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Vesicular Arbuscular Mycorrhizae and *Trichoderma harzianum* as Biological Control Agents against Panama Disease of 'Cavendish' Banana Caused by *Fusarium oxysporum* F. Sp. Cubense Tropical Race 4

Leslie T. Ubaub[1*] and Jermely D. Requina[1]

[1]College of Agriculture, University of Southeastern Philippines, Tagum City, Philippines.

Authors' contributions

This work was carried out in collaboration between both authors. Author LTU designed and contributed the conceptualization of the study with author JDR. She also performed the statistical analysis and wrote and edited the manuscript. While author JDR managed the literature sources, performed the experiment, collected data and wrote the first draft. Both authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to evaluate the effect of Vesicular Arbuscular Mycorrhizae (VAM) and *Trichoderma harzianum* against *Fusarium oxysporum f. sp. cubense*TR4 on 'Cavendish' banana plantlets and to determine the growth performance of 'Cavendish' banana plantlets applied with Vesicular Arbuscular Mycorrhizae and *T. harzianum.*

The experiment was laid-out in Completely Randomized Design (CRD) with seven treatments and replicated three times with five samples per replication. The treatments were as follows: T_1- Negative control (w/out *Foc*), T_2- Positive control (with *Foc*TR4), T_3- 5 g of VAM alone + *Foc* TR4, T_4- 50 g of *T. harzianum*alone + *Foc* TR4, T_5- 3 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4, T_6- 5 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4, T_7- 7 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4.

Based on the result of the study, the different rates of VAM + *T. harzianum* delayed the symptoms

**Corresponding author: E-mail: leslie.ubaub@usep.edu.ph;*

appearance and reduced incidence and severity of Panama disease. Plantlets treated with 7 g of VAM + 50 g of *T. harzianum*reduced *Foc* TR4 development in terms of disease symptom appearance, has been delayed for seven (7) days; disease incidence which had a difference of 53.33%; disease severity had 36.67% compared with the Positive check.
On the effect of the agronomic characteristics, result showed significant differences from the data on the average plant height (cm) of 'Cavendish' banana plantlets at 60 and 90 DAT as well as the diameter of the pseudostem as influenced by VAM and *T. harzianum.*

Keywords: Trichoderma harzianum; vesicular arbuscular mycorrhizae; Fusarium oxysporum f. sp. cubenseTR4; 'Cavendish' banana.

1. INTRODUCTION

Banana is one of the most important fruits because of the role it plays in the global economy, food security and the everyday lives of people around the world. In the Philippines, 'Cavendish' Banana (*Musa acuminata)* is the leading fruit grown and a consistent top dollar earner. 'Cavendish' banana is a dessert banana and mainly for export. 'Cavendish' cultivar contributes about 41.5% for the domestic market [1].

The prospect of Philippine bananas in the domestic and foreign market is still promising. In 2001, the country's banana output reached 5 million tons from a total area of 386, 782 ha. Many workers are employed in the banana industry, both in the farms and in the processing plants. The commercial farms which mostly grow 'Cavendish' cultivars are concentrated in the Mindanao area. Elsewhere in the country are numerous backyard farms, whose products is mainly destined for the local markets [2].

Among the many constraint of banana production in the world especially in Asia are diseases and targeted as the more serious. One of these diseases is the Fusarium wilt caused by the soil-borne fungus, *Fusarium oxysporum*f. sp. *cubense* (*Foc*). Among the four races of *Foc* which attack bananas, race 4 is the most pathogenic and affects many susceptible banana cultivars including 'Cavendish'. *Foc* can be disseminated through suckers, soil, water and by improper farming practices when farmers use contaminated tools. Chemical control, such as soil fumigation, is a promising measure but gives strong negative impact to the environment and to human health. As a result, once the field is invaded by *Foc*, the field cannot be used for banana production up to 30 years. Resistant varieties and/or clones have been produced but not many of them are favourable and marketable in local and international market [3].

There is no satisfactory method to control Fusarium wilt. Chemical control, flood fallowing, crop rotation and the use of organic amendments have not been effective in managing the disease [4,5]. As a soil-borne pathogen producing chlamydospores, the fungus may persist in the soil in the absence of the host for many years [6]. Quarantine and exclusionary procedures are effective in controlling the disease by restricting the movement of plant parts and soil that could be carrying *Fusarium oxysporum* f. sp. *Cubense* from infested to clean areas Jores (2000) as cited by Bagacay, (Philippines Unpublished results).

