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EFFECT OF STORAGE AND CULTURE MEDIA ON PATHOGENICITY OF *Alternaria alternata* CAUSING LEAF SPOT DISEASE OF DATE PALM

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ABSTRACT

This study investigates the influence of culture media and storage duration on the growth, sporulation, and pathogenicity of *Alternaria alternata*, a pathogen causing leaf spot disease in date palms. Six culture media were assessed for fungal growth, with Potato Dextrose Agar (PDA) exhibiting the highest radial growth (9 cm) and sporulation (1.2×10^7 spores/mL), followed by Malt Extract Agar (MEA). Moderate growth occurred on Corn Meal Agar (CMA) and Czapek Dox Agar (CDA), while Sucrose Yeast Peptone Agar (SYPA) and Glucose Yeast Peptone Agar (GYPA) were less effective. After six months of storage at 4°C, PDA maintained superior fungal viability (8.5 cm radial growth and 3.1×10^6 spores/mL), with MEA performing comparably. Pathogenicity tests revealed the PDA-grown inoculum caused the highest infection (39.3 mm² infected area) on date palm leaves after six months. However, storage beyond six months significantly reduced fungal growth, sporulation, and pathogenicity. These findings highlight the importance of selecting nutrient-rich media and optimizing storage conditions to preserve fungal viability and virulence, particularly in pathogen research and agricultural management.

Keywords: Culture media, Fungal growth, Pathogenicity, Storage duration

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Alternaria alternata is a major plant pathogen responsible for leaf spot disease in a wide range of crops, including date palms (Alasadi, 2024; Jassim and Ahmed, 2024). This disease is particularly prevalent in regions where environmental conditions are conducive to fungal growth and dissemination, such as high humidity and moderate temperatures (Rotem, 1994; Gottwald *et al.*, 1997; Timmer *et al.*, 2000). Leaf spot diseases, including those caused by *Alternaria* species, are commonly observed in date palm-growing regions worldwide, with disease severity increasing with the age of the palm (Abdalla *et al.*, 2001; Fayad and Mania, 2006).

The impact of leaf spot disease is both economically and ecologically significant. In particular, regions such as the Arabian Peninsula, which accounts for approximately 34% of global date production, face substantial risks from this disease. Beyond the economic implications of reduced date yield and quality, the disease also threatens the ecological stability of arid and semi-arid regions, where date palms play a crucial role in soil stabilization and habitat provision. *A. alternata* is a cosmopolitan pathogen, and its virulence on date palms is exacerbated in regions with environmental conditions favourable to its proliferation (Manikandan *et al.*, 2025). Recent studies in Iraq have demonstrated that *A. alternata* isolates can be molecularly identified and their pathogenicity assessed, confirming their significant role in causing damage to date palm foliage (Abdul-Hassan *et al.*, 2019).

The preservation of fungi in laboratory settings is essential for conducting precise scientific experiments and long-term studies. The primary goal of preservation is to maintain the biological and behavioural properties of fungal strains without significant alteration (Homolka, 2014; Smith and Ryan, 2012). However, this process poses several challenges, as factors such as storage duration and the type of medium used can significantly affect fungal activity and pathogenicity (Leslie and Summerell, 2006).

Various culture media are employed for fungal storage, with cryopreservation in liquid nitrogen (below -130°C) being one of the most effective methods for ensuring the stability and viability of fungal isolates. For example, research has demonstrated that cryopreservation successfully preserves the biological and molecular characteristics of fungi over extended periods (Nakasone *et al.*, 2004). In addition, some studies have suggested that perlite can serve as a viable long-term preservation medium at low temperatures (4°C) (Homolka and Lisá, 2008).

Furthermore, studies have highlighted the efficacy of using 50% glycerol as a preservation medium for fungal isolates at 4°C, reporting high recovery rates ranging from 86.66% to 100% after 24 to 30 months of storage (Paul *et al.*, 2015).

The present study aims to investigate the effects of different storage durations and commonly

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used laboratory culture media on the activity and pathogenicity of *Alternaria alternata*. These media were chosen based on their widespread use in laboratories for fungal culture and preservation. Each medium offers a unique nutrient composition, which may influence fungal survival in different ways over time.

