

Short Communication: Occurrence of leaf spot diseases on *Aloe vera* (L.) Burm.f. caused by *Curvularia* species from Madhya Pradesh, India

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Abstract. Avasthi S, Gautam AK, Bhadauria R. 2015. Occurrence of leaf spot diseases on *Aloe vera* (L.) Burm.f. caused by *Curvularia* species from Madhya Pradesh, India. *Biodiversitas* 16: 79-83. During 2010-2011, occurrence of leaf spot diseases was observed on *Aloe vera* plants grown in various nurseries of Gwalior, Madhya Pradesh, India. The typical disease symptoms were observed on the abaxial surface, tips and spiny margins of leaves. Disease spots were sunken, dry, necrotic, dark maroon to dark brown in color. On the basis of morphological and microscopic characteristics of the fungus, two species of *Curvularia* i.e. *Curvularia lunata* and *Curvularia ovoidea*, were found to be associated with the leaf spot diseases. Koch's postulate was applied to confirm the causal organisms of the diseases. According to the literature, this is the first report of leaf spot disease on *A. vera* caused by *Curvularia lunata* from Madhya Pradesh, and the first report of *Curvularia ovoidea* from India.

Key words: *Aloe vera*, *Curvularia lunata*, *Curvularia ovoidea*, leaf spot, India

INTRODUCTION

Aloe vera (L.) Burm.f. belongs to family Aloeaceae, is a perennial, succulent, monocotyledonous plant with an average height of 60-100 cm. There are about 400 species of *Aloe*, but the most commercially cultivated species is *A. vera*. It is a native of warm tropical regions, especially North Africa, the Mediterranean region of southern Europe and the Canary Islands. This species was first described by Carl Linnaeus in 1753. *Aloe vera* belongs to a large group of plants known as Xeroids because of its ability to close its stomata completely to avoid loss of water and help in retaining a large amount of water in its tissues (Akinyele and Odiyi 2007). It is commonly referred as a "miracle plant" and has a long history of economic and medicinal uses that spans thousands of years (Daodu 2000). More than 200 chemical components have been identified from the leaf pulp and exudates of *A. vera* (Reynolds and Dweck 1999; Choi and Chang 2003; Ni et al. 2004). Its gel is widely used for the treatment of sores, wounds, skin cancer, cold, constipation, asthma, ulcer, diabetes and fungal infection (Joseph and Justin 2010). The leaf pulp exhibited inhibition of AIDS virus by accemannan and inhibition of the prostaglandin synthesis by anthraquinone-type compound (Hassannuzzaman et al. 2008). In the cosmetic industries *A. vera* is used in the production of soap, shampoo, toothpaste and body lotions (Daodu 2000).

Despite its therapeutic and antimicrobial potential, *A. vera* is susceptible to various fungal diseases. Leaf spot is an important disease that not only affect the leaf texture but also reduce the quality and quantity of mucilaginous gel used for medicinal and commercial purposes. Therefore, it was the objective of this study to identify the causal

agent of leaf spot symptoms on *A. vera*.

MATERIALS AND METHODS

Sample collection and study of symptoms

Various nurseries in Gwalior, India were surveyed for the presence of leaf spot diseases on *A. vera* during the rainy and winter seasons of 2010 and 2011. Ten infected leaves were collected randomly from each nursery, placed into labeled zip lock bags, and brought into the laboratory. The morphology of the symptoms was studied with the help of hand lens and dissecting microscope.

Isolation and purification of pathogen from diseased leaves

Diseased leaves of *A. vera* were taken into the laboratory and washed thoroughly with running tap water to remove the surface dirt. The leaves were cut into small pieces using sterile scalpel blades. These small pieces were then surface sterilized with 2% sodium hypochlorite solution (NaOCl) for 2 mins and then washed three-four times in sterile distilled water. These surface sterilized pieces were then placed between blotting papers and aseptically inoculated onto petridishes containing Potato Dextrose Agar media. The plates were incubated at 25±2 °C for 5 to 6 days, and the growth of fungal colonies were recorded every day.

Characterization and identification of the pathogens

The isolated fungal species were identified on the basis of morphological and cultural characteristics as described by Ellis (1971) and Gilman (2012). Identification of

pathogens was further confirmed at the Indian Type Culture Collection (ITCC), IARI, New Delhi and the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India.

