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Vermicompost Tea with Effective Microorganisms for the Control of Fusarium Wilt of 'Cavendish' Banana caused by *Fusarium oxysporum* f. sp. *cubense* TR4

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Abstract **– Fusarium wilt is increasingly destructive for banana production in the Philippines and abroad. It is caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* TR4 (*Foc* TR4) and alternative management of the disease was the use of microbial agents. This study was conducted to evaluate the effects of different rates of vermicompost tea (VT) with effective microorganisms (EM) on the capacity to control the Fusarium wilt. Vermicompost tea and effective microorganisms were prepared using the standard procedure. After 28 days of application of treatments, inoculation of *Foc* TR4 was done. Different rates of VT (62.50ml/L, 31.25ml/L, 46.88ml/L and 125ml/L) with EM (40ml/L) were tested. The results confirmed that VT at 62.50ml/L with EM (40ml/L) reduced Fusarium wilt incidence (55%), disease severity (Moderately Effective) and delayed the symptom appearance (11.75 days).**

Keywords **– Vermicompost Tea, Effective Microorganisms, *Fusarium oxysporum* f.sp. *cubense* TR4, Cavendish Banana.**

I. INTRODUCTION

Banana (*Musa* sp.) is the fourth most valuable food after rice, wheat, and milk. It is a staple food that represents major dietary sources of carbohydrates, fiber, vitamins A, B6, C, potassium, phosphorus, and calcium [11]. More--over, the domestic and foreign markets are still promising to see that demands for fresh and processed products are increasing. In fact, Philippine Statistics Authority (PSA) recorded a 2.8 percent increase in the production of banana in 2014 as compared to the data which 2013 showed that production increased to 8.88 million metric tons (MT) from January to December 2014 [12].

In the Philippines, the Davao region has been known as the "Banana Capital" for producing 3.36 million metric tons in 2014. Also, Davao Region's Banana Industry Cluster contributes about 75% of the total exports of the country. It comprises the 'Cavendish' for fresh exports, 'Cardaba'/'Saba' for banana chips for export and 'lakatan' for local fresh fruit market [15].

Bananas are attacked by many diseases that affect productivity and sustainable production. Among all of the diseases attacking banana, Fusarium wilt caused by the fungus *Fusarium oxysporum* f.sp *cubense* (*Foc*) is the most devastating disease which affects commercial and subsistence banana production throughout the banana-producing areas of the world [13]. In 2010, Dr. Molina of Biodiversity reported severe infections in 'Cavendish' plantations in China and the Philippines [10].

There are four known races of *Foc*, namely: race 1, race 2, race 3, and race 4. These are identified based on the reaction to specific hosts [14]. In the Philippines, the occurrence of the disease is caused by races 1, 2 and recently, by Tropical Race 4 (TR4). Moreover, *Foc* TR4 damaged 15,507 hectares in Region XI alone, particularly in Davao del Norte and Compostela Valley [4].

Management of *Fusarium* wilt of banana includes the integration of different strategies since no single method is fully effective on its own. Furthermore, many microbial agents and organic fertilizers had recently received much attention since many products were out in the market claiming for sustainable management of the disease. One of these is the use of vermicompost tea and effective microorganisms which were used in Municipality of Asuncion, Davao del Norte (Philippines) where some farmers reported that these products were able to manage the disease. Hence, there is a need to evaluate the effect of selected products containing effective microorganisms and vermicompost tea for the control of Fusarium wilt of 'Cavendish' banana under nursery condition.

II. MATERIALS AND METHODS

A. *Location and Duration of the Study*

The nursery experiment was conducted at the Panama Research Project Area, University of Southeastern Philippines (USeP), Tagum-Mabini Campus, Pindasan, Mabini, Compostela Valley Province from February 2016 to May 2017.

B. *Experimental Design and Treatments*

The experiment was laid out in Completely Randomized Design (CRD) with six treatments and four replications having ten samples per replication. A total of 240 plants were used for the *in vivo* test against *Fusarium oxysporum* f. sp. *cubense* TR4.