MATERIALS AND METHODS

Effect of different culture media on *Alternaria alternata* growth

Alternaria alternata (Accession number, PP330024) grown on Potato Dextrose Agar (PDA) medium was used for this study. Six solid culture media were evaluated for their effect on fungal growth: Corn Meal Agar (CMA) (cornmeal 50 g, agar 15 g), Czapek Dox Agar (CDA) (sucrose 30 g, sodium nitrate 2 g, dipotassium phosphate 1 g, magnesium sulfate 0.5 g, potassium chloride 0.5 g, ferrous sulfate 0.01 g, agar 15 g), Glucose Yeast Peptone Agar (GYPA) (glucose 5 g, peptone 5 g, yeast extract 3 g, agar 20 g), Malt Extract Agar (MEA) (malt extract 30 g, agar 20 g), Potato Dextrose Agar (PDA) (20 g dextrose, 20 g agar, 200 g sliced potato), and Sucrose Yeast Peptone Agar (SYPA) (sucrose 5 g, peptone 5 g, yeast extract 3 g, agar 20 g).

A 5 mm diameter plug of fungal mycelium was inoculated onto the center of Petri dishes containing each of the autoclaved culture media. All Petri dishes were incubated at 25°C for 7 days, after which radial growth and sporulation were recorded. The experiment was repeated twice, with three replicates per medium.

Storage and monitoring of fungal cultures

In this experiment, Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) culture media were used. Glass vials containing 10 ml of autoclaved PDA and MEA were individually inoculated with *Alternaria alternata*. The inoculated vials were then stored in a refrigerator at 4°C for two different storage periods: six months and one year. Six replicates were used for each storage period. To evaluate fungal viability, a 5 mm plug of each stored fungus was transferred to Petri dishes containing fresh PDA medium and incubated at 25°C. Radial growth and sporulation were subsequently measured to assess the fungal growth capability after storage.

Pathogenicity assessment

Pathogen-free date palm leaves were collected and brought to the laboratory, where they were cut into 10 cm long pieces. The leaves were then washed with tap water and sterilized using 10% sodium hypochlorite. A 5 mm plug of fungal colony, grown on PDA and MEA media for two different storage periods, was placed on the surface of each sterilized leaf. The leaves were individually wrapped with parafilm to prevent contamination. The control group was inoculated with a 5 mm plug of PDA alone. All inoculated leaf pieces were placed into autoclaved containers containing sterilized filter paper moistened with sterile water and incubated at 25°C for 10 days under a 12:12:: L:D cycle. The development of fungal infection was monitored daily. The infection area was measured using ImageJ software, which determined the infected percentage by comparing the total leaf area to the infected area, calculated using the following formula:

$$\text{Infection percentage} = \frac{(\text{Number of infected leaves})}{(\text{Total number of leaves})} \times 100$$

Statistical analysis

Data analysis was performed using SPSS software. One-way and two-way ANOVA were performed to evaluate the effects of different variables on the outcomes, and Least Significant Difference (LSD) tests were used to separate the means at a significance level of 0.05.

RESULTS AND DISCUSSION

Effect of different culture media on *Alternaria alternata* growth

The results demonstrated significant differences in fungal growth across the various culture media ($p<0.05$). The greatest radial growth of *A. alternata* was observed on Potato Dextrose Agar (PDA), with a diameter of 9 cm, followed by Malt Extract Agar (MEA) at 8.4 cm. Moderate growth was recorded on Corn Meal Agar (CMA) and Czapek Dox Agar (CDA), with radial growths of 6 cm and 5.6 cm, respectively. The lowest radial growth was observed on Synthetic YpS (SYPA) and Glycerol Yeast Extract Agar (GYPA), with growths of 5 cm and 4.5 cm, respectively. In terms of fungal sporulation, the highest spore concentrations were observed on PDA and MEA, which produced 1.2×10^7 spores/ml and 1×10^7 spores/ml, respectively. On the other hand, the lowest sporulation was recorded on CMA, with a spore concentration of 4.9×10^4 spores/ml (Table 1). These findings suggest that PDA and MEA are nutrient-rich media, providing essential compounds such as sugars and carbohydrates that support both fungal growth and reproduction. In contrast, SYPA and GYPA are nutrient-poor, making them less conducive to fungal growth and sporulation.

However, in some cases, certain culture media may support fungal radial growth without promoting sporulation, as observed with Corn Meal Agar (CMA). The results indicated that CMA supported moderate fungal radial growth but resulted in the lowest

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sporulation. This phenomenon could be attributed to the fact that fungal growth and spore production are not always directly linked and may be influenced by various environmental and structural factors. Several studies have reported that Potato Dextrose Agar (PDA) is the most effective medium for both radial growth and spore production of *A. alternata* compared to other tested media (Apet *et al.*, 2014; Kamei and Singh, 2020), with Malt Extract Agar (MEA) following closely behind (Apet *et al.*, 2014).

