


Identification of *Penicillium aeris* and *Penicillium egyptiacum* in saffron corms from Morocco: Implications for plant health and crop management

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ABSTRACT

In Morocco, saffron production plays a significant role in the local economies of certain regions and relies on high-quality planting material, strict adherence to good agricultural practices and effective post-harvest management. This study aimed to assess the quality of saffron corms used as propagation material and to identify the fungal agents associated with corm rot in the main saffron-growing areas of Taliouine and Taznakht, using an integrated approach combining morphological and molecular analysis. Surveys conducted in 2022 led to the isolation of a fungal complex from necrotic tissues of infected corms. Two species within this complex, *Penicillium aeris* and *P. egyptiacum*, were identified and confirmed as the causal agents of saffron corm rot marking their first report in Morocco. Pathogenicity tests demonstrated their ability to induce typical symptoms characterized by both superficial and internal rotting. These findings fill a major gap in the understanding of saffron diseases, provide new insights into the etiology of corm rot, and lay the foundation for developing targeted strategies to prevent and control this disease, ultimately contributing to the preservation of crop yield and commercial value.

Keywords: saffron, *Penicillium aeris*, *Penicillium egyptiacum*, Morocco, pathogenicity test, molecular analysis.

INTRODUCTION

Saffron is obtained from the dried stigmas of the plant *Crocus sativus* L. (*Iridaceae*), the world's most expensive spice (Plessner and Ziv, 1999; Ourras et al., 2022a). The main producing countries are Iran (90.1%), India (4.5%), Greece (2.8%) and Spain (0.5%) (Cardone et al., 2020; Kothari et al. 2021a, 2021b b; Ourras et al., 2022b, 2025a). with 6.8 tonnes produced over 1800 ha in 2018, Morocco stands as Africa's foremost saffron-producing country (Ourras et al. 2022), with an annual turnover of 50,000,000 MAD (Ben El

Caid et al., 2018). Saffron is extensively grown in the mountainous zones of Taliouine (Taroudant province) and Taznakht (Ouarzazate province), where it represents an important source of income (El Aymani et al., 2023). Saffron cultivation in these regions plays a vital role in enhancing farmers' incomes, serving as a local product that greatly contributes to the livelihoods of the local populations (El Aymani et al., 2019a, b; Ourras et al., 2025a, 2025b).

Improving saffron production requires the use of high-quality plant material, strict control of crop management practices and post-harvest processes

(Devi et al., 2011). The use of plant growth-promoting rhizobacteria (PGPR) as biofertilizers in saffron production has gained considerable attention in recent studies. This approach can help reduce the negative environmental effects associated with the overapplication of synthetic fertilizers and pesticides use (Benjelloun et al., 2021; El Allaoui et al., 2023, 2024, 2025; Laanaya et al., 2025), providing a sustainable strategy for agriculture and environmental protection (Pérez-Montaña et al., 2014; Elbouzaoui et al., 2022a, 2022b).

The propagation of *Crocus sativus* occurs vegetatively through saffron corms or bulbs (Elouark et al., 2025b, 2025c). The cultivation and expansion of this species are influenced by three main limiting factors: 1) a low rate of bulb production, 2) a decline in the vigor and yield of propagated cultivars, and 3) the health status of the corms, particularly the presence of fungal pathogens that can cause diseases affecting saffron (Devi et al., 2011). In general, corms used by farmers for saffron plantations in Taliouine and Taznakht are not carefully selected, this lack of selection has significant implications for the quality and yield of saffron produced in these areas. Furthermore, the absence of a rigorous selection process could perpetuate the use of genetically inferior corms, which might be more susceptible to diseases and environmental stresses. Between 2019 and 2022, corm samples collected from farmers showed different types of symptoms, such as root rot and corm decay, ultimately impacting the saffron vigor (El Aymani et al., 2019b). These authors isolated a fungal complex from affected saffron corms composed of several pathogenic species such as *Fusarium* (*F. solani*, *F. oxysporum*, *F. culmorum*, *F. roseum*), *Aspergillus* (*A. fumigatus*, *A. niger*), *Trichoderma* sp., *Rhizopus oryzae*, and *Penicillium* sp.

