

# KURUKOD

Journal of Agriculture and Technology

Volume 2 Issue No. 1

ISSN: 2815 - 2298



Published by:



Dr. Emilio B. Espinosa, Sr. Memorial State College of Agriculture and Technology

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REGULAR ARTICLE

# Silver Nanoparticle Improves *In Vitro* Germination, Growth and Pigment Accumulation of Coffee (*Coffea arabica* L.)

ARTICLE HISTORY

Received 29 September 2025  
Revised 18 October 2025  
Accepted 23 October 2025

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**Abstract:** This study investigated the effects of silver nanoparticles (AgNPs) on the *in vitro* germination, growth, and pigment accumulation of *Coffea arabica* L. zygotic embryos. Embryos were cultured on Murashige and Skoog (MS) medium supplemented with AgNPs at concentrations of 0.1, 0.5, 1.0, and 1.5 mg L<sup>-1</sup>, arranged in a Randomized Complete Block Design with three replications. Results showed that AgNPs significantly improved the coefficient of velocity of germination and reduced mean germination time, with the most consistent effect observed at 0.5 mg L<sup>-1</sup>. Morphological traits were also enhanced, with 1.0 mg L<sup>-1</sup> of AgNPs producing the longest shoots and 0.5 mg L<sup>-1</sup> of AgNPs inducing the longest roots and the earliest formation of true leaves. Pigment analysis revealed increased chlorophyll and carotenoid content under AgNPs supplementation, indicating enhanced photosynthetic capacity. The findings demonstrate that AgNPs, particularly at 0.5–1.0 mg L<sup>-1</sup>, can accelerate germination, promote early growth, and enhance pigment accumulation in Arabica coffee embryos under *in vitro* conditions.

**Keywords:** arabica coffee; embryo culture; *in vitro* germination; silver nanoparticles; zygotic embryos

## 1. Introduction

Coffee is among the most valuable commodities in global trade. It has four main commercially cultivated species: Robusta, Liberica, Excelsa, and Arabica. However, Arabica is prized for its superior flavor and quality, accounting for 93.87 million bags in global coffee production (International Coffee Organization, 2022). Arabica is the sole self-fertile species within the Rubiaceae family (Gómez et al., 2023). In 2018, Arabica coffee accounted for over 23% of the Philippines' total coffee production, primarily cultivated in the country's highland areas (Philippine Statistics Authority, 2018).

Coffee production in the Philippines has declined over the past decade, despite its significant economic impact. Production decreased from 72,342 MT DC (36,171 MT GCB) to 60,640.95 MT DC (30,320.47 MT GCB) between 2015 and 2020, while yields decreased from 0.64 MT ha<sup>-1</sup> to 0.54 MT ha<sup>-1</sup> (Department of Agriculture, 2021). From 2019 to 2020, coffee production decreased by 4.2% worldwide (ICO, 2022). The same report noted that the country is only 15% self-sufficient in coffee, with imports accounting for about 81% of domestic consumption. Several factors, including uneven and slow seed germination and a lack of high-quality planting materials, cause this decline. These challenges show the pressing need for innovative strategies and the application of cutting-edge technologies to meet the growing demand both domestically and internationally.

The propagation of Arabica coffee presents notable challenges. Although seeds are the primary propagation method, this approach often results in uneven plantation establishment (Pinto et al., 2018). Coffee seeds are also known to germinate slowly, further complicating propagation. Farmers in Bansalan, Davao del Sur, and Pangantucan, Bukidnon, in the Philippines, reported that they are using seeds to propagate Arabica coffee. However, seed germination in the field is slow and uneven, which could be due to the inherent dormancy period of the seeds. Arabica seeds generally germinate in the following order: a) 32 days after harvest, b) 50 days after 8 weeks of storage, and c) 42 to 70 days after harvest (Wintgens & Zamarripa, 2004). *In vitro* propagation, also known as micropropagation, offers a more efficient alternative, enabling the rapid multiplication of high-quality planting materials under controlled, aseptic conditions.

In recent years, nanotechnology has emerged as a promising tool in plant tissue culture. Nanoparticles (NPs), particles smaller than 100 nm, possess unique physicochemical properties influencing plant growth, morphogenesis, pigment accumulation, and secondary metabolite production (Prasad et al., 2024). Among them, silver nanoparticles (AgNPs) are widely studied due to their antimicrobial properties, ability to modulate plant hormonal balance, and influence physiological and biochemical processes. Several studies demonstrate their significant potential in enhancing *in vitro* growth and development when applied at optimal concentrations. For instance, Tamimi and Othman (2023) reported that supplementing culture media with AgNPs improves shoot, root, and photosynthetic pigment accumulation in *Musa acuminata*. Similarly, Şener and Sayğı (2023) found that 0.1 mg L<sup>-1</sup> AgNPs significantly promoted the growth and development of boysenberry under PEG-induced drought stress, demonstrating their ability to alleviate abiotic stress effects. Furthermore, Tung et al. (2021) revealed that adding 0.5 mg L<sup>-1</sup> AgNPs to MS medium markedly improved *in vitro* growth, shoot multiplication, and plantlet survival in strawberry micropropagation, while reducing ethylene accumulation. However, the effects of AgNPs are not universal. Sami et al. (2020) highlight that silver nanoparticles' efficacy is dose-dependent and species-specific.

To the best of our knowledge, no published studies have investigated the effects of AgNPs on *Coffea arabica* zygotic embryo culture. Therefore, this study aims to examine the influence of AgNPs on *in vitro* germination, early growth, and pigment accumulation of arabica coffee. A treatment range of 0.1, 0.5, 1.0, and 1.5 mg L<sup>-1</sup> was selected to capture both the stimulatory and potential inhibitory effects of AgNPs. By integrating nanotechnology into tissue culture, it aims to establish scientific data for nanotechnology-assisted micropropagation that can enhance the production of high-quality and uniform planting materials, thereby advancing plant biotechnology and supporting sustainable coffee production for the benefit of the farmers and the coffee industry.

## 2. Materials and Methods

### 2.1 Explant Collection

Arabica coffee (*Coffea arabica* L. var. Catimor) berries were collected from a reputable farm at Sitio Balutakay, Bansalan, Davao del Sur. The berries were carefully selected for size, maturity, and freedom from visible defects and disease to ensure uniformity. Immediately after harvest, the berries were

packed in jute sacks and transported to the Plant Tissue Culture Laboratory, Department of Horticulture, Visayas State University, Baybay City, Leyte, Philippines.

Upon arrival, one thousand (1000) berries were soaked in tap water, and floating fruits were discarded. The remaining berries were depulped to obtain seeds, which were soaked in tap water containing 10% fungicide (Dithane) for 12 hours. Mucilage was removed by rubbing seeds against each other under running water, and floating seeds were again discarded.

## 2.2 Surface Sterilization of Seeds

Seed disinfection was carried out under aseptic conditions. The seeds were first immersed in 95% ethanol for 5 minutes with continuous shaking, followed by two rinses with sterile distilled water. They were then surface-sterilized in 10% sodium hypochlorite (NaOCl) solution for 10 minutes, rinsed three times with sterile distilled water, and finally treated with 80% ethanol for 20 minutes inside the laminar flow hood. After three additional rinses with sterile distilled water, the sterilized seeds were prepared for zygotic embryo extraction.

## 2.3 Culture Medium Preparation

The medium used was Murashige and Skoog (1962) formulation supplemented with vitamins, Fe-EDTA, 30 g L<sup>-1</sup> sucrose, and 5 g L<sup>-1</sup> agar for solidification. The pH of the medium was adjusted to 5.8 ± 0.1 with either 1.0N HCl or 1.0N NaOH before dispensing. Culture vessels containing 15 mL of the medium were sterilized by autoclaving at 121 °C and 15 psi for 20 minutes (Murashige & Skoog, 1962).

## 2.4 Preparation of Nanoparticle Stock Solution

Silver nanoparticles (AgNPs) were procured from Guangzhou Hongwu Materials Technology Co., Ltd. (Guangzhou, China). Based on the Certificate of Analysis, the AgNPs had the following specifications: particle size 80–100 nm, surface area 4–8 m<sup>2</sup>/g, tap density 1–2.5 g/mL, true density 10.5 g/cm<sup>3</sup>, and purity 99.99%.

A stock solution of 100 mg L<sup>-1</sup> was prepared following the modified protocol of Faraji and Sepehri (2019). Ten (10) mg of AgNPs were suspended in 100 mL of deionized water and sonicated (100 W, 40 kHz) for 30 minutes to ensure uniform dispersion. Working concentrations were prepared by serial dilution using the formula:

$$C_1V_1 = C_2V_2 \quad (1)$$

where  $C_1$  is the concentration of the stock solution,  $V_1$  is the volume of the stock solution required,  $C_2$  is the desired concentration, and  $V_2$  is the final volume of the working solution. Suspensions were vortexed before use to minimize aggregation, sterilized, and immediately incorporated into the culture medium.

## 2.5 Zygotic Embryo Extraction

Under aseptic conditions, zygotic embryos were excised from sterilized seeds by carefully cutting open the endosperm with a sterile razor blade. Excised embryos were transferred individually into culture vessels containing 15 mL of medium corresponding to each treatment. The cultures were incubated at 25 ± 2 °C under cool white fluorescent light at 2,500 lux with an 8 h light/16 h dark photoperiod for four (4) weeks. Germination was monitored daily, using the emergence of cotyledons as the criterion for successful germination.

## 2.6 Plantlet Establishment

After the initial 4-week germination period, the germinated zygotic embryos were subcultured into fresh MS medium supplemented with vitamins, Fe-EDTA, 30 g L<sup>-1</sup> sucrose, 2.0 mg L<sup>-1</sup> BAP, and 5 g L<sup>-1</sup>

agar. Two consecutive passages were conducted at 4-week intervals under the same growth conditions for plantlet establishment.

## 2.7 Data Gathered

### 2.7.1 Germination response

The germination of coffee embryos was assessed using the final germination percentage (FGP), coefficient of velocity of germination (CVG), and mean germination time (MGT). FGP represents the proportion of embryos that successfully germinated, CVG indicates the speed of germination over time, and MGT reflects the average duration required for embryos to germinate throughout the observation period.

### 2.7.2 Morphological characteristics

Morphological characteristics of coffee plantlets were evaluated based on shoot length, root length, the average number of true leaves, days to the formation of the first true leaf, and vigor index (VI). Shoot and root lengths were measured in millimeters from the base to the apex of the shoot and along the entire root, respectively. The number of true leaves per plantlet was counted, and the days to the appearance of the first true leaf were recorded from inoculation to leaf emergence. Vigor index was calculated according to Vashisth and Nagarajan (2010) as the product of the final germination percentage and the total seedling length (root + shoot), providing an integrated measure of both germination performance and early seedling growth.

