DEVELOPMENT OF STRATEGIES FOR MANAGING FUSARIUM WILT OR PANAMA DISEASE OF BANANA

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ABSTRACT

A survey was conducted in Region XI to determine the incidence and distribution of Fusarium wilt disease. Infection was observed in Davao City and the provinces of Davao del Norte, Davao Oriental and Compostela Valley Province on cultivars Lakatan, Latundan, Abuhon, Cavendish and Cardaba. Highest incidence was observed on Latundan at 90%. Fusarium wilt infected plants exhibited yellowing of older leaves, splitting of pseudostem, petiole buckling, vascular discoloration of pseudostem and corm discoloration.

Pathogenicity test was conducted using VCG 01213 Foc isolate from infected Cavendish. Three techniques were compared to come up with an accurate and reliable screenhouse inoculation. The techniques were: (1) root dipping as described by Mohammed *et.al*.(1999); (2) spore pouring by Chao (2006); and (3) corn meal-sand inoculum by Magnaye *et al*. (1969). Twenty plantlets were used as replicates for each of the technique tested. Data from the infected plantlets were taken 1 to 5 weeks after inoculation. Total percentage infection and disease severity index (DSI) based on the leaf symptom index (LSI) and rhizome discoloration index (RDI) were compared for each of the technique used. Results showed that comparing the three techniques based on the computed DSI, the corn meal sand inoculum technique gave the highest percent infection of 90%. Highest LSI of 3.1 and highest RDI of 6.2 were also derived from plantlets inoculated using this technique.

Different control strategies were evaluated using the following treatments: (1) Use of introduced varieties (2) application of commercial microbial fertilizer (Probio Gold) in the nursery (3) application of commercial microbial fertilizer (ProbioGold) upon field infection, (4) application of *Trichoderma harzianum* at the nursery (5) application of *Trichoderma harzianum* upon infection and (6) application of Didecyl dimethyl ammonium bromide (Bestaquam) at planting. Results showed that 73% of the infected mother plants recovered one month after application of *T. harzianum* while 46% recovered one month after application of the microbial fertilizer. No infection was observed on the suckers of these plants.

Keywords: *banana, Fusarium oxysporum f sp. cubense,* microbial fertilizer

INTRODUCTION

Fusarium wilt or Panama disease of banana, caused by *Fusarium oxysporum f. sp. cubense* (E. F. Smith) Snyd. and Hans. (FOC) is considered as one of the most destructive diseases affecting almost all the banana growing regions of the world. The disease was first recognized in Australia in 1874.

In the Philippines, Fusarium wilt was first reported in 1920 by Lee and Serrano on cultivar Latundan which were planted in Los Baños and Calamba,Laguna and in the municipalities of Batangas (as cited by Magnaye 2001).

Lately, commercial productions of cultivars Latundan, Pitogo and Lakatan are being limited by the disease (Magnaye 2001).

Fsarium wilt-infected plants manifest the following symptoms: yellowing of foliage, splitting of psuedostem, changes in new leaves, petiole collapse and discoloration of vascular tissues.

The most devastating FOC belongs to VCG 01213 of the 'tropical' race 4, which is widespread in the islands of Indonesia and peninsular Malaysia.

Currently, there had been a report of its occurrence in the Philippines particularly in Mindanao.

At present the only effective means of controlling Fusarium wilt or Panama disease elsewhere is through the use of resistant cultivars.

In order to help small farmers harvest bananas despite the presence of the disease, control strategies have to be developed, hence this study.

OBJECTIVES

1. To survey and map out the incidence of Fusarium wilt or Panama disease of banana in Region XI.
2. To identify the races and VCGs of *Fusarium oxysporum f.sp. cubense* isolates in Region XI.
3. To determine the virulence of the isolated *Foc* in Region XI using identified susceptible banana cultivars.
4. To compare the inoculation techniques by pathogenicity test of *Foc.*
5. To identify banana cultivars tolerant/resistant to the isolated *Foc.*
6. To develop effective control strategies against Fusarium wilt or Panama disease
7. To identify the most effective control strategy for recommendation to farmers.

MATERIALS AND METHODS

Survey

A survey was conducted in all provinces of Region XI namely Compostela Valley, Davao del Sur, Davao del Norte, Davao Oriental and Davao City where banana grows. In the conduct of the survey, coordination and assistance from the local government units (LGUs) was requested for them to provide guides in going to farms within their areas of jurisdiction. Farmers were interviewed based on questionnaires. The field was surveyed for the incidence of Fusarium wilt and the plants which exhibited typical symptoms with that of the Panama wilt were collected and brought to the laboratory for isolation. Areas in Region XI where Fusarium wilt or Panama disease was found was mapped out.

Collection of samples

Infected banana plants were collected in the field. Discolored strands were excised from the tissues of the pseudostem of the plant. Strands were placed in a sterile envelop with strips of paper and after 3 days strips were removed and transferred to another envelop with sterile filter paper. Duplicate samples were sent to FABI, South Africa for VCG analysis.

