



# **Growth Increment of Tissue Cultured Abaca Seedlings Applied with Conventional Fertilizer and Biostimulant**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

This study was conducted purposely to investigate the growth increment of tissue cultured Abaca seedlings applied with conventional fertilizer and biostimulant. And successfully reject the hypothesis that there is no significant difference on the plant height, pseudostem girth, leaf count per plant, leaf area per plant, shoots length, number of primary roots, and roots length of Abaca to the different combination of conventional and biostimulant fertilizers. And there is no correlation between treatments and parameters.

The application of SDF significantly promoted plant growth and root architecture of Abaca compared with the control, even with a limited amount of mineral nutrient input. Among the four with SDF treatments applied in the study, 100%, 75%, and 2.5g CF combined with SDF proved to be effective in both root development and aboveground growth promotion. There is a positive correlation between treatments and parameters.

**Keywords:** *Biostimulant; conventional fertilizer (CF); peg; seaweed dripping fertilizer (SDF); seaweed extract.*

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## 1. INTRODUCTION

Seaweed that is abundant in coastal towns can be used as source of organic fertilizer to lessen the use of synthetic fertilizer that can cause detrimental effect to the soil. Organic fertilizers are capable of mitigating problems associated with synthetic fertilizers. They reduce the necessity of repeated application of synthetic fertilizers to maintain soil fertility [1-5]. They gradually release nutrients into the soil solution and maintain nutrient balance for healthy growth of crop plants [6-13]. They also act as an effective energy source of soil microbes which in turn improve soil structure and crop growth.

Excessive use of chemical fertilizers has led to several issues such as serious soil degradation, nitrogen leaching, soil compaction, reduction in soil organic matter, and loss of soil carbon [14-16]. In addition, the efficacy of chemical fertilizers on crop yield has been decreasing over time. Many studies have demonstrated the benefits of *Kappaphycus* Drippings or KD Foliar Fertilizer, which is 100% organic, has proven to increase the yield of rice, baby corn, soybean, mungbean, sweet pepper, cauliflower, mango, pechay, and orchid (Argana, R. 2016) yet, there is no study on growth increment of Abaca. The fertilizer has proven to promote enhanced growth in terms of height and diameter.

In modern agriculture, the current trend is to explore for breakthrough technology that would have chemical reduction inputs while maintaining yields [17-19]. KD Foliar, a locally made seaweed fertilizer created by SPAMAST and generated from *Kappaphycus alvarezii* seaweed, was discovered to contain much plant growth and development requires nutrients and minerals [20-26]. This study compared the growth increase of tissue cultured Abaca to conventional fertilizer and biostimulant using the innovative product. This study will shed light on the impacts of Seaweed Dripping Fertilizer and Conventional Fertilizer on Abaca growth increment [27-33]. The findings of this study could help farmers employ seaweed dripping fertilizer in conjunction with traditional fertilizer.

## 2. METHODOLOGY

### 2.1 Research Design

A Completely Randomized Design was used in the study. Each study has five treatments replicated four times with 10 sample plants.

The following are the treatments:

- T<sub>0</sub> – Control (no application)
- T<sub>1</sub> – 5 g CF (RR) + 15 ml SDF
- T<sub>2</sub> – 3.75 g CF + 15 ml SDF
- T<sub>3</sub> – 2.5 g CF + 15 ml SDF
- T<sub>4</sub> – 1.25 g CF + 15 ml SDF

### 2.2 Preparation of the Research Site and Planting Materials

From April to May 2022, the investigation was conducted in the hardened area of Latorre's residents in Sitio Manggahan, Matti, Digos City, Philippines. The research area was encased in a double layer net to provide the seedlings with a more or less uniform environment throughout the growing period and until the study's conclusion. The Tangungon cultivar of Abaca meriplant used in the study was from Calinan, Davao City.

### 2.3 Potting Media Preparation and Bagging Procedure

The potting media was made up of a 1:1 mixture of garden soil and coco coir. For full mixing and even distribution in the potting media, these components were placed out on a flat, cemented surface. Filling perforated plastic bags with at least 400 g of mixed potting media was used for bagging. The nursery was organized using file bags in the recommended layout. Prior to planting, each bag of potted media received at least one-fourth liter of tap water.

