

Comparative pathogenicity of *Fusarium* species isolated from rhizomes and roots of *Iris germanica*

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Abstract

Iris germanica is a valuable ornamental plant with applications in the pharmaceutical, cosmetic, and perfume industries. However, its production is threatened by fungal diseases, particularly those caused by *Fusarium* species. The objective of this study was to investigate the pathogenicity of different *Fusarium* spp. isolates via rhizome inoculation, with emphasis on symptom development, isolate aggressiveness, and consequent damage to the host plant. During this study, thirteen isolates belonging to *Fusarium oxysporum*, *F. solani*, and *Fusarium* sp. were obtained from rhizomes and roots of *Iris* plants collected in the Taza region of Morocco. Pathogenicity tests revealed that all isolates, except S4, induced leaf wilting in rhizomes three months after inoculation, with a wilting index ranging from 30.55 to 50%. Deep brown necrosis and partial root breakdown were observed. The isolate RIZ2B caused the most severe root rot (score 3), while isolates RIZ21, RIZ31, RZ32, RZ26, SP14, R33, and SP21 showed moderate pathogenicity (score 2). All isolates significantly reduced the agronomic performance of *I. germanica* plants. The number of leaves showed a significant reduction compared to an average of 7.66 in the control, with reductions of 73.89% (RZ32) and 65.27% (R'12, RZ'1, S4, RIZ21), and 60.83% (SP21). *In vitro* assays, isolate SP14 achieved 100% colonization of rhizome slices, whereas RIZ2B exhibited the highest sporulation rate (0.32×10^6 spores/mm²). Overall, the results demonstrate that *Fusarium* species, particularly *F. oxysporum*, severely compromise rhizome health and quality, underscoring the urgent need for effective management strategies to control *Fusarium*-induced diseases in *Iris germanica*.

Keywords: *Fusarium* spp.; *Iris germanica*; necrosis; pathogenicity; rhizome rot.

Abbreviations: IAP: Agronomic parameter of the inoculated plants; LSD: Least significant difference; LWI: Leaf Wilting Index; Ndl: Number of wilted leaves; NTI: Number of total leaves; PSA: Potato sucrose agar; RPAP: Percentage reduction of each agronomic parameter; SCc: Surface colonized by the fungus; STr: Total surface of the rhizome slice.

Introduction

Iris germanica L. (Iridaceae), commonly known as bearded iris, is a perennial herbaceous plant. Widely cultivated across the world for its large, vividly colored flowers and strong ornamental appeal, this species is extensively used in landscaping, botanical gardens, and horticultural hybridizations. Its vegetative rhizome multiplication makes it particularly appreciated for its phenotypic stability and hardiness (Rønsted et al. 2012; Mazaheri et al. 2021). Beyond its aesthetic significance, *I. germanica* also possesses notable medicinal properties. Extracts from its rhizomes have shown antioxidant, diuretic, and anti-inflammatory properties, justifying their use in some traditional medicines (Kumar et al. 2015; Morning et al. 2017). However, *I. germanica* is also exposed to numerous biotic threats, particularly soil-borne fungal infections, that severely compromise its growth, health, and propagation capacity. Among the most destructive pathogens are species of the genus *Fusarium* Link, a group of ubiquitous filamentous fungi responsible for severe diseases in a wide range of plant species. Several *Fusarium* species are known to cause ornamental diseases (Hembrom et al. 2019), with *Fusarium oxysporum* and *Fusarium solani* frequently reported to be responsible for rhizome rot in *Iris germanica* (Baayen et al. 1998; Mazaheri et al. 2020). These fungi often penetrate through mechanical or natural wounds and colonize vascular or cortical tissues, causing necrosis, wilting, reduced vegetative vigor, and even rapid plant death (Di Pietro et al. 2003).

In Morocco, iris cultivation is experiencing recent but promising growth, especially in the production of rhizomes (iris roots or 'orris roots') mainly used for the cosmetic industry and luxury perfumery (Khatib et al. 2022). The isolation, identification, and characterization of these phytopathogenic fungi are therefore essential to understand their pathogenicity, adapt cultural practices, and implement effective and sustainable control strategies. In this context, the objective of this study was to investigate the pathogenicity of different *Fusarium* spp. isolates via rhizome inoculation, with emphasis on symptom development, isolate aggressiveness, and consequent damage to the host plant.

