

# First Report of Banana Septoria Leaf Spot Disease Caused by *Septoria eumusae* in Iran

M. Amani<sup>1\*</sup> and Gayane Avagyan<sup>2</sup>

1. Plant Pathology PhD. Student at Armenian State Agrarian University, and Member of Scientific Staff at Date palm and Tropical Fruits Research Institute of Iran
2. Chair of Plant protection at the Armenian State Agrarian University

**Corresponding author:** M. Amani

**ABSTRACT:** Septoria leaf spot is one of the fungal diseases of banana leaves that has been reported in Southern and Southeast Asia. In year 2011, an undescribed leaf spot disease of banana was discovered in Southeast Iran. Symptoms of disease were observed in the Hormozgan, Sistan & Beluchistan areas. Symptoms in the initiation of infection appear as small necrotic, lentis shape and dark brown spots similar Sigatoka symptoms, which soon later enlarge rapidly and become rot. In this research, samples that showed symptoms of Septoria leaf spot were collected from banana orchards in Chabahar, Konarak, Minab and Jask areas and were transferred to laboratory. Leaf samples were first washed and were surface sterilized with 1% sodium hypochlorate and then diseased sections with necrosis were cut into 2x2cm sections. They were stapled to a sterile filter paper and placed in the lid of petri dish on water agar (WA) medium. The obtained spores were transferred to potato dextrose agar (PDA) medium. Pathogenicity tests were carried out with selected isolations on using detached leaves of Harichal cultivar. In this research, fungus, *Septoria eumusae* (Anamorph *Mycosphaerella eumusae*) was isolated from infected tissues. The results showed that, all fungi isolate created symptoms in inoculated leaves and then reisolated from those. This is the first record of Septoria leaf spot disease in Iran. At present, it is a disease of major important affecting banana in Iran.

**Keywords:** Banana, disease, Septoria, Leaf Spot

## INTRODUCTION

Banana (*Musa acuminata* L.) is among the most important crops in tropical and subtropical climates that cultivation in 120 countries of the world. It is one of the most important tropical fruits in Southeast of Iran (Amani, 2002). The total land under cultivation of banana is about 5420 hectares with average production of 35 tons per hectare, which is mainly cultivated in Sistan & Baluchestan and Hormozgan provinces (Anonymous, 2012).

Septoria leaf spot disease of banana previously has been reported in Southern and Southeast Asia. The fungus identified as the causal agent of this disease has a *Mycosphaerella* teleomorph stage and a *Septoria* anamorph stage (Carlier ., 2000). Three species of *Mycosphaerella* are known to Sigatoka leaf diseases in banana. *M. musicola* causes yellow Sigatoka, *M. fijiensis* causes black Sigatoka and *M. eumusae* causes eumusae leaf spot (Stover, ., 1987; Carlier ., 2000; Jones, 2000; Surridge ., 2003).

Yellow Sigatoka (YS) was first recorded from Java in 1902 and then was recorded from Sri Lanka in 1919. Black Sigatoka (BLS) was first recorded from the Sigatoka District, Fiji Island in 1963 which is regarded as the most economically important disease of banana in the Latin American region (Stover, 1972). It is very similar to Yellow Sigatoka but is more virulent and affects a wide range of banana genotypes (Jones, 1990; 2000). Among the fungal diseases, leaf diseases are considered serious in reducing the yield and quality of the fruit. Yellow Sigatoka disease

is well-known and wide spread. Carlier ., 2000 has also confirmed the presence of *M. eumusae*, causing septoria leaf spot disease in the Cavendish cv. Grande Naine in southern India (Selvarajan ., 2001). *M. fijiensis* is currently spreading to new banana-growing areas and replacing *M. musicola*, which has an almost worldwide distribution on banana, as the dominant and most serious leaf spot pathogen. Although this spread is well documented in the Australasian, Pacific, African, Latin American, and Caribbean regions (Mourichon, ., 1990) it is not as well understood in southern and Southeast Asia because of contradictory reports and conflicting evidence (Jones, 1990). *Phaeoseptoria musae*, is the cause of Phaeoseptoria leaf spot, which produces symptoms very similar to Sigatoka, was first described in India and has been detected in other countries (Stover, 1972; Raghunath, 1963; Punithalingam, 1983). Serious damages caused by this minor foliar disease have not been reported (Punithalingam, 1983; Amani ., 2006).

