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First report of *Alternaria* blight of dragon fruit (*Hylocereus undatus*) caused by *Alternaria* sp. In Chhattisgarh

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Abstract

Pathogenicity test were done by mycelial plug and spore suspension method. In mycelial plug method the disease symptoms appeared after three days of inoculation and in spore suspension method the disease symptoms appeared after seven days of inoculation. Based on morphological characters and pathogenicity test, the pathogen was identified as *Alternaria* sp. The vegetative mycelium in the tissues were hyaline at first, later brown or olivaceous and intercellular. Conidiophore arising singly through the stomata, 0-5(4) septate, with the basal cell sometimes slightly swollen, Olivaceous, unbranched, straight and upright often curled when fasciculate, measured 30-110 (79.50) μm in length and 6-10(8) μm in breadth. Conidia borne in chains up to 6 or more, light to dark olivaceous, solitary straight and slightly flexuous, muriform tapering to beak, with the basal cell rounded, the beak usually flexuous, pale or olivaceous brown, length 517.5 (11.32) μm and 2.5-5(2.8) μm breadth, smooth, 25-80 (49.98) μm , with the beak about onesixth the length of the spore and 5-7.5 (6.5) μm breadth, with 2-5 (3) horizonatal septa and 0-2 (1) vertical septa.

Keywords: *Alternaria*, *Hylocereus undatu*, pathogenicity, morphology, conidial suspension

Introduction

Dragon fruit (*Hylocereus undatus*) also known as red pitahaya is herbaceous, perennial, climbing vine cactus and a tropical fruit plant belongs to family Cactaceae. Dragon fruit tree is used in the embellishment as an ornamental vine in gardens and landscapes. It is also used as a natural colouring agent in the preparation of various drinks, juices, smoothie and pastries as a flavour enhancer. Unopened flower buds are cooked and eaten as vegetable. Major commercially cultivated species are *Hylocereus undatus*, *Hylocereus polyrhizus* and *Hylocereus costaricensis*, genus *Hylocereus* are native to Mexico, which were taken to Central America, perhaps by the Europeans (Morton, 1987) ^[1]. As reported from various countries, large number of fungi such as *Alternaria* sp., *Phomopsis* sp., *Bipolaris cactivora*, *Cladosporium cucumerinum* and *Colletotrichum gloeosporioides* cause damage or loss to the plants and fruits of pitahaya (Ngoc *et al.* 2017) ^[2]. Two species of fungi, *Phomopsis* sp. and *Alternaria* sp. have been found on the stems (Oeurn *et al.* 2015) ^[3]. *Alternaria* sp. infects stems and fruits. In the spring, round reddish to orange lesions with dark red centers and no halo were observed on 60% pitahayas stalks with an estimated loss of 10-20% on a farm on the Miami-Dade country farm in Florida. The lesions coalesce and formed larger diseased areas (Patel *et al.* 2017) ^[4].

Materials and Methods

Collection of the diseased sample

Stems of Dragon fruit showing typical blighted symptoms were collected from the growing orchards from district Raipur (C.G.).

Fungal isolation

Stems of Dragon fruit showing typical blighted symptoms were collected from the growing orchards. Isolation process was done under aseptic condition in a laminar air flow. For fungal isolation, small segments of the diseased tissue were cut along with a portion of healthy stem (5 \times 5 mm²) with a and morphological characteristics sterilized blade and the surface of stem segments were sterilized in 0.1% sodium hypochloride solution for 30s.

The stem segments were rinsed three times in distilled sterile water, dried and placed on potato dextrose agar (PDA) medium in sterile petri plates and incubated for 7 days at 26 ± 1 °C. After 7 days, fungal colony observed were identified and purified by hyphal tip method (Pathak, 1972) [5]. The pure culture obtained and maintained with the help of repeated sub culturing. The stock culture grown on potato dextrose agar (PDA) slants were stored at 5 °C in refrigerator. The pathogen was identified based on its cultural as well as pathogenicity test of the fungus.

