



Identification and Characterization of Some Phytopathogenic Fungi in Post-Harvest Potato (*Solanum tuberosum*) in the El Oued Region (Eastern Northern Sahara, Eastern Algeria)



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Abstract

The global staple crop, the potato (*Solanum tuberosum* L.), is susceptible to post-harvest rot caused by various fungal pathogens. These pathogens lead to a significant reduction in potato quality and marketable yield. Our study aimed to isolate and identify different phytopathogenic fungi present on potato tubers after harvest in the El Oued region of Eastern Sahara, Algeria. We utilized Potato's Dextrose Agar (PDA) culture medium for isolating and identifying molds. Macroscopic examination was performed with the naked eye and under a binocular microscope. Microscopic examination allowed us to determine certain morphological characteristics such as spore type and thallus shape. Total genomic DNA was extracted from seven-day-old cultures using a commercial NucleoSpin Plant II kit. Based on morphological features and molecular analyses of spore isolates, using nucleotide sequences from the internal transcribed spacer (ITS), the results of 18S rDNA analysis confirmed that the fungi infesting the tubers were identified as *Fusarium proliferatum*, *Alternaria alternaria*, *Rhizoctonia solani*, and *Wickerhamomyces anomalus*. This study represents the first report of *Fusarium proliferatum*, *Alternaria alternaria*, *Rhizoctonia solani*, and *wickerhamomces anomalus* causing wilt diseases, dry rot, Canker and black scurf of potato tubers in southern Algeria.

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1 Introduction

The potato (*Solanum tuberosum L.*) is considered a staple vegetable for ensuring food security in developing countries (Haverkort & Struik, 2015). In terms of global consumption, it ranks first among non-cereal food crops. Water stress or low fertility can increase the susceptibility of this crop to certain diseases. High soil moisture favors the growth and spread of fungi, while wilting is more severe when soil moisture levels are low.

Fungal diseases play a central role in yield losses and are classified into foliar, soil-borne, and tuber diseases (Large, 1940). Late blight, early blight, and Phoma are among the foliar diseases, while common scab, black scurf, dry rot, and wilt are significant tuber diseases. Strategies for controlling fungal diseases primarily rely on the use of fungicides consisting of synthetic molecules. However, regardless of the recommended method for protecting crops against phytopathogenic agents, it is essential to identify them beforehand. In this context, this study aimed to isolate and identify different species of phytopathogenic fungi that attack potato tubers after harvest (Pedras & Ahiahonu, 2005; Martínez et al., 2017; Termorshuizen, 2007; Hammerschmidt, 1984).

2 Materials and Methods

Isolation and purification of fungi

In 2019 and 2022, potato tubers displaying symptoms such as brown spots, dark necrotic lesions, and sclerotia (Figure 1) were collected from potato plants in El Oued (Southeast Algeria). Microscopic examination of fragments from these infested tubers revealed the presence of spores, indicating a fungal infection. We used Potato's Dextrose Agar (PDA) culture medium, which is commonly recommended for mold research, enumeration, and maintenance of collected and sub-cultured strains (Botton et al., 1990). Small 5 mm diameter pieces of tubers were excised and sterilized in 70% ethanol for 30 seconds. They were then transferred to 1% NaOCl for 1 minute, rinsed with sterile distilled water, and inoculated onto Potato Dextrose Agar (PDA) plates. The plates were incubated at 25°C for 48 hours. After incubation, different fungal colonies were obtained. Each colony was isolated onto a new Petri dish to facilitate further examination. This step involves the purification of the isolated strains through a series of subcultures, which involve the aseptic transfer of the microorganism to a fresh and sterile medium to maintain it in pure culture (Botton et al., 1990).

