EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2026, 27(10), 415–429
https://doi.org/10.12912/27197050/231163
ISSN 2719–7050, License CC-BY 4.0

Received: 2026.07.31
Accepted: 2026.08.31
Published: 2026.09.10

Diversity and pathogenic potential of *Fusarium* species associated with grapevine dieback in Morocco

Soukaina Tayou[1,2*], Karima Selmaoui[1], Samah Ourras[1], My Abdelaziz El Alaoui[1], Mohamed Zarid[1], Amina Ouazzani Touhami[1], Cherkaoui El Modafar[2], Allal Douira[1]

[1] Laboratory of Botany, Biotechnologies, and Plant Protection, Faculty of Science, Ibn Tofail University, Kenitra, Morocco
[2] Laboratory of Excellence in Agrobiotechnology and Bioengineering, AgroBiotech Center, CNRST-Labeled Research Unit (URL05-CNRST), Cadi Ayyad University, Marrakech 40000, Morocco
* Corresponding author's e-mail: soukaina.tayou@uit.ac.ma

ABSTRACT

Grapevine dieback is an increasing concern in Morocco, where fungal pathogens, particularly *Fusarium* species, are involved in disease development. This study aimed to isolate, identify and evaluate the pathogenicity of *Fusarium* species associated with symptomatic grapevine plants. Fungal isolates were obtained from symptomatic tissues and then identified using morphological and molecular approaches, Analyses revealed the presence of isolates belonging to *Fusarium solani* (R1 and R3), *Fusarium falciforme* (R2 and A46), and *Fusarium chlamydosporum* (D11). Pathogenicity was evaluated using herbaceous stem inoculation assay on grapevine. All isolates were pathogenic, inducing progressive vascular lesions and foliar symptoms typical of *Fusarium* dieback. At 14 days post inoculation (dpi), isolate R1 was the most aggressive, with a 25.67 ± 10.52 mm lesion length and 52.1% height reduction compared to the control. This growth reduction was directly associated with the expansion of the necrotic lesion, resulting in stem dieback and structural collapse. The isolate R2, A46 and D11 exhibited intermediate levels of aggressiveness. At this stage, the foliar disease severity was significantly higher in all infected plants. By 21 days, the whole plant disease severity index reached 100%, confirming a lethal systemic infection. The pathogen was successfully re-isolated from symptomatic tissues, fulfilling Koch's postulates. To our knowledge, this study represents the first report of *F. solani* and *F. falciforme* associated with grapevine in Morocco. Overall, this finding highlights the diversity and pathogenicity of *Fusarium* species associated with grapevine dieback in Morocco and demonstrates the effectiveness of herbaceous stem inoculation for pathogenicity assessment.

Keywords: *Vitis vinifera*, pathogenicity test, herbaceous stem inoculation, vascular necrosis, disease severity index, grapevine wood diseases.

INTRODUCTION

The grapevine (*Vitis vinifera*) is an emblematic crop that plays an essential role in the global economy, particularly thanks to the wine industry, which generates billions of dollars each year (Khadatkar et al., 2025). According to the international organization of vine and wine (OIV), viticulture holds a prominent place in many countries, including Morocco, contributing both to the local economy and regional culture (OIV, 2022). In addition to its economic value, the grapevine is also a symbol of cultural heritage, often associated with traditions, rituals, and ways of life (Bustamante et al., 2024).

However, grapevines are highly susceptible to various fungal diseases that threaten their health and productivity. Among these diseases, downy mildew (*Plasmopara viticola*), gray mold (*Botrytis cinerea*), and powdery mildew (*Erysiphe*

necator) are the most studied in the literature (Georgiev et al., 2014). Wood diseases such as Black Dead Arm and Eutypiose, are among the most destructive diseases affecting vineyard sustainability. The agents responsible for these diseases cause the death of the grapevine over a more or less long term (Bertsch et al., 2013; Bruez et al., 2013; Larignon et al., 2009). Grapevine dieback is also a common and significant disease, caused by a multitude of fungal pathogens including *Fusarium* wilt, which primarily manifests as a general weakening of the plants, yellowing and wilting of the leaves followed by dieback (Tayou et al., 2026). It is also manifested by root rot, cankers on the wood (brown/black), and discoloration of the internal vascular tissues, hindering the circulation of the sap (Akgül et al., 2024).

The first studies on grapevine *Fusarium* wilt, caused by *Fusarium oxysporum* f.sp. *herbemontis*, were first reported in 1940 in Brazil in the states of Rio Grande do Sul, Santa Catarina, and São Paulo (de Andrade et al., 1995; Gallotti, 1991; Garrido et al., 2004). Despite the detection of grapevine *Fusarium* wilt in Brazil at that time, research on this disease is still rare worldwide.

In Morocco, *Fusarium* wilt has historically been overlooked, as it is not typically categorized as a major or classic disease of the grapevine (Tayou et al., 2026), unlike its devastating impact other crops such as date palm (Bayoud), cereals (Qostal et al., 2019a; Qostal et al., 2019b; Qostal et al., 2025), legumes (El Hazzat et al., 2023; Elbouazaoui et al., 2022), nursery plants of *Thuja* (EL Haddadi et al., 2019; El Haddadi et al., 2021), or argan trees (El Rhoch et al., 2025).

A recent study conducted in Moroccan vineyards identified a diverse fungal consortium associated with grapevine trunk diseases, compressing ten *Fusarium* species (Kenfaoui et al., 2024). Despite these advances, the diversity of *Fusarium* species associated with grapevine dieback in Morocco, as well as their potential role is symptom development, still requires further investigation.

The present study aims to contribute to a better understanding of this fungal complexes. *Fusarium* isolates were obtained from the roots of grapevines exhibiting wilting symptoms and then identified based on morphological criteria and molecular analyses. A phylogenetic analysis was also conducted to clarify their taxonomic relationship, while pathogenicity test was performed on grapevines via simple inoculation of herbaceous stems to assess their pathogenic potential. This method involves inoculating the herbaceous stems of greenhouse-grown plants with explants derived from cultures of the studied *Fusarium* species, enabling rapid symptom development.

MATERIALS AND METHODS

Isolation of plant pathogenic fungi

During the summer of 2023, in the Marrakech region (Morocco), samples of grapevine plants, traditional local variety (Doukkali, large-grain table grapes), showing foliar symptoms (chlorosis and necrosis), stunting, and especially decline, were collected from five randomly selected fields, totalling 50 plants (10 per vineyard). In the laboratory, the roots showing necrosis and the base of the stems of the diseased plants were cleaned with running water to remove soil particles. A total of 500 fragments of infected vascular tissues (roots and stem bases) were collected and disinfected for 2 minutes in a 0.5% sodium hypochlorite solution, then rinsed several times with sterile distilled water before being dried on sterile filter paper (Boukharta et al., 2012; Meddah et al., 2011; Mouria et al., 2014). Next, the disinfected fragments were transferred to PSA (Potato Saccharose Agar) medium composed of 200 g potato, 15 g agar-agar, 20 g of sucrose, and 1000 mL of distilled water, and supplemented with 100 mg/L chloramphenicol. The cultures were then incubated in the dark at 28 °C for 6 days.

The macroscopic and microscopic observation of the developed colonies allowed for the identification of fungal species and the calculation of their isolation percentages (Pi) (Ouazzani Touhami et al., 2000) using Equation (1)

$$Pi = \frac{NsX}{NT} \times 100 \quad (1)$$

where: *NsX* – number of colonies assigned to an individual species; *NT* – overall number of colonies obtained from all isolated fungal species.

Several fungal species have been obtained. The isolates were purified by single spore culture. A 5 mm agar plug bearing sporulating mycelium was excised from a PSA culture, transferred to 10 mL of sterile distilled water, and vortexed to obtained a spore suspension. 1 mL of these suspension was spread onto water agar plates (15 g

Agare-agare and 1000 mL distilled water), and incubated at 28 °C. The plates were examined every 24 h for spore germination. Germinated spores were individually transferred to separate PSA plates and incubated at the same temperature until colony development, resulting in monosporic cultures. Based on their colony morphology, growth characteristics, sporulation, and microscopic features, five representative isolates of *Fusarium* were selected for morphological and molecular identification.