Hence, this study was conducted to evaluate the efficacy of VAM, and *T. harzianum*when applied in solo and in combination against *Fusarium oxysporum*f. sp. *cubense* (*Foc*) as biological control agents for effective management of the disease and determine the agronomic characteristics of the sample plants as influenced by the different treatments.

2. METHODOLOGY

2.1 Location and Duration of the Study

The nursery experiment was conducted at the Panama Research Project Area, University of Southeastern Philippines, Tagum-Mabini Campus, Pindasan, Mabini, Compostela Valley Province from November 2015 to February 2016. The area was totally cleaned by cutting grasses using bolo. The nursery was constructed using coconut frond as shade and bamboo poles as foundation and support. The test plants were placed in an elevated table with a height of 2 feet and a dimension of 8 m x 6 m.

2.2 Experimental Design and Treatments

The experiment was laid out in a Completely Randomized Design (CRD) with seven

treatments and replicated three times with five samples per replication. The following treatments were used in the study: (T_1) - Negative control (without *Foc*), (T_2) - Positive control (with *Foc*TR4), (T_3) - 5 g of VAM alone + *Foc* TR4, (T_4) - 50 g of *T. harzianum*alone + *Foc* TR4, (T_5) - 3 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4, (T_6) - 5 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4, (T_7) - 7 g of VAM + 50 g of *T. harzianum*+ *Foc* TR4.

2.3 Preparation of Potting Medium and Test Plants

The potting medium was prepared using sandy-loam soil and it was pasteurized in a drum with a temperature of 60 ℃ for about 30 mins. The pasteurized soil was allowed to cool down before putting it in the prepared polyethylene bags with a diameter of 12' x 18'. The bags were placed in bigger plastic bags without holes to make sure that *Foc* TR4 will not spread in the area.

One-month old tissue-cultured 'Cavendish' banana plantlets var. 'Grand Nain' was used in the study. The plantlets were carefully uprooted to avoid root damage during transplanting. Prior to inoculation and transplanting, three root hairs were crushed slowly by hand to facilitate the entry of *Foc*TR4. To avoid intense heat from the sun, the plantlets were placed in a partially shaded area to maintain its moisture.

2.4 Source and Mass production of *Foc* TR4

Pure culture of *Foc* TR4 was acquired from the Crop Research Laboratory of the University of Southeastern Philippines and it was mass-produced on half strength Potato Sucrose Agar medium. Culture disk was obtained from pure culture of *Foc*TR4 and it was planted on fresh half strength PSA medium using a sterile transfer needle.

Mass production of *Foc* TR4 was composed of 200 g of corn grits and 1 kg of sand mixture (Mandariaga, University of Southeastern Philippines, Unpublished results). The mixture was put in a polypropylene bag added with 100 ml of non-chlorinated water. The mixture was tightly closed and sterilized in the pressure cooker at 15 psi for 30 minutes. The prepared sterilized medium was planted with agar blocks of 10-day old *Foc* TR4 pure culture and incubated for two weeks at minimum room temperature.

2.5 Inoculation of the Pathogen

A two-week old inoculum of *Foc* TR4 in corn grits-sand medium was applied at the rate of 100 grams/plant near at the root zone of 'Grand Nain' potted plantlets. Prior to inoculation, the three root hairs of each plantlet were teased to facilitate entry and subsequent infection of *Foc* TR4.

2.6 Preparation and Application of Treatments

2.6.1 Preparation and pre-treatment of plantlets with VAM

VAM was applied before transplanting. It was buried about two inches deep according to the rates per treatment. It was allowed to establish for 2 weeks before transplanting.

2.6.2 Application of *T. harzianum*

In transplanting, *T. harzianum*was applied basally at 50 g per treatment. After the application of *T. harzianum*basally, broadcasting application followed by putting on the surface of the potting medium another 50 g per treatment. The next application was done thrice within 90 days.

For the 1st application of *T. harzianum*, 100 g was used, 50 g for basal application then another 50 g for broadcasting application. For the next month of application 50 g of *T. harzianum*was usedup to the 3rd application.