Pathogenicity test of the pathogens

Pathogenicity test was carried out *in vitro* on the healthy leaves of *A. vera*, according to the method described by Kamalakannan et al. (2008). The isolated fungal species were cultured on Potato Dextrose Agar (PDA) medium at $25\pm 2^\circ\text{C}$ for 8-10 days in an incubator. Conidial concentration was subsequently adjusted to 1×10^9 per ml by using hemocytometer to make a spore suspension. Healthy leaves were surface sterilized for 1 min with 2% sodium hypochlorite solution (NaOCl). Artificial pricks approximately 2 mm deep on the abaxial surface of leaves were made by sterilized needle. Spore suspension of the test organisms was delivered through a sprayer and lined with moist blotting paper. Leaves sprayed with sterile distilled water served as control. Leaves were incubated at $25\pm 2^\circ\text{C}$ for 8-10 days.

RESULTS AND DISCUSSION

Results of the survey revealed that all the nurseries cultivated only a single species of *Aloe*, i.e. *A. vera* and none of the nursery was found free from leaf spot diseases. Microscopic and cultural analysis of the isolated fungi indicates the association of two *Curvularia* species, *Curvularia lunata* and *Curvularia ovoidea*. Isolation result indicates that *C. lunata* and *C. ovoidea* were isolated from all the infected leaf samples. Minor differences were observed in disease symptoms caused by both fungi. The disease symptoms and microscopic characteristics of the pathogens are described as follows.

Symptoms of leaf spot diseases

Leaf spot disease caused by *C. lunata* began in the form of circular, water soaked lesions on the tip and abaxial surface of the leaves. Gradually the size of the lesions expands and became light red in color bordered by water soaked tissues. As the disease progressed, the lesions were sunken, maroon color with an average size of $0.5-1.4 \times 0.4-1.0$ cm. In later stages, lesions became dry, necrotic and turned into dark maroon in color (Figure 1A & B). The disease was observed during both the rainy and winter seasons.

Symptoms of leaf spot disease caused by *C. ovoidea* appeared as elongated, water soaked lesions, generally occurred on the spiny margins and abaxial surface of the leaves. Progressively, these lesions became sunken and light brown in color. Later, these lesions were enlarged, centre of the lesions dark brown in color with maroon red margins, and measured about $0.7-1.7 \times 0.6-1.3$ cm in size. In severe infection, the spiny margin of the leaves was twisted inside due to necrosis of the tissues (Figure 2A & B). Interestingly, the disease was observed only in the winter season.

Identification of fungal pathogens

The fungal colony isolated from diseased tissue was subfloccose, dark olive-gray in color and on the reverse side was greyish black on PDA. Hyphae septate, branched, $3.0-3.6 \mu\text{m}$ in diameter. Conidiophores were erect, long and unbranched. Conidia borne more or less in whorl at the tip of conidiophores, curved and brown in color. Generally conidia have four transverse septa and $18-29 \times 10-8 \mu\text{m}$ in size. The central cell is typically darker and larger as compared to the end cells (Figure 1C & D). Based on morphological and cultural characteristics the fungus was identified as *C. lunata* (Walker) Boedijn (# ITCC - 8185.11).

The growth of another fungal colony on PDA was initially light grey and circular, becoming velvety, pale brown to dark brown in color. The reverse side of the PDA turned into black color. Conidiophores were pale brown, straight, cylindrical and multiseptate. Conidia were ovoid, three septate, straight or curved, $16-29 \times 10-17 \mu\text{m}$, brown with paler end cells (Figure 2C-D). Based on morphological and cultural characteristics the fungus was identified as *C. ovoidea* (Hiroe & N. Watan.) Munt.-Cvetk. (# NFCCI-3053).

Pathogenicity test

Curvularia lunata and *C. ovoidea* were pathogenic under *in vitro* conditions. The symptoms of leaf spot diseases recorded during the pathogenicity test were almost similar to the natural symptoms. Symptoms of leaf spot infection appeared on fourth day of infestation. Initially, round, water soaked lesions were appeared on the abaxial surface of leaves. As the infection progressed, spots became sunken and reddish maroon in color. On the thirteenth day, the lesions become necrotic and turned into dark brown in color. The fungi were re-isolated from the infected leaves and were compared with the original culture of *C. lunata* and *C. ovoidea*.

