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First identification of *Monocillium ligusticum* in Morocco: A newly reported species associated with saffron corms (*Crocus sativus*)

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ABSTRACT

Healthy plant tissues provide a dynamic habitat for endophytes, which are a promising source of secondary metabolites and bioactive natural products. This study explores the fungal diversity of saffron corms to assess its potential impact on plant growth and health. Saffron corms were analyzed in the laboratory, leading to the identification of *Monocillium ligusticum*, a slow-growing isolate (28–33 mm in diameter after one month). Due to the morphological similarity between isolates, molecular techniques were used for precise identification, confirming the presence of *Monocillium* in saffron tissues. The *Monocillium ligusticum* isolate was registered in a national database and deposited in GenBank (OM980234). Pathogenicity tests showed that this strain is non-pathogenic to saffron corms, suggesting it poses no threat to the plant. These results provide new insights into positive fungal/plant interactions and open promising prospects for using endophytic fungi to improve plant production and protection.

Keywords: *Crocus sativus*, endophyte, *Monocillium ligusticum*, plant-fungi interaction, sequencing, DNA.

INTRODUCTION

Endophytes, whether bacterial or fungal, are attracting increasing interest among researchers for their ability to produce bioactive molecules beneficial for medicine, agriculture, industry and the environment (Bogas et al., 2022; Gunjal, 2023; Amallah et al., 2024; Laanaya et al., 2025a). In agriculture, these beneficial microorganisms asymptomatically colonize plant cells (Mouria et al., 2007; Wani et al., 2015; Msairi et al., 2025). Endophytic fungi, in particular, optimize plant growth through their secondary metabolites, which act as growth stimulants, and provide protection against pathogens by serving as biocontrol agents (Settu and Arunachalam, 2020; Gunjal, 2023; El Kaissoumi et al., 2022, 2024; Errifi et al., 2024a, 2024b; Laanaya et al., 2025b; Louark et al., 2025a). They are used as a promising biological approach for sustainable agriculture (El Aymani et al., 2023). The search for these beneficial organisms for plant improvement was the focus of numerous studies and has included many crops (Kribel et al., 2020; Qostal et al., 2020a, 2020b; El Rhoch et al., 2025a).

Like many economically important crops, saffron (*Crocus sativus* L.) occupies a prominent place (Belfiori et al., 2021; Elouark et al., 2025b) as a valuable and desirable spice (El Aymani et al., 2019a, 2019b; Ourras et al., 2021). Saffron, knowing for its culinary, aromatic and medicinal properties, requires intensive work and considerable time investment for cultivation and manual harvesting (Kothari et al. 2021). Beyond these traditional aspects, contemporary research is increasingly interested in its phytochemical and biological properties, in particular, the exploration of beneficial endophytic microorganisms. In this aspect, the analysis of the biodiversity of endophyte fungi associated with saffron generated increasing interest (El Aymani et al., 2019, Ourras et al., 2022a).

Available research on beneficial microorganisms and the analysis of the biodiversity of saffron endophytic fungi revealed remarkable diversity. In Italy, for example, the composition of endophytic communities varies regionally, including genera and species such as *Cadophora*, *Talaromyces pinophilus*, *Alternaria alternata*, *Epicoccum* spp., *Mucor fragilis*, and *Stemphylium vesicarium* (Belfiori et al., 2021). Analysis of the fungal composition of Moroccan saffron, originating from the Taliouine and Taznakht regions (Ourras et al., 2022b), present in the bulbs, roots and soils of saffron revealed the presence of several species, both endophytic and pathogenic, such as *Fusarium solani*, *F. oxysporum*, *F. culmorum*, *F. roseum*, *Fusarium* sp., *Aspergillus fumigatus*, A. niger, *Trichoderma* sp., *Rhizopus oryzae, Botrytis cinereae* and *Penicillium* sp. (El Aymani et al., 2019c; Ourras et al., 2025a, 2025b). Similarly, Chamkhi and colleagues identified *Rhizopus oryzae*, *Aspergillus fumigatiaffinis* and *Aspergillus niger* from saffron roots in 2018. While some microorganisms infect many hosts, specialists are limited to one or a few specific hosts. Some species, such as *Monocillium ligusticum*, are little studied and rarely encountered. According to the bibliography, studies on the genus *Monocillium* in general, and more specifically on the species *Monocillium ligusticum*, are rare. To date, this study represents the first isolation and report of *Monocillium ligusticum* as an endophytic fungus in Taliouine saffron in Morocco, aiming to deepen our knowledge of fungal diversity in saffron corms and propose its potential use to improve saffron growth and health.

