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Research Article Yield Performance and Leaf Nutrient Composition of Bambara Groundnut Under Arbuscular Mycorrhizal Inoculation in a Poultry Manure Amended Ultisol

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Abstract

Background and Objective: Researchers are now targeting the possibility of sustainably producing crops without polluting the soil and groundwater through the integrated use of Poultry Manure (PM) and Arbuscular Mycorrhizal Fungi (AMF). The present study was undertaken to investigate the yield response and leaf nutrient composition of Bambara groundnut under arbuscular mycorrhizal inoculation in poultry manure amended ultisol during the 2016 and 2017 cropping seasons. **Materials and Methods:** The study was a 4×5 factorial experiment consisting of four levels of poultry manure and four inoculums of arbuscular mycorrhizal fungi plus the uninoculated control and was laid out in a Randomized Complete Block design (RCBD) with three replications to give 20 treatment combinations. **Results:** Sole applications of poultry manure and AMF inoculums significantly increased P, K, Ca and Mg contents in the leaves of Bambara groundnut over control plants in both years of study. Soil amended with 8 t ha⁻¹ of poultry manure and at the same time inoculated with *Gigaspora gigantea* gave the highest pod yield in both years, while soil amended with 12 t ha⁻¹ of PM and inoculated with *Glomus clarum* as well as soil amended with 8 t ha⁻¹ of PM and inoculated with *Gigaspora gigantea* and amended with 8 and 12 tons ha⁻¹ of poultry manure in single or combination were more efficient and consistent in the enhancement of growth on marginal soils.

Key words: AMF inoculation, poultry manure, bambara groundnut, nutrients content, yield

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bambara groundnut (Vigna subterranea (L.) Verdc.), a selfpollinating annual legume is native to West Africa, third most important legume after Arachis hypogaea and Vigna unquiculata¹. It is known to be highly tolerant to pest and drought² besides having the capability to thrive and produce some yields where other legumes may fail. Bambara groundnut seeds contain 63% carbohydrates, 6.5% fat^{3,4} and 19% protein (a protein content that is richer than that of peanuts in essential amino acids such as Isoleucine, Lysine, Methionine, Phenylalanine, Threonine and valine)⁵. This distinguishes Bambara groundnut as an important alternative to the expensive animal protein for the resource-poor rural people⁵ such that it is recognized globally as a neglected and under-utilized crop (NUL) that could be exploited to alleviate hunger, improve nutrition and contribute to the overall food security.

The continuous use of chemical fertilizers in plant nutrition is fraught with numerous challenges including its scarcity with attendant high cost, soil nutrient imbalance, soil acidity, pollution of soil and underground water^{6,7}. Researchers are now targeting the possibility of producing crops in a sustainable, eco-friendly manner through organic agriculture^{8,9}, integrated use of poultry manure and biofertilizers such as arbuscular mycorrhizal fungi^{10,11}. These practices could improve the fertility status of soil, plant nutrients uptake and hence minimize the use of chemical fertilizers.

The benefits of poultry manure in stimulating microbial activities, improving soil fertility and soil structure are well documented in literature¹²⁻¹⁵. The use of bio-inoculants such as Arbuscular Mycorrhizal Fungi (AMF) on the other hand is still an emerging sustainable and eco-friendly soil fertility management option with potentials to boost and cheaply improve crop yields¹⁶. AMF is known to provide a direct physical link between soil and plant roots¹⁷ and can promote plant growth by increasing plant access to relatively immobile nutrients¹⁸. Inoculation of a plant with AMF had been found to significantly increase the number of leaves, shoot and root length, nutrient uptake, pod number and pod yield, as compared with non-mycorrhizal plants¹⁹⁻²². The increase in plant growth characters and yield in mycorrhizal inoculated plants were attributed to an increase in the supply of nutrients^{11,23}.

Generally, the response of AMF is expected to be better in slightly acidic soils²⁴ due to improved solubilization of P or extended rhizosphere by fungal hyphae thus increasing the surface area for nutrient acquisition^{25,26}. However, some species of Arbuscular Mycorrhizal (AM) fungi are adapted to acid or alkaline soils, while others occur in both soil conditions²⁷. In acidic soils, where phosphorus is mainly bound with Fe or Al, excretion of chelating agents (citric acid and siderophores), inoculation with mycorrhizae can enhance the bio-available P supply of the soil^{28,29}. Moreover, mycorrhizae also produce phosphatase, which can mobilize P from organic sources³⁰.

Integrated use of AMF and poultry manure may be more beneficial to plant growth apart from ensuring sustainable and eco-friendly farming. Several studies have shown the positive effect of poultry manure in combination with AMF^{10,11,19} on plant growth. However, Jordan et al.³¹ and Varga et al.³² have reported that high content of P in manure could affect AMF colonization potential. There is the need therefore to understand the optimum rates of poultry manure application that could enhance optimum benefits from AMF symbiosis for improved yield and nutrients accumulation in Bambara groundnut. Soils in the study area are classified as ultisols and are known to be low in pH and inherently low in soil essential nutrients³³. The present study was undertaken to investigate the effects of single and combined application of poultry manure and AMF on the performance of Bambara groundnut and its corresponding effects on nutrient uptake.

MATERIALS AND METHODS

The study was conducted during the 2016 and 2017 cropping seasons at the Faculty of Agriculture Teaching and Research Farm, University of Calabar (04°45' and 04°57' N and 08°21' and 08°37' E, 39 m above sea level) (Fig. 1). The mean annual rainfall of the area exceeds 2500 mm with mean annual air temperatures and relative humidity of 26.7°C and 86%, respectively³⁴. The soil is sandy loam, strongly acidic in reaction with low nutrient reserves and is classified as an Ultisol³³.

