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Biodiversity of arbuscular mycorrhizal fungi of pumpkins (*Cucurbita* spp.) under the influence of fertilizers in ferralitic soils of Cameroon and Benin

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ABSTRACT

In Africa, many people suffer from nutrient deficiencies and this enhances the need for yield increase of crops like pumpkins with good nutritional qualities, while safeguarding the environment. The aim of this study was to assess the composition and specific diversity of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of pumpkins under two fertilizer application systems. The experiment was conducted with a completely randomized design with a split-plot of three factors: fertilizers, pumpkin varieties and experimental sites. Dried seeds of pumpkin were sown after field preparation and at flowering and fruiting, samples of soil and root fragments were collected from the rhizosphere. In the laboratory, roots were clarified and coloured before microscopic observations. Soil samples were used for the extraction of spores through humid filtering. Spore suspension was poured into a Petri dish and spores counted with the help of a stereo microscope. The spores of AMF were identified on the basis of morphological descriptions. Results show that chemical fertilizer (T2) significantly reduces ($p < 0.001$) the frequency and intensity of mycorrhization compared with control (T0) and fowl droppings (T1). The hierarchical classification shows two classes ($R^2 = 0.63$): T0 and T1; and T2. Some 15 AMF species of four genera were isolated and identified. Of these, *Glomus* (57.97-85.65%) and *Acaulospora* (12.68-40.42%) were the most abundant with high density (1086 spores/100 g of soil). *Glomus intraradices* was absent in Benin and present in Cameroon. Diversity indices were higher in Cameroon than in Benin.

1. INTRODUCTION

More than 800 million people in the world and more particularly in sub Saharan Africa are undernourished and suffer from insufficient energy intake associated with deficiency in proteins, vitamins and mineral elements 1. In the developing world, life span does not exceed 50 years and malnutrition is the cause of half the number of deaths of children below 5 years of age 1. In addition, the world population is projected to increase beyond agricultural production. Agricultural research is therefore challenged to improve on food security through improved crop yields and soil productivity, while safeguarding the environment. Within this framework, pumpkins (*Cucurbita* spp.) can play an important role since its fruits and leaves are rich in vitamins, minerals and fibers etc. 2. Moreover, pumpkin is an excellent source of vitamin A; its orange color indicates the presence of beta carotene which the organism can transform into vitamin A 3. However, crop yields, particularly those of pumpkins in the countries of sub Saharan Africa are amongst the least in the world. Low yields in rural areas are due to plant diseases, poor cultural practices, pests and especially reduction in soil fertility 4. The role of soil micro-organisms such as mycorrhizal fungi has been

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demonstrated in the development of sustainable agriculture 56. Mycorrhizae are symbiotic associations between plant roots and mycelial filaments of soil fungi which favor plant growth and development 7. Many plant species directly depend on AMF for survival 8. AMF, particularly Endomycorrhizas form associations with approximately 80 percent of all plant species. These fungi cannot be grown in pure culture but must be grown in association with plant roots. Thus they cannot be multiplied on artificial media without a living host 9. They form branched structures called arbuscules within the host's root cells, and thus are known as arbuscular mycorrhizal fungi. The arbuscules are sites of nutrient exchange between the fungus and the host 10.

Despite their importance, these fungi are not exploited and are almost ignored by African farmers. Studies in the humid forest of south Cameroon have shown that most forest species and cultivated plants are highly mycotrophic in their natural habitats 1112. So far, there is no information on mycotrophic species of cultivated pumpkins. Moreover, little data exists on endomycorrhiza biodiversity under pumpkin culture with respect to type of fertilizer used. For these reasons, this work was aimed at studying the biodiversity of endomycorrhiza fungi associated with pumpkin cultivation under the effect of fertilizers in Cameroon and Benin.

2. MATERIAL AND METHODS

2.1. Study sites

The experiment was undertaken during the 2012 and 2013 cropping seasons in Benin and Cameroon. In Cameroon, it was carried out at Mbalgong situated at 11°22'59'' longitude East and 3°46'59'' latitude North. The climate of this locality is of the equatorial Guinean type with an average atmospheric temperature of 23 °C. It has four seasons: two rainy and two dry seasons. Annual rainfall is 1800 mm. Average humidity is 80 %, with daily variation between 35 and 98 %. The preceding plants on the experimental site were cassava (*Manihot esculenta* Crantz) and predominant species like *Chromolaena odorata* (L.), *Mimosa pudica* (L.), and *Imperata cylindrica* (L.).

In Benin, the experiment was realized in the "*Ferme d'Application et de Production* (FAP)" of the Faculty of Agricultural Sciences of the University of Abomey-Calavi (FSA). With reference to information obtained from INSAE [Institut National de la Statistique et de l'Analyse Economique. *Cahier de villages et de quartiers de ville – Département de l'Atlantique*. Cotonou: 2004], the altitude of the locality is 17.4 m; 06°24N, 02°20E). The climate at FAP is the sub Equatorial type marked by two dry and two rainy seasons. The relief is less accidental and annual rainfall in the locality varies between 900 and 1100 mm. Relative humidity varies between 55 and 95 % and annual sunshine is about 2024 h. Average temperature is 27 °C. The site was formerly a fallow dominated by *Panicum maximum* (Jacq.). Physico-chemical analysis of soil from Mbalgong was done in the laboratories of IITA (International Institute for Tropical Agriculture) in Nkolbisson (Cameroon) and Abomey-Calavi at INRAB (National Institute of Agricultural Research of Benin) Agonkanmey in Benin.

