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Diversity of arbuscular mycorrhizal fungi in University of Port Harcourt, Port Harcourt in Southern Nigeria.

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ABSTRACT:

Arbuscular mycorrhizal fungi (AMF) are important obligate symbionts of most cultivated plant species, colonizing roots and supporting plant growth. This study was carried out to identify indigenous arbuscular mycorrhizal fungal spores in selected farms behind the Faculty of Agriculture, University of Port Harcourt, Nigeria. Three test crops namely: cassava, maize, fluted pumpkin were selected for this study while Pigweed (*Amaranthus retroflexus*) was a Control. Rhizosphere soil and root sub-samples were collected per crop from topsoil (0 – 15 cm) and subsoil (15 – 30 cm). The collected soils were prepared and subjected to various analyses to determine soil physicochemical properties. Wet sieving and decanting method were employed for extraction of spores while slide method was used for estimation of degree of colonization. Spore taxonomic identification was conducted by comparison with reference descriptions from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). Data were subjected to analysis of variance (ANOVA) using XLSTAT 2022 version package. Ecological indices: Relative Spore Density (RD), Relative Isolation Frequency (RIF), and Importance Value (IV), were calculated. The results showed that six genera: *Glomus*, *Acaulospora*, *Gigaspora*, *Entrophospora*, *Paraglomus* and *Scutteslopora* were identified. The Soil pH of the entire study area (range = 5.93 to 6.35) falls within the suitable pH range for AMF distribution. The exchangeable cations, soil pH, available phosphorus, potassium, clay content; were among the main factors responsible for the variation in AMF community structure. The percentage root colonization (%) among the crops are as follows: maize (51.94), cassava (48.46), fluted pumpkin (42.93) and control (15.63). Generally, the root mycorrhizal colonization degree and spore density were significantly higher ($P < 0.05$) in topsoil (41.63 and 21.09) than subsoil (8.74 and 6.48) across all identified genera. The superiority of *Glomus* in soils may be related to its functional relevance and ease of propagation in soil ecosystem. AMF source of biofertilizer could be adopted as

alternative source of soil amendment for cultivation of crops especially maize, cassava and fluted pumpkin which are staple food in the sampled location.
Keywords: Mycorrhiza, fungi, isolation frequency, spore density, colonization.

Introduction

Soil is an important natural resource for agriculture and industrial development of a nation. It is very important to human, plants and animals. Due to several anthropogenic and natural factors, the soil may become degraded and its' productivity consequently lowered (Bhardwaj *et al*., 2014). Soil management for improved agricultural productivity is an integral part of land management which may focus on differences in soil types and soil characteristics to define specific interventions aimed at enhancing soil quality. In Port Harcourt city of Nigeria, most farmers make use of organic and inorganic fertilizers in order to enhance good crop and soil productivity. Continuous usage of inorganic fertilizer can lead to ground water pollution which cause eutrophication of water bodies because they are often leached from the soil quickly (Akpan *et al*., 2020). Inorganic fertilizer contains salts which can lead to soil acidity, thereby causing harmful effects to plants, whereas organic fertilizer requires a longer time to produce noticeable effects during plant development.It is in this regard that effort have been channelized towards the production of high-quality crops in sustainable manner without causing damage to the environment through agricultural practices in form of application of Arbuscular mycorrhizal fungal (AMF) which are eco-friendly, encourage plant growth and promote overall wellbeing of microbial community. AMF has also been demonstrated to enhance crop performance under water-stressed conditions by improving the absorption and utilization of essential nutrients, particularly phosphorus thereby increasing overall yield (Anozie *et al*., 2025).

They belong to glomeromycota phylum/division, from the kingdom of fungi. AMF are obligate endophytic symbionts forming an association with a wide variety of host plants. In the association, the plant provides an ecological niche and carbohydrate to the fungus while the fungi improve plant growth and yield through enhanced mineral uptake. It implies that AMF could be a good alternative as natural soil amendment without deleterious effect unlike the inorganic fertilizer. Unfortunately, there is a dearth of information on identification and characterization of indigenous arbuscular mycorrhizae in University of Port Harcourt farms.

