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# Tea tree oil induces activation of defense mechanisms against *Fusarium* wilt infection in cavendish banana – Screenhouse and field set-up

**Merahflor L. Cavite[1]; Dennice G. Catambacan[1]; Benny M. Corcolon[1]; Euberto S. Catayas[1]; Alexis P. Cantil[2]**

[1]Plant Pathology, Postharvest and Tissue Culture R&D Department, Research, Information and Compliance Division (RICD); [2]Agriculture Operations. Tagum Agricultural Development Company, Inc. (TADECO), Brgy. A. O. Floirendo, Panabo City, Davao del Norte, Philippines, 8105.

***Autor de Correspondencia:***
Dennice G. Catambacan
dgcatambacan@anflocor.com

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## ABSTRACT

Evaluation of tea tree oil (TTO) was conducted to determine its efficacy on the enzymatic activity in Cavendish banana when challenged by *Foc*TR4. At screenhouse, there was a significant increased accumulation of pathogenesis-related proteins in plants with sustained application of TTO at 6ml/L. This resulted to induction of resistance manifested by the lowest degree of rhizome discoloration, pseudostem splitting and stunting at 10 weeks after TR4 inoculation. Similar observations were noted in the field wherein there was an increase of enzymatic activity in TTO-treated plants compared to untreated samples. This increase was demonstrated by a significant lower incidence of the disease both in GN and FW-tolerant selection.

**Palabras clave:** tea tree oil, pathogenesis-related proteins, Fusarium Wilt, Cavendish banana, Fitosanidad.

## RESUMEN

Se llevó a cabo una evaluación del aceite del árbol del té (TTO) para determinar su eficacia sobre la actividad enzimática en el plátano Cavendish cuando fue desafiado por *FocTR4*. En el invernadero, hubo un aumento significativo de la acumulación de proteínas relacionadas con la patogénesis en las plantas con la aplicación sostenida de TTO a 6 mL/L. Esto dio lugar a la inducción de la resistencia manifestada por el menor grado de decoloración del rizoma, división del pseudotallo y retraso del crecimiento a las 10 semanas después de la inoculación con TR4. Observaciones similares se observaron en el campo, donde hubo un aumento de la actividad enzimática en las plantas tratadas con TTO en comparación con las muestras no tratadas. Este aumento se demostró por una incidencia significativamente menor de la enfermedad tanto en la selección GN como en la tolerante a FW.

**Keywords:** aceite del árbol del té, proteínas relacionadas con la patogénesis, marchitez por *Fusarium*, plátano Cavendish, phytosanity.

## INTRODUCTION

Induced resistance is a state of enhanced defensive capacity where plant defenses are preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite (Choudhary *et al.*, 2007). The resistance expressed by the plant is associated with increased activity of pathogenesis-related proteins (Hammerschmidt and Smith-Becker, 1999).

*Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc*TR4), causal agent of *Fusarium* Wilt disease, is one of the most destructive strains attacking Cavendish and other cultivars (Cheng *et al*., 2019). On the other hand, tea tree oil (TTO) from plant *Melaleuca alternifolia* which contains mostly terpenes and their alcohols are known for their roles in plant defense against biotic and abiotic stresses (Dalio *et al.*, 2020).

This study was undertaken (1) to determine the effect of TTO on the enzymatic activity in Cavendish banana and relate to disease severity when challenged by *Foc*TR4 at screenhouse set-up; (2) to determine the effectiveness of TTO when applied to Cavendish banana for the control of *Fusarium* wilt in the field.

## MATERIALS AND METHODS

The screenhouse test was carried out from August 2022 to January 2023. Six-week-old FW-susceptible cultivar Gran Naine plantlets were arranged using Randomized Complete Block Design with 3 replications per treatment at 21 samples per replicate (Table 1). Test plants in Treatments 1 and 2 were applied with water only while Treatments 3 to 8 were drenched with respective rates of TTO at 30 days before inoculation (DBI) followed by foliar spraying at 15 DBI and 7 DBI, respectively. Treatments 7 and 8 were applied through foliar spraying at 7 days after inoculation (DAI).