Results

Identification of fungi

Thirteen isolates belonging to the genus *Fusarium* were obtained from rhizomes and roots of *Iris germanica* plants collected from the Muslim cemetery in Taza, North-east (Achajri et al. 2025) (Table 1). Among these, isolates SP14, SP21 and RIZ2B were assigned to *Fusarium oxysporum*, while S4 and R33 were attributed to *F. solani*, according to their morphological characteristics. Species discrimination was based on macroscopic features observed on PSA: *F. solani* typically developing cream to white, woolly colonies with a pale reverse, whereas *F. oxysporum* produced cottony to floccose colonies often showing violet pigmentation, as well as on microscopic traits. *F. solani* was characterized by robust, thick-walled macroconidia (39–75 × 5–9 μm, with 3–5 septa), cylindrical microconidia (5–12 × 2–4 μm), and abundant, coarse-walled chlamydospores (7–12 μm). Conversely, *F. oxysporum* displayed more delicate and slightly curved macroconidia (27–39 × 3–5 μm), smaller oval microconidia (5–12 ×

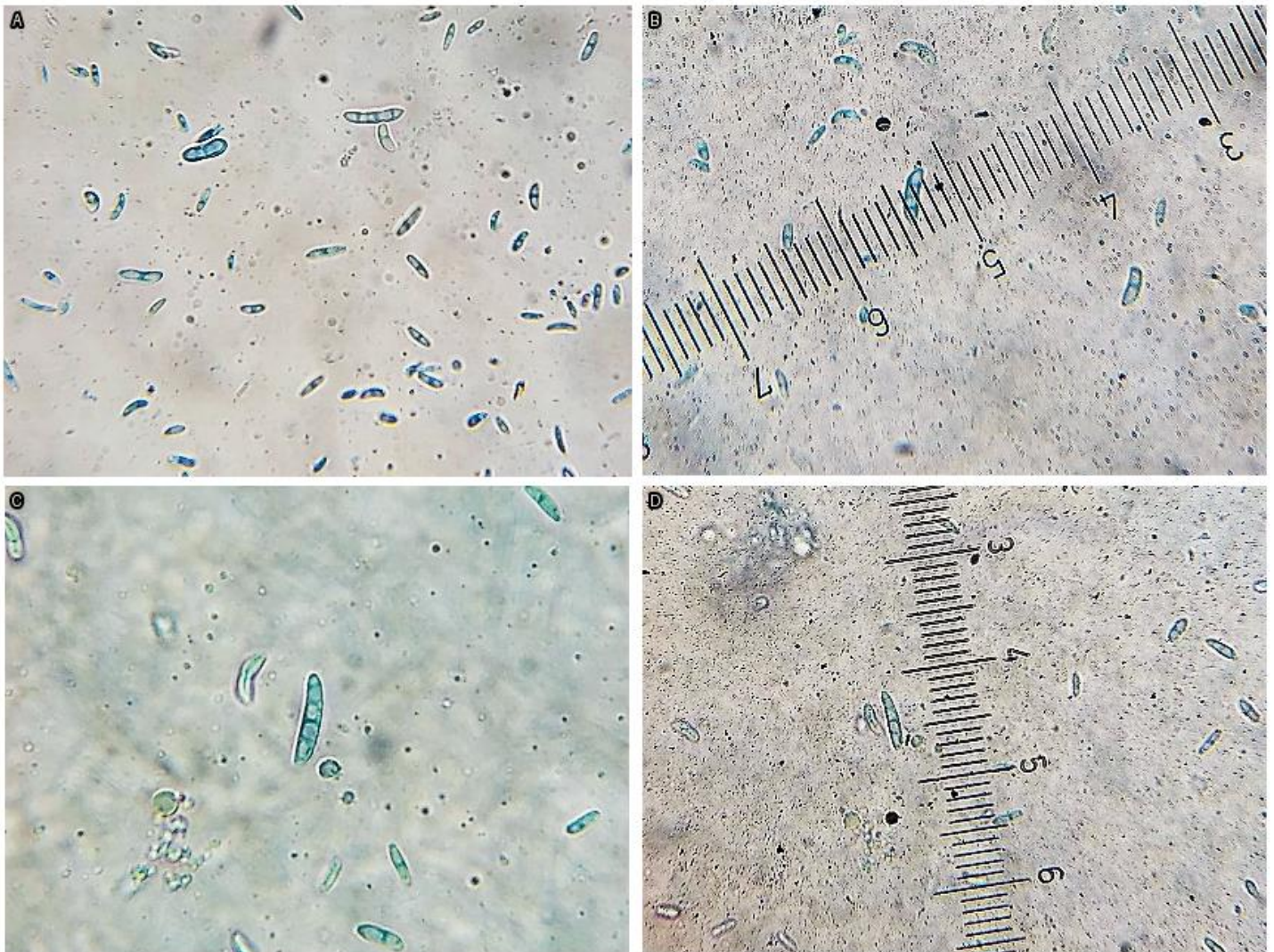


Fig. 1. Morphological characterization of *Fusarium oxysporum* isolates SP14 and SP21. (A–B) SP14: abundant hyaline, mostly non-septate microconidia with regular oval to cylindrical profiles. (C–D) SP21: representative microconidia exhibiting similar morphology. Observed traits align with the species description of *F. oxysporum*. Scale corresponds to ocular micrometer calibration.

Table 1. *Fusarium* spp. isolates from *Iris germanica* rhizomes and roots native to Taza, Morocco.

Fusarium isolates	Isolate codes	Source of isolation
F. oxysporum	SP14	Rhizome
F. oxysporum	SP21	Rhizome
F. oxysporum	RIZ2B	Root
F. solani	S4	Rhizome
F. solani	R33	Rhizome
Fusarium sp.	RIZ31	Rhizome
