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Research Article

HOST PLANT RESISTANCE IN LENTIL GERMPLASM AGAINST *FUSARIUM* WILT AND ITS *IN VITRO* MANAGEMENT USING FUNGICIDES, PLANT GROWTH PROMOTING RHIZOBACTERIA, AND PLANT EXTRACTS

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ABSTRACT

Lentil, locally known as “Masoor” and valued as the “poor man’s meat,” is a protein-rich food crop severely affected by wilt disease caused by *Fusarium oxysporum*, leading to yield losses of 10–100%. The present study was conducted to screen lentil germplasm for resistance to *F. oxysporum* under sick field conditions and to evaluate the efficacy of various chemicals and plant growth-promoting rhizobacteria (PGPRs) against *F. oxysporum* under laboratory conditions. A total of 164 lentil genotypes were screened in the sick field. The results showed that no genotype was highly resistant; however, four genotypes viz. LPP 21133, LPP 21135, LPP 22102, and LPP 22115 were found to be resistant. Four different fungicides were evaluated at concentrations of 50 ppm and 100 ppm using the poisoned food technique. Among them, only score (difenoconazole) and topas (penconazole) were found to be the most effective against *F. oxysporum* at both concentrations. Six PGPR strains were also tested using the poisoned food technique. Of these, *Pseudomonas fluorescens* and *P. aeruginosa* showed effectiveness against *F. oxysporum*. Moreover, five different plant extracts were evaluated for their antifungal activity. Among these, only olive and neem extracts demonstrated some level of fungal growth inhibition *in vitro*. In conclusion, the study identified four resistant lentil genotypes and found that difenoconazole, penconazole, *Pseudomonas fluorescens*, *P. aeruginosa*, and extracts of olive and neem were effective in suppressing *F. oxysporum*, offering promising options for its management.

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) belongs to the family Fabaceae and the genus *Lens* (Lucas and Fuller, 2014). It is a cool-season legume, grown as a winter crop in subtropical regions and as a summer crop in temperate countries (Laskar et al., 2019). The nutritional benefits of lentils have increased their popularity beyond traditional uses in soups and rotis (traditional South Asian bread), making them a common ingredient in baked goods and extruded products. Lentil flour is gaining popularity as a healthier alternative to cereal-based flours in the preparation of baked items and snacks (Chelladurai and Erkinbaev, 2020).

Rich in dietary fiber, folic acid, complex carbohydrates, and protein, lentils are a highly nutritious food. They also contain several bioactive phytochemicals, including flavonoids, total phenolics, phytates, saponins, and tannins, which contribute to their health-promoting properties (Kaale et al., 2023).

Globally, approximately 6.33 million tons of lentils were produced in 2023 on about 6.10 million hectares of land. Canada remained the leading producer, followed by India and Australia (Malik et al., 2022). In Pakistan, lentils were cultivated on an estimated 6,100 hectares, producing around 3,868 tons in 2023 (IndexBox, 2024).

Lentil production is affected by various factors, including pathological problems such as wilt, root rot, collar rot, rust, and blight diseases (Taylor et al., 2007). *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen f. sp. *lentis* (Vasudeva and Srinivasan, 1952) is a plant pathogenic fungus commonly found worldwide. It is a soil-borne ascomycete responsible for causing wilt disease in lentils. Globally, *Fusarium* wilt can result in significant yield losses, ranging from 10% to 50% under favorable environmental conditions (Tosi and Cappelli, 2001). In Pakistan, lentil wilt is a major concern for growers (Altaf et al., 2014). Although the disease incidence typically ranges from 5% to 10%, it can reach up to 100% under conducive conditions (Chaudhary et al., 2008; Tiwari et al., 2018).

The pathogen primarily infects plant roots and later colonizes the vascular tissues, especially xylem vessels, leading to blockage (Bishop and Cooper, 1983). This obstruction results in chlorosis, vascular discoloration, leaf wilting, stunted growth, and, in severe cases, premature plant death (Lal et al., 2024). The disease develops optimally at temperatures between 22-25°C and can affect lentil plants during the seedling, vegetative, and reproductive stages (Tiwari et al., 2018). *F. oxysporum* is also a seed-borne pathogen and can survive in infected plant debris in the soil, indicating its adaptability to harsh environmental conditions. It has a broad host range and can survive on various plant species and substrates as alternate hosts (Tupaki-Sreepurna and Kindo, 2018). The fungus spreads through rain splash and moving water, which can carry chlamydospores and conidia over short distances. Long-distance dissemination occurs through infected planting material and contaminated harvesting equipment (Dubey and Pandey, 2020).

