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First report of leaf spot disease caused by *Polyrostrata indica* on *Aloe vera* from Madhya Pradesh, India

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Abstract: A leaf spot disease was observed on *Aloe vera* plants growing in nurseries and botanical gardens of Gwalior, Madhya Pradesh, India. Symptoms were noticed on the tips and the middle part of the leaves in the form of small, circular, dark brown necrotic spots, with an average diameter of 0.6-1.4×0.5-0.9 cm. Dark brown colonies with granular appearance was consistently isolated from the infected tissue on PDA. Conidia were hyaline, aseptate and rod shaped measuring up to 12.5-20×7.5-15 µm. Based on the morphological and cultural characteristics, fungus was identified as *Polyrostrata indica* Prameela and Nita Mathur. Pathogenicity test conducted on healthy *Aloe* leaves showed typical leaf spot symptoms after fourteen day of infestation. To the best of our knowledge, this is the first record of this pathogen on *A. vera* in India.

Keywords: *Aloe vera*, Leaf spot, *Polyrostrata indica*, Disease, India.

1. Introduction

Genus *Aloe* has a history of economic and medicinal uses that span thousands of years and is the source of some of the oldest known herbal medicines. *Aloe vera* (L.) Burm. fil, is a succulent, perennial, drought resistant medicinal herb of the family Aloaceae. It has naturalized throughout the warm regions around the world including Africa, Asia, China and India. The Egyptians called it "sanctuary plant of immortality" (Park & Lee, 2006). Plant is stemless with triangular, elongated, fleshy leaves ranging in color from light green to bright green and the margin of the leaves are spiny. Most of the active chemical constituents are found in the leaves which composed of rind, juice and gel (Ramachandra & Rao, 2008). The mucilaginous gel at the centre of leaves is also called "aloe gel" is used for various medicinal, cosmetic and nutraceuticals applications. Gel contains 98.5% water and 200 potentially active chemical constituents like vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids (Boudreau & Beland, 2006; Yebpella et al., 2011). Its gel is used both, topically (treatment of wounds, minor burns, and skin irritations) and internally to treat constipation, coughs, ulcers, diabete, headaches, arthritis, immune-system deficiencies (Vogler & Ernst, 1999; Eshun & He, 2004; Steenkamp & Stewart, 2007).

Aloe vera has gained attention of plant pathologist because of various fungal and bacterial diseases like leaf spot, anthracnose, soft rot, dry leaf rot and root rot occur during its cultivation which lead economic loss to the crop. Among the various diseases, leaf spot is one the most devastating disease of *Aloe vera* which causes changes in morphological characteristics as well as in biochemical constituents also. Alteration in the antioxidant potential of *Aloe vera* due to infection of *Alternaria alternata* has also been reported by Pritam & Kale, (2007). Keeping in view the medicinal as well as cosmetic importance of *Aloe vera*, the present study was aimed to isolate and identify fungal pathogen associated with leaf spot disease.

2. Material and methods

An intensive survey of various nurseries and botanical gardens located in five major areas of Gwalior city i.e. Morar, City Centre, Chetakpuri, Kampoo and Lashkar was carried out in 2011 to explore the fungal disease on *A. vera* plants. Infected leaves samples of *A. vera* were collected randomly in sterile polythene bags, brought to the Mycology and Plant Pathology Laboratory at

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School of Studies in Botany, Jiwaji University, Gwalior and kept at room temperature for further analysis. Diseased leaves were washed in running tap water to remove soil & other unwanted contaminants. All the collected plant samples were examined morphologically with the help of magnifying glass. Leaf spot tissues were cut into small pieces, surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 2 min and then washed three-four times in sterile distilled water. The sterilized leaf pieces were then aseptically transferred to petri-plates containing Potato Dextrose Agar and incubate at 27±2 ℃ for 5 to 6 days. The fungal growth on inoculated leaf pieces was sub cultured on fresh Potato Dextrose Medium (PDA) medium for identification purposes.

Microscopic examinations were performed by mounting fungal hyphae in lacto-phenol cotton-blue mixture. The isolated fungus was identified on the basis of cultural characteristics (Shape, size and color of colony) and microscopic features (characteristic of mycelium, diameter of conidia and pycnidia) as described by Devi et al. (2009). The further Identification of pathogens was confirmed at the Indian Type Culture Collection (ITCC), IARI, New Delhi, India.

Pathogenicity of the isolated organism was confirmed on detached healthy leaves of *A. vera*. Three healthy leaves were surface sterilized with 1% sodium hypochlorite solution (NaOCl). Artificial pricks approximately 2 mm deep on the upper surface of leaves were made by sterilized needle. Under aseptic conditions spore suspension (1×10^6 spore ml^{-1}) of the isolated organism was sprayed through sprayer on leaves and lined with moist blotting paper. Leaves sprayed with sterile distilled water served as control. Leaves were incubated at 25±2 ℃ for 12-14 days. The causal organism from the infected leaves was re-isolated on PDA medium.

