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Short Communication

FIRST REPORT OF BLACK ROT OF CARROT CAUSED BY *ALTERNARIA RADICINA* IN PAKISTAN

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ABSTRACT

Dry, black, decayed and sunken lesions of fungus were observed on the surface of infected carrot (*Daucus carota* L.) roots collected from Lahore, Pakistan. The obtained samples were cultured on potato dextrose agar medium in order to obtain the pure fungal colonies and to confirm their pathogenicity by inoculating the fungal mycelia on asymptomatic carrots. On the basis of growth rate, cultural and conidial morphology, the fungus was identified as *Alternaria radicina* Meier, Drechsler & Eddy. Identification of the isolated fungus was further confirmed by rDNA sequencing and the obtained PCR product was deposited in the GenBank with LT799973 accession number. As per our knowledge this is the first report of black rot of carrot in Pakistan.

Key words: *Alternaria radicina,* black rot, causal agent, *Daucus carota*

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INTRODUCTION

Carrot is a renowned and widely consumed vegetable (Rubatsky, 2002). It is an important vegetable in Pakistan as well. Commercial production of carrot is an important economic industry worldwide. In peri-urban areas of Lahore, Karachi and Peshawar, the relative share of carrot production in vegetable area is 2.67%. Because of its good storage attributes and high dietary value, it plays a major role in human nutrition (Leja*et al*., 2013; Umar *et al*., 2015). In addition to vitamins A, C, and E, carrot also possesses β-carotene, flavonoids and phenolics. Due to its multiple nutritional benefits, carrot ranked 10th among fruits and vegetables (Leja *et al*., 2013). Foods that contain natural antioxidants like polyacetylenes, phenolic acids and carotenoids enhance resistance to oxidative damage and have substantial impact on human health (Leiss *et al*., 2013). It is reported that carrots are also beneficial for the heart, blood circulation, eye sight, skin and lungs.

Most common pathogens of carrot are fungi. Several workers in different countries have reported species of the genus *Alternaria* such as *A. carotiincultae* Simmons, *A. dauci* (Kuhn) Groves & Skolko, *A. petroselini* (Neerg.) Simmons, and *A. radicina* Meir, Drechsler & Eddy causing diseases in carrot (Farrar *et al.,* 2004).

A. radicina is a pathogenic species which could be isolated at all growing stages from diseased carrot plants (Stranberg, 2002). Alternaria black rot caused by *A. radicina* is ubiquitous on carrot crops in the world where it is said to cause significant losses (Davis and Raid, 2002). It is a facultative parasite affecting mature tissues, especially those of more or less bruised or wounded roots during harvesting or storage (Meier *et al.,* 1922). In the main carrot producing areas, black rot could be observed. Crown infection, foliar and seedling damping-off are also caused by *A. radicina* (Koike *et al.,* 2009). The purpose of the current investigation was to isolate and identify the causal agent of black rot disease of carrot in Pakistan.

MATERIALS AND METHODS

Sample Collection and Identification: Carrots showing dry, black, decayed and sunken lesions of black rot were collected from local markets of Lahore, Pakistan during November 2016. Small portions of infected carrots were surface sterilized in 1% sodium hypochlorite for 1 min, washed with sterilized water thrice and placed on potato dextrose agar (PDA) in 9-cm diameter Petri plates. Pure culture was obtained by plating a small piece of the mycelium from the margin of a colony and incubating at 23 °C for 10 days. Microscopic examination of 10-day-old culture was performed at 100X magnification to observe cultural and conidial morphology.

Molecular Characterization: DNA isolation was done as per CTAB method (Doyle and Doyle, 1990), by the fungal DNA extraction kit provided by GeneAll Biotechnology Co., Ltd. ITS sequence was used to amplify the 5.8S-ITS segment. Primers used for amplification were ITS1 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4 5′-TCCTCCGCTTATTGATATGC-3′ (Innis *et al*., 2012). Amplified product was submitted to 1st Base Sequencing Singapore Co., Ltd. for bidirectional sequencing. The results of purified, sequenced 18S rDNA sequencing were submitted to NCBI (National Center for Biotechnology Information) database for BLAST alignment search. When

typical strain series were identified, Clustal W software was used to perform homology comparisons (Thompson *et al*., 1994). Different lengths at the start and end of the sequence were trimmed to avoid uneven sequences. The amplified PCR product was subjected to sequencing and the resultant PCR product was deposited to NCBI (National Center for Biotechnology Information) database to obtain accession number LT799973. This sequence of 553 bp showed 100% homology with Accession No. JX418350 isolated from China. The sequences of other isolates of *A. radicina* and *A. alternata*, available in GenBank, were aligned with our sequence at different lengths to construct a neighbor-joining tree using MEGA 6 (Tamura *et al*., 2012).