Morphological description of *Fusarium* isolates

The five isolates, R1, R2, R3, A46, and D11, all obtained from grapevine roots, were first subcultured from isolated conidia and stored on pieces of filter paper at -20 °C (El Hazzat et al., 2022). These *Fusarium* isolates thus collected were replated in Petri dishes containing sterile PSA medium. The examination was conducted based on the development of the cultures on this medium. The macroscopic observations focused on the appearance of the cultures, the density of the *mycelium*, its color, its growth, and the production of spores. The microscopic examination, conducted using an optical microscope at magnifications of ×40, ×100, and ×400, allowed for the observation of the nature of the *mycelium*, conidiophore structure, conidial morphology (shape and size), as well as the presence of survival structures, particularly chlamydospores. For each isolate, approximately 50 conidia were measured from 3 independent plates. The mounting medium used for microscopic observations is cotton blue. Morphological identification was carried out based on the data from (Booth, 1972; Champion, 1997; Messiaen and Cassini, 1968; Nelson et al., 1983; Tivoli, 1988).

DNA-based identification of fungal isolate

Fungal genomic DNA was isolated from 15 mg of fresh mycelial tissue employing the MagPureX magnetic bead-based extraction kit, strictly following the protocols provided by the manufacturer. the Concentration and quality of the recovered DNA were subsequently the manufacturer. The concentration and quality of the recovered DNA were subsequently evaluated by Nano Implant N50 spectrophotometer (Implen GmbH Schatzbogen, München Waltham, Germany) by monitoring the 260/280 and 260/230 absorbance ratio.

For the molecular identification, PCR amplification targeted the ITS rDNA region using the ITS rDNA region using the Multi-Gene OptiMax Thermal Cycler. The amplification was set up in a final volume of 20 µL, consisting of 11 µL milli-Q water, 2 µL 10X PCR buffer (supplemented with $MgCl_2$ and dNTPs), 0.2 µL x-VITA-Taq DNA polymerase, 1 µL pf template gDNA, and specific primers: ITS1 (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC), in accordance with White et al. (1990). The template profile comprised an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, concluded by a final elongation at 72 °C for 7 min 25 s. post-amplification, the resulting PCR products underwent purification via ExoSAP-IT kit. Bi directional sequencing of the ITS rDNA gene was subsequently executed using the ABI PRISM BigDye Terminator kit on an automated ABI PRISM 3130XL Genetic Analyzer.

Sequence alignment and phylogenetic reconstruction

The ITS rDNA sequences obtained from the isolates were deposited in the GenBank database under the accession numbers PV752255.1 (isolate D11), PV752252.1 (isolate A46), PV752253.1 (isolate R2), PV752254.1 (isolate R1), and PV752251.1 (isolate R3). Sequence similarity analyses were performed using the BLASTn algorithm and UNITE Database (Abarenkov et al., 2024) by comparison with reference sequences available in the GenBank database. Multiple sequence alignments were determined with ClustalW algorithm implemented in MEGA11 (Tamura et al., 2021). Phylogenetic relationships were reconstructed using the Neighbor-Joining (NJ) method (Saitou and Nei, 1987), and the robustness of the inferred clades was assessed using 1000 bootstrap replicates.

Pathogenicity assessment

The pathogenicity of the five *Fusarium* isolates, newly obtained from grapevine, traditional local variety (Doukkali), was evaluated under greenhouse conditions. Drawing inspiration from studies that used detached woody shoots from grapevines or citrus, inoculated with explants

of the pathogen, to respectively test the pathogenicity of *Fusarium* (Kenfaoui et al., 2024) or other soil-borne pathogens (Boudoudou et al. 2023; Boudoudou et al. 2024), another simple technique was adopted in this study. This technique was used to shorten the development of symptoms in the inoculated plants. It involves inoculating the herbaceous parts of young grapevine plants growing in plastic bags containing a culture substrate made of Mamora sand and peat (V/V). The herbaceous stems of the 8-month-old plants were inoculated with fungal explants derived from fungal cultures of the studied *Fusarium* species. In this regard, a longitudinal wound of 8 mm in length and 1 mm in width was created in the herbaceous part of the stem (Figure 1). Nine grapevine plants were used for each treatment, including five *Fusarium* isolate and control. Each grapevine plants were considered is one experiments unit, resulting in nine biological replicates per treatment. A square *mycelial* plug from a 10-day-old fungal culture growing on PSA medium is placed on the wounded area of herbaceous stem and then secured with adhesive tape. For the control plants, a piece of sterile PSA plug was applied to the wound in the same manner. Afterward, the plants were brought back to the greenhouse and watering was done every day. The inoculated lesions were individually moistened twice a day. The development of symptoms in the inoculated plants was monitored over time. Lesion length and width were measured using a ruler at 14 days post-inoculation (dpi) and used for statistical analysis.

The Figure 1 illustrates the artificial inoculation process of *Fusarium* isolates into the herbaceous stem, followed by early symptom qualification at 14 dpi including lesion size, foliar disease severity index, and plant height, The final assessment of whole severity plant at 21 dpi (created by Biorender.com).

Assessment of the foliar disease severity index

The foliar disease severity index (FDSI), was used to assess disease intensity at 14 days post inoculation, according to a predefined rating scale reported by Douira et al., (1995) and Mouria et al., (2007) (Table 1).

The notes related to the number of leaves constitute the foliar disease severity index, which was calculated using Equation 2:

$$FDSI = \frac{\sum(i \times Xi)}{6 \times Ntf} \quad (2)$$

where: *FDSI* – foliar disease severity index;
I – foliar damage score (from 0 to 5);
Xi – number of leaves assigned to score *i*;
NtF – total number of evaluated leaves.

Plant height measurement

Plant height was measured from the stem base to the apical meristem using a graduated ruler at 14 dpi. In plants exhibiting structural collapse, height was recorded as the vertical distance from the stem base to the lowest structurally intact point of the stem. These measurements were performed on all replicates across all treatments to quantify the impact of the disease on overall plant growth.

Whole-plant disease severity assessment

Disease severity was evaluated at 21 days post inoculation (dpi) using a qualitative scoring system ranging from 0 to 4 (Table 2). The severity scale was established based on the symptom progression observed on the entire plant.

Disease severity was expressed as mean severity score ± standard deviation. For standardize comparison among treatment, severity score was

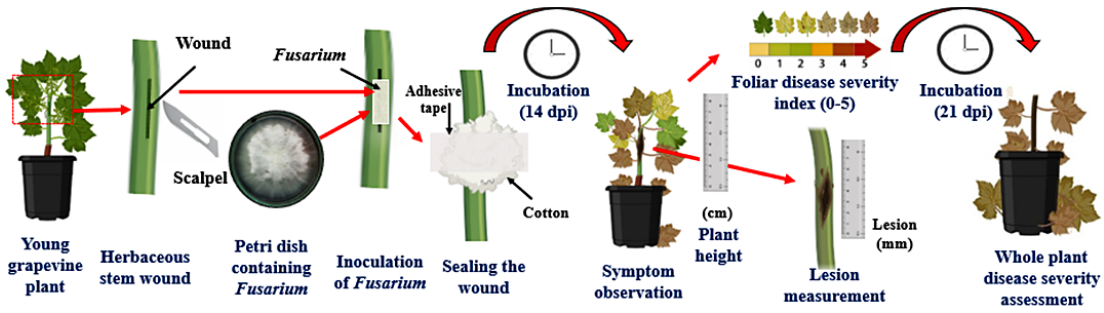

Figure 1. Schematic workflow of the *Fusarium* inoculation protocol on herbaceous grapevine stems

Table 1. Scale for evaluating foliar symptoms on grapevine leaves inoculated with the tested *Fusarium* isolates

Score	Appearance of the leaves
0	Healthy-looking leaf
1	Yellowing of the leaf, less than 50% of the leaf surface
2	More than 50% of the leaf surface is yellowed and the onset of necrosis
3	More than 50% of the leaf surface shows necrosis
4	Leaf desiccation
5	Leaf drop

additionally converted into a disease severity index (WDSI %) using Equation 3:

$$WDSI\ (\%) = \frac{\sum(n \times d)}{N \times D} \times 100 \quad (3)$$

where: n – number of plants assigned of each score; d – represent the disease score; N – total number of plants; D – maximum disease score (4).

Re-isolation

Following disease severity assessment, stem portions adjacent to symptomatic area that remained attached to the inoculated plantlets were excised using a sterile scalpel and cut into small fragments (5–10 mm in length), these fragments were then re-isolating following the procedure previously describe in initial isolation. Briefly, surface sterilization with 0.5% hypochlorite solution, rinsed with sterile water, dried on sterile filter paper, and placed on PSA medium. For each *Fusarium* isolate, 16 stem fragments collected from different inoculated were plated onto four petri dishes containing PSA medium, with four fragments per dish. The plates were incubated for 7 days at 28 °C. Emerging fungal colonies were purified and compared with corresponding inoculated isolates based on their morphological characteristics. The re-isolation rate was calculated as the percentage of inoculated plants from which the corresponding fungus was successfully recovered.

