

Short Paper

First report of *Cladosporium sphaerospermum* causing leaf spot disease of *Aloe vera* in India

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Abstract: This paper deals with the study of a leaf spot disease observed on *Aloe vera* leaves in various nurseries and botanical gardens during the routine surveys of two consecutive years of 2010 and 2011. The symptoms appeared in the form of circular to oval, brown to black, sunken spots on abaxial surface of leaves. The disease was observed only in January to February during the survey. Colonies on PDA were velvety, dark olivaceous to greenish black in colour. The identification of the pathogen was done phenotypically using disease symptoms and microscopic characteristics. Further investigation identified it as *Cladosporium sphaerospermum* Penzig which was also confirmed at Indian Type Culture Collection (ITCC), IARI, New Delhi, India. According to the literature, this is the first report of *Cladosporium sphaerospermum* as causal agent of leaf spot disease on *A. vera* from India.

Keywords: *Aloe vera*, leaf spot, *Cladosporium sphaerospermum*, new report, India

Introduction

Aloe vera is one of the imperative plants of family Aloeaceae. It is perennial succulent plant that grows up to 60-100 cm in height. The rosette or alternate pattern of leaves has made this plant nique in appearance whereas, having wide range of biochemicals made it useful in medicinal and cosmetic industries also. The leaves are long, thick, fibrous and lanceolate, with spiny margins. These are soft, filled with mucilaginous gel, which is most important constituent of the plant. This gel contains more than 75 nutrients and over 200 bioactive compounds including sugars, anthraquinones,

saponins, vitamins, enzymes, minerals, salicylic acid and amino acids (Dureja *et al.*, 2005; Boudreau and Beland, 2006). This wide diversity of bioactive compounds made *A. vera* a key plant used to prepare herbal medicines and cosmetic products. Besides using *A. vera* in the production of soap for bathing, shampoo, hair wash, tooth paste and body creams, it is also found very effective for the treatment of wounds, skin disease, cold and cough, constipation, piles and fungal infection. Many herbal drugs and drinks have also been formulated from *A. vera* plants and can be used for treatment of asthma, ulcer and diabetes (Sahu *et al.*, 2013). Although, *A. vera* is found as wild herb in coastal areas of South India, it now is under cultivation practices due to its huge demand in herbal and cosmetic industries. It is mainly cultivated in Alwar (Rajasthan), Satanapalli (Andhra Pradesh), Rajpipla

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(Gujarat) and some parts of Tamil Nadu, Madhya Pradesh and Maharashtra (Maiti and Chandra, 2002).

During our survey of the various nurseries and botanical gardens in Gwalior region of Madhya Pradesh, presence of a leaf spot disease was noticed. Critical morphological examination of disease symptoms and microscopic analysis of isolated microorganism revealed it a fungal pathogenic disease. Therefore, this study was carried out to identify the causal agent of leaf spot symptoms on *A. vera*.

Materials and Methods

Sample collection

A survey of various nurseries and botanical gardens in Gwalior was carried out for two consecutive years i.e. 2010 and 2011. Symptoms of leaf spot diseases were observed on *A. vera* leaves. Samples showing disease symptoms were collected randomly from surveyed locations, placed into labeled zip lock bags, and brought into the laboratory for isolation studies. Morphology of disease symptoms was studied with the help of hand lens and dissecting microscope.

Isolation and identification of pathogen

Ten infected leaves were collected randomly from each nursery and botanical gardens surveyed, taken into the laboratory and washed thoroughly with running tap water to remove the surface contaminants. The method described by Ayodele and Ilondu (2008) was adopted for pathogen isolation. The infected leaves were cut into small pieces using sterile razor blades. These pieces were surface sterilized with 2% sodium hypochlorite solution for 2mins and rinsed three-four times in sterile distilled water. These disinfected pieces were then placed between blotting papers and aseptically inoculated onto petri dishes containing Potato Dextrose Agar (PDA) media. The plates were incubated at 25 ± 2 °C for 5 to 6 days, and the growth of fungal colonies were recorded every day.

For characterization and identification of the fungus, the mycelia growing from the tissues were

transferred onto fresh PDA medium amended with 1.0 mg/ml chloramphenicol and sub-cultured repeatedly until pure cultures of the isolates were obtained. The identification of the fungus was done microscopically. Slides for microscopic observations were prepared in lactophenol cotton-blue mixture and photographed. Minimum 25 measurements were made for each structure like conidiophores and conidia. Standard mycological manuals by Alexopoulos *et al.* (2002), Ellis (1971) and Bensch *et al.* (2012) were consulted for comparison and confirmation. Identification of fungus was also confirmed at the Indian Type Culture Collection (ITCC), IARI, New Delhi, India.

Pathogenicity test

For pathogenicity test, the Koch postulates were followed. Healthy leaves were surface sterilized for 1min 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 (1×10^9 per ml) of the test organisms was delivered through a sprayer and lined with moist blotting paper. Leaves were incubated at 25 ± 2 °C for 8-10 days. Leaves sprayed with sterile distilled water served as control.