Table 1. Effect of different culture media on the radial growth and sporulation of *Alternaria alternata*

Culture media	Radial growth (cm)	Sporulation (spores/ ml)
CDA*	5.6^{d}	3.8 × 10^{5}
CMA	6.0^{c}	4.9 × 10^{4}
GYPA	4.5^{f}	2.2 × 10^{5}
MEA	8.4^{b}	1.0 × 10^{7}
PDA	9.0^{a}	1.2 × 10^{7}
SYPA	5.4^{e}	3.2 × 10^{5}

*CDA = Czapek Dox Agar, CMA = Corn Meal Agar, GYPA = Glucose Yeast Peptone Agar, MEA = Malt Extract Agar, PDA = Potato Dextrose Agar, SYPA = Sucrose Yeast Peptone Agar

Within a column values followed by same alphabet did not differ significantly at *p≤0.05.*

Storage and monitoring of fungal cultures

The results revealed a significant effect of both culture media and storage duration on the radial growth and sporulation of *A. alternata*. The highest radial growth (8.5 cm) and sporulation (3.1 × 10^{6} spores/ ml) were observed on Potato Dextrose Agar (PDA) after six months, followed by Malt Extract Agar (MEA), which recorded 7.8 cm and 1.1 × 10^{6} spores/ml for the same period. This finding is consistent with the work of Espinel-Ingroff *et al.* (2004), who demonstrated that PDA supports superior fungal growth and viability, likely due to its ability to maintain nutrient stability over extended periods. In contrast, both radial growth and sporulation were reduced after one year of storage. On PDA, radial growth was recorded at 7 cm, with sporulation at 1.4 × 10^{5} spores/ml, while on MEA, radial growth was 6.5 cm and sporulation was 7.9 × 10^{4} spores/ml. These observations are in agreement with Homolka and Lisá (2008), who reported that prolonged storage, particularly beyond one year, can result in nutrient depletion or the accumulation of metabolic by-products, thereby reducing fungal viability and sporulation capacity, especially in less nutrient-rich media such as MEA (Table 2).

Pathogenicity assessment

The results demonstrated a significant effect of culture media, storage time, and their interaction on the pathogenicity of *A. alternata*. The largest infected area (39.3 mm²) was observed on leaves inoculated with the fungus grown on Potato Dextrose Agar (PDA) after six months, compared to the inoculum grown on Malt Extract Agar (MEA), which resulted in a smaller infected area of 23.4 mm² (Fig. 1). The heightened pathogenicity of the PDA-grown inoculum aligns with previous studies highlighting the medium's ability to sustain fungal virulence factors, as noted by Paul *et al.* (2015), who found that glycerol-stabilized media similarly preserved fungal pathogenicity. Furthermore, nutrient-rich media like PDA are known to support enhanced fungal growth and pathogenicity, as demonstrated by Senanayake *et al.* (2020) and Vinzelj *et al.* (2022).

In contrast, the infection areas were reduced when leaves were inoculated with *A. alternata* grown on PDA and MEA for one year, measuring 32.5 mm² and 17.2 mm², respectively. This decline in pathogenicity may be attributed to nutrient degradation in the media or depletion of essential elements over time, as suggested by Paul *et al.* (2015). Their findings indicated that nutrient-rich media like PDA can sustain fungal activity for longer periods compared to simpler media such as MEA.

Regarding the interaction between culture media type and storage time, the results indicated that PDA medium exhibited superior support for fungal growth across both storage periods, while MEA showed a decline in its ability to support fungal growth after one year. The superior performance of PDA compared to MEA can likely be attributed to its richer nutrient content, which provides the necessary energy for fungal growth. The observed decrease in the infection area when using *A. alternata* inoculum grown on culture media for one

Table 2. Effect of various culture media and storage periods on the radial growth and sporulation of *Alternaria alternata*

Culture media	Storage period	Radial growth (cm)	Sporulation (spores/ ml)
PDA*	Six months	8.5^{a}	3.1× 10^{6}
PDA	1 Year	7.0^{c}	1.4× 10^{5}
MEA	Six months	7.8^{b}	1.1× 10^{6}
MEA	1 Year	6.5^{d}	7.9× 10^{4}