### 2.7.3 Pigment accumulation

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids ( $\text{mg g}^{-1}$ ) were determined following the method of Hiscox and Israelstam (1979) with minor modifications. Leaves were collected and cut into thin strips. Leaf samples were incubated in a water bath containing 80% ethanol at 70 °C until the tissues were bleached. The absorbance of the solution was measured using a spectrophotometer at 645 and 663 nm for chlorophylls and at 470 nm for carotenoids. Pigment concentrations were calculated using the equations of Arnon (1949) for chlorophylls and the formula of Lichtenthaler (1987) for carotenoids, as follows:

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{):} \quad \text{Chl}_a = 12.7 (A_{663}) - 2.69 (A_{645}) \quad (2)$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{):} \quad \text{Chl}_b = 22.9 (A_{645}) - 4.68 (A_{663}) \quad (3)$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{):} \quad \text{Chl}_{\text{total}} = 20.2 (A_{645}) + 8.02 (A_{663}) \quad (4)$$

$$\text{Carotenoids (mg g}^{-1}\text{):} \quad \text{Carotenoids} = \frac{1000 (A_{470} - 3.27 (\text{Chl}_a) - 104 (\text{Chl}_b))}{227} \quad (5)$$

Where:

$\text{Chl}_a$  = chlorophyll *a* concentration ( $\text{mg g}^{-1}$ )

$\text{Chl}_b$  = chlorophyll *b* concentration ( $\text{mg g}^{-1}$ )

$\text{Chl}_{\text{total}}$  = total chlorophyll concentration ( $\text{mg g}^{-1}$ )

$A_{645}$ ,  $A_{663}$ ,  $A_{470}$  = absorbance at the indicated wavelength

## 2.8. Statistical Analysis

Data were recorded, consolidated, tabulated, and statistically analyzed through analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) with inoculation days as blocks. The Least Significant Difference (LSD) test was used to determine significant differences among treatment means. Statistical analysis was conducted using the computer software Statistical Tool for Agricultural Research (STAR) version 2.0.1, developed by the International Rice Research Institute (IRRI).

## 3. Results and Discussion

### 3.1 Germination Response

The germination response of coffee zygotic embryos to silver nanoparticles is presented in Table 1. In this study, the emergence of the cotyledon served as a key indicator of germination, although it is widely defined by radicle protrusion (Khan, et al., 2021).

Final germination percentage showed no statistical significance. In contrast, the coefficient of velocity of germination significantly increased at 0.5 mg L<sup>-1</sup> AgNPs with values of 15.21, and is statistically comparable to 1.0 and 1.5 mg L<sup>-1</sup> AgNPs with a CVG of 14.24 and 14.08, respectively. The results suggest improved vigor and more synchronized germination compared to MS alone, with 12.27 CVG. Mean germination time (MGT) also showed a significant reduction, with the shortest duration observed at 0.5 mg L<sup>-1</sup> (6.58 days), considerably lower than MS alone (8.09 days) but statistically comparable to 1.0 and 1.5 mg L<sup>-1</sup> AgNPs with an MGT of 7.05 and 7.11 days, respectively. This indicates a potential acceleration in metabolic activation and cell division due to AgNPs. Faraji and Sepehri (2019) stated that low concentrations of AgNPs can enhance germination by modulating enzymatic activity and stimulating reactive oxygen species (ROS) signaling.

These findings imply that AgNPs at 0.5 to 1.5 mg L<sup>-1</sup> can improve the speed and uniformity of germination, with 0.5 mg L<sup>-1</sup> being the most consistent. The results may be attributed to the influence of AgNPs on starch metabolism. Mahakham et al. (2017) found that nanopriming rice seeds with biosynthesized AgNPs significantly increased  $\alpha$ -amylase activity, an enzyme responsible for converting starch into sugars during early germination. Their study reported a 2.5-to-2.6-fold increase in  $\alpha$ -amylase activity and a 2.0-to-2.2-fold accumulation in total soluble sugars. Interestingly, chemically primed seeds using AgNO<sub>3</sub> or hydropriming did not produce similar results, which suggests a unique action of AgNPs. This supports the hypothesis that AgNPs improve early metabolic processes by promoting enzymatic activities and sugar mobilization.

Table 1. Germination response of arabica coffee to silver nanoparticle applications.

TREATMENTS	FGP (%)	CVG (-)	MGT (day)
MS Alone	70.00	12.37 <sup>c</sup>	8.09 <sup>a</sup>
MS + 0.1 mg L <sup>-1</sup> AgNP	56.67	13.81 <sup>b</sup>	7.25 <sup>b</sup>
MS + 0.5 mg L <sup>-1</sup> AgNP	74.17	15.21 <sup>a</sup>	6.58 <sup>c</sup>
MS + 1.0 mg L <sup>-1</sup> AgNP	70.00	14.24 <sup>ab</sup>	7.05 <sup>bc</sup>
MS + 1.5 mg L <sup>-1</sup> AgNP	80.83	14.08 <sup>ab</sup>	7.11 <sup>bc</sup>
P-value	0.0693	0.0072	0.0048
c.v (%)	11.91	4.55	4.41

Means with the same letter in a column are not significantly different (Fisher's LSD,  $p < 0.05$ ); FGP (Final germination percentage), CVG (Coefficient of germination rate), and MGT (Mean germination time)

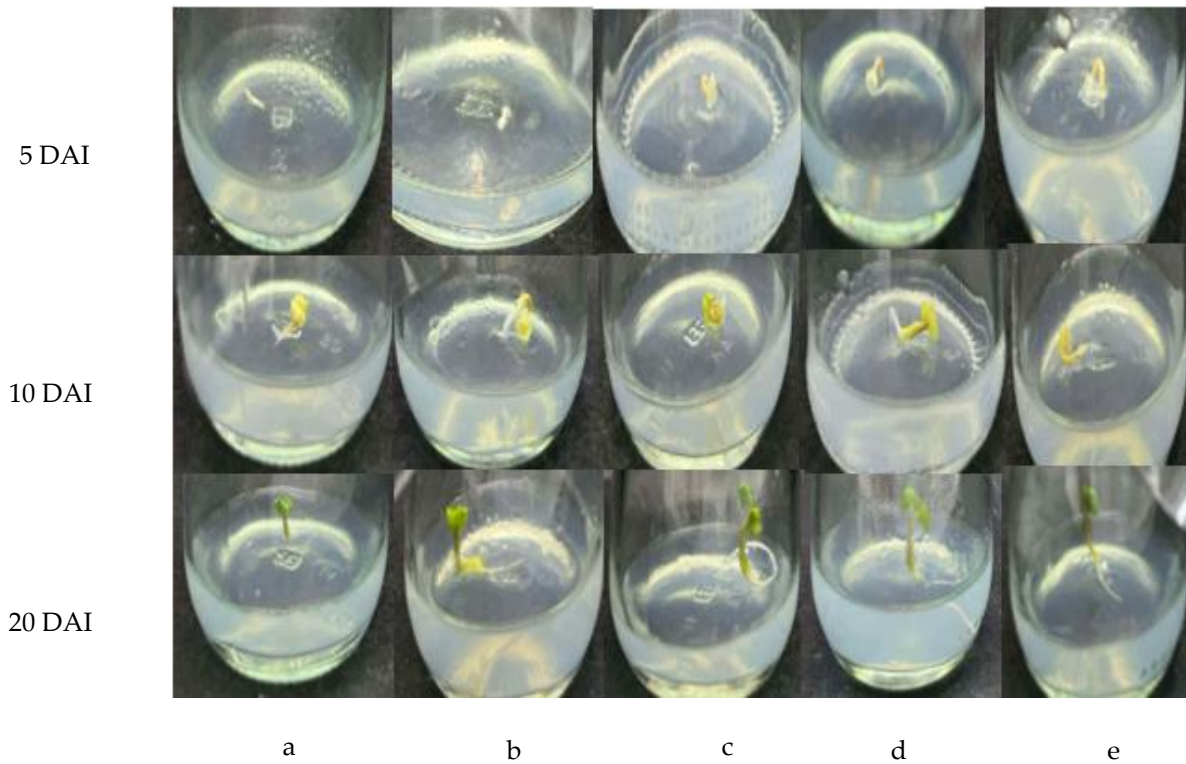


Figure 1. *In vitro* germination of arabica coffee after 5, 10, and 20 days of incubation (DAI) in AgNP-added medium: (a) MS Alone; (b) 0.1 mg L<sup>-1</sup>; (c) 0.5 mg L<sup>-1</sup>; (d) 1.0 mg L<sup>-1</sup>; (e) 1.5 mg L<sup>-1</sup>.

In this study, although the final germination percentage was not statistically significant, the marked reduction in MGT and significant CVG suggest a favorable physiological response in *C. arabica* embryos. These results are in agreement with previous findings that nanoparticles may serve as nanocatalysts, promoting early metabolic activity, enhancing antioxidant defense, and improving overall germination performance (Tymoszuk, 2021; Mahajan et al., 2022).

### 3.2 Shoot Length

The shoot length of *C. arabica* zygotic embryos as affected by varying concentrations of silver nanoparticles (AgNPs) is presented in Figure 2. At 4 weeks after incubation (WAI), no significant differences were observed among treatments, although early trends indicated a slight increase in shoot length in embryos treated with 1.0 mg L<sup>-1</sup> AgNPs compared to the control, which continued at 6 WAI.

By 8 WAI, differences across treatments became statistically significant. The 1.0 mg L<sup>-1</sup> treatment recorded the longest shoot length at 15.39 mm, whereas both MS alone, 0.1 mg L<sup>-1</sup> and 0.5 mg L<sup>-1</sup> treatments remained shorter (11.74 mm, 12.19 mm, and 12.50 mm, respectively). This trend was sustained through 10 and 12 WAI, where the 1.0 mg L<sup>-1</sup> AgNPs continued to promote shoot elongation, reaching a final length of 17.21 mm at 12 WAI. The 1.5 mg L<sup>-1</sup> treatment followed closely (16.05 mm), while MS alone consistently remained the shortest (13.72 mm). These results suggest that shoot growth in *C. arabica* zygotic embryos responded positively to AgNP supplementation, particularly at 1.0 mg L<sup>-1</sup>. The consistent improvement in shoot length over time may reflect enhanced stimulation of cell division and elongation, or improved ion transport across growing tissues. Interestingly, while the 1.5 mg L<sup>-1</sup> treatment also promoted shoot elongation, it did not outperform the 1.0 mg L<sup>-1</sup> level, implying that higher concentrations may yield diminishing returns or even induce mild phytotoxic effects over prolonged exposure.

