut, cassava, cocoyam, fluted pumpkin, okro, maize, millet, pepper and bitter leaf were associated with arbuscular mycorrhizal fungi colonization (Table 2). The results obtained from this study corroborates with that of

Iniobong and Solomon (2014) who obtained similar result in Cross River Basin of Nigeria and also affirms the findings of Sharif and Moawad (2006) who found that most economic crops were colonized by Arbuscular mycorrhizal fungi**.** This finding corroborates with the report of Kheyrodin (2014) and Berruti *et al*. (2016) who found that 80% of tropical crops were associated with mycorrhizal fungi. Iniobong and Solomon (2014) reported that the highest root colonization by fungi were associated with legume. The result of this study is in line with that observation. This study found higher AMF colonization in leguminous than in graminaceous crops. Also, root crops had higher AMF percentage colonization than the other crops investigated with the exception of fluted pumpkin which had similar percent colonization. The highest percentage colonization in groundnut and sweet potato may be due to its rooting morphology. For example groundnut has thick roots with few branches which according to Baylis (1975) in Iniobong and Solomon (2014) have greater dependence on mycorrhizal association. However, the low percent AMF colonization in maize, millet, pepper and bitter leaf is in agreement with the observation of Iniobong and Solomon (2014) who obtained similar result. With that, it indicated that different plant species differ in their degree of AMF support (Grman, 2012) and also their dependency on arbuscular mycorrhizal fungi (Carretero *et al*., 2009).

Mycorrhizal colonization depends on several factors, including the chemical properties of soil (Yusif and Dare, 2016; Tchinmegni *et al.*, 2017). The pH of the study location had no significant impact on root infection. Similar study by Wang *et al*. (2008) on soil pH and vesicular-arbuscular mycorrhizae has

Demonstrated that pH only impacted spores viability and not on mycorrhizal infection. Our results corroborate this report. We found positive correlation between percent colonization and soil phosphorus level (Fig. 1 and 2). This is contrary with other report (Isobe *et al*., 2007; Isobe *et al*., 2008) that soil phosphorus level was negatively correlated with AMF infection but corroborates the finding of Jackson and Masson (1984), Sharif and Moawad (2006) and Tchinmegni *et al*. (2017) who also reported positive effect of P availability on mycorrhizal fungi infection. Figure 2 showed that AMF infection increases with increase in soil available phosphorus, and further increase in soil available phosphorus to a point, above certain level led to a decrease in percent infection. This result corroborates those of several researchers (Isobe *et al*., 2008; Ryan and Ash, 1999; Lekberg and Koide, 2005), that available phosphorus beyond certain level can suppress percent root infection of plant by AM fungi.

The experimented soil had high levels of available phosphorus relative to the moderate value for acid sand soils which is between 18 and 20 mg kg^{-1} (Landon, 1991) but not beyond the level (50 mg kg^{-1}) at or above which root colonization of plant by mycorrhizal fungi are suppress (Swift, 2004). Several studies (Ryan and Ash, 1999; Lekberg and Koide, 2005; Isobe *et al*., 2008) also reported that application of phosphate fertilizer beyond certain level with the aim of increasing soil available phosphorus content were found to suppress percent root infection of plant with AM fungi. Similarly, other report found very high and very low phosphorus levels to reduce mycorrhizal infection/colonization (Koide, 1991). Also, the result obtained in this study found these chemical composition (total nitrogen, exchangeable Ca, Mg, Na, acidity (H^+), ECEC and base saturation) to be negatively correlated with AMF infection but showed no significant correlation ($p>0.05$). However, exchangeable acidity (Al^{3+}) were positively and significant correlation with AMF infection.

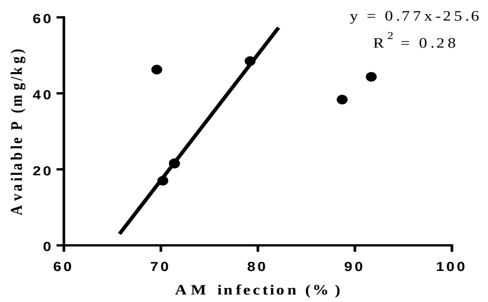

Fig2. *Relationship between AM infection and available P*

CONCLUSION

Based on the result of this study, sweet potato, groundnut, cassava, cocoyam, fluted pumpkin, okro, maize, millet, pepper and bitter leaf were all associated with arbuscular mycorrhizal fungi colonization. The highest percentage AMF colonization of 96.43% was observed in sweet potato while the least colonization of 58.93% was observed in bitter leaf. This study found that different plant species differs in their degree of AMF colonization with sweet potato and groundnut having the highest level to colonization and the level of colonization were strongly affected by exchangeable acidity (Al^{3+}). Root crops had higher AMF percentage colonization than the other crops investigated with the exception of fluted pumpkin which had similar percent colonization with root crops.

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