### 2.4 Transplanting of Meriplants in Media-filled Pots

One meriplant was planted in each pot. The seedlings were hardened for a week after being transplanted. Before applying the prescribed fertilizer treatments, the plants were given another week to stabilize.

### 2.5 Care and Maintenance of the Research Study

To keep the plant moist, it was watered every other day. Because the potting media used has great water holding capacity, daily watering is not required. Weeding was done when weeds were spotted in the bagging bag.

### 2.6 Fertilization Program

The recommended rate and timing of application of fertilizers was based on the recommended practice by Southern Philippine Agribusiness and

Marine and Aquatic School of Technology (SPAMAST) on the fertilization program for Banana or Abaca under nursery condition. The following was the schedule of fertilizer application:

**Table 1. Rate, schedule, frequency, and method of inorganic fertilizer application**

Type/Name of Fertilizer	Rate of Application	Schedule of Application	Method of Application
D.A.P Fertilizer	5 g of water	1, 2, 3,4	Drench
Seaweed Biostimulant	15 ml/L of water	weekly	Foliar

Weekly applications of Seaweed Dripping Fertilizer (SDF) were made to the leaves. For the first to second weeks, one liter of the solution was sprayed on 40 plants each treatment or 40 plants in equal dosages until dripping wet. On the third and fourth weeks, a two-liter solution was used for each of the 40 plants in each treatment. As the size of the leaves grew larger, the application dosage was increased.

The drench method was used to apply the diammonium phosphate (DAP) fertilizer. For the 40 plants per treatment, a liter of fertilizer solution was administered in equal dosages via drench method, similar to the SDF. One week following planting, all essential fertilizing was administered.

## 2.7 Data Gathered and Gathering Procedures

Plant height measurements were taken one week after transplanting and before fertilizer administration. For all parameters that need weekly data collection, subsequent data collection began a week after the initial application of fertilizers and continued a week after each application of the SDF. The following data was gathered:

1. Plant Height (cm). This was measured on weekly basis using a rule. Measurement was located from the mounted peg, installed 1cm above the soil surface to the base of the junction or the base of "V" of the last fully-opened leaf. The peg was set to provide uniform guide in the measurement of the height since soil media is not a stable guide.
2. Pseudostem girth (mm). A caliper was used as a measuring device and measurement area on the pseudostem was located on the tip of the installed guide peg.
3. Morphological Parameters
  - Leaf Count per Plant. All functional leaves were considered at termination day.

- Leaf Area per Plant. Scheduled collection was done at termination day. The leaf length was measured from the tip to the base of the middle lamina. The width was measured from the widest part of the lamina. Area was established by multiplying the length with the width.
- Shoot length. This was measured at termination day. Measurement was started from the base where guide peg was mounted to the point where the last leaf emerged.
- Number of Primary Roots. Counting of primary roots at termination day.
- Root length. All primary roots from every sample were measured from the base of the pseudostem. Average was computed by dividing the total root length over the total number of measured roots. This was gathered on the termination day.

## 2.8 Statistical Procedure

Data gathered was computed using the Analysis of Variance for Completely Randomized Design and significant treatment means were further analyse using the Least Significant Difference (LSD).

## 3. RESULTS AND DISCUSSION

### 3.1 Height of Abaca Plantlet

Height of Abaca plantlets at the initial stage had shown remarkable changes on the weekly growth. Effect of the SDF was again evident at week 4. Although, plantlets with no application showed exponential growth until week 4, SDF in combination with 5g and 3.75g CF significantly produced comparable tall plantlets at week 3 and at week 4 (Fig. 1). Effect of the SDF was again evident at week 4. Although, plantlets with no application showed exponential growth until week 4, SDF in combination with 5 g and 3.75 g CF significantly produced comparable tall plantlets at week 3 and at week 4 (Fig. 3). This is in agreement with Osman et al. (2014) that foliar

application of Seaweed extract increased significantly plant growth such as plant height.

Plantlets that received SDF were consistently comparable with plantlets under control and had even exhibited stunted growth.

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 3,342.901 cm, while within groups have a sum of squares of 189.044 cm, for a total of 3,531.945 cm. The mean square of between groups is 8,355.725 cm, whereas the mean square of within groups is 12.603 cm. The computed F was greater than the significant value, leading to highly significant results with a computed variance of 8.90 percent (Table 2). Thus, it rejected the null hypothesis.

