

# Ginger response and Soil fertility under Inoculation Indigenous Microorganisms (IMO) and Arbuscular Mycorrhizal Fungi (AMF): A Comparative Study in the Highlands (Baboutcha Fongam) and Forest (Tondè) areas of Cameroon

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## ABSTRACT

This study aims to evaluate the impact of Indigenous Microorganisms (IMO) and Arbuscular Mycorrhizal Fungi (AMF) on soil fertility, growth, and yield of ginger (*Zingiber officinale*) in two contrasting agro-ecological zones of Cameroon: the highlands of Baboutcha Fongam and the forest zone with monomodal rainfall of Tondè. The results show that the application of biofertilisers, particularly IMO and AMF, significantly ( $P < 0.05$ ) improves soil fertility by increasing nitrogen content, organic matter, and cation exchange capacity, while maintaining a stable pH. Agronomically, these biofertilisers promote more vigorous vegetative growth, with a notable increase in height, number of leaves, collar diameter, and leaf area. In terms of yield, IMO resulted in the best performance, with significant gains compared to the controls and NPK chemical fertilisers, which showed limited or even negative effectiveness in some cases. The morphological characterization of AMF spores reveals a significant diversity, indicating the ecological richness of the soils and their role in mineral nutrition. These results highlight the importance of integrating biofertilisers into sustainable agricultural practices to improve ginger productivity while preserving soil health in these tropical environments.

**Keywords:** Ginger (*Zingiber officinale*); Indigenous Microorganisms (IMO); Arbuscular Mycorrhizal Fungi (AMF); Biofertilisers; Soil fertility; Crop Yield; Agro-ecological Zones; Cameroon (Baboutcha Fongam and Tondè); Sustainable Agriculture; Nutrient Uptake (Soil-plant Interaction).

## 1. Introduction

Ginger, *Zingiber officinale* Roscoe, is an annual plant of the Zingiberaceae family, widely cultivated in tropical areas primarily for its rhizomes, which play a crucial role in cooking and medicine. Native to Southeast Asia, it is recognized for its numerous medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial effects [1,2,3,4]. During the COVID-19 pandemic, ginger was particularly recommended for its preventive and therapeutic properties.

However, ginger production faces several challenges, including pest pressure, soil diseases, excessive use of chemical fertilisers, which lead to pollution and health risks [5,6], and inappropriate fertilization practices [7,4]. Soil fertility has traditionally been improved through chemical products, but this approach raises environmental concerns [8,9,10].

To address these issues, research is focusing on sustainable alternatives such as biofertilisers. These include beneficial microorganisms like mycorrhizal fungi and indigenous microorganisms, which can improve soil fertility and stimulate plant growth while reducing the need for chemical fertilisers [11,12,13]. Biofertilisers restore the natural nutrient cycle and promote more environmentally friendly agricultural practices.

While several studies have examined the effect of chemical and organic fertilisers on ginger in terms of growth and yield [14,15], there is limited research on the impact of biofertilisers. In Cameroon, ginger is cultivated in various agro-ecological zones, with production significantly increasing from 7,761 tons in 2002 to 79,273 tons in 2016, but

a decline to 65,538 tons was observed in 2018 [16,17]. This raises the question of sustainable agricultural practices that could increase ginger production while maintaining its quality in the face of growing demand, especially during health crises.

### 1.1. Study Objectives

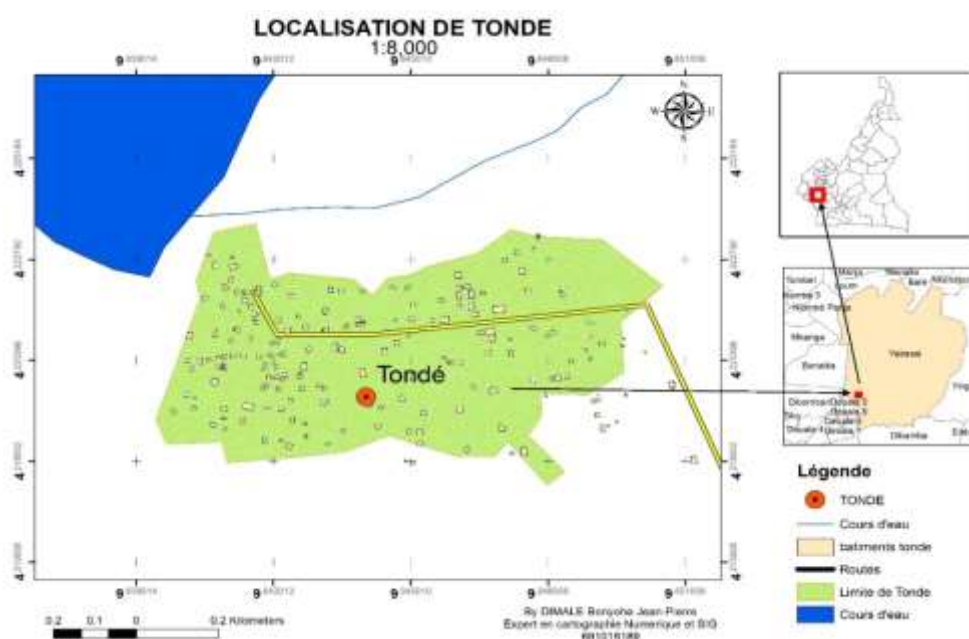
The objective of this work is to:

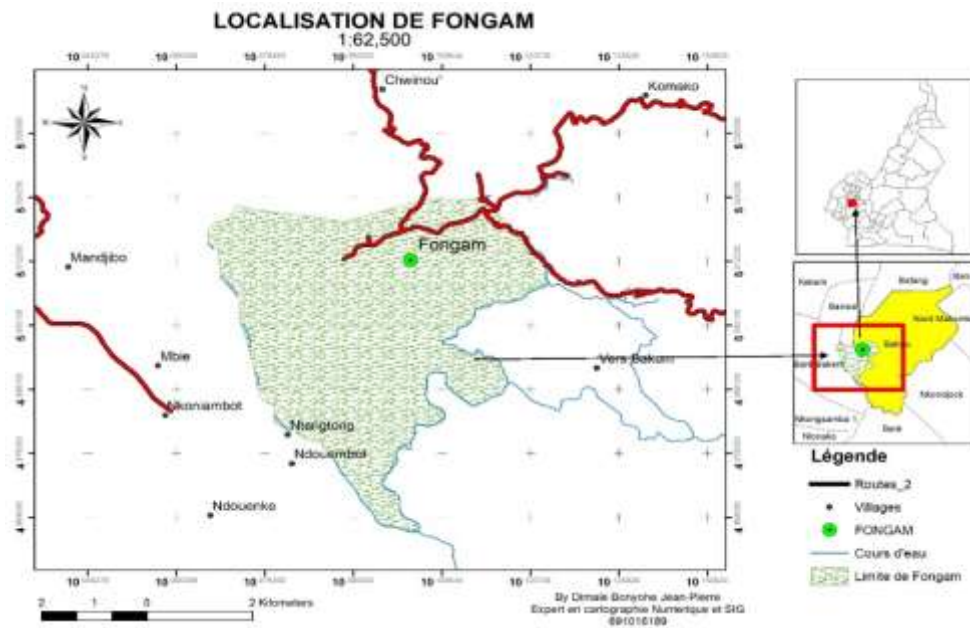
- 1) Evaluate the impact of biofertilisers (IMO and AMF) on the physicochemical properties of the soils of Tondè and Baboutcha Fongam.
- 2) Compare the effectiveness of biofertilisers versus chemical fertilisers (NPK) on ginger growth.
- 3) Measure the influence of treatments on yield parameters (rhizome weight) per hectare.
- 4) Characterize the morphological diversity of AMF spores present in the two study areas.
- 5) Determine the relative abundance of mycorrhizal fungal communities in different pedoclimatic contexts.
- 6) Propose sustainable agricultural practices adapted to the local conditions of Cameroon.

