EFFECT OF DIFFERENT RATES OF LIQUID *TRICHODERMA HARSIANUM* ON GROWTH ENHANCEMENT OF TISSUE CULTURED ABACA SEEDLINGS

ABSTRACT

This study was conducted to evaluate the effect of different rates of liquid *Trichoderma harsianum* on the growth enhancement of tissue-cultured Abaca seedlings. There are six treatments replicated four (4) times with 5 samples per replication. The following were the treatment: T_0-control, T_1- 50 ml of L.T, T_2- 40 ml of L.T, T_3-30 ml of L.T, T_4- 20 ml of L.T, and T_5-10ml of L.T/liter of water. Based on the results, it did not successfully reject the null hypothesis on plant height, pseudostem girth, leaf count per plant, leaf area per plant, and the number of primary roots of Abaca seedlings treated with liquid *Trichoderma harsianum*. While the two parameters successfully rejected the null hypothesis, there is highly significant that developed greater lengths for their shoot and root lengths treated with liquid *Trichoderma harsianum*. When compared to the control, the use of Liquid Trichoderma harsianum can significantly increase abaca growth. Treatment 3 of Abaca seedlings with 30 ml of liquid *Trichoderma harsianum* was the most effective of the five treatments with this substance. The correlation between treatments and parameters is also favorable.

Keywords: Liquid *Trichoderma harsianum*; Growth; Enhancement Tissue-cultured; Morphological;

1. INTRODUCTION

Abaca is a Philippines-native perennial plant. It is one of the Philippines' primary revenue sources because it exports 80 percent to 85 percent of its fiber (Manila hemp) to the global market. As the incidence of Abaca viral diseases increased in the mid-1970s, the production and supply of abaca fiber became uncertain. The apex of the Abaca mosaic is a bunchy Abaca. The impact of these abaca diseases on fiber production and yield quality is significant (Dev, P., 2012).

Trichoderma spp. is widely used in industrial and agricultural processes because it can produce secondary enzymes and metabolites (Jiang, X., Geng, A., He, N., & Li, Q., 2011). This research has the potential to increase the economic benefits of liquid Trichoderma for abaca production, particularly for our beloved Filipino farmers. Trichoderma can combine multiple benefits into a single product, such as the control of multiple plant diseases, the enhancement of plant growth, and the provision of a clean environment for sustainable agriculture (Al-Ani, L. K. T., 2018)). Humans, wildlife, and other beneficial organisms are unaffected by this biological control agent.

Verbal communication from Dr. Juan P. Agudera Jr., who innovated the liquid *Trichoderma harsianum* from his study findings revealed that Trichoderma sp. can be grown in liquid media (coco-water) which was pasteurized using a drum with wood-fire maintaining continuous boiling for three (3) hrs. Further, Trichoderma cannot grow in unpasteurized fresh coco-water and 2-day stocked coco-water (fermented) even

pasteurized for three (3) hrs. Liquid Trichoderma (5 –day old Trichoderma grown in pasteurized coco-water and blended using blender) unable to further colonize the surface of coco-water media (pasteurized for 3 hrs).

could control Fusarium wilt disease, bacterial wilt disease, Compost Fungus Activator (CFA), and root extender, and that liquid Trichoderma was a growth enhancer and highly effective in controlling tomato wilt (*Ralstonia solanacea*). Farmers may be able to use the findings of this study to improve the growth performance of tissue-cultured abaca seedlings.

2. METHODOLOGY

2.1 Research Design

The study was conducted using the Completely Randomized
Design (CRD). There were six treatments replicated four times with 5 sample plants per replication. The treatment used in this study were the following:
T_0- Control (Natural water)
T_1- 50ml of Liquid Trichoderma/1L of water
T_2- 40ml of Liquid Trichoderma/1L of water
T_3-30ml of Liquid Trichoderma /1L of water
T_4- 20ml of Liquid Trichoderma/1L of water
T_5- 10ml of Liquid Trichoderma/1L of water

2.2 Preparation of the Research Site and Planting Materials

The site of the study was located at Barangay Tiguman, Digos City, Davao del Sur. Start on April 4, 2022, and end on May 3, 2022. A double-layer net surrounded the research area to provide a more or less uniform planting environment during the entire growing period until the end of the study. The average temperature was **28°C** and the average humidity was **80%.**

Figure 1. Location of the Research Study Using Google Maps

The Liquid Trichoderma (L.T.) was purchased at Davao del Sur State College (DSSC). Vermicompost, coco coir, and carbonized rice hull were available at Lao Farm Bansalan, Davao del Sur. Two Hundred forty pieces of Tangungon cultivar of Abaca meri-plant seedlings were purchased in Calinan, Davao City. The Polyethylene plastic bag (6" x8" x0.003 mm), a sprinkler can measure cup, graduated cylinder, ruler, Vernier Caliper, record book, and cutting tools were purchased in Digos City public market.