The plot was ploughed and experimental units were mapped. Prior to seedbed preparation, composite soil samples were augered from 0-20 cm depth, thoroughly mixed, airdried and passed through a 2 mm sieve, and then analyzed for Physico-chemical properties using methods outlined by Estefan *et al.*³⁵. The poultry manure sourced from the poultry section of the Teaching and Commercial Farms, University of Calabar, was shed dried, crushed and analyzed for nutrient contents using a method described by Estefan *et al.*³⁵. Bambara groundnut leaf samples collected at eight weeks after planting were oven-dried for 24 hrs at 70°C and milled for leaf tissue analysis as outlined in Estefan *et al.*³⁵.

The experiment was a 5×4 factorial laid out in a Randomized Completed Block Design (RCBD) with three replications. Treatments comprised inoculation with pre-made



Fig. 1: Map showing study area

inoculums of four species of Arbuscular Mycorrhizal Fungi (AMF) namely; *Glomus mosseae*, *Glomus deserticola*, *Glomus clarum* and *Gigaspora gigantea* plus an un-inoculated plant serving and application of Poultry Manure (PM) at four rates of 0, 4, 8 and 12 t ha^{-1}) making 20 treatment combinations and three replications to give a total of 60 experimental units with a distance of 0.5 m² between each unit and 1.0 m² between the replicates on a total land area of 454.25 m².

Poultry manure was broadcasted uniformly on each plot and incorporated into the soil except in the control plots one week before planting whereas planting holes were partially filled with 30 g inoculum of each AMF according to the treatment specifications before planting. Bambara groundnut landrace -TVU 1729 was sourced from the Genetic Resource center of International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, while the inoculums prepared from the starter cultures of arbuscular mycorrhizal fungi obtained from the Soil Microbiology Unit of the Department of Agronomy, University of Ibadan, Nigeria. Two seeds of Bambara groundnut were sown per hole at a distance of 50×20 cm and later thinned to one plant per stand two weeks after emergence, giving a plant population of 100,000 plants ha⁻¹. The plots were hand hoed and earthed at 2 and 4 WAP.

Data were collected from the four middle rows of Bambara groundnut stands from each plot on growth attributes (viz; plant height, leaf area index, number of branches, nodules and leaf number), number of pods per plant, pod and seed yield (t ha^{-1}).

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) procedures for factorial experiment dispersed in a Randomized Complete Block Design (RCBD). Significant means were compared using Duncan's New Multiple Range Test (DNMRT) at 0.05 level of probability.

RESULTS AND DISCUSSION

Properties of the soil and materials used for the experiment: The pre-cropping soil properties of the experimental site are presented in Table 1. The soil was sandy loam, strongly acidic with a pH of 5.2 and 5.0 for 2016 and 2017 cropping seasons. Organic matter (OM), exchangeable acidity, exchangeable cations (K, Ca and Na), total nitrogen and ECEC of the soil were low with moderate Mg content in both years. Previous studies^{33,36}, also reported low values for essential nutrients in the study area. This observed low nutrients status for the present study might be due to coastal plain sand parent material that characterized the soil of the study area and also related to high acidity, immobilization and leaching of soil and continuous cultivation of the land without proper soil amendment.

However, soil enrichment was necessary to support the growth and yield of the Bambara groundnut. Loks *et al.*³⁷ had earlier reported that utilizing mineral fertilizer does not only bring a surge in the production cost but might also create a large number of environmental problems alongside its use. Thus, using AMF alongside with poultry manure may be beneficial in mobilizing nutrients bound to organic matter and soil particles for Bambara groundnut growth. The analysis of Poultry Manure (PM) used in both cropping seasons showed that it was high in N and OC and other basic cations.

Effects of AMF and poultry manure on growth characters of

Bambara groundnut: In both seasons, G. mosseae and Gi. gigantea inoculation resulted in statistically similar plant height, the number of branches, leaf area index (LAI), leaf numbers and nodules, but significantly (p<0.05) higher than expressions of these attributes in G. deserticola and G. clarum inoculated plants, which were, in turn, higher than plants in the control plots (Table 2). The observed increment in plant growth parameters in mycorrhizal inoculated plants could be attributed to an increase in the supply of nutrients by the AMF and improvement of other growth conditions^{19,23,25}. Uko *et al.*²² reported that AMF inoculation increased plant height of groundnut (Arachis hypogaea L.) from 21.86 cm in uninoculated soil to 32.04 cm in inoculated soil at 7 weeks after planting. Similar results have been reported by Anozie and Okereke³⁸ who asserted that okra plant inoculated with AMF causes significant increase in various growth attributes such as plant height, number of leaves and stem girt

compared with the uninoculated soil. The result of this study is in line with several reports^{15,20,22} that inoculation of the plant with AMF had been found to significantly increased the number of leaves, plant height, shoot and root length, nutrient uptake, pod number and pod yield, as compared with nonmycorrhizal plants.

Increasing poultry manure rates significantly (p<0.05) increased the plant height of Bambara groundnut in both years. A similar trend was observed for the number of branches, the number of leaves and the number of nodules in the order of 12>8>4>0 t ha⁻¹ PM in 2016 and 2017 seasons. The LAI among plants that received 8 and 12 t ha⁻¹PM was however statistically at par (p>0.05), significantly higher than LAI among the 4 t ha^{-1} PM (p<0.05) and also plants in the untreated controls in both years (Table 2). The application of poultry manure to significantly increased seed weight, number of pods per plant, 1000 grain weight, pod yield as well as grain yield in Bambara groundnut during the first and second planting seasons could be attributed to high nitrogen content in the poultry manure³⁹. Wamba et al.⁴⁰ also observed improved plant height, dry weight, leaf water contents and foliar chlorophyll of Bambara groundnut in soil amended with poultry manure compared to unamended soil.

