EFFICACY OF MIXED ACTINOMYCETES AGAINST *FUSARIUM* WILT CAUSED BY *Fusarium oxysporum* f.sp. *cubense* IN 'CAVENDISH' BANANA

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Abstract
This study evaluated the potential of three best isolates; AQ6, AQ30 and AQ121 actinomycetes to control *Foc* Tropical Race 4 in Cavendish banana. Combinations of the three isolates yielded an inhibition of 13.5 mm by cup cylinder assay. These findings led to the formulation of the mixed actinomycetes as biocontrol agents against Foc. A field experiment evaluated that preventives method of application of the mixed actinomycetes against *Foc* showed promising results. A 36.67% mortality was observed in control set-up (no biocontrol agent added) compared to 18.33% mortality in preventive method.

Keywords: Actinomycetes, biocontrol agents**,** Cavendish banana, Fusarium oxysporum f. sp. cubense

1. INTRODUCTION

The 'Cavendish' banana (*Musa cavendishii)* is a tropical plant and a triploid (AAA) cultivar of *Musa acuminata* growing in all regions of the Philippines. The Area harvested covered 449,000 hectares (ha) in 2010, up by 1.2 percent per annum from about 429,000 hectares in 2006. 'Cavendish' area is about 80,000 hectares in 2010, or 18 percent of the total banana area (BAS, 2011). Banana is one of the major fruits in the Philippines and is next to coconut in terms of volume of production and export earnings. The Philippines' export of fresh 'Cavendish' banana ranked No. 1 with 22% share in Philippine food exports. Fresh 'Cavendish' bananas are exported to 32 countries basically in Asia and Middle East. The increase in the demand of banana for both domestic and foreign countries could provide lots of investment opportunities in the region such as expansion of banana plantation and related industries (BAS, 2011).

The Philippines's soil and climate are suitable for banana commercial production at the same time is also a paradise for pests and diseases. The latest and major threat to the industry is *Fusarium* Wilt. This disease is caused by a soil-borne pathogen *Fusarium oxysporum* f.sp. *cubense.*

This fungus enters only through the roots and grows and then sporulates abundantly in xylem vessels. The transport of the spores upward in the transpirational stream facilitates the fungal invasion of the entire vascular system. The growth of the fungus blocks the vascular system, causing the plant to wilt. *Fusarium* wilt tops as a major concern of the Philippine industry particularly today in Mindanao (Ploetz and Pegg, 2000).

As a market driven enterprise, the banana industry must maintain and protect its global reputation for quality and sustain its well-established pest control and management systems against banana pests and diseases, particularly for *Fusarium* wilt of Banana. To sustain the operations of banana growers and processors in the region, there is a need for further Research and Development. The search for sustainable and effective control under natural condition is the main focus of the banana industry. The worldwide efforts in the search of natural products or biological control in the crop protection market have increased significantly and actinomycetes appear to be good candidates to control plant diseases (Behal, 2000).

Actinomycetes are known saprophytic bacteria that decompose organic matter, especially biopolymers such as lignocellulose, starch, and chitin in soil (Crawford, et al., 1993). Actinomycetes have characteristic biological features such as a mycelial growth that culminates in sporulation. They also possess the ability to synthesize a wide variety of antibiotics as secondary metabolites (Lechevalier and Waksman, 1962). Actinomycetes are shown as Plant Growth Promoting Rhizotobacteria (PGPR). As PGPR, actinomycetes prevent the harmful effects of the deleterious pathogens by biosynthesis of antibiotics and other secondary metabolites that prevent pathogen invasion. Direct promotion of plant growth by PGPR occurs when the plant is supplied with a compound that is synthesized by the actinomycetes, or when it facilitates plant uptake of soil nutrients (nitrogen fixation, siderophore synthesis and solubilization of minerals to make them available for plant uptake and use) (Glick, 1995).

Several strains of actinomycetes have been found to protect plants against plant diseases and as producer of agroactive compounds (Doumbou *et al*., 2001). A number of studies have reported the antagonism between actinomycetes and diverse of phytopathogens such as *Alternaria*, *Fusarium*, *Macrophomina*, *Phytophthora* and *Rhizoctonia* (Hussain *et al*., 1990 and Heisey and Putnam, 1986) , however, the potential of actinomycetes bacteria to combat

Fusarium wilt TR4 in 'Cavendish' banana planted in naturally infested soil have not yet been explored. Thus, this study.

Objectives of the study

Generally this study aims to determine the efficacy of actinomycetes against *Fusarium* wilt (*Fusarium oxysporum* f.sp. *cubense*) in 'Cavendish' banana.

Specifically, this study was conducted for the following objectives:

1. To assess the efficacy of actinomycetes in controlling *Fusarium* wilt;
2. To assess the population of actinomycetes in the soil before and at termination of the experiment; and
3. To determine the agronomic performance of 'Cavendish' banana applied with actinomycetes;

Conceptual Framework

The researchers were primarily based on the principle of suppresive soil (Doumbou *et al.*, 2001).

Suppressive soils is dominated by antagonistic microbes, which produce number of antibiotics, fungicidal compounds and competition with detrimental microbes, parasitism, induce plant resistance against pathogenic microbes. Antagonistic microbes suppress the pathogen depends on their microbial activities in the soil. The greater the microbial activites, thus rendering the pathogen weak.

Moreover, marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites (Baskaran et al., 2011). Among such microorganisms, actinomycetes are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities (Bredholt et al., 2008). The demand for new antibiotics continues to grow due to the rapid emerging of multiple antibiotic resistant pathogens causing life threatening infection. Now a day's considerable progress is being continuing within the fields of chemical synthesis and in the field of engineered biosynthesis of antibacterial compounds. So, the nature still remains the richest and the most versatile source for new antibiotics (Kpehn and Carter, 2005; Baltz, 2006; Pelaez, 2006).

