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Research Article

GEOGRAPHICAL DISTRIBUTION, MORPHOLOGY, AND PHYLOGENETIC ANALYSIS OF *FUSARIUM PROLIFERATUM*, THE CAUSAL AGENT OF OLIVE WILT IN POTHOHAR, PAKISTAN

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ABSTRACT

A disease survey of olive (*Olea europaea*) orchards was conducted across the Pothohar region, covering 32 locations in the Attock, Chakwal, Jhelum, and Rawalpindi districts. Disease incidence was recorded at five locations in Chakwal and Rawalpindi during 2020 and 2021. Pathogenic fungal isolates were analyzed for morphological characteristics, with spore dimensions ranging from 2.89 µm to 14.03 µm and colony pigmentation varying from white and pink to indigo and pink-indigo. The *Fusarium proliferatum* isolates were tested for pathogenicity against olive, eliciting disease severity ranging from mild to severe, with no significant differences observed within each virulence group. Molecular analyses of the isolates, including PCR amplification of the ITS region, Sanger sequencing, and phylogenetic analysis, confirmed their identity as *F. proliferatum*. These isolates exhibited the closest genetic relationship to those from *Glycine max* (soybean), *Triticum aestivum* (wheat), and *Panax ginseng* (ginseng). These findings highlight the growing threat of *Fusarium* wilt to olive production in Pakistan and emphasize the need for targeted disease management strategies to mitigate its impact on the local olive industry. This study represents the first report of *F. proliferatum* causing olive wilt in Pakistan.

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INTRODUCTION

Olive (*Olea europaea*) is the only member of the Oleaceae family that produces edible oleaginous fruit (Gouvinhas et al., 2017). Although the cultivation of olives began in the early Bronze Age (3150-1200 BC), its exact origin remains unknown, with hypotheses suggesting Syria and sub-Saharan Africa as possible centers of origin (Turecek and Regehr, 2020). The Greeks introduced the olive tree to Italy, particularly in Sicily, around 1000 BC. The Romans further promoted the expansion of olive plantations and processing across the Mediterranean region. Olive cultivation reached

greater levels in the 17th century. According to a 2017-18 communiqué from the International Olive Oil Council, olives are cultivated in more than 30 countries worldwide, producing over 31 million tons of virgin oil. Mediterranean countries such as Spain, Greece, Italy, and Turkey are the leading olive oil producers, with Spain alone accounting for 17 million tons of olive oil production.

In Pakistan, the olive tree was first introduced in 1986 by the Pakistan Agricultural Research Council (PARC) under an Italian project entitled "Fruit, Vegetable, and Olive". A survey conducted under this project identified substantial marginal land in the Pothohar region as suitable for olive cultivation due to its favorable climatic and topographic conditions.

To promote olive farming, the Agriculture Department of Punjab developed a five-year plan (2015-2020) to transform the Pothohar region into an "Olive Valley". As part of this initiative, 3.6 million olive plants were planted across an area of 12,545.25 hectares (Jan et al., 2021).

As a result, olives have become a prominent crop in Pakistan, particularly in the Pothohar region, which is characterized by its semi-arid climate and well-drained soils. The adaptability and economic significance of olive plantations, combined with suitable dry conditions, have led to a substantial increase in olive cultivation in the region over the past few decades (Akhtar et al., 2021).

Olives from this region are consumed locally and also used for olive oil production, which has gained popularity both domestically and internationally. In addition to its economic value, olive cultivation contributes to sustainable agriculture by supporting soil conservation and providing a reliable cash crop for many farmers (Ali et al., 2024).

The successful introduction of olives into Pakistan's agricultural sector has come with challenges, particularly the threat of biotic stresses. One of the most damaging is *Fusarium* wilt, caused by *Fusarium proliferatum*. Olive wilt, characterized by wilting, chlorosis, and vascular discoloration (Figures 1 and 2), could emerge as a major threat to olive plantations in the region, leading to severe yield losses. The pathogen not only poses a risk to the local agricultural economy but also challenges the sustainability of olive farming in the region.

F. proliferatum, a soil-borne fungal pathogen, is known to cause wilt diseases in various economically important crops, including garlic, rice, and soybean (Chang et al., 2015; Ignjatov et al., 2019; Naeem et al., 2020). In olives, *F. proliferatum* causes a devastating vascular wilt disease, often referred to as "olive wilt" (Trabelsi et al., 2017). The disease typically begins with root infections, after which the pathogen spreads through the vascular system, causing characteristic symptoms such as leaf yellowing, early defoliation, and eventual plant death (Figure 1 and 2). Infected trees exhibit stunted growth, reduced fruit yield, and, in severe cases, significant damage to olive orchards (Chliyeh et al., 2017).

In recent years, the economic impact of *F. proliferatum*-induced olive wilt has become increasingly evident in the Pothohar region. The ability of the pathogen to persist in the soil for years, combined with its high virulence, makes its management particularly challenging (Haware et al., 1978). *F. proliferatum* can infect olive trees at various growth stages, from nursery seedlings to mature trees, posing a significant threat to the olive industry.