### 3.2 Pseudostem Girth

Pseudogirth growth was influenced by the application of SDF. Exponential growth of the pseudogirth was maintained in plants receiving 5g CF in combination with SDF in the last week

of the trial. All CF treatments in combination with SDF were significantly different from control.

The trend in plantlet pseudogirth growth, as well as the perfect positive connection between treatments and pseudostem girth (Fig. 2), demonstrated the effectiveness of adding seaweed fertilizer to the chemical fertilizer [34] from week 1 to week 4, because of the soluble organic compounds it contains that, due to their chemical shapes, appear to have direct influence on some plant metabolic processes [35].

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 26.733 mm, while within groups have a sum of squares of 2.352 mm, for a total of 29.086 mm. The mean square of between groups is 6.683mm, whereas the mean square of within groups is 0.157 mm. And the computed F 42.614 was greater than the significant value, leading to highly significant results with a computed variance of 8.86 percent (Table 3). Thus, it was successfully rejected the null hypothesis.

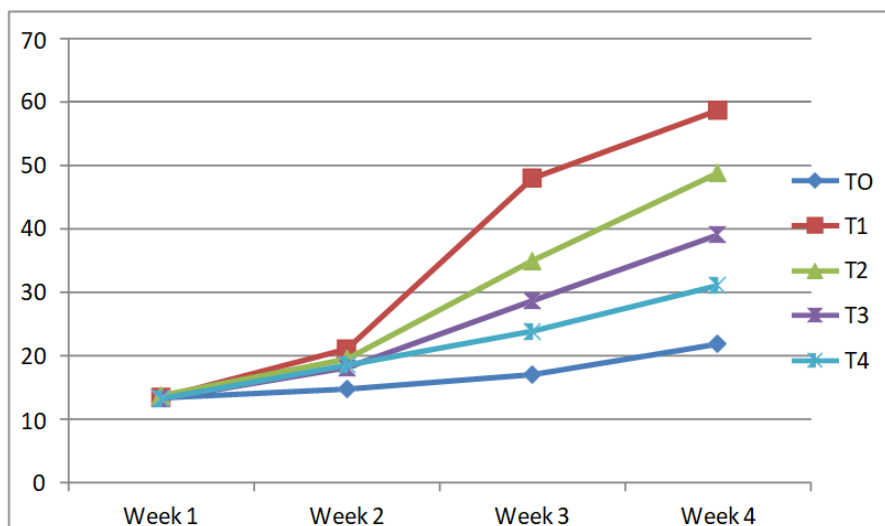


Fig. 1. Changes in plant height (cm) of abaca plantlets within the duration of the study as influenced by conventional fertilizer and biostimulant

Table 2. Analysis of variance on the height (cm) of abaca plantlets at week 4 as influenced by conventional fertilizer and biostimulant

Plant_Height_at_Week4					
Sum of Squares		df	Mean Square	F	Sig.
Between Groups	3342.901	4	835.725	66.312**	.000
Within Groups	189.044	15	12.603		
Total	3531.945	19			

cv=8.90%

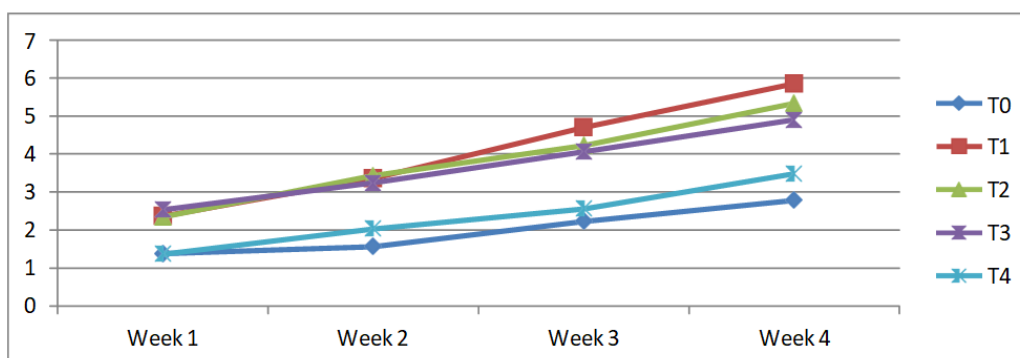


Fig. 2. Changes in pseudostem girth (mm) of abaca plantlets as influenced by conventional fertilizer and biostimulant