Discussion

Curvularia is a filamentous fungus reported to cause various diseases on different plant hosts. Leaf spot disease caused by *C. lunata* and *C. ovoidea* affects the quality and quantity of *A. vera* leaf gel. Jat et al. (2013) reported severe form of leaf spot disease on *A. vera* caused by *C. lunata* and its management from Jaipur. *Curvularia lunata* has been found to cause leaf spot disease on *Amaranthus spinosus* (Sharma et al. 2011), *Clerodendrum indicum* (Mukherjee et al. 2013), *Grewia optiva* (Gautam et al. 2011), *Hordeum vulgare* (Kumar and Singh 2002), *Nelumbo nucifera* (Cui and Sun 2012) and *Zea mays* (Akinbode 2010). Similarly, *C. ovoidea* has been reported as a leaf spot pathogen on *Capsicum annum* (Singh and Tondon 1970) and *Psophocarpus tetragonolobus* (Awurum and Emechebe 2001). Other pathogenic fungi have also been reported to cause leaf spot diseases on *A. vera*, such as *Alternaria alternata* (Kamalakannan et al. 2008), *Colletotrichum gloeosporioides* (Avasthi et al. 2011), *Nigrospora oryzae* (Zhai et al. 2013) and *Phoma betae* (Avasthi et al. 2013). Although, *C. lunata* has already been reported from Jaipur, India, there are no reports available

for *C. ovoidea*. To the best of our knowledge, this is the first report of leaf spot disease on *A. vera* caused by *C. lunata* from Madhya Pradesh, while by *C. ovoidea* from India.

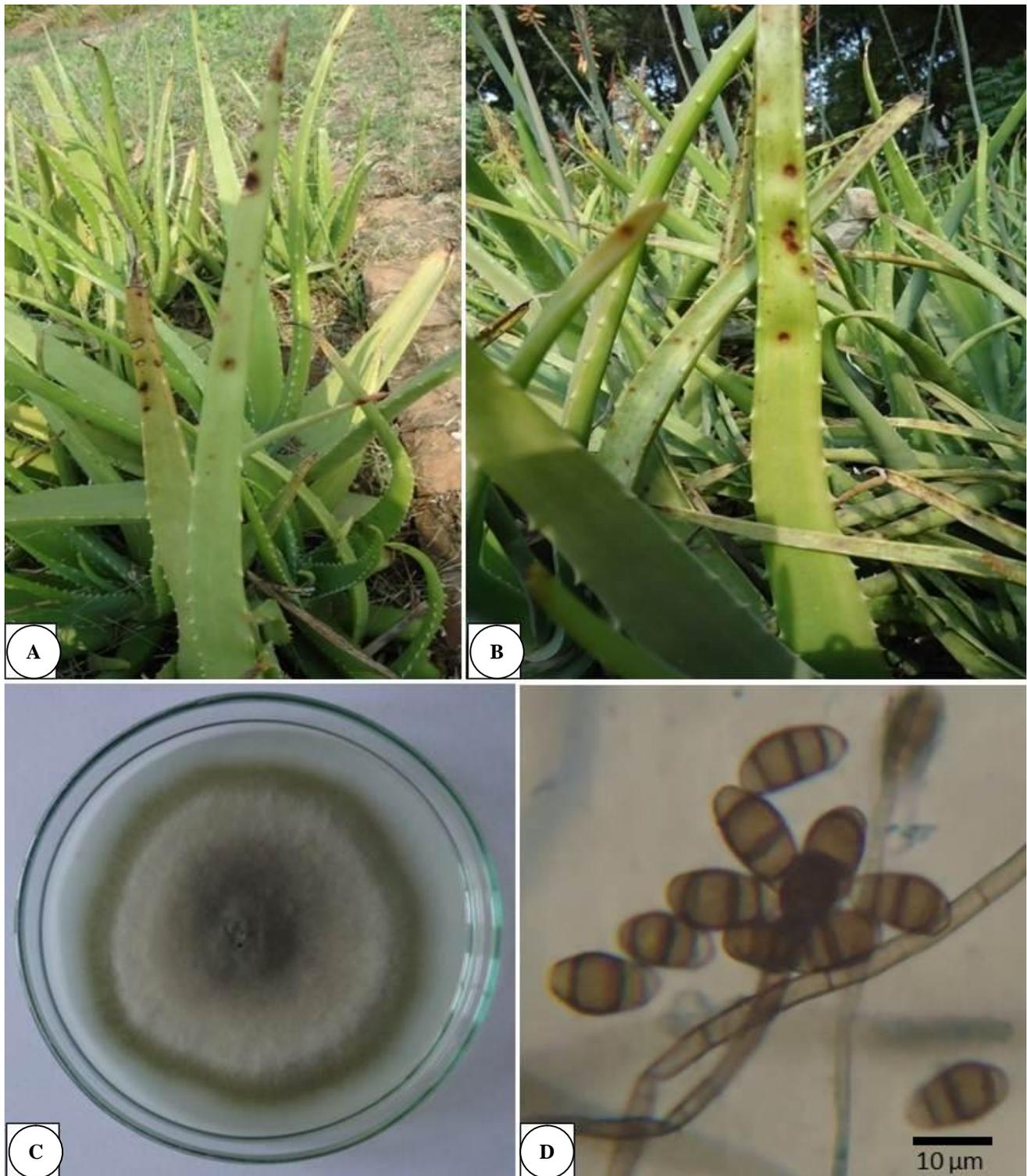


Figure 1. Leaf spot disease on *A. vera* caused by *Curvularia lunata*. A. Initiation of disease symptoms, B. Mature symptoms, C. Ten days old colony of *C. lunata* incubated at 25 ± 2 °C on PDA, D. Conidia of *C. lunata*