Ourras et al. (2023) investigated the mycoflora of dormant saffron corms and found that the lesions contained both pathogenic and saprophytic fungi, including *Fusarium solani*, *F. roseum*, *F. oxysporum*, *F. culmorum*, *Aspergillus ochraceus*, *A. fumigatus*, *A. niger*. The authors of this work have not yet reported the share of each species of the fungal complex affecting corms of saffron. In addition, a number of *Penicillium* species isolated from dormant corms, accounting for 10% to 14%, have not been identified.

In this study, we conducted an in-depth examination of two species from the genus *Penicillium*, specifically focusing on their impact on

saffron corms. We aimed to assess the pathogenicity of these fungi, and understand their effects on saffron crops health and productivity. Initially, we identified the *Penicillium* species present in the saffron corms through morphological and molecular techniques. Following identification, we designed a series of experiments to test their pathogenic potential.

The results of our experiments will provide valuable insights into the role of these *Penicillium* species in saffron cultivation. By understanding their pathogenic characteristics, we can develop better management practices to mitigate their impact, ultimately contributing to the sustainability and productivity of saffron farming.

MATERIALS AND METHODS

Prospecting and sampling

Surveys were conducted in two regions in Morocco known for saffron cultivation: Taliouine and Taznakht, with the objective to identify pathogenic fungi associated with the deterioration of saffron corms destined for planting. Corms were gathered from farmers and were stored in sterile bags to avoid any possible contaminations. Subsequently, the corms were transported to the laboratory for in depth mycological analysis to detect any pathogenic fungi associated with corms deterioration.

Fungal isolation

Both healthy and symptomatic corms were placed in Petri dishes lined with sterile filter paper moistened with distilled water and incubated at 25 °C for 7 days, following the procedure outlined in previous studies (Benkirane et al., 1998, 1999a, 1999b; Ouazzani Touhami et al., 2000; Meddah et al., 2011) to isolate fungal species associated with these propagating organs. Subsequently, the developed mycelial growth were isolated and transferred onto PSA (Potato Sucrose Agar) or water agar media, following the procedure reported by Douira et al. (1989, 1993) and Qostal et al., (2025). Successive transplanting from the developed fungal colonies enabled the purification of isolates (Qostal et al., 2019, 2021). A macroscopic and microscopic description of these colonies was performed, and their pathogenic potential was evaluated.

Molecular analysis

DNA extraction

Genomic DNA was isolated using the OmniPrep fungi kit (G-Biosciences, St. Louis, MO; Cat. # 786-399) following the manufacturer's guidelines. DNA was quantified using the Implen N50 Nano spectrophotometer (Implen GmbH Schatzbogen, München Waltham, Germany).

PCR amplification and sequencing

Amplification of the internal transcribed spacer (ITS) region of the rDNA was performed with a MultiGene OptiMax Thermal Cycler in a total reaction volume mixture of 20 µl containing 11 µl milli-Q water, 2 µl 10X PCR buffer, MgCl₂, dNTPs, 0.2 µl x-VITA-Taq DNA polymerase, and specific primers (ITS1 and ITS4 described by White et al. (1990).), and 1 µl of genomic DNA. The PCR program consisted of an initial denaturation at 95 °C for 3 min, followed by 35 amplification cycles comprising denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s), and extension at 72 °C for 1 min 25 s with a final elongation (7 min 25 s at 72 °C). PCR products were checked on a 1% agarose gel stained with innoQ DNA stain, and visualized under UV light. The products were then purified using the ExoSAP-IT kit, and ITS rDNA gene sequencing was conducted using an ABI PRISM BigDye Terminator kit and analyzed on an ABI PRISM 3130XL Genetic Analyzer.

DNA sequence alignment and phylogenetic construction

DNA sequence alignment and phylogenetic analysis were performed using the sequences generated in this study and other sequences

retrieved from GenBank database. All sequences were aligned with MEGAX software (Kumar et al., 2018). The phylogenetic tree was inferred using the Neighbor-Joining (NJ) method (Saitou et al., 1987; El Alaoui et al., 2021) with 1000 bootstrap replicates to assess branch support.

