Research Article

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Occurrence of arbuscular mycorrhizal fungi spores in soils of some legumes and their response to varying concentrations of phosphorus application

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Abstract: A potted experiment arranged in a 5×3 factorial in a randomized complete block design was undertaken to investigate the occurrence of arbuscular mycorrhizal fungi (AMF) in the soil of five leguminous plants: *Cajanus cajan* (L.) Huth, *Centrosema pascuorum* Martius ex Benth, *Crotalaria ochroleuca* G. Don, *Lablab purpureus* (L.) Sweet and *Mucuna pruriens* (L.) DC. The effects of varying phosphorus concentrations (P0) (0 kg/ha of single superphosphate), P1 (100 kg/ha of single superphosphate) and P2 (200 kg/ha of single superphosphate) on the population of AMF spores under these legumes were also carried out. The AMF spores in soil samples were extracted at 19 weeks after planting, using the wet sieving and decanting method, and enumerated with the aid of a stereoscopic microscope. Spores of different species of genera *Glomus* and *Gigaspora* were encountered in the soils of the five leguminous plants. Spores of *Glomus* species predominated while the spores of *Gigaspora* species were found in lower numbers. The total AMF population was significantly affected by legume species ($p \leq 0.05$). The total AMF spore counts were higher in the soils of *Mucuna pruriens* and *Crotalaria ochroleuca* ($p \leq 0.05$). The populations of *Glomus mossae* in soils decreased with increasing level of applied phosphorus ($p \leq 0.05$). A positive correlation was recorded between the total AMF spores, the predominant AMF spores and soil pH, while the organic matter content and the available phosphorus were negatively correlated with both the total AM spores and the predominant AMF spores.

Keywords: AMF spore, forage, *Glomus* species, *Gigaspora* species and soil

1 Introduction

The *Leguminosae* family is the second largest among worldwide food crops after cereals. Legumes are second to *Poaceae* (the grasses) in agricultural and economic importance (Curiel et al. 2015). Legumes have an important place in crop rotation, provide protein-rich food for man and livestock, are used in admixture with grasses in hay and pastures and are also used as cover crops and green manures. The growth of legumes on soils improves the nutrient status of soil by increasing the nitrogen content of soil. This also has a profound effect on soil characteristics, such as soil structure, soil texture and water content (Peoples et al. 2009; Kopke and Nemecek 2010; Chianu et al. 2011; Lupwayi et al. 2011).

Arbuscular mycorrhiza fungi (AMF) enhanced the uptake of relatively immobile soil ions such as phosphorus (P), magnesium (Mg), calcium (Ca), copper (Cu), manganese (Mn), iron (Fe), potassium (K), zinc (Zn), sulfur (S) and mobile nitrogen (N) ion (Cameron 2010; Smith et al. 2011). They protect crops from the soil-borne pathogens such as nematode (Cavallazzi et al. 2007) and weed (Rinaudo et al. 2010). They improve soil structure development and aggregate stabilization (Gianinazzi et al. 2010). AMF are essential for ecosystem sustainability, plant development and maintenance of biological diversity (Smith et al. 2011).

The major soils of Southern Guinea Savanna of Nigeria have coarse-textured surface and are low in organic matter and chemical fertility (Alori et al. 2012). The productivity of these soils is generally low in most of the existing farming systems. These soil problems have necessitated the intensification of research in this geographical zone. This includes research into biological nitrogen fixing systems as an alternative to chemically produced fertilizer. Certain forage leguminous plants have been considered for integration into Southern Guinea Savanna by a team of scientists (Lupwayi et al. 2011; Okwori and Abdulsalam 2017). It is therefore very important to investigate if forage leguminous plants support this important soil life, i.e., the AMF in Southern Guinea Savanna soil. There is, therefore, a need to obtain more information on the occurrence and distribution of spores of

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different AMF species in soils cropped with forage legumes. Hence, this research has aimed to investigate the occurrence of AMF spore in the soil of Southern Guinea Savanna under different forage legumes and also the effects of soil treatment with varying phosphorus concentrations on the distribution of AM spores.