## 2. Literature Review Materials and Methods

### 2.1. Study Areas

The study was conducted in two regions of Cameroon: the humid forest zone with monomodal rainfall (Coastal: Tondè pk 28) and the highland zone of the West (West: Baboutcha Fongam), which have distinct climatic and pedological conditions (Figure 1). The coastal zone, characterized by a humid climate and high rainfall, has ferrallitic and organic soils, while the western zone, with a more temperate climate and moderate rainfall, features leached ferrallitic soils [18,19]. These environmental differences provide an opportunity to study the impact of pedoclimatic conditions on soil microbiology and ginger growth.





**Figure 1.** Study Sites: Tondè and Baboutcha Fongam

## 2.2. Experimental Design and Treatments

Ginger rhizomes obtained from the markets were divided into four treatments: T0 (control treatment with no amendment); T1 (treatment with Indigenous Microorganisms (IMO)); T2 (treatment with Arbuscular Mycorrhizal Fungi (AMF)); and T3 (treatment with NPK). These treatments were applied in an experimental design consisting of 4 completely randomized blocks with three replications. The blocks were spaced 1 meter apart, covering an area of 4 m<sup>2</sup>. Between the rows, plants were spaced 0.25 meters apart, with 64 plants per block of 4 m<sup>2</sup> [20]. Ginger rhizome pieces, measuring 2.5 cm in length and containing 2 to 3 buds, were planted at a depth of 10 to 15 cm. Fertilisers were applied according to ginger production recommendations [21]. IMOs were prepared according to the method of Ngueuleu [22] and used at a rate of 20 g per plant. The AMF, prepared following the method of Ngonkeu [23] and consisting of a mixture of *Gigaspora margarita* and *Acaulospora tuberculata*, were applied at a rate of 100 g per plant. IMOs were applied one week before planting, while AMF were applied at the same time as planting. As for the NPK, it was applied one month after planting at a rate of 1.5 g per plant. Crop maintenance was carried out monthly until harvest.

## 2.3. Physicochemical Soil Analysis Methods

Soil samples were collected at different depths (0-20 cm, 20-40 cm) and then mixed. Samples were taken both before planting ginger and after harvesting it, using a sterile auger to avoid cross-contamination. Each site was represented by multiple sampling points to ensure statistical representativeness. Samples were stored in sterile plastic bags, labelled, and transported to the laboratory under refrigerated conditions (4°C) for immediate analysis or stored at -20°C for later analysis [24].

These soil samples were analysed at the Soil and Plant Laboratory of IRAD in Nkolbisson (Yaoundé). Physical properties were determined through soil particle size analysis. Soil chemical properties were determined using various methods: total nitrogen, available phosphorus, potassium, organic carbon, magnesium, calcium, iron, pH,

electrical conductivity, cation exchange capacity (CEC), sodium, and organic matter content, according to the methods described by [25].

#### 2.4. Determination of Growth and Yield Parameters

Plant growth was monitored at each site over five weeks at regular intervals. Nine plants per treatment were randomly selected to determine the following growth parameters: stem length, leaf number, collar diameter, and leaf area. Leaf area (S) was calculated using the formula:

$S = 0,8 \times L \times l$ ; where **L** and **l** are the length and width of the last leaf produced [20]. Tubers from all plants were harvested, and the weight and yield of the tubers were calculated per hectare.

#### 2.5. Extraction of Spores and Morphological Characterization

Spore extraction was done using the protocol described by Schenck and Perez [26]. The procedure involves:

- 1) Adding 100 g of soil into a 1-liter Erlenmeyer flask.
- 2) Adding at least 300 ml of tap water and homogenizing the mixture. 1 to 2 drops of 0.05% Tween 20 were added to the mixture (to dissolve aggregates).
- 3) The mixture was then allowed to rest for 15 seconds before being filtered through two sieves with mesh sizes of 710  $\mu\text{m}$  and 45  $\mu\text{m}$ .
- 4) The washing and decanting process was repeated at least 3 times.
- 5) The material in the 45  $\mu\text{m}$  sieve was transferred by washing into a Petri dish with a grid, and spore observation was conducted under a stereomicroscope. Extracted spores were stored at 4°C in distilled water or between glass slides. Morphological characterization was performed according to the protocol described by Morton [27]. The identification of spores, as described by Morton, was based on their size, colour, ornamentation of their hyphal suspensor or bulbous suspensor, sporiferous sacs, and the germination loop.

#### 2.6. Determination of AMF Relative Abundance

The presence of spores is the standard method for estimating the presence of arbuscular mycorrhizal fungal species. The communities of these fungi present in the soil can be estimated in terms of the number of species and the abundance of each species in the community [28]. The abundance estimation can be done by directly observing the number of spores present in the soil [29]. The spore trapping extraction technique was used. The relative abundance (AR) of the spores was calculated using the formula from Johnson [30]:

$AR = 100 (m/M)$ ; where **m** = total number of a genus observed across all plots; **M** = total number of spores observed across all plots.

#### 2.7. Statistical Analyses

Data are presented as mean  $\pm$  standard deviation. All statistical analyses were conducted using R software, version 3.2.1. Data processing and the generation of graphs were performed using Excel 2016. Significant differences between the experimental and control groups were determined using ANOVA. For the comparison of means,

Duncan's multiple range tests were applied at a 5% probability level. Pearson's correlation and regression coefficients were calculated to evaluate the impact of species richness and spore abundance on diversity. Given that the dataset was found to be non-parametric, appropriate non-parametric tests were employed for the analysis.

### 3. Results

#### 3.1. Physicochemical Soil Analysis of the Two Zones

##### 3.1.1. Physical Analyses

Granulometric analyses were carried out to identify the texture of the soil samples. At the Tondè site, soil analysis in 2022 and 2023 showed a drop in clay and silt content in the surface horizon (0-20 cm). Clay decreased from 14.20% to 10%, while silt fell from 32.30% to 27%. Conversely, the sand content rose from 53.50% to 63% (Table 1). Despite these changes, sand and silt levels remained higher than clay levels. This gives the soils of this site a sandy-loam texture according to the soil texture triangle [26].

At the Baboutcha-Fongam site, sand was predominant, increasing from 71.5% in 2022 to 74% in 2023. During the same period, clay and silt contents decreased slightly, averaging 16.25% and 11% respectively. Because sand and clay levels were higher than silt, the soil at this site has a sandy-clay texture [26].