Table 2. Whole-plant disease severity scale used to evaluate symptom progression in inoculated grapevine

Score	Symptom
0	Healthy plant
1	Slight foliar chlorosis or wilting of lower leaves
2	Moderate stem necrosis and leaf wilting
3	Severe defoliation, stem necrosis and stem dieback
4	Complete plant collapse, terminal dieback or plant death

The re-isolation percentage (PR) was determined using Equation 4:

$$PR\ \% = \frac{Ns}{Nt} \times 100 \quad (4)$$

where: Ns – number of stem fragments yielding the target fungal isolate; Nt – total number of stem fragments cultured.

Statistical methods

For the pathogenicity assessment, a completely randomized design was used with six treatments (five isolates and one control). Nine individual plants were evaluated per treatment (n = 9 biological replicates, for a total of plants). Data distribution and homogeneity of variance were systematically checked prior to statistical testing. For non-parametric data resulting for pathogenicity assessment (such as lesion length, lesion width, foliar disease severity index, plant height, and whole-plant disease severity), a Kruskal-Wallis data was conducted, followed by Dunn's pairwise post-hoc test using the dunn. test package in R software (4.4.0), with p-values significant adjustment using the Holm-Bonferroni methods. Conversely, parametric data, such as re-isolation percentages, one-way Analysis of Variance (ANOVA) was performed followed by Tukey test (HSD) test for mean comparison $p < 0.05$ using the agricolae package in R.

RESULTS

Morphological characterization of the isolates

The analysis of 500 fragement of vascular tissus (root and stem bases) allowed the isolation of several fungi, including 50 isolates belonging to the genus *Fusarium*, which was the most represented genus (10%). Among these isolates, five (R1, R2, R3, A46, D11) were selected for in-depth analyzes based on their sporulation capacity and their macro and microscopic characteristics.

The colonies of the R1 and R3 *Fusarium* isolates exhibited a cream-white pigmentation

with a fluffy aerial texture, while microscopic observation revealed the presence of fusiform, slightly curved macroconidia, with 2 – 3 septa, measuring 20 – 80 µm × 10 – 20 µm for R1 and 30 – 85 µm × 10 – 20 µm for R3, septate microconidia measuring 8 – 11 × 2.2 – 2.8 µm for R1 and 8 – 11 × 2.2 – 2.8 µm for R3, and abundant, globular, solitary or paired chlamydospores. Isolates R2 and A46 developed white colonies textures, with R2 exehibiting a cottony texture and A46 a sub-cottony texture. Both isolates exibited falcate (crescent-shaped) macroconidia, with thin walls, 1 to 3 septa, and variable dimensions. Isolate R2 produced macroconidia of 50 – 70 µm × 10 – 11 µm, microconidia of 30 – 50 µm × 10 – 11 µm, and abundant chlamydospores. Isolate A46 developed smaller macroconidia of approximately 40 – 60 µm × 10 – 20 µm and microconidia of 20 – 40 µm × 0.9 – 11 µm. Finally, the D11 isolate exhibited cream-white to brown colonies, dense aerial *mycelium*, with strongly curved macroconidia, 5 – 7 septa, measuring 35 – 50 µm × 3.8 – 5.2 µm, scarce microconidia, 9 – 12 × 2.5 – 3.0 µm, and abundant chlamydospores, often arranged in chains (Table 3; Figure 2).

These morphological differences suggest heterogeneity within the genus *Fusarium*. Specific identification was established by sequencing.

Molecular identification

BLAST analysis of the ITS rDNA sequences, combined with taxonomic assignment using the UNITE database, indicated that all isolates belong to the genus *Fusarium*. Isolate D11 (GenBank accession number PV752255) exhibited the highest sequence similarity to *Fusarium chlamydosporum*, whereas isolates A46 (PV752252) and R2 (PV752253) showed closest affinity to *Fusarium falciforme*. Furthermore, isolates R1 (PV752254) and R3 (PV752251) displayed highest similarity to *Fusarium solani*. Phylogenetic analysis based on ITS rDNA sequences placed the five isolates R1 (PV752254.1), D11 (PV752255.1), R2 (PV752253.1), R3 (PV752251.1), and A46 (PV752252.1) within the genus *Fusarium*, a taxonomically complex and genetically diverse genus comprising several closely related species belonging to the *Fusarium solani* species complex (FSSC), including *F. solani*, *F. falciforme*, and *F. keratoplasticum*. Isolates A46 and R2 clustered with reference sequences of *Fusarium falciforme* providing support for their assignment to this species. Similarly, isolates R1 and R3 grouped with well-characterized *Fusarium solani* reference strains, thereby corroborating their taxonomic identification. In contrast, isolate D11 occupied a distinct phylogenetic position within the same species complex and showed closest affinity to *Fusarium chlamydosporum*, indicating a divergent lineage within the complex (Figure 3).

Evaluation of pathogenicity

Lesions developed and its impacts

The lesion that appeared on the herbaceous grapevine stems depends on the tested. The inoculation, lesions developed on all the inoculated stems. The lesions initially manifested as brown spot canker-like spots. These arears gradually extended in depth, causing necrosis of the vascular tissues. Both the length and width of the lesions were measured at 14 days of post inoculation (dpi) to assess the pathogenicity of the isolates.

Internal lesion length revealed highly significant difference among the strains ($p < 0.001$). Isolate R1 (*F. solani*) produced the most extensive lesion 25.67 ± 10.52 mm, which represents

Table 3. Macroscopic and microscopic characteristics of different *Fusarium* isolates differentiated at the specific level

Code	Colony color	Texture	Shape of macroconidia	Size of macroconidia	Size of microconidia	Presence of chlamydospores
R1	Cream white	Cottony	Fusiform	20 – 80 µm × 10 – 20 µm	8 – 11 µm × 2.2 – 2.8 µm	Present
R3	Cream white	Cottony	Fusiform	30 – 85 µm × 10 – 20 µm	8 – 11 µm × 2.2 – 2.8 µm	Present
R2	White	Cottony	Falciform	50 – 70 µm × 10 – 11 µm	30 – 50 µm × 10 – 11 µm	Present
A46	White	Sub cottony	Falciform	40 – 60 µm × 10 – 20 µm	20 – 40 µm × 0.9 – 11 µm	Present
D11	Creamy white to brown	Cottony	Strongly Curved	35 – 50 µm × 3.8 – 5.2 µm	9 – 12 µm × 2.5 – 3.0 µm	Present

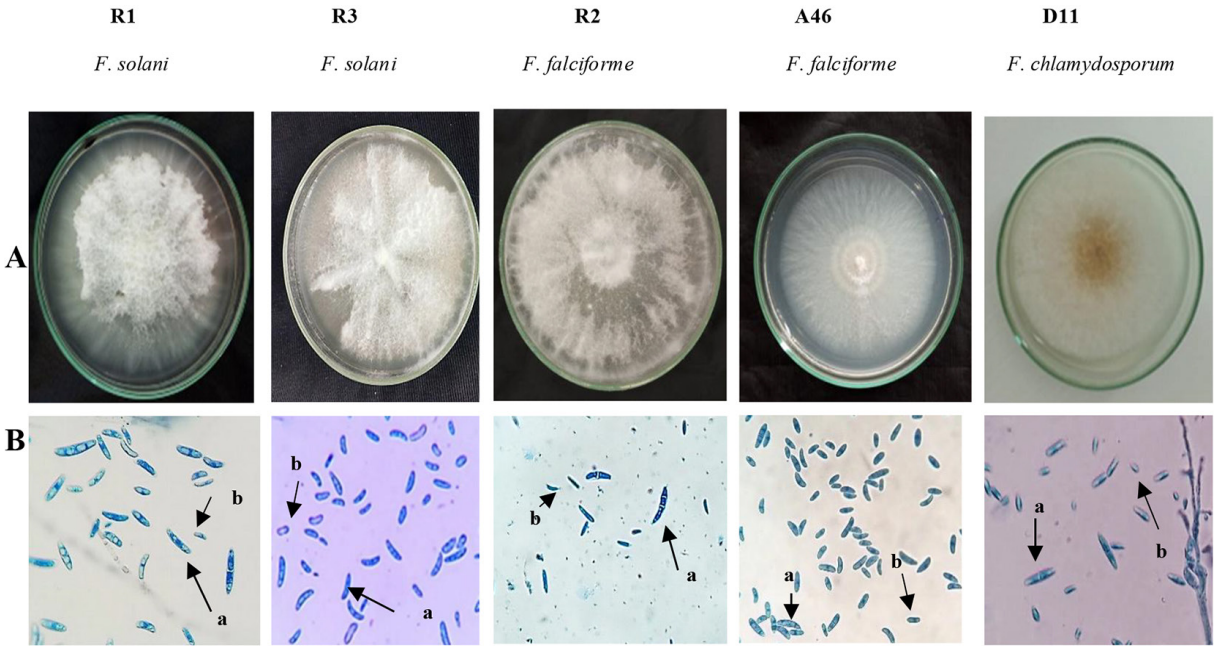

Figure 2. Morphological identification of different *Fusarium* isolates: (A) macroscopic observation of fungal colonies grown on PSA medium, (B) microscopic observation of *Fusarium*, (a) macroconidia, (b) microconidia

a significant increase compared to the control. The isolate R2, A46, and D11 showed intermediate values (from 18.33 ± 3.8 to 20 ± 6.20 mm), while R3 present the limited progression among the pathogens (16.89 ± 3.55 mm).

