

# Mycotrophic Status of Strawberry (*Fragaria* spp.) Suggests High Potential for Sustainable Fruit Production in Sub-Saharan Africa

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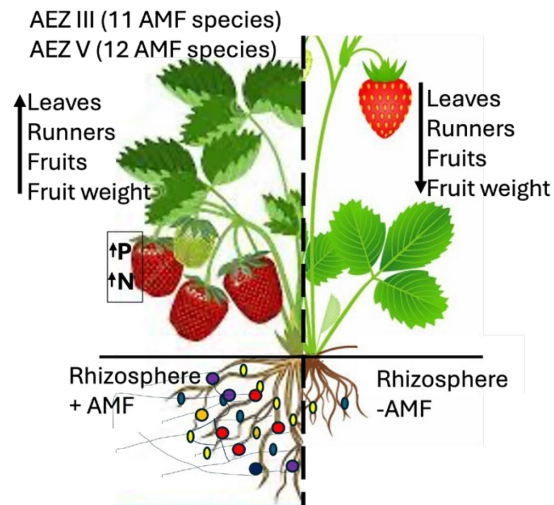
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## Abstract

Little is known about the mycotrophic status and biological production of strawberries in sub-Saharan Africa. This study investigated arbuscular mycorrhizal fungi (AMF) in the rhizospheres of strawberries from two agroecological zones (AEZs) in Cameroon: AEZ III (Western Highlands) and AEZ V (bimodal rainforest). AMF spores were identified from trapping soil, while strawberry roots were assessed for colonization. Two *Fragaria* spp. varieties, “Madame moutot” and “Mara des bois”, were cultivated in both AEZs. The soils were acidic and deficient in available phosphorus (4.9 and 0.88 mg.kg<sup>-1</sup>) with imbalanced Ca:Mg:K ratios (55:22:23 and 46:34:23) respectively. Three fertilization treatments were used: 1) control (unfertilized); 2) mineral (N:P:K 13:13:21); and 3) AMF species complex (at 30 g.plant<sup>-1</sup>) in a split-plot design with four replicates. Thirteen AMF species across seven genera were identified in the rhizospheres (11 in AEZ III and 12 in AEZ V). The spores measured 45 - 250 µm in diameter. Diversity indices (Shannon-Wiener and Simpson’s) indicated no specific dominance of AMF species. AMF fertilizer enhanced strawberry production and fruit quality, improving leaf formation, runners, and fruits per plant, fruit weight, and nutrient contents. This study confirms that strawberries are mycotrophic, and AMF-based fertilizers can enhance biological production in sub-Saharan African soils. **Graphical abstract**



**AMF diversity and strawberry production in tropical SSA**

## Keywords

Arbuscular Mycorrhizal Fungi, Biodiversity, Biofertilizer, Strawberry, Sub-Saharan Africa, Sustainable Agriculture

## 1. Introduction

Cultivated strawberry (*Fragaria* spp.) is a hybrid (of two wild octaploid species, *Fragaria virginiana* and *Fragaria chiloensis*) species of the genus *Fragaria*, an herbaceous perennial plant belonging to the Rosaceae family with leaves in tightly nested rosettes [1]. Strawberries are among the highest-yielding fruit crops and the most consumed globally [1] [2]. Strawberries are highly nutritious, with more antioxidants than other fruits, and increase insulin sensitivity [3] [4]. All these benefits contribute heavily to the strawberry economy [5]. They are a reliable source for nutrients like K, Mg, and Ca [6] [7] and can reduce the occurrence of specific tumors and enhance cardiovascular health [8].

Strawberries are grown over 389,665 hectares in 73 countries, mainly in the Northern Hemisphere. However, strawberries can grow in the Southern part and are not restricted by climate or genetic constraints [9]. China produces the most strawberries worldwide (3,389,620 tonnes), while Egypt is ranked first in Africa, with 470,913.1 tonnes of strawberries produced annually [10]. However, this crop is rare in Cameroon [10], where the high costs on the market reflect an insufficient yield of strawberries, making it a luxury item beyond the average citizen's reach [9].

Necessary factors to optimize strawberry production include good environmental conditions and the availability of healthy, fertile soil, which can become scarce over time [11]. Chemical fertilizers containing nitrogen, phosphorus, and potassium are heavily used to increase agricultural yields, with an annual global use of approximately 53 billion tons [12]. However, the intensive and inappropri-

ate utilisation of chemical fertilizers has been demonstrated to result in detrimental environmental impacts, including pollution and damage to consumers' health [13] [14]. To address these issues, farmers and scientists give more attention to sustainable agriculture practices that incorporate beneficial soil microorganisms [15]. Rhizosphere management optimizes soil nutrient efficiency, promotes plant growth and yield, and may be achieved using microorganisms [16] [17]. Characterizing the functional diversity of microorganisms in the rhizosphere offers the opportunity to understand and improve processes in agricultural biotechnology [18]. Soil scientists widely recognize arbuscular mycorrhizal fungi (AMF) in enhancing nutrient availability in the soil, leading to improved plant nutrition, growth, and production [19] [20]. In Cameroon, there is limited information on the potential diversity, the colonization status of AMF in the strawberry rhizosphere, and the response of crops to AMF inoculation. Unraveling the AMF community is considered of prime importance to understand crop productivity in agroecosystem constraints [21]-[23]. Therefore, the objective of this study was to investigate the biodiversity of AMF in the rhizosphere of strawberries in two agroecological zones in Cameroon and their effect on productivity.

## 2. Materials and Methods

### 2.1. Study Areas and Soil Sampling

The study was carried out in two agroecological zones: the Western Highlands (AEZ III) and the bimodal humid forest zone (AEZ V). AEZ III offers a great diversity of relief, with an altitude range of 1400 - 1600 m [24]. This area is characterized by two seasons of unequal duration, low mean temperatures (19°C), and abundant precipitation (1500 - 2000 mm). Four different types of soil are found in AEZ III: young soils on steeply sloping land, heavily leached soils on old plateaus, soils with an illuvial B horizon in closed depressions, and plateaus enriched in volcanic material (andosol) [25]. The zone V is characterized by precipitation ranging from 1500 to 2000 mm per year, and the vegetation is predominantly southern humid forest of the intertropical type. The climate in Zone V is hot and humid, with four distinct seasons, i.e., two dry seasons and two rainy seasons. The climate in AEZ V is "Guinean" type, with an average temperature of 25°C. The soils in AEZ V are predominantly ferralitic, acidic, and clayey, and their color ranges from red to yellow, depending on the duration of the rainy season [26].

In each zone, the major strawberry production basins were visited, including the localities of Dschang, Mbouda, Bafoussam I, Baham, and Bandjoun in the Western region for AEZ III; and the localities of Omnisport, Nkolfon, Elat, Titi garage, and Obala in the Central region for AEZ V. The experiments took place in these two agro-ecological zones. In AEZ III, the preceding crops were cassava (*Manihot esculenta* Crantz) and groundnut (*Arachis hypogea* L.). In AEZ V, the preceding crop was cassava (*Manihot esculenta* Crantz) with a preponderance of ruderal species like *Chromolaena odorata* L., *Mimosa pudica* L., and *Imperata cylindrica* L.