Pathogenicity test

The pathogenicity test of the isolated species was conducted *in vitro* by inoculating saffron corms with mycelial explants (Msairi et al., 2025). Healthy saffron bulbs were selected, disinfected for three minutes with 5% NaClO (Sodium hypochlorite), rinsed several times with sterile distilled water and air-dried on sterile paper within a laminar flow hood. The surface-sterilized corms were slightly injured at two opposite points using a sterile needle and each wound was inoculated with a 4 mm mycelial plug taken from the actively growing margin of the two *Penicillium* isolates. The explants were placed on the corm wounds with the mycelium side down. Control corms received the same treatment but were inoculated only with an agar disc. All inoculated corms were incubated at 28 °C, and lesion development was periodically observed and recorded.

RESULTS

Morphological analysis

Incubation of symptomatic corms on moistened with sterile filter paper resulted in the appearance of bluish-green mycelial masses and conidia, identifying the isolates as belonging to the *Penicillium* genus (Figure 1).



Figure 1. The appearance of *Penicillium* sp. on saffron corms after 3 days of incubation using the blotter paper method under laboratory conditions

Observations of the colonies cultured on PSA medium provided both macroscopic and microscopic information for describing the two *Penicillium* isolates associated with saffron corms intended for planting.

On PSA medium (25 °C), the *Penicillium* sp.₁ colonies showed a slow growth, a diameter of 19 mm to 24 mm in 8 days (Figure 2 and 3). The colonies generally exhibit a central white cottony mycelium, while the reverse appears yellowish to pale. After 8 to 10 days of incubation, conidiogenesis remains scarcely visible, and the conidiophores show branching structures. The

conidiophores exhibited branching structures that generated groups of monoverticulated, biverticulated or occasionally terverticillate metulae. These metulae measured about $9.50 \times 2.4 \mu\text{m}$ and each supported 4 ampulliform phialides, approximately $6.1 \times 2.2 \mu\text{m}$ in size. The conidia were smooth-walled, globose to subglobose, about $2.1 \mu\text{m}$ in diameter, and arranged in regular chains. Molecular analyses confirmed that this *Penicillium* isolate corresponded to *P. egyptiacum*. *Penicillium* sp.₂ colonies developed slowly on PSA medium at 25 °C (Figure 4 and 5). The surface coloration varied from greenish-white to yellowish-white, while the

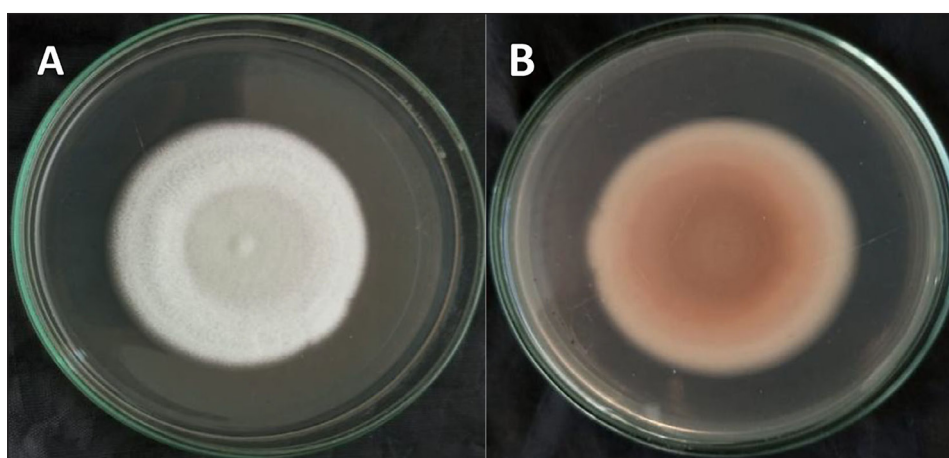


Figure 2. Macroscopic appearance of the colony of *Penicillium* sp.₁ (*Penicillium egyptiacum*) cultivated on PSA for 7 days (A) and (B) reverse side of the culture