2.7 Data Gathered

2.7.1 Days to appearance of pseudostem splitting and yellowing symptoms

Days to appearance symptoms was determined by counting the number of days from inoculation to first appearance of yellowing and splitting symptoms. It was monitored daily.

2.7.2 Fusarium wilt incidence

This was determined by getting the number of plants showing disease symptoms and the total number of assessed plant. It was computed using this formula below.

Diseased Incidence = (Number of plantlets showing disease symptoms/ Total number of sample plantlets) X 100

2.7.3 Disease severity based on the corm discoloration

The data was gathered at the termination of the study. The sample plant will be cut in cross-sectioned and the disease severity was assessed based on the discoloration of the plant corm using the following rating scale (Table 1) proposed by International Network for the Improvement of Banana and Plantain [7].

Table 1. Rating scale for disease severity for rhizome discoloration

Scale	Description
1	No vascular discoloration
2	Discoloration confined in the junction of root and rhizome plexus
3	Discoloration of up to one-third of the vascular tissues
4	Discoloration between one-third and two thirds of vascular tissues
5	Discoloration of greater than two-thirds of vascular tissues
6	Total discoloration of vascular tissues

Based on the standard rating scale, disease severity was computed as follow:

Diseased Severity = [∑ (Number on scale x Number of plantlets in that scale)/ Number of treated seedlings x highest ratings] X 100

Degree of efficacy of the treatment was based on the computed disease severity for rhizome discoloration using a rating scale (Table 2).

Table 2. Rating scale for the degree of efficacy of the treatments based on rhizome discoloration

Disease index	Degree of effectiveness
1 to 1.5	Very Effective (VE)
Between 1.6 and 3	Effective (E)
Between 3.1 and 4.5	Moderately Effective (ME)
Between 4.6 and 6	Not Effective (NE)

2.7.4 Agronomic data

2.7.4.1 Plant height (cm)

This was taken by measuring the basal portion of the plant up to the apical V. It was expressed in centimeter. The height of the plant was measured at 30, 60 and 90 days after transplanting. Initial data of the 'Cavendish' banana plantlet was taken before transplanting.

2.7.4.2 Pseudostem diameter (mm)

This was obtained by measuring the diameter of the pseudostem of six samples 'Cavendish' banana seedlings. This was measured one inch above the potting medium using a caliper at 30, 60 and 90 days after transplanting.

2.8 Statistical Analysis

The different data gathered from the study were analyzed using the Analysis of Variance (ANOVA) following by Completely Randomized Design (CRD) and the difference between treatments was computed using Tukey's HSD when variances are significant.

3. RESULTS AND DISCUSSION

3.1 Number of Days of Symptom Appearance

The typical symptoms of Panama disease such as splitting and yellowing of leaves appeared as early as 19 days after inoculation of *Foc*TR4 (Fig. 1).

Analysis of variance revealed that there was highly significant difference at 1% level on the number of days to symptoms appearance. Results showed that Treatments at 5 g of VAM alone, 50 g of *T. harzianum*alone, 3 g of VAM + 50 g of *T. harzianum,* 5 g of VAM + 50 g of *T. harzianum,* 7 g of VAM + 50 g of *T. harzianum*and Positive control (*Foc*-inoculated only) were comparable with each other with the average ranges from 60 to 67 days (Table 3).

Although among treatments numerical data showed that the combination of 7 g of VAM + 50 g of *T. harzianum*treatments delayed symptoms appearance by one week compared to the Positive check. This delayed in symptoms appearance may be due to the antagonistic effect of *T. harzianum*in combination of VAM which enhanced plant growth. It was already reported that strains of *T. harzianum* showed antagonistic effect on *Foc* while VAM caused few changes in root morphology but physiology of the host plant changes significantly [8,9]. It improved potential for mineral uptake from the soil accounts for changes in the nutritional states of the host biochemical aspects of root cells [10].

The data implied that at higher level of VAM + *T. harzianum*delayed the appearance of yellowing and splitting of 'Cavendish' banana with Panama disease.

