RESEARCH PAPER

Innovating Liquid Trichoderma (LT) and Field Testing Against Fusarium oxysporum f. sp. cubense (Foc) Infecting Cavendish Banana

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ABSTRACT
Fusarium oxysporum f. sp. cubense (Foc) is a serious pathogen that leads to considerable yield reduction in Cavendish banana cultivation. Traditional chemical control approaches have shown limited effectiveness in controlling this disease. For this reason, biological control through beneficial microorganisms such as Trichoderma has received increasing attention as a safer and environmentally sustainable alternative. However, developing a practical and cost-effective liquid formulation of Trichoderma that can be easily used by farmers remains a challenge.
The study titled "Innovating Liquid Trichoderma (LT) and Field Testing Against Fusarium oxysporum f. sp. cubense (Foc) Infecting Cavendish Banana" was conducted at the Trichoderma Laboratory of Davao del Sur State College (DSSC) and at Cagas Banana Farm in Digos City, Davao del Sur, between June 2020 and December 2022. The research aimed to examine the growth performance and spore production of Trichoderma grown in pasteurized coco-water and to determine the bio-efficacy of Liquid Trichoderma under field conditions in banana plantations heavily infested with Foc.
A Completely Randomized Design (CRD) with five replications and several pasteurization treatments was used in the laboratory experiment. The results indicated that Trichoderma successfully developed in fresh coco-water pasteurized for three hours, whereas the fungus did not grow in unpasteurized or fermented media. The harvested culture produced approximately 5.8 million spores per liter within 5–7 days after inoculation. Field evaluation further indicated that banana plants treated with 100–400 ml LT per liter of water showed infection levels ranging from 14.81% to 18.52%. In contrast, higher application rates (500–1000 ml per liter) significantly reduced infection levels to 0–3.70%. Plants that did not receive any treatment showed 100% infection. Based on the results, pasteurization of fresh coco-water for three hours followed by harvesting the culture after seven days can be considered a practical technique for producing Liquid Trichoderma to control Fusarium wilt in Cavendish banana.
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INTRODUCTION

Globally, Fusarium wilt disease (FWD) is considered one of the most destructive diseases impacting Cavendish banana cultivation. In several locations, including Davao del Sur and surrounding provinces, severe infections have forced many farmers to abandon their banana plantations. To address this problem, some farmers temporarily plant alternative crops such as corn to help reduce the population of the pathogen in the soil. However, these practices alone have not been effective in controlling the disease. Among the different strains of the pathogen, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc-TR4) is considered the most aggressive and has caused significant yield losses in Cavendish banana production.

Modern agriculture faces the challenge of increasing crop productivity while also maintaining environmental sustainability. Because of this, there is growing interest in environmentally friendly approaches to disease management, particularly through the use of biological control agents. Microbial antagonists such as *Trichoderma* have shown strong potential in suppressing plant pathogens while also promoting plant growth. These beneficial fungi are widely used in agriculture as biofertilizers, biopesticides, and soil amendments because they produce enzymes and secondary metabolites that help inhibit harmful microorganisms.

In addition, species of *Trichoderma* are generally considered safe for humans, animals, and other beneficial organisms. They can function effectively in both natural and controlled environments without accumulating harmful residues in the food chain. Their use not only helps in controlling plant diseases but also supports plant growth and contributes to more sustainable agricultural practices.

Although *Trichoderma* is commonly produced in solid formulations, liquid culture systems offer certain advantages, particularly in the production of diffusible metabolites that can enhance plant growth and improve

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nutrient availability. However, large-scale production of stable liquid formulations remains challenging due to issues related to cost, shelf life, and consistency in disease control. Therefore, developing a simple and cost-effective method for producing liquid formulations of *Trichoderma* is important for improving the practical use of biological control technologies in agriculture.

OBJECTIVES

To determine the growth performance of *Trichoderma* on pasteurized coco water.

To determine the efficacy of Liquid *Trichoderma* (LT) against *Fusarium* Wilt Disease of Cavendish banana.

METHODOLOGY

The experiment took place at the Trichoderma Laboratory of Davao del Sur State College (DSSC) and at Cagas Banana Farm situated in Matti and Igpit, Digos City, Davao del Sur. The study was conducted from June 2020 to December 2022.