Interactive effects of AMF × PM were significant only for the number of branches and nodules in both years (p<0.05). It was observed that interaction of *G. mosseae* × 12 t ha⁻¹PM resulted in the highest branching in 2016 and 2017 respectively (p<0.05), closely followed by the number of branches when *Gi. giganteax* 12 t ha⁻¹PM interacted (p>0.05)

Table 1: Physicochemical characteristics of the soil and poultry manure used for the experiment

		Soil values		Poultry manure value	es (%)
Physical properties	Units	2016	2017	2016	2017
Sand	g kg ⁻¹	730	710		
Silt	g kg ⁻¹	160	200		
Clay	g kg ⁻¹	110	90		
Texture		Sandy loam	Sandy loam		
Chemical properties					
рН		5.2	5.0		
TN	%	0.10	0.10	3.81	3.80
Av.P	mg kg ^{−1}	33.25	32.2	1.61	1.64
OM	%	1.62	1.58	22.3	21.90
Ca	cmol kg ⁻¹	4.1	3.8	2.30	2.41
Mg	cmol kg ⁻¹	2.0	2.1	0.84	0.87
К	cmol kg ⁻¹	0.11	0.12	1.10	1.12
Na	cmol kg ⁻¹	0.09	0.08	0.42	0.40
Al+++	cmol kg ⁻¹	0.68	0.54		
H+	cmol kg ⁻¹	0.16	0.12		
ECEC	cmol kg ⁻¹	7.14	6.84		
BS	%	88.0	90.35		

Treatments	2016 crop	2016 cropping season					2017 cropping season				
	 PH	NOB	LAI	LN	Nodules	 PH	NOB	LAI	LN	Nodules	
AMF											
AMF ₀	18.31°	46.91 ^c	905.1°	2.58℃	6.57°	18.34 ^c	47.44 ^c	908.7 ^d	2.68°	6.58°	
AMF ₁	18.87ª	55.26ª	975.0ª	3.23ª	8.93ª	18.99ª	54.87ª	973.6ª	3.41ª	8.98 ^b	
AMF ₂	18.56 ^{bc}	49.14 ^b	939.8 ^b	2.88 ^b	7.59 ^b	18.62 ^b	48.87 ^b	935.2 ^b	3.00 ^b	7.61 ^ь	
AMF ₃	18.51 ^{bc}	48.26 ^b	941.2 ^ь	2.97 ^b	7.69 ^b	18.58 ^b	47.93°	950.1°	3.12 ^b	7.72 ^b	
AMF ₄	19.23ª	54.64ª	976.0ª	3.26ª	9.45ª	19.23ª	54.45ª	974.9ª	3.36ª	9.53ª	
PM											
PM ₀	16.99 ^d	42.76 ^d	920.6 ^c	2.33 ^d	7.31 ^d	16.97 ^d	42.55 ^d	915.1°	2.43 ^d	7.11 ^d	
PM ₁	18.65°	50.06 ^c	944.7 ^b	3.05°	7.78°	18.66 [∈]	49.69°	945.4 ^b	3.17℃	7.81°	
PM ₂	19.47 ^b	54.42 ^b	960.1ª	3.23 ^b	8.39 ^b	19.53 ^b	54.27 ^b	963.3ª	3.36 ^b	8.43 ^b	
PM ₃	19.67ª	56.12ª	964.2ª	3.33ª	8.69ª	19.86ª	56.34ª	970.2ª	3.50ª	8.72ª	
AMF×PM											
AMF ₀ PM ₀	16.73ª	37.23 ⁱ	875.4ª	2.18ª	6.43 ^h	16.75ª	37.97 ^j	863.9ª	2.28ª	6.45 ^h	
	18.00ª	46.37 ^h	891.8ª	2.38ª	6.54 ^h	18.17ª	45.95 ⁱ	899.4ª	2.41ª	6.56 ^h	
AMF ₀ PM ₂	19.07ª	51.20 ^{ef}	919.6ª	2.81ª	6.63 ^h	18.86ª	51.97 ⁹	924.0ª	2.93ª	6.65 ^h	
	19.45ª	52.83 ^{de}	933.4ª	2.93ª	6.69 ^h	19.57ª	53.88 ^{ef}	947.6ª	3.10ª	6.69 ^h	
AMF ₁ PM ₀	17.17ª	49.47 ^{fg}	944.4ª	2.44ª	7.62 ^{cd}	17.10ª	49.00 ^h	942.5ª	2.51ª	7.65 ^{cd}	
AMF ₁ PM ₁	18.90ª	55.55 ^{bcd}	977.9ª	3.37ª	8.74 ^b	18.98ª	55.09 ^{cd}	976.1ª	3.57ª	8.77 ^b	
AMF ₁ PM ₂	19.63ª	57.07 ^{abc}	987.4ª	3.52ª	9.66 ^{ab}	19.85ª	56.70 ^b	987.2ª	3.73ª	9.69 ^{ab}	
AMF ₁ PM ₃	19.80ª	58.94ª	990.6ª	3.60ª	9.69 ^{ab}	20.12ª	58.68ª	988.8ª	3.83ª	9.69 ^{ab}	
AMF ₂ PM ₀	16.82ª	39.23 ⁱ	913.9ª	2.27ª	6.71 ^{efg}	16.84ª	38.60 ^j	902.4ª	2.40ª	6.72 ^{efg}	
AMF ₂ PM ₁	18.57ª	47.70 ^{gh}	934.8ª	3.04ª	7.36 ^{cde}	18.48ª	47.28 ^h	928.1ª	3.11ª	7.38 ^{cde}	
AMF ₂ PM ₂	19.40ª	53.80 ^{de}	952.2ª	3.05ª	7.82℃	19.53ª	53.62 ^{ef}	952.1ª	3.15ª	7.81°	
AMF ₂ PM ₃	19.43ª	55.83 ^{bcd}	958.2ª	3.15ª	8.51 ^b	19.65ª	55.98 ^{bc}	958.0ª	3.35ª	8.53 ^b	
AMF ₃ PM ₀	16.87ª	38.67 ⁱ	922.7ª	2.31ª	7.07 ^{def}	16.92ª	38.32 ^j	922.7ª	2.40ª	7.05 ^{def}	
AMF ₃ PM ₁	18.60ª	47.03 ^{gh}	947.2ª	3.14ª	7.36 ^{cde}	18.53ª	46.62 ^{hi}	950.5ª	3.27ª	7.34 ^{cde}	
AMF ₃ PM ₂	19.25ª	52.77 ^{de}	948.7ª	3.20ª	7.82℃	19.36ª	52.06 ⁹	960.4ª	3.37ª	7.85℃	
AMF ₃ PM ₃	19.33ª	54.57 ^{cd}	946.2ª	3.25ª	8.51 ^b	19.50ª	54.72 ^{de}	966.6ª	3.43ª	8.53 ^b	
AMF₄PM₀	17.40ª	49.20 ^{fgh}	946.5ª	2.47ª	8.72 ^b	17.22ª	48.87 ⁹	943.8ª	2.57ª	8.75 ^b	
AMF₄PM₁	19.17ª	53.63 ^{de}	971.8ª	3.31ª	8.89 ^b	19.12ª	53.52 ^f	973.1ª	3.47ª	8.92 ^b	
AMF ₄ PM ₂	20.03ª	57.27 ^{abc}	992.7ª	3.57ª	10.08ª	20.05ª	57.0 ^b	992.9ª	3.60ª	10.17ª	
AMF ₄ PM ₃	20.33ª	58.44 ^{ab}	992.9ª	3.70ª	10.09ª	20.55ª	58.43ª	990.0ª	3.80ª	10.19ª	