The soil was sampled using the method of Sieverding [26]. For each location, an area of 16 m<sup>2</sup> was divided into 10 quadrants of 2 \* 2 m each. A random strawberry plant was selected in each quadrant, three soil and root samples were taken from 0 to 25 cm deep using an auger. At each point, 100 g of soil was collected, and for each site, 500 g of soil per quadrant was mixed to create a composite sample [27]. Fine roots were sampled at the same point in the soil at the base of each plant at a rate of 2 g per point and preserved in 50% alcohol [27].

## 2.2. AMF Trapping, Characterization, and Identification

AMF from the rhizosphere of strawberry plants was trapped using sorghum and cowpea. Sterilized sand and black soil were mixed in a 3:1 ratio, and 250 g of the mixture was layered on top of 5 l pots in five replicates. Sorghum and cowpea seeds were sterilized using sodium hypochlorite, pre-germinated in the dark, and sown in the pots with four seeds per plant species. The soils were watered twice weekly at field capacity with 350 mL of tap water. Two weeks after planting, two plants per species were retained, and the Rorison nutrient solution without phosphorus was applied once a week at a rate of 50 mL per pot for four weeks. Phosphate fertilizer was added to the nutrient solution when the plants showed deficiency symptoms. After 10 weeks of growth, the watering was stopped, and the pots were left at room temperature for two weeks to induce sporulation or massive spore production [27].

Spores were extracted by the rapid spores extraction method of [27]. The spore suspension contained in the sieves was centrifuged on a sucrose gradient of 50 % [28]. To facilitate spore counting, the supernatant was transferred to a gridded petri dish. Spores were counted depending on their size, color, and structure using a binocular loupe. The number of spores was expressed per 100g of dry ground.

The detected spores were collected using forceps and described by microscopic observation. The description characters included their size, color, ornamentation of their suspensory hyphae or bulb, spore saccules, and germination. The morphotypes were mounted between slides. A drop of PVLG mixture (polyvinyl acid, alcohol, lactic acid, and glycerol) and Melzer's reagent was used. A small amount of pressure was applied to crush and break their wall, to reveal their structure [29]. The species identification was based on type specimens at the AMF international collection site (<http://invam.caf.wvu.edu>) as described by other authors [29]-[32].

The root staining was performed using the method described by Charvat [28] and Birhane *et al.* [33]. After the root fragments were discolored, only the structures of the AMF generally remained colored, allowing for microscopic observation of the AMF structures. The technique of description provided by Trouvelot *et al.*, [34] was used to ascertain the mycorrhization frequency, defined as

$$F(\%) = (100(N - no))/N \quad (1)$$

where N is the total number of root fragments observed, and "no" is the number of these fragments exhibiting no evidence of mycorrhization. The intensity of my-

corrhization assesses the level or severity of colonization. It is calculated using the following formula:

$$I(\%) = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / (\text{Total number of fragments observed}) \quad (2)$$

where  $n_5$  = number of fragments ( $n$ ) rated 5,  $n_4$  =  $n$  rated 4,  $n_3$  =  $n$  rated 3,  $n_2$  =  $n$  rated 2 and  $n_1$  =  $n$  rated 1. Rates 0, 1, 2, 3, 4 and 5 indicate the level of mycorrhizal infection corresponding respectively to the percentage of infection 0, < 1%, < 10%, < 50%, 50% and > 50%.

### 2.3. Determination of the Relative Abundance, Density, and Diversity Indices of AMF

The spore relative abundance (RA) was calculated according to the formula stated by Johnson *et al.*, [35] as

$$RA = 100 * (m/M) \quad (3)$$

where  $m$  is the total number of species observed and  $M$  is the total number of spores observed.

The diversity of AMF on each site was analyzed using different indices [36]. The species richness ( $S$ ) was determined as the number of species in the study area. Shannon-Wiener diversity index ( $H'$ ) has been determined by the following formula [37]:

$$H' = - \left[ \sum (p_i * \ln(p_i)) \right] \quad (4)$$

where  $P_i$  is the proportion of the abundance or percentage of the importance of the species

$$P_i = n_i/N \quad (5)$$

Pielou equitability index ( $J'$ ) was calculated as follows [38]:

$$J' = H' / \log(S) \quad (6)$$

where  $H'$  is the Shannon-Wiener diversity index and  $S$  is the species richness. Simpson ( $J$ ) dominance index and Simpson diversity index ( $D_s$ ) were calculated by the following formula [39]:

$$J = \sum [n_i(n_i - 1)] / [N(N - 1)] \quad (7)$$

$$D_s = 1 - J \quad (8)$$

where  $n_i$  is the number of individuals of one species in the sample, and  $N$ , the total number of individuals from all the species that were sampled is hereby stated.

### 2.4. Strawberry Cultivation Conditions

Two field experiments were conducted in the two agroecological zones III and V to investigate the effect of mineral fertilizer and AMF-based biofertilizer on vegetative growth, number of runners, number of fruits, fruit production, and fruit quality of two varieties (Madame Moutot and Mara des Bois) of strawberry

(*Fragaria* spp.). Runners were purchased from a private nursery. The runners of the two strawberry varieties were of the same age (one month), length (5 cm), number of leaves (3), and crown diameter (0.5 cm).

The sites were cleared, cleaned, and plowed. A hoe loosened the soil to facilitate runners' sowing. Each experimental unit was mulched with white wood shavings to conserve soil moisture and limit weed growth [40] [41].

The experimental design was a split plot with the two varieties (*Madame Moutot* and *Mara des Bois*) as main plots, divided by three different fertilization treatments: control without fertilizer application, chemical fertilizer (NPK), and biological fertilizer (arbuscular mycorrhizal fungi). Each treatment had four replicates. In each experimental plot, nine runners were transplanted in three rows, with three plants per row, at intervals of 0.3 m between rows and 0.3 m within rows. The chemical fertilizer was applied to balance cation loads and soil nutrients with plant requirements [13]. Depending on the physicochemical characteristics of the soil, the fertilizer NPK (13:13:21) was applied at a rate of 26.28 g per plant in AEZ V and 27.6 g per plant in AEZ III. The chemical fertilizer was applied in three fractions (seven, ten, and fifteen days after planting). The biological fertilizer was a composite of ubiquitous arbuscular mycorrhizal fungi containing four species (*Glomus hoi*, *Rhizophagus intraradices*, *Gigaspora margarita*, and *Scutellospora dipurpurens*). For each plant, 30 g of biological fertilizer was used. Plots were watered daily (morning and evening with 10 l per m<sup>2</sup>) until harvest.

