Leaf Spot Diseases in Banana (*Musa* spp.) and their Control (*in vitro*)

N.A.T.T. Perera, D.B. Kelaniyangoda and A.S.A. Salgadoe
Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)

Abstract

Banana cultivators encounter difficulties and confusion in distinguishing foliage diseases and controlling them. An experiment was conducted from January to April in 2013, in Sri Lanka focusing on Black Sigatoka, Yellow Sigatoka, Septoria and Cordana leaf spot diseases. Test series were carried out at field level to study symptom development and, at laboratory to observe isolated pathogens under microscope. Symptoms with 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. Also 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 *Cordanamusae* (Cordana disease). For disease control five treatments including Tebuconazole, Bitertatanol, Carbendazim, Chlorothalonilwere tested (*in-vitro*) in a CRD experimental design. 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

Banana (*Musa* spp.) of family Musaceaeis 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. Extent of banana cultivation is 48,044 ha, total production is 383,784 Mt, and 2649 Mt (0.7%) is exported (Anon, 2011).

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 in Sri Lanka.

Currently, Black Sigatoka is the most important disease of banana which is caused by the Ascomycota, *Mycosphaerella fijiensis* (Udugama, 2002). 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 offingers, 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.

Yellow Sigatoka leaf spot disease which is caused by *Mycosphaerellamusicola* was the most important leaf spot disease of banana before the spread of Black Sigatoka. 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 *Cordanamusae*. It was first recorded in 1902 from Java..

In year 2000, Septoria leaf spot disease was identified in Sri Lanka which is caused by *Mycosphaere llaeumusae* (Udugama, 2002). At present, it appears to be the predominant leaf spot disease in Thailand, and is

***Corresponding author:** kelaniyangoda@gmail.com

common in Peninsular Malaysia, Southern India and Sri Lanka (Jones, 2000).

Poor knowledge on proper selection and excessive usage of ineffective chemicals increase the cost of production. Also it is highly harmful to the environment. Objectives of this experiment are; correct identification of diseases in the field, differentiation of the causal organisms and leaf spot symptomsand selection of suitable fungicide 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.

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.

Experimental Design

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

Data Collection

Inhibition radial growth was measured after incubation period of three days until the colony growth was stabilized.

Mycelial plugs of the fungus were placed on agar containing test chemicals. Growth measurements were taken from third day until colony showed stable growth.

Data Analysis

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

Table 01. Treatments used in the experiment for each disease under *in vitro* conditions

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	-

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 10^6spores/ml.

Results and discussion

Field Study

Black Sigatoka Leaf Spot Disease

Field study showed that symptoms of Black Sigatoka leaf spot disease begin 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 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. 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, collapsedand 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 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 become more elliptical and definite spot with a sunkendark 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 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 showed 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

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*.

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*.

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.

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 andinhibits mitosis and cell division. Chlorothalonil is a chloronitrile, non-systemic, foliar fungicide with protective action.

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]

All the fungicides tested showed significant effect on *in vitro* fungal 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 shownsignificantly 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).

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.

The results suggest that Tebuconazole and Carbendazim were capable to completely suppress the *M. fijiensis, M. musicola*and *M. eumusae*under 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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