Its wide host range, saprophytic survival, interaction with other pathogens, and the formation of chlamydospores make *F. oxysporum* a highly versatile and persistent pathogen (Zeeshan et al., 2023; Yaseen et al., 2024). The use of resistant germplasm is the most economical, effective, and environmentally safe strategy to manage this disease (Fatima et al., 2015; Chandra et al., 2020). Mutated lentil germplasm has shown higher resistance to Fusarium wilt compared to recombinant populations. Breeding such resistant genotypes can enhance lentil yield and reduce the impact of the disease (Akhtar et al., 2016).

Several fungicides have shown effectiveness against this fungus *in vitro* and under controlled conditions (Iqbal and Mukhtar, 2020a). Prothioconazole, pyraclostrobin, and ipconazole significantly inhibited mycelial growth and spore germination of *F. oxysporum in vitro* (Bugingo et al., 2024). Lentil yield increased significantly when seeds were treated with a combination of carbendazim (12%) and mancozeb (63%) at 2.5 g per kg of seed (Jadhav et al., 2024).

Biological control agents and plant growth-promoting rhizobacteria (PGPRs) may offer a better alternative to chemical fungicides by improving plant health and inducing systemic resistance (Shahzaman et al., 2015; Azeem et al., 2025). *Trichoderma asperellum* restricted *Fusarium* growth *in vitro*, while seed treatment in combination with *Pseudomonas fluorescens* improved seed quality and increased yield (Iqbal and Mukhtar, 2020b; Jadhav et al., 2024). Plant-based extracts, being safer for human health, offer an eco-friendly alternative to chemical fungicides (Shahbaz et al., 2022). For instance, *Aloe vera* gel coating reduced fungal infection in strawberries by 22-38% (Sogvar et al., 2016), and lemongrass extract exhibited effective antifungal activity against *F. incarnatum* and *F. verticillioides* (Kamsu et al., 2019).

The present study was conducted to screen lentil germplasm in a sick field and to evaluate the relative efficacy of various fungicides, plant extracts, and PGPRs against *F. oxysporum* under laboratory conditions. The findings are expected to provide valuable insights into the genetic resistance of lentil germplasm, aiding the development of more resilient lentil varieties for future cultivation.

MATERIALS AND METHODS

Host plant resistance of different lentil genotypes against *F. oxysporum*

Planting environment

The experiment was conducted at the research field station of the Plant Pathology Section, Plant Pathology Research Institute, Faisalabad, Punjab, Pakistan. The environmental conditions during the growing season in this region are favorable for the development of *Fusarium* wilt of lentil (Fatima et al., 2015).

Sick field preparation

Inoculum was prepared from previously cultured isolates of *F. oxysporum*. The fungus was mass cultured on sterilized (autoclaved) lentil grains supplemented with dextrose and incubated at 25°C for 10-14 days. The inoculated grains were mixed with water, filtered through muslin cloth, and the resulting spore suspension was sprayed onto the soil. The remaining inoculated grain material was also evenly spread in the field. The soil was then rotavated thoroughly. This process was repeated 2-3 times annually. Spore density was quantified and maintained at 10^4-10^6 CFU per gram of soil before sowing (Gordon and Martyn, 1997; Alabouvette, 1999).

Planting material

From 2021 to 2023, screening trials were conducted to identify resistant germplasm among 164 lentil lines against *F. oxysporum*. The germplasm was obtained from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, and the Barani Agricultural Research Institute (BARI), Chakwal under the Punjab Agricultural Research Board (PARB), Lahore, Punjab, Pakistan.

Experimental design

The screening trials were laid out using an Augmented Design, suitable for evaluating a large number of entries with three random replications. The lentil germplasm was sown in the sick field artificially infested with *F. oxysporum*. This design facilitated the effective identification of resistant genotypes by comparing them with standard check varieties under natural disease pressure. Masoor-85 was used as the susceptible check, while PM-2020, NIAB M-2002, Markaz-2009, and Chakwal Masoor were used as breeder's checks.

Disease observations

The total number of plants was recorded after germination. Disease incidence was assessed when the susceptible check (Masoor-85) exhibited more than 80% disease incidence. The infection percentage (IP) was calculated using the following formula.

$$\text{IP} = \frac{\text{Infected plants}}{\text{Total plants}} \times 100$$

The reaction of the germplasm was assessed on the basis of disease rating scale given in Table 1.

Table 1. Disease rating scale for *Fusarium* wilt of lentil.