Table 3. Analysis of variance on the pseudostem girth of abaca plantlets at week 4 as influenced by conventional fertilizer and biostimulant

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	26.733	4	6.683	42.614**	.000
Within Groups	2.352	15	.157		
Total	29.086	19			

cv=8.86%

### 3.3 Morphological Parameters

The morphological characteristics of Abaca in terms of the number of leaves/Plantlet, leaf area, number of primary root, root length, and except for the shoot length were significantly influenced by the application of SDF.

### 3.4 Leaf Count

Number of leaves per plant was remarkably influenced by SDF application. All CF treatments in combination with SDF were significantly different from control. The increasing CF level in combination with SDF showed a better result. And the perfect positive correlation between treatments and leaf count was in agreement with the statement of Zhang & Schmidt cited by

Sangha et al. [34] because of the soluble organic compounds it contains that, due to their chemical shapes, appear to have direct influence on some plant metabolic processes [35] (Fig. 3).

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 31.782 cm, while within groups have a sum of squares of 2.720 cm, for a total of 34.502 cm. The mean square of between groups is 7.945 cm, whereas the mean square of within groups is 0.181 cm. And the computed F 43.817 was greater than the significant value, leading to highly significant results with a computed variance of 9.19 percent (Table 4). Thus, it was successfully rejected the null hypothesis.

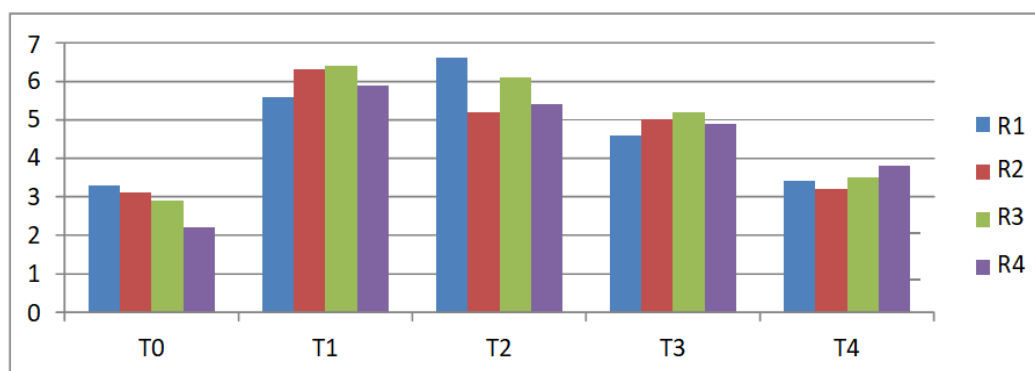


Fig. 3. Leaf count of abaca plantlets as influenced by conventional fertilizer and biostimulant

**Table 4. Analysis of variance on leaf count of abaca plantlets as influenced by conventional fertilizer and biostimulant**

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	31.782	4	7.945	43.817**	.000
Within Groups	2.720	15	.181		
Total	34.502	19			

cv=9.19%

### 3.5 Leaf Area

All CF treatments in combination with SDF were significantly different from control in leaf area. The increasing CF level in combination with SDF showed a better result.

The widest leaf areas was recorded in plantlets applied with 5 g CF combined with 15 ml SDF at 1,073.42 cm<sup>2</sup> however, result was comparable with 3.75 g CF combined with 15ml SDF with leaf area of 995.75 cm<sup>2</sup> but were highly significant different from the rest of the treatments. Compared with control, it implied that SDF had highly significant influence to the leaf area development. The correlation between treatment and leaf area showed perfect positive correlation result (Fig. 4).