**Table 1.** Granulometry of Tondè Granulometry (%)

Year	Clay	Coarse Sand	Fine Sand	Coarse Silt	Fine Silt
2022	14.20 ± 1.20	27.90 ± 2.10	25.60 ± 1.80	26.00 ± 1.60	6.30 ± 0.50
2023	10 ± 1	35 ± 2	28 ± 1	23 ± 2	4 ± 1

**Table 2.** Granulometry of Baboutcha Fongam Granulometry (%)

Year	Clay	Coarse Sand	Fine Sand	Coarse Silt	Fine Silt
2022	17 ± 1	41.5 ± 1.5	30 ± 1	7 ± 1	4.5 ± 1.5
2023	15.5 ± 1.5	44 ± 1	30 ± 1	5.5 ± 1	5 ± 1.5

##### 3.1.2. Chemical Analyses

###### pH levels

The soils at both sites are generally acidic. At Tondè, the average water pH was 6.43 ± 0.05, while at Baboutcha-Fongam, it was 6.3 ± 0.05. The use of fertilisers (IMO, AMF, and NPK) caused a slight decrease in pH. For example, in plots treated with IMO, the water pH dropped from 5.86 ± 0.09 to 5.7 ± 0.00 at Tondè (Table 3), and from 5.4 ± 0 to 4.7 ± 0 at Baboutcha-Fongam (Table 4).

### **Total Nitrogen Content and C/N Ratio**

Before fertilisation, both sites had very low nitrogen levels.

- Tondè: Nitrogen was 0.03% in 2022 and 0.11% in 2023, with high C/N ratios (>14).
- Baboutcha-Fongam: Nitrogen was 0.042% in 2022 and 0.16% in 2023.

After adding fertilisers, the soils became nitrogen-rich. In plots enriched with IMO and AMF, C/N ratios improved significantly at both sites. In contrast, NPK treatment caused the C/N ratio to drop sharply by 2023 (6.66 at Tondè and 7.8 at Baboutcha-Fongam) (Table 3) and (Table 4).

### **Organic Matter Content**

- Tondè: Before fertilisation, the organic matter was stable at 2.8% in 2022 and 2023. After treatment, it increased by 4.42% with IMO and 4.66% with NPK, but decreased by 2.67% with AMF (Table 3).
- Baboutcha-Fongam: The initial average organic matter was 5.1% between 2022 and 2023. After fertilisation, it increased by 5.31% with IMO, but fell with both AMF (4.42%) and NPK (4.75%).

### **Cation Exchange Capacity (CEC)**

At Tondè, the initial CEC increased from  $11.41 \pm 1$  to  $12.8 \pm 0$  cmol/kg between years. After fertilisation, values changed from  $15.71 \pm 1.02$  to  $12.3 \pm 0.5$  cmol/kg with IMO between 2022 and 2023 (Table 3).

At Baboutcha-Fongam, the CEC was  $13.88 \pm 0.6$  cmol/kg in 2022 but dropped to  $10.4 \pm 0$  cmol/kg in 2023 before treatment. Following fertilisation, AMF treatment recorded the highest value at  $15.22 \pm 0.6$  cmol/kg in 2022 and  $13.69 \pm 0.36$  cmol/kg in 2023 (Table 4).

### **Available Phosphorus Content**

At the Tondè site, the baseline available phosphorus content was very low at 4.6 ppm in 2022, rising to 27 ppm in 2023. Following IMO fertilization, levels increased from 24.90 ppm in 2022 to 44.35 ppm in 2023. Conversely, AMF and NPK applications led to declines over the same period, falling from 58 ppm to 44.35 ppm and 31.98 ppm to 30.5 ppm, respectively (Table 3).

At the Baboutcha-Fongam site, initial phosphorus levels were also very low at 4.9 ppm in 2022, increasing to 11.15 ppm by 2023. IMO fertilizer use raised the content from 20.4 ppm in 2022 to 24.9 ppm in 2023. Under AMF and NPK treatments, phosphorus levels showed minor fluctuations, shifting from 35.33 ppm to 24.9 ppm and 20.12 ppm to 22.5 ppm, respectively, between 2022 and 2023 (Table 4).

In summary, the soils at both sites are naturally acidic with low nitrogen and organic matter. However, applying IMO, AMF, or NPK fertilisers improved key parameters such as nitrogen content, organic matter, and CEC, while maintaining stable acidity levels.

**Table 3.** Chemical Analyses at Tondè Before and After Fertilizer Application

Year	Fertilisers	Sampling	Organic Carbon (%)	Total Nitrogen (%)	C/N Ratio	Organic Matter (%)	Available Phosphorus (ppm)	pH (Water)	pH (KCl)	CEC (cmolc/kg)
2022	T0 (Control)	P0	0.75 ± 0.05c	0.032 ± 0.001d	23.4 ± 0.02a	2.8 ± 0.01e	4.6 ± 0.1e	6.45 ± 0.1a	5.2 ± 0	11.41 ± 1c
	T1 (IMO)	P	2.69 ± 0.06b	0.182 ± 0.003b	14.7 ± 0.01c	3.75 ± 0.1c	19.07 ± 2.21d	5.98 ± 0.12b	4.9 ± 0	12.34 ± 0.52b
		IMO	2.96 ± 0.06b	0.35 ± 0.003a	8.45 ± 0.2d	3.98 ± 0.1b	24.90 ± 1.46c	5.86 ± 0.09c	4.95 ± 0	15.71 ± 1.02a
		AMF	4.24 ± 0.01a	0.35 ± 0a	12.11 ± 0.3b	3.32 ± 0.1d	58 ± 0.46a	5.95 ± 0.11b	4.9 ± 0	12.79 ± 0.3b
		NPK	2.63 ± 0.06b	0.13 ± 0.001c	20.23 ± 0.7b	4.47 ± 0.47a	31.98 ± 0.47b	5.9 ± 0.1b	4.85 ± 0	11.63 ± 0.3c
2023	T0 (Control)	P0	1.64 ± 0.1 <sup>e</sup>	0.11 ± 0.003c	14.9 ± 0.9c	2.82 ± 0.1b	27 ± 2.34d	6.4 ± 0.01a	5.2 ± 0	12.8 ± 0b
	T1 (IMO)	P	3.25 ± 0.38a	0.12 ± 0c	27.1 ± 0.5a	2.3 ± 0.2c	28.5 ± 0.3d	5.85 ± 0.01d	4.85 ± 0	13.6 ± 2.1a
		IMO	2.5 ± 0.4c	0.25 ± 0.01c	10 ± 0.3b	4.85 ± 0.21a	44.35 ± 0.52a	5.7 ± 0b	4.95 ± 0	12.3 ± 0.5c
		AMF	2.82 ± 0.8b	0.22 ± 0.02b	12.81 ± 0.7d	2.02 ± 0.22c	39.69 ± 2.16b	5.85 ± 0d	5.05 ± 0	12.8 ± 1.02b
		NPK	2.8 ± 0.2b	0.42 ± 0.03a	6.66 ± 0.4e	4.85 ± 0.31a	30.5 ± 1.45c	5.75 ± 0.01c	4.95 ± 0	10.57 ± 0.3d

The means in the same column followed by different letters are significantly different according to the Duncan test at 5%. SD: Standard deviation; T0: control; T1: treatment after fertilizer amendment; P0: sampling before fertilizer amendment; P: mean of samplings after fertilizer amendment; Phosphor ass: Available phosphorus; CEC: Cation exchange capacity.