Fifty mL of *Foc*TR4 solution at $1x10^6$ spores/mL was drenched to sterilized potted soil of test plant. Regular watering was employed. Assessment for any phytotoxicity symptoms was done every week before inoculation. Six plants were processed for destructive sampling at 15 DBI and 30 DAI for enzymatic evaluation. Guaiacol peroxidase was determined in a mixture consisting of the reaction buffer (10 mM sodium phosphate buffer at pH 7.5, 2.3 mM guaiacol and 2.9 mM $H_2O_2$) and leaf extract, monitored through spectrophotometer at 470 nm. Moreover, β -1,3 glucanase

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assay was done by incubating the leaf extract with laminarin dissolved in sodium phosphate buffer and adding ρ-hydroxybenzoic acid hydrazide (PAHBAH).

**Table 1.** Different rates and timing of application of Tea tree oil at screenhouse set-up.

| **Trt code** | **Treatment application (44 mL solution per plant per application)** | **TTO application at x days before inoculation (DBI)** | | | **TR4 Inoculation at day 0** | **TTO application at 7 days after inoculation (DAI)** |
|---|---|---|---|---|---|---|
| | | **30** | **15** | **7** | | |
| T1 | Healthy Control (– *Foc* TR4/ – tea tree oil) | / | / | / | No | [redacted] |
| T2 | Untreated Control (+ *Foc* TR4/ – tea tree oil) | / | / | / | Yes | [redacted] |
| T3 | Tea tree oil at 3 ml/L (uninoculated) | / | / | / | No | [redacted] |
| T4 | Tea tree oil at 6 ml/L (uninoculated) | / | / | / | No | [redacted] |
| T5 | Tea tree oil at 3 ml/L (before TR4 inoculation) | / | / | / | Yes | [redacted] |
| T6 | Tea tree oil at 6 ml/L (before TR4 inoculation) | / | / | / | Yes | [redacted] |
| T7 | Tea tree oil at 3 ml/L (before and after TR4 inoculation | / | / | / | Yes | / |
| T8 | Tea tree oil at 6 ml/L (before and after TR4 inoculation) | / | / | / | Yes | / |

Glucose concentration was determined using a spectrophotometer at 410 nm. On the other hand, disease severity was evaluated at 10 weeks after inoculation (WAI) with 15 samples per replication. The degree of rhizome discoloration was assessed using modified severity rating below (Table 2). All gathered data were analyzed using two-way Analysis of Variance (ANOVA) following two–factorial Randomized Complete Block Design (RCBD), and the difference among treatments were compared using Tukey's Honest Significant Difference (HSD). The software Statistica Version 10 was used.

**Table 2.** Modified severity rating scale for rhizome discoloration during disease severity assessment.

| **Rating Scale** | **Rhizome discoloration** (Carlier *et al.*, 2003) |
|---|---|
| 0 | Corm completely clean |
| 1 | Isolated points of discoloration in vascular tissue |
| 2 | Discoloration up to 1/3 of vascular tissue |
| 3 | Discoloration between 1/3 and 2/3 of the vascular tissue |
| 4 | Discoloration greater than 2/3 of vascular tissue |
| 5 | Total discoloration of vascular tissue |

Field evaluation was done in a commercial area previously infected with *Fusarium* wilt. Standard procedure of the plantation on land preparation, planting and fertilization was followed. Foliar application of TTO at 6 mL/L was performed to Gran Naine and FW-tolerant selection at 1, 2, and 3 months after planting (MAP) using a calibrated battery-operated knapsack sprayer. Enzymatic activities of guaiacol peroxidase, β -1,3 glucanase and phenol content on the plant was evaluated at 2 MAP and 3 MAP at fifteen randomly sampled plants per treatment. Incubation period and disease incidence were monitored weekly.