2.2. Biological Materials

Three varieties of squashes (pumpkin) were used for the experimentation; two of *Cucurbita moschata* variety (V2: straight neck and V3: egg shaped) and one *Cucurbita maxima* (V1: round shape) (Figure 1). Matured grains were obtained from fruits of the different varieties harvested from the farms of peasants. The grains were dried under sunlight for one month.

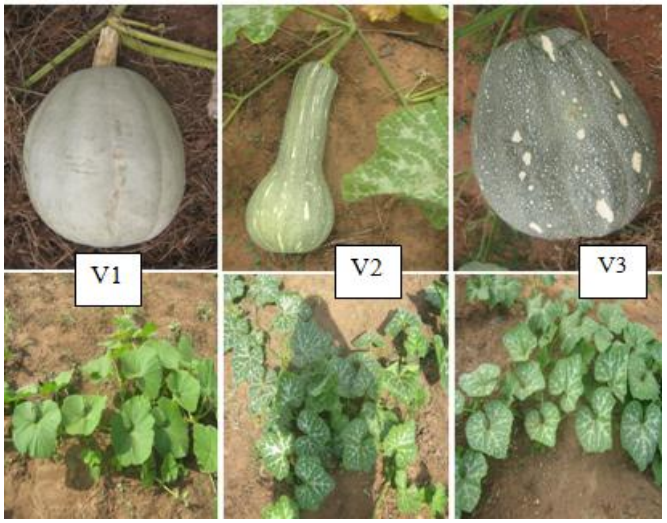

Fig. 1: Varieties of squashes (pumpkin) used for the experimentation. *Cucurbita moschata* variety (V2: straight neck and V3: egg shaped) and one *Cucurbita maxima* (V1: round shape).

2.3. Fertilizers

Two types of fertilizers were used in this study: organic fertilizer (fowl droppings) and chemical fertilizer. Eight months old fowl droppings were obtained from poultries and used to treat experimental plots at the rate of 20 t/ha 13, thus 32 kg for a plot of 16 m². The manure was buried in the soil two weeks before seeds were sown. The chemical fertilizer was used to balance cationic charges and nutritive elements of the soil with respect to plant needs. With reference to physicochemical characteristics of the soil in Benin, 10.4 g magnesium oxide (MgO), 505.6 g potassium chloride (KCl 60 %), 339 g urea (46 %) and 1.5 g of triple superphosphate (TSP 42 %) were applied. In Cameroon 1.48 kg of potassium sulfate (The potassium sulfate contains 50 % K_2O), 0.226 kg kerserite (25 % MgO) and 2.24 kg of ammonium phosphate (18 % N + 45 % P_2O_5) were applied per experimental unit. Chemical fertilizer was applied two weeks after sowing with the exception of urea fertilizer application in Benin which was applied in fractions : 2/3 two weeks after sowing and 1/3 one and a half months later.

2.4. Experimental procedure

The experimental design for this study was a split-plot with three factors: fertilizers (T0: control, T1: fowl droppings, T2:

chemical fertilizer), pumpkin varieties (V1: *Cucurbita maxima* Duch., rounded form, V2 : *Cucurbita moschata* Duch., elongated form with straight neck and V3 : *C. moschata, ovoid* form) and experimental sites. The experiment in each site was organized in complete random blocks. After clearing and tillage, each plot of 51 m x 23.5 m was divided into four parallel blocks of 51 m x 4 m each per site. Spacing between experimental blocks was 1.5 m. With the help of a double decameter, a string and pegs, nine experimental units of 4 m x 4 m were constituted per block, making a total of 36 units per site. Two lines were made per plot and the space between pegs was 2 m. The dried grains of pumpkin were sown (four grains per seed-hole) after field preparation. Two seedlings per seed-hole were eliminated after germination leaving a total of 288 plants per site. Care of experimental plots involved manual weeding, mulching around the plants and watering in the absence of rainfall.

2.5. Sampling

At flowering and fruiting, four samples each of soil and root fragments were collected from the rhizosphere of pumpkin seed-holes. For each experimental unit, the different soil samples were mixed to constitute a composite sample. About 5 g small roots attached to the main root were collected per seed-hole and conserved in 50 % ethyl alcohol.

2.6. Root colonization

In the laboratory, roots were clarified and colored before microscopic observations 14. Selected root samples were rinsed with tap water and reduced to fragments 1-2 cm long. About 1 g of each pumpkin root sample was put into a test tube containing 10 mL of KOH (10 %, W/V) for 24 h. This action will empty the contents of their cytoplasmique cells while keeping intact fungal structures. These roots were later rinsed with tap water and soaked in 10 mL of oxygenated water (10 %) for 45 min in order to clarify and to oxidize the organic material present on the roots. After rinsing with water, the roots were soaked in 10 mL of trypan blue solution (0.05 %) which is a colorant. This solution is prepared using 333 ml of glycerol, 333 ml of lactic acid, 333 mL of distilled water and 0.5 g of trypan blue (powder) to 1 L of solution. The trypan blue solution gives a bluish tint to various infections to facilitate their identification under the microscope. After two to three days, the samples were prepared on glass slides and observed under an electron microscope.

2.7. Isolation, enumeration and identification of AMF

Using the method described by 15, a 100 g soil sample from the pumpkin rhizophere was used for the extraction of spores through humid filtering via a series of sieve meshes between 425 and 432 µm. The suspension of spores in the sieves was centrifuged on a saccharose gradient at 50 % 1617. The suspension of spores was then poured into a Petri dish with cross-ruled surface to facilitate counting of spores with the help of a stereo microscope (Zeiss brand, magnification × 40) with respect to their sizes, color and mode of attachment of hyphae to the spore. The spores of AMF were mounted on glass slide in PVLG with Melzer reagent 19 and identified on the basis of morphological descriptions published by INVAM (http//www.invam.caf.wdu.edu) and BEG (European Bank of Glomales: http//www.bio.ukc.ac.uk.beg).