2.3 Potting Media Preparation and Bagging Procedure

The materials were laid on a flat and cemented surface for thorough mixing for even distribution in the potting medium. Bagging was done by filling the perforated plastic bags with at least 500g of mixed potting medium. Filled bags were arranged in the nursery according to the prescribed layout. Potted media were water at least one-fourth liter of each bag with natural water before planting.

2.4 Transplanting of Meriplants in Mediafilled Pots

Every pot was planted with a meri plant. The transplanted plants were acclimatized for one week in a hardening zone. Another week has been scheduled so that seedlings are stable before applying the recommended fertilizing treatments.

2.5 Care and Maintenance

It was sprinkled every 8:00 in the morning and 5:00 in the afternoon with a bucket. If there is enough rain, no watering takes place. Any weeds noticed growing in the experimental area, particularly inside the polyethylene bag, were immediately removed to minimize underground growth competition.

2.6 **Data Gathering Procedure**

Initial data on plant height and pseudo stem girth were gathered two weeks after transplanting and before the first application of Liquid Trichoderma (L.T.). Succeeding data collection started a week after every three days of application on Liquid Trichoderma (L.T.). After every three days of application to the Liquid Trichoderma, the succeeding collection was done for all parameters that need weekly data collection. The following data will be gathered:

1. Plant Height (cm). This was measured every week by a ruler. Measurement was located from the mounting peg. Installed 1 cm above the soil surface to the base of the junction or the bottom of "V" of the last fully-opened leaf. The mounted peg was set to provide a uniform guide in measuring the height since soil media is not a stable guide.
2. Pseudo stem girth (mm). A caliper was used as a measuring device, and the measurement area on the pseudostem was located on the tip of the installed guide peg. Data was collected every week.

3. Morphological Characteristic

3.1 Leaf Count per Plant. All functional leaves were counted on termination day.

3.2 Leaf Area per Plant. The scheduled collection was done on termination day. The leaf length was measured from the tip to the base of the middle lamina. The width was calculated from the broadest part of the lamina. The area was established by multiplying the length with the width.

3.3 Shoot Length. was measured on termination day. Measurement started from the base where the guide peg was mounted to the point where the last leaf emerged.

3.5 Number of Primary Roots: Counting of primary roots at termination day.

3.6 Root Length. All primary roots from every sample were measured from the base of the pseudostem. The average was computed by dividing the root length by the number of measured roots. This was gathered on the termination day.

2.7 Statistical Analysis

Data were analyzed using the analysis of variance (ANOVA) in Completely Randomized Design (CRD) (Ubaub, L. T., & Requina, J. D.,2016). Least Significant Difference (LSD) was used in comparing treatment means. The IBM Statistical Package for the Social Sciences (SPSS) 26 software was used to analyze the data (George, D., & Mallery, P.,2019).

3. RESULT AND DISCUSSION

3.1 Plant Height

The effects of varying the dosages of liquid *Trichoderma harsianum* weekly result in treatment four (20 ml) having the maximum height with a mean value of 34.1 and treatment zero (Control) having the lowest with a mean value of 28.75. There is no statistically significant difference, according to the analysis of variance data. As a result, it was unable to disprove the null hypothesis. After two months of planting, the height and diameter of the plants kept becoming smaller. This is due to a delay in new root development and water uptake efficiency. A similar pattern in the physiological responses of bananas at various stages of development. After 1 and 2 months of growth, the results show an average increase of 116 cm and 123 cm in plant height, respectively. This translates to a 17-centimeter increase in the first month and a 7-centimeter gain the following month (Bande, M., Asio, V., Sauerborn, J., & Romheld, V. 2016).

3.2 Pseudostem Girth

In terms of pseudostem girth, treatment five (10ml of liquid Trichoderma) has the largest girth with a mean value of 34.1, and treatment zero (Control), with a value of 15.3, has the smallest girth among the six treatments. The effect of varying rates of liquid *Trichoderma harsianum* weekly is shown in the results. There is no discernible difference between the Liquid Trichoderma and Pseudostem of Abaca seedlings, according to the

results of the Analysis of Variance. As a result, it was unable to rule out the null hypotheses. The pseudostem girth was increased proportionally to give the plant the strength to withstand the weight of the bunch. The largest pseudostem circumference was 76 cm (krishnamoorthy & Harif 2017). In developing the pseudo stem girth, it decreased to an average of 2.1 cm per month because of several factors (Bande, M., Asio, V., Sauerborn, J., & Romheld, V. 2016).

