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DIVERSITY AND RELATIVE ABUNDANCE OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH CUCUMBER PLANTS IN KANO STATE, NIGERIA

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms within the phylum Glomeromycota that form mutually beneficial associations with the roots of over 80% of terrestrial plants. In this study, soil samples were collected from the rhizosphere of cucumber plants at three farms in the Kura agroecological region of Kano State, Nigeria. AMF species were isolated and identified using the wet sieving and sucrose centrifugation method. A total of eight morphologically distinct AMF species, representing the genera *Gigaspora, Septoglomus, Archaespora, Acaulospora,* and *Glomus*, were recovered. Spore abundance varied significantly among the sites, with Site A yielding the highest density (53.00 ± 5.89 spores per 50 g of soil), followed by Site B (43.00 ± 5.99) and Site C (33.33 ± 2.89). The variation in AMF diversity and abundance suggests that both biotic and abiotic factors critically influence community structure. These findings provide baseline data that may inform the selection of indigenous AMF species for use as biofertilizers to enhance cucumber growth and soil fertility. Further molecular analyses are recommended to complement the morphological identification and to elucidate the functional roles of AMF in these agro-ecosystems.

Keywords: Arbuscular mycorrhizal, Symbiosis, Photosynthates, Isolation, Identification

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil fungi from the phylum *Glomeromycota* forming mutualistic associations with the roots of more than 80% of terrestrial plants (Smith and Reed 2008; Begum *et al.,* 2019; Iliyas *et al.,* 2024). Through this symbiosis, AMF enhance nutrient uptake, particularly Phosphorus and improve plant tolerance to various abiotic (salinity, drought, heat, cold e.t.c) and biotic stresses (disease, pathogens, pest e.t.c) (Bolandnazaar *et al.,* 2007; Jung *et al.,* 2012; Chen *et al.,* 2017; Ma *et al.,* 2022; Felfoldi *et al.,* 2022; Tian *et al.,* 2023). In return the host plants provide the fungus with photosynthates product in the form of lipid and carbohydrates (Gutjahr *et al.,* 2015; Raj *et al.,* 2023; Martin and vander Heijden, 2024). This symbiotic relationship is mediated by the fungi's extensive extraradical hyphal network, which greatly expands the soil volume exploited for water and nutrient absorption (Albrechtova *et al.,* 2011; Lee *et al.,* 2013; Arcidiacono *et al.,* 2023). Moreover, these hyphae facilitate the uptake of other essential elements, including Nitrogen (N), Potassium (K), Aluminium (Al), Cupper (Cu), Zinc (Zn), and Calcium (Ca) (Baum *et al.,* 2015; Boya *et al.,* 2023; Alrajhi *et al.,* 2024). These benefits have made AMF an increasingly important focus in sustainable agriculture, and researchers are advocating for the isolation and used of AMF as biofertilizers in order to improve plants growth, yield and enhance agricultural ecosystems sustainability (Abdullahi and Sheriff 2013; Jamiolkwaska *et al.,* 2020; Ahammed and Hajiboland 2024). Cucumber (*Cucumis sativus*) is a widely cultivated creeping vine plant belonging to the *Cucurbitaceae* family that bears usually cylindrical fruits valued for its nutritional and economic importance (Swamy, 2023). Cucumber serves as a rich source of vital vitamins and minerals, such as calcium, potassium, silica, phosphorus, and magnesium for human diet (Sabry *et al.,* 2021; Xiang *et al.,* 2024). Conventional cucumber cultivation requires large amount of nutrients supply from the soil to support the plant growth, and improve crop yields (Chen *et al.,* 2017; Xiuxiu et al., 2019; Mohammadnia *et al.,* 2025). In Kano State, Nigeria, agricultural productivity is often constrained by poor soil fertility and irregular rainfall, thus harnessing the potential of AMF could offer a natural and cost-effective means of improving cucumber plants performance. Despite the recognized role of AMF in crop health and soil sustainability, little is known about the diversity and composition of AMF communities associated with cucumber plants in this region.
Understanding the diversity of AMF in cucumber rhizospheres can provide insights into the ecological dynamics of these fungi and inform strategies for improving crop yields through microbial management. The present study was aimed to assess the diversity and distribution of arbuscular mycorrhizal fungi associated with cucumber plants in Kura agricultural region of Kano State Nigeria, as it is the largest production cluster of vegetable crops in Kano state. The findings from the present study may contribute to the broader understanding of plant-AMF interactions in tropical agroecosystems.

