

## Research Article

# Assessment of the impact of applied fertilizers on improving agronomic performance, soil properties, and rhizosphere mycorrhizal community in *Xanthosoma sagittifolium* L. Schott PIF plants in the field

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## Abstract

The objective of this work was to evaluate the effect of applied fertilizers on the growth of *Xanthosoma sagittifolium* PIF plants while highlighting their potential influences on changes in soil quality and the nature of mycorrhizal life in the rhizosphere of plants in the field. The experimental design was a randomized complete block. Seeds of PIF plants and *X. sagittifolium* shoots were used. Four treatments were applied: Control (T), Oyster Shells (OS), Phosphorus Solubilizing Bacteria (PSB), and the mixture of shell + phosphorus solubilizing bacteria (OS+PSB). Agronomic growth parameters, analysis of some metabolites in the leaves, and determination of soil physicochemical parameters were evaluated. The mycorrhizal status of *X. sagittifolium* plants and a morphological characterization of the mycorrhizal types were carried out. The results obtained using the Student Newman and Keuls test showed a significant variation in field growth parameters depending on the treatments applied, with an average plant height value of 48.60±8.47 cm in plants from suckers fertilized with BSP. In the leaves of *X. sagittifolium* in sucker plants, high values of chlorophyll (1.03±0.58 mg g<sup>-1</sup>) (OS), total soluble sugars (41.92±19.68 mg g<sup>-1</sup>) OS+PSB, and total soluble proteins (18.78±3.98 mg g<sup>-1</sup>) (OS), respectively, were noted depending on the treatments applied. In PIF plants, a phenolic compound content of 8.82±0.00 mg g<sup>-1</sup> (OS) was noted. Physicochemical analysis of the soils showed an improvement in soil texture and an increase in available phosphorus in the presence of BSP. Mycorrhization indices were higher in the presence of the BSP treatment. Four AMF genera were identified depending on the treatments: *Acaulospora* spp., *Gigaspora* spp., *Glomus* spp., and *Scutellospora* spp. The genus *Glomus* spp. was the most prevalent in all plots. The fertilization applied influenced the abundance of AMF present.

**Keywords:** *Xanthosoma sagittifolium*, Suckers, PIF plants, Metabolites, Soil, Arbuscular mycorrhizal fungi

## Introduction

In Cameroon, as elsewhere in *Xanthosoma sagittifolium* growing regions in Africa, market prices for *X. sagittifolium* tubers are very high due to the fact that supply is not able to cover the high demand. The primary constraint for Araceae cultivation, particularly for *X. sagittifolium*, is the unavailability of seeds in sufficient quantity and quality. Farmers generally use either tubers or fragments of *X. sagittifolium* rhizomes from previous crops as seeds, also called suckers or conventional seeds (Nzietchueng, 1985; Omokolo *et al.*, 2003). The transfer of these seeds, fragments of rhizomes from one field to another, is responsible for the transfer of microbial diversity from the original soil, which also includes the transfer of pathogenic fungi. This partly justifies the use of chemicals (chemical fertilizers, fungicides, insecticides, etc.). However, the production of seed plants *in vivo* using the PIF method (plants from stem fragments) established in 2021 is an alternative for producing large quantities of *X. sagittifolium* seeds in a short period of time (Djeuani *et al.*, 2021, 2023; Tiki, 2023; Mezimbila, 2025).

This PIF method is therefore an innovative agro-economic approach that makes it possible to quickly

obtain healthy plants from rhizome fragments, thus reducing the costs associated with purchasing seeds and accelerating the production cycle (Djeuani *et al.*, 2021, 2023). With a high multiplication rate ( $\times 7.58$  in three months), it promotes producer autonomy, improves yields, and strengthens farm profitability, while contributing to local food security (Djeuani *et al.*, 2021, 2023; Mezimbila, 2025). The application of this method is a key opportunity to resolve problems of seed shortages or infected soils. The PIF method thus represents a sustainable, accessible, and effective solution to strengthen food security and the resilience of local agricultural systems. By facilitating access to quality plant material using this technique, the aim is to offer solutions to strengthen the autonomy of producers and contribute to improving field yields, while supporting sustainable and competitive agriculture. Associated with adequate fertilizers, therefore, with *X. sagittifolium* PIF seed plants for good growth in the field is a challenge (Djeuani *et al.*, 2021, 2023). However, applying good sustainable fertilization practices around the popularization of *X. sagittifolium* PIF plants would nevertheless aim to maximize agricultural yields while preserving soil health and the environment. Furthermore, it is known that the quality of fertilizers used in fields

would not only influence the nature of the soil, but could also be able to have an impact on microbial life in the rhizosphere of plants while also acting on the interactions of these microbial communities present (Daniel & Kate, 2014; Wang *et al.*, 2025). Therefore, carrying out an identification of arbuscular mycorrhizal fungi present in the rhizosphere of *X. sagittifolium* PIF plants according to the types of fertilization applied in field growing conditions would not only contribute to having information on the richness of arbuscular mycorrhizal fungi present but also to ensure that the fertilizers applied would not contribute to the reduction of mycorrhizal interactions or the alteration of the diversity of these arbuscular mycorrhizal fungi present. It is with this in mind that the objective of this work was to evaluate the effect of fertilizers applied on the growth of *X. sagittifolium* PIF plants in the field, while highlighting the potential changes in soil quality and the nature of mycorrhizal life in the soil following fertilization. More specifically, it was a question of testing the influence of some fertilizers on agronomic growth parameters in PIF plants and plants from field shoots, of determining the contents of some leaf metabolites in *X. sagittifolium* following the fertilizers applied, and of carrying out a physicochemical analysis of the soil and of determining the richness of arbuscular mycorrhizal fungi present following the different fertilizers applied.

## **Materials and methods**

### ***Presentation of the experimental site and plant material used***

The work was conducted in the Centre region, Mfoundi Department, and more specifically at the Higher Teachers' Training College of the University of Yaoundé 1. The field had the geographic coordinates of 03° 51' 36" North latitude and 11° 30' 37.5" East latitude. This sampling site is located in the bimodal rainforest zone (Zone V). The plant material consisted of two-month-old PIF plants, produced using the PIF technique, and rhizome fragments (suckers), a part of *Xanthosoma sagittifolium* generally used by farmers as seeds, harvested from 7- to 12-month-old plants in the field, in the Mbam and Inoubou Departments, more specifically in the Bafia District. These PIF seeds and rhizome fragments were of the white cultivar of *X. sagittifolium*.

### ***Evaluation of the effect of biofertilizers on production in X. sagittifolium plants***

#### *Experimental design and agronomic parameters*

The experimental design used was a completely randomized factorial block design with one replication, consisting of four treatments: Control, Oyster Shell (OS), Phosphorus Solubilizing Bacteria (PSB), and Oyster Shell + Phosphorus Solubilizing Bacteria (OS+PSB). 30 PIF plants and 30 suckers (rhizome fragments) were used for each treatment applied, for a total of 120 PIF plants and 120 suckers. The PIF plants used were two months old. Before sowing, each pocket received 100 g of each fertilizer. The fertilizer mixture was made at a 1:1 ratio.

In each plot unit corresponding to the applied treatments, five plants were randomly selected to evaluate agronomic growth parameters.

### ***Determination of the levels of selected metabolites in X. sagittifolium leaves according to the biofertilizers applied***

For each treatment applied, *X. sagittifolium* leaves were harvested. These leaves were used for physiological and biochemical analyses. The physiological analysis consisted of assessing the total chlorophyll content of the leaves according to Arnon (1949) protocol. For biochemical analysis, the content of total soluble sugars (Babu *et al.*, 2002), total proteins (Bradford, 1976; Pirovani *et al.*, 2008), phenolic compounds (Marigo, 1973; El Hadrami *et al.*, 1997), proline (Counet *et al.*, 2004; Zou *et al.*, 2004), and amino acids (Yemm & Cocking, 1995; Babu *et al.*, 2002) was evaluated.