Actinomycetes have gained prominence in recent years because of their potential for producing antibiotics (Kumar *et al*., 2005). Streptomycin, gentamicin, rifamycin are some of the antibiotics which are in use presently and erythromycin are the product of actinomycetes. The actinomycetes are important in the field of pharmaceutical industries and also the agriculture. Previous study showed that actinomycetes isolated from Malaysia soil have the potential to inhibit the growth of several plant pathogens (Jeffrey *et al*., 2007). Oskay *et al*. (2004) also reported about the ability of actinomycetes isolated from Turkey's farming soil to inhibit *Erwinia amylovora* a bacteria that cause fireblight in apple and *Agrobacterium tumefaciens,* a causal agent of crown gall disease (Jeffrey *et al*., 2008).

On the other hand, *Foc* plays the role of "silent assassin*";* the pathogenic strains of this fungus can be dormant for more than 30 years before resuming virulence and infecting a plant. The *Fusarium* wilt of Banana is lethal to plants and swift- by the time a plant shows any outward sign of infection, it is already too late, and the plant will die. *F.oxysporum* is not

discriminating; they can cause disease in every agriculturally important plant. *Foc* proves to be incredibly tough to eradicate.

The methods of application of actinomycetes and frequency will moderates the symptoms and epidemiology of the disease.

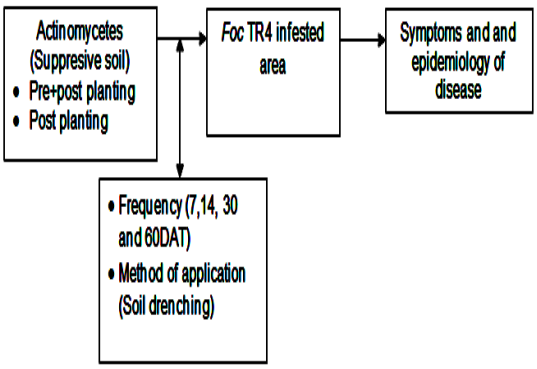

Figure 1. The Conceptual framework of the study

2. RESEARCH DESIGN AND METHODS

Experimental Design and Analysis

The study consists of *in-vitro* bioassay and field-test. The former was laid out using Completely Randomized Design (CRD) while the field study was laid out using Randomized Complete Block Design (RCBD) in *Foc* infested field. There were three treatments and three replications, each block was planted with 20 plants. Data were subjected to Analysis of Variance (ANOVA) and significant means were compared using Tukeys Honest Significant Difference (HSD) test.

Location and Duration of the Study

The optimization of production of the biocontrol agent and formulation of suitable carrier was done by National Institute of Molecular Biology and Biotechnology, University of the Philippines, Los Baños College, Laguna, Philippines.

The test for antagonism of actinomycetes bacteria against *Fusarium oxysporum* f.sp. *cubense* TR4 was conducted at the Crop Protection laboratory of University of Southeastern Philippines (USeP), Tagum-Mabini Campus, Apokon, Tagum City . Pre- planting application of tissue cultured 'Cavendish' meriplants was done at Musa King nursery, Brgy. San Miguel, Tagum City. The field experiment was conducted at Barangay Kinamayan, Sto Tomas, Davao del Norte from March to February 2014.

Experiment 1. *In-vitro* test for antagonism of actinomycetes to *Fusarium oxysporum* f.sp. cubense (*Foc*) Culture Media for Actinomycetes and *Foc*

The Yeast Malt Extract Broth (YMEB) and Yeast Malt Extract Agar (YMEA) were evaluated as production media of the biocontrol agents. YMEB medium was prepared using 5g Malt extract , 20g agar and 1L distilled water. Yeast Malt Extract Agar (YMEA) was prepared using Yeast extract- 4.0g/L, Malt Extract - 10g/L, Glucose - 4g/L and Agar- 17g/L.

Potato Dextrose Agar (PDA) was used as culture medium in culturing *Foc* TR4 in this study. To prepare 1Liter of PDA, the following ingredients were needed, 20g agar, 20 g dextrose powder, and 250 g peeled potato.

The ingredients of each medium were mixed and sterilized in a pressure cooker at 15psi for 20minutes, cooled down and poured into steriled petri plates and allow it to solidify for 10-20minutes.

Isolation of *Foc* TR4

The infected pseudostem strand of 'Cavendish' banana were cut approximately 1 inch, disinfected with 10% sodium hypochlorite, washed for three times with Sterile Distilled Water (SDW) and blotted dry in steriled tissue paper. The dried tissues were planted into plated medium and incubated in an inverted position at 28-30˚C to allow the fungus to grow. As soon as fungal growth appeared, a bit of the growth was examined under the microscope for confirmation if it was *Foc*. Pure culture was obtained by transferring a bit of confirmed *Foc* growth to a PDA slant and incubated using the same temperature.

Test for Antagonism of Actinomycetes to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4

The *in vitro* test for antagonism of the three isolates of Actinomycetes, coded AQ6, AQ3, and AQ121 against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc*TR4) was conducted using the "lawn method". The pathogen, *Foc* TR4 was on the background and the Actinomycetes agar plugs cut from 2 days old pure culture in YEA were laid equidistantly apart over it.

This set-up was prepared by plating 10 mL PDA then allowed to congeal before adding the 7.5 mL spore suspension of *Foc* at 1.8 x 10^{-4} conidia mL^{-1}.in melted warm PDA.

