Plant Protection, 08 (01) 2024. 163-167 DOI: 10.33804/pp.008.01.5021

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

POST-HARVEST FRUIT ROT ON MUSKMELON (*CUCUMIS MELO*) CAUSED BY *ACREMONIUM POTRONII* IN IRAQ

[a,b]Rebwar A. Mustafa, [c]Samir Khalaf Abdullah

[a] *Bakrajo Technical Institute, Sulaimani Polytechnic University, Sulaimani, Iraq.*

[b] *Biology Department, Faculty of Science, University of Zakho, Duhok, Iraq.*

[c] *Department of Medical Laboratory Techniques, Alnoor University College, Nineva, Iraq.*

ARTICLE INFO

Article history

Received: 14th January, 2024

Revised: 26th February, 2024

Accepted: 27th February, 2024

Keywords

Fruit rot

Muskmelon

Acremonium potronii

Post-harvest

ABSTRACT

Muskmelon (*Cucumis melo*) is a vital fruit vegetable thriving in arid and warm climates. Fruit rot is a prevalent issue affecting muskmelon, occurring frequently in fields before harvest and during post-harvest stages. Several pathogenic fungi have been identified as the primary causal agents of fruit rot. In the present study, muskmelon fruits displaying irregular white regions were collected from the Penjwen district, Kurdistan region of Iraq. The isolated causal agent was identified as *Acremonium potronii* based on both phenotypic characteristics and molecular analysis (ITS and LSU gene sequences). A pathogenicity test was conducted to fulfill Koch's postulates. This represents the first documented association of *Acremonium potronii* with muskmelon fruit rot.

Corresponding Author: Samir Khalaf Abdullah

Email: samir.abdullah@alnoor.edu.iq

INTRODUCTION

Muskmelon (*Cucumis melo*) belongs to the Cucurbitaceae family and is a vital fruit vegetable thriving in arid and warm climates. In Iraq, muskmelon enjoys widespread popularity, with both its fully ripened fruits and seeds being consumed. The estimated melon production in Iraq reached approximately 157,834 tons in 2019, cultivated across 12,579 hectares (FAO, 2020).

Fruit rot is a prevalent issue affecting muskmelon, occurring frequently in fields before harvest and during post-harvest stages. Several pathogenic fungi have been identified as the primary causal agents of fruit rot. These include *Phytophthora capsici* (Tompkins and Tucker, 1937), *Fusarium proliferatum* (Garcia et al., 2018), *F. equiseti* (Li et al., 2019), *Neoscytalidium hyalinum* (Mirtalebi et al., 2019), as well as *F. falciforme*, *F. sulawesiense*, *F. pernambucanum*, and *F. kalimantanense* (Aranjo et al., 2021). Furthermore, *F. citrullicola* and *F. melonis* have also been identified (Khuna et al., 2022), along with *F. incarnatum* (Wonglon and Sunpapao, 2022).

The primary objective of the current study is to identify and characterize the fungus, *Acremonium potronii*, responsible for post-harvest fruit rot in muskmelons in Iraq. The study aims to provide valuable information for controlling and preventing fruit rot caused by *A. potronii* in muskmelons to reduce post-harvest losses.

MATERIALS AND METHODS

In August 2021, in the Penjwen Territory of northern Kurdistan region of Iraq, symptoms of fruit rot illness were observed on muskmelons in the field (Figure 1). Infected melon fruits were carefully collected, placed in a clean plastic box, and transferred to the laboratory within a period not exceeding 12 hours. The rotted muskmelon fruits exhibited irregular white lesions that

extensively affected the inner tissues, with the infection reaching the seeds. Seeds extracted from the decayed melons were surface-disinfected with 1% sodium hypochlorite for 2 minutes, rinsed in three successive changes of sterile distilled water, and air-dried under sterile conditions. These seeds were then plated on Potato Dextrose Agar (PDA) medium and incubated at 27°C for five days in darkness (Figure 2a).

Figure 1. Muskmelon fruit in the field showing fruit rot caused by *Acremonium potronii*.

Pure culture colonies were established by cutting the hyphal tips and inoculating freshly prepared PDA medium. Preliminary identification of purified isolates was based on cultural and morphological characteristics assigned to *Acremonium potronii*, matching the description provided by Gams (1971).