Virulence Test

The establishment of the experiment was done under the greenhouse condition. Identified local cultivars were inoculated following the procedure of Roperos and Magnaye (1969). Regular monitoring of inoculated test plants was done as to its symptom development by recording the number of days by which the test plants showed external symptoms like yellowing of leaves, buckling etc. The rhizome discoloration of the plant was recorded and the extent of discoloration of the plant parts. Corm discoloration was also rated based on the rating scale of 1-6 (INIBAP Technical Guidelines).

Comparison of Inoculation Techniques by Pathogenicity Test

Three techniques were compared, namely: root dipping by Mohammed et. al. 2001, spore pouring by Chao 2006 and use of corn meal inoculum by Roperos and Magnaye 1969.

Two trials were conducted for this experiment.

Test Plants

Two month old tissue cultured plants of cultivars Cavendish, Lakatan and Latundan were obtained from the Tissue Culture Laboratory and the National Repository, Multiplication and Distribution Center of BPI-DNCRDC.

Source of Inoculum

Fusarium oxysporum f. sp. cubense was obtained from identified TR4 infected Cavendish banana plants.

Comparison of Inoculation Technique

Technique 1 (Root Dipping) (Mohammed et. al. 2001)

The infected strands of Cavendish banana plants were placed on plated Potato Dextrose Agar (PDA) amended with Streptomycin. Single spores were transferred to a new medium to obtain pure culture of the fungus. The inoculum was then transferred in to flat bottles containing PDA. Cultures were incubated at room temperature (28^0C) for seven days. Inculum of 6 x 10^6 spores/ml was prepared with the aid of a Haemacylometer and was used immediately for root dipping inoculation.

For this trial only Cavendish plantlets were used. Twenty plantlets which were two months old were carefully uprooted and those with healthy white roots were selected and the roots were immersed in the conidial suspension for two hours before replanting to a tray with sand as medium and roots of twenty plantlets were immersed in sterile distilled water to serve as control. Plantlets were maintained inside the screen house and observed until the 5^{th} week after inoculation. The plantlets were then rated accordingly.

Technique 2 (Spore Pouring) (Chao 2006)

Freshly collected infected pseudostem tissues of Cavendish were mixed with the infested soil collected directly from the Fusarium affected area and placed in a container and seated for one month to produce abundant spores including macronidia and chlamydospores for inoculation. The spore density per gram of the mixture was determined using a Haemacylometer. Approximately 6 x 10^6 spores/ml was used for inoculation. Two ml of spore suspensions were poured into pots with *Foc* free soil planted with 2 months old Cavendish plantlets watered one day before inoculation. Twenty plantlets were used as test plants and twenty plantlets served as control. Observations were done daily up to the 5^{th} week after inoculation.

Technique 3 (Corn meal sand inoculum) (Roperos and Magnaye 1969)

The infected strands of Cavendish banana plants were placed onto the plated Potato Dextrose Agar (PDA) amended with Streptomycin. The inoculum used for pathogenicity was grown in corn meal sand medium. The mixture consists of 1:20 of finely ground yellow corn and fine sand autoclaved for one hour. Two hundred grams of 23-day old culture grown in corn meal sand medium was evenly scattered around the

root area of 2-month old tissue culture Cavendish plantlets. Sterilized soil media was added to level the soil surface and to cover the exposed roots. Twenty plantlets were inoculated. Twenty plantlets which were un-inoculated served as the control. Observation on the plants was done daily to the 5th week after inoculation.

Rating Scale

In the first trial, the rating scale used by Mohammed et al. was followed on the external and internal symptoms of the test plants.

Evaluation was done using the following rating scale:

Leaf Symptom Index (LSI)

1- No streaking or yellowing of leaves. Plant appears healthy

2- Slight streaking and/or yellowing of lower leaves

3- Streaking and/or yellowing of most of the lower leaves

4. Discoloration of the younger leaves maybe just beginning to appear

4- Extensive streaking and/or yellowing on most or all of the leaves

5- Dead plants

Rhizome Discoloration Index (RDI)

1- No discoloration of tissue of stellar region of rhizome or surrounding tissue

2- No discoloration of stellar region of rhizome, discoloration at junction of roots and rhizome

3- Trace of 5% of stellar region discolored

4- 8-20% of stellar region discolored

5- 21-50% of stellar region discolored

6- More than 50% of stellar region discolored

7- Discoloration of the entire rhizome stele

8- Dead plants

In the second trial, the INIBAP Technical Guidelines was followed for rating the external symptom:

1- Symptom absent

2- Symptom present

Internal Symptom rating or corm rating:

1- Corm completely clean, no vascular discoloration

2- Isolated points of discoloration in vascular tissue

3- Discoloration of up to1/3 of vascular tissue

4- Discoloration of between 1/3 and 2/3 of vascular tissue

5- Discoloration of greater than 2/3 of vascular tissue

6- Total discoloration of vascular tissue

Pseudostem of inoculated plantlets were cut lengthwise to measure the vascular tissues and measure the length of discoloration.

The best technique identified among the 3 tested was used in the pathogenicity test using the cultivars Cavendish, Lakatan and Latundan.

Selection of Site for Field Experiment

An area known to be infected with Tropical Race 4 *Foc* was for field experiment. Field establishment was done and the management as to the care and maintenance of the plants was also done. The cultivars were planted to the identified area and evaluated their tolerance or susceptibility to the disease. Test plants were monitored regularly and diseased development was recorded.