The aqueous spore suspension was prepared by aseptically adding 10 mL sterile water to the 7-day-old plate culture of *Foc* and scraping the growth with flamed wire loop to dislodge the spores. The suspension was passed through 4 layers of sterile gauze cloth to separate spores from fragments of mycelia and culture medium. One mL of aqueous *Foc* spore suspension was added per 250 mL melted PDA then mixed by rotational shaking before taking aliquots of 7.5mL which were poured over the previously plated 10mL PDA.

After the set-up has congealed and cooled, agar plugs cut from 2-day-old pure cultures of the Actinomycetes were laid equidistantly on its surface. The set-up had five replicates and was incubated at 28-30°C for their growth and observation. The zone of growth inhibition was measured after seven days of incubation (Fig. 2)

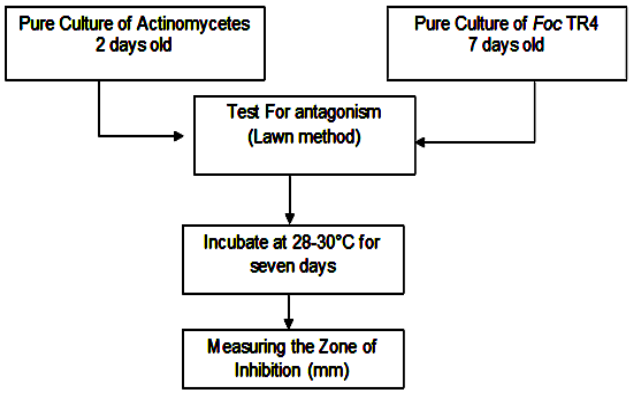

Figure 2. Flow of activities in *in vitro* efficacy test of Actinomycetes against TR4.

Efficacy of the Formulated Actinomycetes Product Against Fusarium Wilt on 'Cavendish' Banana

Selection and Preparation of the Area

The 769.5 sq.m area previously planted to 'Cavendish' banana with 100% reported *Fusarium* wilt incidence was used for this experiment. The area was divided into three blocks. Each block was further subdivided into three plots measuring 9 x 9.5m and separated by half meter canal to avoid water lagging and for ease of identifying the treatments. The planting distance was 2.0 m between hills and 2.5 between rows. Bamboo stakes were used to mark the spot for planting in each plot. A hole measuring 10cm depth was dug on each spot where the seedling was planted.

Soil sampling for determination of Actinomycetes Population

A composite soil sample using "X" pattern was taken from the experimental area to assess the population of Actinomycetes. This was done on October 10, 2013 during the initial counting of actinomycetes in the soil by digging 10inches depth.

Source of Meriplants

To ensure that the experimental plants are disease-free prior to use, the 180 tissue cultured 'Cavendish' meriplants were purchased from a credible source, Musa King Tissue Culture Laboratory, Bermudez Plain Subdivision, Apokon, Tagum City.

The Experimental Treatments

The field evaluation on the efficacy of the Actinomycetes against *Fusarium* wilt in 'Cavendish' banana was conducted in naturally *Foc*-infested area with three treatments which were as follows:

Treatment 1 - Control, no Actinomycetes treatment
Treatment 2 – Pre- and post-planting treatment with Actinomycetes
Treatment 3 - Post-planting treatment with Actinomycetes

Figure 2. Pre-planting root dip treatment of 'Cavendish' banana meriplants with formulated Actinomycetes at the rate of 1 kg/1.5 L water at USeP-Tagum Crop Protection Laboratory.

Treatment Application to 'Cavendish' meriplants

For experimental treatments 1 (Control, no actinomycetes treatment), treatment 2 (pre-and post- planting treatment with Actinomycetes), were treated by root dipping for 12 hours with formulated Actinomycetes product at the rate of one kilogram per 1.5 liter water and treatment 3 (post-planting treatment with Actinomycetes) meriplants were untreated before individual planting in bagged coconut coir peat. All meriplants were acclimatized for one month to provide the plants more prepared in the field conditions. A day prior to transplanting, the individual bagged of treatment 2 (pre-and post- planting treatment with Actinomycetes), with bags intact, at root level were dipped for 12h in actinomycetes suspension at the same rate used ealier (Fig.2).The experimental plants were then brought to the field contained in the plastic crates that were properly labeled.

Experiment 2. Field Treatment Application of Actinomycetes on 'Cavendish' Banana Seedlings

In the field, 'Cavendish' banana seedlings for the untreated control were transplanted in the designated plots at a distance of 2.0 m between

[1]1kg of formulated Actinomycetes / 1.5L water hills and 2.5 between rows.