2.8. Statistical analysis

The AMF diversity of each site 20 was analyzed with the aid of the following indices: specific richness (S = number of species in the study zone), Shannon-Weiner (H') 21 diversity index and Pielou 22 equitability index. Shannon-Wiener index is calculated by the following formula:

$$H' = -\sum_{i=1}^{s} PiLogPi$$

where:

Pi: proportional abundance or percent importance of the species (Pi= ni/N);

S: total number of species;

ni: number of individuals of a species in a sample;

N: total number of individuals of all species in a sample;

The equitability index is calculated using the following formula:

J'= H'/H'max with H'max = log S (S= total number of species).

Data on the frequency and intensity of mycorrhization were treated by analysis of variance (ANOVA) using the SAS (Statistical Analysis Software). Student and Newman & Keuls tests were used to compare means. Relationships between parameters were put into evidence by the Pearson correlation test. Data was also treated by analysis of principal components (ACP) using SAS.

3. RESULTS

3.1. Soil properties of the study sites

Soil analysis results are presented in table 1. The soil structure in Cameroon is limon-clay-sandy while in Benin it is limon-sandy. The pH of the soils were between 4 and 6.

Table 1. Physico-chemical characteristics of the soils (0-15 cm depth) of study sites.

Soil characteristics	Study sites		
	Abomey-Calavi (Benin)	Mbalgong (Cameroon)	
Sand (%)	78.682***	58.232	p<0.001
Clay (%)	12.083	31.490***	p<0.001
Limon (%)	7.000	10.280**	p<0.005
C (%)	0.68	1.880***	p<0.001
OM (%)	1.17	2.680***	p<0.001
pH	6.060 ns	4 .900 ns	p>0.226
N total ($g.kg^{-1}$)	0.620	2.190***	p<0.001
Apparent soil density ($kg.dm^{-3}$)	1.180***	0.755	p<0.001
Ca^{2+} ($g.kg^{-1}$)	0.227	0.741***	p<0.001
K^{+} ($g.kg^{-1}$)	0.022	0.055***	p<0.001
P ($g.kg^{-1}$)	0.498***	0.006	p<0.001
Mg^{2+} ($g.kg^{-1}$)	0.058	0.102***	p<0.001
Na^{+} ($g.kg^{-1}$)	0.053***	0.023	p<0.001
C/N	11.30***	8.59	p<0.001

* : significant difference, ** : highly significant difference, *** : very high significant difference, ns : non-significant, pH : potential of hydronium ion, C % : proportion of total organic carbon, N total : proportion of total nitrogen, C/N : ratio between proportions of total organic carbon and total nitrogen, OM % : organic matter content, P : total phosphorus, Ca^{2+}: calcium content, Mg^{2+}: magnesium content, K^{+}: potassium content, Na^{+}: sodium content.

3.2. AMF colonization

Mycorrhizal structures observed in pumpkin roots are in the form of arbuscules, vesicles, hyphae, spores and axillary bodies (Figure 2).

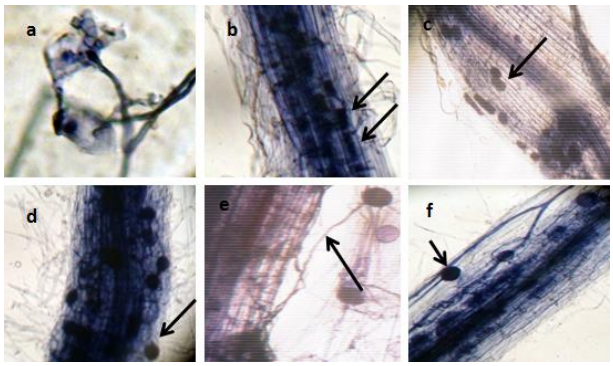

Fig. 2: Mycorrhizal structural colonization in *Cucurbita* spp. a: auxiliary cells; b: arbuscules; c,f: vesicles ; d : spores and e: hyphae.

3.3. Frequency and intensity of mycorrhization

Chemical fertilizer significantly ($p<0.001$) reduces the frequency of AMF as compared to the control (T0) and fowl droppings (T1) at flowering and fruiting of all pumpkin varieties (Figure 3). Whatever the kind of fertilizer used, the site does not influence the rate of mycorrhization in pumpkin culture. Moreover, the variety of pumpkin does not influence the frequency of mycorrhization.

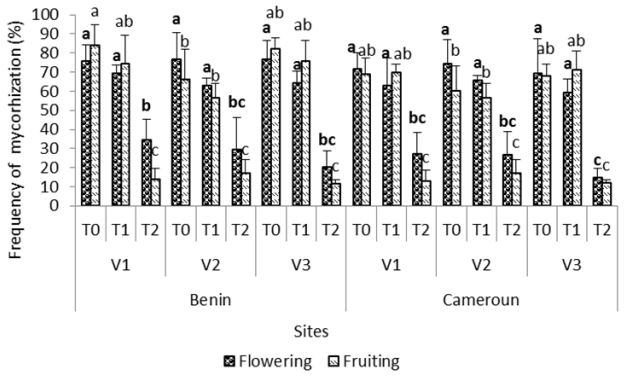

Fig. 3: Frequency of mycorrhization with respect to type of fertilizer application in *Cucurbita* sp. cultivation. V1 : *Cucurbita maxima* ; V2 : *Cucurbita moschata* elongated shape ; V3 : *C. moschata* egg-shaped ; T0 : control ; T1 : fowl droppings ; T2 : chemical fertilizers. For each stage of growth, the means with the same letter are not significantly different at $p<0.05$.