### ***Evaluation of the effect of applied treatments on the diversity of arbuscular mycorrhiza fungi associated with the rhizosphere of X. sagittifolium plants***

#### *Physicochemical properties of the soil used*

The physicochemical and chemical parameters of the soil substrate were evaluated on soils collected before cultivation and after cultivation in the field at 3 months of growth. The method of Pauwels *et al.* (1992) was used according to ISO, AFNOR NF, and EN standards. 1 kg of each soil, depending on the treatments applied, was bagged and then taken for laboratory analysis. Analyses of the physicochemical characteristics of this soil were carried out at the National Laboratory for Diagnostic Analysis of Agricultural Products and Inputs of the Ministry of Agriculture and Rural Development (MINADER). Soil physical parameters (sand, clay, and silt content) and chemical parameters (pH in aqueous (pH - H<sub>2</sub>O) and saline (pH - KCl) media, C/N ratio, Ca<sub>2</sub><sup>+</sup> content, cation exchange capacity (CEC), and available phosphorus content (P Bray II) were determined and analyzed according to the protocols of Calvet and Villemin (1986).

### ***Determination of the mycorrhizal status of X. sagittifolium root fragments: histological analysis and mycorrhizal status assessment***

*X. sagittifolium* root hairs from PIF plants and suckers collected in the field following the applied treatments were cleaned, drained, and sectioned to a size of 0.5 to 1.0 cm. The protocol of Trouvelot *et al.* (1986) was used. These root hairs were soaked in 10 g L<sup>-1</sup> of KOH for 15 minutes at 90 °C, then rinsed with HCl (10%). The dye used was Acid Fuchsin 1 g L<sup>-1</sup>. Microscopic observations were carried out using the HYMEN optical microscope at 400X. 100 of these fragments were observed for each age group and per site. The mycorrhizal status was calculated according to the scale proposed by Trouvelot *et al.* (1986). The parameters F%, M%, m%, a%, A%, v% and V% were calculated.

### Spore extraction, counting, and characterization

The Gerdemann and Nicolson (1963) method was used to extract and count spores from the rhizosphere soils of PIF plants and plants from field-grown seedlings. This method is based on sieving the soil through a set of overlapping sieves with different mesh diameters: 0.5, 0.25, 0.125, and 0.0625  $\mu\text{m}$ . A 10 mL pellet of each sieved soil sample was observed under a binocular microscope with a 40x objective. A complete spore count for each age group was performed through the glasses. This operation was carried out in 10 fields, and an average was calculated based on the number of spores found. Thus, the total number of spores/soil collected was evaluated from the sum of the number obtained in sieves 2, 3, and 4 (0.25, 0.125, 0.0625  $\mu\text{m}$ ). The spore density was determined for each soil sample. The different spores resulting from the extraction and enumeration of the soils were morphologically described according to the identification keys of Schenck and Pérez (1987), of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, 2025), and of the European Glomales Bank. The classification of species was carried out according to the method of Redecker *et al.* (2013). The relative abundance of spores (RA) was calculated according to the formula stated by Johnson *et al.* (2013):

$$AR = 100 * (m/M) \quad (1)$$

where (m) is the total number of species observed and (M) the total number of spores observed. Species diversity was measured using species richness diversity indices (Shannon & Weaver, 1949). The Shannon index (H), ranging from 0 (a single species, or one species overwhelmingly dominant over all others) to  $\log S$  (when all species have the same abundance), was calculated using the formula:

$$H = - \sum_{i=1}^S n_i / N \log(n_i / N) \quad (2)$$

Where: S = total number of species;  $N_i$  = number of individuals of sex (i) in a given category; N = total number of individuals of all sexes in a given category;  $\log_2$  = logarithm to the base 2; H = Shannon diversity index.

### Statistical analysis

The results were analyzed descriptively (mean  $\pm$  standard deviation) and presented in graphs and tables (Microsoft Excel 2013). Statistical analyses were performed using IBM SPSS Version 20.0 software, and mean comparisons were performed using analysis of variance (ANOVA) using the Student-Newman-Keuls test at the 5% threshold.

## Results

### Effect of fertilizers applied to *X. sagittifolium* plants on agronomic parameters in the field

Generally, all treatments applied influenced the growth of PIF plants and plants from suckers (conventional

seeds) using the 5% Student Newman and Keuls test after three months of growth (Table 1). The PSB treatment significantly influenced the parameters of plant size and average plant area. Significant variations in average plant size and average leaf area were observed in the presence of the PSB treatment, with maximum values of  $25.16 \pm 3.19$  cm and  $100.61 \pm 14.85$  cm<sup>2</sup>, respectively, for PIF plants and  $48.60 \pm 8.47$  cm and  $423.86 \pm 95.83$  cm<sup>2</sup>, respectively, for plants from suckers. The OS+PSB treatments present the lowest values of the average leaf area (Table 1). Furthermore, there is no significant difference for the parameter's average diameter at the collar and average number of leaves, for all the treatments applied (Table 1). Although the results show an absence of variation in the average diameter of the rhizome according to the treatments applied, it is noted that the average number of roots appears very high in plants from suckers compared to PIF plants, with values of  $34.33 \pm 4.15$  and  $25.00 \pm 02.35$ , respectively, in the presence of the PSB treatment compared to the other treatments applied (Table 2). We also note a very high average size of the rhizome, and significant at 5% in PIF plants compared to plants from suckers (Table 2). The maximum average rhizome size was  $6.50 \pm 1.08$  cm for PIF plants and  $4.33 \pm 1.25$  cm for suckers, in the presence of the OS+PSB treatment (Table 2). As for the average number of newly formed tubers recorded following the treatments applied, it appears high and significant for the BSP and OS+PSB treatments (Table 2). However, the average weight of these newly formed tubers produced was higher in the presence of the PSB treatment. It appears higher here, with values 13.21 to 14.31 times higher than the maximum values in PIF plants. It is  $60.50 \pm 2.81$  g and  $715.50 \pm 52.79$  g, respectively, for PIF plants and sucker plants.

### Effect of fertilization on fresh and dry biomass in *X. sagittifolium* plants

In PIF plants, the total fresh and dry biomass values were less than 50 g for all treatments (Table 3). However, the maximum values were  $43.25 \pm 5.9$  g and  $18.00 \pm 1.91$  g, respectively, in *X. sagittifolium* PIF plants treated with PSB. Control PIF plants had lower values,  $24.25 \pm 7.02$  g and  $10.75 \pm 2.06$  g. In plants grown from suckers, the maximum values were  $365.25 \pm 26.95$  g and  $132.25 \pm 15.92$  g, respectively, for fresh and dry biomass in the presence of PSB (Table 3). It is observed that the OS treatment applied has the greatest influence on this biomass compared to the OS+PSB mixture. This biomass was evaluated to be 4 to 5 times higher in plants from suckers compared to PIF plants (Table 3).