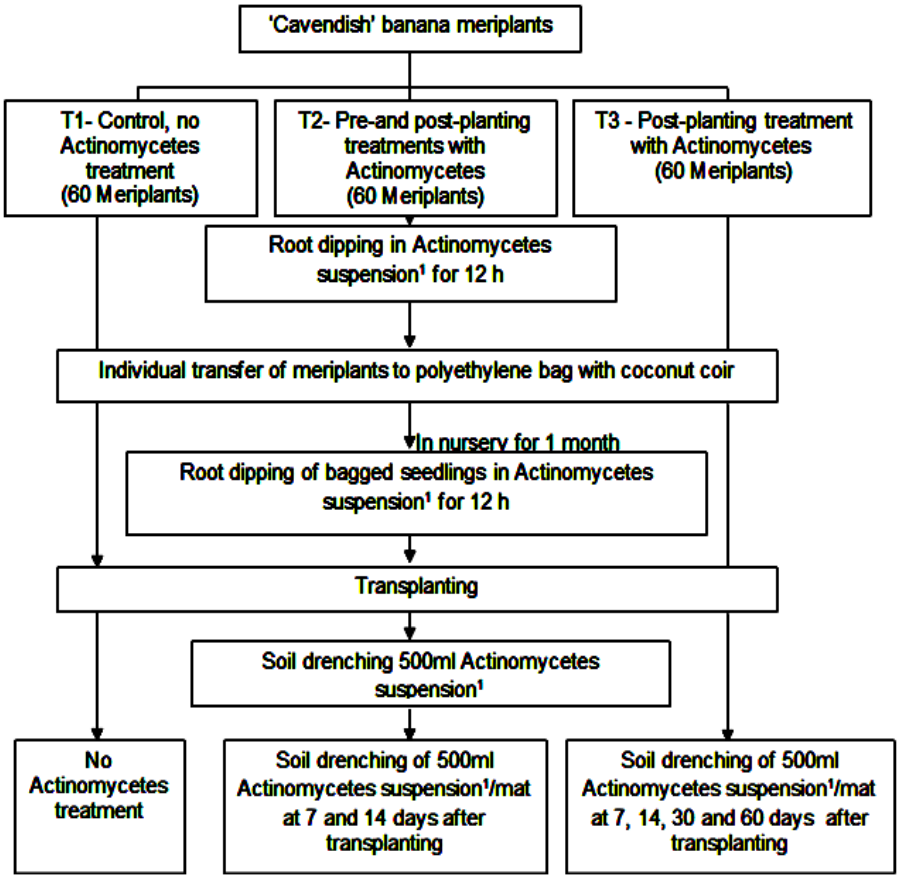

Fig. 3. Flow of experimental treatments application.

For Treatment 2, (pre- and post-planting treatment with Actinomycetes), 500ml of Actinomycetes suspension at the rate of 1kg formulated Actinomycetes per 1.5 liter water was drenched per hill right after transplanting in the designated spots. Augmentation drenching with the same volume of the treatment was done 14 days after transplanting. This was the last Actinomycetes application for treatment 2.

For treatment 3, (post-planting treatment with Actinomycetes), untreated 'Cavendish' banana seedlings were transplanted in the designated spots then soil drenching of 500mL Actinomycetes suspension/hill were done at 7, 14, 30 and 60 days after transplanting. The flow of treatment application is found in Fig. 3.

Transplanting

The tissue cultured 'Cavendish' banana seedlings were one month old at transplanting. Seedlings were set at a distance of 2 x 2.5m using quincunx method. Polyethylene bags were carefully cut using a sharp knife to avoid root injury to seedlings. Transplanting was done late in the afternoon.

Sanitation Practices

Strict sanitation practices are particularly important in the experimental farm. The 0.5m x 0.75m x 5 inches tab with overhead roofing for foothbathing was constructed before entering the experimental area, the tab was covered with thick cellophane and filled with ricehulls. The O-phenylphenol + Linear alkylbenzyl Sulfonic acid (Lasto-zide®) was used as disinfectant. This was placed inside the constructed foothbath. The disposable overshoes(layered cellophane) or boots were disinfected thoroughly before and after soil sampling to avoid introducing/acquiring soil-borne pathogen on footwear. The overshoes were removed 6 meters away from the infested area and sprayed with 70% ethyl alcohol and burned.

Fertilizer Applications

The mixture of 62 grams of urea (46-0-0) , 5 grams Inkabor (Boron) and 1500grams calcitic lime were applied in the hole before planting. This was based on the fertilizer program used by the neighboring farm, Yoshida. The succeeding fertilizer application was likewise patterned from the same farm.

Weed Control

Weeding in the experimental area was done monthly to avoid crop-weed competition, these were done using scythe and grass hook. To avoid transfer of soil from one area to another, the cutting tools were cleaned using a one foot flattened tip of bamboo stakes, the metal parts were sprayed with 70% ethyl alcohol and then burnt. The cut weeds were piled in between rows until decomposed.

Desuckering

To avoid nutrient, water and sunlight competition and provide good air circulation inside the experimental plants desuckering done two months after transplanting by cutting the excess suckers using cutting knife and maintained the 1 sucker per mat. This was done early in the morning.

Leaf trimming

Trimming of black leaf streak – infected leaces was done three months after transplanting and then bi-weekly thereafter and as needed using deleafing knife. These were done early in the morning. The trimmed/cut leaves were piled in between rows.

Bud Injection and Bunch Spray

An insecticide containing the active substance deltamethrin (Decis ® EC 25) was used in bud injection at mixture of 25ml/16L and at the rate of 80-100ml per bud using spotgun delivery. These were injected when bud is at 12 o' clock position to avoid entry of banana flower thrips (*Thrips hawaiiensis*).

Bunch spray was done by mixing Imidacloprid (Confidor® SC) and Mancozeb (Dithane® M-45 fungicides) and sprayed 3-4 days after bud injection to control Red rust thrips (*Chaetanaphothrips signipennis* (Bagnall)). Peel scarring weevils (*Philicoptus iliganis)* and fruit diseases.To avoid miscounted of weeks to harvest, blue crayon was used to label the upper pseudostem indicating the date of delivery.

Deflowering and Defingering

Deflowering and defingering were done one week after bud injection. This were done to performed the fruit obstruction removal by removing the abnormal, center-layered , twins, malformed and excess fingers, flowers and false hands to ensure good hand formation by hand picking and detaching the mature and wilted flowers to avoid insect infestations and reduces abrasion injury.

Bagging and Insertion of plastic hand separator

The impregnated bunch-cover plastic bags were placed in an invidual bunch to protect from flying insects and plastic hand separator was inserted in the whorl hands to prevent point scars.

