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Identification of fungal pathogens associated with fruit rot of *Solanum melongena* L. in Pantnagar (Uttarakhand)

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Abstract
Eggplant, an important vegetable crop in India is prone to several fungal attacks which leads to severe crop loss. Present study was conducted to isolate, characterize and identify the fungi associated with diseased fruits of mature eggplant. Characterization of the recovered fungal isolates was done on the basis of morphological characters, microscopic studies and molecular techniques. Through sequencing of ITS region, recovered fungal isolates F1 and F2 revealed their homology with *Diaporthe vexans* and *Alternaria alternata* respectively. Sequences were submitted to NCBI database and assigned with accession numbers MW425839 for *A. alternata* and MW425838 for *D. vexans*.

Keywords: Brinjal, fungal diseases, yield loss, pathogens

Introduction
Brinjal (*Solanum melongena* L), is a commonly grown non-tuberous vegetable crop of the family solanaceae. It is a native of southern India but grown invariably in tropical, subtropical, temperate and continental regions worldwide including America, Europe and Asia [26]. Tropical and subtropical regions are considered optimal for its cultivation. It is an autogamous diploid plant (2n=24) and majorly cultivated in Asia followed by Africa and Europe and accounts 93%, 3.6% and 2.1% of the total world production respectively [23]. It is a main vegetable of the plains and remain available more or less throughout the year. Brinjal is used in preparation of various dishes in different regions of the world [20].

It is a low calorie vegetable but rich in protein, minerals, fibre, vitamin, antioxidant and anticancerous compounds [21]. Edible portion of the fruit (per 100gm) contains 6.4 g carbohydrate, 0.3 g fat, 0.09 mg nicotinic acid, 200 mg potassium, 1.3 g protein, 120 mg vitamin C and 0.9 mg iron [2]. Global area under it's cultivation is estimated to be 1.85 mha with total production of 32 million metric tones. India is the second largest producer of brinjal after China with total production of 8.7 million metric tones [14]. Being an important vegetable crop for the growers and consumers, attempts must be made to increase its production which is usually hampered by several biotic and abiotic stresses, responsible for loss in yield and quality of the product.

Fruit rot disease, caused by different fungal pathogens is a major limiting factor in the production of various economically viable crops. Eggplant is also susceptible to several fungal diseases which cause prominent loss under field as well as under controlled conditions and hamper its sustainable production [17, 23]. It is severly affected by fungal pathogens like, Alternaria leaf spot (*Alternaria alternata* (Fr.) Keissler), Damping off (*Pythium aphanidermatum* (Eds.); *Phytophthora* spp.; *Rhizoctonia* spp.), Phomopsis blight (*Phomopsis vexans* Sacc. and Syd.) Harter, Cercospora leaf spot (*Cercosporasolani melongenae*), Verticillium wilt (*Verticillium dahliae*), Fusarium wilt (*Fusarium solani*) and foot rot (*Sclerotinia sclerotiorum*) [22]. Out of different fungal pathogens, fruit rot causing pathogens including *Fusarium* sp., *Colletotrichum* sp., *Phytopthora* sp., *Alternaria* sp. and *Phomopsis* sp. are reported to be most destructive for the crop. Fruit rot caused by *P. vexans, Alternaria alternata* is one of the most severe disease in brinjal that damages the fruits during harvest, post-harvest, storage, transportation and marketing [13]. *Phomopsis vexans*, a deuteromycetes responsible for leaf blight and fruit rot of brinjal causes major yield losses causing reduction of its market value by 20-30% [4]. *Phomopsis* sp. is an asexual form of *Diaporthe* sp. and found responsible for several diseases including root and fruit rot, dieback, cankers, leaf spot, blight, decay and wilt in a wide variety of host plants [24, 31].

Seeds are the main infection source of *P. vexans*, which remain on seed coat and causes discoloration in seeds [14]. Spores from fruiting body (pycnidia) are dissiminated by rain splashes, insects and rotten plant parts [11, 12]. Initial symptoms with *P. vexans* appear as small, pulpy, yellow brown, sunken lesions on fruits which later increase in size. Black erumpent pycnidia appear in advanced stage of the rot. Commonly used fungicides against Phomopsis blight are Carbendazim, Mancozeb, Ziram, Copper oxychloride and captafol [10].