F. proliferatum is an emerging threat to olive production in the Pothohar region of Pakistan, causing significant yield losses. Understanding its geographical distribution, morphological traits, and genetic diversity is crucial for effective disease management and the development of resistant olive cultivars. Therefore, this study aims to investigate the geographical distribution, morphological characteristics, and phylogenetic relationships of *F. proliferatum* isolates associated with olive wilt in the Pothohar region of Pakistan to enhance understanding of its epidemiology, evolutionary dynamics, and potential management strategies.

MATERIAL AND METHOD

Sample collection and pathogen isolation

A symptomatic survey was conducted in 32 olive orchards across Attock, Chakwal, Jhelum, and Rawalpindi during 2020-21. The orchards were selected based on the criteria that each covered at least two hectares of land and contained a minimum of 500 plants. Sampling was conducted to document disease incidence and prevalence, which were calculated using the following formulas:

$$\text{Incidence \%} = \frac{\text{Number of fungal infected olive plants}}{\text{Total Number of olive plants examined}} \times 100$$

$$\text{Disease prevalence (\%)} = \frac{\text{Locations displayed disease symptoms}}{\text{Total No. of locations under surveillance}} \times 100$$

Samples of diseased roots were collected in disposable polythene bags, labeled with the farmer's/site name and date of collection, and stored at 4°C for further processing. The longitude and latitude coordinates of each site were recorded using a GPS device (Garmin Mini Etrex 10).

Isolation of the pathogen

Disease samples were processed in the disease diagnostic laboratory at the Barani Agriculture Research Institute, Chakwal. A section of wilted roots from each sample was cut into small pieces (3-4 cm) using a sterile blade. The dissected samples were disinfected with 70% ethanol for 5 min, followed by 3-4 washes with sterilized distilled water, and then spread on a sterilized paper towel to absorb excess moisture (Cherrab et al., 2002).

The infected root samples were plated on 20 ml of potato dextrose agar (PDA) medium in sterilized Pyrex glass Petri plates (88 mm in diameter). The samples were processed in a laminar flow hood, and the plates were incubated at 25°C for seven days under alternating 12-h light and dark cycles provided by a 60 V fluorescent tube (Naeem et al., 2020).

Identification and purification of the pathogen

After seven days of incubation, fungal growth from each sample was examined under a compound microscope by preparing a glass slide with sterilized distilled water. The taxonomic identity of fungal isolates was determined by comparing them with available literature. The isolates were purified on PDA Petri plates using the hyphal tip method for further studies, including pathogenicity tests and morphomolecular characterization (Barnett and Hunter, 1972; Aboul-Nasr and Abdul, 2014).

Morphological characterization

The isolated pathogens were characterized based on their growth patterns and morphological features, both macroscopically and microscopically, and compared with descriptions in taxonomic keys and literature for identification (Barnett, 1955; Dooley, 2007; Bensch et al., 2012).

Pure and mature fungal cultures (15 days old) grown on PDA at 25±°C under alternating light and dark cycles were used for cultural and microscopic studies. Colony characteristics such as color, reverse pigmentation, texture, fruiting body color, and spore mass were examined visually.

Conidial slides for microscopic observations (Nikon YS 100) were prepared by suspending the spores in a drop of lactophenol blue stain. Microscopic features, including spore shape, dimensions (length × width), and hyphal color, were observed and recorded (Markakis et al., 2021). Conidial dimension data were statistically analyzed for mean and standard deviation using SPSS statistical software (version 16.0).

Pathogenicity test

The pathogenicity of *F. proliferatum* isolates was evaluated on one-year-old healthy olive plants of the highly susceptible variety Chemlali (Trabelsi et al., 2017). Conidial suspensions of all isolates were prepared by flooding sterilized distilled water onto seven-day-old fungal colonies grown on PDA, followed by filtration through muslin cloth to remove mycelial fragments. The conidial concentration was adjusted to approximately 1×10^6 conidia/ml by adding sterilized distilled water.

Healthy roots of the plants were immersed in the *Fusarium* inoculum for one hour and then transplanted into 500 ml disposable plastic pots filled with sterilized peat moss. Pathogenicity tests were conducted in triplicate for each isolate, using a single host plant (Tsror and Levin, 2003; Sanei and Razavi, 2017).

The control group consisted of healthy olive plants transplanted into sterilized peat moss in triplicate. The pathogenicity study was conducted in a greenhouse under standard conditions (25 ± 2°C and 70% relative humidity) with a 12-h photoperiod. The experiment was laid out in a Randomized Complete Block Design and plants were monitored weekly to assess disease progression.

DNA extraction and PCR amplification

Pure fungal isolates exhibiting morphological differences and high virulence were processed for DNA extraction using the Zymo Research Fungal/Bacterial Miniprep Kit, following the manufacturer's instructions (Ekpa et al., 2016).

PCR analysis

Purified DNA from each isolate was used to perform PCR amplification of key taxonomic markers using the universal primers ITS1 and ITS4. PCR reactions were conducted in a Veriti™ 96-well thermocycler (Applied Biosystems) with a total reaction volume of 50 µl. The reaction mixture included Phusion High-Fidelity PCR Master Mix (Thermo Fisher), the appropriate primer pair, template DNA, and distilled water (dH_2O).