Harvesting

Eleven weeks after flower initiation bunches were cut using a harvesting knife, each bunch was label using red crayon indicating the treatment and replication for easy identification during packing and weighing of individual bunch.

Data Gathered

Zone of Inhibition (mm)

Zone of inhibition of *Fusarium oxysporum* f. sp. *cubense* in the presence of Actinomycetes was determined 7 days after incubation at 28-30°C.

Population of Actinomycetes in the soil before and after treatment application

Population count of Actinomycetes in the soil was determined using standard dilution technique then plated in Yeast Malt Extract Agar (YMA). Colony count was done following 4 days of incubation at 28-30°C. The Actinomycetes population expressed in colony forming units (cfu mL^{-1}) was computed using the formula below:

$$\text{Population of Actinomycetes (cfu /mL)} = \frac{\text{Average Count x Dilution factor}}{\text{Volume Plated}}$$

Percent (%) Wilt Incidence

The per cent Fusarium wilt incidence was determined by counting and recording the number of plants that were infected with Fusarium wilt throughout the experimental period and computed using the formula below;

% Fusarium wilt incidence =
Number of plants infected x100
Total no. of plants/treatment per replicate

Plant Height (cm)

Plant height was measured from the base up to the candle leaf every other week. This was taken from all plants per treatment per replicate.

Pseudostem Girth (cm)

Pseudostem girth of 'Cavendish' banana was measured six months after transplanting by measuring the girth of the intact pseudostem 1meter above the ground.

Yield (in bunch)

Data on yield was taken by counting all bunches harvested per treatment per replicate. Harvesting was done at 11 weeks after flower initiation.

Severity of *Fusarium* Wilt in "Cavendish' banana treated with Actinomycetes products against *Foc* TR4

Rhizome discoloration index (RDI)

Rhizome discoloration index (RDI) was also recorded at the termination of the trial. The rhizome of the infected plants was cut across to assess discoloration.

RATING	DESCRIPTION
0	No discoloration of stellar region of rhizome
1	Discoloration at junction of root and rhizome
2	Traces to 5 % of stellar region discolored
3	6-20 % of stellar region discolored
4	21-50 % of stellar region discolored
5	More than 50 % of stellar region discolored
6	Discoloration of the entire rhizome stellar
7	Dead plant

3. RESULTS AND DISCUSSIONS

Test for Antagonism of Actinomycetes to *Foc* TR4

The antagonism of Actinomycetes to Foc TR4 was evaluated *in vitro* using the "Lawn" method. The zone of growth inhibiton of Foc TR4 due to the presence of Actinomycetes was measured seven days after incubation at 28-30°C.

Table 1. Inhibition of growth of *Fusarium oxysporum* f. sp. *cubense* by the three isolates of Actinomycetes on PDA. "Halo" zone measured seven days after incubation of culture at 28-32°C[1]

Actinomycetes	Zone of inhibition (mm)					TOTAL	MEAN[ns]
	I	II	III	IV	V		
AQ6	8.50	9.75	12.00	10.25	10.25	50.75	16.92
AQ30	13.70	6.10	5.00	3.75	6.75	35.30	11.77
AQ121	11.00	4.50	3.50	10.25	3.75	33.00	11.70
CV (%)							8.73

[1]Average of 5 plates per replicate

[2] Average zone of inhibition (mm) = Measurement (mm) of 2 narrowest and 2 widest "halo" radii starting from margin of mycelial plug / 4

The mycelial plug of Actinomycetes (AQ6, AQ30 and AQ121) cut from two days old culture in Yeast Malt Extract Agar inhibited the growth of *Foc* TR4 within "halo" zones measuring an average of 16.92 mm, 11.77 mm and 11.70mm, from the mycelial plug margins, respectively. This means that the Actinomycetes used in the set up produces antifungal product deterrent to the growth of Foc TR4. Actinomycetes are gram positive bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds, substrate mycelium and aerial mycelium. These bacteria are best known for their ability to produce antibiotics. Nanjwade et al., (2010) stated that Actinomycetes produce an enormous variety of bioactive molecules that can be sorted into several major structural classes such as amino glycosides (e.g. streptomycin and kanamycin), ansamycins (e.g. rifampin), anthracyclines (e.g. doxorubicin), β-lactam (e.g. cephalosporins), macrolides (e.g. erythromycin), and tetracycline. These compounds are able to kill or inhibit gram negative microorganisms.

The actinobacterial strains that showed the ability to produce antimicrobial compounds belonged to Streptomyces (53%), Micromonospora (13%) and Actinomadura (10%) genera (Indian Journal of Pharmaceutical Science, 2011).

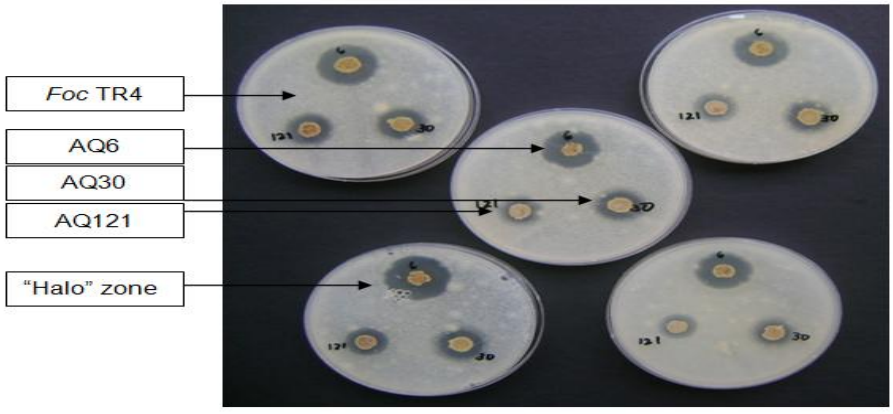

Fig. 4. Test for antagonism of AQ6, AQ30 and AQ121 Actinomycetes against *Foc* TR4 using "Lawn" method. Note the wider "halo" zones between AQ6 and *Foc* TR4.