### Total chlorophyll content of leaves in *X. sagittifolium* following the different treatments applied

The results show significant peaks in the Student Newman and Keuls test (5%) in this total chlorophyll content of leaves in PIF plants ( $0.95 \pm 0.05$  mg/g of PMF) and plants from suckers ( $1.03 \pm 0.10$  mg/g of PMF), in the presence of the OS treatment (Figure 1a). This chlorophyll content in *X. sagittifolium* is

**Table 1:** Agronomic parameters evaluated at the level of the aerial part of *X. sagittifolium* plants in the field. (Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells+Phosphorus solubilizing bacteria (OS+PSB))

Plants	Treatments	Average plant height (cm)	Average collar diameter (cm)	Average number of leaves	Average leaf area (cm <sup>2</sup> )
PIF	Control	19.72±4.41 <sup>a</sup>	2.69±0.23 <sup>a</sup>	3.60±0.89 <sup>a</sup>	070.92±10.02 <sup>b</sup>
	OS	18.84±7.01 <sup>a</sup>	2.95±0.35 <sup>a</sup>	3.08±1.00 <sup>a</sup>	061.94±13.33 <sup>b</sup>
	PSB	25.16±3.19 <sup>b</sup>	2.26±0.23 <sup>a</sup>	3.20±0.83 <sup>a</sup>	100.61±14.85 <sup>c</sup>
	OS+PSB	15.60±6.61 <sup>a</sup>	2.46±0.18 <sup>a</sup>	3.40±0.54 <sup>a</sup>	052.08±16.8 <sup>a</sup>
Suckers	Control	42.40±4.33 <sup>c</sup>	3.67±0.65 <sup>a</sup>	3.25±0.95 <sup>a</sup>	398.06±16.25 <sup>c</sup>
	OS	48.00±6.67 <sup>c</sup>	3.67±0.90 <sup>a</sup>	4.25±1.25 <sup>a</sup>	413.73±25.53 <sup>f</sup>
	PSB	48.60±8.47 <sup>c</sup>	3.85±1.60 <sup>a</sup>	3.25±1.89 <sup>a</sup>	423.86±25.83 <sup>f</sup>
	OS+PSB	45.50±2.51 <sup>d</sup>	4.05±0.73 <sup>a</sup>	3.55±1.00 <sup>a</sup>	283.82±27.85 <sup>d</sup>

Student Newman-Keuls Test

Means followed by different letters in the same column are significantly different (P<0.05) on the Student Newman-Keuls Test

**Table 2:** Agronomic parameters evaluated at the underground part of *X. sagittifolium* plants in the field (Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells+Phosphorus solubilizing bacteria (OS+PSB))

Plants	Treatments	Average number of roots	Average rhizome size (cm)	Average rhizome diameter (cm)	Average number of newly formed tubers	Average weight of newly formed tubers (g)
PIF	Control	12.00±05.77 <sup>a</sup>	4.37±1.18 <sup>b</sup>	9.37±2.42 <sup>a</sup>	07.25±1.70 <sup>a</sup>	40.00±3.16 <sup>a</sup>
	OS	24.25±09.84 <sup>c</sup>	6.37±1.43 <sup>c</sup>	9.62±2.39 <sup>a</sup>	06.75±1.21 <sup>a</sup>	52.50±3.69 <sup>a</sup>
	PSB	25.00±02.35 <sup>c</sup>	6.25±0.64 <sup>c</sup>	8.50±1.68 <sup>a</sup>	10.00±1.94 <sup>c</sup>	60.50±2.81 <sup>a</sup>
	OS+PSB	10.25±01.70 <sup>a</sup>	6.50±1.08 <sup>c</sup>	9.75±0.64 <sup>a</sup>	11.00±1.16 <sup>c</sup>	47.50±3.82 <sup>a</sup>
Suckers	Control	13.50±03.69 <sup>a</sup>	3.00±1.77 <sup>a</sup>	8.87±3.70 <sup>a</sup>	09.00±1.63 <sup>ab</sup>	528.50±40.86 <sup>b</sup>
	OS	22.00±13.22 <sup>c</sup>	3.62±1.25 <sup>a</sup>	8.75±1.70 <sup>a</sup>	10.25±1.62 <sup>c</sup>	528.50±12.73 <sup>b</sup>
	PSB	34.33±04.15 <sup>d</sup>	3.87±0.62 <sup>a</sup>	8.50±1.91 <sup>a</sup>	10.00±1.41 <sup>c</sup>	715.50±52.79 <sup>c</sup>
	OS+PSB	16.50±00.70 <sup>ab</sup>	4.33±1.25 <sup>b</sup>	8.50±2.50 <sup>a</sup>	10.33±2.08 <sup>c</sup>	675.33±47.56 <sup>b</sup>

Student Newman-Keuls Test

Means followed by different letters in the same column are significantly different (P<0.05) on the Student Newman-Keuls Test

**Table 3:** Variation of fresh and dry biomass in *X. sagittifolium* plants (Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells+Phosphorus solubilizing bacteria (OS+PSB))

