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Fungal Leaf Spot Diseases in Banana (*Musa* spp.); Symptom Verification and Their Control (*in vitro*)

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ABSTRACT

An experiment was conducted from January to April in 2013 to identify the causal organisms, to study the proper symptoms and to test the efficacy of chemicals under *in vitro* conditions on banana foliage diseases (Black Sigatoka, Yellow Sigatoka, Septoria and Cordana leaf spot diseases). Banana cultivators encounter difficulties and confusion in distinguishing foliage diseases and control them. A field study was carried out to identify the development of symptoms and causal organisms were verified in the laboratory investigation through isolating pathogens from infected samples. Reddish brown specks which turning black were belonged to Black Sigatoka and oval shape spots were characteristic to Septoria leaf spot disease. Brownish streaks with yellow colour halo can be used to identify Yellow Sigatoka disease and oval shape spots with characteristic concentric zonations were due to Cordana leaf spot disease. Five treatments including Tebuconazole, Bitertatanol, Carbendazim, Chlorothalonil and a control were tested under *in vitro* conditions for control the diseases in CRD design. Statistical analysis of data was done by SAS 9.20 programme. Tebuconazole and Carbendazim were recorded as the most effective chemicals for Yellow Sigatoka, Black Sigatoka and Septoria leaf spot diseases.

KEY WORDS: Cordana, *Mycosphaerella*, Septoria, Sigatoka, Symptoms

INTRODUCTION

The banana (*Musa* spp.) is one of the most ancient food plants that have been used and perhaps cultivated at the dawn of civilization (Arya, 1993). Bananas originated in the South East Asian and Western Pacific regions (Robinson, 1996). The commercial banana is a giant, perennial, herbaceous, monocotyledon plant which belongs to family Musaceae. Edible bananas are derived from either *Musa accuminata* (Dessert type) or *Musa balbisiana* (Cooking type) or a combination of both (Nakasone and Paull, 1998). It is mainly used as a fresh fruit and as a vegetable.

Banana is a major fruit crop in the tropics and subtropics that makes a vital contribution to the economies in many countries. Asia, Latin America, Eastern and Southern Africa are the major banana producers in the world. Banana is the most widely cultivated and consumed fruit in Sri Lanka. Extent of banana cultivation was 48,044 ha, total production was 383,784 Mt, and 2649 Mt (0.7%) were exported in 2010 (Anon, 2011).

Banana is now grown in almost all areas in the world between 30° North and 30° South latitudes that have sufficient water. Diseases are major production constraints wherever banana is grown (Ploetz *et al.*, 2003). Although bananas can adapt efficiently to produce high yields under a wide range of climatic extremes, they are susceptible to a range of serious and debilitating diseases that are caused by fungi, bacteria and viruses (Robinson, 1996).

Fungi are the most important and prevalent pathogens of banana. They affect all organs of the host in the field and are the major causes of pre-harvest yield reductions, postharvest damages and loss of fruit (Ploetz *et al.*, 2003). Among the fungal diseases of banana, Black Sigatoka leaf spot disease, Yellow Sigatoka leaf spot disease, Cordana leaf spot disease, and Septoria leaf spot disease are the most prevalent fungal leaf spot diseases in Sri Lanka. Leaf spot diseases do not kill plants immediately, but increase crop losses gradually with the age of plants. Leaf spot diseases result a reduction of the quality and quantity of fruits. Fruits of infected plants ripen prematurely and do not fill properly (Udugama, 2002).

The most virulent disease of banana is Black Sigatoka leaf spot disease which is found worldwide except for subtropical areas and Caribbean. Currently, it is the most important disease of banana which is caused by the Ascomycota, *Mycosphaerella fijiensis*. It is more destructive than Yellow Sigatoka leaf spot disease and has rapid onset. The disease was first recorded from the Sigatoka district, Fiji Island in 1963 (Mourichon *et al.*, 1997) and in 1995 from Sri Lanka (Udugama, 2002). Black Sigatoka causes early drop of the entire leaf and resulting loss of photosynthetic area leading slower filling of fingers,

premature ripening of fingers, reduced yields and finger size. Yield loss has been estimated to be up to 33% during the first crop cycle and up to 76% in the second (Mobambo *et al.*, 1996).

