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Morphological spore's diversity and agronomic potential of indigenous arbuscular mycorrhizal fungi associated to cowpea (*Vigna unguiculata* L.) in the Adamawa region of Cameroon

Tobolbaï Richard[1*], Ngakou Albert[2], Toukam Takoukam Steve[2], Adamou Souleymanou[3]

[1] Department of Microbiology, Laboratory of Microbiology, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon

[2] Department of Biological Sciences, Laboratory of biodiversity and Sustainable Development. Biofertilizer and Bioinsecticide Unit, Faculty of Science, University of Ngaoundere, Cameroon

[3] Department of Agriculture, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon

Abstract

The present study determines the morphological spore's diversity and the agronomic potential of indigenous arbuscular mycorrhizal fungi from cowpea rhizosphere in the Adamawa region of Cameroon. There for, soils samples were collected in nine sites, distributed in three Divisions. Then, soils from each Division were mixed for a single composite soil sample. Arbuscular mycorrhizal fungus spores were trapped in pots on these soils using three host plants, cowpea, soybean and maize. Maize and soybean rhizosphere spores were characterized by Tobolbaï *et al*. (2018) and Richard *et al*. (2021) respectively. At plants maturity, after the evaluation of mycorrhization, the spores of cowpea rhizosphere were isolated and characterized. Four different treatments were formulated from the spores collection obtained from each host plant rhizosphere: T1 for spores from soybean, T2 for spores from cowpea, T3 for spores from maize, T4 for the mixture of the three treatments and one negative control T0. The agronomic performances of these treatments were tested in field conditions on cowpea plants in Dang locality. Results analysis showed that the rate and degree of mycorrhization varied between 11.66-53% and 27.77-48.66%, respectively; soils spore charge and diversity fluctuate between 145-285% and 3-5%, respectively. Improvements in growth and yield parameters are better with T2 and T3 treatments and weaker with T1 and T4. The data obtained vary from 50.55-63 cm for the size, 36.57-52.37g for the dry biomass, 17-30 for the number of pods, 165-205 for the number of seeds, 30.88-35.22g for the weight of seeds per plant and 165-204 kg for the theoretical yield per hectare. T2 and T3 treatments are therefore recommended for sustainable cowpea production in this part of Cameroon.

Keywords: mycorrhizae, indigenous, diversity, yield, cowpea, adamawa

Introduction

In poor soils, mycorrhizal fungi have the potential to increase plant production. The exploration of a larger soil volume and the possibility of primary minerals solubilization by mycorrhizae allow better plants phosphate nutrition (Landeweert R, *et al*, 2001) [1]. This improvement in inorganic elements acquisition also concerns N, K, Mg, Na, S, B, Br, Cl, Cu, Cr, Cs, Co, Fe, Mo, Mn, Ni, Si, Zn (Caris C, *et al*, 1998) [2]. Likewise, it has been shown that mycorrhizal associations could play a significant role in organic matter decomposition and the mineralization of plant debris (Lambers H, *et al*, 2008) [3]. Many other studies have demonstrated a bio-protective role of mycorrhizae, a reduction or even an inhibition of the negative effect of some phytoparasitic agents (Duponnois R, *et al*, 1994) [4]. In addition, a significant improvement in soil structure has been often noted in the presence of mycorrhizae (Lovelock CE, *et al*, 2004; Wright S, *et al*, 1998) [5-6]. On the other hand, cowpea is one of the oldest plants cultivated by man, and is called the "meat of the poor" in areas of Africa where its cultivation is practiced in association with millet and sorghum (Alzouma, 1995) [7]. The nutritional value of this plant lies in its richness in vegetable protein: 20 to 25% of its dry weight. A food composed of 25% cowpea and 75% cereals (millet, rice or maize) meets human daily needs. In Cameroon and in the Adamawa region in particular, most of the work on cowpea symbiotic microorganisms is essentially based on Rhizobia or on commercial mycorrhizae. The objective of this work is to investigate on the morphological spore's diversity and the agronomic potential of mycorrhizal fungi on cowpea production in this part of Cameroon.

Material and Methods

1. Climate and relief of the study area

The work was carried out in the Adamawa region of Cameroon, in the period from June 2016 to October 2018. This region is located in the ecological zone 2, known as high Guinean savannah (Djoufack-Manetsa V, 2011) [8]. The climate is Sudano-Guinean, characterized by two seasons: a rainy season from April to October and a dry season from November to March. The soils are ferralitic, and red in color.

2. Soil collection and spores isolation

Soil samples were taken in nine localities distributed in three Divisions of the region. Soils from different localities were mixed per Division to obtain a single composite samples. Soils from these localities were also used by (Tobolbaï R, *et*

al, 2018) [9] and (Tobolbaï R, 2018) [10] to respectively assess the morphological diversity of spores of arbuscular mycorrhizal fungi associated with corn and soybeans in this region.