Application of Control Strategies against Fusarium wilt on Banana at Field Condition

Preparation of Experimental Plants

Cavendish (Williams type) was used as experimental plants obtained at the Tissue Culture Laboratory of the Bureau of Plant Industry, Bago Oshiro, Davao City. In-vitro propagated plantlets were transferred to potting trays filled with decomposed coir dust and covered with cellophane for acclimatization. After 2 weeks from potting trays, plantlets were removed and transferred to 6 cm x 8 cm black polyethylene plastic bags individually filled with bagging media. The plantlets were kept in the screen house for two months.

Experimental Design and Field Lay-out

A two-month old Cavendish tissue culture-derived banana plantlets were planted at the experimental site. The plants were arranged in a Ransomized Complete Block Design (RCBD) with 6 treatments with 10 plants per treatment replicated 3 times. The planting distance for the experimental plants was 2.4 m x 1.8 m. Each plant was tagged according to the control strategies to be applied.

Application of Control Treatments

Six (6) treatments were used for the experiment. These are the following:

Control - No treatment

Treatment 1 - Planting of introduced varieties

Treatment 2 - Application of commercial microbial fertilizer (CMF) (ProbioGold) at the nursery

Treatment 3 - Application of commercial microbial fertilizer (CMF) (Probiogold) upon field infection

Treatment 4 - Application of *Trichogramma harzianum* at the nursery

Treatment 5 - Application of *Trichoderma harzianum* upon field infection

Treatment 6 - Didecyl Dimethyl Ammonium Bromide (DDAB) (Bestaquam) application at planting

Application of Commercial Microbial Fertilizer (ProbioGold) at the Nursery

Microbial fertilizer was applied at potting period. It was mixed together with the pure coir dust at a ratio of 1:1, one part for coir dust and 1 part microbial fertilizer. At bagging, individual bags were applied with microbial fertilizer with a ratio of 5:10, 10 parts decomposed coir dust and 5 parts microbial fertilizer.

Application of Commercial Microbial Fertilizer (ProbioGold) upon Field Infection

When infection was observed in the field, microbial fertilizer was applied by broadcasting it around the infected plant at the rate of 1.5 kilos for first application and 1.5 kilos four months after.

Application of *Trichoderma harzianum* at the Nursery

Tissue culture derived Cavendish (Williams) were planted in potting media containing 10 parts rice bran with 1 part yellow cracked corn inoculated with *Trichoderma harzianum* and pure decomposed coir dust. At planting to individual bags, the plantlets were placed with bagging media which contained 1 kilo cracked corn inoculated with *Trichoderma harzianum* and 10 kilos decomposed coir dust.

Application of *Trichoderma harzianum* upon Field Infection

When infection was observed in the field, *Trichoderma harzianum* grown in cracked corn and mixed in rice bran was broadcasted around the infected plant at the rate of 2 kilos for first application and 2 kilos 4 months thereafter.

Application of Didecyl Dimethyl Ammonium Bromide at Planting

At planting 50 ml of the solution was poured around the banana plants.

Maintenance of the Experimental Plants at Screenhouse Condition

The experimental plants were given equal application of Potassium humate at a rate of 1 gram per 1 liter of water and complete fertilizer as basal application with a ratio of 1 g per plant. Application was done every two weeks. Watering of the plants was also done.

Cultural Management in the Field

All of the experimental plants were given equal care like deleafing, de-suckering, weeding and de-belling. Fertilizer was applied 4 weeks after planting in the field. Fertilizers applied were Urea, Complete and Potash.

Data Collection

Monitoring in the field was done three months after planting. Plants which showed infection were recorded and the application of treatments was done. The recurrence of the disease was also recorded according to the treatments applied.

Assessment of Control Strategies

Regular monitoring of the plants for disease incidence and progress of infection was done. The most effective, practical and economical control strategies applied was identified. Regular monitoring and determination of the disease recurrence in the area was done as part of the assessment.

RESULTS AND DISCUSSION

Survey

The survey was conducted in Davao City and the different provinces in Region XI namely Davao del Sur, Davao del Norte, Davao Oriental and Compostela Valley Province. Coordination with the different Provincial Agriculturists was done in going to the different areas of their jurisdiction. Agricultural Technicians served as the guides in going to the areas surveyed. Farmers were interviewed based on the questionnaires provided by INIBAP. Figure 1 shows three of the banana farms surveyed where Fusarium wilt was observed.

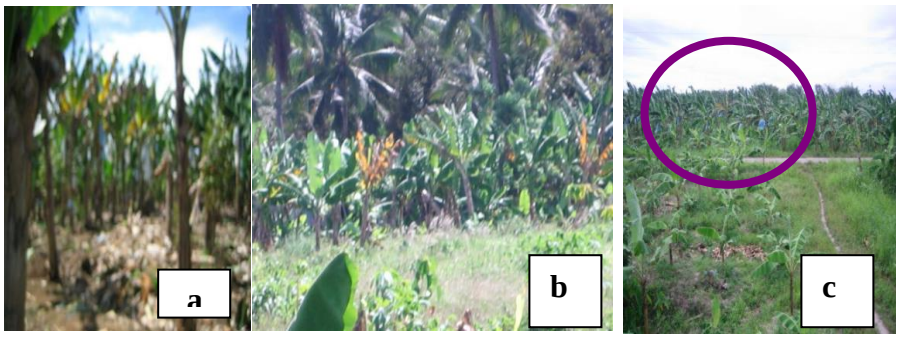


Figure 1. (a) Lakatan (Tamayong, Davao City), (b) Cavendish (Wines, Davao City) and (c) Latundan (Tagum City) farms with Fusarium wilt disease.