**Table 4.** Chemical Analyses at Baboutcha Fongam before and after application of fertilisers

Year	Fertilisers	Sampling	Organic Carbon (%)	Total Nitrogen (%)	C/N	Organic Matter (%)	Available Phosphorus (ppm)	pH (Water)	pH (KCl)	CEC (cmolc/kg)
2022	T0 (Control)	P0	0.88 ± 0.2c	0.042 ± 0.001d	20.95 ± 0.26a	3.8 ± 0.01e	4.9 ± 0.1d	6.5 ± 0a	5.2 ± 0	13.88 ± 0.6b
	T1 (IMO)	P	3.59 ± 0.02a	0.21 ± 0.004b	17.1 ± 0.6b	6.1 ± 0.14b	17.12 ± 0.5c	5.5 ± 0b	4.7 ± 0	13.56 ± 0.25b
		IMO	3.61 ± 0.02a	0.34 ± 0.001a	10.61 ± 0.05c	5.5 ± 0.01c	20.4 ± 1.5b	5.4 ± 0b	4.85 ± 0	13.68 ± 0.3b
		AMF	3.74 ± 0.08a	0.28 ± 0.004c	13.35 ± 0.7a	4.5 ± 0.1d	35.33 ± 1.03a	5.3 ± 0b	4.8 ± 0	15.22 ± 0.6a
		NPK	3.415 ± 0.04b	0.21 ± 0.006b	16.26 ± 0.7b	6.7 ± 0.1a	20.12 ± 12.83b	5.5 ± 0b	5.1 ± 0	12 ± 0.5c
2023	T0 (Control)	P0	3.7 ± 0.1a	0.16 ± 0.01b	23.1 ± 1.3a	6.4 ± 0.1a	11.15 ± 1.78d	6.1 ± 0a	4.9 ± 0	10.4 ± 0d
	T1 (IMO)	P	3.45 ± 0.2b	0.205 ± 0.005a	16.82 ± 1.14b	6 ± 0.3b	16 ± 0.8c	4.8 ± 0c	4.35 ± 0	12.76 ± 0.5c

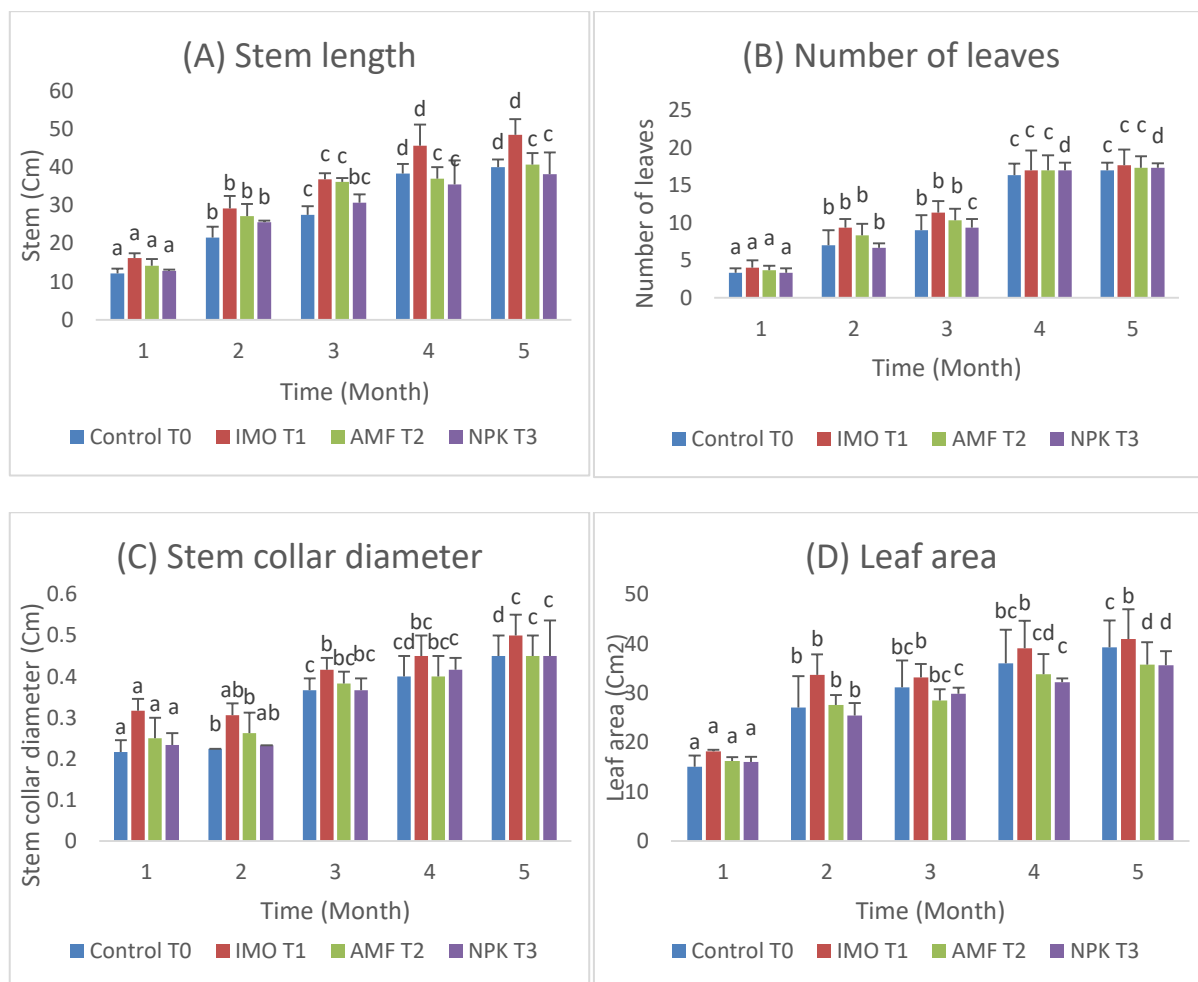
		IMO	2.97 ± 0.05c	0.27 ± 0.006a	11 ± 0.5c	5.12 ± 0.08c	24.9 ± 0.6a	4.7 ± 0c	4.15 ± 0	12.36 ± 1.5c
		AMF	2.5 ± 0.1c	0.236 ± 0.004a	10.59 ± 0.36d	4.35 ± 0.18d	22.5 ± 1.18b	4.75 ± 0c	4.15 ± 0	13.69 ± 0.36b
		NPK	1.6 ± 0.2d	0.205 ± 0.007a	7.8 ± 0.81e	2.8 ± 0.3e	15.14 ± 0.16c	4.75 ± 0c	4.2 ± 0	13.2 ± 2.1a

The means in the same column followed by different letters are significantly different according to the Duncan test at 5%. SD: Standard deviation; T0: control; T1: treatment after fertilizer amendment; P0: sampling before fertilizer amendment; P: mean of samplings after fertilizer amendment; Phosphor ass: Available phosphorus; CEC: Cation exchange capacity.

### 3.2. Growth Parameters

#### 3.2.1. Growth Parameters at Tondè

The analysis of ginger growth parameters at Tondè reveals that different fertilisers had a marked influence on the vegetative development of the plants. Generally, all measured parameters stem length, number of leaves, collar diameter, and leaf area increased progressively over time, showing significant differences ( $P < 0.05$ ) between treatments (Figure 2).