Fusarium sp.	R'12	Rhizome
Fusarium sp.	RZ'1	Rhizome
Fusarium sp.	RZ32	Rhizome
Fusarium sp.	RIZ21	Root
Fusarium sp.	RZ41	Rhizome
Fusarium sp.	RZ'2	Rhizome
Fusarium sp.	RZ26	Rhizome

Table 2. Assessment of how *Fusarium* isolates inoculation affects *Iris germanica* plant development.

Fusarium Isolates	TNHL	RPTNHL (%)	MLA (cm ²)	RPMLA (%)	NrNFR	RPNrNFR (%)	Plant weight (g)	RPPW (%)
SP14	9.33a	-21.80e	52.28c	22.82b	1.33f	75.04a	53.83d	46.43a
RIZ31	8.00b	-4.43d	54.71b	19.75c	0.33f	93.86a	53.83d	48.45a
SP21	3.00d	60.83b	54.98b	19.18c	8.66b	-62.47e	71.26c	29.08b
RIZ2B	7.66b	0d	50.74c	25.41b	2.33e	56.28b	66.6c	33.73b
R'12	2.66d	65.25b	57.52a	15.44d	6.66c	-24.95d	69.6c	30.74b
RZ'1	2.66d	65.27b	56.92a	16.33d	7.66b	-43.71e	75.6c	24.77b
RZ32	2.00e	73.89a	51.86c	23.76b	10.00a	-87.61f	84.7b	15.72c
S4	2.66d	65.27b	49.75c	26.87b	5.00d	6.19c	53.80d	46.46a
RIZ21	2.66d	65.27b	36.50d	46.34a	8.00b	-50.09e	68.76c	31.58b
RZ41	7.33c	4.30c	43.14d	36.58a	1.00f	81.23a	65.3c	35.02b
RZ'2	6.66c	13.05c	55.91b	17.81c	0.66f	87.61a	72.8c	27.56b
RZ26	7.33c	4.30c	57.97a	14.78d	2.00e	62.47b	78.13b	22.25c
R33	3.66d	52.21b	33.40d	50.88a	10.33a	-93.80f	81.26b	19.14c
Control	7.66b	—	68.03a	—	5.33d	—	100.50a	—

Two values in the same column do not differ significantly at the 5% threshold if followed by the same letter. TNHL: Total number of healthy leaves; RPTNHL: Reduction percentage of TNHL; MLA: Mean Leaf Area; RPMLA: Reduction percentage of MLA; NrNFR: Number of Newly Formed Rhizomes; RPNrNFR%: Reduction percentage; PW: Plant weight; RPPW: Reduction percentage of Plant weight.

2–3 μm, generally aseptate), and chlamydospores occurring singly or in pairs (7–11 μm) (Fig. 1). These diagnostic elements are in agreement with the identification criteria outlined by Leslie and Summerell (2006).