Results

Disease symptoms

After survey results analysis it was observed that leaf spot disease is initiated in the winter season, spreads as the winter season progresses (January–February), and lasts up to rainy season. About 8.92-10.0% of the *A. vera* plants surveyed in and around Gwalior, India, were found infected with leaf spot disease. The disease symptoms appeared in the form of circular to oval, brown, sunken spots on the abaxial surface of leaves. Gradually these spots became enlarged ($0.4-0.9 \times 0.3-0.6$ cm), bulged and brown in colour. In later stage sporulation appeared and spots became olive black to dark black in colour. Sometimes spots often joined together and became irregular in shape (Fig. 1 A-B).

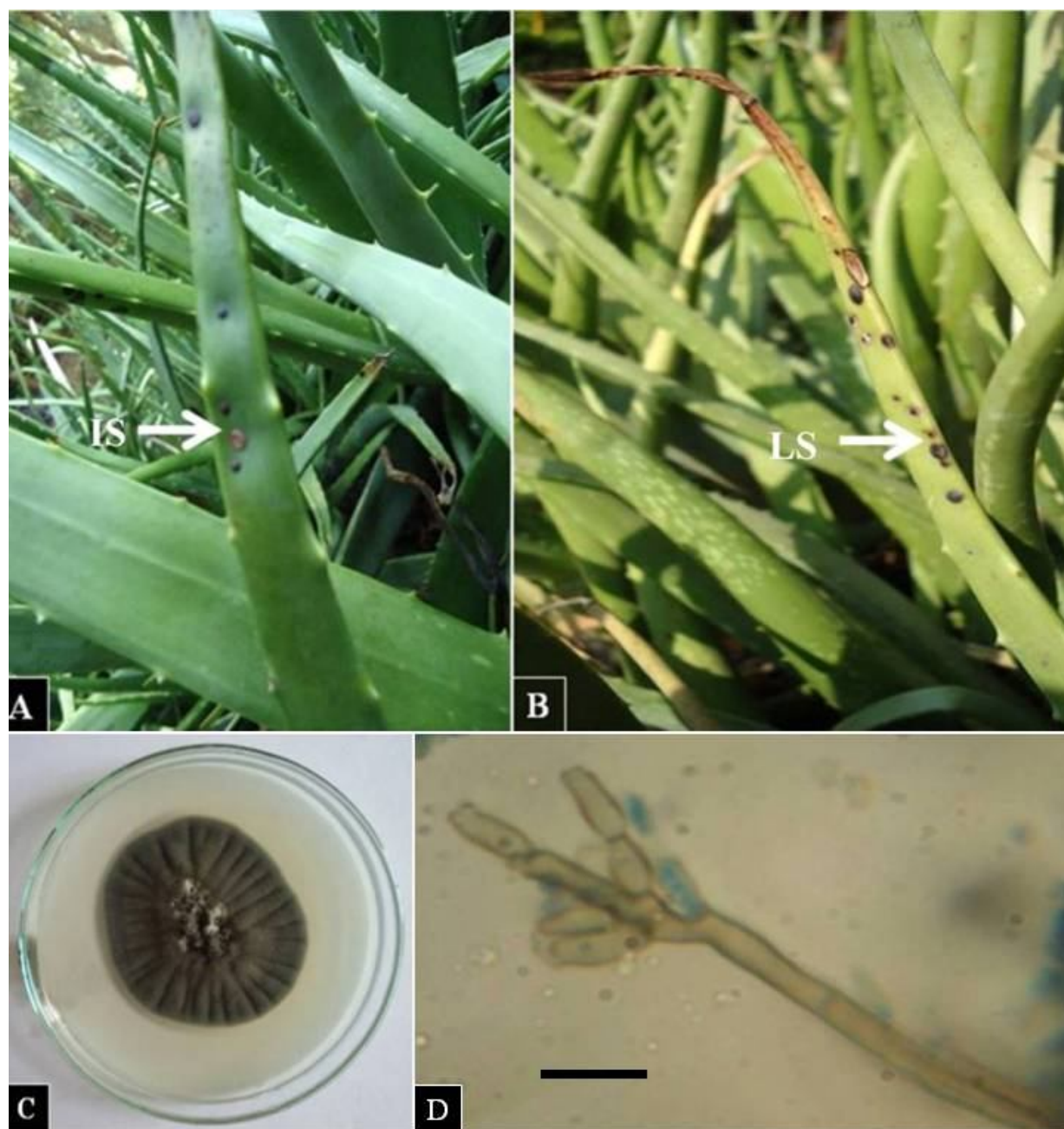


Figure 1. (A-B) Disease symptoms on *Aloe vera*. (C) A mature culture of *Cladosporium. sphareospermum* on PDA. (D) Microscopic view of *C. sphareospermum* showing conidiophore and conidia. (IS=Initial Symptoms; LS = Later symptoms). Scale bar: 10 µm.