Colonies on PDA initially appeared white (Figure 2b), gradually transitioning to pale pink, with the reverse side turning brownish-orange and covering the entire plate within 10 days. The species is characterized by hyaline, smooth vegetative hyphae measuring 2.5 µm in thickness. Conidiophores are hyaline, smooth, and either straight or flexuous, emerging from hyphae ranging from 15.5 to 33.5 µm in length and 1.5 to 2.5 µm in diameter at the base. Conidia are smooth-walled, one-celled, hyaline, ovoid to ellipsoidal, measuring 2.5-4 x 1.5-2.5-5 µm, developed in a mucoid cluster at the end of the conidiophore (Figure 2c). Given the similarity in cultural and morphological characteristics among the isolates, one isolate was selected as a representative for molecular study to confirm the identification of the fungus. Genomic DNA was isolated from the (Re-13) isolate following the manufacturer's instructions, using the Fungi/Yeast Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., Taiwan).

Figure 2. Acremonium potronii a) Primary isolation from seeds on PDA medium, b) colony growth on PDA medium, c) conidiophores ending with cluster of mucoid conidia.

The internal transcribed spacer (ITS) and partial large subunit (LSU) regions were amplified using the primer pairs ITS1/ITS4 and LROR/LR5, respectively (White et al., 1990; Vilgalys and Hester, 1990). Sequencing of the

amplified PCR products was conducted by Macrogen Inc., South Korea. ITS and LSU gene sequences were deposited in the NCBI GenBank nucleotide database with accession numbers OP554770 and OP550098, respectively.

Pathogenicity tests for the pathogen isolate were conducted on healthy muskmelon fruits to confirm Koch's postulates. Five muskmelon fruits underwent two minutes of surface disinfection with a 2% NaOCl solution before being rinsed twice with sterile distilled water. From a 7-day sporulation culture of the pathogen on PDA medium, conidial suspensions were made in sterile water and adjusted to a concentration of 10^6 conidia/ml using a hemocytometer.

The muskmelon fruits were inoculated either by dropping 2 ml of spore suspension at the place where the melon fruit separates from the stem or directly on the side of the melon fruit's surface (n=5). A different batch of five melon fruits was spread with sterilized distilled water, serving as the control. Inoculated fruits and controls were each covered by a nylon sack to maintain dampness and were incubated in the dark at room temperature (28 ± 2°C) for seven days.

RESULTS AND DISCUSSSION

A. potronii was successfully isolated from experimentally infected muskmelon fruits. Symptoms appeared on inoculated muskmelon fruits as white cottony lesions matching the originally observed symptoms, whereas no symptoms were observed on control fruits (Figure 3a, b).

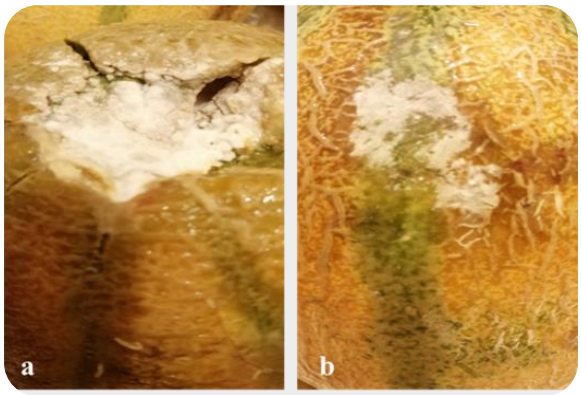

Figure 3: Muskmelon fruit showing white cottony lesions after inoculation with spore suspensions of the fungus: a) inoculation at the place of muskmelon fruit separates from the stem, b) on wounds made on the side surface.

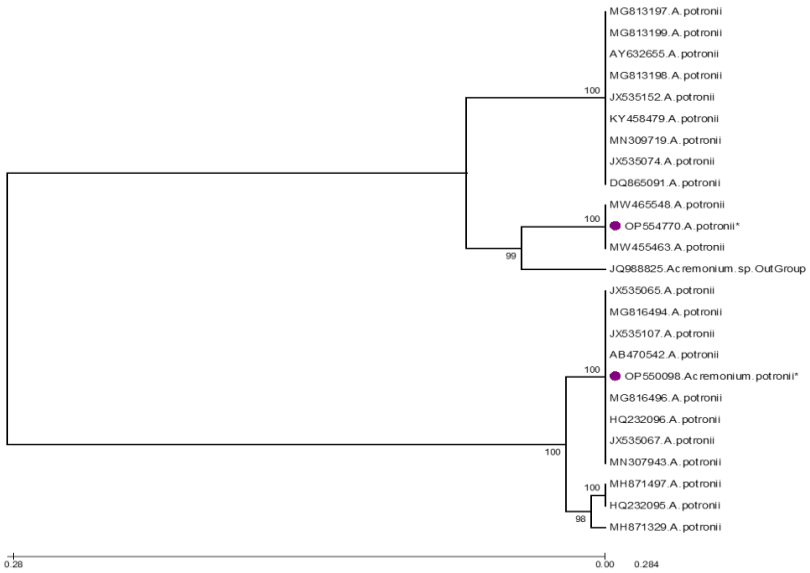

Figure 4: Phylogenetic tree generated from combined ITS and LSU sequences using Neighbor-Joining method by MEGA6.