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Table 2: Effects of Arbuscular Mycorrhizal Fungi (AMF) and Poultry Manure (PM) on growth characters of Bambara groundnut at harvest

PH: Plant height (cm), NOB: Number of branches, LAI: Leaf area index, LN: Leaf number, AMF: Arbuscular mycorrhizal fungi, PM: Poultry manure, AMF₀: Un-inoculated plants, AMF₁: *Glomus mosseae*, AMF₂: *Glomus deserticola*, AMF₃: *Glomus clarum*, AMF₄: *Gigaspora gigantea*, PM₀: 0 tons ha⁻¹, PM₁: 4 tons ha⁻¹, PM₂: 8 tons ha⁻¹, PM₃: 12 tons ha⁻¹, Means within a column not sharing a letter in common differ from other means significantly following Duncan's Multiple Range Test (DMRT) at 5%

with the un-inoculated plants that received zero PM making up the lowest branches in both years (p<0.05). The number of nodules observed from the interaction of *Gi. gigantea* with 4 and 8 t ha⁻¹ PM, respectively was significantly higher in 2016 (10.08 and 10.09) than the number of nodules from plants treated with *Gi. gigantea* ×4 and 0 t ha⁻¹ PM and *G. clarum* ×12 t ha⁻¹ PM, which were statistically at par and significantly higher (p<0.05) than the number of nodules when soil received neither inoculants nor PM applications (Table 2). A similar trend was observed in the 2017 growing season.

The result of the interactive effect is in agreement with the result of Abdullahi *et al.*¹⁹ on the response of maize to poultry manure and AMF inoculations, which in their studies had significantly higher plant height, more leave area, root volume and root and shoot biomass than those grown in natural conditions. The results also confirmed previous findings that the addition of AMF and organic amendments increased the productivity of plant^{10,11}. The vigorous increase in the number of pods could be attributable to improved branching and other growth attributes as a result of an enhanced supply of nitrogen from the poultry manure. This is supported by the opinion of Hayati *et al.*⁴¹ which favors organic fertilizer as the main source of macronutrients as well as essential micronutrients which enhances vegetative growth of plants such as the number of branches and indirectly the number of pods and other yield attributes.

Effects of AMF and PM on yield characters and yield of Bambara nut in 2016 and 2017: Results of yield characters and yield in response to the effects of AMF and PM in both years are presented in Table 3. Inoculation with *G. clarum* resulted in the highest number of pods per plant (10.00), significantly higher than the number of pods from inoculation with all other AMF species, in the order of

	2016 cropping seaso	n		2017 cropping season			
Treatments	No. of pods plant ⁻¹	Pod yield (t ha ⁻¹)	Seed yield (t ha ⁻¹)	No. of pods plant ⁻¹	Pod yield (t ha ⁻¹)	Seed yield (t ha ⁻¹)	
AMF	• •			• •			
AMF	6.42 ^d	1.33 ^c	1.00 ^c	7.08 ^c	1.42 ^d	1.31 ^d	
AMF	7.33 ^{bc}	2.99ª	1.59 ^b	7.00 ^c	3.09 ^a	3.01ª	
AMF ₂	7.00 ^c	2.38 ^b	1.72 ^b	7.67 ^c	2.49 ^c	2.40 ^c	
AMF	10.33ª	2.99ª	2.24ª	11.75ª	3.11ª	3.02ª	
AMF	7.67 ^b	2.88 ^b	2.28ª	9.17 ^b	2.97 ^b	2.86 ^b	
PM							
PM ₀	6.60°	2.36 ^b	1.64 ^b	7.33 ^b	2.45°	2.34 ^c	
PM ₁	7.60 ^b	2.35 ^b	1.67 ^b	8.73ª	2.46 ^c	2.37 ^c	
PM ₂	7.87ª	2.47ª	1.77 ^b	8.60ª	2.58 ^b	2.49 ^b	
PM ₃	8.87ª	2.89ª	1.98ª	9.47ª	2.98ª	2.88ª	
AMF×PM							
	3.33 ^h	0.961	0.78 ^g	3.67 ^g	1.00 ^m	0.78 ^m	
AMF ₀ PM ₁	4.67 ^f	1.09	0.86 ^g	5.67 ^f	1.23 ¹	1.14	
AMF ₀ PM ₂	9.33 ^{bc}	1.27	0.86 ^g	10.33 ^{bc}	1.39 ^k	1.30 ^k	
	8.33°	1.98j	1.51 ^f	8.67 ^d	2.09 ⁱ	2.00 ⁱ	
AMF ₁ PM ₀	8.00 ^{cd}	3.09 ^e	1.67 ^{def}	8.33 ^d	3.20 ^d	3.11 ^d	
AMF ₁ PM ₁	4.33 ^{fg}	3.59 ^c	1.72 ^{def}	4.67 ^f	3.67°	3.58°	
AMF_1PM_2	8.00 ^{cd}	2.50 ⁹	1.47 ^f	7.00 ^e	2.61 ^f	2.52 ^f	
AMF ₁ PM ₃	9.00 ^b	2.79 ^f	1.48 ^f	8.00 ^{de}	2.90 ^e	2.81°	
AMF_2PM_0	9.00 ^b	2.27 ^h	1.50 ^f	9.67°	2.38 ^g	2.29 ^g	
AMF ₂ PM ₁	7.33 ^{de}	1.67 ^k	1.75 ^{def}	8.00 ^d	1.77 ^j	1.69 ^j	
AMF_2PM_2	4.00 ^g	2.13 ⁱ	1.76 ^{def}	4.67 ^f	2.24 ^h	2.15 ^h	
AMF_2PM_3	7.67 ^{de}	3.46 ^d	1.87 ^{cde}	8.33 ^d	3.57°	3.48 ^c	
$AMF_{3}PM_{0}$	11.00ª	3.50 ^{cd}	2.39 ^b	11.00 ^b	3.61°	3.52°	
AMF_3PM_1	11.33ª	2.72 ^f	2.07 ^c	13.00ª	2.84 ^e	2.75 ^e	
AMF_3PM_2	9.67 ^b	1.92 ^j	1.64 ^{ef}	11.67 ^b	2.03 ⁱ	1.94 ⁱ	
$AMF_{3}PM_{3}$	9.33 ^{bc}	3.84 ^b	2.85ª	11.33 ^b	3.96 ^b	3.87 ^b	
AMF_4PM_0	6.67 ^e	1.97 ^j	1.86 ^{cde}	8.33 ^d	2.08 ⁱ	1.99 ⁱ	
AMF_4PM_1	5.67 ^f	2.67 ^f	1.96 ^{cd}	7.33 ^e	2.79 ^e	2.69 ^e	
AMF_4PM_2	8.33°	4.52ª	3.11ª	9.67°	4.62ª	4.54ª	
AMF_4PM_3	10.00 ^b	2.37 ^h	2.16 ^{bc}	11.33 ^b	2.40 ^g	2.25 ^{gh}	