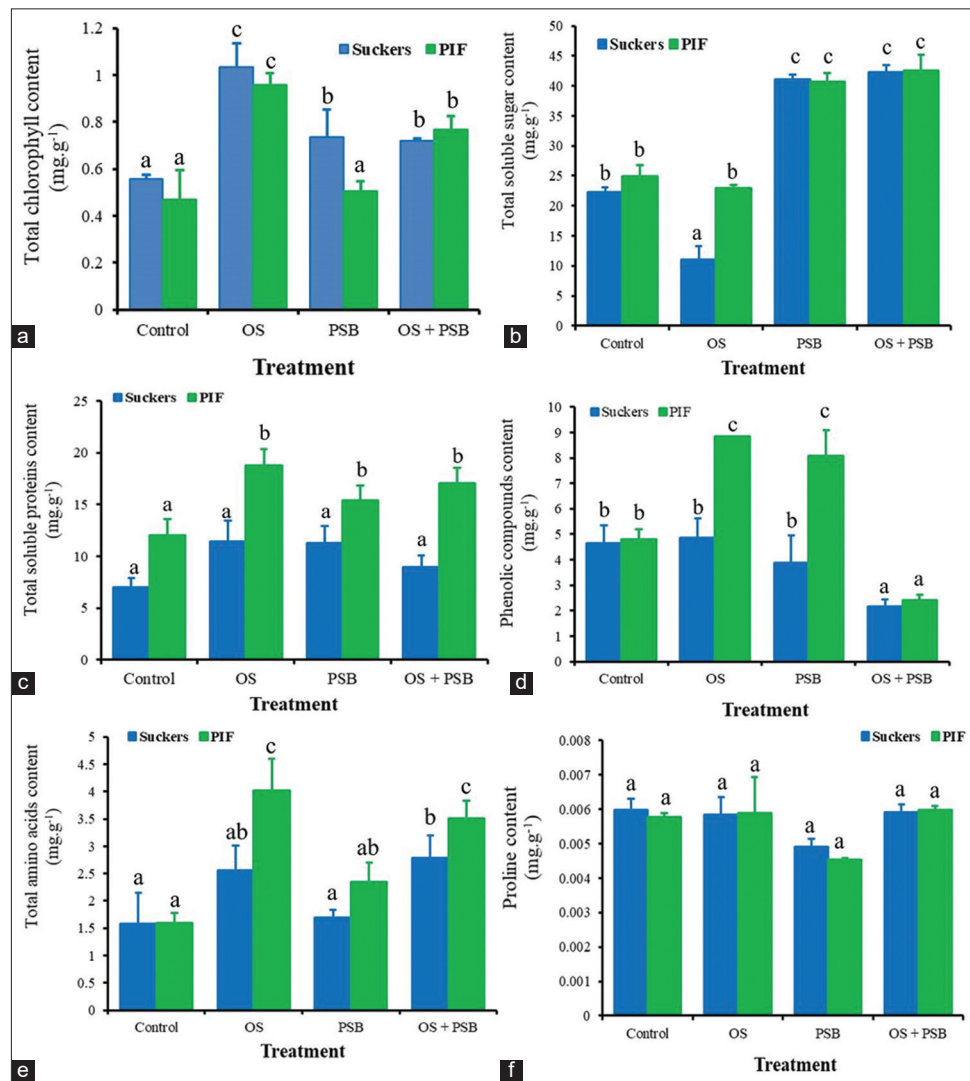
Plants	Treatments	Part of the plant	Parameters			
			Average Fresh Weight (g)	Average dry weight (g)	Total Fresh Weight (g)	Total dry weight (g)
PIF	Control	Leaves	3.00±0.44 <sup>a</sup>	1.00±0.00 <sup>a</sup>	24.25±4.52 <sup>a</sup>	10.75±2.06 <sup>a</sup>
		Rhizomes	20.00±4.08 <sup>d</sup>	9.75±2.06 <sup>d</sup>		
		Roots	1.25±0.50 <sup>a</sup>	0.00±0.00 <sup>a</sup>		
	OS	Leaves	3.75±0.95 <sup>a</sup>	1.00±0.00 <sup>a</sup>	36.25±3.10 <sup>ab</sup>	14.75±2.50 <sup>a</sup>
		Rhizomes	22.50±2.15 <sup>d</sup>	11.75±2.50 <sup>d</sup>		
		Roots	10.00±0.00	2.00±0.00 <sup>a</sup>		
	PSB	Leaves	7.25±1.86 <sup>b</sup>	1.00±0.00 <sup>a</sup>	43.25±5.9 <sup>b</sup>	18.00±1.91 <sup>a</sup>
		Rhizomes	32.75±3.34 <sup>c</sup>	16.00±1.91 <sup>c</sup>		
		Roots	3.25±0.70 <sup>a</sup>	1.00±0.00 <sup>a</sup>		
OS+PSB	Leaves	2.50±0.91 <sup>a</sup>	1.00±0.00 <sup>a</sup>	29.25±4.52 <sup>a</sup>	12.75±1.50 <sup>a</sup>	
	Rhizomes	24.25±2.70 <sup>d</sup>	10.75±1.50 <sup>d</sup>			
	Roots	2.50±0.91 <sup>a</sup>	1.00±0.00 <sup>a</sup>			
Suckers	Control	Leaves	37.75±5.68 <sup>f</sup>	6.50±1.45 <sup>c</sup>	287.50±18.00 <sup>c</sup>	92.75±13.91 <sup>b</sup>
		Rhizomes	241.00±10.73 <sup>j</sup>	84.00±12.21 <sup>g</sup>		
		Roots	8.75±1.59 <sup>b</sup>	2.25±0.25 <sup>a</sup>		
	OS	Leaves	72.50±5.29 <sup>b</sup>	18.00±1.02 <sup>f</sup>	301.00±25.47 <sup>d</sup>	105.50±11.64 <sup>b</sup>
		Rhizomes	215.75±18.55 <sup>i</sup>	85.25±10.12 <sup>g</sup>		
		Roots	12.75±1.63 <sup>c</sup>	2.25±0.50 <sup>a</sup>		
	PSB	Leaves	58.75±5.84 <sup>g</sup>	8.00±1.34 <sup>d</sup>	365.25±26.95 <sup>c</sup>	132.25±15.92 <sup>c</sup>
		Rhizomes	276.25±17.28 <sup>k</sup>	114.50±13.53 <sup>i</sup>		
		Roots	30.25±3.83 <sup>f</sup>	9.75±1.05 <sup>d</sup>		
	OS+PSB	Leaves	45.00±2.52 <sup>g</sup>	6.00±1.46 <sup>c</sup>	290.99±33.76 <sup>c</sup>	110.99±17.74 <sup>b</sup>
		Rhizomes	225.33±27.03 <sup>i</sup>	96.66±15.77 <sup>h</sup>		
		Roots	20.66±4.21 <sup>d</sup>	4.33±0.51 <sup>ab</sup>		

Student Newman-Keuls Test

Means followed by different letters in the same column are significantly different (P<0.05) on the Student Newman-Keuls Test

very low for PIF plants and plants from suckers in the control compared to the other treatments applied. It is noted that the total chlorophyll content, moreover, appears lower than 0.8 mg/g of PMF, for the OS+PSB treatments (0.76±0.05 mg g<sup>-1</sup> of WFM), in the PIF plants and for the PSB (0.73±0.11 mg g<sup>-1</sup>

of WFM) and OS+PSB (0.71±0.00 mg g<sup>-1</sup> of WFM) treatments, in the plants from the suckers (Figure 1a). In the PIF plants, this total chlorophyll content of the leaves is lower for the PSB treatment (0.50±0.04 mg g<sup>-1</sup> of WFM) applied compared to all the others (Figure 1a).



**Figure 1:** Physiological and biochemical analyses of some metabolites in leaves of *Xanthosoma sagittifolium* at three months of growth in the field. a) Total chlorophyll content of leaves, b) Total soluble sugars content, c) Total soluble proteins content, d) Phenolic compounds content, e) Total amino acids content and f) Proline content. Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells + Phosphorus solubilizing bacteria (OS+PSB). Means with different letters are significantly different at 5% using the Student Newman and Keuls test

#### Total soluble sugar content of *X. sagittifolium* leaves according to the different treatments applied

In *X. sagittifolium* plants, the graph in Figure 1b shows that the total soluble sugar content evaluated is less than 25 mg.g<sup>-1</sup> of WFM in both PIF plants and plants from suckers for the Control and OS treatments. This synthesis of total soluble sugars in *X. sagittifolium* leaves is more influenced in the presence of PSB and OS+PSB (Figure 1b). In the presence of the OS+PSB treatment, the maximum values recorded are 42.21±1.28 and 42.56±2.61 mg g<sup>-1</sup> of WFM in plants from suckers and PIF plants, respectively. In PIF plants, the total soluble sugar content appears higher in the treatments: Control (24.87±0.54 mg g<sup>-1</sup> of WFM), OS (22.91±0.54 mg g<sup>-1</sup> of WFM), and OS+PSB (42.56±2.61 mg g<sup>-1</sup> of WFM) applied compared to plants grown from suckers of these same treatments (Figure 1b). However, in general, it should be noted that the total soluble sugar content in *X. sagittifolium* plants is very low in plants grown from suckers (10.99±1.25 mg g<sup>-1</sup> of WFM), compared to all other treatments.

#### Total soluble protein content in *X. sagittifolium* leaves following different treatments

In *X. sagittifolium* plants grown from suckers, the total soluble protein content in leaves did not vary significantly by the Student Newman and Keuls test ( $P < 0.05$ ) (Figure 1c). This level was low, at 6.96±0.94 mg g<sup>-1</sup> of WFM, in control plants grown from suckers and showed values approximately equal to 12 mg g<sup>-1</sup> of WFM in these same plants for the OS (11.45±1.98 mg g<sup>-1</sup>) and PSB (11.25±1.69 mg g<sup>-1</sup> of WFM) treatments. This total soluble protein content in leaves was also higher in PIF plants compared to plants grown from suckers in *X. sagittifolium*. The highest peak of 18.78±3.98 mg g<sup>-1</sup> of WFM was observed in PIF plants fertilized with the OS treatment (Figure 1c). Here, we note that this content also appears significantly lower than or equal to 12 mg g<sup>-1</sup> of PMF for the control (12.03±1.57 mg g<sup>-1</sup> of WFM) and PSB (15.37±1.48 mg g<sup>-1</sup> of WFM) treatments, respectively.