3. Soils samples physico-chemical properties
The Soil samples properties have been evaluated using Palintest 5000 Photometer Kit. The evaluated characteristics included sand content, silt, clay, pH, conductivity, organic carbon (CO), organic matter (OM), phosphorus (P), Magnesium (Mg2 +) and the Calcium (Ca +). These analyzes have been realized at Soil-Water-Plant Analysis Laboratory of the Chadian Institute of Agronomic Research for Development.

4. Spores trapping
The spore trapping was carried out according to the method described by (Brundrette *et al*, 1996) [11]. For this aim, cowpeas, soybeans and maize were cultivated on soil samples to increase spore population for a successful isolation exercise. Each pot had a capacity of 2 liters and five were used per composite soil sample type. The plants in culture were placed on a support and protected from the wind. Watering was achieved by exposure of the test to rainwater. At maturity, the roots and the soil substrate were sent to the Laboratory for analyzes. The growing medium (soil) was used for spore's isolation that has been carried out according to the method of (Gerdemann and Nicolson, 1963) [12].

5. Evaluation of cowpea plants mycorrhization
Mycorrhization rate
The rate of roots mycorrhization was determined according to the following formula:

T (%): $\frac{(N-N0)}{N}$ x100 with N number of fragments observed and N0 number of non-mycrhized fragments, (Arias RM, *et al*, 2012) [13].

Degree of mycorrhization
The degree of mycorrhization was evaluated by assigning each observed root fragment a class score between 0 and 5 according to the colonization estimation of the root cortex by arbuscular fungi: 0 = No infection, 1 = Trace of infection, 2 = less than 10%, 3 = 10 to 50%, 4 = 51 to 90%, 5 = More than 90%.

Deg (%): $\frac{(95n5+70n4+30n3+5n2)}{N}$ where n5, n4, n3, n2 and n1 are the numbers of roots noted from 1 to 5, (Sghir F, *et al*, 2013) [14].

6. Arbuscular mycorrhizal fungi spore's estimation
Mycorrhizal spore charge in soils
The soils mycorrhizal spore charge was evaluated according to the following formula:

C (%): $\frac{N}{100}$ where N is the number of spores counted and 100, the amount of soil used for their isolation (Sghir F, *et al*, 2013) [14].

Diversity of mycorrhizal fungi pores in soils
Soils mycorrhizal spore diversity was estimated according to the following formula:

Div:
$$\frac{100\,(Number\ of\ different\ arbuscular\ fungus\ genus)}{Total\ number\ of\ spore\ counted}$$
(Arias RM, *et al*, 2012) [13].

7. Phenotypic and structural characterization of spores
The spore's shapes were determined according to the observed phenotypes; the colors were determined according to the standard INVAM color chart, while the size was determined according to the method of (Walker, 2008) [15]. The spores were then mounted between slide and coverslip in the compound Polyvinyl alcohol-Lactic Acid-Glycerol (PVGL) and the Melzer's reagent (V: V / 1: 1) to reveal the structure of the different walls of the spores (Koske RE, Tessier B, 1983) [16]. The genera were determined according to the method of Morton and Benny, (Morton B, Benny GL, 1990) [17]. The initial descriptions of the species and the data provided by (INVAM, 2017) [18] were used to determine the species.

8. Production of mycorrhizal fungi inoculum
The microbial Biofertilizer (inoculum) production consisted of a massive multiplication of the spores by cultivating them under cowpeas, soybeans and corn in 2-liter pots. The growing medium is a mixture of sand and soil (v: v / 2: 1) and the mixture has been sterilized. The pots were protected from potential contamination from the ground and wind, and were sprayed directly with rainwater. At maturity, the cultivation soil with the roots were used to formulate crude biofertilizer composed of arbuscular mycorrhizal fungi spores and mycorrhized roots.

9. Treatments formulation
Four different treatments were constituted. The arbuscular fungi species composition of the different formulations was maintained as encountered under natural conditions. The various formulated inoculum were thus as follows: T0: negative control (no treatment applied); T1: Set of spores trapped with soybean, T2: Set of spores trapped with cowpea, T3: Set of spores trapped with corn, T4: Mixture of spores resulting from traps with the three plants.

10. Experimental set-up for field experiments
To assess the impact of the formed biofertilizers on cowpeas plants productivity, an experimental field test was carried out in the locality of DANG. The experimental set-up is a completely randomized block, with 4 treatments and a negative control, each being repeated 3 times. An experimental plot covers an area of 45 m^2 on which there are 1200 cowpea plants separated from each other 50 cm on the line and 70 cm among the lines (density = 37 plants / m2). Figure 1 illustrates the experimental device used.