Table 5 shows a highly significant correlation ($p<0.001$) between the frequency of mycorrhization and the number of spores of *Gigaspora* at fruiting. The intensity of mycorrhization is significantly ($p<0.01$) more important for T0 (29.82±12.78 %^{a}) than for T1 (11.62±7.72 %^{b}) and T2 (2.46±2.79%^{c}) at flowering (Figure 4). The same observation was made at fruiting (T0: 27.89±12.92 %^{a}; T1: 23.50±14.80 %^{a} and T2 : 0.45±0.62 %^{b}). The study site does not influence the intensity of mycorrhization in pumpkin culture (Figure 4). The intensity of mycorrhization at flowering is strongly correlated to the number of spores of *Gigaspora* while at fruiting; it is correlated to the number of spores of *Acaulospora* and *Gigaspora* (Table 5).

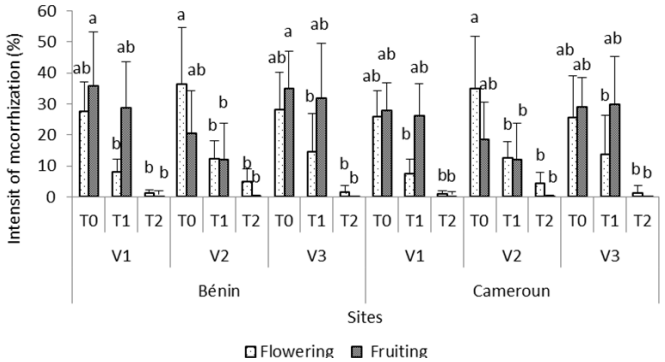

Fig. 4: Intensity of mycorrhization with respect to type of fertilizer application in *Cucurbita* spp. cultivation. V1 : *Cucurbita maxima* ; V2 : *Cucurbita moschata* elongated shape ; V3 : *C. moschata* egg-shaped ; T0 : control ; T1 : fowl droppings ; T2 : chemical fertilizers. For each growth stage, the means with the same letter are not significantly different at $p<0.05$.

3.4. Identification of spores of mycorrhiza fungi

The number of spores of AMF species is presented in table 2 while the images of some species are represented by Figure 5. All the spores of AMF found in the two sites belong to the phylum *Glomeromycota* (Table.3) according to the new classification by 23. The 15 species (Table 3) identified possibly belong to three (3) different families: family *Glomaceae*, family *Acaulosporaceae* and family *Gigasporaceae*. With the exception of *Glomus intraradices* which is present only in Cameroon, the diversity of AMF is identical in the two sites. A signficant correlation ($p<0.05$) is observed between number of spores of the genera *Glomus*, *Gigaspora* and *Scutellospora* at flowering. On the contrary, at fruiting, the genus *Scutellospora* is not correlated to the three others which are correlated amongst themselves (Tab. 5).

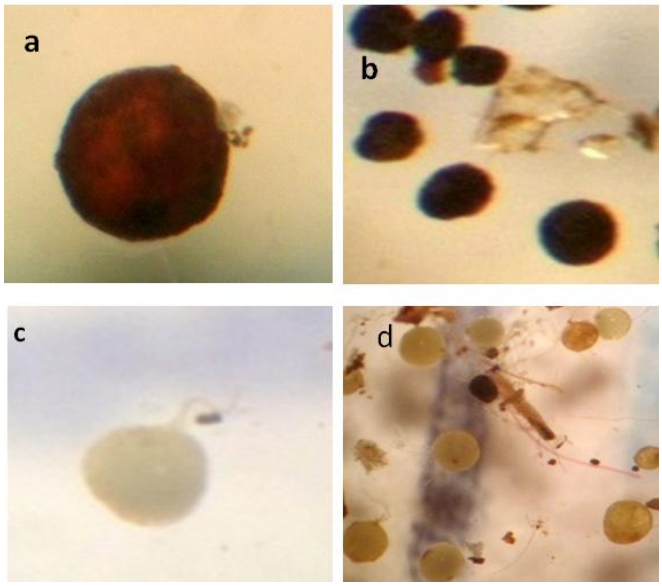

Fig. 5: AMF spores isolated from rhizosphere soil of *Cucurbita* spp. with fertilizer application. a: *Scutellospora gregaria*; b: *Glomus constrictum* and c & d: *Gigaspora margarita*.

Table 2: Mean number of spores in 100 g of soil.

AMF species	Benin						Cameroon					
	Flowering			Fruiting			Flowering			Fruiting		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
G. constrictum	612	626	620	1044	464	1086	562	576	570	1044	1032	1086
G. ambisporum	7	8	8	10	5	17	4	6	7	7	6	6
G. manihotis	31	35	26	55	10	26	31	33	28	57	56	41
G. caledonium	3	3	2	0	0	0	3	3	2	3	2	1
G. geosporum	116	109	94	130	57	108	111	104	89	126	125	107
G. intraradices	0	0	0	0	0	0	15	17	14	29	28	20
Total of*Glomus*	770	781	750	1240	536	1237	727	739	709	1265	1248	1260
A.tuberculata	14	19	17	33	7	35	10	11	11	42	30	49
A. rugosa	10	9	9	92	27	72	6	6	5	76	74	51
A. delicata	3	4	3	26	6	16	36	40	31	19	13	13
A. lacunosa	135	189	212	237	95	212	134	186	209	204	171	176
A. colossica	19	30	23	21	5	11	42	126	33	84	47	29
A. fovea	2	3	2	1	0	2	4	4	2	3	2	2
Total of *Acaulospora*	184	253	266	410	140	347	231	372	290	427	338	320
G. gigantea	10	4	4	32	14	5	11	7	5	38	19	6
G. margarita	1	2	1	13	3	2	4	3	2	12	7	3
Total of *Gigaspora*	11	6	5	45	17	7	15	10	7	50	26	9
S. gregaria	5	7	4	4	1	3	5	5	4	3	2	3
Total of *Scutellospora*	5	7	4	4	1	3	5	5	4	3	2	3

Table 3: Different AMF species found in the two study sites.