Another unrecorded leaf spot disease of banana caused by *M. eumusae*, was recorded from Sri Lanka (Carlier ., 1999). It resembles *M. musicola* and *M. fijiensis* in the telemorph stage and named *S. eumusae* on its anamorph stage. This disease was named Septoria leaf spot or Sigatoka-like disease (Carlier ., 2000).

Between 2012 and 2013, as part of a survey to determine the distribution of leaf spot diseases in Southeast Iran, samples of banana leaf spot were collected from banana orchards and were transferred to laboratory for pathogen identification. In this research, samples, that showed symptoms of Septoria leaf spot were collected from banana orchards in Chabahar, Konarak, Minab and Jask areas and were transferred to laboratory. At present, it is a disease of major important affecting banana in Iran. Our objectives were to study the isolation and identification of the causal agent of Septoria leaf spot disease of banana (*M. acuminata* L.) in the infected orchards in Iran. This is the first record of Septoria leaf spot disease of banana in Iran.

## MATERIALS AND METHODS

### **Sample collection**

More than 100 samples were collected from different regions of banana orchards in Hormozgan, Sistan & Baluchestan provinces between 2011 and 2013 (Table 1). Leaf tissues with advanced symptoms of leaf spot were cut into squares of approximately 20×10 cm and then were sent to laboratory for diagnosis by cool box. These tissues were dried between sheets of newspaper and were kept for diagnosis of pathogen.

### **Microscopic observations of diseased tissues**

Necrotic lesions found on diseased leaf collected were cleared in 10% KOH for overnight and washed in water (Udugama, 2002). To observed conidia, cleared tissues were stained for one minute in 0.5% lacto phenol-cotton blue followed by washing in water. Fungal fruiting bodies associated with these lesions were observed under stereomicroscope for morphological characters were studied, described, and the fungus was identified.

### **Isolation of pathogen**

Leaf samples were first washed and were surface sterilized with 1% sodium hypochlorate and then diseased leaves sections with necrosis were cut into 2cm<sup>2</sup> sections. They were dipped in sterile distilled water for 10-15 min and the sections were stapled to a sterile filter paper and placed in the lid of Petri dish facing 2% water agar (Carlier ., 2000). The plates were incubated at room temperature (25-30°C) and the release of spores was regularly observed for 24 hours under the microscope. The spores were transferred to potato dextrose agar (PDA) medium and then subcultured in PDA slants. After incubating the slants for 15 days, they were maintained at 4°C in the refrigerator.

### **Microscopic observation and identification**

Hyphae, conidia, and other fruiting bodies were observed under the microscope and their sizes were measured. The leaf cross sections were made to observe the fruiting bodies of the pathogens. The mature and immature spots were gently scraped for direct observation of conidia and then the conidial morphology, size, shapes, colour and the number of septa was observed under the microscope at 400x magnification. Fruiting bodies, hyphae, and Conidia were also observed from the slant culture and microphotographs were taken.

### **Pathogenicity test**

Pathogenicity tests were carried out with selected isolations on leaves of the tissue-cultured plants of cv. Robusta (Harichal) cultivar (Dwarf Cavendish subgroup). Conidia produced in the slants were used to inoculate the test plants to study the pathogenicity. Spore concentration of 10<sup>3</sup> was used to inoculate under Mist greenhouse with controlled conditions. Inoculation was done by spraying the spore suspension contained in a hand sprayer on the immediate

leaves. The lower surface of the leaves was covered while spraying. The symptom expression was regularly monitored and the conidia produced in spots were observed for their identity.