Pathogenicity test

The plants grown from these inoculated rhizomes showed leaf wilting and necrosis in the root system. Additionally, there was a decrease in various agronomic parameters (total number of healthy leaves; mean leaf area; number of newly formed rhizomes; plant weight) compared to the control.

In vivo test

Leaf wilting

This allowed for an evaluation of disease severity in *Iris* plants. The isolates RZ32, RIZ21, SP21, and SP14 were the most virulent, causing wilting and leaf drop (Fig. 2), with wilting leaf indices of 50%, 33.33%, 30.55%, and 21.81%, respectively. All other isolates caused only minor or no leaf wilting, ranging from 15.5% to 0% (Fig. 3).

Necrosis symptoms on rhizome

Furthermore, deep brown necrosis was observed on the rhizomes and roots of inoculated plants. The highest rhizome and root rot scores were seen in plants inoculated with RIZ2B, which scored a 3. This was followed by RIZ21, RIZ31, RZ32, RZ26, SP14, R33, and SP21, all of which scored a 2. A score of 1 was given to RZ41. Meanwhile, plants inoculated with other isolates showed no symptoms (Fig. 4). Necrosis symptoms appeared on the rhizomes (Fig. 5), along with deterioration of the root system in the most severe cases (Fig. 6).

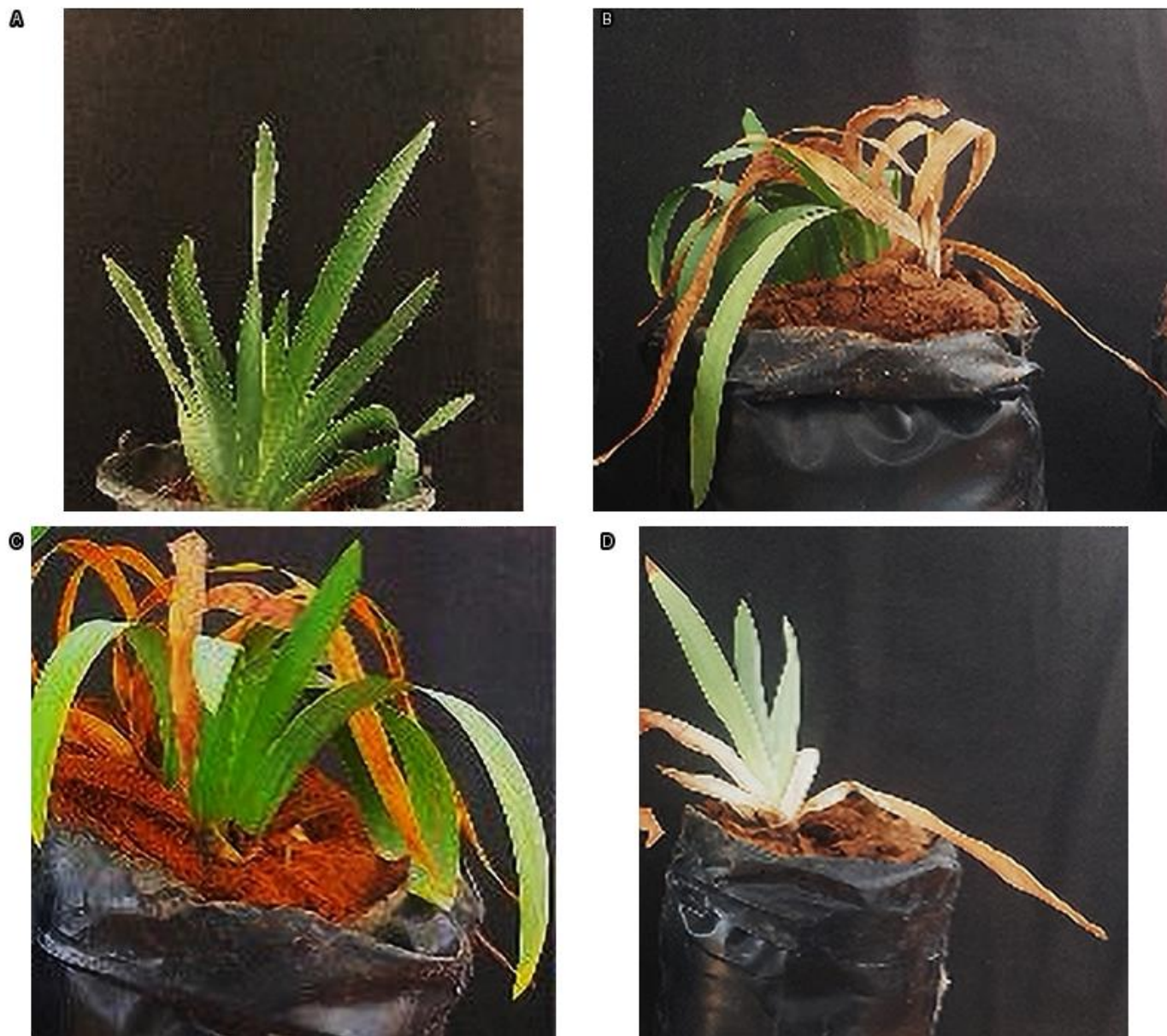


Fig. 2. Symptoms of various strains in leaves. (A) Control. (B–C) Strain SP14. (D) Strain SP21.

Table 1 (severity scale). Rhizome and root rot severity scale used for disease assessment.

Score	Description
0	0% — no obvious symptoms
1	1–30% of the rhizomes and roots affected (with symptoms)
2	30–60% of the rhizomes and roots affected
3	60–100% of the rhizomes and roots affected



Fig. 3. Percentage of leaf wilting in *Iris* plants caused by different *Fusarium* isolates. Two values do not differ significantly at the 5% level if followed by the same letter.



Fig. 4. Rhizome and root rot scores in iris plants inoculated with different *Fusarium* spp. isolates. Two values do not differ significantly at the 5% level if followed by the same letter.

Number of healthy leaves and reduction percentage

The pathogenicity of *Fusarium* isolates caused significant changes in several agronomic traits of *Iris germanica*, indicating major physiological stress. *Fusarium* spp. significantly affected key parameters such as total leaf count, average leaf area, plant fresh weight, and rhizome number (Fig. 7). Data analysis revealed a substantial reduction in the number of leaves, reaching up to 73.89% with RZ32, 65.27% with R'12 and RZ'1, S4, and RIZ21, 60.83% with SP21, and 52.20% with R33. Conversely, isolates SP14, RIZ31, RIZ2B, RZ41, RZ'2, and RZ26 did not affect leaf formation (Table 2).