Identification of the pathogen

The fungus isolated from the inoculated tissues of *A. vera* leaves was identified according to the developed colonies and microscopic characters. Colonies on PDA were velvety,

dark olivaceous to greenish black in colour. Conidiophores were branched and septate. Conidia formed in branched chain, small terminal conidia were globose or subglobose, $2-5 \times 2-4 \mu\text{m}$, apex rounded, base rounded to

slightly attenuated, 4-10×3-5 µm, 0-1-septate, sub hyaline, pale olivaceous to olivaceous-brown and thin-walled. The morphological characteristics of the isolate revealed that pathogen belongs to fungal group. No teleomorph was observed. Further microscopic dimensions were also analysed and the fungal pathogen was identified as *Cladosporium sphaerospermum* Penzig. The Fungal Identification Service, Indian Type Culture Collection (ITCC-7801.10), IARI, New Delhi, India also confirmed the identity (Fig. 1 C-D).

Pathogenicity testing resulted in similar symptoms of leaf spot infection as in natural disease. However, no symptoms were observed on control plants. The fungi were re-isolated from the infected leaves and compared with the original culture of *C. sphaerospermum*.

Discussion

Cladosporium is a hyphomycetes genus proposed by Link (1816). It is one of the largest genera of hyphomycetes comprises more than 807 records (www.indexfungorum.org). The conidiophores and their branching pattern at various stages of development, presence of unique type of scars and conidial hila and basal, intercalary and terminal conidia are the main features of the genus identification. Taxonomically the fungi belong to phylum Ascomycota; class Dothideomycetes; order Capnodiales and family Davidiellaceae. The fungi can grow in extreme to polar environments. *Cladosporium sphaerospermum* the fungal pathogen responsible for leaf spot disease in *A. vera* isolated and identified in the present study, is one of the most common species of genus *Cladosporium*. The fungus is described by Penzig (1882) from decaying *Citrus* leaves and branches in Italy. The main characteristics like numerous globose to subglobose terminal conidia, almost smooth to verruculose; conidiophores often branched described by Bensch *et al.* (2012) were also observed in present study. Besides being phytopathogenic, fungus exhibits saprophytic and symbiotic nature as well. Some of the strains of the fungus are pathogenic to humans and animal also.

Several plant hosts of this pathogen are reported so far. This species of *Cladosporium* is reported to cause foliar diseases of plants in tropical and subtropical regions. It is also reported from leaf spot disease of *Litsea lanuginosa* from India (Pandey and Gupta, 1984). Therefore, this study reports a new host record for the fungus.

A number of leaf spot diseases have been reported on *A. vera* to date from India and other countries. Leaf spot disease caused by *Fusarium phyllophilum* (Kishi *et al.*, 1999); *Alternaria alternata* (Abkhoo, 2014); *Alternaria tenuissima* (Vakalounakis *et al.*, 2015); *Colletotrichum gloeosporioides* (Avasthi *et al.*, 2011); *Curvularia lunata* and *C. ovoidea* (Avasthi *et al.*, 2015); *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) and *Phomopsis* sp. (Avasthi *et al.*, 2016) were reported from various parts of the world including India. However, no record of *C. sphaerospermum* is so far available on *A. vera*. Therefore, to the best of our knowledge this is the first report of *C. sphaerospermum* as causal agent of leaf spot disease on *A. vera* from India.

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اولین گزارش از *Cladosporium sphaerospermum* عامل بیماری لکه‌برگی آلوئه‌ورا در هند

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چکیده: در پژوهش حاضر بیماری لکه‌برگی گیاه *Aloe vera* در گلخانه‌ها و باغ‌های گیاه‌شناسی طی سال‌های ۲۰۱۰ و ۲۰۱۱ مورد بررسی قرار گرفت. علائم به‌صورت لکه‌های گرد تا تخم‌مرغی، قهوه‌ای تا سیاه، آسوخته روی سطح پشتی برگ ظاهر شدند. در بررسی‌های انجام شده بیماری فقط در ماه ژانویه تا فوریه مشاهده شد. برگه روی محیط PDA مخملی، زیتونی تیره تا سیاه مایل به سبز بود. شناسایی بیمارگر به‌صورت فنوتیپی با استفاده از علائم بیماری و خصوصیات میکروسکوپی انجام شد. در ارزیابی‌های تکمیلی عامل بیماری *Cladosporium sphaerospermum* Penzig تشخیص داده شد که توسط کلکسیون گونه‌های هند (ITCC) در دهلی نو، هند، مورد تأیید قرار گرفت. براساس بررسی منابع، این اولین گزارش از وجود *C. sphaerospermum* به‌عنوان عامل لکه‌برگی در گیاه *A. vera* از هند می‌باشد.

واژگان کلیدی: *Aloe vera*، لکه‌برگی، *Cladosporium sphaerospermum*؛ گزارش جدید، هند