## 2.5. Evaluation of Plant Development, Fruit Production, and Quality

The number of leaves was counted at 2-week intervals, from 2 up to 12 weeks after transplantation. The number of runners was counted per plant from the first appearance to the end of the experiment. Strawberry fruits were harvested at the red-ripe stage for yield determination. The total number of fruits and the weight of fresh fruit were determined at harvest. The yield was estimated in tonnes per hectare.

The Dumas method was used to determine the fruits' total nitrogen content. The fruits' phosphorus content was determined after dry ashing at 550 °C, using the colorimetry method described by [42].

## 2.6. Statistical Analyses

Data were subjected to ANOVA using R Studio software version 4.0.4. Means values were compared using the Tukey and Duncan tests at  $p > 0.01$ . Histograms were plotted with Excel software 2013.

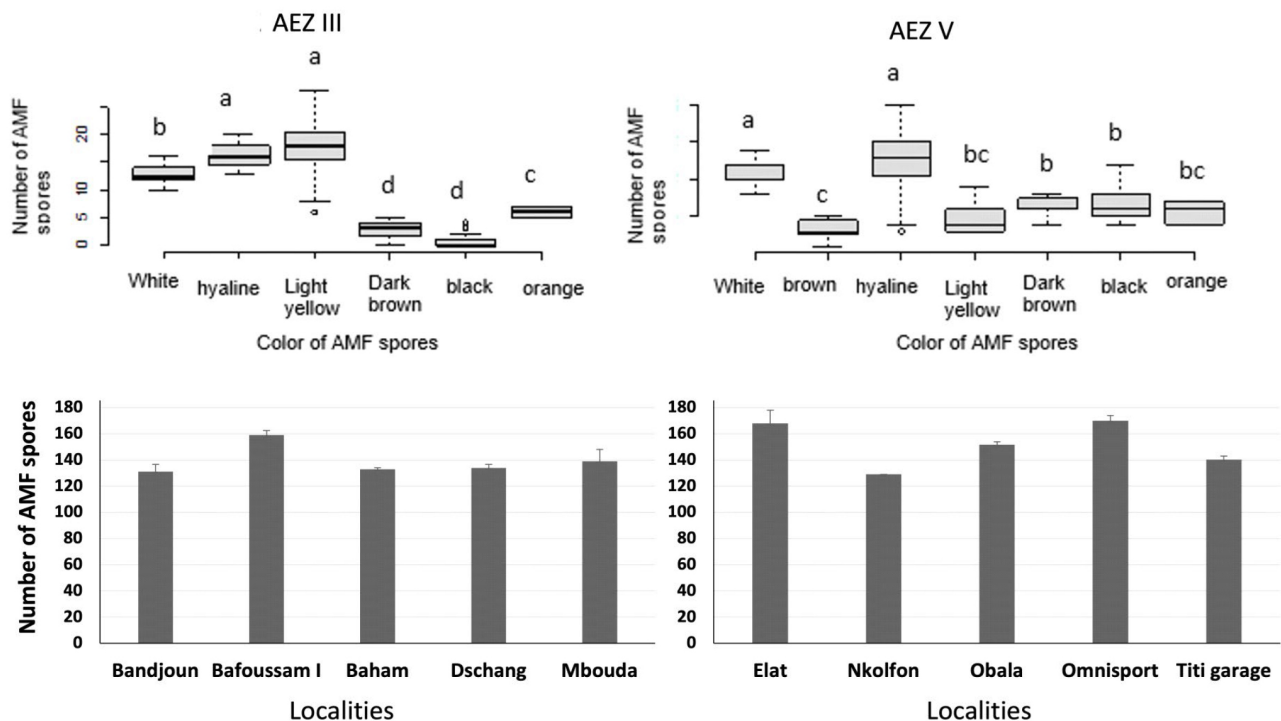
## 3. Results

### 3.1. AMF Spore Characteristics in the Rhizosphere of Strawberry

The AMF spores were characterized by six different colors in AEZ III (light yellow, hyaline, white, orange, dark brown, and black) and AEZ V (hyaline, white,

black, dark brown, orange, light yellow, and brown) (Figure 1). Statistical analysis showed a significant ( $p < 0.001$ ) difference between the number of spores concerning their colors. In AEZ III, no significant difference was found between the number of light yellow and hyaline colors, dark brown and black spores. In AEZ V, there was no significant difference between the number of spores with hyaline and white colors, black and brown colors, and orange and light yellow (Figure 1).

A highly significant difference ( $p < 0.001$ ) was found between the different zones for the number of spores extracted. It was found that the number of AMF spores per 100 g of soil varied according to the type of ecosystem and the sanitary aspect of the rhizosphere soils. The mean number of spores was higher in AEZ V ( $151.60 \pm 3.60$ ) than in AEZ III ( $139.20 \pm 4.36$ ) (Figure 1).

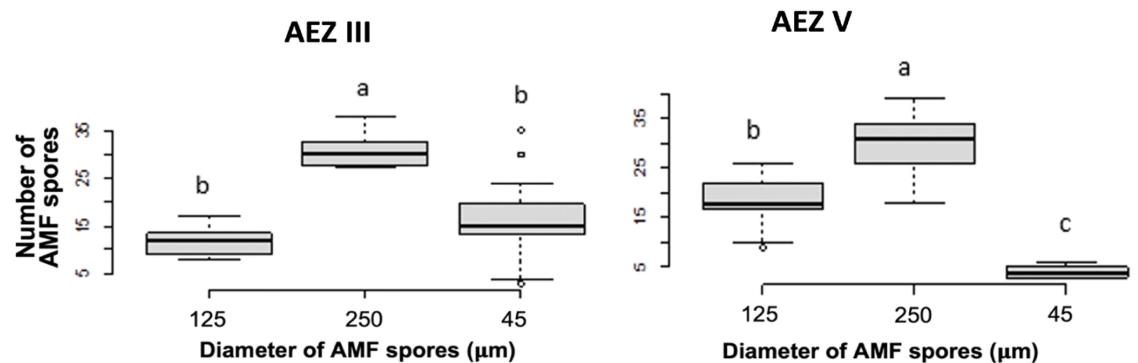


**Figure 1.** Variation of the number of AMF spores in the rhizosphere of strawberry according to color and the sampling localities in the two agroecological zones III and V. Means  $\pm$  Standard errors with the same letters in the same zone are not significantly different at the 0.05 probability level.