## RESULTS AND DISCUSSION

### At screenhouse set-up

**Enzymatic activities of PR proteins.** Samples applied with TTO at 6 mL/L (before and after TR4 inoculation) consistently obtained the highest activity of guaiacol peroxidase among *Foc* TR4-inoculated treatments at 15 DBI (after 2 rounds of TTO application) and 30 DAI. In the absence of the pathogen, highest activity was noted in samples applied with TTO at 6 mL/L at 15 DBI while no significant difference was noted between untreated and treated samples at 30 DAI (Figure 1A).

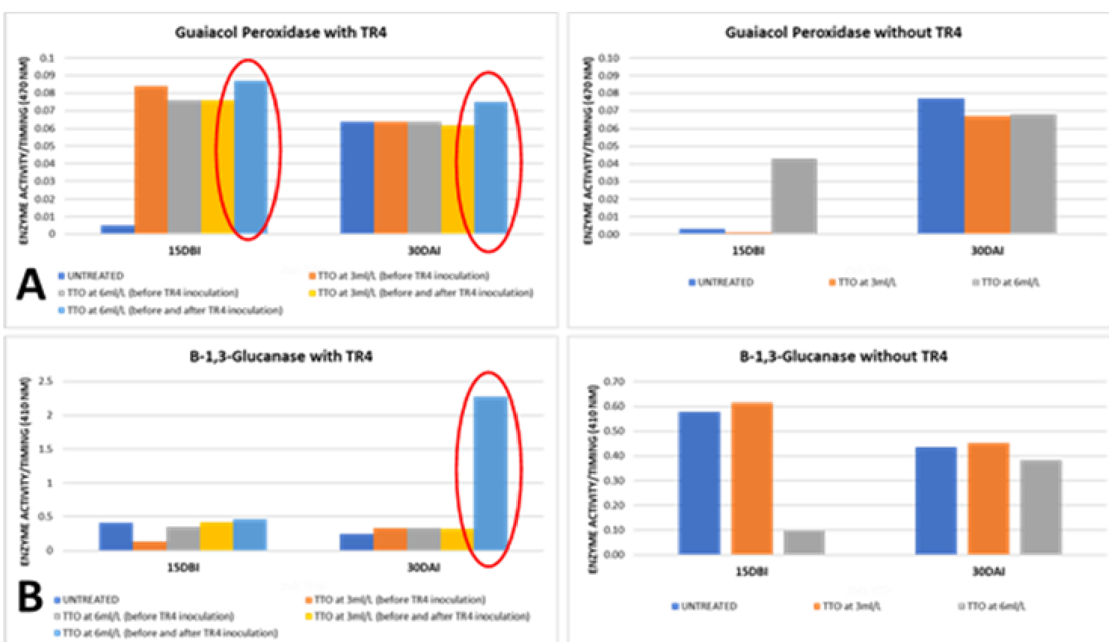


**Figure 1.** Enzymatic activity of guaiacol peroxidase (A), and β-1,3-glucanase (B) of untreated and TTO -treated plants with (left) and without (right) TR4 at 15 DBI (days before inoculation) and 30 DAI (days after inoculation).

Guaiacol peroxidase are widely accepted as “stress” enzyme wherein stressful conditions have been shown to induce its activity (Erofeeva, 2015). This explains the observed activity of the enzyme to untreated samples when exposed to TR4. However, significant induction of its activity to treated plants was noted when the product was applied prior to infection and additional application during post-infection.

With β -1,3 glucanase, comparable results were noted between untreated and treated samples at 15 DBI and 30 DAI except for plants applied with TTO at 6ml/L (before and after TR4 inoculation) where significant increase of activity of the enzyme was observed at 30 DAI in the presence of TR4. Without the pathogen, no significant effect was noted on the application of TTO as comparable results with untreated samples was monitored (Figure 1B). Accordingly, plant β-1,3-glucanases accumulate in the event of a pathogen attack, and some of them have been directly involved in the hydrolysis of pathogen walls, as β-1,3-glucans are found in bacteria, metazoa, viruses, and particularly fungi. In fungi, β-1,3-glucans constitute the most abundant wall structural component existing in the form of linear or branched glucans containing mostly β-1,3 and β-1,6 linkages (Ruiz-Herrera and Ortiz-Castellanos, 2019).