MATERIALS AND METHODS

Soil Sampling

Soil samples were collected from the three different farms of cucumber plants at Kura agro ecological region of Kano state, Nigeria. The sites were Katsinawa, Daneji and Fegin Zabi, coordinates of all the sites were recorded. Soils (500g) were collected from the plants rhizosphere at five location points to obtain 2.5 kg of composite sample. Samples from each farm were mixed thoroughly to make a homogenous composite sample, and it was packed independently in sterile paper bag, sealed, labeled and was transported to the laboratory. Physicochemical assessment of the sampling soils was described by Yakasai and Rabiu (2024).

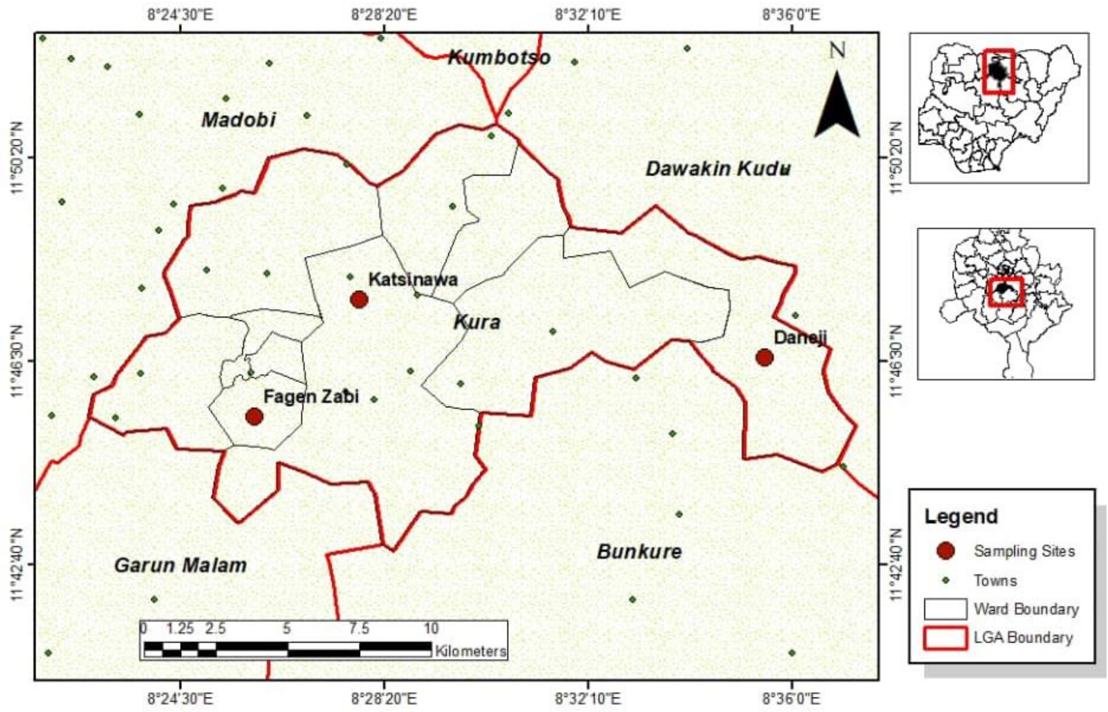

Figure 1: Map of the Sampling Sites

AMF Spore Extraction and Morphological Identification
AMF spores were extracted from the field soil samples using the wet sieving and sucrose centrifugation method of Daniel and Skipper (1982). 50 g of soil samples was mixed with 500 mL of tap water. The resulting mixture was passed through 450, 250, 120 and 45 µm sieves. The fraction retained in the 120 and 45 µm sieve was washed into centrifuge tubes using a small stream of distilled water. Tubes were filled with 60 % sucrose solution and were centrifuged at 4,000 rpm for 5 minutes. The supernatant was filtered through a filter paper Whatman No.1 and was placed on a petri dish for spore counting and morphological identification under a stereo microscope. Morphological identification of the spores was carried out by using the guide of (Schubler and Walker 2010) and the reference database established by International collection for vesicular arbuscular mycorrhizal fungi (INVAM).

Isolation of Abundant AMF Species by Trapping
The remaining collected field soil samples were mixed with sterilized soil (sterilized at 120◦C for 2hour) in a 1:1 ratio (v/v) to create a growth medium for AMF pot culture trapping under Green House conditions (Sery *et al.,* 2016). Cucumber plants were grown as a host plant for the trapping, watering of the plant was done daily up to water holding capacity of the plants and no pesticides or chemical fertilizers was used. The plants were