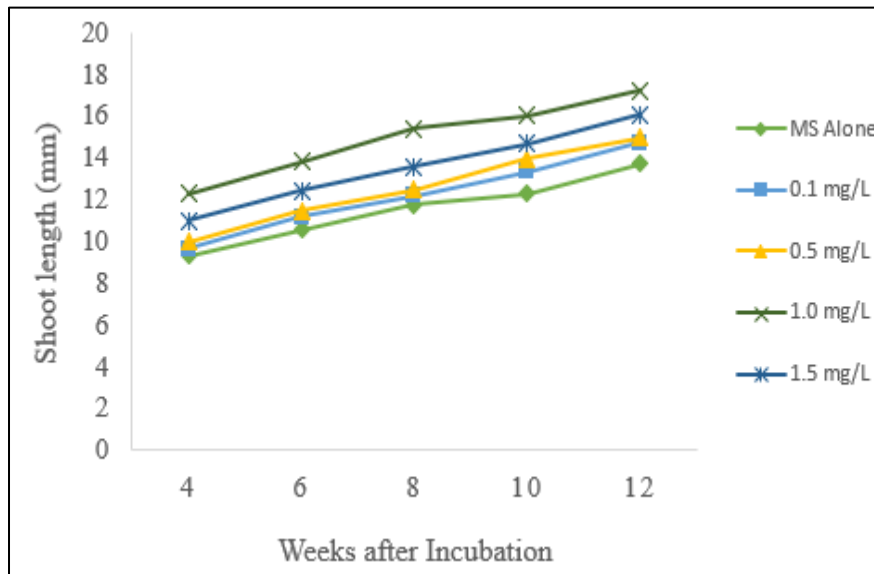


Figure 2. Shoot length (mm) of arabica coffee after 4, 6, 8, 10, and 12 weeks of incubation in AgNP-added medium

Earlier studies support this dose-dependent pattern of growth stimulation. For instance, Aguirre-Noyola (2025) stated that low doses of NPs can elicit positive responses in plants, while high doses can cause nanotoxicity. Sadak (2019) observed increased shoot length in fenugreek after AgNP application. In tomato (*Solanum lycopersicum*), Ansari et al. (2023) reported that 10 ppm AgNPs significantly improved its morphological characteristics, such as its shoot length. However, concentrations beyond 25 ppm resulted in reduced growth, suggesting a threshold beyond which the positive effects of AgNPs decline. These findings support the idea that AgNPs exhibit a hormetic effect, beneficial at low to moderate levels, but potentially inhibitory at higher concentrations. Although these concentrations are considerably higher than those used in the present study, the physiological response appears consistent. The observed enhancement in shoot elongation was attributed to increased pigments, soluble sugars, and proteins, which collectively support metabolic activity and cell proliferation.

### 3.3 Root Length

The effect of AgNPs on the root length of *C. arabica* zygotic embryos at different concentrations is presented in Figure 3. At 4 weeks after inoculation, significant differences were observed among the treatments. Applying 0.5 mg L<sup>-1</sup> AgNPs produced the longest roots with a mean length of 26.20 mm, comparable to those embryos applied with 1.0 mg L<sup>-1</sup> and 0.1 mg L<sup>-1</sup> with root lengths of 23.53 mm and 22.70 mm, respectively. The control (MS alone) and 1.5 mg L<sup>-1</sup> consistently recorded the shortest root length at 16.37 mm and 17.77 mm, respectively. Root elongation was still significantly influenced at 6 WAI, with the 0.5 mg L<sup>-1</sup> and 1.0 mg L<sup>-1</sup> treatments yielding 32.17 mm and 30.68 mm, respectively, compared to 19.83 mm in the control.

A similar trend continued at 10 WAI, where the same treatments recorded the highest values, 39.05 mm and 37.77 mm, respectively, while MS alone remained the lowest at 24.18 mm. Although statistical significance was no longer observed at 12 WAI, the 0.5 mg L<sup>-1</sup> AgNPs produced a root length of 43.83 mm. The results indicate that silver nanoparticles enhanced root elongation over time, particularly at 0.5 mg L<sup>-1</sup>. Accordingly, AgNPs enhanced root elongation through their effects on hormone signaling pathways.

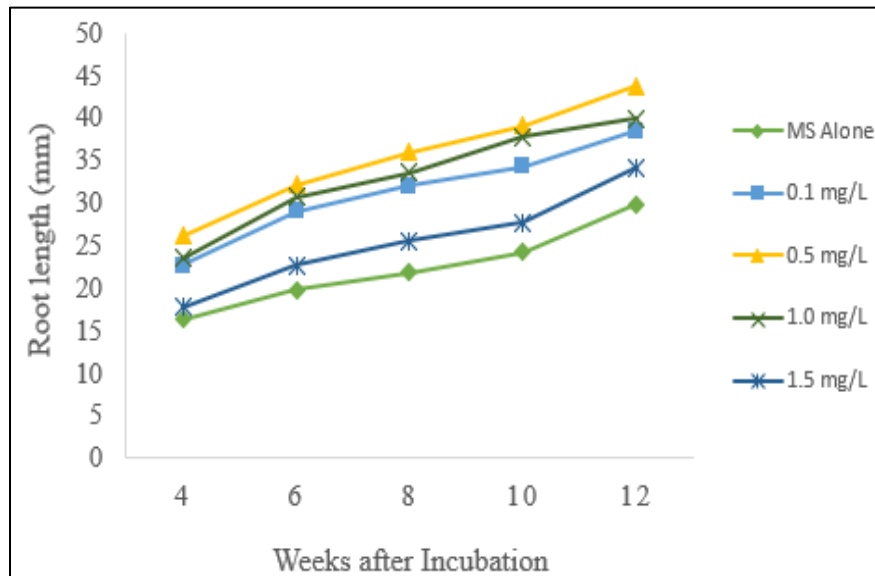


Figure 3. Root length (mm) of arabica coffee after 4, 6, 8, 10, and 12 weeks of incubation in AgNP-added medium

Syu et al. (2014) demonstrated that AgNPs can antagonize ethylene-mediated inhibition of root growth in *Arabidopsis* by reducing the expression of ACC synthase (ACS7) and ACC oxidase (ACO2). This interference with ethylene perception supports better elongation in developing tissues.

At lower concentrations, AgNPs have been found to stimulate moderate production of reactive oxygen species (ROS), particularly hydrogen peroxide ( $H_2O_2$ ), which plays a role in activating the root meristem and promoting cell division. Wang et al. (2020) reported that low levels of ROS can function as signaling molecules involved in root development. In contrast, high concentrations lead to oxidative stress that disrupts the cell cycle, eventually hampering growth. This pattern of response was also evident in the present study, where a higher AgNP concentration ( $1.5 \text{ mg L}^{-1}$ ) led to reduced root length, suggesting a threshold level beyond which toxicity might occur.

The hormetic effect observed in this study, where lower concentrations of AgNPs enhanced root growth while higher concentrations suppressed it, is consistent with the results reported by Guzmán-Báez et al. (2021). They documented a similar response in tomato seedlings, where low levels of AgNPs promoted root length and number, attributed to enhanced auxin activity and nutrient uptake. Similar results were found in this study, where AgNPs, when applied at optimal concentrations, can enhance early root development in *C. arabica* zygotic embryos under *in vitro* conditions. However, exceeding this concentration may lead to a reverse effect due to oxidative stress or possible hormonal imbalances.

### 3.4 Morphological Traits

The morphological traits such as number of leaves, days to formation of true leaves and vigor index of *C. arabica* zygotic embryos cultured *in vitro* is presented in Table 2. The number of true leaves is a vital indicator of early seedling development in *C. arabica*. However, the application of varying levels of Ag nanoparticles showed no significant effect on the number of true leaves formed by the zygotic embryos. On average, the number of true leaves produced across treatments ranged from 2.84 to 3.27 leaves per seedling. Embryos treated with  $1.0 \text{ mg L}^{-1}$  obtained an average number of true leaves of 3.27, while the control obtained an average of 2.94 true leaves.

On the other hand, the application of silver nanoparticles significantly affected the mean number of days to the formation of the first true leaf. The earliest formation was observed in embryos treated with  $0.5 \text{ mg L}^{-1}$  AgNPs, which developed true leaves in just 54 days.

Table 2. Number of leaves, days to formation of true leaves, and vigor index of arabica coffee to silver nanoparticle applications.

Treatments	Number of true leaves	Days to formation of true leaves	Vigor index
MS Alone	2.94	62.00 <sup>c</sup>	305.33 <sup>b</sup>
MS + 0.1 mg L <sup>-1</sup> AgNP	2.84	58.00 <sup>b</sup>	308.80 <sup>b</sup>
MS + 0.5 mg L <sup>-1</sup> AgNP	3.27	54.00 <sup>a</sup>	437.39 <sup>a</sup>
MS + 1.0 mg L <sup>-1</sup> AgNP	3.23	57.00 <sup>b</sup>	396.92 <sup>ab</sup>
MS + 1.5 mg L <sup>-1</sup> AgNP	3.14	58.00 <sup>b</sup>	408.60 <sup>a</sup>
P-value	0.5697	0.0013	0.0394
c.v (%)	11.90	2.25	13.73

Means with the same letter in a column are not significantly different (Fisher's LSD,  $p < 0.05$ )

This was followed by embryos treated with 1.0 mg L<sup>-1</sup>, 1.5 mg L<sup>-1</sup>, and 0.1 mg L<sup>-1</sup>, which formed true leaves at 57 and 58 days, respectively. MS without any supplementation of AgNPs delayed the onset of true leaf formation at 62 days. These findings show that AgNPs, particularly at 0.5 mg L<sup>-1</sup>, promoted faster development of leaf structures in Arabica coffee embryos cultured *in vitro*. The earliest formation of true leaves observed in embryos treated with 0.5 mg L<sup>-1</sup> AgNPs may be attributed to mechanisms similar to those reported by Manickavasagam et al. (2019) in rice calli, where low concentrations of AgNPs enhanced tissue development by regulating reactive oxygen species (ROS), improving antioxidant status, and modulating hormone levels such as abscisic acid and ethylene.

Moreover, the vigor index of *C. arabica* zygotic embryos were significantly influenced by the application of silver nanoparticles (Table 2). Embryos treated with 0.5 mg L<sup>-1</sup> and 1.5 mg L<sup>-1</sup> AgNPs obtained the highest vigor indices at 437.39 and 408.60, respectively. These values were significantly higher compared to the control (305.33) and 0.1 mg L<sup>-1</sup> (308.80), while 1.0 mg L<sup>-1</sup> (396.92) was statistically comparable to both groups.