The ITS sequence (OP554770) was 100% and 99.60% identical to (MW465548 and MW455463) respectively from a marine habitat in Saudi Arabia. The LSU sequence (OP550098) was 100% identical to (JX535107) from peat of slouph in Russia, 99.81% identical to (JX535067 and JX535065) respectively from a littoral zone of slouph in Russia and 99.80% identical to (MG816496 and MG816494) respectively from the sponge *Dysidea fragilis* in the Ireland (Table 1). The phylogenetic tree for ITS sequence grouped our isolate (OP554770) with (MW465548 and MW455463) from Saudi Arabia and the LSU gene sequence grouped with isolates (JX535107, JX535067 and JX535065) from Russia and isolates (MG816496 and MG816494) from the Ireland (Figure 4).

Table 1: Details of DNA sequencing data of *Acremonium potronii* in Genbank that were used for molecular phylogenic analysis.

Isolate/strain	**Accession numbers**		**Similarity**	**Country**	**year**
	ITS	Source			
R-13	OP554770	Melon fruit	100%	Iraq	2022
AUMC9600	MW465548	Marine fungi	100%	Saudi Arabia	2021
Saudi Marine	MW455463	Marine fungi	99.60%	Saudi Arabia	2021
MUT<ITA>:2462	MG813198	Atlantic Ocean	83.90%	Ireland	2018
MUT<ITA>:2439	MG813197	Atlantic Ocean	83.90%	Ireland	2018
3T.309	KY458479	Salt lake	84.01%	Turkey	2013
194	JX535152	Mud littoral zone of slouph	84.28%	Russia	2010
40	JX535074	Mud littoral zone of slouph	84.28%	Russia	2010
MUT<ITA>:2440	MG813199	Atlantic Ocean	85.31%	Ireland	2018
MS02	JN021531	Marine sediment	83.30%	South Africa	2011
CBS 379.70F	AY632655	Marine seaweed	83.64%	Germany	2004
19/2.5.1	DQ865091	Lotus creticus	83.58%	Spain	2021
Isolate/ strain	**Accession numbers**		**Similarity**	**Country**	**year**
	LSU	**Source**			
Re-13	OP550098	Melon root	100%	Iraq	2022
94	JX535107	Peat of slouph	100%	Russia	2019
36	JX535067	Littoral zone of slouph	99.81%	Russia	2019
35	JX535065	Littoral zone of slouph	99.81%	Russia	2019
MUT<ITA>:2462	MG816496	Dysidea fragilis	99.80%	Ireland	2018
MUT<ITA>:2360	MG816494	Dysidea fragilis	99.80%	Ireland	2018
CBS 379.70F	HQ232096		99.63%	Netherland	2016
795_3_CP03	AB470542	Methane cold seep	99.63%	Japan	2009
CBS 379.70D	MH871497		96.20%	Netherlands	2022
CBS 379.70D	HQ232095		96.13%	Netherlands	2016
CBS 189.70	MH871329		95.43%	Morocco	2022
AMF022	MN307943	Marine Yeast	99.44%	USA	2019
G-Cla1-LUS-CTU0-	MF331970	G-cla rhizome-associated	91.88%	Taiwan	2019

Acremonium potronii was previously reported from houseflies (*Musca domestica*) on cattle infected with ringworm in South Texas (Ysquierdo et al., 2017) and as an opportunistic pathogen causing human keratitis (Foster et al., 1975). The fungus was frequently isolated from saline environments (Samadi et al., 2013; Grum-Grzhimaylo et al., 2016; Bovio, 2018; Khusnullina et al., 2018; Kocabiyik and Ilhan, 2021) and as an endophyte colonizing plants under saline stress (Macia-Vicente et al., 2008).

AUTHORS' CONTRIBUTIONS

RAM collected muskmelon infected fruit samples, isolated the fungus, morphologically and molecularly identified the fungus and conducted pathogenicity tests; SKA supervised the work, compiled the literature, wrote and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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