**SEASONAL EFFECTS ON ARBUSCULAR MYCORRHIZAL FUNGI DIVERSITY AND COLONIZATION IN COWPEA (*Vigna unguiculata* L. Walp) RHIZOSPHERE OF RAINFOREST SOILS**

**ABSTRACT**

This study investigated the impact of seasonal variation on the richness and composition of Arbuscular mycorrhiza fungi (AMF) communities in soil from rainforest vegetation in Rivers State, Nigeria. Samples of top soil (0-15cm) and subsoil (15-30cm) were collected from rainforest vegetation in Port-Harcourt, and sampling months were categorized into rainy season, peak of rainy season, dry season and peak of dry season based on the data obtained from NiMET during the study period. A potted experiment was carried out at the teaching and research farm of the Rivers State University from January to April (dry season) and April to July (rainy season) to determine the colonization status of AMF in rhizosphere soils planted with cowpea. Morphology and microscopy were used for AMF identification. A total of eight genera of AMF namely: *Glomus, Acaulospora, Gigaspora, Paraglomus, Rhizophagus*, *Dentiscutata, Archeaospora, and Clariodeglomus* were isolated and identified in rhizosphere soils of cowpea cultivated, and the mycorrhiza genera followed the order: *Acaulospora* > *Glomus* > *Archeaspora* > *Clariodeoglomus* > *Rhizophagus* > *Paraglomus* > *Dentiscutata* > *Gigaspora*. AMF colonization and population was higher in the dry season than in the rainy season indicating that seasonal variations has an impact on AMF diversity, population, and colonization of cowpea rhizosphere. Seasons can be taken advantage of to improve plant productivity and health.

**Keywords:** Colonization, Arbuscular mycorrhiza Fungi, Cowpea, Seasonal Variations, Tropical Rainforest

**INTRODUCTION**

One of the primary challenge faced by agriculturist in Africa is the continuous reduction in crop yields due to dwindling soil fertility (Houngnandan *et al*., 2022). In order to boost crop yield, farmers frequently use chemical fertilizers, whether or not they are registered, and although this increased use of chemical fertilizer increases crop yields, it has detrimental effects on the ecosystem and has become a serious concern (Ashoka *et al*., 2017; Meena *et al*., 2017).

As an alternative to chemical fertilizers, legumes which have the ability to symbiose with nodule bacteria (rhizobia), that are found in most tropical soils, if not all of them, have been proposed and used (Houngnandan *et al*., 2022). This is because rhizobia have an enzyme called the nitrogenase complex that can convert atmospheric nitrogen (N2) into substances that the host plant can absorb, and when non-nodulated crops like cereals fail in Nitrogen-deficient soils, this trait fertilizes the soil to provide for a sufficient yield (Vertès *et al*., 2015). In tropical nations where costly nitrogenous fertilizers are scarce, optimizing N2 fixation is a cost-effective solution, and the biological nitrogen fixation by legume crops benefits succeeding cereal crops when rotated or intercropped (Houngnandan *et al*., 2022).

Cowpea (*Vigna unguiculata*) is one of the leguminous crop that is widely grown in Nigeria, and contributes significantly to the safe supply of proteins in food systems by raising protein levels necessary for appropriate human nutrition (Vertès *et al*., 2015). The crop is a members of the Fabaceae family and Faboideae subfamily (Sindhu *et al*., 2019), it is a major grain legume that has been adapted to sub-Saharan Africa (SSA), where it helps with the nutrition, health, and income of people living in rural and suburban areas (Boukar *et al*., 2015; Horn *et al*., 2022). Hence, finding the best methods to boost cowpea productivity without harming the environment or soil is essential (Houngnandan *et al*., 2022).

