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Valorization of Some Endophytic Fungi Isolated from Rhizome Fragments of *Xanthosoma sagittifolium* L. Schott, and Evaluation of Their Fertilizing Potential in Improving Growth in PIF Plants

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Abstract

Background: To improve the growth of *Xanthosoma sagittifolium*, farmers use chemical fertilizers to compensate for the limited availability of soil nutrients, particularly phosphorus. Alternatives involving the use of beneficial endophytic fungi capable of mineralizing this phosphorus are an avenue that is increasingly being explored. **Aim:** This study aimed to evaluate the effect of biofertilizers produced from phosphate-solubilizing endophytic fungi (PSF) on certain growth parameters in *Xanthosoma sagittifolium* PIF plants, based on their phosphate-solubilizing capacity. **Methods:** Four endophytic fungi isolated from rhizome fragments of *X. sagittifolium* was used to produce formulations. Each formulation, produced from the culture of these fungi on a sawdust or rice bran substrate, represented one treatment. Six treatments were applied: a control treatment and five treatments consisting of fungal strains from the genera *Trichoderma asperellum, Aspergillus niger, Aspergillus sp, Fusarium oxysporum*, and the mixture, which were applied as fertilizer to three-month-old *X. sagittifolium* PIF seedlings. The experimental design was a completely randomized block design where each treatment was replicated twice. Thirty PIF seedlings were used for each treatment. Growth and the expression of potential disease symptoms were assessed. **Results:** All selected fungal strains were capable of solubilizing phosphorus. *T. asperellum* proved to be the most effective at phosphorus solubilization. The *T. asperellum* + rice bran fertilizer substrate improved plant growth parameters and limited disease occurrence. The treatment Rice bran + *T. asperellum* unveiled the highest plant height (43.17 ± 3.74 cm), number of leaves (3.3 ± 1.3), leave area (366.07 ± 55.43 cm^2), and collar diameter (10.61 ± 1.15). Disease incidence and severity were higher in treatments using sawdust + *Aspergillus* sp, with rates of 62.162% and 58.11%, respectively. **Conclusion:** These results suggest that *T. asperellum* derived from rhizome fragments could be proposed as a suitable and reliable inoculant for improving the growth of *X. sagittifolium*.

Keywords: rhizome fragment; phosphorus-solubilizing fungi; biofertilizer; PIF plants; *X. sagittifolium*

1. Introduction

Biofertilizers are a class of fertilizers that are formed by incorporating beneficial living microorganisms into carrier materials, which are applied in the soil to boost nutrient availability and accessibility. These beneficial living microorganisms carry out biological, physical, and chemical processes that elevate soil and plant characteristics (Lee et al., 2018; Abbey et al., 2019). E.g., bacteria, fungi, actinomycetes and algae, depending on the purpose of application (Chaudhary et al., 2022a). Microorganisms used as biofertilizers carry out processes such as nitrogen fixation, phosphate solubilization, resistance to biotic and abiotic stress, and the release of plant growth hormones (Okur, 2018; Chaudhary et al., 2021, 2022b). The classification of biofertilizers is done based on the type of microorganisms used, the function, and the mode of action.

According to microbial type, they can be classified as endophytic bacteria biofertilizers and endophytic fungi biofertilizers. Whereas, based on function, they are divided into nitrogen-fixing biofertilizers, phosphate-solubilizing biofertilizers, and

potassium-solubilizing biofertilizers (Nosheen et al., 2021; Somagaini et al., 2024). Phosphorus-solubilizing biofertilizers could be made up of either bacteria or fungi, which are responsible for solubilizing and mobilizing phosphorus found within the soil (Mahanty et al., 2017). These microbes, when inoculated as biofertilizers, may remain in the soil as rhizospheric microbes, create relationships with the host plant at the level of the roots, or colonize the intra/intercellular system of their plants as endophytic fungi. In all, the mechanism of solubilization involves the release of organic acids or enzymes, which dissociate phosphorus from ions such as aluminum and calcium in a process known as solubilization, or breakdown phytic acid in a process known as mineralization (Kirui et al., 2022). Among them, *Penicillium, Trichoderma* or *Aspergillus spp* are more identified (Abdallah *et al.,* 2016). The action of these microbes within the soil is known to be an economically attainable solution to the use of chemical fertilizers, more eco-friendly (adaptable to soil and environmental changes) and agronomically valuable (Rastegari et al., 2020; Singh and Yadav, 2020). These microorganisms are often inoculated in biodegradable liquid or solid media (carrier materials) such as wheat bran, rice bran, peat and lignite, which function in easing the handling of microbial inoculants and increasing the shelf life of the microbes (Bhattacharjee and Dey, 2014).

The application of endophytic fungi to plants as microbial fertilizers is increasingly valued and is, moreover, an approach with multiple advantages, as it contributes to improved growth while promoting plant nutrition (Oteino et al., 2015; Nosheen et al., 2021; Ramachandran et al., 2026). However, no studies highlight such trials in the context of improving growth and yield in tuberous plants, or even in *Xanthosoma sagittifolium*. Some studies, such as those by Djeuani et al. (2017, 2018), have highlighted the applications of arbuscular mycorrhizal fungi in improving the growth of *X. sagittifolium*, and those by Mbarga et al. (2012) on the applications of T. asperellum in protecting plants against *Pythium myriotylum* attacks. However, to limit the use of chemical fertilizers for improving *X. sagittifolium* production, trials applying fertilizers formulated from endophytic fungal strains appear to be a promising avenue to explore.

X. sagittifolium is a tuberous plant widespread in tropical areas, particularly in Africa, where its cultivation and consumption are significant. In Cameroon, rhizome fragments and tubers are the parts of the plant used as seeds (Omokolo *et al.,* 2003). The transfer of these rhizome fragments across fields as seeds also explains the transfer of fungi, some of which can be beneficial or harmful (pathogens). Among the various seed production methods proposed, the PIF method allows for faster and more rapid seed multiplication (Djeuani et al., 2023). This technique involves using *X. sagittifolium* rhizome fragments from which live plants are produced. The work of Djeuani et al. (2023), Tiki (2023), and Ziem et al. (2026) had already shown that supplementing PIF seedling propagators with beneficial fertilizers facilitates rapid seed production. However, growth in *X. sagittifolium* requires the availability of soil nutrients, such as phosphorus, to ensure optimal tuber and leaf development. It is known that deficiencies in certain soil nutrients, such as phosphorus, due to unavailability, poor uptake, and insufficient fertilizer use, lead to stunted shoot growth, reduced root exploration, and decreased yields (Asante et al., 2017). Therefore, developing reliable and environmentally friendly approaches for fertilization trials is a promising avenue to explore. Evaluating the potential impacts of applying isolated endophytic fungi from plants as fertilizers would require studying their ability to solubilize phosphorus. With this in mind, the overall objective of this work was to produce biofertilizers from phosphate-solubilizing endophytic fungi isolated from

rhizome fragments of *X. sagittifolium*, while also highlighting their ability to influence growth in PIF plants.