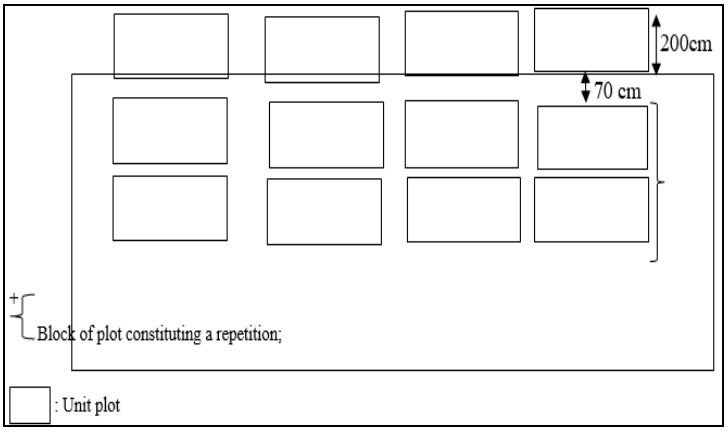


Fig 1: Experimental setup in the field

11. Field application of endomycorrhizal inoculum

The application of the endomycorrhizal inoculum to the cultures was carried out twice. Firstly, the seeds were directly coated with mycorrhizal biofertilizer during the sowing period, and secondly, the plants roots were infested with mycorrhizal biofertilizer after 4 weeks of development. Normally, applying the biofertilizer twice is not necessary; but may be beneficial in the way of a higher chance of successful inoculation.

12. Evaluation of growth and yield parameters of cowpea plants

Growth parameters were taken after three months of development. The plants were harvested and dried in shade conditions for 90 days, at the Agronomic Research Institute for Development, Wakwa Ngaoundéré Regional Centre. A total of twenty plants were used to evaluate each parameter: Size, total biomass, number of pods, weight of pods, number of seeds, weight of seed and seed yield per hectare. The weights were measured using a precision electronic scale. The formula used by (Tobolbaï R, 2018) [10] was used to determine the yield per hectare:

YSH = (WANS/1000) × 90000 Where 90 is the theoretical number of plants per hectare, WNS is the weight of the average number of seeds per plant in grams.

13. Statistical analyses

The data were statistically analysed using the "Statgraphic.5.0" program which performs analysis of variance (ANOVA). The results average from different localities were separated using the least significant difference (LSD) at the threshold of the indicated probabilities.

Results

1. Soils sample physicochemical properties

Table 1 indicates that the soils of Faro et Deo (pH = 4.32) are more acidic while those of Vina are less acidic (pH = 5). The soils of Vina Division are more clayey (56.19%) compared to those of the other two Divisions, Mbéré (40.44%), Faro et Déo (42.47). Regarding organic matter, the highest value is one of Faro et Déo (0.112%), followed by that of Mbéré (0.105%) and it is in Vina that the lowest value is recorded (0.095%). Regarding fertilizer parameters, the phosphorus content is higher in Vina Division (94 ppm), lower in Faro et Déo (17 ppm) and intermediate in Mbéré (26 ppm). Conversely, it is the Faro et Deo samples (560 ppm) which are richer in potassium, while those of the Vina are poorer (330 ppm); the value recorded in the Mbéré (450 ppm) is intermediate.

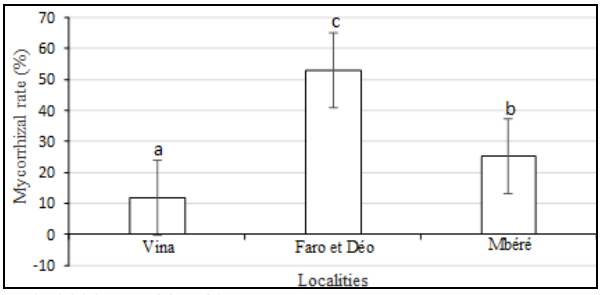
Table 1: Physico-chemical characteristics of soil samples

	pH	Sand	Silt	Clay	Cond	O.C	O.M	P (pmm)	K (ppm)	Mg2+
Vina	5	20,28	23,52	56,19	212	0,055	0,095	94	330	185
Mbéré	4,92	35,89	23,66	40,44	271	0,061	0,105	26	450	95
Faro et Déo	4,32	48,74	10,78	42,47	152,1	0,065	0,112	17	560	125

Cond: conductivity, O.C: Organic Carbone; O.M: Organic Maters, P (ppm): available phosphorus, K (ppm): Available potassium, Mg2+: Magnesium.

2. Mycorrhization rate

The rate of mycorrhization varies between the three Divisions (Figure 2) (P = 0.0000). The highest rate was observed in Faro et Déo (53%), unlike that recorded in Vina (11.66%) which is lower. The value recorded in the Mbéré is intermediate (23.55%).

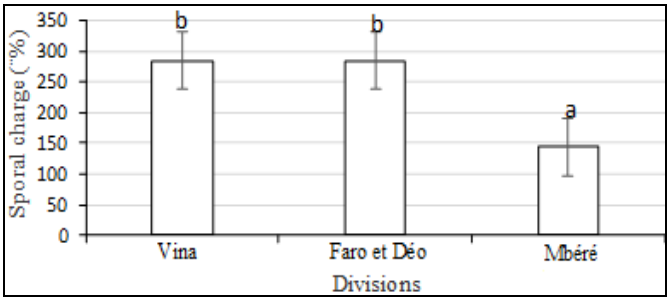


P=0, 0000, F=2394, 60

Fig 2: Mycorrhization rate

3. Degree of mycorrhization

The degree of mycorrhization is variable among the Divisions, (P = 0.0000). The highest value was obtained in Faro and Déo (48.68%), and the lowest in Mbéré (27.77%). The value of the Vina Division is intermediate (33.5%).