Morphology of the fungal pathogen

For morphological characterization of the fungus infecting pitahaya plant, plant sample was collected in a sterilized polythene bag and brought to the lab. After surface sterilization by 0.1% sodium hypochloride solution, infected plant part was cut by sterilized sharp blade and kept in a moist chamber for 24–48 hrs. for sporulation. Cultures slide were made and observed under the compound microscope. The microscopic measurement were taken with the help of micrometry technique (Mahesha).

Pathogenicity test

To fulfill Koch's postulates, fresh disease free samples were brought to the laboratory and surface sterilized with 5% Ethanol. Two methods were employed for the pathogenicity test of the fungus 1.Mycelial plug method 2.Conidial suspension method.

Mycelial plug Methods

Pathogenicity tests were done on red X red cultivar of dragon fruit. Stems were used for the inoculation method. The healthy stem were collected from an orchard then were rinsed with tap water and gently wiped with cotton soaked in 75% ethyl alcohol for 30–40 seconds, then dried in the air before inoculation. After incubating the culture for one week in the dark at 26 ± 1 °C, a 0.5 cm diameter margin containing hyphae and conidia was cut from the colony's active growth margin and placed in the surface of the wounded stems (two plugs per stem). Sterilized water and agar plugs without the fungus on stems were used as control. The inoculated and control stems were wrapped in individual plastic bags with moist cotton (soaked in sterile water) to provide continuous high relative humidity (> 85%) and incubated at 26 ± 1 °C in B.O.D. Incubater.

Conidial suspension method

The suspensions of conidia were prepared by flooding the culture plates with sterile distilled water and interrupting the spores using a sterile scalpel, then filtering them with muslin

cloth to remove hyphae and transfer conical suspensions into a beaker. The suspensions were then refrigerated until use, which was within 2 hours of preparation. The inoculations were performed on wounded stems. All the stems were rinsed with pure water and gently wiped with cotton soaked in 75% ethyl alcohol for 30 to 40 seconds to detach any attached microbes before inoculation. Sterile pins for the inject have been used to make eight-ten wounds (epidermal punctures) placed randomly on the stems. Conidial suspensions (1ml/stem) were applied to the stem surface with a sterile calligraphy brush and kept in open field. The development of symptoms was photographed after ten days of inoculation.

Results

Morphology: Colonies were greyish in the obverse and brownish white margin in the reverse, and they were 68.8 mm diameter when grown on PDA after 192 hours after incubation at 25°C (Fig. 1A-B). Mycelium were aerial, irregular and thick when cultured on PDA.

Mycelium hyaline at first, later brown or olivaceous, intercellular. Conidiophore arising singly through the stomata, septate 0-5 (4), with the basal cell sometimes slightly swollen, olivaceous, unbranched, straight and upright often curled when fasciculate, measured 30-110(79.50) µm in length and 6-10(8) µm in breadth (Fig. 1C).

Conidia borne in chains up to 6 or more, light olivaceous to dark olivaceous, solitary straight and slightly flexuous, muriform tapering to beak, with the basal cell rounded, the beak usually almost flexuous, pale or olivaceous brown, length 5-17.5 (11.32) µm and 2.5-5 (2.8) µm breadth, smooth, 25-80(49.98) µm, with the beak about one-sixth the length of the spore and 5-7.5 (6.5) µm breadth, with 2-5 (3) horizontal septa and 0-2 (1) vertical septa. The characteristics features of species are: the shape of the conidia which is quite variable but in culture is chiefly cylindrical to oblong, the poor developed beak, the pre dominantly smooth surface and the readily visible pores in the septa (Fig. 1D-E).

Pathogenicity

Small orange spots (1-2 mm diameter) appeared after 3 days of inoculation in mycelial plug methods (incubated at 25 ± 2 °C temperature) as compared to spore suspension spray method which took 7 days for the initiation of disease symptoms on stems of red X red variety of red pitahaya. After inoculation, symptoms appeared on inoculated stems as orange to brown and round or oval necrotic spots. The fungus was re-isolated and purified culture from these artificially infected stems was similar to that of the original culture. Hence, the causal agent of the blight disease was confirmed as *Alternaria* sp.