2 Materials and methods

2.1 Experimental layout and collection of soil samples

A 5×3 factorial experiment in a randomized block design with two replicates to make a total of 30 pots was laid out in the screen house at the Faculty of Agriculture University of Ilorin, Ilorin, Nigeria. Water-soluble single super phosphate with 14.5% P_2O_5 with additional nutrients of 11% sulfur and 21% calcium was used. The 30 experimental pots were filled with 10 kg each of soil presieved with 2 mm sieve size. These pots were treated with three phosphorus levels: P0 (0 kg/ha single superphosphate), P1 (100 kg/ha of single superphosphate) and P2 (200 kg/ha of single superphosphate) in two replicates. Seeds of *Cajanus cajan* (L.) Millsp., *Centrosema pascuorum* Martius ex Benth, *Crotalaria ochroleuca* (G.) Don, *Lablab purpureus* (L.) Sweet. and *Mucuna pruriens* (L.) DC were sown at 2 per pot, and this was thinned to 1 per pot 10 days after germination. Crops were irrigated to soil moisture capacity at 6.00 and 18.00 h daily using a watering can. These crops were allowed to grow to maturity (19 weeks). Soil samples were collected from the soil from these pots cropped with different legumes: *C. cajan*, *C. pascuorum*, *C. ochroleuca*, *L. purpureus* and *M. pruriens* with the aid of small hoe blade and transported to the laboratory in the well-labeled polythene bags for analysis.

2.2 Determination of some selected soil chemical properties

The chemical properties determined include pH using Kent pH meter model 7020, soil organic matter (SOM), using wet oxidation method as described by Shamshuddin *et al.* (1994). Available phosphorus in soil samples was determined using the modified No. 1 method of Bray and Kurtz (1945).

2.3 Extraction and morphological characterization of isolates of AMF spores in soils

The wet sieving and decanting technique for the extraction of AMF spores developed by Brundrett *et al.* (1996) was employed for the extraction of AMF spores. The isolated spores were then picked up with a needle under a dissecting microscope and were mounted in both polyvinyl alcohol lactic acid-glycerol (PVLG), Meltzer's reagent and PVLG mixed with Meltzer's reagent (1:1 v/v). All spores were examined using a compound microscope. The morphological properties of these spores were determined according to the key proposed by Trappe (1982). The characteristics used include shape, size, color, distinct wall layer, attached hyphae, sporocarps, bulbous attachment, clustering and surface ornamentation of spores. Characterization was made by using the description provided by the international collection of vesicular and AMF (INVAM 2001).

3 Statistical analysis

The percentage frequency of occurrence of AMF spores in each legume soil was calculated using the formula below:

$$\frac{\text{No of an AMF sp}}{\text{Total no AMF spores}} \times 100$$

The correlation between the AMF spore counts and variables such as pH, organic matter content and available phosphorus in soil of each legume was analyzed.

Data collected were subjected to analysis of variance using Genstat statistical package. Means were separated using Duncan's multiple range test.

4 Results

4.1 The percentage frequencies of occurrence of the predominant AMF spores under different legumes

The percentage frequencies of occurrence of the predominant AMF spores under different legumes are

shown in Figure 1. The spores of *Glomus fasciculatus* were predominant in soils of the legumes except *M. pruriens* in which the spores of *Glomus macrocarpus* var. *macrocarpus* were predominant. The spores of *Glomus macrocarpus* var. *macrocarpus* were the next in abundance to *Glomus fasciculatus* in soils of *C. pascuorum*, *C. ochroleuca* and *L. purpurens*, respectively. The spores of *Gigaspora coralloidea*, however, had lowest counts in all the five legumes soils.

4.2 Total AMF spore count and some chemical properties of soil under different leguminous plants

The total AMF spore count and some chemical properties of soil under different leguminous plants as reported in Table 1 show that most of the soils at different levels of applied phosphorus were slightly acidic, with the soils under *C. ochroleuca* at 200 kg/ha of applied phosphorus level having the lowest pH (5.0). The soils of *C. cajan* at 200 kg/ha level of applied phosphorus and *M. pruriens* at zero (0 kg/ha) level of applied phosphorus were, however, neutral (7.0 and 7.1).