This longitudinal progression was closely mirrored by traversal expansion of the lesions (p = 0.014). Isolate R1 again showed the highest capacity of lateral incision, with a mean width of 2.78 ± 1.09 mm being significant to the control (1.33 ± 0.50 mm), followed by R2 (*F. falciforme*) with 2.56 ± 0.73 mm. where the other isolates did not differ from it. No significant differences were observed between the isolates (Figure 4 and 5A-B).

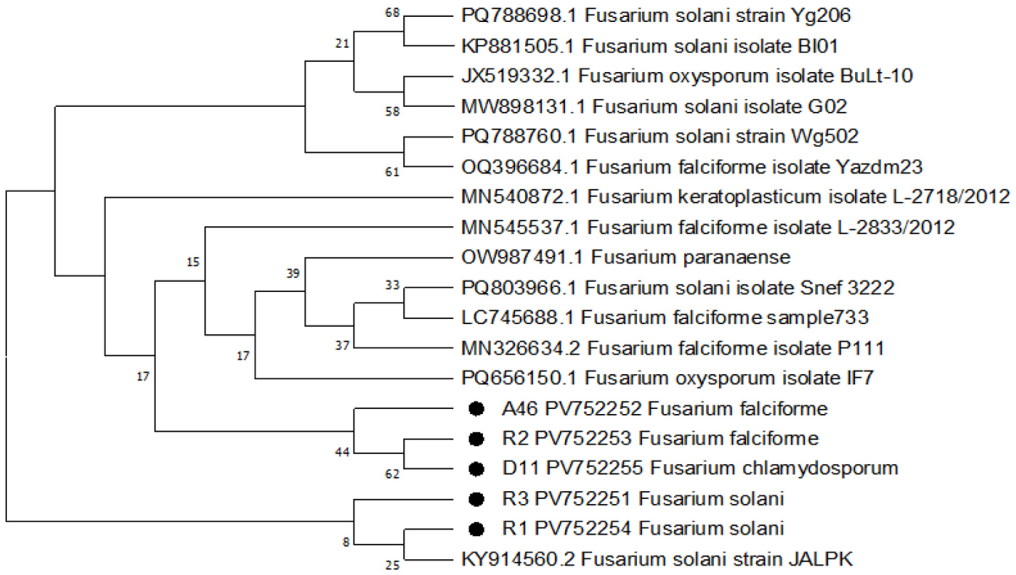

Figure 3. Phylogenetic tree showing the relationships between isolates of *Fusarium solani*, *Fusarium falciforme*, and *Fusarium chlamydosporum* (R1_PV752254, D11_PV752255, R2_PV752253, R3_PV752251, and A46_PV752252) based on ITS region sequences. The phylogenetic tree was constructed using the Neighbor-Joining method with the Kimura 2-parameter model and 1000 bootstrap replicates

Leaf symptoms and foliar disease severity index

In addition to the lesions observed on the herbaceous stems, foliar symptoms were observed on the inoculated grapevines. The leaves exhibited progressive chlorosis, starting at the margins and extending toward the central blade, followed by the appearance of necrosis and wilting, and ending with the drying or dropping of the leaves. The evaluation of symptom severity conducted using the foliar disease severity index (FDSI) was significantly affected by the inoculations. The control plants exhibited the lowest values (0.084 ± 0.07), while all the studied isolates induced a significant increase the FDSI (0.55 ± 0.19 to 0.76 ± 0.10; $p < 0.001$), with no significant difference among them (Figure 4 and 5C).

Figure 4. Morphological and symptomatic progression of *Fusarium* infection: (A) lesion on the herbaceous stem at 14 dpi, showing characteristic vascular browning; (B) specific leaf symptoms, including marginal chlorosis and necrotic spots; (C) general appearance of inoculated plants versus the control at 14 dpi, displaying initial wilting and growth reduction; (D) final disease outcome at 21 dpi illustrating terminal dieback and total desiccation of all inoculated plants. The chorological sequence from (C) to (D) highlight the rapid systemic dieback of the host following infection

Plant height and stem stability

Simultaneously, all inoculated plants showed a significantly lower plant height compared to the controls ($p < 0.001$). This reduction in growth was directly linked to progressive lesion expansion and the initiation of stem collapse induced by the fungal infection. Among the tested strains, isolate R1 induced the most marked reduction in plant height, decreasing the height to 21.33 ± 6.42 cm, which corresponds to a 52.1% reduction compared to the control group. This was followed by isolate A46 with a 45.0% reduction. The isolates R3, A46, and D11 showed intermediate effects on plant height, with no significant differences between them ($p > 0.05$) (Figure 5D). The progression of symptoms, particularly stem collapse, was