3. Results

During survey it was revealed that pathogen found associated with leaf spot disease on *A. vera* was observed only in winter season i.e. December to January. Leaf spot infection was recorded from almost all the nurseries and botanical gardens. Results exhibited that *Polyrostrata indica* isolated from the infected leaf samples.

3.1. Symptoms of the Disease

Morphological examination revealed changes in terms of colour, texture and appearance of the leaves. Infected leaves were light green in colour, mushy and less fleshy, margin of the leaves were distorted. The disease appeared as small, circular, light maroon spots on tips and the middle portion of leaves. Progressively, the size of spots enlarged, sunken, and became brown colour bordered with water soaked margins. At the maturity, spots became dry, necrotic and turned into brownish black in colour with an average size of 0.6-1.4×0.5-0.9 cm (Fig. 1 A-B).

3.2. Identification of Causal Organism

The fungal colonies on PDA showed dark brown colour with granular appearance. Mycelium was thin, hyaline turned into thick walled. Rough septate mycelium attached to the pycnidia, long thick hairs were immersed or semi immersed. Pycnidia were black having beak through which spores were discharged in cirrus form. Conidiogenous cells were hyaline and smooth with large globose guttulae. Conidia were hyaline, aseptate, smooth and rod shaped measuring up to12.5-20×7.5-15 µm in diameter (Figure 2 A-D). Based on the cultural and microscopic characters, the fungus was identified as *Polyrostrata indica* Prameela and Nita Mathur (#ITCC-8188.11).

3.3. Pathogenicity Test

Initiation of symptoms was appeared on the fifth day of infestation. Initially, round, water soaked spots were appeared on the upper surface of leaves. As the infection progressed, spots got sunken and brown in color. On the fourteen day, the spots become necrotic and turned into brownish black in color. The symptoms of disease noticed during pathogenicity were almost similar to the natural symptoms. The pathogen was re-isolated from all inoculated leaf samples. However, no symptoms were observed on control plants. The fungi were re-isolated from the infected leaves and were compared with the original culture of *Polyrostrata indica.*

4. Discussion

Aloe vera, being like a cactus plant has enjoyed a long history as a herbal remedy and is perhaps the most popular herb in use today. It is highly

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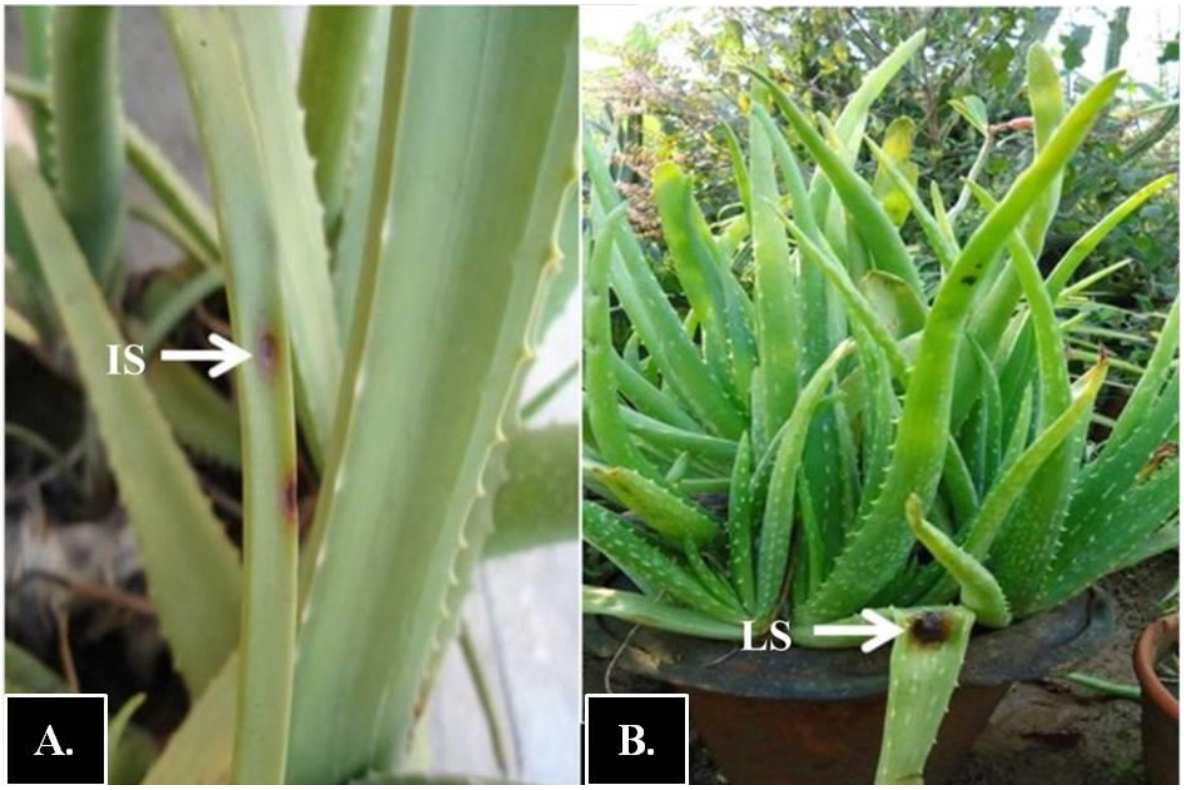