Mean leaf area and reduction percentage

A marked decrease in average leaf area (50.88%) was observed in plants inoculated with *Fusarium solani* R33 (35.41 cm²), followed by RIZ21 (36.58 cm²) and RZ41 (43.4 cm²), representing reductions of 46.34% and 36.58%, respectively, from the control's 68.03 cm². The other isolates resulted in moderate reductions, with leaf areas ranging from 49.75 to 57.97 cm², not exceeding 26.87% (Table 2).

Number of newly formed rhizomes

Additionally, there was a significant decrease in new rhizome production in plants inoculated with RIZ31, RZ'2, RZ41, and SP14, with reductions of over 75%. The number of rhizomes per plant dropped by 93.86% to 75.04%, leaving about one rhizome. RZ26 and RIZ2B followed, each with around 2 rhizomes per plant. In contrast, other isolates maintained the same or even higher number of rhizomes than the control (averaged 5.33), with some plants producing up to 10 rhizomes, such as RZ32 and R33 (Table 2).



Fig. 5. (A–B) Necrosis symptoms seen in rhizomes after inoculation.



Fig. 6. (A–B–C) Necrosis observed in roots inoculated with the isolate RIZ2B of *Fusarium oxysporum*.

Plant weight (fresh biomass) and reduction percentage

A significant reduction of over 46% was observed in the total fresh biomass of inoculated plants with isolates SP14, S4, and RIZ31 (53.83 g, 53.80 g, and 51.8 g, respectively) compared to healthy controls at 100.5 g. The biomass with RIZ2B was 66.6 g. Conversely, RZ32 and R33 (*F. solani*) retained relatively high plant mass at 84.28 g and 81.26 g, respectively, indicating lower virulence for this trait (Fig. 7).

In vitro test

Colonization rates of Fusarium

After seven days, all *Fusarium* isolates colonized and sporulated on *Iris* rhizome slices, showing variable colonization levels (Table 3). SP14 reached 100% colonization, followed by RIZ2B (56%), S4 (49%), and SP21 (36%). RIZ2B produced the highest conidial density (0.32×10^6 conidia·mm⁻²), while the others ranged from 0.06 to 0.07×10^6 . Yellow-brown discoloration and dry rot developed after ten days, confirming tissue degradation and differences in isolate virulence (Fig. 8).