2. Methods

2.1. Identification of phosphate-solubilizing fungi (PSF) strains and test of their ability to solubilize phosphorus

Four strains of endophytic fungi isolated from rhizome fragments of adult *Xanthosoma sagittifolium* plants were used. These rhizome fragments were collected in Bambili at the following geographic coordinates: N 6°0'37.62050" and E 10°15'35.933340", in the Western Highlands of Cameroon. These four fungal strains were collected in rhizome fragments of the white cultivar of *X. sagittifolium*. Those four strains were *Aspergillus niger*, *Aspergillus* sp, *Fusarium oxysporum*, and *Trichoderma asperellum*. To test their ability to solubilize phosphorus, these strains were cultured on Pikovskaya medium (Pikovskaya, 1948). This medium was sterilized at 121 °C in a pressure cooker for 15 minutes. Each isolated endophytic fungus was then sub-cultured into Petri dishes containing 25 ml of Pikovskaya medium and incubated for 3 to 5 days. The formation of a halozone served as a selection criterion for their ability to solubilize phosphorus, followed by the calculation of the phosphate solubilization index. The diameter of the halozone and that of the colonies were measured using calipers. Isolates exhibiting a high phosphate solubilization capacity (PSF) were then selected and used for the preparation of biofertilizers (Manzoor et al., 2017; Mayadunna et al., 2023).

$$\text{Solubilization index} = \frac{\text{Colony diameter + Halozone diameter}}{Colony\ diameter}$$

2.1.1. Preparation of PSF inoculum

The multiplication of phosphate-solubilizing fungi was done in rice media using the method of Rajashekara et al. (2016) and Panguepko et al. (2025). Each isolated PSF strain was sub-cultured in individual bottles containing rice media for 14 days at a temperature of 27°C. Ten grams of each strain were transferred into a 250 mL conical flask containing 100ml of sterilized distilled water and Tween 80 (0.05%). Each mixture was homogenized for 10 min using a mechanical shaker before the suspension was filtered into a clean conical flask using a double-layered muslin cloth (Sahayaraj et al., 2008).

2.1.2. Inoculation of fungal strains into the carrier material

Two carriers (rice bran and sawdust) were used for each isolated PSF strain and for the combined strain (*Aspergillus niger*, *Aspergillus* sp, *Fusarium oxysporum*, and *Trichoderma asperellum*). Twenty-five kilograms of each substrate were weighed and sealed in sterile polythene bags. They were then sterilized using the autoclave method described by FNCA (2006) for an hour at 121°C. Sterilized substrates were separated into five compartments of 5 kg in different sealable containers with respect to the individual and combined PSF strains to be cultured. A ten (10) ml suspension of each PSF strain containing spores at different concentrations (Trichoderma asperellum at 10^8 CFU/ml, Aspergillus niger at 10^7 CFU/ml, Aspergillus sp. at 10^7 CFU/ml, Fusarium oxysporum at 10^8 CFU/ml, and the mixture of the four PSF strains at 10^8 CFU/ml) was then inoculated into the respective substrates and incubated for 3 weeks at room temperature and in the dark. A total of 10 treatments were formulated by combining each PSF strain and the mixture on the substrates (rice bran and sawdust).

2.2. PIF Plant Production in *X. sagittifolium*

Propagation and seed production in *X. sagittifolium* using the PIF technique was carried out according to the protocol of Djeuani et al. (2021) and Amama (2021). 500 g rhizome fragments from plants of the white cultivar of *X. sagittifolium* was used. In each propagator, the culture substrate consisted solely of a mixture of white wood chips and white sawdust in a 2:1 ratio. These rhizome fragments, after disinfection and preparation according to the protocol of Djeuani et al. (2021), were placed at a 30x30 cm distance in a right angle within the propagators. The propagators were covered with transparent plastic throughout the entire process. The inoculated propagators were watered once a day with 5 L of water every 72 hours. Fifty rhizome fragments were used to produce 400 PIF plants after two months. These plants were transferred to a substrate of sterile black soil and coarse sand (2:1 ratio) and acclimated for one month.

2.3. Application of the different formulated biofertilizer treatments, and evaluation of growth and disease parameters of *X. sagittifolium* treated

The experimental design was a randomized complete block design. The treatments consisted of biofertilizers formulated and produced on rice bran and sawdust substrates, using the four fungal strains employed. Six treatments were applied: the control, rice bran or sawdust + (*Aspergillus niger, Aspergillus sp., Fusarium oxysporum, Trichoderma asperellum,* and the mixture), and the mixture. The control treatment consisted of *X. sagittifolium* plants cultured only in bags containing a mixture of sterile black soil and coarse sand. Each treatment was replicated twice in a random manner. For each treatment, 30 PIF plants of *X. sagittifolium* were used. For each treatment, 38 g of the formulated fertilizers were applied following the protocols of Djeuani et al. (2018) and Muyang et al. (2014) in the substrate (mixture of sterile black soil + coarse sand) around the roots, at 10 cm from the plant. During a period of 5 weeks, growth parameters such as average plant height, average number of leaves, average collar diameter, and average leaf area were collected weekly (Omokolo *et al.,* 2003).

2.4 Estimation of disease incidence and severity

The disease incidence in the plant was calculated based on the number of infected plants using the method of Agrios (2005) and Kranz & Rotem (1988).

$$\text{Disease incidence (\%)} = \frac{\textbf{Number of diseased plant}}{\textbf{Number of total plants observed}} \; x \; 100$$

The rating scale of 0 – 100 was then used to rank the level of disease severity from onset, which was then calculated using the formula described by Horsfall (1945) below.

$$\text{Disease severity (\%)} = \frac{\textbf{Sum total of disease rating x 100}}{\textbf{Sum of overall observation x highest grade in the scale}}$$

2.5 Data Analysis

Collected data were structured and stored in Microsoft office LTSC Professional plus 2021. Analysis of the various growth parameters was done using IBM SPSS statistics version 24. The results were expressed as means ± SD. Significant differences between mean values were determined using analysis of variance (ANOVA) with respect to the Student-Newmann-Keuls test. Principle component analysis of growth parameters per treatment was done using R-analysis software. R Version 4.5.2.

3. Results and Discussion

3.1. Microscopic characterization of the different phosphate-solubilizing fungi used

Microscopic analysis of the four endophytic fungal strains used (*Aspergillus niger, Fusarium oxysporum, Trichoderma asperellum,* and *Aspergillus* sp.) revealed the form of their conidia (Figure 1). The morphological aspects of the isolates revealed that the genus *Aspergillus* sp. exhibited a radial (Figure 1A) and eccentric (Figure 1D) shape, with abundant, granular, black aerial mycelium (Figures 1A and D). The *Aspergillus* head, black (Figure 1E) and cinnamon-brown (Figure 1H), bore globose, unicellular conidia, black (Figure 1E) and brown (Figure 1H). In the genus *F. oxysporum*, this mycelium is cottony and abundant (Figure 1B), with fusiform, hyaline macroconidia having one to five septa with a tapered and curved apical end (Figure 1F). Whereas in the genus *T. asperellum*, there is a powdery texture which tends towards a paste, dark green in color, showing under the microscope unicellular ovoid conidia of pale green color (Figure 1G). These four fungi, on Pikovskaya media, showed the ability to solubilize phosphorus, exhibiting colonial growth and the formation of variable clear zones at different time intervals (Figure 2).

