

Original Research Article

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Identification of *Alternaria alternata*

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ABSTRACT

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The present study was on identification of *Alternaria alternata* which responsible for *Alternaria* leaf spot in Asalio. The disease was found to be very severe in all Asalio growing areas causing heavy destruction. The main aim of this study clearly demonstrated that *A. alternata* is a primary pathogen in Asalio, causing a leaf-spot disease. Isolates of *Alternaria sp.* were collected from leaf spot disease infected samples of Asalio in the fields around Udaipur. The pathogen was isolated from sample on PDA media in slants. These cultures were sent for final identification to "Indian Type Culture Collection Identification/Culture supply Services, Division Of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012". These cultures were identified as *Alternaria alternata*.

Introduction

Asalio is mainly affected by diseases like *Alternaria* leaf spot & white rust. The Rabi season Asalio crop in India is commonly affected by *Alternaria* leaf spot. The disease causes extensive damage to the quality and quantity of the foliage and grains. If persistent cloudy and cool weather prevails the blight appears after flowering stage of the crop. *Alternaria* genus is the largest genus. It is distributed worldwide and has been reported

on 115 plant genera from 43 plant families that cause blight and leaf spot disease (Neergaard, 1945).

Alternaria genus has a worldwide distribution. Its species have a wide host range fungi and responsible for causing leaf spot diseases in number of economically important crop plants. *Alternaria* causes disease among the most common of many plants throughout the world and the total yield losses caused by the fungus on its wide range

of host sometimes exceeds the total yield loss caused by any other pathogen (Agrios, 1997). A great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk *et al.*, 2008)

Presently, *Alternaria* leaf spot is the most destructive disease of Asalio. In the year 1967 *Alternaria* blight of Asalio caused by *Alternaria brassicae* was first reported from Kaffa province (Stewart and Dagnalechew, 1967), Ethiopia.

In India for the first time Melkania (1980) reported *Alternaria alternata* as instant of *Alternaria* leaf spot on leaves of cress at Almora and in the same year Singh (1980) reported that *A. alternata* as causal agent of *Alternaria* leaf spot on cress at College Farm, Banaras Hindu University, Varanasi (U.P.).

Alternaria is included in order Hyphomycetes, family Dematiaceae, genus dictyosporic and it is a Fungi Imperfecti. *Alternaria* genus was first reported by Nees (1816). *A. alternata* belongs to Longicatenatae according to Neergaard (1945). Melkania (1980) reported that *Alternaria* leaf spot on leaves of Asalio was caused by *A. alternata* and Utikar and Padule (1980) described its morphology.

He reported that conidiophores of *A. alternata* were simple, light brown, variable in length ranging from 17.10 to 61.56 μm and mostly 2-3 septate rarely 4-5 septate. Conidia were found light to dark brown in colour, uniform with 0-2 longitudinal septa and 1-6 transverse septa, and variable in shape and size, mostly oval shape with rudimentary beak and in size measuring about 10.26-77.52 x 4.56-14.82 μm .

Simmons and Roberts (1993) observed three-dimensional sporulation patterns of *A.*

alternata in electron microscope at 50 magnifications. The symptoms produced by *Alternaria brassicae* on *Lepidium sativum* L. are given in detail by (Singh and Upadhyaya, 1971).

The initiation of disease symptom is from basal leaves in the form of small, yellow, circular patches which becomes necrotic, having 2-5 concentric rings and light brown in colour (1-7 mm in diameter).

Sometimes these spots coalesce with each other and occupy large blighted area. In later stage, spots become larger in size with distinct concentric rings and dark in colour. The severity of the disease increases upto mid-January.

At this stage, the stem and floral parts of plant also become diseased. Finally, whole plant shows typical blight symptoms. Fungal colonizes the in xylem of the host plant, and as a result, blockage and breakdown of the xylem lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant.

Materials and Methods

The infected samples of Asalio showing symptoms of *Alternaria* leaf spot disease were collected from the farmer's fields around Udaipur. Fungal pathogen was isolated from diseased samples of Asalio using standard methodology on potato dextrose agar (PDA) medium.

Small bits of infected portions were surface sterilized for 1 minute in mercuric chloride solution (0.1%) and washed thrice in sterilized distilled water under totally aseptic conditions in a laminar air flow. These were then dried by keeping in two folds of sterilized filter papers then aseptically transferred to PDA in Petri plates.

Plate.1 Pathogenicity of *A. alternata* on Asalio



Plate 1: Pathogenicity of *A. alternata* on Asalio
a. Field view of disease
b. Diseased plant
c. Typical symptom generated by *A. alternata*
d. Close view of disease symptoms
e. Pure culture of *A. alternata*
f. Conidia at 40X

The plates were incubated at $27\pm 1^{\circ}\text{C}$ for 7-8 days. For Sub-culturing 5 mm bit of the culture were cut from the periphery of the mycelial growth of 6-7 days old colonies and transferred on to the (PDA) slants. The cultures were incubated at $27\pm 1^{\circ}\text{C}$ for growth and sporulation and further purified by single spore/hyphal tip method.

Pure cultures were maintained on PDA slants. The microscopic examination of cultures indicated that the fungus belongs to the genus *Alternaria*. The culture was maintained at 4°C . The culture of *A. alternata* thus isolated and identified was further tested on pot grown plants for its pathogenicity and fulfilling the Koch's postulates.

These Asalio plants were raised in Soil: FYM (3:1) mixture from field. Ten plants were maintained per pot. The inoculum prepared

and also by periodical transfers on PDA slants for further use.

Temporary mount were prepared from freshly subcultured sporulating culture on the slides in lacto phenol and cotton blue for observing the morphological characters of the fungus such as mycelium, conidiophores, conidia and various cultural characters on PDA media these slides were examined thoroughly under the microscope. The standard references of *Alternaria alternata* were used for identification of culture (Simmons, 2007). from the pure culture. The pure culture of pathogen obtained by culturing of isolate on PDA for 10 days on $27\pm 1^{\circ}\text{C}$ in Petri plates so as to allow profuse sporulation.

The Petri plates were filled with sterile distilled water and spores were harvested with the help of a sterilized plastic loop by gently

scrapping the colony and spore suspension was then strained through muslin cloth. An inoculum with load of 1×10^3 conidia ml^{-1} concentration of the spores was prepared. 30-days old pot grown plants were then inoculated with this spore suspension using a hand held atomizer.

The inoculated plants were kept in humid chamber for 24 hrs or periodically spraying distilled water on inoculated plant and covered by 2-3 pored polythene bags and then transferred to cage house and high humidity was maintained throughout the disease development period by frequent irrigations. Un-inoculated plants served as control.

The typical concentric ring symptoms appeared within 7-10 days after inoculation. Re-isolation was done from infected plant parts collected 10 days after inoculation. The resultant cultures were compared with the original ones to confirm the pathogenicity and then these cultures were sent for final identification to "Indian Type Culture Collection Identification/Culture supply Services, Division Of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012".

Identification

Dr. T. Prameela Devi and Dr. Deeba Kamil identified these cultures as *Alternaria alternata*. The identification number is 10,856.18 is allotted by ITCC. These cultures were used further research of *Alternaria alternata*.

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