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Colletotrichum gloeosporioides on *Adhatoda vasica* in India

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Adhatoda vasica is a medicinally important perennial shrub used in traditional as well as modern systems of medicines to treat cough and cold, asthma, dysentery, rheumatic pain and many other disorders. A severe leaf spot disease was observed on *A. vasica* leaves collected from different regions of Bilaspur, Himachal Pradesh, India. The disease was further investigated and the pathogen was identified in the present study. Small necrotic lesions on leaves which later developed in to circular light to dark grey spots were observed when disease started. New dark grey spots encircled the older one by giving concentric ring appearance along with leaf shrinkage around the necrotic spots were also recorded. Hyaline, straight, dumbbell shaped, aseptate and oblong or cylindrical conidia with rounded or bulbous ends having size ranging between 10.4-18.6×3.4-5.5 µm and brown coloured setae of 25-130 × 3-5 µm size were observed during microscopy. *Colletotrichum gloeosporioides* was identified as the causal agent based on disease symptoms, the morphological and microscopic characteristics of the isolated fungus and pathogenicity tests. To our knowledge, this is the first report of *C. gloeosporioides* on leaves of *A. vasica* in India.

Keywords: Leaf spot, *Colletotrichum gloeosporioides*, concentric ring, *Adhatoda vasica*, India

Introduction

Adhatoda vasica Nees is a beautiful, evergreen, gregarious, medicinally important perennial shrub used in traditional as well as modern systems of medicines. The plant is also known as *Adhatoda zeylanica* or *Justicia adhatoda* belongs to the family acanthaceae and distributed throughout India, mainly in sub-Himalayan regions up to the height of 1300 m. The plant is 1-2.5 m in height and having lance shaped, oppositely arranged, smooth-edged leaves containing contain several alkaloids like vasicine and vasicinone responsible for medicinal activity. All the plant parts are generally possesses expectorants, antiseptic and antispasmodic activity and have been reported to treat cough and

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cold, asthma, dysentery and rheumatic pain (Singh *et al*., 1996; Jain and Defilipps, 1991).

The leaves of *A. vasica* contain potent phytochemicals responsible for their antimicrobial effect. The essential and the fragrant volatile oils extracted from plant leaves are rich in borneol which is an excellent antimicrobial compound (Santoyo et al. 2005). There are relatively few reports of disease of *A. vasica*; these include leaf spot caused by *Rhizoctonia solani* (Verma *et al.*, 2006), *Alternaria* blight (Singh and Verma, 2009) and rust (Yadav and Sharma, 2006) caused by *Puccinia thwaitesii*.

A severe leaf spot disease of *A. vasica* was observed in different regions of Bilaspur, Himachal Pradesh, India in 2011. Since plant is medicinally important and used in many ayurvedic formulations, the disease was further investigated and reported in the present study.

Material and methods

Sample collection

Diseased leaves exhibiting different types of typical leaf spot symptoms were collected from the *A. vasica* plants and carried to the laboratory in individual snap lock plastic bags. In the laboratory they were examined for visible symptoms as well as for microscopic examination and isolation.

Isolation and Identification of pathogen

Leaves showing the typical disease symptoms were cut into small fragments aseptically, washed thoroughly in tap water, then surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 2 min and washed three to four times in sterile distilled water. The surface sterilized leaf pieces were then aseptically plated on potato dextrose agar (PDA) media and incubated at 25 ± 2^{o} C for 6-7 days under 12 h light and dark conditions. Hyphal tips from the margin of each developing colony were subculture on PDA to study morphological and cultural characteristics. Microscopic examinations were carried out to study dimensions like conidiophore and conidia. The pathogen was identified from all infected leaf samples.

Pathogenicity test

A spore suspension of isolated pathogen was prepared in sterile distilled water and adjusted to approximately 10^5 conidia/ ml. Randomly selected healthy leaves of *A. vasica* were pin pricked, spray inoculated with spore

suspension of the pathogen and covered with ploythene bags to maintain temperature and relative humidity (average temp. 25±2 °C and 60-80% RH). Leaves sprayed with sterile distilled water served as a control. Symptoms appeared after 6-8 days consistently re-isolated and compared with original pathogen.