Table 1. Distribution of Septoria leaf spot disease of banana in Iran

Isolate code	Province	Location	Cultivar	Date of observation
3	Sistan & Baluchestan	Eslam Abad	Dwarf Cavendish	2011
6	Sistan & Baluchestan	Eslam Abad	Robusta	2011
8	Sistan & Baluchestan	Eslam Abad	Robusta	2011
11	Sistan & Baluchestan	Eslam Abad	Wallery	2011
12	Sistan & Baluchestan	Zar Abad	Robusta	2011
15	Sistan & Baluchestan	Zar Abad	Robusta	2011
18	Sistan & Baluchestan	Bahookalat	Wallery	2011
21	Sistan & Baluchestan	Pollan	Robusta	2011
24	Sistan & Baluchestan	Pollan	Robusta	2011
27	Sistan & Baluchestan	Uoraki	Wallery	2011
31	Sistan & Baluchestan	Uoraki	Dwarf Cavendish	2011
34	Hormozgan	Jask	Robusta	2011
35	Sistan & Baluchestan	Bahookalat	Robusta	2012
38	Sistan & Baluchestan	Sarbaz	Robusta	2012
42	Hormozgan	Jask	Robusta	2012
44	Sistan & Baluchestan	Zar Abad	Robusta	2012
47	Sistan & Baluchestan	Zar Abad	Robusta	2012
51	Sistan & Baluchestan	Bahookalat	Dwarf Cavendish	2012
53	Sistan & Baluchestan	Uoraki	Robusta	2012
59	Sistan & Baluchestan	Kahir	Robusta	2012
62	Sistan & Baluchestan	Eslam Abad	Robusta	2012
66	Sistan & Baluchestan	Bahookalat	Robusta	2012
68	Sistan & Baluchestan	Eslam Abad	Robusta	2012
72	Sistan & Baluchestan	Eslam Abad	Robusta	2012
76	Sistan & Baluchestan	Pollan	Robusta	2012
78	Sistan & Baluchestan	Eslam Abad	Dwarf Cavendish	2013
80	Sistan & Baluchestan	Pollan	Robusta	2013
81	Sistan & Baluchestan	Eslam Abad	Robusta	2013
83	Sistan & Baluchestan	Uoraki	Dwarf Cavendish	2013
84	Sistan & Baluchestan	Bahookalat	Dwarf Cavendish	2013
91	Sistan & Baluchestan	Eslam Abad	Robusta	2013
92	Sistan & Baluchestan	Kahir	Robusta	2013
93	Sistan & Baluchestan	Zar Abad	Robusta	2013
96	Sistan & Baluchestan	Uoraki	Wallery	2013
99	Hormozgan	Jask	Robusta	2013

## RESULTS AND DISCUSSION

### Results

The leaf spot diseases observed on the three cultivars Dwarf Cavendish, Robusta (Harichal) and Wallery in the Hormozgan, Sistan & Beluchistan areas. Disease symptoms observed initially were first visible as faint brown streaks on the lower side of the leaf. When infection density was low, streaks developed into oval lesions with gray centers, as small necrotic, and dark brown spots similar Sigatoka symptoms, which soon later when infection density was high, developing lesions coalesced and large areas of leaf tissue enlarge rapidly and became rot and necrotic (Fig. 1). Spots with bright grey centre and with size 2×3 mm to 2×12 mm appeared on upper surface of the leaf (Fig. 2A). Yellow streaks with size 3×0.4 mm to 12×1 mm appeared on upper surface of the leaf. Some spots looked unusually dark, light grey to bright grey centers with brown margin surrounded by yellow halo was seen on mature spots (Fig. 2B). These symptoms were observed on cultivars belonging to different genomic groups (Table 1).