Arbuscular mycorrhizal fungi (AMF), which inhabit the rhizosphere of crops are known to improve crop yield in soil through mineral absorption in general and phosphorus absorption in particular, and their presence have been known to boost cowpea productivity (Kpenavoun *et al*., 2018; Houngnandan *et al*., 2022). AMF improves crop phosphorus absorption in two ways: first, by mineralizing organic phosphorus via the action of phosphatases found in mature arbuscular and intercellular hyphae; and second, by producing acid, which dissolves insoluble phosphorus (tricalcium phosphate, rock powder) (Kpenavoun *et al*., 2018). Also, AMF are seen as an essential part of the plant–soil system because they form symbiotic relationships with the majority of land and cultivated plants and enhance vital ecosystem processes and plant productivity (Johnson *et al*., 2016; Dong *et al*., 2025).

Furthermore, AM fungal populations are particularly sensitive to environmental changes acting as "indicators" in ecosystems (Dong *et al*., 2025). For instance, the distribution and dominating species of AM fungus provide a reliable indicator of soil health and the degree of ecosystem degradation (Vasconcellos *et al*., 2016; Wu *et al*., 2021), and because of their sensitivity, AM fungi are now more valuable in ecological restoration and environmental monitoring projects (Asmelash *et al*., 2016; Singh *et al*., 2022).

Additionally, numerous factors, including vegetation communities, soil physicochemical parameters, climate change, and human activities, have been reported to affect the composition, diversity, and functional aspects of AM fungal communities (Jamiołkowska *et al*., 2018; Jerbi *et al*., 2021). Also, numerous studies have demonstrated that seasonal change can impact AM fungal diversity, spore density, root colonization, and community composition (Lara-Pérez *et al*., 2020; Liu *et al*., 2020; Singh *et al*., 2022), but the impact of seasonal variation on the richness and composition of AM fungal communities, particularly in soil from rainforest vegetation in Rivers State, Nigeria, has received little attention. This study aimed to assess Arbuscular mycorrhizal fungi diversity, population, and colonization status in cowpea rhizosphere in rainforest soil during the two seasons.

**MATERIALS AND METHODS**

**Study Area**

The study area was Port Harcourt metropolis; Port Harcourt is situated in the humid rainforest region of Southern Nigeria. It lies between latitude 4.50N and longitude 7.00E on an elevation of 18m above sea level. The climate of the area is tropical with two prominent seasons, the wet (rainy) and dry seasons. The dry season is short, usually lasting for 4 months, from November to March, with little rains during this period; while the longer wet season prevails during the remaining months. The mean annual rainfall in Port Harcourt ranges from about 3,000mm to 4,500mm. Annual maximum temperature ranges from 220C to 290C while relative humidity varies between 75% and 95%.

**Collection of Soil Samples**

Four kilograms of soil was collected from individual sampling points in the rainforest and mangrove vegetation in both rainy and dry seasons which were transferred to the Rivers state University school farm, and placed into experimental pots for the cultivation of Vigna unguiculata (Cowpea). Soil samples were collected from the experimental pots, just before the cultivation of cowpea seeds, and transferred to the laboratory, for analysis of physical, chemical and microbial properties before cultivation of cowpea. The experimental pots containing soils from the two vegetation types were laid out in a complete randomized complete block design on the school farm.

**Planting**

Seeds of cowpea local variety (Iron beans) was obtained from the market and used for this experiment four seeds were planted per pot, after germination, the seedlings were thinned to two seedlings per pot to allow for vigorous growth. Cultivation of cowpea seeds was carried in the months of January and April, for dry and rainy season respectively. Daily irrigation was carried out in the dry season to prevent death of the crop.

**Rhizosphere Soils**

Rhizosphere soils containing both soil and root samples were collected on the 12th week during each season and transferred to the laboratory for estimation of Arbuscular mycorrhiza colonization status, and population. Rhizosphere soils were also collected at 12 weeks for analysis of soil physical, chemical and microbial properties after cultivation of cowpea.

**Evaluation of AM Fungal Colonization**

Fresh fine roots were selected from cowpea plants. The roots were put inside KOH10% solution for 24hr at room temperature. The KOH solution was discarded afterwards, and the root samples were washed with flowing water till they became clean. Roots were then soaked in HCl 2% for 30min. The cowpea roots were soaked in staining solution (trypan blue 0.05% + glycerol 70% + aquadest 30%) for 24hr. The roots were washed again and put into glycerol 50% solution. Roots were cut into pieces of 1cm and placed on slides for microscopic observation at 400x magnification. Fields of view which showed colonization were marked with (+) while those without colonization were marked with a negative sign (-).