Pathogenicity Test: Pathogenicity was tested on 10 carrots, which were surface sterilized by 1% sodium hypochlorite solution (Okigbo *et al.,* 2009). Carrots were inoculated with 5 mm discs of freshly grown *A. radicina* on PDA and control was prepared by placing sterile PDA discs. Carrots were placed in autoclaved beakers at room temperature for establishment of the pathogen and *A. radicina* was re-isolated from the inoculated carrots after 10 days. Further identification was done as described earlier from infected carrots in comparison to control which were symptomless.

RESULTS AND DISCUSSION

Morphological identification revealed that colonies of the fungus were fast growing, reached 4-5 cm in diameter in 14 days on PDA. Colonies appeared to be dark green to blackish on PDA of Merck (Fig. 1). Conidia were single, or sometimes in two chains, and were dark olive-brown to natal brown, broadly ellipsoid to ovoid, 42–50 × 19–37 µm, with 4-5 trans-septa and 1–3 longisepta in some or all segments, except the apical and basal cells. Morphological characterization of the examined isolates on PDA were similar with explanations of Ellis (1970, 1971), Rotem (1994) and Simmons (2007).

Inoculated carrots showed the typical symptoms of black rot 10 days after inoculation. Initially, small chlorotic spots were which joined together by expanding. All the inoculated carrots showed symptoms of the disease (Fig. 1). Re-isolation of the fungus from inoculated carrots on PDA produced mycelia and conidia with the same characteristics as the inoculated fungus. Sequence alignment revealed that both the isolates (originally isolated and re-isolated) were similar to each other. BLAST analysis of ITS region of the isolated pathogen revealed 100% sequence similarity with *A. radicina* sequences. Phylogenetic analysis was performed using neighbor-joining method in MEGA 6 (Tamura *et al.,* 2012). In the phylogenetic tree, the characteristic isolate was placed within a clade including reference isolates of *A. radicina* (Fig. 2). These results indicated that *A. radicina* is the causal agent of the disease. As per our knowledge this is the first report of black rot of carrots in Pakistan.

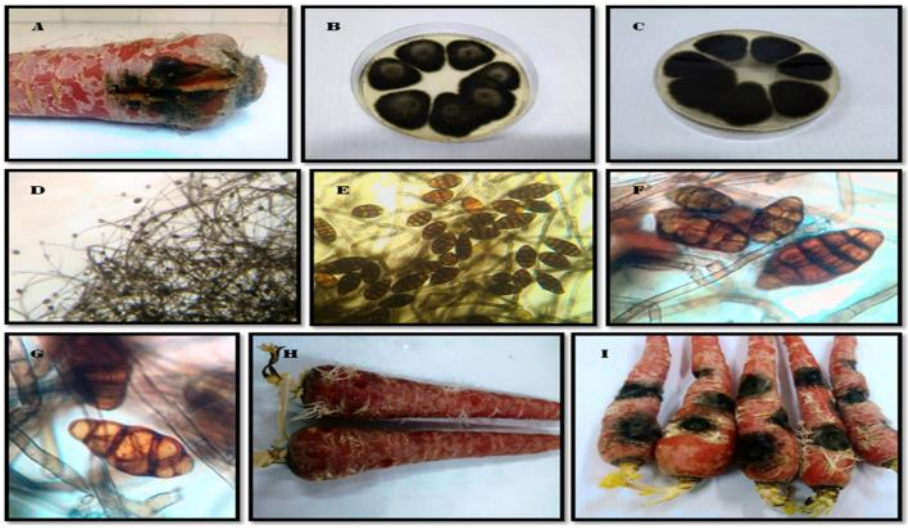

Fig. 1. A)- Carrot showing symptoms of black rot. Morphological characterization of *Alternaria radicina* Meir, Drechsler & E. D. Eddy. (B)- Colony morphology on PDA. (C)- Colony reverse on PDA. (D)- Conidia at 10X. (E)- Conidia at 40X. (F & G)- Conidia showing trans-longisepta at 100X. (H)-Control carrots which are symptomless. (I)-Typical symptoms of *Alternaria radicina* after inoculation on carrots.