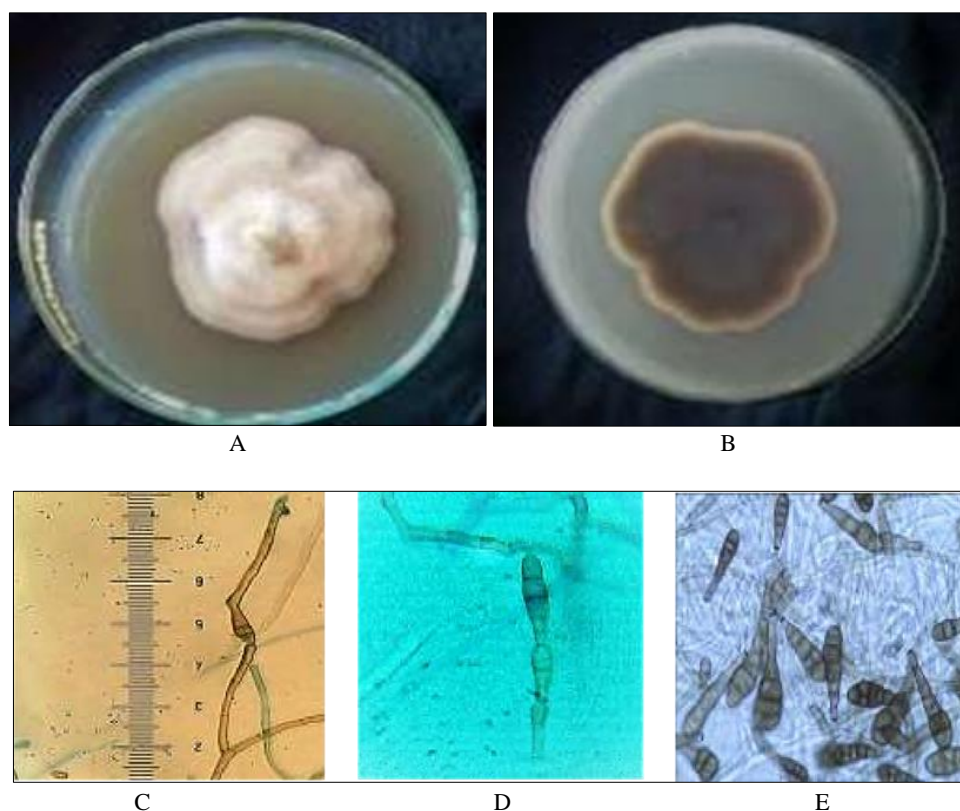


Fig 1: Morphology of *Alternaria* sp. Colonies grown on potato dextrose agar (A obverse, B reverse) for 7 days at 26 ± 1 °C; conidiophores and conidia (C-E) produced on tissue.



Fig 2: Pathogenicity test of *Alternaria* sp. on the stems of Dragon fruit

Conclusions

From the above investigation it was conducted that the vegetative mycelium of the pathogen causing *Alternaria* blight was hyaline, brown or olivaceous and intercellular. Conidiophore arising singly, septate 0-5(4), olivaceous, unbranched, straight and upright, fasciculate, length 30-110 (79.50) μm . and 6-10(8) μm in breadth. Conidia borne in chains, light olivaceous to dark olivaceous, solitary straight and muriform or slightly flexuous or ellipsoidal. The beak usually flexuous, olivaceous brown, length 5-17.5 (11.32) μm and 2.5-5 (2.8) μm breadth. Length of conidia was 25-80 (49.98) μm and 5-7.5(6.5) μm breadth, with 2-5(3) horizontal septa and 0-2(1) vertical septa. On the basis of morphological characters the fungus *Alternaria* sp. was identified.

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