**Isolation and Extraction of Arbuscular Mycorrhizal Fungal Spores**

The AM fungal spores were separated from the soil by wet sieving and decanting technique. Fifty gram of rhizospheric soil sample was mixed in 200ml of distilled water in a large beaker. After 1hr, the contents of the beaker were decanted through sieves which were arranged in a descending order from 200um to25um size. The process was repeated thrice, until the upper layer of soil suspension was transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out.

**Identification of Mycorrhizal Fungi**

Identification of AMF spore was performed by morphological observation of color, shape, size, hyphal attachment, spore ornamentation and spore reaction towards Meizer’s solution. Spore enumeration was conducted under a stereo microscope and spore identification was conducted under a microscope with 200x magnification.

**Statistical Analysis**

Data collected from the various parameters was subjected to analysis of variance (ANOVA) at P ≤ 0.05. Means were separated using Tukey’s Pair Wise Comparison at 95% confidence intervals.

**RESULTS**

**Seasonal Classification of Climate Parameters**

Based on the data obtained from the Nigerian Meteorological Agency (NIMET) during the study period, June 2023 – May 2024, the period was classified into four seasons: Peak of Rainy season (PRS), Rainy season (RS), Peak of Dry season (PDS) and Dry season (DS). The monthly and seasonal classification of climate parameters recorded during the months of sampling are presented in Table 1. During the Peak of Rainy season (June-August), Minimum and maximum atmospheric temperature ranged from 22.830C-31.170C, with a mean value of 23.090C and 29.980C respectively. Rainfall ranged from 202.69mm - 401.83mm, with a mean value of 299.72mm; Relative humidity and Soil Temperature ranged from 86.39%-87.77% and 26.900C - 28.940C with mean values of 87.30% and 28.240C respectively. In the Rainy season (September - November), minimum and maximum temperature ranged from 22.460C-31.290C, with mean values of 22.930C and 30.920C respectively. Values for rainfall ranged from 94.23mm- 364.24mm, with a mean value of 203.96mm, relative humidity and soil temperature ranged from 85.52% - 86.23% and 27.170C-28.250C with mean values of 85.94% and 27.770C respectively (Table 1). During the Peak of Dry season (December-February), minimum and maximum temperature ranged from 21.320C-22.600C and 33.350C-33.950C with mean values of 21.930C and 33.400C respectively. Rainfall ranged from 0.00mm - 63.75mm, with a mean value of 40.39mm; while relative humidity and soil temperature ranged from 73.29%-79.86%, and 29.440C-29.900Cwith mean values of 76.83% and 29.680C respectively. In the Dry season (March-May), minimum and maximum temperature ranged from 23.860C- 32.880C with mean values of 23.960C and 32.530C. Values for rainfall ranged from 41.15mm-288.80mm, with a mean rainfall value of 140.72mm. Relative humidity and soil temperature ranged from 82.74%-83.65% and 29.460C- 29.830C with mean values of 83.23% and 29.640C respectively.