The treatments were as follows:

T1 – Negative Control (Untreated and uninoculated)
T2 – Positive control (Inoculated and untreated)
T3 – Vermicompost Tea (66.67ml RR per 1000ml of water) + EM (40ml/L)
T4 – Vermicompost Tea (33.34ml per 1000ml of water) + EM (40ml/L)
T5 – Vermicompost Tea (50.00ml per 1000ml of water) + EM (40ml/L)
T6 – Vermicompost Tea (133.34ml per 1000ml of water) + EM (40ml/L)

C. *Source of Foc* TR4

A pure culture of *Foc* TR4 was acquired from USeP PCR Laboratory, Tagum City and was sub-cultured on half strength Potato Dextrose Agar (PDA).

D. *Preparation and Sterilization of Culture Media*

Half strength Potato dextrose agar (PDA) medium was prepared following the standard procedure using 10g dextrose, 20g agar, 100g potatoes (unpeeled and diced) and 1000ml distilled water [1]. The potatoes were washed and cooked for 15-20 minutes or until soft in 500ml distilled water. It was poured on a container and set aside. The dextrose-agar mixture was cooked in 500ml distilled water. The potato and dextrose-agar mixture were mixed with distilled water to make a total volume of one liter and sterilized in a pressure cooker for 30 minutes at 15 psi.

E. *Pathogenicity Test*

The *Foc* TR4 acquired from USeP PCR laboratory was subjected to a rapid test of pathogenicity using tissue-cultured 'Grand Nain' 'Cavendish' banana in a test tube to ensure that the pathogen is virulent before the study (Fig 1).

Fig. 1. Pathogenicity test of *Foc* TR4 using tissue-cultured 'Grand Nain' 'Cavendish' banana in a test tube. (A)*Foc* TR4 inoculated tissue-cultured 'Grand Nain'; (B) Uninoculated tissue-cultured 'Grand Nain'.

F. *Preparation of Potting Medium*

The potting medium was prepared using a sandy-loam soil and coco coir dust. This was heat sterilized at a temperature of 82°C for about 30 minutes to ensure that it is free from other disease-causing microorganisms before inoculation [2]. The sterilized soil was allowed to cool before putting it in the prepared plastic pot (18cm diameter). Five kilograms of the sterilized potting medium was placed in every pot.

G. *Preparation of Test Plants*

Two-month-old tissue-cultured 'Cavendish' banana plantlets var. 'Grand Nain' were used in the study. It was transferred from the original bag to the plastic pot (18cm diameter) filled with five kilograms of sterilized potting medium [6]. The plantlets were properly cared to avoid excessive root damage during transplanting.

H. *Inoculation of Foc* TR4

Corn grits and sand medium was prepared and inoculated with two weeks old inoculum of *Foc* TR4 [8]. One hundred fifty grams per pot of soil medium was applied near the root zone of 'Grand Nain' Cavendish banana potted plantlets. Before inoculation, the root hairs of each seedling were teased to facilitate entry and subsequent infection of *Foc* TR4.

I. *Treatment Application*

Vermicompost tea and Effective Microorganisms (EM) were applied 28 days before *Foc* TR4 inoculation and follow up application every seven days after *Foc* TR4 inoculation.

J. *Care and Maintenance*

Management practices such as fertilization, watering, weed control, leaf pruning, pseudostem sanitation, and other practices were done in the experiment.

K. *Biosafety Protocol*

The potting medium and infected banana plantlets used in the experiment was decontaminated using drum at 82ºC for 30 minutes in the Panama Facility in University of Southeastern Philippines-Mabini Unit.

J. *Data Gathered*

Incubation Period was recorded as soon as the earliest symptoms of Fusarium wilt appeared. Splitting of the pseudostem was monitored daily as well as the appearance of yellow streaks and wilting symptoms. Also, disease incidence was monitored daily and recorded until termination of the study and computed using the formula:

$$\text{Percent Disease Incidence} = \frac{\text{Number of Infected Plants}}{\text{Total Number of Plants Assessed}} \times 100$$

Moreover, the severity of infection was recorded at the termination of the study using a rating scale for the leaf yellowing and rhizome discoloration index was based on Mohammed and co-workers (1999) with some modifica--tions [9].

Table 1. Rating scale for severity of leaf yellowing.

Scale	Description
1	No yellow discoloration of leaves
2	Yellowing of older leaves
3	Yellowing of all older leaves and slight discoloration of younger leaves
4	All leaves turn yellow
1	No yellow discoloration of leaves

Table 2. Rating scale for rhizome discoloration.