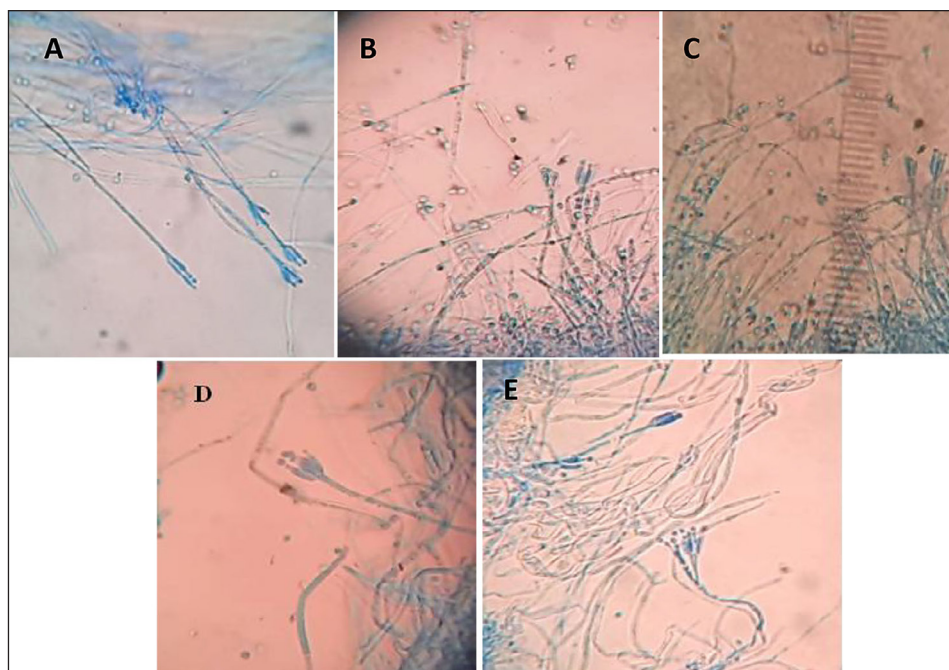


Figure 3. Microscopic observation of conidiophores, phialides and conidia of *P. egyptiacum* (x400)

reverse sides appeared greenish-orange to greyish-white. The conidiophores bear flask-shaped phialides whose dimensions range from $3.2\text{--}7.1 \times 1.9\text{--}3.2 \mu\text{m}$ (average $4.8 \pm 0.5 \times 2.3 \pm 0.2 \mu\text{m}$, $n = 50$). Conidia were globose with rough to echinulate walls, ranging from $1.8\text{--}2.3 \times 1.8\text{--}2.4 \mu\text{m}$ (mean = $1.9 \pm 0.2 \times 2.1 \pm 0.23 \mu\text{m}$, $n = 50$), with a width-to-length ratio close to 0.97. No sclerotia, Conservation organs, were detected. Molecular analyses confirmed the isolat as *Penicillium aeris*.

Molecular analysis

The rDNA ITS sequences from *Penicillium* isolates were deposited in GenBank under the accession numbers (GenBank: OP389143.1; OP389142.1; OP379573.1; OP389145.1; OP389144.1; OP389133.1) (Figure 6).

Pathogenicity test

Each of the fungal isolates was able to alter the inoculated corms on which light to dark brown lesions gradually developed, reaching an average diameter of 1.46 cm and 1.15 cm for *P. aeris* and *P. egyptiacum* respectively (Figure 7). No symptoms of disease were observed on the control. Corm rot, observed after 5 days of incubation, progressed significantly and the diameter of the lesions formed significantly enlarged transversely and longitudinally in the deep tissues of the corms, 15 days after inoculation. Over time, lesions expanded to cover about 30% to 80% of the corm surface, turning from brownish to bluish-grey as *P. aeris* and *P. egyptiacum* progressively colonized the tissues. The interior of the corms has also become necrotic (Figure 8). These

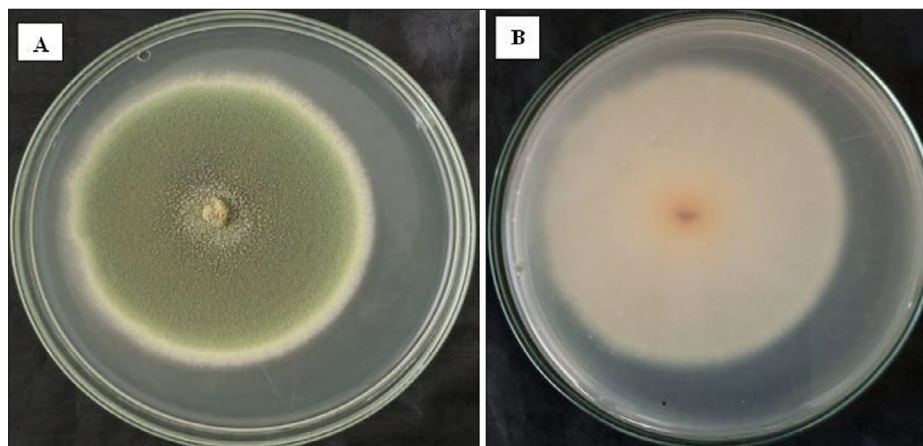


Figure 4. Macroscopic morphology of *Penicillium aeris* colony after 7 days of growth on PSA medium: (A) obverse and (B) reverse sides