**Disease Severity.** TR4-inoculated treatments had 100% incidence of vascular internal discoloration expressed by *Fusarium* wilt. This shows the susceptibility of Gran Naine to the disease. Nevertheless, the degree of rhizome discoloration was numerically lowest in TTO at 6ml/L (before and after TR4 inoculation) with 22.66% at 10 weeks after *Foc* TR4 inoculation (Table 3 and Figure 2).

**Table 3.** Disease incidence (%) and rhizome discoloration (%) of Cavendish banana applied with different rates and timing of application of TTO at 10 weeks after *Foc* TR4 inoculation.

| TREATMENT | FW INCIDENCE (%) | RHIZOME DISCOLORATION (%) |
|---|---|---|
| T1 - Healthy Control (– *Foc* TR4/ – TTO) | 0.00 | 0.00 $^{b}$ |
| T2 - Untreated Control (+ *Foc* TR4/ – TTO) | 100.00 | 29.78 $^{a}$ |
| T3 - TTO at 3ml/L (uninoculated) | 0.00 | 0.00 $^{b}$ |
| T4 - TTO at 6ml/L (uninoculated) | 0.00 | 0.00 $^{b}$ |
| T5 - TTO at 3ml/L (before TR4 inoculation) | 100.00 | 28.39 $^{a}$ |
| T6 - TTO at 6ml/L (before TR4 inoculation) | 100.00 | 30.22 $^{a}$ |
| T7 - TTO at 3ml/L (before and after TR4 inoculation) | 100.00 | 27.55 $^{a}$ |
| T8 - TTO at 6ml/L (before and after TR4 inoculation) | 100.00 | 22.66 $^{a}$ |
| *P* - value | - | 0.00 |
| CV | - | 25.04 |