**Table 1: Seasonal Classification of Monthly Climate Parameters (2023-2024)**

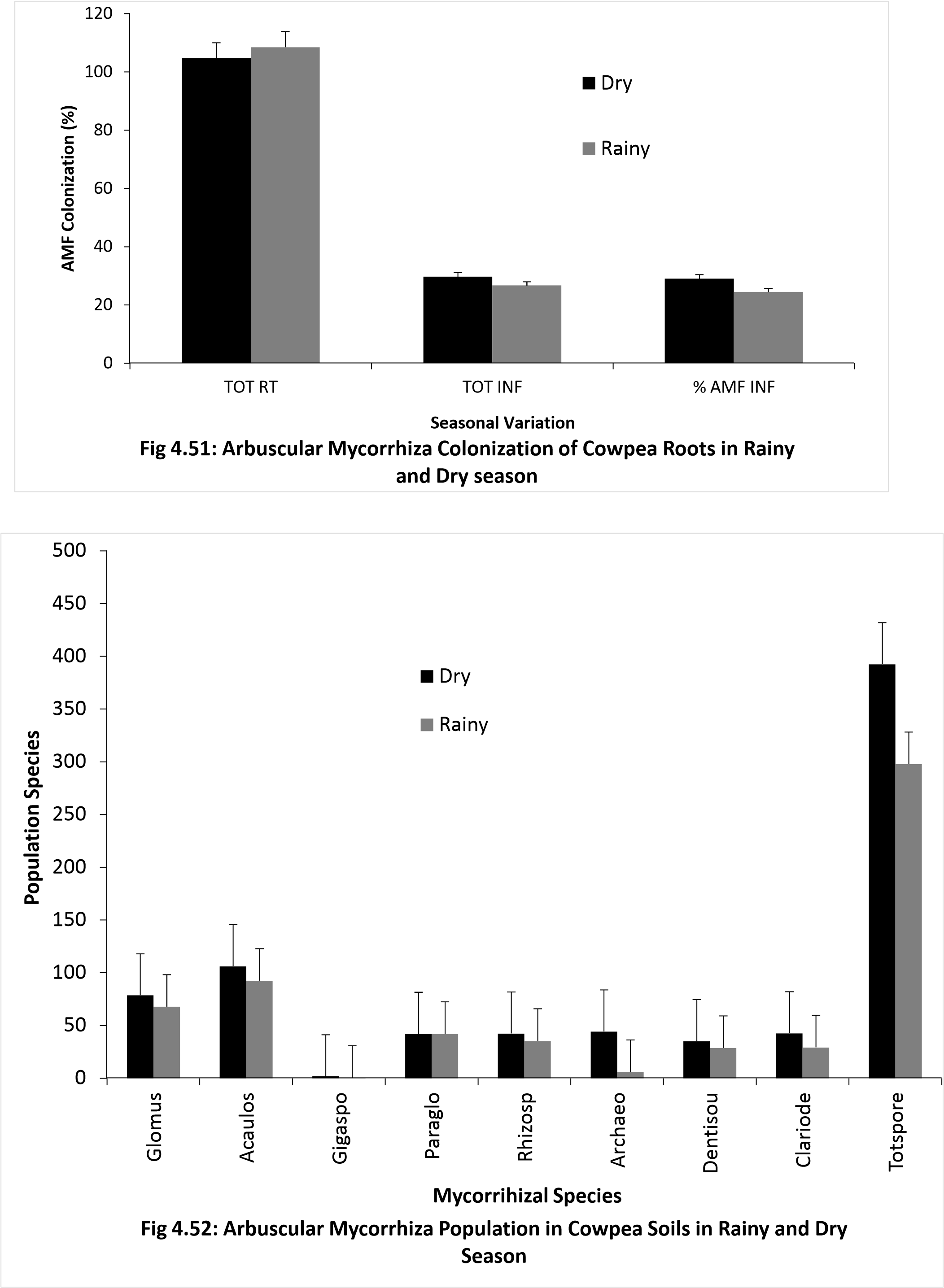
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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Seasons** | **Months** | **Max**  **Temp.**  **(0C)** | **Min**  **Temp.**  **(0C)** | **Rainfall**  **(Inches) (mm)** | | **Soil**  **Temp.**  **(0C)** | **Relative**  **Humidity**  **(%)** |
| Peak of  Rainy  Season | June  July  August | 31.17  29.29  29.48 | 22.95  22.83  23.50 | 15.82  8.61  7.98 | 401.83  218.69 202.69 | 28.87 28.94  26.90 | 87.77 87.74  86.39 |
| Mean | 29.98 | 23.09 | 11.80 | 299.72 | 28.24 | 87.30 |
| Rainy Season | September | 30.32 | 22.46 | 14.34 | 364.24 | 27.17 | 86.23 |
| October | 31.15 | 22.98 | 6.04 | 153.42 | 28.25 | 85.52 |
| November | 31.29 | 23.34 | 3.71 | 94.23 | 27.90 | 86.07 |
| Mean | 30.92 | 22.93 | 8.03 | 203.96 | 27.77 | 85.94 |
| Peak of  Dry  Season | December  January  February | 33.48  33.95  33.35 | 21.32  21.86  22.60 | 0.00  2.26  2.51 | 0.00  57.40  63.75 | 29.69  29.90  29.44 | 73.29  77.35  79.86 |
| Mean | | 33.40 | 21.93 | 1.59 | 40.39 | 29.68 | 76.83 |
| DRY  SEASON | March  April | 32.88  32.78 | 23.92  24.11 | 3.64  1.62 | 92.46  41.15 | 29.46  29.83 | 82.74  83.30 |
| May | 31.93 | 23.86 | 11.37 | 288.80 | 29.64 | 83.65 |
| Mean | 32.53 | 23.96 | 5.54 | 140.72 | 29.64 | 83.23 |

