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Isolation, purification, identification and pathogenicity of *Alternaria burnsii* (Uppal, Patel and Kamat) causing blight of cumin

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Abstract
Blight of cumin caused by *Alternaria burnsii* (Uppal, Patel and Kamat) is one of the more severe yield destabilizing factors causing serious yield losses each year. In recent years, blight of cumin is becoming more prevalent in agricultural areas where growing crop is predisposed to cloudy weather and severe rain due to changing climate. An experiment was conducted in which, a series of fungal isolation, purification, identification and pathogenicity test from the infected plant parts were done. Infected plant parts with typical disease symptoms were collected from ARS and Institutional Farm, Collage of Agriculture, SKRAU, Bikaner. These infected plant samples were brought to the laboratory for microscopic examinations and for further investigations. For the identification of fungus, koch's postulates were proven. Isolation was made from infected plant parts. The culture of *A. burnsii* was purified by hyphal tip culture method. The identification of the test pathogen was confirmed by cultural characteristics as well as shape of conidia and conidiophores. The pathogen *Alternaria burnsii* causing cumin blight was isolated from the diseased plant, purified on PDA and pathogenicity was proven under screen house condition. Disease appeared after the flowering stage of the plant when leaf tip became necrotic and purple or brownish in colour and ultimately black. All the above ground plant parts were severely affected as there was no seed formation and if formed they were shrivelled and non- viable. On re-isolation it was found that the fungus was identical to the original isolate. It was observed that the infection was much higher in inoculated plants as compared to control.

Keywords: Identification, purification, *Alternaria burnsii*, pathogenicity, blight

Introduction
Cumin (*Cuminum cyminum* L.) is an important seed spice and one of the earliest known major spices used by mankind and Indispensible condiment consumed in every Indian home. Cumin locally known as Jeera or Jiru is belongs to the family Apiaceae (formerly called Umbelliferae) is an annual herb. Sustainable cumin cultivation is continuously challenged by diseases that cause quantitative and qualitative losses in yield. Crop quality is badly affected by different diseases, and is reflected in a lower price once the seeds have turned black. Wilt, blight and powdery mildew are most important diseases in cumin cultivation.

Among the major diseases of cumin, Alternaria blight caused by *Alternaria burnsii* is most serious threat in major cumin growing areas in Rajasthan and Gujarat (Lodha and Mawar, 2007) [8]. This disease is quite prevalent and destructive as it affects all above ground plant parts including seed, thus, causing direct yield loss. *A. burnsii* affects cumin plant only after flowering stage and causes complete failure of the crop in some years depending on climatic conditions (Sastry and Anandaraj, 2013) [11]. *A. burnsii* causing blight of cumin was recorded for the first time in Pakistan (Shakir *et al.*, 1995) [12]. It was first reported by Joshi (1955) [6] from the state of Rajasthan. The disease occurs in all the aerial parts of cumin plants. Although infection is initially confined to the tips of the leaves, but rapidly extends to branches and stems under favorable weather conditions (Patel, 1968) [9]. Blights appear as very minute and brownish necrotic spots on leaves and stems, which later turn to blackish, whereas the stem tips bend downwards. Mostly diseased plants fail to produce seeds, if seeds are produced, they remain shrivelled, light in weight and dark in colour. Cloudy weather and warm-wet conditions after flowering increase the incidence of disease and spread in the whole field within a short period causing complete failure of the crop (Jadeja and Pipliya, 2008) [5]. High humidity during flowering & fruit setting is conducive for disease development. Mostly diseased plants fail to produce seeds (Abdul Wadud *et al.*, 2017) [1]. (Bandoupadhayay *et al.*, 1980) [2] reported that the incidence of blight pathogen on cumin seed was seed borne.

In severe epiphytotic of blight there was no seed formation. The disease severity varied from 16- 65% causing serious damage to the crop (Kalpana, 1993) [7]. Alternaria blight causes losses up to 85 per cent in this crop (Gemawat and Prasad, 1969; Bhatnagar *et al*., 1995) [4, 3]. So, the present investigation was conducted to isolate, purify, identify and pathogenicity test of blight of cumin.

Material and Methods

Collection, isolation and purification of the pathogen

Alternaria blight infected cumin plants showing disease symptoms were collect from ARS and Institutional Farm, Collage of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. These infected plant samples were brought to the laboratory for microscopic examinations and for further investigations. All the plastic ware and glassware *viz.*, Petri plates, flasks, beakers, test tubes, pipettes and measuring cylinders were dipped in chromic acid solution (Sulphuric acid 400 ml, water 300 ml and potassium dichromate 80 g) for 12 hrs., then washed with tap water and subsequently with sterile water. All glass wares were sterilized in hot air oven at 180°C for one hour. Laminar air flow chamber that sterilized previously with 95 per cent spirit for 30 minutes of U.V. light radiation was used for isolation of pathogen. The cork - borer, forceps and inoculation needle were sterilized directly over spirit lamp flame after dipped in spirit. Soil used for experiment was sterilized at 1.045 kg/cm² pressure for 90 minutes for two consecutive days. The earthen pots were dipped in 10% formaldehyde solution for sterilization.