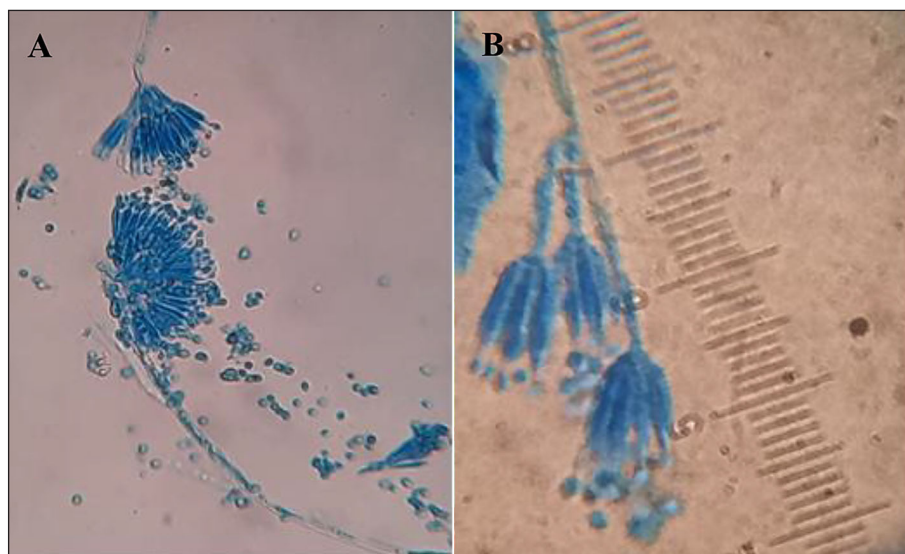


Figure 5. (A) and (B) Microscopic observation of conidiophores, phialides and conidia of *P. aeris* (×400)

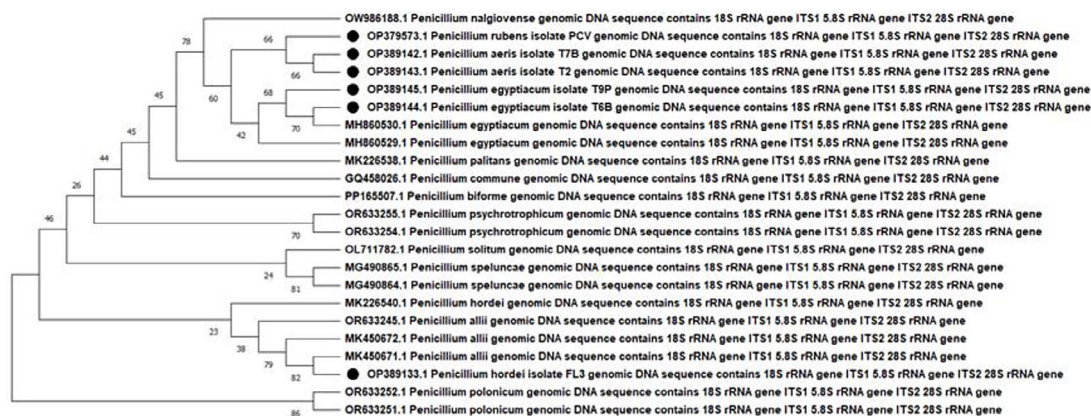


Figure 6. Neighbor-joining phylogenetic tree constructed from complete 18S SSU rDNA nucleotide sequences and other sequences retrieved from GenBank. The black circle represents the isolates of the current study (GenBank: OP389143.1; OP389142.1; OP379573.1; OP389145.1; OP389144.1; OP389133.1)