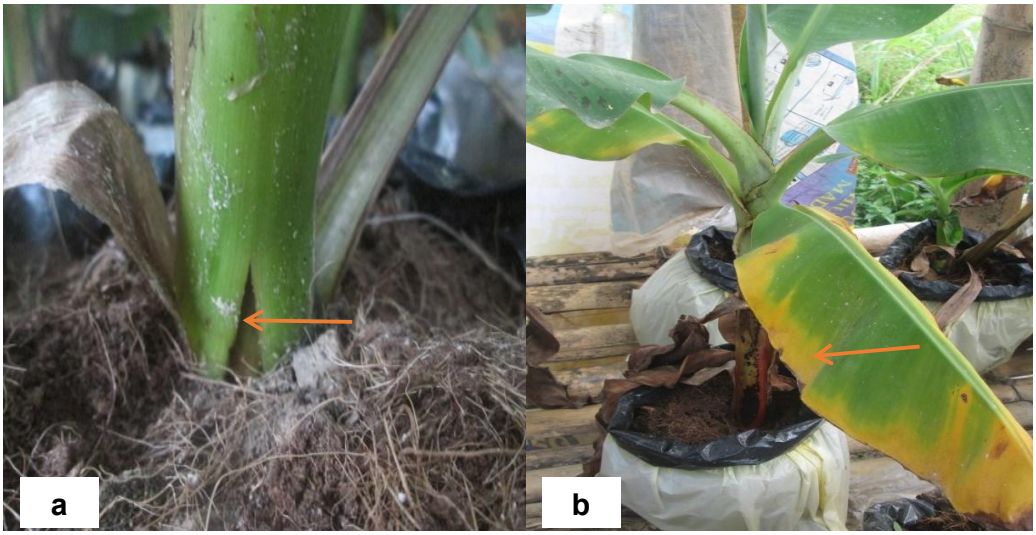

Fig. 1. Typical symptoms of 'Cavendish' banana inoculated with *Foc*TR4 under nursery condition (a) splitting of pseudostem; (b) yellowing of leaves

Table 3. Mean number of days to symptom appearance of panama disease on 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition

Treatment	Replication			Total	Mean**
	I	II	III		
Negative control (Without Foc)	0	0	0	0	0[a]
Positive control (With Foc)	56	59	65	179	60[b]
5 g of vam alone + Foc Tr4	66	64	63	193	65[b]
50 g of *T. Harzianum*Alone + Foc	58	64	69	188	63[b]
3 g of vam + 50 g of *T. Harzianum*+ Foc	69	59	66	194	65[b]
5 g of vam + 50 g of *T. Harzianum*+ Foc	60	67	64	191	64[b]
7 g of vam + 50 g of *T. Harzianum*+ Foc	74	68	59	201	67[b]

*CV (%) =8.43%; ** Significant at 1% level; Means having common letter superscript is not significantly different at 1% level using Tukeys' HSD*

3.2 Disease Incidence

The Fusarium wilt incidence on 'Cavendish' banana plantlets as influenced by the application of VAM and *T. harzianum*was monitored weekly after *Foc*inoculation (Fig. 2). Lowest disease incidence was observed in plantlets treated with 7 g of VAM + 50 g of *T. harzianum*wherein at week 8, 6.67% was recorded and it progressed to 13.33% at week 9, 26.67% at week 10 and at week 11 it reached 46.67%. Disease incidence of all treatments with a combination of different level of VAM and *T. harzianum* were recorded starting week 7 with 13.33%.

Among treatments both 5 g of VAM alone and 50 g of *T. harzianum*alone had disease incidence of 86.67% at 12 and 10 WAI, respectively. At 5 g of VAM + 50 g of *T. harzianum*had disease incidence of 53.33% at week 12 after *Foc*inoculation. Disease incidence of plantlets treated with 7 g of VAM + 50 g of *T. harzianum,* 5 g of VAM + 50 g of *T. harzianum,* 3 g of VAM + 50 g of *T. harzianum,* 50 g of *T. harzianum*alone and 5 g of VAM alone have stabilized at 46.67%, 53.33 %, 73.33 %, 86.67%, respectively. While at week 10, all plantlets in Positive control died and none in Negative control. Table 4 showed the mean percentage of disease incidence of 'Cavendish' banana plantlets 12 weeks after inoculation (WAI).