Figure 1. Symptoms of Septoria leaf spot on naturally infected banana leaves.



Figure 2. Septoria leaf spot of banana in cv. Robusta

A, Yellow spots/streaks; B, Mature spots with bright grey centre.

**Fungal description and identification:**

Pycnidia structure, was closely associated in all of the infected banana leaf samples. This structure was more prevalent in lesions on upper leaf surfaces. Young pycnidia were ostiolated, pear-shaped, 30 to 40  $\mu\text{m}$  wide, more or less erumpent (Fig. 3A), and mature pycnidia were acervular-like (Fig. 3B). Conidia were hyaline, Fusiform, with 3-5 septate and measured between 21-40  $\mu\text{m}$  long (Fig. 3C & D). These conidia were different from those of *Phaeoseptoria musae*. The imperfect state of the fungus was identified as *Septoria* that agreed with Sutton, 1980.

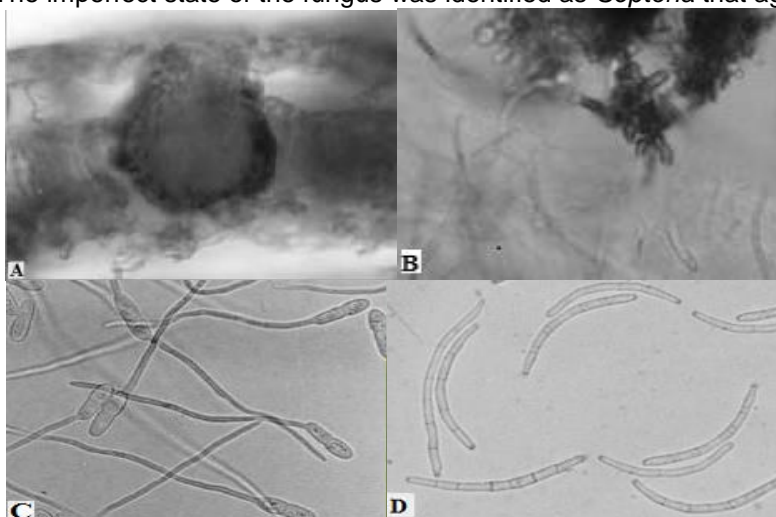


Figure 3. Photographs of *Septoria eumusae*, cause of Septoria leaf spot of banana. A, view of pycnidia-like structures; B, view of hyphae; C, germination spores on medium. Bars = 20  $\mu\text{m}$ .

### **Pathogenicity:**

*Septoria eumusae* isolates inoculated on banana cv. Robusta were pathogenic. Symptoms were observed in all repetitions, and the spot appeared on the inoculated leaves. Initial symptoms were small yellow specks that appeared about 6-7 weeks after inoculation. These lesions enlarged and turned pale brown or yellowish gray. Mature lesions developed, were dark brown and usually surrounded by narrow yellow halos.

### **Isolation and microscopic observation:**

In the Petri dish containing 2% water agar, released spores from the leaf spots were observed with 400 x magnification. Spores had one septum, germination of them was observed on PDA media. After one month, the culture grew about 0.5 cm in diameter. The colonies were light grey, pink, and light green color. Behind of the colony was light brown to black in color.

### **Conclusion**

The results of this research show that most samples of Sigatoka-like leaf spot collected on banana in southern Iran, during the 2012-2013 were caused by an undescribed pathogenic fungal species. The preliminary study performed in Southeast Iran confirmed the occurrence of *Septoria* leaf spot disease caused by *S. eumusae*. The symptoms of disease varied with the season of occurrence. This agreed with Selvarajan., 2001.

Although *Mycosphaerella* stage was not present in the leaf samples, the *Septoria* stage with pycnidial conidiomata and fusiform, hyaline, and septate conidia observed. This agreed with Stover., 1987; Sutton, 1980; Jones 2000. Therefore, the fungus identified, *S. eumusae* (telemorph *M. eumusae*), was the causal agent of the leaf spots samples.