Table 1. Morphological characteristics and halozone diameter of phosphate-solubilizing fungi screened from isolated endophytic fungi.

Isolate	Colony Diameter (cm)	Halozone Diameter (cm)	Phosphate Solubilization Index
Aspergillus niger	8.4 ± 0.01^{c}	3.3 ± 0.06^{b}	1.34 ± 0.04^{b}
Aspergillus sp	2.8 ± 0.01^{a}	0.4 ± 0.03^{a}	1.12 ± 0.01^{a}
Fusarium oxysporum	4.5 ± 0.02^{ab}	1.3 ± 0.02^{a}	1.26 ± 0.02^{ab}
Trichoderma asperellum	6.3 ± 0.01^{b}	4.1 ± 0.06^{c}	1.65 ± 0.07^{c}

Note: Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test.

3.2. Solubilization of Phosphorus by PSF strains

Trichoderma asperellum released phosphorus on the 2nd day of their inoculation, while *Aspergillus* and *Fusarium* species expressed clear zones as a form of phosphorus solubilization on the 3rd and 5th day, respectively. *Aspergillus* isolates later decreased their phosphate solubilization activity on the 5th day of their inoculation, whereby the halozone area became coagulated and undetectable. The solubilization of phosphorus by endophytic fungi is dependent on several mechanisms, such as organic acid production (citric acid, oxalic acid, gluconic acid), enzymatic hydrolysis and chelation (Oteino et al., 2015; Varga et al., 2020; Pan & Cai, 2023). However, studies by Belay et al. (2022) revealed that only 39-43% of endophytic isolates tested can solubilize tricalcium phosphate effectively with limited activity against aluminum phosphate or iron phosphate, as a result of pH sensitivity, acid production deficits or the inability to handle metal deficits. However, *T. asperellum* persisted in the solubilization of phosphorus right up to the 7th day, while *F. oxysporum,* on the other hand, released phosphorus from the 3rd day to the 6th day. It was observed that the endophytic fungi strain with the highest solubilization index was *T. asperellum* with an index of 1.65 ± 0.07 cm when compared with other fungal strains and the control on the 5th day. This was followed by *Aspergillus niger* with an index of 1.34 ± 0.04 cm. It was observed that *Aspergillus* sp had the smallest index (1.12 ± 0.01 cm). Therefore, the phosphate solubilizing index of isolate *T. asperellum* was significantly different ($p < 0.05$) from that of other strains (Table 1). *Trichoderma asperellum* was

observed to commence phosphate solubilization on the second day of inoculation on Pikovskaya agar right up to the 7th day, during which complete solubilization was observed on the entire petri dish.

Contrary to this, *Aspergillus niger* and *Fusarium oxysporum* began phosphate solubilization on the 3rd day after inoculation till the 5th and 6th day respectively. This indicates the variation in phosphate solubilizing capacity among phosphate solubilizing fungi isolates and their solubilization rate. The ability of *T. asperellum* to solubilize phosphate faster than the other strains may be due to its rapid production of organic acids such as gluconic acid, which could lower pH quickly, together with high levels of acid phosphatase and phytase enzymes. The phosphate solubilization indices obtained for each PSF strain used highlight that the high index observed in *T. asperellum* allows it to be classified as the fungus with the greatest phosphate solubilization capacity in this case of study. These results are concordant with the aforementioned data as they highlight the ability of *T. asperellum* to solubilize larger amounts of phosphorus. Research experiments have unveiled the ability of *Trichoderma* to fully dissolve tricalcium phosphate in 2 days through high phosphatase activity and organic acid production, with isolates reaching 143.33 µg/ml soluble phosphorus in 5 days (Ahmed & Zaim, 2023). Contrary to this, *Aspergillus* species were noted to solubilize up to 778.77 µg/ml of tricalcium phosphate after 15 days, indicating a strong but slower performance when compared to *Trichoderma,* while *F. oxysporum* emerged with lower efficiency, limited to rock phosphate and minimal in tricalcium phosphate (Elias et al., 2016).

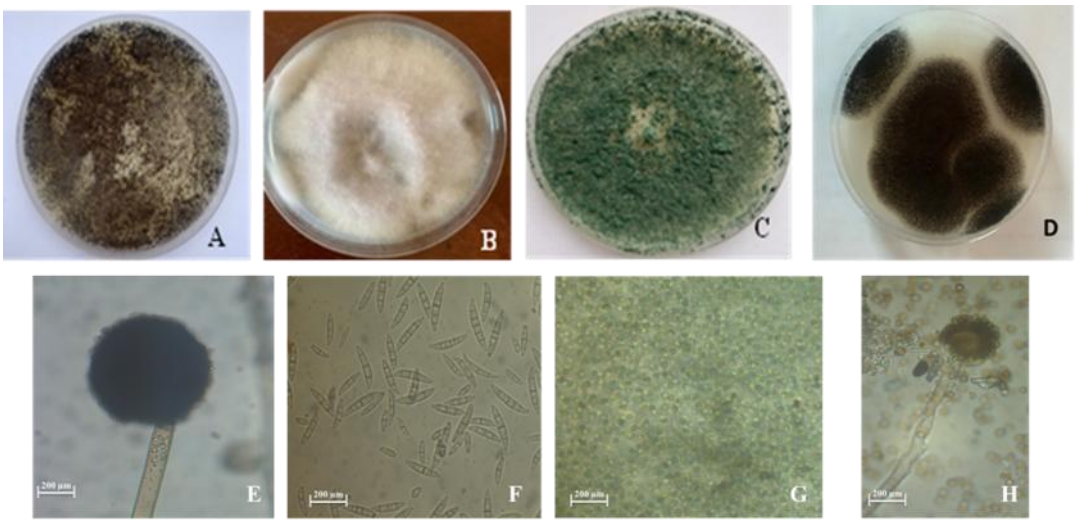

Figure 1. Macroscopic and microscopic representation of identified phosphate-solubilizing fungi. A-E) *Aspergillus niger*, B-F) *Fusarium oxysporum*, C-G) *Trichoderma asperellum*, D-H) *Aspergillus* sp.