Therefore, the objective of this study was to identify and characterize the AMF in soils of the study area. The outcome will serve as a recommendation tool for peasant farmers regarding crop adaptability and performance based on the type and availability of indigenous AMF in the region.

Material and Methods

The experiment was conducted at the selected farms behind Department of Crop and Soil Science, University of Port Harcourt which is located at Latitude 4^{o} 54N and longitude 06^{o} 55E with an average temperature of 27 ^{0}C, relative humidity of 78 % but decreases slightly in dry season and an average rainfall ranging from 2500 mm – 4000 mm per annum (Atijegbe *et al.,* 2013). The area has a rainfall pattern with a long rainfall season between March and July and a short rainy season from September to October after a short spell of dryness in August and a longer period from December to February (Akande *et al.,* 2010).

Sample Collection

Three test crops namely: Cassava, Maize, Fluted Pumpkin were selected for this study while Pigweed (*Amaranthus retroflexus*) was the Control. Three healthy plants were randomly sampled per crop. Three sub-samples were collected per variety on each spot in the farm, with each sub-sample obtained from different points within the same plot to ensure adequate representation of within-site variability and to minimize sampling bias.The choice of *Amaranthus retroflexus* as Control was based on the work of Pagliarani *et al.,* (2025) who observed the absence of mycorrhizal colonization and the lack of arbuscules in *Amaranthus retroflexus*.

While sampling, care was taken to trace the crop roots, which were identified by its white colour and distinct phenolic odor emitted upon crushing. For each crop, approximately 1 kg of soil, including associated roots, was collected separately from two depth intervals: 0–15 cm for topsoil and 15–30 cm for subsoil. At each sampling point, four 250 g sub-samples were collected within about 5 m^2 each, mixed and pooled to produce composite soil sample. In each Location, three spots were sampled; both root and soil samples were collected and composite samples were obtained after thorough mixing for analysis. The samples were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed whereas soil samples were stored at 4 ^oC until analyzed. The 100 g air-dried soil was employed for extraction of AM fungal spores while 250 g was utilized for nutrient analysis.

Soil Analysis

Particle size distribution was carried out using the hydrometer method as modified by Udo *et al.,* (2009). Soil pH was determined in 1:1 (soil: water) ratio using a glass electrode pH meter. Total Nitrogen was estimated using the Kjedahl digester method (IITA, 1979). Organic matter content was determined by the wet oxidation methods of Walkey and Black as outlined by Nelson and Somers, (1982). Available Phosphorus by Turner *et al.,* (2015). Exchangeable bases Ca, Mg, K and Na were leached from the soil sample using NH_4O_4 buffered at pH 7.0. The exchangeable Na and K contents in the extracts were determined using the atomic absorption spectrophotometer (Sparks, 2003). Exchangeable acidity (Al^{3+} and $H^{+)}$ by method of IITA (1979). Effective Cation Exchangeable Capacity by summing up the exchangeable bases and exchangeable Al^{3+} as outlined by Udo *et al.,* (2009)

Processing of Roots and estimation of Root Colonization (RC)

Roots clearing and staining were done according to method provided by Phillips and Hayman (1970). Hundred root segments for each sample were randomly selected for microscopic observation and the estimation of the degree of colonization was carried out using slide method (Giovannetti and Mosse, 1980). Total root colonization by AM fungi was expressed in percent.

Extraction, quantification and identification of AM fungi

Wet sieving and decanting method (Brundrett *et al.,* 1994) was employed for extraction of AM fungal spores and sporocarps from the rhizosphere soil sampled while the Separation of AMF spores into morphological groupings after extraction from soil was done as described by Brundrett *et al.,* (1994).

Spore Density Quantification

Spore density of AM fungi was quantified using Gaur and Adholeya (1994) method. Spore density was expressed as total number of spores recorded/100g rhizosphere soil. Taxonomic identification of spores was also carried out by matching the descriptions

provided by International Collection of Vesicular AM fungi (http://invam.caf.wvu.edu).