Production of Liquid *Trichoderma* (LT)

A Completely Randomized Design (CRD) with five replications was employed in this study. The treatments applied were as follows:

Treatment 1 – 0 hr pasteurization (fresh) ,inoculated with solid *Trichoderma*

Treatment 2 – 1 hr pasteurization (fresh) ,inoculated with solid *Trichoderma*

Treatment 3 –2 hrs pasteurization (fresh), inoculated with solid *Trichoderma*

Treatment 4 –3 hrs pasteurization (fresh), inoculated with solid *Trichoderma*

Treatment 5 –4 hrs pasteurization (fresh), inoculated with solid *Trichoderma*

Treatment 6 – 3 hrs pasteurization (fermented for 2 days), inoculated with solid *Trichoderma*

Treatment 7 - 3 hrs pasteurization (fresh), inoculated with liquid *Trichoderma*

Collection of the Coco-water

Fresh coco-water was taken from Digos City public market at coco grating store. The coco-water was a 100% waste in the said store, only the grated coco meat was sold to costumers. After collection, the coco-water was placed in a clean plastic container and delivered to the DSSC Trichoderma Laboratory for further use.

Dispensing of the Fresh Coco-water to Flat Bottles

Rhum flat bottles were used as the containers filled with 100 ml coco-water and plugged with cotton plugs. The flat bottles were labelled based on the treatments and were arranged in the drum for pasteurization.

Pasteurization

Pasteurization was done using drum with water at the bottom levelled in its tray. Burning woods were done to bring and maintain the water boiling. One (1) hour from boiling, flat bottles labelled treatment 2 were taken out from the drum, two (2) hours from boiling flat bottles labelled treatment 3 were taken out from the drum, three (3) hours from boiling flat bottles labelled treatment 4, 6 (filled with coco-water stocked for 2 days) and 7 were taken out from the drum, four (4) hours from boiling flat bottles labelled treatment 5 were taken out from the drum. Treatment 1 was left unpasteurized.

***Trichoderma* Inoculation/Planting**

A puncher- hole size of a five (5) day old pure culture of *Trichoderma sp*. grown in Potato Dextrose Agar (PDA) was transferred to pasteurized coco-water aseptically using sterilized transfer needle. Inoculated flat bottles were slanted to increase the surface for the *Trichoderma* to grow. Growing *Trichoderma* was incubated under room temperature.

For Treatment 7, liquid *Trichoderma* was prepared by continuous blending for five (5) minutes the five (5) day old *Trichoderma* culture grown in coco-water using sterilized blender. Ten (10) ml of the liquid *Trichoderma* was inoculated aseptically using sterilized syringe to the pasteurized coco-water.

Harvesting

Fully grown *Trichoderma* was harvest 7 days after inoculation including the liquid medium coco water and process to liquid *Trichoderma* by one (1) minute blending using portable blender.

Spore Counts

Samples were taken from LT. Serial dilution was employed and spores were counted using haemocytometer.

Data were analyzed through Analysis of Variance (ANOVA) following the Completely Randomized Design (CRD), while the Least Significant Difference (LSD) test was used to identify significant differences among treatment means.

Field Testing of LT

A Randomized Complete Block Design (RCBD) was employed in the experiment, including eleven treatments with three replications each. Seven (7) test plants were included in every replication. The treatments applied in the study were as follows:

T1 (100 ml/L),
T2 (200 ml/L),
T3 (300 ml/L),
T4 (400 ml/L),
T5 (500 ml/L),
T6 (600 ml/L),
T7 (700 ml/L),
T8 (800 ml/L),
T9 (900 ml/L),
T10 (1000 ml/L).

T11 – no application.

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) based on the Randomized Complete Block Design (RCBD), while the Least Significant Difference (LSD) test was used to identify significant differences among treatment means.

Application Method

Liquid *Trichoderma* (LT) was mixed in one liter water based on treatments using water sprinkler and drenched slowly around the base of the banana plant. Newly applied LT was covered with dried banana leaves available in the area. Application was done late in the afternoon.

RESULTS AND DISCUSSION

Table 1 shows the number of liquid medium (coco-water) with *Trichoderma* growth seven (7) days after incubation. All the five (5) Liquid media per treatment pasteurized for 2 hrs (T3), 3 hrs (T4), 4 hrs (T5) and 3 hrs (inoculated with LT) had *Trichoderma* growth after seven (7) days incubation under room temperature. Only one (1) among the five (5) liquid media pasteurized for 1 hr (T2) had growth. For T1 (unpasteurized liquid media), the *Trichoderma* was unable to grow. Same in T6 (3 hrs pasteurization of coco-water fermented for 2 days) with zero (0) *Trichoderma* growth.