The thermal cycling conditions were as follows: an initial denaturation at 95°C for 10 min, followed by 30-40 cycles of denaturation (95°C for 30 sec), annealing (58°C for 30 sec), and extension (72°C for 30 sec), with a final extension at 72°C for 5 min.

DNA fragment elution

The amplified PCR products for each sample were purified using the Gel Purification Kit (FavorPrep, Favorgen Biotech Corp.), following the manufacturer's protocol. The PCR products were first separated on a 2% high-resolution agarose gel, alongside a DNA ladder, using a gel electrophoresis apparatus. The DNA band corresponding to the target amplicon was carefully excised. The gel slice was then processed using a gel extraction kit to elute the DNA fragment, which was subsequently prepared for Sanger sequencing at the Genomics Facility, Biodesign Institute, Arizona State University, USA.

Phylogenetic analysis

The amplified DNA sequences were trimmed and aligned using bioinformatics tools, specifically the Gblocks server. To assess sequence homology, BLASTn searches were performed for each high-quality, trimmed sequence. Phylogenetic relationships were determined using the Maximum Likelihood method, as implemented in the MEGA 7 software package (Kumar et al., 2016). The resulting sequences were deposited in the National Center for Biotechnology Information (NCBI) database to obtain GenBank accession numbers.

RESULTS

The surveys conducted across 32 locations in the four districts of Pothohar (Attock, Chakwal, Jhelum, and Rawalpindi) detected wilt pathogen infection at only five locations. These locations included Kalar Kahar, PEL Farm, and BARI in Chakwal, as well as Dhok Chaudhrian Gujar Khan and the University Research Farm, Koont, in Rawalpindi. *F. proliferatum* was identified in samples collected from olive plants exhibiting wilting (Figure 2 and 3).

Figure 1. Disease symptoms caused by *F. proliferatum* on olive roots in Pothohar, Pakistan.

Figure 2. Disease symptoms of the pathogen affecting olive roots in (a) Gujar Khan, (b) BARI, (c) PEL Farm, (d) Kalar Kahar, and (e) University Research Farm, Koont.

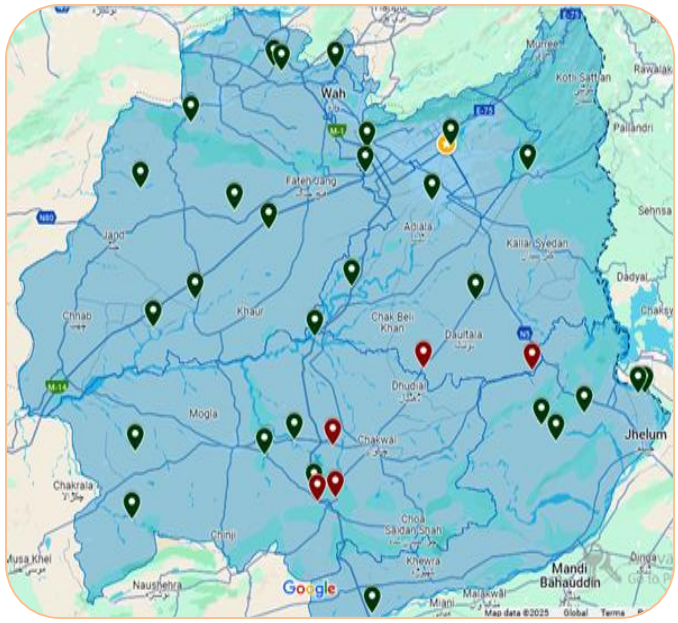

Figure 3: Geographical distribution of sampling sites for the survey on *F. proliferatum* causing olive wilt in Pothohar. (Red indicates diseased sites, while green indicates disease-free sites).

The prevalence of olive wilt in Chakwal was found to be 33.33%, indicating that approximately one-third of the surveyed locations were affected by the disease. This high infection rate suggested a significant presence of *F. proliferatum* in the district, warranting attention from both growers and agricultural researchers. In Rawalpindi, the prevalence of the pathogen was slightly lower at 28.57%, meaning that around one-quarter of the surveyed olive orchards were infected. Although the infection rate is lower compared to Chakwal, it still represents a substantial threat to olive production in the district.

In Attock and Jhelum districts, no orchards were found to be affected by *Fusarium* wilt. The prevalence in Chakwal and Rawalpindi is attributed to intercropping in olive orchards. The widespread presence of *F. proliferatum* in these districts raises concerns about the long-term impact of olive wilt on production and highlights the need for immediate intervention.

The incidence of olive wilt in the two key districts of the Pothohar region, Chakwal and Rawalpindi, indicates a concerning presence of the pathogen, while the other two districts, Attock and Jhelum, remain disease-free (Figures 3 and 4).