Table 3. Colonization and conidia production by *Fusarium* isolates on *Iris germanica* rhizome slices after 10 days of incubation.

Isolates	Colonization (%)	Sporulation (10 ⁶ conidia/mm ²)
SP14	100a	0.07b
RIZ2B	56b	0.32a
SP21	36d	0.06c
S4	49c	0.06c

Two values do not differ significantly at the 5% level if followed by the same letter.

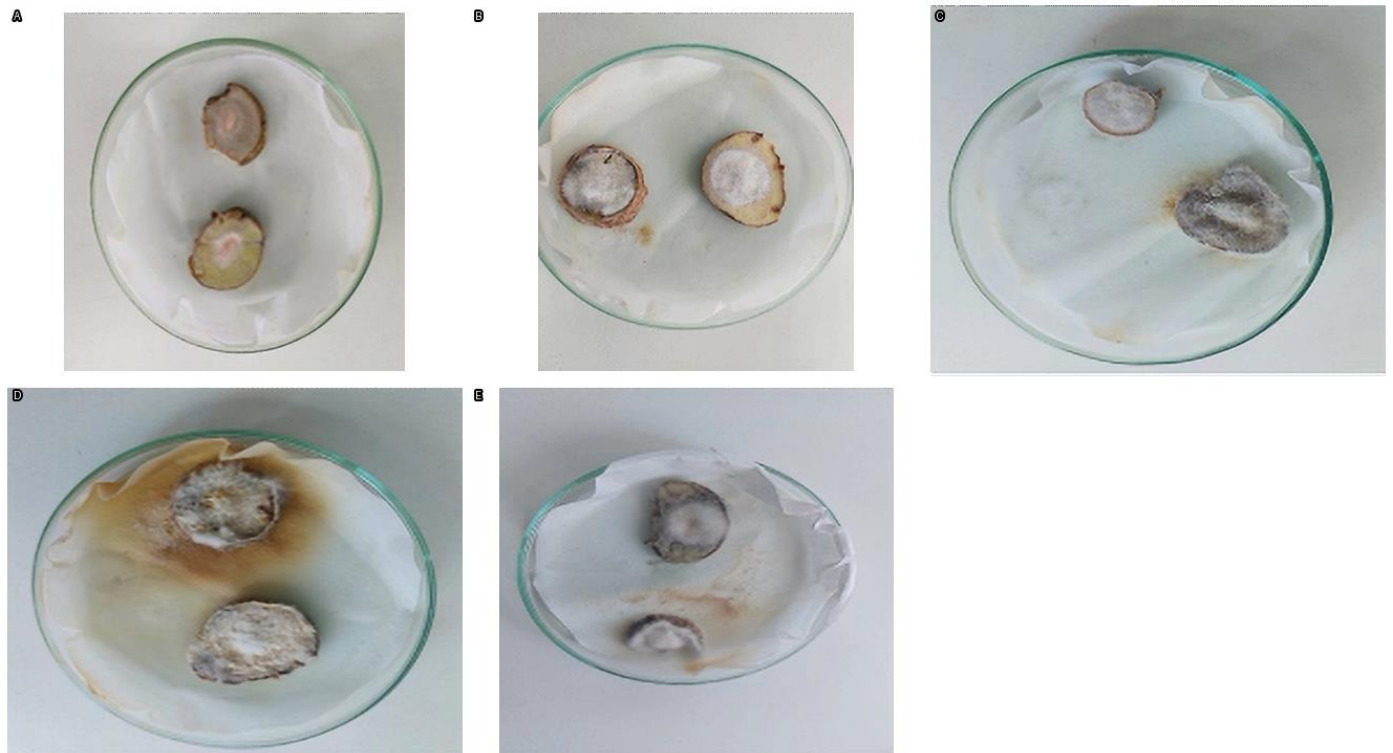


Fig. 7. Symptoms observed on the iris plant after inoculation with different isolates of *Fusarium*. (A) Control plant. (B) RZ31. (C) SP14 (*Fusarium oxysporum*). (D) Strain R33 (*Fusarium solani*). (E) R'12. (N) Necrosis.



