



University of Zabol
Graduate school
Faculty of Agriculture
Department of Plant Protection

The Thesis Submitted for M.Sc. Degree in Plant pathology

**Identification of small- spored *Alternaria* species
based on morphological characteristics and *rpb2* gene
sequencing in Sistan fields**

Supervisors

Dr. M. Salari

Advisor

Dr. N. Panjehkeh

Dr. M. Pirnia

By

R. Rezaei

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Abstract

Alternaria (Hyphomycetous), is one of the genus large and complex in terms of morphology. Several species of the genus *Alternaria* have been identified on agricultural production, that often causing leaf spot and fruit rot before and after harvest. In this study, for identification of *Alternaria* small-spored species in sistán region, for the first time, *Alternaria* species from various host plants were isolated and studied according to morphological and molecular characteristics. A total, more than 250 fungal isolates showing *Alternaria* characteristics were isolated from different hosts. Morphological studies of the isolates were performed on PCA cultural under standard conditions (8 hr light, 16 hr dark and temperature 20-25°C). Nine species *Alternaria alternata*, *A. solani*, *A. arborescens*, *A. triticina*, *A. tenuissima*, *A. dumosa*, *A. porii*, *A. destruens* and *A. mimicula* of this genus were identified. *A. alternata* was found the most common species. To molecular studies were extracted DNA number of isolates and *rpb2* gene region amplified using Polymerase Chain Reaction (PCR) and PCR products were purified and sequenced. Blast analyses showed that there was significant variation in *rpb2* gene sequence among different isolates. According to morphological characteristics and nucleotide sequence data, this is the first report of *A. alternata* on *Medicago sativa*, *Raphanus sativus*, *Verbascum thapsus* and *Sansvieria trifasciata*; *A. arborescens* on *Plantago lanceolata* L. and *A. tenuissima* on *Aglaonema commutatum*, *Calendula persica*, *Petroselinum sativum* Hoffm *Malva neglecta* Wallr and *Cardaria draba* in Iran which verified by morphology and molecular data. In between, *A. arborescens* on *Plantago lanceolata* L. and *A. tenuissima* on *Aglaonema commutatum* and *Cardaria draba* for the first time in the world were isolated from the hosts. Based on analysis DNA sequescens of *rpb2* gene region and phylogenetic tree drawn, the isolates were grouped in two clades and 14 sub-clade. *A. alternata* and *A. tenuissima* isolates were grouped in two sub-clades. Isolates from *Mangifera indica* and *Mentha aquatica* showed maximum similarity with *A. tenuissima* and *A. destruens* based on morphological characteristics respectively, but according to *rpb2* gene sequence, isolate of *Mangifera indica* was placed in separate clade far from other isolates and isolate of *Mentha aquatica* was grouped near isolates with similar sequence with *A. alternata*. According to the results, *rpb2* gene sequence is useful for separation small-spored species.

Key words: *Alternaria*, Taxonomy, *rpb2* gene, Morphological characteristics