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Table 2. Variations in the height of plants within different treatments over time

Substrate	Treatment	Time of growth evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	20.92 ± 9.42^{a}	32.99 ± 7.16^{a}	34.00 ± 6.39^{a}	35.38 ± 8.12^{ab}	34.26 ± 4.97^{ab}
	Aspergillus niger	27.02 ± 4.94^{a}	30.51 ± 8.80^{a}	33.16 ± 6.35^{ab}	33.30 ± 5.66^{ab}	32.91 ± 6.32^{ab}
	Aspergillus sp	24.86 ± 8.73^{a}	27.67 ± 9.47^{a}	31.11 ± 7.02^{ab}	32.29 ± 6.57^{ab}	33.16 ± 5.46^{ab}
	Fusarium oxysporum	27.92 ± 8.48^{a}	30.55 ± 7.51^{a}	30.27 ± 7.01^{ab}	29.86 ± 6.83^{ab}	31.31 ± 7.47^{ab}
	Trichoderma asperellum	28.98 ± 6.56^{a}	34.42 ± 4.94^{a}	39.05 ± 4.36^{b}	41.93 ± 4.99^{b}	45.20 ± 5.31^{b}
	Mixture	27.45 ± 6.72^{a}	27.23 ± 6.15^{a}	27.93 ± 7.89^{a}	27.08 ± 6.99^{a}	32.58 ± 7.72^{ab}
Sawdust	Control	24.72 ± 2.07^{a}	28.45 ± 3.49^{a}	26.00 ± 2.14^{a}	25.19 ± 2.36^{a}	25.22 ± 1.55^{a}
	Aspergillus niger	24.58 ± 7.89^{a}	26.04 ± 4.78^{a}	29.96 ± 5.86^{ab}	25.75 ± 5.84^{a}	24.70± 6.46^{a}
	Aspergillus sp	26.75 ± 1.50^{a}	29.61 ± 3.94^{ab}	30.37 ± 4.42^{b}	31.28 ± 7.85^{b}	30.34 ± 7.11^{b}
	Fusarium oxysporum	26.47 ± 9.49^{a}	29.61 ± 10.13^{a}	32.03 ± 7.82^{ab}	32.94 ± 8.80^{ab}	33.78 ± 8.42^{ab}
	Trichoderma asperellum	26.73 ± 6.13^{a}	30.93 ± 4.57^{a}	28.75 ± 6.58^{ab}	31.68 ± 7.94^{ab}	32.30 ± 7.82^{ab}
	Mixture	24.99 ± 7.07^{a}	28.65 ± 7.49^{a}	25.00 ± 7.14^{a}	23.19 ± 6.36^{a}	24.38 ± 4.55^{a}

Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test.

Table 3. Variations in the collar diameter of *X. sagittifolium* plants within treatments over time

Substrate	Treatment	Time of growth evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	8.92 ± 2.01^{ab}	9.82 ± 1.72^{ab}	9.65 ± 1.43^{b}	9.58 ± 1.93^{ab}	10.30 ± 1.35^{b}
	Aspergillus niger	8.73 ± 2.91^{ab}	9.18 ± 2.68^{ab}	9.29 ± 2.70^{ab}	8.40 ± 1.53^{ab}	7.48 ± 1.16^{ab}
	Aspergillus sp2	9.30 ± 2.28^{ab}	8.62 ± 2.69^{a}	7.61 ± 1.72^{ab}	7.37 ± 2.09^{ab}	8.55 ± 2.11^{ab}
	Fusarium oxysporum	9.39 ± 1.39^{ab}	9.13 ± 2.48^{ab}	8.5 ± 2.52^{ab}	8.55 ± 2.61^{ab}	8.15 ± 2.91^{ab}
	Trichoderma asperellum	10.61 ± 2.56^{b}	10.46 ± 2.82^{b}	9.86 ± 1.46^{b}	10.06 ± 1.94^{b}	10.15 ± 1.85^{b}
	Mixture	7.86 ± 1.79^{a}	7.93 ± 1.83^{a}	7.07 ± 3.16^{a}	7.30 ± 2.79^{ab}	7.00 ± 2.00^{ab}
Sawdust	Control	6.91 ± 1.21^{a}	7.26 ± 1.02^{a}	7.47 ± 1.76^{a}	7.40 ± 1.32^{a}	5.67 ± 2.45^{a}
	Aspergillus niger	8.33 ± 2.45^{ab}	7.92 ± 1.12^{a}	8.05 ± 2.91^{ab}	6.67 ± 2.37^{a}	5.93 ± 2.12^{a}
	Aspergillus sp2	9.00 ± 1.52^{ab}	9.73 ± 1.21^{ab}	9.11 ± 1.98^{a}	8.41 ± 3.25^{ab}	8.01 ± 2.87^{ab}
	Fusarium oxysporum	8.32 ± 3.05 ^{ab}	8.98 ± 2.55^{ab}	9.77 ± 2.86^{b}	9.14 ± 2.92^{ab}	8.75 ± 2.29^{ab}
	Trichoderma asperellum	7.91 ± 2.21^{a}	9.06 ± 2.02^{a}	8.47 ± 1.76^{ab}	7.20 ± 3.92^{a}	8.67 ± 2.45^{ab}
	Mixture	8.51 ± 1.94^{ab}	7.78 ± 1.68^{a}	6.57 ± 2.00^{a}	5.71 ± 2.14^{a}	4.82 ± 1.64^{a}

Note: Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test.

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Table 4. Variations in the number of leaves of *X. sagittifolium* plants within treatments

Substrate	Treatment	Time of growth evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	2.61 ± 0.62^{a}	3.00 ± 0.93^{a}	3.38 ± 1.14^{ab}	3.2 ± 1.49^{b}	3.30 ± 1.33^{b}
	Aspergillus niger	2.22 ± 0.84^{a}	2.61 ± 2.24^{a}	2.27 ± 0.45^{ab}	2.33 ± 0.81^{ab}	2.00 ± 0.78^{ab}
	Aspergillus sp2	2.26 ± 0.56^{a}	2.38 ± 0.71^{a}	2.27 ± 0.64^{ab}	2.44 ± 0.74^{ab}	2.66 ± 0.67^{ab}
	Fusarium oxysporum	2.33 ± 0.55^{a}	2.44 ± 0.48^{a}	2.33 ± 0.75^{ab}	2.44 ± 0.95^{ab}	2.44 ± 1.11^{ab}
	Trichoderma asperellum	2.50 ± 0.62^{a}	2.66 ± 0.60^{a}	3.44 ± 0.61^{b}	3.27 ± 0.69^{b}	3.16 ± 0.64^{b}
	Mixture	2.16 ± 0.76^{a}	2.44 ± 0.61^{a}	2.05 ± 0.79^{ab}	2.16 ± 1.01^{ab}	1.77 ± 0.91^{a}
Sawdust	Control	2.00 ± 0.58^{a}	2.18± 0.44^{a}	1.86 ± 0.35^{a}	1.80 ± 0.47^{a}	1.88 ± 0.66^{a}
	Aspergillus niger	2.44 ± 0.41^{a}	2.44 ± 0.60^{a}	2.27 ± 0.69^{ab}	2.27 ± 1.03^{ab}	1.88 ± 0.87^{a}
	Aspergillus sp2	2.27 ± 0.13^{a}	2.50 ± 0.62^{a}	2.77 ± 0.80^{ab}	2.72 ± 1.11^{ab}	2.05 ± 0.94^{ab}
	Fusarium oxysporum	2.50 ± 0.54^{a}	2.55 ± 0.63^{a}	2.88 ± 0.67^{ab}	2.94 ± 0.80^{ab}	3.05 ± 0.76^{b}
	Trichoderma asperellum	2.33 ± 0.49^{a}	2.50 ± 0.56^{a}	2.83 ± 0.66^{ab}	2.38 ± 1.07^{ab}	2.11 ± 0.86^{ab}
	Mixture	2.00 ± 0.68^{a}	2.14 ± 0.64^{a}	1.66 ± 0.55^{a}	1.50 ± 0.48^{a}	1.38 ± 0.66^{a}

Note: Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test.