Yellow Sigatoka leaf spot disease which is caused by *Mycosphaerella musicola* was the most important leaf spot disease of banana before the spread of Black Sigatoka. Although Yellow Sigatoka is slower to develop than Black Sigatoka it has distributed worldwide. Yellow Sigatoka was first recorded from Java in 1902 (Mourichon *et al.*, 1997) and in 1919 from Sri Lanka (Udugama, 2002).

Cordana leaf spot disease is a common, but usually innocuous disease of banana which is caused by *Cordana musae*. It was first recorded in 1902 from Java (Nakasone and Paull, 1998).

In year 2000, Septoria leaf spot disease was identified in Sri Lanka which is caused by *Mycosphaerella eumusae* (Udugama, 2002). At present, it appears to be the predominant leaf spot disease in Thailand, and is common in Peninsular Malaysia, Southern India and Sri Lanka (Jones, 2000).

Management strategies of leaf spot diseases vary with cultivars. Various fungicides and chemical compounds are used with high dosage in commercial level production. Poor knowledge on proper selection and excessive usage of ineffective chemicals increase the cost of production. Also it is highly harmful to the environment. Symptoms of diseases mentioned above are more similar to each other and have difficulties to distinguish resulting confusion. Correct identification of diseases in the field, differentiation of the causal organisms and symptoms related to above leaf spot diseases are vital for commercial level production and also selection of suitable chemical to control is necessary for long term solution.

MATERIALS AND METHODS

Location

This experiment was carried out at the Department of Horticulture and Landscape Gardening of the Faculty of Agriculture and Plantation Management of Wayamba University of Sri Lanka, Makandura, Gonawila from January to April in 2013.

Sample Collection

Infected leaves of each disease were randomly collected from the Regional Agricultural Research and Development Centre, Makandura, Gonawila.

Field Study

Development of characteristic symptoms of each disease was observed under the field conditions from initial stage to severe stage.

Laboratory Investigation

Microscopic Observation

Infected leaves were chopped in fresh form and examined under stereo microscope for their spores.

Humid Chamber Method

Infected leaves were kept in moist chambers at room temperature (25°C) under normal light conditions for five days. The mycelia and spore shapes were observed under the microscope.

Culture Media Performance

Collected samples were washed with 1% NaOCl solution and then three times with distilled water. Sections of two millimeter of sterilized leaves were placed on petri dishes containing Potato Dextrose Agar (PDA). All the *in vitro* steps were carried out under aseptic conditions. Cultured Petri dishes were incubated at 27±2°C for seven days. Pure isolates of each fungus were obtained through a series of sub culture.

Efficacy of Chemicals

Foliar fungicides that were commonly used in commercially were tested to compare its efficacy on each disease. The treatments used in the experiment for each disease under *in vitro* conditions are given in Table 1.

Table 1. Chemical treatments and their dosages

Number	Treatments	Dosage
T1	Tebuconazole	0.35 ml/L
T2	Bitertatanol	1.00 ml/L
T3	Carbendazim	0.70 g/L
T4	Chlorothalonil	2.00 ml/L
T5	Control	-

As described by Maloy (1993), mycelial plugs of the fungus were placed on agar containing test chemicals. Growth measurements were taken from third day until colony showed stable growth.

Experimental Design

Complete Randomized Design (CRD) was used with five treatments and seven replicates.

Data Collection

Width of mycelia was measured after incubation period of three days until stabilize the colony growth.

Data Analysis

The data generated from the experiment were statistically analyzed using SAS 9.20 programme.

Koch's Postulation

Healthy leaves were kept in moist chamber and inoculated with the spore suspension of the each disease to confirm the respective causal organism. The spore concentrations of the solutions were greater than 10^6.