Fig. 2. Transformation in Plant Height (cm) of Abaca Plantlets threatened by Liquid *Trichoderma harsianum*

Table 1. Analysis of Variance on Plant Height of Abaca Plantlet at week 4 Threatened by Liquid *Trichoderma harsianum*

ANOVA

Plant Height

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68.333	5	13.667	1.067^{ns}	0.411
Within Groups	230.540	18	12.808		
Total	298.873	23	26.474		

CV=11.12%
ns=*Not Significant*

Fig. 3. Transformation in Pseudostem Girth (mm) of Abaca Plantlets threatened by Liquid Trichoderma

Table 2. Analysis of Variance on Pseudostem Girth of Abaca Plantlet at week 4 Threatened by Liquid *Trichoderma harsianum*

ANOVA

Pseudo Stem Girth

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62.000	5	12.400	0.852^{ns}	0.532
Within Groups	262.125	18	14.563		
Total	324.125	23	26.963		

CV=8.87%
ns= *Not Significant*

3.3 Morphological Parameters

3.3 Leaf Count

Fig. 4 shows the Average leaf count of applying Liquid *Trichoderma harsianum* in Treatment four (40 ml of Liquid Trichoderma) has the highest mean value of 35.75 while treatment (Control) has a mean value of 31.25 is the lowest among the six treatments. Based on the result of the Analysis of Variance represents that there is no statistically significant difference (p-0.05). Thus, it could not able to reject the null hypothesis. The

results indicate that Liquid Trichoderma and Natural water has no potential in increasing the number of leaves within 30 days of observation. There is a statistically significant difference (p0.05) in the total number of functional leaves between blocks. From the first to the sixth month after planting, the number of functional leaves increased, then decreased after seven months (Bande, M. M., Asio, V. B., Sauerborn, J., & Römheld, V. 2016).

Fig. 4. Transformation in Leaf count of abaca plantlets threatened by Liquid Trichoderma Harsianum

Table 3. Analysis of Variance on Leaf Count of Abaca Plantlet Threatened by Liquid *Trichoderma harsianum*

ANOVA

Leaf_Count

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44.000	5	8.800	1.576^{ns}	0.217
Within Groups	100.500	18	5.583		
Total	144.500	23	14.383		

CV=7.11%

ns= *Not Significant*

Leaf Area

Fig. 5 shows the effect of Liquid Trichoderma in terms of Leaf Area per plant. Treatment five (10 ml of Liquid Trichoderma) has the highest mean with a value of 37394 while treatment zero (Control) has a value of 24955.5 is the lowest among the six treatments.

The Analysis of Variance on Leaf Area per plant with different levels of Liquid Trichoderma and natural water. Based on the results, the p-value is 0.219 and the F-value is 1.569 which means there is no statistically significant difference (0.05). Thus, it could not able to reject the null hypothesis. The results indicate that Liquid Trichoderma and natural water has no potential in increasing the Leaf Area per plant of Abaca Seedlings within 30 days. The slight decrease in cumulative leaf area is due to the decline in the number of functional leaves which is due to the combined effect of nutrient deficiency (Bande, M. M., Asio, V. B., Sauerborn, J., & Römheld, V. 2016).

Fig. 5. Transformation in Leaf Area of Abaca Plantlets Threatened by Liquid *Trichoderma harsianum*

Table 4. Analysis of Variance on Leaf Area of Abaca Plantlet Threatened by Liquid *Trichoderma harsianum*

ANOVA

Leaf_Area

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	331179798.708	5	66235959.742	1.569^{ns}	0.219
Within Groups	760030781.250	18	42223932.292		
Total	1091210579.958	23	108459892		

CV=20.61%
ns= *Not Significant*

Shoot Length

Fig. 6 shows the Average Shoot length with different levels of Liquid Trichoderma. Treatment three (30 ml of Liquid Trichoderma) has the highest mean with a value of 561.75 while treatment zero (Control) has a mean value of 369 is the lowest among the six treatments.

The analysis of variance revealed that the results are statistically significant differences (p0.05). Thus, it was successfully rejected the null hypothesis. It demonstrates that liquid Trichoderma can lengthen the shoot length of tissue cultured abaca seedlings, and indicates that has the potential to increase within 30 days. The growth rate of shoot length and dry weight of the whole plant in the Trichoderma treated plants were 1.6 and 1.5 times, respectively, the rate of the noninoculated control plants, from 7 to 28 days post-emergence (Yedidia, I., Srivastva, A. K., Kapulnik, Y., & Chet, I. 2001). The Peat moss medium produced the highest values for shoot diameter, shoot length, shoot dry weight, and fresh root weight (2.52 mm, 248.42 mm, 2.23 g, and 0.83 g, (Shekhany, H., 2017).