The increased vigor index at 0.5 and 1.5 mg L<sup>-1</sup> suggests enhanced seedling performance as a result of AgNP application. This improvement likely reflects faster germination and early seedling growth, potentially due to the stimulatory effects of silver nanoparticles on metabolic processes during *in vitro* development. Similar effects have been reported in rice seeds, where low concentrations of green-synthesized AgNPs enhanced seedling vigor by boosting  $\alpha$ -amylase activity, increasing soluble sugar accumulation, upregulating aquaporin transporters, and optimizing reactive oxygen species (ROS) signaling, all of which support vigorous early seedling growth (Mahakham et al., 2017).

### 3.5 Photosynthetic Pigments

The application of silver nanoparticles (AgNPs) significantly influenced the photosynthetic pigment profile of *C. arabica* regenerants (Figure 4). The chlorophyll *a* content was highest at 0.5 mg L<sup>-1</sup> AgNPs (5.90 mg g<sup>-1</sup>), followed by 0.1 mg L<sup>-1</sup> (5.67 mg g<sup>-1</sup>), both significantly higher than the control (4.37 mg g<sup>-1</sup>). For chlorophyll *b*, the 0.1 mg L<sup>-1</sup> treatment recorded the highest value (2.48 mg g<sup>-1</sup>), closely followed by 0.5 mg L<sup>-1</sup> (2.40 mg g<sup>-1</sup>), while the control was lower (2.03 mg g<sup>-1</sup>). A similar pattern was observed for total chlorophyll, where 0.5 mg L<sup>-1</sup> showed the highest content (8.29 mg g<sup>-1</sup>), followed by 0.1 mg L<sup>-1</sup> (8.15 mg g<sup>-1</sup>). In contrast, the 1.5 mg L<sup>-1</sup> treatment recorded the lowest total chlorophyll (3.04 mg g<sup>-1</sup>), suggesting possible phytotoxicity at higher concentrations. Carotenoid content followed the same trend, where the highest value was observed at 0.5 mg L<sup>-1</sup> (1.01 mg g<sup>-1</sup>), followed by 0.1 mg L<sup>-1</sup> (0.95 mg g<sup>-1</sup>), whereas 1.5 mg L<sup>-1</sup> showed the lowest level (0.46 mg g<sup>-1</sup>), even lower than the control (0.67 mg g<sup>-1</sup>). These results indicate that low to moderate concentrations of AgNPs enhance pigment biosynthesis, while excessive doses reduce it.

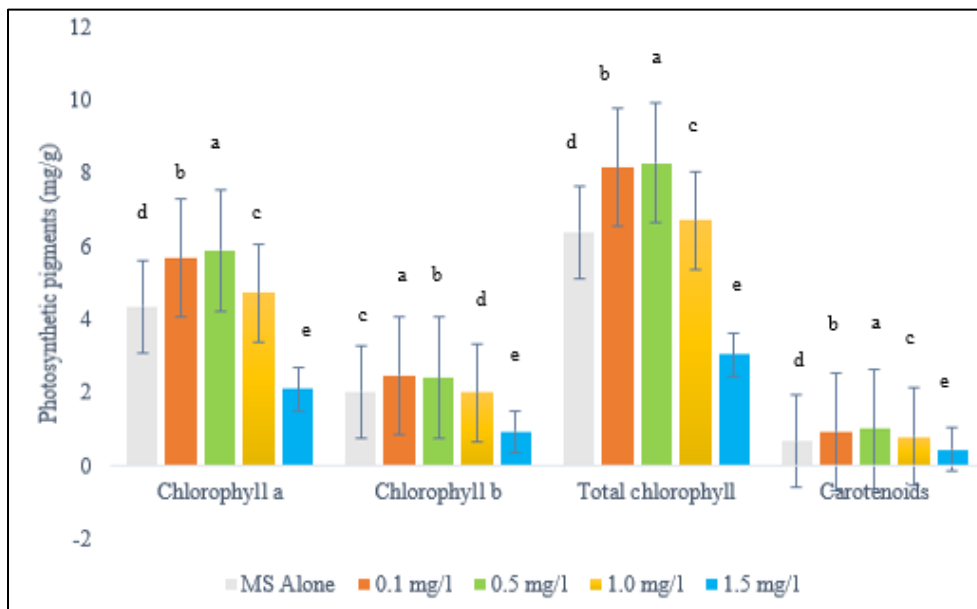


Figure 4. Photosynthetic pigments (mg g<sup>-1</sup>) of arabica coffee in AgNP-added medium

Comparable findings were reported by Tejada-Alvarado et al. (2023), who showed that AgNPs significantly enhanced chlorophyll *a*, *b*, and carotenoids in *Ananas comosus* seedlings grown *in vitro* under AlCl<sub>3</sub>-induced stress. In their study, pigment enhancement was most evident at moderate doses (e.g., 0.025 g L<sup>-1</sup>), whereas higher concentrations reduced pigment accumulation. The authors attributed the stimulatory effect of AgNPs to their antioxidant activity, which neutralized reactive oxygen species (ROS) and preserved chloroplast integrity. Similarly, Sadak (2019) observed a dose-dependent response in *Trigonella foenum-graecum*, where foliar application of AgNPs up to 40 mg L<sup>-1</sup> significantly increased chlorophyll and carotenoid contents. However, pigment levels declined at 60 mg L<sup>-1</sup>. The stimulatory effect was attributed to enhanced indole acetic acid (IAA) activity and modulation of ethylene signaling, which are mechanisms that may also contribute to the responses observed in *C. arabica*, though further study is required.

#### 4. Conclusion

The application of silver nanoparticles significantly enhanced germination, shoot and root elongation, vigor index, and pigment accumulation in arabica coffee embryos cultured *in vitro*. The optimal response was observed at 0.5–1.0 mg L<sup>-1</sup>, where seedlings exhibited faster germination, more vigorous growth, and earlier true leaf development. These results suggest that AgNPs can serve as effective growth stimulants in coffee tissue culture by modulating physiological and metabolic processes. Further studies are recommended to validate these findings and to investigate potential synergistic effects with other nanoparticles or plant growth regulators for improved propagation of coffee.

#### 5. Acknowledgement

The authors would like to sincerely thank the Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program Scholarship (DOST-ASTHRDP) and the J.H. Cerilles State College for funding this research. Appreciation is also extended to the VSU Administration for providing laboratory facilities.

## 6. Competing Interests

The authors have declared that no competing interests exist.

## 7. Authors' Contributions

ALL Paler designed the study, conducted the experiments, performed the statistical analysis, and wrote the first draft of the manuscript. CC Arradaza supervised the study, provided guidance on the methodology, and critically reviewed the manuscript. All authors read and approved the final manuscript.

## 8. Use of Artificial Intelligence (AI)-Assisted Technology

The authors of this manuscript affirm that in the writing process of this work, no generative artificial intelligence (AI) or AI assisted technologies were used to generate scientific content, ideas, or references. AI tools were solely employed for enhancing readability and refining language, specifically Grammarly for grammar checking, QuillBot for paraphrasing guidance, and ChatGPT for phrasing suggestions. This use was strictly supervised and controlled by humans. Following the implementation of these AI-assisted technologies, the manuscript meticulously examined and revised by the authors to guarantee its precision, coherence, and scientific integrity. The authors are aware that AI can generate content that may sound authoritative yet might be incorrect, incomplete, or biased. In light of this, human judgment was used to review the manuscript thoroughly.

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REGULAR ARTICLE

## Growth and Yield Performance of High-Yielding Sweet Potato (*Ipomoea batatas* L.) Varieties Applied with Biofertilizers

ARTICLE HISTORY

Received 05 June 2025

Revised 05 August 2025

Accepted 07 September 2025

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**Abstract:** Sweet potato (*Ipomoea batatas* L.) is an important root crop valued for its adaptability, nutritional content, and economic significance, especially in developing countries. However, optimal yield and quality are often constrained by poor soil conditions and excessive reliance on chemical fertilizers. This study investigated the growth and yield performance of high-yielding sweet potato varieties under different biofertilizer applications. A total of 864 slips were planted across 72 plots (1.70 × 2.60 m<sup>2</sup> each) in a 6 × 3 factorial experiment arranged in a Randomized Complete Block Design (RCBD). Treatments included six fertilizer options: negative control (no biofertilizer), Indigenous Microorganisms (IMO), Lactic Acid Bacteria Serum (LABS), Seaweed Extract, Bokashi, and positive control (complete fertilizer), combined with three sweet potato varieties: NSIC SP 25, NSIC SP 30, and NSIC SP 35. Results revealed that both fertilizer treatments and varieties had significant effects ( $p < 0.05$ ) on vine length, tuber length, and tuber yield. However, their interaction showed no significant effect on the parameters tested. Among the fertilizer treatments, the complete fertilizer exhibited the highest performance, while Bokashi and LABS produced comparable results. Among the varieties, NSIC SP 35 consistently performed best in tuber size, weight, and overall yield, achieving 14.24 tons/ha, while NSIC SP 25 and NSIC SP 30 produced 8.32 tons/ha and 6.95 tons/ha, respectively. Among the fertilizer treatments, complete fertilizer produced the highest vine length and tuber yield, while seaweed extract also enhanced performance. Bokashi and IMO showed potential as sustainable alternatives, though their effects varied numerically across varieties without significant interaction. Cost and return analysis indicated that NSIC SP 35 applied with LABS was the most profitable combination, yielding the highest return on investment (ROI) at 248.93%. The study concluded that combining a high-performing variety such as NSIC SP 35 with a suitable fertilizer source can result in notable gains in productivity and profitability.

**Keywords:** biofertilizer; growth and yield performance; sustainable agriculture; sweet potato (*Ipomoea batatas*); variety evaluation

## 1. Introduction

Sweet potato (*Ipomoea batatas* L.), a member of the morning-glory family, is a globally significant food crop. It ranks sixth in importance after rice, wheat, potatoes, maize, and cassava, and fifth in developing countries. Unlike true tubers such as potatoes, sweet potatoes are storage roots that grow from sea level to elevations of up to 2,500 meters. They require minimal inputs and thrive in marginal environments, including poor soils and dry conditions (International Potato Center, 2017). Nutritionally, sweet potatoes are rich in vitamins B, C, and E, and contain moderate iron and zinc. Recent studies also highlight the cancer-preventive potential of anthocyanins found in purple varieties (Clt, 2024).

Highly versatile, sweet potatoes are consumed as food, used as animal feed, and friendly animal feed, as they help reduce methane emissions compared with conventional feed sources (Varalakshmi et al., 2022). In the Philippines, sweet potato farming is economically promising. Farmers can earn up to a 144% return per hectare, with an estimated net income of around Php 48,400.00 (Lirag, 2019; ISP Platform, n.d.).

Despite these benefits, several environmental and agronomic challenges limit the crop's full potential. Sweet potatoes grow best in warm climates with temperatures between 24°C and 29°C. Yield can be reduced by extreme temperatures, inconsistent rainfall, waterlogging, root rot, and drought stress. Pest and disease pressures, particularly during the rainy season, further constrain production. The availability of healthy planting material, especially during the dry season, is also crucial and can be ensured through methods such as sand storage (Abram et al., 2020; Sapakhova et al., 2023).