Ecological AMF Diversity Indices

To determine differences in the structure of the AMF communities under different crops, the following parameters were calculated: The Relative Isolation Frequency (RIF) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all samples and it reflects the distribution status. RIF represents how widely a species is distributed across sampling units, RF = (frequency of a specie/sum frequency of all species) X 100/1. Relative Spore Density (RD) was defined as the ratio between the spore densities of a particular genus or specie to the total AMF spore densities and it shows the degree of sporulation ability of different AMF in a given soil.

The importance Value (IV) was used to evaluate the dominance of the AMF species based on IF and RD and was calculated as IV = (RIF + RD). An IV ≥ 50 % indicates that a genus or species is dominant; 10 % < IV < 50 % applies to common genera or species; an IV ≤ 10 % indicates that a genus or species is rare (Chen *et al.,* 2012).

Statistical Analysis

Statistically, the data obtained were subjected to analysis of variance (ANOVA) using XLSTAT 2022 version package and means were separated using Duncan Multiple Range test (DMRT) at 5 % level of significance.

Results and Discussion

Spore Density of Arbuscular mycorrhizal fungi

The spore density (SD) of the identified genera is presented in Table 1. A total of six genera were identified and isolated in this study, namely: *Acaulospora, Paraglomus, Entrophospora, Gigaspora, Glomus and Scuttelospora*. The present results with respect to quantitative distribution of AM fungi spore of various studied sites showed a great deal of variations across all the data obtained. The results of this study are favoured by the findings of Ye *et al.,* (2024) who reported that variations in the quantitative distribution of soil organisms and nutrients are closely linked to soil physico-chemical conditions—such as pH, texture, organic matter, and mineral nutrient status—which influence soil nutrient availability, microbial activity, and spatial heterogeneity of soil properties. Similarly, some scientists, such as Sharief *et al.* (2005) and Nasrullah *et al.* (2010), have confirmed that AM fungal spore abundance varies considerably from place to place, depending on the physical and chemical properties of the soil.

Generally, the topsoil of maize (30.04) and cassava (18.83) recorded the highest SD which is significantly higher than the rest SD values among the sampled crops. The anatomical differences in root structure may influence AMF development and be responsible for the differences observed in RC percentages especially as maize recorded the highest average RC among the sampled crops. This is in tandem with the findings of Brundrett and Kendrick (1990) who reported that characteristics of root surface besides the anatomical structure of the roots may also influence the early stages of the plant-fungus interaction.

Table 1: Spore Density of identified Genera

Crop	Depth	*Acaulospora*	*Paraglomus*	*Entrophospora*	*Gigaspora*	*Glomus*	*Scuttelospora*
Fluted	Top	8.833c	5.69c	4.07d	13.22d	11.13f	9.82b

Pumpkin							
	Sub	11.52b	5.46c	7.00b	9.19e	14.17e	7.24c
Maize	Top	21.09a	9.43a	6.94b	24.65a	30.04a	18.13a
	Sub	8.74c	7.85b	5.65c	16.37c	23.69b	17.50a
Cassava	Top	19.80a	8.75a	8.78a	18.83b	21.89c	9.35b
	Sub	12.72b	7.41b	3.35e	9.20e	17.78d	6.61c
Control	Top	7.57d	5.16d	2.33f	11.50d	17.17d	6.56c
	Sub	6.26d	4.80d	1.74f	3.28f	8.43g	4.35d

Means followed with the same alphabets were not significantly different at $p < 0.05$

Percentage Colonization of identified Genera (% RC)

The percentage colonization of identified genera is presented in Table 2. In the present investigations, the extent of root colonization varied among the sampled crops from Control in Scuttelospora (4.87 %) to maize in *Glomus* (51.94 %). These variations may be attributed to the differential preference of the AM fungi to various species as reported by Khade and Rodrigues (2009). It was observed that Glomus recorded its highest % RC in topsoil maize (51.94) followed by that of topsoil cassava (48.46). However, *Acaulospora* recorded its highest % RC in topsoil of maize (41.63) followed by that of cassava (37.46), likewise *Gigaspora* recorded highest % RC in topsoil maize (32.63) followed by that of cassava topsoil cassava (30.22) . This result indicates that both the maize and cassava are more dependant on the AM fungi associations compared with fluted pumpkin and the control. This observation agrees with findings of Bennie (1996) who reported that maize and cassava have greater ability for nutrient absorption, to attend to the nutrient needed for plant growth. Characteristics of root surface besides the anatomical structure of the roots may also influence the early stages of the plant-fungus interaction (Brundrett and Kendrick, 1990). The anatomical differences in root structure may influence AMF development and be responsible for the differences observed in RC percentages especially as maize recorded the highest average RC among the sampled crops.