Figure 1. (A-B) Symptoms of leaf spots on *Aloe vera* caused by *P. indica***. (**IS-Initial symptoms; LS- Later symptoms).

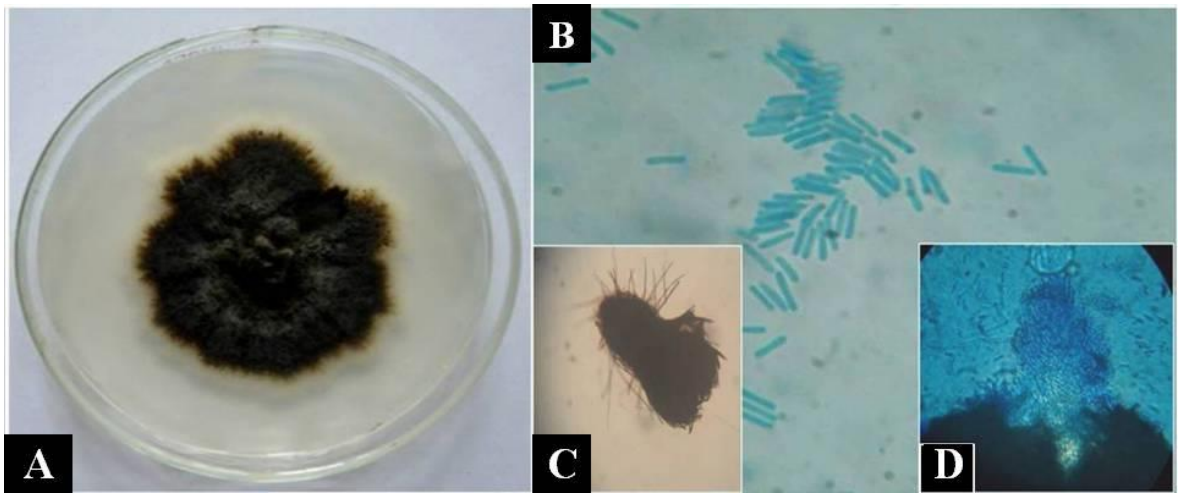

Figure 2. A. Culture of *P. indica* on PDA (ten days old); **B**. Microscopic view of Conidia, **C** Hairy pycnidia; **D** Pycnidia releasing conidia

appreciated due to its multifarious uses and high medicinal values. Besides having therapeutic potential, *A. vera* has been reported to infect with different leaf spot pathogens. Various fungal pathogens have been reported to cause leaf spot disease on *A. vera* such as *Fusarium phyllophilum* (Kishi et al., 1999); *Alternaria alternata* (Manjul et al., 2008; Kamalakannan et al., 2008; Bajwa et al., 2010; Abkhoo, 2013), *Colletotrichum gloeosporioides* (Avasthi et al., 2011); *Fusarium oxysporum* (Kawuri et al,. 2012); *Nigrospora oryzae* (Zhai et al., 2013) and *Phoma betae* (Avasthi et al., 2013), *Sphaeropsis sapinea* (Kamil et al., 2014), *Curvularia lunata* & *C. ovoidea* (Avasthi et al., 2015); *Alternaria tenuissima* (Vakalounakis et al., 2015) *Phomopsis* sp. (Avasthi

et al., 2016), *Cladosporium sphaerospermum* (Avasthi et al., 2016) and *Phoma eupyrena* (Avasthi et al., 2017).

A new genus *Polyrostrata* was described in the family Sphaeropsidaceae in 2009 (Uma et al., 2009). There are scarce published reports on this pathogen. *Polyrostrata indica*, has been reported from the stem of *Erianthus munja* (Devi et al., 2009). To the best of our knowledge, this is the first report of leaf spot disease on *A. vera* caused by *P. indica* in the India.

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