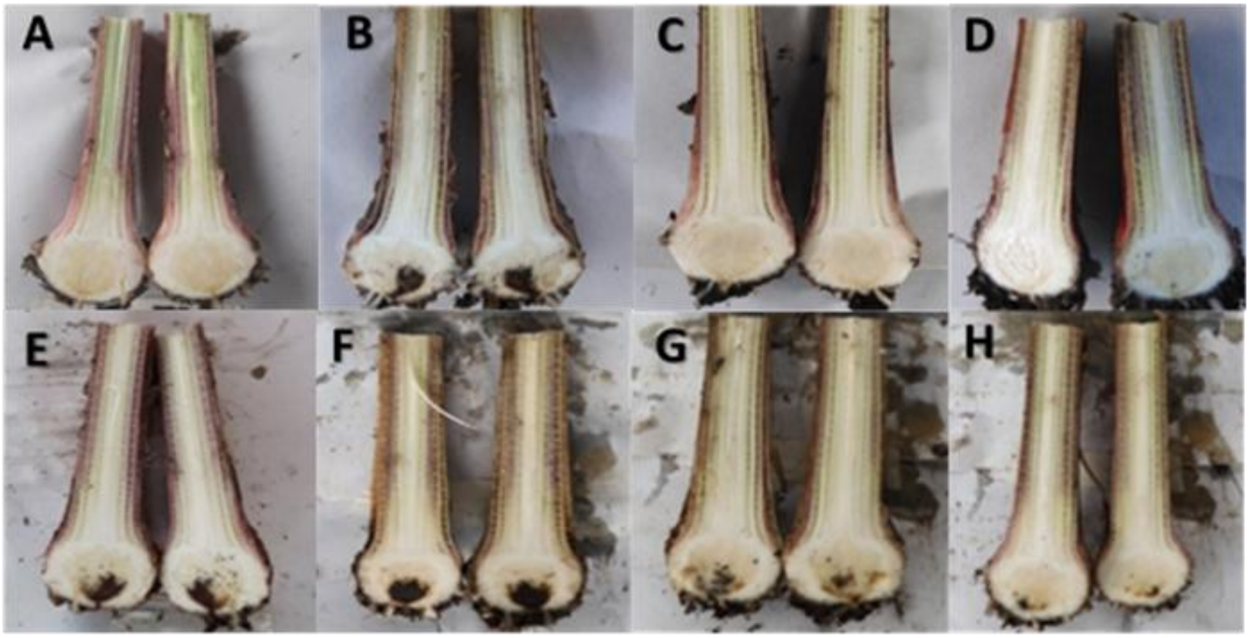


**Figure 2.** Effect of TTO on the degree of rhizome discoloration caused by *Foc* TR4 on Gran Naine at 10 WAI. A) Healthy Control (– *Foc* TR4/ – TTO), B) Untreated Control (+ *Foc* TR4/ – TTO), C) TTO at 3mL/L (uninoculated), D) TTO at 6mL/L (uninoculated), E) TTO at 3mL/L (before TR4 inoculation), F) TTO at 6mL/L (before TR4 inoculation), G) TTO at 3mL/L (before and after TR4 inoculation), and H) TTO at 6mL/L (before and after TR4 inoculation). Pictures photographed by the authors, 2023.

In terms of external symptoms, samples treated with TTO at 6ml/L (before and after TR4 inoculation) showed significantly lowest mean of 8.88% and 4.44% for pseudostem splitting and stunting, respectively (Table 4).

**Table 4.** Percentage of pseudostem splitting and stunting or choking on Cavendish banana applied with different rates and timing of application of TTO at 10 weeks after *Foc* TR4 inoculation.

| Treatment | Pseudostem splitting (%) | Stunting or choking (%) |
|---|---|---|
| T1 - Healthy Control (– *Foc* TR4/ – TTO) | 0.00 c | 2.22 c |
| T2 - Untreated Control (+ *Foc* TR4/ – TTO) | 22.22 ab | 37.78 a |
| T3 - TTO at 3ml/L (uninoculated) | 0.00 c | 0.00 c |
| T4 - TTO at 6ml/L (uninoculated) | 0.00 c | 0.00 c |
| T5 - TTO at 3ml/L (before TR4 inoculation) | 26.67 a | 31.11 ab |
| T6 - TTO at 6ml/L (before TR4 inoculation) | 20.11 ab | 24.17 ab |
| T7 - TTO at 3ml/L (before and after TR4 inoculation) | 11.11 bc | 13.33 bc |
| T8 - TTO at 6ml/L (before and after TR4 inoculation) | 8.88 bc | 4.44 c |
| *P* - value | 0.00 | 0.00 |
| CV | 43.48 | 38.99 |

### At Field Set-up

**Enzymatic activities of PR proteins.** Results showed that enzymatic activities specifically for β -1,3 glucanase and phenols in TTO-treated plants were higher compared to untreated plants at 2 MAP and 3 MAP. Further, no significant difference was noted in guaiacol peroxidase between untreated and TTO-treated plants. In general, there was an increase of enzymatic activities of PR proteins at 3 MAP (Figure 3).