All the % RC of the sampled genera were significantly higher than the Control % RC value (5.02). This observation is in tandem with the findings of Gupta *et al.,* (2002) who reported increased percentage of root colonization in plants inoculated with AMF compared with respective controls of non-inoculated plants.

Table 2: Percentage colonization of identified Genera

Crop	**Depth**	***Acaulospora***	***Paraglomus***	***Entrophospora***	***Gigaspora***	***Glomus***	***Scuttelospora***
Fluted Pumpkin	Top	24.94d	12.50d	16.48c	26.27c	42.93d	22.13 c
	Sub	18.50e	18.63c	12.00d	6.48e	29.89f	20.07d
Maize	Top	41.63a	33.65a	35.33a	32.63a	51.94a	27.32a
	Sub	24.85d	26.26b	24.98b	8.037	38.13e	24.32b
Cassava	Top	37.46b	29.35b	35.72a	30.22b	48.46b	15.00e
	Sub	31.31c	21.82c	16.82c	8.26d	45.83c	13.33e
Control	Top	8.70f	12.54d	8.20e	10.69d	15.63g	6.48f
	Sub	6.48f	8.37e	5.02f	9.74d	5.44h	4.87g

Means followed with the same alphabets were not significantly different at $p < 0.05$

Ecological Indices for the identified Genera (Ecological indices is presented in Table 3 below).

Relative Isolation frequency (IF)

The range of Isolation frequency which reflects the distribution status of the identified organism recorded its highest value in *Glomus* (66.87), followed by *Gigaspora* (49.19), *Scuttelospora* (36.83) *Acaulospora* (44.69), Paraglomus (25.25) while the least was in *Entrophospora* (18.45).The Dominance of *Glomu*s as observed in the present study is in agreement with the findings of many other workers Bi *et al*., (2019) and Xu *et al*., (2017) who reported that *Glomus* was dominant genus across the entire their sampled locations. The predominance of *Glomus spp.* under varying soil conditions might be due to the fact that they are widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004). Previous reports have shown that the dominant AMF species will continue to maintain its leading position across a continuous gradient from open sand prairie to closed oak-hickory forest (Benjamin *et al*., 1989). The superiority of *Glomus* in soils may be related to its functional relevance, host-specificity preference, adaptation, and ease of propagation in the soil ecosystem (Cui *et al*., 2016; Wang *et al*., 2019). In fact, *Glomus* is the most common and largest genus within the phylum Glomeromycota (Rodrigues *et al.,* 2020).

Important Value (IV)

The result of the Important Value in the study area implies that Paraglomus (IV = 35.72) and Entrosphospora (IV = 26.10) were classified as common genera while *Glomus* (IV = 94.56), Gigaspora (IV = 69.58), Scuttelospora (IV = 52.10) and Acualospora (IV = 63.22) were all classified as dominant genus as described by Chen *et al.,* 2012.

Mycorrhizal Relative Density

The Relative Density which reflects the degree of sporulation ability of different AMF in a given soil, recorded the highest value in *Glomus* (27.69 %), followed by *Gigaspora* (20.39 %), *Acaulospora* (18.53 %), *Scuttelospora* (15.27 %), *Paraglomus* (10.47) while the least was *Entrophospora* (7.65 %) and *Diversispora* (2.3 %)

Table 3: Selected Ecological Indices of the study Area

AMF GENERA	RD	RIF	IV
Glomus	27.69	66.87	94.56
Gigaspora	20.39	49.19	69.58
Acaulospora	18.53	44.69	63.22
Scutellospora	15.27	36.83	52.10
Paraglomus	10.47	25.25	35.72
Entrophospora	7.65	18.45	26.10

RD = Relative Spore Density, RIF = Relative Isolation frequency, IV = Important Value, AMF = Arbuscuar Mycorhizal Fungi.