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Table 3: Effects of Arbuscular Mycorrhizal Fungi (AMF) and Poultry Manure (PM) on yield of Bambara groundnut

AMF: Arbuscular mycorrhizal fungi, PM: Poultry manure, AMF₀: Un-inoculated plants, AMF₁: *Glomus mosseae*, AMF₂: *Glomus deserticola*, AMF₃: *Glomus clarum*, AMF₄: *Gigaspora gigantea*, PM₀: 0 tons ha⁻¹, PM₁: 4 tons ha⁻¹, PM₂: 8 tons ha⁻¹, PM₃: 12 tons ha⁻¹, Means within a column not sharing a letter in common differ from other means significantly following Duncan's Multiple Range Test (DMRT) at 5%

G. clarum>Gi. gigantea > G. mosseae >= Control. In 2017 however, G. clarum produced number of pods (11.75) higher (p<0.05) than Gi. gigantea which in turn outstripped statistically similar number of pods among the G. mosseae, G. deserticola and un-inoculated plants respectively. The observed increase in the number of pods relative to the control in this study corroborates with the findings of previous study⁴² that Bambara groundnut like others in Fabaceae family is mycorrhizal. Ellah and Singh⁴³ and Uko et al.¹² have reported enhanced growth, biomass yield and seed yield in AMF treated plants relative to untreated plants. Pod yield of plants inoculated with G. mosseae and G. clarum was statistically similar (p>0.05) and significantly higher (p<0.05) than pod yield from inoculation with G. deserticola and Gi. gigantea which was also higher than the un-inoculated control in 2016. In 2017, pod yield of G. mosseae and G. clarum were statistically similar (p>0.05), greater than pod yield with *Gi. gigantea* inoculant which was in turn higher than pod yield among un-inoculated plants and plants inoculated with *G. deserticola.* In 2016 the seed yield of plants inoculated with *G. clarum* and *Gi. gigantea* (2.24 and 2.28 t ha⁻¹) was statistically at par (p>0.05), significantly higher than seed yield of *G. mosseae* and *G. deserticola* inoculated plants (1.72 and 1.59 t ha⁻¹) which were also at par (p>0.05) and significantly higher than control (1.00 t ha⁻¹). During 2017 however, *G. deserticola* and *G. clarum* had statistically similar (p>0.05) seed yield of 3.01 and 3.02 t ha⁻¹ respectively but higher than seed yield of *Gi. gigantea* > *G. deserticola* > un-inoculated control (p<0.05). The results from previous studies by Uko *et al.*²² on groundnut; Hidayat *et al.*¹⁰ on sweet corn; Abdullahi *et al.*¹⁹ on maize, all recorded results that are in good agreement with that obtain herein.

The number of pods, pod and seed yield in both years increased significantly with each increment in PM rates from

0 to 12 t ha^{-1} (Table 3). During 2016, the number of pods at 8 and 12 t ha^{-1} was statistically at par (p>0.05) and significantly higher than pods recorded from 4 and 0 t ha⁻¹ PM respectively. In the 2017 season, the application of 4-12 t ha⁻¹ PM resulted in the number of pods that were statistically at par (p>0.05) and only higher than control. Pod yield in 8 and 12 t ha⁻¹ PM treated plots was at par and significantly higher than pod yield of plants treated with 0 and 4 t ha⁻¹ PM which were also at par. During 2017 however, pod yield in plots that got 12 t ha⁻¹ PM (2.98 t ha⁻¹) was higher than those that received 8 t ha⁻¹ PM (2.58 t ha⁻¹) which outstripped both 0 and 4 t ha⁻¹ PM (2.45 and 2.46 t ha⁻¹) respectively in yield (p<0.05). The increase in yield under this study may be ascribed to an increase in the number of pods and nodules, which could be attributable to N, P, K, Ca, Mg contents of the poultry manure utilized. The results agreed with those obtained by Akpan *et al.*³⁶ and Gyapong *et al.*¹⁴. Wamba et al.40 also observed improvement in the number of pods, number of seed, 1000-grain weight, grain yield and pod yield of Bambara groundnut in soil amended with poultry.