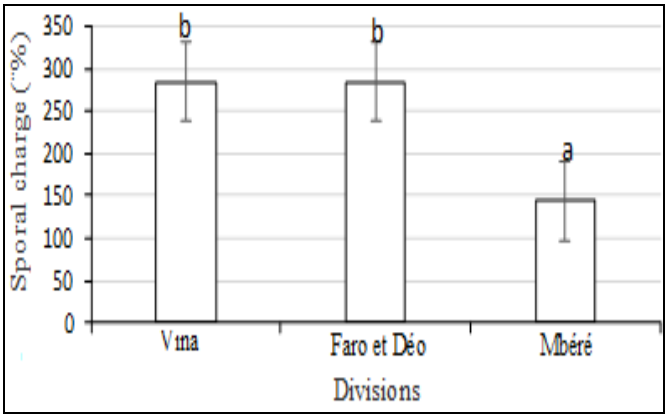


P = 0.0000, F = 137.66

Fig 3: Degree of mycorrhization in the different division

4. Soils spore's charge

Figure 3 shows the variation of spore charge between the différents Division (P = 0.0000). It emerges that the soils of Vina (285%) and those of Faro et Déo (285%) have similar and significantly higher sporal charge than that of Mbéré (145%).

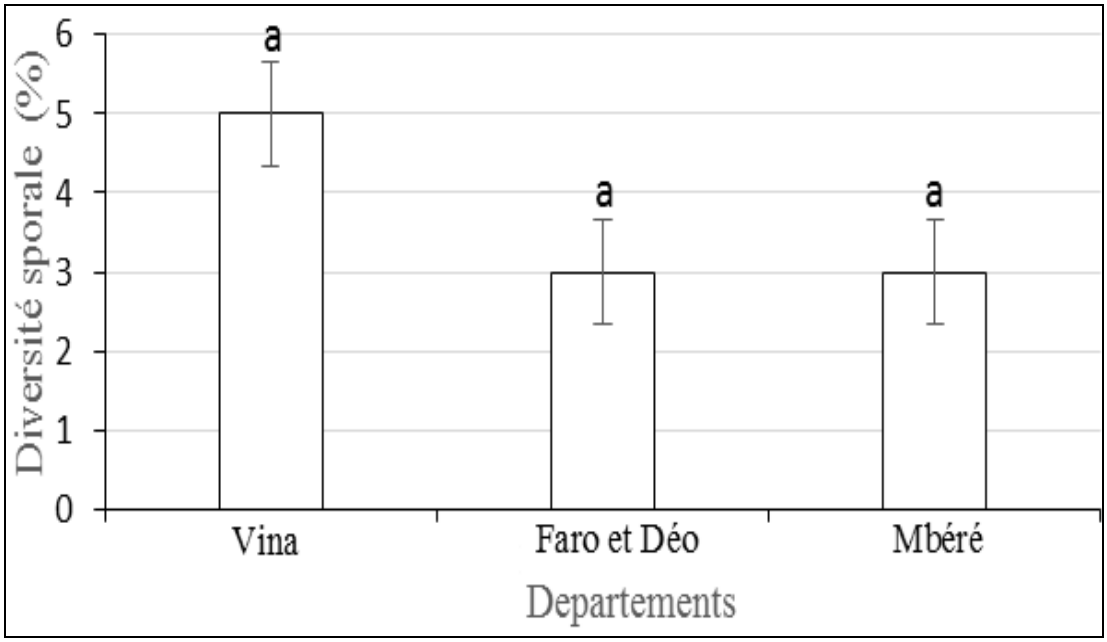


P=0,0000, F=159,88

Fig 4: Spore load of soil samples from different Departments

5. Arbuscular mycorrhizal fungi spore diversity

The analysis of Figure 5 reveals that there is no significant difference (P = 0.1250) between the spore diversity of the soil samples from the different Departments, Vina (5%), Faro and Déo (3%) and Mbéré (3%).



P=0,1250, F=3

Fig 5: Spore diversity of arbuscular mycorrhizal fungi in the three division

6. Characterized spores

The morpho-anatomical characterization of the spores revealed the presence of 5 different specimens divided into 3 genera: *Diversispora epigae*, *Glomus* (*G. constrictum*, *G. maculosum and G. manihotis*), and *Rhizophagus intraradices.*