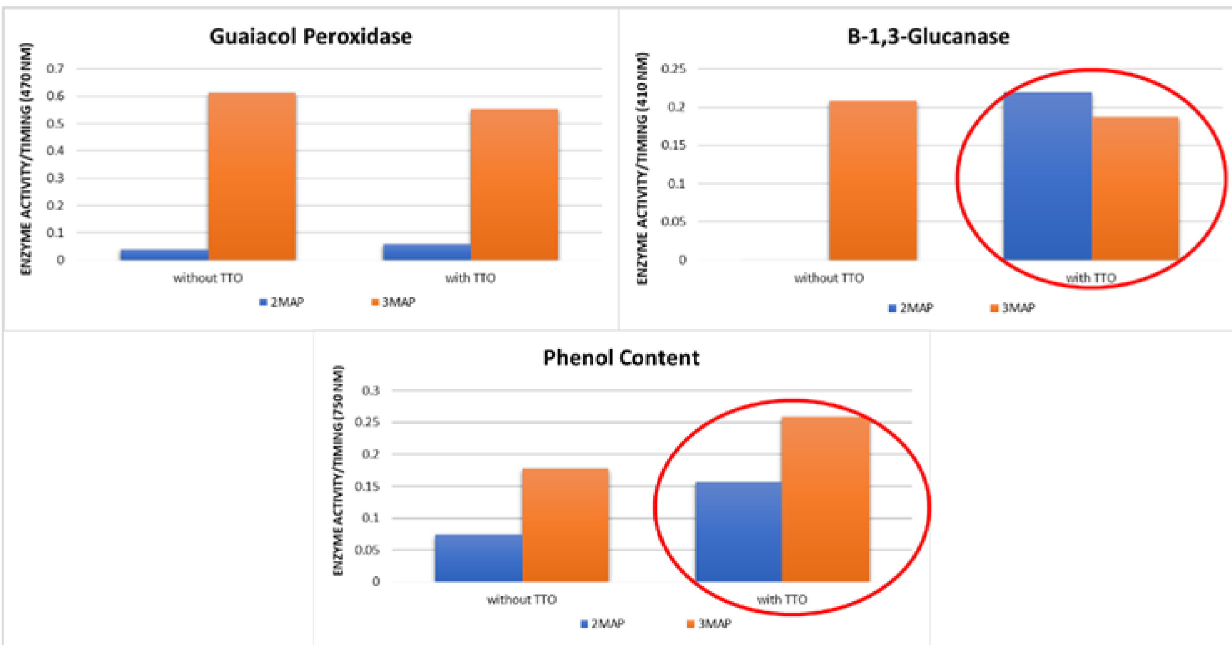


**Figure 3.** Enzymatic activity of guaiacol peroxidase (A), β-1,3-glucanase (B), and phenols (C) of untreated and TTO -treated plants at 2 months after planting (MAP) and 3 MAP.

**Disease Incidence and Incubation.** From July 2022 to September 2023, susceptibility of Gran Naine to *Fusarium* Wilt was evident with higher number of cases than FW-tolerant selection. Comparing between TTO-applied and untreated, lower FW cases was noted in TTO-treated plants with 1.93% in Gran Naine and 0.12% in FW-tolerant selection. In terms of incubation period, earliest manifestation of the disease was earlier in Gran Naine at 13 weeks after planting while longer incubation was noted in FW-tolerant selection at 20 weeks (Table 5). Further, FW incidence peaked at five months after planting or two months after the last TTO application particularly for susceptible cultivar as shown in Figure 4.

**Table 5.** Disease incidence (%) and incubation of *Fusarium* wilt in TTO-applied Gran Naine and FW-tolerant selection in the field (July 2022 to September 2023).

| Treatment | No. of FW cases (Disease Incidence, %) | | Earliest Incubation Period (Week) | |
|---|---|---|---|---|
| | Gran Naine | FW-tolerant selection | Gran Naine | FW-tolerant selection |
| Without TTO (untreated) | 33/828 (3.98%) | 7/810 (0.86%) | 13 | 14 |
| With TTO at 6 mL/L (1, 2, 3 months after planting) | 16/828 (1.93%) | 1/810 (0.12%) | 13 | 20 |