Family	Genus	Species
Glomeraceae	Glomus	*Glomus constrictum* Trappe *G. ambisporum* Smith &Schenck *G. manihotis* R.H. Howeler, Sieverd. & N.C. Schenck *G. caledonium* Nicolson & Gerdemann *G. geosporum* (Nicol. & Gerd.) Walker *G. intraradices* Schenck & Smith*
Acaulosporaceae	Acaulospora	*Acaulospora tuberculata* Janos & Trappe *A. rugosa* J.B. Morton *A. delicata* Walker, Pfeiffer & Bloss *A. lacunosa* Morton *A. colossica* P.A. Schultz, Bever & J.B. Morton *A. fovea*
Gigasporaceae	Gigaspora	*Gigaspora gigantean* (Nicol. & Gerd.) Gerd. & Trappe *G. margarita* Becker & Hall
	Scutellospora	*Scutellospora gregaria* (N.C. Schenck& T.H. Nicolson) C. Walker & F.E. Sanders
Total	4	15

*: AMF species found only in Cameroon.

Table 4: AMF species richness and diversity indices in *Cucurbita* sp. under fertilizer application.

Zones	Variety	Fertilizer	Diversity index					
			(S)		Shannon-Weiner(H')		Piélou (E)	
			Flowering	Fruiting	Flowering	Fruiting	Flowering	Fruiting
Benin	V1	T0	12.50±1.73a	13.25±0.50bc	1.85±0.18bc	1.64±0.26bc	0.51±0.70bc	0.44±0.07b
		T1	12.50±1.00a	13.50±1.00b	1.93±0.21bc	1.77±0.08bc	0.53±0.06ab	0.47±0.08b
		T2	14.00±0.00a	13.00±0.00bc	1.81±0.05bc	1.41±0.03c	0.47±0.01bc	0.38±0.09b
	V2	T0	13.50±1.00a	12.25±0.50c	1.54±0.07c	2.01±0.13ab	0.41±0.01c	0.55±0.03ab
		T1	14.00±0.00a	12.75±0,50bc	1.76±0.05bc	1.98±0.02ab	0.46±0.01bc	0.54±0.01ab
		T2	11.75±1.50a	12.00±0.00c	1.81±0.14bc	1.63±0.02bc	0.51±0.04bc	0.45±0.05b
	V3	T0	13.50±0.57a	13.00±0.00bc	2.11±0.23ab	2.13±0.08ab	0.56±0.06ab	0.57±0.02ab
		T1	13.00±1.15a	13.00±0.00bc	2.11±0.33ab	1.81±0.39bc	0.57±0.10ab	0.49±0.10ab
		T2	13.00±0.81a	12.25±0.957c	1.63±0.07c	1.98±0.07ab	0.44±0.02bc	0.55±0.01ab
Cameroon	V1	T0	14.00±1.41a	15.00±0.00a	2.17±0.18ab	1.82±0.39bc	0.57±0.05ab	0.46±0.09b
		T1	14.25±0.95a	15.00±0.00a	2.27±0.22ab	1.87±0.13b	0.59±0.07ab	0.47±0.03b
		T2	15.00±0.00a	14.75±0.50a	2.09±0.11b	1.53±0.04bc	0.53±0.03ab	0.39±0.01b
	V2	T0	14.75±0.50a	15.00±0.00a	1.98±0.09bc	2.14±0.19ab	0.48±0.02bc	0.55±0.04ab
		T1	15.00±0.00a	15.00±0.00a	2.06±0.01b	2.18±0.04ab	0.52±0.01b	0.55±0.01ab
		T2	13.75±0.95a	13.25±0.50bc	2.05±0.13b	1.71±0.24bc	0.54±0.02ab	0.46±0.06b
	V3	T0	15.00±0.00a	15.00±0.00a	2.45±0.20a	2.26±0.09a	0.69±0.05a	0.58±0.02a
		T1	14.25±0.95a	14.50±0.57a	2.14±0.35ab	1.90±0.45ab	0.56±0.08ab	0.49±0.11ab
		T2	13.25±0.95a	13.50±1.29b	1.85±0.14bc	2.05±0.19ab	0.48±0.02bc	0.54±0.05ab

V1 : *Cucurbita maxima* ; V2 : *Cucurbita moschata* elongated shape ; V3 : *C. moschata* egg-shaped ; T0 : control ; T1: fowl droppings ; T2 : chemical fertilizers. For each growth stage, the average with the same letter are not significantly different at $p<0.05$.

Table 5: Pearson correlation test between different parameters evaluated.