RESULTS AND DISCUSSION

Field Study

Black Sigatoka Leaf Spot Disease

Field study showed that symptoms of Black Sigatoka leaf spot disease were beginning as yellowish colour tiny specks that quickly turn reddish brown on the lower surface of the leaf lamina. As they progress, tiny specks elongated, widen, becoming streaks and more clearly visible on lower leaf surface than upper surface. The streaks were expanded in size and change colour to very dark brown or almost black and clearly visible from the upper side of the leaf. The streaks were continued to enlarge and become more elliptical in shape as it broaden and a water-soaked border developed around the edges. The spots were becoming slightly depressed and water soaked border was developed in to a yellow halo around it. Finally, centre of each spot becoming dry and pale grey in colour with a distinctive black border surround it. Mourichon *et al.* (1997) mentioned that the spots remain visible even after the death and desiccation of the leaf due to the dark border encircling each of the individual spots. Where infection was heavy, the streaks were overlapped and fused to give a black appearance to large areas of the leaf, collapsed and become necrotic.

Yellow Sigatoka Leaf Spot Disease

Appearance of very small light green dots or dashes was identified as initial symptoms of Yellow sigatoka leaf spot disease in the field. These small dots or dashes were elongated into a light green streak of several millimeters long and change the colour to rusty brown. The streaks became elongated and widen slightly with a poorly defined border. As described by Udugama (2002), the streaks were become more elliptical and definite spot with a sunken dark brown centre. It was often surrounded by a yellow halo. Finally, spots had grey, dried out centre and an obvious black margin which remains even after the leaf had dried out.

Septoria Leaf Spot Disease

At initial stage, tiny brown streaks of Septoria leaf spot disease helps to distinguish it from Sigatoka diseases. Streaks were continued to expand and become oval to elliptical shape. They were darkened and a grey colour center with dark border was developed as they mature. The mature lesions were larger and oval shaped (Udugama, 2002).

Cordana Leaf Spot Disease

Cordana leaf spot had shown pale brown, oval shape spots of several centimeters long. Udugama (2002) indicated that, light grey colour necrotic center of the spot had characteristic concentric zonations and these lesions were surrounded by bright yellow colour haloes.

Laboratory Investigation

Microscopic Observation

Spores of *Cordana musae* were clearly observed but, spores of *Mycosphaerella fijiensis*, *Mycosphaerella musicola* and *Mycosphaerella eumusae* were not observed on chopped fresh leaves.

Humid Chamber Method

Cordana musae had developed both mycelia and spores, where *M. fijiensis, M. musicola* and *M. eumusae* had mycelia only.

Culture Media Performance

Mycosphaerella fijiensis, *M. musicola* and *M. eumusae* were successfully grown on PDA. But *C. musae* was not grown on PDA. Therefore it was cultured on Sweet Potato Sucrose Agar and PDA broth culture. The attempts were not successful. Therefore, *C. musae* was not able to test for the chemicals.

Black Sigatoka Leaf Spot Disease

Black colour mycelia were visible on cultures and sub hyaline, obclavate to cylindrical shape spores were observed under the microscope. They were straight or slightly curved at apex with six to eight septa. As reported by Udugama (2002), dark, basal scar on the apex in spores confirmed *M. fijiensis*.

According to Rutter *et al.* (1998), conidia and ascospores of the fungus are both infective. They are formed under high moisture conditions and are disseminated by wind.

Yellow Sigatoka Leaf Spot Disease

White fungal mycelia were developed and sub hyaline, cylindrical to obclavate shape conidia was observed on cultures. According to Ploetz *et al.* (2003), they were straight or slightly curved at apex, with eight to ten septa that confirmed the presence of *M. musicola.* These conidia were larger than conidia of *M. fijiensis.*

Both conidia and ascospores of the fungus are infective. They are formed under high moisture conditions and are disseminated by wind and conidia, also by rain and irrigation water (Ploetz *et al.*, 2003).

Septoria Leaf Spot Disease

Black colour mycelia were developed in the cultures. As described by Ploetz *et al.* (2003), sub hyaline, cylindrical and straight conidia with four to five septa were observed under microscope. Conidia were shorter than other *Mycosphaerella* spp. which were studied in the experiment.