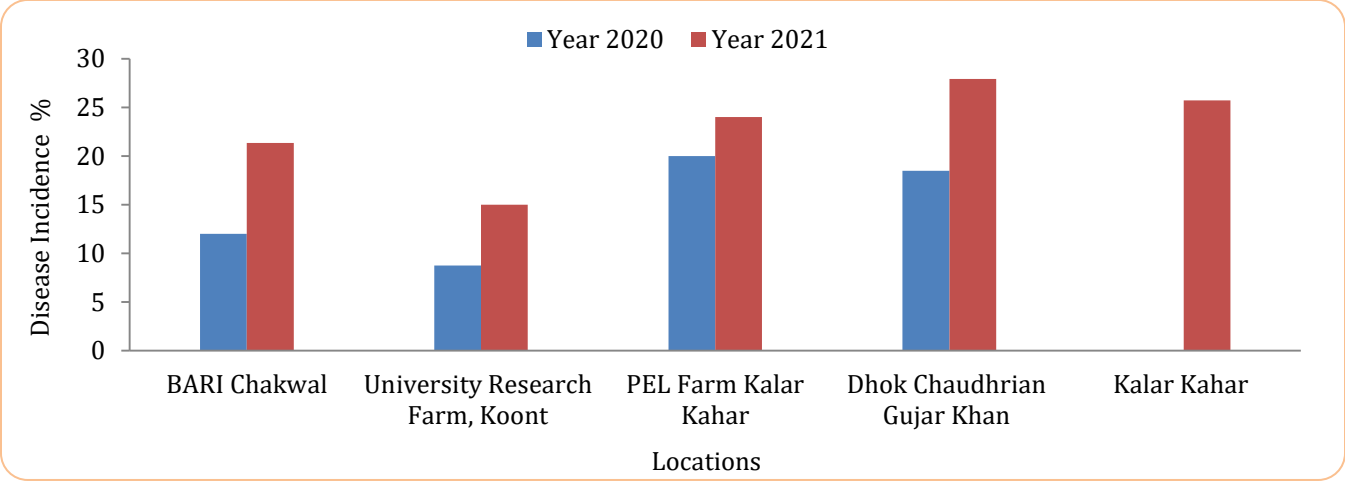

Figure 4. Incidence (%) of olive wilt in Chakwal and Rawalpindi during 2020-2021.

In 2020, PEL Farm Kalar Kahar had the highest wilt incidence of 20.00%, while Dhok Chaudhrian, Gujar Khan reported the highest incidence of 27.90% in 2021, identifying these locations as critical areas for intervention. Notably, Kalar Kahar, which recorded no incidence in 2020, showed a significant increase to 25.71% in 2021, highlighting it as a new area of concern. All locations experienced an increase in wilt incidence from 2020 to 2021, with the most significant rises observed in Kalar Kahar and Dhok Chaudhrian, Gujar Khan (Figure 4). Although the infection rates remain moderate compared to other olive diseases, *F. proliferatum* poses a considerable threat to olive cultivation in the region. Immediate efforts are needed to manage and control the spread of this pathogen to ensure sustainable olive production in Pothohar. These findings emphasize the need for targeted disease management and preventive measures to curb the spread of wilt in these regions.

Morphological and cultural characteristics of *F. proliferatum* isolated from olive roots

Detailed morphological characteristics of *F. proliferatum* isolates from olive roots are provided, with a focus on

colony coloration, spore type (macroconidia or microconidia), and spore dimensions (length and width). The colony coloration of *F. proliferatum* isolates varied, ranging from white and pink to indigo and pink-indigo. Unique combinations were observed in isolates such as W25c, W27b, and W20 (pink-indigo). Moreover, a light pink-whitish coloration was noted in isolates such as W26b (Figure 5). All isolates produced microconidia, while no macroconidia were observed (Figure 6). Spore dimensions varied significantly among isolates, with spore length ranging from 6.60 µm (W25b) to 14.03 µm (W27c), and spore width ranging from 2.89 µm (W20b) to 6.19 µm (W27c and W26).

The morphological diversity observed among *F. proliferatum* isolates suggested potential variability in pathogenicity and environmental adaptability. The consistent production of microconidia across all isolates supports their classification within the *Fusarium* genus. Notably, isolate W25b exhibited the widest spores (14.03 µm), which may indicate an adaptation or a unique morphological trait (Table 1). The significant variation in spore dimensions among isolates highlights the need for molecular studies to confirm species-level identification.