There were also variations in the level of available phosphorus of the soils of the five legumes 19 weeks

after planting. *C. cajan* at zero (0 kg/ha) level of applied phosphorus had the highest available phosphorus of 32 ppm, while *M. pruriens* at the same level of applied phosphorus had the lowest available phosphorus of 3.16 ppm.

4.3 Effect of the applied phosphorus levels and different legumes on the total AMF spores

Table 2 shows the effect of the applied phosphorus levels and legume types on the total AM spores. The population of AMF spores was significantly higher in the soils of *M. pruriens* and *C. ochroleuca* than the soils of other legumes. The lowest AM spore count was recorded in the soils of *L. purpurens*.

Higher AMF counts were recorded in the soil of *M. pruriens* when no phosphorus was applied than with applied phosphorus. A decline in the spore count was recorded with increase in the applied phosphorus. On the other hand, *C. ochroleuca* had lower AM counts in soils with no applied phosphorus, whereas an increase in AMF count was observed with increasing level of applied phosphorus.

Figure 1: Percentage frequency of occurrence of predominant AMF spores in the soil of studied legume species. Bars are % error bars.

Table 1: Total counts of AMF spores in the soil of studied legume species and some physicochemical properties of soils

Legumes	P treatment (kg/ha)	Organic matter (%)	pH	Available phosphorus 19WAP (PPM)	Total AM spores (g soil ⁻¹)
<i>C. cajan</i>	P0	3.36	6.5	3.2	98
	P1	4.49	6.2	7.9	80
	P2	4.01	7.0	3.90	101
<i>C. pascuorum</i>	P0	3.52	6.2	5.53	154
	P1	3.49	5.6	12.40	98
	P2	3.32	6.5	9.25	103
<i>C. ochroleuca</i>	P0	2.49	5.4	4.70	78
	P1	3.24	5.4	11.62	161
	P2	3.36	5.0	29.69	209
<i>L. purpureus</i>	P0	3.93	5.9	22.40	92
	P1	2.31	6.5	26.00	95
	P2	3.38	6.7	12.00	48
<i>M. pruriens</i>	P0	2.26	7.1	3.16	283
	P1	3.97	6.3	15.08	124
	P2	4.46	6.3	15.54	55

Note: P0 = 0, P1 = 100 and P2 = 200 kg/ha.

4.4 Effects of different legumes and varying phosphorus levels on the population of *Glomus mossae*

The population of *G. mossae* spores was significantly higher in the soil of *M. pruriens* than in the soils of the other legumes. *G. mossae* spores were more numerous in soils with zero (0 kg/ha) level of applied phosphorus. The number of spores in soils when phosphorus was not applied was in fact significantly higher than the number of spores in soils of 100 and 200 kg/ha levels of applied phosphorus (Table 3).

4.5 Effects of different legumes and varying phosphorus levels on the population of *Glomus macrocarpus* var. *geosporus* and *Glomus macrocarpus* var. *macrocarpus* spores

As revealed by Tables 4 and 5, the populations of *G. macrocarpus* var. *geosporus* and *G. macrocarpus* var. *macrocarpus* spores were significantly higher in the soil of *M. pruriens* than in the soils of the other legumes. However, the varying P levels have no significant effect on the spore population of both AMF species.

Table 2: Effect of phosphorus levels and studied legume species on total AM spores

Legumes	P. treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	Amount of spores/g soil ⁻¹			
<i>C. cajan</i>	98.0	88.0	101.0	95.67a
<i>C. pascuorum</i>	154.0	98.0	103.0	118.33a
<i>C. ochroleuca</i>	78.0	161	209.0	149.33b
<i>L. purpureus</i>	92.0	94.5	48.0	78.17a
<i>M. pruriens</i>	283.0	124.0	55.0	154.0ab
P. level mean	140.8a	411.3a	103.1a	

Means with the same alphabets in the same column for mean legume and in the same row for mean P. level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 100 and P2 = 200 kg/ha.