Cordana Leaf Spot Disease

White mycelium was produced on leaf samples in the moist chamber. Solitary, pyriform, two celled, sub hyaline and smooth conida were observed under microscope which were similar to characters described by Ploetz *et al.* (2003). Conidia are infective and disseminated by wind (Ploetz *et al.*, 2003).

Efficacy of Chemicals

Bitertatanol and Tebuconazole are broad spectrum, systemic, triazole fungicides with a protective, curative and eradicative activity. Triazoles inhibit ergosterol biosynthesis. Ergosterol is the major sterol in the most fungi which essential for membrane structure and function. Carbendazim is a systemic fungicide belongs to the benzimidazole group and inhibits mitosis and cell division. Chlorothalonil is a chloronitrile, non-systemic, foliar fungicide with protective action. It inhibits spore germination and toxic to fungi cell membrane (Anon, 2013).

All the fungicides tested had shown significant effect on disease development. Results revealed that Tebuconazole and Carbendazim had capability of completely suppressing the pathogens of *M. fijiensis, M. musicola* and *M. eumusae.* The highest colony diameter was recorded in control treatment for each disease. Bitertatanol and Chlorothalonil had shown significantly less colony growth compared to control. The colony diameter of *M. fijiensis* and *M. eumusae* on Bitertatanol were significantly lesser than Chlorothalonil. There was no significant difference of colony diameter of *M. musicola* on Bitertatanol and Chlorothalonil (Table 2).

According to Carlier *et al.* (2000), *M. fijiensis* is closely related to *M. musicola* and *M. eumusae,* DNA sequence analyses suggest that these pathogen may have evolved from a common ancestor. This could be the fact that chemicals show similar effect on all pathogens.

Selection of several effective fungicides for alternative application is important to avoid resistant development. Cultural Practices such as removal of infected leaves, increase space between plants to reduce humidity and providing efficient drainage system are important to control leaf spot diseases in commercial production (Ploetz *et al.*, 2003)

Koch's Postulation

The characteristic symptoms were developed by each inoculation and indicate the presence of respective pathogens.

Table 2. Effect of chemicals on colony development of pathogen

	Treatment	Colony Diameter (cm)		
		M. fijiensis	*M. musicola*	*M. eumusae*
T1	Tebuconazole	0.00	0.00	0.00
T2	Bitertatanol	1.17^{c}	1.04^{b}	1.10^{c}
T3	Carbendazim	0.00	0.00	0.00
T4	Chlorothalonil	3.32^{b}	2.50^{b}	3.02^{b}
T5	Control	11.00^{a}	7.17^{a}	11.00^{a}

Means followed by the same letter in each column are not significantly different at 0.05 levels

CONCLUSIONS

The field study revealed that these diseases can be identified at initial stage by their symptoms. It helps to solve the confusion of distinguishing these diseases. Reddish brown specks which turning black were belonged to Black Sigatoka and oval shaped spots were characteristic to Septoria leaf spot disease. Brownish streaks with yellow colour halo can be used to identify Yellow Sigatoka disease. Oval shape spots with characteristic concentric zonations make the identification easy of Cordana leaf spot disease.

This study indicates that spore shapes of pathogens can help to confirm the causal organisms as well as the symptoms. Cylindrical shaped spores with basal scar on the apex were produced by *Mycosphaerella fijiensis* (Black Sigatoka disease) and it has six to eight septa. Larger, cylindrical shaped spores with eight to ten septa that were developed by *M. musicola* (Yellow Sigatoka disease). Spores of *M. eumusae* (Septoria disease) were cylindrical and straight with four to five septa. Solitary, pyriform conidia were belonged to *Cordana musae* (Cordana disease).

According to the study, *C. musae* was not able to culture on PDA, Sweet Potato Sucrose Agar or PDA broth culture. The results suggest that Tebuconazole and Carbendazim were capable to completely suppress the *M. fijiensis*, *M. musicola* and *M. eumusae* under *in vitro* conditions. However, the chemical control along with proper cultural practices in the field could boost the results in managing the foliage diseases. Therefore, it is necessary to carry out further testing to determine the effectiveness of Tebuconazole and Carbendazim in the field.

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