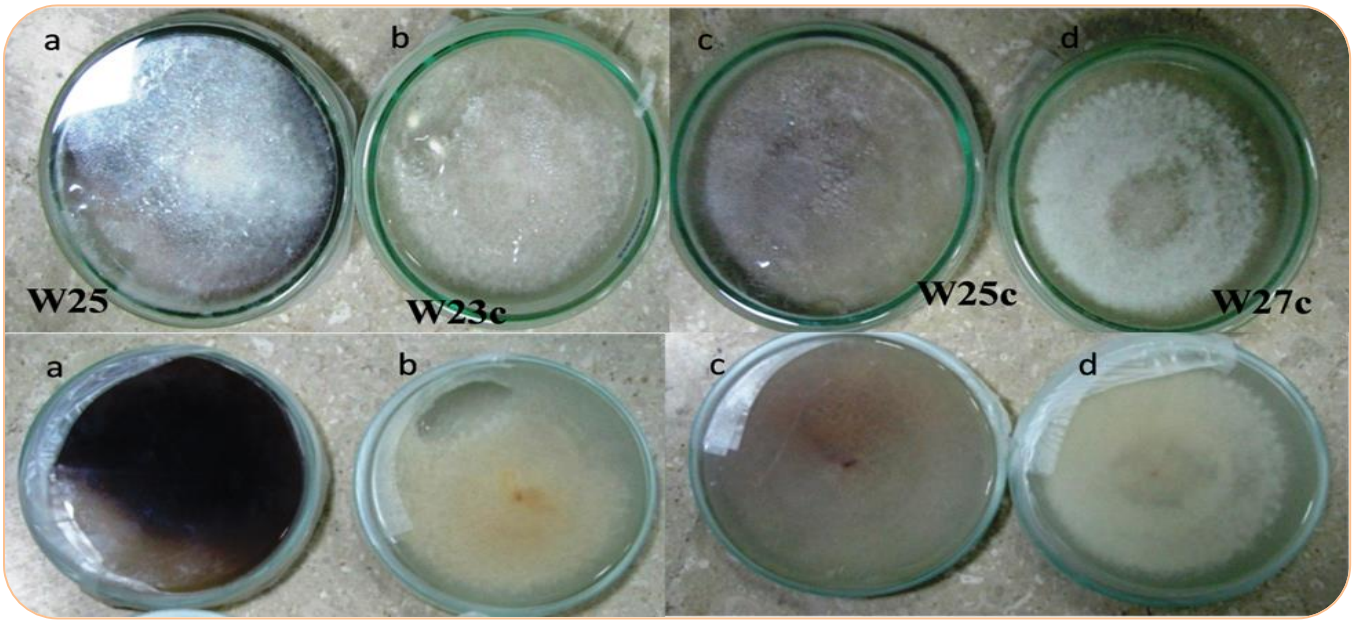

Figure 5. Cultural characteristics of *F. proliferatum* causing wilt; Top panel = Front view of different isolates growing on petri plates; Bottom panel = Basal view of different isolates growing on petri plates. (a) Irregular colony with purple pigmentation; (b) Irregular colony with pinkish pigmentation; (c) Regular colony with light purple pigmentation and (d) Irregular colony with no pigmentation.

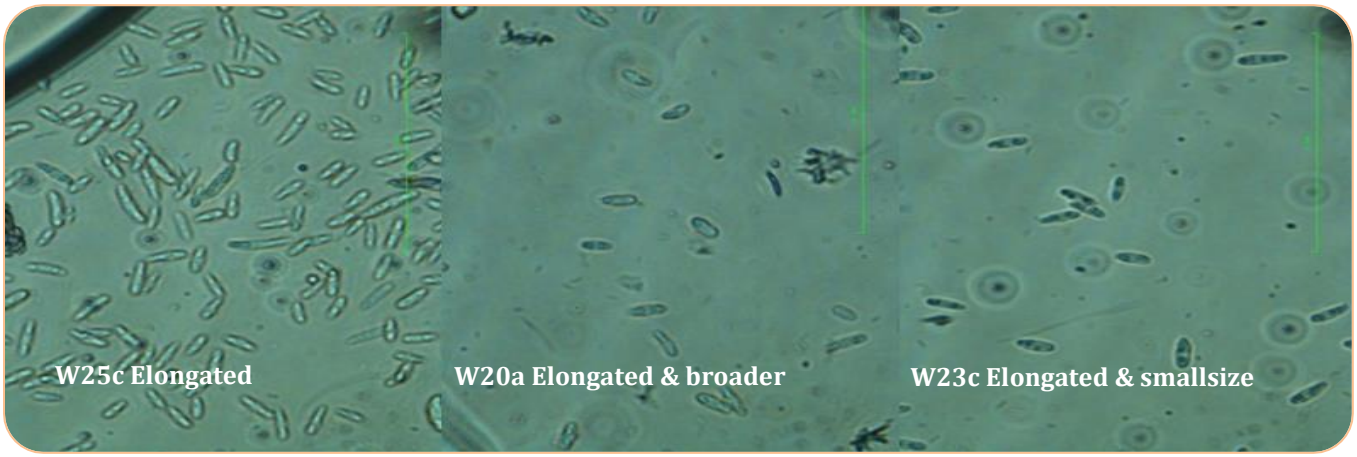

Figure 6. Microscopic examination of *F. proliferatum* spores.

Table 1. Cultural and morphological characteristics of *F. proliferatum* causing olive wilt.