Rating scale	Disease response	Symptom on plants
0	Immune	No plant wilting
1	Highly Resistant	1% plants wilted
3	Resistant	2-20% plants wilted
5	Moderately Resistant	20.1-30% plants wilted
7	Susceptible	30.1-50% plants wilted
9	Highly Susceptible	50.1% or more plants wilted

Evaluation of different fungicides against *F. oxysporum*

Using the poisoned food technique, four fungicides, Cabrio Top (Pyraclostrobin 5% + Metiram 55%), Rally (Myclobutanil 40%), Score (Difenoconazole), and Topas (Penconazole), were evaluated at concentrations of 50 ppm and 100 ppm. After sterilizing the Potato Dextrose Agar (PDA) medium, the fungicides were added to the medium while it was lukewarm. The control plates contained PDA without any fungicide. Streptomycin sulfate (1 g/L) was also added to the medium to prevent bacterial contamination before pouring it into Petri plates. From the pure culture of *F. oxysporum*, 5 mm mycelial discs were cut using a sterile cork borer and placed upside down at the center of each Petri plate. The plates were incubated at 28°C for seven days. Each treatment was replicated three times, and one separate plate served as a control without fungicide.

Evaluation of different PGPRs against *F. oxysporum*

Six Plant Growth-Promoting Rhizobacteria (PGPR) strains, *Pseudomonas aeruginosa*, *P. fluorescens*, *Agrobacterium fabrum*, *Enterobacter cloacae*, *Gluconacetobacter liquefaciens*, and *P. putida*, were evaluated against *F. oxysporum* using the poisoned food technique. These PGPR strains were obtained from the Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad.

The PGPR cultures were added to lukewarm PDA medium at the same concentration for each treatment, while the control plates contained PDA without any PGPR. The medium was poured into Petri plates and allowed to solidify. Mycelial discs (5 mm) from a pure culture of *F. oxysporum* were placed at the center of each plate as described previously. Each PGPR treatment was replicated three times, and a separate control plate was maintained without PGPR. The plates were sealed and incubated at 28°C. Radial mycelial growth was recorded in millimeters after 3 and 7 days.

Evaluation of different plant extracts against *F. oxysporum*

Fresh, healthy leaves of neem (*Azadirachta indica*), kaner (*Nerium oleander*), aak (*Calotropis gigantea*), olive (*Olea europaea*), and tummah (*Citrullus colocynthis*) were collected, washed under tap water, and air-dried on tissue paper. The leaves were surface-sterilized using ethanol. A 5 g sample of each was macerated in 50 ml of double-distilled water (ddH$_2$O). The extract was filtered, and three concentrations (5%, 10%, and 15%) were prepared by diluting the stock with the appropriate volume of ddH$_2$O.

These plant extracts were evaluated using the poisoned food technique by incorporating them into lukewarm PDA medium, which was then poured into Petri plates. After the medium solidified, 5 mm discs from a pure culture of *F. oxysporum* were placed at the center of each plate using a sterile cork borer. The plates were sealed and incubated at 28°C. Radial mycelial growth was measured in millimeters after 3 and 7 days.

Data analysis

Radial mycelial growth was recorded in millimeters on the 3rd and 7th days after inoculation. Data were subjected to Analysis of Variance (ANOVA) following the procedure described by Steel and Torrie (1960) using SAS software (version 9.4, Edition 2014). Treatment means were compared using Fisher's Least Significant Difference (LSD) test. The data were organized and tabulated using Microsoft Excel (version 2021) (Wahyuni and Kusumawati, 2021).

RESULTS AND DISCUSSIONS

Screening of lentil germplasm against *F. oxysporum* under sick field conditions

Different lentil germplasm entries exhibited varying responses to *F. oxysporum* under sick field conditions (Table 2). None of the germplasm lines showed immune or highly resistant reactions. Only four lines (LPP 21133, LPP 21135, LPP 22102, and LPP 22115) were categorized as resistant, with disease incidence ranging from 2% to 20%. Lines with a disease incidence of 20.1% to 30% were classified as moderately resistant, and this response was observed in 60 germplasm lines. Seventy-three entries were found to be susceptible, exhibiting a disease incidence of 30.1% to 50%. Twenty-seven lines were highly susceptible, with disease incidence exceeding 50.1%.