Papa, et. al, 2013 screened 199 Actinomycetes and these three isolates they coded as AQ6, AQ30 and AQ121 demonstrated antifungal activities higher than 18mm zones of inhibition against black leaf streak of banana/ black Sigatoka caused by *Mycospharella fijiensis* and Sheath blight of corn caused by *Rhizoctonia solani.*

Actinomycetes Population Count (cfu/ml) on the Soil

The assessment of population of Actinomycetes in the experimental area were done at 120, 160 and 330 days after last treatment application(DALTA). These were done using serial dilution.

Table 2. Actinomycetes population count (x10^{5}cfu/ml) in the experimental area during the three soil sampling periods[1]. This were done 120, 160 and 330 DALTA in Barangay Kinamayan, Sto. Tomas, Davao del Norte.

Actinomycetes	[2](CFU/ml^{-1}) x 10^5		
	120DALTA*	160DALTA**	330DALTA^{ns}
Control	0.71^b	2.10^b	7.83
Pre+Post Planting Treatment	3.52^a	1.33^c	6.17
Post Planting Treatment	3.30^a	5.83^a	5.67
HSD (α=0.05)	1.37	1.64	-
HSD (α=0.01)	-	2.94	-
CV (%) =	42.10	40.94	34.95

Means having the same letter superscript are not significantly different at 5% or 1% level of significance using HSD.
[1]Average of 5 plates per replicate
[2]Cfu of Actinomycetes/ml = $\frac{\text{Average count x dilution factor}}{\text{Volume Plated}}$

Results showed that pre+post planting treatment and post planting treatment statistically comparable in terms of Actinomycetes population. This means that Actinomycetes applied 7, 14, 30 and 60DAT where found at most the same numbers and significant compared to control, no Actinomycetes treatment at 120 days after treatment application (DATA). Moreover, 160DATA post planting obtained the highest Actinomycetes population because of additional application of Actinomycetes at 60DAT but crossing over of the Actinomycetes was observed in control, no Actinomycetes treatment and pre+post planting treatment during heavy rains (rain splashed) and transfer of overshoes from one block to another. Likewise, the crossing overs of the antagonists were observed and true to all treatments at 330DATA (Table 2).

Spread can also occur in infested soil adhering to vehicles and footwear. There is reason to believe that infested soil adhering to the feet of animals is another way by which spread occurs. The fungus can move over short distances within an infected area of a property through the interconnecting network of roots and in surface water (http://www.nt.gov.au).

Percent (%) Wilt Incidence of *Fusarium* Wilt
'Cavendish' Banana Applied with Actinomycetes

The efficacy of Actinomycetes against *Fusarium* wilt in 'Cavendish' banana was assessed using percent (%) incidence of *Fusarium* wilt as a parameter.The putative biological control agent applied at the rate of 1kg formulated Actinomycetes per 1.5 liter water was used as pre+post planting treatment, and post-planting treatment only in the field naturally infested with *Foc.*

Banana plants that were treated with Actinomycetes before transplanting at transplanting and at 7 and 14 days after transplanting(DAT) showed symptoms of *Fusarium* wilt 8 months after transplanting (MAT). Banana plants that were treated with Actinomycetes before transplanting at transplanting and at 7 and 14DAT showed *Fusarium* wilt 6MAT. However, on the eight to eleven months old, *Fusarium* wilt was observed higher in banana with pos-planting treatment of Actinomycetes with a mean of 45%, likewise with the control having a mean of 36.67% but those in the pre-planting treatment had only 18.33%. The mean differences however were not statistically significant.

Table 3. Percent (%) incidence of Fusarium wilt on 'Cavendish' banana applied with formulated Actinomycetes at the rate of 1 kg/1.5 L water as pre + post-planting and post-planting treatment alone. The experiment was conducted at Brgy. Kinamayan, Sto. Tomas Davao del Norte from April 13 to February 20, 2014.

Actinomycetes	% Fusarium wilt incidence[1]			Total	Mean[ns]	% Red,n of Fw[3]
	I	II	III			
Control	20.00	40.00	50.00	110.00	36.67	0.00
Pre- + post-planting Treatment	15.00	10.00	30.00	55.00	18.33	50.00
Post-planting Treatment	45.00	15.00	75.00	135.00	45.00	-22.00
CV (%)					45.80	

[1] Data based on 20 plants per treatment per replicate

[2] % Fusarium wilt Incidence = $\frac{\text{No. of plants with Fusarium Wilt}}{\text{Total no. of plants per treatment}} \times 100$

[3] % Reduction of Fw = $\frac{\text{Incidence in Control} - \text{Incidence in Treated}}{\text{Incidence in Control treatment}} \times 100$

ns – not significant

While the *in vitro* test of the Actinomycetes against *Foc* demonstrated a high degree of antagonism, the Actinomycetes was only able to reduce occurrence of *Fusarium* wilt in 'Cavendish' banana by 50% compared to the plants that were untreated and only when it was applied twice as pre-planting and thrice as post planting treatment. It should be noted that the post-planting application of Actinomycetes to the 'Cavendish' banana seedlings resulted in numericallyhigher number of plants with *Fusarium* wilt.