Genus *Alternaria* comprises of saprophytic fungi that is known to cause severe damage to several crops including vegetables, oils and pulses [1]. *Alternaria alternata* causes severe damage to the crop by developing necrotic lesions on leaves as well as on fruits. Disease causes leaf drop, necrosis as well as rotting of fruits at harvesting or post-harvesting stages of brinjal [8]. Fungus grows and sporulates on plant debris during periods of rain, heavy dew, or under good moisture conditions. This disease is severe causing heavy losses to the crop. Balai and Ahir reported upto 25% yield losses from Jaipur district due to leaf spot of brinjal [3]. The disease first makes its appearance in young seedling and later attacks leaves and then spreads to fruits which subsequently rot and become unfit for consumption [5]. Symptoms of *A. alternata* infection on fruits appear as dark brown black concentric rings Initially disease appears as small, dark brown and sunken spots on the fruits which subsequently get converted into concentric rings and then become olivaceous dark brown lesion due to spore formation [22]. Infected plant debris, soil and seeds are primary source of infection. Secondary spread of the pathogen occurs through air borne conidia present on infected leaves [28]. Carbendazim, Thiram, Difolatan, Thiophanate methyl are some fungicides reported to inhibit the growth of *A. alternata* [7, 15, 27].

Diagnosis of the disease and identification of the causative agent is vital because without proper identification of the disease, correct control measures cannot be taken. Keeping in view of the economic importance of the crop and damage caused by fungal pathogens, present study was conducted to isolate and identify the pathogens of eggplant causing fruit rot in brinjal grown at Pantnagar (Uttarakhand) using morpho-cultural as well as molecular characterization using internal transcribed spacer (ITS) sequencing and comparative analysis of their rot pattern.

Materials and Methods

Collection of sample

Symptomatic mature fruits of brinjal were collected in the month of September from the fields of Vegetable Research Centre (VRC), Govind Ballabh Pant University of Agriculture and Technology Pantnagar located in Tarai region of shivalik hills of Himalayan range at 29.50^{O}N and 79.30^{O}E latitude and longitude respectively. Diseased of fruits with apparent symptoms were brought to the laboratory to isolate relevant phytopathogens.

Isolation of pathogenic fungi

Diseased fruits were washed with running water and dried in a laminar air flow chamber for 30min. After surface sterilization, infected fruit tissues were cut into pieces (5 mm) and disinfected by dipping in 1% Sodium hypochlorite (NaClO) for 3 minutes, rinsed 3 times with sterile distilled water and dried on sterilized tissue paper [18]. After drying, sterilized tissue samples were placed on PDA plates supplemented with chloramphenicol (0.05g/L) to inhibit bacterial growth. Inoculated plates were incubated for 6-7 days at 28 ^{O}C. Fungal isolates appeared on the plates were sub-cultured and pure cultures obtained from single spore were stored at 4 ^{O}C [1].

Identification of fungal isolates

Fungal isolates were primarily characterized on the basis of morpho-cultural characterstics by observing their growth and colony morphology on Potato Dextrose agar plates. Hypha and spores were visualized after staining with lactophenol (cotton blue) under microscope. For molecular characterization, DNA was extracted from the fungal isolates according to the method of Zhang and coworkers [32]. Internal transcribed spacer (ITS) region was amplified using ITS -1 and ITS-4 primers followed by sequencing of the amplified product. Sequencing services were provided by Bioserve Biotechnologies Pvt. Ltd.

Phylogenetic analysis

ITS-rDNA sequences of related species showing maximum similarity with the query sequences were retrived from Genebank database. Similarity search was performed using Basic local alignment tool (BLAST) available at National Centre for Biotechnology Information (NCBI) http://www.ncbi.n1m.nih.gov/blast/Blast.cgi. Isolates were identified on the basis of percent similarity of the query sequences with the available sequences in the database. Thereafter, sequences were submitted to NCBI GenBank database and accession numbers were obtained. Sequences were aligned using clustalW programme [16]. The evolutionary history of the fungal isolates was inferred using the Neighbor-Joining method. Tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analysis of the recovered fungal isolates was conducted using MEGA 6.0 software [29].