No	Isolate	Cultural characteristics		Morphological characteristics	
		Colony color	Type of conidia	Length (µm)	Width (µm)
1	W27	Indigo	Microconidia	10.73 ± 1.43	5.36 ± 0.71
2	W27a	Pink	Microconidia	10.73 ± 1.43	5.36 ± 0.71
3	W26	White	Microconidia	11.14 ±2.14	6.19 ± 0.00
4	W20	Pink Indigo	Microconidia	11.55 ± 1.43	5.78 ± 0.71
5	W25	Pink	Microconidia	11.55 ± 1.43	5.78 ± 0.71
6	W23	White	Microconidia	10.73 ± 3.78	4.95 ± 2.48
7	W26a	White	Microconidia	9.08 ± 3.78	4.54 ± 1.89
8	W23a	Indigo	Microconidia	10.73 ± 5.15	5.36 ± 2.58
9	W25a	Pink	Microconidia	9.90 ± 0.00	4.54 ± 0.71
10	W23b	White	Microconidia	7.43 ± 2.48	4.13 ± 0.71
11	W20a	Pink	Microconidia	13.20 ± 2.86	5.78 ± 1.43
12	W20b	Pink	Microconidia	9.08 ± 3.78	2.89 ± 0.71
13	W20c	White	Microconidia	8.25 ± 1.43	4.27 ± 0.14
14	W27b	Pink Indigo	Microconidia	8.25 ± 2.86	4.68 ± 0.98
15	W26b	Light Pink Whitish	Microconidia	11.55 ± 2.86	6.08 ± 2.34
16	W26c	Pink	Microconidia	8.25 ± 1.43	4.60 ± 0.71
17	W25b	Pink	Microconidia	6.60 ± 2.86	2.89 ± 0.71
18	W23c	Indigo	Microconidia	9.08 ± 3.78	4.54 ± 1.89
19	W27c	White	Microconidia	14.03 ± 3.78	6.19 ± 1.24
20	W25c	Pink Indigo	Microconidia	9.90 ± 4.95	4.95 ± 2.48

Pathogenicity of *F. proliferatum* isolates on olive roots

The results of the pathogenicity study on *F. proliferatum* isolates collected from olive roots are presented as Disease Severity Index (DSI%) values, based on a disease assessment scale (0-3) adapted from Trabelsi et al. (2017). Twenty isolates of *F. proliferatum* were tested on olive seedlings under controlled conditions. Disease symptoms were monitored weekly, and DSI% values were recorded.

The isolates were categorized based on pathogenicity levels: mild (1-20% necrosis), moderate (21-50%), and severe (>50%). The results indicate variability in isolate virulence and their corresponding effects on roots. Notably, all isolates exhibited consistent pathogenic behavior across experimental replicates, with no significant differences within each virulence group.

The DSI% values for all isolates ranged from 51.66% to 93.33%, whereas control plants showed no symptoms (DSI% = 0), further validating the virulence of *F. proliferatum* isolates (Table 2).

Table 2. Pathogenicity profile of *F. proliferatum* isolates from olive roots.

Sr. No.	Isolate	DSI%	Pathogenic potential	Sr. No	Isolate	DSI%	Pathogenic potential
1	W23c	93.33 a	+++	11	W26a	70.66 bcdefgh	+++
2	W25c	92.00 a	+++	12	W23b	69.66bcdefgh	+++
3	W20b	88.00 ab	+++	13	W20	65.66cdefgh	+++
4	W23	86.00 abc	+++	14	W26c	64.33defgh	+++
5	W26	85.66 abc	+++	15	W20c	63.66efgh	+++
6	W26b	85.33 abcd	+++	16	W23a	61.00efgh	+++
7	W27a	79.66 abcde	+++	17	W25a	59.00efgh	+++
8	W25b	75.66 abcdef	+++	18	W27b	56.66fgh	+++
9	W27	74.66 abcdef	+++	19	W20a	52.33gh	+++
10	W27c	73.00 abcdefg	+++	20	W25	51.66h	+++

The symbol "+++" denote isolates that induced notable disease symptoms, confirming the pathogenic potential of all tested isolates.

The isolates exhibited distinct pathogenic behaviors, as reflected in their DSI% and disease scoring. Isolates such as W23c and W25c caused severe root necrosis (>90%) and rapid symptom progression (Figure 7). Above-ground symptoms included pronounced chlorosis, wilting, and plant death. These isolates likely secrete potent toxins and enzymes, facilitating aggressive tissue colonization and damage. In contrast, isolates such as W27b, W20a, and W25 induced moderate necrosis and symptoms. Although less aggressive, they significantly disrupted plant water and nutrient transport, leading to visible chlorosis and wilting. These isolates pose a significant risk under favorable environmental conditions.