*PDA = Potato Dextrose Agar, MEA = Malt Extract Agar
Values with the same letter are not significantly different at *p≤0.05*

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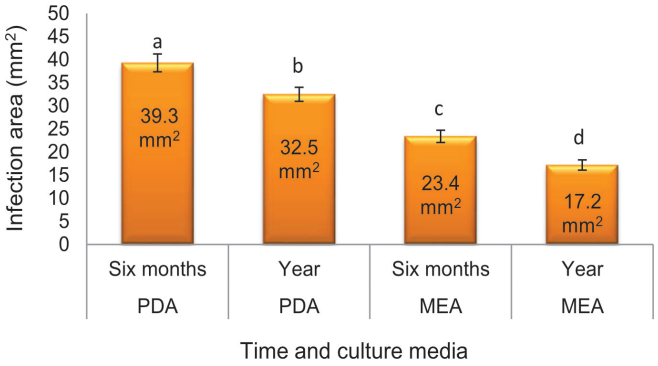

Fig. 1. Effect of storage time and culture media on the pathogenicity of *Alternaria alternata*
PDA = Potato Dextrose Agar, MEA = Malt Extract Agar
Six months and a year are the two storage periods

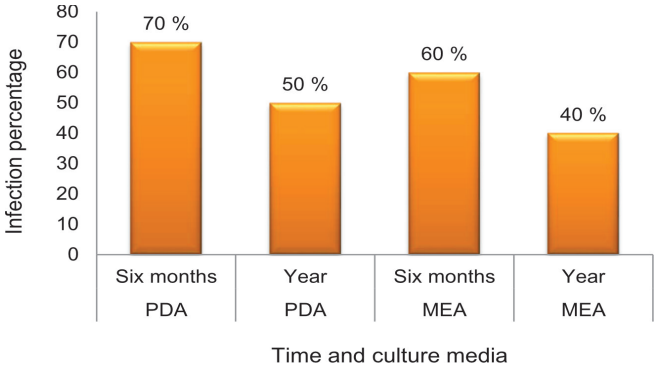

Fig. 2. Effect of Time and Culture Media on Infection Percentage of *Alternaria alternata*
PDA = Potato Dextrose Agar, MEA = Malt Extract Agar
Six months and a year are storage periods

year may be due to nutrient degradation or depletion over time, negatively affecting fungal activity.

Furthermore, the inoculum grown on PDA and MEA for six months resulted in higher infection percentages on leaves, recorded at 70% and 60%, respectively. After one year, the activity of the inoculum declined to 50% and 40%, respectively (Fig. 2). This reduction in infection potential is consistent with long-term storage studies, such as those by Espinel-Ingroff *et al.* (2004), which highlighted that nutrient availability and storage conditions significantly influence fungal pathogenicity and infection potential over time. Additionally, long-term storage of fungi may lead to a deficiency in pathogenic factors (e.g., enzymes and toxins) necessary for penetrating plant tissues and causing infection, likely due to fungal stress or reduced vitality. These findings align with Liu *et al.* (2024), who reported that long-term storage can result in DNA damage and a reduction in enzyme activity within spores, further compromising fungal pathogenicity.

CONCLUSION

This research demonstrates the influence of culture media type and storage duration on the growth, sporulation, and pathogenicity of *Alternaria alternata*. Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were identified as the most effective media, with PDA exhibiting the highest radial growth and sporulation, likely due to its nutrient-rich composition. Storage for six months preserved fungal viability and infection potential, especially on PDA. However, storage beyond six months, particularly for one year, led to reductions in growth, sporulation, and pathogenicity. Pathogenicity tests further revealed that inoculum grown on PDA resulted in higher infection rates compared to MEA. This study highlights the importance of selecting nutrient-rich media and optimizing storage conditions to sustain fungal growth and virulence, with PDA emerging as the most reliable medium for *A. alternata*.

Looking forward, future research should explore advanced fungal preservation techniques, such as cryopreservation, freeze-drying, and encapsulation, to maintain long-term viability and pathogenicity. A comparative evaluation of these preservation methods across different fungal species would contribute to the development of reliable storage techniques, benefiting research, agriculture, and industry.

Authors' contribution

Conceptualization of research work (RMSA); Data collection (RMSA); Statistical analysis (RMSA); Analysis of data and interpretation (RMSA); Review of manuscript (RMSA); Preparation of manuscript (RMSA).

Conflicts of interest

The author declares no conflicts of interest.

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