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FUNGAL PATHOGENS OF *OPUNTIA* SPP. CLADODES AND THEIR CONTROL USING COMMERCIAL FUNGICIDES

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ABSTRACT

Cladodes of *Opuntia* spp. are affected by diverse fungal pathogens that cause important yield losses. This study aimed to isolate, identify, and evaluate the pathogenicity of fungi associated with diseased cladodes and to assess the efficacy of three commercial fungicides; AZOXI 25 SC, ORTIVA TOP, and AGRIZOLE, applied at 0.5, 1.0, 1.5, and 2.0 L hL^{-1} under laboratory (27±2 °C) and screenhouse (25±4 °C) conditions. Six fungal isolates; *Alternaria sp.*, *Fusarium sp.*, *Rhizopus sp.*, *Aspergillus sp.*, *Cladosporium sp.*, and *Penicillium sp.* were recovered from symptomatic cladodes and identified based on morphological characteristics. Pathogenicity tests confirmed lesion development for all isolates, with lesion diameters increasing from 20.0 to 45.2 mm for *Alternaria sp.*, 22.1 to 50.1 mm for *Fusarium sp.*, 18.3 to 38.6 mm for *Rhizopus sp.*, 25.0 to 55.4 mm for *Aspergillus sp.*, 21.5 to 40.7 mm for *Cladosporium sp.*, and 17.8 to 35.4 mm for *Penicillium* sp. Disease incidence at 30 days ranged from 88.6% (*Rhizopus sp.*) to 95.4% (*Fusarium sp.*), and all fungi were successfully reisolated, fulfilling Koch's postulates. In vitro antifungal activity increased with fungicide concentration; at 0.5 L hL^{-1}, antifungal index (AI) values ranged from 24.8-37.4% for AZOXI 25 SC, 33.1-45.6% for ORTIVA TOP, and 20.5-36.8% for AGRIZOLE, increasing to 71.7-77.3%, 74.1-78.4%, and 71.2-76.8%, respectively, at 2.0 L hL^{-1}. Screenhouse trials conducted 20 days post-inoculation showed reductions in lesion severity from 3.1-3.6 to 1.1-3.0, accompanied by marked decreases in disease incidence: *Alternaria sp.* (45.3-3.4%), *Fusarium sp.* (50.2-4.1%), *Rhizopus sp.* (55.2-6.1%), *Aspergillus sp.* (41.9-1.6%), *Cladosporium sp.* (50.2-4.2%), and *Penicillium* sp. (53.9-5.2%). Overall, AZOXI 25 SC, ORTIVA TOP, and AGRIZOLE effectively reduced fungal growth, lesion severity, and disease incidence, providing practical management options for fungal diseases of *Opuntia* spp.

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INTRODUCTION

The family *Cactaceae* (order Caryophyllales) comprises about 130 genera and 2,000 species worldwide, with Mexico recognized as the main center of diversity, particularly for *Mammillaria*, *Echinopsis*, and *Opuntia* (Novoa *et al*., 2015; Van Steenderen, 2021). Most species are diploid (2n = 22), although polyploidy is frequent in the Opuntioideae, and the four subfamilies; Maihuenioideae, Opuntioideae, Pereskioideae, and Cactoideae are cultivated in more than 30 countries (Labra *et al*., 2003; Van Steenderen, 2021). Commercial production of cactus fruits is mainly concentrated in Chile, Mexico, Italy, the United States, and South Africa (Mondragón and Perez, 2001). In Morocco, the prickly pear, introduced in the 16th century (Arba, 2002), rapidly expanded due to its diverse uses in human and animal nutrition, soil conservation, biodiversity enhancement, beekeeping, cosmetics, and pharmaceuticals, helping the

country reach 160,000 ha of plantations by 2014 (Sbaghi *et al*., 2019). This sector was severely impacted in 2014 by the first detection of the wild cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), which spread quickly across major production areas and caused substantial socio-economic and environmental losses (Sbaghi *et al*., 2019). Comparable outbreaks were reported in Brazil, where 100,000 ha of *Opuntia ficus-indica* (L.) Mill. were destroyed, resulting in losses of 25 million USD (Lopes *et al*., 2009). In response, the Moroccan Ministry of Agriculture implemented an emergency program combining control actions and targeted research to develop biological control strategies, assess plant- and microbe-based biopesticides, and identify resistant varieties. This program led to the identification of eight resistant cactus varieties (Sbaghi *et al*., 2019), now incorporated into the national "Génération Green" strategy to rehabilitate 120,000 ha by 2030. Cactus remains a highly valuable crop due to its nutrient-rich fruits and its multiple ecological, economic, and industrial applications (Rodriguez-Felix, 2002; Shetty *et al*., 2012; El Kharrassi *et al*., 2016).

The cactus industry is constrained by insect pests and diseases (Swart and Swart, 2000). Approximately 122 insect species are associated with Opuntia spp., with Syrphidae, Otitidae, and Ephydridae acting as vectors of *Erwinia carotovora* subsp. carotovora, the causal agent of cladode soft rot (Fucikovsky, 1990; Longo and Rapisarda, 1995). Major fungal pathogens include *Alternaria tenuissima* (Kunze) Wiltshire (Pleosporales: Pleosporaceae), causing chlorotic spots and superficial necrosis; *Alternaria alternate* (Fr.) Keissl. (Pleosporales: Pleosporaceae), responsible for leaf and fruit rot; *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Botryosphaeriales: Botryosphaeriaceae) and Botryodiplodia theobromae Pat. (Botryosphaeriales: Botryosphaeriaceae), causing black cankers; *Fusarium sporotrichoides* Sherb. (Hypocreales: Nectriaceae) and *Fusarium solani* (Mart.) Sacc. (Hypocreales: Nectriaceae), inducing dry lesions and stem rot; *Diplodia opuntiae* Cooke & Harkn. (Botryosphaeriales: Botryosphaeriaceae), causing cladode rot; *Phomopsis cacti* (Thüm.) W. W. Rossi (Diaporthales: Diaporthaceae), inducing cladode spots; *Phyllosticta concave* (Sacc.) Sacc. (Botryosphaeriales: Botryosphaeriaceae), causing leaf spot; and *Pseudocercospora* sp. and *Pseudocercospora opuntiae* (Speg.) Deighton (Capnodiales: Mycosphaerellaceae), responsible for black and leaf spots, respectively (Swart and Kriel, 2002; Ammar *et al*., 2004; Quezada-Salinas *et al*., 2006; Ayala-Escobar *et al*., 2006). Additional pathogens reported in Mexico and Brazil include *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Glomerellales: Glomerellaceae) and *Colletotrichum fructicola* Prihastuti, L. Cai & K. D. Hyde (Glomerellales: Glomerellaceae) (anthracnose), *Fusarium lunatum* (Nirenberg & O'Donnell) (Hypocreales: Nectriaceae) and *Curvularia lunata* (Wakker) Boedijn (Pleosporales: Pleosporaceae) (rot), *Alternaria longipes* Neerg. (Pleosporales: Pleosporaceae) (leaf spot), *Lasiodiplodia euphorbicola* Abdollahz., Phillips & Abdollahz. (Botryosphaeriales: Botryosphaeriaceae), *Lasiodiplodia* iraniensis Abdollahz., Phillips & Khod, *Lasiodiplodia jatrophicola* Abdollahz., Phillips & Khod. (Botryosphaeriales: Botryosphaeriaceae) (canker and rot), *Neofusicoccum batangarum* Abdollahz., A.J.L. Phillips & Khod. (Botryosphaeriales: Botryosphaeriaceae), *Neofusicoccum hyalinum* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (Botryosphaeriales: Botryosphaeriaceae), and *Neopestalotiopsis* sp. (stem and cladode rot) (Flores-Flores *et al*., 2013; Ochoa *et al*., 2015; Conforto *et al*., 2019; Feijo *et al*., 2019). Collectively, these agents cause major diseases, including cactus anthracnose, charcoal spot, dry rot, foot rot, sunscald, soft rot, root rot, and stem rot, which significantly reduce cactus productivity worldwide (Carballo *et al*., 2000; Granata and Sidoti, 2000; Swart and Kriel, 2002). Recent field studies in Opuntia spp. production areas documented that fungal pathogens such as *Alternaria* sp. and *Fusarium* sp. are responsible for extensive cladode and fruit rot symptoms that translate into measurable yield impacts (Souza *et al.,* 2010; Bertetti *et al*., 2017; Bertetti *et al*., 2020). In affected Moroccan orchards, these diseases were associated with significant economic losses and declines in plant health, with growers reporting noticeable reductions in marketable yield and quality where infection incidence was high, particularly when interacting with insect pests such as *D. opuntiae*. In some production systems, disease pressure from rot-causing fungi contributed to reductions in productive cladode output by an estimated 20-50% in unmanaged plantations, highlighting the quantifiable effect of these phytopathogens on crop performance (Bertetti *et al*., 2017). These rot diseases compromise photosynthetic surface area and structural integrity of cladodes, lowering biomass accumulation and reducing overall productivity. Such quantified impacts emphasize the critical role of plant pathology research and integrated disease management to sustain *Opuntia spp*. cultivation.