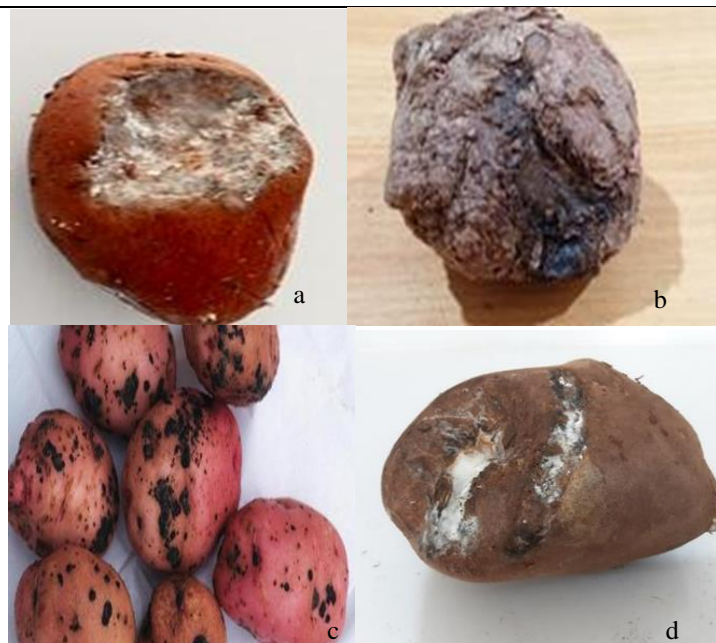


Figure 1. Symptoms of fungal diseases on potato tubers
a: Dry rot, b: Canker, c: Black scurf, d: Wilt

Morphological characterization of fungi

The purpose of identification is to classify fungal strains based on genus and species using identification criteria. It relies on two aspects: macroscopic and microscopic examination (Botton et al., 1990). Macroscopic examination of the Petri dishes was conducted with the naked eye and a binocular microscope. We carefully observed the external appearance of the fungi in a well-lit area, checked if all the colonies were identical, and noted their consistency (cottony, woolly, fluffy, powdery, etc.). Microscopic examination was based on morphological characteristics, including fruiting bodies, spore type, thallus shape, size, color, and spore arrangement (Bourgeois & Leveau, 1980).

DNA extraction, PCR amplification, and identification

Total genomic DNA was extracted from seven-day-old cultures using a commercial kit, NucleoSpin Plant II (Macherey-Nagel, Germany). The primers ITS1 CTTGGTCATT TAGAGGAA GTA A / ITS4 TCCTCCGCTTATTGATATGC (Gardes & Bruns, 1993) and EF2/EF-728F (Carbone & Kohn, 1999), were used for PCR amplification and sequencing of the translation elongation factor 1-alpha gene. The different steps involved in the process were as follows:

- a) Initial denaturation for 5 minutes at 95°C, followed by a second denaturation step for 30 minutes (35 cycles) at 95°C.
- b) Hybridization at 52-55°C for 30 minutes.
- c) Extension at 72°C for 45 seconds.
- d) Final extension at 72°C for 7 minutes.

The PCR reaction system consisted of 14.1 µl of ultra-pure water, 5 µl of Promega Taq Buffer (X5), 1.5 µl of 25 mM buffer (with MgCl₂), 0.2 µl of dNTP (25 mM), 0.5 µl of forward primer (10 µM), 0.5 µl of reverse primer (10 µM), 0.2 µl of Promega Taq Polymerase (5 U), and 2 µl of genomic DNA. The amplification products were visualized after electrophoresis on a 1.5% agarose gel with the addition of 10 µl of PCR products. Migration

was followed by staining in an ethidium bromide bath (0.5 µg/ml). Subsequently, the DNA was visualized and photographed under UV light using the Bio-Rad Gel Doc system (USA).

The PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany). The isolated and purified PCR products were sequenced using the Sanger sequencing technique (Sanger et al., 1977), with the Applied Biosystems BigDye v3.1 kit. PCR primers were used to amplify the sequencing fragments. The obtained sequences were analyzed using the CHROMAS PRO software, and the final sequences were then compared to sequences in the GeneBank database using the BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) from NCBI. This comparison was done to identify the studied isolates based on the percentage of similarity with reference strains (Bharadwaj et al., 2008; Singh et al., 2022; van der Wolf & De Boer, 2007; Karnata & Putra, 2017).