A total of 201 barangays from 41 municipalities were visited and 691 farmers interviewed from Davao City and the four Provinces of Region XI. Table 1 shows the areas surveyed for Fusarium wilt on banana

Table 1. Areas surveyed in Region XI

Province	No. of Municipalities	No. of Barangays	No. Barangays with Fusarium Wilt Incidence	No. of Respondents
Davao City		34	23	165
Davao del Sur	8	37	0	140
Davao del Norte	13	38	4	151
Davao Oriental	11	47	3	36
Compostella Valley	9	45	11	199
TOTAL	**41**	**201**	**41**	**691**

In Davao City banana cultivars like Cardaba, Latundan, Cavendish, Pisang Mas and Lakatan were observed to be infected with the disease. As high as 80% infection was observed on the Latundan cultivar in barangay Malagos and as low as 3% disease infection was observed in barangay Atan-awe. The Cavendish cultivar was observed to have a low percentage infection at 5% and .09% in the barangays of Sirib and Manambulan, respectively. Lakatan was observed to have as high as 22% infection in barangay Tamayong and the lowest percentage infection at 10% was observed in barangay Tibuloy. In Cardaba, the highest percentage infection was only 5.82% which was observed in barangay Ilam and the lowest was 0.36%, as observed in barangay

New Carmen. In Pisang Mas only barangay Eden was observed to have Fusarium wilt infection at 1.14% (Table 2).

Table 2. Banana cultivars observed with Fusarium wilt in the barangays of Davao City.

Barangay	**Cultivar Grown**	**Total Mats**	**Plants infected**	**Percentage Infection**
1. Tamayong	Lakatan	9,000	2,000	22.22
	Cavendish	8,250	300	3.64
2. Sirib	Cavendish	10,000	500	5.00
3. Wangan	Cardaba	410	3	0.73
4.Upper Lacson	Lakatan	12,185	21	0.17
	Latundan	860	61	7.09
5. Dacudao	Lakatan	21,290	356	1.67
	Latundan	60	20	33.33
6. Eden	Pisang Mas	13,500	154	1.14
7.Gumalang	Lakatan	11,187	30	0.27
8. Ilam	Cardaba	550	32	5.82
9. Malagos	Lakatan	9,500	110	1.16
	Latundan	150	120	80.00
10. Baracatan	Latundan	25	15	60.00
11. Atan-awe	Latundan	200	6	3.00
12. Tibuloy	Lakatan	6,020	6	0.10
13. Catigan	Latundan	700	25	3.57
14. Talandang	Latundan	60	6	10.00
15. Tacunan	Latundan	400	20	5.00
16. Ula	Latundan	50	10	20.00
17. New Valencia	Cardaba	2,800	20	0.71
	Latundan	50	25	50.00
18. Biao Escuela	Cardaba	4,450	90	2.00
19.Manambulan	Cavendish	25,000	22	0.09
	Cardaba	400	20	5.00
20. Langub	Latundan	850	253	30.00

21. New Carmen	Cardaba	1,400	5	0.36
	Latundan	200	10	5.00
22. Wines	Cavendish	4,700	200	4.00
23. Tagluno	Latundan	5	1	20.00

In Davao del Sur, no incidence of Fusarium wilt was observed during the survey.

In Davao del Norte the highest percentage infection of 90% was observed in cultivar Latundan and lowest in Lakatan which is 0.16% only (Table 3). It shows that the infection varied with the particular variety infected.

Table 3. Banana cultivars observed with Fusarium Wilt in the Barangays of Davao del Norte

Municipality	Barangay	Cultivar Infected	Total Mats	No. of Plants Infected	Percentage Infection
1.. Tagum City	Mankilam	Cardaba	7,300	215	2.9
		Latundan	800	720	90.00
	San Miguel	Latundan	500	50	10.00
		Lakatan	12,175	20	0.16
	Magdum	Cardaba	3,000	60	2.00
2. Carmen	Cebulano	Latundan	530	35	6.60

In Davao Oriental, the disease was observed only from three barangays, namely Don Salvador and Don Enrique in Mati City and in barangay Central, Manay. Highest infection of 10% was observed on cultivar Latundan and the lowest infection on Cardaba with only 0.01% (Table 4).

Table 4. Banana cultivars observed with Fusarium Wilt in the barangays of Davao Oriental Province

Municipality	Barangay	Cultivar Grown	Total Mats	No. of Plants Infected	Percentage Infection
1.Mati	Don Salvador	Lakatan	2,580	5	0.19
		Latundan	350	35	10.00
	Don Enrique	Cardaba	6,200	65	1.05
2.Manay	Central	Cardaba	12,300	1	0.01

In Compostela Valley Province, Fusarium wilt was observed to be highest in barangay Hijo on cultivar Latundan with 16% infection and lowest infection was observed on Cardaba at 0.2%. Infection was observed in the barangays of Dumlan and Salvacion.