Cactus is an important component of Moroccan

agroecosystems, yet few studies have examined the fungal pathogens affecting the cladodes of *O. ficus-indica*, a species widely cultivated throughout the country. Identifying the fungi responsible for diseases in economically important Opuntia plantations is essential for developing effective management strategies that improve crop productivity and sustainability. Consequently, it is necessary to evaluate the use of antifungal compounds to control fungal diseases in prickly pear. Azoxystrobin, a broad-spectrum fungicide used in agriculture since 1988, blocks electron transfer in mitochondria, inhibiting adenosine triphosphate (ATP) production and inducing oxidative stress in fungi, thereby disrupting cellular respiration (Wang *et al*., 2016; Imura *et al*., 2019; Poznanski *et al*., 2024). These properties make azoxystrobin an efficient fungicide for protecting plants during the vegetative stage (Poznanski *et al*., 2023). However, repeated and excessive applications can lead to the development of resistant pathogen strains, reducing its effectiveness, and there are concerns regarding its toxicity to vertebrates and invertebrates (Hahn, 2014). The objectives of this study were to investigate the diversity of fungi causing cladode spots in Moroccan cactus plantations, determine their pathogenicity on prickly pear, and evaluate the antifungal activity of azoxystrobin both in vitro and in vivo.

MATERIAL AND METHODS

Sampling Site and Fungal Isolation Procedures

Cladodes showing symptoms of rot or necrotic spots were collected weekly between October and December 2025 from *Opuntia spp*. plants exhibiting circular or map-shaped lesions (Figure 1).

Figure 1. Disease symptoms on *Opuntia* spp. observed in the field.

Sampling was conducted in the *Opuntia* sp. platform (2ha) containing *Opuntia sp*. genotypes resistant to *D. opuntiae* (Karama, Ghalia, Belara, Marjana, Cherratia, Angad, and Melk Zhar), located at the Experimental Domain of the National Institute of Agricultural Research (INRA) in Zemamra-Doukkala, along National Road No. 1 (El Jadida-Agadir), approximately 40 km from the Atlantic coast. A total of 120 symptomatic cladodes were collected during the study period, with approximately 10 cladodes sampled per week from each of the seven genotypes. The

site lies at latitude 32°21' N, longitude 8°22' W, at an elevation of 168 m, within a semi-arid zone. Annual rainfall ranges from 112.6 to 607 mm, with a long-term average of about 330 mm. Temperatures fluctuate from -1°C in winter (December-January) to 40-45°C in summer (July-August). The soil is characterized as sandy-clay hydromorphic with an alkaline pH (Khattabi *et al*., 2004). According to Eljebri *et al*. (2019), the typical soil parameters of the irrigated Doukkala plain include a pH of 8.15; silt, sand, and clay contents of 14.25%, 56.40%, and 29.31%, respectively; CEC of 22.40 meq/100 g; P_2O_5 and K_2O levels of 45.27 and 90.40 mg/kg, respectively; and 1.46% organic matter.

Fungal isolation was performed following the protocol of Flores-Flores *et al*. (2013). Small tissue fragments (~0.5 cm^2) were excised from the diseased areas of the cladodes and surface-disinfected with 1.5% sodium hypochlorite for 1 min 30 s. The fragments were then rinsed three times with sterile distilled water and air-dried on sterile paper towels. Each tissue piece was placed onto 90-mm glass Petri dishes containing potato dextrose agar (PDA). The plates were incubated at 27±2 °C, 55±10% relative humidity, and an 8:16 h (light:dark) photoperiod for 10 days in a growth chamber to allow fungal colonies to develop. Colony growth, color, and morphology were monitored daily.

Pathogenicity Assessment

Healthy cladodes of *Opuntia spp*. were thoroughly washed under running tap water and surface-disinfected with 1.5% sodium hypochlorite for 1 min 30 s, followed by three rinses in sterile distilled water. The cladodes were then air-dried on sterile paper towels. Small wounds (~5 mm in length and depth) were made on the cladode surface using a sterile scalpel. Mycelial plugs (5 mm diameter) from each fungal isolate, grown on 90-mm Petri plates containing PDA, were placed onto these wounds, with ten inoculation points per cladode to ensure reproducibility. Each fungal isolate, including controls inoculated with sterile PDA plugs, was tested on 20 cladodes randomly. Each inoculation site was covered with sterile cotton moistened with distilled water for 24 hours to maintain high humidity. Inoculated cladodes were incubated in a controlled growth chamber at 27±2 °C, 55±10% relative humidity, and an 8:16 h (light:dark) photoperiod until visible symptoms developed. Lesion development, including size and progression, was assessed at 10, 20, and 30 days after inoculation by measuring lesion diameters (Abo-El-Ela *et al*., 2001; Swart *et al*., 2003). Disease incidence (%) was calculated 20 days after treatments using Badawy and Rabea (2009) formula:

$$\text{DI (\%)} = \frac{\text{Number of infected wounds}}{\text{Total wounds per replicate}} \times 100$$

DI = Disease incidence

To confirm Koch's postulates, fungi were reisolated from lesion margins onto 90-mm PDA plates. Emerging colonies were purified via serial dilution and monosporic culture to obtain single-spore isolates, which were stored at 4 °C for subsequent experiments.

Morphological Identification of Fungal Isolates

Mycelial plugs (5 mm) from each isolate were placed at the center of 90-mm glass Petri dishes containing PDA and incubated at 27±2 °C, 55±10% relative humidity, and an 8:16 h (light:dark) photoperiod for 7-10 days to allow colony growth. Colony characteristics including diameter, color, texture, margin shape, and growth rate were recorded every 24 hours. Morphological features of the mycelium and conidia were examined using standard taxonomic keys (Leslie and Summerell, 2006; Simmons, 2007) and the MycoBank database (2011). For microscopic analysis, conidia and hyphae were mounted on glass slides and observed under a phase-contrast microscope (Canon INC DS126311, Taiwan) at ×40 magnification. Identification was based on diagnostic features including conidial shape, size, color, septation, and arrangement. Purified isolates were subsequently subcultured onto fresh PDA and stored at 4 °C for further experiments. Although molecular identification is recommended for precise species delimitation in complex fungal genera, the present study relied on detailed morphological characterization using standardized taxonomic keys. Molecular confirmation using ITS will be undertaken in future studies.

