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Isolation, culturing and identification of the fungus *Cordana musae* (Zimm.) Hohn causing leaf spot of banana

Gaikwad CB, Thorat BS and Bhokare KR

Abstract
The present study was conducted for isolation, culturing and identification of the fungus causing leaf spot of banana in the year 2011-13. Disease samples showing typical symptoms of the new leaf spot of banana were collected from banana plantation of the Department of Horticulture, College of Agriculture, Dapoli. Banana was affected by many fungal pathogens causing significant economic damage to quality and productivity in all banana growing regions of the world. Leaf spot diseases can reduce the productivity and quality of the produce and can hinder the market value if not managed properly. Recently a new leaf spot caused by *Cordana musae* (Zimm.). The pathogen was isolated on PDA (9.00 cm) and identified as *Cordana musae* on the basis of morphological characters. The symptoms of this disease were different from those of sigatoka leaf spot disease. Maximum colony diameter was recorded on PDA (9.00 cm), which was followed by Malt extract (7.57 cm), King's agar (6.67 cm), Host leaf extract agar (5.97 cm) and Czapek's dox agar (3.90 cm). Though the maximum colony diameter was recorded on PDA, the fungal growth was not cottony and profuse as in case of Host leaf extract agar and King's agar. On malt extract the colony diameter was 7.57 cm but the mycelia growth was sparse. On the basis of the findings of present study it can be concluded that, leaf spot of banana caused by *Cordana musae* is a new disease of banana in Konkan region.

Keywords: Isolation, culturing, disease, fungus, leaf spot, *Cordana musae*, banana, PDA

1. Introduction
Banana is considered as the second most important fruit crop grown in the tropical and subtropical regions of the world (FAO, 2011) [1]. Banana is grown over an area of 4843774 thousand hectares in the world with annual production of 93390721 thousand MT with productivity of 19.3 MT/ ha (Annonymous, 2011). In India banana is grown over an area of 830 thousand hectares with production of 29780 thousand MT with productivity 35.88 MT/ ha. (Annonymous, 2011). Major banana producing states of the country are Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh and Karnataka. Maharashtra ranks second in area with an annual production of 5200MT (Annonymous, 2011) [2]. The soil and climatic conditions of Konkan are suitable for banana. Many local verities such as Harisal, Basrai, Konkan safed velchi, Lal velchi are traditionally cultivated in the region. Since last three to four years, a promising commercial cultivar- Grand-nain is gaining popularity in the region due to its high yield potential (Jones 1999 [3], Ploetz *et al.* 2003 [4]). Banana is affected by many fungal pathogens causing significant economic damage to quality and productivity in all banana growing regions of the world (Arzanlou *et al.* 2008) [5]. Leaf spot diseases can reduce the productivity and quality of the produce and can hinder the market value if not managed properly (Thangavelu *et al.*, 2006) [6]. Recently a new leaf spot caused by *Cordana musae* (Zimm.) Hohn, is also being observed in moderate forming many banana growing areas of Konkan (Joshi, 2008) [7]. The aim of this work was to isolate, culturing and identification the fungus *Cordana musae* (Zimm.) Hohn causing leaf spot of banana.

2. Materials and Methods
The present study was conducted for isolation, culturing and identification of the fungus causing leaf spot of banana in laboratory of Department of Plant Pathology, College of Agriculture, Dapoli in the year 2011-13. The infected leaves showing typical symptoms of the new leaf spot disease of banana were collected in the paper bags from the banana plantation of the Department of Horticulture of College of Agriculture, Dapoli. Potato dextrose agar (PDA),

the common laboratory medium was used for isolation of the causal organism from the infected leaf samples. All the chemicals used in different experiments were of analytical grade. The laboratory equipments *viz.*, autoclave, laminar-airflow bench, BOD incubator, refrigerator, research microscope, sintered glass filter, centrifuge *etc*. were used. Electronic balance, cork borer, polythene bags, forceps, inoculation needles, spirit lamp, cotton etc. were used during the course of investigation.

Table 1: Effect of culture media on growth and sporulation of *Cordana musae.*

Tr. No.	Culture media
T_1	Potato dextrose agar medium
T_2	Malt extract agar medium
T_3	Czapeks dox agar medium
T_4	Asthana & Hawkars agar medium
T_5	Richard's agar medium
T_6	Kings medium
T_7	Host extract agar medium

Visual observations on initial and final disease symptoms were recorded in the field to know the development of the disease in a plant population under natural conditions. Fresh diseased samples showing typical symptoms of leaf spot were collected and brought to the laboratory. These samples were then washed under tap water to remove extraneous material. Temporary mounts were prepared from the diseased specimens in lacto phenol cotton blue and examined under compound microscope for presence of microorganism if any (Crous *et al.,* 2009) [8].

Small pieces of infected leaf tissues were placed on surface sterilized glass slides and each slide was kept separately on a pair of glass rods in a sterilized Petri plate internally lined with sterilized, moist blotting paper. Such Petri plates were then incubated at room temperature and examined periodically for fungal growth.

3. Statistical analysis

The data obtained in all the experiments were statistically analyzed using methods suggested by Panse and Sukhatme (1967) [9]. The percentage values were transformed into Arcsine values. The standard error (S. EM.) and critical difference (C.D.) at level P = 0.01 were worked out and results obtained were compared statistically.

4. Results and Discussion

4.1. Visual

The leaf spot of banana incited by *Cordana musae* was observed during kharif 2011 in banana plantation of Department of Horticulture, College of Agriculture, Dapoli. The disease manifested initially in the form of small oval spots and later large, pale-brown oval patches on the surface of the lamina. Young infections on the upper leaf surface were at first brown; with delicate concentric zonation with a light brown colour margin. On the underside the spots were greyish-brown. The zonation and border were less clearly defined. Colony colors were treated according to the colour chart of Rayner (1970) [10]. Such results were reported by Joshi (2008) [7].