Overall, among the tested lentil germplasm, 2.38% exhibited a resistant response, 37.5% showed moderate resistance, 43.45% were susceptible, and 16.6% were highly susceptible to *F. oxysporum*. In a recent study, three genotypes (IC201561, EC714243, and EC718238) out of 100 accessions were reported to be resistant to seven races of *F. oxysporum* f. sp. *lentis* (Nishmitha et al., 2023). The occurrence of different isolates of *F. oxysporum* f. sp. *lentis* in lentil-growing regions highlights the need to identify region-specific pathogens in order to develop effective resistance strategies tailored to specific agro-climatic zones (Belabid et al., 2004).

At the Regional Agricultural Research Station in Sagar, India, only six of 90 lentil germplasm entries were found to be resistant, while 24 were moderately resistant (Kharte et al., 2023). In a greenhouse pot experiment conducted in Kumarganj (Ayodhya), India, 100 lentil germplasm entries were screened; 41 were classified as highly resistant and 30 as resistant (Pandey et al., 2024). *F. oxysporum* can cause severe disease under hot and dry conditions, with an optimal temperature for disease development between 22°C and 25°C. Consequently, significant yield losses have been reported in regions such as Moghan, Ardebil, and Khorasan (Mohammadi et al., 2012).

With the emergence of new fungal races, most lentil cultivars currently grown by farmers globally have lost their resistance. Therefore, there is a pressing need to develop an increasing number of resistant cultivars. Given the ongoing changes in climate, resistant cultivars should be suitable for a wide range of environments. It is also essential to evaluate advanced breeding material annually to ensure the stability of resistance across multiple years, allowing promising lines to be directly released to farmers or used in breeding programs.

Furthermore, the identification of functional R-genes is crucial for understanding the molecular mechanisms of resistance in lentils against different *F. oxysporum* races. The resistant germplasm identified in our study provides a valuable resource for future breeding of wilt-resistant cultivars and aligns with findings from previous studies.

Table 2. Total number of lines categorized by disease ratings for *Fusarium* wilt (2021-2023).

Ratings	Incidence (%)	Response	Lines	No. of lines
0	No plant wilting	I	-	-
1	1% plants wilted	HR	-	-
3	2-20% plants wilted	R	LPP 21133, LPP 21135, LPP 22102, LPP 22115	4
5	20.1-30% plants wilted	MR	LPP 21102, LPP 21104, LPP 21106, LPP 21107, LPP 21112, LPP 21113, LPP 21114, LPP 21115, LPP 21117, LPP 21118, LPP 21120, LPP 21123, LPP 21125, LPP 21129, LPP 21132, LPP 21134, LPP 21152, LPP 21154, LPP 21156, LPP 21158, LPP 21161, LPP 21163, LPP 21164, LPP 21166, LPP 21170, LPP 21173, LPP 21175, LPP 22101, LPP 22103, LPP 22104, LPP 22105, LPP 22106, LPP 22107, LPP 22108, LPP 22109, LPP 22110, LPP 22112, LPP 22117, LPP 22121, LPP 22122, LPP 22128, LPP 22134, LPP 22143, LPP 22144, LPP 22145, LPP 22146, LPP 22147, LPP 22148, LPP 22160, LPP 22162, LPP 22163, LPP 22164, LPP 22167, LPP 22168, LPP 22169, LPP 22170, LPP 22171, LPP 22172, LPP 22175 and PM-2020	60
7	30.1-50% plants wilted	S	LPP 21101, LPP 21103, LPP 21105, LPP 21108, LPP 21109, LPP 21110, LPP 21111, LPP 21116, LPP 21121, LPP 21124, LPP 21124, LPP 21127, LPP 21128, LPP 21130, LPP 21131, LPP 21138, LPP 21139, LPP 21140, LPP 21143, LPP 21151, LPP 21153, LPP 21155, LPP 21157, LPP 21159, LPP 21160, LPP 21162, LPP 21165, LPP 21167, LPP 21168, LPP 21171, LPP 21174, LPP 21176, LPP 21177, LPP 21178, LPP 21179, LPP 21180, LPP 22111, LPP 22113, LPP 22116, LPP 22118, LPP 22119, LPP 22120, LPP 22123, LPP 22124, LPP 22125, LPP 22126, LPP 22127, LPP 22129, LPP 22130, LPP 22131, LPP 22133, LPP 22135, LPP 22136, LPP 22138, LPP 22139, LPP 22140, LPP 22141, LPP 22142, LPP 22149, LPP 22150, LPP 22151, LPP 22153, LPP 22154, LPP 22161, LPP 22165, LPP 22166, LPP 22173, LPP 22176, LPP 22177, LPP 22178, LPP 22180, Markaz-2009 and Chakwal Masoor.	73
9	50.1% or more plants wilted	HS	LPP 21119, LPP 21122, LPP 21136, LPP 21137, LPP 21141, LPP 21142, LPP 21144, LPP 21145, LPP 21146, LPP 21147, LPP 21148, LPP 21149, LPP 21150, LPP 21169, LPP 21172, LPP 22114, LPP 22132, LPP 22137, LPP 22152, LPP 22155, LPP 22156, LPP 22157, LPP 22158, LPP 22159, LPP 22174, LPP 22179 and NIAB M-2002.	27
			Total	164