Comparative Assessment of Fungicides for the Control of Fungal Growth and Disease

Tested Fungicides

Three commercial fungicides were evaluated for their efficacy against fungal pathogens of *Opuntia ficus-indica*. The products included AZOXI 25 SC (Azoxystrobin 250 g/L; NANJING AGROCHEMICAL CO., LTD., Nanjing, China), ORTIVA TOP (Azoxystrobin 200 g/L + Difenoconazole 125 g/L; SYNGENTA CROP PROTECTION AG, Basel, Switzerland), and AGRIZOLE (Difenoconazole 250 g/L; GLOBACHEM, Johannesburg, South Africa). Each fungicide was tested at five concentrations: 0.5 L hL^{-1}, 0.8 L hL^{-1} (the recommended field dose approved by the

National Office for Food Safety [ONSSA], Morocco), 1.0 L hL^{-1}, 1.5 L hL^{-1}, and 2.0 L hL^{-1}. The lower and higher concentrations were included to evaluate dose-dependent efficacy and to determine whether doses above the recommended rate provide additional control without exceeding legal or safety limits. Distilled water was used as the control treatment.

Laboratory Trials

The in vitro antifungal activity of each fungicide was evaluated using 90-mm glass Petri dishes containing potato dextrose agar (PDA). One milliliter of each fungicide solution was incorporated into the medium at the designated concentrations prior to solidification. Mycelial plugs (5 mm in diameter) obtained from 7-day-old, actively growing fungal cultures were placed at the center of each Petri dish. Control Petri dishes were inoculated with the fungal mycelial plugs but contained no fungicide, allowing normal fungal growth. All Petri dishes were incubated in a growth chamber at 27±2 °C, 55±10% relative humidity, and an 8:16 h (light:dark) photoperiod. Colony diameters were measured daily along two perpendicular axes for 20 days. Each treatment was replicated two times, with ten Petri dishes per replicate. The antifungal index (AI, %) was calculated following Guo *et al*. (2006) formula:

$$AI(\%) = 1 - (\frac{Da}{Db}) \times 100$$

where *Da* represents the fungal colony diameter in the treated Petri dishes and *Db* represents the colony diameter in the control Petri dishes. This index was used to quantify the inhibitory effect of the fungicides on fungal growth.

Screenhouse Trials

Experiments were conducted in a climate-controlled screenhouse (11 m × 7 m × 3 m) maintained at 25±4 °C and 60±10% relative humidity under natural light conditions. Two-year-old *Opuntia ficus-indica* plants bearing 3-6 healthy cladodes were used. For inoculation, three cladodes per plant were selected. Plants were washed under running tap water, surface-sterilized with 70% ethanol, and air-dried for 24 h. Small wounds (5 mm in diameter) were made on each cladode using a sterile cork borer, with ten wounds per cladode. Mycelial plugs (5 mm in diameter) obtained from 7-day-old fungal cultures were inserted into the wounds, covered with the removed tissue disks, and allowed to develop for 10 days. Fungicide treatments were then applied as follows: 50 µL of each fungicide solution was applied directly to each wound using a sterile micropipette, ensuring complete coverage of the inoculation site. Treated wounds were left to air-dry for 5 min. Control plants consisted of wounded cladodes inoculated with mycelial PDA plugs but receiving no fungicide treatment. The experiment was arranged as a Randomized Complete Block Design (RCBD) with ten plants per treatment per replicate, and the entire trial was repeated twice as independent biological replicates. Plants were randomly distributed within blocks to account for potential environmental gradients in the screenhouse.

Disease development was assessed at 5, 10, and 20 days post-inoculation by recording the number of infected wounds per replicate. Lesion areas were photographed using a digital camera and quantified using ImageJ software (version 1.45g). Lesion severity was rated on a scale of 1 to 5, where 1 = 0 mm^2, 2 = 1-50 mm^2, 3 = 51-100 mm^2, 4 = 101-150 mm^2, and 5 = >151 mm^2 (Flores-Flores *et al*., 2013). Disease incidence (%) was calculated 20 day after treatments using Badawy and Rabea (2009) formula.

Statistical Analysis

Data from the pathogenicity assessment (Koch's Postulates), including lesion diameters and disease incidence (%) on *Opuntia ficus-indica* cladodes, were analyzed separately using one-way ANOVA to compare the pathogenic potential of different fungal isolates. When ANOVA indicated significant differences ($p < 0.05$), means were separated using Tukey's HSD test for multiple comparisons. All quantitative data, including in vitro antifungal activity (AI, %), lesion diameters, and disease incidence (%), were first checked for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Percentage data were arcsine square-root transformed prior to analysis to meet the assumptions of ANOVA (Snedecor and Cochran, 1982). The effects of fungicide type, fungicide concentration, and their interaction (fungicide × concentration) on antifungal activity, disease incidence, lesion severity, and Pv (%) were analyzed using a two-way ANOVA. This analysis allowed assessment of (i) the overall effect of fungicide type across all fungal isolates, (ii) the effect of different concentrations, and (iii) whether the efficacy of a fungicide depended on its concentration. All statistical analyses were performed using Statistica version 6 (StatSoft Inc., Tulsa, OK, USA), and results are presented as mean±standard error (SE).

RESULTS

Fungal Isolates

Six fungal isolates were obtained from diseased cladodes of *Opuntia* spp. and selected based on distinct

morphological characteristics observed on potato dextrose agar (PDA). As illustrated in Figure 2, the isolates showed clear phenotypic variation in colony colour, texture, margin shape, and growth pattern after 10 days of incubation. Isolate A produced an olive-brown to dark brown colony with a velvety to powdery texture, irregular margins, and concentric zonation, and induced circular necrotic lesions with dark margins on cladodes, consistent with *Alternaria Sp. (Pleosporales: Pleosporaceae)*. Isolate B was characterized by a yellowish-brown colony with a granular surface and moderate aerial mycelium and caused soft rot accompanied by internal tissue degradation, suggesting *Fusarium sp.* (Hypocreales: Nectriaceae). Isolate C formed fast-growing white cottony colonies with smooth margins and induced pale necrotic lesions with a water-soaked appearance, typical of *Rhizopus sp.* (Mucorales: Mucoraceae).