Fig. 6. Transformation in Shoot Lenght of Abaca Plantlets Threatened by Liquid *Trichoderma harsianum*

Table 5. Analysis of Variance on Shoot Lenght of Abaca Plantlet Threatened by Liquid *Trichoderma harsianum*

ANOVA

Shoot_Length

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	105996.375	5	21199.275	3.695**	0.018
Within Groups	103267.25	18	5737.069		
Total	209263.625	23	26936.344		

CV=15.51

**= *Highly Significant*

Number of Primary Roots

Fig. 7 shows the average number of primary roots with different levels of Liquid Trichoderma. Treatment four (20 ml of Liquid Trichoderma) with a value of 30 is the highest number of primary roots while treatment five (10 ml of Liquid Trichoderma) with a value of 25 is the lowest.

The Analysis of Variance revealed the number of primary roots with different levels of Liquid Trichoderma was not statistically significant. Thus, it could not able to reject the null hypothesis. It shows pure water and Liquid Trichoderma has no potential in increasing the number of primary roots within 30 days. Only 2.8 percent of the Trichoderma strains evaluated in the study promoted the growth of primary and lateral roots as compared to untreated plants, implying that only around 3.6 percent of the strains tested could enhance the number of lateral roots (Lo, C. T., & Lin, C. Y. 2002.

Fig. 7. Transformation in Number of Primary Roots of Abaca Plantlets Threatened by Liquid *Trichoderma harsianum*

Table 6. Analysis of Variance on Number of Primary Roots of Abaca Plantlet Threatened by Liquid *Trichoderma harsianum*

ANOVA

Number of Primary Roots

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68.208	5	13.642	1.878^{ns}	0.148
Within Groups	130.75	18	7.264		
Total	198.958	23	20.906		

CV=9.73

ns= *Not Significant*

Root Length

Fig. 8 shows the average Root Length with different levels of Liquid Trichoderma. Treatment three (30 ml of Liquid Trichoderma) with a value of 2433 was the highest Root length while treatment zero (Control) with a value of 1065.5 is the lowest. All treatments with Liquid Trichoderma are better than pure water (T0).

Based on the Analysis of Variance indicates that the results are a statistically significant difference. Thus, it was successfully rejected the null hypothesis. It reveals that liquid Trichoderma can lengthen the Root length of tissue cultured abaca seedlings within 30 days. Trichoderma showed increased root and shoot growth in a pot experiment. A stronger root system leads to improved uptake of water, minerals, and nutrients when the root surface area responds to nutrient limitation circumstances (Mahato, S., Bhuju, S., & Shrestha, J. 2018).

Fig. 8. Transformation in Root Length of Abaca Plantlets Threatened by Liquid *Trichoderma harsianum*

Table 7. Analysis of Variance on Root Length of Abaca Plantlet Threatened by Liquid *Trichoderma harsianum*

ANOVA

Root_Length

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4795056.708	5	959011.342	7.062**	0.001
Within Groups	2444504.250	18	135805.792		
Total	7239560.958	23	1094817.133		

CV=18.58

**=*Highly Significant*

Correlation Between Treatment and the Parameters

Tab.8 shows the correlation between treatment and the parameters. The Pseudo stem girt, leaf area, shoot length, and root length have a perfect Pearson correlation at the 0.01 level. It indicates that Liquid Trichoderma has the potential to enhance the growth of Abaca seedlings. The leaf count has a Pearson correlation significant at the 0.05 level which indicates the relationship of Liquid Trichoderma. The plant height and the number of primary roots have a very weak Pearson correlation at the level of 0.01 and 0.05 in 2-

tailed. It represents that Liquid *Trichoderma harsianum* between plant height and the number of primary roots has no potential in terms of enhancing the growth of Abaca seedlings.