Table 5. Variations in the leaf area of *X. sagittifolium* plants within treatments over time

Substrate	Treatment	Time of growth evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	201.11 ± 93.68^{a}	225.31 ± 35.56^{a}	217.04 ± 45.73^{bc}	222.79 ± 12.89^{ab}	227.45 ± 22.13^{ab}
	Aspergillus niger	216.39 ± 93.68^{a}	235.53 ± 79.51^{a}	240.04 ± 99.93^{bc}	242.19 ± 92.56^{ab}	267.03 ± 99.13^{ab}
	Aspergillus sp2	234.70 ± 96.90^{a}	247.98 ± 94.06^{ab}	232.18 ± 82.11^{bc}	250.80 ± 84.68^{ab}	259.92 ± 96.10^{ab}
	Fusarium oxysporum	228.98 ± 80.63^{a}	229.43 ± 91.80^{a}	238.31 ± 89.74^{bc}	230.22 ± 99.78^{ab}	228.50 ± 88.30^{ab}
	Trichoderma asperellum	246.43 ± 84.14^{a}	294.73 ± 89.35^{b}	351.28 ± 48.08^{c}	335.56 ± 95.86^{b}	366.07 ± 88.03^{b}
	Mixture	216.39 ± 93.68^{a}	235.53 ± 79.51^{a}	240.04 ± 99.93^{bc}	242.19 ± 72.56^{ab}	267.03 ± 99.13^{ab}
Sawdust	Control	194.73 ± 78.75^{a}	216.72 ± 52.01^{a}	128.31 ± 85.22^{a}	198.09 ± 45.37^{a}	173.13 ± 74.62^{a}
	Aspergillus niger	216.96 ± 80.61^{a}	234.98 ± 74.89^{ab}	276.30 ± 91.06^{bc}	286.68 ± 99.96^{ab}	293.05 ± 93.38^{ab}
	Aspergillus sp2	227.13 ± 57.39^{a}	273.22 ± 65.67^{ab}	267.07 ± 67.67^{bc}	285.89 ± 90.19^{ab}	252.15 ± 87.04^{ab}
	Fusarium oxysporum	239.25 ± 91.14^{a}	225.11 ± 84.95^{a}	253.36 ± 92.54^{bc}	242.81 ± 82.30^{ab}	258.37 ± 88.33^{ab}
	Trichoderma asperellum	209.71 ± 63.82a	247.71 ± 53.98^{ab}	232.18 ± 50.96^{bc}	198.77 ± 96.58^{a}	232.21 ± 77.13^{ab}
	Mixture	204.74 ± 69.75^{a}	226.72 ± 62.91^{a}	116.31 ± 77.22^{a}	184.09 ± 65.37^{a}	163.13 ± 74.62^{a}

Note: Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test.

3.3. Growth response of *X. sagittifolium* plants to biofertilizers

3.3.1. Impact of biofertilizers on the height of *X. sagittifolium* plants

No significant difference was observed in the height of *X. sagittifolium* plants with respect to treatments two weeks after biofertilizer application (Table 2). This showed that the effects of formulated biofertilizers on the height of *X. sagittifolium* seedlings were rather slow or insignificant when compared to the control treatment. The exceptional difference in the height of the plant for treatments made up of Rice bran + *T. asperellum* and sawdust + *F. oxysporum* suggests that the effectiveness of a biofertilizer is not solely dependent on its microbial composition but is also influenced by the carrier material of the inoculant. According to Sohaib et al. (2020), organic substrates are organic carriers that aid in maintaining larger populations of microbes in the rhizosphere, which may influence plant root development through the improvement of nutrient availability. Notably, plants treated with Rice bran + T. asperellum showed a steady and significant increase in height, exceeding those of all other treatments at weeks 3, 4, and 5. From week 2 to Week 5, Rice bran + *T. asperellum* recorded the highest height at 34.42 ± 3.4 to 43.17 ± 3.74 cm, whereas the lowest height recorded in week 2 was Sawdust + *Aspergillus sp* at 26.04 ± 4.29 cm, with Sawdust mixture recording the lowest height from week 3 to week 5 at 25.00 ± 3.29 cm to 24.39 ± 2.46 cm, respectively.

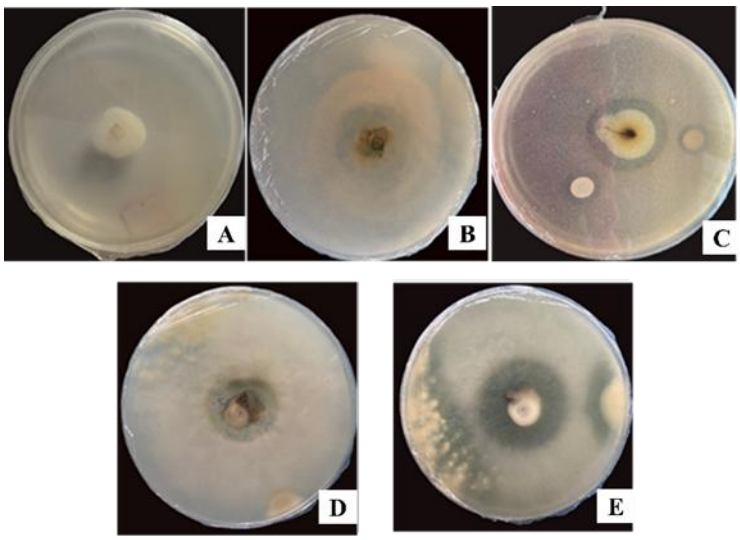

Figure 2. Isolated Phosphorus-solubilizing endophytic fungi expressing clear zones in Pikovskaya agar plates. A) Control plate, B) *Aspergillus niger*, C) *Fusarium oxysporum*, D) *Trichoderma asperellum*. F) *Aspergillus* sp.

The physicochemical properties derived from this formulation were probably suitable to promote the rapid growth of *Trichoderma*, influence the physicochemical properties of the soil, making the environment suitable for plant growth while easing the accessibility to essential nutrients and promoting plant growth hormones. Results obtained by Kapri & Tewari (2010); Ahmed & Zaim (2023), agreed with those of this study, as it showcases the effectiveness and reliability of *Trichoderma* in enhancing the growth parameters of chickpea plants. The treatment Sawdust mixed recorded the lowest number of leaves, leaf area and collar diameter within 5 weeks. The minimum effectivity of the treatment, Sawdust mixed, on the growth parameters of *X. sagittifolium* seedlings could be influenced by the incompatibility of the selected phosphate-solubilizing endophytic fungi strains when mixed. Among these phosphate-solubilizing endophytic fungi strains,

Trichoderma is typically identified as a biocontrol agent against *Aspergillus* spp and *F. oxysporum* as it inhibits their growth through mycoparasitism, enzyme secretion and competition (Modrzewska et al., 2022). However, the presence of poor outcomes could be triggered by antagonistic failures when *T. asperellum* isolates do not inhibit the pathogenic activity of *F. oxysporum*, *Aspergillus* sp and *Aspergillus niger* strains, leading to the accumulation of mycotoxins that damage plants (Wadhwa et al., 2024; Modrzewska et al., 2025). Ibrahim et al. (2026) and Huertas et al. (2026) highlight the potential of some specific endophytic fungi as multifunctional bioinoculants to improve plant growth and health for sustainable agriculture.