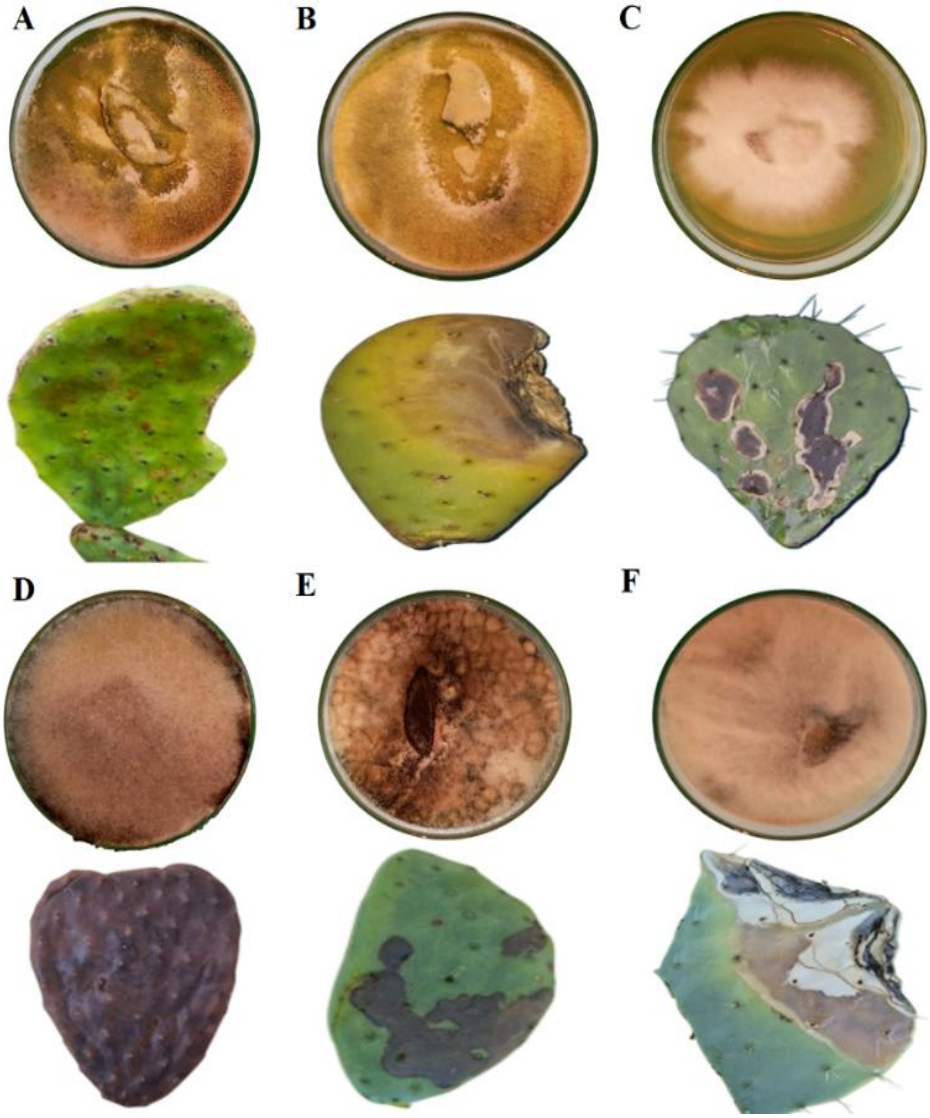

Figure 2. Morphological characteristics of fungal isolates on PDA and corresponding symptoms on *Opuntia spp*. cladodes. A = *Alternaria sp.*; B = *Fusarium sp.*; C = *Rhizopus sp.*; D = *Aspergillus sp.*; E = *Cladosporium sp.*; F = *Penicillium* sp.

Isolate D developed dark brown to black, compact colonies with slow growth and caused extensive black rot and tissue collapse, indicative of *Aspergillus sp.* (Eurotiales: Trichocomaceae). Isolate E exhibited grayish-brown colonies with fluffy mycelium and irregular pigmentation and induced large, irregular necrotic patches on cladodes, corresponding to *Cladosporium sp.* (Cladosporiales: Cladosporiaceae). Isolate F produced

light brown to beige colonies with a smooth texture and diffuse margins and caused internal necrosis with translucent tissue decay, consistent with *Penicillium sp*. The morphological features of the colonies on PDA and the associated disease symptoms on *Opuntia spp*. cladodes are summarized in Table 1.

Table 1. Morphological identification of fungal isolates isolated from diseased *Opuntia spp*. cladodes.

Isolate code	Colony morphology on PDA (10 days)	Associated cladode symptoms	Putative identification
A	Olive-brown to dark brown colony, velvety to powdery texture, irregular margins, concentric zonation	Circular necrotic lesions with dark margins	*Alternaria sp.*
B	Yellowish-brown colony, granular surface, moderate aerial mycelium	Soft rot with internal tissue degradation	*Fusarium sp.*
C	White cottony colony, fast-growing, smooth margins	Pale necrotic lesions with water-soaked appearance	*Rhizopus sp.*
D	Dark brown to black colony, compact texture, slow-growing	Extensive black rot and tissue collapse	*Aspergillus sp.*
E	Grayish-brown colony with fluffy mycelium and irregular pigmentation	Large irregular necrotic patches	*Cladosporium sp.*
F	Light brown to beige colony, smooth texture, diffuse margins	Internal necrosis and translucent tissue decay	*Penicillium* sp.

Across the 120 cladodes sampled, a total of 85 fungal isolates were obtained. These included 20 isolates of *Alternaria sp*. (23.5%), 15 of *Fusarium sp*. (17.6%), 12 of *Rhizopus sp*. (14.1%), 10 of *Aspergillus sp*. (11.8%), 14 of *Cladosporium sp*. (16.5%), and 14 of *Penicillium sp*. (16.5%). The frequency of isolation was calculated as the proportion of cladodes yielding each fungus, providing a systematic overview of the fungal community associated with cladode diseases in the experimental platform.

Pathogenicity Test

The pathogenicity test indicated that all six fungal isolates caused lesions on *Opuntia* spp. cladodes (Table 2). Different pathogenicity symptoms were observed during the incubation period, and lesion size increased significantly with time for all tested fungi. *Alternaria* sp. caused circular necrotic lesions on cladodes, with lesion diameters increasing from 20.0 mm at 10 days to 45.2 mm at 30 days after inoculation. *Fusarium* sp. induced soft rot associated with internal tissue degradation and produced lesions reaching 50.1 mm at 30 days. *Rhizopus* sp. provoked pale, water-soaked necrotic lesions, with lesion diameter increasing to 38.6 mm at 30 days. *Aspergillus* sp. caused extensive black rot and tissue collapse and showed the highest aggressiveness, with lesions reaching 55.4 mm at 30 days.

Table 2. Pathogenicity tests on *Opuntia spp*. cladodes.

Isolate	Lesion Diameter at 10 days (mm±SE)	Lesion Diameter at 20 days (mm±SE)	Lesion Diameter at 30 days (mm±SE)	Disease Incidence at 30 days (%)	Symptoms	Koch's Postulates
AA	20.0±0.5 BCa	35.5±0.6 BCb	45.2±0.7 BCc	90.1±0.9 BC	Circular necrotic lesions	Confirmed
FS	22.1±0.6 Ba	38.1±0.6 Bb	50.1±0.8 ABc	95.4±0.8 A	Soft rot, internal tissue decay	Confirmed
RS	18.3±0.4 Ca	28.3±0.5 Cb	38.6±0.4 Cc	88.6±0.6 C	Pale water-soaked lesions	Confirmed
AN	25.1±0.7 Aa	40.2±0.5 Ab	55.4±0.6 Ac	95.2±0.5 A	Extensive black rot	Confirmed
CC	20.2±0.4 BCa	30.3±0.6 BCb	40.7±0.8 BCc	91.7±1.0 BC	Large irregular necrotic patches	Confirmed
PS	15.5±0.6 Da	25.1±0.5 Db	35.4±0.4 Dc	92.2±0.5 B	Translucent tissue decay	Confirmed

Different uppercase letters within columns indicate significant differences at $P < 0.05$ according to Tukey's HSD test. Different lowercase letters within rows for lesion diameter indicate significant differences at $P < 0.05$ according to the same test. AA = *Alternaria* sp.; FS = *Fusarium* sp.; RS = *Rhizopus* sp.; AN = *Aspergillus* sp.; CC = *Cladosporium* sp.; PS = *Penicillium* sp.