Table 3: Effect of phosphorus levels and studied legume species on spores of *G. mossae*

Legumes	P. treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	No of <i>G. mossae</i> spores (g soil ⁻¹)			
<i>C. cajan</i>	16.5	7.50	10.0	11.33a
<i>C. pascuorum</i>	17.5	7.0	11.5	11.83a
<i>C. ochroleuca</i>	10.2	16.50	11.5	14.17a
<i>L. purpureus</i>	12.0	6.0	15.5	9.83a
<i>M. pruriens</i>	44.5	9.5	11.5	21.83b
Mean P level	20.1b	9.3a	11.5a	

Means with the same alphabets in the same column for mean legume and in the same row for mean P. level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 100 and P2 = 200 kg/ha.

Table 4: Effect of phosphorus levels and studied legume species on spores of *G. macrocarpus* var. *geosporus*

Legumes	P. treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	No of <i>G. macrocarpus</i> spores (g soil ⁻¹)			
<i>C. cajan</i>	7.5	7.5	11.0	8.67a
<i>C. pascuorum</i>	17.0	9.5	11.0	12.5a
<i>C. ochroleuca</i>	7.50	15.0	10.5	11.17a
<i>L. purpureus</i>	12.0	18.0	10.5	13.5a
<i>M. pruriens</i>	14.5	22.0	12.0	16.17b
Mean P level	11.7a	14.5a	11.0a	

Means with the same alphabets in the same column for mean legume and in the same row for mean *p*. level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 100 and P2 = 200 kg/ha.

4.6 Effects of different legume species at varying P levels on *Glomus fasciculatus* spore population

G. fasciculatus spores in soils of the different legumes at varying P levels in Table 6 reveal that the population of *G. fasciculatus* spores encountered at the different legume species was not significantly different from one another at $p \leq 0.05$. However, the application of 0 kg/ha of P resulted in a significantly higher *G. fasciculatus* spore compared to the application of 200 kg/ha of P at $p \leq 0.05$.

4.7 Effects of different legumes at varying P levels on *Gigaspora coralloidea* spore population

G. coralloidea spore count in soils under different legumes at varying levels of applied P in Table 7 shows that the population of *G. coralloidea* spores was not affected significantly either by the legume species or by the P level ($p \leq 0.05$).

4.8 Correlations between some soil properties and AMF spore population in soil

Table 8 shows the correlations between some soil properties and AMF spore population in soil. Significant negative correlations were observed between AMF spore population

Table 5: Effect of phosphorus levels and studied legume species on spores of *G. macrocarpus* var. *macrocarpus*

Legumes	P treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	No of <i>G. macrocarpus</i> var. <i>macrocarpus</i> spores (g soil ⁻¹)			
<i>C. cajan</i>	7.0	17.0	7.0	10.33a
<i>C. pascuorum</i>	18.5	9.5	8.5	12.17a
<i>C. ochroleuca</i>	22.5	24.5	26.0	24.33a
<i>L. purpureus</i>	8.0	15.5	17.0	13.5a
<i>M. pruriens</i>	75.0	24.5	31.5	43.67b
Mean P level	26.2a	18.2a	18.0a	

Means with the same alphabets in the same column for mean legume and in the same row for mean P. level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 100 and P2 = 200 (kg/ha)

and available P and between AMF spore population and SOM content.

5 Discussion

5.1 The percentage frequencies of occurrence of the predominant AM spores under different legumes

The occurrence of *Glomus* spores reported in this research (Figure 1) was also reported by Alori et al.