Table 5. Banana cultivars observed with Fusarium wilt in the barangays of Compostela Valley Province.

Municipality	Barangay	Cultivar Grown	Total Mats	Plants infected	Percentage Infection
1.Laak	Kilagding	Latundan	100	2	2.00
2.Maragusan	Coronobe	Cardaba	800	20	2.50
		Latundan	50	1	2.00
3.Mabini	Cabuyoan	Latundan	6,750	40	0.59
4.Maco	Sangab	Latundan	1,400	35	2.50
	New Visayas	Latundan	4,000	100	2.50

	Mapaang	Latundan	6,000	75	1.25
	Hijo	Latundan	100	16	16.00
	Dumlan	Cardaba	3,000	6	0.20
5.Monkayo	Union	Lakatan	18,000	1,000	5.56
	Salvacion	Cardaba	9,780	20	0.20
6.Mawab	Manipungol	Latundan	1,400	20	1.43

Symptoms of the disease like yellowing of leaves, wilting, splitting of the pseudostem and discoloration of the corm (Figure 2) were observed in the areas surveyed with Fusarium wilt .

Figure 3 shows the areas in Region XI where the disease was observed.

a **b** **c** **d**

Figure 2. Banana plant showing external (a and b) and internal symptoms (c and d) of Fusarium infection as observed in the field.

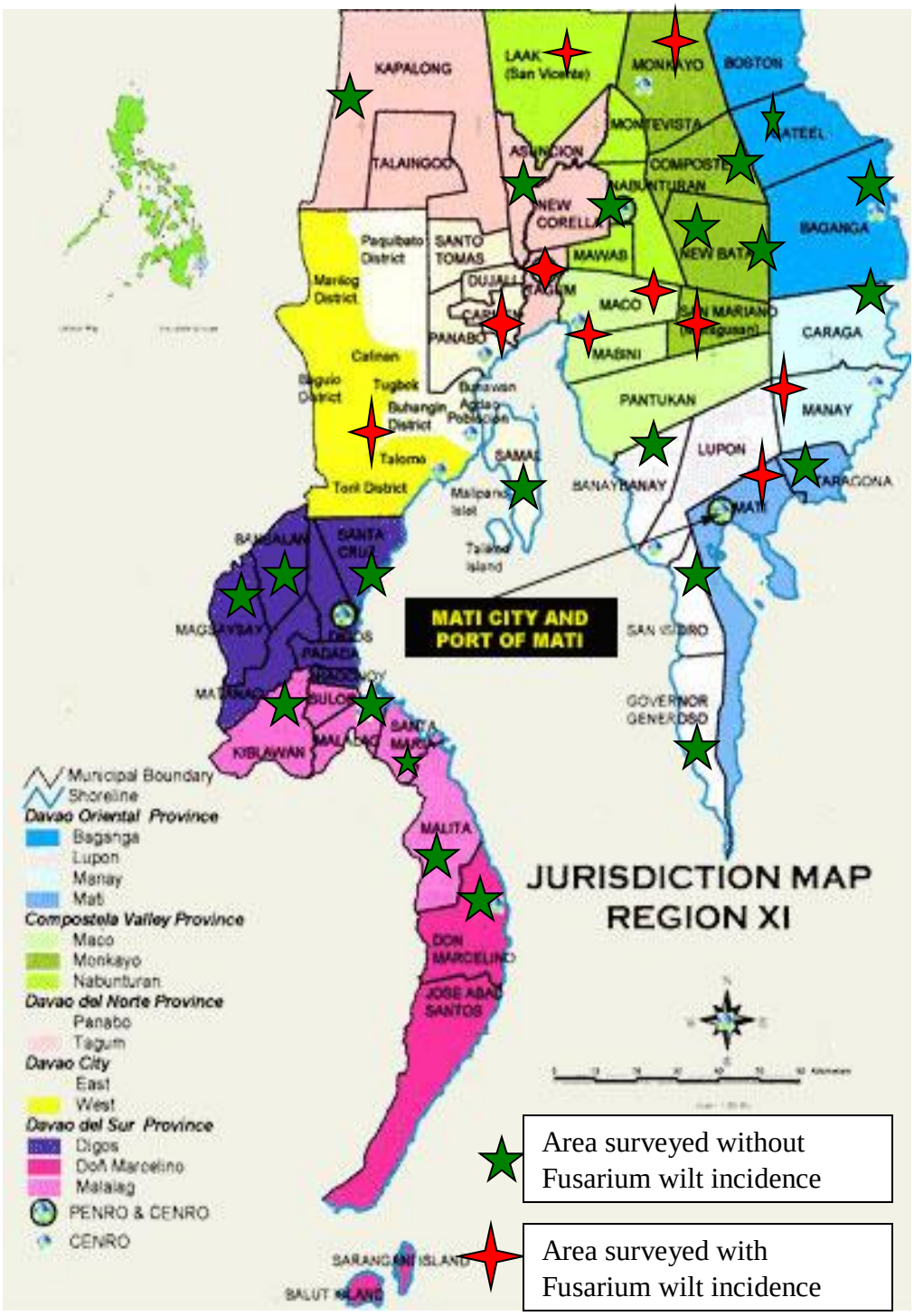


Figure 3.. Map of areas surveyed in Region XI

Collection of Samples

Discolored strands from the pseudostems of infected plants were collected. Samples were duplicated and sent to FABI, South Africa for VCG analysis. A total of 48 samples were collected from the different cultivars of infected banana plants from the various areas surveyed.