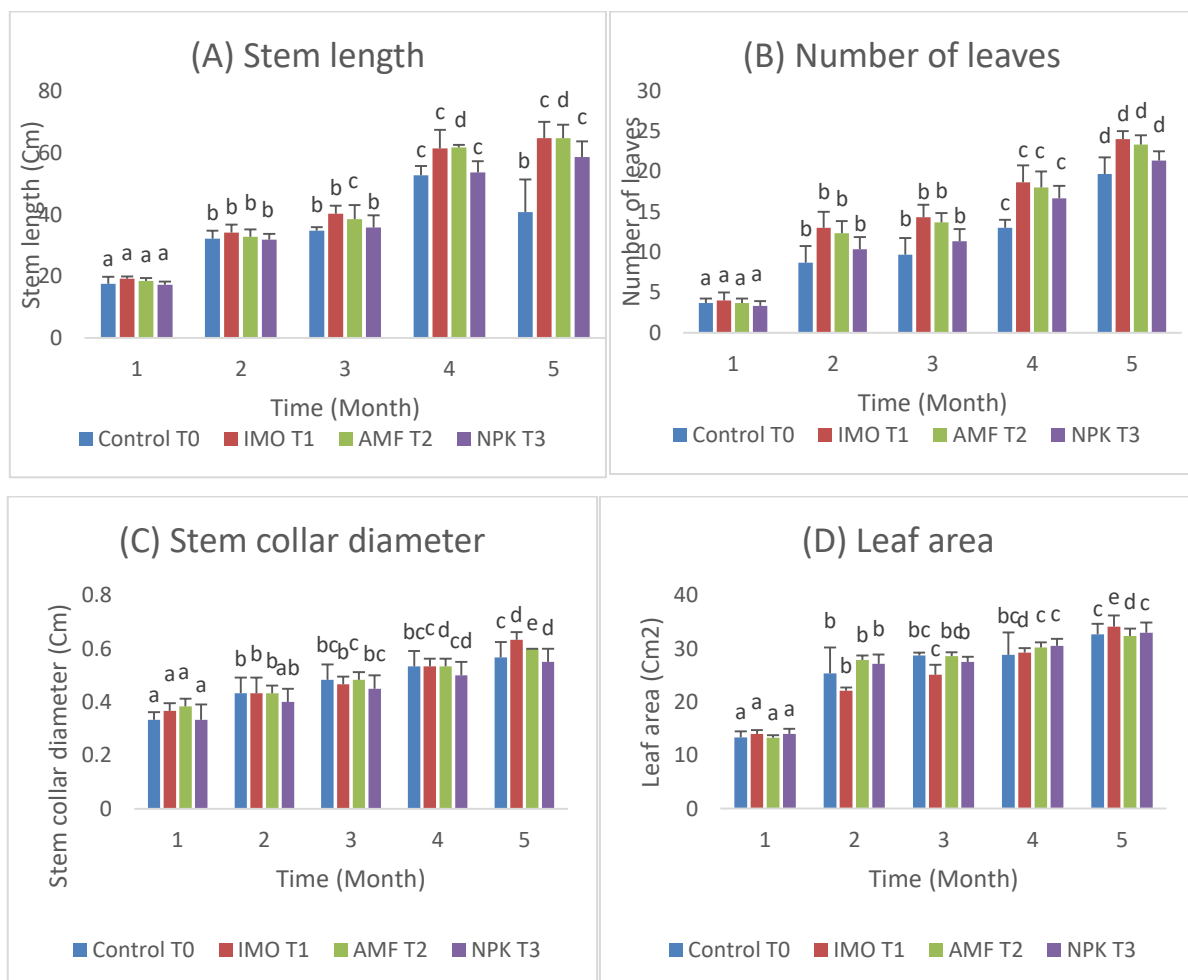


**Figure 2.** Growth parameters at Tonde: (A) stem length, (B) number of leaves, (C) collar diameter, (D) leaf area

From the third month, stem length showed a noticeable improvement under the influence of (AMF) and NPK, suggesting enhanced nutrient absorption that favours cell elongation (Figure 2A). Similarly, the number of leaves was significantly higher ( $P < 0.05$ ) under AMF and NPK compared to the control and (IMO) treatments; this indicates superior foliar branching and a potential increase in photosynthetic activity (Figure 2B). The collar diameter followed a similar trend, with the highest values recorded under AMF and NPK, reflecting better plant vigour and stability (Figure 2C). Furthermore, leaf area, a parameter directly linked to photosynthetic capacity, was strongly stimulated by AMF and NPK, surpassing the control and IMO treatments as early as the second month (Figure 2D). Overall, these results confirm that mycorrhizal fertilisers significantly ( $P < 0.05$ ) improve ginger growth, performing similarly to synthetic chemical fertilisers (NPK).

### 3.2.2. Growth Parameters at Baboutcha Fongam

The analysis of ginger growth parameters at Baboutcha Fongam reveals a steady progression in stem length, leaf count, collar diameter, and leaf area throughout the vegetative cycle. However, significant differences ( $P < 0.05$ ) were observed between the various treatments (Figure 3).



**Figure 3.** Growth parameters at Baboutcha Fongam: (A) stem length, (B) number of leaves, (C) collar diameter, (D) leaf area

Regarding stem length (Figure 3A), all treatments showed a progressive increase over the five-month observation period. Notably, plants treated with NPK and IMO exhibited significantly longer stems ( $P < 0.05$ ) from the fourth

month onwards, indicating superior vegetative vigour compared to the control group. The number of leaves (Figure 3B) followed a similar trend; while natural growth occurred across all treatments, the application of fertilisers particularly NPK and IMO, resulted in a higher leaf count. This confirms that both mineral and organic nutrition stimulate leaf production, thereby enhancing photosynthetic potential. Similarly, collar diameter (Figure 3C) showed an upward growth trend. Although differences between treatments were initially less pronounced, NPK and IMO treatments demonstrated significant superiority from the fourth month, reflecting improved plant robustness. Finally, leaf area (Figure 3D) showed the most distinct variations, with fertilised plants (especially those receiving NPK and IMO) developing a significantly larger leaf area ( $P < 0.05$ ) than the controls. This offers a major physiological advantage, as a larger leaf area correlates directly with more efficient photosynthesis and, consequently, a higher yield.

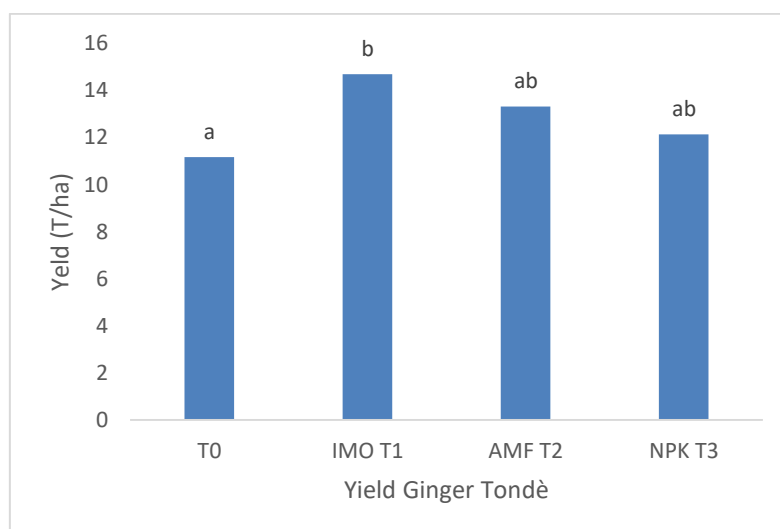
### 3.3. Ginger Yield in the Cultivation Sites

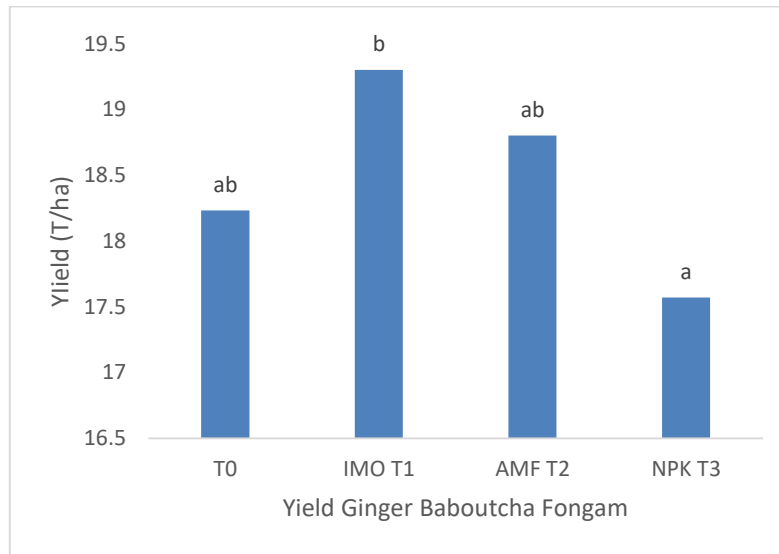
The analysis of ginger yields at the two study sites shows a significant variation ( $P < 0.05$ ) depending on the treatments applied (Figure 4).