The *Cladosporium* sp. induced large, irregular necrotic patches, with lesion diameters of 40.7 mm at 30 days. *Penicillium* sp. caused translucent tissue decay and produced the smallest lesions among the isolates (35.4 mm at 30 days) (at 30 days $F_{5,114}$= 36.2, $P<0.0001$)). Disease incidence at 30 days was high for all isolates, ranging from 88.6% (*Rhizopus* sp.) to 95.4% (*Fusarium* sp.) ($F_{5,114}$= 13.4, $P<0.0001$). After symptom development, all fungi were successfully reisolated from the lesion margins onto PDA, thereby confirming their pathogenicity and fulfilling Koch's postulates.

***In vitro* Evaluation of the Antifungal Effects of Fungicides**

The in vitro antifungal activity of the tested fungicides is presented in Table 3**.** In general, all fungicides significantly inhibited mycelial growth of the six phytopathogenic fungi compared with the control, and the antifungal index (AI, %) increased progressively with increasing concentration. Significant differences were observed among fungicides, concentrations, and fungal isolates. AZOXI 25 SC exhibited a dose-dependent inhibitory effect on all isolates, with AI values ranging from 24.8-37.4% at 0.5 L/hL to 71.7-77.3% at 2L/hL. The highest inhibition with this fungicide was recorded against *Alternaria sp.*and *Aspergillus sp.*at 2 L/hL. ORTIVA TOP showed higher overall efficacy than AZOXI 25 SC, producing AI values above 70% at 1L/hL and reaching maximum inhibition (74.1-78.4%) at 2 L/hL for all isolates. AGRIZOLE displayed comparatively lower antifungal activity at low concentrations but still caused substantial inhibition at higher doses, with AI values exceeding 71% at 2 L/hL across all fungi. Among the isolates, *Aspergillus sp.*and *Alternaria sp.* were generally the most sensitive to the tested fungicides, whereas *Rhizopus sp.* and *Penicillium* sp. showed relatively lower inhibition at the lowest concentrations. Nevertheless, at 2L/hL, all fungicides resulted in high and statistically similar levels of growth inhibition for most isolates. The two-way ANOVA (Table 4) revealed that fungicide type and concentration had highly significant effects on the antifungal index for all fungal isolates ($P < 0.0001$). The interaction between fungicide and concentration was also significant for *Alternaria sp., Fusarium sp.*, *Rhizopus sp.*, *Aspergillus sp.*, and *Penicillium* sp., indicating differential responses of these fungi to increasing fungicide concentrations. In contrast, this interaction was not significant for *Cladosporium sp.*, suggesting a more uniform response across fungicides and concentrations. Overall, the results demonstrate strong, concentration-dependent antifungal activity of the tested fungicides against all six fungal pathogens under laboratory conditions.

Table 3. In vitro antifungal activity of fungicides against fungal isolates.

Fungicide	Concen-tration (L/hL)	The antifungal index (AI, %) 20 days post-inoculation (Mean± SE)					
		AA	FS	RS	AN	CC	PS
AZOXI 25 SC	0.5	35.5±0.5 M	31.1±0.9 I	28.5±0.7 I	37.4±1.0 I	29.4±1.1 I	24.8±0.7 K
	0.8	54.2±0.5 J	51.8±0.9 F	50.2±1.4 F	55.2±0.8 G	53.5±1.0 F	47.8±0.9 H
	1	68.5±0.6 G	65.1±1.2 E	62.6±1.1 CD	69.4±0.7 DE	65.6±1.1 D	61.7±0.7 E
	1.5	73.1±1.0 DE	70.8±1.0 C	68.2±1.0 B	73.3±0.9 BC	70.3±0.6 C	68.3±0.8 C
	2	77.3±0.5 AB	74.5±0.8 A	72.5±0.7 A	76.7±1.2 AB	73.3±1.0 B	71.7±0.6 B
ORTIVA TOP	0.5	41.0±1.1 L	35.4±0.7 H	32.3±0.7 H	43.7±1.3 H	34.9±0.7 H	30.4±0.6 J
	0.8	59.6±1.1 I	58.3±0.6 E	54.4±1.1 E	62.5±0.9 F	57.4±1.4 E	51.8±0.6 G
	1	70.4±1.0 FG	67.7±0.6 D	64.2±1.2 C	71.3±0.9 CD	68.2±0.9 C	64.6±1.0 D
	1.5	74.5±0.8 CD	73.7±1.1 AB	71.3±0.7 A	75.9±1.0 AB	74.4±0.6 B	71.2±0.6 B
	2	78.4±0.2 A	76.3±0.7 A	74.6±1.0 A	78.3±1.4 A	77.2±0.7 A	74.1±0.9 A
AGRIZOLE	0.5	30.8±0.7 N	25.6±1.1 J	20.7±0.8 J	34.3±1.0 J	28.4±0.6 I	20.5±0.6 L
	0.8	49.8±0.9 K	48.4±0.6 G	44.3±1.2 G	52.8±1.0 G	50.1±0.7 G	45.3±1.1 I
	1	64.2±0.6 H	63.4±0.9 D	60.1±1.3 D	67.5±1.2 E	63.4±1.2 D	59.4±0.7 F
	1.5	71.6±0.6 EF	70.1±1.2 CD	67.7±0.9 B	73.3±1.0 BC	70.3±0.5 C	66.2±1.0 CD
	2	75.8±0.5 BC	73.3±0.8 A	72.4±1.1 A	75.9±0.6 AB	73.6±0.5 B	71.3±0.6 B

Different capital letters within columns indicate significant differences at $P < 0.05$ according to Tukey's HSD test. AA = *Alternaria sp.*; FS = *Fusarium sp.*; RS = *Rhizopus sp.*; AN = *Aspergillus sp.*; CC = *Cladosporium sp.*; PS = *Penicillium* sp.

Table 4. Two-way ANOVA results for the effects of fungicide, concentration, and their interaction on the antifungal index (AI, %) under laboratory conditions.

Factors		Isolates of phytopathogenic fungi					
		AA	FS	RS	AN	CC	PS
Fungicide	d.f	2	2	2	2	2	2
	F	90.1	59.8	49.9	40.8	48.5	72.5
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Levene's test	df	2	2	2	2	2	2
	F	2.0	0.100	0.421	0.352	0.609	1.435
	P-value	0.13	0.89	0.66	0.70	0.55	0.24
Concentration	df	4	4	4	4	4	4
	F	1516.3	1217.6	1038.2	738.0	1224.2	1821.8
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Levene's test	df	4	4	4	4	4	4
	F	0.97	0.037	0.452	0.178	0.576	0.753
	P-value	0.09	0.97	0.64	0.84	0.56	0.47
Fungicide × Conc.	df	8	8	8	8	8	8
	F	6.2	4.0	5.1	3.7	1.3	2.9
	P-value	<0.0001	1.7×10^{-4}	6.0×10^{-5}	4.6×10^{-4}	0.20	4.3×10^{-3}
Levene's test	df	8	8	8	8	8	8
	F	5.2	0.037	0.428	0.330	0.550	1.433
	P-value	0.42	0.88	0.65	0.72	0.58	0.24

AA= *Alternaria sp.*; FS= *Fusarium sp.*; RS= *Rhizopus sp.*; AN= *Aspergillus sp.*; CC= *Cladosporium sp.*; PS= *Penicillium* sp.