### **Phenolic compounds content in *X. sagittifolium* leaves following the different treatments applied**

The phenolic content recorded in the leaves of *X. sagittifolium* was generally lower in plants grown from suckers compared to PIF plants (Figure 1d). In the sucker plants, this content of phenolic compounds is less than 5 mg g<sup>-1</sup> of WFM for all the treatments applied and presents the lowest value in the presence of the OS+PSB treatment (2.16±0.27 mg g<sup>-1</sup> of WFM). However, it is noted that this content value does not vary significantly in the Student Newman and Keuls test at 5%, in the control plants, whether in the plants from the suckers (4.64±0.70 mg g<sup>-1</sup> of WFM) or in the PIF plants (4.85±0.40 mg g<sup>-1</sup> of WFM), in *X. sagittifolium* (Figure 1d). In the PIF plants, maxima greater than 5 mg g<sup>-1</sup> of WFM are noted respectively for the treatments applied with OS (8.82±0.0 mg g<sup>-1</sup> of WFM) and PSB (8.08±4.25 mg g<sup>-1</sup> of WFM) (Figure 1d).

### **Total amino acid and proline content in leaves of *X. sagittifolium* following the different treatments applied**

The results obtained show significant variation in leaf amino acid content. In *X. sagittifolium* plants grown from suckers, the values of this amino acid content are all below 3 mg g<sup>-1</sup> of WFM, despite the maximum observed in the leaves of plants fertilized with the OS+PSB treatment (2.78±0.41 mg g<sup>-1</sup> of WFM) (Figure 1e). For the control treatment, it should be noted that the results obtained show no significant variation at the 5% level in the Student Newman and Keuls test in plants grown from suckers (1.57±0.57 mg g<sup>-1</sup> of WFM) and PIF plants (1.59±0.18 mg g<sup>-1</sup> of WFM) in *X. sagittifolium*. In PIF plants, this total amino acid content appears higher and varies significantly for the OS, PSB, and OS+PSB treatments (Figure 1e). However, the OS fertilizer treatment had the greatest influence on leaf total amino acid synthesis, with a maximum of 4.02±0.57 mg g<sup>-1</sup> of WFM (Figure 1e). However, leaf proline content (Figure 1f) appears very low, with values below 1 mg g<sup>-1</sup> of WFM for all treatments, despite the maximum values of 0.0074±0.0002 and 0.0083±0.0001 mg g<sup>-1</sup> of WFM, respectively, observed in plants from suckers and PIF plants receiving the OS+PSB fertilizer mixture in the field (Figure 1f).

### **Evaluation of the effect of applied treatments on the diversity of AMF associated with the rhizosphere of *X. sagittifolium* plants in the field**

#### *Physicochemical characteristics of soils before and after sowing*

The analysis of the soil substrate of the cultivation site (Figure 2) revealed that, physically, this soil was rich in 46.25% clay, 16% silt, and 37.75% sand, which allows it to be assigned a clayey nature based on the texture triangle. Chemically, the pH is acidic, with a pH (HCl) of 4.8. A C/N ratio of 4.44 (C/N < 6) and a high cation exchange capacity (18.25%) were also recorded. It should be noted that the soil of the cultivation site is very poor in available phosphorus (18.89±1.56%) (Figure 2a-i). It should be noted that three months after the application of the different fertilizers and

the growth of plants from the shoots and PIF plants of *X. sagittifolium*, the soil used became of silty-clayey texture (Figure 2a, b, and c), showing a decrease in the clay content and an increase in the silt and sand content. The water pH varied between 5.6 and 5.9, while the hydrochloric acid pH was between 4.5 and 4.8. For the CEC, it is noted that it is between 13.5 and 15.25 after sowing (Figure 2i). An increase in calcium content was recorded in the presence of the PSB treatment in the rhizosphere soil of PIF plants and shoots after sowing, compared to the content in the soil before sowing (Figure 2i). Regarding the available phosphorus content, which corresponds to the most biologically available phosphorus (labile organic and inorganic forms and microbial phosphorus), the results show that it appears very high in the rhizosphere soil of PIF plants and plants from *X. sagittifolium* shoots for the PSB and OS + PSB treatments (Figure 2g).

#### *Appearance of mycorrhizal hyphae, arbuscules, and vesicles in roots*

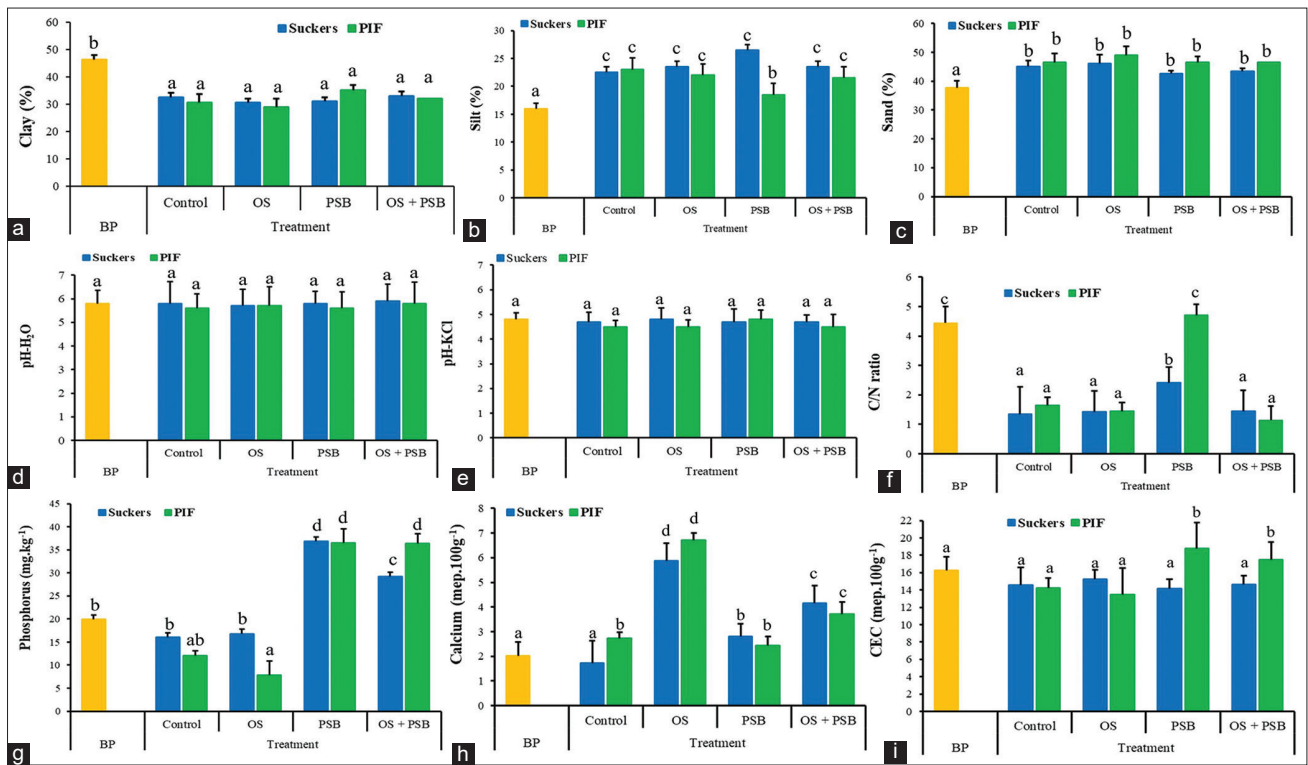
Microscopic observations of the cytological organization of *X. sagittifolium* root hair fragments from PIF plants and suckers collected from each plot revealed endomycorrhizal structures such as intra- and inter-root hyphae characteristic of endomycorrhizae, crossing the root cortex on both sides (Figure 3). Arbuscular structures appear filling the plant cells on both sides of the plant roots (Figure 3). These structures, stained dark or light red with Fuchsin, are characterized by their richness in chitin.