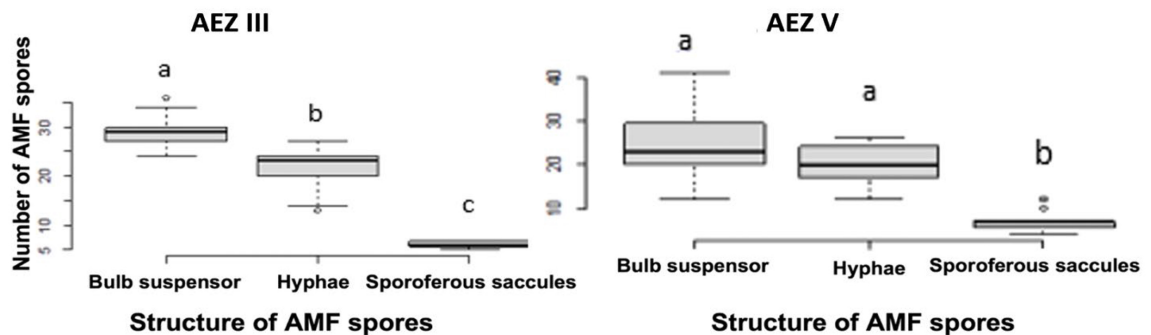
Three class diameters were identified in AEZs III and V (250, 125, and 45  $\mu\text{m}$ ). The largest number of AMF spores were those of 250  $\mu\text{m}$  in diameter, followed by those of 125  $\mu\text{m}$ , and finally 45  $\mu\text{m}$ . The statistical analysis of variance of AMF spore diameters in AEZ III did not show significant differences between the number of AMF spores of 125  $\mu\text{m}$  and 45  $\mu\text{m}$ . There were significant differences ( $p < 0.001$ ) between the number of spores of the three class diameters in AEZ V (Figure 2).

In AEZs III and V, three main AMF structures were distinguished (bulb suspensor, hyphae, and sporiferous saccule). It was found in AEZ III that the number

of AMF spores bearing suspensor ( $28.80 \pm 3.21$ ) was significantly ( $p < 0.001$ ) higher than that of AMF spores with hyphae ( $21.33 \pm 4.18$ ) and that of those having sporiferous saccules ( $6.07 \pm 0.73$ ) (**Figure 3**). In AEZ V, results showed no significant difference between the number of AMF spores having bulb suspensors ( $24.33 \pm 8.67$ ) and that of those having hyphae structures ( $20.47 \pm 4.48$ ). However, the number of spores with sporiferous saccules ( $6.80 \pm 2.34$ ) was significantly lower than the others.



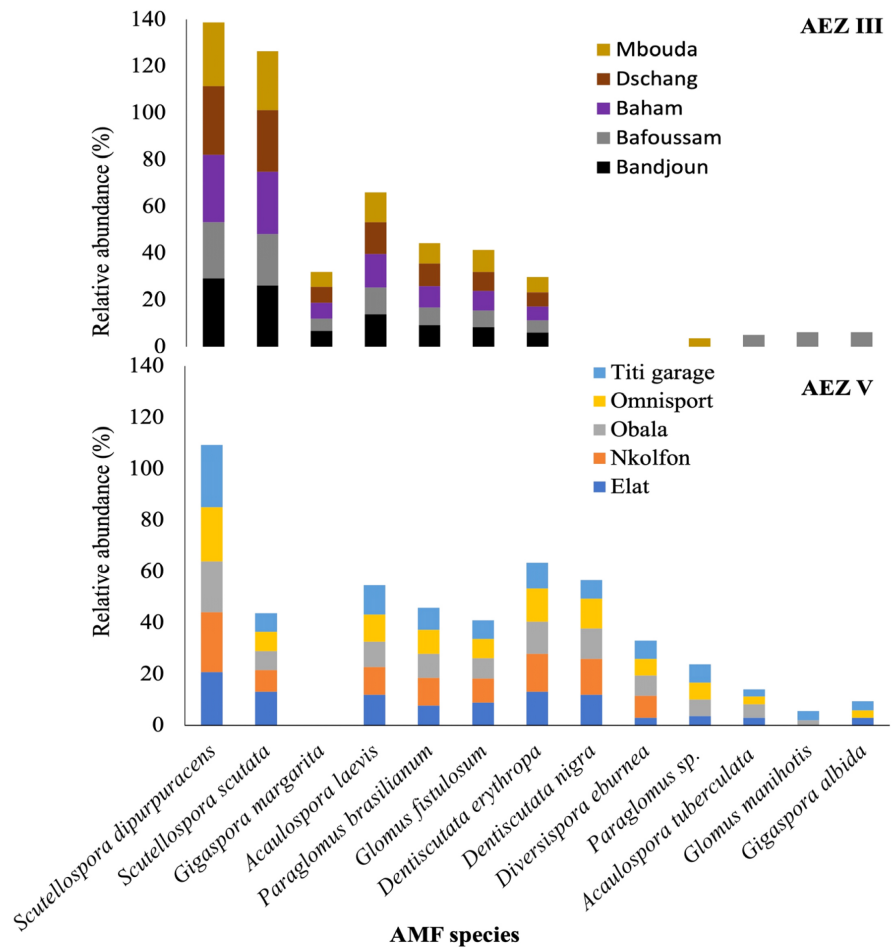
**Figure 2.** Variation of the number of AMF spores in the rhizosphere of strawberry according to the class diameter in the two agroecological zones III and V. Means  $\pm$  Standard errors with the same letters in the same zone are not significantly different at the 0.05 probability level.



**Figure 3.** Variation of the number of AMF spores in the rhizosphere of strawberry depending on characteristic structures in the two agroecological zones III and V. Means  $\pm$  Standard errors with the same letters in the same zone are not significantly different at the 0.05 probability level.

### 3.2. Relative Abundance of AMF Species in the Rhizosphere of Strawberry

Thirteen AMF morphotypes were observed in the strawberries' rhizosphere in the two AEZs, showing an important relative abundance. In AEZ III, 11 different morphotypes of seven genera were identified. *Gigaspora margarita* was found only in AEZ III. Twelve different morphotypes of seven genera were found in AEZ V and *Dentiscutata nigra*, and *Diversispora eburnea* were found only in AEZ V (**Figure 4**). *Scutellospora dipurpuracens* was the prevalent AMF species in the two AEZs. *Paraglomus* sp. and *Glomus manihotis* were the less abundant AMF species in AEZ III and V, respectively.



**Figure 4.** Relative abundance of AMF species in the strawberry’s rhizospheres in the different sites in the two AEZs III and V.

### 3.3. Shannon-Wiener, Simpson’s, and Pielou’s Equitability Indices

An overall observation of AMF species diversity showed that Shannon-Wiener index values were generally between 0.77 and 1 (**Table 1**). Simpson’s index varied between 0.81 and 0.89, while Pielou’s equitability index varied between 0.89 and 0.96. These indices indicate that both sites had AMF species diversity with no actual dominance of any one species.

**Table 1.** Diversity indices for AMF species in the strawberry’s rhizosphere of the different localities of the two AEZs III and V.