MATERIALS AND METHODS

Fungal isolation

To achieve the study objective, saffron corms were brought to the laboratory for diagnosis. The corm fragments were first superficially disinfected in 70% alcohol for 3 min, rinsed three times in sterile distilled water. Then, dried completely on sterile absorbent paper and inoculated into PSA medium (potato-sucrose agar). Petri dishes were incubated in the dark at 25 °C. After seven days of incubation, isolates developed around corm fragments were purified by successive recultivation of fungal colonies onto Petri dishes containing PSA or PDA medium, preceded by microscopic observations (Meddah et al., 2011; Boukharta et al., 2012).

DNA extraction and sequencing

The isolated strains were cultured seven days on PSA medium. After lyophilization, mycelium (10–40 mg) were ground using mortar and pistil. The total genomic DNA was extracted using the OmniPrep kit for fungi (G-Biosciences, St. Louis, MO; Cat. #786-399) following to the manufacturer's guidelines and quantified later with a Implen N50 Nano spectrophotometer (Implen GmbH Schatzbogen, München Waltham, Germany). The absorbance ratios at 260/280 and 260/230 were measured to check DNA purity.

PCR Amplification and sequencing: PCR Amplification was performed using a MultiGene OptiMax Thermal Cycler in a 20 µl reaction mixture containing 11 µl milli-Q water, 2 µl 10X PCR buffer, MgCl2, dNTPs, 0.2 µl x-VITA-Taq DNA polymerase, specific primers (ITS1 and ITS4 described by White et al. (1990).), and 1 µl gDNA. The thermal cycle, involved in the amplification phase, includes cycles that start with an initial denaturation at 95 °C for 3 min and a second denaturation phase of 35 cycles (30 s at 95 °C). The denaturation phase is followed by annealing (30 s at 55 °C) and extension (30 s at 72 °C), and ends with a final elongation (7 min 25 s at 72 °C). Following amplification, the resulting PCR 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 sequences generated during this study, along with sequences from the GenBank database, were used for alignment and phylogenetic analysis. All sequences were aligned with MEGAX software (Kumar et al., 2018). The phylogenetic tree was generated by the Neighbor-Joining (NJ) method developed by Saito et al., (1987) with 1000 bootstrap replicates to assess branch support.

Pathogenicity test

The pathogenic potential of the isolated species was tested in vitro by inoculating saffron bulbs with mycelial explants. Healthy saffron bulbs were selected to be disinfected using 5% pure sodium hypochlorite (NaClO) for 3 min, traces of NaClO were removed by successive washes with sterile distilled water. The corms were then dried on sterile absorbent paper under a laminar flow hood. The disinfected corms were slightly injured with a sterile needle at two equidistant points, and each wound was inoculated with mycelial explants (4 mm) taken from the area of active fungal growth. 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. Inoculated bulbs were maintained at 28 °C and observed daily to monitor lesion progression over time.

RESULTS AND DISCUSSION

Among the colonies developed on PSA medium, those of *Monocillium ligusticum* reached 28 to 33 mm maximum in diameter, one-month after incubation, demonstrating a relatively slow mycelial growth rate. Colonies show a variation in color, from white cream, pale yellow or pale orange at first to dark grey or even blackish shades over time (Figure 1). This change in color is attributed to the formation of chlamydospore and sclerotia, resistant structures often observed at the periphery of colonies. These sclerotia being lighter at the periphery and darken towards the center with aging, hardening and becoming blackish (Figure 2). Colonies are generally dry, with a sparse aerial mycelium, often flaky or slightly cotton, and sometimes powdery. Mycelial senescence is observed after 20 days of culture, indicating a phase of maturity and aging of the colonies (Figure 1).