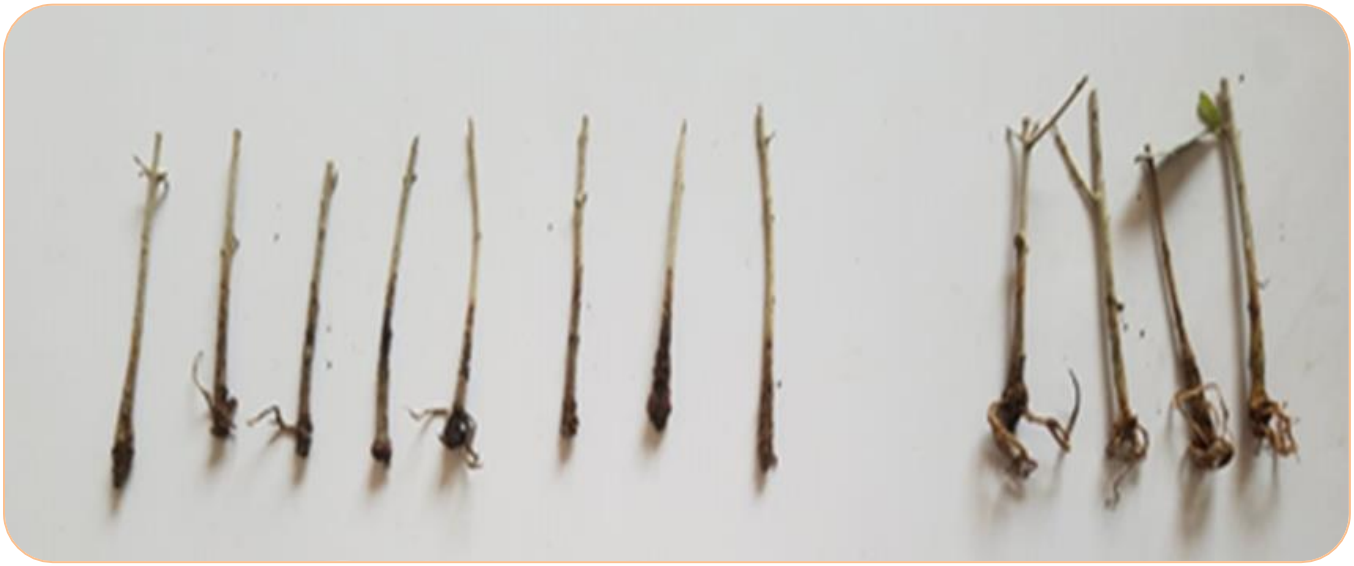

Figure 7. Pathogenicity behavior of *F. proliferatum* isolates from olive roots.

Although not observed in this study, such isolates could cause limited necrosis and minimal above-ground symptoms while maintaining partial plant functionality. Control plants remained healthy (DSI% = 0), highlighting the pathogenic potential of the isolates under study. The absence of disease symptoms in the controls underscores the specificity of the observed damage caused by the tested isolates.

Disease correlation

The correlation between DSI% and disease scoring validates the utility of the assessment scale. High DSI% consistently aligned with severe disease symptoms, reaffirming the reliability of the scale for characterizing pathogenicity. Highly virulent isolates (>60% DSI) pose a significant threat to olive cultivation, necessitating targeted management strategies. Future research should focus on resistance breeding and the role of environmental factors in disease expression.

Molecular characterization of highly virulent fungal pathogens

Fungal isolates were grouped based on their cultural characteristics. Highly virulent isolates from each group were sequenced and cataloged in the NCBI database. Nucleotide sequence analysis confirmed the identity of these highly virulent isolates at the species level. Phylogenetic analyses demonstrated close relationships with previously documented sequences from various hosts, providing understanding for the diversity and broad host spectrum of these pathogens in the context of evolution.

Phylogenetic analysis of *F. proliferatum*

The phylogenetic tree presented here illustrates the genetic relationships among various isolates of *F. proliferatum*. This analysis utilizes sequences obtained from the NCBI GenBank database to understand the genetic diversity and relationships of *F. proliferatum*, with a particular focus on isolates from olive in Pakistan (W25b). The tree reveals a single clade, in which the Pothohar isolate is closely positioned with other isolates from around the world, indicating a strong genetic relationship and suggesting a shared evolutionary lineage. Among the isolates, W25b from Pakistan is most closely related to *Glycine max* isolates from the USA (MN452347.1 and MN452230.1), clustering together and reflecting their genetic similarity (Figure 8).

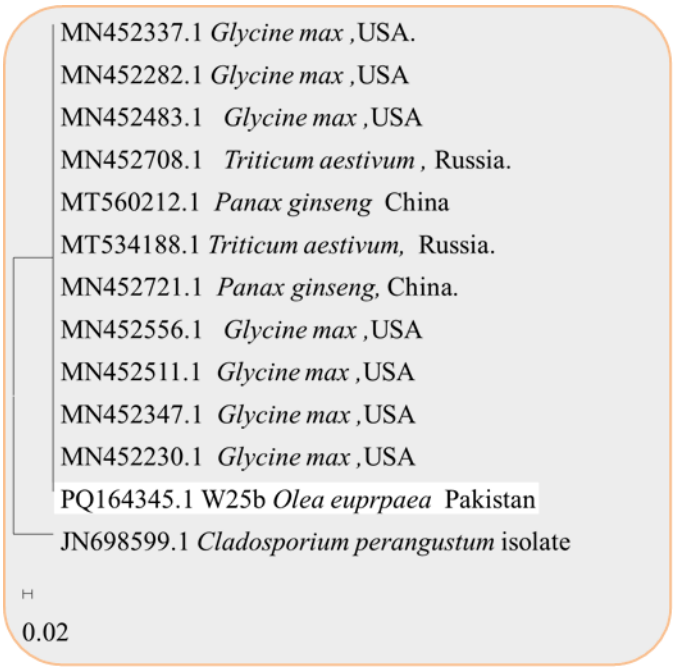

Figure 8. Cladogram showing the genetic relationship between the *F. proliferatum* isolate from olive in Pakistan and *F. proliferatum* isolates from other plants in the USA, China, and Russia associated with olive wilt.