All test plants applied with different rates of VAM and *T. harzianum* were infected with Panama disease but percentage of disease incidence was significantly different with Positive control with a mean of 100.00%. Plantlets treated with 7 g of VAM + 50 g of *T. harzianum*has the lowest

disease incidence of 46.67% which was comparable with Negative control (Uninoculated, untreated plantlets). Both treatments at 3 g of VAM + 50 g of *T. harzianum*and 5 g of VAM + 50 g of *T. harzianum*were comparable to 7 g of VAM + 50 g of *T. harzianum*with a percent mean of 73.33% and 53.33%, respectively. Disease incidence on plantlets treated with 5 g of VAM alone and 50 g of *T. harzianum*alone were statistically comparable with Positive control (*Foc*-inoculated, untreated).

T. harzianum fungi used as biological control agents due to their modes of action: competition, parasitism, production of inhibitory compounds and enzymes or inactivation of the pathogen's enzymes systems (Donald, 2010). Whereupon, at 7 g of VAM + 50 g of *T. harzianum* (46.67%) applied on inoculated *Foc*TR4 'Cavendish' banana plantlets affect a high difference of 53.33% to positive control (100%). Noted that pretreatment of *T. harzianum*prior to transplant was not practiced.

The result implied that T. *harzianum* has a potent ability to reduce disease incidence and better results were attained with VAM in combination. Pretreatment at early stage before transplanting is also suggested.

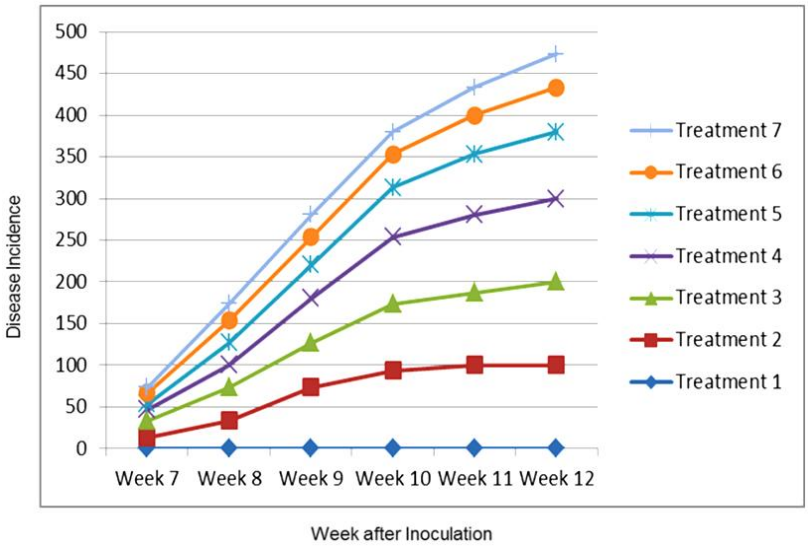

Fig. 2. Disease incidence of 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition

Table 4. Mean incidence of Panama disease based on corm discoloration on 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition 12-Weeks after inoculation (WAI)

Treatment	Replication			Total	Mean*
	I	II	III		
Negative control (Without *Foc*)	0.00	0.00	0.00	0.00	0.00[a]
Positive control (With *Foc*)	100.00	100.00	100.00	300.00	100.00[c]
5 G of vam alone + *Foc* Tr4	80.00	80.00	100.00	260	86.67[bc]
50 G of *T. Harzianum* Alone + *Foc*	100.00	80.00	80.00	260.00	86.67[bc]
3 G of vam + 50 G of *T. Harzianum* + *Foc*	80.00	60.00	80.00	220.00	73.33[b]
5 G of vam + 50 G of *T. Harzianum* + *Foc*	80.00	40.00	40.00	160.00	53.33[b]
7 G of vam + 50 G of *T. Harzianum*+ *Foc*	60.00	40.00	40.00	140.00	46.67[ab]

*CV (%) = 19.35%; ** Significant at 1% level; Means having common letter superscript is not significantly different at 1% level using Tukeys' HSD*

3.3 Disease Severity

The severity of infection of Panama disease in 'Cavendish' banana plantlets treated with VAM and *T. harzianum*at 90 days observation period is presented in Table 5. Disease severity was assessed based on the discoloration of the plant corm (Fig. 3) using the rating scale proposed by International Network for the Improvement of Banana and Plantain [7].