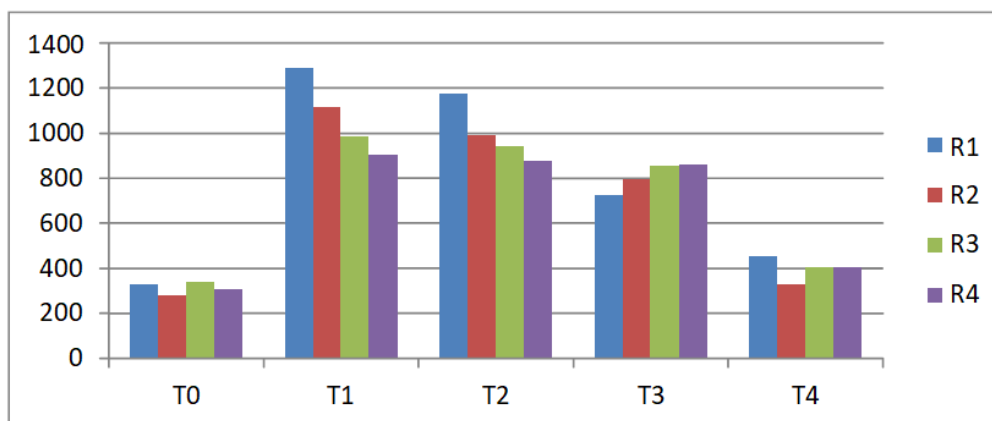
Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of

1,912,719.556 cm<sup>2</sup>, while within groups have a sum of squares of 154,049.056 cm<sup>2</sup>, for a total of 2,066,768.612 cm<sup>2</sup>. The mean square of between groups is 478,179.889 cm<sup>2</sup>, whereas the mean square of within groups is 10269.937 cm<sup>2</sup>. And the computed F 46.561 it was greater than the significant value, leading to highly significant results with a computed variance of 14.12 percent (Table 5).

Thus, it was successfully rejected the null hypothesis.

### 3.6 Number of Primary Roots

All CF treatments in combination with SDF were significantly different from control in Primary Roots. The increasing CF level in combination with SDF showed a better result. The number of primary root was pronouncedly enhanced by the application of SDF in addition to 5 g CF combined with 15 ml SDF (26.7 pieces) with an



**Fig. 4. Leaf area of abaca plantlets as influenced by conventional fertilizer and biostimulant**

**Table 5. Analysis of variance on the leaf area of abaca plantlets as influenced by conventional fertilizer and biostimulant**

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	1,912,719.556	4	478179.889	46.561	.000
Within Groups	154,049.056	15	10269.937		
Total	2,066,768.612	19			

cv=14.12%

increase of 2.3 pieces over the plantlets applied with 3.75 g CF combined with 15 ml SDF (24.4 pieces). Primary root count in 5 g CF combined with 15 ml SDF applied plantlets showed highly significant differences to plantlets under treatment with decreasing CF, from 75%, 50%, and 25% (Fig. 5).

This showed that decreasing the CF had effect to the production of primary roots structure but was enhanced through the application of SDF which was in agreement with the statement of Calvo et al. (2014) that applying seaweed extract to the leaves improves root development. The study's findings on root growth were in agreement with Jeannin cited by Khan et al. [36].

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 35.622 cm, while within groups have a sum of squares of 7.570 cm, for a total of 43.192 cm. The mean square of between groups is 8.906cm, whereas the mean square of within groups is 0.505 cm. And the computed F 17.646 which is greater than the significant value, leading to highly significant results with a computed variance of 14.12 percent (Table 6). Thus, it was successfully rejected the null hypothesis.

### 3.7 Root Length

All CF treatments in combination with SDF were significantly different from control in Root Length. The increasing CF level in combination with SDF showed a better result. Similar trend was observed in terms of root length, however, in an inverse manner, where 5 g CF combined with 15 ml SDF significantly produced the longest main roots structure at 69.98 cm compared to the rest

of the plantlets in other CF combinations with SDF treatments and control. This is because, in order to survive, plants explore their rhizosphere in quest of nutrition, as one of the key roles of the roots is to give water and nutrients [37] and the rate of root extension varies by species (Watt et al., 2006) (Fig. 6).

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 7,452.273 cm, while within groups have a sum of squares of 606.384 cm, for a total of 8,058.657 cm. The mean square of between groups is 1,863.068 cm, whereas the mean square of within groups is 40.426 cm. And the computed F 46.086 which is greater than the significant value, leading to highly significant results with a computed variance of 13.68 percent (Table 7). Thus, it was successfully rejected the null hypothesis.