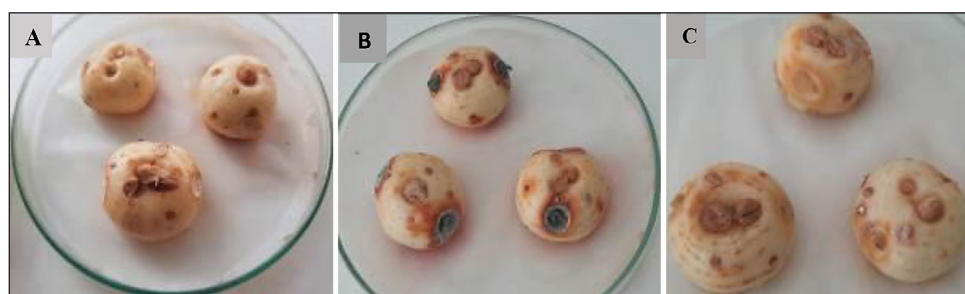


Figure 7. Appearance of necrotic lesions developed around bulb inoculation sites by *Penicillium* isolates tested after 7 days of incubation. Lesions on corms inoculated with saffron corms control (A), *Penicillium aeris* (B), and *P. egyptiacum* (C)

results show that *P. aeris* and *P. egyptiacum*, isolated from corms intended for planting saffron, are endowed with a significant pathogenic power on these seeds. Both species have been shown to induce necrotic lesions, rots, superficial and untenable, which develop, decolorize and deform, over time, the inoculated corms.

DISCUSSION

In their inventory of saffron-associated fungi, Mouden et al. (2024) reported 142 species belonging to three phyla: Ascomycota, Basidiomycota, Mucoromycota. Among these, 26 *Fusarium* species were identified, making this genus the most abundant, followed by *Aspergillus* with 15 species and *Penicillium* with 14. Almost all *Penicillium* species isolated from saffron corms are considered either true pathogens or facultative parasites of these propagation organs. Previous studies have reported several *Penicillium* species associated with saffron corms, including

P. crocicola (Yamamoto et al., 1954), *P. corymbiferum* (Gupta et al., 2011), *P. canescens* (Taheri et al., 2021; Wani et al., 2016, 2017), *P. citrinum* (Belfiori et al., 2021; Tian et al., 2022), *P. citreosulfuratum* (Tian et al., 2022; Hu et al., 2022), *P. cyclopium* (Cappelli and Di Minco, 1999; Fiori, 2002), *P. chrysogenum* (Shuwen et al., 2019), *P. digitatum* (Saeedizadeh, 2014, 2016; Najjar et al., 2017; Zheng et al., 2012) and *P. pinophilum* (Wani et al., 2016, 2017). A number of these species have been reported to compromise postharvest quality, as well as the vitality, propagation and yield of saffron (Rubio-Moraga et al., 2013). worldwide saffron production has been negatively affected by come rot caused by pathogenic fungi, particularly species belonging to the genera *Fusarium*, *Rhizoctonia* and *Penicillium* (Belfiori et al., 2021; Mansotra et al., 2023). Other studies have also identified a broader range of fungi associated with corm decay, including *Macrophomina*, *Aspergillus*, *Sclerotium*, *Phoma*, *Stromatinia*, *Rhizoctonia*, *Rhizopus*, *Penicillium*, *Fusarium*, *Cochliobolus*, *Safostereum*, *Talaromyces*, as well