VCG Analysis

A total of 48 samples were collected from infected banana plants and were sent to Dr. Altus of FABI, South Africa. The samples were from different cultivars from the different areas surveyed (Table 6).

Table 6. Samples from different cultivars in areas of Region XI.

Place of Collection	Abuhon	Latundan	Lakatan	Cardaba	Cavendish
Davao City	1	8	8	5	5
Davao del Norte		2	1	4	
Davao Oriental		1	2	2	
Comval Province		4	1	4	
Total	1	15	12	15	5
Grand Total					**48**

Based on a national survey conducted by Magnaye in 1994-1997, Fusarium Wilt was present in Luzon, Visayas and Mindanao, with Latundan being highly susceptible followed by Lakatan.

The result of the VCG analysis done by Ken Pegg on the samples sent by Magnaye showed that most of the isolates were found to belong to VCG 0123 and has been regarded as being race 1 type pathogen. Only one isolate from Grand Naine belonged to race 4 VCG 0122. One isolate each from Cardaba and Latundan were also characterized as belonging to VCG 0126.

Comparison of the Three Inoculation Techniques on Pathogenicity Test

Disease symptoms were manifested by the inoculated Cavendish banana plantlets (Figures 4 and 5). Yellowing of the older leaves was first observed followed by petiole collapse and splitting of the pseudostem. Infection was observed on 8 inoculated plants one week after inoculation on the techniques of rot dipping and 7 inoculated plants with corn meal sand inoculum. Infection of inoculated plants were

observed up to the third week. In the spore pouring technique the infection on inoculated plants were observes on the second week after inoculation (Table 7).

Figure 4. External symptoms exhibited by the inoculated test plants.

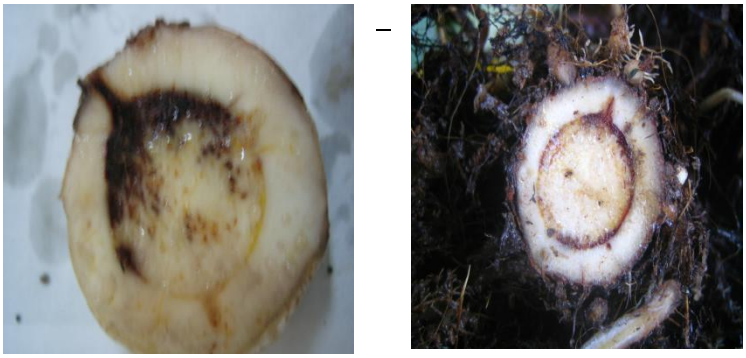


Figure 5. Rhizomes of inoculated plants showing discolorations

Table 7. Cummulative Percentage infection of TR4 on Cavendish plantlets from 1st to 5th week of inoculation.

Technique	*1st week*	*2nd week*	*3rd week*	*4th week*	*5th week*	*Total Plants infected*	*Percentage Infection*
Root dipping	8	16	18	0	0	18	90.0
Spore pouring	0	8	16	0	0	16	80.0
Corn meal sand inoculum	7	16	19	0	0	19	90.0

In both root dipping and corn meal sand techniques, pure culture of *Foc* were used as inoculum while in the spore pouring, the purity of the isolate was not determined since the pathogen was not isolated using artificial media. The roots of the test plants also had direct contact with the inoculum used. This observation is consistent with the previous observations that Foc enters only through roots. Other microorganisms might have grown and interacted with Foc.

After 5 weeks of inoculation, 90 percent infection using root dipping and corn meal sand techniques was obtained while with spore suspension pouring only 80 percent infection. This means that the ability of the pathogen to produce infection would be dependent on the technique being used. Higher infection was observed on the two techniques root dipping and corn meal sand inoculum while spore pouring had lower infection. The roots were also directly attached to the inoculum, thus observed to have higher infection.

At the end of the experiment, 19 plants were recorded to be infected using corn meal sand technique; 18 were infected using root dipping while only 16 were observed infected using spore pouring technique. Nelson and Toussoun (2006) observed that corn meal sand medium can be used for the mass production of inoculum of *Fusarium* spp. in large amounts to be added to the soil. The result of this experiment is corroborated by their observation.

The inoculated plantlets were observed to have different degrees of infection as computed in the Disease Severity Index (Table 8).

Table 8. Disease Severity Index (DSI) of cultivar Cavendish using the three techniques for inoculation of TR4.