Effects of Fungicides on Lesion Severity and Disease Incidence under Screenhouse Conditions

The effects of fungicide treatments on lesion severity and disease incidence of six fungal pathogens on *Opuntia* spp. at 20 days post-inoculation are summarized in Tables 5-7. Lesion severity decreased progressively with increasing fungicide concentrations for all fungal isolates. For *Alternaria sp.* (AA), AZOXI 25 SC reduced lesion severity from 3.1 at 0.5 L/hL to 1.3 at 2 L/hL, ORTIVA TOP from 2.2 to 1.1, and AGRIZOLE from 3.3 to 1.4. *Fusarium sp.* (FS) showed lesion severity reductions from 3.3 to 1.6 (AZOXI 25 SC), 3.1 to 1.5 (ORTIVA TOP), and 3.5 to 1.7 (AGRIZOLE). For *Rhizopus sp.* (RS), lesion severity decreased from 3.2 to 2.5 (AZOXI 25 SC), 3.1 to 2.2 (ORTIVA TOP), and 3.4 to 2.8 (AGRIZOLE). For *Aspergillus sp.* (AN), lesion severity decreased from 3.5 at 0.5 L/hL to 2.7 at 2 L/hL with AZOXI 25 SC, from 3.3 to 2.5 with ORTIVA TOP, and from 3.6 to 3.0 with AGRIZOLE. *Cladosporium sp.* (CC) showed a similar reduction pattern, with lesion severity declining from 3.3 to 2.5 (AZOXI 25 SC), 3.1 2 to 2.3 (ORTIVA TOP), and 3.5 to 2.9 (AGRIZOLE) across increasing concentrations. For *Penicillium sp*. (PS), lesion severity decreased from 3.3 to 2.5 with AZOXI 25 SC, from 3.1 to 2.3 with ORTIVA TOP, and from 3.5 to 2.9 with AGRIZOLE, showing a clear dose-dependent response similar to that observed for the other fungal isolates. Disease incidence (%) also declined markedly with increasing fungicide concentration. For AA, incidence decreased from 45.3% at 0.5 L/hL AZOXI 25 SC to 3.4 % at 2 L/hL, ORTIVA TOP from 40.1 % to 1.4 %, and AGRIZOLE from 47.9% to 4.1%. *FS* incidence decreased from 50.2% to 4.1% (AZOXI 25 SC), 45.5% to 1.4% (ORTIVA TOP), and 52.3 % to 5.2% (AGRIZOLE). For RS, disease incidence was reduced from 55.2% to 6.1% with AZOXI 25 SC, 50.1% to 2.1% with ORTIVA TOP, and 58.4% to 6.3% with AGRIZOLE. AN incidence declined from 41.9% to 1.6% (AZOXI 25 SC), 35.1% to 0.8% (ORTIVA TOP), and 45.3% to 2.1% (AGRIZOLE). CC incidence decreased from 50.2% to 4.2% with AZOXI 25 SC, 45.1% to 1.4% with ORTIVA TOP, and 52.2% to 5.4% with AGRIZOLE. PS incidence was reduced from 53.9 % to 5.2 % (AZOXI 25 SC), 50.4% to 2.0% (ORTIVA TOP), and 58.1% to 6.1% (AGRIZOLE). Two-way ANOVA (Table 7) showed that both fungicide type and concentration significantly influenced lesion severity and disease incidence across all tested fungi. Lesion severity was predominantly affected by concentration for all isolates, while fungicide type had significant effects on severity for PS, CC, AN, and AA. The interaction between fungicide and concentration was generally not significant for lesion

severity, indicating mostly independent effects. In contrast, disease incidence was highly responsive to both fungicide and concentration, with extremely high F-values for concentration. Significant fungicide × concentration interactions were observed for all fungal isolates, indicating that the effectiveness of specific fungicides depended on their concentration, particularly in reducing infection rates.

A summary of the relationships between pathogen, symptom, and fungicide efficacy is presented in Table 8.

Table 5. Lesion severity on *Opuntia spp*. plants inoculated with fungal isolates and treated with fungicides at 20 days post-inoculation.

Fungicide	Concentration (L/hL)	Lesion severity (Scale 1-5) (Mean± SE)					
		AA	FS	RS	AN	CC	PS
AZOXI 25 SC	0.5	3.1±0.2 B	3.3±0.2 A	3.2±0.2 AB	3.5±0.1 AB	3.3±0.2ABC	3.3±0.1 AB
	0.8	2.1±0.2 CDE	2.3±0.2 BC	3.0±0.2 AB	3.3±0.2 ABC	3.1±0.2 BCD	3.1±0.1 BC
	1	1.6±0.2 FG	1.9±0.2 BCD	2.8±0.2 ABC	3.1±0.2 ABC	2.9±0.1 CDE	2.9±0.1 CD
	1.5	1.4±0.1 FG	1.7±0.2 CD	2.7±0.2 ABC	2.9±0.2 BCD	2.7±0.1 CDE	2.7±0.1 DE
	2	1.3±0.1 FG	1.6±0.2 D	2.5±0.2 BC	2.7±0.2 CD	2.5±0.1 DE	2.5±0.1 DE
ORTIVA TOP	0.5	2.2±0.2 C	3.1±0.2 A	3.1±0.2 AB	3.3±0.1 ABC	3.1±0.2 BCD	3.1±0.1 BC
	0.8	1.6±0.1 FG	2.1±0.2 BCD	2.9±0.1 ABC	3.1±0.1 ABC	2.9±0.2 BCD	2.9±0.1 CD
	1	1.3±0.1 FG	1.8±0.2 CD	2.7±0.2 ABC	2.9±0.1 BCD	2.7±0.1 CDE	2.7±0.1 DE
	1.5	1.2±0.1 G	1.6±0.2 D	2.5±0.2 BC	2.7±0.1 CD	2.5±0.1 DE	2.5±0.1 DE
	2	1.1±0.1 G	1.5±0.1 D	2.2±0.1 C	2.5±0.1 D	2.3±0.1 E	2.3±0.1 E
AGRIZOLE	0.5	3.3±0.1 B	3.5±0.1 A	3.4±0.2 A	3.6±0.1 A	3.5±0.2 A	3.5±0.1 A
	0.8	2.1±0.1 E	2.5±0.2 B	3.3±0.2 A	3.5±0.1 AB	3.4±0.2 AB	3.3±0.1 AB
	1	1.8±0.2 EF	2.0±0.2 BCD	3.1±0.1 AB	3.3±0.2 ABC	3.2±0.1 ABC	3.2±0.1 AB
	1.5	1.6±0.2 FG	1.8±0.2 CD	3.0±0.1 ABC	3.2±0.2 ABC	3.1±0.1 BCD	3.0±0.1 BC
	2	1.4±0.1 FG	1.7±0.2 CD	2.8±0.2 ABC	3.0±0.2 ABC	2.9±0.1 BCD	2.9±0.1 CD

Different capital letters within columns indicate significant differences at $P < 0.05$ according to Tukey's HSD test. AA = *Alternaria sp.*; FS = *Fusarium sp.*; RS = *Rhizopus sp.*; AN = *Aspergillus sp.*; CC = *Cladosporium sp.*; PS = *Penicillium* sp.

Table 6. Disease incidence (%) on Opuntia spp. plants inoculated with fungal isolates and treated with fungicides at 20 days post-inoculation.