Results

Identification of fungal isolates

Infected brinjal fruits collected from sampling site showed two kinds of symptoms, i.e. brown water-soaked lesion with concentric rings along with the presence of black pycnidia and fruits with black necrotic lesions (Fig 1). Two morphologically distinct fungal isolates were obtained from the diseased samples. One isolate showed fluffy, white to pale pink mycelial growth with wavy margins on PDA while other showed olive green colouration later changing into grayish fluffy growth (Figure 2a, 2c). Fungal spores were observed under microscope after lactophenol staining (Fig 2b, 2d). Conidia of *Alternaria alternata* were of ellipsoidal shape, golden brown coloured and septate with transverse as well as vertical walls whereas alpha and beta conidia of *D. vexans* were visible under compound microscope.

Fig 1: Diseased fruit samples recovered from sampling site

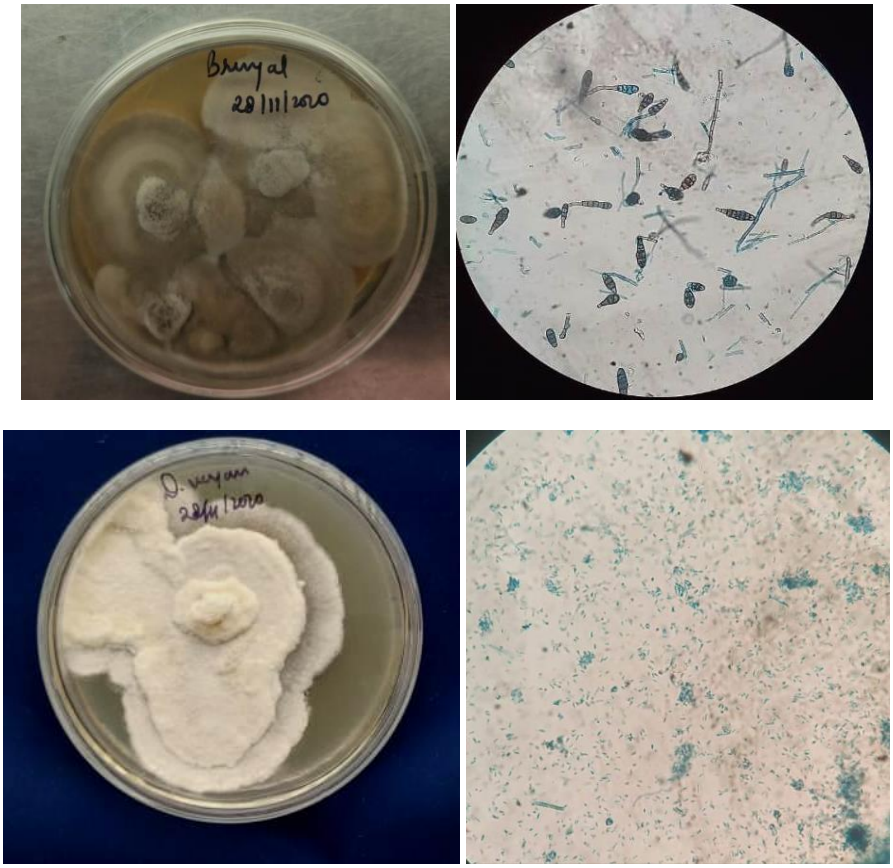
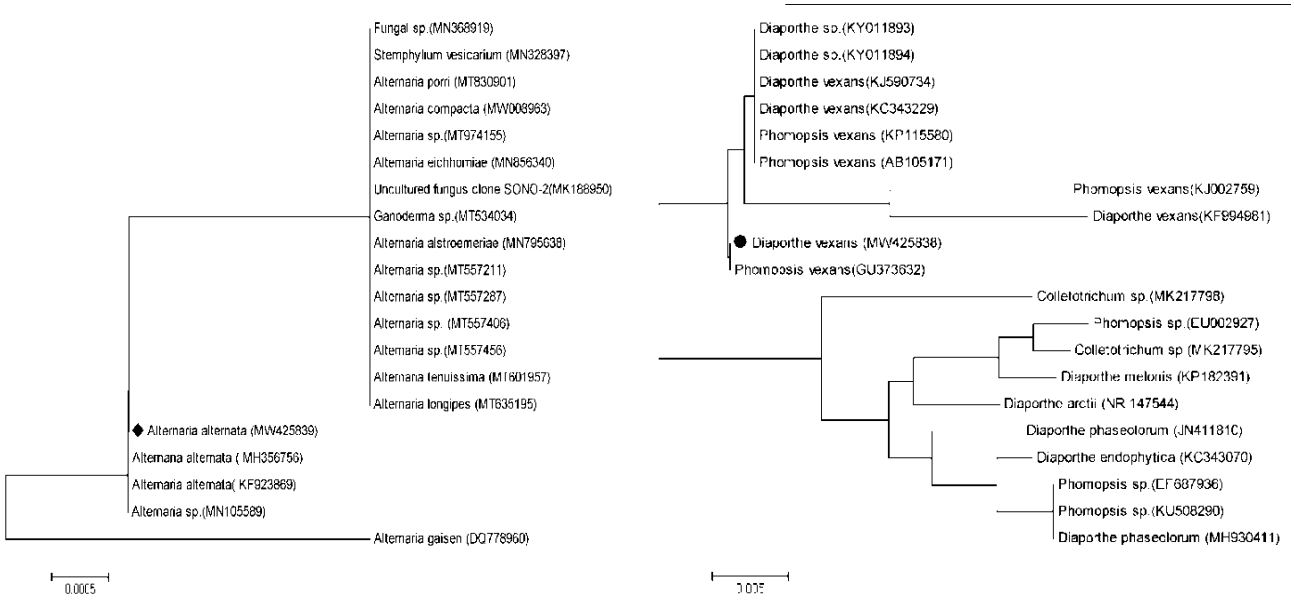