Efficacy of different PGPRs against *F. oxysporum*

There were significant differences in the inhibition of fungal growth by various PGPRs. Among them, *P. fluorescens* showed the strongest suppression of mycelial growth, limiting it to 9.22 mm after three days. In contrast, *F. oxysporum* exhibited 14.11 mm of mycelial growth when grown against *P. aeruginosa*. *A. fabrum* and *P. putida* demonstrated moderate inhibition, with *F. oxysporum* mycelial growth measured at 21.78 mm and 21.11 mm, respectively. With a mycelial growth of 28.89 mm, *G. liquefaciens* showed less effectiveness. The least suppression was observed in media enriched with *E. cloacae*, where *F. oxysporum* grew up to 42.89 mm. In the control plates, where no PGPR was applied, maximum mycelial growth was recorded at 50.89 mm.

After seven days, a similar trend in mycelial growth inhibition was observed. *P. fluorescens* continued to exhibit the highest efficacy, limiting fungal growth to 11.00 mm. *P. aeruginosa* also maintained considerable inhibition, with mycelial growth of 14.67 mm. *E. cloacae* showed minimal inhibition, with fungal growth increasing to 51.89 mm. The control plates demonstrated maximum mycelial development, reaching 69.89 mm (Table 3 and Figure 1).

Recently, biological control agents isolated from soil have gained attention for their ability to suppress plant pathogens and promote plant health. *P. fluorescens* inhibits *F. oxysporum* growth through the production of antibiotics and extracellular lytic enzymes that degrade the fungal cell wall (Gao et al., 2012; Majumder et al., 2014). *P. aeruginosa* also produces antifungal compounds such as pyocyanin, phenazines, and rhamnolipids, which are effective against fungi like *Candida albicans* and *Aspergillus fumigatus* (Xu et al., 2014). Moreover, it has shown significant inhibition of dermatophytes including *Trichophyton mentagrophytes* and *T. rubrum*, via secretion of proteins and enzymes that degrade fungal filaments (Treat et al., 2007; Xu et al., 2014).

Using the dual culture technique, *Rhizobium pusense* and *Burkholderia contaminans* have been reported to inhibit *F. oxysporum* growth by 72.57% and 63.42%, respectively. In addition to disease suppression in pot experiments, both strains significantly improved shoot and root growth in lentil plants (Ayub et al., 2024). PGPRs, when used as seed treatments or soil amendments, can suppress pathogens and enhance plant growth. Our findings are consistent with previous literature, which shows that these bacteria are effective in reducing fungal mycelial growth through the production of antifungal metabolites, competitive exclusion, or parasitism.

Table 3. Efficacy of different PGPRs against *F. oxysporum*.

PGPRs	Mycelial growth (mm)	
	3 Days	7 Days
P. fluorescence	9.22 H	11.00 H
P. aeruginosa	14.111 GH	14.667 GH
P. putida	21.778 FG	32.889 E
A. fabrum	21.111 FG	35.44 DE
G. liquefaciens	28.889 EF	45.556 BC
E. cloacae	42.889 CD	51.889 B
Control	50.889 BC	69.889 A
LSD	8.56	

Efficacy of different fungicides against *F. oxysporum*

Both fungicides, score (difenoconazole) and topass (penconazole), demonstrated significant inhibition of mycelial growth at concentrations of 50 ppm and 100 ppm. After 3 days of inoculation, Score restricted fungal growth to 18.33 mm at 50 ppm and 18.11 mm at 100 ppm, while Topass limited growth to 38.33 mm and 17.67 mm at the respective concentrations. Rally (myclobutanil) showed moderate inhibition, with fungal growth measuring 41.89 mm at 50 ppm and 38.00 mm at 100 ppm. Cabrio top (pyraclostrobin + metiram) was the least effective, with fungal growth of 49.22 mm at 50 ppm and 49.21 mm at 100 ppm. In the control Petri dishes, the average mycelial growth reached 52.50 mm.