grown for three month, and two weeks prior to harvesting the plants were subjected to water stress by watering the plants twice a week instead of everyday to allow for maximum sporulation of the existing AMF spores (Ogoma *et al.,* 2021). The soil from each pot was used to extract and identify abundant and ubiquitous AMF morphotypes using the method described above.

Statistical Analysis
The data reported in this study were expressed as mean values of three replications. Differences among the mean values were tested by ANOVA at $p<0.05$.

RESULTS AND DISCUSSION
Table 1 is the diversity and morphological Identification of AMF Spores isolated from cucumber plants rhizosphere. Eight different AMF species belonging to 5 genera were isolated from cucumber rhizosphere at the 3 sites. The Genera were *Gigaspora, Septoglomus, Acaolospora, Archaeospora* and *Glomus.* The 3 sites were found to have 5 AMF species in common, and the species were *Septoglomus constrictum, Gigaspora magariata, Acaolospora capsicula, Archaeospora trappei* and *Glomus botryoides. Glomus etunicatum* was found to be present in site B and site C cucumber soils, while *Glomus mossae* was found in site A and site B, and *Glomus macrocarpaum* was only found in site A cucumber soils. Thus in the present study, site A cucumber soils was associated with 7 AMF species, site B was also found to have 7 AMF species while site C had 6 AMF species. Also, Site A had the highest AMF density of 53.00 spores per 50g of soil, site B had 43.00 spores and site C had the lowest value of 33.33 spores per 50g of soil.
Table 2 is the diversity and morphological Identification of AMF Spores isolated from cucumber soil trap culture. The diversity of the AMF species in each pot decreased to 4 species. The 4 species that were obtained from the trap culture are *Gigaspora sp, Septoglomus sp, Achaeospora sp, and Rhizophagus sp.* However the total number of spores per 50g of soil increased, with site A having the highest value of 150.7 spores and site C had the lowest value 105.0 spores per 50g of soil.

Table 1: Diversity and Morphological Identification of AMF Spores isolated From Cucumber Plants Rhizosphere