Technique	*Number of plants inoculated*	*Number of plants infected*	**DSI**	
			LSI	**RDI**
Root dipping	20	18	2.6 [b]	4.8 [b]
Spore pouring	20	16	1.8 [b]	1.9 [b]
Corn meal sand inoculum	20	19	3.1 [a]	6.2 [a]

LSI- Leaf Severity Index; RDI – Rhizome Discoloration Index

In the second trial, it was observed that the three techniques had the same rating on external symptoms (Figure 6) but the internal symptoms (Figure 7), i.e. corm discoloration ratings were different using the INIBAP Technical Guidelines (Table 9).

Comparing the two rating scales used in each of the trials of the experiment, it was observed that the INIBAP Technical Guidelines was easier to use in terms of data

recording and the scale used in corm rating was better than that used by Mohammed *et al*. (2001).

Figure 6. Inoculated Cavendish showing yellowing of older leaves and splitting of psuedostem

Figure 7. Corm of Inoculated Cavendish showing discoloration

Table 9. Disease Rating of cultivar Cavendish using the three techniques.

Technique	Number of plants inoculated	Number of plants infected	Disease Rating	
			ES	CR
Root dipping	20	20	2 ^{ns}	3.4 ^{ab}
Spore pouring	20	20	2	2.9 ^{b}
Corn meal sand inoculum	20	20	2	4.0 ^{a}

ES - External symptoms; CR - Corm rating

The highest values of LSI (Leaf Severity Index) and RDI (Rhizome Discoloration Index) was also obtained using the corn meal sand inoculum, followed by root dipping. The least values were from the spore pouring technique.

Virulence Test

In the pathogenicity, three cultivars namely Cavendish, Lakatan and Latundan were tested using the best observed inoculation technique which is the corn meal sand inoculum.

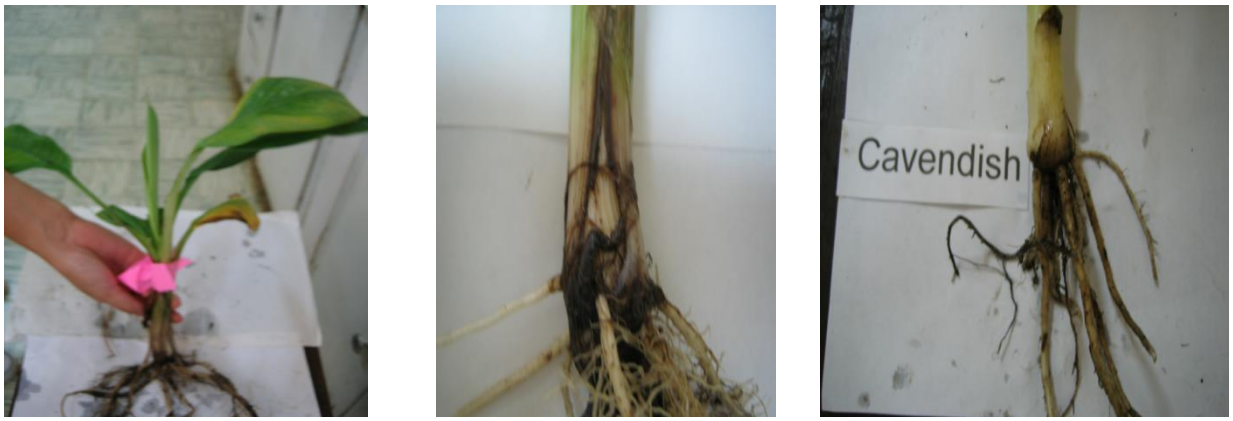
Table 10 shows the disease severity index of the different banana cultivars varied in their Leaf Severity Index (LSI) and Rhizome Discoloration Index (RDI).

Table 10. Disease Severity Index (DSI) of three banana cultivars using corn meal sand inoculum technique for inoculation of TR4.

Cultivar	*Number of plants inoculated*	*Number of plants infected*	**DSI**	
			LSI	RDI
Cavendish	20	20	3.1	6.4[b]
Lakatan	20	18	3.2	6.4[b]
Latundan	20	20	3.3	7.0 [a]

DSI - Disease Severity Rating; LSI – Leaf Severity Index; RDI – Rhizome Development Index

The highest LSI and RDI were observed on cultivar Latundan. External symptoms like yellowing of older leaves, splitting of the pseudostem were expressed by the inoculated plants typical to the plants infected in the field and is shown in Figure 8.



a **b** **c**

Figure 8. External symptoms of inoculated test plants of cultivars (a) Lakatan, (b) Latundan and (c) Cavendish

Rhizome discoloration of inoculated plantlets ranged from slight to severe, as shown in Figure 9.

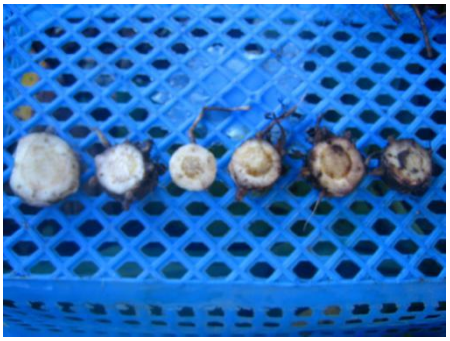


Figure 9. Degrees of discoloration on the rhizomes of inoculated plantlets

Pseudostems of inoculated plantlets were cut lengthwise to examine the vascular tissues and measure the length of discoloration (Figure 10). The length of vascular discoloration varied from each cultivar (Table 11). Latundan exhibited the longest vascular discoloration while Cavendish, the shortest.

a **b** **c**

Figure 10. Vascular tissues of inoculated *Lakatan* (a) *Latundan* (b) and uninoculated plantlet (c)

Table11. Average length of vascular discoloration (cm) on inoculated banana cultivars.