The typical symptoms of Alternaria blight showing on infected plants were gently washed in tap water and subsequently with sterile water to remove the soil and other debris materials adhering on stem surface. Alternaria blight infected stems tissues were washed thoroughly with water and cut into small pieces followed by sterilization with 0.1 per cent sodium hypochlorite (1 glt^{-1}) solution in Petri plates for 1 to 2 minutes. Three washings were done with distilled water in laminar air flow chamber. Under laminar air flow chamber, diseased stem, leaves and branches pieces were aseptically transferred into Petri dishes containing 20 ml potato dextrose agar media. Then, plates were incubated in BOD for seven days at 28 ± 2°C for growth of the pathogen. The mycelium developed from diseased tissues was sub cultured aseptically into tubes and Petri plates containing PDA media. The culture of *A. burnsii* was further purified by hyphal tip technique (Rangaswami and Mahadevan, 1999) [10]. Pure culture of *A. burnsii* was maintained periodically on PDA containing slants and taken for further investigations.

Identification of pathogen

A. burnsii causing cumin Alternaria blight was characterized on cultural attributes of mycelium growth appearance till the period of 15 days and pure culture were used further for the studies.

Pathogenicity test

The seeds of cumin (GC-4) obtained from local market of Bikaner were sown in earthen pots after surface sterilized with 0.1 per cent sodium hypochlorite solution for 1 minute and subsequently three washings with water under aseptic conditions. Proper temperature and moisture conditions were maintained in pots till germination of seeds under glasshouse. After germination, 4-5 seedlings were maintained in each pot for further inoculations of test pathogen. The inoculum was prepared from 7 days old actively growing pure culture of *A. burnsii*. For inoculums, sand maize meal medium was prepared in 2:1 proportion suitably moistened and transferred in 250 ml Erlenmeyer flasks which were sterilized at 15 psi for 30 minutes. Sterilized media was prepared and inoculated with inoculums of *A. burnsii* cultures and uphold at 28 ± 2 °C for 10 days. The inoculum of *A. burnsii* was added @ 20 g kg^{-1} soil near the stem zone and covered the inoculated area with soil. Pots were regularly watered to keep suitable moisture regime. Each earthen pot containing 4-5 young seedlings were inoculated with pathogen and one pot without inoculums served as a control. The appearance of disease symptoms on leaves and stem of seedlings were recorded periodically. Infected seedling stems were taken for re-isolation of pathogen on PDA media and compared with original isolates of *A. burnsii* to prove the koch's postulates theory.

Results and Discussion

Isolation, purification and pathogenicity of *Alternaria burnsii*

The diseased plant parts of cumin showing typical symptoms of blight were collected from infested fields during *Rabi* season 2019-20 and 2020-21. Isolation was made from infected plant parts. The culture of *A. burnsii* was purified by hyphal tip culture method. The causal fungus was isolated on potato dextrose agar medium and pure culture [Plate 1(A)] was maintained. The identification of the test pathogen was confirmed by cultural characteristics as well as shape of conidia and conidiophores. The fungus on potato dextrose agar medium produced brown to olive green coloured aerial mycelium with brownish discoloration of substratum. The sporulation was started after seven days of incubation. The pathogen was identified on the basis of spore morphology. Conidia was oval and oblong, 3 to 6 transverse septa and septate longitudinally, having 3 to eight spores long chain with a rounded base. Conidiophores were 3 to 5 celled, branched and septate (Plate 1(B)]. This culture was periodically sub-cultured and maintained on the same medium. The morphology of the pathogen are in conformity with the earlier results given by (Uppal *et al.*,1938; Joshi, 1955; Patel, 1968) [13, 6, 9].

Pathogenicity: The isolated pathogen was inoculated at 55 days old cumin (variety GC-4) plants and kept in humid chamber for 48 hours. There after pots were kept in the open in cage house. The first symptom of the disease appeared after three days of inoculation in the form of white necrotic region on the aerial part of the plant especially on the tip of the young leaf and gradually enlarged and coalesced ultimately black in colour. The pathogen was re-isolated and compared, hence the Koch's postulate was proved. Thus the Pathogenicity of *Alternaria burnsii* (Plate 2) was tested. Disease symptoms were appeared after the flowering stage of the plant when leaf tip became necrotic and purple or brownish in colour and ultimately black. All the above ground plant parts were severely affected as there was no seed formation and if formed they were shriveled and non- viable. Several other workers have reported earlier also the occurrence of the disease only at flowering stage (Uppal *et al*., 1938; Gemawat and Prasad, 1972 and Bandopadhayay *et al*., 1980) [13, 4, 2]. Infection started as minute necrotic lesion on

all the above ground plant parts which turned purple at advance stages and later turn brown to black during 36-71 hours and the affected parts of the plant got blighted. These typical leaf blight symptoms appeared after 7 days of 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.

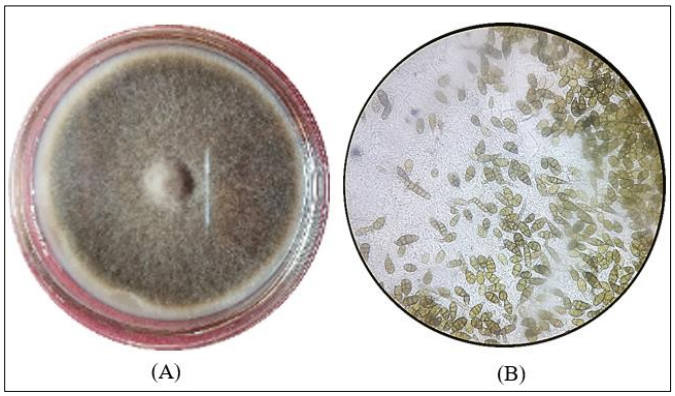

Plate 1: (A) Pure culture of *Alternaria burnsii* (B) Microscopic view of *Alternaria burnsii*

(C) Healthy plant (D) Diseased plant

Plate 2: Pathogenicity test of *Alternaria burnsii* on cumin

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