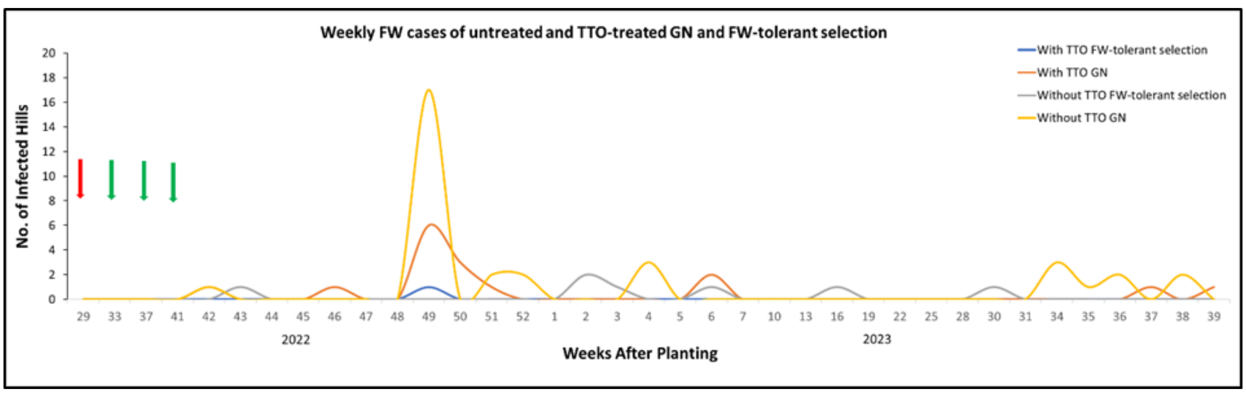


**Figure 4.** Weekly monitoring on Fusarium Wilt incidence from planting (red arrow) and TTO application at 1, 2, and 3 months after planting (green arrows) of untreated and TTO-treated Gran Naine and FW-tolerant selection.

Cost-Benefit Analysis. Based on % FW infection, equivalent number of FW cases per hectare, number of boxes lost due to FW as well as the corresponding value of boxes were computed. With TTO application, about 4,116 US $ and 1,442 US $ per hectare will be saved for Gran Naine and FW-tolerant selection, respectively. These savings are far higher when compared to the cost of TTO product and labor at 249 US $ per hectare only (Table 6 and 7).

**Table 6.** Cost-benefit analysis of TTO application against FW incidence in Gran Naine cultivar.

| Treatment | Gran Naine | | | | |
|---|---|---|---|---|---|
| | FW Infection (%) | Equivalent No. of FW cases/ha | No. of boxes lost due to FW infection/ha | Value of boxes lost due to FW (US $) | Cost of TTO application/ha + labor (US $) |
| Without TTO | 3.98 | 78 | 1147 | 8,029.00 | 249 |
| With TTO | 1.93 | 38 | 559 | 3,913.00 | |
| *Difference* | *2.05* | *40* | *588* | *4,116.00* | |

**Table 7.** Cost-benefit analysis of TTO application against FW incidence in FW-tolerant selection.

| Treatment | FW-tolerant selection | | | | |
|---|---|---|---|---|---|
| | FW Infection (%) | Equivalent No. of FW cases/ha | No. of boxes lost due to FW infection/ha | Value of boxes lost due to FW (US $) | Cost of TTO application/ha + labor (US $) |
| Without TTO | 0.86 | 17 | 250 | 1,750.00 | 249 |
| With TTO | 0.12 | 3 | 44 | 308.00 | |
| *Difference* | *0.73* | *14* | *206* | *1,442.00* | |

## CONCLUSIONES

At screenhouse set-up, there was a significant increased accumulation of pathogenesis-related proteins (guaiacol peroxidase and β-1,3 glucanase) specifically in plants with sustained application of TTO at 6mL/L. The increased accumulation of PR proteins resulted to induction of systemic resistance which was manifested primarily by the lowest degree of rhizome discoloration among treatments at 10 weeks after TR4 inoculation. Moreover, TTO at 6ml/L (before and after TR4 inoculation) obtained significantly lowest incidence in terms of external symptoms of the disease (pseudostem splitting and choking or stunting).

In the field, similar observations were noted where there was an increase of enzymatic activity of β-1,3 glucanase and phenols in TTO-treated plants compared to untreated samples at 2 MAP and 3 MAP. This increase was demonstrated by a significant lower incidence of the disease both in GN and FW-tolerant selection.

With this study, tea tree oil increases the activity of pathogenesis- related proteins and induces systemic resistance of Cavendish banana in response to *Foc*TR4 infection. Application of tea tree oil to newly-planted Cavendish banana could be a best strategy in managing *Fusarium* wilt in the field.

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