Table 6: Effect of phosphorus levels and studied legume species on spores of *G. fasciculatus*

Legumes	P treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	No of <i>G. fasciculatus</i> spores (g soil ⁻¹)			
<i>C. cajan</i>	30.0	29.5	33.0	30.83a
<i>C. pascuorum</i>	38.0	18.0	25.0	27.0a
<i>C. ochroleuca</i>	25.0	29.5	42.5	32.17a
<i>L. purpureus</i>	29.0	31.5	24.5	31.67a
<i>M. pruriens</i>	60.0	44.5	15.0	39.83a
Mean P level	38.5b	30.6ab	27.8a	

Means with the same alphabets in the same column for mean legume and in the same row for mean P level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 0.5 and P2 = 1.0 kg/ha.

Table 7: Effect of phosphorus levels and studied legume species on spores of *G. coralloidea*

Legumes	P treatment level/pot (kg/ha)			Mean legume
	P0	P1	P2	
	No. of <i>G. coralloidea</i> spores (g soil ⁻¹)			
<i>C. cajan</i>	2.5	2.0	8.5	4.33a
<i>C. pascuorum</i>	6.0	1.5	3.0	3.5a
<i>C. ochroleuca</i>	2.5	3.0	2.0	2.17a
<i>L. purpureus</i>	3.5	8.0	8.0	6.5a
<i>M. pruriens</i>	10.5	4.0	8.0	7.33a
Mean P level	4.8a	3.7a	5.8a	

Means with the same alphabets in the same column for mean legume and in the same row for mean *p*. level are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

Note: P0 = 0, P1 = 100 and P2 = 200 kg/ha.

(2012) from the soils of Ilorin. Invam database (INVAM 2001) reported the genus *Glomus* to be the most diverse in Glomales order. Hindumathi and Reddy (2011) also reported abundance of *Glomus* sp. accompanied with a fewer species of *Gigaspora*.

5.2 Total AMF spore count and some chemical properties of soil under different leguminous plants

Alori *et al.* (2012) reported a marked variation between the abundance of mycorrhizal spores and the physico-chemical characteristics of soils among species of mycorrhizal fungi. The difference in the available phosphorus under different legumes could probably be due to varying abilities of legumes to produce phosphates. According to Amba *et al.* (2013), some plant species are known to produce more extracellular

phosphates in response to low levels of available phosphorus. The total AMF spores count in the soils of *M. pruriens* was higher than that of the soils of *C. cajan*. There are slight variations in the quantity of the organic matter of the soils studied. This probably had an effect on the level of available phosphorus in the soils. A soil with high organic matter content will likely have a higher microbial population. Immobilization of phosphorus by these organisms will cause reduction in the available phosphorus of that particular soil. The reduction in pH in soils under *Crotalaria ochroleuca* at 200 kg is most likely due to the extrusion of H^+ , following the uptake of ammonium by the fungal hyphae.

5.3 Effect of the applied phosphorus levels and different legumes on the total AMF spores

Variation in the abundance of AMF under different legumes and varying P levels could probably be due to the differences in morphological structures, growth characteristics and nitrogen-fixing ability of the legumes. Hindumathi and Reddy (2011) also reported a significantly varying population of AM spore under different legumes. The ability of some crop types to support high AMF population may be attributed to the root exudates of these plants, which stimulate the germination of mycorrhizal spores and increase the infection percentage. Some crop root exudates may also encourage growth and activity of other beneficial microbes such as the sulfur-oxidizing bacteria. Mohamed *et al.* (2014) reported increased AMF spores count by dual inoculation with AMF and sulfur-oxidizing bacteria. Mathimaran *et al.* (2007) discovered that spore densities of AM fungi were significantly affected by plant species identity. Mahesh and Selvaraj (2008) reported

Table 8: Correlation of some soil variables with total number of AMF spores and predominant AMF spores extracted from soils of studied legume species

Variable	Correlation coefficient					
	Total AMF spores	<i>G. mossea</i>	<i>G. fasciculatus</i>	<i>G. macrocarpus</i> var. <i>geosporus</i>	<i>G. coralloidea</i>	<i>G. macrocarpus</i> var. <i>macrocarpus</i>
pH	0.038	0.344	0.190	0.214	0.650 ^a	0.213
Available P	-0.230	-0.262	-0.218	0.027	0.458	-0.210
Organic matter	-0.463	-0.483	-1.199	-0.107	-0.091	-0.388

^a Correlation was significant at $p \leq 0.05$.