AEZ	Localities	Indices				
		S	Ds	L	H'	J'
III	Bandjoun	131 ± 5.57 <sup>cd</sup>	0.81 ± 0.002 <sup>f</sup>	0.19 ± 0.002 <sup>a</sup>	0.77 ± 0.003 <sup>g</sup>	0.913 ± 0.003 <sup>d</sup>
	Bafoussam I	159 ± 3.61 <sup>b</sup>	0.86 ± 0.002 <sup>d</sup>	0.14 ± 0.002 <sup>c</sup>	0.92 ± 0.003 <sup>d</sup>	0.92 ± 0.002 <sup>d</sup>
	Baham	133 ± 1 <sup>cd</sup>	0.81 ± 0.002 <sup>f</sup>	0.19 ± 0.002 <sup>a</sup>	0.77 ± 0.003 <sup>g</sup>	0.91 ± 0.003 <sup>d</sup>
	Dschang	134 ± 2.65 <sup>cd</sup>	0.81 ± 0.002 <sup>f</sup>	0.19 ± 0.002 <sup>a</sup>	0.77 ± 0.003 <sup>g</sup>	0.91 ± 0.003 <sup>d</sup>
	Mbouda	139 ± 9 <sup>c</sup>	0.82 ± 0.006 <sup>e</sup>	0.18 ± 0.006 <sup>b</sup>	0.81 ± 0.009 <sup>f</sup>	0.89 ± 0.01 <sup>e</sup>

## Continued

	Elat	168 ± 10 <sup>a</sup>	0.88 ± 0.001 <sup>c</sup>	0.12 ± 0.002 <sup>d</sup>	0.96 ± 0.001 <sup>c</sup>	0.92 ± 0.001 <sup>cd</sup>
	Nkolfon	129 ± 0 <sup>d</sup>	0.86 ± 0.001 <sup>d</sup>	0.14 ± 0.001 <sup>c</sup>	0.88 ± 0.001 <sup>e</sup>	0.96 ± 0.001 <sup>a</sup>
V	Obala	151.67 ± 2.08 <sup>b</sup>	0.89 ± 0.001 <sup>a</sup>	0.11 ± 0.001 <sup>f</sup>	0.99 ± 0.003	0.95 ± 0.003 <sup>b</sup>
	Omnisport	170 ± 4 <sup>a</sup>	0.89 ± 0.002 <sup>b</sup>	0.11 ± 0.002 <sup>e</sup>	0.98 ± 0.007 <sup>b</sup>	0.94 ± 0.006 <sup>b</sup>
	Titi garage	140.33 ± 2.52 <sup>c</sup>	0.89 ± 0.01 <sup>b</sup>	0.11 ± 0.005 <sup>e</sup>	1.004 ± 0.01 <sup>a</sup>	0.93 ± 0.012 <sup>c</sup>

S, Ds, I, H', and J' indicate the total number of spores, Simpson diversity index, Simpson dominance index, Shannon-Wiener diversity index, and Pielou equitability index, respectively. According to the Duncan test, means ± standard errors followed by the same letters in a column and the same zone are not significantly different at  $P < 0.05$ .

### 3.4. Family Diversity of AMF

**Table 2.** Different families of AMF species and their spores' characteristics in the strawberry's rhizosphere.

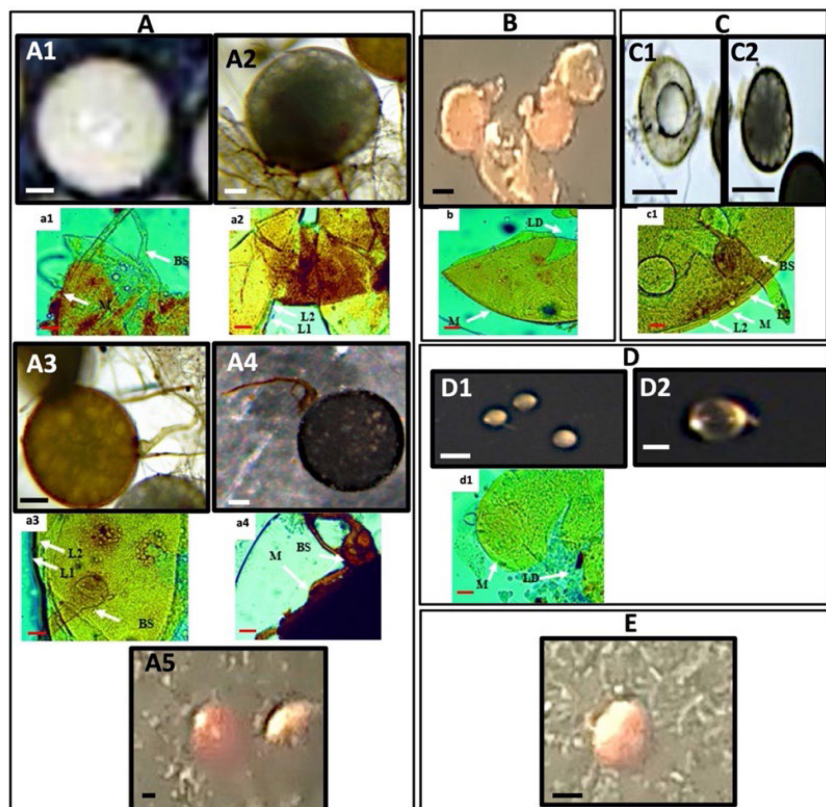
Morphotype codes	Spore characteristics			Identified species, genera, and families	
	Color	Structures	Diameters	AMF species	Families
M1	White	bulb suspensor + single layer	250	<i>Scutellospora dipurpurascens</i>	Gigasporaceae
M2	Hyaline	bulb suspensor + double layers	250	<i>Scutellospora scutata</i>	
M3	Light yellow	Bulb suspensor + double layers	250	<i>Gigaspora margarita</i>	
M13	Hyaline	Bulb suspensor	250	<i>Gigaspora albida</i>	
M7	Dark brown	Hyphae + single layer	250	<i>Dentiscutata erythropha</i>	
M8	Black	Bulb suspensor	250	<i>Dentiscutata nigra</i>	
M4	Orange	Sporiferous saccule + lipid droplets + distinctive membrane	125	<i>Acaulospora laevis</i>	Acaulosporaceae
M11	Black	Bulb suspensor	125	<i>Acaulospora tuberculata</i>	
M5	Hyaline	Hyphae + double layers + distinctive membrane	125	<i>Paraglomus brasilianum</i>	Paraglomaceae
M10	Hyaline	Hyphae	125	<i>Paraglomus</i> sp.	
M6	Light yellow	Hyphae + lipid droplets + distinctive membrane	45	<i>Glomus fistulosum</i>	Glomeraceae
M12	Light yellow	Hyphae	125	<i>Glomus manihotis</i>	
M9	Brown	Sporiferous saccule	125	<i>Diversispora eburnea</i>	Diversisporaceae

M: Morphotypes.

Thirteen AMF morphotypes were isolated from the strawberry's rhizosphere and identified based on the morphological characters (Table 2). The identified species belong to seven genera and five families (Gigasporaceae, Paraglomeraceae, Diversisporaceae, Glomeraceae, and Acaulosporaceae). The family Gigasporaceae was the most represented, with six species belonging to 03 genera: *Scutellospora* (*S. dipurpurascens* and *S. scutata*), *Gigaspora* (*G. margarita* and *G. albida*), and *Dentiscutata* (*D. erythropha* and *D. nigra*). The Acaulosporaceae family was represented by 02 species of the genus *Acaulospora* (*A. laevis* and *A. tuberculata*). Two species of the genus *Paraglomus* (*P. brasilianum* and *Paraglomus* sp.) represented

the family Paraglomaceae. The genus *Glomus* of the Glomeraceae family was represented by 02 species (*G. fistulosum* and *G. manihotis*). The Diversisporaceae family was represented by a single species, *Diversispora eburnea* (Table 2).