While sweet potatoes are recognized for their adaptability and resilience, optimizing their yield, especially in areas with limited access to synthetic fertilizers, remains a challenge. Growing interest in sustainable and cost-effective farming practices has brought attention to biofertilizers as alternatives to chemical fertilizers (Ibrahim et al., 2024; Li et al., 2022). Biofertilizers enhance soil fertility and promote plant growth by improving nutrient availability through microbial activity (Wei et al., 2024). However, limited research has examined the combined effects of high-yielding sweet potato varieties and biofertilizer application under localized farming conditions.

Most existing studies focus on either high-yielding varieties or biofertilizers in isolation, often under controlled conditions. Little is known about how these components interact under varying environmental and resource settings, particularly in regions such as Masbate. Moreover, the economic implications of adopting biofertilizer-based cultivation methods remain underexplored.

From April to June 2023, the Bicol Region, including Masbate, produced approximately 43.87 thousand metric tons of sweet potato, representing 28.9% of the national output. The harvested area from January to June 2023 totaled 38.33 thousand hectares, reflecting a slight increase compared with the previous year (Philippine Statistics Authority, 2023).

Despite this notable production, a research gap remains in identifying cultivation strategies that enhance both agronomic performance and profitability. This study addresses these gaps by evaluating the growth and yield performance of selected high-yielding sweet potato varieties with various biofertilizers. It also includes a cost and return analysis to assess the profitability and practical viability of biofertilizer use under local farming conditions at DEBESMSCAT. The findings are expected to provide insights for sustainable sweet potato production, improved land-use efficiency, and enhanced food security in Masbate province.

This study aims to evaluate the vegetative performance of high-yielding sweet potato varieties applied with different biofertilizers, specifically in terms of vine length. It also seeks to assess the yield performance based on several parameters, including the number of tubers per hill, the total weight of tubers per hill, the average weight of each tuber, and the diameter and length of each tuber. In addition, the study conducts a cost and return analysis to determine the economic viability of the treatment combinations.

## 2. Materials and Methods

### 2.1 Experimental Design

This study employed a Randomized Complete Block Design (RCBD) in a 6 x 3 factorial arrangement with 4 replications. The first factor was the biofertilizer treatments: F1 – Negative control (no Biofertilizer); F2 - Indigenous microorganism (IMO), F3 - Lactic Acid Bacteria Serum (LABS), F4 - Seaweed Extract, F5 - Bokashi, and F6 - Positive Control (Complete fertilizer). The second factor was the sweet potato variety: V1 - NSIC SP 25, V2 - NSIC SP 30, and V3 - NSIC SP 35. All experimental plots were amended with Carbonized Rice Hull (CRH) and planted with six experimental plants each.

### 2.2 Production Practices Employed

The experiment was conducted in a well-drained, sandy loam soil area with ample sunlight (6–8 hours/day). A total of 72 plots were established, each properly spaced to ensure drainage and ease of management. Three high-yielding sweet potato varieties (NSIC SP 25, SP 30, SP 35) were sourced from Visayas State University (VSU), Baybay, Leyte, and propagated at DEBESMSCAT. Planting materials were trimmed to 30cm in length and planted using the L-shape method, with a spacing of 75 cm between hills and 50 cm between rows.

Biofertilizers were applied at recommended rates. IMO, LABS and seaweed extract were applied at 106 L/ha through drench application at two-week intervals (Keliikuli et al., 2019). Bokashi was applied at 3 t/ha and was incorporated with CRH to improve soil structure and aeration. The complete fertilizer was prepared in a 1:10 dilution and applied biweekly at the recommended NPK rates for sweet potato (40 kg N, 50 kg P, and 70 kg K per hectare) to support vegetative growth, root development, and tuber quality (NPK Fertilizer Calculator, n.d).

Irrigation was provided manually using water from a nearby deep well, and weeding was done regularly. Standard Cultural practices were employed to minimize pest and disease incidence. Harvesting took place 90 days after planting, as indicated by leaf yellowing. Tubers were harvested manually using hand tools.

### 2.3 Data Gathering Procedure

Vine length was measured monthly from planting until the flowering stage using a tape measure, with three samples taken per plot. Only the main vine of each sampled plant was measured to minimize disturbance. Yield was assessed by recording the number of tubers per hill, the total weight of tubers per hill, the average weight per tuber, and the diameter and length of tubers.

### 2.4 Cost and Return analysis

Cost and return analysis was conducted using formulas adapted from the Department of Agriculture (2012). Total expenses were calculated as the sum of fixed and variable costs. Net income was obtained by subtracting total expenses from gross income. Return on investment (ROI) was computed by dividing the net income by the total expenses and multiplying the result by 100.

### 2.5 Data Analysis

Data were subjected to normality and homogeneity tests using the Shapiro-Wilk and Bartlett's tests, respectively. When assumptions were met, a two-factor RCBD ANOVA was performed to determine treatment effects. Significant means were further separated using Tukey's test. All statistical analyses were carried out using the Statistical Tool for Agricultural Research (STAR), Version 2.0.1, developed by the International Rice Research Institute.

### 3. Results and Discussion

#### 3.1 Length of Vines

Table 2 presents the mean vine length of sweet potato varieties under different biofertilizer treatments. Analysis showed no significant interaction between variety and biofertilizer, but the main effects of both factors were statistically significant.

Table 2. Average length of vines (cm) of high-yielding sweet potato varieties applied with biofertilizers at 30 and 60 days after planting

Factors		Length of vines (cm)	
		30 days	60 days
Variety	NSIC SP 25	56.71 <sup>b</sup>	117.00 <sup>b</sup>
	NSIC SP 30	93.35 <sup>a</sup>	185.42 <sup>a</sup>
	NSIC SP 35	84.00 <sup>a</sup>	172.17 <sup>a</sup>
Fertilizer	No Biofertilizer	73.59 <sup>abc</sup>	148.75 <sup>b</sup>
	IMO	63.63 <sup>c</sup>	147.69 <sup>b</sup>
	LABS	75.59 <sup>abc</sup>	154.11 <sup>b</sup>
	Seaweed Extract	66.50 <sup>bc</sup>	157.33 <sup>ab</sup>
	Bokashi	85.78 <sup>ab</sup>	155.28 <sup>ab</sup>
	Complete Fertilizer	103.03 <sup>a</sup>	186.00 <sup>a</sup>
Interaction	Variety × Fertilizer	ns	ns

Means in a column for a given factor with the same letter are not significantly different ( $p > 0.05$ ).

Among fertilizer treatments, the complete fertilizer (F6) recorded the longest vine length of 103.03 cm at 30 days and 186.00 cm at 60 days, significantly outperforming the other treatments. This indicates that balanced NPK inputs enhance vegetative growth, as nitrogen supports vine development, phosphorus promotes root and tuber initiation, and potassium contributes to disease resistance and tuber quality (Jagdish, 2023). Similar findings were reported by Oycha et al. (2023), who noted that complete fertilizers improve vine length through readily available nutrients that stimulate photosynthesis and vigor.

Among the varieties, NSIC SP 30 (V2) exhibited significantly longer vines at both 30 and 60 days (93.35 cm and 185.42 cm, respectively) compared with V1 (NSIC SP 25), which had the shortest vines. This performance is likely due to the favorable genetic traits of V2 that promote vigorous vegetative growth and adaptability (Quindara et al., 2020).

#### 3.2 Yield Response

Table 3 presents the effects of different sweet potato varieties and fertilizer treatments on key yield parameters, including tuber length, diameter, number, and weight per hill. Significant differences were observed among varieties, with NSIC SP 35 showing the highest yield performance. The effects of fertilizer treatments and their interactions with variety were not significant.

##### 3.2.1 Length of tubers

Fertilizer type significantly influenced tuber length, while no interaction effects were observed (Table 3). The complete fertilizer produced the longest tubers (10.55 cm), followed by Bokashi. This suggests that balanced mineral nutrition, particularly phosphorus and potassium, promotes root elongation and tuber development (Jagdish, 2023).

Table 3. Yield parameters of sweet potato as influenced by variety and fertilizer treatments.

Factors		Length of tubers (cm)	Diameter of tubers (cm)	Number of tubers per hill	Weight of tuber per hill	Weight of each tuber (g)
Variety	NSIC SP 25	10.41 <sup>a</sup>	4.19 <sup>b</sup>	2.97 <sup>b</sup>	308.68 <sup>b</sup>	92.57 <sup>a</sup>
	NSIC SP 30	9.34 <sup>b</sup>	3.98 <sup>b</sup>	3.53 <sup>b</sup>	260.59 <sup>b</sup>	71.12 <sup>b</sup>
	NSIC SP 35	9.34 <sup>b</sup>	4.67 <sup>a</sup>	5.09 <sup>a</sup>	533.99 <sup>a</sup>	97.36 <sup>a</sup>
Fertilizer	No Biofertilizer	9.17 <sup>b</sup>	4.22	3.29	300.45 <sup>b</sup>	84.27
	IMO	9.66 <sup>ab</sup>	4.19	3.47	301.01 <sup>b</sup>	86.16
	LABS	9.41 <sup>ab</sup>	4.28	3.73	362.32 <sup>ab</sup>	86.89
	Seaweed Extract	9.37 <sup>b</sup>	4.23	3.56	314.05 <sup>ab</sup>	79.88
	Bokashi	10.01 <sup>ab</sup>	4.36	4.19	407.17 <sup>ab</sup>	87.07
	Complete Fertilizer	10.55 <sup>a</sup>	4.39	4.93	521.52 <sup>a</sup>	97.82
Interaction	Variety × Fertilizer	ns	ns	ns	ns	ns

Means in a column for a given factor with the same letter are not significantly different ( $p > 0.05$ ).

Similarly, Oycha et al. (2023) reported that balanced fertilization enhances tuber growth by improving nutrient uptake and root system expansion, which supports greater tuber elongation. Bokashi's effectiveness may be attributed to its microbial content, which improves nutrient cycling and root expansion (Christel, 2017).

NSIC SP 25 produced the longest tubers among the varieties tested, whereas NSIC SP 30 and NSIC SP 35 did not differ significantly and exhibited shorter tuber lengths. This may reflect genetic differences in assimilate allocation, where NSIC SP 25 favors tuber elongation (Oni & Olaniyi, 2025). Although NSIC SP 30 excelled in vine length, NSIC SP 25 appears to possess traits that promote tuber development. These results support the view that genetic makeup determines how nutrients are partitioned between vegetative and storage organs in sweet potatoes, with some varieties allocating more to tuber growth than vine elongation (Hammes & Belehu, 2005).