At the Tondè site, the use of IMO (T1) produced the highest yield at approximately  $14.67 \text{ t/ha}^2$ . This was significantly higher ( $P < 0.05$ ) than the control group (T0), which yielded about  $11.15 \text{ t/ha}^3$ . While the AMF and NPK treatments did improve yields compared to the control, these differences were not statistically significant. These results suggest that indigenous microorganisms are more effective at increasing ginger productivity in Tondè than other treatments.

At Baboutcha Fongam, a similar trend was observed. The IMO treatment (T1) achieved the best yield at approximately  $19.30 \text{ t/ha}$ . The AMF (T2) and control (T0) followed with yields of  $18.80 \text{ t/ha}$  and  $18.23 \text{ t/ha}$ , respectively. There was a significant difference ( $P < 0.05$ ) between the IMO treatment and the other groups.

Overall, the application of indigenous microorganisms (IMO) led to a significant increase in ginger yield at both study sites. These findings highlight the superior performance of biological fertilisers, particularly IMO, in this region. In contrast, chemical fertilisers (NPK) did not lead to a notable improvement in yield and actually had a negative effect at the Baboutcha site.



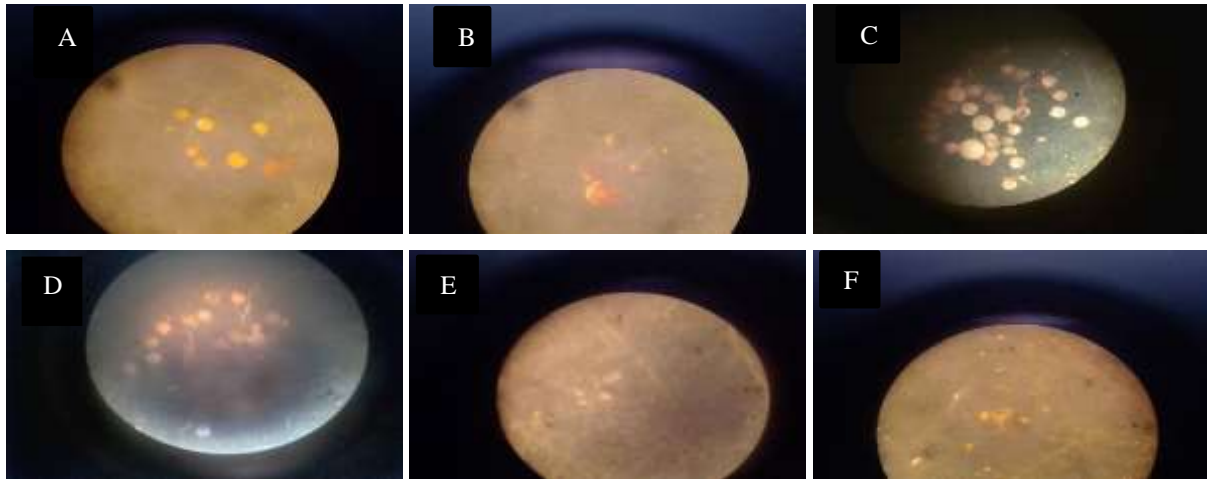


**Figure 4.** Ginger Yield in Tondè and Baboutcha Fongam

### 3.4. Morphological Characterization and Identification of AM Fungi from Spores

The morphological identification of mycorrhizal spores from the two study sites was based on standard criteria: diameter, colour, spore structure, and the presence of suspensor bulbs. Seven morphotypes were identified across the Tondè and Baboutcha zones. These include six known genera (*Gigaspora*, *Glomus*, *Scutellospora*, *Diversispora*, *Paraglomus*, and *Dentiscutata*) and one unidentified genus. This diversity is typical of tropical and cultivated soils and may support plant mineral nutrition. The morphological description of some of the morphotypes is as follows:

- 1) **Morphotype T1 (*Gigaspora* sp):** These are solitary, smooth, yellow spores with a spherical shape and a diameter exceeding 250  $\mu\text{m}$ . The spore wall consists of a single group of membranes topped with a suspensor bulb (**Figure 5A**).
- 2) **Morphotype T2 (*Scutellospora* sp):** These spores are solitary, white, smooth, and spherical, with a diameter greater than 250  $\mu\text{m}$ . The wall is formed by one group of membranes and features both a suspensor bulb and a germination shield (**Figure 5B**).
- 3) **Morphotype T3 (*Dentiscutata* sp):** These are solitary, black, smooth, and spherical spores with a diameter over 250  $\mu\text{m}$ . They have a single group of wall membranes and a suspensor bulb (**Figure 5C**).
- 4) **Morphotype T4 (*Diversispora* sp):** These spores range from spherical to ellipsoid and are hyaline (translucent) with a diameter of approximately 125  $\mu\text{m}$ . The spore wall is made of two groups of membranes and is topped with suspensor hyphae (**Figure 5D**).
- 5) **Morphotype T6 (*Glomus* sp):** These are light yellow, globular or spherical spores with a diameter between 45 and 125  $\mu\text{m}$ . The wall consists of a single group of membranes with very little variation (**Figure 5E**).
- 6) **Morphotype T7 (*Paraglomus* sp):** These are hyaline, spherical to ellipsoid spores with a small diameter of about 45  $\mu\text{m}$ . The wall is formed by two groups of membranes topped with suspensor hyphae (**Figure 5F**).



**Figure 5.** Morphological structures of arbuscular mycorrhizal fungal spores. (A- *Gigaspora sp.* spores showing a suspensor bulb; B- *Scutellospora sp.* spores with suspensor bulb and germination loops; C- *Dentiscutata sp.* showing characteristics; D- *Diversispora sp.* showing a suspensor hypha; E- *Glomus sp.* spores showing a suspensor hypha; F- *Paraglomus sp.* spores showing a suspensor hypha).

### 3.5. Diversity and Abundance of AM Fungi

#### 3.5.1. Species Richness of AM Fungi

Following the trapping, extraction, and characterisation processes, the morphological identification of Arbuscular Mycorrhizal (AM) fungal spores was performed across the various soil samples. The relative abundance (RA) of AM spores per gram of soil was found to vary depending on the specific ecosystem type of the rhizosphere soils.

Similarly, the species richness (S) showed variation, with a value of 18 recorded for both the Tondè and Baboutcha zones. The Shannon diversity index ( $H'$ ) ranged from 1.51 to 2.78 in Tondè and 1.54 to 2.79 in Baboutcha. These figures, alongside the Pielou evenness index (E), reflect a diversity of AM fungi within these soils (Table 5).