Microscopic observation revealed a septate, hyaline, and branched mycelium. The phialides, measuring 35–75 µm of length and 1.2–1.4 µm of breadth, produce cylindrical hyaline conidies of 3 to 4 µm in length (Figure 3). Sclerotia, constituted by filament aggregated mycéliens, measure between 12 to 90 µm length and 8 to 60 µm of diameter (Figure 2).

The morphological and microscopic description of this study is consistent with those reported by Ashrafi et al. (2017) and Gams et al. (2019). Slow growth, coloration variation due to sclerotia formation, and structure of phialides and conidia are critical distinguishing features for the precise identification of this species, distinguishing *Monocillium ligusticum* from other similar species of the genus *Monocillium*.

Due to a strong morphological similarity between the different isolates, it was necessary to use molecular biology techniques for precise identification. Sequencing the ribosomal DNA (ITS) region of several isolates identified the genus *Monocillium* confirming its presence in the internal tissues of saffron. The isolate

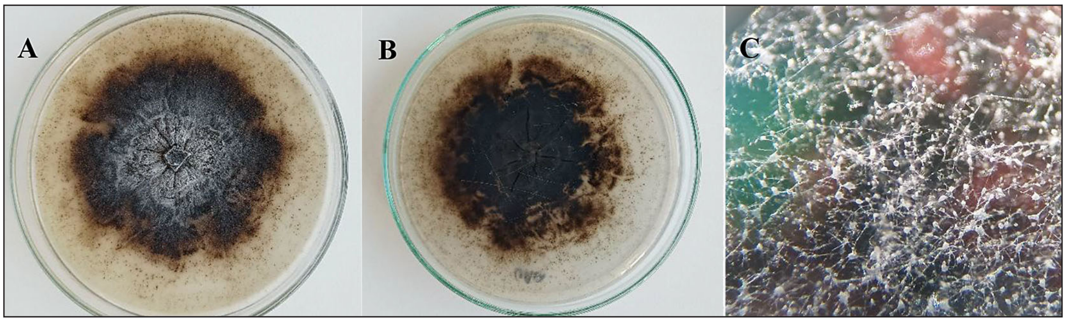

Figure 1. Macroscopic criteria of *Monocillium ligusticum:* A, B – *Monocillium ligusticum* on PSA medium; C – fertile mycelial heads under the microscope at magnification x100

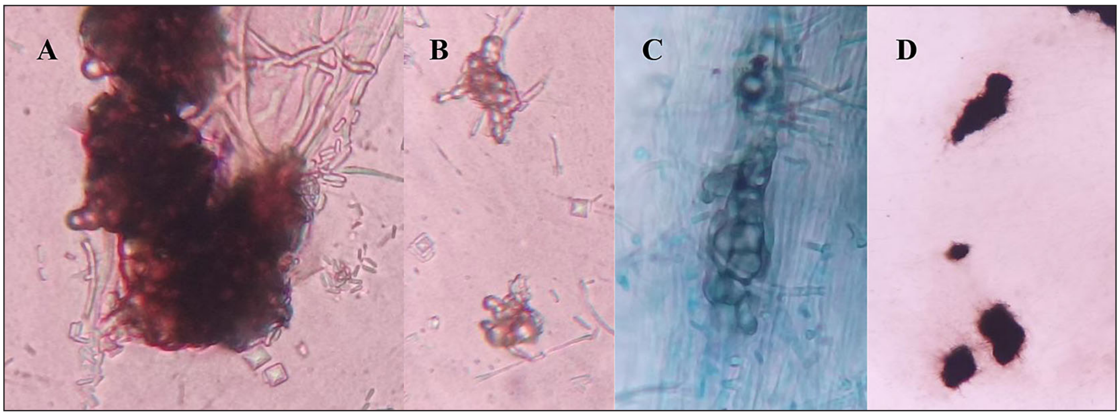

Figure 2. A–B – condensed chlamydospore formation; C – early formation of microsclerotia; D – mature pigmented sclerotia; magnification x400; mounting fluid: cotton blue