#### *Assessment of the mycorrhizal status of *X. sagittifolium* plant roots in PIF plants and suckers*

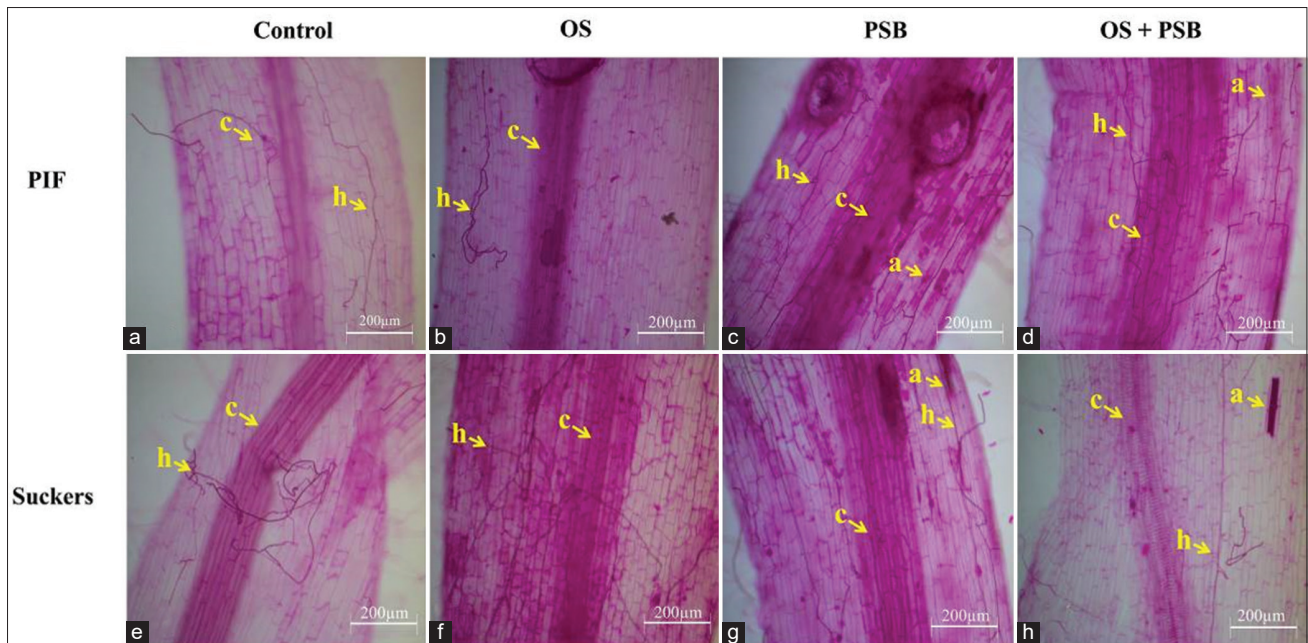
Mycorrhization frequency and intensity were lower than 75% and 13%, respectively (Table 4). Results show that the highest mycorrhization frequency (F%) was 72.66% in the root hairs of *X. sagittifolium* PIF plants fertilized with the BSP treatment compared to the other treatments. Mycorrhization intensity (M), with a maximum of 22.84% in plants from offshoots and 21.56% for PIF plants at 3 months of growth, respectively, in the presence of the BSP treatment. For mycorrhizal intensity in the root system (m%), a maximum value of 2.57% and 5.39% was noted in plants from suckers and PIF plants under the BSP treatment. A low presence of arbuscules and vesicles was also noted in the roots (Figure 3 and Table 4).

#### *Relative abundance of AMF spores by harvest site and determination of the Shannon-Weiner index*

The results of the relative abundance of AMF spores in 50 g of rhizosphere soil from PIF plants and *X. sagittifolium* suckers according to the treatments applied allowed us to assess the variations in spore count from one treatment to another (Figure 4). The abundance was 21 spores in 50 g before sowing (Figure 4). For all cultivation conditions, it appears higher than 21 spores/50 g of soil after three months of cultivation, for the treatments, control, and OS+PSB, and in the PIF plants, only for the OS and BSP treatments. In the PIF plants, the maximum recorded is 40 spores for the OS+PSB treatment (Figure 4). While for the sucker plants,



**Figure 2:** Physicochemical analyses of plant rhizosphere soils obtained from shoots and PIF plants in *X. sagittifolium*, before and after three months of field growth following the treatments applied. a) Clay contents, b) silt contents, c) sand contents, d) pH in aqueous media (pH - H<sub>2</sub>O), e) in acidic media (pH-KCl), f) available phosphorus content (mg kg<sup>-1</sup>), g) C/N ratio, h) exchangeable calcium and i) cation exchange capacity (CEC). Before sowing (BP) and treatments applied after sowing: Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells + Phosphorus solubilizing bacteria (OS+PSB). Means with different letters are significantly different at the 5% level in the Student Newman-Keuls test



**Figure 3:** Appearance of mycorrhizal structures present in the roots of PIF plants and *X. sagittifolium* suckers stained with fuch sine and observed under an OLSTROM (Leica) optical microscope at 200 μm. Treatments applied: T, COQ, BSP, and COQ+BSP: a, b, c and d) roots of PIF plants, e, f, g and h) roots of sucker plants. Arbuscules (a), cortex (c), and hyphae (h)

the maxima are recorded at the level of the rhizosphere soil of the control plants, with 32 spores. As for the calculated Shannon index, to assess the diversity of AMF following the treatments applied, the analysis of the diversity of AMF through the Shannon index showed that the rhizosphere soil

of *X. sagittifolium* plants, having received the BSP treatment, appears the richest in AMF with an index of 1.17 in the PIF compared to the other treatments (Figure 5). However, in plants grown from suckers, the highest value was 0.92 recorded in the presence of the OS+PSB treatment (Figure 5).

**Table 4:** Mycorrhizal status of *Xanthosoma sagittifolium* plant roots, in PIF and suckers

Plants	Treatments	Mycorrhizal status						
		F (%)	M (%)	m (%)	a (%)	A (%)	v (%)	V (%)
Suckers	Control	34.33	16.52	2.29	2.00	0.01	1.40	0.00
	OS	32.66	11.60	1.02	1.90	0.00	0.80	0.00
	PSB	64.33	22.84	2.57	2.60	0.02	1.20	0.02
	OS+PSB	34.33	11.59	1.03	1.02	0.00	0.90	0.00
PIF	Control	37.66	12.05	2.00	2.40	0.01	2.00	0.02
	OS	42.66	19.40	1.98	4.00	0.02	2.00	0.01
	PSB	72.66	21.56	5.39	1.70	0.03	1.80	0.07
	OS+PSB	42.66	10.00	2.00	1.40	0.00	0.50	0.00

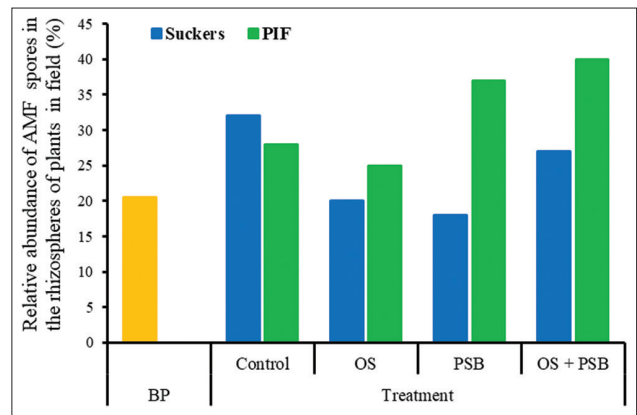
F% = Frequency of mycorrhizal colonization, M% = Intensity of mycorrhizal colonization, m% = Intensity of mycorrhizal colonization in the root system, a% = Arbuscular content, A% = Arbuscular intensity in the root system, v% = Vesicular intensity of the mycorrhizal part, V% = Vesicular intensity in the root system

**Morphological descriptions of spores of AMF species from the rhizosphere soils of *X. sagittifolium* before and after sowing, depending on the treatments applied**