S/N	Color	Shape	Spore Surface	Morphological Identification	Mean no of Spores per 50g of Cucumber plants Soil		
					Site A	Site B	Site C
1	Milk	Globose, Subglobose	Smooth	*Gigaspora sp*	9.00 ± 1.00^{a}	7.62 ± 1.15^{ab}	6.00 ± 1.00^{b}
2	Reddish Black	Globose	Smooth	*Septoglomus constrictum*	10.67 ±1.53^{a}	7.67 ± 1.53^{ab}	6.62 ± 1.53^{b}
3	Light Brown	Globose	Smooth	*Archaespora trappei.*	8.33 ± 1.15^{a}	7.00 ± 1.00^{ab}	5.33 ± 0.58^{b}
4	Brown	Globose	Grainy	*Acaolospora capsicula*	7.67 ± 0.58^{a}	5.33 ± 0.58^{b}	4.33 ± 0.58^{b}
5	Black	Globose	Rough	*Glomus botryoides*	6.60 ± 1.00^{a}	6.67± 0.58^{a}	6.00 ± 1.00^{a}
6	Orange	Globose	Smooth	*Glomus etunicatum*	0.00	6.33 ± 1.53^{a}	4.33 ± 0.58^{b}
7	Brown	Globose	Smooth	*Glomus Mossae*	5.67 ± 0.58^{a}	3.33 ± 0.58^{b}	0.00
8	Brown	Globose	Smooth	*Glomus macrocarpum*	7.00±1.00	0.00	0.00
	Total no of spores at each site				53.00 ± 5.89^{a}	43.00 ± 5.99^{b}	33.33 ± 2.89^{c}

Values are expressed as mean ± standard deviation. n=3.
Values with different superscript in a row are significantly different at $P < 0.05$

Table 2: Diversity and Morphological Identification of AMF Spores isolated From Cucumber Pots Trap Culture

S/N	Color	Shape	Spore Surface	Morphological Identification	Mean no of Spores per 50g of Soil		
					Site A	Site B	Site C
1	Milk/white	Globose, Subglobose	Smooth	*Gigaspora sp*	41.67± 1.00^{b}	45.00± 1.00^{a}	27.00 ± 1.00^{c}
2	Reddish Black	Globose	Smooth	*Septoglomus Sp*	37.67± 1.53^{a}	31.33 ±1.53^{b}	25.67 ± 1.15^{c}
3	Light Brown	Globose	Smooth	*Archaespora sp*	37.67± 0.58^{a}	38.67 ±0.58^{a}	28.00 ± 1.00^{b}
4	Golden	Globose, cluster	Smooth	*Rhizophagus Sp.*	34.33 ± 1.15^{a}	32.33 ±1.53^{a}	24.33± 0.58^{b}
	Total no of spores per 50g of pot culture				150.67± 4.26^{a}	137.33 ± 4.60^{b}	105.00± 3.73^{c}

Values are expressed as mean ± standard deviation. n=3.
Values with different superscript in a row are significantly different at $P < 0.05$

Discussion

The present study revealed diverse community of arbuscular mycorrhizal fungi (AMF) associated with cucumber plants at Kura agro-ecological region of Kano sate, Nigeria, with notable variations in species composition and abundance across the three study sites. A total of eight morphologically distinct AMF species were identified, belonging to genera including *Gigaspora*, *Septoglomus*, *Archaespora*, *Acaulospora*, and *Glomus*. Genus *Glomus* had the most abundant species representatives among at all the three sites. High abundance of *Glomus* in cucumber fields have also been documented by (Gai *et al.*, 2010; Hu *et al.*, 2015). The high abundance of *Glomus* species in cultivation fields is attributed to the ability of the species to produce a relatively high number of spores within a very short period of time (Khade and Rodriguez, 2009; Oyediran *et al.,* 2018) and high frequency of hyphal fusions of *Glomus* genus to plug into compatible extraradical networks and hence immediate access to host plants and subsequent spore formation (Mukhongo *et al.,* 2023). The dominance of Glomus species in agricultural fields may also be as a consequence of the strong selection pressure imposed by agricultural fields leading to the predominance of disturbance resistance AMF species (Belay *et al.,* 2015; Welemariam *et al.,* 2018; Masebo *et al.,* 2023). The presence of *Septoglomus constrictum, Gigaspora margarita, Achaeospora trappei*, *Acaolospora capsicula* and *Glomus botryoides,* at all the three sites may be attributed to the fact that most AMF species could be considered as generalists within a geographical region, as they can be present in each soil type investigated, irrespective of land use intensity and host plant species composition (Oehl and Koch, 2018; Furrazola *et al.,* 2020; Mausse-Sitoe and Dames, 2024). Moreover the variation in AMF diversity and abundance across the three sites could be attributed to both biotic and abiotic factors which significantly influence AMF community structure (Zhang *et al.,* 2021; Kumar and Tapwal 2022; Martin and vander Heijden, 2024).