The result for interactive effect showed that the least expressions of the number of pods, pod yield and seed yield were observed among plants that neither received PM nor inoculation. The number of pods increased significantly (p<0.05) from G. clarum \times 0 and 1 t ha⁻¹ PM interaction (11.00 and 11.33), respectively, closely followed by Gi. gigantea ×12 t ha⁻¹ PM (10.00) in 2016. In 2017 (Table 3), G. clarum \times 4t ha⁻¹ PM interaction gave the highest (p<0.05) number of pods (13.00), closely followed by each of Gi. gigantea and G. clarum \times 12 t ha⁻¹ PM (p<0.05) respectively. The increase in pod yield was highest (p<0.05) when Gi. gigantea interacted with 8 t ha^{-1} PM (4.52 t ha^{-1}), followed by *G. clarum* \times 12 t ha⁻¹ PM (3.48 t ha⁻¹) in 2016. A similar trend was observed in 2017 with *Gi. gigantea* x 8 t ha^{-1} PM (4.62 t ha^{-1}) giving the highest in pod yield of Bambara groundnut (Table 3). The seed yield of *Gi. gigantea* \times 8 t ha⁻¹ PM (3.11 t ha⁻¹) was highest (p<0.05), statistically similar to seed yield of G. clarum \times 12 t ha⁻¹ PM (2.85 t ha⁻¹). During the 2017 season, however, Gi. gigantea x 8 t ha-1 PM with seed yield of 4.54 t ha⁻¹ was significantly the highest (p<0.05) than yield in G. clarum \times 12 t ha⁻¹ PM interaction (3.87 t ha⁻¹). Positive growth and yield response of the crop to AMF inoculation in combination with organic manure under field conditions have been reported in Zea mays^{11,23} and Zingiber officinale⁴⁴. The result obtained for yield of seed and pod yield of Bambara groundnut is also in agreement with previous studies²².

Correlation between growth, yield attributes and yield of Bambara groundnut: The correlation matrix plot between growth, yield parameters and yield is presented in Fig. 2 The clustered variables showed that the variables are either positively or negatively correlated with each other (Fig. 2a,b); the closer the variable to each other the higher the relationship between them and vice versa. The colour of the line represents the direction of the correlation while the line shade and thickness represent the strength of the relationship. Similarly, the deep blue coloured boxes showed a positive and strong correlation, light blue boxes showed strong to weak positive correlation (Fig. 2c,d).

As revealed in the matrix plot, in 2016 cropping season, strong and positive correlations were observed between seed yield and pod yield (r = 0.83, p<0.01), Leaf Area Index (LAI) (r = 0.56, p<0.05), nodules (r = 0.57, p<0.05). Similarly, strong and positive correlations were observed between seed yield and pod yield (r = 1.0, p<0.01), Leaf Area Index (LAI) (r = 0.65, p<0.05), nodules (r = 0.59, p<0.05) in 2017 cropping season. The positive relationships between seed yield and growth and yield attributes of Bambara groundnut in this study imply that a unit increase in growth and yield attributes of Bambara groundnut would result to a corresponding increase in seed yield. In addition, positive correlations were also found between plant height and, number of branches, leaf area index, leaf number and nodules; number of branches and, leaf area index, leaf number and nodules; leaf area index and, leaf number and noules in both 2016 and 2017 cropping seasons. The increase in seed yield under this study may be ascribed to an increase in other growth and yield attributes such as the number of leaves, leaf area index, nodules in addition to N, P, K, Ca, Mg contents of the poultry manure utilized⁴⁵.

Nutrient concentration of BBG leaf tissue in 2016 and 2017:

The effects of AMF and PM on leaf tissue nutrient content in both years of the study are presented in Table 4. Leaf tissue of un-inoculated BBG plants had the lowest N, P and Ca in both years. Inoculation with *Gi. gigantea* and *G. mosseae* resulted in the highest N (p<0.05) in both years, significantly higher than N among *G. deserticola* and *G. clarum* inoculated plants which were statistically at par (p>0.05). Available P from *G. mosseae*, *G. deserticola* and *G. clarum* inoculation was statistically at par (p>0.05), significantly higher than P in plants that received *G. deserticola* and un-inoculated plants which were statistically at par (p>0.05) in 2016 (Table 4). Similar trend was observed in 2017.



Fig. 2(a-d): (a, b) Correlation matrix plot and (c, d) coefficient showing relationship between yield of Bambara groundnut and, growth and yield attributes for 2016 and 2017 growing seasons NOB: Number of branches, LAI: Leaf area index, NOP: Number of pod: Plant height (cm), Pod yield (t ha⁻¹), Seed yield (t ha⁻¹)

Inoculation with *G. clarum* gave significantly higher K content in both years (p<0.05), above *G. mosseae* and *G. deserticola* which were at par (p>0.05) but higher than leaf tissue K in *Gi. gigantea* inoculated plants (Table 4). Ca content in *G. deserticola* and *G. clarum* was at par (p>0.05), significantly higher than Ca in the tissue of *G. mosseae* inoculated and un-inoculated plants, which were also at par but significantly outstripped *Gi. gigantea* leaf tissue Ca (p<0.05). Inoculation with *Gi. gigantea* increased Mg content in leaves (p<0.05) above un-inoculated plants, *G. mosseae* and *G. deserticola* inoculated plants which were at par but higher than Ca in plants that received *G. clarum* (Table 4). Liu *et al.*²³ reported high plant K, Ca and Mg accumulation in tissue and

their uptake by maize due to AMF symbiosis at low soil nutrients concentration. Perner *et al.*⁴⁵ also observed a similar increase in nutrient content in *Pelargonium* plants due to AMF and levels of compost supply.

