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First report of *Phomopsis* sp. on *Aloe vera* in India

Avasthi S[1], Gautam AK[2] and Bhadauria R[3*]

[1]School of Studies in Botany, Jiwaji University, Gwalior- 474012 (M.P.) India.
[2]Faculty of Agriculture, Abhilashi University, Mandi 175045, Himachal Pradesh, India

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Abstract

In rainy season of 2010 and 2011, leaves of *Aloe vera* were found infected with leaf spot disease. The leaves with typical symptoms of disease were collected from different nurseries and botanical gardens of Gwalior, Madhya Pradesh, India. Disease spots were observed on the adaxial surface and tip of leaves. Based on its morphological and cultural characteristics, the pathogen was identified as *Phomopsis* sp. This is the first report of leaf spot caused by *Phomopsis* sp. on *A. vera* in India.

Key words – *Aloe vera* – India – leaf spot – *Phomopsis* sp.

Introduction

Aloe vera is a green cactus-looking plant with beautiful rosette pattern of leaves. The average height of plant is about 80–100 cm with prickly leaves that hold translucent gel. The raw pulp contains about 98.5% water, whereas inner fillet gel has approximately 99.5% water (Eshun & He 2004). The remaining 0.5-1% solid material contains more than 200 bioactive chemical compound like vitamins, minerals, enzymes, mono and polysaccharides, sugar, lignin, phenolic compounds and organic acids (Boudreau & Beland 2006, Lanjhiyana et al. 2011). Aloe gel is widely used in beauty products, diet supplements, health juices and pharmaceutical purposes.

A survey of various nurseries and botanical gardens of Gwalior, Madhya Pradesh, India was carried out in 2010–11 to study the diversity of diseases associated with *A. vera*. During the survey a leaf spot disease was observed from various surveyed localities. The occurrence of leaf spot infection on *A. vera* poses a concern not only over the morphology of the plant but also on quality and quantity of the mucilaginous gel, which is used in herbal and cosmetic industries. The main objective of the present study was to isolate and identify fungal pathogen associated with leaf spot disease.

Materials & Methods

Collection of disease samples and isolation of pathogen

Diseased samples were collected from various *A. vera* growing nurseries and botanical gardens of Gwalior, Madhya Pradesh and brought into the laboratory for further isolation studies. Infection on living leaves was photographed with a digital camera (Sony DSC-X80) and then

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Corresponding Author: Rekha Bhadauria – e-mail – rekhabhadauria@yahoo.com

subjected to mycological and plant pathological analysis. Collected diseased samples were washed thoroughly with running tap water to remove the surface contaminants. These were then cut into small pieces with a sterilized scalpel and surface sterilized with 2% sodium hypochlorite solution for 90 seconds. These tissues were washed 3–4 times with sterilized distilled water and aseptically transferred onto autoclaved potato dextrose agar (PDA, containing 1.0 mg/ml chloramphenicol) medium in petri plates. Inoculated petri plates were incubated at 25±2 °C for 5 to 6 days and the growth of fungal colonies were recorded every day. The cultural and morphological characteristics of the isolates were studied to identify the fungus associated with the disease. For microscopic observations, fungal tissues were mounted in lactophenol cotton-blue mixture and observed using a routine compound microscope.

Isolated fungi were identified on the basis of their morphological/cultural characteristics (shape, size and colour of colony) and microscopic features (characteristics of mycelium, shape, size of conidia). Micrometry was also performed to identify the isolated fungus in terms of length, breadth and diameter of conidia. The identification of the pathogen was also confirmed at Indian Type Culture Collection (ITCC), IARI, New Delhi, India, where the cultures were deposited. Pathogenecity test was done to complete Koch's postulates.

Results

After the survey of two consecutive years it was noted that, leaf spot disease on *A. vera* was observed only in rainy season (July–August). The percentage frequency of isolated fungus was ranged between 5–10.5%.

Spots were observed on the adaxial surface and tip of leaves during the rainy season. Initially spots were small and maroon-brown in colour. Gradually spots became enlarged, sunken, dark brown in colour with a maroon margin. Later, these spots turned black, many small black spots were speckled on the centre of the spots, and extended up to 0.4–0.9 × 0.4–0.6 cm. Disease was found only in survey period from July to August (Fig. 1).

Developing colonies on PDA were extremely variable, woolly to cottony, white or whitish, pale to light brown. Two types of conidia were observed upon microscopic examinations. The alpha (α) conidia were hyaline, fusiform to ovate, straight, aseptate and frequently biguttulate, measuring 2.5–3.7 × 2–2.5 µm. The beta (β) conidia were filiform, sigmoidal, and hyaline, measuring 3.7–4 × 2.3–3 µm (Fig. 2A, B).