**Table 5.** Species Richness of AM Fungi

Zone	Treatment	Species Richness	Shannon	Pielou
Tondè	AMF T2	7	2.787	0.993
	IMO T1	4	1.997	0.999
	Control T0	4	1.99	0.995
	NPK T3	3	1.514	0.955
Baboutcha	AMF T2	7	2.795	0.996
	Control T0	4	1.995	0.998
	IMO T1	4	1.995	0.998
	NPK T3	3	1.543	0.974

#### 3.5.2. Average Number of Spores per Treatment and per Zone

The analysis of the average spore count in the Tondè and Baboutcha experimental zones revealed similar trends, though some statistical nuances were noted between the two environments (Table 6).

In Tondè Zone, both the AMF and IMO treatments yielded the best results with statistically equivalent values, indicating high microbial activity. The negative control (T0) produced lower results but did not differ significantly from these treatments. In contrast, the NPK treatment resulted in a significantly lower spore count, suggesting a weaker effectiveness of microorganisms in this environment.

In the Baboutcha Fongam Zone, the AMF treatment maintained its superiority, producing a high and statistically significant number of spores. The IMO treatment showed slightly reduced performance compared to the Tondè zone, suggesting more moderate effectiveness. The control (T0) was statistically less effective than the IMO, while the NPK treatment remained the least effective.

Overall, the AMF treatment proved to be the most effective in both zones for stimulating both root colonisation and spore production. While the IMO treatment remained effective, its performance was slightly diminished in the Baboutcha zone. The control treatment (T0), representing natural soil activity without inoculation, produced intermediate results. Finally, the chemical treatment (NPK) was consistently the least favourable, suggesting that it may inhibit or compromise the natural colonisation of mycorrhizal fungi.

**Table 6.** Number of Spores in Tondè and Baboutcha According to Treatments

Treatments	Spores Tondè	Spores Baboutcha
AMF T2	9.10a	9.33a
IMO T1	8.58a	8.50ab
Control T0	7.75ab	7.41bc
NPK T3	5.66b	5.77b

## 4. Discussion

### 4.1. Physicochemical Parameters of Soils and Impacts of Microorganisms

The physicochemical analysis of the soils from the two study areas, Tondè and Baboutcha-Fongam, reveals distinct characteristics that influence fertility and crop productivity. Granulometric analysis shows that the soil in Tondè has a sandy-loam texture, predominantly sand, while the soil in Baboutcha-Fongam is sandy-clayey, with a high sand proportion of 71.5%. These textures affect water retention, drainage, and nutrient availability [26].

In Tondè, clay and silt content decreased over the years, possibly due to erosion or poor farming practices [31]. Conversely, the increase in sand content may indicate soil degradation, as sandy soils have lower water retention and are more prone to nutrient loss [32].

The soil pH (averaging 6.43 in Tondè and 6.3 in Baboutcha-Fongam) are slightly acidic to neutral. While this acidity suits some crops, a slight decrease in pH was observed after applying fertilisers like Indigenous Microorganisms (IMO), Arbuscular Mycorrhizal Fungi (AMF), and NPK. This aligns with research showing that biofertilisers can cause acidification through the decomposition of organic matter and the release of organic acids [33]. Before fertilization, the soils were nitrogen-poor with high C/N ratios, indicating slow organic matter decomposition. Fertilization significantly increased nitrogen levels, particularly with IMO, which led to very high

contents (8.45% to 10.86%). This increase is attributed to microorganisms in IMO that promote the mineralisation of organic matter [34]. This supports the results of [35], who also found that organic biofertilisers increase nitrogen availability in the soil.

Organic matter results varied by treatment. In Tondè, IMO caused a significant increase, while AMF plots showed a slight decrease. This may be due to faster decomposition in AMF amended plots, as suggested by Zarea [36], who indicated that the application of organic fertilisers influence organic matter dynamics. Cation exchange capacity (CEC), a key fertility indicator, improved after fertilization especially with IMO, which significantly increased calcium and magnesium levels. These results align with those of Ngueuleu [25], who demonstrated that biofertilisers increase CEC by improving soil structure and organic matter accumulation. Finally, assimilable phosphorus remained very low at both sites despite fertilization, highlighting the need for better nutrient management in tropical soils where phosphorus is often limited [25].

In summary, applying fertilisers, especially IMO, positively impacts soil parameters by increasing nitrogen, organic matter, and CEC while maintaining stable acidity. These effects show the importance of sustainable practices for soil health and productivity.

## **4.2. Growth Parameters and Ginger Yield in the Study Sites**

### **4.2.1. Growth Parameters**

The results of this study highlight the significant impact of fertilisers, particularly mycorrhizal fertilisers (AMF) and chemical fertilisers (NPK), on the vegetative growth of ginger. The observed improvements in growth parameters, such as stem length, number of leaves, collar diameter, and leaf area, align with previous studies that emphasise the importance of nutrient supply for optimal plant development [38].

The notable improvement in stem length from the third month under AMF and NPK in Tondè indicates efficient nutrient absorption, which promotes cell elongation. These results support the findings of [39], who demonstrated that mycorrhizal fertilisers can stimulate growth by improving nutrient availability, especially phosphorus, which is essential for cellular growth. The ability of AMF to enhance nutrient uptake is also supported by studies. Recent studies by Thompson [40], suggest that the integration of AMF with nano-fertilisers creates a synergistic effect that surpasses traditional NPK applications. This supports your observation that AMF significantly enhances root absorption surfaces, particularly when phosphorus mobility is traditionally a limiting factor showing that mycorrhizal inoculants increase the root absorption surface, leading to more vigorous growth.

An increased number of leaves under AMF and NPK indicates better foliar branching, which can lead to higher photosynthetic capacity. According to Lee and Kim [41], Increased leaf growth is directly linked to enhanced biomass production, which is crucial for overall plant development. A similar trend observed for collar diameter indicates greater vigour and better plant stability, which is essential for resistance to environmental stresses [42].

The analysis of ginger growth in Baboutcha reveals a steady progression of several indicators, such as stem length, number of leaves, collar diameter, and leaf area, during the vegetative cycle. These results align with literature stating that proper nutrition is vital for optimal plant development. For example, Sarker and colleagues [43] showed

that applying organic and mineral fertilisers promotes more vigorous ginger growth, particularly by increasing stem length and leaf numbers, which is consistent with our observations.

Significant differences observed between treatments, particularly with NPK and IMO, highlight the positive impact of these fertilisers on vegetative growth. The superiority of these treatments from the fourth month onwards indicates effective growth stimulation, likely due to better nutrient availability. A balanced supply of macro and micronutrients, such as those provided by NPK, optimises vegetative growth by improving chlorophyll synthesis and photosynthesis [44].

Regarding collar diameter, the upward growth seen from the fourth month aligns with the results of Kadir and colleagues [45], who reported that plant robustness is enhanced by adequate nutrition through organic and mineral fertilisers. The stability of this growth, with less pronounced differences between treatments later on, may indicate a physiological limit or nutrient saturation at a certain stage. Leaf area is a key parameter for productivity. A larger leaf area, as observed with NPK and IMO, enhances photosynthetic capacity, which can result in higher yields. According to Choudhury [46], increased leaf area is directly related to better light capture, which improves photosynthesis and biomass production. These results emphasise the importance of balanced nutrition to maximise leaf area and yield.