Correlations

		PLANT HEIGHT	Pseudo Stem_Girth	Leaf Count	Leaf_Area	Shoot Length	Number of Primary Roots	Root_Length
PLANT_HEIGHT	Pearson Correlation	1	.442*	.506*	.728**	.650**	.178	.701**
	Sig. (2-tailed)		.031	.012	.000	.001	.406	.000
	N	24	24	24	24	24	24	24
Pseudo_Stem_Girth	Pearson Correlation	.442*	1	.498*	.416*	.274	-.060	.482*
	Sig. (2-tailed)	.031		.013	.043	.195	.782	.017
	N	24	24	24	24	24	24	24
Leaf_Count	Pearson Correlation	.506*	.498*	1	.286	.377	.264	.392
	Sig. (2-tailed)	.012	.013		.176	.069	.213	.058
	N	24	24	24	24	24	24	24
Leaf_Area	Pearson Correlation	.728**	.416*	.286	1	.569**	-.014	.668**
	Sig. (2-tailed)	.000	.043	.176		.004	.949	.000
	N	24	24	24	24	24	24	24
Shoot_Length	Pearson Correlation	.650**	.274	.377	.569**	1	.048	.784**
	Sig. (2-tailed)	.001	.195	.069	.004		.824	.000
Number_of_Primary_Roots	Pearson Correlation	.178	-.060	.264	-.014	.048	1	.082
	N	24	24	24	24	24	24	24
Root_Length	Pearson Correlation	.701**	.482*	.392	.668**	.784**	.082	1
	Sig. (2-tailed)	.000	.017	.058	.000	.000	.704	
	N	24	24	24	24	24	24	24

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 8. Correlation Between Treatment and the Parameters

Fig. 9. The Average of Temperature and Humidity *Source of https://ww.weatheravenue.com/en/asia/ph/davao-del-sur/tigumok-almanac.html*

4. SUMMARY

The study was conducted to evaluate the effect of different rates of liquid Trichoderma on the growth enhancement of tissue cultured abaca seedlings. The study was carried out in a completely randomized design experiment. There were six treatments replicated four (4) times with 5 five samples per replicate. The following treatments were T_0- control, T_1- 50 ml of L.T., T_2- 40 ml of L.T., T_3- 30 ml of L.T., T_4- 40 ml of L.T., and T_5- 10 ml of L.T. The abaca seedling was monitored for 30 days before applying liquid Trichoderma, and the initial data on plant height and pseudostem girth was collected for the basis. The data collection of plant height and pseudostem are collected every week. The application of liquid Trichoderma was every three days. The various effects of liquid Trichoderma were not observed in the first week of abaca seedlings. The control and Liquid Trichoderma appear to have different effects in the second week.

The leaves of abaca seedlings treated with Liquid Trichoderma were healthier than the natural water. In the third-week observation of T_0 (control or natural water), there was an insect found on the tissue-cultured Abaca Seedlings.

Based on the results, Plant Height has the highest mean in treatment five (10 ml of Liquid Trichoderma) with a value of 34.1. Pseudo stem girth has the larger in treatment four (20 ml of Liquid Trichoderma). Leaf Count has the highest mean in treatment four (20 ml of Liquid Trichoderma)with a value of 35.75. Leaf Area has the highest mean in treatment five (10 ml of Liquid Trichoderma)with a value of 37394. Shoot Length has the

highest mean in treatment three (30 ml of Liquid Trichoderma) with a value of 561.75. The number of primary roots has the highest mean in treatment four (20 ml of Liquid Trichoderma) with a value of 30, and Root Length has the highest mean in treatment three (30 ml of Liquid Trichoderma) with a value of 2433.

5. CONCLUSION

Based on the findings of the study, the following conclusion is drawn:

1. Plant height and pseudo stem girth of Abaca seedlings treated with various concentrations of liquid Trichoderma are comparatively higher than those treated with natural water (control), but the findings are insufficient to rule out the null hypotheses.
2. Abaca seedlings exposed to various concentrations of liquid Trichoderma have more leaves, more leaf area, and more primary roots than those exposed to natural water (the control), but the results do not rule out the null hypotheses. While Abaca seedlings with various rates of liquid Trichoderma strongly rejected the null hypothesis and developed greater lengths for their shoot and root lengths.
3. The relationship between treatments and parameters is positive.
4. Among the six treatments applied in the study, 30ml of Liquid Trichoderma is suited for Abaca seedlings.

5. RECOMMENDATION

Based on the results, T_3 (30 ml of L.T.) for shoot length and root length are the most effective treatments, although T2 (40 ml of L.T.), T4 (20 ml of L.T.), and T5 (10ml of L.T) also produce better results too.

- It is recommended to increase the duration of the study to observe the excellent results of Liquid Trichoderma in the seedling stage.
- Liquid Trichoderma is recommended for growth enhancement.
- To keep pests and diseases from the plant, it is advised that the plants be treated with liquid Trichoderma.
- Due to its inexpensive cost, the 10 ml of liquid Trichoderma was suggested to give the research study more significance.

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