**Source: Nigerian Meteorological Society (NIMET) (June 2023- May 2024)**

***Arbuscular Mycorrhiza* Spore Abundance (Population), Species richness and Diversity in Cowpea Rhizosphere soils**

*Arbuscular mycorrhiza* spore abundance varied between the rainy and dry season (Figure 1). Total number of spores was significantly higher (P ≤ 0.05) in dry season (392.5spores/100g of soil) than in the rainy season (297.7spores/100g of soil). At both seasons, the highest spore numbers were recorded for *Acaulospora s*p, with 92.3 and 106.1 spores per 100g of soil, for rainy season and dry season respectively. This was followed by *Glomus s*p. with 67.7 and 78.5spores/100g of soil for rainy season and dry season. The lowest spore numbers were recorded for *Gigaspora sp* with values of 0.3 and 1.8spores/100g of soil, in rainy season and dry season, respectively (Figure 1).

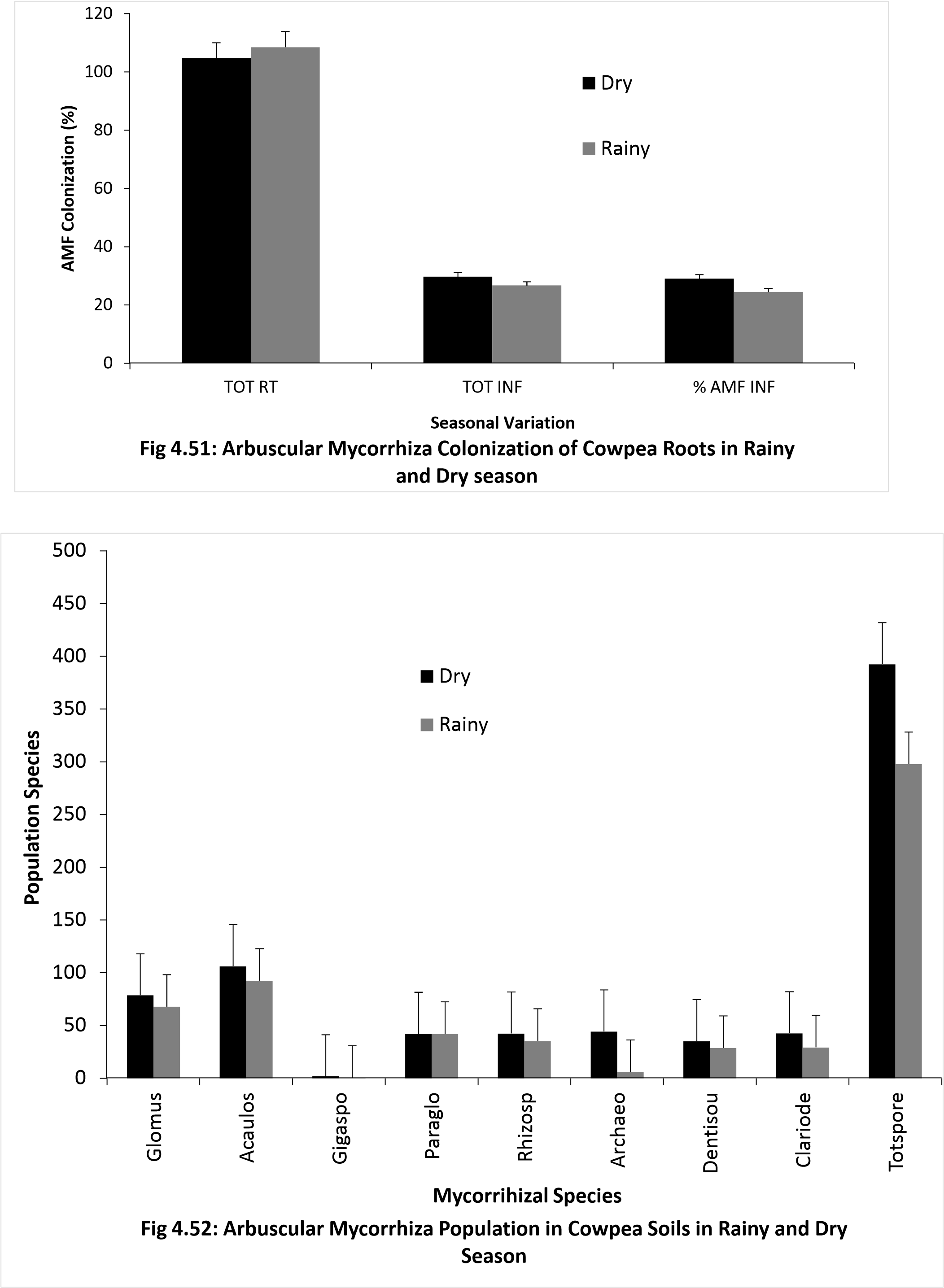
During both seasons, a total of eight arbuscular mycorrhiza fungal morphological types were observed, belonging to eight different genera, which include: *Glomus,* *Acaulospora,* *Gigaspora*, *Paraglomus,* *Rhizophagu*s, *Archaeospora,* *Dentiscutata,* and *Clariodeoglomus* (Figure 1). The mycorrhiza genera followed the order *Acaulospora* > *Gomus* > *Archaeospore* > *Clariodeoglomus* > *Rhizophagus* > *Paraglomus* > *Dentiscutata* > *Gigaspora* for spore abundance (spores/100g) of soil, in both rainy season and dry season.