Fig 2: Colony morphology of (a) *Alternaria alternata* colony morphology (b) Spores of *A. alternata* (c) Colony morphology of *D. vexans* on PDA plate (d) Spores of *D. vexans*

Molecular analysis of both the fungi based on rDNA- ITS nucleotide homology revealed the identity of recovered fungi with *Alternaria alternata* and *Daporthe vexans* respectively. Neighbour joining tree reflected phylogenetic relationships among different fungal species (Figure 3a, c).



(a) (b)

Fig 3: Phylogenetic tree representing evolutionary relationship of (a) *A. alternata* and (b) *D. vexans.*

Genetic relatedness was calculated using Neighbor-joining method using MEGA 7.0 software. The evolutionary distances were computed using the Maximum Composite Likelihood method and were in the units of the number of base substitutions per site.

Discussion

It is evident from our observation that *D. vexans* (MW425838) and *A. alternata* (MW425839), were two rot causing pathogens of brinjal in VRC Pantnagar. Both are important phytopathogens and reported to cause severe yield losses in various important crops including brinjal [4, 8]. Pathogens were characterized on the basis of morphological characters, microscopic properties followed by ITS sequencing as the nuclear ribosomal internal transcribed spacer (ITS) region is a formal fungal barcode and in most cases, marker of choice for the exploration of species-level identification in ecological and taxonomic studies of fungi [9]. It offers several advantages over other species-level markers in terms of high information content and ease of amplification. It was recently designated as official barcode for fungal [25].

Considerable morphological variations in terms of colony structure, texture and microscopic properties were observed in both the fungal isolates. *D. vexans* appeared as white profuse mycelium with simultaneous appearance of black coloured pycnidia in later stages. Similar observations were made by Jayaramaiah and co-workers [14]. Phylogenetic analysis based on ITS sequence of both the fungal isolates of this study along with the reference sequences retrieved from NCBI database revealed their homology with *A. alternata* and *D. vexans* respectively. Besides, typical rot symptoms appeared on second day in the fruits inoculated with *A. alternata*. whereas it took six days for *D. vexans* to show the symptoms. Findings of this study will be helpful for better understanding of etiology of fruit rot causing phytopathogenic fungi in brinjal. It also opens avenues for developing sustainable solutions for control of fruit rot of brinjal as studying plant diseases and their causative agents is important to prevent the loss caused by them.

Conflict of interest

The authors declare that they have no conflict of interest

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