### 3.8 Shoot Length

All CF treatments in combination with SDF were significantly different from control in Primary Roots. The increasing CF level in combination with SDF showed a better result. Plantlets during the termination day had produced significant result among treatments and have perfect positive correlation between treatments and shoot length (Fig. 7). SE has been shown to increase root mass or root-to-shoot ratio in a number of crops, such as cucumber [38].

Based on the results of the analysis of variance on Abaca plant height during the study, the between groups have a sum of squares of 2,734.567cm , while within groups have a sum of squares of 1451.129 cm, for a total of

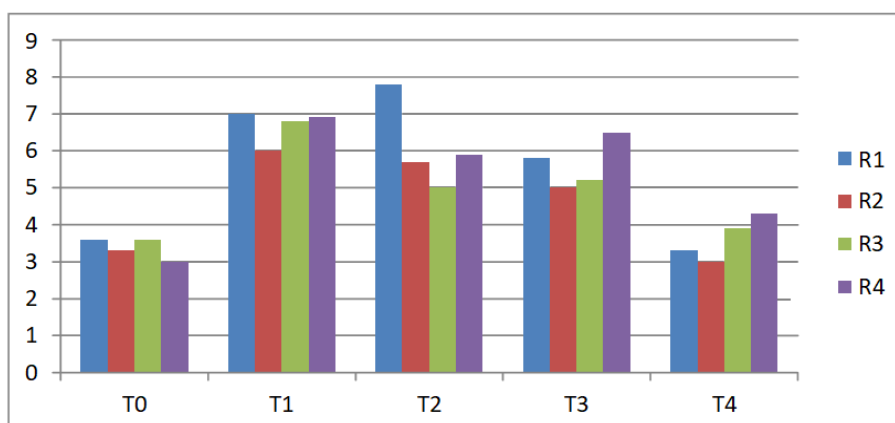
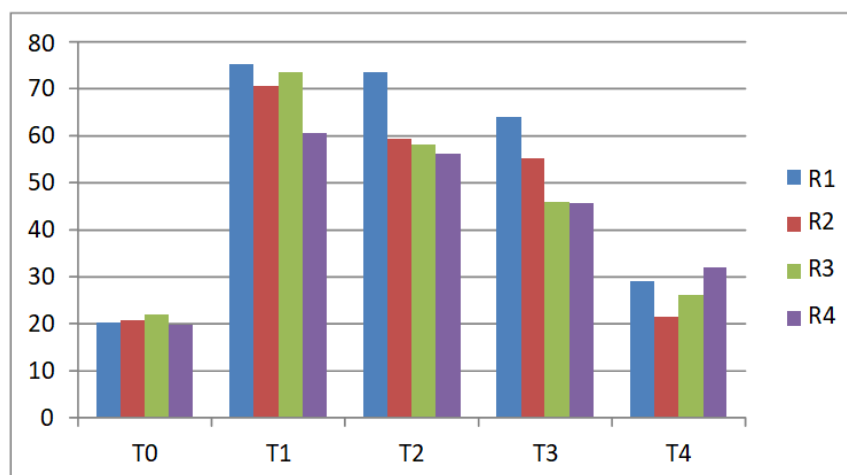


Fig. 5. Number of primary roots of abaca plantlets as influenced by conventional fertilizer and biostimulant

**Table 6. Analysis of variance on the number of primary roots of abaca plantlets as influenced by conventional fertilizer and biostimulant**

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	35.622	4	8.906	17.646**	.000
Within Groups	7.570	15	.505		
Total	43.192	19			