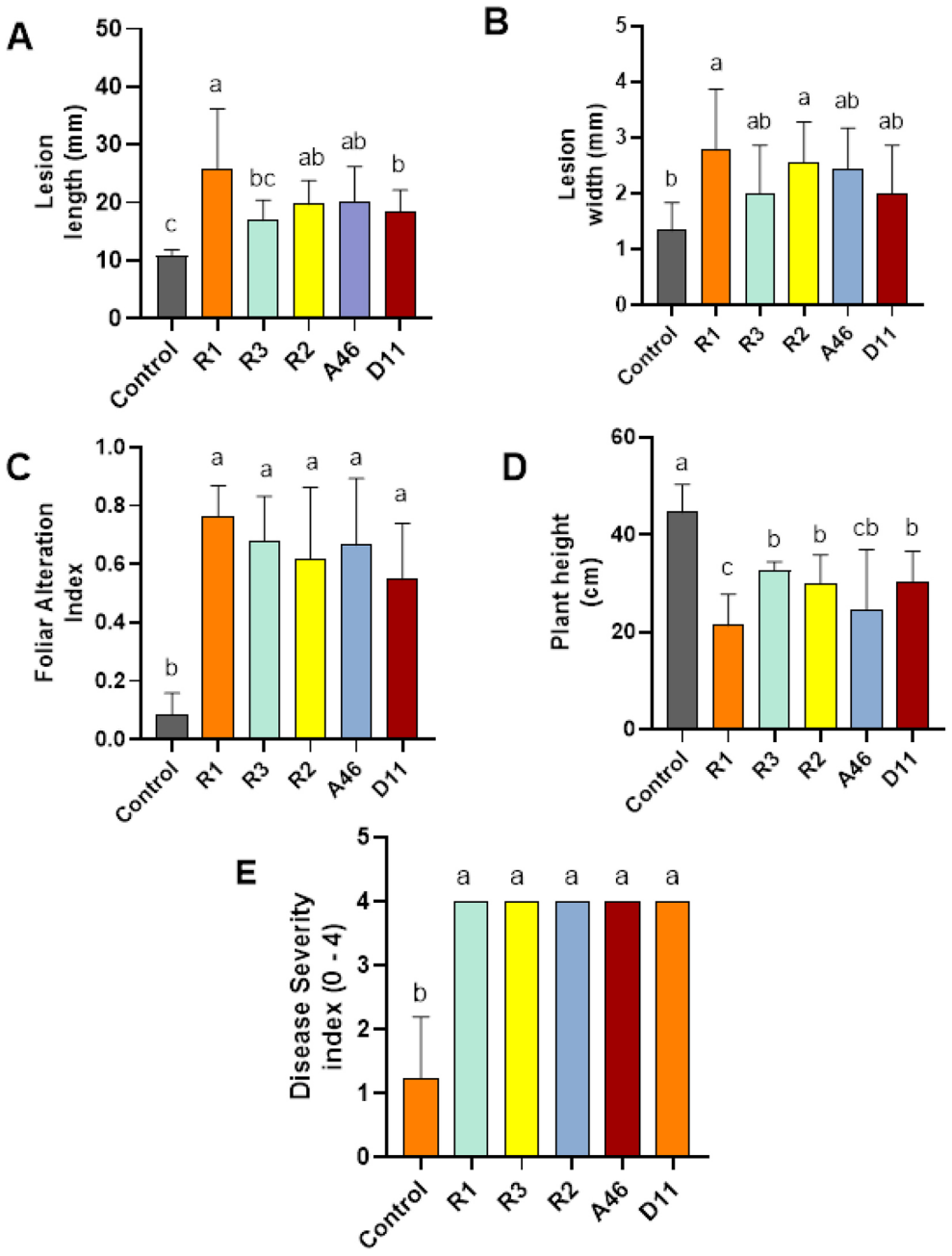

Figure 5. Pathogenicity and disease developement of *Fusarium* isolates tested on grapevine: (A) average lesion length on the herbaceous stem in mm, (B) average of lesion width in mm. (C) foliar disease severity index, (D) plant height in cm, (E) whole plant disease severity index based on a 0 – 4 qualitative scale. Data represents means ± SD ($n = 9$). Different letters indicate significant differences according to Kruskal-Wallis test following by Dunn's multiple comparison ($p < 0.05$)

ultimately reflected in the overall disease severity assessed at a later stage.

Whole-plant disease severity index (WDSI) and terminal symptom

Symptom progression was definitively captured by the disease severity assessment at 21 dpi. Control plants maintained a low baseline severity score of 1.22 ± 0.97, corresponding to a WDSI of 30.6%. In stark contrast, all *Fusarium*-inoculated plants reached the maximum score of 4.0 ± 0.0 (100% WDSI; $p = 0.003$), characterized by complete plant collapse, terminal dieback and severe tissue necrosis (Figure 4D; 5E).

Re-isolation

The re-isolation of infected grapevine stems showed variable re-isolation rates depending on the tested isolates. Isolate R1 was re-isolated in 87.5% of the analyzed fragments. Isolate R3 showed a re-isolation rate of 68.75%. The isolates R2, A46, and D11 showed average re-isolation rates of 87.5%, 81.25%, and 56.25%, respectively (Figure 6). This re-isolation confirmed the penetration and establishment of these pathogens in the plant tissues and the induction of the various observed symptoms, thus meeting the criteria of Koch's postulates.

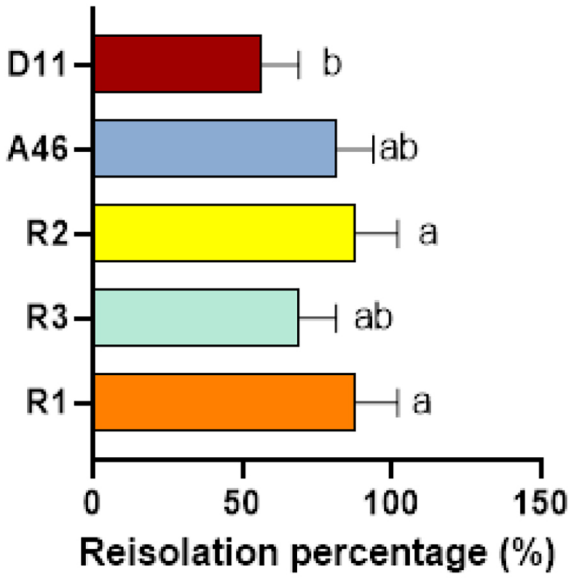

Figure 6. Percentage of re-isolation of *Fusarium* isolates from stem tissues. Mean followed by the same letter do not differ significantly according to the Tukey's HSD test ($p < 0.05$)

DISCUSSION

Inoculation test on herbaceous stems of *Vitis vinifera* showed that all tested isolates are capable of inducing cankers on the stems, and cause foliar symptoms, characteristic of *Fusarium* wilt (de Sain and Rep, 2015; Qostal et al., 2025; Ramchandra and Bhatt, 2012). Morphological and molecular analyses allowed the isolates to be assigned to the *Fusarium solani* species complex (FSSC), including *Fusarium solani* (R1 and R3), *Fusarium falciforme* (R2 and A46), and *Fusarium Chlamydosporum* (D11). Although all were pathogenic. Significant variability in pathogenicity was observed among *Fusarium* isolate.

Isolate R1 (*F. solani*) showing the highest virulence, associated with more extensive longitudinal lesions (25.67 ± 10.52 mm). This aggressive colonisation of vascular tissues resulted in severe inhibition of vegetative development. In comparison, isolate R2, A46, D11 exhibited intermediate pathogenicity, while R3 (16.89 ± 3.55 mm) proved to be least virulence than R1.

The foliar disease severity index, evaluated at 14 dpi was significantly higher in all isolates compared to the control, reflects an overall physiological effect of the infection, which is likely to affect photosynthesis and, consequently, plant vigor (Adnani et al., 2024; Huang and Luo, 2021; Mendonça De Souza et al., 2023; Nogués et al., 2002). Our results highlight a direct link between the extension of necrotic lesions and impaired host function. In particular, the extension of cankers causing a loss of structural integrity in the stem at the inoculation point, leading to the onset of collapse, which explains the drastic reduction in plant height 52.3% for R1 compared to the control.

The symptomatic progression, ranging from leaf chlorosis to stem dieback, suggests a disruption in the plant's water balance (Adu-Acheampong et al., 2012; Guo et al., 2020; Li et al., 2025). Although all isolates reached lethal pathogenicity at 21 dpi (100% WDSI), a marked variability in virulence kinetics was captured at 14 dpi. This distinct chronological difference suggests that isolate R1 exhibited superior aggressiveness, likely associated to faster vascular colonization on increased toxin production, thereby causing an earlier collapse of vital physiological functions compared with the other strains (Bernardos et al.,

2025; Guo et al., 2020; Li et al., 2025). On the other hand, controls plants exhibited a baseline severe score of 1.22 ± 0.97 (30.6% WDSI) at 21 dpi. This mild symptomatic response non inoculated control is primarily interpreted as a physiological reaction to mechanical stress caused by stem wounding during inoculation (Fotios et al., 2021; Gramaje et al., 2018). Young herbaceous grapevine tissues are particularly fragile and may develop localized chlorosis or slight wilting following physical injury. Nevertheless, the contrast remains highly significant ($p = 0.003$); while control plants tolerated this localized stress without further disease progression, all *Fusarium* isolates induced systemic, irreversible dieback, confirming that plant death was strictly driven by pathogen virulence (Mirghasempour et al., 2022).

Furthermore, the direct responsibility of the isolates in this process was confirmed by the systemic re-isolation of the pathogens from the stem tissues, thus validating Koch's postulates for all tested species (Beccari et al., 2022; Ekwomadu and Mwanza, 2023). The overall system progression, resulting in the complete dieback of the plant, is the consequence of a major physiological failure that can be explained by the isolate variable ability to produce cell wall-degrading enzymes such as pectinases and cellulases, facilitating tissue penetration and progression, as well as by the secretion of phytotoxins, several of which have been identified in *F. oxysporum*, *F. graminearum*, and *F. solani*. These compounds allow for the colonization of the xylem, leading to vessel blockage (vascular occlusion) and disruption of water transport, which results in leaf wilting, necrosis, and general plant weakness (Castillo et al., 2025; Ekwomadu and Mwanza, 2023; Gutiérrez-Sánchez et al., 2023; Jackson et al., 2024; López-Díaz et al., 2018).

Fusarium on grapevines fits into a pattern already described in several wine-growing regions. In Turkey, Akgül et al. showed a high diversity of *Fusarium* in nurseries, particularly *F. solani, F. annulatum, F. brachygibbosum,* and *F. nirenbergiae*, with some species causing wood necrosis, basal and root rot, as well as a marked reduction in root biomass, while others behaved more like opportunistic parasites (Akgül et al., 2024). In California, Bustamante et al. (2022) also highlighted a complex of ten *Fusarium* species associated with the decline of young vines, including the *F. solani* complex, capable of producing significant woody lesions on rootstocks when conditions are favorable for infection (Úrbez-Torres et al., 2023). On grapevines in Egypt, *F. solani* has been identified among the main agents of root rot and decline of young grapevines, with highly aggressive isolates on roots and collars (Ziedan et al., 2020; Hemida et al., 2017; Hemida et al., 2024; Shehata et al., 2019). Other species, such as *F. commune*, *F. acutatum, or F. proliferatum*, have recently been reported as emerging agents of root rot, vascular wilt, or cluster burn in various countries (China, India, Russia), confirming the growing role of the *Fusarium* genus in grapevine diseases on a global scale (Bustamante et al., 2022; Holkar et al., 2025; Volynchuk, 2023; Yurchenko et al., 2020; Zhang et al., 2023).