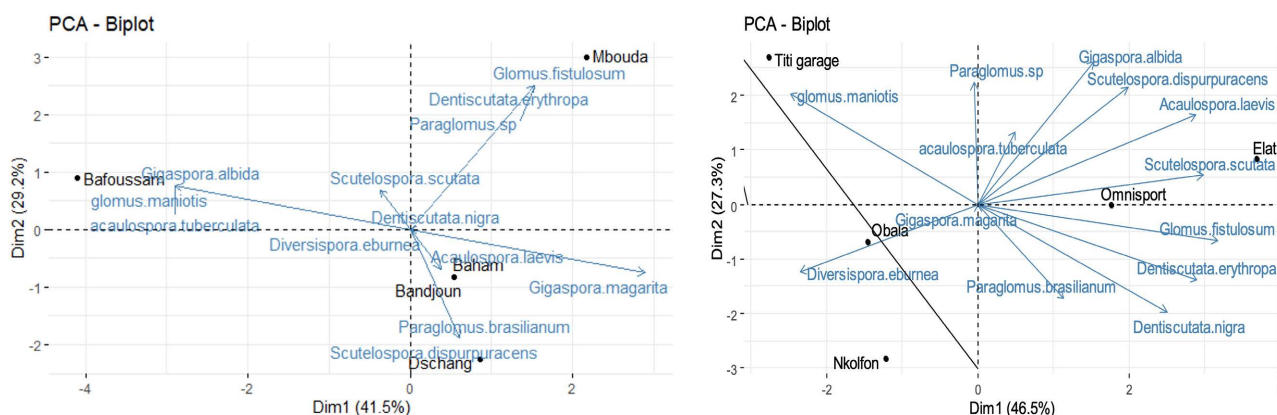
The different shapes, colors, and sizes can be distinguished for the Gigasporaceae (Figure 5(A)), the Acaulosporaceae (Figure 5(B)), Paraglomaceae (Figure 5(C)), the Glomeraceae (Figure 5(D)), and the Diversisporaceae (Figure 5(E)). The different membrane structures, as well as the content of the AMF spores, are presented. A single layer and the suspensory bulb can be observed on *Scutellospora dipurpurascens* (Figure 5(A1)) and *Dentiscutata erythropha* (Figure 5(A4)). The double layers are observed on *Scutellospora scutata* (Figure 5(A2)) and *Gigaspora margarita*, in which the suspensory bulb is also present (Figure 5(A3)). *Acaulospora laevis* shows lipid droplets and a membrane (Figure 5(B)), while *Paraglomus brasilianum* (Figure 5(C1)) presents a suspensory bulb, two layers, and a membrane. *Glomus fistulosum* shows lipid droplets and a membrane (Figure 5(D1)).



**Figure 5.** Some of the diversity of AMF spores (capital letters), membrane structures and contents (small letters) of spores isolated from the rhizosphere of *Fragaria × ananassa* Duch. A, Gigasporaceae; A1 + a1, *Scutellospora dipurpurascens*; A2 + a2, *Scutellospora scutata*; A3 + a3, *Gigaspora margarita*; A4 + a4, *Dentiscutata nigra*; A5, *Dentiscutata erythropha*; B, Acaulosporaceae (*Acaulospora laevis*); C, Paraglomaceae; C1 + c1, *Paraglomus brasilianum*; C2, *Paraglomus* sp.; D, Glomeraceae; D1 + d1, *Glomus fistulosum*; D2, *Glomus manihotis*; E, Diversisporaceae (*Diversispora eburnea*); BS, Bulb suspensor; LD, Lipid droplet; M, membrane; L1, first layer; L2, second layer; Scale bars, 50 µm.

### 3.5. Distribution of AMF Species in the Two Studied AEZs

After performing the principal component analysis, it appears that in AEZ III, the genera *Gigaspora*, *Glomus*, *Paraglomus*, *Acaulospora*, and *Scutelospora* were the most abundant in the different sites. In AEZ V, the genera *Gigaspora*, *Glomus*, *Paraglomus*, *Acaulospora*, and *Denticutata* were the most abundant in the different sites (Figure 6).



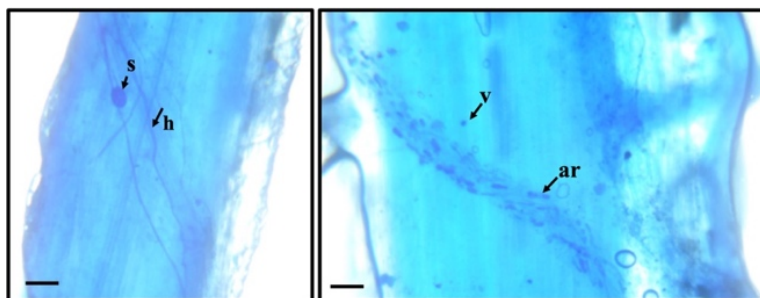
**Figure 6.** Principal component analysis biplot of AMF species collected in the different localities in the two agroecological zones III and V.

### 3.6. Strawberry Roots' Mycorrhization and Colonization

The intensity of mycorrhization was not significantly different in all localities. Still, it varied from 1.13 to 1.19, with an average of 1.15 in AEZ III, and from 1.17 to 1.24, with an average of 1.20 in AEZ V. The frequency of mycorrhization was 34.67 on average in AEZ III and 23.33 in AEZ V (Table 3). Root staining revealed the presence of arbuscular mycorrhizal fungi (Figure 7). Characteristic structures of AMFs, such as hyphae and spores (Figure 7(A)), as well as arbuscules and vesicles, have been observed in the roots (Figure 7(B)).

**Table 3.** Intensities and frequencies of mycorrhization in the different studied sites.

AEZ	Localities	Intensity of mycorrhization	Frequency of mycorrhization
III	Bandjoun	1.19	27.78
	Bafoussam I	1.18	27.78
	Baham	1.13	21.11
	Dschang	1.13	21.11
	Mbouda	1.13	18.89
	Average	1.1520.03	23.3344.159
V	Elat	1.24	35.56
	Nkolfon	1.23	35.56
	Obala	1.19	35.56
	Omnisport	1.18	33.33
	Titi garage	1.17	33.33
Average	1.2030.03	34.6681.221	



**Figure 7.** Root staining of trap plants. h, hyphae; s, spore; ar, arbuscules; v, vesicles; scale bar, 95  $\mu$ m.

### 3.7. Soil Characteristics in the Experimental Field of Strawberry Cultivation

The soils of AEZs III and V were clay-sand and acidic (**Table 4**). The soil organic carbon and nitrogen contents were low, giving relatively low soil C/N ratios of 9.56 and 9.18 in AEZs III and V, respectively. The soils could be considered relatively unfertile with a weak nitrogen mineralization potential. Conversely, the available phosphorus content was found to be particularly low (4.90 mg P/kg of soil) in AEZ III and critically low in AEZ V (0.88 mg P/kg of soil). Also, the soils were critically poor in calcium (1.06 mEq/100 g of soil in AEZ III and 1.36 mEq/100 g of soil in AEZ V). The exchangeable cation values indicated that the soils were profoundly unbalanced in favor of potassium and magnesium and had insufficient levels of calcium [Ca: Mg: K was 55:22:23 in AEZ III and 46:34:23 in AEZ V].