cv=13.99%

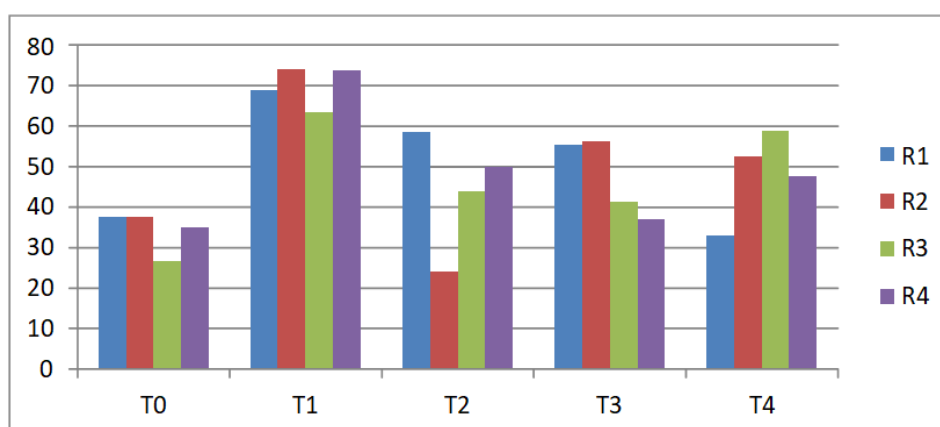


**Fig. 6. Root length of abaca plantlets as influenced by conventional fertilizer and biostimulant**

**Table 7. Multiple comparisons on the root length of abaca plantlets as influenced by conventional fertilizer and biostimulant**

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	7452.273	4	1863.068	46.086**	.000
Within Groups	606.384	15	40.426		
Total	8058.657	19			

cv=13.68%



**Fig. 7. Shoot length of abaca plantlets as influenced by conventional fertilizer and biostimulant**

4,185.696 cm. The mean square of between groups is 683.642 cm, whereas the mean square of within groups is 96.742 cm. And the computed F 7.067 which is greater than the significant

value, leading to highly significant results with a computed variance of 20.19 percent (Table 8). Thus, it was successfully rejected the null hypothesis.



**Table 8. Analysis of variance on the shoot length of abaca plantlets as influenced by conventional fertilizer and biostimulant**

Sum of Squares		df	Mean Square	F	Sig.
Between Groups	2734.567	4	683.642	7.067	.002
Within Groups	1451.129	15	96.742		
Total	4185.696	19			

cv=20.19%

#### 4. SUMMARY

This study focused on the growth increment of tissue cultured Abaca seedlings applied with conventional fertilizer and Biostimulant. Generally, the study was conducted purposely to investigate the growth increment of tissue cultured Abaca seedlings applied with conventional fertilizer and biostimulant. Specifically it aims to investigate the significant difference on the plant height, pseudostem girth, leaf count per plant, leaf area per plant, shoot length, number of primary roots, and roots length of Abaca to the different combination of conventional and biostimulant fertilizers [39-46]. And Determine which of the different combination of conventional fertilizers and biostimulants suited to Abaca seedlings.

There were five treatments including control and each treatment replicated into four times with ten samples each replication, thus, forty meriplants per treatment, a total of two hundred meriplants of more or less uniform in height.

Parameters gathered were: Height, Pseudostem Girth, Morphological Parameters (Leaf Count, Leaf Area, Number of Primary Roots, Root length, and Shoot Length).

The average maximum height and pseudostem girth were observed in the application of 100%, 75%, and 2.5 g CF combined with 15 ml SDF. And both had perfect positive correlation between treatments. All the morphological parameter were great influenced by the addition of SDF to 5 g CF. And had perfect positive correlation between treatments and parameters.

Over-all results on the parameters of Abaca plantlets were influenced by the application of SDF. This is in agreement with Thirumaran et al. (2009) that seaweed liquid fertilizer applied to crop plant showed better results. The effect of commercial seaweed extracts on growth of plant is reminiscent of activity of phytohormones.

#### 5. CONCLUSION

Based on the findings, it is concluded that fertilizer management is of great important in Abaca especially under the nursery. In this study, four sources of SDF in combination with CF were applied to promote the growth of the Abaca seedlings at nursery stage.

Based on the results:

- The application of SDF significantly promoted plant growth and root architecture of Abaca compared with the control, even with a limited amount of mineral nutrient input.
- Among the four with SDF treatments applied in the study, 100%, 75%, and 2.5g CF combined with SDF proved to be effective in both root development and aboveground growth promotion.
- There is a positive correlation between treatments and parameters.
- The study was succeeding in rejecting the entire hypothesis.

#### 6. RECOMMENDATIONS

As perspective, the researcher believe that at the rate of 3.75 g CF combined with SDF is beneficial rate to be recommended but it is also better to carry out more research including characterization of growth hormones and other metabolites which may have effect to plants' total growth and development.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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