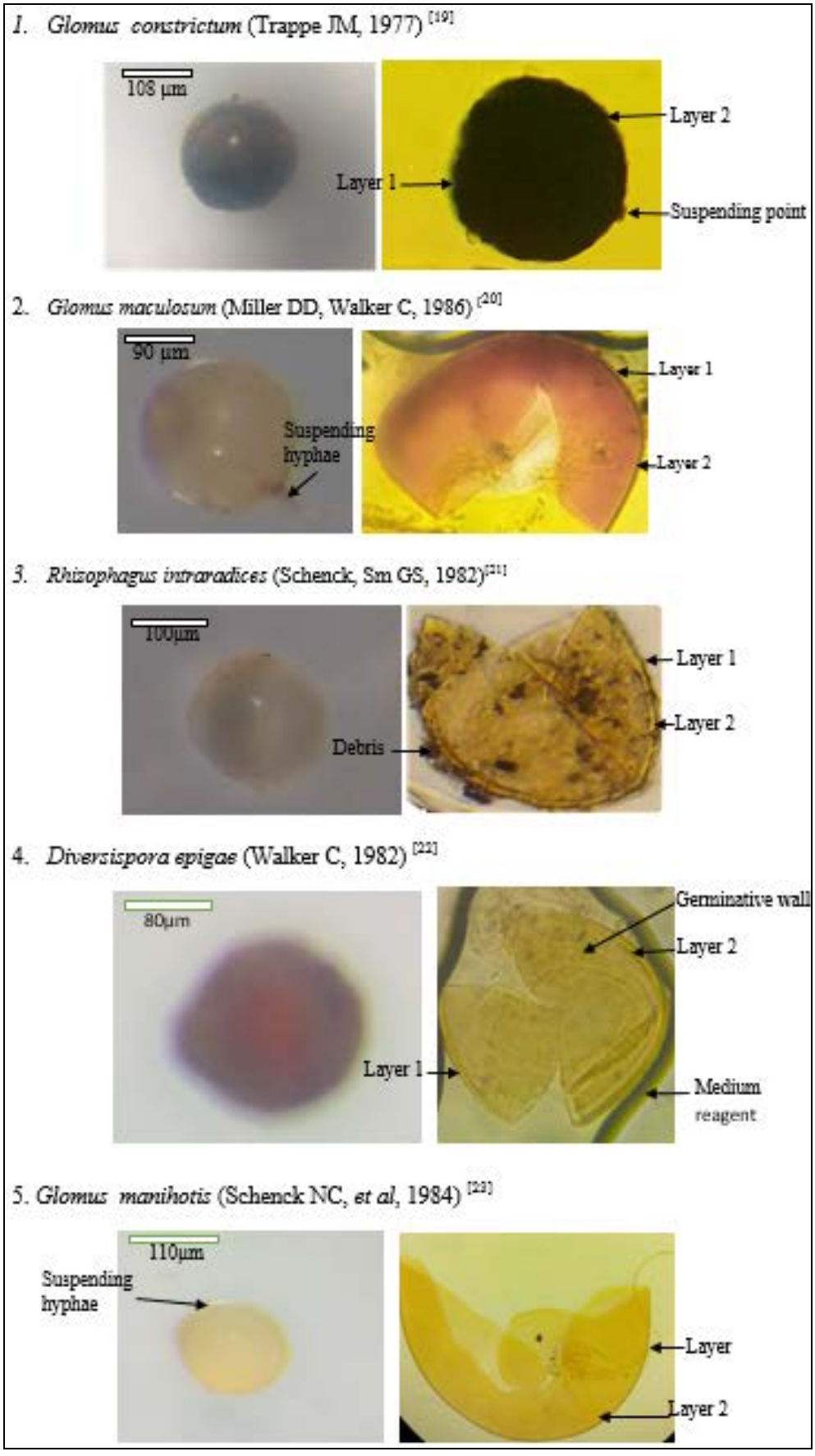

1. Glomus constrictum (Trappe JM, 1977) [19]
108 µm
Layer 2
Layer 1
Suspending point
2. Glomus maculosum (Miller DD, Walker C, 1986) [20]
90 µm
Suspending hyphae
Layer 1
Layer 2
3. Rhizophagus intraradices (Schenck, Sm GS, 1982)[21]
100µm
Debris
Layer 1
Layer 2
4. Diversispora epigae (Walker C, 1982) [22]
80µm
Layer 1
Germinative wall
Layer 2
Medium reagent
5. Glomus manihotis (Schenck NC, et al, 1984) [23]
110µm
Suspending hyphae
Layer
Layer 2

Fig 6

7. Distribution of mycorrhizal fungi specimens in the study area

Table 2 shows the distribution of arbuscular mycorrhizal fungal spores in the study area and indicates that the spores were not uniformly distributed in the three Division. *Glomus constrictum* is the most abundant specimen, Vina (986), Faro et Déo (611) and Mbéré (760), followed by *Rhizophagus intraradices* (Vina: 3; Faro et Déo: 6; Mbéré: 11). *Glomus maculosum* is the least representative (Vina: 1; Mbéré: 2).

Table 2: Distribution of arbuscular fungal spores in the three Departments

	Vina	Faro et Déo	Mbéré
G. constrictum	986	611	760
G. maculosum	11	0	2
G. manihostis	1	5	2
R. intraradices	3	6	11
D. epigae	46	2	0
H’	0,99	0,095	0,64

8. Effects of treatments on cowpea plants growth

Table 3 shows that all the treatments have significantly (P = 0.0001) improved the size of cowpea plants in the field. The best effects were recorded with the T2 (63 ± 1.3) and T3 (62.1 ± 3.82) treatments, while the T1 treatment shows a lower performance. Regarding the biomass, the best increase was obtained with the T2 treatment (52.37 ± 3.96), while the effects of T4 (36.57 ± 2.93) are significantly (P = 0.0006) lower. Data obtained with T1 (40.18 ± 3.10) and T3 (41.32 ± 4.28) are similar and intermediate to those of T2 and T4.