**Table 4.** Physicochemical characteristics of the studied soils in the two agroecological zones III and V.

Soils characteristics	Agroecological zones	
	III	V
Sand (%)	43	36
Silt (%)	15.5	18
Clay (%)	41.5	46
pH	5.3	5.1
Organic C (%)	2.57	1.53
Total N (%)	0.28	0.16
Available P (ppm)	4.9	0.88
CEC (meq/100 g)	9.96	13.5
Ca (meq/100 g)	1.06	1.36
Mg (meq/100 g)	0.42	1.08
K (meq/100 g)	0.45	0.74
Na (meq/100 g)	0.31	0.32

### 3.8. Influence of AMF on Strawberry Leaf Formation

**Table 5.** Relationship between the number of leaves per plant ( $y$ ) and the time ( $x$  in weeks) up to 12 weeks after planting of strawberry plants, for control, mineral fertilizer, and AMF-based biofertilizer. Values are means from four plants per experimental plot. \* Significant at the 0.05 probability level. Zone III: Western Highlands zone; Zone V: bimodal rainforest zone.

AEZ	Strawberry varieties	Fertilizer treatments	Time (weeks after planting)	Regression equation for number of leaves	R <sup>2</sup>
III	Madame Moutot	Control		$y = 2.004x - 0.1073$	0.9922
		Mineral fertilizer	$x = 4; x = 8$	$y = 2.1209x + 0.282$ (*)	0.9964
		AMF-based biofertilizer		$y = 1.9514x + 0.3733$	0.9942
	Mara des Bois	Control		$y = 1.914x + 0.0227$	0.9895
		Mineral fertilizer	$x = 2$	$y = 2.4143x - 1.1167$ (*)	0.9917
		AMF-based biofertilizer	$x = 2$	$y = 1.9203x + 0.8907$ (*)	0.9953
V	Madame Moutot	Control		$y = 2.1154x - 0.3673$	0.9877
		Mineral fertilizer	$x = 4$	$y = 0.9835x + 0.7842$ (*)	0.98
		AMF-based biofertilizer		$y = 1.1615x - 0.8766$	0.9513
	Mara des Bois	Control		$y = 0.8832x + 0.655$	0.9869
		Mineral fertilizer	$x = 12$	$y = 1.2606x - 1.0758$ (*)	0.9929
		AMF-based fertilizer		$y = 1.0424x + 0.6267$	0.9894

Regarding the number of leaves per plant, there were weakly significant effects ( $p < 0.05$ ) of fertilization in the two AEZs with the two strawberry varieties (Table 5). In AEZ III, significant increases in the “Madame Moutot” strawberry number of leaves were observed at 4 and 8 weeks after transplantation under mineral fertilizer. The number of leaves per plant was significantly increased under mineral fertilizer and AMF-based biofertilizer at 2 weeks after transplantation in “Mara des Bois”. The highest slopes (2.12 for “Madame Moutot” and 2.41 for “Mara des Bois”) of increasing number of leaves per plant were observed between 2 and 12 weeks after transplantation, under mineral fertilizer. In AEZ V, a significant increase in “Madame Moutot” and “Mara des Bois” strawberries’ number of leaves per plant was observed with mineral fertilizer at 4 and 12 weeks after transplantation, respectively.

### 3.9. Influence of AMF on Strawberry Productivity and Fruit Quality

The maximum number of runners per plant ( $15.22 \pm 2.17$ ) was obtained with Madame Moutot variety in the mineral fertilizer treatment in AEZ V (Table 6). The highest fruit weight ( $13.7 \pm 1.42$  g) was obtained with Madame Moutot variety in the mineral fertilizer treatment in AEZ V. The maximum number of fruits per plant was obtained with Mara des Bois variety using the mineral fertilizer, in the AEZs III ( $20.33 \pm 0.58$ ) and V ( $20.33 \pm 0.57$ ), followed by the AMF-based biofer-

tilizer treatment (**Table 6**).

**Table 6.** Effect of fertilization on *Fragaria* spp. production in two agroecological zones.

AEZ	Strawberry varieties	Fertilization treatments	Number of runners	Number of fruits	Fruit weight (g)	Phosphorus content (g per 100 g)	Nitrogen content (g per 100 g)
III		Control	13.89 ± 2.03a	15 ± 1b	7.3 ± 1.32 b	0.233 ± 0.003d	1.37 ± 0.04b
		Madame Moutot	14.67 ± 2.6a	19 ± 1a	10.9 ± 1.08a	0.252 ± 0.002c	1.35 ± 0.05b
		AMF based fertilizer	14.78 ± 2.95a	16.33 ± 0.58b	8.7 ± 1.1ab	0.276 ± 0.002a	1.53 ± 0.03a
		Control	12.44 ± 1.94ab	14 ± 1.73c	7.2 ± 0.87b	0.282 ± 0.002c	1.61 ± 0.01b
	Mara des Bois	Mineral fertilizer	13.44 ± 1.01a	20.33 ± 0.58a	11.2 ± 1.73a	0.294 ± 0.004b	1.6 ± 0.02b
		AMF based fertilizer	13.33 ± 2.18a	16.67 ± 0.58b	9.4 ± 1.6ab	0.305 ± 0.005a	1.71 ± 0.01a
V		Control	14.67 ± 2.83a	15 ± 1b	8.9 ± 1.74 b	0.231 ± 0.001c	1.36 ± 0.02b
		Madame Moutot	15.22 ± 2.17a	19 ± 1a	13.7 ± 1.42a	0.25 ± 0.02b	1.34 ± 0.04b
		AMF based fertilizer	15 ± 3.28a	16.33 ± 0.57b	12 ± 1.32a	0.269 ± 0.002a	1.51 ± 0.01a
		Control	13.56 ± 1.59ab	14 ± 1.73c	8 ± 1.73b	0.281 ± 0.001a	1.65 ± 0.05ab
	Mara des Bois	Mineral fertilizer	14.67 ± 0.87a	20.33 ± 0.57a	11.9 ± 1.15a	0.292 ± 0.002a	1.61 ± 0.01b
		AMF based fertilizer	14.56 ± 1.59a	16.67 ± 0.57b	9.8 ± 1.73ab	0.299 ± 0.001a	1.69 ± 0.03a

For each agroecological zone, the values followed by the same letter do not differ significantly at the 5% threshold in the same column.