82% AM colonization in *Sorghum* compared to 20% in *Saccharum*. Saif (1987) observed a great variation in dependence on mycorrhiza among forage species. *Crotalaria ochroleuca* enhanced the multiplication of mycorrhizal propagule in soil (Benkhroua et al. 2017). Khakpour and Khara (2012) also reported negative correlation between soil P and AMF spore population of soil.

5.4 Effects of different legumes and varying phosphorus levels on the population of *Glomus mossae*

Glomus mossae in soils of different legumes at varying phosphorus levels (Table 3) shows that *Glomus mossae* spores were significantly affected by the legume species and the phosphorus level ($p \leq 0.05$). This agrees with the observations of Ghorbani et al. (2012) who reported that soil chemical treatment may directly influence the occurrence of AMF and that low P favored spore production in soils. According to Yang et al. (2014), increasing soil nutrient reduces the effectiveness of AMF.

5.5 Effects of different legumes and varying phosphorus levels on the spore population of *Glomus macrocarpus* var. *geosporus* and *Glomus macrocarpus* var. *macrocarpus*

G. macrocarpus var. *geosporus* and *G. macrocarpus* var. *macrocarpus* spore populations were significantly influenced by legume species but not by the varying P levels. This implies that all the varying P contents of the soil support the mycorrhizae. This agrees with the report of Emmanuel et al. (2012) that fertilizer application did not influence AMF spore abundance in the maize/*Centrosema* soil.

5.6 Effects of different legumes at varying P levels on *Glomus fasciculatus* spore population

The lack of significant difference in the spore population of *G. fasciculatus* under different legumes implies that exudate from the legumes studied favors the growth of *G. fasciculatus*. The significant decline in *G. fasciculatus*

spore population observed with increasing level of the applied P is consistent with the report of the study by Johnson et al. (2013). Increased phosphorus supply has previously been reported to exert strong inhibition of arbuscular mycorrhizal development (Nouri et al. 2015).

5.7 Effects of different legumes at varying P levels on *Gigaspora coralloidea* spore population

Metabolites from all the legumes and the existing soil factors in this ecological zone probably have a positive influence on the *G. coralloidea* spore population. These probably accounts for an insignificant difference in the spore population of *G. coralloidea* under different legumes and varying P. levels. Plant roots excrete quite a number of organic compounds to attract and select microorganisms in the rhizosphere (Huang et al. 2014).

5.8 Correlations between some soil factors and AMF spore population in soil

Isobe et al. (2007) reported a negative correlation between soil P and AMF population of soil and between SOM and AMF spore population, respectively. The positive correlation observed between soil pH and AMF spore count corresponds to the findings of Isobe et al. (2007).

6 Conclusion

This study has revealed that spores of *Glomus* sp. and *Gigaspora* sp. occur in the soils of Southern Guinea Savanna, ecological zone of Nigeria. However, spores of *Glomus* sp. are abundant than spores of *Gigaspora* sp. in soils of this zone.

The occurrence of AMF spores in soils under leguminous plant – *C. cajan*, *C. pascuorum*, *C. ochroleuca*, *L. purpureus*, and *M. pruriens* was also established. *M. pruriens* soil was observed to have more AMF spores than the other legume soils. The use of *M. pruriens* in crop rotation could help to improve soil fertility in the study area. The mere presence of mycorrhizal spores does not necessarily reflect the abundance of mycorrhizal infection. There is, therefore, the need for further

studies on the rate of infection of these legumes with the existing mycorrhizal spores.

This study has also shown that soil properties such as pH, organic matter content, and the available P do have effects on the AMF population. Further work is required to observe the correlation between the presence of AMF and the amount of P and N in tissues of different legumes. This information will enable the manipulation of AMF symbiosis for the benefit of a minimum input agricultural system in Southern Guinea Savanna Zone of Nigeria.

Conflict of interest: The authors declare no conflict of interest.

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