Previous studies in Morocco reported ten *Fusarium* species associated with grapevine trunk diseases, including *F. clavum, F brachygibbosum, F. chlamydosporum, F. equiseti, F. foetens, F. culmorum, F. oxysporum, F. proliferatum, F. verticillioides*, and *F. thapsinum* (Kenfaoui et al., 2024). In the present study, *F. chlamydosporum* was also identified, whereas the other two *Fusarium* species recovered in our study were not previously reported in Moroccan vineyards. To the best of our knowledge, this study represents the first report of *Fusarium solani* and *F. falciforme* associated with grapevine dieback in Morocco. Moreover, pathogenicity was assessed differently between the two studies. The previous study evaluated detached grapevine twigs mainly based on lesion length (Kenfaoui et al., 2024), whereas the present study used herbaceous stems that remained attached to the plant, allowing pathogenic effects to be assessed to both the local and whole-plant levels through lesion development, plant height, foliar disease, and overall whole plant disease severity.

The inoculation test on the herbaceous stem is a simple and quick method (14 days) to assess the pathogenic potential of a species. On the other hand, root soaking inoculation or inoculation via the culture medium requires at least 3 to 5 months for symptoms to appear (Ziedan et al., 2011). The use of this method allows for preliminary assessment of the pathogenic potential and facilitates preventives control measures.

However, some limitations of the present study should be acknowledged. While the pathogenicity of these *Fusarium* isolates was successfully demonstrated under controlled condition, field environments introduce complex biotic and abiotic interaction that may influence real-world disease dynamics. Furthermore, although

the identified species provide critical insights, a broader screening involving a larger number of isolates from diverse agro-ecological zones in Morocco is required to fully map the genetic diversity and widespread prevalence of this complex. In addition, the use for ITS regions alone may provide limited resolution for distinguished closed related species.

Nevertheless, the detection of highly aggressive isolates particularly *F. solani*, suggests a potential risk for young grapevine plants, especially under Mediterranean conditions. These pathogens may contribute to grapevine dieback and reduce plant vigour, highlighting the need for improved phytosanitary monitoring and the development of integrated disease management strategies.

CONCLUSIONS

In conclusion, this study provides critical insights into the diversity and pathogenic potential of *Fusarium* species associated with grapevine dieback in Morocco. Through a robust polyphasic approach, *Fusarium solani*, *F. falciforme,* and *F. chlamydosporum* were successfully isolated from symptomatic root tissues and accurately characterized. Compared with the ten *Fusarium* species previously reported to be associated with grapevine trunk diseases in Morocco, *F. chlamydosporum* was the only species in common, whereas for the other two species identified in the present study were distinct. This species composition provides new evidence for the occurrence of *F. solani* and *F. falciforme* in Morocco and, to the best of our knowledge, represents the first report of their involvement in this context, further documenting their ability to induce typical vascular necrosis and severe stem desiccation, thereby supporting their role as active trunk pathogens. Our findings demonstrate that all evaluated isolates are highly pathogenic, culminating in a 100% whole-plant disease severity index and complete plant dieback by 21 dpi. Notably, the distinct kinetics captured at 14 dpi highlight that isolate virulence is a key determinant of the speed and severity of stem dieback. Ultimately, these results fill a critical knowledge gap and underscore the urgent need to integrate these *Fusarium* species into future epidemiological surveillance and integrated management strategies to protect the regional viticulture industry.

Acknowledgements

The authors sincerely acknowledge the financial support provide by the Partnership for Research and Innovation in the Mediterranean Area, the Ministry of Higher Education, Research and Innovation (Morocco), the Ministry of University and Research (Italy), the Scientific and Technological Research Council of Turkiye, and the European Union provided in the development of the VineProtect project. The author also thanks the Laboratory of Botany, Biotechnologies, and Plant Protection in Ibn Tofail University, for their technical assistance and support during this study.

This work was carried out as part of the VineProtect project. N° 10.54499/PRIMA/0011/2021.

REFERENCES

1. Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., May, T. W., Frøslev, T. G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T. E., Ishigami, Y., … Kõljalg, U. (2024). The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. *Nucleic Acids Research*, *52*(D1), D791–D797. https://doi.org/10.1093/nar/gkad1039
2. Adnani, M., El Hazzat, N., Msairi, S., El Alaoui, M. A., Mouden, N., Selmaoui, K., Benkirane, R., Ouazzani Touhami, A., Douira, A. (2024). Exploring the efficacy of a *Trichoderma asperellum*-based seed treatment for controlling *Fusarium equiseti* in chickpea. *Egyptian Journal of Biological Pest Control*, *34*(1), 7. https://doi.org/10.1186/s41938-024-00771-x
3. Adu-Acheampong, R., Archer, S., Leather, S. (2012). Resistance to dieback disease caused by *Fusarium* and *Lasiodiplodia* species in cacao (*Theobroma cacao* L.) genotypes. *Experimental Agriculture*, *48*(1), 85–98. https://doi.org/10.1017/S0014479711000883
4. Akgül, D. S., Önder, S., Savaş, N. G., Yıldız, M., Bülbül, İ., Özarslandan, M. (2024). Molecular identification and pathogenicity of fusarium species associated with wood canker, root and basal rot in Turkish grapevine nurseries. *Journal of Fungi*, *10*(7), 444. https://doi.org/10.3390/jof10070444
5. Beccari, G., Hao, G., Liu, H. (2022). Editorial: *Fusarium* pathogenesis: Infection mechanisms and disease progression in host plants. *Frontiers in Plant Science*, *13*, 1020404. https://doi.org/10.3389/FPLS.2022.1020404
6. Bernardos, M., Cosoveanu, A., Sierra Cornejo, N., Jiménez, I. A., Arévalo, J. R., Cabrera, R. (2025).

Fusaric acid as physiological stress trigger in Rumex lunaria. *Biological Invasions 2025 27:6*, *27*(6), 135. https://doi.org/10.1007/S10530-025-03594-5

7. Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C., Fontaine, F. (2013). Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology*, *62*(2), 243–265. https://doi.org/10.1111/j.1365-3059.2012.02674.x
8. Booth, C. (1972). The genus *Fusarium. Mycologia*, *64*(5), 1199. https://doi.org/10.2307/3758092
9. Boudoudou, D., El Marrakchi, S., Talha, A., Kerroum, B., Ouazzani Touhami, A., Douira, A., Benyahia, H. (2023). Effect of some derivatives of pyridazin-3 (2h) – ones on the in vitro and in situ development of different pathogenic fungi on citrus fruits. *Lecture Notes in Networks and Systems*, *713 LNNS*, 536–551. https://doi.org/10.1007/978-3-031-35248-5_49
10. Boudoudou, Dalal, Douira, A., Benyahia, H. (2024). Evaluation of the resistance of 10 new citrus rootstocks to root rot caused by Phytophthora parasitica. In: M. Azrour, J. Mabrouki & A. Guezzaz (Eds.), *Sustainable and Green Technologies for Water and Environmental Management* (World Sust, pp. 253–266). Springer. https://doi.org/10.1007/978-3-031-52419-6_18
11. Boukharta, N., Ennaffah, B., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2012). Effect of salinity on the sensitivity of tomato to *Verticillium wilt*. *Bulletin de La Société Royale Des Sciences de Liège*, *81*, 75–81.
12. Bruez, É., Lecomte, P., Grosman, J., Doublet, B., Bertsch, C., Fontaine, F., Ugaglia, A., Teissèdre, P., Costa, J. D., Guerin-Dubrana, L., Rey, P. (2013). Overview of grapevine trunk diseases in France in the 2000s. *Phytopathologia Mediterranea*. https://doi.org/10.14601/phytopathol_mediterr-11578
13. Bustamante, M. I., Elfar, K., Smith, R. J., Bettiga, L. J., Tian, T., Torres, G. A., Eskalen, A. (2022). First report of *Fusarium annulatum* associated with young vine decline in California. *Plant Disease*, *106*(10), 2752. https://doi.org/10.1094/PDIS-12-21-2790-PDN
14. Bustamante, Marcelo I., Todd, C., Elfar, K., Hamid, M. I., Garcia, J. F., Cantu, D., Rolshausen, P. E., Eskalen, A. (2024). Identification and pathogenicity of *Fusarium* species associated with young vine decline in California. *Plant Disease*, *108*(4), 1053–1061. https://doi.org/10.1094/PDIS-07-23-1362-RE
15. Castillo, V. C.-D., Imbachi, J. J. M., Benito, E., Díaz-Mínguez, J. (2025). FoG4MAT Is a new virulence factor in *Fusarium oxysporum* also involved in growth, differentiation and sporulation. *Phytopathology*. https://doi.org/10.1094/phyto-02-25-0069-r
16. Champion, R. (1997). *Identifier les champignons transmis par les semences* (Editions Quae, Ed.). Inra.
17. de Andrade, E. R., Dal Bó, M. A., Schuck, E., Gallotti, G. J. M. (1995). Evaluation of grapevine (Vitis Spp) resistance to *Fusarium oxysporum* f.sp. *herbemontis* in Rio Do Peixe Valley, Santa Catarina State, Brazil. *Acta Horticulturae*, *388*, 65–70. https://doi.org/10.17660/ACTAHORTIC.1995.388.9
18. de Sain, M., Rep, M. (2015). The role of pathogen-secreted proteins in fungal vascular wilt diseases. *International Journal of Molecular Sciences*, *16*(10), 23970. https://doi.org/10.3390/IJMS161023970
19. Douira, A., Ben Kirane, R., Ouazzani Touhami, A., Okeke, B., Elhaloui, N. E. (1995). *Verticillium wilt* of pepper (*Capsicum annuum*) in Morocco. *Journal of Phytopathology*, *143*(8), 467–470. https://doi.org/10.1111/J.1439-0434.1995.TB04556.X
20. Ekwomadu, T. I., Mwanza, M. (2023). Fusarium fungi pathogens, identification, adverse effects, disease management, and global food security: A review of the latest research. *Agriculture*, *13*(9), 1810. https://doi.org/10.3390/agriculture13091810
21. EL Haddadi, R, Errifi, A., Msairi, S., Ouazzani Touhami, A., Douira, A. (2019). First report of *Fusarium solani* causing damping-off disease on *Tetraclinis articulata* seedlings. *Plant Cell Biotechnology and Molecular Biology*, *20*(23–24), 1106–1114.
22. El Haddadi, Rachid, Errifi, A., Msairi, S., Ouazzani Touhami, A., Douira, A. (2021). Effect of interaction between *Fusarium solani* and *Rhizoctonia solani* on damping-off and root rot disease of *Tetraclinis articulata* seedlings. *Forestry Studies*, *75*(1), 166–175. https://doi.org/10.2478/fsmu-2021-0018
23. El Hazzat, N., Adnani, M., Msairi, S., El Alaoui, M. A., Mouden, N., Chliyeh, M., Boughribil, S., Selmaoui, K., Ouazzani, T. A., Douira, A. (2022). *Fusarium equiseti* as one of the main *Fusarium* species causing wilt and root rot of chickpeas in Morocco. *Acta Mycologica*, *57*. https://doi.org/10.5586/AM.576
24. El Hazzat, Naila, Adnani, M., Berber, F., Boughribil, S., Mouden, N., Errifi, A., Chliyeh, M., Selmaoui, K., Benkirane, R., Ouazzani Touhami, A., Douira, A. (2023). Comparative pathogenesis of *Fusarium* spp. obtained from diseased chickpea plants in Morocco. *Notulae Scientia Biologicae*, *15*(3), 11361. https://doi.org/10.55779/nsb15311361
25. El Rhoch, M., Maazouzi, S., Ouajdi, M., Sellal, Z., Mouden, N., Selmaoui, K., Ouazzani Touhami, A., Rabhi, A., Douira, A. (2025). *Fusarium solani* associated with dieback disease of argan trees in nurseries (Morocco). In *Technical Innovation and Modeling in the Biological Sciences* (pp. 217–228). https://doi.org/10.4018/979-8-3693-9450-2.ch012
26. Ziedan, El- S. H., Saad, M. M., Hemida, K. A., El- Aziz El -Naggar M. A., Mostafa M. H., El-Rab El- Samman M. G. (2020). In vitro: stem cutting a simple technique for determination aggressive potential of fungal isolates causing root rot disease of grapevine. *GSC Biological*

and Pharmaceutical Sciences, *11*(1), 141–147. https://doi.org/10.30574/gscbps.2020.11.1.0094

27. Elbouazaoui, A., Douira, A., Maafa, I., Seid, A. (2022). integrating sowing date with chickpea genotypes in managing *Fusarium Wilt* in Morocco. *Agriculture*, *12*(6), 773. https://doi.org/10.3390/agriculture12060773
28. Fotios, B., Sotirios, V., Elena, P., Anastasios, S., Stefanos, T., Danae, G., Georgia, T., Aliki, T., Epaminondas, P., Emmanuel, M., George, K., Kalliope, P. K., Dimitrios, K. G. (2021). Grapevine wood microbiome analysis identifies key fungal pathogens and potential interactions with the bacterial community implicated in grapevine trunk disease appearance. *Environmental Microbiome*, *16*(1), 23. https://doi.org/10.1186/S40793-021-00390-1
29. Gallotti, G. J. M. (1991). Resistance of *Vitis* spp to *Fusarium oxysporum* f sp herbemontis. *Fitopatologia Brasileira*, *16*(1), 74–77. https://eurekamag.com/research/007/749/007749100.php
30. Garrido, L. da R., Sônego, O. R., Urben, A. F. (2004). *Cylindrocarpon destructans* causador do "pé-preto" da videira no Rio Grande do Sul. *Fitopatologia Brasileira*, *29*(5), 548–550. https://doi.org/10.1590/S0100-41582004000500014
31. Georgiev, V., Ananga, A., Tsolova, V. (2014). Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients*, *6*(1), 391–415. https://doi.org/10.3390/nu6010391
32. Gramaje, D., Úrbez-Torres, J. R., Sosnowski, M. R. (2018). Managing grapevine trunk diseases with respect to etiology and epidemiology: Current strategies and future prospects. *Plant Disease*, *102*(1), 12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE
33. Guo, M., Li, B., Wang, R., Liu, P., Chen, Q. (2020). Occurrence of dieback disease caused by *Fusarium equiseti* on *Dendrobium officinale* in China. *Crop Protection*, *137*, 105209. https://doi.org/10.1016/J.CROPRO.2020.105209
34. Gutiérrez-Sánchez, A., Plasencia, J., Monribot-Villanueva, J., Rodríguez-Haas, B., Ruíz-May, E., Guerrero-Analco, J., Sánchez-Rangel, D. (2023). Virulence factors of the genus Fusarium with targets in plants. *Microbiological Research*, *277*, 127506. https://doi.org/10.1016/j.micres.2023.127506
35. Hemida, K. A., Ziedan, E.-S. H., El-Samman, M. G., Khattab, A. E.-N. A., Mohamed, M. H. (2024). Morphological, molecular biology and pathological characterization of fungi causes root rot on grapevine. *Egyptian Pharmaceutical Journal*, *23*(2), 368–381. https://doi.org/10.4103/epj.epj_286_23
36. Hemida, K., Ziedan, E., El-Saman, M., El-Naggar, M., Mostafa, H. (2017). Etiology of fungi associated with grapevine decline and their pathological potential. *Arab Universities Journal of Agricultural Sciences*, *25*(2), 355–365. https://doi.org/10.21608/ajs.2017.13618
37. Holkar, S. K., Bhondave, S. B., Patil, S. J., Nanekar, S. C., Bhosale, R. K., Khade, Y. P., Khandagale, K. S., Radhakrishna, A., Mainkar, P., Saha, S., Banerjee, K. (2025). First report of *Fusarium acutatum* causing root rot and vascular wilt disease in grapevine (*Vitis vinifera*) in India. *Plant Disease*, *109*(10), 2213. https://doi.org/10.1094/PDIS-03-25-0621-PDN
38. Huang, Q., Luo, P. (2021). Effects of leaf cutting on fusarium head blight disease development, photosynthesis parameters and yield of wheat under *F. graminearum* inoculation condition. *Agriculture, 11*(11). https://doi.org/10.3390/agriculture11111065
39. Jackson, E., Li, J., Weerasinghe, T., Li, X. (2024). The ubiquitous wilt-inducing pathogen *Fusarium oxysporum*—A review of genes studied with mutant analysis. *Pathogens*, *13*. https://doi.org/10.3390/pathogens13100823
40. Kenfaoui, J., Amiri, S., Goura, K., Radouane, N., Mennani, M., Belabess, Z., Tahiri, A., Fontaine, F., Barka, E. A., Ghadraoui, L. El., Lahlali, R. (2024). Uncovering the hidden diversity of fungi associated with grapevine trunk diseases in the Moroccan vineyards. *Tropical Plant Pathology*, *0123456789*. https://doi.org/10.1007/s40858-024-00656-2
41. Khadatkar, A., Sawant, C. P., Thorat, D., Gupta, A., Jadhav, S., Gawande, D., P, M. A. (2025). A comprehensive review on grapes (*Vitis* spp.) cultivation and its crop management. *Discover Agriculture*, *3*, 9. https://doi.org/10.1007/S44279-025-00162-2
42. Larignon, P., Fontaine, F., Farine, S., Clément, C., Bertsch, C. (2009). Esca et black dead arm : deux acteurs majeurs des maladies du bois chez la Vigne. *Comptes Rendus. Biologies*, *332*(9), 765–783. https://doi.org/10.1016/j.crvi.2009.05.005
43. Li, Z., He, F., Gai, X., Zhu, H., He, S., Hu Xuan, Y., Bai, L. (2025). The growth, development and infection process of the plant pathogen Fusarium. *Plant Signaling & Behavior*, *2025*(1), 2573097. https://doi.org/10.1080/15592324.2025.2573097
44. López-Díaz, C., Rahjoo, V., Sulyok, M., Ghionna, V., Martín-Vicente, A., Capilla, J., Di Pietro, A., López-Berges, M. S. (2018). Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. *Molecular Plant Pathology*, *19*(2), 440–453. https://doi.org/10.1111/mpp.12536
45. Meddah, N., Ouazzani Touhami, A., Benkirane, R., Douira, A. (2011). Etude du pouvoir pathogène de quelques espèces de *Fusarium* sur le bananier sous serre au Maroc. *Bulletin de La Société Royale Des Sciences de Liège*, *80*, 939–952.
46. Mendonça De Souza, M., Luciana, M., Vitorino, C., Márcio, Bruno, R., Mendes, M., Fabiano, D., Silva, G., Layara, Bessa, A., De, M. M., Marques, S., Vitorino, L C, Rosa, M., Dário, B. M. M., Silva, F. G., Bessa, L. A. (2023). Leaf physiology and histopathology of the interaction between the opportunistic phytopathogen