This clustering suggests that *F. proliferatum* has a narrow host range and that the genetic traits observed in *O. europaea* may be shared with these related hosts. The clade also includes isolates from *Triticum aestivum* (wheat) from Russia (MN452708.1 and MT534188.1) and *Panax ginseng* (ginseng) from China (MN452721.1 and MT560212.1), demonstrating the adaptability of *F. proliferatum* across various host plants. Intercropping of wheat and leguminous crops is a common agricultural practice in the Pothohar region (Arif and Malik, 2009). Phylogenetic analysis suggests that intercropping these crops within olive orchards can significantly increase the risk of spreading wilt disease due to their shared susceptibility to a common pathogen, which facilitates disease transmission and proliferation. The outgroup, *Cladosporium perangustum*, highlights the evolutionary distance between *F. proliferatum* and other species, providing a reference point for understanding the evolutionary trajectory of this fungus.

DISCUSSION

F. proliferatum is a widespread soil-borne pathogen known to infect various crops worldwide. It has been reported as a cause of soybean root rot in Canada, Korea, and the United States (Diaz et al., 2011; Chang et al., 2015; Kang et al., 2024). In Serbia and Spain, it has been identified as the causal agent of root rot and clove rot in garlic (Palmero et al., 2010; Maja et al., 2018; Ignjatov et al., 2019). In Brazil, China, Pakistan, and Turkey, *F. proliferatum* has been reported as a root rot pathogen affecting cowpea, gerbera, Ficus, and rice (Palmero et al., 2010; Egerci et al., 2021; Farias et al., 2022; Naeem et al., 2020; Zhao et al., 2020).

F. proliferatum is pathogenic to a wide variety of plants, causing significant yield losses by inducing root rot and symptoms such as wilting and brown lesions (Diaz et al., 2011; Chang et al., 2015; Kang et al., 2024). A study reported that *Allium sativum* (spring varieties Labud and Sedef) and *A. ampeloprasum* (cv. Biser) were susceptible to all tested isolates of *F. proliferatum*, whereas mycelial growth inhibition was observed in cv. Ranko. No fungal growth was observed on the control cloves (Maja et al., 2018).

Another study found that infection by this fungal species causes vein clearing and marginal necrosis on the younger leaves of *Ficus benjamina*, while older leaves exhibit yellowing. As the infection progresses, drooping and wilting occur in plants artificially inoculated with *F. proliferatum* (Naeem et al., 2020). A study on the rice variety Baldo reported that at 24 ± 2°C, *F. proliferatum* infection produced early symptoms (10 days post-infection), including pale-green leaves, thinning, browning, and plant death, with severity ranging from 27.11% to 58.33%. Later symptoms (30 days post-infection) included white-pink mycelia on stems and necrotic roots, with severity ranging from 33.16% to 66.66% (Egerci et al., 2021).

Similarly, a study on one-month-old gerbera plants reported that 49 days after inoculation with *F. proliferatum* strain FZJf3-1, root rot, chlorosis, and other symptoms developed, while control plants remained healthy. *F. proliferatum* was reisolated from infected plants and confirmed to be genetically identical to the original strain, fulfilling Koch's postulates (Zhao et al., 2020). The findings from these studies are consistent with the results of the current research, as all *F. proliferatum* isolates demonstrated highly virulent behavior in olive plants. This similarity underscores the pathogenicity of *F. proliferatum* across different studies.

The isolates from this study produced abundant, aerial, white mycelia that turned purple in older cultures. Microconidia were formed in chains from polyphialides, clavate or oval, usually single-celled with a flattened

base. These characteristics align with descriptions of *F. proliferatum* in the literature (Kang et al., 2024). At the early stages of growth, *F. proliferatum* colonies exhibit white mycelium, which gradually turns violet with aging. Microconidia were abundant, usually non-septate, occasionally septate, oval, club-shaped, or kidney-shaped, measuring 6 to 10 × 2 to 4 µm (average 7.6 × 3.0 µm, n = 100) (Zhao et al., 2020). Aerial white mycelium with violet to dark pigments was also observed by Ignjatov et al. (2019).

When grown on PDA at 25°C, *F. proliferatum* isolates were classified into three morphological groups based on pigmentation: Type I (salmon-orange pigmentation, white to light rose mycelium), Type II (yellow to light brown pigmentation, cottony white mycelium), and Type III (white, fast-growing mycelium with dark violet pigments) (Maja et al., 2018). The cultural and morphological features observed in these studies closely align with the findings of the present research.

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AUTHORS' CONTRIBUTIONS

SKH conducted the experiments and collected data; FN, MIH, and ZA conceived and supervised the study; RA wrote the initial manuscript and provided the research facilities; AA and MA supervised the molecular study of the pathogen and finalized the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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