Fungicide	Concentration (L/hL)	Disease incidence (%) (Mean± SE)					
		AA	FS	RS	AN	CC	PS
AZOXI 25 SC	0.5	45.3±0.9 B	50.2±1.0 B	55.2±0.7 B	41.9±0.6 B	50.2±0.8 A	53.9±1.0 B
	0.8	12.2±0.4 E	15.1±0.5 E	20.6±0.7 E	10.2±0.3 E	15.1±0.5 E	18.4±0.3 E
	1	8.4±0.3 G	10.2±0.4 G	15.5±0.7 F	6.4±0.4 G	10.6±0.9 FG	12.1±0.4 G
	1.5	5.2±0.3 HI	7.5±0.3 HI	10.3±0.3 H	3.2±0.3 H	6.6±0.4 I	8.3±0.3 I
	2	3.4±0.3 J	4.1±0.3 KL	6.1±0.3 J	1.6±0.2 IJ	4.2±0.2 JK	5.2±0.2 J
ORTIVA TOP	0.5	40.1±1.0 A	45.5±0.9 C	50.1±0.7 C	35.1±0.5 C	45.1±0.8 C	50.4±1.0 C
	0.8	8.3±0.3 FG	10.2±0.4 G	12.2±0.6 G	6.4±0.4 G	10.6±04 G	12.2±0.3 G
	1	5.1±0.3 HI	6.5±0.3 IJ	8.1±0.5 I	3.4±0.2 H	6.4±0.4 I	8.5±0.3 I
	1.5	3.1±0.1 J	3.5±0.3 L	5.6±0.3 J	1.6±0.2 IJ	3.3±0.2 K	5.1±0.2 J
	2	1.4±0.1 K	1.4±0.1 M	2.1±0.2 K	0.8±0.1 J	1.4±0.12 L	2.0±0.2 K
AGRIZOLE	0.5	47.9±0.6 A	52.3±0.7 A	58.4±0.7 A	45.3±0.6 A	52.2±0.8 A	58.1±0.6 A
	0.8	15.2±0.5 D	18.0±0.4 D	22.1±0.6 D	12.1±0.3 D	18.4±0.9 D	20.8±0.8 D
	1	9.8±0.3 F	12.1±0.4 F	16.5±0.4 F	8.2±0.3 F	12.2±0.3 F	15.1±0.4 F
	1.5	6.1±0.4 H	8.1±0.2 H	10.1±0.2 H	4.3±0.2 H	8.4±0.3 H	10.2±0.3 H
	2	4.1±0.3 IJ	5.2±0.3 JK	6.3±0.3 J	2.1±0.1 I	5.4±0.2 IJ	6.1±0.2 J

Different capital letters within columns indicate significant differences at $P < 0.05$ according to Tukey's HSD test. AA = *Alternaria sp.*; FS = *Fusarium sp.*; RS = *Rhizopus sp.*; AN = *Aspergillus sp.*; CC = *Cladosporium sp.*; PS = *Penicillium* sp.

Table 7. Two-way ANOVA results for the effects of fungicide, concentration, and their interaction on lesion severity and disease incidence (%) under screenhouse conditions.

Isolates	Factors	Lesion severity (s)			Disease incidence (%) (DI)			Levene's test (S)			Levene's test (DI)		
		df	F-value	P-value	df	F-value	P-value	df	F	P-value	df	F	P-value
AA	Fungicide	2	23.6	<0.0001	2	141.9	<0.0001	2	3.5	0.12	2	0.1	0.91
	Concentration	4	67.9	<0.0001	4	3854.5	<0.0001	4	2.2	0.12	4	0.1	0.98
	Fungicide × Conc.	8	1.5	0.1	8	5.7	1.0×10-6	8	4.2	0.16	8	0.1	0.94
FS	Fungicide	2	3.5	0.03	2	178.3	<0.0001	2	1.5	0.23	2	0.5	0.61
	Concentration	4	58.8	<0.0001	4	4363.5	<0.0001	4	0.2	0.85	4	0.3	0.73
	Fungicide × Conc.	8	0.1	1.0	8	2.8	4.9×10-3	8	1.8	0.17	8	0.4	0.68
RS	Fungicide	2	9.9	6.9×10-5	2	271.1	<0.0001	2	1.4	0.25	2	0.5	0.63
	Concentration	4	9.1	1.0×10-6	4	4540.7	<0.0001	4	1.6	0.20	4	0.1	0.89
	Fungicide × Conc.	8	0.1	1.0	8	7.4	<0.0001	8	0.8	0.45	8	0.3	0.72
AN	Fungicide	2	13.4	3.0×10-6	2	263.7	<0.0001	2	0.4	0.67	2	0.1	0.98
	Concentration	4	13. 04	<0.0001	4	6654.6	<0.0001	4	0.8	0.45	4	0.1	0.96
	Fungicide × Conc.	8	0.1	1.0	8	25.0	<0.0001	8	0.8	0.99	8	0.1	0.98
CC	Fungicide	2	18.9	<0.0001	2	171.6	<0.0001	2	4.3	0.14	2	0.5	0.63
	Concentration	4	13.5	<0.0001	4	3883.6	<0.0001	4	0.5	0.63	4	0.5	0.63
	Fungicide × Conc.	8	0.1	1.0	8	2.3	2.0×10-2	8	3.8	0.22	8	0.4	0.66
PS	Fungicide	2	30.2	<0.0001	2	199.5	<0.0001	2	5.1	0.60	2	0.2	0.84
	Concentration	4	24.1	<0.0001	4	4635.3	<0.0001	4	1.5	0.22	4	0.1	0.88
	Fungicide × Conc.	8	0.2	1.0	8	4.2	1.0×10-4	8	4.5	0.12	8	0.1	0.88

AA = *Alternaria sp.*; FS = *Fusarium sp.*; RS = *Rhizopus sp.*; AN = *Aspergillus sp.*; CC = *Cladosporium sp.*; PS = *Penicillium* sp.

Table 8. Summary of pathogen–symptom–fungicide efficacy on Opuntia spp. at 20 days post-inoculation.

Pathogen	Typical Symptom on Cladodes	Fungicide	Relative Efficacy
Alternaria sp.	Necrotic circular spots, tissue sunken	AZOXI 25 SC	High
		ORTIVA TOP	High
		AGRIZOLE	Moderate
Fusarium sp.	Necrotic, elongated lesions	AZOXI 25 SC	High
		ORTIVA TOP	High
		AGRIZOLE	Moderate
Rhizopus sp.	Soft rot, spreading lesions	AZOXI 25 SC	Moderate–High
		ORTIVA TOP	Moderate–High
		AGRIZOLE	Moderate
Aspergillus sp.	Dark, sunken lesions	AZOXI 25 SC	High
		ORTIVA TOP	High
		AGRIZOLE	Moderate
Cladosporium sp.	Dark green-brown coalescing spots	AZOXI 25 SC	High
		ORTIVA TOP	High
		AGRIZOLE	Moderate
Penicillium sp.	Grayish mold, surface mycelium	AZOXI 25 SC	High
		ORTIVA TOP	High
		AGRIZOLE	Moderate

High: Lesion severity ≤ 2.0 and/or disease incidence ≤ 15%; Moderate: Lesion severity 2.1–3.0 and/or disease incidence 16–30%; Low: Lesion severity > 3.0 and/or disease incidence > 30%.

All tested pathogens caused characteristic lesions on Opuntia cladodes, ranging from necrotic circular or elongated spots (*Alternaria sp*. and *Fusarium sp*.), soft rot (*Rhizopus sp*.), to dark sunken lesions (*Aspergillus*), coalescing green-brown spots (*Cladosporium sp*.), and surface mycelium (*Penicillium sp*.). Among the fungicides tested, AZOXI 25 SC and ORTIVA TOP consistently showed the highest efficacy across most isolates,

reducing both lesion severity and disease incidence, particularly at concentrations ≥1 L/hL. AGRIZOLE exhibited moderate activity, with reductions in lesion severity and disease incidence becoming more pronounced at higher concentrations (≥1.5 L/hL).