P and N contents were found to be considerably elevated in the strawberries harvested under AMF-based biofertilizer treatment than those from the other fertilizer treatments. The highest strawberry fruit phosphorus ( $0.305 \pm 0.005$  g/100 g) and nitrogen ( $1.71 \pm 0.01$  g per 100 g) contents were observed in the Mara des Bois variety cultivated with the AMF-based biofertilizer in AEZ III (**Table 6**).

#### 4. Discussion

*Fragaria* spp. is a hybrid strawberry with promising production potential in sub-Saharan Africa. Its mycotrophic status can predict a high capacity for biological production in previously considered hostile ecosystems, such as Cameroon's agroecological zones (AEZ). Regarding the number of spores, relative abundance, and diversity of arbuscular mycorrhizal fungi (AMF) in two AEZs, the diversity of AMF in the rhizosphere of strawberries from the study locations showed a low spore density of AMF 139.2/100 g of dry soil in AEZ III (pH 5.7) and dry soil in AEZ V (pH 5.48). The potential for external acidification of the soils could explain the low proportion of spores observed in certain AMF species [43]. Additionally, the monoculture cultivation practice promotes a decrease in spore density compared to crop rotation [44]. The monoculture employed in the study locations can

account for the obtained results.

In the two AEZs, large-diameter spores were prevalent. These results were different with the findings of [35] [45], who noted that the number of AMF spores was inversely proportional to their size in their respective studies. The AMF spores from the strawberry rhizosphere were black, dark brown, white, hyaline, brown, and light yellow. These findings revealed a greater variety of colors than those reported by [45], who identified spores with only black, brown, white, and yellow colors in the *Zea mays* rhizosphere in Benin. The relative abundance of AMF in the *Fragaria* spp. rhizosphere revealed 11 different morphotypes in AEZ III and 12 in AEZ V, with some species specific to each AEZ. This discrepancy in species richness could be attributed to the fact that the two AEZs were situated at different altitudes and possessed distinct ecological conditions. These results aligned with those of [46], who demonstrated that altitude influences the diversity of AMF in the potato rhizosphere.

Thirteen species from seven genera and five families (Gigasporaceae, Paraglomeraceae, Diversisporaceae, Glomeraceae, and Acaulosporaceae) were identified in the strawberry rhizosphere across both AEZs III and V. The species richness was greater than that reported in Cameroon by [47] for *Carica papaya*. However, the species richness was lower than that noted by [48] for voandzou (16 species) and by [49] for pumpkins (15 species). In the present study, the genera *Gigaspora*, *Glomus*, *Paraglomus*, *Acaulospora*, and *Scutellospora* were most abundant in AEZ III, while in AEZ V, the same genera were the most prevalent. The dominance of the genus *Glomus* in the tropics may result from its high competitiveness and adaptation that enables it to establish itself better than other AMF genera under tropical conditions. This could also be because its development cycle remains unaffected by crop repetition on land compared to non-dominant genera (*Acaulospora*, *Gigaspora*, and *Scutellospora*) [50]. The study also examined the impact of fertilization on the growth and fruit production of strawberries (var. Madame Moutot and var. Mara des Bois) in AEZ III and V, revealing that both mineral fertilizers and AMF-based biofertilizers enhanced leaf formation, the number of runners per plant, the number of fruits per plant, the average fruit weight, and the P and N contents under specific conditions. Therefore, Mara des Bois variety exhibited the highest leaf count in AEZ V when treated with mineral fertilizer. This result aligned with that of [51], who reported the greatest number of strawberry leaves from mineral fertilization. Tripathi *et al.*, [52] also noted that biological fertilization led to higher leaf count compared to the control in strawberry. Madame Moutot variety produced the highest number of runners under mineral fertilization in AEZ V, which contradicts the findings of Rahman *et al.*, [53], who observed that organic fertilization yielded the most runners compared to mineral options. The highest average fruit weight was recorded with Madame Moutot variety harvested under mineral fertilizer, followed closely by those treated with AMF-based biofertilizer in AEZ V. This outcome was consistent with [51], who noted that AMF-based biofertilizer led to greater strawberry fruit weights, as well

as with findings from [54], which indicated that soybean productivity improved (by 74%) due to mycorrhizal colonization. The Mara des Bois variety yielded highest fruits when grown with mineral fertilizer, followed by AMF-based biofertilizer across both AEZs. This matched the results of [55], who found that mineral fertilization was advantageous for producing the highest fruit counts compared to organic fertilization. The P and N contents were significantly elevated in strawberry fruits grown with AMF-based biofertilizer this could be due to the fact that, the wood chip mulching promotes AMF by improving soil structure, retaining moisture (without excess), and providing carbon substrates for symbionts, followed by those treated with mineral fertilizer. The highest levels of P and N were observed in the Mara des Bois variety in AEZ III. Variations in P and N content among varieties could be attributed to genetic differences in nutrient absorption capabilities [56]. It is well acknowledged that the function of mycorrhizal networks plays a crucial role in facilitating nutrient transfer between plants [57]. This finding corroborates those who found that AMF significantly enhanced N, P, and K uptake in wheat [58] and P uptake in fenugreek [59].

## 5. Conclusion

Strawberry (*Fragaria* spp.) is mycotrophic in agroecological zones III and V of Cameroon, located in sub-Saharan Africa. Thirteen (13) morphotypes of arbuscular mycorrhizal fungi (AMF) belonging to seven genera and five families have been identified in the strawberry rhizosphere and its roots. The AMF spores were characterized by seven colors, three distinctive structures, and diameters. The AMF species richness, relative abundance, and diversity varied with altitude, locality, and strawberry variety. Mineral fertilizer and AMF-based biofertilizer enhanced strawberry leaf formation, the number of runners per plant, the number of fruits per plant, the average fruit weight, and the P and N contents in Cameroon agroecological zones III and V. These findings indicate that strawberry cultivation using arbuscular mycorrhizal fungi in Cameroon's agroecological zones III and V can produce fruits with high phosphorus and nitrogen content. This study highlights the potential of AMF to enhance strawberry production and fruit quality in this specific context and opens the perspective for evaluating the effects of associated fertilizers. Therefore, local farmers must be aware of the economic, health, and environmental benefits of using AMF instead of chemical fertilizers. This study opens the perspective of assessing the effects of associated fertilizers on strawberry production and fruit nutrient contents in sub-Saharan Africa. Also, a molecular characterization of AMF will be performed to obtain more accurate results, as we have already performed morphological characterization.

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## Conflicts of Interest

The authors declare no competing interests.

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## Glossary

AEZ III	Agroecological zone of the Western Highlands of Cameroon
AEZ V	Agroecological zone of the bimodal rainforest area of Cameroon
AMF	Arbuscular mycorrhizal fungi
Ds	Simpson diversity index
H'	Shannon-Wiener diversity index
J'	Pielou equitability index
l	Simpson dominance index
S	Total number of spores