Based on its morphological and cultural characteristics, the pathogen was identified as *Phomopsis* sp. The Indian Type Culture Collection (ITCC), IARI, New Delhi India (# ITCC-7802.10) confirmed the identity. Artificially inoculated leaves showed symptoms similar to natural diseased leaves, and the fungus was re-isolated from the leaves, thus confirming pathogenicity of *Phomopsis* sp. No symptoms appeared on control leaves sprayed with sterile distilled water.

Discussion

Taxonomically the genus *Phomopsis* is placed under class Sordariomycetes, order Diaporthales, family Diaporthaceae. To date, almost 1000 *Phomopsis* epithets have been recorded (www.indexfungorum.org) on a wide variety of plant hosts like cane and grape in Ohia (Nita et al. 2008), leaf spots on strawberry (Eshenaur & Milholland 1989), Colorado blue spruce in Michigan (Igoe et al. 1995), Phomopsis blight of brinjal (Thippeswamy et al. 2006), cucurbits (Shishido et al 2006) and many more. Various leaf spots of *A. vera* have been reported to be caused by *Fusarium phyllophilum* (Kishi et al. 1999), *Alternaria alternata* (Manjul et al. 2008, Bajwa et al. 2010, Abkhoo 2014), *A. tenuissima* (Vakalounakis et al. 2015), *Colletotrichum gloeosporioides* (Avasthi et al. 2011), *Curvularia lunata* and *C. ovoidea* (Avasthi et al. 2015a), *Fusarium oxysporum* (Kawuri et al. 2012), *Nigrospora oryzae* (Zhai et al. 2013), *Phoma betae* (Avasthi et al. 2013), and *Sphaeropsis sapinea* (Kamil et al. 2014) from various parts of the world including India. A collar and root rot disease caused by *Penicillium purpurogenum* was also found associated with this plant (Avasthi et al. 2015b). No record of *Phomopsis* sp. is so far available on *A. vera.* Therefore, this is the first report of leaf spot disease on *A. vera* caused by *Phomopsis* sp. in India. Since *A. vera* is

known as the source of several important products of nutritional and therapeutic value, the leaf spot disease caused by *Phomopsis* sp. deserves special attention.

Fig 1 – Symptoms of *Phomopsis* leaf spot disease on *Aloe vera*.

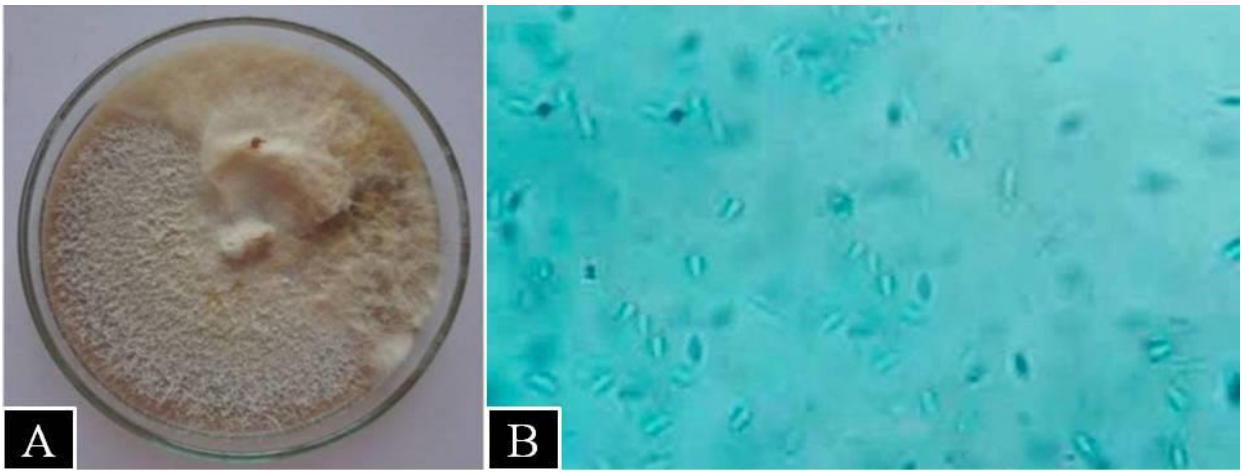

Fig 2 – *Phomopsis* sp. A: Mature colony on PDA. B: Microscopic view of conidia.

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