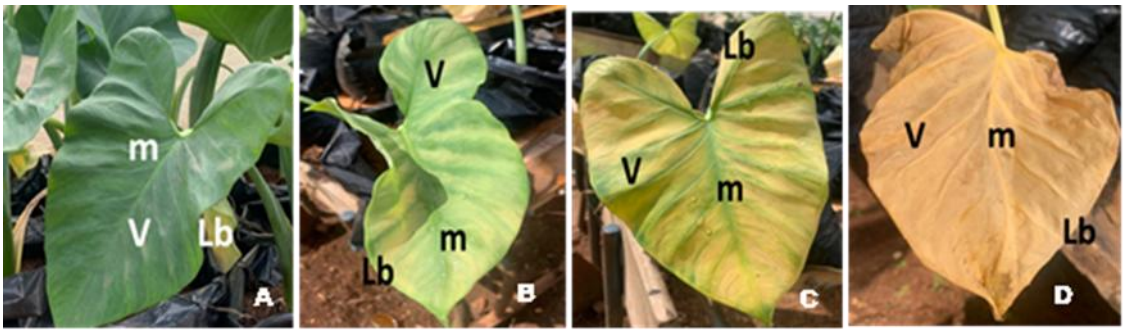

Figure 3. Various intensities of disease symptoms in *X. sagittifolium* leaves when compared to healthy leaves (m = mid rib, v = vein, lb =leaf blade). A) Healthy leave, B) Mild disease symptom, C) Severe disease symptom, D) leave dead

Table 6. Disparities in the degree of disease incidence in *X. sagittifolium* within treatments over. Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test

Substrate	Treatment	Time of evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	00.00	00.00	03.27	08.47	04.73
	Aspergillus niger	20.00	17.02	39.02	35.71	29.57
	Aspergillus sp	00.00	02.22	10.00	16.32	18.14
	Fusarium oxysporum	16.66	36.36	19.04	00.00	19.86
	Trichoderma asperellum	00.00	04.16	04.83	08.47	03.49
	Mixture	15.38	13.63	00.00	12.82	18.36
Sawdust	Control	00.00	00.00	05.25	08.27	08.00
	Aspergillus niger	28.94	33.33	17.07	04.87	26.84
	Aspergillus sp	10.25	30.23	34.14	13.63	19.32
	Fusarium oxysporum	2.22	06.52	09.61	09.44	08.10
	Trichoderma asperellum	00.00	0.000	09.80	34.88	21.04
	Mixture	13.88	21.62	26.66	22.22	28.87

3.3.2. Impact of biofertilizers on the collar diameter of *X. sagittifolium* plants

The changes in the collar diameter of *X. sagittifolium* seedlings were not significantly different at 5% with respect to treatments over time. The highest collar diameter obtained in all weeks over time was in the treatment Rice + *T. asperellum,* even though no significant increase in collar diameter was observed (Table 3). Contrary results were obtained in *Cannabis sativa* plants fertilized with *Macrophomina phaseolina*, which showed an increase in collar diameter compared to the control plant (Seemakram et al., 2026). The

treatments Sawdust + *Aspergillus* sp, Rice + *Aspergillus* sp, Sawdust mixed, and Rice mixed displayed a continuous decrease in collar diameter from week 1 to week 5. The lowest collar diameter was obtained in the treatment Sawdust mixed at week 5, while the highest was in Rice bran + *T. asperellum* at week 1. Other treatments like Rice bran + *Aspergillus* sp, Sawdust + *T. asperellum*, Rice + *F. oxysporum,* and Rice mixed were noted to have an inconsistent rise and fall in the size of their collar diameters, while the treatments Rice bran + *T. asperellum* and control were distinct from every other treatment by the sustenance of a larger size in collar diameter from week 1 to week 5 (Table 3).

Table 7. Disparities in disease severity in *X. sagittifolium* within treatments over time. Means with the same letter for the same parameter are not significantly different at 5% for the Student Newman-Keuls Test

Substrate	Treatment	Time of evaluation (Week)				
		1	2	3	4	5
Rice bran	Control	00.00	00.00	01.63	05.08	02.97
	Aspergillus niger	15.00	11.70	23.78	11.30	28.47
	Aspergillus sp2	00.00	02.22	05.50	11.73	58.11
	Fusarium oxysporum	10.71	23.29	11.31	00.00	16.47
	Trichoderma asperellum	00.00	02.60	03.63	05.93	00.00
	Mixture	12.82	07.38	00.00	08.97	42.96
Sawdust	Control	00.00	00.00	01.10	02.11	03.33
	Aspergillus niger	28.94	31.41	9.14	04.87	38.91
	Aspergillus sp2	08.54	25.58	26.83	03.40	08.33
	Fusarium oxysporum	02.22	05.97	8.17	07.07	08.64
	Trichoderma asperellum	00.00	00.00	2.45	33.13	52.63
	Mixture	09.72	19.59	6.66	16.66	22.00

3.3.3. Impact of biofertilizers on the number of leaves and the leaf area in the *X. sagittifolium* plant

The number of leaves obtained per treatment displayed similar dynamics as the height of the plant. All treatments showed no significant difference at 5% in the number of leaves from week 1 to 2 **(**Table 4). The control and Rice bran +*T. asperellum* had the highest significant ($P<0.05$) number of leaves at weeks 3, 4 and 5, with the addition of Sawdust + *F. oxysporum* at week 5. Plants treated with sawdust mixed had the lowest number of leaves from week 1 to week 5, while those treated with Rice bran + *T. asperellum* had the overall highest (3.44 ± 0.61) number of leaves at week 3. *X. sagittifolium* seedlings responded differently to different treatments with time. This could be caused by the presence of disease incidence, which led to an increase in leaf loss over time. The inconsistent effect of these formulated biofertilizers on the growth parameters of *X. sagittifolium* suggests that they can still be beneficial for the growth of plants, but could be limited in this experiment due to the environmental conditions induced by their carriers. According to Debolina & Rishi, (2023), phosphorus deficiency in plants is expressed by stunted growth and the development of dark green or purplish discoloration on leaves. However, this condition was not observed in the treatments during this study, thereby eliminating phosphorus deficiency as the reason for poor or rapid growth development. Inconsistency in growth development could be caused by common reasons, or specific reasons to each strain or formulation, or could be due to the gradual change in

physicochemical properties of the soil. In the context of biofertilizers, leaf yellowing could be caused by microbial imbalances, toxin production, or unfavorable formulation conditions that increase deficiency and stress (Chaudhary et al., 2022b). This can also explain the insignificant differences in the leaf area of *Xanthosoma* plants observed among all treatments in week 1. Plants treated with sawdust mixed unveiled an inconsistent increase in leaf area and had the lowest values across all weeks except at week 2 (Table 5).