The low number of spores per gram of soil of the sampling sites is probably as a result of conventional farming practice and the negative effect of agrochemicals, high-dosage use of systemic fungicides, and synthetic fertilizers may cause decreased in AMF spores abundance (Lee *et al.,* 2013; Toh *et al.,* 2018; Masebo *et al.,* 2023). Soil management practices such as tillage, might also cause a decrease in AMF relative abundance due to destruction of AMF spores and extra radical hyphal network (Hijri *et al.,* 2006; Oehl and Koch 2018; Avila-salem *et al.,* 2020). Even though the relative abundance of the AMF spores in the present study were low however the diversity of the AMF species is in accordance with the normal scale of AMF species diversity proposed by sieverding (1991) of 6–9 AMF species in high-input intensive agriculture, 10–15 species in low-input systems and 16–21 species in natural ecosystems (Straker *et al.*, 2010). The high diversity of AMF species in the present study may be attributed to the positive effect of nitrogen in the soil which might have been indirectly involved in the sustainance of the AMF species through the increased supply of photosynthates from the host-crop to the fungi (Cavagnaro *et al.,* 2006; Ingraffia *et al.,* 2019).

The results of pots culture trapping revealed a decreased in AMF species diversity identified directly from field soils. Similar observations of decreased in AMF species diversity after pot culture trapping were made by Belay *et al*. (2015), Chairul *et al.* (2019) and Danesh *et al.* (2022). Pot culture trapping is an important technique for obtaining abundant healthy spores of different species so as to establish mono specific cultures that can be used as AMF inoculum or biofertilizers which can be used for plants inoculation (INVAM, 2021). The procedure is widely used in AMF ecological studies and usually provides sufficient quantities of viable spores with almost all morphological traits that can aid in accurate identification and detection of non sporulating AMF species (Ogoma *et al.*, 2021). Trap cultures act as a filter allowing sporulation of only AMF species that are aggressive enough to colonize and sporulates in a fast growing host under greenhouse conditions in short time span (Songachan and Kayang, 2013; Mukhongo *et al.*, 2023). A likely reason for failure in production of new spores by the remaining species during trapping of AM fungi may perhaps be due to the fact that spores may have not been viable when isolated from the field samples or the spores may have extended dormancy or their quiescence was not broken in the conditions and time span used (Khade and Rodrigues, 2009). Although the remaining species were lost during pot culture trapping, however another species *Rhizophagus irregularis* was obtained from all the three pots cultures which were not initially observed during identification of AMF species from farm soils. Similar observation of new AMF species from pot culture trapping was documented by Burrows & Pfleger (2002), Stracker *et al.* (2010) and Trejor-aguilar, (2013) who noted that field spores could not be maintained in trap cultures after subculturing. The sporulation of *Rhizophagus irregularis* in pot culture only is an indication that the species were at the vegetative stage inside the roots during sampling and then subsequently began sporulating under favorable green house condition.

CONCLUSION

This study highlights the diversity and site-specific distribution of arbuscular mycorrhizal fungi (AMF) associated with cucumber plants in Northern Nigeria. Eight distinct AMF species were morphologically identified, with variations in abundance and composition across the study sites. Site A recorded the highest spore density and species richness, suggesting favorable ecological conditions for AMF proliferation. These findings provide valuable baseline data for the development of sustainable agricultural practices, particularly in selecting indigenous AMF species for used as bio-fertilizer in order to enhance cucumber growth and soil fertility. Further research using molecular tools is recommended to complement morphological identification and to better understand the functional roles of AMF in these agro-ecosystems.

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