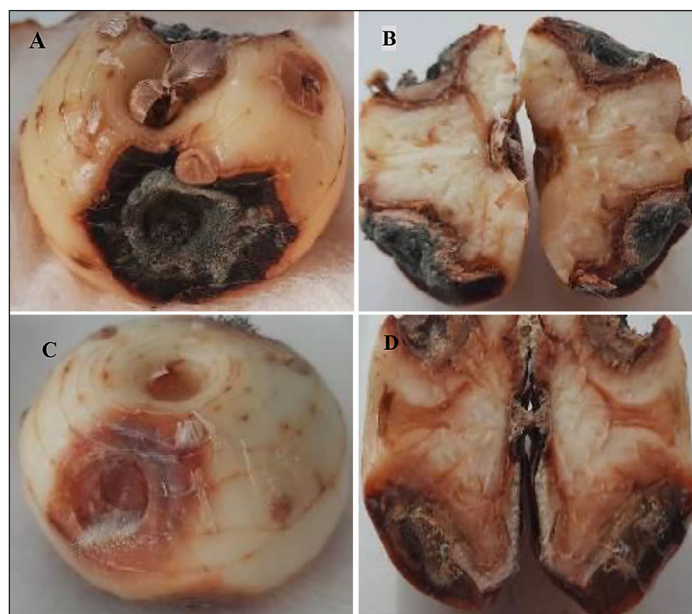


Figure 8. Rot developed after inoculation of corms by *Penicillium aeris* and *P. egyptiacum*: (A) superficial rot of corms, inoculated pat *P. aeris*, manifested by dark brown lesions, with development of a blue-greyish mycelial felting protecting from fructification; after 15 days of incubation; (B) rot spread to deep tissues, with an onset of atrophy of corm edges; (C) development of superficial dry rot, accompanied by discoloration of corms, reddish gray, and the onset of appearance of an airborne mycelial bearing fruit; (D) alteration of corms inoculated with *P. egyptiacum*, superficial and internal rot, with discoloration of corms, after 15 days of incubation

as *Penicillium aeris* and *P. egyptiacum*. These last two species, isolated from saffron corms cultivated in Morocco, have not previously been reported in the literature as saffron-related fungi (Wani et al., 2016, 2018; Ahrazem et al., 2010) and can be considered as two new species for saffron.

Penicillium aeris and *Penicillium egyptiacum* have been shown to infect healthy, seemingly inoculated saffron corms. The necrotic lesions induced after inoculation developed both on the surface and within the corm tissues. Over time, these lesions caused bleaching and deformation of the corms, accompanied by the appearance of greenish mycelial felting and carriers of organs of asexual reproduction of *Penicillium*. Gupta and Vakhlu (2015) also isolated *Penicillium* sp. from infected corms with typical rot symptoms. Several *Penicillium* species have been identified as pathogens of saffron corms. For instance, *Penicillium citreosulfuratum* and *Penicillium citrinum* isolated within a fungal complex that also included *Fusarium solani*, *Stromatinia gladioli* and *Rhizopus oryzae*, have been reported to cause rot symptoms in saffron (Hu et al., 2021; Belfiori et al., 2021). Additionally, *P. digitatum* (Saeedizadeh, 2014, 2016), *P. pinophilum* (Wani et al., 2016, 2017) and *P. solitum* (Zhang et al., 2020) are known to contribute to corm rot. Studies by

Wani et al. (2016, 2017) and Ahmad et al. (2022) noted that *P. pinophilum* can induce more severe rot in saffron corms. Moreover, *P. crocicola* and *P. chrysogenum* have been associated with seedling collapse, wilting of saffron shoots, and development of dark lesions beneath the plant sheaths (Ahrazem et al., 2010; Cappelli et al., 1991). In contrast, other species of the genus *Penicillium*, isolated from corms, are considered to be optional parasites, capable of inducing moderate corm rot, such as *Penicillium cyclopium* (Cappelli and Di Minco, 1999) and *Penicillium canescens* (Wani et al., 2016; 2017). Ambardar et al. (2016) noted that other *Penicillium* species are beneficial, acting as growth biostimulants and biological control agents (Hassine et al., 2022). Some species, such as *P. rugulosum* and *P. bilaiae*, are also known to solubilize phosphate (Wakelin et al., 2004a,b; Fankem et al., 2014). Extending these findings, filamentous *Penicillium* species in general are recognized for colonizing the rhizosphere, where they interact beneficially with plant roots and contribute significantly to the phosphate cycle (Mehta et al., 2019). Recently, a strain of *P. guanacastense* was reported to solubilize aluminum phosphate, highlighting the continued discovery of functional traits in this genus that support plant nutrition (Qiao et al., 2020).

CONCLUSIONS

This study enriches current knowledge on saffron corm rot by identifying *Penicillium aeris* and *P. egyptiacum* as new pathogens and confirming their pathogenicity. The combined application of morphological and molecular tools proved effective for the accurate identification of the two fungal species. The results obtained provide a solid foundation for future epidemiological studies of saffron fungal pathogens and for understanding the role each pathogen plays in damaging the corms and consequently the plants that will grow from these corms. The presence of *Penicillium aeris* and *P. egyptiacum* on the corms suggests that serious economic losses are to be expected in the coming years. Therefore, it is important to consider these pathogens in future integrated pest management programs to reduce saffron yield losses in Morocco.

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