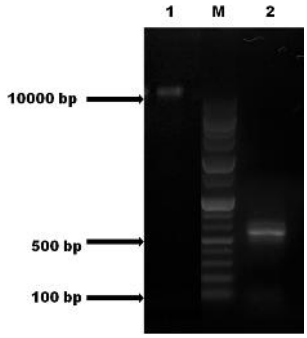

Fig. 2. Agarose gel electrophoresis. 1: Total genomic DNA isolated from Carrot. 2: Amplified PCR product of approximately 553bp by universal primer pair ITS1/ITS4. M: DNA size marker.

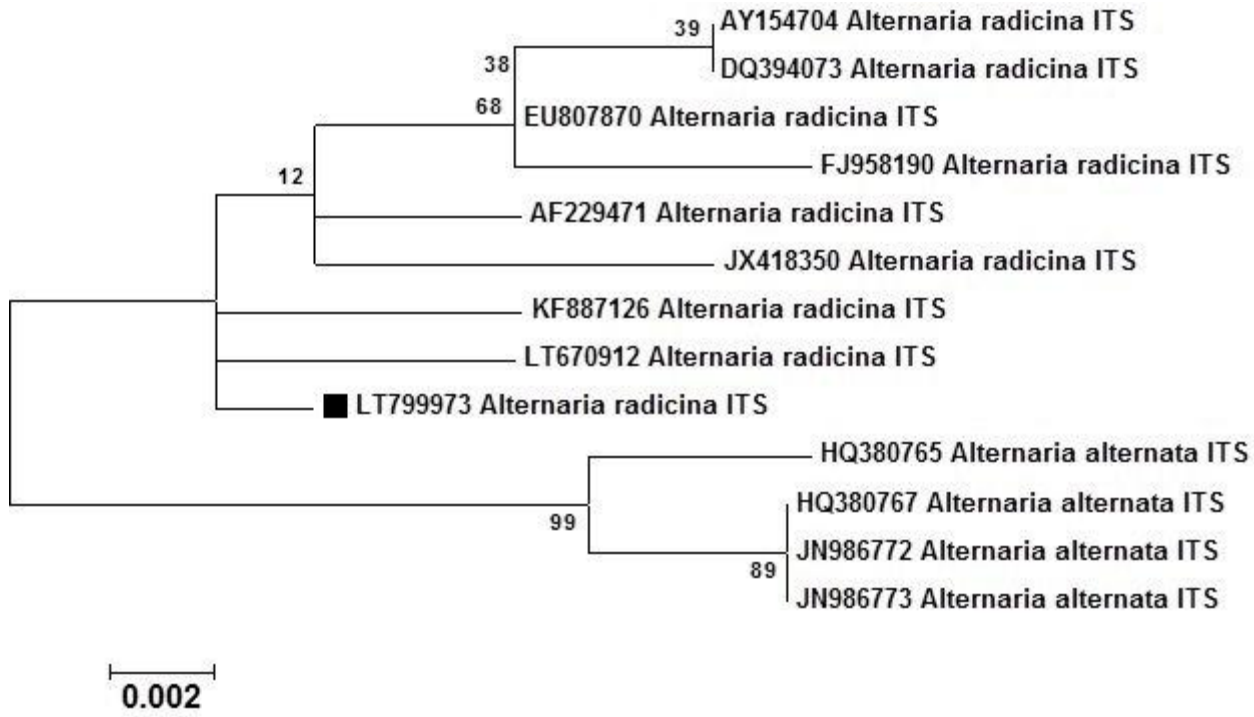

Fig. 3. The ITS1 gene sequence of the isolate from this study was aligned with reference sequences of *Alternaria radicina* isolates from GenBank using Clustal W© program. The phylogenetic tree was constructed using the neighbor-joining method in MEGA 6 version 6.0 (Tamura *et al.*, 2012).

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