Table 3: Effects of treatments on cowpea plants growth

Localities	Sizes	Biomass
T0	46,47±2,45a	34,11±2,35a
T1	55,75±3,05c	40,18±3,10c
T2	63±1,3d	52,37±3,96d
T3	62,1±3,82d	41,32±4,28c
T4	50,55±2,9b	36,57±2,93b
P-value	0,0001	0,0006
F-value	21,38	12,78

9. Effects of treatments on pods and seeds yield

Table 4 shows that all the four treatments have significantly (0.0001) induced an increase in cowpea plants pods number in field. Plots that received the T2 (30 ± 2) and T3 (27 ± 2) treatments showed a significantly higher number of pods compared to plots that received the others treatments. The numbers of pods obtained with T1 (17 ± 4) and T4 (17 ± 3) treatments are similar and lower. Regarding the number of seeds, it is always the T2 (205 ± 6) and T3 (204 ± 5) treatments which show a better performances, (P = 0.0000); the smallest improvement is the one noted with T1 treatment (165 ± 4).

Table 4: Effects of treatments on pods and seeds yield

Localities	Pods number	Seeds number
T0	9±2a	100±5a
T1	17±4b	165±4b
T2	30±4c	205±6d
T3	27±2c	204±5d
T4	17±3b	185±5c
P-value	0,0001	0,0000
T-value	22,04	221,89

10. Effects of treatments on seeds weight and yield per hectare

The weight of the seeds varied significantly (0.0014) with the treatments (Table 5). It is the T2 treatment (35.22 ± 1.38) which is more efficient in improving the weight of the seeds compared to the other treatments while T1 (31.71 ± 1.31) is the least efficient. The same trend is observed with the yield per hectare where T2 (223.2 ± 2.8) and T3 (222.4 ± 4.9) give better results compared to the other treatments, (P = 0.0000). The T4 treatment (207.8 ± 4.4) shows the smallest improvement.

Table 5: Effects of treatments on seed weight and yield per hectare

Localities	Seeds weight	Yield per hectar
T0	24,55±1,31a	146,5±3,1a
T1	31,71±1,31b	214,1±2,1c
T2	35,22±1,38d	223,2±2,8d
T3	34,14±2,79c	222,4±4,9d
T4	30,88±2,34b	207,8±4,4b
P-value	0,0014	0,0000
T-value	10,42	241,64

Discussions

Our results establish that the rate of mycorrhization varies between 11.66-53%, and the degree of root colonization, 27.77-48.68%. These data are lower than those of (Ogou A, *et al*, 2018) [24] who reported mycorrhization rates between 41-65.67% under soybeans in Togo; at the contrary, these results are close to those of (Tobolbaï R, *et al*, 2018) [9] who obtained mycorrhization rates of 20.5% and a mycorrhization degree of 15.38% in maize rhizosphere in Northern Cameroon. Our values are superior to those of (Nadjilom Y, *et al*, 2019) [25] who recorded a mycorrhization rate between 4.33-7.33%, a mycorrhization degree between 0.8-2.9 percent under rice in Chad, as well as those of (Richard T, *el al*, 2021) [26] who reported a mycorrhization rate between 1.33-4.6%, a mycorrhization degree of 1.22-3.95% with soybean in Cameroon.

The soil samples spore charge fluctuates between 145-285%. These values greatly exceed those of (Ouallal I, *et al*, 2018) [27] who obtained a spore number less than 100 in the rhizosphere of argan tree in south-western Morocco; in contrast, these data are similar to those of (Gnamkoulamba A, *et al*, 2018) [28] who recorded spore numbers varying between 100 and 400 under rice in Togo. The species diversity of

arbuscular mycorrhizal fungi is between 3-5%. These values are very low compared to those of (Yuriko P, *et al*, 2018) [29] who reported a diversity of 21 species of arbuscular mycorrhizal fungi in the tomato rhizosphere in Calakmul, Mexico.

The prevalence of *Glomus* in our study site is similar to the data reported by Kariman (KH, *et al*, 2005) [30] who showed that the genus *Glomus* was the most representative in the rhizosphere of sugar cane in Iran. The predominance of this genus in cultivated land may be justified by its ability to establish a hypha network more quickly or to sporulate rapidly. (Voetes L, *et al*, 2006) [31] explains that Glomerales rapidly form anastomoses between different mycelial branches of the same genotype or of a similar genotype. This gives these fungi the ability to establish an interconnected network after mechanical disturbance such as ploughing.