**Figure 1: *Arbuscular Mycorrhiza* Spore Abundance (Population), Species richness and Diversity**

***Arbuscular Mycorrhiza* Colonization Status**

Cowpea roots were infected by *mycorrhiza* fungal hyphae and arbuscules in both rainy and dry season (Figure 2). Total number of roots in rainy season ranged from 106.4-109.6 with a mean value of 110 while total number of roots in dry season ranged from 103.4-105.6, with a mean value of 105. Total root infection ranged from 13.6 - 49.0 in rainy season with a mean value of 27.0, while values in the dry season ranged from 26.2 - 33.0 with a mean value of 30. Percentage AMF infection ranged from 12.8% - 44.7% in the rainy season with a mean value of 24.5%, while percentage infection in the dry season ranged from 27.3% -31.3%, with a mean value of 29% (Figure 2). AMF root colonization was higher during the dry season than at the rainy season, though not significantly different (P ≤ 0.05).



**Figure 2: *Arbuscular Mycorrhiza* Colonization Status**

**DISCUSSION**

Numerous factors affects AM fungal populations, and some of these factors include the type of host plant (López-García *et al*., 2017), the pH of the soil (Li *et al*., 2021), changes in soil nutrient content, especially nitrogen and phosphorus (Ceulemans *et al*., 2019), brought on by management practices or climate change (Xiao *et al*., 2021; Shi *et  al*., 2022). In this study, we investigated the impacts of seasonal variations on AMF diversity, population and colonization status, and found that AMF spore abundance varied between the rainy and dry season, and the total number of spores was significantly higher (P ≤ 0.05) in dry season (392.5spores/100g of soil) than in the rainy season (297.7spores/100g of soil) (Figure 1). The spore density under cowpea in this study aligns with those reported by Balogoun *et al*. (2015), under cashew plantations in central Benin, and under *Isoberlinia doka* in the Wari-Maro classified forest in northern Benin (237 to 258 spores per 100g soil), but is lower than those reported by Bossou *et al*. (2019) under maize (6260 spores per 100g soil), and. The plant species itself may be the cause of this variation in spore density levels. In fact, the quantity and makeup of mycorrhizal fungal spores can be directly influenced by a plant species, environmental factors, and floristic composition (Houngnandan *et al*., 2022). Moreover, legumes thrive in impoverished soils, in part because to the symbiotic microbes like rhizobia and mycorrhizal fungi that colonize their root system, and through the release of exudates into their rhizosphere, they can encourage the growth of microorganisms, particularly mycorrhizal fungi, and the generation of fungal propagules (mycelial hyphae, spores) (Houngnandan *et al*., 2022).