Physicochemical properties of Soil

The clay content was highest in Control (9.965) followed by that of fluted pumpkin (8.111) and then maize (4.665) and cassava (5.687). The clay value for control is significantly higher than that

of fluted pumpkin which is also higher than those of cassava and maize respectively. However, there is no significant different in clay value of maize and that of cassava. It was observed in the results of the study that sandy soil stimulated the development of mycorrhizal association while clayey soil inhibited it. The good soil aeration is a prerequisite for optimum AMF development (Saif, 1981). This observation is in tandem with the findings of Sylvia and Williams (1992) who reported that sandy soils are usually more porous, warmer, drier, and less fertile than those of a finer texture and these conditions have direct and indirect effects on AM fungi. In general, clayey soils are more fertile than sandy soil because clay has a higher capacity for adsorbing ions from the soil solution (Malavolta, 1980). In this study high cationic exchange capacity was observed in the clayey soil and this greater nutrient concentration could have limited AMF development, as shown in several studies (Weissenhorn and Leyval 1996; Eason *et al.,* 1999). Additionally, the mechanical impediment, caused by a finer soil texture, favors the deposition of suberin on the epidermis (Wilson and Robards, 1978), which increases resistance to infection by AM fungi (Koske and Gemma, 1995). With reduced space between the soil particles, mechanical stress on the roots is increased, so that breakage of the cortical layers is increased and the colonization sites are lost. This could have reduced the dependence of the host plants on the mycorrhiza.

As evident from the results, the soil pH of studied sites is slightly acidic (5.93 - 6.35) among all three sampled locations which falls within the suitable pH range for AM fungi distribution. This favours the findings of Kamal and Prasad, (1995) who reported that the optimum pH for spore germination is between slightly acidic (5.5 - 6.5) to alkaline (8.6) which is suitable for AMF spore population.

It has been reported by Porter and Robson, (1987) also that soil pH is a major determinant of the distribution of AM fungal spores. The varying soil pH may affect the development and functioning of AM by altering the concentration of many nutrients and toxic ions in soil solutions as well as hydrogen ions (Muthukumar *et al.*, 1994). This can be explained by the fact that *Glomus* which dominated the study area prefers slightly acidic or neutral soil pH (Da Silva *et al.*, 2004) and this preference might be explained by the generally slightly soil pH in the study area as aforementioned which often leads to AM fungi communities being dominated by *Glomeraceae* species (Wang *et al.*, 2019). The Scholars, Guo and Gong (2014) also observed soil pH as the principal factor in shaping the AM fungi composition in a salt-stressed ecosystem because soil pH affected spore germination and hyphal growth (Gai *et al*., 2004). The available phosphorus recorded highest value in maize (11.750), followed by cassava (11.368), fluted pumpkin (11.255) while the Control (11.090) had the least value. All the values of Phosphorus were significantly higher as arranged above. Significant differences in the community structure of soil AMF were observed between the various plants Phosphorus and Control.

Recent research demonstrates that arbuscular mycorrhizal fungi (AMF) can significantly increase soil available phosphorus and enhance plant P uptake, both directly through hyphal phosphorus transport and indirectly via interactions with rhizosphere bacteria, leading to improved plant phosphorus nutrition and soil fertility (Zhang et al., 2025; Li et al., 2024; Olayode et al., 2024).

The Al^{3+} in the study area varied as follows: 0.192 $cmolkg^{-1}$ (Cassava) to 0.661 $cmolkg^{-1}$ (Control). This implies that there was a variation of Al^{3+} among the various evaluated crops. The variation is in agreement with the findings of Fageria and Baligar, (2008) who reported that the degree of toxicity by Al^{3+}varies widely depending on the species of grasses, growth conditions, Al concentrations and exposure time (Fageria and Baligar, 2008). Another explanation is that AM fungi is more sensitive to all the changes that take place in the legume-grass mycorrhizosphere as production of exudates and pH changes, which lead to Al chelation and retention, favoring mycorrhizal crops (especially maize and cassava) more than fluted pumpkin and control.