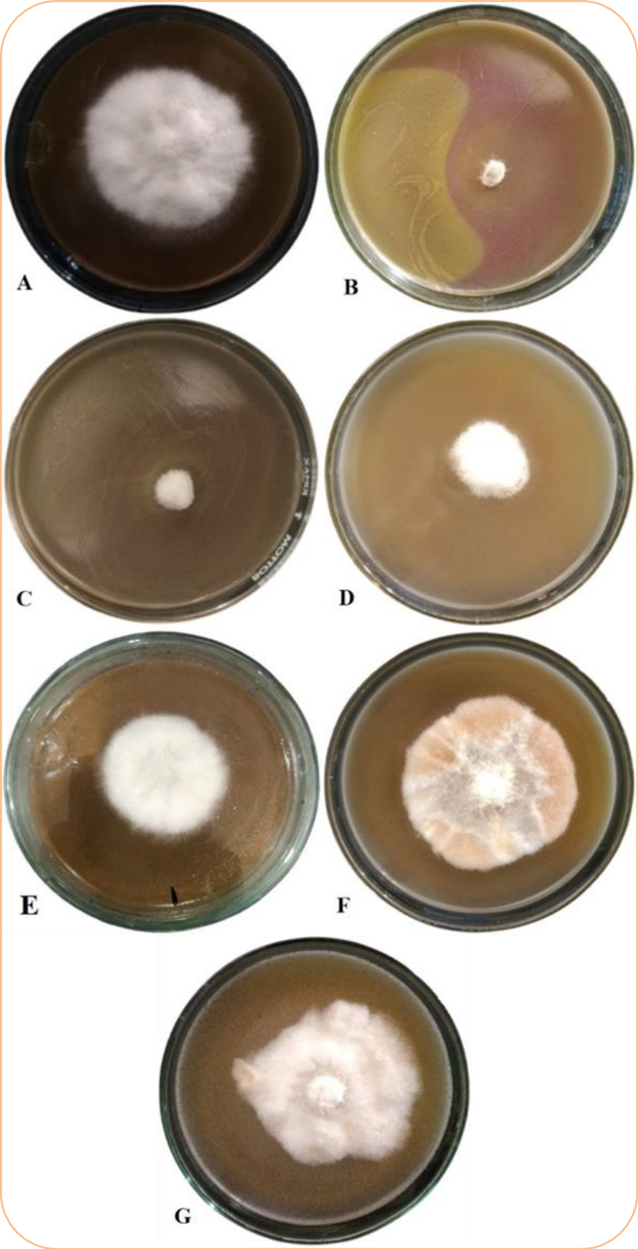

Figure 1. Effect of different PGPR strains on the growth of *F. oxysporum*: (A) Control, (B) *P. fluorescens*, (C) *P. aeruginosa*, (D) *P. putida*, (E) *A. fabrum*, (F) *G. liquefaciens*, and (G) *E. cloacae.*

After 7 days of incubation, a similar trend was observed. Score continued to show significant inhibition, with fungal growth measuring 37.78 mm at 50 ppm and 37.00 mm at 100 ppm. Topass restricted growth to 75.22 mm at 50 ppm and 37.00 mm at 100 ppm. Rally exhibited increased fungal growth, with measurements of 90.00 mm at 50 ppm and 89.11 mm at 100 ppm. Cabrio Top showed the highest fungal growth at both concentrations (90.00 mm), equivalent to the control group (Table 4 and Figure 2).

Table 4. Efficacy of different chemicals against *F. oxysporum*.

Treatments		Mycelial growth (mm)			
		3 Days		7 Days	
Trade Name	Chemical Name	50 ppm	100 ppm	50 ppm	100 ppm
Score	Difenoconazole	18.33 F	18.11 F	37.778 DE	37.000 E
Topass	Penconazole	38.33 DE	17.67 F	75.222 B	37.000 E
Rally	Myclobutanil	41.89 D	38.00 DE	90.000 A	89.111 A
Cabrio Top	Pyraclostrobin+Metiram	49.22 C	49.21 C	90.000 A	90.000 A
Control		53.44 C	51.56 C	90.000 A	90.000 A
LSD			4.46		