The effects of the treatments on the growth and yield of cowpeas indicate that the size fluctuates between 50.55-63 cm, the biomass varies from 36.57-52.37 cm, the number of pods, from 17-30, the number seed from 165-205, seed weight of 30.88-35.22, yield per hectare of 165-204 kg/h. The ability of arbuscular mycorrhizal fungi to induce increased growth and yield parameters in cowpea plants may be justified by the fact that they establish mutually beneficial interactions with the roots of most terrestrial plants (Strullu DG, 1991; Van DHMGA, *et al*, 1998a) [32-33]. In return for the carbohydrates obtained from the host plant, arbuscular mycorrhizae boost the hydro-mineral nutrition of plants and particularly in phosphorus (Bolan NS, 1991) [34], improves resistance to drought (Hardie K, Leyton L, 1981) [35] and minimize the negative impacts caused by pathogens (Duponnois R, *et al*, 1993; Duponnois R, Cadet P, 1994; Abdalla ME, Abdel-Fattah GM, 2000) [36-4 and 37]. Mycorrhization also increases the adaptation of plants in an environment polluted by heavy metals (Leyval C, Joner EJ, 2001) [38] and pollution of organic matter (Joner EJ, Leyval C, 2003) [39]. (Duponnois R, Garbaye J, 1990) [40], have demonstrated that there are positive interactions between mycorrhizal fungi and soil bacterial flora. In addition, mycorrhizal fungi develop a network of mycelial hyphae, which expands the volume of soil explored by the host plant, thus allowing it to improve its hydro-mineral nutrition (Rhodes LH, Gerdemann JW, 1975) [41].

Conclusion

This work determines the morphological spore's diversity and agronomic potential of indigenous arbuscular mycorrhizal fungi from cowpea rhizosphere in the Adamawa region of Cameroon. The results analyses reveal the presence of five different arbuscular mycorrhizal fungi specimens grouped into three genera: *Diversispora epigae*, *Glomus* (*G. constrictum*, *G. maculosum*, *and G. manihotis*) and *Rhizophagus intraradices*. The genus *Glomus* is the most representative and *Glomus constrictum* is the most representative while *G. maculosum* is the rarest. Field testing of treatments performances, formulated from these spores showed positive effects with all the treatments; T2 and T3 treatments are more efficient than T1 and T4 treatments. Therefore, T2 and T3 treatments can be recommended for an ecological cowpea production in the Adamawa region of Cameroon.

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References

1. Landeweert R, Hoffland E, Finlay RD, Kuyper TW, Van BN. Linking plant to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution*,2001:16:248-255.
2. Caris C, H¨ordt W, Hawkins HJ, R¨omheld V, George E. Studies of iron transport by arbuscular mycorrhizal hyphae to peanut and sorghum plants. *Mycorrhiza*,1998:8:35-39.
3. Lambers H, Raven JA, Shaver GR, Smith SE. Plants nutrient-acquisition strategies change with soil age. *Trends in ecologies and Evolution*,2008:23:95-103.
4. Duponnois R, Cadet P. Interactions of *Meloidogyne javanica* and *Glomus* sp. on growth and N2 fixation of *Acacia seyal*. Afro Asian Journal of Nematology,1994:4:228-233.
5. Lovelock CE, Wright SF, Clark DA, Ruess RW. Stocks of glomalin produced by arbuscular mycorrhizal fungi in soil across a tropical rain forest landscape. J. Ecol,2004:92:278-287.
6. Wright S, Upadhyaya F. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*,1998:198:97-107.
7. Alzouma. Connaissance et contrôle des coleoptères Bruchidae ravageurs de legumineuses alimentaires au sahel,1995:1(4):10-11.
8. Djoufack-Manetsa V. Étude multi-échelle des précipitations et du couvert végétal au Cameroun : Analyses spaciales, tendances temporelles, facteurs climatiques et anthropiques de variabilité du Normalized Différence Végétation Index. Thèse de Doctorat d'État, Université de Yaoundé I-Université de Bourgogne, Bourgogne, 2011, 322.
9. Tobolbaï R, Adamou S, Ngakou A. Morphological and structural diversities of indigenous endomycorrhiza communities associated to maize [*Zea mays* (L.)] in Northern Cameroonian soils. Journal Animal and Plants Sciences,2018:1:6057-6073.
10. Tobolbaï R. Taxonomic diversity of endomycorrhizal fungi associated with à *Vigna unguiculata* (L.), *Glycine max* (L.) and *Zea mays* (L.) cultivated in Northern Cameroon: Production and application of inoculants in the field. University of Ngaoundere, 2018, 181.
11. Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. Working Ylith Mycorrhizas in Forestry and Agriculture. AClAR Monograph. Mycorrhizas for Forestry and Agriculture, 1996, 374.
12. Gerdemann JW, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological*