The data showed that all treatments with VAM and *T. harzianum*are comparable with each other however, treatments at 5 g of VAM alone and 50 g of *T. harzianum* alone as well as the Positive check were significantly different with the Negative control while all treatments with VAM and *T. harzianum*combination were comparable with Negative control. Numerically, the plantlets treated with 7 g of VAM + 50 g of *T.harzianum*had the lowest disease severity (50%) with the difference of 36.67% on the Positive check. Apparently, all treatments with VAM and *T. harzianum*obtained lower disease severity compared to the Positive check.

Literature suggest that biological control of pathogenic *Fusarium oxysporum*in the root rhizosphere can be enhanced by using combination of biocontrol agents, particularly if they exhibit different or complementary modes of actions [11]. The results suggest that the application of biological control organism in combinations has often yielded better results than the organisms applied individually.

While Arbuscular Mycorrhiza (AM) fungi may also contribute to protection of the host plant against soil borne plant pathogens. Combinations of AM fungi and biocontrol agents like *T. harzianum* could, therefore, provide levels of disease control which are superior to the effects of the organisms when they are used alone.

3.4 Degree of Efficacy

The degree of efficacy of VAM, *T. harzianum*and the combination of two at different level of VAM was assessed based on the disease severity. The data showed that only 5 g of VAM applied alone was not effective while 50 g of *T. harzianum*alone and with the combination of different levels of VAM were all moderately effective (Table 6).

This implied that VAM alone cannot control *Foc*TR4 and affirmed that VAM's effects to plants are purely on the nutritional aspect.

Table 5. Mean of disease severity due to Panama disease on 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition

Treatment	Replication			Total	Mean**
	I	II	III		
Negative control (without *Foc*)	16.67	16.67	16.67	50.01	16.67[a]
Positive control (with *Foc*)	96.67	90	73.33	260.00	86.67[b]
5 g of VAM alone + *Foc* TR4	66.67	76.67	76.67	220.01	73.34[b]
50 g of *T. harzianum*alone + *Foc* TR4	80.00	66.67	50.00	196.67	65.56[b]
3 g of VAM + 50 g of *T. harzianum*+ *Foc*	53.33	36.67	76.67	66.67	55.56[ab]
5 g of VAM + 50 g of *T. harzianum*+ *Foc*	70.00	53.33	40.00	163.33	54.44[ab]
7 g of VAM + 50 g of *T. harzianum*+ *Foc*	36.67	40.00	73.33	150.00	50.00[ab]

*CV= 22.35%; ** Significant at 1% level; Means having common letter superscript is not significantly different at 1% level using Tukeys' HSD*

Table 6. Degree of efficacy of different levels of VAM and *T. harzianum* due to panama disease on 'Cavendish' banana plantlets cv. 'Grand Nain' under nursery condition

Treatment	Disease index	Degree of efficacy
Negative control (without Foc)	1.11	------
Positive control (with Foc)	5.78	------
5 g of VAM alone + Foc TR4	4.89	Not effective
50 g of *T. harzianum*alone + Foc	4.37	Moderately effective
3 g of VAM + 50 g of *T. harzianum* + Foc	3.70	Moderately effective
5 g of VAM + 50 g of *T. harzianum* + Foc	3.63	Moderately effective
7 g of VAM + 50 g of *T. harzianum* + Foc	3.33	Moderately effective

Fig. 3. Severity of *Foc* TR4 infection characterized by leaf yellowing and corm discoloration; (a) T1 (b) T2 (c) T3 (d) T4 (e T5 (f) T6 (g) T7

3.5 Plant Height (cm)

The height of 'Cavendish' banana plantlets inoculated with *Foc*TR4 at 30, 60 and 90 days after transplanting (DAT) as affected by Vesicular-Arbuscular Mycorrhiza (VAM) and *T. harzianum*was measured (Fig. 4).

At 30 DAT, no significant differences in mean of plant height were noted which ranged from 20.03 cm to 22. 13 cm. On the 60 DAT, however, plant height was highly significantly different with each other. It showed that all plantlets treated with VAM were significantly comparable with the Negative control (without *Foc)* with an average ranged from 26.63 to 33.13 cm. VAM alone and 50 g of *T. harzianum*alone were significantly different with the Negative control but comparable with all other treatments. Lowest plant height (25.00 cm) was obtained by plantlets treated with *T. harzianum*alone.