In summary, these results confirm that applying fertilisers, particularly NPK and IMO, significantly stimulates ginger growth by improving vegetative vigour, robustness, and photosynthetic capacity. These observations align with the work of several authors who emphasise the positive impact of balanced fertilisation on growth and productivity [44,45,46].

#### **4.2.2. Yield**

The results of this study show that using indigenous microorganisms (IMO) significantly increased ginger yields at both study sites, Tondè and Baboutcha-Fongam. In Tondè, the highest yield was recorded in plots treated with IMO (14.5 t/ha). At Baboutcha-Fongam, the yield reached 19 t/ha. These findings align with the research of Sturz and Christie [47], who demonstrated that beneficial microorganisms improve soil health by making nutrients more available, which encourages plant growth.

While AMF treatments and NPK fertilisers also improved yields, they were less effective than IMO. The superior performance of IMO may be due to their ability to work symbiotically with ginger roots, helping the plants absorb more water and nutrients [48]. Furthermore, these microorganisms might stimulate the plant's natural defences, making them more resilient to environmental stress and pests. Davies and Gupta [49] suggesting that organic amendments like IMO promote superior resistance to environmental stressors compared to traditional regimes. Ultimately, these observations corroborate the current consensus in the literature that balanced, sustainable nutrition is essential to maximise leaf area and total biomass production in rhizomatous crops. At Baboutcha-Fongam, the yields for AMF and control treatments (18.8 and 18 t/ha) were similar and did not significantly exceed the IMO results. This suggests that indigenous microorganisms create a synergy that promotes both root growth and the breakdown of organic matter, improving nutrient availability for the ginger. This supports the findings of Muyang [35], who noted that organic fertilisers enhance soil nutrients, leading to higher yields.

Interestingly, chemical fertilisers (NPK) performed relatively poorly. They did not lead to a notable improvement in yield and even had a negative impact at Baboutcha-Fongam. This may be because excessive chemical fertiliser use can cause salt to build up in the soil, which harms plant health through osmotic stress [51]. Additionally, relying on these fertilisers can reduce the variety of microbes in the soil, which are vital for decomposition and nutrient cycling [25].

As noted by [53], sustainable practices using biofertilisers like IMO can increase yields while protecting long-term soil health.

In conclusion, applying IMO is an effective strategy for boosting ginger production. These results highlight the value of sustainable farming that promotes soil biodiversity over chemical fertilisers, which may harm productivity in the long run. Future research should further investigate how IMO influence plant growth and soil nutrient dynamics.

#### 4.3. Diversity of AMF

Morphological identification of mycorrhizal spores from the Tondè and Baboutcha-Fongam sites revealed six genera of arbuscular mycorrhizal fungi (AMF): *Gigaspora*, *Glomus*, *Scutelospora*, *Diversispora*, *Paraglomus*, and *Dentiscutata*. The diversity and abundance of spores observed indicate a significant ecological richness typical of tropical cultivated soils. These soils are often linked to beneficial interactions between plants and mycorrhizal fungi, which improve plant mineral nutrition [50].

The presence of various morphotypes, such as *Gigaspora* sp. and *Glomus* sp., is particularly notable. These genera are well-known for enhancing nutrient absorption, especially phosphorus and improving plant resistance to environmental stress [54]. Morphological descriptions show variations in size and structure, suggesting that the spores have adapted to the different environments of the two sites. These variations may also be influenced by soil properties, such as pH and nutrient availability [55].

The relative abundance (AR) and species richness (S) of AMF spores, which reached 18 in both study areas, demonstrate interesting diversity. A Shannon diversity index ( $H'$ ) ranging from 1.05 to 1.94 indicates good AMF diversity, which is vital for maintaining the resilience of rhizosphere ecosystems [56]. Additionally, the Pielou evenness index is close to 1, suggesting that the species present are relatively balanced in terms of abundance.

Results regarding the average number of spores show that AMF and IMO (Indigenous Micro-organism) treatments performed best in both areas. These treatments were significantly efficient at stimulating AMF colonisation and sporulation. This is consistent with research by Zarea [36], which showed that applying AMF can increase spore density and enhance root colonisation.

In contrast, the use of chemical fertilisers (NPK) was less favourable. This supports the work of Hodge and the University of California [58], which indicated that excessive chemical fertiliser use can inhibit mycorrhizal colonisation by disrupting symbiotic interactions. This phenomenon may be caused by an increase in soil salts, which can impair the ability of mycorrhizal hyphae to explore the soil and absorb nutrients [51].

In the Baboutcha area, although the AMF treatment remained the most effective, the IMO treatment was slightly less successful than in the Tondè area. This could be due to differences in soil properties between the two sites, which may influence microbial activity and diversity. Rillig [59] also showed that environmental conditions, including soil moisture and texture, can affect the diversity and abundance of mycorrhizal spores.

In conclusion, this study highlights the importance of AMF in tropical cultivated soils. It emphasises the benefits of agricultural practices that promote colonisation, such as using AMF and IMO biofertilisers. The results suggest that integrated approaches, combining organic fertilisers and sustainable management, are essential for improving soil health and crop productivity. Future research should focus on how different types of fertilisers interact and impact AMF community dynamics in various environments.

## 5. Conclusion

This study highlights the significance of sustainable agricultural practices for ginger (*Zingiber officinale* Roscoe) production in Cameroon, specifically within the regions of Tondè and Baboutcha-Fongam. Physicochemical analyses of the soil revealed varying characteristics that influence both fertility and overall crop productivity.

The application of biofertilisers, particularly indigenous microorganisms (IMO) and arbuscular mycorrhizal fungi (AMF) was shown to have a positive impact on soil health. These treatments increased nitrogen content, organic matter, and cation exchange capacity. Furthermore, the diversity and abundance of mycorrhizal spores found at both sites reflect the ecological richness of tropical cultivated soils. This confirms how vital beneficial plant-fungus interactions are for optimising agricultural yields.

This research demonstrates that integrating biofertilisers (IMO and CMA) is a viable strategy for improving ginger productivity while preserving the health of tropical soils in Cameroon.

### 5.1. Future Suggestions

- Promote the use of locally produced IMO to reduce dependence on chemical fertilisers.
- Further investigate the synergistic mechanisms between CMA and IMO under controlled conditions.
- Disseminate these results to agricultural cooperatives in the Tondè and Baboutcha areas.
- Evaluate the long-term economic impact of replacing NPK with biofertilisers.
- Study the resistance of inoculated plants to soil pathogens specific to ginger.
- Test these biofertilisers on other local ginger varieties to verify performance stability.

### Declarations

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### Competing Interests Statement

The authors declare that they have no competing interests related to this work.

### **Consent for publication**

The authors declare that they consented to the publication of this study.

### **Authors' contributions**

All the authors read and approved the submitted version of the manuscript. Vaugelas Duthie Tefouet conceived the study and wrote the manuscript; Oscar Simplicie Wamba Fotsop and Alphonse Ervé Nouck supervised the analyses; Armand Deuheula Ngueuleu, Paule Danielle Madjouko, Victor Désiré Taffouo, and Eddy Léonard Mangapché Ngonkeu contributed to the fieldwork and review. All the authors reviewed the manuscript and provided with critical input and corrections.

### **Informed Consent**

Not applicable for this study.

### **Availability of data and material**

Data are available upon request from the corresponding author.

### **Research Ethics Board Statement**

Not applicable.

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### **Declaration regarding artificial intelligence**

Artificial intelligence was used solely to assist with the formatting and linguistic correction of the manuscript.

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