*Septoria* leaf spot does not kill banana plants immediately, but reduces crop with the gradually increase of plant age. The disease results in a reduction of the fruit quality and quantity (Jones 2000; Stover, 1983). Further investigations on pathogen diversity through intensive survey and resistance sources among *Musa* spp., to the different pathogens are necessary

## **REFERENCES**

- Amani M. 2002. Cultivation & Production of Banana in Iran. Published by Agricultural Research and Education Organization. Iran. 168pp.
- Amani M, Amani H, Ershad J & Rezaee S. 2006. Isolation and identification of banana leaf spot (*Alternaria alternata*) in Iran. 17<sup>th</sup> Iranian Plant Protection Congress.
- Anonymous. 2012. Statistics from FAO website [http:// apps. Food and Agriculture Organization](http://apps. Food and Agriculture Organization).
- Carlier J, Zapater MF, Lapeyre F, Jones DR and Mourichon X. 2000. *Septoria* leaf spot of banana: A newly discovered disease caused by *Mycosphaerella eumusae* (Anamorph *Septoria eumusae*). *Phytopathology* 90(8): 884-890.
- Carlier J, Lebrun MH, Zapater MF, Dubois C and Mourichon X. 1996. Genetic structure of the global population of banana black leaf streak fungus, *Mycosphaerella fijiensis*. *Mol. Ecol.* 5:499-510.
- Jones DR. 1990. Black Sigatoka in the southeast Asian-Pacific region. *Musarama* 3:2-5. 8. Mourichon, X., and Fullerton, R. A. 1990. Geographical distribution of the two species *Mycosphaerella musicola* Leach (*Cercospora musae*) and *M. fijiensis* Morelet (*C. fijiensis*), respectively, agents of Sigatoka disease and black leaf streak disease in bananas and plantains. *Fruits* 45: 213-218.
- Jones DR. 2000. Diseases of Banana, Abaca & Enset. CAB International. 544pp.
- Mourichon X and Fullerton RA. 1990. Geographical distribution of the two species *Mycosphaerella musicola* Leach (*Cercospora musae*) and *M. fijiensis* Morelet (*C. fijiensis*), respectively, agents of Sigatoka disease and black leaf streak disease in bananas and plantains. *Fruits* 45: 213-218.
- Punithalingam, E. 1983. *Phaeoseptoria musae*: Description of pathogenic Fungi and Bacteria No.772. Commonw. Mycol. Inst., Kew, England.
- Ragunath T. 1963. A new leaf spot of banana from India. *Plant Dis. Rep.* 47:1084-1085.
- Stover RH. 1972. Banana, plantain and abaca diseases. Commonw. Mycol. Inst. Kew, England.
- Selvarajan R, Uma S & Sathiamoorthy S. 2001. Etiology and survey of banana leaf spot diseases in India. *Advancing banana and plantain R & D in Asia and the Pacific*, Vol. 10.p. 94-102.
- Stover RH and Simmonds NW. 1987. Bananas. Longman, New York. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol* 215:403-410.
- Stover RH and Simmonds NW. 1993. Bananas. Longman scientific & technical Newyork. T.edi-486 pp.
- Sutton BC. 1980. The Coelomycetes: Fungi imperfecti with pycnidia, acervuli and stromata. CAB, Commonw. Mycol. Inst. Kew, England.
- Udugama S. 2002. *Septoria* leaf-Spot Disease of banana *Mycosphaerella eumusae*: A new record for Sri Lanka. *Annals of Sri Lanka Department of Agriculture*, 4: 337-343.
- Surridge AKJ, Viljoen A, Crous PW and Wehner FC. 2003. Identification of the Pathogen associated with Sigatoka disease of banana in South Africa. *Australasian Plant Pathology*, 32: 27-31.