Fig. 8. *In vitro* inoculation of *Iris germanica* rhizome slices. (A–B) *Fusarium solani* S4. (C) *Fusarium oxysporum* RIZ2B. (D) SP21. (E) SP14.

Discussion

This study evaluated *Fusarium* spp. isolates from the rhizomes and roots of *Iris germanica*, an ornamental medicinal plant, for their ability to cause disease and induce changes in morphology and physiology. Inoculation with these isolates caused various symptoms, including necrosis and decay of underground tissues and reduced vegetative growth.

The findings describe various plant responses to *Fusarium* spp. To our knowledge, this is the first report comparing the pathogenicity of different *Fusarium* strains infecting the underground parts of *Iris germanica* and recording the severity of symptoms. *Fusarium oxysporum* isolates (notably SP14, SP21, and RIZ2B) significantly hindered the growth of the host plant. Their activity included a marked suppression of aerial biomass for SP14 and a net loss of formed bulbs, which may affect vegetative propagation. These strains also caused necrotic leaf spots and root rot areas, common symptoms of vascular wilt caused by *F. oxysporum* (Di Pietro et al. 2003; Qostal et al. 2019a, 2019b; Elbouazaoui et al. 2022; Mazaheri et al. 2020). Their symptoms closely resemble those seen in saffron, where *F. oxysporum* is a primary pathogen causing corm rot and leaf necrosis (Hu et al. 2021; Elouark et al. 2025).

According to Salehi and Safaie (2015), *Fusarium oxysporum* f. sp. *lilii* is the primary species associated with rot disease in lily bulbs. Su (2002) reported that the pathogen causing rot spots in *Iris japonica* Thumb. in Chongqing was identified as *Fusarium oxysporum* Schl. based on its morphology, cultural characteristics, and pathogenicity. Caligiore-Gei et al. (2023) also demonstrated that some *Fusarium oxysporum* isolates are highly virulent, causing severe rot symptoms in healthy saffron corms after experimental inoculation. *Fusarium solani* isolates (R33 and S4) showed varying levels of pathogenicity; they caused fewer necrotic symptoms but still produced notable morphological changes, such as smaller leaves, finer leaf structure, and slight biomass reductions. Although less severe, these symptoms suggest a background infection, likely confined to cortical tissues, as observed in other sensitive cultures (El Haddadi et al. 2019, 2021; Mazaheri et al. 2022; El Hazzat et al. 2023; El Rhoch et al. 2025).

Notably, isolates RZ32 and R33 caused a slight increase in new bulb formation. This increase may reflect an adaptive response of the plants to infection, a phenomenon observed in other plant–*Fusarium* interactions (Mazaheri et al. 2020). Zare et al. (2011) confirmed that *Fusarium oxysporum* is the dominant species associated with this disease, although other *Fusarium* species have also been found.

Palmero et al. (2014) collected isolates from infected corms and tested their pathogenicity on different species within the Iridaceae family. The differences observed between rhizome isolates (RZ32, RZ26, RZ'1) and root isolates (RIZ2B, RIZ31) highlight the importance of the original organ in the pathogen's profile. Similar to what has been seen in saffron (Iridaceae family), where *Fusarium* isolates from corms cause symptoms ranging from superficial rot to deep, sporulating lesions (Shah et al. 2018), *Iris germanica* isolates also displayed similar behaviors depending on their origin.

According to Yadav et al. (2024), many plants in the Iridaceae family, such as *Crocus*, *Gladiolus*, *Iris*, and *Freesia*, are cultivated worldwide for their valuable flowers and blooms. All these plants are susceptible to a variety of diseases caused by different pathogens, resulting in decreased yields and significant economic losses. Corm and rhizome rot are the most damaging diseases across all major species of this family worldwide. *Fusarium oxysporum* has been identified as a primary cause of these diseases.

Materials and Methods

Plant material collection

Plants of *Iris germanica* were collected with their rhizomes from the Muslim cemetery of Lamsalla Taza in northeastern Morocco (Achajri et al. 2025). The collected rhizomes were placed in plastic bags and transported to the laboratory for further processing.

Isolation and preservation of fungi from *Iris* rhizomes

Rhizome fragments were cut into small pieces, disinfected with 75% ethanol for 5 min, rinsed in sterile water, blotted dry on sterile paper, and aseptically placed in Petri dishes with Potato Sucrose Agar (PSA) medium, according to the method described by El Aymani et al. (2019) and Ourras et al. (2025). The preserved fungal species were kept on filter paper discs at -20°C in a freezer.