### 3.2.2 Diameter of tubers

Among the fertilizer treatments, complete fertilizer produced the highest mean tuber diameter (4.39 cm), followed closely by Bokashi (4.36 cm) and LABS (4.28 cm). However, the differences were not statistically significant ( $P > 0.05$ ), indicating that although biofertilizer application visibly affected tuber diameter, the variations were not strong enough to establish treatment superiority at the 5% level.

In terms of variety, NSIC SP 35 consistently produced the widest tubers, significantly outperforming NSIC SP 25 and NSIC SP 30. ANOVA confirmed a significant varietal effect, and Tukey's test further showed that V3 differed significantly from the other two varieties. This suggests that the superior tuber diameter in NSIC SP 35 may be attributed to genetic characteristics that favor storage organ expansion through enhanced assimilate allocation to the tuber. While NSIC SP 30 excelled in vine length and NSIC SP 25 in tuber length, NSIC SP 35 appears genetically inclined toward producing larger tuber diameters—an important market trait in sweet potato production. Rahman et al. (2015) similarly emphasized that genetic variation plays a key role in determining root size and shape.

The present results align with the findings of Mukhongo et al. (2017), who reported that biofertilizers enhancing nutrient uptake and microbial activity significantly improved root crop biomass. Likewise, Boubaker et al. (2023) found that organic amendments, including biofertilizers, increased tuber diameters in root crops, underscoring their value in sustainable agriculture. Duan et al.

(2024) also noted that biofertilizers can match or even surpass conventional fertilizers in improving crop growth and yield parameters.

### 3.2.3 Number of tubers per hill

The analysis showed no significant interaction between variety and biofertilizer, but the main effect of variety was statistically significant. Among the fertilizer treatments, complete fertilizer recorded the highest average number of tubers per hill (4.93). However, ANOVA indicated no significant differences among biofertilizer treatments at the 5% level. This means that although numerical differences were observed, they were not statistically significant, and no treatment can be considered superior in influencing tuber number per hill.

Across varieties, NSIC SP 35 produced the highest average number of tubers per hill (5.09), while NSIC SP 25 had the lowest (2.97). ANOVA confirmed that V3 significantly outperformed both V1 and V2 (NSIC SP 30). This suggests that V3 has stronger genetic potential for tuber initiation and development, possibly due to more efficient assimilate partitioning toward multiple tuber formation. These results are consistent with the findings of Hartemink (2003) and Mwangi et al. (2017), who emphasized that varietal differences in sweet potato tuber production are largely governed by genetic traits such as sink strength, tuber initiation capacity, and overall yield architecture.

### 3.2.4 Weight of tubers per hill

The analysis showed no significant interaction between variety and biofertilizer (Table 3), but the main effects of both factors were statistically significant. Among the fertilizer treatments, complete fertilizer produced the highest mean tuber weight per hill (521.52 g), significantly outperforming the other treatments. This indicates that F6, a balanced fertilizer, enhanced tuber development by providing essential macronutrients such as nitrogen, phosphorus, and potassium, which are critical for root crop productivity. The significant difference was confirmed by ANOVA and further supported by Tukey's test, which showed F6 to be statistically superior at the 5% level.

The higher tuber weight under F6 may be attributed to increased photosynthetic activity and assimilate partitioning, particularly the role of potassium in tuber bulking and carbohydrate transport (Chowdhary et al., 2021). This finding is consistent with Fageria (2008), who reported greater yield potential with complete fertilizers due to improved nutrient availability and uptake efficiency.

Across varieties, NSIC SP 35 consistently produced the highest tuber weight per hill across all fertilizer treatments, significantly surpassing NSIC SP 25 and NSIC SP 30. ANOVA confirmed a significant varietal effect at the 5% level, and Tukey's test indicated that NSIC SP 35 differed significantly from the other varieties.

The results suggest that NSIC SP 35 has superior genetic potential for tuber yield compared with the other varieties under study, regardless of fertilizer treatment. The consistently higher tuber weight in V3 indicates traits such as improved nutrient uptake efficiency and better biomass allocation to storage organs, which contribute to its yield advantage. This aligns with the findings of Ebem et al. (2021), who reported that sweet potato varieties differ significantly in yield performance due to genetic and environmental interactions, and that integrated nutrient management enhances productivity while maintaining soil health. The study also supports the findings of Higashikawa et al. (2025), who reported that high-yielding sweet potato varieties paired with efficient nutrient management can significantly improve food security and farm income, particularly in developing regions.

### 3.3.5 Weight of each tuber

The statistical analysis indicated that while there was no significant interaction between variety and biofertilizer, the main effect of variety showed a significant influence on tuber weight. Analysis of variance indicated that the interaction between variety and biofertilizer was not significant; however,

the main effect of variety exhibited a statistically significant influence on tuber weight. This suggests that varietal differences played a more critical role in determining individual tuber size than fertilizer treatments.

Although complete fertilizer recorded the highest average weight per tuber (97.82 g), statistical analysis confirmed that the differences among biofertilizer treatments were not significant. The superior numerical performance of F6 may be associated with its balanced nutrient composition, providing an optimal supply of nitrogen, phosphorus, and potassium—elements essential for carbohydrate synthesis and tuber enlargement.

In contrast, varietal performance showed clearer distinctions. NSIC SP 35 consistently produced heavier individual tubers than the other varieties, while NSIC SP 25 also exhibited competitive results under most fertilizer regimes. The relatively lower tuber weight of NSIC SP 30 suggests that this variety may channel assimilates toward producing a greater number of smaller tubers, reflecting its inherent genetic tendency for higher tuber count rather than size.

These observations highlight genetic variation in assimilate partitioning and storage organ development among the varieties. NSIC SP 35 appears to possess stronger sink strength and greater efficiency in allocating carbohydrates toward tuber enlargement, a trait that directly contributes to its yield advantage. This aligns with the findings of Tumwegamire (2011), who emphasized that varietal differences in genetic makeup influence the synchronization of tuber growth and the efficiency of assimilate distribution. Such traits are crucial not only for yield potential but also for improving crop quality and post-harvest handling characteristics.

### 3.3 Economic Viability

Table 4 presents a detailed computation of the cost and return analysis. The analysis assumes uniform land area, labor, and management practices across all treatment combinations. Fixed and labor costs remain constant, while variable costs vary according to fertilizer application. All produce was sold at a fixed price of ₱60/kg, and net income and ROI were used to evaluate the economic performance of each treatment.

The economic evaluation revealed that complete fertilizer produced the highest mean return on investment (ROI) at 155.64%, followed by LABS at 107.13% and Seaweed Extract at 82.89%. The lowest ROI was observed with Bokashi at only 22.98%, largely due to the negative return recorded in NSIC SP 25 (-25.63%), indicating its inconsistent effectiveness. These results indicate that complete fertilizer substantially outperformed the organic-based fertilizers overall, suggesting that commercially formulated nutrient inputs remain more effective in enhancing profitability under the conditions of this study.

Table 4. Average return on investment (%) in the production of selected sweet potato varieties under different biofertilizer treatments.

Fertilizers	Return on investment (%)			Mean
	NSIC SP 25	NSIC SP 30	NSIC SP 35	
No Biofertilizer	12.46	2.98	212.70	76.04
IMO	46.70	19.54	122.96	63.07
LABS	55.12	17.34	248.93	107.13
Seaweed Extract	92.19	24.14	132.33	82.89
Bokashi	-25.63	24.47	70.09	22.98
Complete Fertilizer	165.56	64.99	236.38	155.64
Mean	57.73	25.58	170.56	

When evaluating varietal performance, NSIC SP 35 achieved the highest mean ROI (170.56%), followed by NSIC SP 25 (57.73%) and NSIC SP 30 (25.58%). NSIC SP 35 consistently generated strong returns across fertilizer treatments, particularly when combined with LABS, which yielded an ROI of 248.93%. This outcome reflects the variety's inherent advantages, including efficient nutrient utilization, high tuber yield, and adaptability to different fertilization strategies, making it a promising candidate for commercial cultivation.

Overall, profitability is maximized when an effective fertilizer is paired with a highly responsive variety. While complete fertilizer provided the greatest returns overall, the high ROI achieved by the NSIC SP 35 + LABS combination highlights that microbial biofertilizers can be economically viable when matched with varieties that efficiently convert available nutrients into yield. These results align with Raman et al. (2022), who reported that the effectiveness of LABS depends on crop responsiveness, microbial activity, and soil nutrient dynamics.

#### **4. Conclusion**

This study showed that sweet potato growth, yield, and profitability depend strongly on both variety selection and fertilizer input. Among the varieties, NSIC SP 35 consistently produced the largest tubers and highest yield at 14.24 t/ha, demonstrating superior genetic potential compared to NSIC SP 25 and NSIC SP 30, which yielded 8.32 and 6.95 t/ha, respectively. Complete fertilizer promoted vigorous growth and larger tubers, while LABS also supported high returns due to its lower production cost, particularly when paired with NSIC SP 35. In contrast, the NSIC SP 25 and Bokashi combination resulted in economic loss, highlighting that fertilizer effectiveness varies by varietal responsiveness. Overall, matching a high-performing variety such as NSIC SP 35 with an efficient fertilizer source can significantly improve productivity and profitability. Further research should refine nutrient management strategies and test more varieties under different field conditions.

#### **5. Conflict of Interest**

The author declares that there is no conflict of interest regarding the publication of this paper.

#### **6. Use of Artificial Intelligence (AI)-Assisted Technology**

During the preparation of this work, the author used ChatGPT and Grammarly to paraphrase and ensure accurate grammar. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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REGULAR ARTICLE

# Survivability and Growth Performance of Abaca (*Musa textilis* Née) using Biofertilizers in Masbate, Philippines Condition

ARTICLE HISTORY

Received 21 June 2025  
Revised 18 October 2025  
Accepted 12 November 2025

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**Abstract:** The study was conducted to evaluate the effects of biofertilizers on the survivability and growth performance of abaca under Masbate conditions. A trial was carried out following a Randomized Complete Block Design (RCBD) with four treatments and four replications. The effects of different fertilizers, applied at their recommended rates, served as treatments, namely: Treatment A – 10 g of complete fertilizer per plant (Control); Treatment B – 10 g of complete fertilizer per hill combined with a foliar application of 15 mL of ANAA per 4 L of water; Treatment C – 20 g of Mykovam per plant; and Treatment D – 20 g of Mykoplus per plant. Abaca plants were monitored under field conditions for three months, and soil chemical properties were analyzed before and after treatment application. Growth parameters—including height increment, leaf development, pseudostem size, sucker production, and survival rate—showed no significant differences ( $p > 0.05$ ) among treatments. These findings suggest that biofertilizers such as Mykovam and Mykoplus remain practical alternatives when inorganic fertilizers are limited, as their beneficial microbes help support nutrient uptake and root development. The consistently high survival rate across treatments also indicates that Masbate provides favorable conditions for establishing abaca. Further research should extend into later growth stages, focusing on fiber yield and quality, and evaluating whether combined or reduced fertilizer rates with biofertilizers can provide long-term benefits for plant performance and soil health.