The results of this experiment demonstrated that the efficacy of the biological control agents is affected by the natural soil microflora, soil factors, climatic factors and its interaction with the host. Actinomycetes is an endophytic bacteria that have been reported to inhibit the growth of soil-borne plant pathogens through the production of inhibitory antifungal metabolites (Coombs et al, 2004 as cited by El-Tarabily et al, 2008), siderophores (Cao et al, 2005 as cited by El-Tarabily et al, 2008) and induction of systemic resistance (Conn et al, 2006 as cited by El-Tarabily et al, 2008).

Sivasithamparam (2006) and El-Tarabily, 2003 as cited by El-Tarabily et. al, 2008, also reported that Actinomycetes in the rhizopheres are competent and adapted to endophytic life in root cortices. Moreover, actinomycetes that showed potential to suppress soil-borne

fungal plant pathogens, are able to employ one or more mechanisms of antagonism including antibiosis, hyperparasitism and the production of cell-wall degrading enzymes.

Plant Height (cm)

The plant height of 'Cavendish' banana as affected by pre-planting and post-planting treatments of Actinomycetes was measured 6 months after transplanting.

Results showed that the height of 'Cavendish' bananas were similar regardless of the treatment.

This shows that the application of Actinomycetes to bananas have no effect on the plant growth. This is an indicator that Actinomycetes can be a good potential antagonist/biocontrol agent since growth of 'Cavendish' banana is not affected by its application.

Table 4. Plant height(cm) of 'Cavendish' banana applied with formulated Actinomycetes at the rate of 1 kg/1.5 L water as pre + post-planting and post-planting treatment alone. The experiment was conducted at Brgy. Kinamayan, Sto. Tomas Davao del Norte from April 13 to February 20, 2014.

Actinomycetes	Plant height (cm)[1]			Total	Mean[ns]
	I	II	III		
Control	316.20	294.88	307.30	918.38	306.13
Pre- + post-planting Treatment	211.91	334.92	343.92	890.57	296.86
Post-planting Treatment	309.52	295.63	307.10	904.25	301.42
CV (%)					12.53

[1] Based on the 163 of surviving banana plants at 180 days from transplanting.

Pseudostem Girth (cm)

The pseudostem of 'Cavendish' banana treated with Actinomycetes as pre-planting and post-planting treatments was measured 180 days after transplanting.

Results showed that the girth of the 'Cavendish' bananas did not vary with a mean that ranged from 36.13cm to 40.43cm (Table 4). Pseudostem girth is another parameter for determining growth. With this data in can be inferred that the putative biocontrol agent, Actinomycetes, applied to 'Cavendish' banana did not hinder its growth, a desirable characteristics of a biocontrol agent.

Table 5. Pseudostem girth (cm) of 'Cavendish' banana applied with formulated Actinomycetes at the rate of 1 kg/1.5 L water as pre + post-planting and post-planting treatment alone[1]. The experiment was conducted in Brgy. Kinamayan, Sto. Tomas Davao del Norte, April 13 to February 20, 2014.

Actinomycetes	Pseudostem girth (cm)[1]			Total	Mean[ns]
	I	II	III		
Control	38.60	38.40	42.00	119.00	39.67
Pre- + post-planting Treatment	47.80	36.90	47.50	121.30	40.43
Post-planting Treatment	42.90	45.70	34.40	108.40	36.13
CV (%)					15.24

[1]Average of 18 surviving plants per treatment per replicate.
[ns]- not significant

Yield (in bunch)

The bunch yield of 'Cavendish' banana treated with Actinomycetes as pre-planting + post-planting and post-planting treatments only, was measured 330DAT.

Results of Analysis of Variance revealed not significant differences on the number of banana bunch that were harvested in all the three treatments. From bananas that were treated with Actinomycetes twice before and thrice after planting, an average 5.67 bunches out of 20 hills that were planted. The bananas that were untreated had an average of 2.33 bunches while those that were applied with Actinomycetes four times after planting produced an average of 1.67 bunches.

The harvested bunches have no direct effect on percent(%) wilt incidence as of second harvest. Moreover, even in severe cases, *Foc* may even enter the leaf petioles and the peduncle (bunch stalk) of bunched plants. However, infection has not been shown to progress into the fruit (Ploetz and Pegg, 2000). Actinomycetes interact with plant roots and consequently influence plant health and soil fertility (Kloepper and Schroth, 1978) but contradicted to powerful technology to enhance plant growth and yield as reported by Glick (1995); Yeole and Dube (1997) and Glick et. al (1999).

Table 6. Yield (in Bunch) of 'Cavendish' banana applied with formulated Actinomytes at the rate of 1 kg/1.5 L water as pre+post-planting and post-planting treatment alone1. The experiment was conducted in Brgy. Kinamayan, Sto. Tomas, Davao del Norte, April 13 to February 20, 2014.

Actinomycetes	Yield (in Bunch)[1]			Total	Mean[ns]
	I	II	III		
Control	4.00	0.00	3.00	7.00	2.33
Pre- + post-planting Treatment	2.00	11.00	4.00	17.00	5.67
Post-planting Treatment	2.00	1.00	2.00	5.00	1.67
					11.10
CV (%)					

[1]Bunch yield based on the standing healthy plants at harvest.
Data were transformed using square root of transformation.
[ns] – not significant

Severity of *Fusarium* Wilt in 'Cavendish' banana treated with Actinomycetes products against *Foc* TR4

Pseudostem and Rhizome Discoloration

The RDI of 'Cavendish' banana as affected by pre+ post planting and post-planting treatments of Actinomycetes in Brgy. Kinamayan, Sto. Tomas, Davao del Norte is presented in Table 7.