3 Results and Discussions

Morphological and molecular identification/characterization of fungi

The colonies rapidly developed a white aerial mycelium that turned violet in the older culture. This is due to the production of a light violet pigment after 7 days on Potato Dextrose Agar (PDA) at 25°C (Figure 2) with a fluffy appearance covering the entire Petri dish. The results of the microscopic examination revealed that *F. proliferatum* had a large number of small microconidia without septa, measuring between 10 and 15 µm (Figure 2). These microconidia were abundant in the aerial mycelium and formed chains of variable length on both monophialides and polyphialides. These findings are consistent with those described by Leslie & Summerell (2008).



Figure 2. a- *Fusarium proliferatum* on PDA agar b
b- *Fusarium proliferatum* under optical microscope at 40X magnification

Figure 3 displays a colony with colors ranging from olive green to black. The majority of colonies exhibit a fuzzy or cottony appearance and a notably slow growth rate on PDA medium. Microscopic observation of *A. alternata* colonies revealed significant sporulation, with numerous multicellular, greenish conidia surrounding the mycelium. Fungal identification was based on the morphological characteristics of the sporulation mode and conidia (Simmons, 1967).

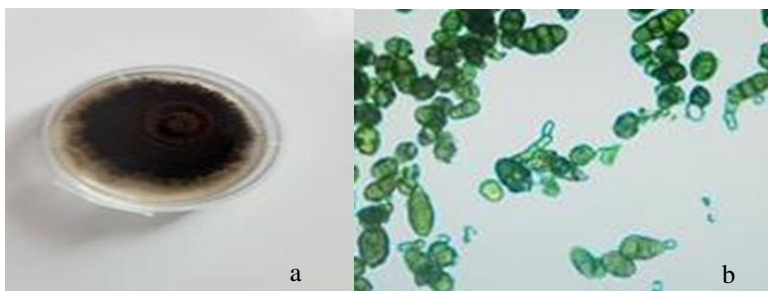


Figure 3. a- *Alternaria alternata* on PDA agar
b- *Alternaria alternata* under optical microscope at 40X magnification

The mycelial growth in Figure 4 exhibits a whitish appearance and rapidly covers the entire Petri dish. It gradually turns brown over time and displays dark brown sclerotia. Microscopic observation of pure colonies revealed the typical morphological characteristics of the *Rhizoctonia* genus, including colorless septate hyphae, a branching pattern at right angles, multinucleate hyphal cells, and hyphal constriction near their point of origin. These characteristics align with the description provided by [Sneh et al. \(2013\)](#).

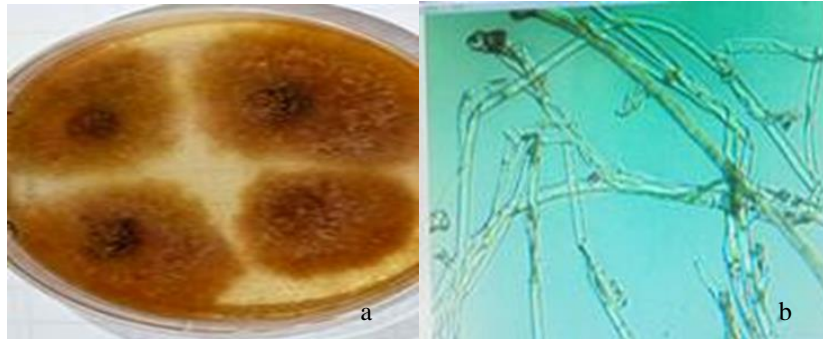


Figure 4. a- *Rhizoctonia solani* on PDA agar
b- *Rhizoctonia solani* under optical microscope at 40X magnification

The isolated white colonies in Figure 5 exhibited rapid cottony growth with a diameter of approximately 2 mm. Under the microscope, the cells appeared round, budding, dispersed, and reproductive. This description corresponds to that provided by [Ma et al. \(2021\)](#), for the *Wickerhamomyces* genus.