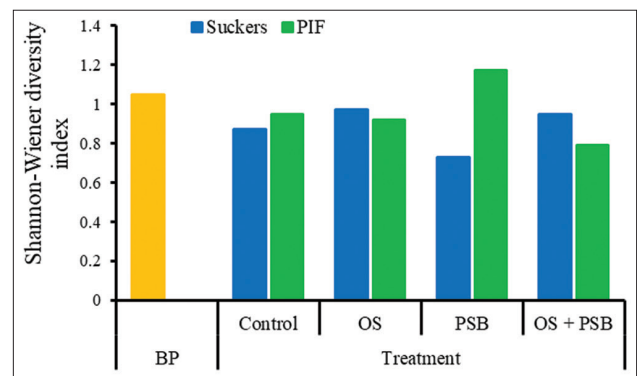
The spores of the different species of arbuscular mycorrhizal fungi were described according to the INVAM (2025) identification key. Significant variations were observed between spores of the same or different species, particularly in terms of shape, color, cell walls, and spore cleft (Figure 6). Microscopic observations of these AMF spores isolated from the soils of the different plants allowed the distinction of four genera: *Acaulospora* spp., *Gigaspora* spp., *Glomus* spp., and *Scutellospora* spp. Spores of the genus *Glomus* spp. was the most abundant and were found in the rhizosphere of both plants (PIF and sucker). The rhizosphere of PIF plants appears to have more AMF spores than that of plants from suckers (Figure 6).

**Discussion**

The objective of this work was to evaluate the effect of applied fertilizers on the growth of *X. sagittifolium* PIF plants, while highlighting their influence on potential changes in soil quality and mycorrhizal diversity in the rhizosphere of field plants. The morphological growth parameters assessed increased significantly after three months of cultivation. Whether in plants grown from suckers or *X. sagittifolium* PIF plants, this growth improvement was more significant in the Student-Keuls t-test (at 5%) in the presence of the PSB treatment. This would thus reflect the positive impact of the nature of this applied biofertilizer. PSB releases available phosphorus into the soil, which is an important bio stimulant molecule for growth (Liu *et al.*, 2025). However, pre-sowing soil analyses showed a lack of available phosphorus, which suggests that, since the work was carried out in the field, these bacteria would have acted in synergy with other indigenous soil microorganisms, contributing to plant development. Generally speaking, the dry mass of leaves, rhizomes, roots in PIF, and shoots has decreased considerably. In general, the dry masses of leaves, rhizomes, roots in the PIF, and suckers decreased considerably. This decrease is due to the loss of water after drying. The largest masses were observed in the sucker



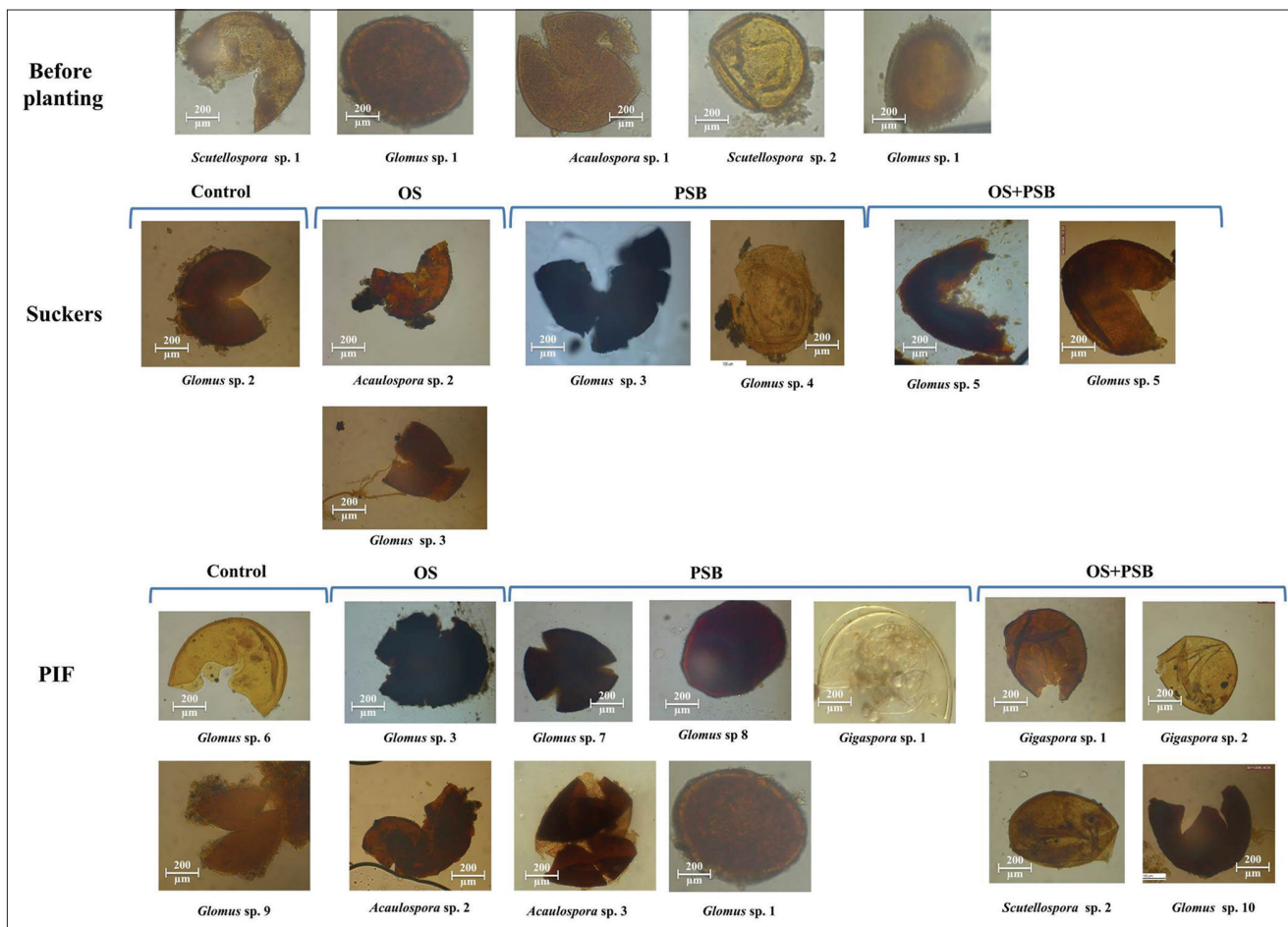
**Figure 4:** Average spore abundance in 50 g of rhizosphere soil from plants obtained from suckers and PIF plants in *X. sagittifolium*. Before sowing (BP) and treatments applied after sowing: Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells + Phosphorus solubilizing bacteria (OS+PSB)



**Figure 5:** Shannon-Wiener index of spores in 50 g of rhizosphere soil from plants obtained from suckers and PIF seedlings in *X. sagittifolium*. Before sowing (BP) and treatments applied after sowing: Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells + Phosphorus solubilizing bacteria (OS+PSB)

plants for rhizomes and roots for the PSB treatment. The treatments applied do not significantly influence the number of leaves. Djeuani *et al.* (2021, 2023) emphasize that, whether in propagator or in field, the treatments applied do not influence the number of leaves emitted by the plant. Furthermore, the very high number of roots in the presence of PSB would also reflect the establishment of good nutrient adsorption, and the consequence of which would be partly at the origin of the increase in the average weight of newly formed tubers, thus observed in plants treated with PSB.