The effect of PM on nutrient content in both years was significant. The 12 t ha⁻¹ PM significantly increased leaf N and P (p<0.05), higher than 8 and 4 t ha⁻¹ PM which were also at par (p>0.05) but higher in content than plants tissue in control in both years. The 8 and 12 t ha⁻¹ PM application resulted in statistically similar K content, significantly higher than in 4 and 0 t ha⁻¹ PM fertilized plants which were also statistically at par (p>0.05) in both years. Ca was highest (p<0.05) among untreated plants and decreased as PM rates increased up to

	2016 cro	2016 cropping season					2017 cropping season			
Treatments	 N	P	K	Са	Mg	 N	Р	K	Ca	Mg
AMF					-					
AMF ₀	2.58°	0.15 ^b	1.84 ^{bc}	1.25 ^b	0.68 ^c	2.68 ^c	0.17 ^ь	1.94 ^{bc}	1.27 ^b	0.69℃
AMF ₁	3.23ª	0.17ª	1.76 ^{bc}	1.14 ^b	0.77 ^{bc}	3.41ª	0.19ª	1.90 ^{bc}	1.16 ^b	0.79 ^{bc}
AMF ₂	2.88 ^b	0.15 ^b	2.06 ^b	1.65ª	0.87 ^b	3.00 ^b	0.17 ^ь	2.20 ^b	1.66ª	0.88 ^b
AMF ₃	2.97 ^b	0.16 ^{ab}	2.63ª	1.55ª	0.45 ^d	3.12 ^b	0.19ª	2.76ª	1.56ª	0.46 ^d
AMF ₄	3.26ª	0.16 ^{ab}	1.66 ^c	0.83°	1.24ª	3.36ª	0.18 ^{ab}	1.80 ^d	0.85°	1.25ª
PM										
PM ₀	2.33c	0.14 ^c	1.67 ^b	1.62ª	0.61 ^c	2.43°	0.16 ^c	1.78 ^b	1.63ª	0.63°
PM ₁	3.05 ^b	0.15 ^b	1.70 ^b	1.34 ^b	0.79 ^b	3.17 ^b	0.18 ^b	1.83 ^b	1.35 ^b	0.82 ^b
PM ₂	3.25 ^b	0.15 ^b	2.44ª	1.25 ^b	0.86 ^{ab}	3.36 ^b	0.17 ^b	2.57ª	1.27 ^b	0.88 ^{ab}
PM ₃	3.33ª	0.19ª	2.15ª	0.94°	0.92ª	3.50ª	0.20ª	2.28ª	0.95°	0.94ª
AMF×PM										
	2.18ª	0.19 ^b	1.45 ^d	1.87 ^b	0.19 ^m	2.28ª	0.19 ^{bc}	1.45 ^e	1.87 ^b	0.20 ^m
AMF ₀ PM ₁	2.38ª	0.10 ^h	2.00 ^{bcd}	1.33 ^{de}	0.38 ^{jklm}	2.41ª	0.12 ^g	2.13 ^{cde}	1.35 ^{cd}	0.39 ^{jklm}
AMF ₀ PM ₂	2.81ª	0.18 ^{bc}	1.95 ^{bcd}	1.10 ^{efd}	0.96 ^{de}	2.93ª	0.20 ^b	2.08 ^{cde}	1.11 ^{def}	0.98 ^{de}
AMF ₀ PM ₃	2.93ª	0.14 ^{defg}	1.95 ^{bcd}	0.72 ^{ij}	1.20 ^{cd}	3.10ª	0.16 ^{cdef}	2.08 ^{cde}	0.73 ^{hi}	1.22 ^{cd}
AMF ₁ PM ₀	2.44ª	0.18 ^{bc}	1.90 ^{bcd}	0.47 ^{jk}	1.20 ^{cd}	2.51ª	0.20 ^b	2.03 ^{cde}	0.48 ^{ij}	1.22 ^{cd}
AMF ₁ PM ₁	3.37ª	0.18 ^{bc}	1.25 ^d	1.67 ^{bc}	0.53 ^{hijk}	3.57ª	0.20 ^b	1.38 ^e	1.68 ^b	0.55 ^{hijk}
AMF ₁ PM ₂	3.52ª	0.15 ^{cdef}	2.30 ^{bcd}	0.87 ^{ghi}	0.58 ^{fghij}	3.73ª	0.17 ^{bcde}	2.43 ^{bcd}	0.88 ^{fgh}	0.59 ^{fghij}
AMF_1PM_3	3.60 ^a	0.16 ^{cde}	1.60 ^{cd}	1.57 ^{cd}	0.77 ^{efg}	3.83ª	0.18 ^{bcd}	1.73 ^{de}	1.58 ^{bc}	0.79 ^{efgh}
AMF ₂ PM ₀	2.27ª	0.17 ^{bcd}	1.50 ^{cd}	2.93ª	0.48 ^{ijkl}	2.40ª	0.19 ^{bc}	1.63 ^{de}	2.95ª	0.49 ^{ijkl}
AMF ₂ PM ₁	3.04ª	0.14 ^{defg}	2.00 ^{bcd}	1.03 ^{fgh}	2.02ª	3.11ª	0.16 ^{cdef}	2.13 ^{cde}	1.04 ^{efg}	2.04ª
AMF_2PM_2	3.05ª	0.15 ^{cdef}	1.95 ^{bcd}	1.83 ^{bc}	0.29 ^{klm}	3.15ª	0.17 ^{bcde}	2.08 ^{cde}	1.85 ^b	0.31 ^{klm}
AMF_2PM_3	3.15ª	0.12 ^{fgh}	2.80 ^b	0.79 ^{hi}	0.67 ^{fghi}	3.35ª	0.14 ^{efg}	2.93 ^b	0.80 ^{gh}	0.69 ^{fgh}
AMF_3PM_0	2.31ª	0.23ª	2.05 ^{bcde}	2.41ª	0.19 ^m	2.40ª	0.25ª	2.18 ^{bcde}	2.43ª	0.21 ^m
AMF ₃ PM ₁	3.14ª	0.17 ^{bcd}	1.40 ^d	1.68 ^{bc}	0.24 ^{Im}	3.27ª	0.19 ^{bc}	1.53 ^e	1.69 ^b	0.26 ^{Im}
AMF_3PM_2	3.20ª	0.14 ^{defg}	4.45ª	1.22 ^{ef}	0.82 ^{ef}	3.37ª	0.16 ^{cdef}	4.58ª	1.23 ^{de}	0.84 ^{ef}
AMF_3PM_3	3.25ª	0.11 ^{gh}	2.60 ^{bc}	0.89 ^{ghi}	0.53 ^{hijk}	3.43ª	0.13 ^{fg}	2.73 ^{bc}	0.90 ^{fgh}	0.55 ^{hijk}
AMF_4PM_0	2.47ª	0.16 ^{cde}	1.45 ^d	0.41 ^k	1.0 ^{de}	2.57ª	0.18 ^{bcd}	1.58 ^e	0.42 ^j	1.03 ^{de}
AMF_4PM_1	3.31ª	0.17 ^{bcd}	1.85 ^{bcd}	0.97 ^{fghi}	0.82 ^{ef}	3.47ª	0.19 ^{bc}	1.98 ^{cde}	0.98 ^{efgh}	0.84 ^{efg}
AMF_4PM_2	3.57ª	0.13 ^{efgh}	1.55 ^{cd}	1.23 ^{ef}	1.68 ^b	3.60ª	0.15 ^{defg}	1.68 ^{de}	1.25 ^{de}	1.69 ^b
AMF ₄ PM ₃	3.70ª	0.16 ^{cde}	1.80 ^{bcd}	0.72 ^{ij}	1.44 ^{bc}	3.80ª	0.18 ^{bcd}	1.93 ^{cde}	0.73 ^{hi}	1.46 ^{bc}