Society,1963:46:235-244.
13. Arias RM, Heredia-Abarca G, Sosa VJ, Fuentes-Ramı´rez LE. Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee production systems and in a tropical montane cloud forest patch in Veracruz, Mexico. *Agroforest Syst*,2012:85:179-193.
14. Sghir F, Chliyeh M, Kachkouch W, Khouader M, Touhami A, Benkirane R, *et al*. Mycorrhizal status of *Olea europaea* spp. oleaster in Morocco. Journal of Applied Biosciences,2013:61:4478-4489.
15. Walker C. *Ambispora* and Ambisporaceae resurrected. *Mycology Research*,2008:112:297-298.
16. Koske RE, Tessier B. A convenient, permanent slide mounting medium. *Newsletter American Mycological Society*,1983:34:59.
17. Morton B, Benny GL. Revised classification of arbusculars mycorrhizal fungi (Zygomycètes): New order, Glomales, two new families, Acaulosporaceae and Gigasporaceae, with an amendation of Glomaceae. *Mycotaxon*,1990:37:471-491.
18. INVAM. International culture collection of VA Mycorrhizal fungi, 2017.
19. Trappe JM. Three new Endogonaceae: *Glomus constrictus*, *Sclerocystis clavispora* and *Acaulospora scrobiculata*. *Mycotaxon*,1977:6:359-366.
20. Miller DD, Walker C. *Glomus maculosum* sp. nov. (Endogonaceae): an endomycorrhizal fungus. *Mycotaxon*,1986:26:217-227.
21. Schenck NC, Sm GS. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia*,1982:74:77-92.
22. Walker C. Complexipes moniliformis: A new genus and species tentatively placed in the Endogonaceae. *Mycotaxon*,1979:12:99-104.
23. Schenck NC, Spain JL, Sieverding E, Howler RH. Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia*,1984:76:685-699.
24. Ogou A, Tchabi A, Tounou AK, Agboka K, Sokame BM. Effet de quatre souches de champignons mychoriziens arbusculaires sur Meloidogyne spp., principal nématode parasitaire du soja (*Glycine max*, L.) au Togo. Journal of Applied Biosciences,2018:127:2758-12769.
25. Nadjilom Y, Toukam TS, Tobolbaï R, Ngakou A. Morphological and structural characterization of rhizospheric endomycorrhiza communities associated with rice grown in the sahelian zone (Chad). International Journal of Soil & Plant Science,2019:31(5):1-14.
26. Richard T, Albert N, Steve TT. Prevalence and morpho-anatomical diversity of arbuscular mycorrhizal fungi Spores, from soybean (*Glycine max* L.) Rhizosphere in the agro-ecological zone 1 of Cameroon. International Journal of Sciences,2021:10(2):30-40.
27. Ouallal I, Abbas Y, Ech-cheddadi S, Ouajdi M, Ouhadach M, El YH, *et al*. Diversité des champignons endomycorhiziens de l'arganier et potentiel mycorhizogène des sols rhizosphériques des arganeraies du Sud-Ouest marocain. Bois et Forêts des Tropiques,2018:338:73-86.
28. Gnamkoulamba A, Tounou AK, Tchabi A, Agboka K, Adjévi AKM, Batawila K. Prévalence et diversité des spores des champignons mycorhiziens arbusculaires en culture de riz sous les différents systèmes de culture de riz dans cinq zones agro-écologiques au Togo. Journal of Applied Biosciences,2018:126:12647-12664.
29. Yuriko P, Alayon-Gamboa JA, Moron-Rios A, Castellanos-Albores J, Aguilar-Chama A, Guevara R. Effects of organic and chemical agriculture systems on arbuscular mycorrhizal fungi and green tomato production in Calakmul, Mexico. Agricultural Sciences,2018:9:1145-1167.
30. Kariman KH, Goltapeh EM, Minassian V. Arbuscular mycorrhizal fungi from Iran. Journal of Agricultural Technology,2005:1(2):301-313.
31. Voets L, De la Providencia IE, Declerck S. Glomeraceae and Gigasporaceae differ in their ability to form hyphal networks. *New Phytologist*,2006:172:185-188.
32. Strullu DG. Les mycorhizes des arbres et des plantes cultivées. Techniques et Documentation Lavoisier. Paris, 1991, 242.
33. Van DHMGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, *et al*. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature,1998:396:69-72.
34. Bolan NS. A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil*,1991:134:189-130.
35. Hardie K, Leyton L. The influence of VA mycorrhiza on growth and water relations of red clover in phosphate deficient soil. *New Phytologist*,1981:89:599-608.
36. Duponnois R, Garbaye J, Bouchard D, Churin JL. The fungus-specificity of mycorrhization helper bacteria (MHBs) used as an alternative to soil fumigation for ectomycorrhizal inoculation of bare-root Douglasfir planting stocks with Laccaria laccata., Plants and Soil,1993:157:257-262.
37. Abdalla ME, Abdel-Fattah GM. Influence of the endo-mycorrhiral fungus *Glomus mosseae* on the development of peanut pod rots disease in Egypt. *Mycorrhiza*,2000:10:29-35.
38. Leyval C, Joner EJ. Bioavailability of heavy metals in the mycorrhizosphere. Dans: Trace elements in the rhizosphere, CRC Press,2001, 165-185.
39. Joner EJ, Leyval C. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environmental Science and Technology,2003:37:2371-2375.
40. Duponnois R, Garbaye J. Some mechanisms involved in growth stimulation of ectomycorrhizal fungi by bacteria. Canadian Journal of Botany,1990:68:2148-2152.
41. Rhodes LH, Gerdemann JW. Phosphate uptake zones of mycorrhizal and non mycorrhizal onions. *New* Phytologist,1975:75,555-561.