Results

Symptoms

The earlier symptoms of the disease were observed in early rainy season. Disease started on young leaves as small necrotic lesions which later developed in to circular light to dark grey spots. These lesions gradually enlarged and turned brown or dark grey, resulting in early leaf senescence. In the advance stage of infection the inner necrotic spots looses brown or grey coloration and new dark grey spots encircled the older one by giving concentric ring appearance. The shrinkage around the necrotic spots in infected leaves was also observed which ultimately leading to leaf drying. Interestingly the disease infection was not observed systematic; but it was found localised to various regions. The infection spreads severely as the rainy season progressed when temperatures ranged from 25 to 30℃ and lasts up to winter season. The progressive stages of leaf spot infection are depicted in Figure 1(c) whereas, severe infection in natural field conditions and on collected leaves is showed in Figure 1 (a & b).

Isolation and Identification of pathogen

Fungal colonies were circular and greyish to black in colour on PDA with greenish margin and blackish on the reverse side (Figure 2 a & b). Microscopic observations of the fungus revealed simple, septate, hyaline and branched mycelium. The conidia were found to be hyaline, straight, dumbbell shaped, one celled (aseptate) and oblong or cylindrical with rounded or bulbous ends. Their size ranging between 10.4-18.6×3.4-5.5 µm. Conidiophores were long, hyaline, septate and unbranched. Setae were brown and ranged in size from 25-130×3-5 µm (Figure 2 c). On the basis of symptoms, conidial and morphological characteristics, the pathogen was identified as *Colletotrichum gloeosporioides*. This identification was also confirmed by Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi, India (ITCC- 8607.11).

During the pathogenicity test, similar symptoms also appeared on inoculated leaves of *A. vasica* after 6-8 days of inoculation. No symptoms were

observed in control leaves. Diseased leaf specimens from artificially inoculated plant also yielded same fungus that confirmed pathogenicity of *C. gloeosporioides.*

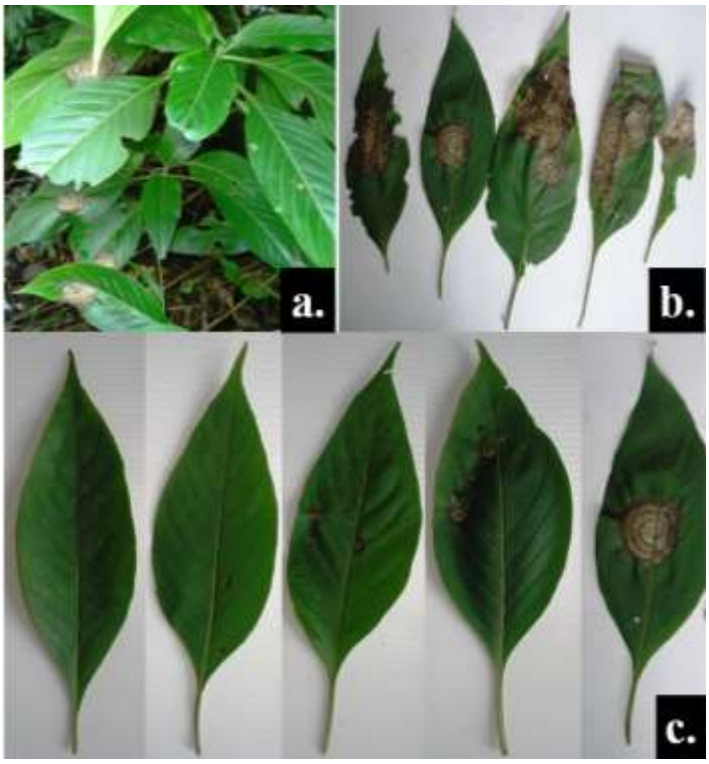

Fig. 1. (a) *A. vasica* leaves infected with leaf spot disease in field (b) severely infected leaves (c) leaf spot symptoms showing the progressive stages of disease infection, healthy leaf (very left).