During both seasons, a total of eight arbuscular mycorrhiza fungal morphological types were observed, belonging to eight different genera, which include: *Glomus,* *Acaulospora,* *Gigaspora*, *Paraglomus,* *Rhizophagu*s, *Archaeospora,* *Dentiscutata,* and *Clariodeoglomus* (Figure 1). The mycorrhiza genera followed the order *Acaulospora*> *Gomus> Archaeospore*> *Clariodeoglomus> Rhizophagus> Paraglomus> Dentiscutata> Gigaspora* for spore abundance (spores/100g) of soil, in both rainy season and dry season. These species of AMF are among the 97 species of AM fungi, including 937 operational taxonomic units (OTUs) from 10 genera and 9 families that were identified in the study by Dong *et al*. (2025), and also by Houngnandan *et al*. (2022) whose study involved soybean. However, *Glomus* and *Paraglomus* were the most prevalent genera reported Dong *et al*. (2025) which is contrary to findings in this study in which *Acaulospora s*p had the highest spore numbers at both seasons (Figure 1). Also, the genus *Glomus* had the highest representation (40 percent of all OTU) in the study by Diop *et al*. (2015), and was the most prevalent genus in alpine meadow and alpine steppe, according to the spatial distribution of AM fungal communities in 15 alpine grasslands on the Tibetan Plateau by Zhang *et al*. (2024). Glomus has consistently been found to be widely dispersed in different ecosystems worldwide and to be extremely adaptable to severe settings (Coutinho *et al*., 2015; Bonfim *et al*., 2016).

Seasonal variation has a significant impact on AM fungal communities, and still plays a small part in ecology of the rainforest in Rivers State. We discovered that species, community structure, composition, and diversity were all impacted by seasonal variation in this study (Figures 1 and 2). Cowpea roots were infected by *mycorrhiza* fungal hyphae and arbuscules in both rainy and dry season (Figure 2). Total number of roots in rainy season had a mean value of 27.0, while a mean value of 30 was recorded for dry season, percentage AMF infection in the rainy season had a mean value of 24.5%, while in the dry season a mean value of 29% was recorded (Figure 2). AMF root colonization was higher during the dry season than at the rainy season, though not significantly different (P ≤ 0.05) (Figure 2). Similarly, Dong *et al*. (2025) reported that seasonal fluctuation had a substantial impact on the diversity, distribution, and composition of AM fungal communities in the alpine grassland of the eastern Tibetan Plateau (p < 0.05), with geographic distance acting as a determining factor. Nevertheless, some research has shown that seasonal fluctuations have little effect on the spore density, community composition, and richness of AM fungus (Pereira *et al*., 2018; Álvarez-Lopeztello *et al*. 2019). Seasonal variation's impact on AM fungal communities' features is therefore complicated and impacted by a number of environmental factors. To better understand how climate and environmental factors affect the characterization of AM fungal communities in various habitats, more research is therefore required.

**CONCLUSION**

AMF are an essential part of the plant–soil system because they form symbiotic relationships with the majority of land and cultivated plants and enhance vital ecosystem processes and plant productivity. Numerous factors affect AMF population in soil and seasonal variation also has a significant impact on AM fungal communities, and still plays a small part in ecology of the rainforest in Rivers State. In this study, species, community structure, composition, and diversity of AMF were all impacted by seasonal variation.

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