4.2. Microscopic examination

Microscopic examination of temporary mounts prepared from fresh diseased samples revealed the presence of typical two celled, obovate, hyaline conidia.

4.3 Incubation of the disease sample in the humid chamber

The disease samples incubated at room temperature for 48 hrs in sterilized humid chamber lined with moist blotting paper, produced whitish, septate mycelial growth.

4.4. Isolation of the causal organism

The test fungus was repeatedly isolated from the infected leaves of banana on PDA in the laboratory. The visual mycelial growth was observed after 3-4 days of isolation around the surface of sterilized pieces. The colony was circular and greyish white in colour. Reference strains were deposited in Laboratory of Department of Plant Pathology, nomenclature and taxonomic information in MycoBank (www.mycobank.org) (Crous *et al.* 2004) [11].

4.5 Cultural characteristics of *Cordana musae* on various solid media

It is evident from Table 2 that, the different media markedly affected the growth of *C. musae* in terms of colony diameter and appearance.

Table 2: Cultural characters of *Cordana musae* on various solid media.

Sr. No.	Medium	Average colony diameter (cm)	Colony characters	Sporulation
1.	Potato dextrose agar	9.00	Colony greyish white, with concentric rings, sporulation at the periphery of concentric rings.	+++
2.	Malt extract agar	7.57	Colony circular, whitish, withsparse growth.	++
3.	Czapek's dox agar	3.90	Colony circular, cottony compact growth**.**	+
4.	Asthana and Hawker's agar	-	No growth	-
5.	Richard's agar	-	No growth	-
6.	King's agar	6.67	Colony circular, white with brownish tinge, cottony compact growth with a distinct circular ring at the periphery.	++
7.	Host leaf extract agar	5.97	Colony circular, white, with fluffy, compact growth**.**	++

S.EM. +/- = 0.14 C.D. at 1% = 0.58 Sporulation: + + + Abundant, + + Good, + Poor, - Ni

Maximum colony diameter was recorded on PDA (9.00 cm), which was followed by Malt extract (7.57 cm), King's agar (6.67 cm), Host leaf extract agar (5.97 cm) and Czapek's dox agar (3.90 cm). Growth of the fungus did not occur on two media *viz*. Asthana and Hawker's agar and Richard's agar. Though the maximum colony diameter was recorded on PDA, the fungal growth was not cottony and profuse as in case of Host leaf extract agar and King's agar. On malt extract the colony diameter was 7.57 cm but the mycelia growth was sparse. Similar result reported by Zimmermann (1902) [12] and Joshi (2008) [7].

4.6. Establishment of Pure Culture

Pure culture of test fungus was obtained by preparing single

spore culture on PDA, which was subculture regularly at an interval of one week and used as stock culture for further studies.

4.7. Identification of causal organism

The morphological characters of the pure culture of the test fungus re-isolated from diseased tissues were compared with the available information on morphology of *Cordana musae* in the literature as well as on the standard websites for fungal identification.

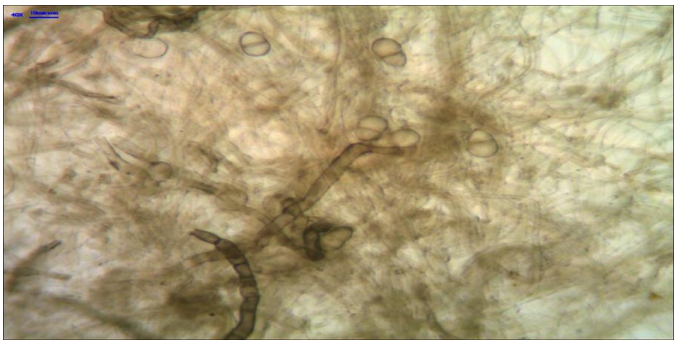

Fig. 1: Mycellium of fungus *Cordana musae* (Zimm.) Hohn observed under Microscope.

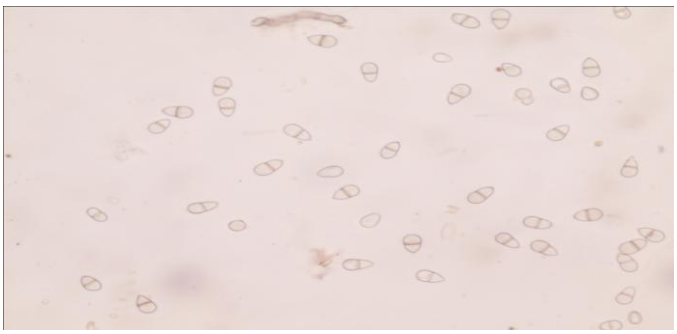

Fig. 2: Spores of fungus *Cordana musae* (Zimm.) Hohn observed under Microscope.

The morphology of isolated fungus were found to be similar with that of *Cordana musae*. Hence the pathogen was identified as *Cordana musae*. Furthermore this isolated fungus is morphologically similar to the Cordana musae (Zimm.) Hohn fungus described and illustrated by Herradura and Carreon (1990) [13] and Soares *et al.* (2005) [14].

5. Conclusion

On the basis of the findings of present study, it can be concluded that, leaf spot of banana caused by *Cordana musae* is a new disease of banana in Konkan region. As the climatic conditions of Konkan are favourable for development and spread of this disease, even though, at present the disease is not severe in the region, it may pose a major threat to banana plantations in the region in forthcoming years.

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