Table 1. Number of liquid media (coco-water) with *Trichoderma* growth seven (7) days after incubation.

TREATMENT	MEAN**
T1 - 0 hr	0.00 [b]
T 2 – 1 hr	0.20 [b]
T 3 – 2 hrs	1.00 [a]
T 4 - 3 hrs	1.00 [a]
T 5 – 4 hrs	1.00 [a]
T 6 – 3 hrs (fermented)	0.00 [b]
T7 – 3 hrs (liquid)	1.00 [a]

CV= 28.17%; ** = *highly significant*

Treatment means with identical superscript letters indicate no significant difference at the 5% level based on the HSD test.

Table 2 shows the percent of liquid medium (coco- water) occupied by *Trichoderma* growth seven (7) days after incubation. The liquid media (coco-water) pasteurized for 3 hrs (T4) and 4 hrs (T5) had a 100% growth. Only 70% growth had been observed in T3 (2 hr pasteurization) but comparable to T4 and T5. Liquid media pasteurized for 3 hrs but inoculated with LT (T7) had only 25% growth. The T1 (unpasteurized) and T6 (3 hrs pasteurization but coco-water used was left fermented for 2 days), the *Trichoderma* unable to grow.

Table 2. Percent of liquid medium (coco- water) occupied by *Trichoderma* growth seven (7) days after incubation.

TREATMENT	MEAN**
T1 - 0 hr	0 [d]
T 2 – 1 hr	10 [bc]
T 3 – 2 hrs	70 [ab]
T 4 - 3 hrs	100 [a]
T 5 – 4 hrs	100 [a]
T 6 – 3 hrs (fermented)	0 [d]
T7 – 3 hrs (liquid)	25 [cd]

CV= 43.72% ; ** = *highly significant*

Treatment means with identical superscript letters indicate no significant difference at the 5% level according to the HSD test.

Table 3 shows the percent infection of *Fusarium* Wilt Disease (FWD) on Cavendish banana plants applied with different levels of LT. The lowest infection percentage (14.81%) was observed in Treatment 3 (300 ml per liter of water). In comparison, Treatments 1 (100 ml/L), 2 (200 ml/L), and 4 (400 ml/L) showed a higher infection level of 18.52%. However the effects of treatments 1, 2, 3 and 4 are comparable. On the other hand, treatments 6 (600 ml/liter water), 7 (700 ml/liter water) and 8 (800 ml/liter water) had 3.70 percent infection, respectively. While treatment 5 (500 ml/liter water), 6 (600 ml/liter water) and 10 (1000 ml/liter water) had 0 infections, respectively. The effects, however of treatments 6, 7, 8, 9 and 10 are comparable. Treatment 11 (no application of LT) had a 100 percent application. According to Wong et al. (2021), the use of bio-formulations with *Trichoderma harzianum* can activate the plant's defense system by elevating phenolic and proline contents, which helps minimize root damage caused by Foc-TR4 and reduces malondialdehyde (MDA) levels in infected plants. In addition, Khan et al. (2017) confirmed the potential of *Trichoderma harzianum* as a biological control agent against the banana wilt pathogen.

Table 3. Percent infection of *Fusarium* Wilt Disease (FWD) on Cavendish banana plants applied with different levels of LT.

TREATMENT		MEAN*
T1	100 ml/liter water	18.52[b]
T2	200 ml/liter water	18.52[b]
T3	300 ml/liter water	14.81[b]
T4	400 ml/liter water	18.52[b]
T5	500 ml/liter water	0.00[c]
T6	600 ml/liter water	0.00[c]
T7	700 ml/liter water	3.70[c]
T8	800 ml/liter water	3.70[c]
T9	900 ml/liter water	3.70[c]
T10	1000 ml/liter water	0.00[c]
T11	no application	100.00[a]

CV = 31.02 % ; *- *significant*

Means having similar letter superscripts are not significantly different at 5% LSD test.

RECOMMENDATIONS

Based on the results, it is recommended to grow Trichoderma in pasteurized fresh coco-water (PFCW) for three (3) hours. The *Trichoderma* grown on PFCW can be harvested and processed as Liquid *Trichoderma* (LT) 5-7 days after inoculation with 5.8 million spores per liter. To manage *Fusarium* wilt disease of cavendish banana, it is recommended to drench 500 ml LT mixed in liter water per banana plant slowly and cover with dried banana leaves available in the area.

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