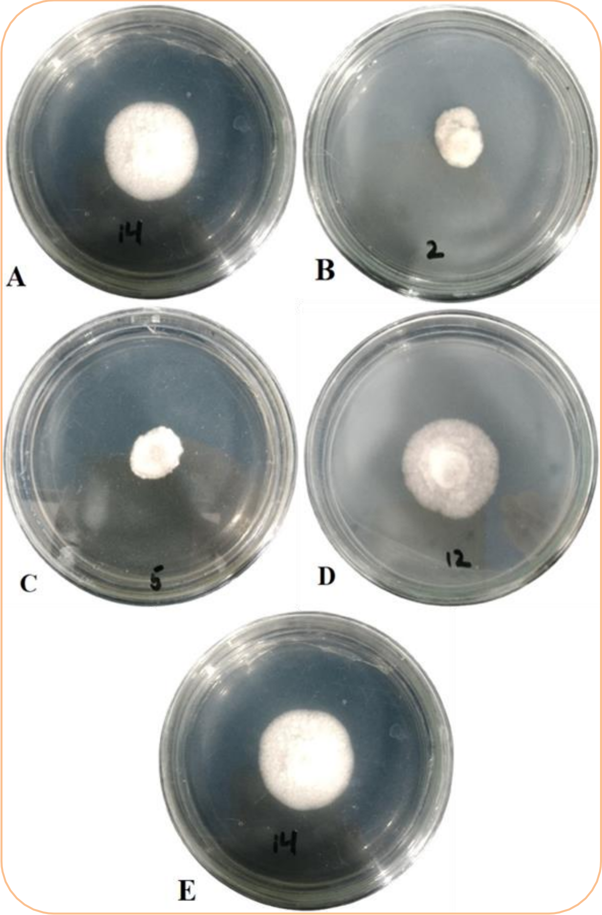

Figure 2. Effect of different chemical treatments on the growth of *F. oxysporum*: (A) Control, (B) Score (Difenoconazole), (C) Topas (Penconazole), (D) Rally (Myclobutanil), and (E) Cabrio Top (Pyraclostrobin + Metiram). The images show the fungal growth after 3 days of incubation with each chemical applied at a concentration of 100 ppm.

Score has proven effective in controlling the growth of *F. oxysporum*, the causative agent of *Fusarium* wilt in various crops. Several studies have shown that Difenoconazole can significantly reduce the mycelial growth of *F. oxysporum* under *in vitro* conditions. For instance, Bashir et al. (2018) reported that Difenoconazole effectively inhibited fungal growth at multiple concentrations.

Similarly, Topass also demonstrated strong antifungal activity. Research has confirmed that Penconazole significantly suppresses the mycelial growth of *F. oxysporum*. For example, Ibrahim et al. (2023) reported that Penconazole exhibited high toxicity against *F. oxysporum*, with an IC50 value of 0.989 ppm, indicating its effectiveness even at low concentrations. Moreover, Nguyen et al. (2024) observed that Penconazole stunted the growth of *F. oxysporum* strains across various tested concentrations.

Efficacy of different plant extracts against *F. oxysporum*

After 3 days of treatment, most plant extracts at 15% concentration showed similar results, with the mycelial growth of *F. oxysporum* ranging from 51.520 mm to 53.133 mm. The control Petri plates exhibited mycelial growth of 51.520 mm, which was comparable to most treatments. However, the olive extract at 15% concentration significantly inhibited mycelial growth, with fungal growth measured at 41.500 mm. This indicates a notably higher effectiveness compared to other plant extracts and the control. Neem extract at 15% also showed inhibitory effects, with mycelial growth measured at 45.800 mm, though it was less effective than olive extract.

Other treatments, including olive and neem extracts at 5% and 10%, as well as kaneer, tummah, and aak extracts at 5% and 10%, produced similar results to the control and were not significantly effective.

After 7 days, all treatments, including the control, reached maximum mycelial growth of 90 mm, except for the olive and neem extracts at 15% concentration. The olive extract at 15% showed continued inhibition, with fungal growth measured at 61.500 mm, while neem at 15% recorded 75.800 mm. Though neem was less effective than olive, it still outperformed all other treatments at all concentrations (Table 5 and Figure 3).

Table 5. Efficacy of various plant extracts against *F. oxysporum*.

Treatments	After 3 days			After 7 days		
	5%	10%	15%	5%	10%	15%
Olive	52.200 D	52.267 D	41.500 F	90.000 A	90.000 A	61.500 C
Neem	52.167 D	52.167 D	45.800 E	90.000 A	90.000 A	75.800 B
Kaneer	52.167 D	52.167 D	52.167 D	90.000 A	90.000 A	90.000 A
Tummah	52.167 D	52.600 D	52.167 D	90.000 A	90.000 A	90.000 A
Aak	52.567 D	52.933 D	51.500 D	90.000 A	90.000 A	90.000 A
Control	51.520 D	53.133 D	51.520 D	90.000 A	90.000 A	90.000 A
LSD			2.76			