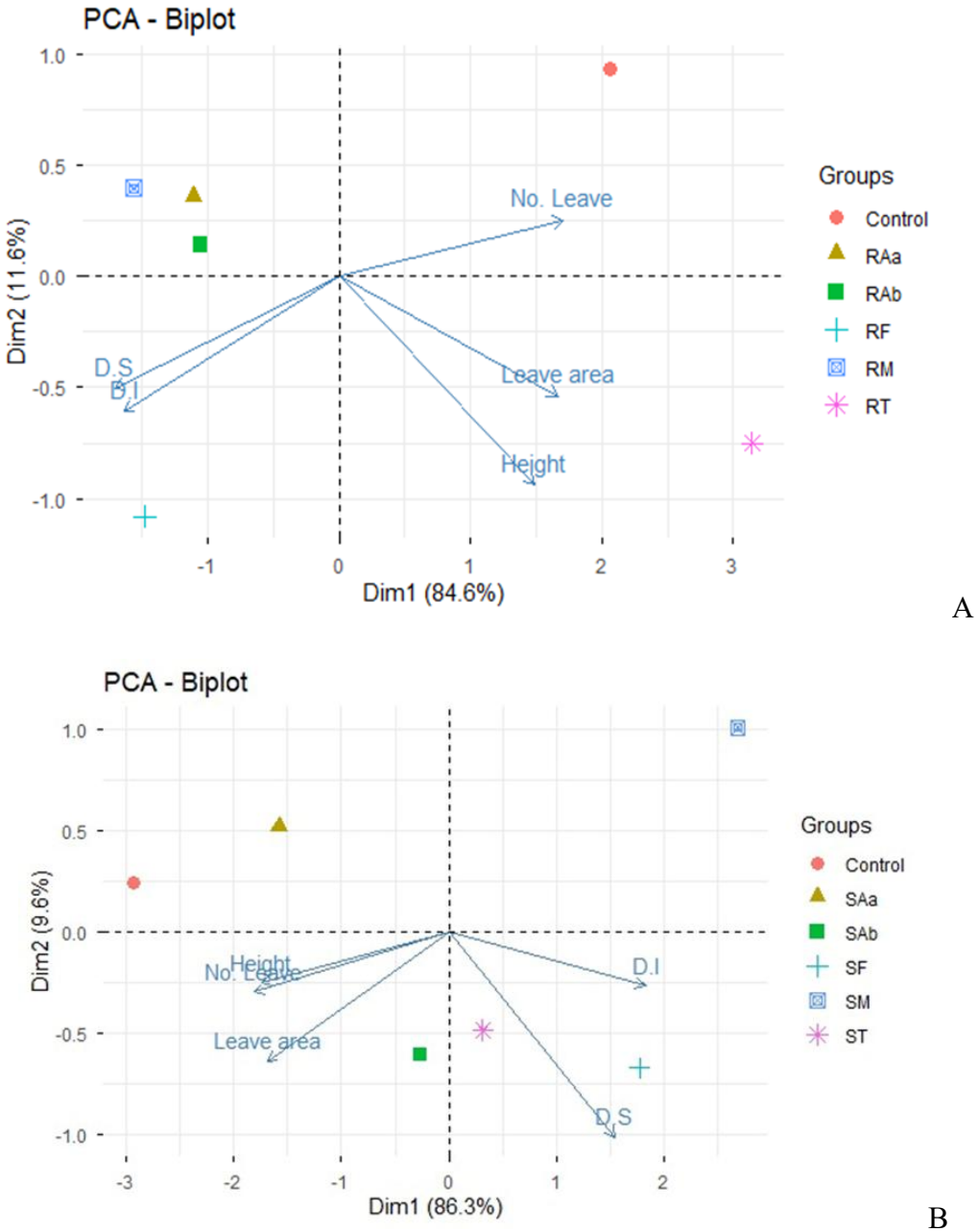

Figure 4. Principal component analysis factorial diagrams of data relative to the effect of different biofertilizers on some agronomic parameters of *X. sagittifolium* plant. D.S = disease severity, D.I = Disease incidence, C.D = Collar diameter, No. Leaves = Number of leaves, RAa = Rice bran + *Aspergillus niger*, Rab = Rice bran + *Aspergillus* b, RF = Rice bran + *F. oxysporum,* RM = Rice Mix, RT = Rice bran + *T. asperellum,* SAa = Sawdust + *A. niger*, SAb = Sawdust + *Aspergillus* b, SF = *Sawdust + F. oxysporum*, SM = Sawdust mix, ST = Sawdust + *T. asperellum.*

The treatments Rice bran + *Aspergillus niger*, Rice bran + *T. asperellum,* and the control exhibited a steady increase in leaf area from week 1 to 5, while every other treatment was marked by an intermittent increase and decrease in leaf area from week 3 to 5. Rice bran + *T. asperellum* recorded the highest leaf area in all weeks except week 1, which was topped by the control treatment. The lowest recorded leaf area was in the sawdust mixed treatment at week 3 (116.31 ± 77.22 cm^2), while the highest was in Rice bran + *T. asperellum* at week 5 (366.07 ± 88.03 cm^2).

3.4. Degree of disease incidence and severity after biofertilizer application

The results obtained unveiled the manifestation of disease symptoms common to all treatments at different time intervals and intensities. This was characterized by the partial or complete yellowing of leaves before their withering (Figure 3). Different intensities of disease symptoms expressed by the leaves of *X. sagittifolium* plants upon application of some biofertilizer treatments were observed as early as the first week. This was expressed mainly by the yellowing of leaves at intensities ranging from mild to severe, and eventually, cell death. The expression of the disease was manifested by a yellowing of the plant leaves (Figure 3). According to the evaluation of disease incidence within treatments, it was realized that the treatments, Rice bran + *Aspergillus* sp, Sawdust bran + *F. oxysporum*, Rice bran + *Aspergillus niger*, Sawdust bran + *Aspergillus niger,* and Sawdust mixed, had manifested disease symptoms in all five weeks (Table 6). Here, the highest disease incidence for Rice bran + *Aspergillus* sp. was in week 3 at 34.146 %, Sawdust + *A. niger* in week 5 at 50%, Rice bran + *A. niger* in week 3 at 39.024%, and Sawdust + *F. oxysporum* and Rice bran + *F. oxysporum* in week 5 at 12.72%, and 60%, respectively. For the treatments Sawdust + *Aspergillus* sp, Rice bran + *F. oxysporum,* and Rice bran mixed, the presence of disease was observed solely in 4 different weeks, with the highest disease incidence occurring in week 5 for Sawdust + *Aspergillus* sp (62.162%) and Rice bran mixed (50%), and in week 2 for Rice bran + *F. oxysporum* (36.364%). Nevertheless, the treatments Sawdust + *T. asperellum*, Rice bran + *T. asperellum,* and control recorded disease incidence within 3 weeks, with the highest intensity in week 5 for Sawdust + *T. asperellum* (60.526%) and Control (11.904%), and in week 4 for Rice bran + *T. asperellum* (8.47%). The highest and lowest disease incidence were obtained in Sawdust + *Aspergillus* sp2 at weeks 5 and 1, respectively. This shows that treatment made up of *Aspergillus* spp had a rapid increase in disease incidence across all weeks when compared to other treatments. It could be that the physicochemical conditions of this formulation triggered the production of toxins by *Aspergillus*, thereby activating its pathogenicity. This study indicates that the yellowing of leaves could be influenced by the physicochemical properties of formulated biofertilizers or by the pathogenic properties of *A. niger* and *F. oxysporum*. Regardless of this, the presence of disease incidence in all treatments could also be a sign of contamination.