	Glomus Fl	Acaulo sporaFl	Giga sporaFl	Scutello sporaFl	Glomus Fr	Acaulo spora Fr	Giga spora Fr	Scutellospora Fr
Glomus Fl	1							
AcaulosporaFl	0.147ns	1						
GigasporaFl	0.415***	0.065 ns	1					
ScutellosporaFl	0.706***	-0.039ns	0.371**	1				
Glomus Fr	-0.021ns	0.364***	0.082ns	-0.085ns	1			
Acaulospora Fr	-0.105 ns	0.113ns	0.199ns	-0.185ns	0.496***	1		
Gigaspora Fr	-0.066ns	-0.188ns	0.256*	0.129ns	0.247*	0.328**	1	
Scutellospora Fr	0.003ns	-0.356ns	0.124ns	-0.037ns	-0.106ns	-0.006ns	0.062ns	1
H'Fl	-0.423***	0.060ns	-0.045ns	-0.304**	0.046ns	0.175ns	0.164ns	0.050ns
E Fl	-0.491***	0.021ns	-0.152ns	-0.395 ***	0.013ns	0.098ns	0.113ns	-0.001ns
H' Fr	-0.078ns	-0.196ns	0.275*	-0.029ns	-0.563***	0.203ns	0.250*	0.093ns
E Fr	-0.061ns	-0.253*	0.242*	-0.001ns	-0.618***	0.175ns	0.212ns	0.139ns
FM Fl	0.055ns	-0.077ns	0.196ns	0.118ns	0.101ns	0.172 ns	0.416***	0.011ns
FM Fr	-0.123ns	-0.068ns	0.224ns	0.039ns	0.033ns	0.162ns	0.495***	0.036ns
IM Fl	0.101ns	-0.088ns	0.316**	0.110ns	0.101ns	0.189ns	0.499***	0.037ns
IM Fr	-0.307**	0.016ns	0.146ns	-0.130 ns	0.161ns	0.237*	0.546***	-0.013ns

	H'Fl	E Fl	H' Fr	E Fr	FM Fl	FM Fr	IM Fl	IM Fr
Glomus Fl								
AcaulosporaFl								
GigasporaFl								
ScutellosporaFl								
Glomus Fr								
Acaulospora Fr								
Gigaspora Fr								
Scutellospora Fr								
H'Fl	1							
E Fl	0,965 ***	1						
H' Fr	0.151ns	0.092ns	1					
E Fr	0.022ns	-0.013ns	0.975***	1				
FM Fl	0.124 ns	0.128ns	0.263*	0.218ns	1			
FM Fr	0.287 *	0.309 **	0.305**	0.262*	0.801***	1		
IM Fl	-0.078ns	-0.093ns	0.291*	0.270*	0.776 ***	0.558***	1	
IM Fr	0.233 *	0.256*	0.231*	0.192ns	0.676 ***	0.879***	0.527***	1

Fl : Flowering, Fr : fruiting, H' : Shannon-Weiner diversity index, E: Pielou equitability index, IM : Intensity of mycorrhization, FM : Frequency of mycorhization, * : significant difference, ** : highly significant difference, *** : very high significant difference, ns : non-significant.

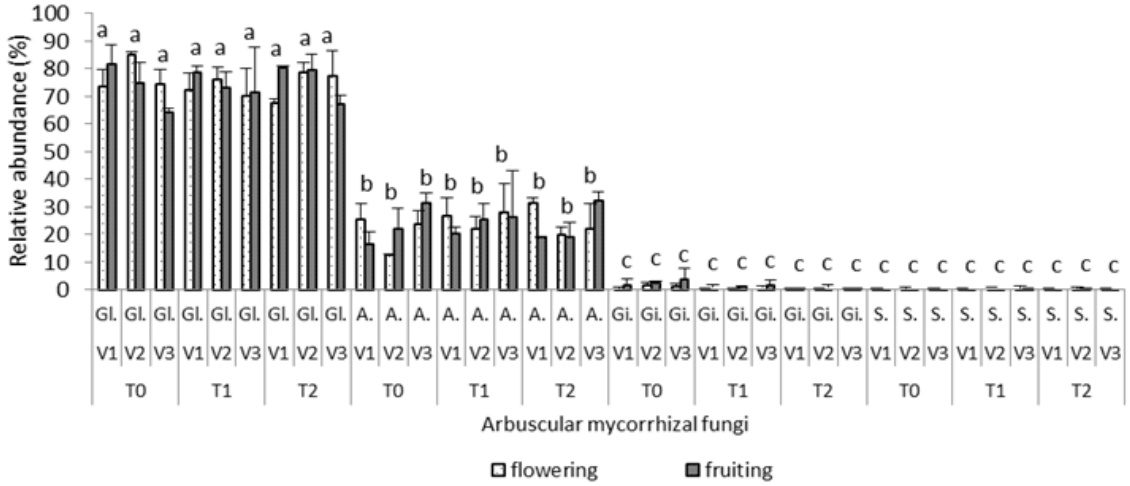

Fig. 6: AMF species relative abundance from Cucurbitaspp. under fertilizer application in Benin.Gl: Glomus; A: Acaulospora; Gi: Gigaspora; S: Scutellospora. V1 : Cucurbita maxima ; V2 : Cucurbita moschata elongated shape ; V3 : C. moschata egg-shaped ; T0 : control ; T1 : fowl droppings ; T2 : chemical fertilizers. For each growth stage, the means with the same letter are not significantly different at $p<0.05$.