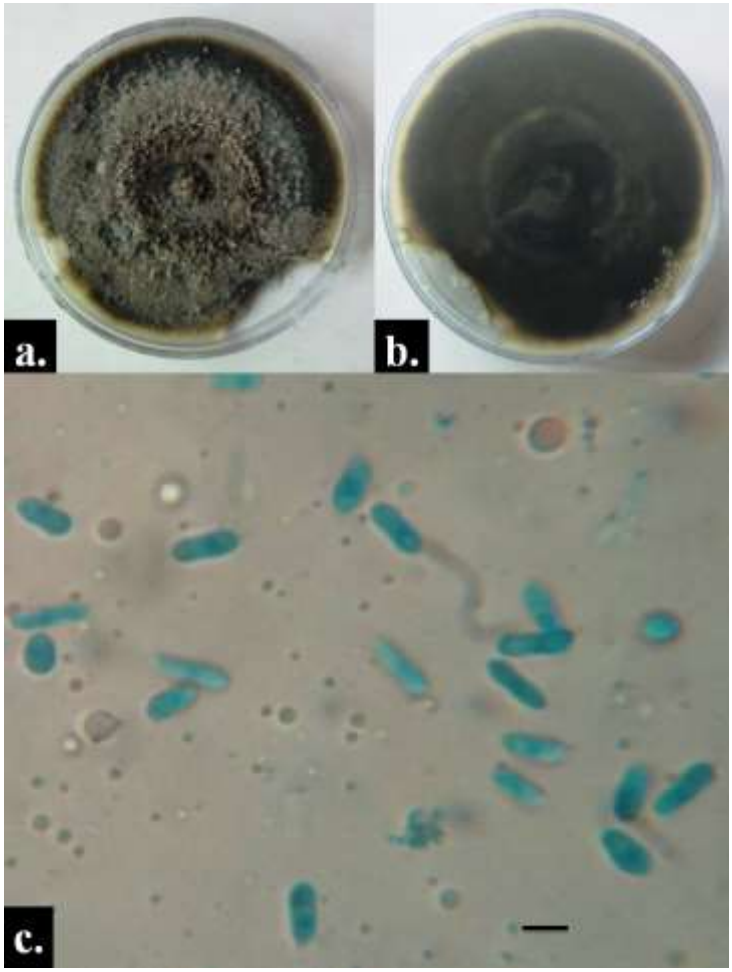

Fig. 2. (a) Mycelial colony grown on potato dextrose agar media (b) reverse side of culture (c) conidia of *C. gloeosporioides* in cotton blue stain at 40X (bar = 20 μm).

Discussion

The results obtained in the present study indicated *Colletotrichum gloeosporioides* as the disease causing fungus on *Adhatoda vasica* leaves. A number of fungal diseases of *A. vasica* have been reported earlier. A leaf spot disease caused by *Rhizoctonia solani* was observed from Jaipur (Verma *et al.,* 2006), covering an area of 8-10 km^2. A severe leaf rust of *A. zeylanica* (*Justicia gendarussa*) was observed from central India (Yadav and Sharma, 2006). The pathogen was identified as *Puccinia thwaitesii* (HCIO No. 46262). Later on, Singh and Verma (2009) studied the incidence (26.25 and 32.25%) of alternariablight (caused by *Alternaria alternata*) in *Adhatoda vasica* (*Justiciaadhatoda*) plantations in Jaipur and Sikar districts, Rjasthan, India.

Similarly, Sutare and Kareppa (2010) studied the fungal diseases of *Adhatoda zeylanica.*

Colletotrichum gloeosporioides was also recorded as a disease causing fungal pathogen on a variety of hosts. Anthracnose caused by *C. gloeosporioides* on plants like Indian fig cactus (Kim *et al.,* 2000) and *Jatropha curcas* (Kwon *et al.,* 2012) was recorded form Korea whereas, on *Olea europaea* (Sergeeva *et al.*,2008) from Australia; *Blepharocalyx salicifolius* (Larran *et al.,* 2011) from Argentina and *Allium cepa* (Sikirou *et al.,* 2011) from Benin during last couple of years.

The pathogen is reported to cause a number of plant diseases in India also. *C. gloeosporioides* causing anthracnose in bell pepper seed crop was recorded by Gupta *et al.* (2009). Similarly, anthracnose of *Aloe vera* leaves caused by *C. gloeosporioides* was reported by Avasthi et al. (2011). They observed the loss of mucilaginous gel in affected area which ultimately leads the death of infected leaves. Occurrences of *C. gloeosporioides* on noni (Hubballi *et al.,* 2012) and on *Jasminum grandiflorum* form Jaipur, Rajasthan (Sharma *et al.,* 2012) and on *Pedilanthus tithymaloides* (Gautam *et al.*, 2012 a&b) were recorded recently from India.

After the detailed analysis of diseased leaves and isolated pathogen it was concluded that present disease found on *Adhatoda vasica* leaves was caused by *Colletotrichum gloeosporioides*. According to the literature, this is the first report of *C. gloeosporioides* on leaves of *A. vasica* in India.

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