DISCUSSION

The results of the present study confirm that cladode diseases of *Opuntia spp*. are caused by a diverse complex of phytopathogenic fungi and that disease expression varies markedly according to the causal agent. The isolation of *Alternaria sp., Fusarium sp., Rhizopus sp., Aspergillus sp., Cladosporium sp., and Penicillium sp.*, sp. highlights the heterogeneity of the fungal community affecting prickly pear, consistent with previous reports describing a rich and varied mycobiota in cladodes(Ammar *et al*., 2004; Bezerra *et al*., 2012; Flores-Flores *et al*., 2013; Chavarría-Cervera *et al*., 2014). Notably, the pathogenic fungi identified here differ from previously documented endophytes in *O. ficus-indica*, highlighting the distinction between endophytic colonization and pathogenic infection (Flores-Flores *et al*., 2013). This distinction emphasizes that the mere presence of fungi in cladode tissues does not necessarily imply pathogenicity, a pattern observed in earlier studies (Bezerra *et al*., 2012; Flores-Flores *et al*., 2013; Chavarría-Cervera *et al*., 2024).Pathogenicity tests clearly demonstrated that all six isolates were capable of inducing lesions on *Opuntia* cladodes, although symptom type and aggressiveness differed among species. These findings align with numerous studies conducted in different prickly pear-growing regions worldwide, including South Africa, Egypt, Brazil, and Mexico, where cladode spot and rot diseases were attributed to a range of fungal pathogens rather than a single species (Morales and Hernández, 2002; Swart and Kriel, 2002; Swart *et al*., 2003; Ammar *et al*., 2004; de Souza *et al*., 2010). In particular, the pronounced aggressiveness of *Alternaria sp.* and *Fusarium sp.* observed in this study agrees well with previous reports describing these fungi as major causal agents of necrosis, spots, and rots on cladodes and fruits of prickly pear (Abo-El-Ela *et al*., 2001; Swart and Kriel, 2002). Similarly, *Aspergillus sp.* caused extensive black rot, whereas *Fusarium sp.* induced soft rot, illustrating that symptom expression is pathogen-specific, even when field symptoms may appear similar. These observations reinforce the concept that cladode spot is a disease syndrome caused by multiple fungal species, with variation in lesion morphology and severity influenced by pathogen identity, host variety, and environmental conditions (Turner, 1981; Abo-El-Ela *et al*., 2001).. Other fungi, such as *L. theobromae*, *C. lunata*, *C. gloeosporioides*, *F. lunatum*, *Pseudocercospora* spp., and *P. opuntiae*, have similarly been reported as causal agents of cladode spot diseases (Ayala-Escobar *et al*., 2006; Quezada-Salinas *et al*., 2006; Souza *et al*., 2010), further highlighting the multi-species nature of the disease.

The high disease incidence recorded for all isolates in the pathogenicity assays emphasizes the potential risk posed by these fungi, particularly because cladodes are widely consumed fresh. This reinforces the importance of effective disease management strategies aimed at reducing fungal populations on cladodes. Chemical control remains a commonly adopted approach, and the in vitro antifungal assays demonstrated that all tested fungicides significantly inhibited mycelial growth of the six pathogens. When analyzed according to FRAC classes, the efficacy of the tested fungicides varied according to their specific mode of action and cellular targets. QoI fungicides (FRAC group 11, e.g., AZOXI 25 SC) inhibit mitochondrial respiration by blocking electron transfer at the cytochrome bc1 complex, which disrupts ATP production and rapidly affects fast-growing necrotrophic fungi such as *Alternaria sp.* and *Cladosporium sp.* (Fraaije et al., 2021; White and Bradley, 2022). SDHI fungicides (FRAC group 7, e.g., ORTIVA TOP) target succinate dehydrogenase in the fungal mitochondrial respiratory chain, impairing energy production and showing higher activity against slow- to moderate-growing fungi such as *Fusarium sp.* and *Rhizopus sp.* (FRAC, 2024). AGRIZOLE, a multisite contact fungicide (FRAC group M02), acts on multiple cellular sites, including membrane integrity, enzyme function, and spore germination, providing moderate but consistent inhibition across all tested fungal sp. (van den Bosch et al., 2014; Kaur et al., 2024). These differences in efficacy reflect both the biochemical targets of the fungicides and the inherent susceptibility of each pathogen. FRAC-based interpretation of these results enables rational disease management, including rotation among fungicide classes to reduce resistance risk and maintain long-term effectiveness (van den Bosch et al., 2014; Ceresini et al., 2024). The observed differences among fungicides, concentrations, and fungal species are consistent with earlier studies reporting that fungal responses to fungicides vary depending on several factors, including cell wall and membrane permeability,

resistance mechanisms of the fungal cell, and the chemical composition and mode of action of the fungicides (Ammar *et al*., 2004; Flores-Flores *et al*., 2013). Similar variability in fungicide efficacy against prickly pear pathogens was reported by Abo-El-Ela *et al*. (2001), and Ammar *et al*. (2004) who noted that each pathogen reacted differently to specific fungicides and concentrations.

Under screenhouse conditions, fungicide applications significantly reduced lesion severity and disease incidence for all tested fungi, with concentration emerging as the most influential factor. These findings corroborate earlier reports showing that appropriate fungicide selection and dosing are critical for effective control of cladode and fruit rots of prickly pear (Granata and Sidoti, 2000; Swart *et al*., 2003). The significant fungicide × concentration interactions observed for disease incidence indicate that the effectiveness of a given fungicide depends strongly on its applied rate, a pattern also noted in previous studies on cactus diseases. Successful control of fungal spot and rot diseases on cacti using fungicides has been recommended by several authors, who emphasized the importance of integrating chemical control with cultural practices such as avoiding wounds, removing and destroying infected tissues, and promptly treating damaged surfaces with protective fungicides, including copper-based compounds (Chase, 1992; Abo-El-Ela *et al*., 2001).

CONCLUSION

This study confirms that cladode diseases of *Opuntia* spp. are caused by a complex of phytopathogenic fungi with variable aggressiveness and sensitivity to fungicides. Among the tested products, AZOXI 25 SC and ORTIVA TOP were the most effective in reducing lesion severity and disease incidence under the tested conditions applied, while AGRIZOLE showed moderate efficacy. These results indicate the significant sspotential of fungicide applications for managing cladode diseases in controlled environments. However, due to the diversity of causal agents and the influence of host and environmental factors, including host surface characteristics, effective disease control should rely on an integrated approach combining accurate pathogen identification, appropriate cultural practices, and judicious fungicide use. Although morphological identification provided valuable information in this study, future research should incorporate molecular tools to confirm species identity, detect cryptic pathogens, assess population structure, and better understand fungicide sensitivity under field-relevant conditions. Overall, this work provides a baseline framework for improving integrated management strategies aimed at reducing losses and ensuring the quality and safety of *Opuntia* cladodes intended for consumption.

AUTHOR CONTRUBUTIONS

Mohamed El Aalaoui: Writing - review & editing, Writing - original draft, Visualization, Resources, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Anass bella: Investigation, Formal analysis, Data curation. Abdelilah Bougamaz: Investigation, Formal analysis, Data curation. Mohamed Sbaghi: Writing - review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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