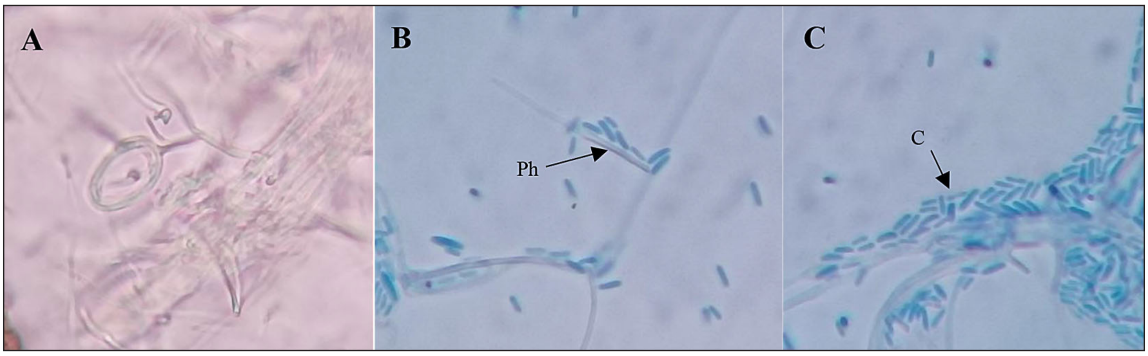

Figure 3. A – coiled hyphae; B – phialide (Ph); C – conidia (c); magnification x400; mounting fluid: cotton blue

Monocillium ligusticum was registered in a national database and deposited in GenBank under number OM980234. These morphological, microscopic, and molecular analyses confirmed the identity of the species *Monocillium ligusticum*.

After identification, the pathogenicity of *Monocillium ligusticum* was tested on healthy saffron corms. The test was negative, *Monocillium ligusticum* did not induce any symptoms on saffron bulbs, indicating that this strain was not pathogenic and does not pose a threat to the saffron plant under the experimental conditions tested, suggesting a possible potential as biological control agent for the management of plant diseases. In this context, the genus *Monocillium* has proven parasitic activity on the eggs of phytopathogenic nematodes. In 1984, Grant and Elliott isolated *Monocillium* sp. from the cysts of *Heterodera glycines*, a soybean nematode. Years later, Achrafi and his team (2017) reported the parasitic activity of *Monocillium gamsii* on the eggs of *Heterodera filipjevi*, a very common cyst nematode in cereals. They showed that *Monocillium gamsii* hyphae penetrate nematode eggs and completely colonize the larva, forming multicellular chlamydospores and microsclerotia. *M. bulbillosum* was also tested against *H. filipjevi* and showed similar infection symptoms to *Monocillium gamsii* over a period of 2 to 4 weeks.

To date, this study presents *Monocillium ligusticum* as the first documented isolation of the genus *Monocillium* on saffron in Morocco and worldwide. The search for potentially important fungal species within the endophytic fungal diversity of cultivated plants was the subject of numerous studies. These studies included important crops such as saffron (Ourras et al., 2025c, 2025d), strawberry (El Kaissoumi et al., 2025), cannabis (Laanaya et al., 2025a ; Msairi et al., 2025), argan tree (Maazouzi et al., 2023; Sohaib et al., 2024; El Rhoch et al., 2025b ; Sellal et al., 2025), and tomato (Sinno et al., 2020; Dong et al., 2021; Albatnan et al., 2025 ;Ouazzani Chahdi et al., 2025a, 2025b).

CONCLUSIONS

In conclusion, the objective of the study, which aimed to deepen our knowledge on the fungal diversity of saffron corms, was largely achieved, with the discovery of *Monocillium ligusticum* as an endophytic fungus of corms intended for cultivation. This discovery should be exploited in future research on the use plants endophytes as biostimulants and bioprotectants of crops, in order to better understand the interactions between fungi and plants. Exploring plant-endophyte interactions could be a relevant approach to initiate the adoption of scientific practices for sustainable agriculture.

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