On the metabolic level, physiological analysis showed that the total chlorophyll content was higher in sucker plants and PIF plants in the presence of OS treatment. This increase would be the result of the richness of the treatment in calcium ions (Ca<sup>2+</sup>). It is known that calcium plays an important role in photosynthesis (Nechi, 2016). The sucker plants used in growth had a greater weight than the PIF plants and a very high leaf surface in the presence of PSB. Furthermore, their very high number of roots in the latter would have contributed to a significant assimilation of mineral elements by osmosis and consequently led to good photosynthesis. This explains the sugar content, more



**Figure 6:** Morphological characterization of arbuscular mycorrhizal fungal spores in plants obtained from suckers and PIF seedlings in *X. sagittifolium*. Before sowing (BP) and treatments applied after sowing: Control, Oyster shells (OS), Phosphorus solubilizing bacteria (PSB), and Oyster shells + Phosphorus solubilizing bacteria (OS+PSB)

concentrated in the leaves of *X. sagittifolium*, of the sucker plants compared to the PIF plants. Good hydromineral nutrition leads to good photosynthesis (Lin *et al.*, 2024). According to Zouine and El Hadrami (2004), total soluble sugars are involved in two metabolic pathways in plants: the phenylalanine ammonia lyase (PAL) pathway and the precursor amino acid pathway for phenol and protein biosynthesis. The results show that protein contents have the highest values in Pif plants in all treatments. This suggests that in PIF plants, the sugars produced would be directly involved in the protein synthesis pathway, which are nitrogenous macromolecules composed of an ordered assembly of amino acids linked by peptide bonds (Stayanarayana & Chakrapati, 2007). In addition to their role as protein constituents, amino acids are also involved in a multitude of cellular reactions (Fagard *et al.*, 2014) and therefore influence several physiological processes such as plant growth and development, intracellular pH control, energy generation, and resistance to abiotic and biotic stress (Pratelli & Pilot, 2014). This observed protein synthesis and the decrease in amino acids, and similarly in proline, suggest that in PIF and *X. sagittifolium* plants, the synthesized amino acids may have favored the protein biosynthesis pathway over that of phenolic compounds.

Chemical analyses of the soils at the cultivation site show that these soils have acidic pHs, with values ranging

from 5.6 to 5.8 for pH ( $H_2O$ ) and from 4.5 to 4.8 for pH (HCl). The treatments applied did not surely influence the pH variation, perhaps because the duration of the experiment was just three months. Similarly, Cameroonian soils are acidic (Tsufac *et al.*, 2020). According to Boakye (2018), *X. sagittifolium* plants exhibit optimal growth and production at pH values ranging between 5.5 and 6.5 (pH -  $H_2O$ ). However, it has been shown that pH (5.8 to 6.3) is satisfactory for good biological activity and nutritional exchanges in the soil (Neina, 2019). The soils in the harvest site had a C/N ratio (4.71) < 6 (Calvet & Villemin, 1986), which would reflect rapid decomposition of organic matter. Furthermore, the clayey soil we used before sowing presented a silty-clayey nature after three months of fertilization. This change in texture would be the result of the action of plant root growth and also of the microbial activity present. According to the fertility scale for available phosphorus of Boyer (1982), the soil of the cultivation site is very poor in available phosphorus before sowing. The results showed an increase in the presence of this available phosphorus for the PSB and OS+PSB treatments. The application of these two fertilizers would promote ideal conditions for the activity of microorganisms responsible for the assimilation of unavailable phosphorus in the environment. The cation exchange capacity evaluated did not vary before and after sowing, this surely because of the texture of the silty-clayey soil. But the slight decrease

in this CEC observed for the control and OS treatments also appears to be linked to the decrease in clay content for these two treatments. The percentage of calcium ( $\text{Ca}^{2+}$ ) is higher with OS. It is noted here that the main source of their presence observed for the OS treatment is the richness in calcium carbonates of the oyster shells used. This is an important mineral because it is involved in physiological processes important for plants: photosynthesis, fruiting, cell permeability, and ionic balance (Nechi, 2016).

The mycorrhizal status of the roots of *X. sagittifolium* plants, determined according to the different treatments applied, showed that the frequency of mycorrhization is higher in the presence of PSB. These different treatments applied would have an impact on the activation of soil microbial activities, like the activity of arbuscular mycorrhizal fungi. The presence of mycorrhizal structures in the roots made it possible to assess the symbiosis between *X. sagittifolium* and the types of AMF present in the soil of the site. The work of Djeuani *et al.* (2018) showed that *X. sagittifolium* is a mycotrophic plant. This variation in the parameters of the mycorrhizal status observed would be influenced not only by root renewal in plants but also by soil properties. The observed root colonization would surely have been influenced by the density and diversity of spores of the AMF species present in the rhizosphere, having responded to the hormone effect emitted by the plants from the suckers or the PIF plants of *X. sagittifolium*, despite the fertilizers applied. Because the variability of response thus observed would be due to the fact that the efficiency of the symbiosis does not depend only on the AMF but on the compatibility between the plant and the fungus (Al Raish *et al.*, 2025; Qi *et al.*, 2025). The results of the relative abundance of spores showed that the values obtained varied from one plot to another. It was very high in the PIF plants, with a value of 40 spores/50 g of soil in the plants having received the OS+PSB treatment. This very high abundance would reflect the ability of PIF plants to establish symbiosis with native AMF species in the soil, but also, this could be qualified as an adaptation strategy developed by PIF plants to improve the assimilation of mineral elements, given their size upon leaving the propagators and after acclimatization. The analysis of this abundance made it possible to understand the richness and diversity of the arbuscular mycorrhizal fungal communities present in the rhizosphere of *X. sagittifolium* plants. This diversity would be attributed to environmental conditions, the age of the plants, and even to the quality of the soil. The availability of nutrients in the soil would also influence this presence of spores. Moreover, Touré *et al.* (2021) report that this abundance of spores depends on the physicochemical properties of the soil. The variation in spore density could also be attributed to the presence of plant cover, which contributes to the creation of a vast network of hyphae and interconnections with the roots of host plants, improving not only biomass but also soil microbial activity (Graves *et al.*, 1997).

Based on morphological characteristics and the INVAM (2025) identification key, four genera were identified in the cultivated plot: *Acaulospora* spp., *Gigaspora* spp., *Glomus*

spp., and *Scutellospora* spp. These four AMF genera belong to three families: Acaulosporaceae (*Acaulospora* spp.), Gigasporaceae (*Gigaspora* spp. and *Scutellospora* spp.), and Glomeraceae (*Glomus* spp.). The genus *Glomus* spp. was the most represented in the rhizosphere of plants from shoots and PIF plants of *X. sagittifolium*. The contribution of phosphorus-solubilizing bacteria and oyster shells would have facilitated the AMF to achieve symbiosis. The work of Ngonkeu (2009) showed that Cameroonian soils are richer in AMF of the genus *Glomus* spp. This abundance of the genus *Glomus* spp. could be explained by the fact that this species of AMF would have a capacity to multiply and adapt not only to extreme conditions but also to acidic soils.

## Conclusion

The PSB treatment applied during growth to *X. sagittifolium* sucker plants or PIF seedlings significantly influenced growth parameters, showing an improvement in the weight of newly formed tubers after three months of growth. Soil texture changed from loamy before sowing to sandy-clay loam after three months for all treatments applied, with a pH-KCl ranging between 4.5 and 4.8. The available phosphorus content was very low before sowing and appeared significant for the PSB and OS+PSB treatments. These physicochemical properties of the assessed soils had an impact on the diversity of AMF associated with the rhizosphere of *X. sagittifolium* sucker plants and PIF seedlings, and thus the four genera identified were *Acaulospora* spp., *Gigaspora* spp., *Glomus* spp., and *Scutellospora* spp.

## Authors' contribution

All authors have significantly contributed to the realization of the activities of this research project, encompassing all stages, from conducting the experiments to writing and revising the final manuscript, which is now submitted for publication.

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