**Keywords:** abaca; biofertilizer; growth; Masbate condition; survival rate

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## 1. Introduction

Abaca (*Musa textilis* Née), commonly known as Manila hemp, is an economically significant crop native to the Philippines. Its fiber is widely valued for its exceptional strength and flexibility—three times stronger than cotton and twice as strong as sisal (Armecin et al., 2014). The Philippines remains the world's largest producer of abaca fiber, supplying key industries such as specialty paper, textiles, handicrafts, furniture, cosmetics, meat casing, and even composite materials for the automotive and construction sectors (Philippine Fiber Industry Development Authority, 2015).

However, despite its strong market demand, the abaca industry continues to face serious constraints. The growth and productivity of abaca are highly dependent on environmental conditions such as rainfall distribution, temperature, radiation, and soil fertility (Bureau of Agriculture and

Fisheries Standards, 2019). While the crop thrives in areas with evenly distributed rainfall and moderate temperatures (Type IV climate) (PhilFIDA, 2016), provinces such as Masbate fall under a Type III climate classification, where rainfall patterns and temperature levels may not readily support optimal abaca performance. This raises uncertainty regarding the adaptability and survivability of abaca under Masbate's agro-climatic conditions.

In addition, abaca is a shallow-rooted, heavy nutrient feeder that requires abundant nitrogen and potassium to sustain growth and fiber quality (Bande et al., 2012). Many abaca-growing areas rely heavily on inorganic fertilizers, which can lead to soil degradation, reduced soil biological activity, and long-term decline in productivity. This concern highlights the need for more sustainable nutrient management strategies in abaca cultivation.

Biofertilizers present a potential solution to this challenge. Products such as Mykovam and MykoPlus, developed by UPLB-BIOTECH, contain beneficial microorganisms that improve soil health, enhance nutrient uptake, increase plant vigor, and reduce susceptibility to environmental stress (Aggangan et al., 2013; Sindhu et al., 2010; Abdelaal et al., 2021).

While the Bicol Region is currently the country's leading abaca-producing region, contributing 35% of national output in 2019 (Magno-Ballesteros & Ancheta, 2022), Masbate has no documented research confirming whether abaca can survive or perform well under its local environmental conditions. This lack of information presents a critical knowledge gap. Without empirical evidence on abaca's adaptability and the effectiveness of biofertilizers in Masbate, farmers, researchers, and development agencies are unable to make informed decisions regarding potential abaca expansion in the province.

Therefore, this study was conducted to evaluate the survivability and growth performance of abaca in Masbate using biofertilizers and Alpha-Naphthalene Acetic Acid (ANAA). Specifically, it assessed (1) soil chemical properties before and after the study, (2) growth parameters such as plant height, leaf number and size, pseudostem length and diameter, and sucker formation, and (3) the survival rate of abaca under field conditions. Findings from this research aim to support future academic studies, guide farmer awareness and adoption of biofertilizers, and contribute to developing sustainable fiber-based livelihood opportunities in the province.

## **2. Materials and Methods**

### *2.1 Research Design and Experimental Unit*

The experiment was arranged in a Randomized Complete Block Design (RCBD) with four treatments, each replicated four times, for a total of 16 plots. Each plot contained six hardened abaca plantlets and was located in the High Value Commercial Crops (HVCC) area of DEBESMSCAT under Masbate growing conditions. The treatments were: A (Control) – complete fertilizer (14-14-14); B – complete fertilizer (14-14-14) with foliar ANAA (15 mL per 4 L water); C – Mykovam; and D – Mykoplus.

### *2.2 Land and Planting Materials Preparation*

The experimental area was prepared after the collection of a soil sample and a week prior to planting. Plowing was done to break the soil clods and remove the weeds present in the area to create a suitable environment for the abaca to grow well. A total of 96 hardened plantlets of the abaca variety 'Musa Tex 51' were collected from the Provincial Plant Nursery, Tissue Culture Laboratory. The plantlets were planted in a polyethylene bag and cared for 21 days before transplanting in the field.

### *2.3 Field Management and Treatment Application*

The abaca plantlets were transplanted in the late afternoon using the square planting method, maintaining a plant-to-plant distance of 2 m. The hardened plantlets were irrigated twice daily (morning and afternoon) for one week to allow recovery from transplanting shock, and thereafter irrigation was

carried out every other day. Ring weeding was performed around each plant before fertilizer application.

For Treatment A (Control), 10 g of complete fertilizer was applied basally at transplanting, followed by 10 g every 15 days, applied in a ring 10 cm from the base of the plant. For Treatment B, 10 g of complete fertilizer was applied at transplanting, followed by 10 g every 15 days (ring method), along with a foliar application of ANAA at 100 mL per plant every seven days. For Treatment C, 20 g of Mykovam was placed into each planting hole at transplanting, followed by drenching 650 mL of a Mykovam solution (1 kg in 16 L of water) per plant every 10 days. For Treatment D, 20 g of Mykoplus was applied into each planting hole at transplanting, followed by drenching 650 mL of a Mykoplus solution (200 g in 16 L of water) per plant every 10 days.

#### 2.4 *Pest and Disease Management*

The occurrence of pests and diseases was closely monitored from planting until termination. Shrubs, bushes, and grasses growing in the experimental site were removed to prevent them from providing shelter for insect and animal pests. Green-labeled pesticides were used to control leaf spot disease and insect pests.

#### 2.5 *Data Gathering*

##### 2.5.1 *Soil sampling and analysis*

Soil sampling was conducted before and after the experiment using a random sampling method. Prior to land preparation, composite soil samples were collected from the experimental area to determine the initial soil properties. After the study, soil samples were collected from each experimental plot and subsequently combined to form one composite sample per treatment.

All soil samples were air-dried under shade, pulverized, thoroughly mixed, and passed through a sieve (2 mm mesh) to obtain uniform particle size. The processed samples were then stored and prepared for chemical analysis. A total of 1 kg of soil per treatment, consisting of equal portions of fine and coarse fractions, was submitted to the Central Analytical Services Laboratory, Visca, Baybay City, Leyte, for the determination of soil pH, organic matter, total nitrogen, and available phosphorus.

##### 2.5.2 *Growth parameters*

All growth parameters were recorded at the initial measurement and subsequently measured at 30-day intervals throughout the duration of the study. Plant height increment was determined by measuring the increase in height from the base of the plant to the highest point. The number of leaves was obtained by counting only the fully developed leaves on each plant. Leaf size was assessed by measuring leaf length from the tip to the end of the leaf blade. The diameter of the pseudostem was measured one centimeter above the plant base using a caliper, while the length of the pseudostem was taken from the base of the plant up to the topmost petiole. The number of suckers per plant was determined by visually counting all suckers present in the surviving plants at the end of the study.

##### 2.5.3 *Survival rate*

The percentage of survival was determined at the end of the experiment by counting the number of surviving plants. It was computed by dividing the number of surviving plants by the total number of plants initially planted, then multiplying the result by 100.

#### 2.6 *Data Analysis*

The experimental data were analyzed using Analysis of Variance (ANOVA) to evaluate differences among treatments. Before analysis, the assumptions of ANOVA—normality and homogeneity of variances—were tested to ensure the validity of the results. All statistical analyses were conducted using

the Statistical Tool for Agricultural Research (STAR) software, version 2.0.1, developed by the International Rice Research Institute (IRRI).

### 3. Results and Discussion

#### 3.1 Soil Chemical Properties

The initial soil pH, organic matter, total nitrogen, and available phosphorus were measured prior to fertilizer application and compared with the final values after treatment. The treatments included: the recommended inorganic fertilizer alone (TA), the combination of inorganic fertilizer and ANAA (TB), Mykovam (TC), and Mykoplus (TD). The table below (Table 1) shows the changes in soil chemical properties from the initial levels to the final values after the application of the respective treatments.

The application of the different fertilizer treatments resulted in slight variations in soil pH. The recommended inorganic fertilizer resulted in a pH of 5.28, while the combination of inorganic fertilizer and ANAA produced a pH of 5.26. Mykovam similarly decreased the pH slightly to 5.33. In contrast, Mykoplus increased the soil pH to 5.44. The slight increase associated with Mykoplus may be attributed to microbial activity that can buffer soil acidity or exert a mild liming effect. This trend aligns with the findings of Yasa et al. (2023), who reported that biofertilizers tend to increase soil pH, whereas continuous application of inorganic fertilizers may lead to gradual soil acidification.

The initial soil organic matter (OM) content was 1.689%, which falls within the commonly reported range for cultivated soils. After treatment application, OM content ranged from 1.762% to 2.481%. The recommended inorganic fertilizer resulted in the highest OM value (2.481%), followed by Mykovam (2.116%) and Mykoplus (2.011%). The combination of inorganic fertilizer and ANAA showed the lowest increase (1.762%). The increase in OM may be linked to enhanced plant growth and subsequent return of leaf residues, which contribute to organic matter buildup through decomposition. Pardo et al. (2010) noted that microbial inoculants can support nutrient cycling and organic matter turnover, which may explain the moderate increases under Mykovam and Mykoplus.

The initial soil nitrogen content was low at 0.139%. Mykovam-treated soil showed the highest nitrogen increase at 0.172%, while both the recommended inorganic fertilizer and Mykoplus increased nitrogen to 0.148%. The combination treatment did not result in any noticeable increase from the initial nitrogen level. The increase associated with Mykovam may be related to the role of vesicular arbuscular mycorrhiza in enhancing nutrient uptake by converting unavailable nitrogen into plant-available forms. This is consistent with Mulyani et al. (2017), who reported that endomycorrhizal biofertilizers can significantly improve soil nitrogen availability.

The initial available phosphorus level was 13.537 mg kg<sup>-1</sup>. Mykoplus resulted in the highest increase to 20.737 mg kg<sup>-1</sup>, followed by the recommended inorganic fertilizer, which increased phosphorus to 14.316 mg kg<sup>-1</sup>. In contrast, Mykovam and the combination treatment produced slightly lower values (11.600 and 13.011 mg kg<sup>-1</sup>, respectively). The substantial increase in phosphorus under Mykoplus may be attributed to the activity of P-solubilizing microorganisms that enhance phosphorus availability, particularly when soil pH is slightly improved. Fitriatin et al. (2021) observed similar effects with biofertilizers containing P-solubilizing microbes.