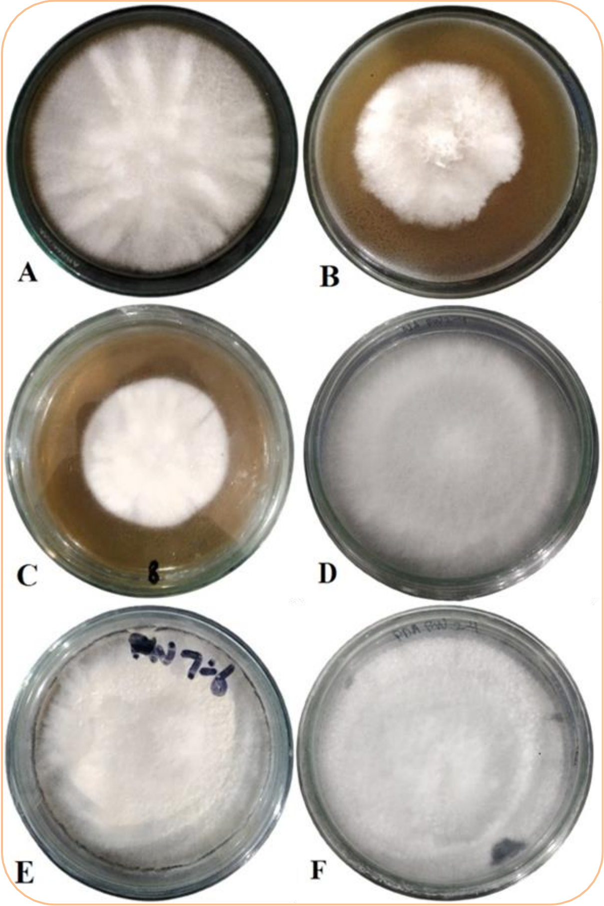

Figure 3. Effect of different plant extracts on the growth of *F. oxysporum*. (A) Control, (B) Olive, (C) Neem, (D) Kaneer, (E) Tummah, and (F) Aak. The images show *F. oxysporum* growth after 7 days of incubation with 15% concentrations of the respective plant extracts.

Overall, olive extract at 15% demonstrated the most significant inhibitory effect on *F. oxysporum* growth at both 3 and 7 days, suggesting a unique antifungal property in olive leaf extract. The control and other treatments consistently allowed full fungal growth, reaching 90.000 mm by day 7.

Neem extracts have previously shown significant efficacy in suppressing *F. oxysporum* growth. For example, neem leaf extract has been reported to reduce mycelial growth by up to 78.19% (Sunderrao et al., 2017). Active compounds in neem, such as azadirachtin, nimbin, and quercetin, possess strong antifungal properties contributing to this inhibition (Yi et al., 2021). Furthermore, neem extract has been shown to reduce or inhibit the production of fusaric acid, a toxin produced by *F. oxysporum* (Geraldo et al., 2010).

Although less extensively studied than neem, olive extracts have also demonstrated antifungal properties. Olive leaf extracts contain antimicrobial compounds effective against various pathogens, including *Fusarium* species (Minz et al., 2012). For example, olive pomace compost has been found to reduce the incidence of *Fusarium* root rot and promote plant growth. Similarly, olive oil cakes used as soil amendments have shown effectiveness in suppressing *F. oxysporum* (Abd-El-Khair and El-Nagdi, 2021).

Our findings, together with previous studies, suggest that olive extract, particularly at higher concentrations, could serve as a promising natural treatment for managing *Fusarium* wilt disease.

CONCLUSION

Fusarium species are significant plant pathogens that adversely affect field crops and contaminate food and feed through mycotoxin production. Developing and utilizing resistant germplasm remains the most sustainable and eco-friendly strategy for managing such diseases. In the present study, four lentil lines demonstrated resistance to *Fusarium* wilt and may serve as potential candidates for future breeding programs. For effective disease management, *P. fluorescens*, difenoconazole, penconazole, and olive plant extract showed promise against *Fusarium* wilt in lentils. We conclude that plant growth-promoting rhizobacteria (PGPR) such as *P. fluorescens*, along with native plant extracts or related biological products, offer environmentally friendly and sustainable approaches for managing plant diseases, thereby contributing to global food security.

AUTHORS' CONTRIBUTIONS

MUS, MJA and MEH designed the study; MUS, MK and MEH prepared the materials, MEH, MG and MS collected and analyzed the data; MUS and KPA helped in disease scoring; MUS and MJA supervised the studies; MUS, MEH and MK wrote the manuscript; All the authors proofread and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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