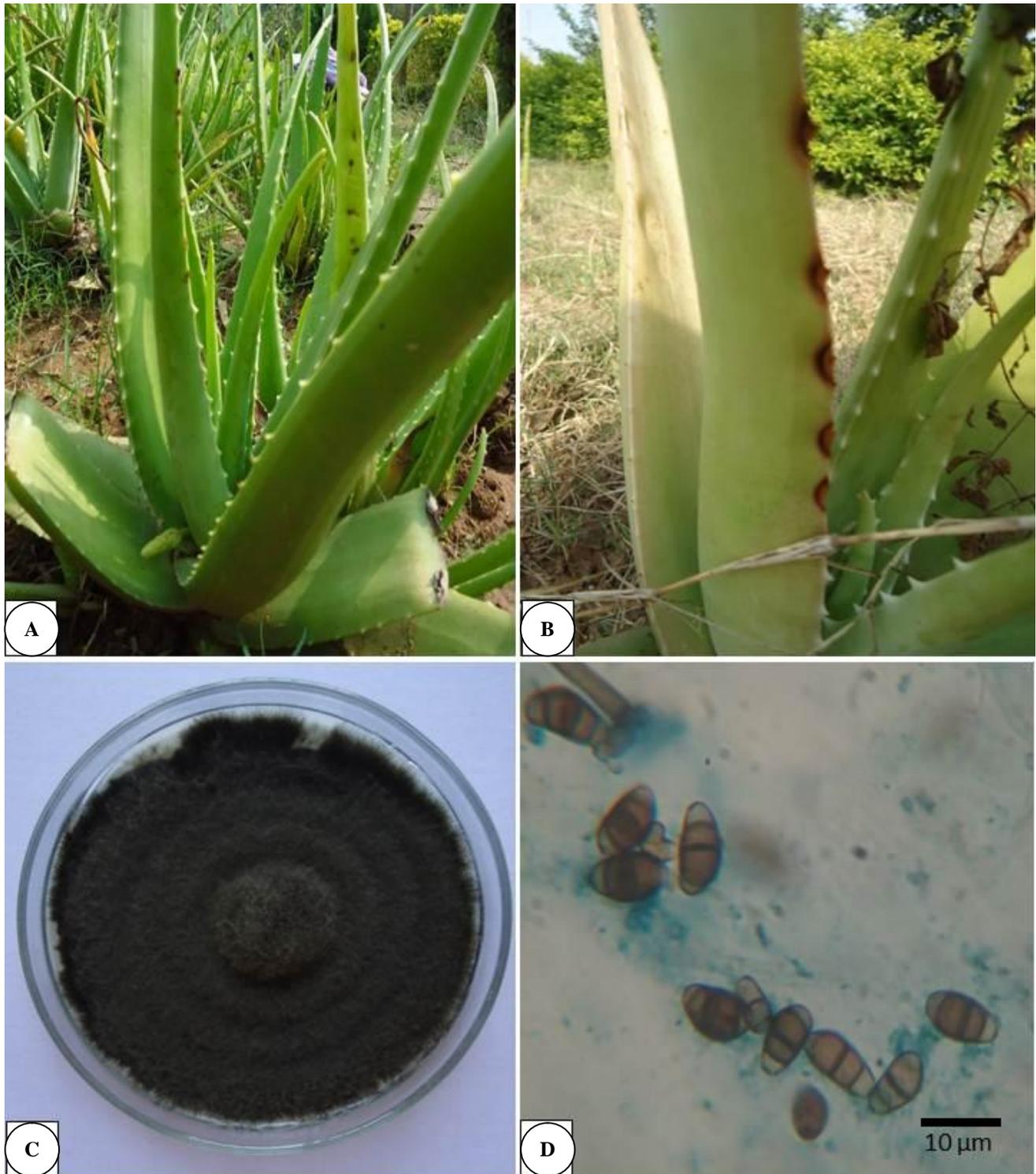


Figure 2. Leaf spot disease on *A. vera* caused by *Curvularia ovoidea*. A. Initiation of disease symptoms, B. Mature symptoms, C. Ten days old colony of *C. ovoidea* incubated at 25 ± 2 °C on PDA, D. Conidia of *C. ovoidea*

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