Table 1. Soil chemical properties as affected by different fertilizer treatments

Soil Properties	Initial Content	Final Content			
		TA	TB	TC	TD
pH	5.36	5.28	5.26	5.33	5.44
Organic Matter (%)	1.689	2.481	1.762	2.116	2.011
Total N (%)	0.139	0.148	0.139	0.172	0.148
Available P (mg kg <sup>-1</sup> )	13.537	14.316	13.011	11.600	20.737

### 3.2 Growth Response

Figure 1 presents the mean values of the measured growth parameters, including plant height increment, number of leaves, leaf length, pseudostem diameter, pseudostem length, and number of suckers per plant under the different fertilizer treatments. In general, all treatments supported plant growth throughout the duration of the study, with observable but generally not significant ( $p>0.05$ ) variations among treatments. The treatments with microbial inoculants (Mykovam and Mykoplus) showed a tendency toward improved growth responses in several parameters compared to the recommended inorganic fertilizer alone and the combination of inorganic fertilizer with ANAA.

The growth performance of abaca under the different fertilizer treatments showed numerical variations among parameters measured, although these differences were not statistically significant. The plant height increment ranged from 11.25 cm to 19.53 cm, with the highest value observed in the recommended inorganic fertilizer (19.53 cm), followed by the combination of inorganic fertilizer and ANAA (17.00 cm), Mykovam (14.78 cm), and Mykoplus (11.25 cm). The number of leaves per plant showed very minimal variation, ranging only from 2.81 to 2.92, where Mykoplus obtained the highest mean (2.92), followed closely by Mykovam (2.90), ANAA + inorganic fertilizer (2.84), and the inorganic fertilizer alone (2.81). Leaf length ranged from 27.18 cm to 33.33 cm, with the longest leaves recorded in the inorganic fertilizer treatment (33.33 cm), followed by ANAA + inorganic fertilizer (30.42 cm), while Mykovam and Mykoplus produced shorter leaves (27.76 cm and 27.18 cm, respectively).

Similarly, pseudostem length increment ranged from 8.13 cm to 12.29 cm, wherein the inorganic fertilizer (12.29 cm) and the ANAA combination (12.21 cm) showed slightly higher increments than Mykoplus (8.79 cm) and Mykovam (8.13 cm). Pseudostem diameter ranged from 0.79 cm to 0.93 cm, with the largest mean diameter recorded in the inorganic fertilizer treatment (0.93 cm), followed by the ANAA combination (0.89 cm), while both Mykovam and Mykoplus resulted in the lowest and similar values (0.79 cm).

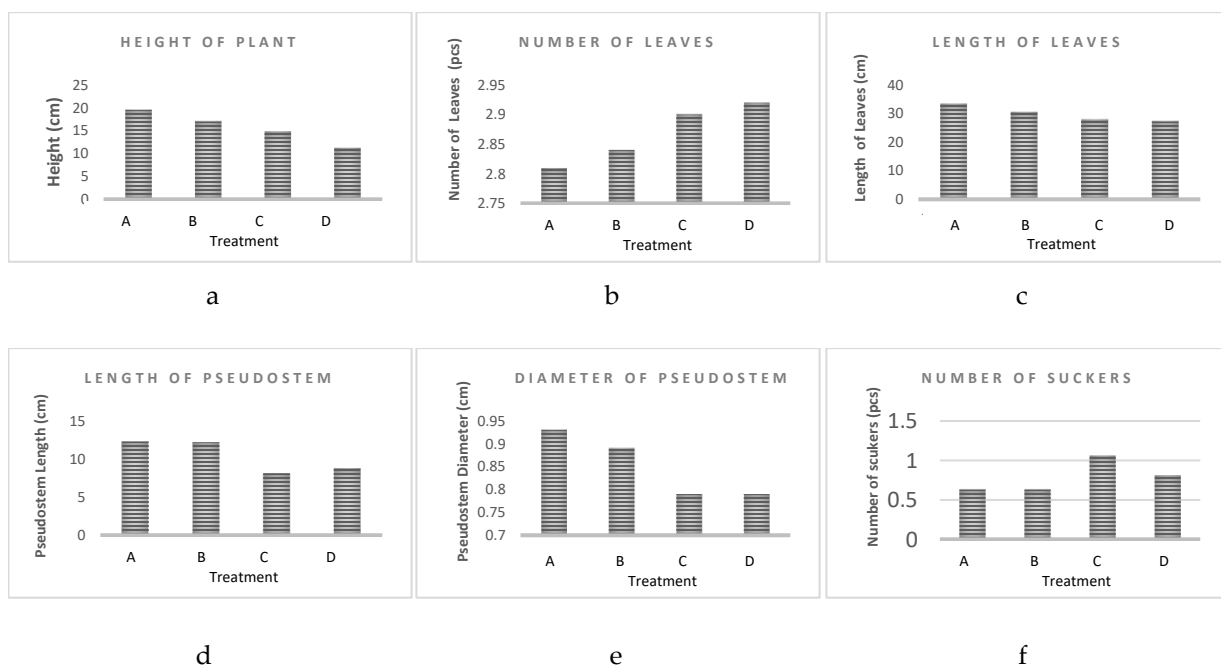


Figure 1. Growth performance of abaca under different fertilizer treatments: (a) plant height increment, (b) number of leaves, (c) leaf length, (d) pseudostem diameter, (e) pseudostem length, and (f) number of suckers per plant.

Meanwhile, the number of suckers per plant varied from 0.63 to 1.06, where Mykovam produced the highest average (1.06), followed by Mykoplus (0.81), while both inorganic fertilizer and the ANAA combination produced the lowest means (0.63).

Despite these numerical variations in all growth parameters, the ANOVA results indicated no significant differences ( $p > 0.05$ ) among treatments, suggesting that the growth responses were generally comparable across fertilizer types. This may be attributed to the sufficient nutrient availability provided by all treatments. The inorganic fertilizer supplied readily available N, P, and K for immediate uptake (Asadu et al., 2024), while the biofertilizers contributed plant growth-promoting microorganisms that enhanced nutrient accessibility through nitrogen fixation, phosphorus solubilization, and improved mycorrhizal colonization (Hartman & Six, 2023; Bora et al., 2016). Such microorganisms are also known to produce natural growth regulators like auxins, which promote cell elongation and shoot development (Keswani et al., 2020; Kumar et al., 2021), helping supplement nutrient uptake even when inorganic input levels are lower. It is also proven that the biofertilizers used (MykoVam and MykoPlus) are effective in enhancing the growth of abaca and other similar crops (Abadayan & Coracero, 2024; Aguilar et al., 2018)

### 3.3 Survivability

The survival rates of abaca under different fertilizer treatments showed no significant differences despite numerical variation (Figure 2). Mykoplus recorded the highest survival (100%), followed by Mykovam (91.67%), while both the no-fertilizer treatment and the complete fertilizer + ANAA combination showed the lowest survival (75%). Since these differences were not statistically significant ( $p > 0.05$ ), all treatments were considered adequate to support early plant establishment, suggesting that essential nutrients in the soil were sufficient to maintain vigor during the initial growth stage (Neves & Costa, 2020).

These findings indicate that abaca can establish successfully even under varying levels of fertilizer input. The comparable survival rates across treatments support earlier studies showing that abaca's early growth is not highly dependent on external nutrient supplementation (Bande et al., 2016). Although biofertilizers such as Mykovam and MykoPlus can enhance nutrient uptake through root colonization, their benefits are often more pronounced in later growth phases when nutrient demand increases (Aggangan et al., 2013; Hou et al., 2025; Inocencio et al., 2025; Zarate & Jardeniano., 2022). The influence of biofertilizers also depends on soil microbial activity and plant physiological response, which may explain the limited observable differences in survival (Abdelaal et al., 2021).

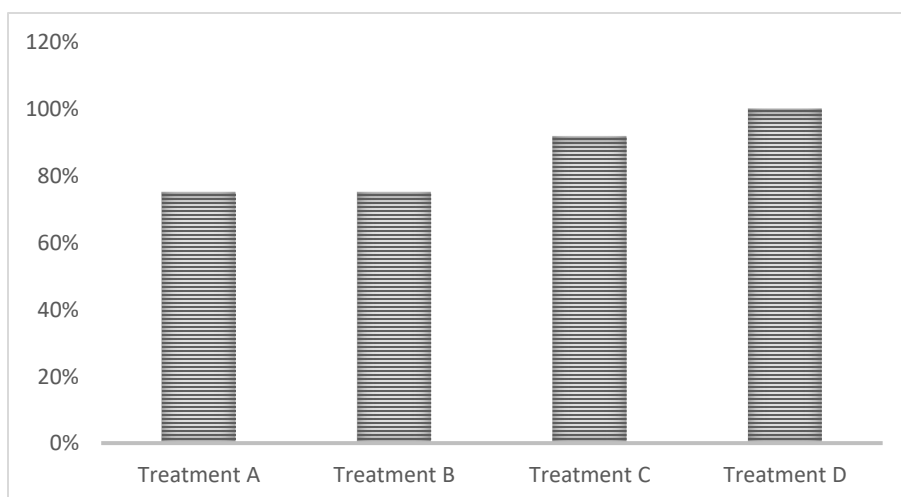


Figure 2. Survivability of Abaca Per Treatment Using Biofertilizer

The consistently high survival rates further demonstrate that Masbate provides a favorable environment for abaca cultivation. The province's warm, humid climate and relatively uniform rainfall align well with the ecological requirements of abaca, while its well-drained loamy soils help prevent waterlogging during early establishment (Armezin et al., 2011; Gagula et al., 2024; Shahri et al., 2014). These environmental conditions, combined with abaca's natural adaptability to diverse agroecological settings (Bande et al., 2012), likely contributed to the successful establishment observed.

#### **4. Conclusion**

The study found that the application of biofertilizers did not significantly affect the height increment, number of leaves, leaf size, pseudostem length and diameter, number of suckers, and survival rate of abaca compared to inorganic fertilizer and the control, indicating that abaca can establish well even under varying nutrient inputs. This suggests that biofertilizers can be used as an effective alternative when inorganic fertilizers are limited, as the beneficial microbes in products such as Mykovam and Mykoplus support nutrient uptake and root development. The consistently high survival across treatments further demonstrates that Masbate provides favorable environmental conditions for abaca cultivation. Future studies are recommended to extend the evaluation into later growth stages, particularly fiber yield and quality, and to explore combined or reduced fertilizer rates with biofertilizers to determine potential synergistic effects and long-term impacts on soil health.

#### **5. Conflict of Interest**

The author declares that there are no conflicts of interest associated with this manuscript.

#### **6. Use of Artificial Intelligence (AI)-Assisted Technology**

The author confirms that no artificial intelligence (AI)-assisted technologies were used in the preparation of the scientific content of this manuscript. ChatGPT and Grammarly were used solely to improve grammar and writing flow; all writing and intellectual content are the work of the author.

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