Scale	Description
1	No vascular discoloration
2	Isolated points of discoloration in vascular tissue
3	Discoloration up to between 1/3 of vascular tissue
4	Discoloration of between 1/3 and 2/3 of vascular tissue
5	Discoloration greater than 2/3 of vascular tissue
6	Total discoloration of vascular tissue

Based on the rating of the severity of leaf yellowing and rhizome discoloration manifested by *Foc* TR4, the disease index for each of the symptoms was computed using the formula below:

$$\text{Disease Severity Index (DSI)} = \frac{\Sigma(\text{Number on scale x Number of seedlings in that scale})}{\Sigma\ (\text{Number of treated seedlings})} \times 100$$

The degree of control was computed using the formula below:

Percent degree of control =
Percent DI of Untreated Plants – Percent DI Treated Plants x 100
Percent DI Untreated Plants

Where,

DI = Disease Incidence

The degree of effectiveness of the treatments was based on the computed percent degree of control (% DC) using the following arbitrary scale (Table 3).

Table 3. Scale for the degree of control and effectiveness.

Percent Degree of Control	Degree of Effectiveness
1-20%	Not Effective
21-40%	Less Effective
41-60%	Moderately Effective
61-80%	Effective
81-100%	Very Effective

III. Results and Discussion

A. *Incubation Period*

After *Foc* TR4 inoculation on 'Cavendish' banana plantlets, days to the first appearance of yellow streaks on leaves, pseudostem splitting, yellowing and wilting were recorded.

Different rates of VT with EM significantly prolonged the incubation period of Fusarium wilt on 'Cavendish' banana plantlets ranging from 9.66 days to 13.45 days compared to the positive control, which recorded the earliest symptoms of 7.70 days (Table 4). Based on the trend, as the rate of VT with EM increases, the numbers of days to symptom appearance were delayed. Plantlets treated with VT (125 ml/L) + EM (40 ml/L) symptoms appeared at 13.45 days in the average, followed by VT (62.50 ml/L) + EM (40 ml/L) and VT (46.88 ml/L) + EM (40 ml/L) then VT (31.25 ml/L) + EM (40 ml/L) with the mean of 11.75, 10.82, and 9.66 days, respectively. As expected, negative control (uninoculated and untreated) did not show any symptoms of Fusarium wilt.

Table 4. The incubation period (days) of Fusarium wilt on 'Cavendish' banana as affected by different rates of Vermicompost Tea (VT) with Effective Microorganisms[1]

Treatments (Per liter water)	Days After Inoculation				Mean**
	Rep 1	Rep 2	Rep 3	Rep 4	
Negative Control	0.00	0.00	0.00	0.00	0.00^{d}
Positive Control	8.70	6.80	10.00	5.40	7.70^{c}
VT (62.50ml) + EM (40ml)	13.00	11.00	9.00	14.00	11.75^{ab}
VT (31.25ml) + EM 40ml)	9.50	8.00	10.14	11.00	9.66^{bc}
VT (46.88ml) + EM (40ml)	11.28	9.28	12.12	10.57	10.82^{abc}
VT (125ml) + EM (40ml)	14.60	14.37	11.43	13.40	13.45^{a}
CV = 17.27%					

[1]Means with the same letter superscripts are not significantly different at 5% level by Tukey's HSD. Data are means of four replicate at ten plantlets per replicate.
**Significant at 1% level

According to Edwards *et al*., (2006) possible mechani-sm for disease control of VT was on competition for nutrient and energy of beneficial microorganisms against the pathogen [5]. Moreover, they reported that disease suppression could also be indirectly associated with host-mediated induced resistance which may be due to the presence of beneficial microorganisms and nutrients. Also, since VT was made in liquid form, thus it can easily be absorbed by the plants resulting to increase in the level of plants' immunity.

Likewise, competition for pathogen infection sites of beneficial microorganisms helps delayed the appearance of Fusarium wilt [5]. It should be noted that four treatment applications were given ahead before to *Foc* TR4 inoculation allowing a head start against the pathogen.

As a result revealed, plantlets which were treated with VT with EM displayed a delayed symptoms appearance of Fusarium wilt compared to positive control.

B. *Rhizome and Leaf Severity Rating*

The typical symptoms of Fusarium wilt of 'Cavendish' banana are rhizome discoloration and leaf yellowing. The disease severity of 'Cavendish' banana plantlets as influenced by different rates of VT with EM and *Foc* TR4 inoculation was presented in Table 5.