Effect of Soil Depth on AM Fungi Population

The present study revealed sudden decrease in AMF spore counts with depth due to the loamy sand texture which reflects the occurrence of spore density. So, soil texture is an important

characteristic for the occurrence and colonization of AM fungi spore density. In this study, root mycorrhizal colonization degree and spore density were significantly higher in the topsoil (0 – 15 cm) soil than in the subsoil (15 – 30 cm) across all the identified genera. Similar results were earlier obtained by Wu *et al.*, (2012) in the rhizosphere of *Citrus unshiu*. Such differences are accountable to the fact that AMF are aerobic fungi and the surface soil possesses higher capacity of air exchange than sub-surface soil (Wu *et al.*, 2014). The possible factors behind depleted propagule density may be, the low soil fertility, organic matter, soil texture, soil moisture and severe soil compaction.

TABLE 4
Soil properties of the Study area

ID	Sand	Clay	Silt	pH	Av.P	TN	OC	K	Na	Mg	Ca	CEC	Al^{3+}	H+	ECEC	BS
		%										$Cmolkg^{-1}$				%
Crop																
C1	86.317 a	8.111 b	7.643 b	6.35a	11.255c	0.135a	1.330 a	0.292a	0.488 a	1.836 a	3.894 a	6.514a	0.196b	0.959a	7.669 a	85.084a
C2	87.589 a	4.665 c	8.007 b	6.24a	11.750a	0.134a	1.071 b	0.239b	0.415 b	1.594 b	4.099 a	6.274b	0.358b	0.951a	7.583 a	81.136b
C3	81.426 b	5.687 c	7.304 b	6.20a	11.368b	0.122b	1.013 bc	0.300a	0.531 a	1.941 a	3.896 a	6.638a	0.192b	0.991a	7.821 a	84.396a
C4	82.787 b	9.965 a	10.611a	5.93b	11.090d	0.116b	0.946 c	0.261ab	0.386 b	1.904 a	4.022 a	6.474ab	0.661a	0.756b	7.891 a	82.762b
Soil Depth																
D1	85.541 a	7.633 a	8.195 a	6.23a	11.599a	0.127a	1.099 a	0.291a	0.478 a	1.768 a	4.034 a	6.511a	0.405a	0.991a	7.801 a	82.286a
D2	83.519 b	6.581 b	8.587 a	6.13a	11.132a	0.127a	1.081 a	0.256b	0.432 a	1.869 a	3.921 a	6.439b	0.299b	0.837b	7.681 a	84.286b

Means followed by the same letter within a column are significantly different according to Duncan's New Range Test at 5% probability level. Soil, ID = Identity, D1 = Topsoil, D2 = Subsoil, C1 = Fluted pumpkin, C2 = maize, C3 = Cassava, C4.

Conclusion

In conclusion, the results of the study revealed that AMF abundance and diversity were high in the area studied. *Acaulospora, Gigaspora, Entrophospora, Scuttelospora* were classified as common genera, *Glomus* as a dominant genus and *Diversispora* as a rare genus. Also, it was observed that *Glomus* was the most common genus in all surveyed crops. Maize and Cassava are more mycotropic and will respond to mycorrhizal inocula than fluted pumpkin. The study revealed that crop type, soil texture, soil pH, phosphorus, clay, Al^{3+} levels were identified as the factors influencing the intra-radical development of AM fungi. This study provides insights into the potentially functionally active genera in soil which can be adopted for the selective application of AMF species as soil reclamation biofertilizer inocula.

Declaration of Conflict of Interest*:* The authors declare that no known conflict of interest or personal relationships that have influence the work reported in this paper.

Author Contributions**:**

1st Author contributed to the conceptualization, methodology and supervision.

2nd Author contributed to investigation, data collection and data curation.

3rd Author contributed to formal analysis, interpretation of results, and manuscript writing (original draft preparation).

All authors contributed to manuscript revision and approved the final version.

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3