Isolated fungi were examined according to the identification key of Leslie and Summerell (2006), at magnifications of 40 \times , 100 \times , 400 \times and 1000 \times . Observations included mycelial morphology, conidiophore structure, conidial shape and size, presence of chlamydospores or other resting organs. Mounting fluids were either tap water or lactophenol cotton blue stain (Ourras et al. 2023).

Identification of fungi

The identification of fungal isolates was conducted based on both macroscopic and microscopic examinations. Macroscopically, colony characteristics such as color, texture, and general morphology on PSA were carefully assessed. For microscopic observation, a small fragment of mycelium was mounted on a glass slide and examined under a light microscope at various magnifications to visualize reproductive structures, including conidia and conidiophores. The morphological traits observed were then compared with authoritative taxonomic descriptions using the identification key of Leslie and Summerell (2006).

Preparation of the inoculum

Thirteen *Fusarium* isolates were grown on PSA medium. The cultures were kept at 28°C in the dark for 15 days. Spores were aseptically scraped from the medium surface using a metal spatula, rinsed with distilled water, and filtered through muslin gauze to remove mycelial fragments (Meddah et al. 2011; Boukharta et al. 2012; Mouria et al. 2014). The resulting spore suspension was adjusted to 10^6 conidia mL^{-1} using a Malassez cell for counting.

In vivo inoculation

A total of 52 *I. germanica* rhizomes were washed with running water, disinfected with 2% sodium hypochlorite (NaOCl), rinsed thoroughly in sterile distilled water, and then immersed for 24 h in 100 mL of spore suspension at a concentration of 10^6 conidia mL^{-1} . Non-inoculated rhizomes served as negative controls. Four replicates were performed for each isolate and for the control.

Three months after inoculation, several morphological and agronomic parameters were assessed. The percentage reduction of each agronomic parameter (RPAP %) relative to the un-inoculated control was calculated using:

$$\text{RPAP \%} = (\text{C} - \text{IAP}) / \text{C} \times 100$$

The wilting of *Iris* plants was evaluated using the Leaf Wilting Index (LWI %):

$$\text{LWI (\%)} = \text{Ndl} / \text{NTI} \times 100$$

In addition, rot of rhizomes and roots was assessed using a modified 0–3 severity scale (Aoyagi et al. 1998) based on the percentage of affected underground tissue showing discolouration and necrosis (Table 1).

Inoculation of rhizome slices in vitro

Rhizomes from *I. germanica* plants (average diameter ~ 3 cm, width ~ 2 cm) were washed multiple times with running water, disinfected using a 2% sodium hypochlorite solution, and then rinsed three times with sterile distilled water. They were cut with a sterile scalpel into discs approximately 2.5 cm in diameter, and two slices were placed in 90 mm Petri dishes containing three disks of sterile filter paper. Each rhizome slice was inoculated with a 5 mm diameter piece cut from a 7-day-old fungal culture. The plates were kept in a growth-chamber at 28°C under a 12-h light/12-h dark cycle.

After 10 days of incubation, the lesion diameters on the rhizome slices were measured using a dual-scale decimeter. The percentage of rhizome slice colonization (% C) was calculated using:

$$\% C = \text{SCc} / \text{STr} \times 100$$

Statistical analysis

Data processing centered on variance analysis using the 5% ANOVA I test. Significant differences between means were assessed with the least significant difference (LSD) test at $P < 0.05$.

Conclusion

I. germanica plants infected with *Fusarium oxysporum* and *Fusarium solani* showed severe root and rhizome rot, reduced growth, and poor bulb formation. These results reveal the high pathogenic risk of *Fusarium* species and stress the need for integrated control strategies using prevention, rapid detection, and biological control to protect plant quality and reduce economic losses.

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None.

Author contributions

NA conceived and designed the study, performed the experiments, and wrote the manuscript. **BK** was responsible for data curation and processing. **ST** contributed to writing the manuscript, prepared the figures, and revised the text and language. **HE** conducted the statistical analyses. **KA** and **AH** provided supervision and scientific guidance. **AOT** and **AD** contributed to manuscript revision, language verification, and final approval of the version to be published.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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