It was found out that VT with EM-treated plants got a rating of effective and moderately effective on Rhizome Discoloration Index (RDI) and Leaf Severity Index (LSI). The severity of rhizome discoloration ranged from 1.97 to 3.05, meanwhile severity of leaf yellowing ranged from 1.70 to 2.15 which is much lower than positive control, where the mean RDI is 4.10 and LSI of 2.33 (Figure 2 and 3). Among the VT with EM-treated plants, VT (62.50 ml/L) +EM (40ml/L) has been rated as effective (E) due to lowest rating of RDI (1.97) and LSI (1.70). Moreover, the VT (46.88ml/L) + EM (40ml/L)-treated plants also rated effective (E) in minimizing the severity of *Foc* TR4 on 'Cavendish' banana but compared to VT (125ml/L) + EM (40ml/L), it gains much less severity despite of the increased rate of VT. Treatment with the lowest rate of VT + EM showed a rate of effective (E).

Table 5. Disease severity index (DSI) of rhizome discoloration and leaf yellowing of *Foc* TR4 applied with different rates of VT with EM on inoculated banana plantlets.

Treatments (Per liter water)	Rhizome Discoloration Index (RDI)	[1]Translation	Leaf Severity Index (LSI)	[2]Translation
Negative Control	1.00^{a}	-	1.00^{a}	-
Positive Control	4.10^{d}	-	2.33^{c}	-
VT (62.50ml) + EM (40ml)	1.97^{b}	E	1.70^{b}	E
VT (31.25ml) + EM 40ml)	3.05^{c}	ME	2.07^{c}	ME
VT (46.88ml) + EM (40ml)	2.23^{bc}	E	1.70^{b}	E
VT (125ml) + EM (40ml)	2.70^{bc}	E	2.15^{c}	ME

[1]RDI Translation: 1 = Very Effective (VE);
Between 1.1 and 3 = Effective (E);
Between 3.1 and 5 = Moderately Effective (ME);
Between 5.1 and 8 = Less Effective (LE).
[2]LSI Translation: 1 = Very Effective (VE);
Between 1.1 and 2 = Effective (E);
Between 2.1 and 3 = Moderately Effective (ME);
Between 3.1 and 4 = Less Effective (LE).

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Among the VT with EM-treated plants, VT (62.50ml/L) +EM (40ml/L) has been rated as effective (E) due to lowest rating of RDI (1.97) and LSI (1.70). Moreover, the VT (46.88ml/L) + EM (40ml/L)-treated plants also rated effective (E) in minimizing the severity of *Foc* TR4 on 'Cavendish' banana but compared to VT (125ml/L) + EM (40ml/L), it gains much less severity despite of the increased rate of VT. Treatment with the lowest rate of VT + EM showed a rate of moderately effective (E).

According to Ingham (2005), the dilution of compost tea to water is important because too little compost tea will result in too dilute suspension which results to limited nutrients and few microorganisms [7]. This might be one of the reasons which lowest rate of VT with EM got the highest rating of disease severity index (DSI) and rated as moderately effective.

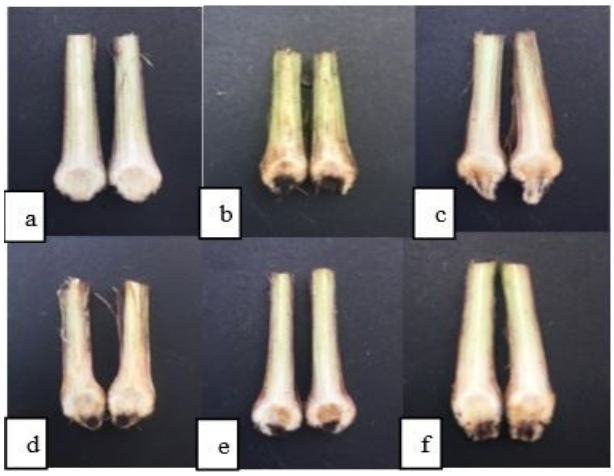

Fig. 2. Rhizome discoloration index (RDI) of Fusarium wilt on 'Cavendish' banana as affected by different rates of VT supplemented by EM; a. Negative Control; b. Positive control; c. Vermicompost Tea (66.67ml) + EM (40ml/L); d. Vermicompost Tea (33.34ml) + EM (40ml/L); e. Vermicompost Tea (50.00ml) + EM (40ml/L); f. Vermicompost Tea (133.34ml) + EM (40ml/L)