Cultivar	No.of inoculated plantlets	No of plants with vascular discoloration	Length (cm) of discoloration
Cavendish	20	6	7.0
Lakatan	20	5	9.6
Latundan	20	12	20.0

This means that young tissue-cultured plants can also be infected by a virulent pathogen especially if the cultivars are susceptible. Tissue culturing is not a guarantee of freedom from infection. On the contrary, they are easily infected when subjected to high inoculum pressure.

Symptom manifestation on the different cultivars inoculated increased with time. (Table 12)

Table 12. Cumulative weekly observations on the number of plants infected

Cultivar	N	1st week	2nd week	3rd week	4th week	5th week	Total
Cavendish	20	7	16	20	0	0	20
Lakatan	20	0	8	16	18	0	18
Latundan	20	8	16	20	0	0	20

The three cultivars exhibited external symptoms meaning external symptoms were present but the three cultivars varied in their internal corm rating where Latundan had the highest observed internal corm rating of 6 followed by Cavendish which is 3 and Lakatan a rating of 4. This shows the susceptibility of cultivar Latundan to the disease based on the corm rating. (Table 13)

Table 13. Disease Rating (DR) of three banana cultivars using corn meal sand technique by (2nd trial)

Cultivar	Number of plants inoculated	Number of plants infected	Disease Rating	
			ES	CR
Cavendish	20	20	2	3
Lakatan	20	7	2	2
Latundan	20	20	2	6

ES – External Symptom; CR – Corm Rating

Establishment of Experimental Plants in the field

A total of 180 three months old tissue-culture derived plantlets of cultivar Cavendish obtained from the Tissue Culture Laboratory of the Bureau of Plant Industry, Bago Oshiro, Davao City were planted in the field where Fusarium wilt infection is high. (Figure 11)

Figure 11. Establishment of experimental plants in the field.

Observation on the experimental plants in the field

External symptoms were observed on the plants as early as two months after planting particularly on the control plants. Table 14 showed disease incidence in the field after planting. Application of *Trichoderma* upon infection was also done to the plants that exhibited external symptoms at the rate of 2 kilos per mat and it was observed that 73% of the plants applied with *Trichoderma* (Figure 12) had recovered from yellowing of leaves (Figure 13) one month after application. No infection was observed on the introduced varieties in the field. It was also observed that earlier application of commercial microbial fertilizer resulted to a reduction of infection by 13% as compared to the plantlets with no application at the nursery stage.

Table 14. Disease occurrence in the field at monthly observation after planting from September 2010- April 2010.

Treatments	Total # of Plants	2 Sep.	3 Oct.	4 Nov.	5 Dec.	6 Jan.	7 Feb.	8 Mar.	9 Apr.	Total	% infection
Control	30	1	8	6	5	1	1	1	0	23	77
Introduced varieties	30	0	0	0	0	0	0	0	0	0	0
CMF at nursery	30	0	2	0	4	0	3	0	0	9	30
CMF upon	30	0	0	5	6	0	2	0	0	13	43

infection											
T. harzianum at nursery	30	0	2	4	1	1	6	1	0	15	50
T.harzianum upon infection	30	1	5	0	1	2	3	0	3	15	50
DDMB	30	0	1	0	2	2	2	0	0	7	23

Figure 12. Plant observed in the field (a) showing yellowing of leaves and applied with *Trichoderma harzianum* upon infection.

Figure 13. Banana plant recovered 1 month after application of *Trichoderma harzianum*

Application of commercial microbial fertilizer was also done to the plants infected in the field and observed that 46% of the total treated plants had recovered one month after application.(Figure 14) This microbial fertilizer contains various types of beneficial microorganisms (Appendix 1) that may have combated the infection of the pathogen thus resulted to the recovery of the infected plant. Furthermore, it also contains nutrients that are needed by the plant that boosted the immunity of the plant.

Figure14. Commercial microbial fertilizer applied on the infected plant

CONCLUSIONS

1**.** Fusarium wilt disease on banana could infect not only Cavendish but also the different local cultivars and is widely distributed in the different provinces of Region XI.

2. The best inoculation technique to be used for pathogenicity test is using the corn meal sand inoculum.

3. The virulence of the isolates differed when inoculated to different banana varieties.

4. The application of Commercial microbial fertilizer at the nursery could contribute to the decrease in disease incidence in the field and promotes vigor of the plant.

5. Both *Trichoderma harzianum* and commercial microbial fertilizer are potential control against Fusarium wilt.

RECOMMENDATIONS

1. Plant banana in a non Fusarium infested soil.
2. In conducting pathogenicity test it is best to use corn meal sand inoculum.
3. Early application of potential control strategies must be done to reduce disease incidence.

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