At 90 DAT, mean height of plantlets likewise significantly increased in all treatments. Uninoculated + untreated plantlets were significantly taller (33.13 cm) and were comparable to Foc-inoculated plantlets treated with 5 g of VAM alone and 3 g of VAM + 50 g of *T. harzianum*with the plant height of 32.03 cm and 31.60 cm, respectively, 50 g of *T. harzianum*alone, 5 g of VAM + 50 g of *T. harzianum*and 7 g of VAM + 50 g of *T. harzianum* had a plant height of 31.60 cm, 29.45 cm and 28.09 cm, respectively, which were comparable to Positive control (*Foc*- inoculated) with the plant height of 27.02 cm. It could be noted that 'Cavendish' banana seedlings, even if inoculated with Foc TR4, increased in height significantly three months after transplanting but not as much as those treated with VAM and *T. harzianum.*

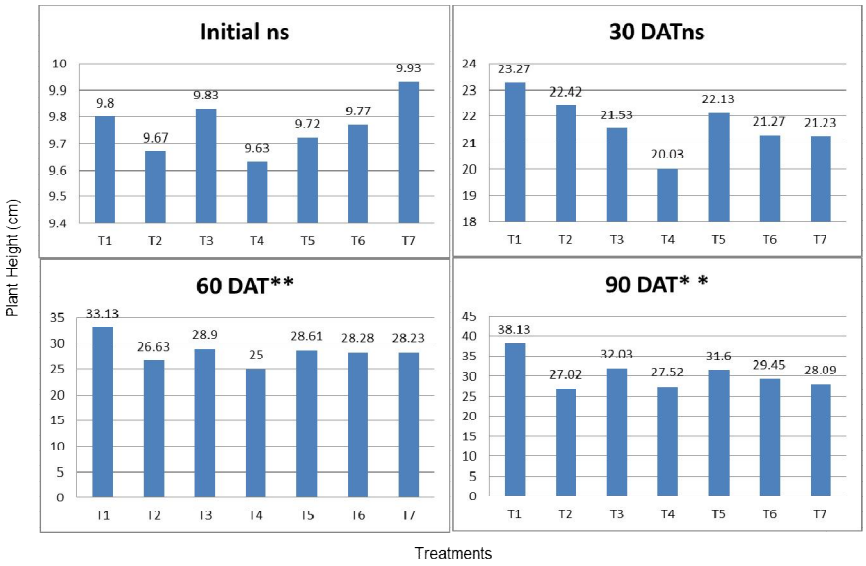

Fig. 4. Mean height (cm) of 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition

3.6 Plant Pseudostem (mm)

The average pseudostem diameter (mm) of sample 'Cavendish' banana plantlets under nursery condition at 30, 60 and 90 DAT was likewise measured (Fig. 5). At 30 DAT, no significant differences among means of plant pseudostem diameter were noted. Pseudostem of 'Cavendish' banana plantlets ranged from 4.82 mm to 5. 23 mm.

The analysis of variance showed significantly difference between treatments at 60 DAT. *Foc*-inoculated test plantlets applied with 5 g of VAM alone, and 5 g of VAM + 50 g of *T. harzianum,* 7 g of VAM + 50 g of *T. harzianum* were significantly comparable with the Negative check with the mean pseudostem ranged from 5.52 to 6.21 mm. *Foc*-inoculated treatment with 50 g of *T. harzianum*alone were significantly shorter (4.99 mm) than those treated with VAM alone and VAM + *T. harzianum.* The positive control (Foc TR4- inoculated), on the other hand, had the lowest mean pseudostem of 4. 88 mm.

At 90 DAT, mean pseudostem of plantlets likewise significantly different among treatments although all treatments measured lower than the diameter at 60 days. Uninoculated + untreated test plantlets had the biggest pseudostem diameter (2. 99 mm) and were comparable to the inoculated plantlets treated with VAM alone, *T. harzianum*alone and the combination of VAM +*T. harzianum* with means pseudostem ranged from 2.50 mm to 2.80 mm. The positive control (Foc TR4- inoculated) on the other hand, had the lowest mean pseudostem of 2.46 mm.