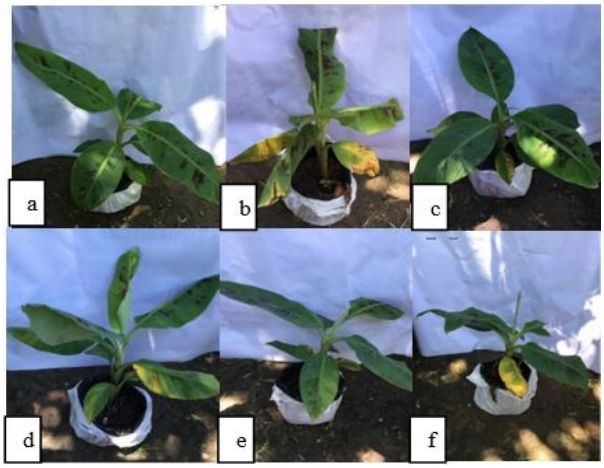

Fig. 3. Leaf severity index (LSI) of Fusarium wilt on 'Cavendish' banana as affected by different rates of VT supplemented by EM1; a. Negative Control; b. Positive control; c. Vermicompost Tea (66.67ml) + EM (40ml/L); d. Vermicompost Tea (33.34ml) + EM (40ml/L); e. Vermicompost Tea (50.00ml) + EM (40ml/L); f. Vermicompost Tea (133.34ml) + EM (40ml/L)

C. *Fusarium Wilt Incidence and Degree of Control*

Common symptoms of Fusarium wilt such as the presence of yellow streaks on leaves, pseudostem splitting, yellowing and wilting were checked daily until the termination of the study.

Results showed lowest incidence of Fusarium wilt on plantlets treated with VT (62.50ml/L) + EM (40ml/L) with 55% followed by VT (31.25ml/L) + EM (40ml/L) with 77.50% and was comparable to VT (46.88ml/L) + EM (40ml/L) with 77.50% (Table 6).

Table 6. Percentage of disease incidence and degree of control of Fusarium wilt on 'Cavendish' banana plantlets treated with different rates of VT with EM[1]

Treatments (Per liter water)	Disease Incidence[2]	Degree of Control	Degree of Effectiveness
Negative Control	0.00a	-	-
Positive Control	100.00d	-	-
VT (62.50ml) + EM (40ml)	55.00b	45.00	Moderately Effective
VT (31.25ml) + EM 40ml)	72.50bc	27.50	Less Effective
VT (46.88ml) + EM (40ml)	77.50bc	22.50	Less Effective
VT (125ml) + EM (40ml)	82.50c	17.50	Not Effective

[1]Means with the same letter superscripts are not significantly different at 5% level by Tukey's HSD. Data are means of four replicate at ten seedlings per replicate.
[2]Data subjected to ANOVA were transformed using Arc sine Transformation.

However, highest disease incidence was noted on plantlets treated with VT (125ml/L) + EM (40ml/L) which was comparable to the positive control (inoculated and untreated) plantlets with 82.50% and 100%, respectively. According to Arancon *et al*. (2007), vermicompost tea added with carbon substrates such as molasses encourage the growth of beneficial microorganisms as well as the pathogenic microorganisms [3]. Thus, the increase of the application rate of VT may also contribute to the increase of the *Foc* TR4 due to the higher amount of molasses, the source of food for microorganisms. This conforms to the reports of Edwards *et al*. (2006), which the addition of the molasses or sugars during preparation may also multiply the pathogens [5].

Based on this result, it is evident that VT (62.50ml/L) + EM (40ml/L) was the most effective among the different rates of VT with EM on reducing the Fusarium wilt incidence on 'Cavendish' banana plantlets.

IV. Conclusion

The application of different rates of vermicompost tea (VT) with Effective Microorganisms (EM) on 'Cavendish' banana inoculated with *Foc* TR4 revealed longer symptom appearance particularly on plantlets treated with VT (62.50ml/L) + EM (40ml/L) and VT (125ml/L) + EM (40ml/L) with 11.75 days and 13.45 days, respectively. Also, lower severity index was recorded on plantlets treated with VT (62.50ml/L) + EM (40ml/L) and VT (46.88ml/L) + EM (40ml/L) of which rhizome severity index and leaf severity index was rated effective (E).

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