Fusarium equiseti and *Gossypium hirsutum* plants. *European Journal of Plant Pathology, 168*(2), 329–349. https://doi.org/10.1007/S10658-023-02759-Z

47. Messiaen, C. M., Cassini, R. (1968). Recherches sur les fusarioses. IV. La systématique des Fusarium. *Ann. Epiphyt*, *19*(3), 387–454.
48. Mirghasempour, S. A., Michailides, T., Chen, W., Mao, B. (2022). *Fusarium* spp. associated with dendrobium officinale dieback disease in China. *Journal of Fungi, 8*(9). https://doi.org/10.3390/JOF8090919
49. Mouria, B, Ouazzani-Touhami, A., Douira, A. (2007). Effect of various strains of *Trichoderma* on the growth of a greenhouse tomato crop and their ability to colonize roots and substrate. *Phytoprotection*, *88*(3), 103–110.
50. Mouria, Btissam, Ouazzani-Touhami, A., Douira, A. (2014). Effets in vitro et in vivo du compost sur *Verticillium dahliae*, agent causal de la verticilliose de la tomate. *Bulletin de La Société Royale Des Sciences de Liège*, *83*, 10–34.
51. Nelson, P. E., Toussoun, T. A., Marasas, W. F. O. (1983). *Fusarium Species: An Illustrated Manual for Identification.* Pennsylvania State University Press. https://books.google.co.ma/books?id=qJIMAQAAMAAJ
52. Nogués, S., Cotxarrera, L., Alegre, L., Trillas, M. I. (2002). Limitations to photosynthesis in tomato leaves induced by *Fusarium wilt*. *New Phytologist*, *154*(2), 461–470. https://doi.org/10.1046/J.1469-8137.2002.00379.X;page:string:article/chapter
53. OIV. (2022). *International Organisation of Vine and Wine Intergovernmental Organisation. Statistical Report on World Vitiviniculture*. https://www.oiv.int/what-we-do/statistics %0A%0A
54. Ouazzani Touhami, A., Ennaffah, B., El Yachioui, M., Douira, A. (2000). Pathogenie comparee de 4 especes d'Helminthosporium obtenues a partir des plantes malades du riz au Maroc. *Journal of Phytopathology*, *148*(4), 221–226. https://doi.org/10.1046/J.1439-0434.2000.00491.X
55. Qostal, S, Kribel, S., Chliyeh, M., Ouazzani Touhami, A Serghat, S., Benkirane, R., Douira, A. (2019). Study of the fungal complex responsible for root rot of wheat and barley in the northwest of Morocco. *Plant Archives*, *19*(3), 2143–2157.
56. Qostal, S., Kribel, S., Chliyeh, M., Serghat, S., Ouazzani Touhami, A., Zaarati, H., Benkirane, R., Douira, A. (2019). Study of the fungal complex responsible for root rot of wheat and barley in the north-west of Morocco. *Plant Archives 19*(2), 2143–2157.
57. Qostal, Safae, Kribel, S., Mouden, N., Chliyeh, M., El Alaoui, M. A., Selmaoui, K., Benkirane, R., Ouazzani Touhami, A., Douira, A. (2025). *Fusarium falciforme*, another causal agent of root rot of wheat and barley varieties in Morocco. *3 Biotech*, *15*(6). https://doi.org/10.1007/S13205-025-04333-2
58. Ramchandra, S., Bhatt, P. N. (2012). First report of *Fusarium equiseti* Causing *Vascular Wilt* of cumin in India. *Plant disease 96*(12), 1821. https://doi.org/10.1094/PDIS-03-12-0236-PDN
59. Saitou, N., Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
60. Shehata, A., Hussein, N.-E., Abdou, E.-S., Galal, A. (2019). Prevalence and possibility of management of grapevine root-rot in Minia Governorate, Egypt. *Egyptian Journal of Phytopathology*, *47*(1), 223–235. https://doi.org/10.21608/ejp.2019.120385
61. Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120
62. Tayou, S., Selamoui, K., Zarid, M., El Alaoui, M. A., Ouazzani Touhami, A., El Moadar, C., Douira, A. (2026). Fungal diseases of grapevine in Morocco: current knowledge, limitations, and future perspectives. *Notulae Scientia Biologicae*, *18*(1), 12763. https://doi.org/10.55779/nsb18112763
63. Tivoli, B. (1988). Guide d'identification des différentes espèces ou variétés de *Fusarium* rencontrées en France sur la pomme de terre et dans son environnement. *Agronomie*, *8*(3), 211–222. https://doi.org/10.1051/AGRO:19880306
64. Úrbez-Torres, J. R., Boulé, J., Hrycan, J., O'gorman, D. T. (2023). Potential role of *Fusarium* spp. in grapevine decline. *Phytopathologia Mediterranea*, *60*(2), 269–281. https://doi.org/10.36253/phyto-14679
65. Volynchuk, N. N. (2023). Grape *Fusarium*: From screening to biocontrol by yeast fungi. *Fruit Growing and Viticulture of South Russia*, *5*(83), 116–134. https://doi.org/10.30679/2219-5335-2023-5-83-116-134
66. White, T. J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols* (pp. 315–322). Elsevier. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
67. Yurchenko, E. G., Savchuk, N. V., Porotikova, E. V., Vinogradova, S. V. (2020). First report of grapevine (*Vitis* sp.) cluster blight caused by *Fusarium proliferatum* in Russia. *Plant Disease*, *104*(3), 991–991. https://doi.org/10.1094/PDIS-05-19-0938-PDN
68. Zhang, J., Zhou, Y. Y., Li, X. H., Zhang, W., Li, Y. H., Wang, X. D., Yan, J. Y. (2023). First report of *Fusarium* commune associated with a root rot of grapevine in China. *Plant Disease*, *107*(4), 1238. https://doi.org/10.1094/PDIS-08-22-1740-PDN
69. Ziedan, E.-S. H., Embaby, E.-S. M., Farrag, E. S. (2011). First record of *Fusarium vascular* wilt on grapevine in Egypt. *Archives of Phytopathology and Plant Protection*, *44*(17), 1719–1727. https://doi.org/10.1080/03235408.2010.522818