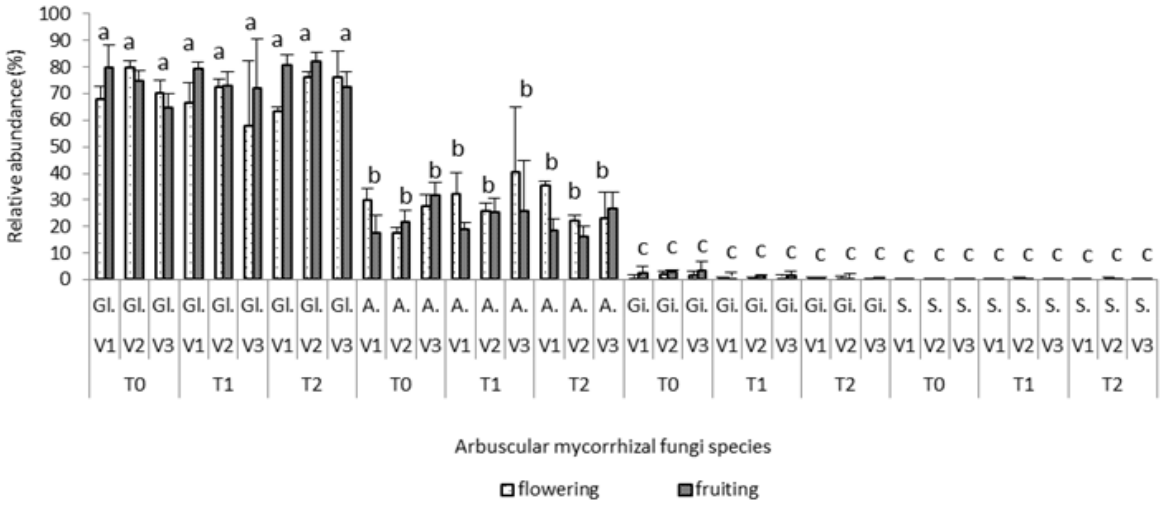

Fig. 7: AMF species relative abundance from *Cucurbita* spp under fertilizer application in Cameroon.Gl: *Glomus*; A: *Acaulospora*; Gi: *Gigaspora*; S: *Scutellospora*. V1 : *Cucurbita maxima* ; V2 : *Cucurbita moschata* elongated shape ; V3 : *C. moschata* egg-shaped ; T0 : control ; T1 : fowl droppings ; T2 : chemical fertilizers. For each growth stage, the means with the same letter are not significantly different at $p<0.05$.

3.5. Specific richness and diversity indices

The specific richness, Shannon-Weiner (H') diversity index and Pielou (E) equitability index are higher in Cameroon than in Benin (Table 4). AMF are less diversified in Benin both at flowering and fruiting. Chemical fertilizer reduces the diversity of AMF.

In Cameroon at flowering, Shannon-Weiner index of variety 3 is significantly ($p<0.05$) low for T2 (1.85±0.14bc) compared to T0 (2.45±0.20a) and T1 (2.14±0.35ab). Shannon-Weiner diversity index and Pielou equitability index are strongly correlated ($p<0.001$) both at flowering and fruiting (Table 5). At flowering, the number of spores of the genera *Glomus* ($p< 0.001$) and *Scutellospora* ($p<0.05$) are correlated to these two indices. The number of spore of the genera *Glomus* ($p<0.001$) and *Gigaspora* ($p<0.05$) are correlated to these indices at fruiting (Table 5).

3.6. Relative Abundance of AMF

The spores of *Glomus* (57.97-85.65 %) and *Acaulospora* (12.68-40.42 %) are more abundant than those of *Gigaspora* (0.24-3.93 %) and *Scutellospora* (0.07-0.82 %) in the two study sites. In fact, spores of *Glomus constrictum*, *G. geosporum* and *Acaulospora lacunosa* are more abundant in the two sites (Figure 6 and 7, Table 5). Hierarchical ascending classification (CAH) regroups the types of fertilizer in statistically homogenous classes on the basis of the number of AMF spores isolated from the rhizosphere of pumpkins (Figure 8). The hypothesis tested through this analysis is that the specific composition of endomycorrhizae in the sites helped to discriminate the fertilizers. Analysis of the dendrogramme shows two classes at a distance of $R^2 = 0.63$ (Figure 8). The first class is made of two fertilizers, the non-fertilized control (T0) and fowl droppings (T1). The chemical fertilizer (T2) constitutes the second class.

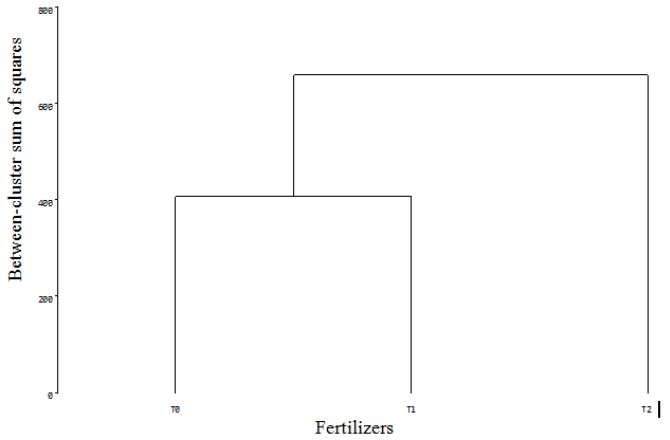

Fig. 8: Dendrogram showing the clustering of fertilizers based on presence of AMF morphotypes associated with rhizosphere soil of *Cucurbita* spp. in Benin and Cameroon. T0 : control ; T1 : fowl droppings ; T2 : chemical fertilizers.

4. DISCUSSION

The soil from Benin experimental site is slightly acidic (pH 6.06) while that in Cameroon is acidic (pH 4.90) in reference to the Canadian information system on soils 24. Interpretation of the effect of pH on the population of AMF is very difficult, since a majority of chemical properties of the soil change with variations of soil pH 25. The soil of Abomey-Calavi (Benin) is poor in nitrogen contrary to the soil in Mbalgong (Cameroon). According to 26, a soil is poor in nitrogen if the quantity of total N is less than 0.75 g/kg. A ratio of C/N greater than 25 can block any mineralization process without external input of nitrogen 27. Low C/N ratios in the two study sites indicate a rapid decomposition of organic matter. It leads to a poor functioning of the clay-humic complex. Chemical analysis also shows that these soils are deficient in organic matter.