The plant height and pseudostem diameter of 'Cavendish' banana plantlets treated with VAM and *T. harzianum* increased due to the effect of VAM fungi and *T. harzianum.* When VAM inoculated to plantlets which was infect the roots. It helps in the absorption of water and nutrients particularly phosphorus, prevent root infection by pathogens and can increase plant tolerance to drought and heavy metalsto the plantlets.

VAM application has positive effects on plant growth, health and protection against biotic and abiotic stresses. Arbuscular Mycorrhizae Fungi (AMF) are said to improve nutrient uptake and growth of bananas [12].

In addition, mycorrhizal root inoculants assist plant roots in absorbing more water and nutrients. It can substantially reduce the chemical requirement of crops and can also increase the resistance and tolerance of the plants against root pathogen by secreting growth promoting substance to improved soil structure and soil aggregation (Brown, A.M, University of California Davis, USA, Unpublished results).

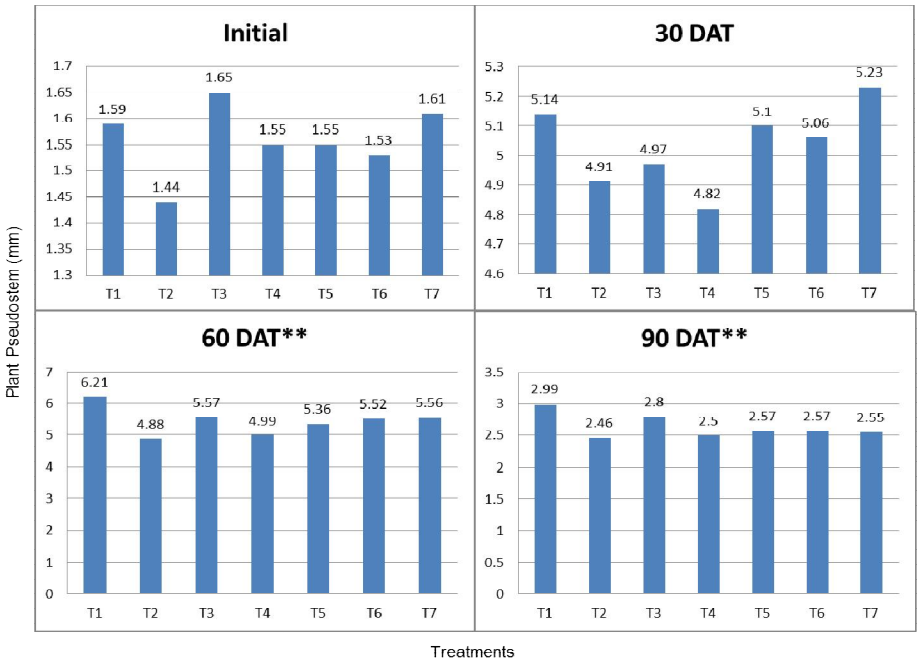

Fig. 5. Mean Plant Pseudostem (mm) on 'Cavendish' banana plantlets cv. 'Grand Nain' as affected by different levels of VAM and *T. harzianum* under nursery condition

While the fungus *T. harzianum* is a biological control organism against a wide range of soil-borne pathogens and has the capacity to promote plant growth. *Trichoderma* are generally, soil dwelling saprophytes. They have a rapid growth rate, sporulate abundantly, compete well with other soil microorganism, show resistance to chemical pesticides and produce various antibiotics.

They also exist in many other diverse habitats. *Trichoderma* readily colonizes plant roots and some strains are rhizosphere competent in able to grow on roots as they develop. *Trichoderma* spp. also attacks, parasitize and otherwise gain nutrition from other fungi. They have numerous mechanisms for both attack of other fungi and for enhancing plant root growth.

4. CONCLUSION

Based on the results, it can be concluded that VAM and *T. harzianum*and their combinations had positively influenced growth of 'Cavendish' banana plantlets as shown by the increased in plant height and pseudostem diameter. *T. harzianum*and VAM + *T. harzianum* were moderately effective in suppressing the development of the Foc TR4 in 'Cavendish' banana plantlets.

Among the plantlets, those treated with 7 g of VAM + 50 g of *T. harzianum*were the best among the treatments in terms of the number of days to symptoms appearance, disease incidence and disease severity. Plantlets treated with 5 g of VAM alone obtained the highest plant height and pseudostem diameter.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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