According to Zacharia (2024), Aspergillus contains several species that act as plant pathogens, which affect growth, development and yield. These species influence the yellowing of leaves, increase mycotoxin production in plant tissues and contaminate agricultural products. *F. oxysporum* is rated as one of the most important pathogens that contribute to crop loss due to infections associated with the biosynthesis of mycotoxins (Rozewicz et al., 2021; Del Palacios et al., 2023; and Modrzewska et al., 2022). The results of disease severity for each treatment were concordant to that of the disease incidence derived (Table è). According to the analyzed data, the lowest disease severity ranged from 2.22% to 11.309%, while the highest ranged from 5.085% to 58.11%. Disease severity was more abundant in the treatments Sawdust + *Aspergillus* sp (58.11%), Sawdust + *T.*

asperellum (52.632%), Rice Mixed (42.968%), and Sawdust + *A. niger* (38.911%), all at week 5 (Table 7). Meanwhile, the medium occurrence of disease severity was in Rice bran + *Aspergillus* sp (26.83%) at week 3, Rice bran + *F. oxysporum* (23.295%) at week 2, and Rice bran + *A. niger* (28.472%), and Sawdust Mixed (22%) at week 5. The treatments Rice bran + *T. asperellum* and control were not observed as highly affected by disease severity due to the fact that their highest registered severity was 5.932% and 5.082%, respectively.

3.5. Principal component analysis between growth parameters, disease incidence and severity

The principal component analysis (PCA) reveals relationships between treatments according to carrier material (rice bran and sawdust) and agronomic variables across two dimensions. Treatments containing rice bran as a carrier had Dimensions 1 (84.6%) and Dimensions 2 (11.6%), accounting for 96.2% variance (Figure 4A). The control treatment and Rice bran + *T. asperellum* were strongly associated with the growth parameters height of plant, number of leaves and leaf area of plants. The treatments Rice + *A. niger*, Rice + *Aspergillus* sp2, Rice + *F. oxysporum* and Rice + mixture, clustered on the negative side of dimension 1, revealing strong associations with disease incidence and severity (Figure 4A). Sawdust treatments (Figure 4B) rather had a variance of 95.9% with Dimension 1(86.3%) and Dimension 2 (9.6%). In this case, Sawdust + *A. niger* and the control treatment were strongly associated with the observed growth parameters except for disease incidence and severity. Sawdust + *F. oxysporum* and Sawdust + *T. asperellum* were strongly associated with disease incidence and severity. Positive correlations observed from the PCA visuals suggest that the rise in a single growth parameter directly leads to an increase in other auxiliary parameters. Nevertheless, negative correlations between disease incidence and growth parameters visualize the reductive effects of disease incidence and severity on the proper growth of plants. Shutt et al. (2022) reveal the negative effects of disease incidence caused by *Phytophthora colocasiae,* which eventually led to retarded growth, leaf wilting, and impaired plant vigor in *Colocasia esculenta*.

4. Limitations and Future Directions

Despite the promising potential of phosphate-solubilizing endophytic fungi as biofertilizers for *Xanthosoma sagittifolium*, several limitations should be considered. The inconsistent effects observed among the formulations indicate that biofertilizer performance may be strongly influenced by the choice of carrier material, the microenvironment created by the formulation, and prevailing environmental conditions. The contrasting responses of *T. asperellum* formulated with rice bran and sawdust particularly highlight the importance of carrier selection in determining biofertilizer effectiveness. Furthermore, the occurrence of disease symptoms in several treatments, especially those containing *Aspergillus* spp. and *Fusarium oxysporum*, indicates that phosphate-solubilizing ability does not necessarily exclude pathogenic potential. These findings emphasize the importance of appropriate strain selection, formulation optimization, and stringent quality-control measures to minimize potential adverse effects before these fungal inoculants are considered for wider agricultural application.

Future studies should focus on validating the performance and safety of the most promising formulation, particularly rice bran + *T. asperellum*, through field trials under diverse environmental conditions. Molecular characterization of the fungal strains would be important for confirming strain identity and improving the selection of effective and safe inoculants. In addition, soil microbiome analysis could provide a better

understanding of the interactions between the introduced fungi and indigenous soil microbial communities. Long-term assessments are also needed to determine the stability and consistency of the biofertilizer during storage and following application to the soil. Such investigations would contribute to the development of more stable, effective, and safe fungal biofertilizers for sustainable *X. sagittifolium* production.

5. Conclusion

T. asperellum had the highest phosphate-solubilizing index and showed higher sustenance in phosphate-solubilizing activity. It could therefore be used as a highly reliable biofertilizer inoculant for soil amelioration and crop enhancement. The high level of disease incidence was observed in *X. sagittifolium* plants treated with *F. oxysporum* and *A. niger* biofertilizers. This shows that the ability of fungi to solubilize phosphorus does not limit their pathogenic potential. Nevertheless, the effectiveness and consistency of Rice bran + *T. asperellum* in increasing growth parameters make it a prospective bioinoculant for the industrial production of biofertilizer and an effective agricultural amendment. The contradictory performance of Sawdust + *T. asperellum* in promoting plant growth suggests that factors such as carrier choice, microenvironment and environmental conditions may limit the effectiveness of biofertilizers or trigger a negative impact. It also highlights rice bran as a good carrier material for biofertilizer production using *T. asperellum*. This study, therefore, emphasizes the importance of carrier material in biofertilizer production and the possibility of biofertilizers acting as pathogenic agents when quality control measures and selection processes are not implemented. Future studies should include the field trials, the molecular characterization of our different strains, the soil microbiome analysis, this to understand the long-term biofertilizer stability.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that ChatGPT was used only as an auxiliary tool during manuscript preparation, mainly for language polishing, wording refinement, and translation support. All outputs generated with AI assistance were critically evaluated, verified, and substantially modified by the authors. The authors remain fully accountable for the accuracy, originality, integrity, and academic content of the manuscript.

Authorship Contribution Statement

Drafting of the first draft of the article (Theresa Akinimbom Moma); Methodology and data analysis (Theresa Akinimbom Moma; Mbouobda Hermann Désiré and Djeuani Astride Carole); Supervision, review, and corrections for publication (Mbouobda Hermann Désiré and Djeuani Astride Carole); Fundraising (Djeuani Astride Carole)

Declaration of Conflicts of Interest

The authors declare that they have no conflict of interest.

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