Table 7. Rhizome Discoloration Index (RDI) of 'Cavendish' banana applied with formulated Actinomycetes by pre + post-planting and post-planting treatment in Brgy. Kinamayan, Sto. Tomas Davao del Norte, Philippines[1]. 2014.

Actinomycetes	Rhizome Discoloration Index (RDI) [1]			Total	Mean*
	I	II	III		
Control	5.60	6.40	6.80	18.80	6.27[b]
Pre- + post-planting Treatment	4.60	5.00	5.60	15.20	5.07[a]
Post-planting Treatment	6.60	6.00	7.40	20.00	6.67[b]
HSD ($_{0.05}$) =					0.87
CV (%) =					6.23

[1]Rhizome Discoloration Index (RDI) of five plants per treatment per replicate 330 DAT: 0) No discoloration of stellar region of rhizome; 1) Discoloration at junction of root and rhizome; 2) Traces to 5 % of stellar region discolored; 3) 6-20 % of stellar region discolored; 4) 21-50 % of stellar region discolored; 5) More than 50 % of stellar region discolored; 6) Discoloration of the entire rhizome steler; and 7) Discoloration of the entire rhizome stellar.
[2]Means having the same letter superscript is not significantly different at 5% level of significant using HSD.

Cavendish' banana plant infected with *Foc* was cut 330DAT. This was done by cutting across and in the middle along with the pseudostem to determine the presence of vascular discoloration and to assess the *Fusarium* wilth severity following the Rhizome Discoloration Index (RDI) demonstrated by Brake et.,al (1995).

Results revealed that 'Cavendish' banana plant treated with actinomycetes at the rate of 1kg/1.5 L of water at 7, 14, 30 and 60 DAT obtained the highest disease severity with RDI of 6.67 which is statistically comparable to control, no application of actinomycetes product with RDI of 6.27 (more than 50% of stellar region discolored). The lowest disease severity with RDI of 5.07 (21-50% of stellar region discolored) was observed in 'Cavendish' banana that received pre+post planting treatment (7 and 14 DAT) with actinomycetes.

Result of Analysis of Variance revealed significant in terms of RDI. This showed significance compared to control. Symptoms in all treatments in terms of rhizome discoloration appearance were visible in control and less severe on pre+post and post planting treatment with actinomycetes.

This fungus enters only through the roots and grows and then sporulates abundantly in xylem vessels. The transport of the spores upward in the transpirational stream facilitates the fungal invasion of the entire vascular system. The growth of the fungus blocks the vascular system, causing the plant to wilt. *Fusarium* Wilt is the most and notorious disease of banana and the pathogen stays for more than 30 years in the soil (Ploetz and Pegg, 2000).

Occasionally, the discoloration first appears yellow in plants showing early stages of infection. When a cross-section is cut, the discoloration appears in a circular pattern around the center of the rhizome where the infection concentrates due to the arrangement of the vessels. As symptoms progress into the pseudo-stem, continuous lines of discoloration are evident when the plant is cut longitudinally. The infection may travel all the way up to the top of the pseudo-stem (Ploetz and Pegg, 2000).

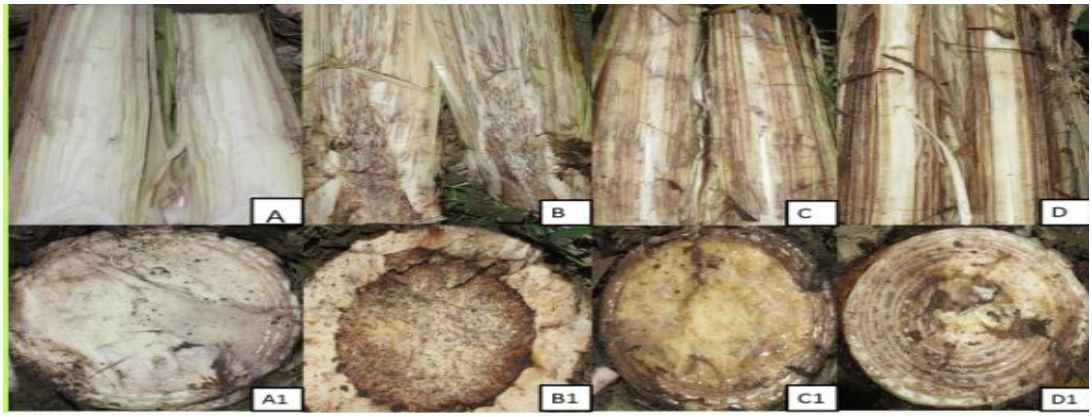

Figure 5. ections of pseudostems and rhizomes of 'Cavendish' banana treated with actinomycetes products and cut 330DAT. A and A1 – Healthy plants, B and B1 – contro (untreated with actinomycetes), C and C1 – pre+post planting treatment and D and D1 – post planting treatment. Note: The presence of greater rhizome discoloration appeared in B and B1.

CONCLUSION

The test for antagonism of Actinomycetes (AQ6, AQ30, and AQ121) to *Foc* TR4 were statistically comparable. On the other hand, actinomycetes population count ($x10^5$cfu/ml) in the experimental area during the three soil sampling periods, these were done 120, 160 and 330 DALTA. The first actinomycetes population count were significant and highly significant in second count while on the third count insignificant result were obtained since crossing over of the antagonist was also observed. Moreover, pseudostem and Rhizome discoloration index also found significant. However, all agronomic performances; plant height, pseudostem girth and yield were not significant, a good characteristics of a biological control since growth parameters were not affected.

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