

First Report of *Ocimum gratissimum* as a New Host for Pathogenic Fungus *Alternaria alternata* Causing Leaf Spot Disease in Uttar Pradesh, India

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Abstract

A new leaf spot disease was observed during the 2014 on *Ocimum gratissimum* in the BSIP Garden of Uttar Pradesh (India), causing significant damage to the host. The symptoms produced by the associated fungus consisted of necrotic dark brown, circular to sub-circular, up to 5 mm spots on both surfaces of the leaves. A fungus having distinct dictyospores (muriform) similar to those produced by *Alternaria* spp. was observed. The morphological characteristics of the fungus as well as the pathogenicity on the host confirmed that the pathogenic fungus is *Alternaria alternata*. To the best of our knowledge, *Ocimum gratissimum*, is here for the first time reported as a new host for the pathogenic fungus *A. alternata* causing leaf spot disease in Uttar Pradesh, India.

Keywords: Foliar fungi; Fungal disease; *Alternaria alternata*; New host record

Introduction

Ocimum gratissimum L. is an aromatic and medicinal plant belongs to family Lamiaceae, commonly called clove basil, African basil and wild basil. The plant is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicine for the treatment of different diseases such as upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, cough fever and conjunctivitis [1]. It is sometime also used as an antibiotic. The essential oil is extracted from the plant leaves and stems of the plant that contain Eugenol and a lesser amount of Thymol, which used as substitutes for clove oil and thyme oil. The essential oil of the plant is also used as insect repellent. It has been demonstrated that the essential oil showed fungicidal activity against all of the *Candida* species [2] and had significant fungistatic activity against all the species investigated [3]. Many research studies showed that the oil has antifungal activities against different food-borne pathogens. It was also reported that the volatile oil of the plant had significant antimicrobial effects against both fungi and bacteria [4].

Objectives: The aim of the study was to identify the pathogenic micromycetes species inhabiting and damaging the living leaves of *Ocimum gratissimum* by producing leaf spot symptoms using morphological methods.

In the continuation of disease observation during 2014, the garden plant (*Ocimum gratissimum*) leaves showing leaf spots were collected from BSIP campus, Lucknow (26.8656° N, 80.9364° E), Uttar Pradesh. The samples were placed in separate polyethylene bags and transported to the laboratory and processed by following the standard techniques [5,6]. Photographs of infection spots on host leaves were taken by using a Sony DSC-5730 camera. The specimens for microscopic observation were prepared by hand sectioning, scraping and culturing in PDA, after the slide preparations mounted on clear lacto-phenol cotton blue mount mixture. The observations were made with the help of an Olympus BX-51 light microscope by using the Syntek USB camera. Detailed observations of morphological characters were carried out at different magnification through a light microscope (400X and 1000X). Measurements were done with the help of micrometer. Morphotaxonomic determinations were made with the help of current

literatures. The Koch's postulate was performed for pathogenicity. The dried specimen of *Alternaria alternata* has been deposited in the Ajrekar Mycological Herbarium, (AMH-MACS-ARI), Agharkar Research Institute, Pune, India (Accession No. AMH-9720) and a part of the same was retained in BSIP Herbarium (BSIPMH-66), Lucknow, India.

The host symptoms, sporulation pattern, conidiophores and conidial morphology were examined and upon morphological observation the fungus shows following characteristics as:

The symptoms of disease initially began from the almost middle of the leaves as small light brown circular to sub circular spots (Figures 1a-1d). In severe infection it forms a larger necrotic area.

Anamorphic fungus, Follicolous, Infection spots amphigenous, circular to sub circular, brown, 5 mm in diam. Colonies amphiphylous, effuse, brown. Mycelium internal. Stromata present, but not clearly seen. Conidiophores macronematous, fasciculatus, 2-3 in a fascicle, straight to flexuous, geniculate, simple, cylindrical, unbranched, thick walled, smooth, 2-3 septate, brown, 30-50 × 3-5 µm. Conidiogenous cells integrated, terminal, polytetric, scars thickened. Conidia simple, acropleurogenous, solitary to catenate, dry, obclavate to ellipsoidal to ovoid, rostrum present (upto 20 µm), 3-7 transversely septate and 2-5 obliquely septate, brown, base obtuse, hilum thickened, 15-60 × 7-18 µm (Figure 2).

Material Examined

India, Uttar Pradesh, Lucknow, BSIP Garden, on living leaves of

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Figure 1: Leaf spot symptoms on *Ocimum gratissimum*
a: Host plant habitat; b: Enlarge view of host plant
c: Leaf spots on upper surface d: Leaf spots on lower surface
c-d = 20 mm.



Figure 2: *Alternaria alternata*, leaf spot pathogen of *Ocimum gratissimum* plant.
a, b: host-pathogen interaction showing conidiophores formed on host epidermis;
c, e, f, g: conidia d: attachment of conidia Scale bars a-g = 20 µm.

Ocimum gratissimum (Lamiaceae), 5th December, 2014, Coll., Shambhu Kumar, AMH-9720, BSIPMH-066.

The disease bearing leaves (infected leaves) were cut into smaller size and then sterilized in sodium hypochlorite (2 min.). After this, they washed with sterile distilled water and then plated on PDA media, and incubated at 25°C for 5-7 days under a 12 h light and dark condition. The fungal colonies were developed on the media, were initially white but becoming brown later.

While determining the pathogenicity test, the inoculations were performed on detached, surface sterilized, healthy leaves following by the [7]. 10 leaves per isolates were used to experiment. A 5-µl drop of conidial suspension containing 1×10^5 CFU/ml was placed on each leaf. The leaves were incubated in a growth chamber (90% relative humidity and a 12-h photoperiod). After 5-7 days, leaf spots similar to the original symptoms developed on all inoculated leaves and the pathogen *A. alternata* was consistently re-isolated from symptomatic tissue,

fulfilling Koch's postulates. The control leaves, however inoculated with sterile distilled water remained asymptomatic (symptomless) or without infection.

On the basis of external symptomatology, fungus morphological characteristics, and pathogenicity, the pathogen was identified as *Alternaria alternata*, is an ubiquitous fungal pathogen reported globally known as the cause of leaf spot and other diseases in over 100 host species of plants [8], particularly wide range of agronomic plants, as well as wild species [9] but also as postharvest disease in various crops [10].

According to the current literatures, no any fungus has been reported to cause leaf spot diseases on *Ocimum gratissimum* from all over the world [11-15] particularly from India [16-23].

Although, *A. alternata* reported on *Ocimum* sp. from Kenya [24], *Alternaria* sp. on *Ocimum basilicum* from California [25] and Florida [26] and *A. tenuissima* from Pakistan [27] and from India, *A. alternata* has been reported on leaves of *Ocimum sanctum* from Poona, M.S. [28], on seeds of *Ocimum* sp., from Solan, H.P. [29] and leaves of *Ocimum basilicum* from Nagarjun University, A.P. [30].

Therefore, to the best of our knowledge, the leaf spot disease of *Ocimum gratissimum* caused by *A. alternata* is the first report from Uttar Pradesh (India) as well as for science and incidence of present leaf spot disease is very interesting and significant to control measures.

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