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Table 4: Effects of Arbuscular Mycorrhizal Fungi (AMF) and Poultry Manure (PM) on nutrient concentration (%) in leaf tissue of Bambara groundnut

AMF: Arbuscular mycorrhizal fungi, PM: Poultry manure, AMF₀: Un-inoculated plants, AMF₁: *Glomus mosseae*, AMF₂: *Glomus deserticola*, AMF₃: *Glomus clarum*, AMF₄: *Gigaspora gigantea*, PM₀: 0 tons ha⁻¹, PM₁: 4 tons ha⁻¹, PM₂: 8 tons ha⁻¹, PM₃: 12 tons ha⁻¹, Means within a column not sharing a letter in common differ from other means significantly following Duncan's Multiple Range Test (DMRT) at 5%

12 t ha⁻¹ (p<0.05) in 2016 and 2017. Mg was highest at 12 and 8 t ha⁻¹ PM, significantly higher than 4 t ha⁻¹ PM with the control bringing up the lowest Mg content (p<0.05) in both years. Abdullahi *et al.*¹⁹ recorded higher shoot nutrients content (N, P and K) in inoculated and organic manured plants compared to un-inoculated ones.

The interaction effect of AMF × PM was not significant for N content in leaf tissue alone (Table 4). P in leaf tissue increased significantly (p<0.05) as plants received *G. clarum* × zero PM (0.23 and 0.25) in both years, with *G. clarum* × 12 t ha⁻¹ PM (0.11 %) in 2016 and *G. mosseae* × 4 t ha⁻¹ PM (0.12%) in 2017 bringing up the least P content. The K, Ca and Mg content in leaf tissue were significantly increased at the interaction of *G. clarum* × 8 t ha⁻¹ PM (4.45 %), *G. clarum* × zero PM (2.41%) and *G. deserticola* × 4 t ha⁻¹ PM (2.02%), respectively in 2016 (p<0.05) over other interactions, with *G. mosseae* × 4 t ha⁻¹ PM (1.25%), *Gi. gigantea* × zero PM (0.41%) and control (0.19%) bringing up the least in K, Ca and

Mg content respectively (Table 4). A similar trend was observed in 2017 growing season. During this period, the K, Ca and Mg content in leaf tissue were significantly higher at the interaction of *G. clarum* \times 8 t ha⁻¹ PM (4.58%), *G. clarum* \times zero PM (2.43%) and *G. deserticola* \times 4 t ha⁻¹ PM (2.04%), respectively in both years (p<0.05) over other interactions, with *G. mosseaex* 4 t ha⁻¹ PM (1.38%), *Gi. gigantea* \times zero PM (0.42%) and control (0.20%) bringing up the least in K, Ca and Mg content respectively. Enhanced nutrient concentrations (P, K, Ca and Mg) significantly over control plants due to both AM application and organic manure application have also been observed by several researchers^{11,19}.

CONCLUSION

The results of this study showed that soil inoculated with *Glomus clarum* and *Gigaspora gigantea* and amended with 8 and 12 tons ha⁻¹ of poultry manure in single or combination

were more efficient and consistent in the enhancement of growth parameters (plant height, number of branches, leaf number, leaf area index and nodules), leaf nutrient uptake, yield and yield components of Bambara groundnut. Bearing in mind the marginal nature of the soil of the study area, utilizing AMF and poultry manure as single and in combinations compared to no application will be beneficial in the Bambara groundnut yield.

SIGNIFICANCE STATEMENT

This research study has shown the inoculation of *Glomus clarum* and *Gigaspora gigantea* alongside with 8 and 12 ton ha⁻¹ poultry manure to be beneficial in mobilizing nutrients bound to organic matter and soil particles for Bambara groundnut growth. The current research has demonstrated that the application of poultry manure at 8 and 12 tonnes per hectare in single or conjunctively with either *Glomus clarum* or *Gigaspora gigantea* significantly enhances growth parameters, leaf nutrient uptake, yield and yield components of Bambara groundnut. Hence, the result of this study will be beneficial to agronomists, growers and researchers at large looking for reliable means of optimizing crop yield over a long time without environmental pollution.

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