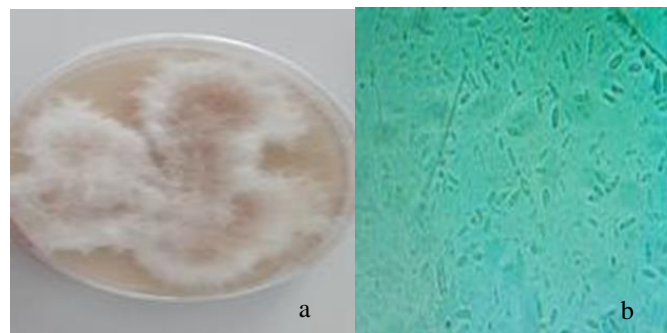


Figure 5. a- *Wickerhamomyces anomalus* on PDA agar
b- *Wickerhamomyces anomalus* under optical microscope at 40X magnification

The internal transcribed spacer (ITS) region of the fungal sequences was amplified by PCR and subjected to sequencing data in the GenBank NCBI (GenBank accession numbers OQ606246.1, OQ860003.1, OQ771178.1, and OQ606247.1). A BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the sequenced PCR products revealed that the isolated fungal strains were *Fusarium proliferatum* with a 99.77% identity, *Alternaria alternata* and *Rhizoctonia solani* with a 100% identity, and *Wickerhamomyces anomalus* with a 99.83% identity.

4 Conclusion

Fungal pathogens pose a significant threat and result in massive yield losses in potato cultivation, which is economically important both globally and in Algeria. In this study, we found that dry rot of potatoes was caused by *F. proliferatum*. It has been reported that this fungus causes rot in garlic roots ([Leyronas et al., 2018](#)), in France, Codonopsis ([Gao et al., 2017](#)), gerbera ([Zhao et al., 2020](#)), alfalfa ([Cong et al., 2016](#)) in China,

Aloe vera (Avasthi et al., 2018) in India, soybean (Díaz Arias et al., 2011) in the USA, and blueberry (Pérez et al., 2011) in Argentina. Other studies have revealed the presence of *Fusarium proliferatum* on apricot trees in Turkey (Ören et al., 2023), on *Cyrtoneura polygonatum* causing leaf blight in China (Zhou et al., 2021), on bananas in China (Huang et al., 2019), preserved garlic in Slovakia (Horáková et al., 2021), *Syagrus romanzoffiana* in Punjab-India (Faraz et al., 2020), chickpeas in Cuba (Duarte-Leal et al., 2020), and zucchinis in Morocco (Ezrari et al., 2020). *Alternaria* is an important fungal genus with a global distribution. This pathogenic Ascomycete can be found in plants as well as in humans (Thomma, 2003).

Studies have reported the presence of *Alternaria alternata* on peach trees in Pakistan (Alam et al., 2019), and *Gerbera Jamesonii* in Brazil (Bellé et al., 2019), *Xanthium strumarium* L (Abdessemed et al., 2019), in Algeria, pear trees (Chen et al., 2020), rose plants (Fang et al., 2020), and peanut crops (Zhang et al., 2021), in China. Garibaldi et al. (2023) mentioned its presence in *Hydrangea paniculata* in Italy, as well as in soybeans in Pakistan (Buzdar et al., 2023). Rhizoctonia, a potato disease, causes significant losses in marketable yield, which can reach up to 30% (Carling et al., 1989). Several studies have reported the presence of *Rhizoctonia solani*, such as on white cabbage in Turkey (Saygi et al., 2020), *Campanula* plants in Italy (Garibaldi et al., 2019), pepper plants in Kyrgyzstan (Erper et al., 2021), and sugar beet crops in Turkey (Avan et al., 2021).

Concerning *Wickerhamomyces anomalus*, few studies have been reported regarding this fungus to date. Only simple reports have been mentioned on the NCBI platform. Potatoes are typically harvested in February and March. Initially infected tubers in the fields can also develop rot during their storage in cold rooms. This results in significant losses for farmers as well as processing industries (Morrell & Rees, 1986; Fernandez-San Millan et al., 2021; Pedras et al., 2009; Suriani, 2019). The severity of the infection varies depending on the predominant species in a given area of potato cultivation. Species identification is the critical first step that provides a scientific basis for investigating the disease cycle, epidemiology, and management strategies of this important pathogen. To our knowledge, this is the first report confirming various diseases (dry rot, black scurf, canker, and wilting) on potato tubers caused by *Fusarium proliferatum*, *Rhizoctonia solani*, *Alternaria alternata*, and *Wickerhamomyces anomalus* in the El Oued region (Eastern Northern Sahara) of Algeria.

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