Chemical fertilizer significantly reduces the frequency and intensity of mycorrhization. Its application certainly increased soil acidity. Extreme soil acidity could be the cause of low proportions of spores observed in certain AMF species 28. Studies by 29 have shown that in the long run, chemical fertilizer application leads to reduction of the total number of AMF spores. They observed that only *Acaulospora* sp. of the nine species sampled on maize maintained its spore number with fertilizer application. This species has been classified as being insensitive to chemical fertilizer application. On the contrary, these workers noticed that the absolute number of *Glomus* sp. and *G. geosporum* diminished in response to fertilizer application; but not their relative abundance. *Glomus* sp. and *G. geosporum* were classified as being slightly sensitive to fertilizer application 29. Results obtained in this study show that *Glomus* is insensitive, *Acaulospora* slightly sensitive, *Gigaspora* and *Scutellospora* very sensitive to chemical fertilizer application. Contrary to chemical fertilizer, fowl droppings (organic fertilizer) did not reduce the frequency of mycorrhization by AMF in soil under pumpkin culture.

Fowl droppings had a mycorrhization frequency similar to that of the control without fertilizer. The hierarchical classification diagram shows two classes of fertilizer; one constituted of the control and fowl droppings and the other constituted of chemical fertilizer. These results corroborate with those of 30 who found that fungal communities of the soil are less influenced by application of compost from domestic waste. The results are equally similar to those of 31. These workers found that the use of natural phosphate (rock phosphate) is more efficient in mycorrhized jujubes (*Ziziphus jujube*) compared to the control. In fact, organic fertilizers positively influence physical properties of the soil as compared to chemical fertilizers 32. Soil amendment with organic fertilizers have shown increase of the activity 33, diversity 34 and soil microbial biomass 35. Some workers 36 have also observed that cotton, maize and soya bean farms fertilized with organic matter present higher specific richness of AMF.

The present study has put into evidence the fact that soils on which pumpkin is cultivated both in Benin and Cameroon show a high diversity of AMF (15 species). This result is similar to those obtained by 37 working on 15 species in the north of Tibet. These 15 species identified under pumpkin culture were grouped into four genera (*Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*). However, the culture conditions for pumpkin certainly favour the genus *Glomus* and *Accaulospora as well as* adaptation of species such as *Glomus constrictum* and *Acaulospora lacunosa*. The latter seem to be more competitive than the other species vis-à-vis pumpkins. In fact, the genus *Glomus* is considered as the most abundant of all the AMF in the tropical arid zone 38. The *Acaulospora* species are usually associated with acid soils 39. They are adapted to a wide range of soils at different levels of nutrient availability 40. This could justify their abundance in the two study sites. The predominance
of the *Glomus* and *Acaulospora* genera in the tropical zone seem to be due to their high competitiveness and to adaptation which permits them to be better established than the other genera of AMF in tropical conditions 41. This could equally be due to the fact that their developmental cycle is not affected by repetition of cultures on the soils as compared to the minority genera such as *Gigaspora* and *Scutellospora* 17. The genera *Glomus* and *Acaulospora* are propagated more by spores which constitute a form of resistance of AMF in difficult conditions while the genera *Gigaspora* and *Scutellospora* are propagated more by the other types of propagules such as hyphae, and mycelial fragments on root surfaces 18.

The production of AMF spores vary significantly with different ecosystems. It can be influenced by various factors such as environment, host species, the fungus and the density of spores which tends to diminish during root growth but increases during inactivity or senescence of roots 42. A low difference in specific richness was observed in the two study sites. *Glomus intraradices* was absent in Benin and present in Cameroon. The difference in physicochemical properties in the two soils could justify this result. In fact, the irregular spatial distribution of AMF spores and the complex structure of the rhizospheric constituents affect density of spores 43. Both at flowering and fruiting, the values of Shannon-Wiener index are lowest in the plots treated with chemical fertilizer. In the two sites, average values of Shannon-Wiener index vary from 1.41 to 2.47. This result is similar to those of 44 who observed an index in the order of 2.3 in the tropical forest. *Cucurbita moschata* ovoid form (2.04) and *C. moschata* elongated form (1.90) had Shannon-Weiner index values higher than that of *C. maxima* (1.85). The diversity of AMF under pumpkin culture seems to be influenced by the plant's genotype.
The study highlighted the predominance of *Glomus* and *Acaulospora* genera in pumpkin rhizosphere. The genera *Glomus* and *Acaulospora* are more appropriate for the preparation of inocula for pumpkin cultivation in the study sites (Benin and Cameroon). For sustainable production of pumpkins, these fungi can be isolated, purified, multiplied and used as organic fertilizer without necessarily being associated with the chemical fertilizers.

5. CONCLUSION

The objective of this study was to assess the biodiversity of arbuscular mycorrhiza fungi (AMF) associated with pumpkin culture under the effect of fertilizers in Cameroon and Benin. Chemical fertilizer reduced the frequency and intensity of mycorrhization but not the specific diversity. On the basis of number of spores observed, the hierarchical classification diagram shows two classes of fertilizer. The control and fowl droppings constitute the first class while chemical fertilizer constitutes the second class. In these soils, four genera of AMF (*Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*) constituting 15 species were recorded. The genera *Glomus* and *Acaulospora* and particularly *Glomus constrictum*, *G. geosporum* and *Acaulospora lacunosa* are specific to pumpkins in the two sites.

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